



UNIVERSITAT
POLITÈCNICA
DE VALÈNCIA

DOCTORAL PROGRAMME
IN BIOTECHNOLOGY

Doctoral thesis

Deciphering Mip6 multifaceted role across eukaryotic gene expression

Ana Tejada Colón

Supervisor:
Susana Rodríguez Navarro

Tutor:
Lynne Paula Yenush

September, 2024



INSTITUTO DE
BIOMEDICINA DE
VALENCIA CSIC

This thesis was accomplished thanks to a predoctoral fellowship (ACIF/2019/212) granted by the Conselleria de Educació, Cultura, Universitats y Empleo from the Generalitat Valenciana, and a predoctoral contract associated with the project funded by AICO/2020/296, also given by the Conselleria de Educació, Cultura, Universitats y Empleo from the Generalitat Valenciana. All the work described here was performed in the Instituto de Biomedicina de Valencia (IBV-CSIC).

An international research stay was carried out in Rowan University School of Osteopathic Medicine, in Stratford, New Jersey (USA) under the supervision of Dr. Randy Strich. The international research stay was accomplished thanks to a pre-doctoral fellowship for research stays outside the Valencian Community (CIBAFP/2021/49), also granted by the Conselleria de Educació, Cultura, Universitats y Empleo from the Generalitat Valenciana.



INSTITUTO DE
BIOMEDICINA DE
VALENCIA CSIC



ROWAN UNIVERSITY
School of
Osteopathic Medicine

AGRADECIMIENTOS





Hace ya casi seis años que mi camino se cruzó con el del laboratorio de Expresión Génica y Metabolismo de RNA. Primero, como estudiante de TFM y, un año más tarde, con esta gran aventura que ha sido la tesis doctoral. La verdad es que todavía me cuesta pensar que esta etapa ya está llegando a su fin. Y eso que hace ya unos meses que la situación no es la misma. Han sido seis años en los que han pasado infinidad de cosas, teniendo en cuenta que ni siquiera llevaba un año de tesis cuando llegó una pandemia mundial. Seis años de trabajo, de aprender, de mejorar, como científica y como persona, pero también de risas, de fiestas, de amistades. Seis años de subidas y de bajadas. Pero lo más importante de todo es que siempre he tenido el apoyo de toda la gente que ha querido acompañarme en esta aventura.

La primera persona a la que quiero dar las gracias es a mi directora de tesis. Gracias, Susana, por abrirme las puertas del laboratorio y ofrecerme la oportunidad de hacer el doctorado aquí. Cuando solicité la ayuda para la tesis, aún no había comenzado mi TFM en el laboratorio, así que no tenía claro si aquello terminaría siendo una buena o una mala idea. Pero apenas unos meses después comprendí que no podría haber elegido mejor grupo. Así que gracias, no solo por haberme acogido, sino también por todo lo que he podido aprender y crecer estando aquí.

Por supuesto, todo esto no hubiera sido lo mismo sin mis compañeros de viaje. Los que se han ido, los que han llegado a mitad de camino y los que han estado siempre. Carme y Joan, me alegro muchísimo de haber compartido esta aventura con vosotros de principio a fin. Esta tesis tiene mucho vuestro. Muchas de las técnicas, análisis y razonamientos que he adquirido en este viaje os lo debo a vosotros. Se me hizo hasta raro terminar enseñándoos yo cosas en la recta final, con todo lo que he aprendido yo gracias a vosotros. Y no solo en lo laboral. Os habéis convertido también en muy buenos amigos. No habría podido tener mejores compañeros. Lola, empezamos prácticamente a la vez, dos novatas que apenas sabían usar una pipeta. Y mira a dónde estamos ahora. Y todo lo que nos queda por delante. ¿Ahora quién va a ser mi compañera de congresos? No sé si voy a encontrar una tan buena como la que tenía. Ali, mi madre del laboratorio. No sabes cuánto me alegro del día que decidiste mudarte a nuestro grupo. Recuerdo cuando decías que os tenía que dejar todo bien enseñado antes de irme de estancia. Y al final has sido tú la que



AGRADECIMIENTOS

me has enseñado muchísimo más. Gracias a ti también comencé en el maravilloso mundo del coro. No sabes lo feliz que me hacía ese ratito a la semana dedicado a la música. Muchísimas gracias también por eso. Malena, aún no entiendo lo rápido que congeniamos con la mala primera impresión que tuve que darte. Supongo que el estar lado a lado en el laboratorio ayudó. Gracias por dejarme ser un poco pesada con la música pop y por las mil veces que te he robado las pipetas. Gracias en general a todos por las risas, los viajes, las fiestas, las cenas, los almuerzos, los planes, y por dejarme cantar mientras trabajaba. Y a todos los que han pasado por el laboratorio durante estos seis años: Lolo, Jesús, Clara, Afef, JR, Sergi, Andrea, Bianca, Marta, Camille. Todos, de una u otra forma, habéis contribuido a esto.

Quiero agradecer también a los doctores Jerónimo Bravo y José Manuel Pérez Cañadillas por las colaboraciones conjuntas que han enriquecido esta tesis. A la doctora Ethel Queralt y a todo el laboratorio de División Celular y Cohesinopatías por todo el *feedback* recibido en los seminarios conjuntos. Y a todas las personas que trabajan en el IBV, ya sea por los seminarios, incluidos los *juniors*, las jornadas de resultados, los planes después del trabajo de la CDF o simplemente las charlas por los pasillos o en el comedor.

Gracias también al doctor Randy Strich, por abrirme las puertas de su laboratorio en Rowan University durante los tres meses que estuve allí de estancia. Prácticamente todo lo que sé sobre esporulación es gracias a él. Gracias a todo su grupo, por acogerme y ayudarme en todo lo que necesitaba, en especial a Brittany y, sobre todo, a Alicia. Es un placer llegar a un grupo nuevo con gente como vosotras. Y no me olvido de todos aquellos que, fuera del ámbito científico, hicieron que, a pesar de estar a miles de kilómetros lejos de casa, mi estancia en Philadelphia fuera todo un sueño americano. Gracias a Mia, María, Jesús, Irati, Jenn, María, Jaz, Fernanda, Sebas y todos los del Spanish group.

Y, por supuesto, no puedo dejar de mencionar a mi segunda familia, la que se elige, que, aunque ellos no hayan tenido tanto peso científico, me han ayudado muchísimo a despejarme en los momentos duros que, desgraciadamente, vienen en todas las etapas de la vida. Gracias a Raquel y a Bea, con las que yo creo que más tiempo he compartido en estos años, y a Cristian, a María y



a Beatriz. Gracias por eurovisión, las preparties, los viajes, las cenas, las verbenas, las noches en Picca y los mil momentos que hemos compartido juntas. Me alegra haber coincidido en el master y, mucho más, haber hecho por seguir juntas después (y a los nuevos integrantes en nuestro tan exclusivo grupo que se han ido uniendo por el camino). Gracias a Rubén y a Isabel, mis papis adoptivos. Os juro que no sé qué hubiera hecho este tiempo en Valencia sin vosotros. Me habéis apoyado siempre que lo he necesitado y a veces siento que ni en toda la vida seré capaz de devolvéroslo. La Ana de veinte años que se fue de Erasmus a Rennes no es consciente de todo lo que va a ganar con esa experiencia. Y, por último, a mis piñas, que, a pesar de no estar en Valencia, se han sentido igual de cerca. Nunca imaginé que una amistad surgida en la distancia pudiera aportarme tanto. Prometo que, ahora que esta locura de la escritura ha llegado a su fin, haré un intento por volver a darle a las teclas. Echo de menos las historias que creamos juntas.

Para terminar, mil gracias a mi familia y, sobre todo, a mi madre. Sé que no lo digo a menudo, pero estaré siempre agradecida por teneros. Me habéis apoyado siempre en cualquier nueva aventura que haya decidido vivir, aunque eso implicase irme a vivir a otra comunidad autónoma, a otro país o incluso a otro continente. Pensaba que volver a casa después de tantos años quizás no iba a ser tan buena idea, pero no sabía lo equivocada que estaba. Gracias por estar ahí siempre que lo he necesitado.

ABSTRACT





ABSTRACT

The interconnection of the different steps that comprise gene expression is essential for the regulation of the process. Mip6 is a protein that participates in some of these steps, regulating the expression of the stress-response genes dependent on Msn2/4. Mip6 shuttles between the nucleus and the cytoplasm due to its interaction with Mex67. Therefore, under optimal conditions, Mip6 binds to the Msn2/4-dependent transcripts, transports them to the cytoplasm, and participates in keeping their levels low. However, upon stress, Mip6 rather interact with ribosomal protein transcripts and accumulates in stress granules, so that the stress response mRNAs are metabolized to perform their function.

In this work, we study Mip6 role in gene expression under both optimal and stress conditions, focusing on its participation in transcription regulation. Besides, Mip6 Mex67-independent export pathways were also characterized.

We demonstrated genetic interactions between Mip6 and Msn2/4 under both severe heat stress and oxidative stress. The growth of the *msn2Δmsn4Δmip6Δ* mutant is affected in both conditions, suggesting the participation of Mip6 in a different cell survival mechanism than Msn2/4. Besides, Mip6 interacts with histone deacetylase Rpd3 both physically and genetically, and Rpd3 is essential for Mip6's ability to regulate the levels of stress response transcripts. This suggests that Mip6 functions as a negative regulator of Rpd3, given that their effects on transcript regulation are opposite.

Additionally, we studied yeast transcriptional memory and the ability of yeast cells to respond to a second heat stress exposure characterizing both WT and *mip6Δ*. Both strains show two-thirds of the genome differentially expressed in a first and a second heat exposure, presenting both strains differences in the expression of a certain subset of genes.

A putative Nuclear Export Signal (NES) in Mip6 RRM4 was already suggested to be a Mex67-independent export pathway. However, its functionality was unclear, since point mutations in

the sequence also lead to an impairment in the Mip6-Mex67 interaction which could indirectly affect Mip6 export. Here, we characterized Mip6 NES export signal, demonstrating its capacity to transport Mip6 from nucleus to cytoplasm under both optimal and stress conditions. We also studied cysteine residues that surround this NES sequence and C₄₅₀ functionality. However, the homologous sequence present in Pes4 RRM4, a Mip6 paralogous protein, does not participate in Pes4 export, but it seems to be important for Pes4 localization in cytoplasmic granules. In addition, we found a second NES sequence in the Mip6 C-terminal domain, showing its role in Mip6 transport upon H₂O₂-induced oxidative stress.

Finally, the formation of stress granules was also studied, focusing on the importance of some intrinsically disordered regions in Tif4631 N-terminal domain. The deletion of both RNA1 and BOX1 regions affects Tif4631 levels and cell growth during the diauxic shift, which suggests the importance of these regions in Tif4631 function during the diauxic shift and, likely, with the formation of stress granules.



RESUMEN

La conexión entre las diferentes etapas que comprenden la expresión génica es esencial para la regulación del proceso. Mip6 es una proteína que participa en varias de estas etapas, regulando la expresión de los genes de respuesta a estrés dependientes de Msn2/4. Mip6 viaja entre el núcleo y el citoplasma gracias a su interacción con Mex67. De esta forma, en condiciones óptimas, Mip6 se une a los transcritos dependientes de Msn2/4, los transporta hacia el citoplasma y participa en mantener sus niveles bajos. Sin embargo, en condiciones de estrés, Mip6 se une preferentemente a los transcritos de proteínas ribosomales y se acumula en gránulos de estrés. Así, los mRNA de respuesta a estrés se metabolizan para ejercer su función.

En este trabajo, se ha estudiado el papel de Mip6 en la expresión génica tanto en condiciones óptimas como en condiciones de estrés, centrándonos en su participación en la regulación de la transcripción. Además, también se han caracterizado otras vías de exporte de Mip6 independientes de Mex67.

Se han demostrado que Mip6 y Msn2/4 interaccionan genéticamente tanto en condiciones de estrés térmico severo como en estrés oxidativo. El crecimiento del mutante *msn2Δmsn4Δmip6Δ* está afectado en ambas condiciones, lo que sugiere que Mip6 participa en un mecanismo de supervivencia celular diferente del de Msn2/4. Además, Mip6 interacciona con la histona desacetilasa Rpd3 física y genéticamente y la presencia de Rpd3 es esencial para el papel de Mip6 en la regulación de los niveles de los transcritos de respuesta a estrés. De esta forma, Mip6 actuaría como un regulador negativo de Rpd3, puesto que sus efectos en la regulación de los niveles de los transcritos son opuestos.

Por otro lado, también se ha estudiado la memoria transcripcional en levaduras y la habilidad de las células de responder a una segunda exposición a estrés térmico. Se ha caracterizado tanto la cepa WT como el mutante *mip6Δ*. En ambas cepas, se ha observado que dos tercios del genoma se expresa diferencialmente dependiendo de si se trata de una primera o una segunda exposición



ABSTRACT

a estrés térmico. Además, un determinado grupo de genes también presentan diferencias de expresión entre WT y *mip6Δ*.

Existe una posible señal de exporte nuclear (NES) en el RRM4 de Mip6 que se había sugerido como una vía de exporte independiente de Mex67. Sin embargo, su funcionalidad no estaba clara, dado que al mutar dicha secuencia afectaba a la interacción entre Mip6 y Mex67 y, por tanto, podría afectar al exporte de Mip6 de forma indirecta. En este trabajo, se ha caracterizado la NES de Mip6 y se ha demostrado su capacidad de transportar a Mip6 entre el núcleo y el citoplasma tanto en condiciones óptimas como en estrés. También se han estudiado unos residuos de cisteínas que rodean la secuencia NES y, en concreto, la funcionalidad del residuo C₄₅₀. Por otro lado, la secuencia homóloga presente en el RRM4 de Pes4, una proteína paróloga de Mip6, no participa en el exporte de Pes4, pero parece que es importante para la localización de Pes4 en gránulos citoplasmáticos. Además, se ha encontrado una segunda secuencia NES en el dominio C-terminal de Mip6 que participa en el transporte de Mip6 en estrés oxidativo inducido por H₂O₂.

Por último, también se ha estudiado la formación de los gránulos de estrés. En concreto, la importancia de algunas regiones intrínsecamente desordenadas del dominio N-terminal de Tif4631. La delección de las regiones RNA1 y BOX1 afectan los niveles de Tif4631 y el crecimiento celular durante el cambio diáuxico. Esto sugiere la importancia de estas regiones para la función de Tif4631 durante el cambio diáuxico y, probablemente, en la formación de gránulos de estrés.



RESUM

La connexió entre les diferents etapes que comprenen l'expressió gènica és essencial per a la regulació del procés. Mip6 és una proteïna que participa en diverses d'aquestes etapes, regulant l'expressió dels gens de resposta a estrés dependents de Msn2/4. Mip6 viatja entre el nucli i el citoplasma gràcies a la seua interacció amb Mex67. D'aquesta forma, en condicions òptimes, Mip6 s'uneix als transcrits dependents de Msn2/4, els transporta cap al citoplasma i participa en mantindre els seus nivells baixos. Malgrat això, en condicions d'estrés, Mip6 s'uneix preferentment als transcrits de proteïnes ribosomals i s'acumula en grànuls d'estrés. D'aquesta manera, els mRNA de resposta a estrés són metabolitzats per a exercir la seua funció.

En aquest treball, s'ha estudiat el paper de Mip6 en l'expressió gènica, tant en condicions òptimes com en condicions d'estrés, en concret en la seua participació en la regulació de la transcripció. A més a més, també s'han caracteritzat altres vies d'exportació de Mip6 independents de Mex67.

S'ha demostrat que Mip6 i Msn2/4 interaccionen genèticament tant en condicions d'estrés tèrmic agut com en estrés oxidatiu. El creixement del mutant *msn2Δmsn4Δmip6Δ* es veu afectat en ambdues condicions, suggerint que Mip6 participa en un mecanisme de supervivència cel·lular diferent a Msn2/4. A més a més, Mip6 interacciona amb la histona desacetilasa Rpd3 física i genèticament i la presència de Rpd3 és essencial per al paper de Mip6 en la regulació dels nivells dels transcrits de resposta a estrés. D'aquesta manera, Mip6 actuaria com un regulador negatiu de Rpd3, ja que els seus efectes en la regulació dels nivells dels transcrits són oposats.

D'altra banda, també s'ha estudiat la memòria transcripcional en llevat i l'habilitat de les cèl·lules per a respondre a una segona exposició a estrés tèrmic. S'ha caracteritzat tant la soca WT com la mutant *mip6Δ*. En ambdues, s'ha observat que dos terços del genoma s'expressa diferent depenent de si es tracta de una primera o una segona exposició a estrés tèrmic. A més, un determinat grup de gens també presenten diferències d'expressió entre WT i *mip6Δ*.



ABSTRACT

Existeix una possible senyal d'exportació nuclear (NES) en el RRM4 de Mip6 que s'havia suggerit com una via d'exportació independent de Mex67. Malgrat això, la seua funcionalitat no era del tot clara ja que mutar aquesta seqüència afectava la interacció entre Mip6 i Mex67 i, per tant, podia afectar a l'exportació de Mip6 de manera indirecta. En aquest treball, s'ha caracteritzat la NES de Mip6 i s'ha demostrat la seua capacitat per a transportar Mip6 entre el nucli i el citoplasma tant en condicions òptimes com en estrès. També s'han estudiat uns residus de cisteïna que rodegen la seqüència NES i, en concret, la funcionalitat del residu C₄₅₀. Per tant, la seqüència homòloga present en el RRM4 de Pes4, una proteïna paràloga de Mip6, no participa en l'exportació de Pes4, però sembla que és important per a la localització de Pes4 en grànuls citoplasmàtics. A més a més, s'ha trobat una segona seqüència NES en el domini C-terminal de Mip6 que participa en el transport de Mip6 en estrès oxidatiu induït per H₂O₂.

Per últim, també s'ha estudiat la formació de grànuls d'estrès. En concret, la importància d'algunes regions intrínsecament desordenades del domini N-terminal de Tif4631. La deleció de les regions RNA1 i BOX1 afecten els nivells de Tif4631 i el creixement cel·lular durant el canvi diàuxic. Açò suggereix la importància d'aquestes regions per a la funció de Tif4631 durant el canvi diàuxic i, probablement, en la formació de grànuls d'estrès.

INDEX





ABBREVIATIONS	25
INTRODUCTION.....	31
Eukaryotic gene expression.....	33
Histone post-translational modifications.....	33
RNA polymerase II-mediated transcription	37
Transport between the nucleus and the cytoplasm.....	41
Translation.....	44
Mip6, a protein participating in gene expression at different levels	46
Cellular response to stress	52
Stress granules formation.....	56
Transcriptional memory	58
Sporulation	62
OBJECTIVES.....	67
MATERIALS AND METHODS	71
MATERIALS.....	73
Yeast strains	75
Plasmids.....	77
Oligos	79
Antibodies.....	82
Commercial kits.....	83
METHODS	85
Microbiological techniques	87
Bacteria culture and transformation.....	87
Yeast culture and transformation	88
Stress treatments	90
Sporulation.....	91
Growth assays	92
Nucleic acids techniques	93
Polymerase Chain Reaction (PCR).....	93
PCR products purification.....	94
Plasmid isolation (Miniprep)	94
Genomic DNA extraction	94
Agarose DNA/RNA electrophoresis.....	95
Site-directed mutagenesis	95
RNA extraction	98
cDNA synthesis	99
Quantitative PCR.....	99
RNA-seq	99
Protein techniques	100



Whole cell extracts	100
Protein immunoprecipitation (GFP-TRAP).....	101
TAP purification.....	102
Coomassie blue dyeing	103
Mass spectrometry for protein identification	103
Western blotting.....	103
Other techniques.....	104
Confocal microscopy	104
Statistics.....	105
CHAPTER I: Mip6 ROLE IN GENE EXPRESSION UNDER OPTIMAL AND STRESS CONDITIONS.....	109
Background	111
Mip6 interaction with the transcriptional machinery	112
Mip6 physically interacts with the RNAPolII subunit Rpb1.....	112
Inactivation of the RNAPolII subunit Rpb1 does not affect the Mip6-Mex67 interaction ...	113
Mip6 role in the metabolism of transcripts depending on the transcription factors Msn2 and Msn4.....	114
Growth under optimal conditions does not require the coexistence of Mip6 and transcription factors Msn2 and Msn4.....	114
<i>MSN2</i> and <i>MSN4</i> genetically interact upon heat stress	115
The triple mutant <i>msn2Δmsn4Δmip6Δ</i> is sensitive to severe heat stress and oxidative stress	115
No genetic interaction between <i>MIP6</i> , <i>MSN2</i> and <i>MSN4</i> is observed in cells growing using glycerol as the carbon source.....	119
Crosstalk between Mip6 and HDAC Rpd3 and Hda1	120
Mip6 physically interacts with histone deacetylase Rpd3 which is dispensable for its interaction with Mex67.....	120
Rpd3 and Gcn5 are dispensable for Mip6-Mex67 interaction	122
<i>MIP6</i> genetically interacts with <i>RPD3</i> and <i>HDA1</i> under optimal conditions and heat stress	122
Oxidative stress specifically affects the survival of <i>hda1Δmip6Δ</i> mutant.....	123
Analysis of the synergistic effect of HDAC deletion with <i>mip6Δ</i> in the expression levels of <i>HSP12</i> and <i>CTT1</i>	127
Mip6 role in transcriptional memory	129
Ribosomal Protein (RP) genes are sensitive to transcriptional memory upon heat shock at 39°C and Mip6 presence contributes to it.....	129
Global analysis of transcriptional memory upon mild heat shock	131
CHAPTER II: CHARACTERIZATION OF Mip6 EXPORT PATHWAYS FROM THE NUCLEUS TO THE CYTOPLASM	135
Background	137

Point mutations in the putative NES in the RRM4 of Mip6 affect the Mip6-Mex67 interaction.....	138
Mip6 does not physically interact with karyopherin Msn5.....	138
Point mutations in the NES _{RRM4} lead to Mip6 nuclear retention independent of the W ₄₄₂ A mutation under optimal conditions.....	139
Point mutations in the NES _{RRM4} do not lead to nuclear retention under heat shock.....	141
Nuclear retention caused by point mutations in the NES _{RRM4} is independent of the Mip6W ₄₄₂ A mutation under oxidative stress	142
Mip6 NES sequence I ₄₂₇ RFSEIKI ₄₃₄ can partially export the SV40 NLS <i>in vivo</i>	144
Study of the putative NES sequence present in Pes4, Mip6 homologous protein	146
Mip6 Cys450 residue is important for Mip6 presence in SG under stress.....	150
A second putative NES in Mip6 C-terminal domain is activated by oxidative stress.....	153
CHAPTER III: STUDY OF FACTORS THAT PARTICIPATE IN STRESS GRANULES FORMATION	159
Background	161
Most of the interactions of Tif4631 with stress granules proteins are still present in the absence of its paralogous protein Tif4632	162
Tif4631 protein levels depend on different regions of the N-terminal domain	165
Deletion of the N-terminal domain of Tif4631 causes an increase in the duplication time under optimal conditions.....	166
Deletions of the BOX1 and RNA1 regions of the Tif4631 N-terminal domain affect cell growth after the diauxic shift	166
DISCUSSION.....	169
Mip6 interacts with both the transcription and the mRNA degradation machinery.....	171
Mip6 role under optimal conditions is not essential for cell viability.....	172
Mip6 contribution in cell survival depends on the kind of stress and the transcription factor ..	173
Is Mip6 a negative regulator of HDAC Rpd3?	176
Yeast cells present a transcriptional memory mechanism to respond to heat stress exposure...	180
May Mip6 regulate the expression of the early meiotic genes?.....	182
There is a nuclear export signal in the RRM4 of Mip6.....	183
Mip6 export is dependent on its interaction with mRNAs.....	187
Is the putative NES in the Mip6 CTD a functional export signal?	187
Regions of the Tif4631 N-terminal domain would be important for its function in SG formation	188
CONCLUSSIONS	189
BIBLIOGRAPHY	193

ABBREVIATIONS





A site	Aminoacyl Site
aa-tRNA	Aminoacyl-Transfer RNA
ANOVA	Analysis of Variance
APC/C	Anaphase Promoting Complex/Cyclosome
APS	Ammonium Persulphate
Cas	CRISPR Associated
cDNA	Complementary DNA
CPF	Cleavage and Polyadenilation Factor
CRISPR	Clustered Regularly Interspaced Short Palindromic Repeats
crRNA	CRISPR RNA
CTD	C-Terminal Domain
C-ter	C-Terminal
Cys	Cysteine
DAPI	4',6-Diamidino-2-Phenylindole
DMSO	Dimethyl Sulfoxide
DNA	Desoxiribonucleic Acid
dNTP	Deoxynucleotide Triphosphate
dsDNA	Double Strand DNA
DTT	Dithiothreitol
DUB	Deubiquitination
E site	Exit Site
EDTA	Ethylenediaminetetraacetic Acid
EGTA	Ethylene Glycol-bis(b-aminoethyl ether)-N,N,N',N'-Tetraacetic Acid
EMG	Early Meiotic Genes
FG	Phenylalanine-Glycine
FW	Forward
GDP	Guanosine Diphosphate
GFP	Green Fluorescent Protein
GTP	Guanosine Triphosphate
H4K12ac	Acetylated H4 at lysine 12
HAT	Histone Acetyltransferase
HDA	Histone Deacetylase
HDAC	Histone Deacetylase Complex
HIS	Histidine



ABBREVIATIONS

HPH	Hygromycin B
HS	Heat Stress
HSE	Heat Shock Element
HSR	Heat Shock Response
IDR	Intrinsically Disordered Region
IP	Immunoprecipitated
LB	Lysogeny Broth
LB	Loading Buffer
LB	Lysis Buffer
LBA	Lysogeny Broth supplemented with Ampicillin
LC-MS/MS	Liquid Chromatography-Mass Spectrometry
LEU	Leucine
LLPS	Liquid-Liquid Phase Separation
lncRNA	Long non coding RNA
LRR	Leucine-Rich Region
Lys	Lysine
Met-tRNA	Methionine-Transfer RNA
mRNA	Messenger RNA
mRNP	Messenger RNP
NES	Nuclear Export Signal
NLS	Nuclear Localization Signal
NNS	Nrd1 Nab3 Sen1 Complex
NP-40	Nonidet P-40
NPC	Nuclear Pore Complex
N-ter	N-Terminal
NTF2	Nuclear Transport Factor 2
OD	Optical Density
ORF	Open Reading Frame
OxS	Oxidative stress
OxSR	Oxidative Stress Response
P site	Peptidyl Site
PAR-CLIP	Photoactivatable-Ribonucleoside-enhanced Crosslinking and Immunoprecipitation
PAS	Polyadenylation Signal



PBS	Phosphate Buffered Saline
PCR	Polymerase Chain Reaction
PEG	Polyethylene Glycol
PIC	Preinitiation Complex
PKA	Protein Kinase A
PMSF	Phenylmethylsulfonyl Fluoride
PSM	Prospore Membrane
PTC	Peptidyl Transfer Center
qPCR	Quantitative PCR
RBP	RNA Binding Protein
RNA	Ribonucleic Acid
RNAPolIII	RNA Polymerase II
RNA-seq	RNA-sequencing
RNP	Ribonucleoparticle
ROS	Reactive Oxygen Species
Rpd3L	Rpd3 Large
Rpd3S	Rpd3 Small
RPG	Ribosomal Protein Genes
RRE	Rme1 Repressor Element
RRM	RNA Recognition Motif
rRNA	Ribosomal RNA
RT	Real Time
RT	Room Temperature
RV	Reverse
SAGA	Spt/Ada/Gcn5 Acetyltransferase
SC	Synthetic Complete
SDS	Sodium Dodecyl Sulfate
SDS-PAGE	Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis
SG	Stress Granule
sgRNA	Single Guide RNA
SOC	Super Optimal medium with Catabolic Repressor
SPII	Sporulation Liquid Medium
SPO	Sporulation Solid Medium
STRE	Stress Responsive Element



ABBREVIATIONS

TAE	Tris-Acetate-EDTA
TAg	T Antigen
TAP	Tandem Affinity Purification
TBP	TATA-Binding Protein
TBS	Tris-Buffered Saline
TCA	Trichloroacetic Acid
TEMED	N,N,N',N'-Tetramethylethane -1,2-diamine
TEV	Tobacco Etch Virus
TREM	Transcriptional Repression Memory
TREX	Transcription Export
tRNA	Transfer RNA
UBA	Ubiquitin-Associated
URA	Uracile
UTR	Untranslated Region
W₄₄₂	Tryptophan-442
WCE	Whole Cell Extract
WT	Wild Type
YNB	Yeast Nitrogen Base
YPA	Yeast Extract-Peptone-Acetate
YPD	Yeast Extract-Peptone-Dextrose
YPDA	Yeast Extract-Peptone-Dextrose supplemented with Adenine
YPGal	Yeast Extract-Peptone-Galactose
YPGly	Yeast Extract-Peptone-Glycerol

INTRODUCTION





EUKARYOTIC GENE EXPRESSION

Eukaryotic gene expression is a highly regulated process which consists in the “reading” of the information coded in genes in order to synthesize a protein, as a final product, with a specific function. Far to be a simple process, gene expression is comprised by several steps, taking place in both the nucleus and the cytoplasm. As a brief summary, the process starts in the nucleus where, as a response to a specific stimulus, chromatin is remodeled to make it more accessible through epigenetic modifications. Thus, transcriptional machinery can access genes and the mRNA is synthesized by the RNAPolIII. Newly synthesized mRNAs must undergo a maturation process before their export to the cytoplasm, where they can follow three different fates: degradation by the mRNA decay machinery, even though this process can also happen before their export from the nucleus, storage in specific foci or translation into proteins by ribosomes. Different factors participate in gene expression, not only by taking part in the process itself, but also in the regulation and coupling of the different levels, which makes it a complex network rather than a linear sequence of events as it was classically believed (Galán & Rodríguez-Navarro, 2012; A. Lin et al., 2020; Soffers & Workman, 2020; Stewart, 2019a; C. Wang et al., 2020). Mip6, as an example of a protein that participates in some of these different steps, would take part in events that diverse as transcription, mRNA export, decay or storage in cytoplasmic granules, interconnecting the different levels that comprise gene expression (Martín-Expósito et al., 2019; Nuño-Cabanes et al., 2020; **Figure 1**).

HISTONE POST-TRANSLATIONAL MODIFICATIONS

In order to allow the transcription machinery to enter to the genes, chromatin must be remodeled from a condensed conformation, called heterochromatin, into a more flexible and dynamic state, known as euchromatin (Bi, 2014). In this process of chromatin reorganization, histone proteins play an important role. Thus, an octamer of histones, composed by histones H2A, H2B, H3 and H4, two molecules of each one, is located among the chromatin, so that a DNA fragment of 145-147bp is wrapped around the octamer, forming the nucleosomes, the chromatin structural basic unit (Chou et



INTRODUCTION

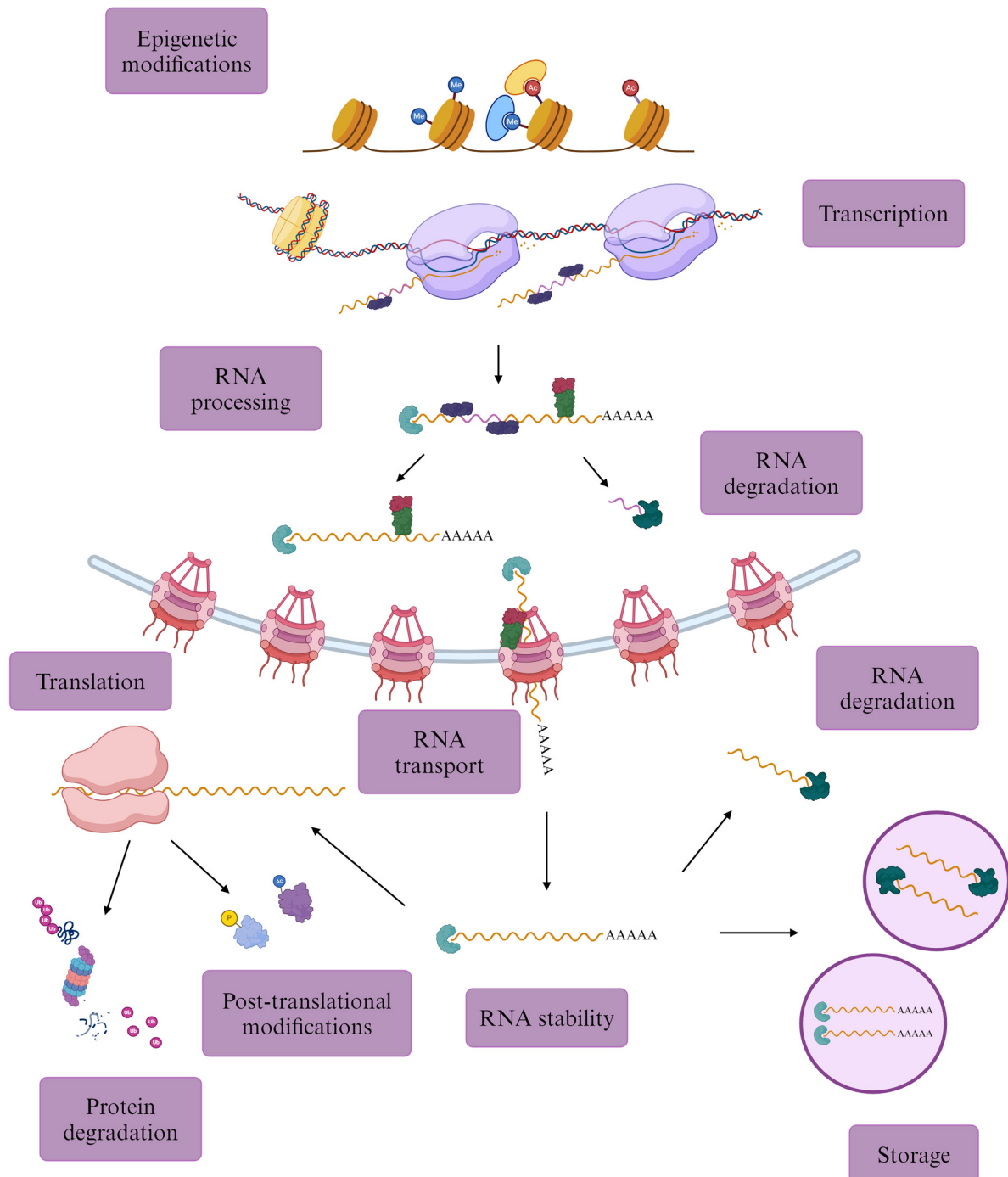


Figure 1. The eukaryotic gene expression cycle. The process starts in the nucleus where, as a response to a specific stimulus, chromatin is remodeled to make it more accessible due to epigenetic modifications. Thus, transcriptional machinery can access the genes and the mRNA is synthesized by the RNAPolIII. Newly synthesized mRNAs must undergo a maturation process before their export to the cytoplasm, where they can follow three different fates: degradation by the mRNA decay machinery, storage in specific foci or translation into proteins by ribosomes. Created with BioRender.com.

al., 2023; A. Lin et al., 2020). Post-translational modifications, which occur predominantly in the terminal tails of histones, are key not only in gene expression regulation, but also in other processes such as chromosome segregation, DNA repair or cell fate. Among the most studied post-

translational modifications of histones, we can find acetylation, methylation, phosphorylation or ubiquitination (Chou et al., 2023; Kouzarides, 2007).

The first histone modification discovered to be linked to gene expression was acetylation (Allfrey et al., 1964), which consists in the transfer of an acetyl group to diverse lysine residues in histone tails. This modification provokes the removal of the positive charge of these residues, leading to less stable interactions between histones and the negatively charged DNA and, therefore, making the chromatin more flexible so that gene transcription could be activated (Chou et al., 2023; Grunstein, 1997). Two kinds of protein groups participate in histone deacetylation: the “writers” histone acetyltransferases (HATs) and the “erasers” histone deacetylases (HDAs), which are numerous even in simple eukaryotes such as yeast. These proteins not only take a role in the acetylation/deacetylation of Lys residues in histones, but they can also modify certain residues of other kind of proteins, regulating various biological processes (Kurdistani & Grunstein, 2003; Seto & Yoshida, 2014; Yeheskely-Hayon et al., 2013).

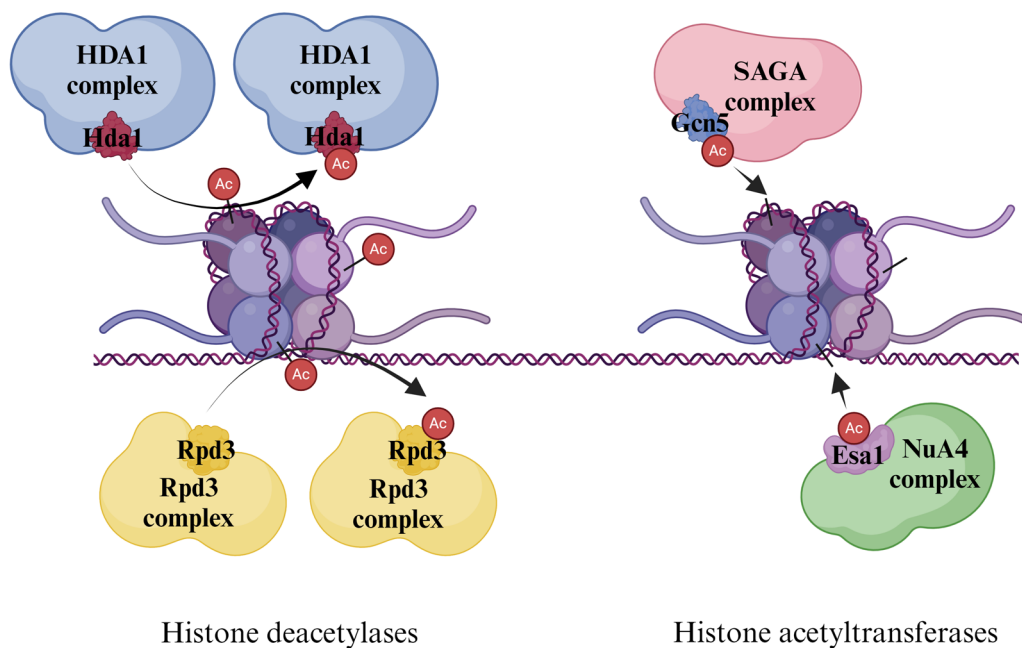


Figure 2. Complexes and proteins participating in the acetylation and deacetylation of histones. On the one hand, Hda1 and Rpd3 are the catalytic subunits of the histone deacetylase complexes Hda1C and Rpd3, respectively. On the other hand, Gcn5 and Esa1 are proteins with histone acetyltransferase activity belonging to the complexes SAGA and NuA4, respectively. Created with BioRender.com.



INTRODUCTION

Some of the HAT complexes are also transcriptional coactivators, as in the case of the SAGA complex (Knutson & Hahn, 2011; Martin et al., 2021). Highly conserved among eukaryotes, the Spt/Ada/Gcn5 acetyltransferase (SAGA) complex is composed of 19 subunits grouped in four different functional modules, as well as the Tra1 protein, which would conform a module by itself: the CORE structural module, the TBP-interaction module, the H2B deubiquitination (DUB) module and the H3 acetylation (HAT) module (Grant et al., 2021; Soffers & Workman, 2020; H. Wang et al., 2020). It is this last module, the HAT one, which, as its name says, has the HAT activity. The Gcn5 protein is the catalytic subunit of the module and acetylates histone H3 (Cieniewicz et al., 2014; Sterner et al., 1999; **Figure 2**); while the other subunits, Ada2, Ada3 and Sgf29, function as modulators of the activity and the specificity of Gcn5 (Balasubramanian et al., 2002; Ringel et al., 2015). Another protein with HAT activity is Esa1, which is the catalytic subunit of the NuA4 and the Piccolo complexes (**Figure 2**). Esa1 acetylates histones H2A and H4 (Kurdistani & Grunstein, 2003).

Regarding the HDAs, in yeast, they can be grouped into three different classes: class-I, comprised of Rpd3, Hos1 and Hos2; class-II, which includes Hda1 and Hos3; and class-III, where Sir2, Hst1, Hst2, Hst3 and Hst4 can be found (Sauty et al., 2021; Seto & Yoshida, 2014; Yang & Seto, 2008). Rpd3 protein is the catalytic subunit of three different HDA complexes with specific functions: Rpd3L, Rpd3S and Rpd3 μ (**Figure 2**). While Rpd3L and Rpd3S share two more subunits (Sin3 and Ume1), these two complexes only have in common with the Rpd3 μ complex the Rpd3 protein (X.-F. Chen et al., 2012; McDaniel & Strahl, 2013). Rpd3L mainly participates in ribosomal biogenesis and heat stress response, being recruited to the promoters of the genes to be repressed by the action of different DNA-binding proteins (Carrozza, Florens, et al., 2005; McDaniel & Strahl, 2013; Ruiz-Roig et al., 2010; Sauty et al., 2021). Rpd3S, on the other hand, has a role in the chromatin integrity maintenance and the repression of cryptic transcription; and its recruitment to the ORFs of the transcribed genes depends on histone methylated residues (Carrozza, Li, et al., 2005; Sauty et al., 2021; Wagner & Carpenter, 2012). Finally, Rpd3 μ has been shown to be important for the cellular response to osmotic and oxidative stress. This complex is recruited to two different kinds of promoters: one that has the Rpd3 μ subunits Snt2 and Ecm5 bound in a



constitutive manner, called “superenriched” promoters, and another where Rpd3 is recruited only in oxidative stress conditions in an Snt2/Ecm5-independent way (Baker et al., 2013; McDaniel & Strahl, 2013). Besides, Rpd3 does not only participate in the modification of histones tails, but also by acetylating other kinds of proteins, such as transcription factors or repressors, as it is the case of Rpd3’s role in repressing the expression of early meiotic genes (Law et al., 2014; Mallory et al., 2012). Lastly, although deacetylation has always been linked to the repression of gene expression, Rpd3 has also been discovered to take part in the activation of certain genes, such as the ones participating in heat shock, osmotic stress response or DNA damage (Pérez-Martínez et al., 2019; Ruiz-Roig et al., 2010; Sadeh et al., 2011; Sauty et al., 2021).

Hda1, a class-II HDA, is part of the Hda1 complex (**Figure 2**), together with Hda2, Hda3 and Arb2. This complex is known to deacetylate lysine residues in histones H2B and H3 by cooperating with the general corepressor Tup1 so that transcription of stress-response genes is repressed (M. K. Lee & Kim, 2020; Wu et al., 2001). Recently, Hda1C has also been found to take part in deacetylation of histone H4 in active genes. The complex is recruited to the ORFs of hyperactive genes by means of its interaction with histones and the transcription machinery in order to deacetylate Lys residues in H4 and maintain the optimal histone acetylation. Although, Rpd3S is also known for being recruited to ORFs of certain genes, both pathways are independent (Ha et al., 2019; M. K. Lee & Kim, 2020). However, Hda1 and Rpd3 do seem to have redundant roles at certain promoters, since the deletion of genes that coded for both proteins affects the acetylation of the same intergenic regions in some cases (Rowlands et al., 2019).

RNA POLYMERASE II-MEDIATED TRANSCRIPTION

Transcription is a tightly regulated and essential process where RNAs are synthesized by means of DNA-dependent RNA polymerases. In eukaryotes, there are three different RNA polymerases that, even though they differ in the kind of RNA they produce, share many of its features, such as a similar structure or the three phases that comprise the transcription process: initiation, elongation and termination (Engel et al., 2018; Girbig et al., 2022; Khatter et al., 2017). The RNA



INTRODUCTION

Polymerase II (RNAPolIII) is the one that synthesizes the mRNA, which is going to be translated into proteins (Jacobs & Schneider, 2024; **Figure 3**).

RNAPolIII transcription initiation starts with the recruitment of all the proteins and complexes that are necessary for the assembly of the preinitiation complex (PIC) (H. Chen & Pugh, 2021). There are two main transcriptional activators in yeast: TFIID and SAGA, both with the ability to recruit the TATA-binding protein (TBP) (Donczew et al., 2020; Timmers, 2021). SAGA was classically

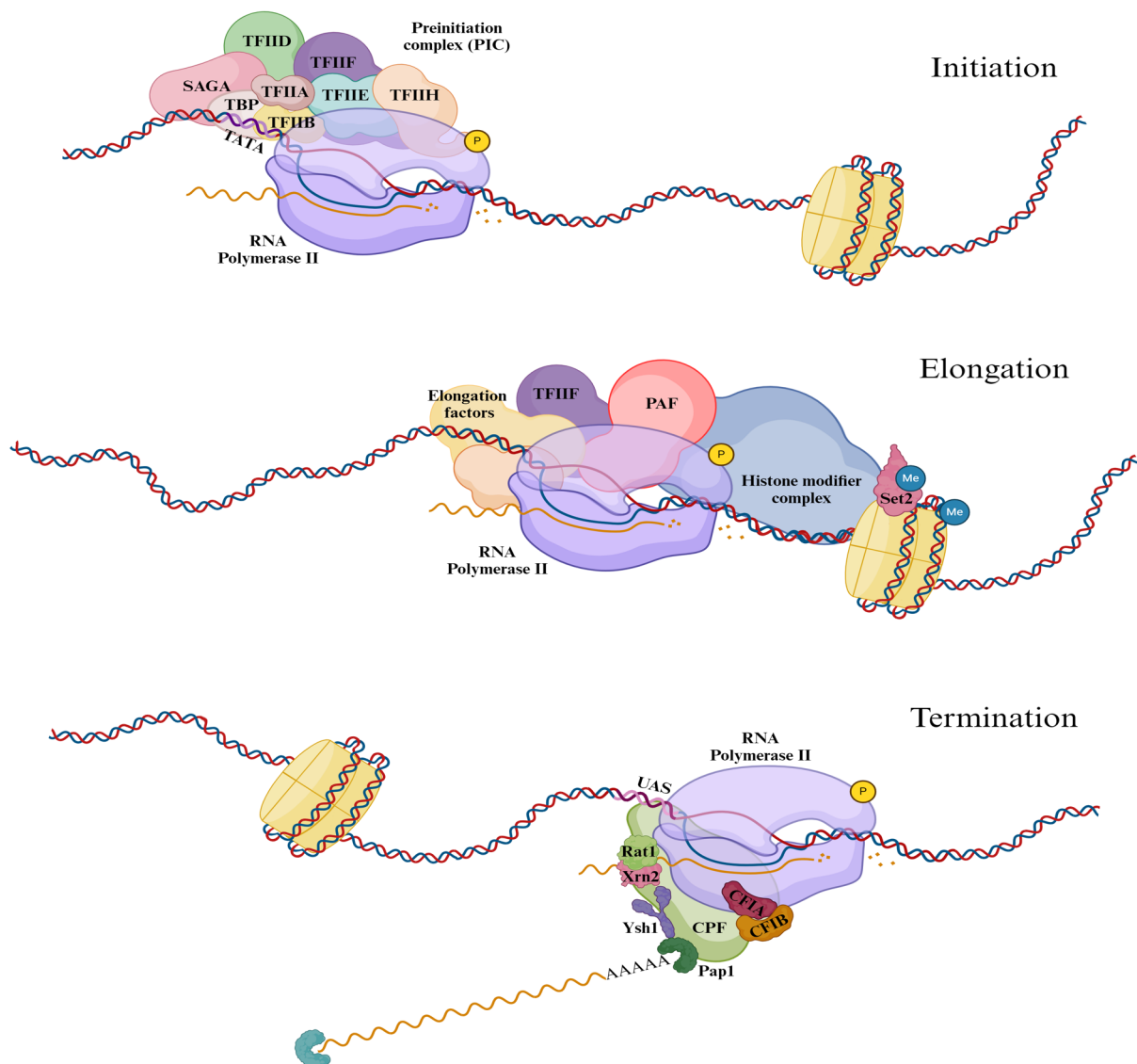


Figure 3. mRNA transcription mediated by RNA Polymerase II. Transcription initiation starts with the assembly of the preinitiation complex (PIC). RNAPolIII, together with other transcription initiation factors, is recruited to the promoter of the gene. Once the RNAPolIII disassembles from the PIC, elongation starts and the elongation machinery sets in motion. As the polymerase leaves the PIC and the nascent RNA is synthesized, the elongation transcription factors substitute the initiation factors, which regulate RNAPolIII activity. Transcription ends with transcription termination, where the RNA is released from the RNAPolIII once the polymerase reaches the 3' UTR. Finally, the RNAPolIII dissociates from the nascent RNA. Created with BioRender.com.



believed to participate in the activation of a reduced amount of genes, usually related to the cellular stress response, which show TATA boxes in their promoters (thymine and adenine-rich regions). On the other hand, TFIID was associated with housekeeping genes whose promoters present TATA-like or TATA-less sequences (de Jonge et al., 2017; Yella & Bansal, 2017). However, some recent studies have demonstrated that SAGA and TFIID function as coactivators of some TATA box genes (Donczew et al., 2020). Once the TBP is recruited to the promoters of the genes, the recruitment of the general transcription factors starts. First, TFIIB binds to the TBP, with the aid of TFIIA that stabilizes the interaction, and this bound is necessary for the recruitment of the RNAPolIII, together with TFIIF. Later, TFIIE and TFIIH are also recruited for the PIC assembly and the beginning of the transcription, since TFIIH phosphorylates the C-terminal domain (CTD) of the RNAPolIII subunit Rpb1, which facilitates the dissociation of the polymerase so that transcription can start (Hahn, 2004; Jacobs & Schneider, 2024; Singh et al., 2022; Thomas & Chiang, 2006).

Once the RNAPolIII disassembles from the PIC, elongation starts and the elongation machinery sets in motion. Transition from initiation to elongation is strongly regulated, being important the phosphorylation of the RNAPolIII CTD, since this modification promotes the interaction of the polymerase with other complexes participating in the process. It is the case of certain histone modifiers, such as the methyltransferase Set2, or other complexes needed for elongation, like the Paf1 complex (Krogan et al., 2003; Oliete-Calvo et al., 2018; Singh et al., 2022; Squazzo et al., 2002). As the polymerase leaves the PIC and the nascent RNA is synthesized, the elongation transcription factors substitute the initiation factors, which regulate RNAPolIII activity. Besides, the nascent RNA itself appears to take part also in the recruitment of those factors participating in elongation (Battaglia et al., 2017). However, the initiation of the process is not a guarantee of its completion. Polymerase sometimes pauses during elongation due to the presence of obstacles or backward movements: another regulatory mechanism for the proper execution of the process (Mustaev et al., 2017; Nudler, 2012; Singh et al., 2022).



INTRODUCTION

Transcription ends with transcription termination, where the RNA is released from the RNAPolIII once the polymerase reaches the 3' untranslated region (UTR), and it is a process tightly coupled with RNA processing. There are two different mechanisms regulating transcription termination: one for short non-coding RNAs and small mRNAs and another one for the majority of protein-coding genes (Creamer et al., 2011; Schulz et al., 2013). The first mechanism is dependent on the NNS complex, comprised of two RNA-binding proteins (RBPs), Nrd1 and Nab3, and the helicase Sen1. The Nrd1-Nab3 heterodimer is recruited to the transcription machinery by the phosphorylation in the CTD of the RNAPolIII and by the nascent RNA. Later, the Sen1 helicase is also recruited, by binding both RNAPolIII and the Nrd1-Nab3 heterodimer, and it is the protein responsible for the elongation machinery dissociation (Porrua & Libri, 2013; Rodríguez-Molina et al., 2023; Singh et al., 2022). In the second mechanism, on the other hand, 3' end processing mechanisms are involved. Once the RNAPolIII has arrived at the 3'UTR of the transcribed gene, the polymerase added a polyadenylation signal (PAS) sequence (Eaton & West, 2020). Then, the cleavage and polyadenylation factor (CPF), together with its accessory factors CFIA and CFIB, recognizes this PAS sequence and, through the endonuclease Ysh1, cleaves the nascent RNA between 10 and 30 nucleotides downstream the PAS sequence (Mandel et al., 2006; Rodríguez-Molina et al., 2023). Besides, the poly(A) polymerase Pap1 from the CPF protects the 3' end of the recently cleaved RNA by adding a poly(A) tail, which is important for the RNA stability and its subsequent export to the cytoplasm (Hill et al., 2019).

For the dissociation of the RNAPolIII from the nascent RNA, which ends the transcription process, two different models have been proposed. On the one hand, the allosteric model suggests that, when the RNAPolIII transcribes the PAS sequence, the polymerase suffers a conformational change, provoking the slow-down of the elongation and the final dissociation of the elongation machinery from the nascent RNA (L. Davidson et al., 2020; Zhang et al., 2015; Žumer et al., 2021). On the other hand, the torpedo model proposes that the ribonucleases Rat1 and Xrn2 co-transcriptionally degrade the nascent RNA beyond the cleavage and the polyadenylation site until they reach the RNAPolIII. Due to this “collision”, the polymerase would dissociate from the



RNA, ending the transcription (Jacobs & Schneider, 2024; Rodríguez-Molina et al., 2023). Both models are not incompatible with each other.

The 3' end cleavage and the polyadenylation are not the only maturation processes the nascent RNA must undergo. 5' end of the RNA must also be protected from degradation by the addition of a 7-methylguanosine cap (Kiledjian, 2018; Zhai & Xiang, 2014). Besides, even though a little amount of genes present introns in yeast, splicing is also essential because of the importance of the expression of these genes (Wilkinson et al., 2020). All these maturation processes occur together with the transcription, since the factors implicated are recognized and recruited by the nascent RNA as it emerges from the RNAPolIII (Bentley, 2005).

TRANSPORT BETWEEN THE NUCLEUS AND THE CYTOPLASM

While transcription and RNA processing occur in the nucleus, translation happens in the cytoplasm, so the newly synthesized and processed mRNA must be exported from the nucleus to the cytoplasm through the nuclear pore complex (NPC). NPCs are cylindrical complexes in the nuclear membrane comprised of multiple copies of around 30 proteins, called nucleoporins, that serve as channels for the transport of RNAs and proteins (Kim et al., 2018; D. H. Lin & Hoelz, 2019). Their structure is conserved among eukaryotes, even though yeast NPCs are smaller than the ones in metazoans (Stewart, 2019a). NPCs are composed of two rings on each side of the nuclear envelope, connected by a channel filled with peptides that present phenylalanine-glycine (FG) repeats and a basket located in its nuclear face and fibrous extensions from the cytoplasmic ring (Alber et al., 2007; Terry & Wente, 2009).

The main export factor in yeast is Mex67, which forms a heterodimer with Mtr2 and facilitates the transport of most of the mRNAs (Katahira et al., 1999; Segref et al., 1997; **Figure 4**). Mex67 is formed by four different domains: an RNA recognition motif (RRM) in its N-terminal domain, a leucine-rich region (LRR), a nuclear transport factor 2 (NTF2)-like domain, through which it binds Mtr2, and an ubiquitin-associated (UBA) domain in its C-terminal end (Santos-Rosa et al., 1998; Strässer et al., 2000; Xie & Ren, 2019). The NTF2-like and UBA domains are able to interact with the FG-nucleoporins of the NPC, as well as with other proteins, while the RRM



INTRODUCTION

and LRR domains bind the mRNA together with RNA-binding proteins (RBPs; Gwizdek et al., 2006; Hobeika et al., 2009; Martín-Expósito et al., 2019; Valkov et al., 2012). Despite its ability to bind RNAs, Mex67 presents low affinity for them, so that it must interact with RBPs that serve as adaptors in order to be able to properly perform its function (Katahira et al., 1999).

Yra1 is one of these RBPs acting like adaptors for Mex67. This protein associates, together with the DEAD-box helicase Sub2, with the THO complex, composed of the proteins Tho2, Hpr1, Mft1, and Thp2. All these proteins together form the Transcription-Export (TREX) complex that couples transcription and mRNA export (Niño et al., 2013; Strässer et al., 2002). The THO complex interacts with the phosphorylated CTD of the RNAPolIII and recruits Sub2 (Meinel et al., 2013), which in turn binds Yra1 and both proteins cooperate in the interaction with the mRNA (Ren et al., 2017). Yra1, in contrast, is not exported, since it is removed when the TREX complex binds Mex67-Mtr2 due to a conformational change in the secondary structure of the mRNA and Yra1 ubiquitination by Tom1 (Iglesias et al., 2010). Thus, the mRNA remains attached to the heterodimer Mex67-Mtr2, which facilitates the messenger ribonucleoparticles (mRNPs) to dock the NPC.

For the translocation of the mRNPs through the NPC channel from the nucleus to the cytoplasm, the TREX-2 complex comes into play. This complex is composed of Sac3, Thp1, Sem1, Sus1, and Cdc31 and also couples transcription and mRNA export, since it shares the Sus1 subunit with the transcription coactivator complex SAGA (Galán & Rodríguez-Navarro, 2012; Rodríguez-Navarro et al., 2004). TREX-2 is associated with the nuclear basket of the NPCs and mediates the recruitment of the mRNA actively transcribed into the NPCs (Cabal et al., 2006). The Sac3 subunit acts as a scaffold, interacting with all the other subunits comprising the complex and recognizing the Mex67-Mtr2 heterodimer by its N-terminal domain that shows FG repeats (Stewart, 2019b).

The mRNA export platform in the cytoplasmic face of the NPC is composed of the helicase Dbp5 and the nucleoporins Gle1, Gle2, Nup42, Nup159 and Nup116. The last three nucleoporins present FG repeats, which facilitate the transport of the mRNPs through the NPC channel (Fernandez-Martinez et al., 2016). Then, Gle1 activates the DEAD-box helicase Dbp5, a process mediated

by Nup42 and, when active, Dbp5 dissociates the mRNA from Mex67 once in the cytoplasm, preventing the mRNA from coming back to the nucleus, while Mex67-Mtr2 is recycled by shuttling back through the NPCs (Xie & Ren, 2019; **Figure 4**).

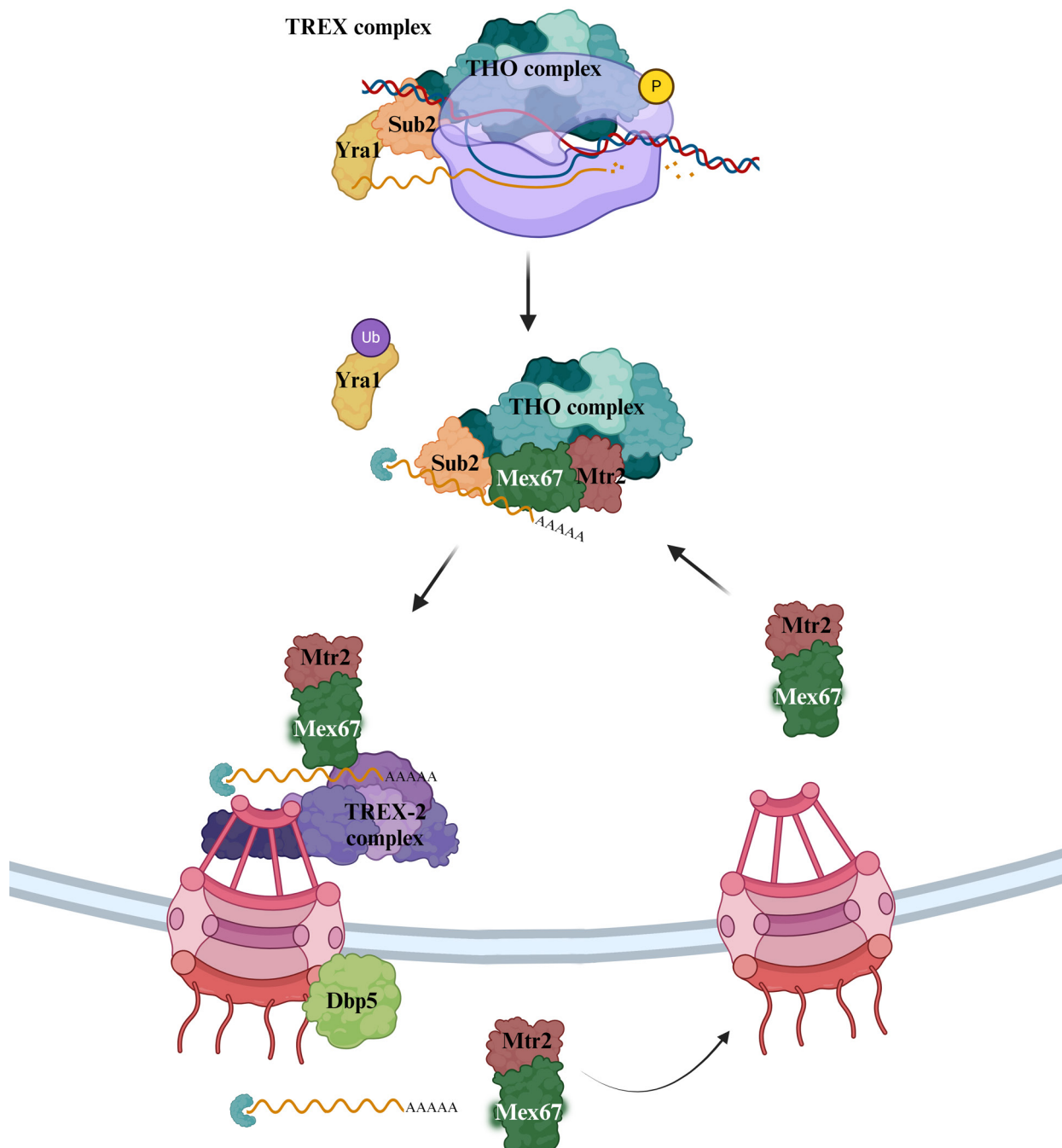


Figure 4. mRNA transport from the nucleus to the cytoplasm. Mex67 is the main export factor in yeast, which forms a heterodimer with Mtr2 and facilitates the transport of most of the mRNAs. However, its affinity for the mRNA is low, so that this factor needs other proteins that act as adaptors, such as Yra1. This protein, together with the THO complex, couples transcription and mRNA export and binds to the newly synthesized mRNA. Then, TREX2 complex recruits the mRNA to the NPC and the helicase Dbp5, together with other nucleoporins, mediates the transport of the mRNP through the NPC channel. Finally, the Mex67-Mtr2 heterodimer is recycled by shuttling back through the NPCs. Created with BioRender.com.



INTRODUCTION

Mex67-dependent transport is the most common for mRNA export, although there are other pathways for the shuttling of proteins and small RNAs, regulated by karyopherin- β -family carriers and GTP-dependent. For protein import, the karyopherin interacts with the protein to be transported in the cytoplasm and facilitates its import into the nucleus. There, the karyopherin binds RanGTP, producing the release of the protein, and the karyopherin-RanGTP complex goes back to the cytoplasm, where the GTP is hydrolyzed into GDP and the complex disengages. Then, RanGDP returns to the nucleus thanks to NTF2 and is recharged into GTP again (Christie et al., 2016). In the case of proteins and small RNAs export, the mechanism is similar. The interaction of the karyopherin with the protein transported requires the bound with the RanGTP and, once in the cytoplasm, the protein is released when GTP is hydrolyzed into GDP (Stewart, 2019a). Karyopherins are able to recognize their cargo proteins thanks to the presence of different signals in the protein sequence. In the case of proteins to be imported, they present Nuclear Localization Signals (NLS), while the proteins transported from the nucleus to the cytoplasm contain Nuclear Export Signals (NES). There are different classes of both NLS and NES sequences, which could be linear or conformational. Karyopherins are able to interact with these signals, directly or by means of an adaptor protein, so that the cargo proteins can be properly transported (Soniati & Chook, 2015).

TRANSLATION

mRNA translation into proteins occurs in the cytoplasm where the ribosomes “read” the information in the mRNA codon by codon and, by means of an aminoacyl-tRNA, assign each one an amino acid, based on the genetic code, in order to form polypeptides chains (Blanchet & Ranjan, 2022). In eukaryotes, the 80S ribosomes are formed by two different subunits: the 60S large subunit and the 40S small subunit. While the small subunit takes part in the decoding of the mRNA, the large subunit participates in the formation of the peptide bond between the different amino acids (Vanden Broeck & Klinge, 2024; Yusupova & Yusupov, 2017). Even if a ribosome is able to produce a polypeptide chain at a time, one single mRNA can be translated by several ribosomes simultaneously in order to produce a higher amount of protein, forming structures called polyribosomes (Afonina & Shirokov, 2018).

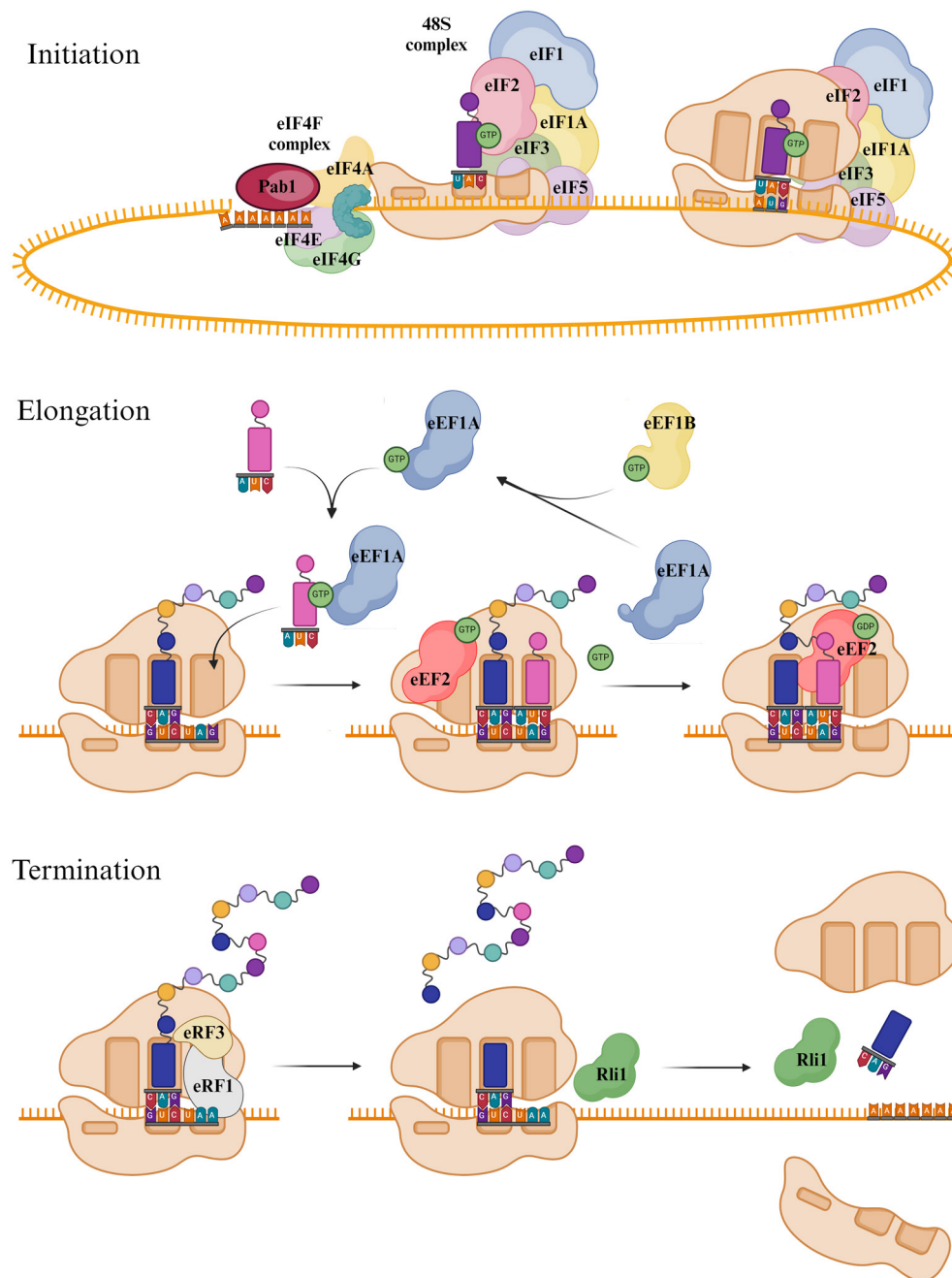


Figure 5. mRNA translation into proteins. Translation begins when the ribosomal subunits, together with the initiation factors and the Met-tRNA, are recruited to the mRNA. The complex starts scanning the sequence in search for the AUG codon, the one that initiates the process. Then, the elongation factor eEF1A interacts with a new aa-tRNA and places it in the A site. The bond between the aa-tRNA in the A site and the Met-tRNA in the P site is formed and a conformational change allows the movement of both tRNAs and mRNA, so that the aa-tRNA is now in the P site and the A site is free for a new tRNA. This process is repeated until the ribosome reaches a STOP codon. eRF1 released factor produces the release of the newly synthesized protein and the complex is disassembled. Created with BioRender.com.

Translation comprises three different phases: initiation, elongation and termination (**Figure 5**).

Initiation begins when the small ribosomal subunit recruits, together with other initiation factors, eIF2, which is bound by a GTP to a Met-tRNA. The assembly of all these molecules produces



INTRODUCTION

a conformational change in the 40S subunit that allows the mRNA to enter (Llácer et al., 2015). Thus, the complex starts scanning the mRNA in search for the AUG codon (Brito Querido et al., 2020). Once the initiation codon is recognized, the large ribosomal subunit is recruited and the 80S initiation complex is assembled, with the Met-tRNA in the peptidyl (P) site of the ribosome (Lapointe et al., 2022).

In the translation elongation phase, a new aminoacyl-tRNA bound to GTP is placed in the aminoacyl (A) site of the ribosome. When the next codon is recognized by this aa-tRNA, GTP is hydrolyzed and the aa-tRNA is properly accommodated in the A site (Xu et al., 2021). Then, the amino acid of the A site attacks the methionine in the P site by its carbonyl carbon, so that the tRNA in the P site loses its amino acid while in the A site there is a peptidyl-tRNA with two amino acids (Beringer & Rodnina, 2007) and the eEF2 factor facilitates the movement of both tRNAs and mRNA. Therefore, the deacylated tRNA goes to the exit (E) site, the peptidyl-tRNA is now located in the P site and a new codon is placed in the A site waiting for an aa-tRNA to arrive and start another round of elongation (Blanchet & Ranjan, 2022). This phase is repeated in a cycle until an STOP (UGA, UAG or UAA) codon is placed in the A site, leading to translation termination.

When one of the three STOP codon enters the A site of the ribosome, the released factor eRF1 is recruited to the ribosome (Pisareva et al., 2006) to produce the hydrolysis of the bond between the peptidyl chain and the tRNA in the P site releasing the newly synthesized protein (Alkalaeva et al., 2006). Released proteins may suffer some structural changes or post-translational modifications in order to become active proteins, while the recently translated mRNAs will be degraded by the mRNA decay machinery (Matsuo & Inada, 2023).

Mip6, A PROTEIN PARTICIPATING IN GENE EXPRESSION AT DIFFERENT LEVELS

Mip6 was initially identified as a Mex67 interactor in a yeast two-hybrid (Y2H) screen, so that it was named Mex67 Interacting Protein 6 (Segref et al., 1997). However, the characterization of Mip6 remained elusive. In a study looking for dosage-sensitive proteins prone to liquid-liquid



phase separation, it was shown that overexpression of Mip6 produces a toxic accumulation of the protein in liquid-like cytoplasmic foci (Bolognesi et al., 2016). Nevertheless, this work did not address the molecular function of Mip6. Through a TAP purification of Sus1, our lab found Mip6 as a copurifying Sus1 protein. This, together with our interest on Mex67, makes the functional study of Mip6 very attractive. In recent years and in collaboration with other groups, we have addressed structural and functional Mip6 characterization, focusing on the role of this protein at the different levels of gene expression (Martín-Expósito et al., 2019; Nuño-Cabanes et al., 2020). In additions to my work, two other PhD Thesis have centered their effort in Mip6 cellular function (Martín Expósito, 2019; Nuño Cabanes, 2024).

Mip6 is a RNA binding protein, located in both the nucleus and the cytoplasm, and contains four RRM domains, each one contributing in a different way to Mip6 function. While the RRM1 and RRM2 domains show a higher affinity for RNA, which suggests that RNA interaction would be carried out mostly by them, even as a tandem (Martín Expósito, 2019), the RRM4 is the domain that binds Mex67. Specifically, the Mex67 UBA domain interacts with the tryptophan-442 residue (W_{442}) in Mip6 RRM4. This Mip6 domain can bind both Mex67 and RNA, but the Mip6-Mex67 interaction prevents the binding of RRM4 with the RNA (Martín-Expósito et al., 2019). The connection between these two proteins may suggest a role of Mip6 in mRNA export from the nucleus to the cytoplasm, but it is not the only clue, since Mip6 also physically interacts with other factors of the export machinery, such as the TREX-2 subunits Sus1 and Sac3 and the helicase Dbp5 (Martín-Expósito et al., 2019).

The role of Mip6 within the cell seems to be more related to the stress response. On the one hand, the protein changes its location upon different kinds of stress, such as heat shock, glucose deprivation or sodium azide-induced oxidative stress. On these conditions, Mip6 is accumulated in stress granules and p-bodies, since it colocalizes with proteins present in these foci like Pab1, Pbp1 or Dcp2 (Grousl et al., 2022; Martín-Expósito et al., 2019). On the other hand, a photoactivatable-ribonucleoside-enhanced crosslinking and immunoprecipitation (PAR-CLIP) assay performed at both 30°C and after a heat shock at 39°C for 20min demonstrates that Mip6 preferential mRNA



INTRODUCTION

targets under optimal conditions are transcripts codified by genes dependent on the transcription factors Msn2 and Msn4 (Martín-Expósito et al., 2019). These two homologous transcription factors that are also functionally redundant take part in the activation of the expression of many genes that participate in the cellular response to stress (Martínez-Pastor et al., 1996). However, under stressful conditions, Mip6 binds preferentially to non-stress-induced transcripts, such as the ones of genes that coded for ribosomal proteins (Martín-Expósito et al., 2019).

Not only Mip6 has a preference for binding the Msn2/4-dependent transcripts, which suggests that this protein participates in the export from the nucleus to the cytoplasm of these stress response mRNA, but it also takes part in the regulation of the homeostasis of these transcripts. An RNA-seq study of both WT and *mip6Δ* strains under optimal and stressful conditions has shown that many of the Msn2/4-dependent genes are overexpressed in the mutant strain, as well as other genes related with metabolism, such as the ones that take part in the trehalose biosynthesis (Nuño-Cabanes et al., 2020). Trehalose is a disaccharide with importance in the protection of the cell from stress and whose levels have already been discovered to be altered in cells lacking Mip6 (N. K. Jain & Roy, 2009; Martín Expósito, 2019). In the case of the genes regulated by transcription factor Hsf1, the main regulator of the cellular heat stress response (Morano et al., 2012), some of them are shown to be overexpressed in the mutant, while others remain underexpressed. Nevertheless, genes involved in ribosomal biogenesis, such as the ones that code for ribosomal proteins, are underexpressed in the *mip6Δ* strain (Nuño-Cabanes et al., 2020). Thus, all these results would indicate that Mip6 has a role in the regulation of the mRNA levels of the transcripts it binds.

If Mip6 is involved in transcripts levels regulation, this would mean that this protein takes part whether in transcription, in mRNA degradation or both. Mip6 genetically interacts with proteins of the mRNA decay machinery, such as the exonucleases Rrp6 and Xrn1 (Braun & Young, 2014; Fox & Mosley, 2016), as well as with the RNAPolIII subunit Rpb1 (Martín-Expósito et al., 2019). However, comparing Msn2/4-dependent transcripts levels in *rpb1-1* and *rpb1-1 mip6Δ* strains, they decrease at the same rate in both strains, suggesting Mip6 would be mainly involved in transcription of those mRNA than in their degradation (Martín-Expósito et al., 2019). Besides, it

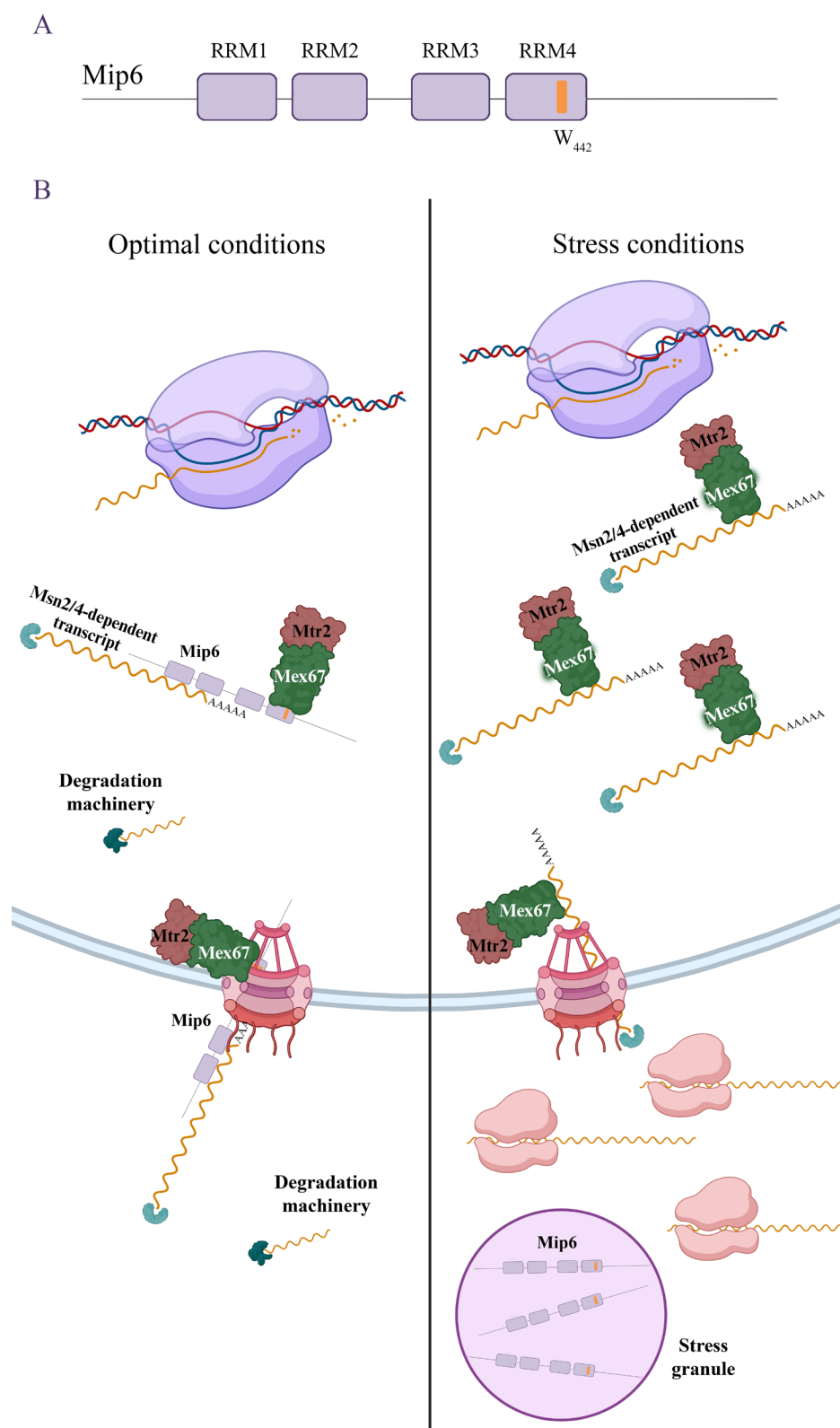


Figure 6. Mip6 regulates the metabolism of the Msn2/4-dependent transcripts. (A) Mip6 is comprised of 4 RRMs. The W₄₄₂ residue, placed in its RRM4, is the residue that directly binds to the export factor Mex67. (B) When the cell is growing under optimal conditions, Mip6 binds preferentially to the Msn2/4-dependent transcripts and participates in keeping the levels of these transcripts low, since they are not needed. Nevertheless, when the cell suffers a stressful condition such as heat shock, Mip6 rather interacts with other kinds of transcripts, like the ones coded by ribosomal proteins genes, and is accumulated in stress granules, so that the stress response mRNAs can be translated into proteins and perform their function. Created with BioRender.com.



INTRODUCTION

has also been shown that, in the *mip6Δ* mutant, there is slightly more occupancy of the RNAPolIII in the genes dependent on Msn2/4 than in the WT strain, which confirms a possible role of Mip6 regulating the transcription of these genes (Nuño Cabanes, 2024).

Thus, as a brief summary, when the cell is growing under optimal conditions, Mip6 binds preferentially to the Msn2/4-dependent transcripts and participates in keeping the levels of these transcripts low, since they are not needed. Nevertheless, when the cell suffers a stressful condition such as heat shock, Mip6 rather interacts with other kinds of transcripts, like the ones coded by ribosomal proteins genes, and is accumulated in stress granules, so that the stress response mRNAs can be translated into proteins and perform their function (**Figure 6**).

As it was already said, Mip6 is a shuttling protein, being transported between the nucleus and the cytoplasm, and this export is partially mediated by Mex67 (**Figure 7**), since when the Mip6-Mex67 interaction is impaired, by the deletion of either the Mex67 UBA domain or the Mip6 RRM4, Mip6 remains partially retained in the nucleus (Martín-Expósito et al., 2019). Nevertheless, as this retention is partial and not total, there must be at least another export mechanism for this protein.

One possible Mip6 alternative export pathway could be related to a putative Nuclear Export Signal (NES) present in the RRM4 domain of the protein, just some residues upstream the W₄₄₂ that interacts with Mex67 (I₄₂₇RFSEIKI₄₃₄). Point mutations in this sequence also lead to Mip6 nuclear retention, as well as the deletion of the whole sequence, which would indicate this NES sequence acts as a Mip6 export mechanism (Martín Expósito, 2019). This pathway would be mediated by the karyopherin Msn5 (**Figure 7**), which regulates the export from the nucleus to the cytoplasm of other proteins with unconventional NES sequences, as well as the transport of the two stress response transcription factors Msn2 and Msn4 (Bakhrat et al., 2008; DeVit & Johnston, 1999; Görner et al., 2002). Deletion of Msn5 provokes a Mip6 nuclear retention similar to the one observed with point mutations in the NES sequence (Martín Expósito, 2019).

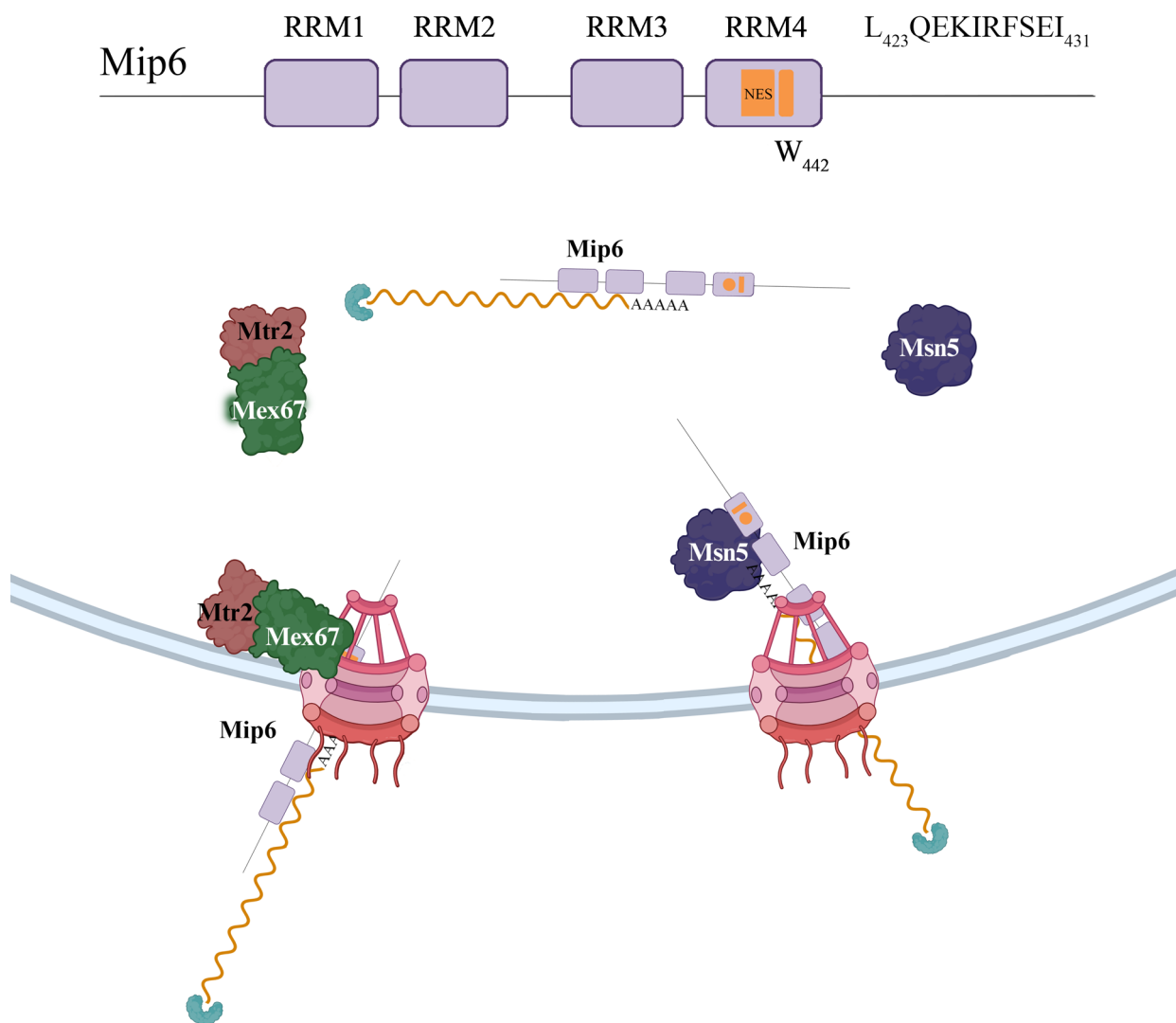


Figure 7. Mip6 export mechanism. Mip6 shuttles between the nucleus and the cytoplasm by means of different pathways. On the one hand, the W₄₄₂ residue present in its RRM4 interacts with the UBA domain of the export factor Mex67 and this bound mediates the transport of the protein. On the other hand, there is a NES sequence present in its RRM4 that would suppose another export mechanism of the protein, mediated by the karyopherin Msn5. Created with BioRender.com.

The stress response is not the only cellular process in which Mip6 protein is involved, since it has been described to have a role in sporulation too, together with its paralogous protein Pes4 (Jin et al., 2017). Little is known about this protein, beyond the fact that it is also an RNA binding protein comprised of four RRMs. No interaction of Pes4 with Mex67 has been described. Both proteins take part in the regulation of the expression of certain genes necessary for yeast sporulation, specifically, a certain group of genes regulated by the meiotic transcriptional activator Ndt80 which are transcribed early in the meiotic process but they are not translated until the end of meiosis II. Pes4 and Mip6 protect the mRNA of these genes to avoid their degradation (Jin et al.,



2017). Besides, Pes4 seems to be also necessary to regulate meiotic commitment, since both the absence of this protein and its overexpression alters the levels of uncommitted cells (Gavade et al., 2022).

CELLULAR RESPONSE TO STRESS

Throughout their lives, cells are exposed to many changes in their environment that can cause malfunction or damage, so that they must be able to respond to them and reprogram their genetic expression patterns in order to be adapted to these changes. One of the adaptation mechanisms that has been studied is the one due to temperature changes. Yeast's optimal growth temperature is between 25°C and 30°C; thus, when it rises over 36-37°C, the cell suffers proteotoxic damage due to the aggregation of misfolded and denatured proteins. In order to survive these conditions, cells activate the heat shock response (HSR; Dea & Pincus, 2024; Morano et al., 2012; Santiago et al., 2020). Most of the genes activated by HSR code for protein chaperones that prevent or reverse this toxic protein aggregation. Chaperones are also expressed under non stress conditions, although their levels rise dramatically when cells suffer a stress condition (Santiago et al., 2020). However, HSR also triggers the expression of other molecules with a protective role, such as the disaccharide trehalose, which stabilizes both proteins and membranes upon diverse kinds of stresses (Shen et al., 2022).

As it was already said, cells must undergo genetic expression reprogramming to respond to heat shock. There are two families of transcription factors that function as the main regulators of the HSR: the heat shock factor 1 (Hsf1) and the proteins Msn2 and Msn4. Hsf1 recognizes the heat shock element (HSE), a canonical motif that consists of repeated units of the sequence 5'-nGAAn-3' (Sorger & Pelham, 1987), in the promoter of the HSR genes and binds to them as a trimer, activating its expression (Sorger & Nelson, 1989). Hsf1 is bound to the heat shock protein Hsp70 when the cell grows under optimal conditions, Hsp70 acting as an inhibitor of Hsf1 activity. However, when the HSR is triggered, Hsp70 dissociates, thus Hsf1 can bind the HSE motifs in the HSR genes (Masser et al., 2019; Zheng et al., 2016; **Figure 8**). Besides, under heat shock, Hsf1 forms transcriptional condensates in the HSR genes with a high concentration

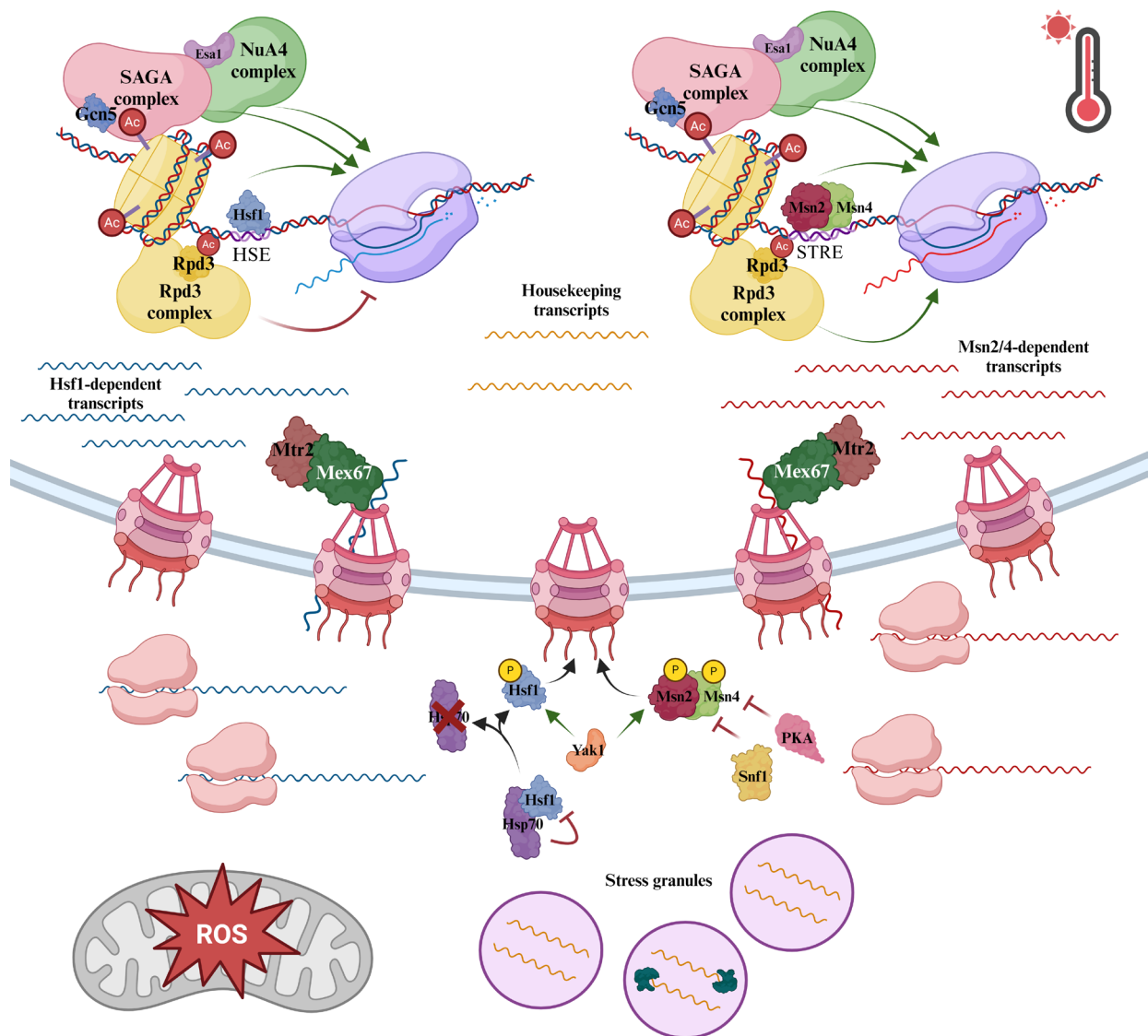


Figure 8. Proteotoxic damage produced by a high temperature triggers the heat stress response in yeast cells. Transcription factors Hsf1 and Msn2/4, whose expression and activity are tightly regulated under optimal and stress conditions, activate the expression of stress response genes. HATs and HDAs complexes also regulate the expression of these genes, by either activating or inhibiting them, depending on the complex. Upon heat shock, Mex67 dissociates from the mRNAs needed for growth under optimal conditions and facilitates the export of the HSR transcripts without the need for any adaptor. Besides, proteins and mRNAs needed for optimal growth are kept in stress granules so that their expression can be easily restored once growth conditions are optimal again. In addition, heat shock stress under aerobic conditions is tightly related to oxidative stress, since it can produce an unbalanced ROS equilibrium. Created with BioRender.com.

of the factors comprising the transcriptional machinery, which ensures a rapid and robust HSR activation (Chowdhary et al., 2022).

Msn2/4 transcription factors are necessary for the activation of several genes in different kinds of stresses. These proteins also recognize a specific sequence in the promoters of the genes they regulate: a stress responsive element (STRE), consisting of the sequence 5'-CCCCT-3' (Martínez-



INTRODUCTION

Pastor et al., 1996). There are multiple pathways that regulate the activation of these factors upon heat shock. Msn2/4 are phosphorylated by the protein kinase A (PKA) and the kinase Snf1 under optimal conditions, which blocks their import to the nucleus, preventing them from activating the HSR genes (De Wever et al., 2005; Görner et al., 2002; P. Lee et al., 2013). However, under stress conditions, the kinase Yak1 phosphorylates Msn2/4, which activates these factors and allows the expression of the HSR genes (P. Lee et al., 2008; **Figure 8**). Interestingly, Hsf1 activity is also regulated by phosphorylation with these kinases. Another regulatory mechanism of the Msn2/4 activity is mediated by the rapamycin-sensitive TOR signaling pathway. The kinases Tor1 and Tor2 stimulate the binding of the Bmh2 protein to Msn2/4, inhibiting them under optimal conditions (Beck & Hall, 1999).

Chromatin remodeling and post-translational histone modifications are also key for the activation of the HSR gene expression, especially histone acetylation and deacetylation. Both SAGA and NuA4 HATs regulate gene transcription upon heat shock, since the deletion of their catalytic subunits, Gcn5 and Esa1 respectively, supposes a decrease in HSR genes transcription. Besides, both complexes are recruited to the promoter of the Hsf1 and Msn2/4-dependent genes in stress conditions and, in the case of SAGA, even in the coding regions (Kremer & Gross, 2009; **Figure 8**). The three HDA Rpd3 complexes also play an important role in HSR regulation. The Rpd3L complex seems to have opposite effects depending on the transcription factor that activates the HSR genes. In the case of the Hsf1-dependent genes, Rpd3L is recruited to the promoters of these genes in both optimal and stress conditions, its occupancy increasing in HS, and acts as a repressor of the expression of these genes (Kremer & Gross, 2009). Whereas in the case of the genes regulated by Msn2/4, Rpd3L is only recruited upon heat shock and is essential for the transcription initiation of these genes (Ruiz-Roig et al., 2010; Sadeh et al., 2011). The Rpd3S complex also plays a role as a repressor of the Hsf1-regulated genes transcription elongation. The HDA binds to the promoters of these genes by recognizing methylated histone residues in both non-stress and stress conditions, even though its recruitment is highly increased upon heat shock (Keogh et al., 2005; Kremer & Gross, 2009; Pinskaya et al., 2009; **Figure 8**). Finally, Rpd3 μ has also been shown to be important for the cellular stress response; nevertheless, it seems to take



part more in the osmotic and oxidative stress response than in heat shock (Baker et al., 2013; McDaniel & Strahl, 2013).

HSR gene expression is not only regulated at transcriptional level, but mRNA Mex67-mediated transport is also altered in these conditions. On the one hand, upon heat shock, Mex67 dissociates from the mRNAs needed for growth under optimal conditions, so that their export to the cytoplasm is blocked and, thus, their translation. On the other hand, Mex67 interacts with Hsf1 for binding HSR transcripts without the aid of any adaptor protein and exports them through the NPCs overpassing quality control (Zander et al., 2016; **Figure 8**).

Heat shock stress under aerobic conditions is tightly related to oxidative stress (Morano et al., 2012; **Figure 8**). Oxygen in its molecular state (O_2) is not harmful since it is not a very reactive molecule. However, its partial reduction can suppose the formation of reactive oxygen species (ROS), such as the superoxide anion (O_2^-) or hydrogen peroxide (H_2O_2). ROS, unlike molecular oxygen, can damage a great variety of cellular components, including lipids, proteins and DNA (Halliwell, 2006; Liguori et al., 2018). Exposure to ROS under aerobic conditions is usual in all organisms and the cell has mechanisms to ensure ROS levels are kept at a bearable threshold. However, when the cells are exposed to high levels of these toxic compounds, ROS equilibrium is imbalanced and the cell is unable to maintain ROS concentration below the threshold. It is then that the oxidative stress response (OxSR) is triggered (Liguori et al., 2018; Santiago et al., 2020). In the case of cells suffering heat shock, the elevated temperature impairs the mitochondrial respiratory electron carriers, so that it provokes the disruption of the electron flow through the electron transport chain, inducing oxidative stress (J. F. Davidson & Schiestl, 2001). For this reason, HSR and OxSR overlap at many points.

The main regulator for the expression of the genes involved in OxSR is Yap1 (Harshman et al., 1988). This protein presents two different cysteine (Cys)-rich domains, which are key in the sensing of oxidative stress and the activation of the OxSR. In response to the presence of H_2O_2 , the Yap1 Cys residues interact through a disulfide bridge with the glutathione peroxidase Gpx3, which activates Yap1 (Delaunay et al., 2002). Once activated, Yap1 enters the nucleus and



induces the expression of a variety of antioxidant genes, such as the thioredoxin Trx2 (Coleman et al., 1999). This protein also presents Cys residues in its structure that are prone to being oxidized, which provokes a conformational change that mediates transcription factors Msn2/4 activation. Therefore, Msn2/4 enter the nucleus and trigger the expression of the genes they regulate (Boisnard et al., 2009; Collet et al., 2003). Hsf1 is also important in the OxSR, since Hsp70, the protein that regulates Hsf1 activation through its binding, also responds to high ROS levels by oxidation of their Cys residues (Ahn & Thiele, 2003; Y. Wang et al., 2012).

STRESS GRANULES FORMATION

Another characteristic of the cellular stress response is the assembly of membrane-less organelles in the cytoplasm composed of both proteins and mRNA. There are two different kinds of these compartments: stress granules and p-bodies (van Leeuwen & Rabouille, 2019).

Stress granules (SGs) formation in yeast is triggered by a great variety of stresses, such as glucose deprivation, heat shock or sodium azide-induced oxidative stress (Buchan et al., 2008, 2011; Wallace et al., 2015). When the cell suffers from a stress condition, one of its first survival mechanisms is the inhibition of the translation, which leads to the disassembly of the polyribosomes (Anderson & Kedersha, 2002; Riggs et al., 2020). Thus, proteins from the translation machinery and mRNA whose translation has been stalled are sequestered in stress granules, so that, when the cell returns to optimal conditions or adapts to the stress, SGs release their components to restart translation (Guzikowski et al., 2019; Ivanov et al., 2019). Therefore, the principal proteins located in SGs are translation factors, especially those involved in translation initiation: eIF4G, eIF4E, eIF3, Pab1 or the 40S ribosomal subunit (Brengues & Parker, 2007; Hoyle et al., 2007; Kato et al., 2011). However, other kinds of proteins are also present in these organelles, such as RBPs (Pub1 and Pbp1) or signaling proteins (Anderson & Kedersha, 2002; Tian et al., 2020). Interestingly, SG composition may vary depending on the kind of stress (Grousl et al., 2022).

The assembly of SGs is a two-phase process, since first translation is stalled, leading to mRNA accumulation, and then mRNA and proteins condense forming the SGs due to liquid-liquid phase separation (LLPS) (Hofmann et al., 2021; Kedersha et al., 2002). In mammals, the mechanism



by which SGs are assembled is well characterized, being triggered by the phosphorylation of the alpha subunit of the translation initiation factor eIF2, which leads to translation inhibition (Anderson & Kedersha, 2002). Nevertheless, SG formation in yeast seems to be independent of eIF2 α phosphorylation (Buchan et al., 2008, 2011). A recent study of the initiation translation factor eIF4G from a structural point of view has proposed an SGs assembly mechanism focusing on the amyloid-like properties of this protein (Chaves-Arquero et al., 2022). eIF4G presents two different isoforms in yeast, being the Tif4631, also known as eIF4G1, the most abundant (Goyer et al., 1993). The N-terminal part of this protein is structurally very interesting, since it contains several domains with intrinsically disordered regions (IDRs), such as a conserved RNA binding domain (RNA1) and some boxes of 15-20 conserved amino acids (BOX1, BOX2). Furthermore, these regions present multiple RBPs binding sites, through which Tif4631 interacts with the major SGs components Pub1 and Pab1 (S. Jain et al., 2016; Park et al., 2011; Tarun & Sachs, 1996). The BOX1 region presents amyloid-like properties that lead to self-assembly and self-aggregation of the protein. Thus, when translation is inhibited by the cellular stress response, Tif4631 would interact with Pub1, which triggers a remodeling of the protein that allows BOX1 self-assembly. This would facilitate the interaction with other Pab1 and Pub1 regions that are also amyloid-like, leading to SG assembly (Chaves-Arquero et al., 2022; **Figure 9**).

P-bodies are also membrane-less ribonucleoprotein assemblies whose formation is triggered by stress conditions. However, unlike stress granules, P-bodies could also be present in unstressed cells in some organisms (Souquere et al., 2009). Among the components of these granules, besides mRNA, there are also proteins participating in the repression of translation and the mRNA decay machinery, such as the 5' decapping proteins Dcp1 and Dcp2 or exonucleases like Xrn1 (Parker & Sheth, 2007; Teixeira & Parker, 2007). For this reason, p-bodies have always been proposed to be organelles whose function is the regulation of mRNA degradation and turnover. However, recent data has proved that degradation of mRNA is not dependent on p-bodies (Horvathova et al., 2017; Hubstenberger et al., 2017).



INTRODUCTION

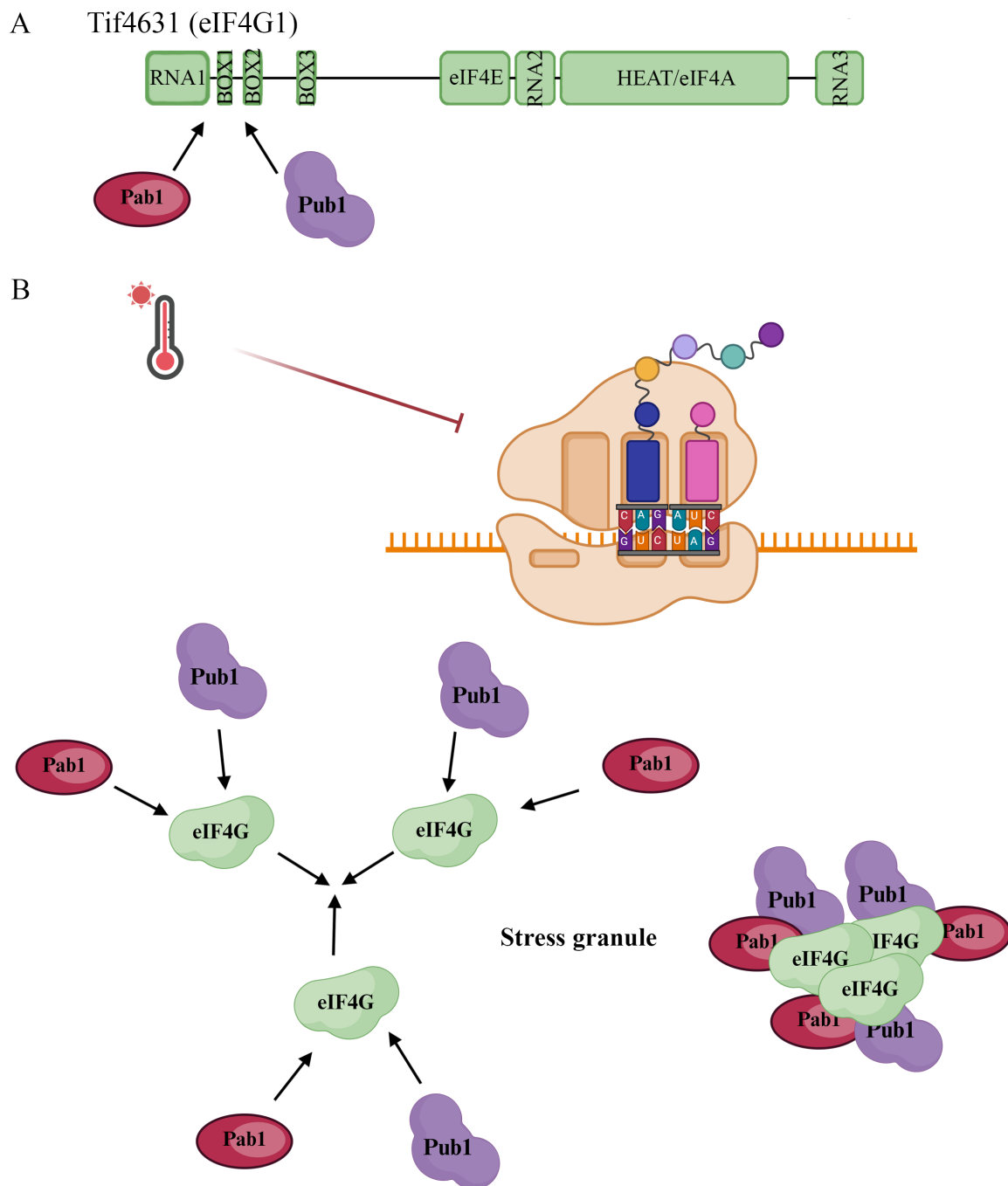


Figure 9. eIF4G participates in the assembly of stress granules. (A) The N-terminal of the Tif4631 isoform of eIF4G contains several domains with intrinsically disordered regions (IDRs), such as a conserved RNA binding domain (RNA1) and some boxes of 15-20 conserved amino acids (BOX1, BOX2). Furthermore, these regions present multiple RBP binding sites, through which Tif4631 interacts with the major SGs components Pub1 and Pab1. (B) When translation is inhibited by the cellular stress response, Tif4631 would interact with Pub1, which triggers a remodeling of the protein that allows BOX1 self-assembly. This would facilitate the interaction with other Pab1 and Pub1 regions that are also amyloid-like, leading to SG assembly. Created with BioRender.com.

TRANSCRIPTIONAL MEMORY

Another mechanism cells rely on to respond to changes in their environment is a phenomenon called transcriptional memory. Cells are constantly being exposed to stimuli that trigger a stress

response, so it is not rare that they are exposed to the same stimulus a second or successive times. Thus, they are capable of “remembering” that they have already responded to that stimulus and trigger the stress response swifter than the first time, presenting then transcriptional memory (Fabrizio et al., 2019; Pascual-Ahuir et al., 2020; Sump & Brickner, 2022). Besides, cells are able to inherit this ability even up to 14 generations later, depending on the kind of stimulus (Brickner et al., 2007; D’Urso et al., 2016; Light et al., 2013; Sump & Brickner, 2022).

This phenomenon is widely spread among eukaryotic organisms. For instance, the treatment with interferon gamma in HeLa cells promotes the activation of certain genes that are swiftly reactivated with a second exposure (Gialitakis et al., 2010; Light et al., 2013; Siwek et al., 2020). In *Drosophila*, there is also transcriptional memory when the fly is exposed to ecdysone, a molting hormone (Pascual-Garcia et al., 2017, 2022). Furthermore, in *Arabidopsis*, transcriptional memory has been studied related to thermal stress and dehydration, allowing the plants to resist longer periods of water scarcity (Ding et al., 2013; Liu et al., 2014).

Transcriptional memory studies in yeast focus on the response to two different stimuli. On the one hand, the lack of inositol in the medium, which provokes the transcription activation of the gene *INO1*. When there is no inositol, chromatin suffers a remodeling and is relocated so that the gene is placed near the nuclear periphery and interacts with the NPCs, allowing a rapid expression of the gene. However, when inositol levels return to optimal, the gene remains located next to the NPCs; thus, when a second period of inositol scarcity happens, the gene could be expressed faster (Brickner et al., 2007; D’Urso et al., 2016; Light et al., 2013; Randise-Hinchliff et al., 2016). Besides, dimethylation of the histone H3K4 residue by the methyltransferase complex Set1/COMPASS is also a key feature for the maintaining of transcriptional memory (D’Urso et al., 2016).

On the other hand, the expression of the *GAL* genes when there is a shift of the carbon source in the medium from glucose to galactose. When there is no galactose, the protein Gal80 inhibits the transcription factor Gal4, so that *GAL* genes are repressed. Nevertheless, with the addition of galactose, Gal80 entry to the nucleus is blocked and Gal4 can activate *GAL* genes expression



INTRODUCTION

(Peng & Hopper, 2002). The protein Gal1 is responsible for the Gal80 blockage in the presence of galactose. This protein is highly accumulated in the cytoplasm in these conditions and is inherited by mitosis to the daughter cells in glucose repression conditions, allowing a faster reactivation of the *GAL* genes in a second galactose induction (Kundu & Peterson, 2010). Another protein that takes part in the regulation of the transcriptional memory of these genes is Tup1, which functions as both an activator and a repressor of gene transcription. Tup1 regulates the dimethylation of the H3K4, necessary for the recruitment of the RNAPolII to the promoter of the *GAL* genes (Sood et al., 2017). Interaction with the NPCs and dimethylation of H3K4 seems to be a conserved mechanism for transcriptional memory regulation, not only in yeast, but also among other eukaryotic organisms (Liu et al., 2014; Pascual-Garcia et al., 2022; Siwek et al., 2020).

Interestingly, transcriptional memory does not only involve gene activation, but also the repression of certain genes that need to be inhibited in response to the stimulus. In the case of the *GAL* genes induction in the presence of galactose, there are also other genes that are repressed in these conditions and present transcriptional repression memory (TREM), since their expression is rapidly inhibited in successive galactose inductions. This mechanism has been studied to be regulated by the HDAC Rpd3L. Thus, at the beginning of the transcription, the RNAPolII recruits Set1/COMPASS for the H3K4 methylation. Then, the Pho23 subunit of the Rpd3L complex binds these methylation marks in H3 and triggers the histone deacetylation and the repression of the genes. In the absence of the Pho23 subunit, the cells do not present TREM, which suggests this mechanism is key for maintaining the transcriptional memory (B. B. Lee et al., 2018; **Figure 10**).

Nevertheless, although chromatin remodeling and histone modification play an important role in the regulation of transcriptional memory, post-transcriptional mechanisms may also modulate this phenomenon. A recent work searching for proteins whose depletion alters *GAL* genes transcriptional memory has shown that the mRNA degradation machinery seems to take part in the process too. Indeed, depletion of the exosome factor Rrp6 enhances both activation and repression of a subset of genes in memory conditions. Besides, genes with higher activation in a second exposure to the stimulus present a higher association with mRNA degradation

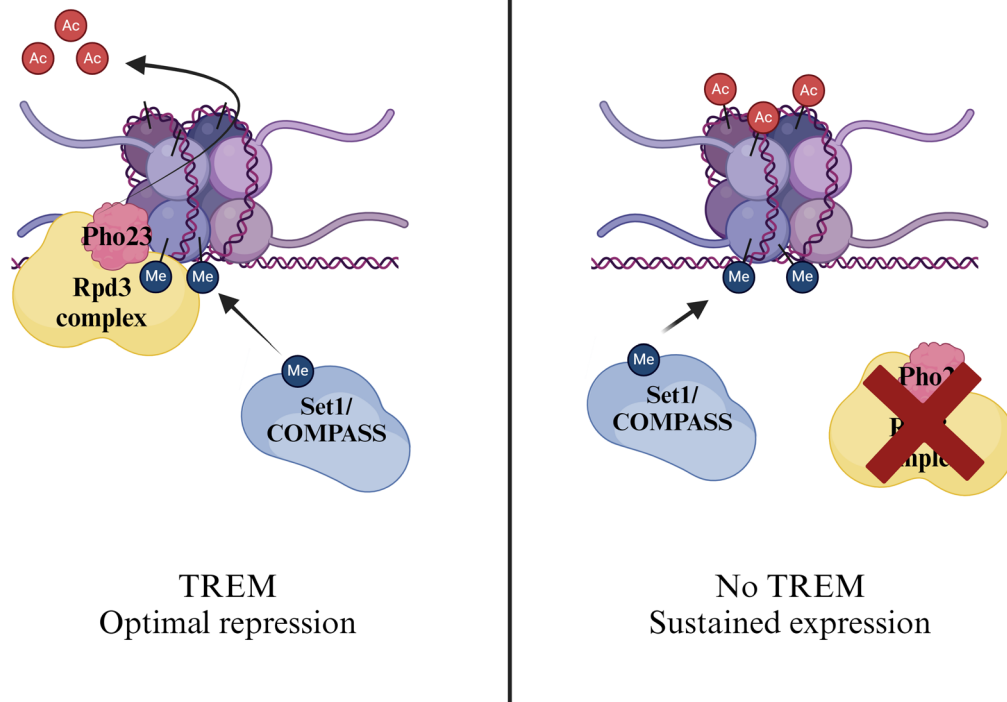


Figure 10. Rpd3 HDA complex participates in transcriptional repression memory. RNAPolIII recruits Set1/COMPASS for the H3K4 methylation. Then, the Pho23 subunit of the Rpd3L complex binds these methylation marks in H3 and triggers the histone deacetylation and the repression of the genes. However, in the absence of the Pho23 subunit, the cells do not present TREM. Created with BioRender.com.

complexes, such as TRAMP or NNS, while this is not observed in repressed genes. Interestingly, this association is clearly decreased even after 3h of the first exposure, increasing mRNA stability and facilitating a faster response for a second induction. However, for the subset of genes whose activation is enhanced by Rrp6 depletion, the observed phenotype is different, since those mRNAs are less stable in a second induction. Thus, changes in mRNA stability are involved in transcriptional memory regulation. Besides, this role is not reserved for exosome factors, since the mRNA cytoplasmic degradation machinery also seems to take part in the process. In the absence of Xrn1, the expression pattern is similar. However, focusing on genes that show a higher repression memory in *rrp6Δ*, they present less mRNA stability in a second induction. Therefore, the transcriptional memory of these genes would be regulated by Xrn1. These evidences confirm that the mechanisms regulating transcriptional memory do not only focus on the earlier steps in gene expression, but mRNA stability and turnover are also significant regulatory mechanisms of the process (Li et al., 2023).



SPORULATION

Sporulation is a process occurring in diploid cells triggered during starvation whose aim is the survival of the cell in these conditions. Four haploid spores are generated, surrounded by a four-layered wall that confers the spores protection from environmental stressors (Durant et al., 2024; Lee-Soety et al., 2024). An important part of the formation of spores is the cell division, carried out by a specialized process called meiosis in which from a single diploid cell, through two cell divisions, four haploid gametes are formed (Neiman, 2011; Piekarska et al., 2010; Serrano-Quílez et al., 2020). Cell decision to undergo a meiotic process depends on several factors: the expression of an $a1/\alpha2$ heterodimer, only possible in diploid cells that present both $MATa$ and $MAT\alpha$ alleles (Piekarska et al., 2010), as well as nutritional conditions such as nitrogen scarcity and the presence of a poor carbon source (Chu et al., 1998; Gao et al., 2024; Sou et al., 2021). The combination of these factors triggers a cascade of transcriptional events that lead to the beginning of the meiotic process, which includes changes in chromatin dynamic and organization due to, among other mechanisms, histones and chromatin remodelers post-translational modifications (Bannister & Kouzarides, 2011; Hillers et al., 2017; Jin & Neiman, 2016; Tessarz & Kouzarides, 2014).

Meiosis initiates with the expression of the master regulator *Ime1*, a transcription factor that is necessary for the activation of several meiotic genes expression. This protein presents many regulatory mechanisms. First, in haploid cells, the *Rme1* repressor promotes the expression of the long non-coding RNA (lncRNA) *IRT1*. This lncRNA binds the *IME1* promoter, inhibiting the transcription of the gene (van Werven et al., 2012). However, in diploid cells, the $a1/\alpha2$ heterodimer inhibits *Rme1* expression, which allows it to bind to a specific sequence in the *IME1* promoter called the *Rme1* Repressor Element (RRE; Mitchell & Herskowitz, 1986; Sagee et al., 1998). Besides, another *Ime1* repression mechanism in haploid cells involves the expression of a non-coding anti-sense RNA, called *IME4-AS* or *RME2*, which leads to *IME4* mRNA degradation. *Ime4*, the product of this mRNA, is a protein that participates in the induction of *Ime1* expression too. Thus, the $a1/\alpha2$ heterodimer of diploid cells inhibits the transcription of *IME4-AS*, so that the protein could be formed and carry out its function (Clancy et al., 2002; Hongay et al., 2006). *Ime1*

expression is also subjected to nutritional regulation. PKA and TORC1 activation by glucose presence and nitrogen availability, respectively, also leads to Ime1 repression. In contrast, nutrient scarcity triggers the inhibition of these regulatory pathways with the subsequent phosphorylation of Ime1 and Ume6. Both proteins form a complex that activates early meiotic genes (EMG) expression, starting the meiotic process (Malathi et al., 1997; Weidberg et al., 2016; **Figure 11**).

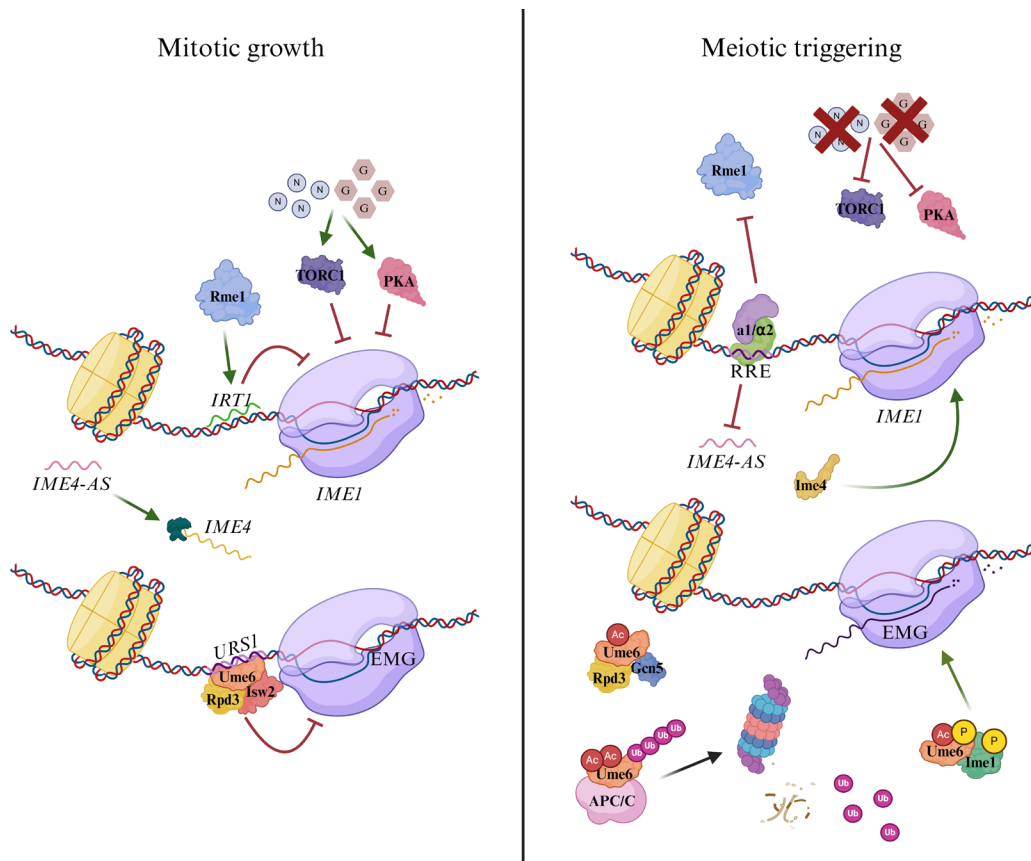


Figure 11. Nitrogen scarcity and the presence of a poor carbon source in yeast diploid cells trigger sporulation. In haploid cells, the Rme1 repressor promotes the expression of *IRT1*, which binds the *IME1* promoter and inhibits its transcription. Besides, *IME4-AS* leads to *IME4* mRNA degradation, which also leads to Ime1 inhibition. PKA and TORC1 activation by glucose presence and nitrogen availability, respectively, also leads to Ime1 repression. In addition, Ume6 binds to the *URS1* site of the EMG, preventing their transcription together with Rpd3 and Isw2. However, in diploid cells, the $\alpha 1/\alpha 2$ heterodimer inhibits Rme1 expression and the transcription of *IME4-AS*, so that Ime1 can be expressed. Furthermore, nutrient scarcity triggers the phosphorylation of Ime1 and Ume6, promoting EMG expression. Glucose and nitrogen starvation also leads to Ume6 degradation in a two-step process, mediated by both acetylation regulated by Gcn5 and Rpd3 and ubiquitination by APC/C, which enables EMG transcription. Created with BioRender.com.

Ume6 acts as both an activator and a repressor of the EMG. These genes present a common regulatory element in their promoters known as the *URS1* site. Under non meiotic conditions, Ume6 binds to the *URS1* site, preventing the transcription of the genes by the recruitment of HDA Rpd3 and the chromatin remodeler factor Isw2 (Goldmark et al., 2000; Mallory & Strich, 2003; Strich et al., 1994). Glucose and nitrogen starvation produces Ume6 degradation in a



INTRODUCTION

two-step process, which enables EMG transcription. First, the presence of a poor carbon source instead of glucose triggers Ume6 acetylation, regulated by both the SAGA HAT catalytic subunit Gcn5 and the HDA Rpd3 (Law et al., 2014; Mallory et al., 2012). This acetylation supposes the partial dissociation of Ume6 from the EMG promoters, leading to the ubiquitination of the free protein by the anaphase-promoting complex/cyclosome (APC/C) ubiquitin ligase activated by Cdc20 and its partial destruction (Mallory et al., 2007). Then, nitrogen starvation induces Ime1 expression and the Ume6-Ime1 complex is assembled. This interaction serves as an activator of EMG transcription, but it also enhances the later Ume6 acetylation in a different cluster of Lys residues, which triggers Ume6 total degradation (Harris & Ünal, 2023; Law et al., 2014; Mallory et al., 2007; **Figure 11**).

EMG expression promotes the cell entry in a premeiotic S phase, where DNA is replicated, as well as the genetic recombination caused by the pairing and the segregation of homologous chromosomes (Börner et al., 2023). This process happens in the early steps of meiosis I and it is also extremely regulated, since it involves DNA double-strand breaks that could produce mutations or aberrant chromosomal rearrangements if the process is not properly conducted (Cooper & Strich, 2011; Hochwagen & Amon, 2006; Lane & Kauppi, 2019). As recombination is close to the end, Ndt80 expression starts, another meiotic general transcription factor whose activation is induced by Ime1 (Chu & Herskowitz, 1998). This protein regulates the expression of the middle meiotic genes, activating them once the cell is fully committed to meiosis and during meiosis I and II (Winter, 2012).

For the completion of the sporulation process, apart from the meiotic program, cells must also undergo spore morphogenesis. Thus, during meiosis II, the prospore membrane (PSM) is produced and surrounds each of the four haploid cells in formation (Neiman, 2024). When meiosis II comes to an end, the four layers comprising the spore wall are generated and encircle the PSMs thanks to the action of the proteins coding by midlate genes. Finally, the process culminates with the spore maturation, induced by the late genes expression, and the total formation of four spores able to resist several environmental stress conditions (Durant et al., 2024).

OBJECTIVES





Throughout this thesis, the main aim has been the study and characterization of Mip6, a protein participating in gene expression at different levels. Thus, considering its multifaceted role, since it takes part in processes that diverse as transcription, mRNA export, degradation or storage, the study of this protein may shed more light on how the different gene expression steps are interconnected and regulated. Therefore, for the functional and structural characterization of Mip6, we can state the following specific objectives:

1. Deepen on Mip6 interaction with the stress response transcription factors Msn2/4.
2. Analyze the crosstalk of Mip6 with histone deacetylase proteins.
3. Study heat shock transcriptional memory and Mip6 participation.
4. Deepen on Mip6's role in sporulation and meiosis.
5. Characterization of Mip6 export mechanisms from the nucleus to the cytoplasm.
6. Study of factors that participate in stress granule formation.

MATERIALS AND METHODS



MATERIALS





YEAST STRAINS

Three different *Saccharomyces cerevisiae* backgrounds were used: BY4741 (Baker Brachmann et al., 1998), W303-1A (Thomas & Rothstein, 1989), SK1 (Kane & Roth, 1974) and a background obtained by crossing an SK1 and a W303 strains (Cooper, 1997). The yeast strains employed are listed in the following table:

BY4741 background		
Strain	Genotype	Source
WT	MATa <i>his3Δ1 leu2Δ0 met15Δ0 ura3Δ0</i>	(Baker Brachmann et al., 1998)
<i>mip6Δ</i>	MATa <i>his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 mip6::KANMX4</i>	EUROSCARF
<i>msn2Δ</i>	MATa <i>his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 msn2::KANMX4</i>	EUROSCARF
<i>msn2Δmip6Δ</i>	MATa <i>his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 msn2::KANMX4 mip6::HIS3</i>	(Martín-Expósito et al., 2019)
<i>msn4Δ</i>	MATa <i>his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 msn4::KANMX4</i>	EUROSCARF
<i>msn4Δmip6Δ</i>	MATa <i>his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 msn4::KANMX4 mip6::HIS3</i>	(Martín-Expósito et al., 2019)
<i>msn2Δmsn4Δ</i>	MATa <i>his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 msn2::NATNT2 msn4::KANMX4</i>	(Zapater et al., 2007)
<i>msn2Δmsn4Δmip6Δ</i>	MATa <i>his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 msn2::NAT msn4::KANMX4 mip6::HIS3</i>	(Martín-Expósito et al., 2019)
<i>rpd3Δ</i>	MATa <i>his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 rpd3::KANMX4</i>	EUROSCARF
<i>rpd3Δmip6Δ</i>	MATa <i>his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 rpd3::LEU2 mip6::KANMX4</i>	Constructed in Gene expression and mRNA metabolism laboratory
<i>gcn5Δ</i>	MATa <i>his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 gcn5::KANMX4</i>	EUROSCARF
<i>gcn5Δmip6Δ</i>	MATa <i>his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 gcn5::KANMX4 mip6::LEU2</i>	This work
<i>hda1Δ</i>	MATa <i>his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 hda1::KANMX4</i>	EUROSCARF



MATERIALS AND METHODS

Strain	Genotype	Source
<i>hda1Δmip6Δ</i>	MATa <i>his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 hda1::KANMX4 mip6::HIS3</i>	Constructed in Gene expression and mRNA metabolism laboratory
Rpd3-TAP	MATa <i>his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 RPD3-TAP-HIS3</i>	OPEN BIOSYSTEM
Rpd3-TAP <i>mip6Δ</i>	MATa <i>his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 RPD3-TAP-HIS3 mip6::LEU2</i>	This work
<i>pes4Δ</i>	MATa <i>his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 pes4::KANMX4</i>	EUROSCARF
Tif4631-TAP	MATa <i>his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 TIF4631-TAP-KANMX6</i>	(Martín Expósito, 2019)
<i>tif4632Δ</i>	MATa <i>his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 tif4632::HPHNT1</i>	This work
<i>tif4632Δ</i> Tif4631-TAP	MATa <i>his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 TIF4631-TAP-KANMX6 tif4632::HPHNT1</i>	This work
<i>tif4632Δ</i> Tif4631ΔNter	MATa <i>his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 tif4632::HPHNT1 TIF4631ΔNter</i>	This work
<i>tif4632Δ</i> Tif4631ΔNter-TAP	MATa <i>his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 TIF4631ΔNter-TAP-KANMX6 tif4632::HPHNT1</i>	This work
<i>tif4632Δ</i> Tif4631ΔBOX1	MATa <i>his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 tif4632::HPHNT1 TIF4631Δbox1</i>	This work
<i>tif4632Δ</i> Tif4631ΔBOX1-TAP	MATa <i>his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 TIF4631Δbox1-TAP-KANMX6 tif4632::HPHNT1</i>	This work
<i>tif4632Δ</i> Tif4631ΔBOX2	MATa <i>his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 tif4632::HPHNT1 TIF4631Δbox2</i>	This work
<i>tif4632Δ</i> Tif4631ΔBOX2-TAP	MATa <i>his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 TIF4631Δbox2-TAP-KANMX6 tif4632::HPHNT1</i>	This work
<i>tif4632Δ</i> Tif4631ΔRNA1	MATa <i>his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 tif4632::HPHNT1 TIF4631ΔRNA1</i>	This work
<i>tif4632Δ</i> Tif4631ΔRNA1-TAP	MATa <i>his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 TIF4631ΔRNA1-TAP-KANMX6 tif4632::HPHNT1</i>	This work
W303-1A background		
Strain	Genotype	Source
<i>rpb1-1 mip6Δ</i>	MATa <i>ade2-1 ura3-1 his3-11,-15 leu2,3-112 trp1 rpb1-1^{ts} mip6::HIS3</i>	(Martín-Expósito et al., 2019)
Msn5-MYC <i>mip6Δ</i>	MATa <i>ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 MSN5-9MYC-TRP1</i>	(Alepez et al., 1999)
SK1+W303 background		
Strain	Genotype	Source
WT	MATa/MATα <i>cyh2r/cyh2r ho::LYS2/ho::LYS2 leu2::hisG/leu2::hisG trp1::hisG/trp1::hisG lys2/lys2 ura3/ura3</i>	(Cooper, 1997)



Strain	Genotype	Source
<i>mip6Δ</i>	MATa/MATα <i>cyh2r/cyh2r ho::LYS2/ho::LYS2</i> <i>leu2::hisG/leu2::hisG trp1::hisG/trp1::hisG lys2/lys2</i> <i>ura3/ura3 mip6::KANMX4/mip6::KANMX4</i>	This work
<i>pes4Δ</i>	MATa/MATα <i>cyh2r/cyh2r ho::LYS2/ho::LYS2</i> <i>leu2::hisG/leu2::hisG trp1::hisG/trp1::hisG lys2/lys2</i> <i>ura3/ura3 pes4::TRP1/pes4::TRP1</i>	This work
<i>mip6Δpes4Δ</i>	MATa/MATα <i>cyh2r/cyh2r ho::LYS2/ho::LYS2</i> <i>leu2::hisG/leu2::hisG trp1::hisG/trp1::hisG lys2/</i> <i>lys2 ura3/ura3 mip6::KANMX4/mip6::KANMX4</i> <i>pes4::TRP1/pes4::TRP1</i>	This work
14a	MATa <i>his1</i>	(Trueheart et al., 1987)
17α	MATα <i>his1</i>	(Trueheart et al., 1987)
SK1 background		
Strain	Genotype	Source
WT	MATa/MATα <i>ura3/ura3 his3ΔSK/his3ΔSK leu2/leu2</i> <i>trp1::hisG/trp1::hisG lys2/lys2 ho::LYS2/ho::LYS2</i>	(Jin et al., 2017)
Mip6-GFP	MATa/MATα <i>ura3/ura3 his3ΔSK/his3ΔSK leu2/leu2</i> <i>trp1::hisG/trp1::hisG lys2/lys2 ho::LYS2/ho::LYS2</i> <i>MIP6-3xsfGFP::KANMX6/MIP6-3xsfGFP::KANMX6</i>	This work
Pes4-GFP	MATa/MATα <i>ura3/ura3 his3ΔSK/his3ΔSK leu2/leu2</i> <i>trp1::hisG/trp1::hisG lys2/lys2 ho::LYS2/ho::LYS2</i> <i>PES4-3xsfGFP::KANMX6/PES4-3xsfGFP::KANMX6</i>	This work

Table 1. Yeast strains used during this study, indicating their background, their genetic description and their source.

PLASMIDS

The different plasmids employed in this study are listed in the table below:

Plasmid	Description	Source
pADH1pr-GFP	Yeast plasmid for protein expression regulated by <i>ADH1</i> promoter and tagged in the C-terminal with a GFP epitope (<i>URA3</i> marker)	(Taberner et al., 2009)
pADH1pr-Mip6-GFP	Yeast plasmid for Mip6 expression regulated by <i>ADH1</i> promoter and tagged in the C-terminal with a GFP epitope (<i>URA3</i> marker)	(Martín-Expósito et al., 2019)
pADH1pr-Mip6W₄₄₂A-GFP	Yeast plasmid for Mip6W ₄₄₂ A expression regulated by <i>ADH1</i> promoter and tagged in the C-terminal with a GFP epitope (<i>URA3</i> marker)	(Martín-Expósito et al., 2019)
pADH1pr-Mip6I₄₂₇R-GFP	Yeast plasmid for Mip6I ₄₂₇ R expression regulated by <i>ADH1</i> promoter and tagged in the C-terminal with a GFP epitope (<i>URA3</i> marker)	Constructed in Gene expression and mRNA metabolism laboratory



MATERIALS AND METHODS

Plasmid	Description	Source
pADH1pr-Mip6I₄₃₂R-GFP	Yeast plasmid for Mip6I ₄₃₂ R expression regulated by <i>ADH1</i> promoter and tagged in the C-terminal with a GFP epitope (<i>URA3</i> marker)	Constructed in Gene expression and mRNA metabolism laboratory
pNOP1pr-GFP	Yeast plasmid for protein expression regulated by <i>NOP1</i> promoter and tagged in the N-terminal with a GFP epitope (<i>LEU2</i> marker)	(Sikorski & Hieter, 1989)
pNOP1pr-GFP-Pes4	Yeast plasmid for Pes4 expression regulated by <i>NOP1</i> promoter and tagged in the N-terminal with a GFP epitope (<i>LEU2</i> marker)	Constructed in Gene expression and mRNA metabolism laboratory
pNOP1pr-GFP-Pes4I₄₁₈R	Yeast plasmid for Pes4I ₄₁₈ R expression regulated by <i>NOP1</i> promoter and tagged in the N-terminal with a GFP epitope (<i>LEU2</i> marker)	This work
pNOP1pr-GFP-Pes4ΔI₄₂₃	Yeast plasmid for Pes4ΔI ₄₂₃ expression regulated by <i>NOP1</i> promoter and tagged in the N-terminal with a GFP epitope (<i>LEU2</i> marker)	This work
pADH1pr-SV40-TAgNLS-GFP	Yeast plasmid for the expression of the NLS of the SV40 large T antigen by <i>ADH1</i> promoter and tagged in the C-terminal with a GFP epitope (<i>LEU2</i> marker)	(Koch et al., 2012)
pADH1pr-SV40-TAgNLS-NES_{RRM4}-GFP	Yeast plasmid for the expression of the NLS of the SV40 large T antigen and the NES present in the Mip6 RMM4 by <i>ADH1</i> promoter and tagged in the C-terminal with a GFP epitope (<i>LEU2</i> marker)	This work
pADH1pr-SV40-TAgNLS-NES_{Cter}-GFP	Yeast plasmid for the expression of the NLS of the SV40 large T antigen and the NES present in the Mip6 C-terminal by <i>ADH1</i> promoter and tagged in the C-terminal with a GFP epitope (<i>LEU2</i> marker)	This work
pADH1pr-Mip6W₄₄₂A;I₄₂₇R-GFP	Yeast plasmid for Mip6W ₄₄₂ A;I ₄₂₇ R expression regulated by <i>ADH1</i> promoter and tagged in the C-terminal with a GFP epitope (<i>URA3</i> marker)	This work
pADH1pr-Mip6W₄₄₂A;I₄₃₂R-GFP	Yeast plasmid for Mip6W ₄₄₂ A;I ₄₃₂ R expression regulated by <i>ADH1</i> promoter and tagged in the C-terminal with a GFP epitope (<i>URA3</i> marker)	This work
pADH1pr-Mip6ΔNES_{Cter}-GFP	Yeast plasmid for Mip6ΔNES _{Cter} expression regulated by <i>ADH1</i> promoter and tagged in the C-terminal with a GFP epitope (<i>URA3</i> marker)	This work
pADH1pr-Mip6C₄₅₀A-GFP	Yeast plasmid for Mip6C ₄₅₀ A expression regulated by <i>ADH1</i> promoter and tagged in the C-terminal with a GFP epitope (<i>URA3</i> marker)	This work
pRS405	Yeast plasmid for gene deletion with the <i>LEU2</i> marker integration	(Sikorski & Hieter, 1989)



Plasmid	Description	Source
pRS403	Yeast plasmid for gene deletion with the <i>HIS3</i> marker integration	(Sikorski & Hieter, 1989)
pRS404	Yeast plasmid for gene deletion with the <i>TRP1</i> marker integration	(Sikorski & Hieter, 1989)
pRS400	Yeast plasmid for gene deletion with the <i>KANMX4</i> marker integration	(Baker Brachmann et al., 1998)
pFA6a- <i>HPHNT1</i>	Yeast plasmid for gene deletion with the <i>HPHNT1</i> marker integration	(Janke et al., 2004)
pGFP- <i>KANMX4</i>	Yeast plasmid for the GFP epitope integration in the C-terminal with the <i>KANMX4</i> marker	Dr. Ethel Queralt (Instituto de Biomedicina de Valencia)
pMEL10	Yeast plasmid designed to clone and express a sgRNA for CRISPR technology (<i>KIURA3</i> marker)	(Mans et al., 2015)
pJH001	Yeast plasmid for Cas9 expression (<i>LEU2</i> marker)	(Laughery et al., 2015)
p <i>GAL1-HO-URA3</i>	Yeast plasmid for Ho expression regulated by <i>GAL1</i> promoter (<i>URA3</i> marker)	Dr. Juan Carlos Igual (Universidad de Valencia)

Table 2. Yeast plasmids used during this study, indicating their description and their source.

OLIGOS

Oligos designed and used during this study, either for a qPCR experiment or for the creation of a new mutant strain or plasmid, are listed in the following table:

Oligo	5' → 3' sequence	Assay
<i>HSP12</i> ORF FW	CTTCCAAGGTGTCCACGACT	qPCR
<i>HSP12</i> ORF RV	ACATATTCGACGGCATCGTT	qPCR
<i>CTT1</i> ORF FW	GAACCATGACTGGGTCTTCA	qPCR
<i>CTT1</i> ORF RV	GAAGGAATGACCAGAGTACG	qPCR
<i>RPL6B</i> ORF FW	AACATCTAGAAGACAACACT	qPCR
<i>RPL6B</i> ORF RV	GAACAAGTTAGCTTCCTTCT	qPCR
<i>RPL23A</i> ORF FW	TATCGCCGTCAAAGGCTCTG	qPCR
<i>RPL23A</i> ORF RV	CGATGACACCAGCATTGTCT	qPCR
<i>RPS19A</i> ORF FW	CTTCTGGTAACGAAATGCCA	qPCR
<i>RPS19A</i> ORF RV	TTGCAAAACCTTTCTGTTGA	qPCR
<i>RPS27A</i> ORF FW	CCCAACTGCAGCTTCTGAAG	qPCR
<i>RPS27A</i> ORF RV	CCTCAGAAAGCTTTGCCTTA	qPCR
<i>SCR1</i> FW	GAGTTTTATCCAGCGTCAGC	qPCR
<i>SCR1</i> RV	GGTTCAGGACACACTCCATC	qPCR
CRISPR plasmid backbone FW	GATCATTTATCTTTCACTGCGGAGAAG	CRISPR
CRISPR plasmid backbone RV	GTTTTAGAGCTAGAAATAGCAAGTTAAA ATAAGGCTAGTC	CRISPR



MATERIALS AND METHODS

Oligo	5' → 3' sequence	Assay
Tif4631ΔBOX1/ Tif4631ΔNter crRNA FW	TGCGCATGTTTCGGCGTTCGAAACTTC TCCGCAGTGAAAGATAAATGATCGCT GGGGCTGGTAGTAAACGGTTTTAGAGC TAGAAATAGCAAGTTAAAATAAG	CRISPR
Tif4631ΔBOX1/ Tif4631ΔNter crRNA RV	GTTGATAACGGACTAGCCTTATTTTAA CTTGCTATTTCTAGCTCTAAAACCGTTT ACTACCAGCCCCAGCGATCATTATCT TTCCTGCGGAGAAGTTTCGAACGCCG AAACATGCGCA	CRISPR
Tif4631ΔBOX2 crRNA FW	TGCGCATGTTTCGGCGTTCGAAACTTC TCCGCAGTGAAAGATAAATGATCATA GAAATCACTACCAAGTCGTTTTAGAG CTAGAAATAGCAAGTTAAAATAAG	CRISPR
Tif4631ΔBOX2 crRNA RV	GTTGATAACGGACTAGCCTTATTTTAA ACTTGCTATTTCTAGCTCTAAAACGA CTTGGTAGTGATTTCTATGATCATTTA TCTTTCCTGCGGAGAAGTTTCGAA CGCCGAAACATGCGCA	CRISPR
Tif4631ΔRNA1 crRNA FW	TGCGCATGTTTCGGCGTTCGAAACTT CTCCGCAGTGAAAGATAAATGATCGC TTTGAAGCAGATTGTGTCGTTTTAGAG CTAGAAATAGCAAGTTAAAATAAG	CRISPR
Tif4631ΔRNA1 crRNA RV	GTTGATAACGGACTAGCCTTATTTTAA CTTGCTATTTCTAGCTCTAAAACGACA CAATCTGCTTCAAAGCGATCATTATC TTTCACTGCGGAGAAGTTTCGAAC GCCGAAACATGCGCA	CRISPR
Tif4631ΔNter dsDNA repair FW	CCTTCTTGCTCATAAATAAGCAAGGTA AGAGGACAACTGTAATTACCTATTACA ATAATGGGATCGGACAAACCTGAGGT AACCGAAAAAACCAAGCCTGCTGAAG AAAAATCTGCTGAG	CRISPR
Tif4631ΔNter dsDNA repair RV	CTCAGCAGATTTTTCTTCAGCAGGCTT GGTTTTTTCGGTTACCTCAGGTTTGTCC GATCCCATTTATTGTAATAGGTAATTAC AGTTGTCCTCTTACCTTGCTTATTTATG AGCAAGAAGG	CRISPR
Tif4631ΔBOX1 dsDNA repair FW	AACTATAATGGTGGCGGTAGTTTCAGA GGTGGACACATGGGAGCCAACAGCTC AAACGTGGCGGCCGCTGGTAGTGCCC CTGCTAATCCAATTCCTGTCGAAGAGA AGTCACCTGTTCCA	CRISPR



Oligo	5' → 3' sequence	Assay
Tif4631ΔBOX1 dsDNA repair RV	TGGAACAGGTGACTTCTCTTCGACAGG AATTGGATTAGCAGGGGCACTACCAG CGGCCGCCACGTTTGAGCTGTTGGCTC CCATGTGTCCACCTCTGAAACTACCGC CACCATTATAGTT	CRISPR
Tif4631ΔBOX2 dsDNA repair FW	CAGCAAATGGCGGCCGCTGGTAGTGC CCCTGCTAATCCAATTCCTGTCGAAGA GAAGTCAAAAGCCAAGCTACAGTCTC AGGAAAGATCTACTGTGTCTCCGCA ACCAGAGTCAAAGTTA	CRISPR
Tif4631ΔBOX2 dsDNA repair RV	TAACTTTGACTCTGGTTGCGGAGACA CAGTAGATCTTTCCTGAGACTGTAGC TTGGCTTTTGACTTCTCTTCGACAGGA ATTGGATTAGCAGGGGCACTACCA GCGGCCGCCATTGCTG	CRISPR
Tif4631ΔRNA1 dsDNA repair FW	CCTTCTTGCTCATAAATAAGCAAGGT AAGAGGACAACCTGTAATTACCTATT ACAATAATGGGTGGACACATGGGA GCCAACAGCTCAAACGTGCCATG GACTGGTTACTATAATAACTAC	CRISPR
Tif4631ΔRNA1 dsDNA repair RV	GTAGTTATTATAGTAACCAGTCCA TGGCACGTTTGAGCTGTTGGCTCCC ATGTGTCCACCCATTATTGTAATAG GTAATTACAGTTGTCTCTTACCTTGCT TATTTATGAGCAAGAAGG	CRISPR
Pes4I₄₁₈ R FW	AAGAAAATCGTAGACCGTTCAAGATT CAGATAGATGGATA	QuikChange™
Pes4I₄₁₈ R RV	GAACGGTCTACGATTTTCTTGGTGACA TAAATATTTAAGA	QuikChange™
Pes4ΔI₄₂₃ FW	ACCGTTCAAGCAGATAGATGGATATG ACGAAAATTCTTCT	QuikChange™
Pes4ΔI₄₂₃ FW	CATCTATCTGCTTGAACGGTCTAATAT TTTCTTGGTGACA	QuikChange™
Mip6W₄₄₂A;I₄₂₇ R FW	CAAGAAAAACGAAGATTCAGTGAAAT TAAGATCACAAAC	QuikChange™
Mip6W₄₄₂A;I₄₂₇ R RV	CTGAATCTTCGTTTTTCTTGTAACATA GTTGAGAAATAA	QuikChange™
Mip6W₄₄₂A;I₄₃₂ R FW	CAGTGAACGTAAGATCACAAACTATA ACTCATTAAATGCG	QuikChange™
Mip6W₄₄₂A;I₄₃₂ R RV	TTGTGATCTTACGTTCACTGAATCTTA TTTTTCTTGTA	QuikChange™
Mip6ΔNES_{Cter} FW	ATCACAGAAAACAGTTGGATCCAGTAA AGGAGAAGAACTT	QuikChange™
Mip6ΔNES_{Cter} RV	ATCCAACGTGTTTTCTGTGATTCTAATG AAGTATTTGTCAT	QuikChange™
Mip6C₄₅₀ A FW	CATGTTGAGGCTTTTTCTCGTAGTGAT GCTGAGAGATTAT	QuikChange™



MATERIALS AND METHODS

Oligo	5' → 3' sequence	Assay
Mip6C ₄₅₀ A RV	CGAGAAAAAGCCTCAACATGTCCACA AAATGTCCAATTTA	QuikChange™
pADH1pr-SV40- TAgNLS-GFP linearization FW	GGATCCGGAGCTGGAGCTG	Cloning
pADH1pr-SV40- TAgNLS-GFP linearization RV	GACCTTTCTCTTCTTTTTTGGAGCCATA TG	Cloning
NES _{RRM4} FW	CAAAAAAGAAGAGAAAGGTCGGATC CGGAGCTGGAGCTGGTGCAGGTGCTG GTGCTATAAGATTCAGTGAAATTAAG ATCGGATCCGGAGCTGGAGCTGG	Cloning
NES _{RRM4} RV	CCAGCTCCAGCTCCGGATCCGATCTT AATTTCACTGAATCTTATAGCACCAGC ACCTGCACCAGCTCCAGCTCCGGATC CGACCTTTCTCTTCTTTTTTG	Cloning
NES _{Cter} FW	CAAAAAAGAAGAGAAAGGTCGGATC CGGAGCTGGAGCTGGTGCAGGTGCTG GTGCTATTCTACAAAAACAAGTCACT AGAGCTGCTGAAAGCCTCGGATTTGG ATCCGGAGCTGGAGCTGG	Cloning
NES _{Cter} RV	CCAGCTCCAGCTCCGGATCCAAATCC GAGGCTTTCAGCAGCTCTAGTGACTT GTTTTGTAGAATAGCACCAGCACCT GCACCAGCTCCAGCTCCGGATCCGA CCTTTCTCTTCTTTTTTG	Cloning

Table 3. Oligos employed during this study, indicating their sequence in the 5'→3' direction and the kind of assay they were used in.

ANTIBODIES

Both primary and secondary antibodies used in this work are listed in the table below:

Primary antibodies			
Antibody	Host	Dilution (if use for Western Blot)	Source
α-GFP	Mouse	1:7500	ROCHE
α-Mex67	Rabbit	1:10000	Dr. C. Dargemont (Paris, France)
α-TAP	Rabbit	1:5000	INVITROGEN
α-MYC	Mouse	1:2000	CLONTECH
α-Pgk1	Mouse	1:15000	INVITROGEN
α-Rpb1 (8WG16)	Mouse	1:1000	ABCAM
Secondary antibodies			
Antibody		Dilution	Source
α-Rabbit IRDye 800CW		1:10000	LI-COR
α-Mouse IRDye 680LT		1:10000	LI-COR

Table 4. Primary and secondary antibodies employed, indicating their host, the dilution used in Western Blot and their source.



COMMERCIAL KITS

The commercial kits employed during this study are listed in the following table:

Kit	Manufacturer
NZYGelpure	NZYTECH
NZYMiniprep	NZYTECH
PrimeScript™ RT reagent Kit (Perfect Real Time)	TAKARA
NEBuilder HiFi DNA Assembly Cloning Kit	NEW ENGLAND BIOLABS
GFP-TRAP®	CHROMOTEK
Library Efficiency™ DH5α	INVITROGEN

Table 5. The commercial kits used during this study, indicating their manufacturer.

METHODS





MICROBIOLOGICAL TECHNIQUES

Bacteria culture and transformation

DH5α *Escherichia coli* competent cells were used for the production and storage of the plasmids created and employed during the study. The medium used for *E. coli* culture is Lysogeny Broth (LB; 1% (w/v) tryptone, 0,5% (w/v) yeast extract, 1% (w/v) NaCl; Miller, 1972), supplemented with 0,00075% (w/v) of Ampicilin (LBA) as a marker for plasmid selection. For bacteria growth, the cells were incubated in liquid medium at 37°C and 160rpm or in solid plates with 2% (w/v) agar at the same temperature. For the storage and preservation of the new *E. coli* transformed strains, 300μL of sterilized 50% (v/v) glycerol was added to 700μL of the overnight bacteria culture and the mixture was kept at -80°C.

E. coli cells were transformed using two different strategies that depend on the plasmid origin. For the plasmids created during the development of the study by the QuikChange™ approach, a heat shock-dependent transformation was carried out, using Library Efficiency™ DH5α competent cells (INVITROGEN) and following the instructions provided by the manufacturer. 3μL of the new plasmid was added to 100μL of the competent cells and the cells were incubated on ice for 30min. Then, they were heat-shocked for 45s at 42°C without shaking and straightaway placed on ice for 2min. Later, 900μL of Super Optimal medium with Catabolic repressor (SOC) at room temperature (RT) was added and the cells were incubated for 1h at 37°C at 300rpm. Finally, 200μL of the cells were spread on an LBA plate and incubated overnight at 37°C.

For the plasmids created in this work by cloning, a similar heat shock-dependent transformation was performed with NEB® 5-alpha Competent *E. coli* (High Efficiency; New England Biolabs) following the manufacturer's instruction. 2μL of the assembled plasmid were added to 50μL of the competent cells and the cells were incubated on ice for 30min. Then, they were heat-shocked for 30s at 42°C without shaking and straightaway placed on ice for 2min. Later, 950μL of SOC medium at RT were added and the cells were incubated during 1h at 37°C at 300rpm. Finally, 100μL of the cells were spread on an LBA plate and incubated overnight at 37°C.



MATERIALS AND METHODS

For the new plasmids purchased or sent from other laboratories, electrotransformation with electrocompetent cells was carried out. 50µL of the cells were thawed on ice, 1µL of the plasmid was added and the mix was placed on an electroporation cuvette (BIO-RAD) previously sterilized. For the electroporation, the Electroporator 2510 (EPPENDORF) was used and the voltage was set to 1,7kV. Quickly after the pulse, 450µL of the LB medium were added and the mix was transferred to a 1,5mL tube. Then, the cells were incubated for 1h at 750rpm at 37°C. Finally, 100µL of the cells were spread on an LBA plate and incubated overnight at 37°C.

Yeast culture and transformation

Different media were employed for the *Saccharomyces cerevisiae* culture and growth depending on the experimental necessities: Yeast extract-Peptone-Dextrose (YPD; 1% (w/v) yeast extract, 2% (w/v) bacterial peptone, 2% (w/v) dextrose), Yeast extract-Peptone-Galactose (YPGal; 1% (w/v) yeast extract, 2% (w/v) bacterial peptone, 2% (w/v) galactose), Yeast extract-Peptone-Glycerol (YPGly; 1% (w/v) yeast extract, 2% (w/v) bacterial peptone, 2% (w/v) glycerol) and Synthetic Complete (SC; 2% (w/v) dextrose, 0,17% (w/v) Yeast Nitrogen Bases (YNB), 0,5% (w/v) ammonium sulfate (NH₄)₂SO₄, 0,2% (w/v) Drop-out mix). When needed for marker selection, 0,02% (w/v) of Geneticin G418 (*KANMX4* resistance marker) or 0,02% (w/v) of Hygromycin B (*HPHMXNT1* resistance marker) were added to the YPD medium and the different amino acids were added to the SC medium. For yeast growth, the cells were incubated in liquid medium at 30°C and 160rpm or in solid plates with 2% (w/v) agar at the same temperature. For the storage and preservation of the new *S. cerevisiae* created strains, 400µL of sterilized 50% (v/v) glycerol was added to 600µL of the overnight yeast culture and the mixture was kept at -80°C.

S. cerevisiae cells were transformed using different strategies that depend on the nature of the DNA (either plasmid or cassette) and the background of the yeast strains. For transformation with plasmids in any background, a LiAc/SS carrier DNA/PEG protocol modified from (Gietz & Schiestl, 2007) was followed. 30mL of cells were grown until exponential phase and centrifuged at 3000rpm for 3min at 4°C. Then, the pellet was washed twice, transferred into a 1,5mL tube and resuspended in 1mL of cold milli-Q water. 100µL of the cells were transferred to new 1,5mL tubes,



centrifuged for 1min at 13000rpm at 4°C and resuspended in a sterile mix composed of 240μL 50% (w/v) poly(ethylene glycol; PEG), 36μL LiAc 1M, 34μL milli-Q water, and 50μL salmon sperm DNA carrier (2mg/mL dissolved in TE (10mM Tris-HCl pH 8, 1mM ethylenediaminetetraacetic acid (EDTA) pH 8)). 200ng of the plasmid to be transformed were added (a negative control with no DNA added was also performed) and the cells were incubated for 40min at 42°C at 800rpm. Later, the cells were centrifuged at 3000rpm for 2min, resuspended in 100μL of sterile milli-Q water and transferred to the SC selection plate.

For transformation with a cassette in BY4741, yeasts were transformed using a high efficiency transformation method. 30mL of cells were grown until the exponential phase and centrifuged at 3000rpm for 3min at 4°C. Then, the pellet was washed twice, transferred into a 1,5mL tube and resuspended in 1mL of cold milli-Q water. 100μL of the cells were transferred to new 1,5mL tubes, centrifuged for 1min at 13000rpm at 4°C and resuspended in a sterile mix composed of 240μL 50% (w/v) PEG, 36μL LiAc 1M, 34μL TE 10X, and 50μL salmon sperm DNA carrier (2mg/mL dissolved in TE). 2μg of the cassette to be transformed were added (a negative control with no DNA added was also performed) and the cells were incubated first at 30°C for 30min at 800rpm, later at 42°C for 20min at 800rpm and finally on ice for 2min. After the incubations, two different steps are followed depending on the selection marker. On the one hand, if the selection marker is an auxotrophy, the cells were centrifuged at 3000rpm for 2min, resuspended in 100μL of sterile milli-Q water and transferred to the SC selection plate. On the other hand, if the selection marker is an antibiotic, the cells were centrifuged at 3000rpm for 2min, resuspended in 100μL of sterile milli-Q water, transferred to a YPD plate and incubated at 30°C overnight. The next day, the lawn of cells grown was transferred using a replicaplate (Treco & Winston, 2008) to a plate with the corresponding antibiotic.

For transformation with cassettes in SK1 or SK1+W303, a modified high efficiency yeast transformation protocol was followed (Gardner & Jaspersen, 2014). 50mL of cells were grown until the exponential phase and centrifuged at 3000rpm for 3min at 4°C. Then, the pellet was washed twice with LiAc 100mM, transferred into a 1,5mL tube and resuspended in 1mL of LiAc



MATERIALS AND METHODS

100mM. 100 μ L of the cells were transferred to new 1,5mL tubes and a sterile mix composed of 560 μ L 50% (w/v) PEG, 70 μ L LiAc 1M, 70 μ L TE 10X, and 50 μ L salmon sperm DNA carrier (2mg/mL dissolved in TE) was added. 2 μ g of the cassette to be transformed were added (a negative control with no DNA added was also performed) and the cells were incubated first at room temperature for 30min. Later, 48 μ L of dimethyl sulfoxide (DMSO) and the cells were incubated at 42°C for 15min. After the incubations, two different steps are followed depending on the selection marker. On the one hand, if the selection marker is an auxotrophy, the cells were centrifuged at 3000rpm for 1min, resuspended in 100 μ L of sterile milli-Q water and transferred to the SC selection plate. On the other hand, if the selection marker is an antibiotic, the cells were centrifuged at 3000rpm for 1min, resuspended in 50 μ L of sterile milli-Q water, transferred to a YPD plate and incubated at 30°C overnight. The next day, the lawn of cells grown was transferred using a replicaplate (Trecó & Winston, 2008) to a plate with the corresponding antibiotic.

Stress treatments

For heat shock treatments, cells were grown at optimal conditions until the exponential phase and were split into two different flasks: one for the heat shock treatment and the other one for the non-stress control. In the heat shock treatment flask, one part of the volume of the corresponding medium at 51°C was added so that the resulting temperature of the mixture was 39°C. Immediately, the cells were incubated at 39°C for 20min and, then, they were collected depending on the protocol to be followed. In the non-stress control flask, one part of the volume of the corresponding medium at 30°C was added, not altering the culture temperature and the cells were collected depending on the protocol to be followed.

For the oxidative stress treatment, two different methods were used depending on the protocol to be performed. In the case of a viability assay, a yeast cell survival protocol was followed (Tran & Green, 2019). Cells were grown at optimal conditions until the exponential phase and a volume equivalent to 12 OD₆₀₀ was transferred to a 50mL centrifuge tube. Then, the cells were centrifuged for 3min at 3900xG, resuspended in 20mL of YPD and split into two different tubes: one for the oxidative stress treatment and the other one for the non-stress control. In the oxidative



stress treatment tube, H_2O_2 4mM was added and both tubes were incubated at 30°C for 30min. Immediately after the incubation, 1mL was removed from each culture, transferred to a 1,5mL tube, centrifuged for 2min at 12200xG and resuspended in 1mL of YPD. Finally, the cells were treated according to the protocol to be followed.

For microscope visualization, 20mL of cells were grown at optimal conditions until the exponential phase and were split into two different flasks: one for the oxidative stress treatment and the other one for the non-stress control. In the oxidative stress treatment flask, H_2O_2 3mM was added and, immediately, the cells were incubated at 30°C for 15min. Then, both cultures were washed with phosphate buffered saline 1X (PBS; 13,7mM NaCl, 0,27mM KCl, 0,4mM Na_2HPO_4 , 0,14mM KH_2PO_4 in water) and the cells were fixed with methanol.

Sporulation

Different media were employed in order to induce *Saccharomyces cerevisiae* sporulation: Yeast extract-Peptide-Acetate (YPA; 1% (w/v) yeast extract, 2% (w/v) bacterial peptone, 1% (w/v) potassium acetate) and sporulation liquid medium (SPII; 2% (w/v) potassium acetate, pH 7) as liquid media and sporulation solid medium (SPO; 0,3% (w/v) potassium acetate, 0,02% (w/v) raffinose, 2% (w/v) agar) and YPD plates supplemented with 0,002% (w/v) adenine (YPDA) as solid media.

For the cross of two different haploid cells of different mating (MATa and $\text{MAT}\alpha$) in order to obtain a heterozygous diploid cell, they were put together in a YPD plate and were incubated at 30°C for 24h. For the selection of the diploids, the cells were transferred using a replicaplate to selection plates and, later, to YPDA plates. Then, the cells were incubated in SPO plates at 30°C for at least 48h or 72h, depending on the background, in order for them to sporulate. Once the formation of tetrads was confirmed by optical microscopy, they were prepared for dissection by incubating them with 20 μL of Zymolias 20T 20mg/mL in 180 μL of sorbitol 1M 8min at 37°C or 5min at 30°C, depending on the background of the strains. Then, tetrad dissection was carried out using the MSM 300 System dissection microscope™ (SINGER™) and the spores dissected were incubated in YPD plates at 30°C for 48-72h. For the genotyping of the haploid cells obtained



from the spores, they were transferred using a replicaplate to selection plates and, later, to YPGly plates, in order to avoid petite mutants, unable to sporulate (Küenzi et al., 1974). Finally, the mating type of the cells was determined by crossing them with the tester strains 14a and 17 α (with the *his1* marker (Trueheart et al., 1987)). The cells were incubated with each of the tester strains in liquid media for at least 4h at 30°C and 850rpm and, later, they were transferred to SC-all plates, so that only the MATa/MAT α strains, obtained of the cross between the tester and the cells to be tested, could grow in the selection plate.

To obtain a diploid cell from the corresponding haploid one, two different methods were performed. On the one hand, if both haploid cells with mating type MATa and MAT α were already obtained, they were mixed in a YPD plate and incubated at 30°C to conjugate. Later, isolated colonies were tested to be diploid crossing them with the tester strains 14a and 17 α as explained above. On the other hand, if only one mating type was obtained, the cells were transformed with a plasmid that expresses *HO*, which codes for an endonuclease required for homothallic switching (Nasmyth, 1993), under the control of a galactose-induced promoter. Then, the transformed cells were incubated in YPGal for at least 4h and, later, transferred to SC selection plates. Finally, isolated colonies were tested to be diploid crossing them with the tester strains 14a and 17 α as explained above.

For sporulation assays, a meiotic cell synchrony protocol previously described was used (Cooper & Strich, 2002). The cells were grown until the exponential phase at 30°C and 250rpm in YPA five times the final volume. Then, the cells were washed twice with 500mL of milli-Q water and resuspended in SPII to obtain a cell concentration of 3,35 OD₆₀₀ (5x10⁷ cells/mL approximately). Finally, cells were collected at different time points depending on the protocol to be followed.

Growth assays

Two different growth assay protocols were used. On the one hand, the cells were grown in YPD until the exponential phase and, if necessary, a stress treatment was performed. Then, they were diluted to an OD₆₀₀ 0,1 and a series of 1:10 dilutions were applied, spotted on YPD plates and incubated at 30°C, 33°C or 37°C. Images of the dot spot assay were taken after 24h and 48h of



incubation. On the other hand, the cells were also grown in YPD until the exponential phase and, if necessary, a stress treatment was performed. Then, they were diluted to an OD_{600} 0,1 and, if a media change is necessary, they were centrifuged at 13000rpm for 1min and resuspended in the new media. Later, 260 μ L of the cells were transferred to a 96-well plate and the plate was inserted into a TECAN Spark® Multimode Microplate Reader, where the cells were incubated at 30°C, 37°C or 42°C until they reached stationary phase and the OD_{600} of the samples were measured every 20min.

NUCLEIC ACIDS TECHNIQUES

Polymerase Chain Reaction (PCR)

DNA amplification was carried out either for the creation of transformation cassettes or for checking the genotype of new mutant strains. In the case of cassette creation, the PCR mix used was composed of 1X NZYTaq II DNA polymerase Reaction Buffer (NZYTech), 0,4mM dNTPs (INVITROGEN), 2,5mM $MgCl_2$, 0,5 μ M of each primer, 25mU/ μ L NZYTaq II DNA polymerase (NZYTech), 10ng of DNA template and milli-Q water in a set volume of 50 μ L. The PCR program used is described in the table below:

Step	Temperature	Time
Initial denaturation	95°C	3min
Denaturation	95°C	15s
Annealing	The set temperature depends on the pair of primers used	30s x35
Amplification	72°C	1min/Kb
Final extension	72°C	7min

Table 6. PCR program used for the creation of transformation cassettes.

In the case of checking the new mutant strains, the PCR mix used was composed of 1X NZYTaq II DNA polymerase Reaction Buffer (NZYTech), 2,5mM $MgCl_2$, 0,4mM dNTPs (INVITROGEN), 0,5 μ M of each primer, 50mU/ μ L NZYTaq II DNA polymerase (NZYTech), 100ng of DNA template and milli-Q water in a set volume of 20 μ L. The PCR program used is described in the table below:



Step	Temperature	Time	
Initial denaturation	94°C	2min	
Denaturation	94°C	15s	
Annealing	The set temperature depends on the pair of primers used	30s	x30
Amplification	72°C	1min/Kb	
Final extension	72°C	5min	

Table 7. PCR program used for the verification of the new mutant strains.

PCR products purification

The amplified DNA, product of a PCR, was purified, either directly after the performed PCR or from an agarose gel, using the NZYGelpure kit (NZYTECH) according to the manufacturer’s guidelines. Following the purification, DNA concentration was measured using a NanoDrop™ 2000c spectrophotometer (THERMO SCIENTIFIC).

Plasmid isolation (Miniprep)

Plasmids used in this study were stored in DH5α *Escherichia coli* cells. For the purification of those plasmids, the NZYMiniprep kit (NZYTECH) was used following the instructions the company provided. Following the purification, DNA concentration was measured using a NanoDrop™ 2000c spectrophotometer (THERMO SCIENTIFIC).

Genomic DNA extraction

Genomic DNA was purified using two different strategies that depend on its later use. If the DNA was used for checking the genotype of a new mutant strain, a patch of cells was placed in a 1,5mL tube and resuspended in 5% Chelex® 100 sodium. Then, 0,5mm acid-washed glass beads were added and the cells were first vortexed for 4min at 4°C and later incubated for 4min at 100°C. Finally, they were centrifuged at 13000rpm for 1min and the supernatant was transferred to a new 1,5mL tube. Following the purification, DNA concentration was measured using a NanoDrop™ 2000c spectrophotometer (THERMO SCIENTIFIC).

If the DNA was used for other purposes or if the genotype checking of the new mutant strain required Sanger sequencing, a patch of cells was placed in a 1,5mL tube and resuspended in Breaking Buffer (2% (v/v) Triton™ X-100, 1% (w/v) sodium dodecyl sulfate (SDS), 100mM NaCl, 10mM Tris-HCl pH 8). Then, 0,5mm acid-washed glass beads were added together with the same



volume of phenol:chloroform:isoamyl alcohol (25:24:1) the one previously added of Breaking Buffer and the cells were vortexed for 5min at 4°C. After a centrifugation for 5min at 13000rpm, the supernatant was transferred to a new 1,5mL tube and 2,5 times the volume of 100% (v/v) ethanol and 1/10 of the volume of 3M sodium acetate pH 5,3 were added. In order to precipitate the DNA, the sample was incubated for at least 30min at -20°C and, after a centrifugation for 15min at 13000rpm at 4°C, was washed with 70% (v/v) ethanol. Finally, the pellet was let dry and resuspended in nuclease free water. Following the purification, DNA concentration was measured using a NanoDrop™ 2000c spectrophotometer (THERMO SCIENTIFIC).

Agarose DNA/RNA electrophoresis

Separation of DNA/RNA fragments was performed in 0,8-2% (w/v) agarose gels, prepared with 1% (w/v) agarose in 1X TAE buffer (40mM Tris-Acetate, 1mM EDTA pH 8). For the visualization of the nucleic acids in the gel, GreenSafe Premium (NZYTECH) was added before polymerization. For loading the DNA/RNA samples, they were mixed with 6X DNA Loading Buffer (30% (v/v) Glycerol, 0,25% (w/v) Blue xylene cyanol, 0,25% (w/v) Bromophenol blue). The gels were run at a constant voltage of 120V for the time necessary for the samples to be separated and imaged using an Ultima 16si-Plus (ISOGEN).

Site-directed mutagenesis

Different strategies were carried out for direct mutagenesis depending on the nature of the DNA to be mutated. In the case of plasmid mutations, two different methods were performed. On the one hand, a modified protocol from the QuikChange™ mutagenesis (AGILENT) was performed. The plasmid to be mutated was linearized by PCR using primers that included the mutation. The PCR mix used was composed of 1X Phusion HF Buffer (THERMO SCIENTIFIC), 200μM dNTPs (INVITROGEN), 0,5μM of each primer, 20mU/μL Phusion™ High-Fidelity DNA Polymerase (THERMO SCIENTIFIC), 10ng of DNA template and milli-Q water in a set volume of 50μL. The PCR program used is listed in the table below:



MATERIALS AND METHODS

Step	Temperature	Time
Initial denaturation	98°C	30s
Denaturation	98°C	10s
Annealing	The set temperature depends on the pair of primers used	30s
Amplification	72°C	30s/Kb
Final extension	72°C	5min

Table 8. PCR program used for the QuikChange™ mutagenesis protocol.

After the PCR, the linearized plasmid was treated with DpnI (THERMO SCIENTIFIC) and incubated for 3h at 37°C, in order to degrade the methylated DNA. Finally, DH5α competent cells were transformed with the plasmid as described above in order to circularize and store the plasmid. In order to check if the mutation was correctly incorporated into the plasmid, Sanger sequencing was performed in the DNA analysis service at the Biomedical Institute of Valencia (IBV-CSIC).

On the other hand, the NEBuilder HiFi DNA Assembly Cloning Kit (NEW ENGLAND BIOLABS) was used, following the guidelines the manufacturer provided, both for the assembly protocol and the chemically competent cells transformation protocol. In order to check if the mutation was correctly incorporated into the plasmid, Sanger sequencing was performed in the DNA analysis service at the Biomedical Institute of Valencia (IBV-CSIC).

In the case of mutations in genomic DNA, a CRISPR technology modified protocol was performed (Mans et al., 2018). First, a plasmid that expresses the sgRNA and where the crRNA was going to be cloned (pMEL10) was linearized by PCR. The PCR mix used was composed of 1X Phusion HF Buffer (THERMO SCIENTIFIC), 200μM dNTPs (INVITROGEN), 0,2μM of each primer, 20mU/μL Phusion™ High-Fidelity DNA Polymerase (THERMO SCIENTIFIC), 1ng of DNA template and milli-Q water in a set volume of 50μL. The PCR program used is listed in the table below:

Step	Temperature	Time
Initial denaturation	98°C	30s
Denaturation	98°C	10s
Annealing	67°C	20s
Amplification	72°C	3min
Final extension	72°C	5min

Table 9. PCR program performed for the linearization of the pMEL10 plasmid used for the CRISPR technology protocol.



After the PCR, the linearized plasmid was treated with DpnI (THERMO SCIENTIFIC) and incubated for 3h at 37°C, in order to degrade the methylated DNA, and later purified using the NZYGelpure kit (NZYTECH).

Second, the crRNA was designed using the web tool CRISPOR (Concordet & Haeussler, 2018). The creation of the crRNA cassette was carried out by hybridization. Both oligos were put together in a tube in a 1:1 volume ratio. Then, they were incubated at 95°C for 5min and later they were cooled down at room temperature.

Third, the DNA donor was designed: two 120-nucleotides complementary oligos, homologous to the genomic region where the mutation was going to be introduced, including the said mutation. The creation of the DNA donor cassette was carried out by hybridization. Both oligos were put together in a tube in a 1:1 volume ratio. Then, they were incubated at 95°C for 5min and later they were cooled down at room temperature.

Once all the constructs were created, a highly efficient transformation protocol was performed for the transformation of the yeast strain to be mutated with all the constructs. The yeast strains must be already transformed with a plasmid expressing Cas9 (pJH001) as described above. 30mL of cells were grown until the exponential phase and centrifuged at 3000rpm for 3min at 4°C. Then, the pellet was washed twice, transferred into a 1,5mL tube and resuspended in 1mL of cold milli-Q water. 100μL of the cells were transferred to new 1,5mL tubes, centrifuged for 1min at 13000rpm at 4°C and resuspended in a sterile mix composed of 240μL 50% (w/v) PEG, 36μL LiAc 1M, 25μL salmon sperm DNA carrier, 100ng of the pMEL10 linearized plasmid, 300ng of the crRNA cassette, 0,2-1μg of the DNA Donor cassette and milli-Q water in a set volume of 351μL. A negative control with no DNA added was also performed. Next, the cells were incubated first at 30°C for 30min at 800rpm, later at 42°C for 20min at 800rpm and finally on ice for 2min. After the incubations, the cells were centrifuged at 3000rpm for 2min, resuspended in 100μL of sterile milli-Q water and transferred to the SC-LEU-URA plates for selection. Finally, in order to check if the mutation was correctly incorporated into the plasmid, Sanger sequencing was performed in the DNA analysis service at the Biomedical Institute of Valencia (IBV-CSIC).



RNA extraction

For RNA purification a modified protocol using hot acidic phenol was performed (Collart & Oliviero, 2001). 10mL of cells were grown until the exponential phase and centrifuged at 3000rpm for 3min at 4°C. Then, the pellet was washed twice, transferred into a 1,5mL tube and frozen with liquid N₂. Without thawing the samples, 450μL of acidic phenol were added and the tubes were incubated at 1400rpm for 5min at 65°C. Then, 450μL of TES Buffer (10mM Tris-HCl pH 7,5, 10mMEDTA, 0,5% SDS) were added, and the samples were kept at 65°C for 30min, shaking them at 1400rpm for 1min every 5min. After 10min in ice, the cells were centrifuged at 1300rpm for 10min and the aqueous phase was transferred to a new 1,5mL tube with the same volume of acidic phenol. This step was repeated twice, the first one with acidic phenol:chloroform:isoamyl alcohol (25:24:1) instead of phenol and the second one with chloroform:isoamyl alcohol (24:1). After a centrifugation at 1300rpm for 10min, the aqueous phase was transferred to a new 1,5mL tube with 2,5 times the volume of 100% (v/v) ethanol and 1/10 of the volume of 3M sodium acetate pH 5,3. In order to precipitate the DNA, the sample was incubated at -20°C and, after a centrifugation for 15min at 13000rpm at 4°C, was washed with 70% (v/v) ethanol. Finally, the pellet was let dry and resuspended in nuclease free water. Following the purification, DNA concentration was measured using a NanoDrop™ 2000c spectrophotometer (THERMO SCIENTIFIC) and RNA integration was checked in an 0,8% agarose gel.

In order to degrade the DNA present in the samples, 10μg of the sample were taken and they were incubated with 1x DNase I recombinant RNase free Incubation Buffer and 200mU/μL DNase I recombinant RNase free (ROCHE) in a set volume of 50μL at 30°C for 20min. For the purification of the RNA, 50μL of 3M sodium acetate pH 5,3, 400μL of nuclease free water and 500μL of acidic phenol:chloroform:isoamyl alcohol (25:24:1) were added. After a centrifugation at 1300rpm for 10min, the aqueous phase was transferred to a new 1,5mL tube with the same volume of phenol:chloroform:isoamyl alcohol (25:24:1). This step was repeated with chloroform:isoamyl alcohol (24:1) instead of phenol:chloroform:isoamyl alcohol (25:24:1). After a centrifugation at 1300rpm for 5min, the aqueous phase was transferred to a new 1,5mL tube with 1mL of 100% (v/v) ethanol and 1μL of glycogen RNA grade (THERMO SCIENTIFIC).



In order to precipitate the DNA, the sample was incubated at -20°C and, after a centrifugation for 15min at 13000rpm at 4°C, was washed with 70% (v/v) ethanol. Finally, the pellet was let dry and resuspended in nuclease free water. Following the purification, DNA concentration was measured using a NanoDrop™ 2000c spectrophotometer (THERMO SCIENTIFIC) and DNA degradation was checked by amplifying *SCR1* by PCR.

cDNA synthesis

In order to synthesize cDNA from the RNA extracted, the PrimeScript™ RT reagent Kit (TAKARA) was used and the company's instructions were followed.

Quantitative PCR

Relative RNA levels were measured by quantitative PCR (qPCR) using the QuantStudio™ 5 Real-Time PCR System (THERMO SCIENTIFIC) or the CFX Opus 96 Real-Time PCR System (BIO-RAD). The PCR mix used was composed of 5μL of TB Green® Premix Ex Taq™ (Tli RNase H Plus; TAKARA), 0,37μL of each primer, 0,2μL of the ROX Reference dye 1:5 diluted if necessary, 3μL of the cDNA 1:10 diluted and nuclease free water in a set volume of 10μL. The PCR program used is listed in the table below:

Step	Temperature	Time
Initial denaturation	95°C	10min
Denaturation	95°C	10s
Annealing	58°C	10s
Amplification	72°C	12s
	95°C	5s
Melt curve	50°C	1min
	95°C	1min

Table 10. qPCR program performed for the quantification of RNA levels.

The relative quantity of each PCR amplicon was measured following the $2^{-\Delta\Delta CT}$ method (Livak & Schmittgen, 2001) using *SCR1* as a reference gene for normalization. Three technical replicates were performed for each sample.

RNA-seq

For RNA-seq analyses, RNA purified samples as described above were submitted to Macrogen, Inc to be processed, using the Illumina TruSeq protocol. Between 40 and 60 million reads were



MATERIALS AND METHODS

obtained from each sample. The quality of the raw sequencing data was checked by fastQC and a trimmomatic program was used to withdraw the adapter sequences and the bases with a low base quality from the ends. Moreover, the bases of reads which had a window size lower than 4 and a mean quality lower than 15 and the reads shorter than 36bp were also trimmed. After trimming, the quality of the samples was checked again by fastQC. Trimmed reads were mapped using sacCer3 as a reference genome with HISAT2, splice-aware aligner, and StringTie. The abundance of each gene and transcript was calculated in the read count for each sample.

The NOISeq R package (Tarazona et al., 2015) was used to perform the quality control of count data. Counts were normalized via TMM (Bullard et al., 2010) and a low count filtering was applied with the NOISeq cpm method (with cpm = 1). Hidden batch effects were removed by ARSyN (Nueda et al., 2012). In total, we obtained gene expression values for 6,172 genes. A PCA was applied to these normalized counts to get a first glimpse of the transcriptomic changes.

The maSigPro package (Conesa et al., 2006) was used for differential expression analysis using default parameters.

PROTEIN TECHNIQUES

Whole cell extracts

Whole cell extracts (WCE) were prepared following three different strategies. One of them consists of a modified yeast NaOH WCE protocol (Kushnirov, 2000). Cells were grown until exponential phase and a volume equivalent to $OD_{600} 2$ was collected. After a spin, the cells were resuspended in 1mL of cold milli-Q water and 150 μ L of NaOH/ β -mercaptoethanol (1,85M NaOH and 7,5% (v/v) β -mercaptoethanol) were added. Then, the tube was centrifuged at 13000rpm for 1min at 4°C and the pellet was resuspended in 2X Loading Buffer (LB; 112,5mM Tris-HCl pH 6,8, 4,14% (w/v) SDS, 0,9% (w/v) Bromophenol blue, 18% (v/v) Glycerol and 50mM DTT). Next, samples were incubated for 2min at 95°C and later centrifuged at 13000rpm for 5min at 4°C. Finally, the supernatant was stored at -20°C (protein extract).



The other two strategies consist of modified Trichloroacetic acid (TCA) protocols (Keogh et al., 2006). On the one hand, 50mL of cells were grown until the exponential phase. They were collected by centrifugation at 3000rpm for 3min at 4°C, washed with 20% (v/v) TCA and frozen with liquid N₂. Later, the cells were resuspended in 500μL of 20% (v/v) TCA with 200μL of 0,5mm acid-washed glass beads and the tubes were vortexed for 5min (1min ON/ 1min OFF) at 4°C. Then, the lysate was transferred to a new 1,5mL tube and the beads were washed with 5% (v/v) TCA in order to retrieve the remaining supernatant. After a centrifugation at 3000rpm for 10min at 4°C, the pellet was resuspended with Laemmli Buffer (60mM Tris-HCl pH 6,8, 10% (w/v) SDS, 5% (v/v) β-Mercaptoethanol, 0,0025% (w/v) Bromophenol blue) and 2M Tris and the tubes were incubated at 95°C for 3min. The cells were centrifuged at 3000rpm for 5min at 4°C, the supernatant was transferred to a new 1,5mL tube and 4X LB (225mM Tris-HCL pH 6,8, 8,28% (w/v) SDS, 0,18% (w/v) Bromophenol blue, 36% (v/v) Glycerol and 100mM DTT) was added to a final concentration of 1X. Finally, the supernatant was stored at -20°C (protein extract).

On the other hand, cells were grown until the exponential phase and a volume equivalent to OD₆₀₀ 1,65 was collected by centrifugation at 3000rpm for 5min at 4°C and resuspended in 20% (v/v) TCA. After a centrifugation at 13000rpm for 1min, the pellet was washed with 1M Tris and resuspended with 2X SDS and 0,5M DTT. 100μL of 0,5mm acid-washed glass beads were added and, after an incubation at 95°C for 4min, the cells were broken in a cell breaker (6 cycles of 25s ON and 60s OFF). Then, samples were incubated at 95°C for 2min and centrifuged at 4000rpm for 5min. Finally, the supernatant was stored at -20°C (protein extract).

Protein immunoprecipitation (GFP-TRAP)

Protein immunoprecipitation was performed using GFT-TRAP® (CHROMOTEK), following the guidelines the company provided with certain modifications. 50mL of cells were grown until the exponential phase. The cells were centrifuged at 3000rpm for 3min at 4°C, washed first with cold milli-Q water and later with Dilution Buffer (10mM Tris-HCl pH 7,5, 150mM NaCl, 0,5mM EDTA, 0,5X cOmplete™ (ROCHE)) and the pellet was frozen with liquid N₂. Then, 200μL of Lysis Buffer (10mM Tris-HCl pH 7,5, 150mM NaCl, 0,5mM EDTA, 0,5% (v/v) NP-40,



MATERIALS AND METHODS

0,5X cOmplete™ (ROCHE)) and 200µL of 0,5mm acid-washed glass beads were added and the samples were vortexed at 4°C for 8min (1min ON/1min OFF). The cell lysate was transferred to a new 1,5mL tube avoiding the beads, and the samples were centrifuged at 13000rpm for 10min at 4°C. 10µL of the supernatant were taken as input and the rest was incubated for 1h at 4°C with 25µL of GFT-TRAP® beads, previously equilibrated by washing them with Dilution Buffer twice. After the incubation, the beads were washed twice with Dilution Buffer and they were incubated at 95°C for 10min with LB 4X for elution. After a centrifugation at 2500xG for 2min at 4°C, the supernatant was transferred to a new 1,5mL tube, obtaining 30µL of IP volume.

TAP purification

Tandem Affinity Purification was carried out as previously described (Puig et al., 2001) with certain modifications. 3L of cells were grown until OD₆₀₀ 2-2,5 and they were collected by centrifugation at 4000rpm for 5min at 4°C. Then, the pellets were washed first with cold milli-Q water and later with Lysis Buffer (LB; 100mM NaCl, 50mM Tris-HCl pH 7,5, 1,5mM MgCl₂, 0,15% (v/v) NP-40) supplemented with 0,5mM PMSF and 0,5X cOmplete™ (ROCHE) and frozen with liquid N₂. Later, the pellets were thawed by incubating them at 37°C and resuspended in 25mL of LB supplemented with 1mM DTT, 0,5mM PMSF and 0,5X cOmplete™ (ROCHE) and 25mL 0,5mm acid-washed glass beads were added. The cells were broken using a BeadBeater (FRITSCH-PULVERISETTE) with a program consisting of 3 cycles of 4min of shaking and 1min of rest. The samples were centrifuged at 4000rpm for 10min at 4°C and the supernatant was centrifuged again at 4000rpm for 1h at 4°C. Then, the supernatant was incubated with 500µL of IgG-Sepharose 6 Fast Flow™ (CYTIVA) for 1h at 4°C and, after the incubation, the beads were washed twice with LB supplemented with 0,5mM DTT and later they were incubated with 3-4µg at 4°C overnight. For the purification of the TEV eluate, the samples were incubated with 400µL of calmodulin-coated beads (Calmodulin Affinity Resin™; STRATAGENE) and 4mM CaCl₂ for 1h at 4°C. After the incubation, the beads were washed with LB supplemented with 2mM CaCl₂ and 1mM DTT and the elution was performed by incubating the beads with 350µL of elution buffer (5mM EGTA, 10mM Tris-HCl pH 8,0 and 50mM NaCl) for 10min at 37°C three times. Once the eluate



was obtained, TCA was added to a final concentration of 10% (v/v) for its precipitation and the pellet was stored at -20°C.

Coomassie blue dyeing

In the case of samples coming from a TAP purification, Mini-PROTEAN TGX Gels (BIO-RAD) were used for SDS-PAGE and the electrophoresis was carried out in Mini-Protean Tetra Cell cuvettes (BIO-RAD) using 1X Running Buffer (25mM Tris-Base, 190mM Glycine and 0,01% (w/v) SDS in water) at a constant voltage of 100V. Then, coomassie blue dyeing was performed by immersing the gel in BlueSafe (NZYTECH) for at least 1h. Finally, the gel was washed several times with distilled water until the different bands of proteins could be seen.

Mass spectrometry for protein identification

The pellets obtained from TAP purification and the protein bands cut from a gel stained with coomasie were sent to the mass spectrometry service (*Servei Central de Suport a la Investigació Experimental* (SCSIE)) of the University of Valencia (UV). Protein identification was carried out by LC-MS/MS analysis.

Western blotting

In the case of samples coming from other protocols of protein extraction and purifications, they were run on SDS-PAGE gels with different concentrations depending on the protein size, as described previously (Sambrook & Russell, 2006). The separating part of the gels was composed of 37,5mM Tris-HCL pH 8,8, 0,1% (w/v) SDS, 1,45mM Ammonium persulfate (APS), 0,003% N,N,N',N'-tetramethylethane-1,2-diamine (TEMED), water and acrylamide/bisacrylamide (37,5:1), the concentration depending on the protein size. The stacking part of the gels was composed of 4% (w/v) acrylamide/bisacrylamide (37,5:1), 125mM Tris-HCL pH 6,8, 0,1% (w/v) SDS, 1,45mM APS, 0,003% N,N,N',N'-tetramethylethane-1,2-diamine (TEMED). The electrophoresis was carried out in Mini-Protean Tetra Cell cuvettes (BIO-RAD) using 1X Running Buffer (25mM Tris-Base, 190mM Glycine and 0,01% (w/v) SDS in water) at a constant voltage of 100V.



Once the electrophoresis was finished, the proteins separated in the gel were transferred to 0,45µm nitrocellulose Amersham™ Protean® Western blotting membranes (CYTIVA) following a previously described protocol (Green et al., 2012). The buffer used was composed of 25mM Tris-HCl pH 8,3, 192mM glycine, 0,1% SDS (w/v) and 20% methanol (v/v) and the transfer was performed in Mini-Protean Tetra Cell cuvettes (BIO-RAD) at a constant voltage of 100V for 1h. After the transfer, the membranes were incubated in Ponceau staining (1% (w/v) Ponceau, 1% (v/v) Acetic acid) and washed several times with distilled water. For blocking, the membranes were incubated with Tris-Buffered Saline (TBS; 0,02M Tris-HCl pH 7,6 and 0,8% (w/v) NaCl in water) with 0,1% Tween-20 and 5% (w/v) milk powder for 1h at room temperature or overnight at 4°C with rocking. Then, the membranes were incubated first with the primary antibody (listed in a table above) for 1h at room temperature with rocking and, after three washes with TBS-Tween, with the secondary antibody (listed in a table above) for 1h at room temperature with rocking. The membrane was washed again three times with TBS-Tween and once with TBS. Finally, visualization of the membrane was performed with the Odyssey Imaging system (LI-COR).

OTHER TECHNIQUES

Confocal microscopy

Fluorescent proteins localization was visualized by confocal microscopy. 10mL of cells were grown in SC medium until the exponential phase and, if necessary, a stress treatment was performed. The cells were washed with PBS, fixated with methanol 100% and incubated at 4°C for up to 1 month. Then, the cells were centrifuged at top speed for 1min, washed twice and resuspended in PBS. DAPI staining was carried out following two different methods: on the one hand, using ProLong™ Diamond Antifade Mountant with DAPI (THERMO FISHER) as liquid mounting and, on the other hand, incubating the cells with 1µg/mL DAPI for 15min. Sample visualization was conducted using Leica DM6 B Thunder® (LEICA BIOSYSTEMS) and Leica SP8 confocal system with inverted DMI 6,000 microscope (LEICA BIOSYSTEMS), both running LASX software, and Zeiss Axio Observer Z1® (ZEISS), running ZEN software. Fiji (Schindelin et al., 2012), LASX software (LEICA BIOSYSTEMS) and ZEN software (ZEISS) were used for the processing of the images.



Statistics

For the statistical analyses, ANOVA Test, following Tukey's Multiple Comparison Test, or t-test were performed, assuming normality and homoscedasticity of the samples, for the detection of statistically significant differences. GraphPad Prism 10 was used for the statistical analysis. In the case of genomic data, other statistics tools and R packages were used, as described above.

RESULTS



CHAPTER I: Mip6 ROLE IN GENE EXPRESSION UNDER OPTIMAL AND STRESS CONDITIONS



BACKGROUND

Mip6 is a multifaceted protein that participates in several of the levels that comprise gene expression, having been studied its interaction with factors that are present in the transcription, export and mRNA decay machineries, as well as in histone modifier complexes (Martín-Expósito et al., 2019; Nuño Cabanes, 2024; Nuño-Cabanes et al., 2020).

Mip6 function within the cell seems to be tightly related to the stress response, since it participates in the homeostasis of stress response genes, especially those regulated by transcription factors Msn2 and Msn4. This protein does not only bind the transcripts of these genes under optimal conditions, but it also regulates its levels, keeping them low when the stress response is not triggered. Whether Mip6 is able to mediate the expression of the Msn2/4-dependent genes by means of the inhibition of their transcription or the activation of its degradation is not well known yet, since it presents genetic interactions with both factors of the mRNA decay machinery (the exonucleases Rrp6 and Xrn1) and the RNAPolII subunit Rpb1. However, recent evidence seems to tip the scales in favor of transcription. On the one hand, Msn2/4-dependent transcripts levels decrease at the same rate in both *rpb1-1* and *rpb1-1 mip6Δ* strains, which suggests mRNA degradation is not affected in the absence of Mip6 (Martín-Expósito et al., 2019). And, on the other hand, there is slightly more occupancy of the RNAPolII in the genes dependent on Msn2/4 in the *mip6Δ* mutant than in the WT strain (Nuño Cabanes, 2024).

Besides, Mip6 could be involved directly or indirectly in post-translational modification of histones, which regulates transcription machinery accessibility to the genes, playing a key role in the activation of several genes during the heat stress response, the Msn2/4-dependent genes among them (Kremer & Gross, 2009; Ruiz-Roig et al., 2010; Sadeh et al., 2011). *MIP6* genetically interacts with some histone deacetylases coding genes, such as class II HDAC *HDA1*, and its deletion supposes a general increase in the acetylation of H4K12 (Nuño Cabanes, 2024). Furthermore, in a previous work performed in our group, Mip6 was observed to physically interact with class I HDAC Rpd3 and HAT catalytic subunit of the SAGA complex Gcn5 by TAP purification (our unpublished data).

Thus, in this chapter, I focus on the Mip6 role at the transcriptional level. The crosstalk of this protein with histone modifiers, specifically HATs and HDAs, as well as with the transcription factors Msn2 and Msn4 was also studied, since they take part in the regulation of the transcription activation. Part of this work is available on BioRxiv and under revision in the G3 journal (Tejada-Colon et al., under revision).

Mip6 INTERACTION WITH THE TRANSCRIPTIONAL MACHINERY

Mip6 physically interacts with the RNAPolIII subunit Rpb1

In order to study Mip6 involvement in transcription, the physical interaction of Mip6-GFP with the RNAPolIII subunit Rpb1 was analyzed. Mip6 is a very low abundance protein during mitotic cell growth and it cannot be detected in its endogenous levels neither by western blotting nor by confocal microscopy (Ghaemmaghani et al., 2003; Martín-Expósito et al., 2019). For this reason, throughout this work, Mip6-GFP is expressed by means of a plasmid under the control of the promoter of the alcohol dehydrogenase 1 (*ADH1_{pr}*), which increases Mip6 levels to a detectable amount without being toxic for the cell (Martín-Expósito et al., 2019).

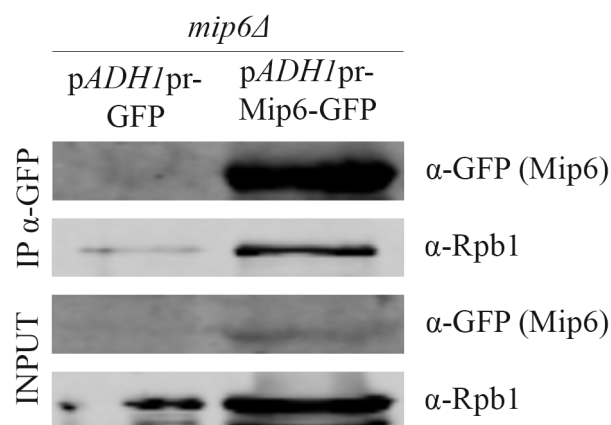


Figure 12. Mip6 physically interacts with the RNAPolIII subunit Rpb1. Immunoprecipitation of Mip6-GFP expressed from plasmid in *mip6Δ* strain. An empty plasmid was used as a negative control. Mip6-GFP and Rpb1 were detected by Western blotting using the indicated antibodies.

Mip6 physically interacts with the RNAPolIII subunit Rpb1 (**Figure 12**). Thus, Mip6 interaction with this subunit of the transcription machinery, together with the previous evidence, would indicate the participation of this protein in the transcription process.

Inactivation of the RNAPolIII subunit Rpb1 does not affect the Mip6-Mex67 interaction

Not only Mip6 genetically and physically interacts with the transcription machinery, but the location of this protein is also affected when the transcription is altered. In the thermosensitive *rpb1-1* mutant, in which the transcription is inhibited when the cells are grown at 39°C, Mip6 is partially retained in the nucleus (Martín-Expósito et al., 2019). Thus, transcription would be necessary for Mip6 export, for instance, by promoting Mip6 interaction with the export factor Mex67. To test this, the Mip6-Mex67 interaction was studied by a pull-down assay using a plasmid that expresses Mip6-GFP in in *rpb1-1 mip6Δ* strain under optimal conditions and following a heat shock at 39°C for 20min.

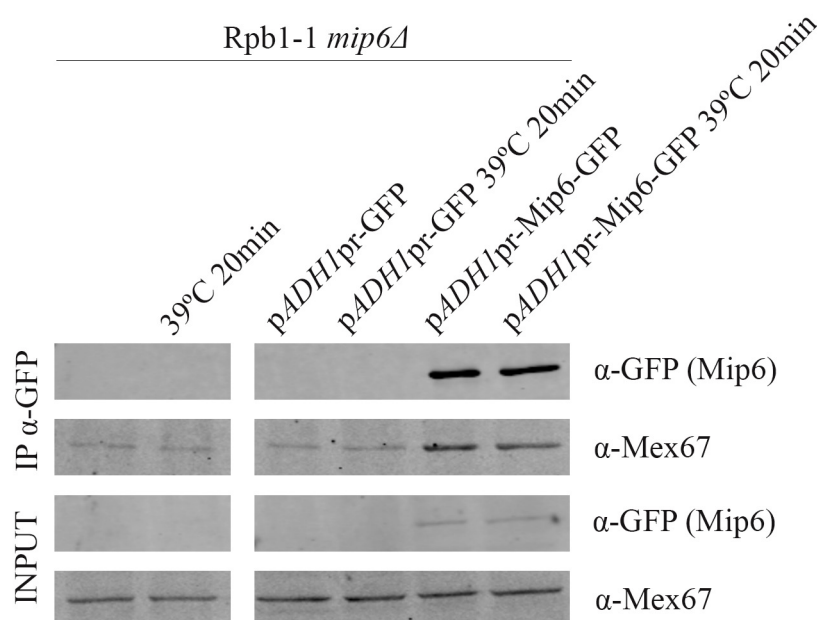


Figure 13. Inactivation of the RNAPolIII subunit Rpb1 does not affect the Mip6-Mex67 interaction. Immunoprecipitation of Mip6-GFP expressed from a plasmid in *Rpb1-1 mip6Δ* strain under optimal conditions or following a heat shock at 39°C for 20min. An empty plasmid and a strain without a plasmid were used as negative controls. Mip6-GFP and Mex67 were detected by Western blotting using the indicated antibodies.

Rpb1 inactivation, by increasing the temperature, does not impair Mip6-Mex67 interaction (Figure 13), hence this interaction is not dependent on transcription. Thus, since Mip6 is retained in the nucleus in the same mutant (Martín-Expósito et al., 2019), transcription mediates, either directly or indirectly, Mip6 export in a Mex67-independent manner.

Mip6 ROLE IN THE METABOLISM OF TRANSCRIPTS DEPENDING ON THE TRANSCRIPTION FACTORS Msn2 AND Msn4

Growth under optimal conditions does not require the coexistence of Mip6 and transcription factors Msn2 and Msn4

In order to deepen the crosstalk of Mip6 with Msn2/4 transcription factors, whether there is a genetic interaction between the genes coding these proteins or not was studied by growth assay in WT, *mip6Δ*, *msn2Δ*, *msn2Δmip6Δ*, *msn4Δ*, *msn4Δmip6Δ*, *msn2Δmsn4Δ* and *msn2Δmsn4Δmip6Δ* strains under optimal growth conditions.

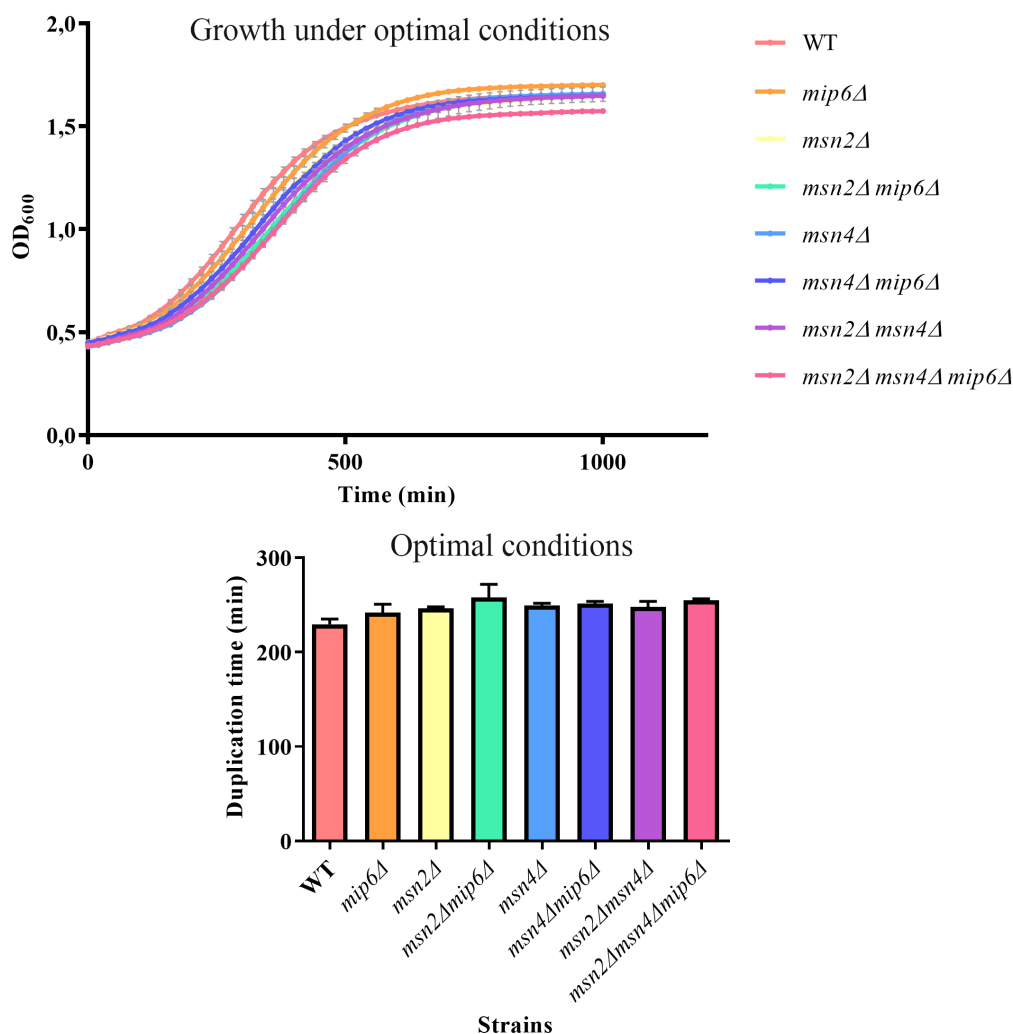


Figure 14. No genetic interaction between *MIP6* and *MSN2* and *MSN4* is observed when grown under optimal conditions. Growth curve of wild-type (WT), *mip6Δ*, *msn2Δ*, *msn2Δmip6Δ*, *msn4Δ*, *msn4Δmip6Δ*, *msn2Δmsn4Δ* and *msn2Δmsn4Δmip6Δ* strains under optimal conditions. Data represent the mean and the standard error (SE) of three biological replicates (up). Duplication time of wild-type (WT), *mip6Δ*, *msn2Δ*, *msn2Δmip6Δ*, *msn4Δ*, *msn4Δmip6Δ*, *msn2Δmsn4Δ* and *msn2Δmsn4Δmip6Δ* strains under optimal conditions. Data represent the mean and the standard error (SE) of three biological replicates (down).

There are no significant differences in any of the studied strains when the cells were grown under optimal conditions (**Figure 14**). Thus, neither of these genes are essential for cell viability under optimal growth and no genetic interaction was observed between them.

MSN2 and *MSN4* genetically interact upon heat stress

Although there is no genetic interaction between *MSN2*, *MSN4* and *MIP6* under optimal growth conditions (**Figure 14**), Msn2 and Msn4 are transcription factors that regulate the expression of stress response genes, hence they gain importance upon stress (P. Lee et al., 2008, 2013; Martínez-Pastor et al., 1996). Besides, Mip6 also takes part in the HSR (Martín-Expósito et al., 2019). Thus, *MSN2*, *MSN4* and *MIP6* could genetically interact upon heat stress. In order to check this, the growth of WT, *mip6Δ*, *msn2Δ*, *msn2Δmip6Δ*, *msn4Δ*, *msn4Δmip6Δ*, *msn2Δmsn4Δ* and *msn2Δmsn4Δmip6Δ* strains at 37°C was studied.

Both the *MSN2* and the *MSN4* individual deletions lead to a growth defect upon heat stress, which shows the importance of these factors in heat stress survival. Unexpectedly, when both deletions are combined, the cells grow at the same rate as the WT strain in our experiments (**Figure 15**). By contrast, no genetic interaction with *MIP6* was observed under heat stress conditions. However, we observed a tendency (not statistically significant) of the *msn2Δmip6Δ* strain to present a slight increase in the growth rate compared with the *msn2Δ* single mutant, which is also the case for the triple mutant (**Figure 15**).

The triple mutant *msn2Δmsn4Δmip6Δ* is sensitive to severe heat stress and oxidative stress

The tendency observed above for the triple mutant prompted us to test the genetic interaction between *MIP6*, *MSN2* and *MSN4* increasing the severity of the nature of the stress, as for instance growing at 42°C.

The *msn2Δmsn4Δmip6Δ* mutant presents a growth defect comparing not only with the WT strain, but also with its corresponding single mutants (**Figure 16**), which indicates a genetic interaction between *MSN2*, *MSN4* and *MIP6* upon a severe heat shock. Besides, in this case, the absence of

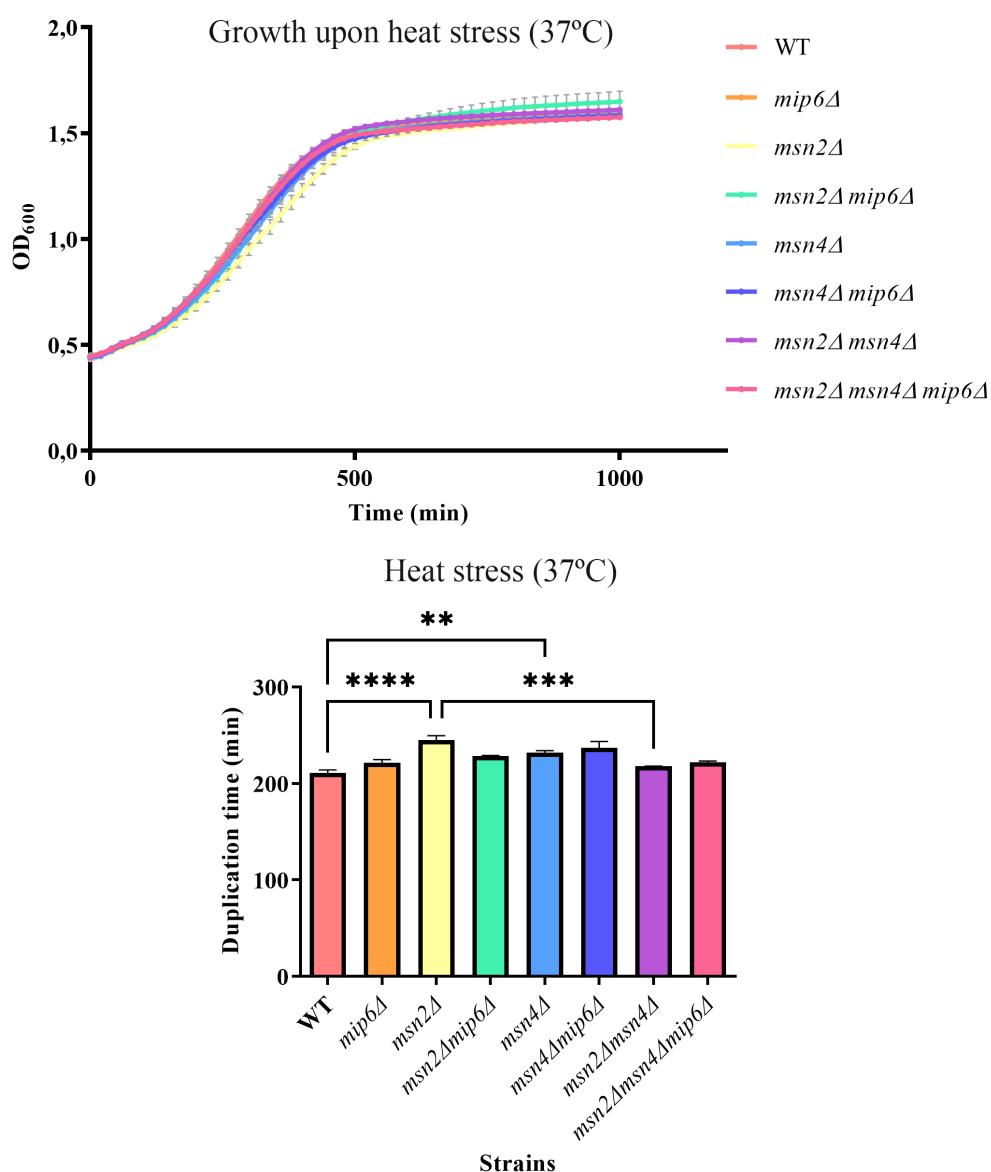


Figure 15. *MSN2* and *MSN4* genetically interact upon heat stress. Growth curve of wild-type (WT), *mip6Δ*, *msn2Δ*, *msn2Δmip6Δ*, *msn4Δ*, *msn4Δmip6Δ*, *msn2Δmsn4Δ* and *msn2Δmsn4Δmip6Δ* strains upon heat stress (37°C). Data represent the mean and the standard error (SE) of three biological replicates (up). Duplication time of wild-type (WT), *mip6Δ*, *msn2Δ*, *msn2Δmip6Δ*, *msn4Δ*, *msn4Δmip6Δ*, *msn2Δmsn4Δ* and *msn2Δmsn4Δmip6Δ* strains upon heat stress (37°C). Data represent the mean and the standard error (SE) of three biological replicates. **** p value < 0,001; *** p value < 0,005; ** p value < 0,01 (down).

only one of the transcription factors does not imply an alteration in cell survival, which suggests that the presence of only one of them is enough to properly trigger the HSR.

Transcription factors Msn2/4 also regulate other kinds of stresses, such as oxidative stress (Boisnard et al., 2009; Collet et al., 2003). Besides, Mip6 seems to have a role in the fatty acid oxidation occurring in peroxisomes and in the maintaining of the redox balance (Ugidos Guerrero, 2023).

Taking all these evidences together, Mip6 might participate also in the OxSR, which is tightly

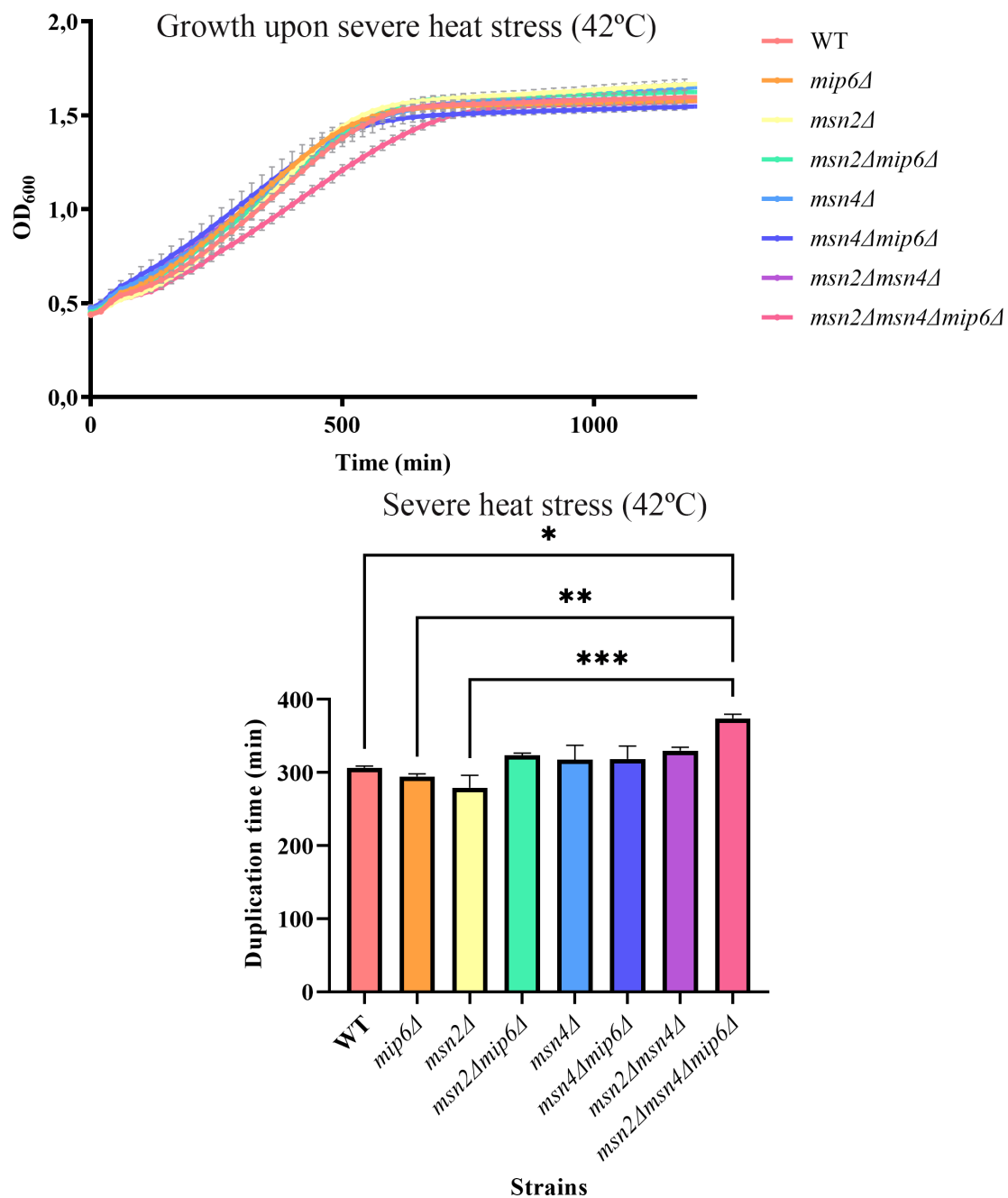


Figure 16. The *msn2Δmsn4Δmip6Δ* mutant presents a growth defect in severe heat stress. Growth curve of wild-type (WT), *mip6Δ*, *msn2Δ*, *msn2Δmip6Δ*, *msn4Δ*, *msn4Δmip6Δ*, *msn2Δmsn4Δ* and *msn2Δmsn4Δmip6Δ* strains upon severe heat stress (42°C). Data represent the mean and the standard error (SE) of three biological replicates (up). Duplication time of wild-type (WT), *mip6Δ*, *msn2Δ*, *msn2Δmip6Δ*, *msn4Δ*, *msn4Δmip6Δ*, *msn2Δmsn4Δ* and *msn2Δmsn4Δmip6Δ* strains upon severe heat stress (42°C). Data represent the mean and the standard error (SE) of three biological replicates. *** p value < 0,005; ** p value < 0,01; * p value < 0,05 (down).

related to HS (Morano et al., 2012). Thus, the genetic interaction between *MIP6*, *MSN2* and *MSN4* under oxidative stress was studied by a growth assay of WT, *mip6Δ*, *msn2Δ*, *msn2Δmip6Δ*, *msn4Δ*, *msn4Δmip6Δ*, *msn2Δmsn4Δ* and *msn2Δmsn4Δmip6Δ* strains after an oxidative shock with 4mM H₂O₂ for 30min.

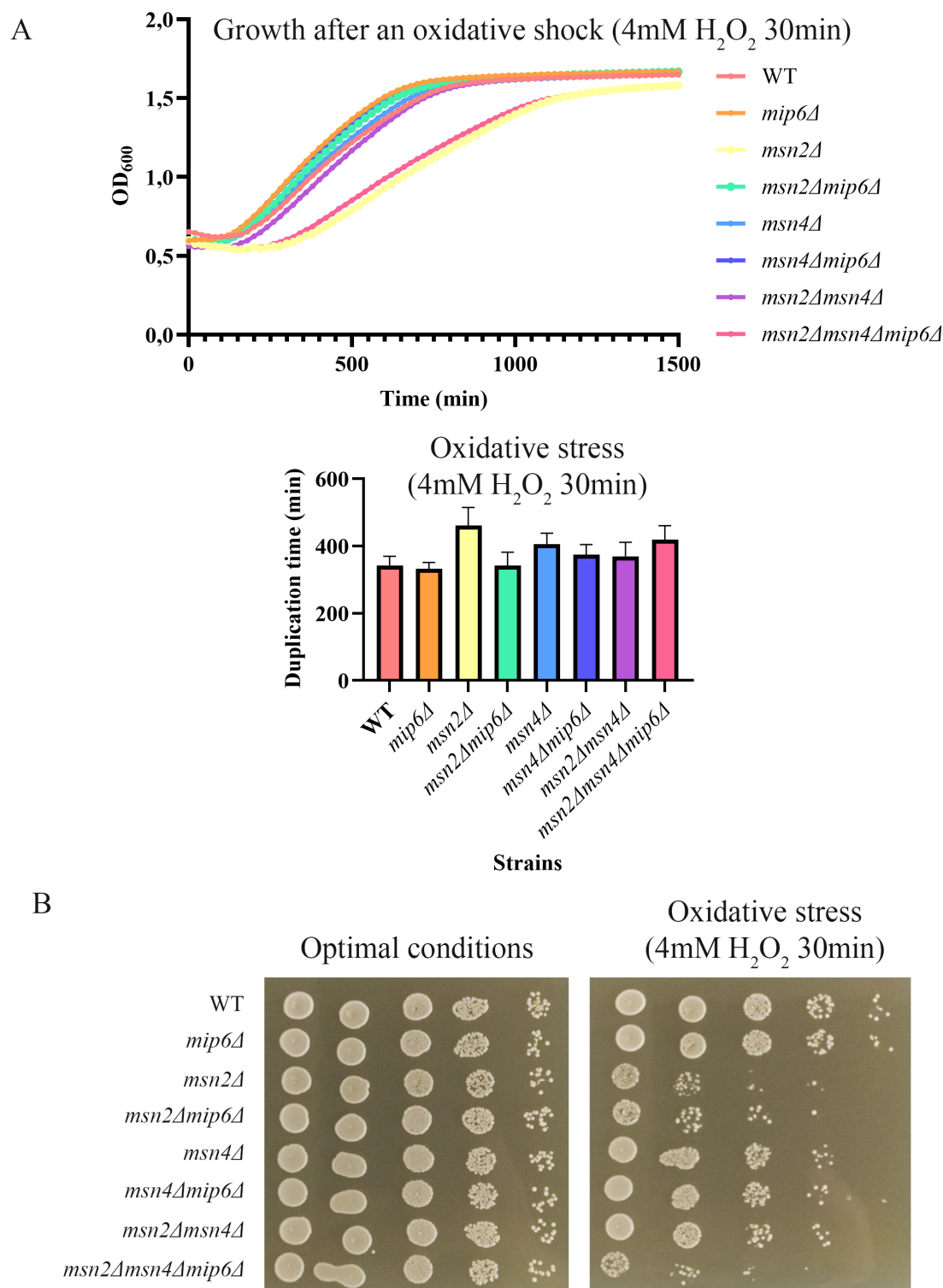


Figure 17. *MIP6*, *MSN2* and *MSN4* genetically interact upon oxidative stress. (A) Growth curve of wild-type (WT), *mip6Δ*, *msn2Δ*, *msn2Δmip6Δ*, *msn4Δ*, *msn4Δmip6Δ*, *msn2Δmsn4Δ* and *msn2Δmsn4Δmip6Δ* strains after an oxidative shock (4mM H₂O₂ 30min). One representative replicate is shown (up). Duplication time of wild-type (WT), *mip6Δ*, *msn2Δ*, *msn2Δmip6Δ*, *msn4Δ*, *msn4Δmip6Δ*, *msn2Δmsn4Δ* and *msn2Δmsn4Δmip6Δ* strains after an oxidative shock (4mM H₂O₂ 30min). Data represent the mean and the standard error (SE) of six biological replicates (down). (B) Dot spot growth assay of wild-type (WT), *mip6Δ*, *msn2Δ*, *msn2Δmip6Δ*, *msn4Δ*, *msn4Δmip6Δ*, *msn2Δmsn4Δ* and *msn2Δmsn4Δmip6Δ* strains under optimal conditions and after an oxidative shock (4mM H₂O₂ 30min). Precultures were diluted in liquid YPD medium, and ten-fold dilutions of cells were spotted onto YPD plates and incubated for two days at 30°C. One representative replicate is shown.

Although they are not statistically significant, we observed differences in the duplication time of the studied strains when they were grown after an oxidative shock (**Figure 17A, down**). The *msn2Δ* mutant presents a growth defect that is recovered with the deletion of either *MIP6* or *MSN4*. However, the triple mutant grows worse than any combination (**Figure 17A, up** and **Figure 17B**). Thus, there is a genetic interaction between *MIP6*, *MSN2* and *MSN4* upon oxidative stress.

No genetic interaction between *MIP6*, *MSN2* and *MSN4* is observed in cells growing using glycerol as the carbon source

When yeast cells are grown using glycerol instead of glucose as the only carbon source, cell metabolism must shift from fermentative to oxidative and, thus, aerobic. In these circumstances, the expression of genes involved in the mitochondrial electron transport and respiration is altered, which affects the ROS equilibrium and, hence, can trigger the OxSR (Liguori et al., 2018; Mattenberger et al., 2017; Santiago et al., 2020). As above, a growth assay on glycerol was followed in WT, *mip6Δ*, *msn2Δ*, *msn2Δmip6Δ*, *msn4Δ*, *msn4Δmip6Δ*, *msn2Δmsn4Δ* and *msn2Δmsn4Δmip6Δ*.

No growth difference is observed in any of the studied strains when the cells were grown with glycerol as the only carbon source nor any genetic interaction between *MIP6*, *MSN2* and *MSN4* (**Figure 18**). Thus, Mip6 may not participate in the metabolic shift when the carbon source in the media changes from glucose to glycerol.

As a summary of the growth assays carried out (**Figure 19**), Mip6 importance in cell survival, as well as its interaction with Msn2/4, seems to be different depending on the kind of stress cells are exposed to. While under mild heat stress, *MIP6* does not seem to genetically interact with *MSN2* and *MSN4*, the situation changes upon a severe heat shock or an oxidative shock. Besides, when cells suffer from oxidative stress due to the metabolic shift when the carbon source in the media changes from glucose to glycerol, neither of these genes seem to be essential for cell survival upon this environmental change.

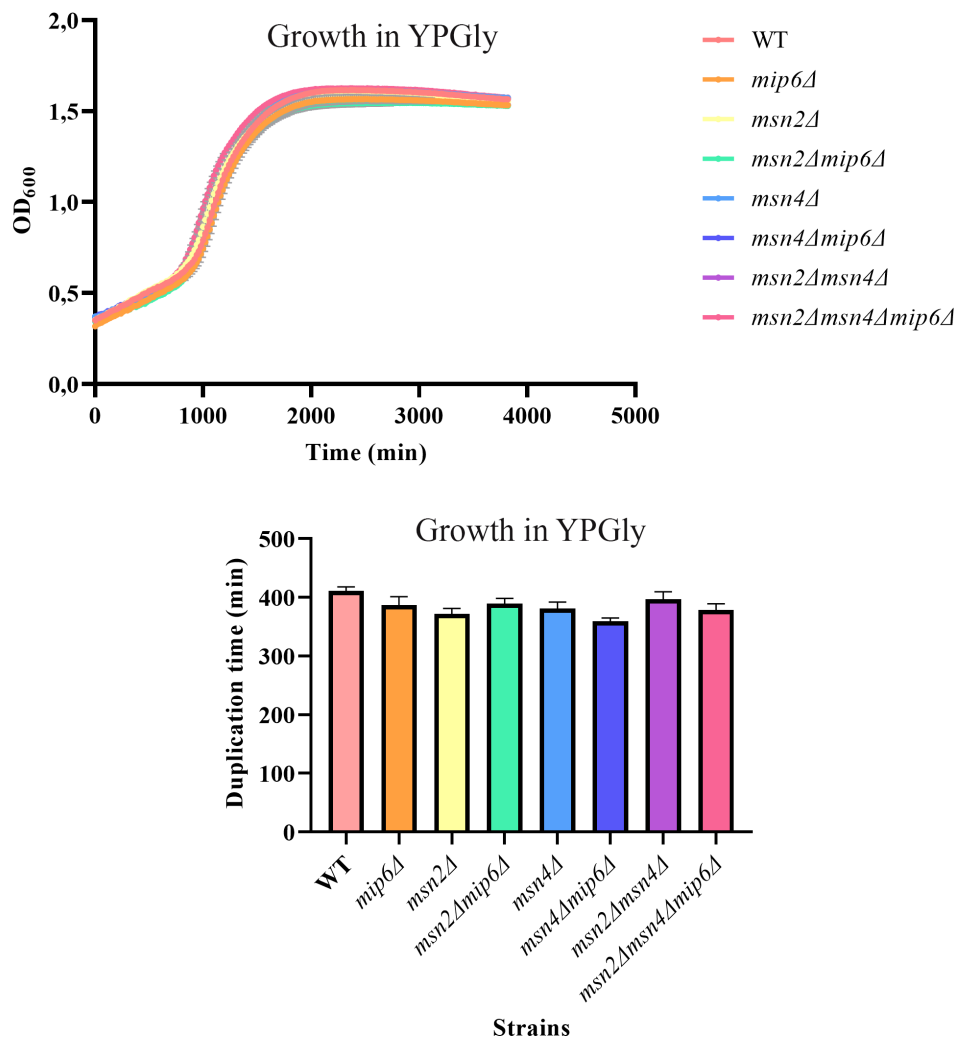


Figure 18. No genetic interaction between *MIP6*, *MSN2* and *MSN4* is observed with glycerol as the only carbon source. Growth curve of wild-type (WT), *mip6Δ*, *msn2Δ*, *msn2Δmip6Δ*, *msn4Δ*, *msn4Δmip6Δ*, *msn2Δmsn4Δ* and *msn2Δmsn4Δmip6Δ* strains in YPGly. Data represent the mean and the standard error (SE) of three biological replicates (up). Duplication time of wild-type (WT), *mip6Δ*, *msn2Δ*, *msn2Δmip6Δ*, *msn4Δ*, *msn4Δmip6Δ*, *msn2Δmsn4Δ* and *msn2Δmsn4Δmip6Δ* strains in YPGly. Data represent the mean and the standard error (SE) of three biological replicates (down).

CROSSTALK BETWEEN *Mip6* AND HDAC *Rpd3* AND *Hda1*

Mip6 physically interacts with histone deacetylase *Rpd3* which is dispensable for its interaction with *Mex67*

Chromatin remodelers, especially HATs and HDAs, play an important role in regulating the stress response at the epigenetic level (Kremer & Gross, 2009; Ruiz-Roig et al., 2010). Indeed, HDAs *Rpd3* and *Hda3* are strictly necessary for *Msn2/4* activity, since their absence leads *Hsp12* levels to decrease in the same way as the lack of *Msn2/4* (Sadeh et al., 2011). Besides, *Mip6* also seems to be related to these kinds of proteins. Genetic interaction between *MIP6* and *HDA1* was studied in our group, being H4K12 acetylation levels altered in *mip6Δ* (Nuño Cabanes, 2024). Moreover,

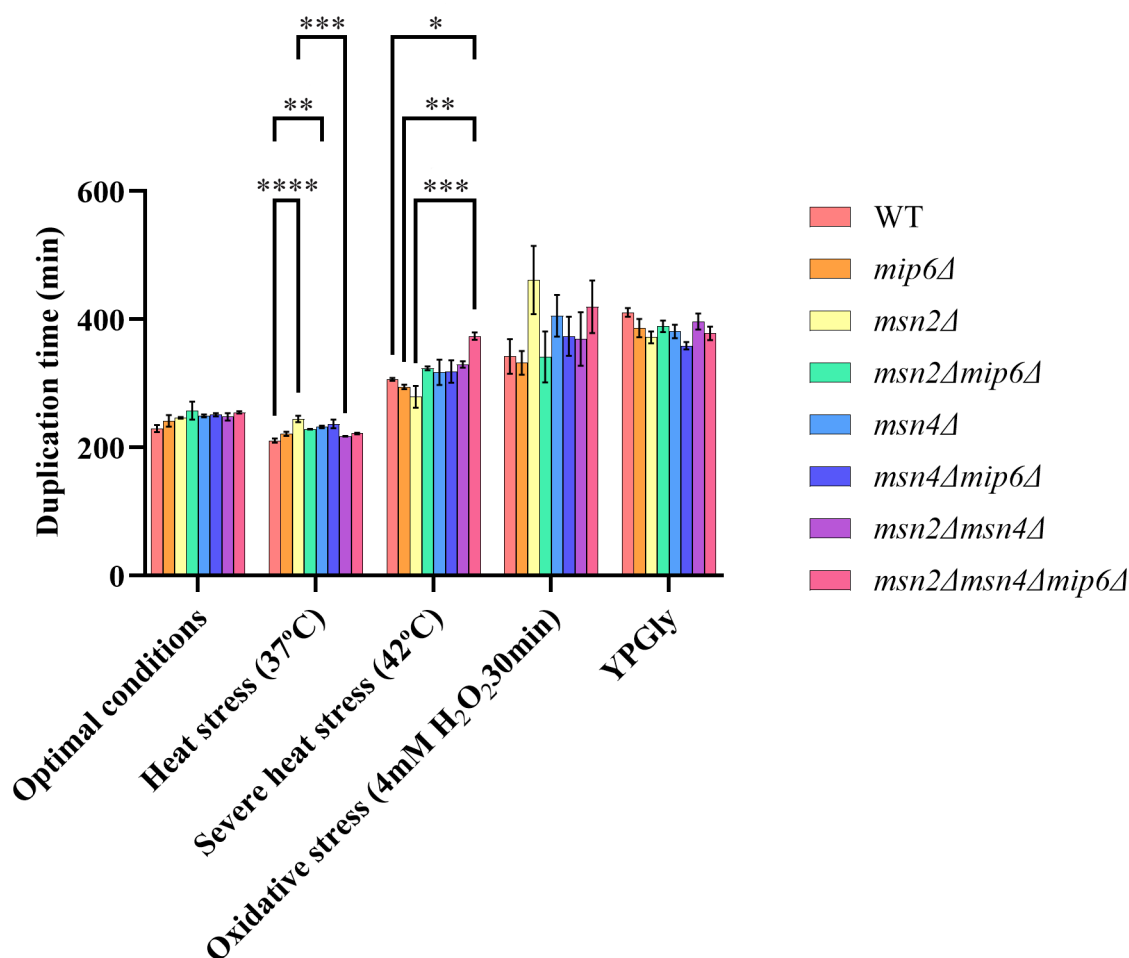


Figure 19. Summary of the growth assay performed in order to study genetic interaction between *MIP6*, *MSN2* and *MSN4*. Duplication time of wild-type (WT), *mip6Δ*, *msn2Δ*, *msn2Δmip6Δ*, *msn4Δ*, *msn4Δmip6Δ*, *msn2Δmsn4Δ* and *msn2Δmsn4Δmip6Δ* strains under optimal conditions and upon different kind of stresses: heat stress (37°C), severe heat stress (42°C), after an oxidative shock (4mM H₂O₂ 30min) and growth in YPGly. Data represent the mean and the standard error (SE) of at least three biological replicates. **** p value < 0,001; *** p value < 0,005; ** p value < 0,01; * p value < 0,05.

a previous TAP purification assay carried out by us shows Rpd3 and Gcn5 among Mip6-TAP copurifying factors (our unpublished data).

In order to confirm the Mip6-Rpd3 physical interaction, a coimmunoprecipitation assay was performed expressing Mip6-GFP using a plasmid in Rpd3-TAP *mip6Δ*. The experiment was carried out under optimal conditions, upon heat shock (39°C 20min) and impairing the Mex67-Mip6 interaction using the Mip6W₄₄₂A mutant.

As shown in **Figure 20**, Mip6 physically copurifies with HDA Rpd3. Besides, this copurification is not impaired upon heat stress nor when the interaction between Mip6 and Mex67 is affected

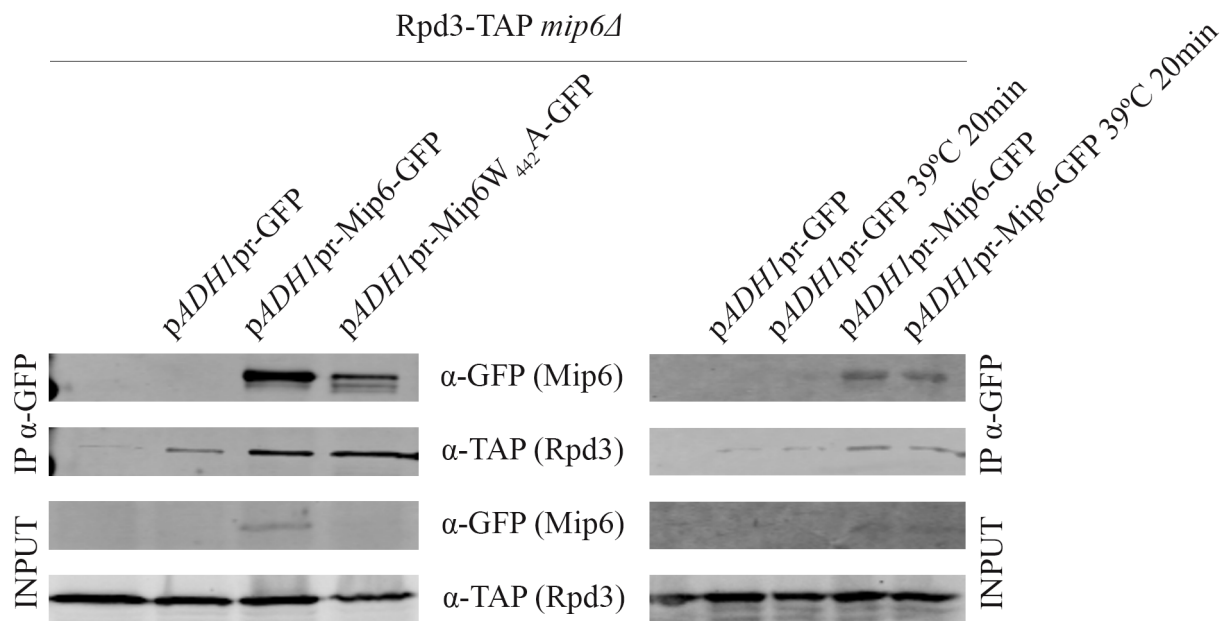


Figure 20. Mip6 physically interacts with histone deacetylase Rpd3. Immunoprecipitation of Mip6-GFP or Mip6W₄₄₂A-GFP expressed from plasmid in Rpd3-TAP *mip6Δ* strain under optimal conditions or following a heat shock at 39°C for 20min. An empty plasmid and a strain without a plasmid were used as negative controls. Mip6-GFP and Rpd3-TAP were detected by Western blotting using the indicated antibodies.

(Mip6W₄₄₂A; **Figure 20**). Thus, Mip6 interacts with Rpd3 under both optimal and heat stress conditions in a Mex67-independent manner.

Rpd3 and Gcn5 are dispensable for Mip6-Mex67 interaction

Mip6-Mex67 interaction is Rpb1 independent (**Figure 13**). Thus, one possibility is that the Mip6-Mex67 interaction may occur more upstream and depend on histone regulators that show a functional link with Mip6. For this reason, a pull-down assay of Mip6-GFP in both *rpd3Δmip6Δ* and *gcn5Δmip6Δ* strains was performed to study how the lack of Rpd3 or Gcn5 affect the Mip6-Mex67 interaction.

Neither *RPD3* nor *GCN5* deletions affect the interaction between Mip6 and Mex67 (**Figure 21**). Therefore, the Mip6-Mex67 interaction is independent of the presence of Rpd3 and Gcn5.

MIP6 genetically interacts with *RPD3* and *HDA1* under optimal conditions and heat stress

Growth assays of WT, *mip6Δ*, *rpd3Δ*, *rpd3Δmip6Δ*, *gcn5Δ*, *gcn5Δmip6Δ*, *hda1Δ* and *hda1Δmip6Δ* strains were carried out under both optimal conditions and heat stress (37°C), comparing the growth curve and the duplication times.

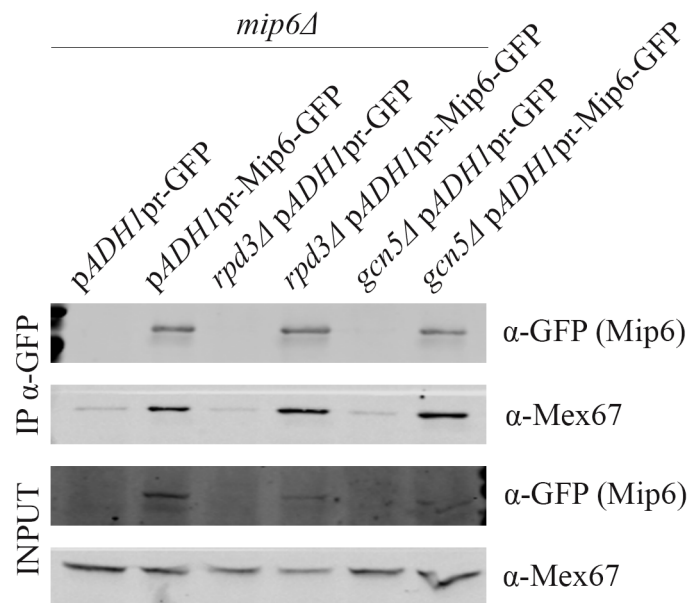


Figure 21. The Mip6-Mex67 interaction does not depend on histone deacetylase Rpd3 or histone acetyltransferase Gcn5. Immunoprecipitation of Mip6-GFP expressed from a plasmid in *mip6Δ*, *rpd3Δmip6Δ* and *gcn5Δmip6Δ* strains. An empty plasmid was used as a negative control. Mip6-GFP and Mex67 were detected by Western blotting using the indicated antibodies.

As illustrated here, both *rpd3Δmip6Δ* and *hda1Δmip6Δ* strains grow worse than their corresponding single mutants (**Figure 22** and **Figure 23**), confirming a negative genetic interaction between *MIP6* and *HDA1* and *RPD3*. This result may indicate that the absence of Mip6 is more relevant when combined with the “eraser” HDAs rather than the “writer” HATs.

Oxidative stress specifically affects the survival of *hda1Δmip6Δ* mutant

As part of this work, we found genetic interaction between *MIP6* and *Msn2/4* upon oxidative stress (**Figure 17**), hence, as above, a growth assay in WT, *mip6Δ*, *rpd3Δ*, *rpd3Δmip6Δ*, *gcn5Δ*, *gcn5Δmip6Δ*, *hda1Δ* and *hda1Δmip6Δ* strains was carried out after an oxidative shock with 4mM H_2O_2 for 30min. As shown in **Figure 24**, the *hda1Δmip6Δ* double mutant grows much worse than the others upon oxidative stress, indicating that *MIP6* together with *HDA1* is required to cope with the OxSR. In contrast, even if *MIP6* genetically interacts with *RPD3* under both optimal and heat stress conditions, this interaction is not observed after an oxidative shock (**Figure 24**).

As a summary (**Figure 25**), Mip6 shows genetic interaction with the HDAs Rpd3 and Hda1, both under optimal and heat stress conditions but with only *HDA1* upon oxidative stress. On the contrary, no genetic interaction with the HAT coding gene *GCN5* is observed in any of the tested conditions.

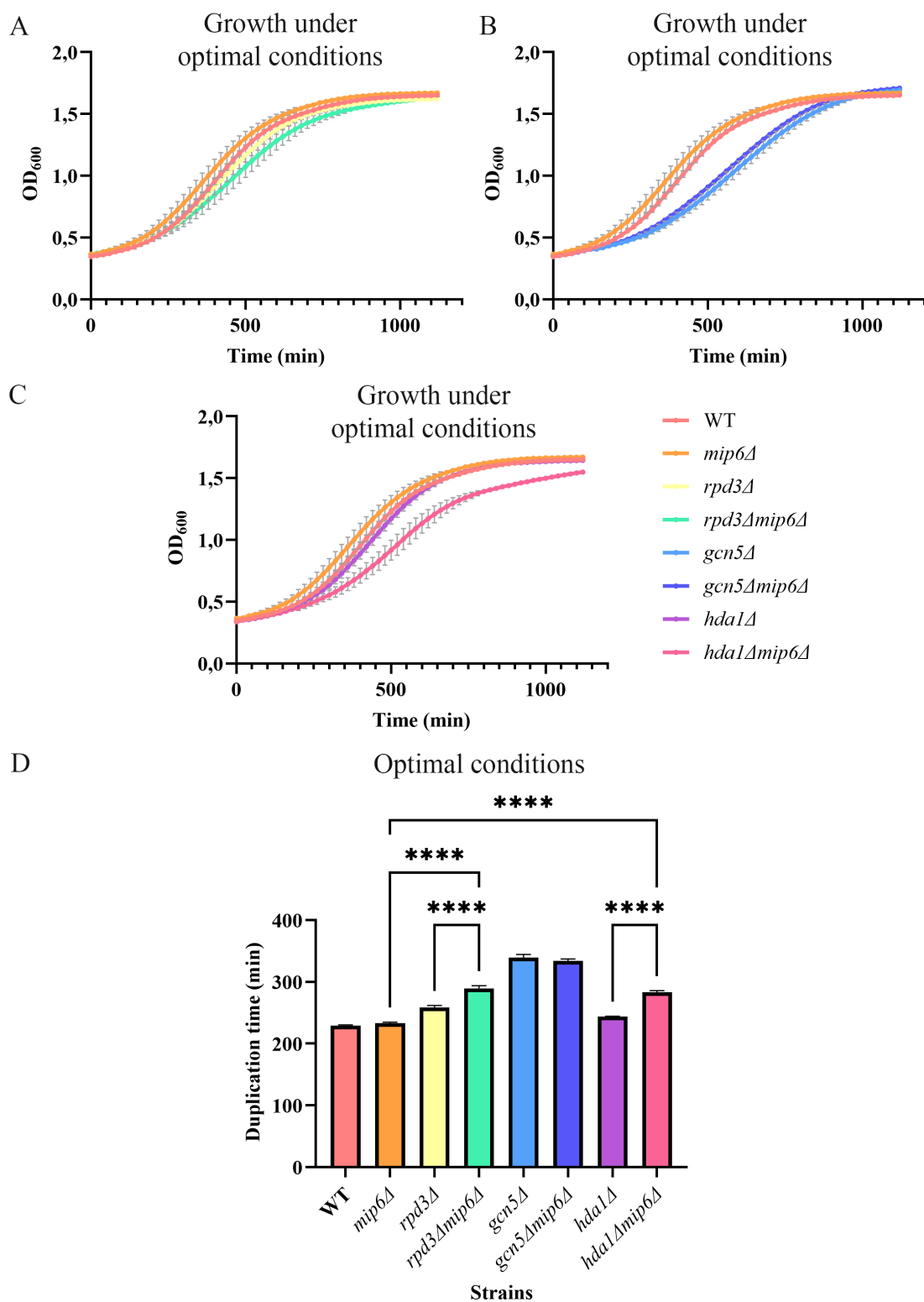


Figure 22. MIP6 genetically interacts with RPD3 and HDA1 under optimal conditions. (A) Growth curve of WT, *mip6Δ*, *rpd3Δ* and *rpd3Δmip6Δ* strains under optimal conditions. Data represent the mean and the standard error (SE) of three biological replicates. (B) Growth curve of WT, *mip6Δ*, *gcn5Δ* and *gcn5Δmip6Δ* strains under optimal conditions. Data represent the mean and the standard error (SE) of three biological replicates. (C) Growth curve of WT, *mip6Δ*, *hda1Δ* and *hda1Δmip6Δ* strains under optimal conditions. Data represent the mean and the standard error (SE) of three biological replicates. (D) Duplication time of WT, *mip6Δ*, *rpd3Δ*, *rpd3Δmip6Δ*, *gcn5Δ*, *gcn5Δmip6Δ*, *hda1Δ* and *hda1Δmip6Δ* strains under optimal conditions. Data represent the mean and the standard error (SE) of three biological replicates. **** p value < 0,001.

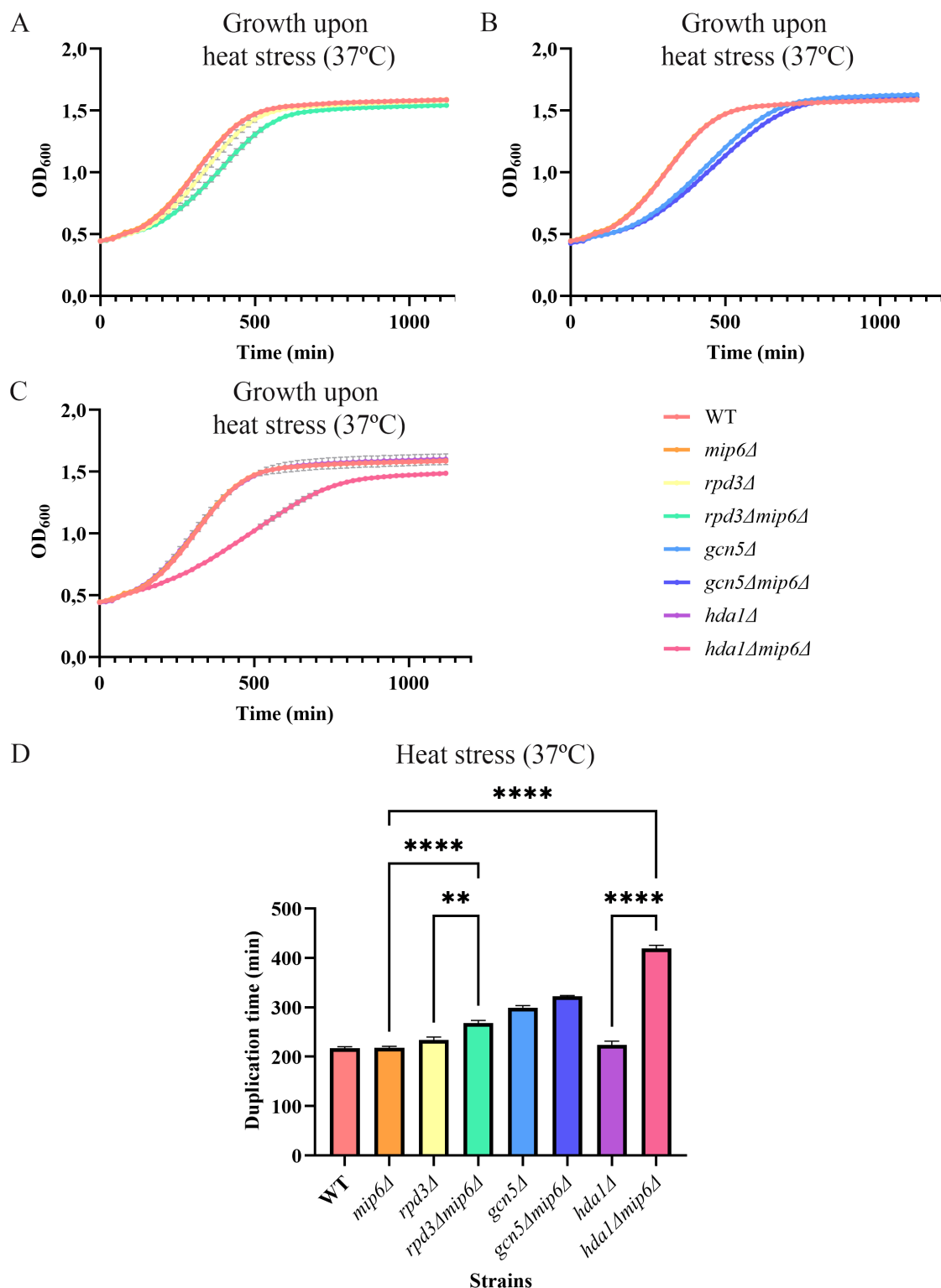


Figure 23. A genetic interaction of *MIP6* with *RPD3* and *HDA1* is observed upon heat stress. (A) Growth curve of WT, *mip6Δ*, *rpd3Δ* and *rpd3Δmip6Δ* strains upon heat stress (37°C). Data represent the mean and the standard error (SE) of three biological replicates. (B) Growth curve of WT, *mip6Δ*, *gcn5Δ* and *gcn5Δmip6Δ* strains upon heat stress (37°C). Data represent the mean and the standard error (SE) of three biological replicates. (C) Growth curve of WT, *mip6Δ*, *hda1Δ* and *hda1Δmip6Δ* strains upon heat stress (37°C). Data represent the mean and the standard error (SE) of three biological replicates. (D) Duplication time of WT, *mip6Δ*, *rpd3Δ*, *rpd3Δmip6Δ*, *gcn5Δ*, *gcn5Δmip6Δ*, *hda1Δ* and *hda1Δmip6Δ* strains upon heat stress (37°C). Data represent the mean and the standard error (SE) of three biological replicates. **** p value < 0,001; ** p value < 0,01.

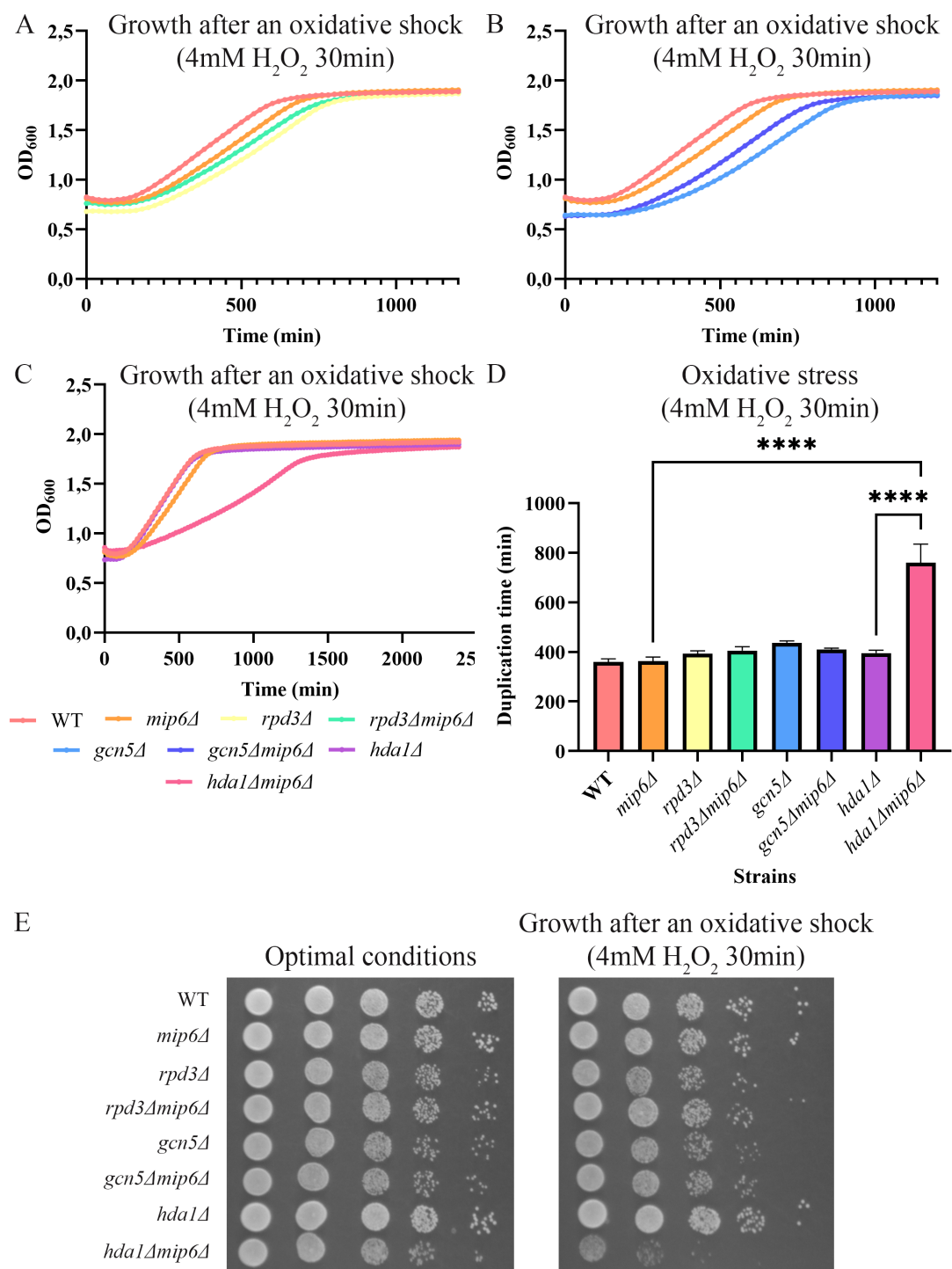


Figure 24. The *hda1Δmip6Δ* mutant presents a growth defect after an oxidative stress induction. (A) Growth curve of WT, *mip6Δ*, *rpd3Δ* and *rpd3Δmip6Δ* strains after an oxidative shock (4mM H₂O₂ 30min). One representative replicate is shown. (B) Growth curve of WT, *mip6Δ*, *gcn5Δ* and *gcn5Δmip6Δ* strains after an oxidative shock (4mM H₂O₂ 30min). One representative replicate is shown. (C) Growth curve of WT, *mip6Δ*, *hda1Δ* and *hda1Δmip6Δ* strains after an oxidative shock (4mM H₂O₂ 30min). One representative replicate is shown. (D) Duplication time of WT, *mip6Δ*, *rpd3Δ*, *rpd3Δmip6Δ*, *gcn5Δ*, *gcn5Δmip6Δ*, *hda1Δ* and *hda1Δmip6Δ* strains after an oxidative shock (4mM H₂O₂ 30min). Data represent the mean and the standard error (SE) of three biological replicates. **** p value < 0,001. (E) Dot spot growth assay of WT, *mip6Δ*, *rpd3Δ*, *rpd3Δmip6Δ*, *gcn5Δ*, *gcn5Δmip6Δ*, *hda1Δ* and *hda1Δmip6Δ* strains under optimal conditions and after an oxidative shock (4mM H₂O₂ 30min). Precultures were diluted in liquid YPD medium, and ten-fold dilutions of cells were spotted onto YPD plates and incubated for two days at 30°C. One representative replicate is shown.

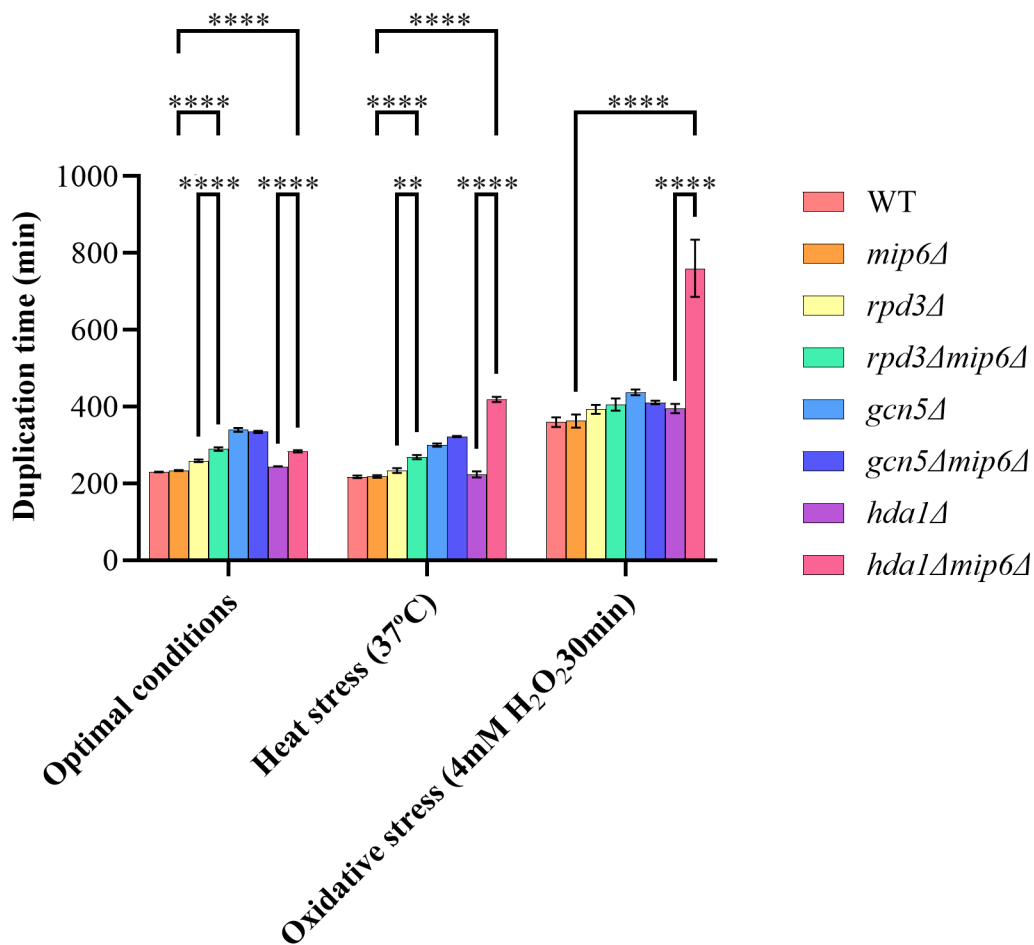


Figure 25. Summary of the growth assay performed in order to study genetic interaction between *MIP6*, *RPD3*, *GCN5* and *HDA1*. Duplication time of wild-type (WT), *mip6Δ*, *rpd3Δ*, *rpd3Δmip6Δ*, *gcn5Δ*, *gcn5Δmip6Δ*, *hda1Δ* and *hda1Δmip6Δ* strains under optimal conditions and upon different kind of stresses: heat stress (37°C) and after an oxidative shock (4mM H₂O₂ 30min). Data represent the mean and the standard error (SE) of at least three biological replicates. **** p value < 0,001; ** p value < 0,01.

Analysis of the synergistic effect of HDAC deletion with *mip6Δ* in the expression levels of *HSP12* and *CTT1*

Previous experiments performed in our group showed that Mip6 would also present a functional interaction with Rpd3 regarding histone H3 and H4 acetylation levels (our unpublished data). Keeping this in mind and knowing that histone acetylation and deacetylation are regulatory mechanisms for gene expression (Chou et al., 2023), this functional interaction between Mip6 and Rpd3 may be also reflected in the levels of the Msn2/4 transcripts, since the absence of both proteins individually affects their levels (Martín-Expósito et al., 2019; Ruiz-Roig et al., 2010). Thus, mRNA levels of *HSP12* and *CTT1*, two Msn2/4-dependent transcripts, were analyzed in WT, *mip6Δ*, *rpd3Δ*, *rpd3Δmip6Δ*, *hda1Δ* and *hda1Δmip6Δ* by qPCR.

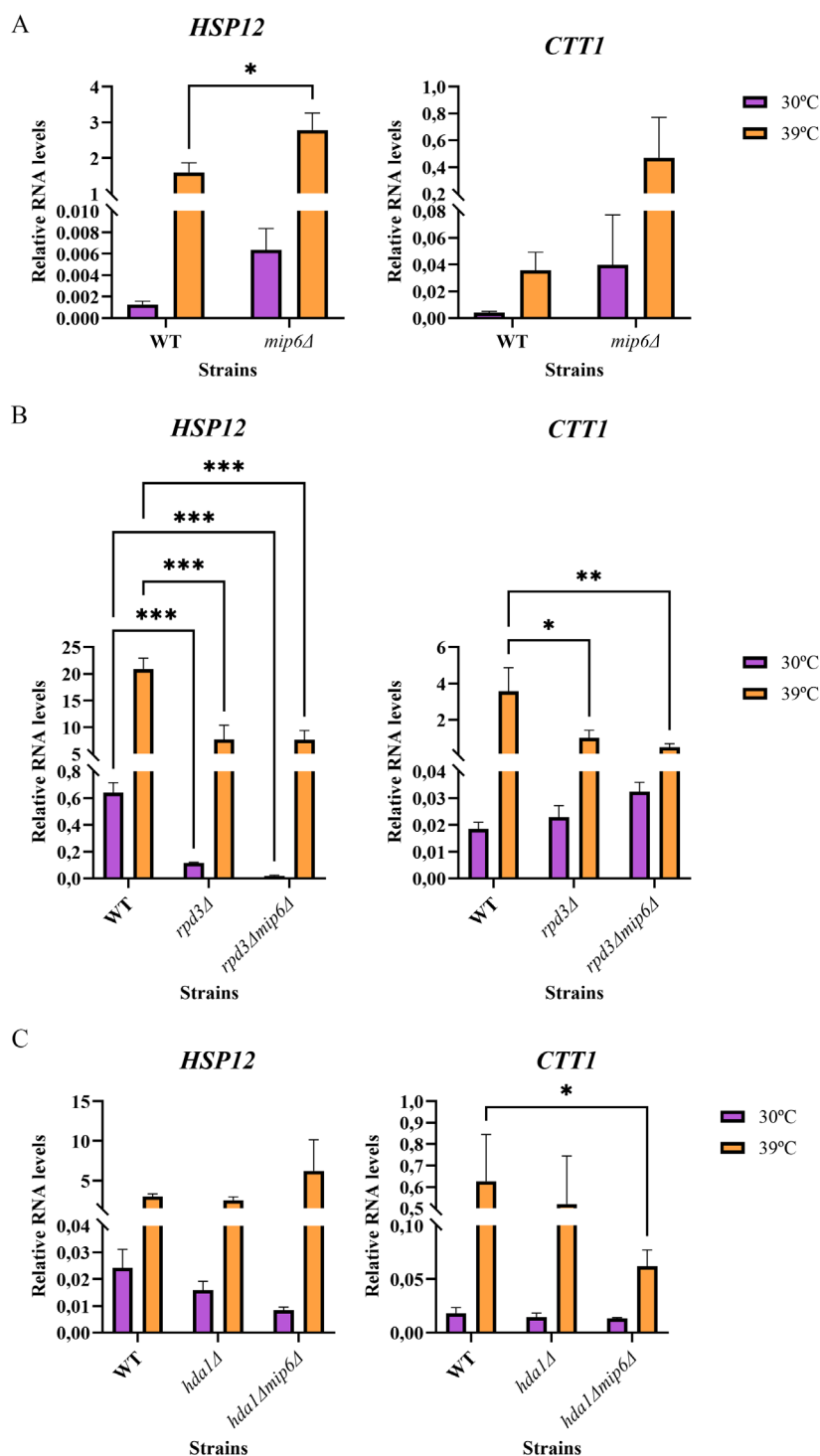


Figure 26. Analysis of the synergistic effect of HDAC deletion with *mip6Δ* in the expression levels of *HSP12* and *CTT1*. (A) *HSP12* and *CTT1* RNA levels measured by qRT-PCR experiments from WT and *mip6Δ* strains. Total RNA was obtained from yeast cultures under optimal conditions and after a heat shock of 20min at 39°C. Values were calculated by using the $\Delta\Delta C_t$ method. Data represent the mean and the standard error (SE) of three biological replicates. * p value < 0,05. (B) *HSP12* and *CTT1* RNA levels measured by qRT-PCR experiments from WT, *rpd3Δ* and *rpd3Δmip6Δ* strains. Total RNA was obtained from yeast cultures under optimal conditions and after a heat shock of 20min at 39°C. Values were calculated by using the $\Delta\Delta C_t$ method. Data represent the mean and the standard error (SE) of three biological replicates. *** p value < 0,005; ** p value < 0,01; * p value < 0,05. (C) *HSP12* and *CTT1* RNA levels measured by qRT-PCR experiments from WT, *hda1Δ* and *hda1Δmip6Δ* strains. Total RNA was obtained from yeast cultures under optimal conditions and after a heat shock of 20min at 39°C. Values were calculated by using the $\Delta\Delta C_t$ method. Data represent the mean and the standard error (SE) of three biological replicates. * p value < 0,05.

Both *HSP12* and *CTT1* mRNA levels decrease when Rpd3 is lacking (**Figure 26B**), in contrast with the increase observed when *MIP6* is deleted (**Figure 26A**). Besides, the double mutant *rpd3Δmip6Δ* shows a reduction in the mRNA levels similar to the *rpd3Δ* single mutant. This could indicate that both proteins may take part in the same pathway, being Rpd3 presence essential for Mip6 intervention. In contrast, Hda1 absence does not affect *HSP12* and *CTT1* mRNA levels, but double mutant *hda1Δmip6Δ* lowers *CCT1* levels, unlike *HSP12* levels (**Figure 26C**).

This difference in the phenotype depending on the studied gene is also observed in the Rpd3 mutants under optimal growth conditions. Rpd3 absence leads to a reduction in *HSP12* mRNA levels, just as shown in the *rpd3Δmip6Δ* double mutant. While *CTT1* levels are not altered in any of the mutants (**Figure 26**). These results suggest that the Msn2/4-dependent genes might present different HDAC regulation patterns. Thus, analyzing more stress response genes regulated by Msn2/4 would be interesting in order to check if there is any specific set of genes with each one of the phenotypes to better study this difference.

Mip6 ROLE IN TRANSCRIPTIONAL MEMORY

Ribosomal Protein (RP) genes are sensitive to transcriptional memory upon heat shock at 39°C and Mip6 presence contributes to it

Transcriptional memory facilitates yeast cells to respond faster to a subsequent stimulus when they have already suffered a previous one. This mechanism is behind the cell response to the nutritional status of yeast being the histone deacetylation complex Rpd3 a key player in the process, participating in the regulation of the expression of the *GAL* genes (B. B. Lee et al., 2018). Interestingly, even if heat shock-induced transcriptional memory has been studied in other organisms, such as *Arabidopsis* (Ohama et al., 2017), transcriptional memory upon heat stress is scarcely described. Since Mip6 interacts with Rpd3 (**Figure 20** and **Figure 25**), we speculate that it could play a role in this process.

We first investigated whether yeast cells present a transcriptional memory induced by heat stress, and then, whether Mip6 presence is required. With this aim, two flasks coming from the same culture were grown in parallel. One of them was subjected to a heat shock at 39°C for 20min,

a subsequent 60min recovery treatment at 30°C (optimal conditions), and then to a second heat shock at 39°C. Meanwhile, the second flask was only subjected to heat stress once at 39°C for 20min. Samples were taken every 5min in both the second heat shock of the first flask, which was called “memory”, and the unique heat shock of the second flask, which was called “no memory” (Figure 27A). Samples were processed and mRNA levels of a subset of ribosomal protein (RP) genes were analyzed by qPCR. The experiment was carried out in both WT and *mip6Δ* strains.

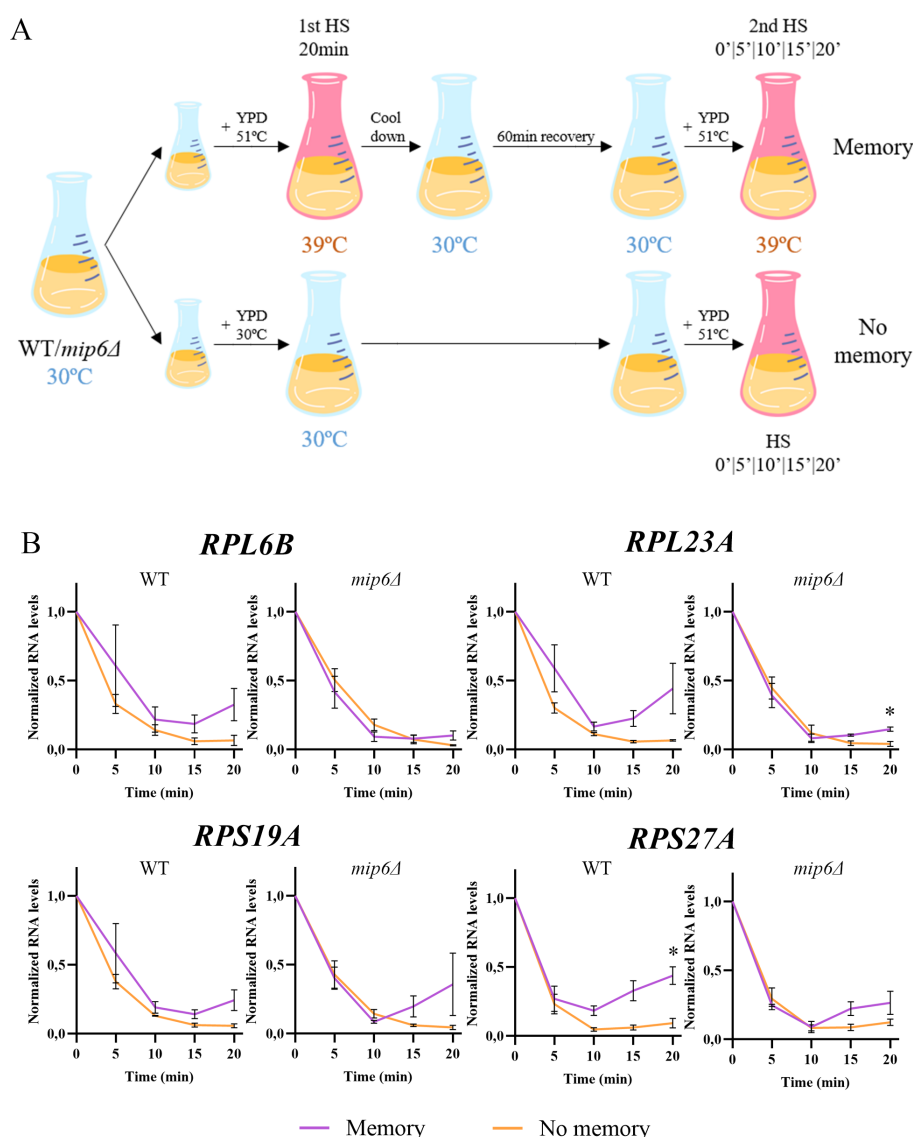


Figure 27. Ribosomal Protein (RP) genes are sensitive to transcriptional memory upon heat shock at 39°C and Mip6 presence contributes to it. (A) Experimental design for the study of the transcriptional memory associated with heat shock (HS). A cell culture was split into two flasks. One of them (memory) was exposed to a first 20min heat shock and, after a 60min recovery phase at optimal conditions, to a second 20min heat shock. In the meantime, the other flask (no memory) was only exposed to a single 20min heat shock. The heat shocks were performed by adding media at 51°C. (B) *RPL6B*, *RPL23A*, *RPS19A* and *RPS27A* RNA levels measured by qRT-PCR experiments from WT and *mip6Δ* strains following the experiment previously described. Values were calculated by using the $\Delta\Delta C_t$ method and were normalized so that values of time 0 were equal to 1. Data represent the mean and the standard error (SE) of three biological replicates. * p value < 0,05.

In the studied RP genes, mRNA levels decrease similarly after 5min and 10min upon HS. However, in the “memory” samples there is a tendency to reverse this behavior and, in the 20min time point, there is an increased expression that is not observed in the “no memory” samples (**Figure 27B**). It was reported that RP genes expression decreases under many stressful conditions, including heat stress (Causton et al., 2001; Gasch et al., 2000; Mühlhofer et al., 2024) and interestingly Mip6 binds to their transcripts during the HSR (Martín-Expósito et al., 2019). Here, we show that in the *mip6Δ* mutant, some of the studied RP genes showed a different memory effect than in the WT strain (**Figure 27B**), which suggests a possible Mip6 role in transcriptional memory regulation.

Global analysis of transcriptional memory upon mild heat shock

In order to deepen the study of the changes observed in gene expression when cells have been already exposed to a previous heat shock and the role of Mip6 in this mechanism, an RNA-Seq analysis was performed, following the experimental design described above (**Figure 27A**) and collecting samples at 0min, 15min and 20min after the beginning of the heat stress exposure.

At first glance at the RNA-Seq results, a principal components analysis (PCA) was performed in order to identify the principal variables that explain the differences between the analyzed samples (**Figure 28A**). On the one hand, the main variable, which explains 55,5% of the differences, divides the samples depending on the growth conditions: optimal (time point 0min) or stress (time points 15min and 20min). Therefore, more than half of the differences were due to the environmental conditions in which the cells were grown. On the other hand, the second component, corresponding to 16,3% of the differences, splits the samples regarding whether the cells were previously exposed to heat stress (“memory”) or not (“no memory”). This indicates that almost a fifth of the differences were due to transcriptional memory. Thus, other possible variables, such as the strain or the different biological replicates, are less significant when it comes to explain the observed differences, since almost 75% of them were due to stress and memory.

In both WT and *mip6Δ*, more than two-thirds of the yeast genes show differential expression during the HSR depending on the existence of a previous exposure to heat stress. Besides, although most of the genes that show transcriptional memory in the mutant also present this memory in the WT

strain, there are 580 genes with a differential behavior only in WT and 588 genes in the mutant (Figure 28B). The overrepresented Gene Ontology (GO) biological processes among these 588 genes are related to oligosaccharide and disaccharide metabolic processes. These differences may indicate a possible Mip6 role in the transcriptional memory regulation, since when this protein is lacking, genes implicated in these processes show a memory pattern that is not observed in the WT strain.

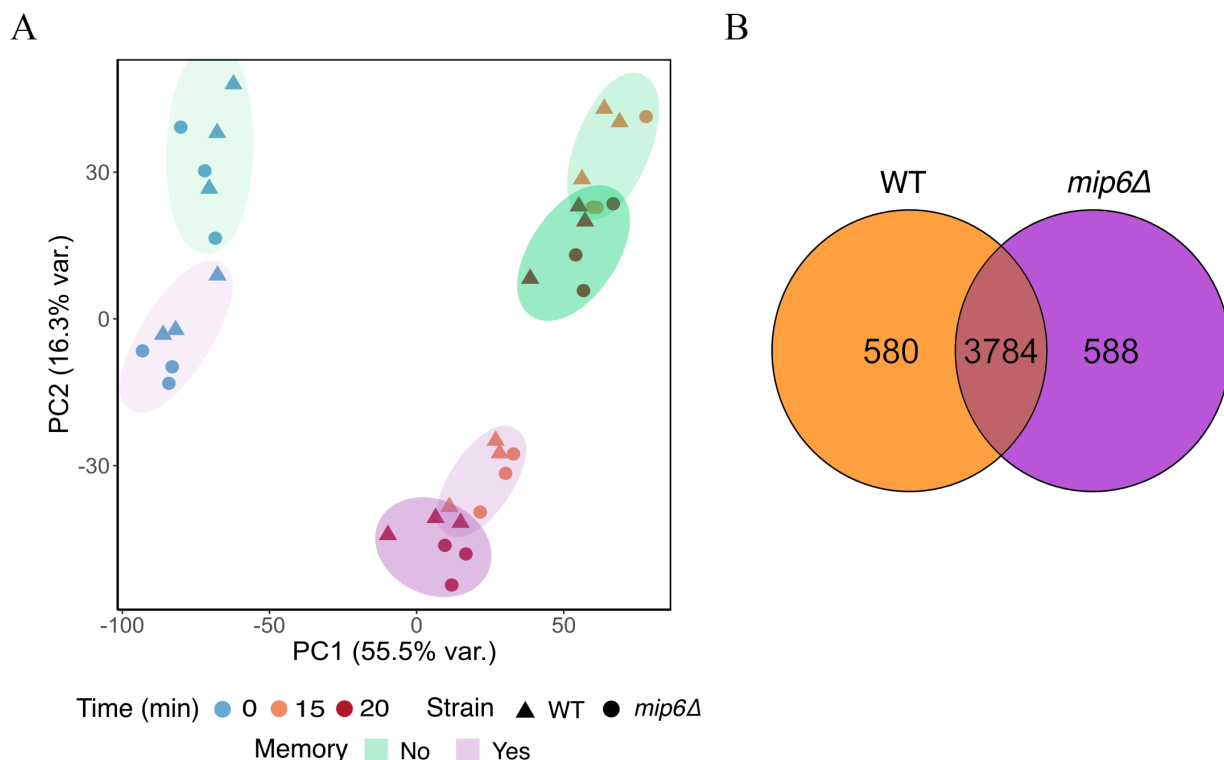


Figure 28. There is a difference in the heat shock stress response in yeast cells when they have already suffered a previous heat shock. (A) PCA score plot from the RNA-Seq analysis of the WT and *mip6Δ* collected at different times during a heat shock treatment (0min, 15min or 20min at 39°C). The samples were previously exposed to a 20min heat shock at 39°C with a 60min recuperation period at 30°C (memory) or were previously grown only under optimal conditions (no memory). Three biological replicates were carried out. (B) Venn diagram of genes with significant differences in mRNA levels comparing a first and a second exposition to heat shock in WT and *mip6Δ* strains.

In order to deeply analyze transcriptional memory in response to heat stress, the dynamic behavior of the differentially expressed genes throughout the heat shock time course and how their expression changes were studied using the maSigPro R package (Conesa et al., 2006). This package facilitates the study of differential expression profiles in time-course experiments. Hence, differentially expressed genes (DEG) in the WT strain were grouped in four different clusters depending on the changes in their expression profile (Figure 29). For each cluster, GO terms related to biological processes are shown (Table 11).

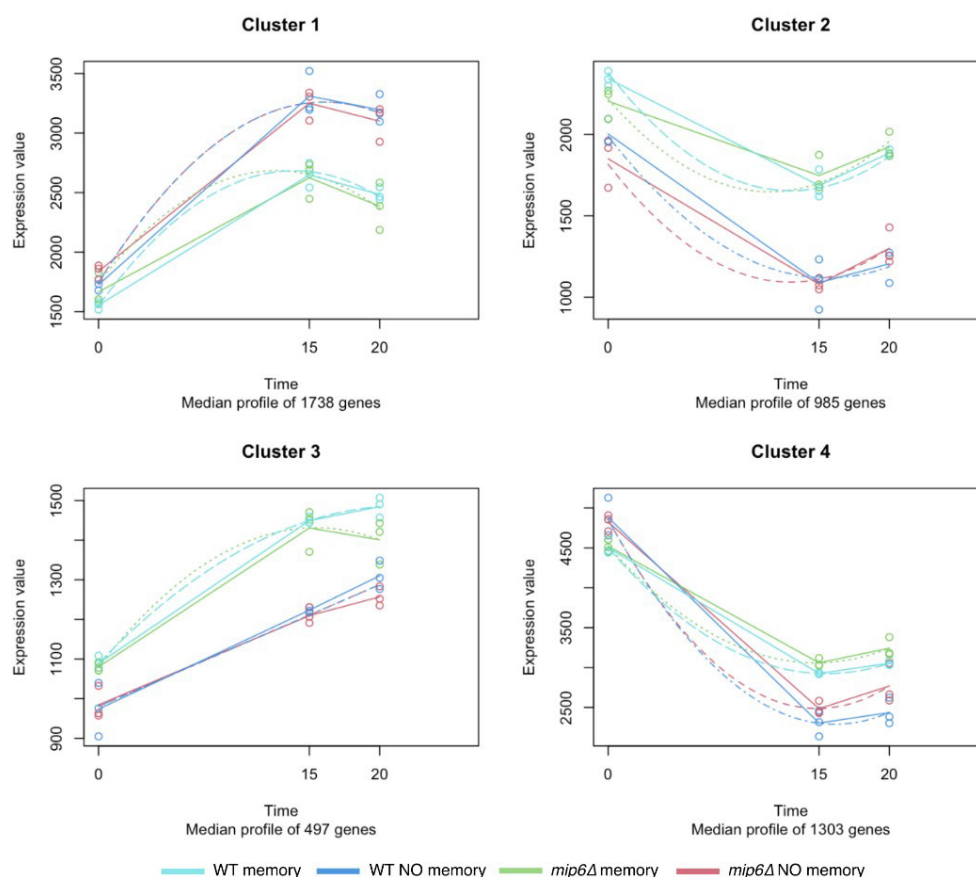


Figure 29. Clustering of the genes that present differential expression between memory and no memory in WT and *mip6Δ* strains. Genes with differential expression between memory (light blue) and “no memory” (dark blue) in WT and between memory (green) and “no memory” (red) in *mip6Δ* were plotted in four different clusters according to their expression profile along the heat shock response. Data represent the mean value of three biological replicates.

Half of the differentially expressed genes are activated upon heat stress in both “memory” and “no memory” samples, being grouped in clusters 1 and 3; while clusters 2 and 4 represent genes whose expression is repressed during the HSR (**Figure 29**). All the genes being clustered in these latter groups show higher repression in the “no memory” samples than in the “memory” ones, either because they already present lower expression levels under optimal conditions (985 genes in cluster 2) or because, even if their optimal mRNA levels are similar in both samples, their reduction upon heat shock softens with memory (1303 genes in cluster 4). Besides, among the GO biological processes overrepresented in these clusters, processes that are inhibited during the HSR can be found, such as translation and amino acid biosynthesis (**Table 11**).

Focusing on the clusters with HS activated genes, they could also be classified into two different groups. On the one hand, the 1738 genes of cluster 1, in which optimal mRNA levels are similar

in both “memory” and “no memory” samples, but activation is less intense when the cells have already suffered from previous heat exposure (Figure 29). This cluster is more functionally heterogeneous, but most of the overrepresented processes are related to obtaining energy, such as catabolic metabolism or respiration. Interestingly, most of the genes that participate in trehalose biosynthesis are grouped in this cluster (Table 11). On the other hand, the 497 genes grouped in cluster 3 show a higher and faster activation when cells are exposed to heat stress a second time (Figure 29). Indeed, what is traditionally known as transcriptional memory is precisely this phenotype: a more significant and quick activation of certain genes in a second response to the same stimuli (Pascual-Ahuir et al., 2020; Sump & Brickner, 2022). Strikingly, the only process overrepresented in this cluster is mitochondrial translation (Table 11). *mip6Δ* mutant presents a similar pattern compared to WT. However, differences in specific sets of genes are observed by heatmap (Tejada-Colon et al., under revision).

GO Biological Process Overrepresented	
Cluster 1	Monosaccharide catabolic process Aerobic electron transport chain Trehalose metabolic process Ubiquitin-dependent protein metabolic process Negative regulation of gluconeogenesis Ketone catabolic process Aldehyde catabolic process Fatty acid catabolic process
Cluster 2	tRNA metabolic process Poly(A)-dependent snoRNA 3'-end processing Ribosomal large subunit assembly tRNA transcription by RNA polymerase III Ribosomal small subunit biogenesis mRNA catabolic process Nucleolar large rRNA transcription by RNA polymerase I
Cluster 3	Mitochondrial translation
Cluster 4	Biogenetic amine biosynthetic process Amino acid metabolic process Protein N-linked glycosylation Ergosterol biosynthetic process Ribonucleoside monophosphate metabolic process

Table 11. Gene Ontology annotation overrepresented in the nine clusters of genes with differential expression between memory and no memory in WT strain.

CHAPTER II: CHARACTERIZATION OF Mip6 EXPORT PATHWAYS FROM THE NUCLEUS TO THE CYTOPLASM



BACKGROUND

One important feature of Mip6 is its location. Mip6 shuttles between the nucleus and the cytoplasm in a Mex67-mediated manner, since the impairment of the Mip6-Mex67 interaction, whether by the deletion of the Mex67 UBA domain or the RRM4 of Mip6, supposes its nuclear retention (Martín-Expósito et al., 2019). However, other alternative export pathways might exist, considering that the nuclear retention observed in both mutants is partial (Martín Expósito, 2019).

One of these alternative mechanisms proposed was the presence of a Nuclear Export Signal (NES) that could be recognized by a karyopherin (Martín Expósito, 2019; Soniat & Chook, 2015). Indeed, the NetNES 1.1 tool, a server able to predict leucine-rich NES (la Cour et al., 2003), shows that a putative NES sequence is located in the RRM4 of Mip6, close to the W₄₄₂, the residue by which the protein interacts with Mex67. The predicted NES sequence (I₄₂₇RFSEIKI₄₃₄) presents an amino acid pattern that corresponds to one of the class 2 hydrophobic isoleucine spacing, an unconventional class of NES sequences (Kosugi et al., 2008; Martín Expósito, 2019). When point mutations are performed in this putative NES sequence and when it is completely deleted, Mip6 partial nuclear retention is observed, which suggests its role in the correct export of the protein (Martín Expósito, 2019). Besides, the absence of karyopherin Msn5, which regulates the transport of other proteins with unconventional NES sequences (Bakhrat et al., 2008; Görner et al., 2002; Quilis & Igual, 2012; Taberner et al., 2012), seems to provoke also a Mip6 nuclear retention at the same level than the point mutations in this sequence. Thus, Msn5 could be the karyopherin that mediates this Mip6 NES-dependent transport (Martín Expósito, 2019).

In this chapter, we deepened the study and characterization of the NES sequence present in the RRM4 of Mip6 as a possible Mex67-independent transport. Furthermore, we studied other possible alternative export mechanisms, such as the presence of a different NES sequence in the C-terminal region of the Mip6 sequence.

Point mutations in the putative NES in the RRM4 of Mip6 affect the Mip6-Mex67 interaction

To study whether Mex67 accessibility to the W₄₄₂ residue is affected when the NES sequence is mutated, an immunoprecipitation assay was carried out using plasmids that express different versions of Mip6-GFP expressing point mutations (both in the NES sequence and the W₄₄₂) in *mip6Δ* strain. The experiment was performed under optimal conditions and following a heat shock at 39°C for 20min.

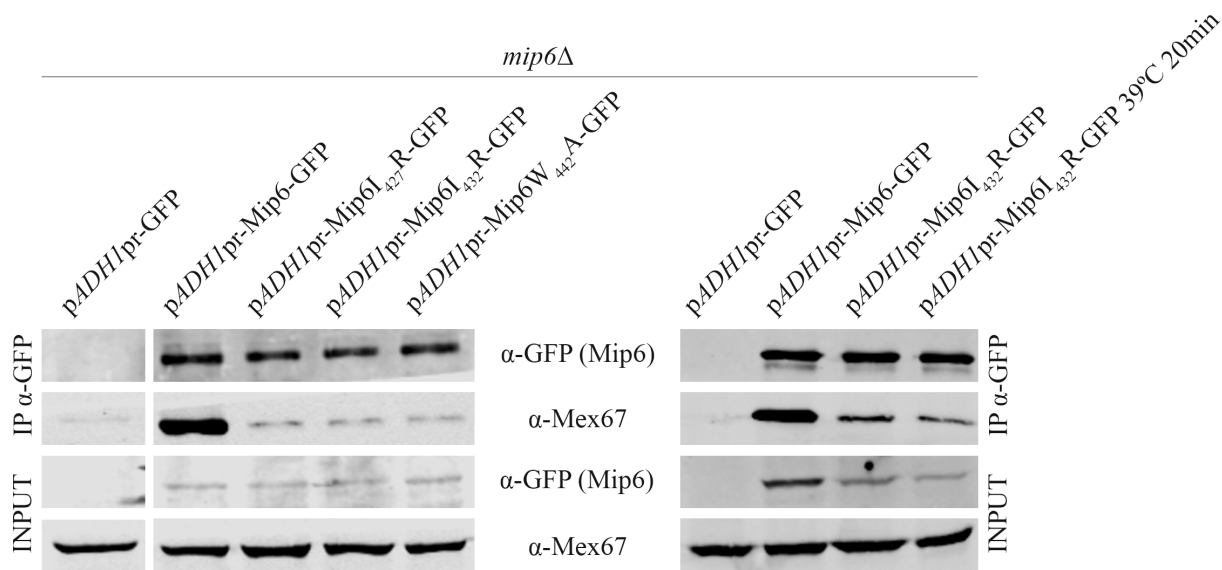


Figure 30. Point mutations in the putative NES in the RRM4 of Mip6 affect the Mip6-Mex67 interaction. Immunoprecipitation of different versions of Mip6-GFP with point mutations expressed from plasmids in *mip6Δ* strain under optimal conditions or following a heat shock at 39°C for 20min. An empty plasmid was used as a negative control. Mip6-GFP and Mex67 were detected by Western blotting using the indicated antibodies.

Point mutations in the putative NES sequence (both Mip6I₄₂₇R and Mip6I₄₃₂R) provoke a reduction of the interaction between Mip6 and Mex67 at the same level as the Mip6W₄₄₂A mutation under optimal and heat shock stress conditions (**Figure 30**). Therefore, the Mip6 nuclear retention in these mutants could be due, at least partially, to the impairment of Mip6-Mex67 interaction. This result is not incompatible with the NES sequence being a Mip6 export pathway, since the observed nuclear retention could be due to defects in several transport mechanisms.

Mip6 does not physically interact with karyopherin Msn5

It was shown that Mip6-GFP is partially nuclear in the *msn5Δ* mutant and the Mip6-Mex67 interaction is not affected (Martín Expósito, 2019). Msn5 participates in the import and the

export of multiple proteins through different NLS and NES sequences (Hu et al., 2016; Quilis & Igual, 2012; Sugiyama et al., 2014; Taberner et al., 2012), including the HSR transcription factors Msn2 and Msn4 (Görner et al., 2002; Sunnåker et al., 2013). Taking all this together, a coimmunoprecipitation assay was performed expressing Mip6-GFP through a plasmid in Msn5-MYC *mip6Δ* strain to analyze their physical interaction. In our hands, Mip6 does not copurify with karyopherin Msn5 (**Figure 31**), suggesting that Mip6 may not be transported in a Msn5-mediated manner.

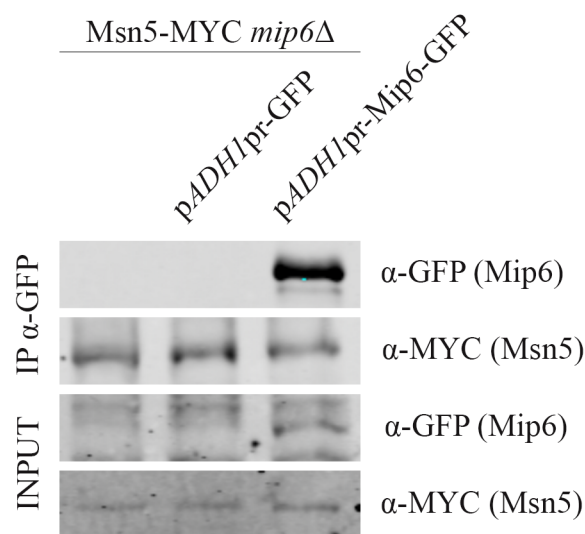


Figure 31. Mip6 does not physically interact with karyopherin Msn5. Immunoprecipitation of Mip6-GFP expressed from a plasmid in Msn5-MYC *mip6Δ* strain. An empty plasmid and a strain without a plasmid were used as negative controls. Mip6-GFP and Msn5-MYC were detected by Western blotting using the indicated antibodies.

Point mutations in the NES_{RRM4} lead to Mip6 nuclear retention independent of the W₄₄₂A mutation under optimal conditions

To study how mutants that combine NES sequence mutations with the impairment of the Mip6-Mex67 binding (Mip6W₄₄₂A), double mutants (Mip6W₄₄₂A;I₄₂₇R and Mip6W₄₄₂A;I₄₃₂R) were created. First, the Mip6 protein stability of the mutants was checked by immunoblotting.

As shown in **Figure 32**, the mutations do not seem to alter much Mip6 stability under optimal conditions, since protein levels in each mutant are similar to the WT strain. Then, the location of the different Mip6-GFP versions expressed from plasmids in *mip6Δ* strain was studied and the ratio of Mip6 presence in both the nucleus and the cytoplasm was analyzed.

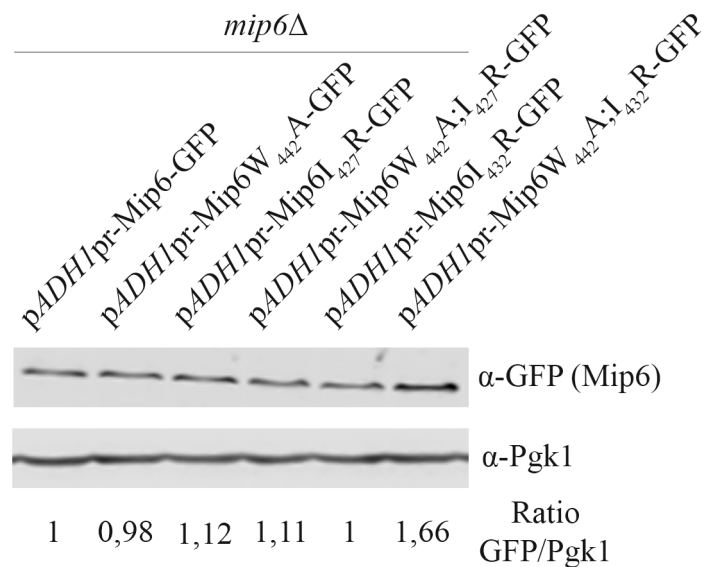


Figure 32. Combining the point mutations in the putative NES sequence in the Mip6 RRM4 domain and the Mip6W₄₄₂A mutation does not affect protein stability when growing under optimal conditions. Immunoblotting for the detection of different versions of Mip6-GFP with point mutations expressed from plasmids in *mip6Δ* strain using the indicated antibodies. Pgk1 was also detected using the indicated antibody as a load control. For the quantification, the Fiji software was used; the ratio GFP/Pgk1 is shown and the normalization was performed by assigning a value of 1 to the WT version of Mip6.

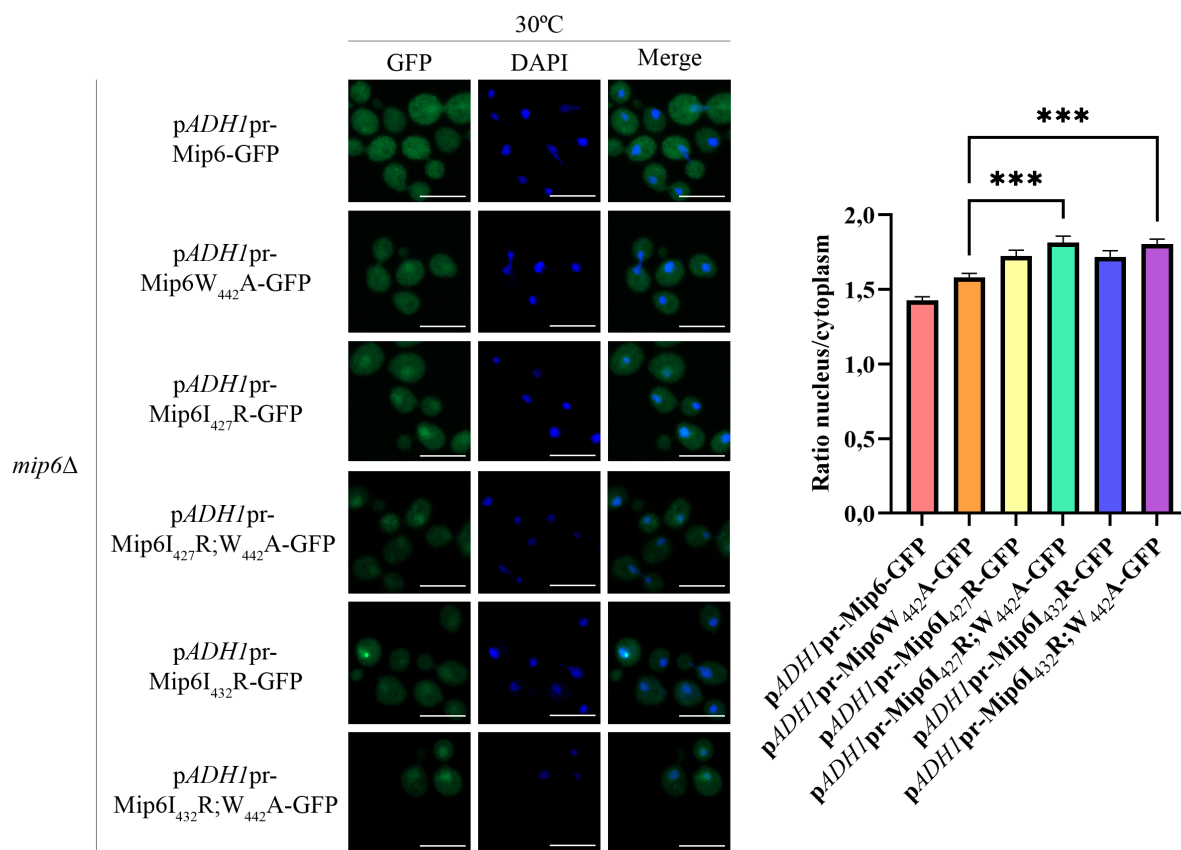


Figure 33. Point mutations in the NES_{RRM4} lead to nuclear retention independent of Mip6W₄₄₂A mutation under optimal conditions. Confocal images displaying different versions of Mip6-GFP with point mutations expressed from a plasmid in *mip6Δ* strain under optimal conditions. Nuclei were stained with DAPI. Scale bar: 5μm (left). Bar graphs representing the ratio of GFP intensity between the nucleus and the cytoplasm. Data represent the mean and the standard error (SE) of at least 60 cells coming from three biological replicates. *** p value < 0,005 (right).

The combination of the Mip6W₄₄₂A mutation and point mutations in the NES sequence supposes a higher Mip6 nuclear retention than the one observed in the Mip6W₄₄₂A single mutant (**Figure 33**). This would indicate that point mutations in the Mip6 NES sequence lead to nuclear retention of the proteins independent of the Mip6-Mex67 interaction, which suggests this sequence is a functional Mip6 export signal.

Point mutations in the NES_{RRM4} do not lead to nuclear retention under heat shock

Since Mip6 accumulates in stress granules upon HS (Martín-Expósito et al., 2019), it was analyzed whether the mutations of both the Mip6 W₄₄₂ residue and the NES sequence affected Mip6 location under heat shock. Except for the Mip6I₄₃₂R mutant, in which a slight nuclear retention is observed, the Mip6W₄₄₂A and Mip6ΔNES_{RRM4} mutants were not affected (Martín Expósito, 2019). Here, we extended this analysis and studied the localization of the new double mutants under heat shock conditions. First, an immunoblot of the different mutants after a heat shock at 39°C for 20min was performed in order to check protein stability.

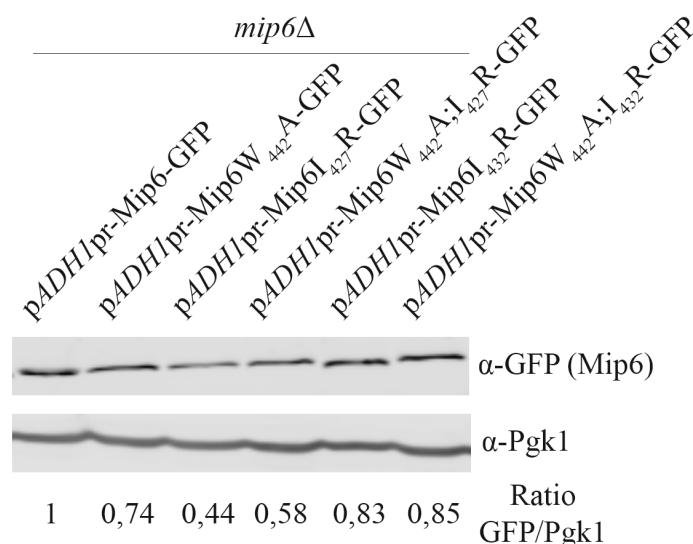


Figure 34. The Mip6I₄₂₇R mutation supposes a decrease in the protein levels until half the amount of the WT when growing under heat shock conditions. Immunoblotting for the detection of different versions of Mip6-GFP with point mutations expressed from plasmids in *mip6Δ* strain after a heat shock at 39°C for 20min using the indicated antibodies. Pgk1 was also detected using the indicated antibody as a load control. For the quantification, the Fiji software was used, the ratio GFP/Pgk1 is shown and the normalization was performed by assigning a value of 1 to the WT version of Mip6.

Under the heat stress conditions studied, Mip6 remains detectable, although the Mip6I₄₂₇R mutation supposes a decrease in protein levels compared to the WT (**Figure 34**). However, more

replicates of the experiment would be necessary to confirm the statistical significance of this decrease. Besides, during HSR, there is no difference according to Mip6 location in any of the mutants, unlike what happens under optimal conditions (**Figure 35**).

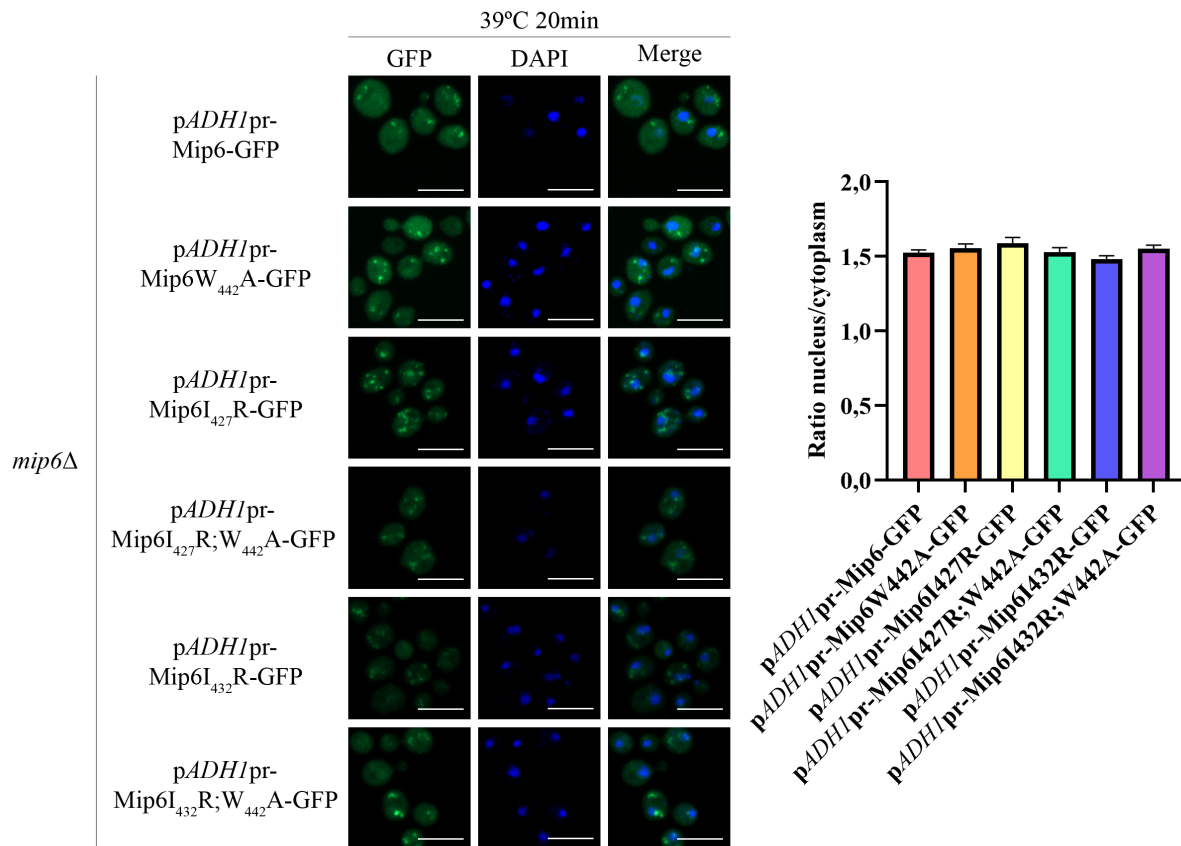


Figure 35. Point mutations in the NES_{RRM4} do not lead to nuclear retention under heat shock. Confocal images displaying different versions of Mip6-GFP with point mutations expressed from a plasmid in *mip6Δ* strain after a heat shock at 39°C for 20min. Nuclei were stained with DAPI. Scale bar: 5μm (left). Bar graphs representing the ratio of GFP intensity between the nucleus and the cytoplasm. Data represent the mean and the standard error (SE) of at least 60 cells coming from three biological replicates (right).

Nuclear retention caused by point mutations in the NES_{RRM4} is independent of the Mip6W₄₄₂A mutation under oxidative stress

Most of the studies carried out in our laboratory to study Mip6 location have been performed either under optimal growth conditions or under heat stress (Martín Expósito, 2019; Martín-Expósito et al., 2019; Nuño Cabanes, 2024; Nuño-Cabanes et al., 2020). However, recent experiments led to believe that Mip6 implication in stress response could also be important during oxidative stress, since there is a strong genetic interaction between *MIP6* and *MSN2/4* during H₂O₂ oxidative stress (OxS; **Figure 17**). Thus, the location of Mip6 and the different Mip6 mutants was studied

upon OxS. As a control to check protein stability, an immunoblot of the different mutants after an oxidative shock with 3mM H₂O₂ for 15min was performed.

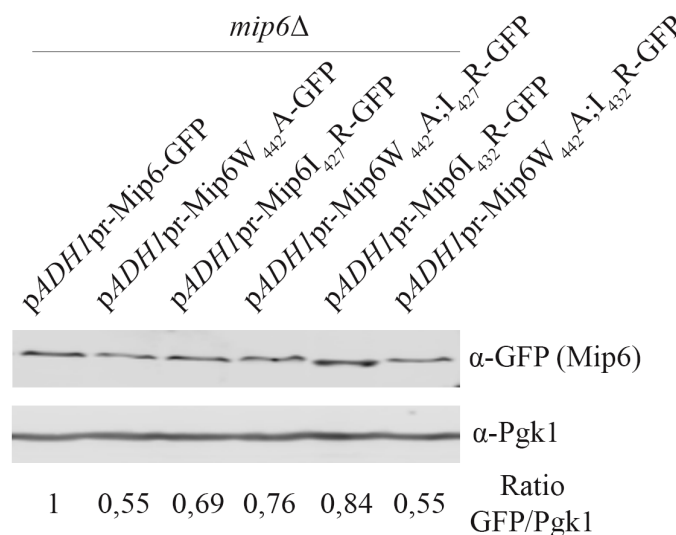


Figure 36. Some point mutations in Mip6 RRM4 lead to a decrease in protein levels when growing under oxidative stress conditions. Immunoblotting for the detection of different versions of Mip6-GFP with point mutations expressed from plasmids in *mip6Δ* strain after an oxidative shock with 3mM for 15min using the indicated antibodies. Pgk1 was also detected using the indicated antibody as a load control. For the quantification, the Fiji software was used, the ratio GFP/Pgk1 is shown and the normalization was performed by assigning a value of 1 to the WT version of Mip6.

In contrast to HS, most of the studied mutations seem to affect protein stability when the cells grow under H₂O₂ oxidative stress conditions, since protein levels decline in all the mutants studied (**Figure 36**). Nevertheless, the experiment should be repeated in order to obtain more biological replicates and confirm the statistical significance of the result.

Regarding localization, under H₂O₂-induced oxidative stress conditions, Mip6 and the mutants are localized similarly to optimal conditions. Indeed, both the point mutations in the NES sequence and the combinations of these mutations with the Mip6W₄₄₂A one lead to a stronger nuclear retention when compared to the Mip6W₄₄₂A single mutant (**Figure 37**). This result suggests that the NES sequence would be a Mip6 export signal also under OxSR. Besides, no Mip6 accumulation in cytoplasmic granules is observed (**Figure 37**). Even if stress granules are formed in a great variety of stress conditions, such as sodium azide-induce oxidative stress, the foci observed in the cell cytoplasm during H₂O₂-induced oxidative stress correspond to p-bodies (Buchan et al., 2008, 2011; Wallace et al., 2015). Thus, Mip6 does not seem to be accumulated in p-bodies under oxidative stress conditions.

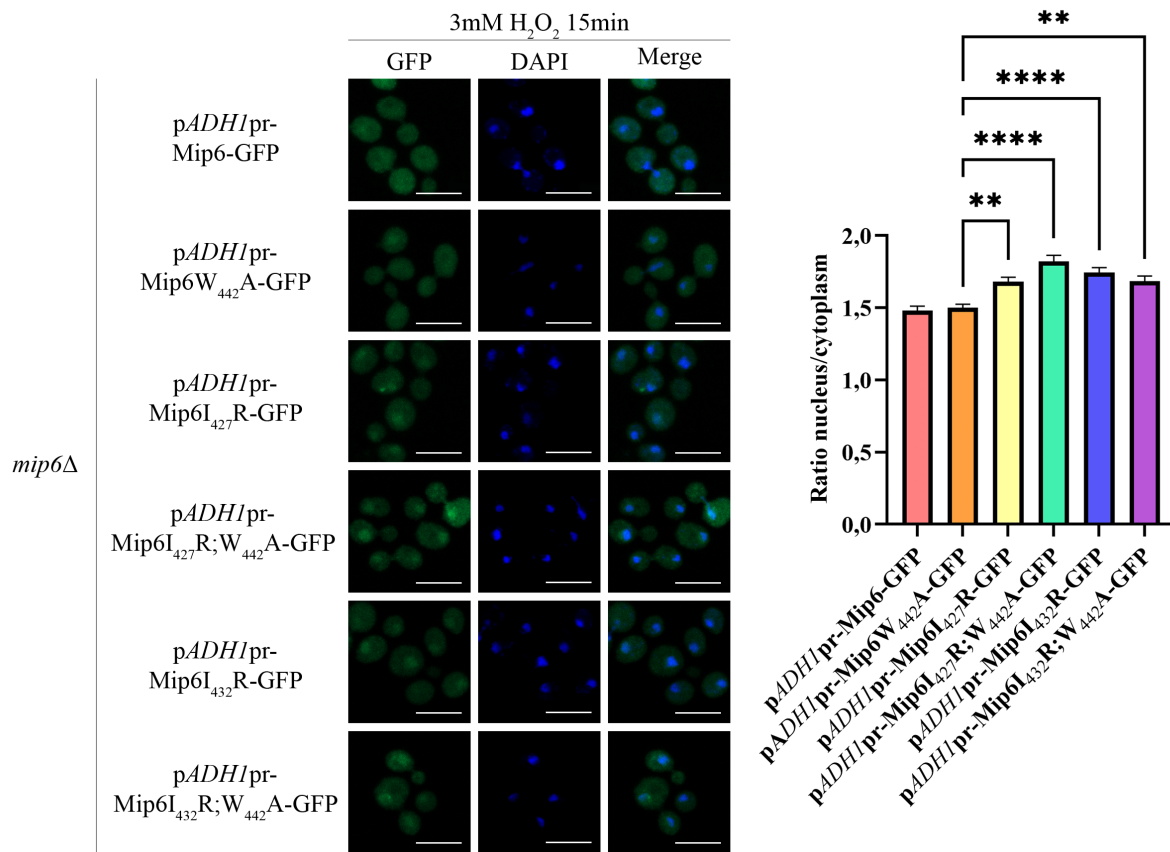


Figure 37. Nuclear retention caused by point mutations in the NES_{RRM4} is independent of the Mip6W₄₄₂A mutation under oxidative stress. Confocal images displaying different versions of Mip6-GFP with point mutations expressed from a plasmid in *mip6Δ* strain after an oxidative shock with 3mM H₂O₂ for 15min. Nuclei were stained with DAPI. Scale bar: 5μm (left). Bar graphs representing the ratio of GFP intensity between the nucleus and the cytoplasm. Data represent the mean and the standard error (SE) of at least 60 cells coming from three biological replicates. ** p value < 0,01; **** p value < 0,001 (right).

Mip6 NES sequence I₄₂₇RFSEIKI₄₃₄ can partially export the SV40 NLS *in vivo*

The NES sequence in the Mip6 RRM4 region functions as an export signal for the protein independent of the Mex67-dependent transport, since mutations that alter both mechanisms lead to a higher Mip6 nuclear accumulation than the single ones (Figure 33 and Figure 37). However, in order to finally confirm the ability of this sequence to be a NES by itself, a GFP construct that expresses a strong nuclear localization signal (NLS) present in the SV40 virus T antigen (TAg) was used (Koch et al., 2012). Controls of protein levels of the different constructs upon different growth conditions were checked by immunoblotting in order to study the stability of the fusion protein.

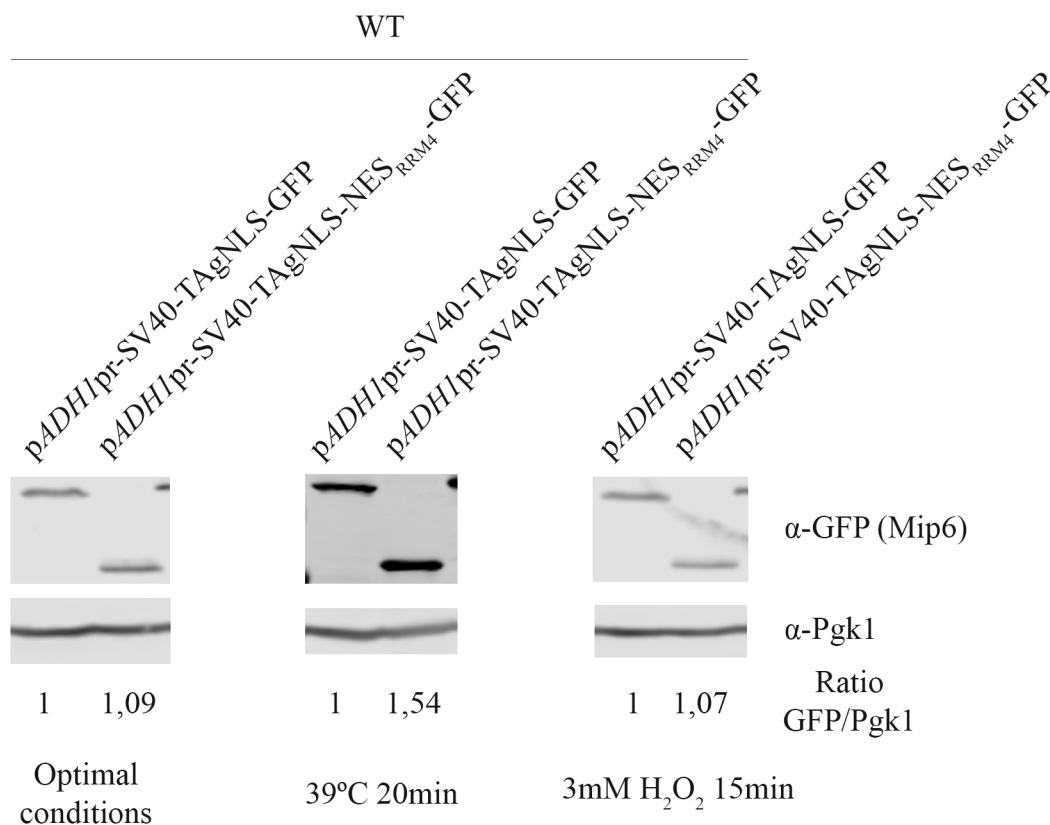


Figure 38. The cloning of the NES sequence in the Mip6 RRM4 into a construct that expresses the NLS present in the SV40 Tag does not compromise the stability of the expressed product. Immunoblotting for the detection of SV40-TAgNLS-GFP and SV40-TAgNLS-NES_{RRM4}-GFP expressed from plasmids in WT strain using the indicated antibodies. The experiment was performed under optimal conditions, after a heat shock at 39°C for 20min and after an oxidative shock with 3mM H₂O₂ for 15min. Pgk1 was also detected using the indicated antibody as a load control. For the quantification, the Fiji software was used, the ratio GFP/Pgk1 is shown and the normalization was performed by assigning a value of 1 to the original construct.

The SV40-TAgNLS-GFP expression is not affected by the incorporation of the cloned Mip6 NES sequence. However, the fusion protein has a lower molecular weight than the one expected (**Figure 38**). The construct used is tagged with three molecules of GFP, hence the different sizes observed for the fusion protein could be due to a non-sense mutation originated when the NES sequence was cloned, leading to the expression of only two of the three GFP molecules. In any case, the NES sequence was properly cloned and checked by Sanger sequencing.

Locations of both SV40-TAgNLS-GFP and SV40-TAgNLS-NES_{RRM4}-GFP were observed by confocal microscopy. The experiments were performed under optimal growth conditions and both heat shock stress and H₂O₂-induced oxidative stress. As shown in **Figure 39**, in all cases the SV40-TAgNLS-NES_{RRM4}-GFP is partially exported compared to the SV40-TAgNLS-GFP.

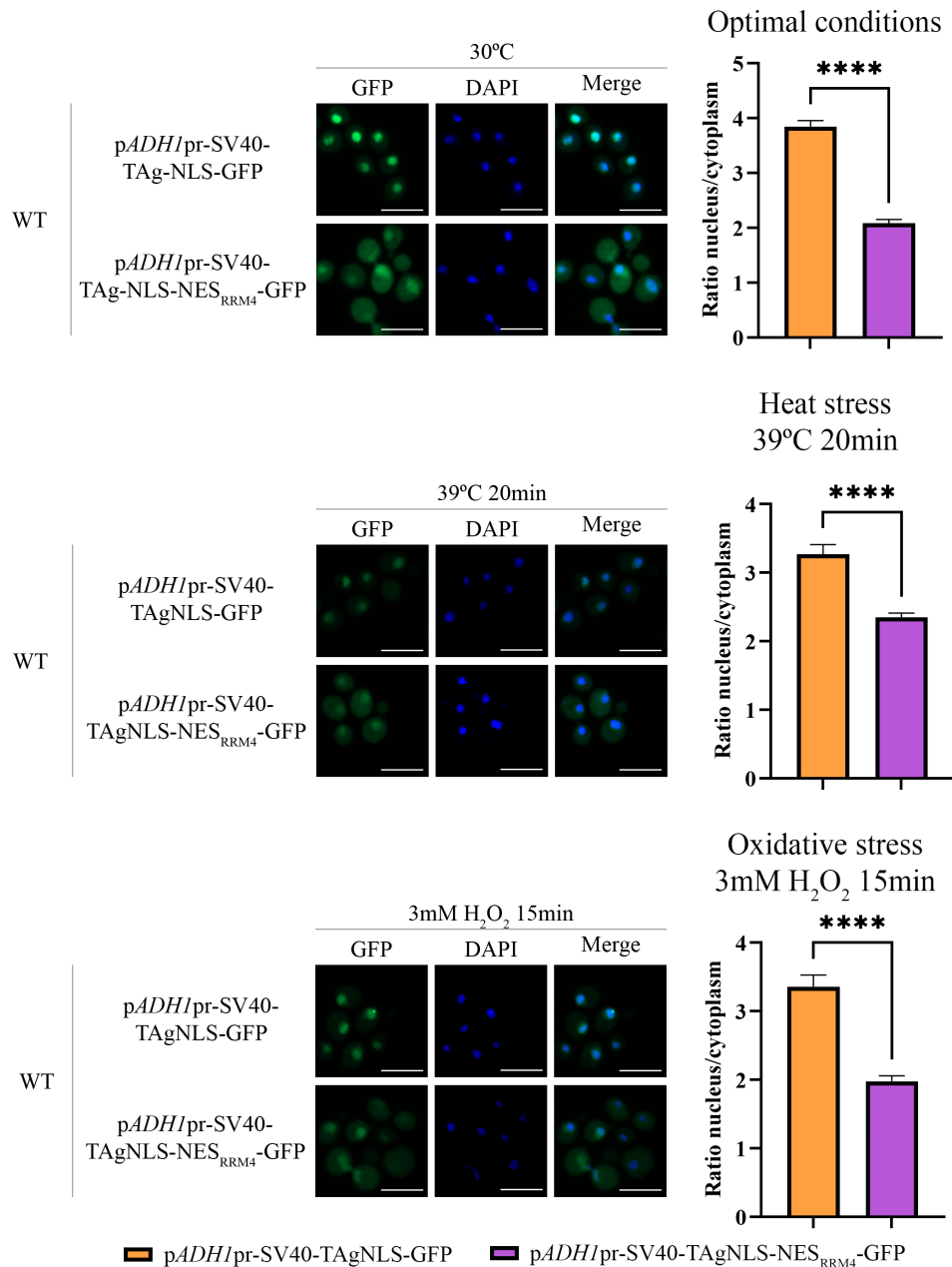


Figure 39. The NES in the RRM4 of Mip6 is able to export a construct with an NLS from the nucleus to the cytoplasm. Confocal images displaying the NLS from the antigen T of the SV40 virus and the same NLS together with the NES_{RRM4} of Mip6, both tagged with GFP, expressed from a plasmid in the WT strain. The experiment was performed under optimal conditions, after a heat shock at 39°C for 20min and after an oxidative shock with 3mM H₂O₂ for 15min. Nuclei were stained with DAPI. Scale bar: 5µm (left). Bar graphs representing the ratio of GFP intensity between the nucleus and the cytoplasm. Data represent the mean and the standard error (SE) of at least 60 cells coming from three biological replicates. **** p value < 0,001 (right).

Study of the putative NES sequence present in Pes4, Mip6 homologous protein

Mip6 has a paralogous protein, the RNA binding protein Pes4, which shows a similar domain organization. Four RRM4s are positioned in Pes4, resembling Mip6 organization. However, Pes4 function seems to be more relevant for yeast sporulation than in coping with heat stress (Gavade et al., 2022; Jin et al., 2017). Pes4 localizes in the prospore membrane during sporulation (Jin

et al., 2017), but its location during mitotic cell growth remains unknown. Interestingly, using the EMBOSS Needle sequence alignment software (Madeira et al., 2024), a putative NES sequence homologous to the one in Mip6 was discovered in the Pes4 RRM4 (**Figure 40**). Additionally, instead of the Mip6 tryptophan that interacts with Mex67, Pes4 presents a serine (S₄₃₂) residue (**Figure 40**), which suggests that Pes4 does not physically interact with Mex67.

```

MIP6_YEAST      404 VYLKNSLFNCNKEFISQLCLQEKIRFSEIKITNYNSLNWTFCGHVECFSR      453
                  ::|||...|||:..||.||.||...:|...|...:|...|...:
PES4_YEAST      395 LFLENLSAVCNKEFLKYLCHQENIRPFKIQIDGYDENSSTYSGFIKFRNF      444

```

Figure 40. Pes4, a Mip6 paralogous protein, also presents a putative NES in its RRM4. Pairwise sequence alignment of a part of the RRM4 of both Mip6 and Pes4 by EMBOSS Needle software. | fully conserved residue; : conservation between groups of strongly similar properties; . conservation between groups of weakly similar properties. Putative NES sequences are shown in purple and the W₄₄₂ residue of Mip6 and its homologous residue in Pes4 are shown in orange.

In order to check whether Pes4 is a shuttling protein or not in mitotic cells, GFP-Pes4 location was studied under both optimal and heat shock conditions. Pes4 is located in both the nucleus and the cytoplasm of yeast cells (**Figure 41**), like its paralogous protein Mip6. Interestingly, accumulation in cytoplasmic granules was observed not only after a heat shock, but also under optimal conditions (**Figure 41**), while Mip6 distribution in stress granules was solely described in different stress conditions (Martín-Expósito et al., 2019).

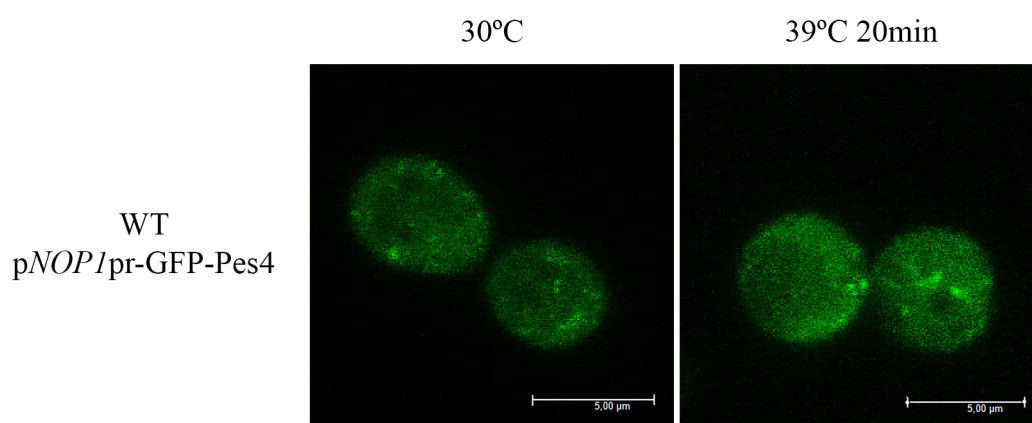


Figure 41. Pes4 shuttles between the nucleus and the cytoplasm. Confocal images displaying GFP-Pes4 expressed from a plasmid in *pes4Δ* strain under optimal conditions or following a heat shock at 39°C for 20min. Scale bar: 5μm.

To confirm that Pes4 does not interact with Mex67, a pull-down assay was carried out expressing GFP-Pes4 or Pes4 NES point mutants (**Figure 30**). The experiment was performed under both optimal conditions or following a heat shock at 39°C for 20min. Indeed, no interaction between

Pes4 and Mex67 was observed either in the WT version or the NES mutants (**Figure 42**). Thus, only Mip6 binds to Mex67 upon the conditions tested in our study. However, we observed that in Pes4 mutants Pes4I₄₁₈R and Pes4ΔI₄₂₃, protein levels decreased when compared to WT (**Figure 42**). In fact, NES mutants affect protein levels, being the mutation in the I₄₂₃ residue the one showing the most drastic effect (**Figure 42, INPUT** and **Figure 43**).

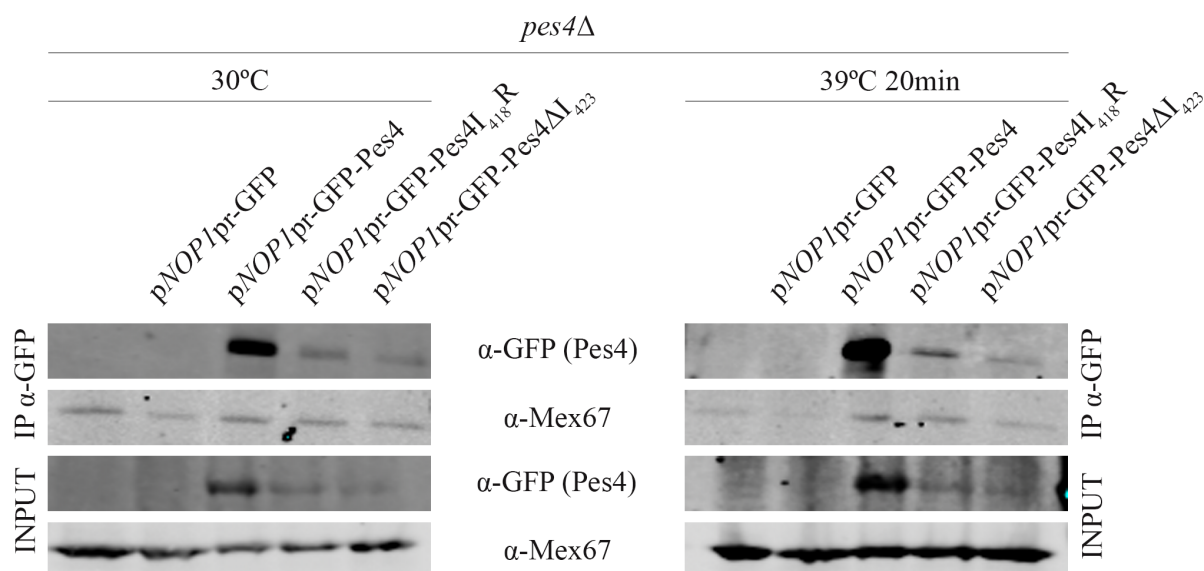


Figure 42. Pes4 does not bind to the export factor Mex67. Immunoprecipitation of different versions of GFP-Pes4 with point mutations expressed from plasmids in *pes4Δ* strain under optimal conditions or following a heat shock at 39°C for 20min. An empty plasmid and a strain without a plasmid were used as negative controls. GFP-Pes4 and Mex67 were detected by Western blotting using the indicated antibodies.

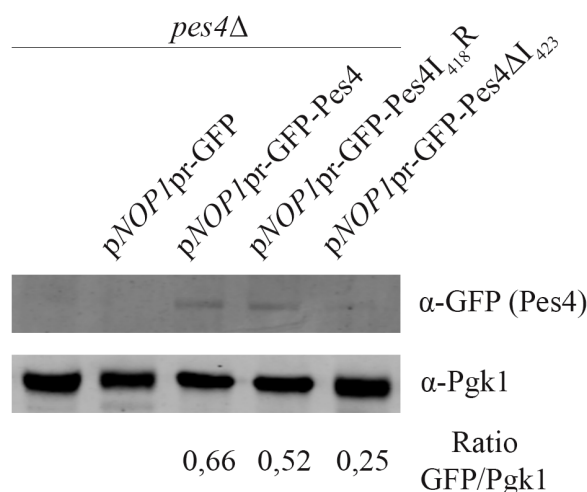


Figure 43. Point mutations in the putative NES sequence in the RRM4 of Pes4 affect protein stability. Immunoblotting for the detection of different versions of GFP-Pes4 with point mutations expressed from plasmids in *pes4Δ* strain using the indicated antibodies. Pgk1 was also detected using the indicated antibody as a load control. For the quantification, the Fiji software was used and the ratio GFP/Pgk1 is shown.

To finally assess whether the location of GFP-Pes4I₄₁₈R and GFP-Pes4ΔI₄₂₃ mutants is different from the WT Pes4, Pes4 localization was studied under optimal and heat shock conditions. Under



optimal conditions, no difference in Pes4 location was observed in the mutants in relation to the WT version of the protein (**Figure 44**), except for a decrease in the intensity of the signal, fitting with its effect in Pes4 protein stability (**Figure 43**). In contrast, after a 20min heat shock, Pes4 accumulates in cytoplasmic granules which is more evident in Pes4_{I₄₁₈}R and Pes4 Δ I₄₂₃ mutants (**Figure 44**). These foci do not seem to be stress granules, since they are also present under optimal conditions. Further work is required to understand the nature of these foci and if, either directly or indirectly, the residues mutated affect localization beyond its stability.

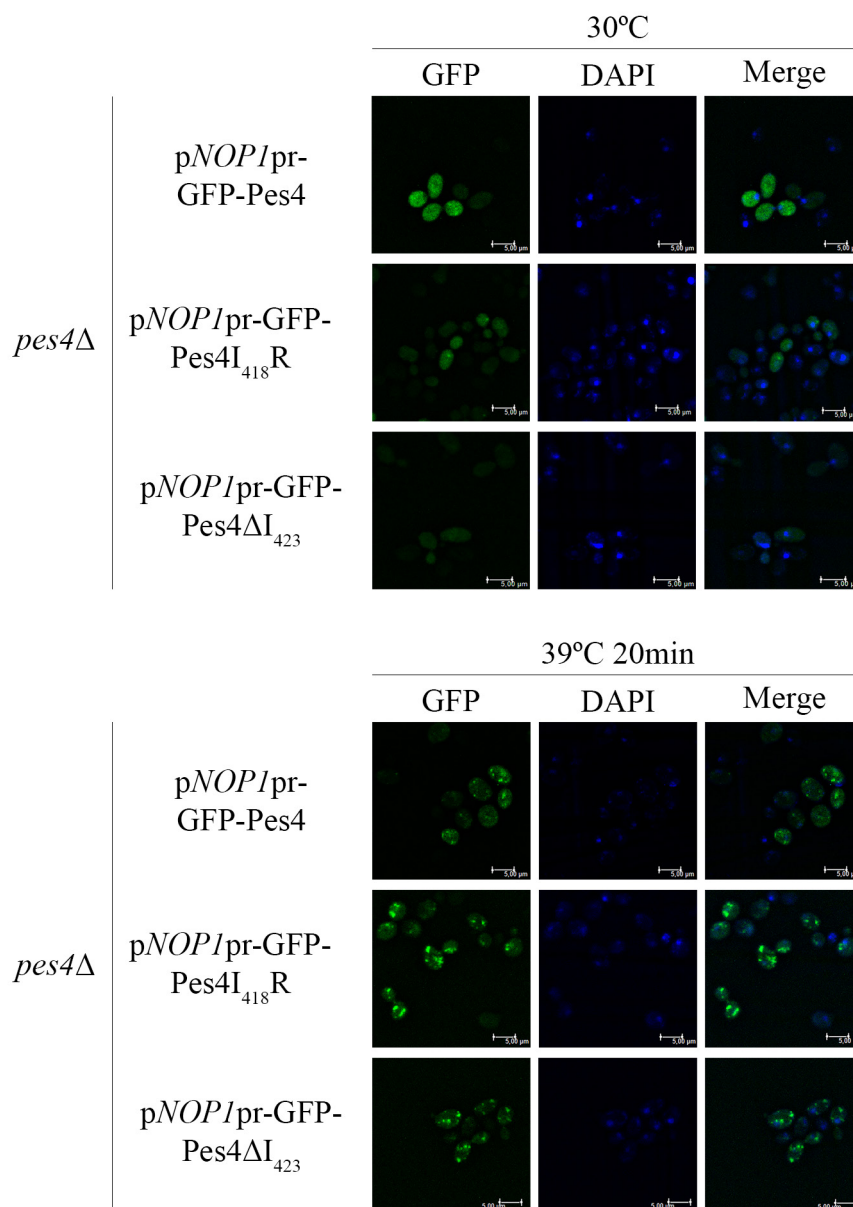


Figure 44. Point mutations in the putative NES of Pes4 do not lead to Pes4 nuclear retention. Confocal images displaying different versions of Pes4-GFP with point mutations expressed from a plasmid in *pes4* Δ strain under optimal conditions or following a heat shock at 39°C for 20min. Nuclei were stained with DAPI. Scale bar: 5 μ m.

In addition to the differences already described between Mip6 and Pes4 RRM4, the differential positions of specific cysteine residues got our attention. As shown in **Figure 45**, four cysteine residues were discovered flanking the NES sequence and the Mex67 binding site (Mip6W₄₄₂). Two of them were upstream of the NES and the other two downstream, whereas Pes4 lacks

MIP6_YEAST 404 VYLKNSLFCNKEFISQLCLQEKIRFSEIKITNYNSLNWTFCGHVECFSR 453
::|:|||.|||||:.||.|.||.:|:|.:.:..:|.|.:.:..
PES4_YEAST 395 LFLENLSAVCNKEFLKYLCHQENIRPFKIQIDGYDENSSTYSGFIKFRNF 444

Figure 45. There are four Cys residues that surround the NES in the RRM4 of Mip6. (A) Mip6 predicted structure by PyMOL software. I₄₂₇ and I₄₃₂ residues of the NES sequence are shown in pink, the W₄₄₂ residue is shown in yellow, the residues C₄₁₃ and C₄₂₂, the two cysteines residues upstream the NES sequence, are shown in blue and the residues C₄₄₅ and C₄₅₀, the two cysteines residues downstream the NES sequence, are shown in red. (B) Pairwise sequence alignment of a part of the RRM4 of both Mip6 and Pes4 by EMBOSS Needle software. | fully conserved residue; : conservation between groups of strongly similar properties; . conservation between groups of weakly similar properties. Putative NES sequences are shown in purple, the W₄₄₂ residue of Mip6 and its homologous residue in Pes4 are shown in orange and the four Cys residues and its homologous counterpart in the Pes4 sequence are shown in blue.

the two more downstream Cys in its RRM4. We hypothesized that the two Mip6 Cys residues downstream of its NES signal (absent in Pes4) might have a regulatory function in contrast to Pes4. Interestingly, during the HSR in aerobic conditions, mitochondrial respiration is altered, leading to an imbalance in the ROS equilibrium (J. F. Davidson & Schiestl, 2001). The cell is able to sense this kind of stress by the formation of disulfide bridges between Cys residues of different proteins that takes part in the OxSR (Boisnard et al., 2009; Coleman et al., 1999; Delaunay et al., 2002; Y. Wang et al., 2012). Thus, it is possible that, under heat stress conditions, these Cys residues in Mip6 may create disulfide bonds, either between them or with Cys residues of another sensing protein, which could suppose the NES sequence to be “protected” and hence, less accessible.

In order to study whether these residues are important for the Mip6 NES-dependent export pathway, the cysteine-450 (C₄₅₀) residue, one of the two that are not present in Pes4, was mutated to generate the Mip6C₄₅₀A mutant. Then, Mip6C₄₅₀A protein stability and location were analyzed. Both experiments were performed under optimal growth conditions, after a heat shock at 39°C for 20min and after an oxidative shock with 3mM H₂O₂ for 15min. As shown in **Figure 46**, the new Mip6C₄₅₀A mutation does not affect Mip6 levels under any of the tested conditions.

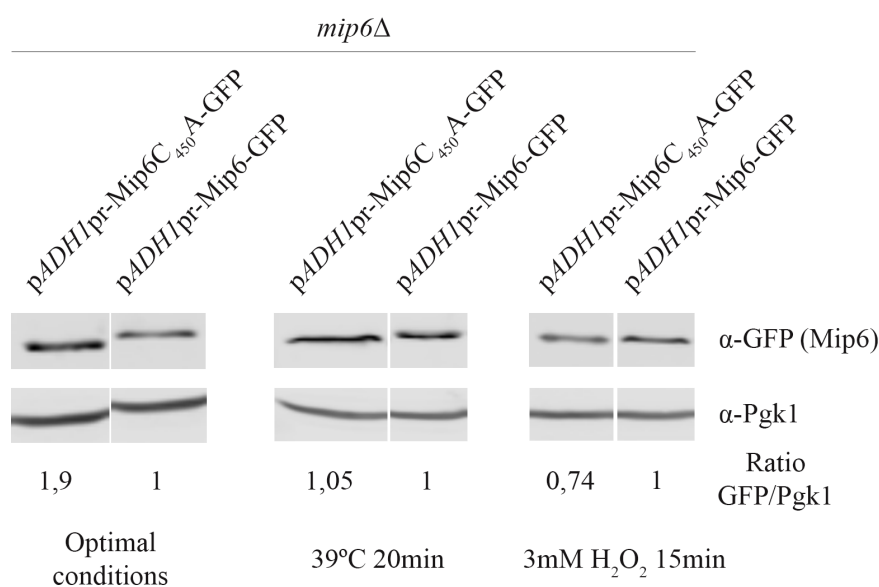


Figure 46. The Mip6C₄₅₀A mutation does not seem to affect protein stability. Immunoblotting for the detection of Mip6-GFP and Mip6C₄₅₀A-GFP expressed from plasmids in *mip6Δ* strain using the indicated antibodies. The experiment was performed under optimal conditions, after a heat shock at 39°C for 20min and after an oxidative shock with 3mM H₂O₂ for 15min. Pgk1 was also detected using the indicated antibody as a load control. For the quantification, the Fiji software was used, the ratio GFP/Pgk1 is shown and the normalization was performed by assigning a value of 1 to the WT version of Mip6.

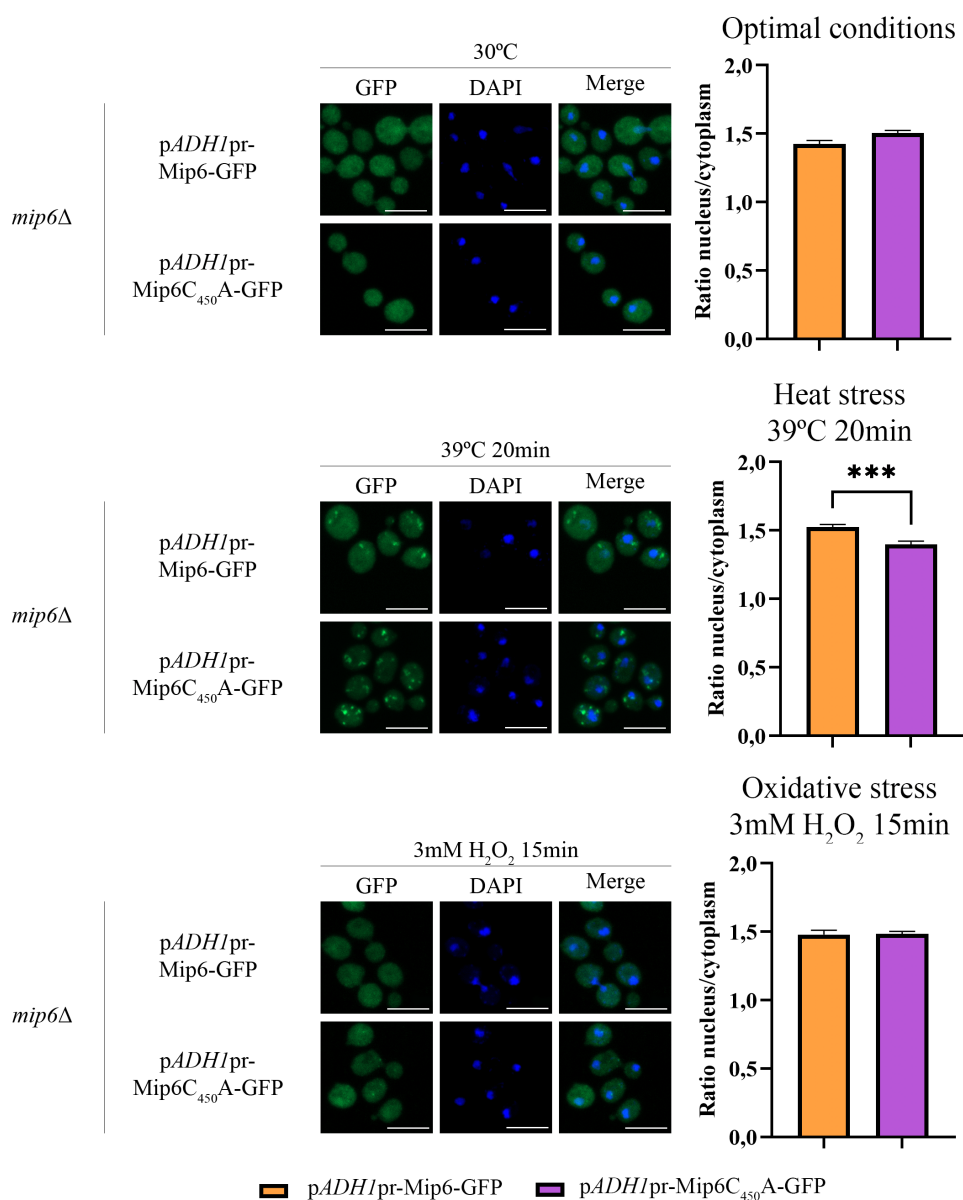


Figure 47. The Mip6C₄₅₀A mutation does not lead to nuclear retention. Confocal images displaying Mip6-GFP and Mip6C₄₅₀A-GFP expressed from a plasmid in *mip6Δ* strain. The experiment was performed under optimal conditions, after a heat shock at 39°C for 20min and after an oxidative shock with 3mM H₂O₂ for 15min. Nuclei were stained with DAPI. Scale bar: 5μm (left). Bar graphs representing the ratio of GFP intensity between the nucleus and the cytoplasm. Data represent the mean and the standard error (SE) of at least 60 cells coming from three biological replicates. *** p value < 0,005 (right).

When studying the Mip6C₄₅₀A location upon HS, a significant decrease in the nucleus/cytoplasm ratio was observed (Figure 47). This result might indicate that the C₄₅₀ contributes to Mip6 transport. HS leads to Mip6 accumulation in stress granules in the cytoplasm (Martín-Expósito et al., 2019); hence, the Cys residue mutated could alter Mip6 presence in these foci, leading to either the formation of a higher number of granules or a higher accumulation of Mip6 into them without altering the SGs number. To shed light on this, the number of granules in which Mip6 is

present per cell was counted in both Mip6-GFP and Mip6C₄₅₀A under HS. There is a tendency in the mutant to present more granules per cell (**Figure 48**). However, no statistically significant difference was observed between both strains.

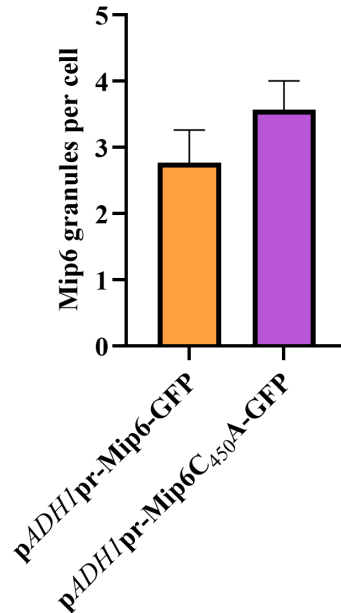


Figure 48. The Mip6C₄₅₀A mutation does not suppose Mip6 accumulation in a higher number of granules. Bar graphs representing the number of Mip6 granules per cell in *mip6Δ* strain with plasmids expressing Mip6-GFP and Mip6C₄₅₀A-GFP after a heat shock at 39°C for 20min. Data represent the mean and the standard error (SE) of at least 80 cells coming from two biological replicates.

A second putative NES in the Mip6 C-terminal domain is activated by oxidative stress

By using a different tool designed for predicting classical NES signal patterns likely recognized by the exportin Crm1 (D. Xu et al., 2015), we obtained a high score for a region of Mip6 spanning from the I₆₄₃ residue to the F₆₅₇ residue (ILQKQVTRAAESLGF). Interestingly, this region is predicted to be accessible as a C-terminal alpha helix (**Figure 49**). To study the relevance of this additional sequence, two different strategies were carried out. On the one hand, the whole sequence of this NES signal was deleted, creating the Mip6ΔNES_{Cter} mutant. And, on the other hand, the sequence was cloned in a construct expressing the SV40 TAg NLS, as it was already performed for the NES in the RRM4 (**Figure 39**). In both cases, the stability of the expressed products was checked by immunoblotting and, then, their locations were studied by confocal microscopy.

As shown in **Figure 50**, Mip6 stability is not compromised by deleting this NES_{Cter} nor the expression of the SV40-TAgNLS-GFP (**Figure 50**). In contrast to the upstream NES_{RRM4}, under



Figure 49. Mip6 presents a second putative NES sequence in its C-terminal alpha helix domain. Mip6 predicted structure by PyMOL software. The NES sequence located in its RRM4 is shown in red and the NES sequence present in its C-terminal domain is shown in green.

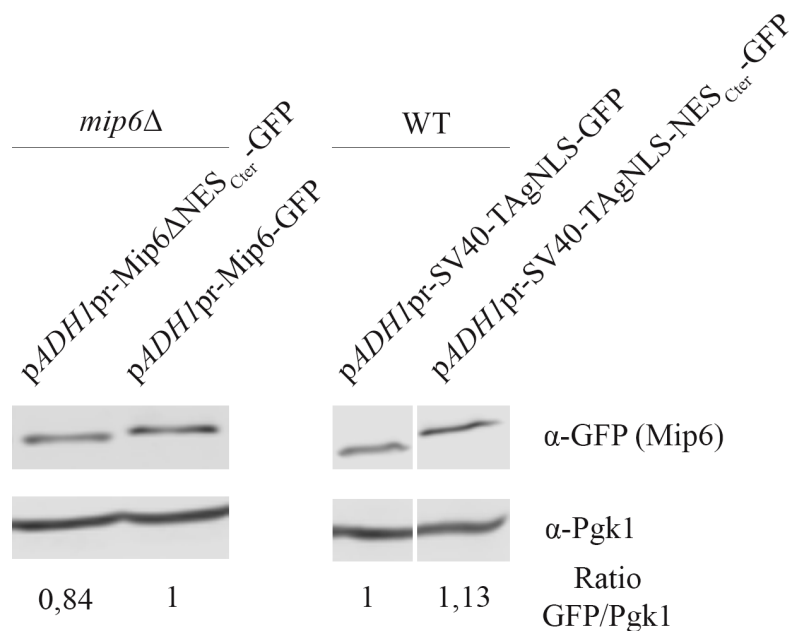


Figure 50. Neither the deletion of the Mip6 NES_{Cter} nor its clonation in a construct expressing the SV40 Tag NLS affect the stability of the expressed product under optimal conditions. Immunoblotting for the detection of Mip6-GFP and Mip6ΔNES_{Cter} expressed from plasmids in *mip6Δ* strain using the indicated antibodies. Pgk1 was also detected using the indicated antibody as a load control. For the quantification, the Fiji software was used; the ratio GFP/Pgk1 is shown and the normalization was performed by assigning a value of 1 to the WT version of Mip6 (left). Immunoblotting for the detection of SV40-TAgNLS-GFP and SV40-TAgNLS-NES_{Cter}-GFP expressed from plasmids in WT strain using the indicated antibodies. Pgk1 was also detected using the indicated antibody as a load control. For the quantification, the Fiji software was used; the ratio GFP/Pgk1 is shown and the normalization was performed by assigning a value of 1 to the original construct (right).

optimal conditions, Mip6 does not change its location when this sequence is deleted (**Figure 51A**), suggesting that it is not a functional NES. Furthermore, the putative NES sequence by itself is not capable of exporting a construct bearing the strong NLS (**Figure 51B**).

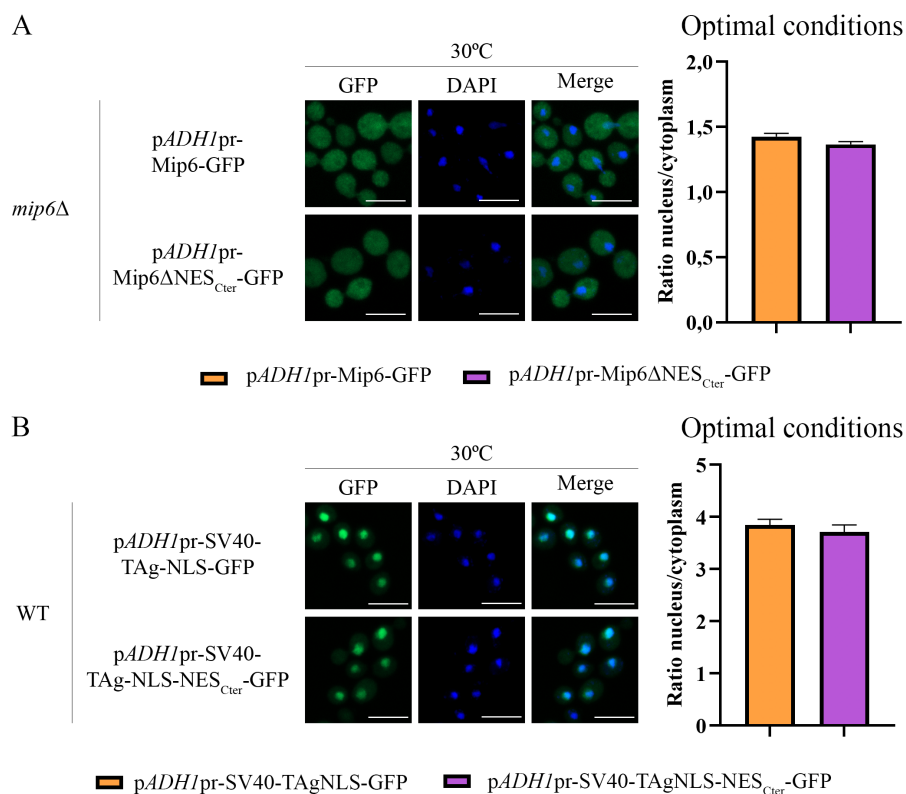


Figure 51. The NES_{Cter} does not seem to be a Mip6 export pathway under optimal conditions. (A) Confocal images displaying Mip6-GFP and Mip6ΔNES_{Cter}-GFP expressed from a plasmid in *mip6Δ* strain under optimal conditions. Nuclei were stained with DAPI. Scale bar: 5μm (left). Bar graphs representing the ratio of GFP intensity between the nucleus and the cytoplasm. Data represent the mean and the standard error (SE) of at least 60 cells coming from three biological replicates (right). (B) Confocal images displaying the NLS from the antigen T of the SV40 virus and the same NLS together with the NES_{Cter} of Mip6, both tagged with GFP, expressed from a plasmid in WT strain. The experiment was performed under optimal conditions. Nuclei were stained with DAPI. Scale bar: 5μm (left). Bar graphs representing the ratio of GFP intensity between the nucleus and the cytoplasm. Data represent the mean and the standard error (SE) of at least 60 cells coming from three biological replicates (right).

Next, the effect of the deletion of the NES_{Cter} sequence was also studied upon stress; after a heat shock at 39°C for 20min or an oxidative shock with 3mM H₂O₂ for 15min. Notably, the deletion of the NES sequence present in Mip6 CTD supposes a significant decrease in its protein levels specifically during the OxSR (**Figure 52**).

Similar to WT Mip6, HS leads to Mip6ΔNES_{Cter} mutant accumulation in stress granules and a significant decrease in its nuclear/cytoplasmic ratio (**Figure 53A**). However, the number of granules per cell was not statistically different compared to WT (**Figure 53B**). Strikingly, under oxidative stress, the same sequence is able to partially export SV40-TAg-NLS-GFP (**Figure 54A**).

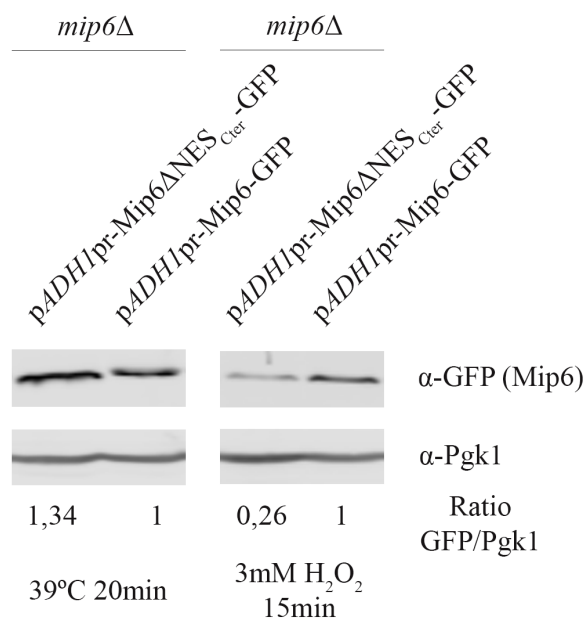


Figure 52. The deletion of the Mip6 NES_{Cter} decreases Mip6 levels during the OxSR. Immunoblotting for the detection of Mip6-GFP and Mip6ΔNES_{Cter}-GFP expressed from plasmids in *mip6Δ* strain after a heat shock at 39°C for 20min or after an oxidative shock with 3mM H₂O₂ for 15min using the indicated antibodies. Pgk1 was also detected using the indicated antibody as a load control. For the quantification, the Fiji software was used; the ratio GFP/Pgk1 is shown and the normalization was performed by assigning a value of 1 to the WT version of Mip6.

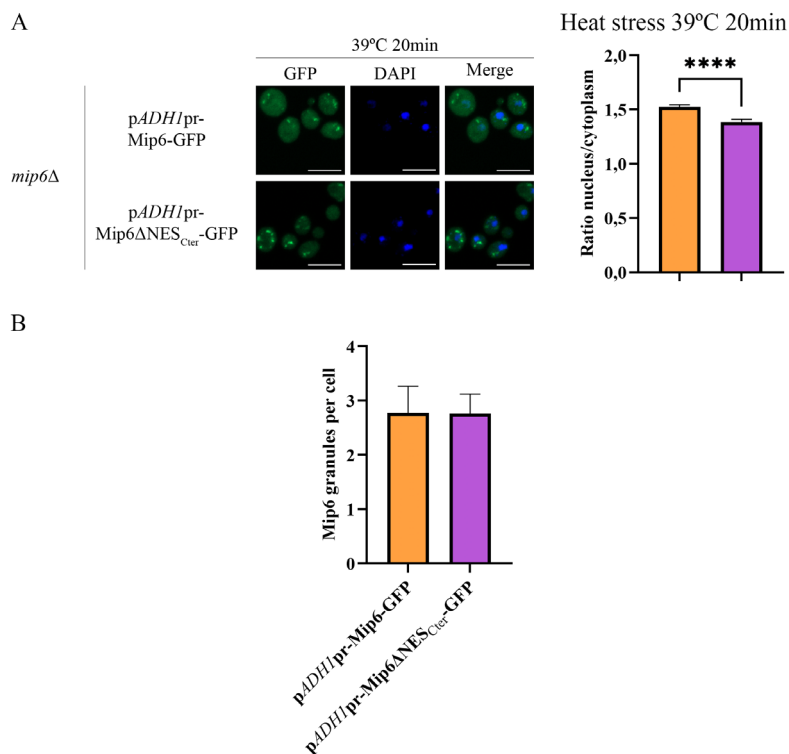


Figure 53. The Mip6ΔNES_{Cter} mutation supposes Mip6 higher accumulation in stress granules, without altering their number. (A) Confocal images displaying Mip6-GFP and Mip6ΔNES_{Cter}-GFP expressed from a plasmid in *mip6Δ* and after a heat shock at 39°C for 20min. Nuclei were stained with DAPI. Scale bar: 5μm (left). Bar graphs representing the ratio of GFP intensity between the nucleus and the cytoplasm. Data represent the mean and the standard error (SE) of at least 60 cells coming from three biological replicates. **** p value < 0,001 (right). (B) Bar graphs representing the number of Mip6 granules per cell in *mip6Δ* strain with plasmids expressing Mip6-GFP and Mip6ΔNES_{Cter}-GFP after a heat shock at 39°C for 20min. Data represent the mean and the standard error (SE) of at least 80 cells coming from two biological replicates.

This result suggests that the Mip6 NES_{Cter} could act as an export signal during OxSR; however, its role must be redundant, since Mip6 nuclear retention is not observed for Mip6ΔNES_{Cter} in the same conditions (Figure 54B).

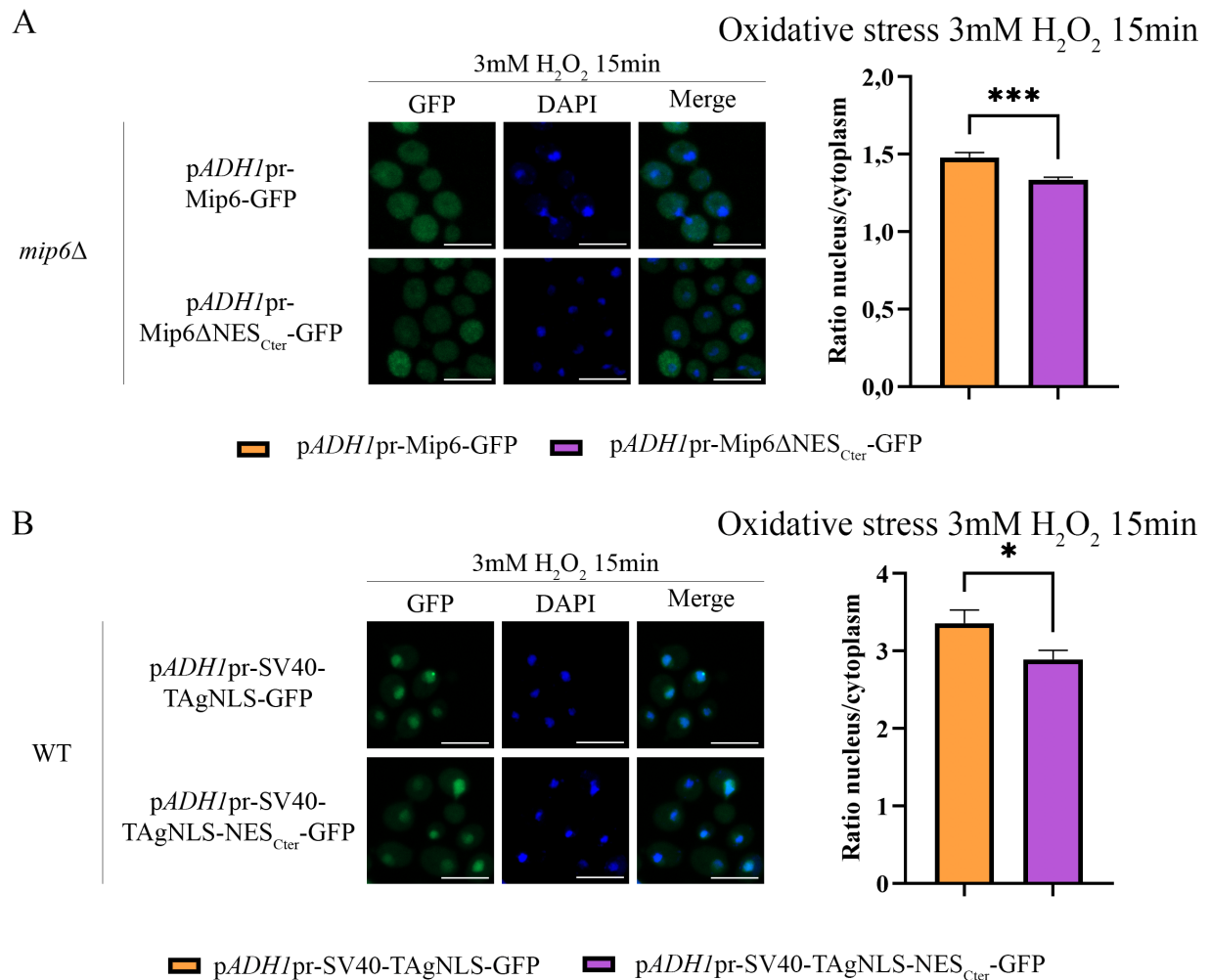


Figure 54. The NES_{Cter} sequence seems to be a functional nuclear export signal under oxidative stress. (A) Confocal images displaying the NLS from the antigen T of the SV40 virus and the same NLS together with the NES_{Cter} of Mip6, both tagged with GFP, expressed from a plasmid in WT strain after an oxidative shock with 3mM H₂O₂ for 15min. Nuclei were stained with DAPI. Scale bar: 5μm (left). Bar graphs representing the ratio of GFP intensity between the nucleus and the cytoplasm. Data represent the mean and the standard error (SE) of at least 60 cells coming from three biological replicates. * p value < 0,05 (right). (B) Confocal images displaying Mip6-GFP and Mip6ΔNES_{Cter}-GFP expressed from a plasmid in *mip6Δ* and after an oxidative shock with 3mM H₂O₂ for 15min. Nuclei were stained with DAPI. Scale bar: 5μm (left). Bar graphs representing the ratio of GFP intensity between the nucleus and the cytoplasm. Data represent the mean and the standard error (SE) of at least 60 cells coming from three biological replicates. *** p value < 0,005 (right).

CHAPTER III: STUDY OF FACTORS THAT PARTICIPATE IN STRESS GRANULES FORMATION



BACKGROUND

Mip6 accumulates in cytoplasmic granules when cells suffer from different kinds of stresses, such as heat shock, sodium azide-induced oxidative stress or glucose deprivation. Besides, this protein colocalizes with components present in both stress granules (Pab1 and Pbp1) and p-bodies (Dcp2), which indicates that Mip6 would also be a component of these cytoplasmic foci (Martín-Expósito et al., 2019). Furthermore, in a previous work performed in our group, using a TAP purification assay, Mip6 was observed to physically interact with SGs proteins, such as Pab1, Pbp1 and the translation factor Tif4631, also known as eIF4G1 (our unpublished data). As part of a collaboration with the group of Dr. Pérez-Cañadillas, we explore how structural features of Tif4631 are required for its physical interactions, SG formation and Tif4631 *in vivo* expression.

Tif4631 is a protein which does not only participate in translation initiation, but it is also a component of stress granules, coupling both processes (Bregues & Parker, 2007; Grousl et al., 2022; Hoyle et al., 2007). Interestingly, from the structural point of view, this protein presents some structural features that suggest its possible role in SG formation (Chaves-Arquero et al., 2022). In the N-terminal (N-ter) domain of Tif4631, there are some domains that contain intrinsically disordered regions (IDRs); a conserved RNA binding domain (RNA1) and two boxes of 15-20 conserved amino acids (BOX1, BOX2). Besides, these regions also present multiple RBP binding sites, through which Tif4631 interacts with the major SGs components Pub1 and Pab1 (S. Jain et al., 2016; Park, Walker, et al., 2011; Tarun & Sachs, 1996). A structural study focusing on these Tif4631 domains has shed light on the amyloid-like properties of the BOX1 region, which confers the protein the ability of self-assembly and self-aggregation. Thus, when cells suffer from stress conditions and the translation machinery is inhibited, Tif4631 interaction with Pub1 would trigger a Tif4631 conformational change that allows BOX1 self-assembly. This remodeling of the protein would also facilitate the binding of Tif4631 with Pab1 and Pub1, which also present amyloid-like regions, leading to the SGs assembly (Chaves-Arquero et al., 2022).

Most of the interactions of Tif4631 with stress granules proteins are still present in the absence of its paralogous protein Tif4632

In *Saccharomyces cerevisiae*, there is a Tif4631 paralogous protein, known as Tif4632 or eIF4G2. Both proteins are functionally overlapping, being the absence of both Tif4631 and Tif4632 lethal (Clarkson et al., 2010; Goyer et al., 1993). Thus, Tif4632 presence could mask the effect of Tif4631 mutant phenotypes. For this reason, *TIF4632* should be deleted in the mutant strains to study Tif4631 phenotypes. However, Tif4632 is not only involved in translation, but it is also present in SGs (Clarkson et al., 2010; Grüner et al., 2018; S. Jain et al., 2016; Park, Zhang, et al., 2011), so this deletion may interfere in the SGs assembly. Bearing this in mind, the effects of *TIF4632* deletion in Tif4631-TAP copurification with SG components were studied. Physical interactions of Tif4631-TAP with proteins participating in the stress response or that are present in both SGs and PBs were studied by TAP purification in both Tif4631-TAP and Tif4631-TAP *tif4632Δ* strains.

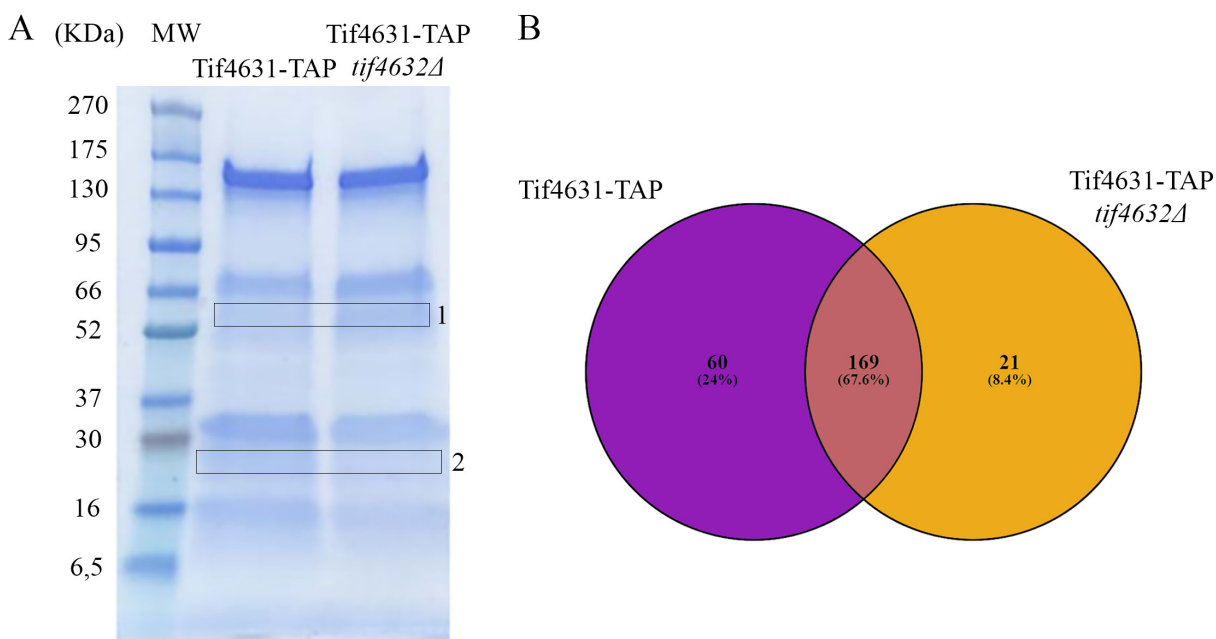


Figure 55. Most of Tif4631 interactions are still present in the absence of its paralogous protein Tif4632. (A) Tif4631-TAP precipitation from wild type (WT) and *tif4632Δ* strains. Tif4631-TAP and co-precipitating proteins were analyzed on an SDS 4-12% gradient polyacrylamide gel and stained with Coomassie blue. Boxes 1 and 2 indicate the bands where changes in protein levels were observed. (B) Venn diagram that shows the protein interactions identified in both strains.

The banding pattern observed of the proteins identified by Tif4631-TAP purification in both WT and *tif4632Δ* strains is very similar (Figure 55A). However, there are some significant differences that can be detected. On the one hand, proteins around the 55-60kDA are enriched in the *tif4632Δ*

mutant (**Figure 55A, band 1**). This could indicate that there are proteins of this size that interact with Tif4631 only in the absence of Tif4632, that these interactions become more significant in these conditions, or that the expression of these proteins increases in the mutant. On the other hand, there is a band around 25KDa that also seems to change between the two strains, being more intense in the WT (**Figure 55A, band 2**). Proteins present in each of the purified extracts, as well as the ones present in each of the mentioned bands, were identified by mass spectrometry.

Interactions identified in both WT and <i>tif4632Δ</i>	
Protein	Gene Ontology term
Tif4631	Biological process: Stress granule assembly Cellular component: Cytoplasmic stress granule Cellular component: P-body
Pab1	Cellular component: Cytoplasmic stress granule
Ded1	Cellular component: Cytoplasmic stress granule
Ssd1	Cellular component: Cytoplasmic stress granule Cellular component: P-body
Sro9	Cellular component: Cytoplasmic stress granule
Cdc33	Cellular component: Cytoplasmic stress granule
Xrn1	Cellular component: Cytoplasmic stress granule Cellular component: P-body
Ubp3	Biological process: Stress granule assembly
Sbp1	Cellular component: P-body
Psp2	Cellular component: P-body
Yef3	Cellular component: Cytoplasmic stress granule
Pbp1	Biological process: Stress granule assembly Cellular component: Cytoplasmic stress granule
Bre5	Biological process: Stress granule assembly Cellular component: P-body
Rbg1	Cellular component: Cytoplasmic stress granule
Pat1	Cellular component: Cytoplasmic stress granule Cellular component: P-body
Ksp1	Cellular component: Cytoplasmic stress granule
Ahp1	Biological process: response to stress
Gbp2	Biological process: response to stress Cellular component: Cytoplasmic stress granule
Npl3	Cellular component: Cytoplasmic stress granule
Slf1	Cellular component: Cytoplasmic stress granule
Pbp4	Cellular component: Cytoplasmic stress granule
Nab6	Cellular component: Cytoplasmic stress granule
Bmh1	Cellular component: Cytoplasmic stress granule
Rpo21	Cellular component: Cytoplasmic stress granule
Lsm12	Cellular component: Cytoplasmic stress granule
Ecm32	Cellular component: Cytoplasmic stress granule
Vts1	Cellular component: P-body



RESULTS

Protein	Gene Ontology term
Tef4	Cellular component: Cytoplasmic stress granule
Lsm6	Cellular component: P-body
Ssb2	Cellular component: Cytoplasmic stress granule
Rqc2	Cellular component: Cytoplasmic stress granule
Interactions identified only in WT	
Protein	Gene Ontology term
Ssb1	Biological process: response to stress
Kar2	Biological process: response to stress
Gpp1	Biological process: response to stress
Dpb2	Biological process: response to stress
Rpg1	Cellular component: Cytoplasmic stress granule
Vma2	Cellular component: Cytoplasmic stress granule
Interactions identified only in <i>tif4632Δ</i>	
Protein	Gene Ontology term
Lsm4	Cellular component: P-body
Hrb1	Biological process: response to stress
Pol2	Biological process: response to stress
Proteins identified in band 1 of the gel	
Protein	Gene Ontology term
Slf1	Cellular component: Cytoplasmic stress granule
Gbp2	Biological process: response to stress
	Cellular component: Cytoplasmic stress granule
Sro9	Cellular component: Cytoplasmic stress granule
Hrb1	Biological process: response to stress
Sup45	Cellular component: Cytoplasmic stress granule
Npl3	Cellular component: Cytoplasmic stress granule
Tef4	Cellular component: Cytoplasmic stress granule
Rio2	Cellular component: Cytoplasmic stress granule
Hrr25	Cellular component: P-body
Proteins identified in band 2 of the gel	
Protein	Gene Ontology term
Cdc33	Cellular component: Cytoplasmic stress granule
Lsm12	Cellular component: Cytoplasmic stress granule
Pbp4	Cellular component: Cytoplasmic stress granule
Tsa1	Biological process: response to stress
Ahp1	Biological process: response to stress
Gpp1	Biological process: response to stress

Table 12. Stress response-related proteins identified by mass spectrometry in a Tif4631-TAP purification in WT and *tif4632Δ* strain and their Gene Ontology terms assigned taken from the Saccharomyces Genome Database (SGD), <https://www.yeastgenome.org/>.

Notably, Tif4631-TAP interactors are mainly kept in the absence of Tif4632. Nevertheless, there are 60 proteins whose interaction is affected in the mutant, while the interaction with other 21 proteins seems to gain importance when *TIF4632* is deleted (**Figure 55B**). Since this work focuses on SG formation and the relevance of the Tif4631 N-ter region in the process, the proteins

involved in the cellular stress response or present in either SGs or PBs were studied (Table 12). Almost every stress-related protein copurifies with Tif4631 in both WT and *tif4632Δ* strains, including all the proteins participating in SGs assembly. Regarding the proteins identified in the bands that present different intensities between both strains (Figure 55, bands 1 and 2), the result is similar. Although some proteins involved in the stress response or located in both SGs and PBs have been identified in these bands, suggesting either its interaction with Tif4631 or their levels are affected in the mutant strain, there are not specific differences. Thus, the decision was to follow with the study in *TIF4632*-deleted strains.

Tif4631 protein levels depend on different regions of the N-terminal domain

To gain insights into the *in vivo* characterization of Tif4631 structural features, deletions of the different regions present in the N-terminal domain of Tif4631 (BOX1, BOX2 and RNA1), as well as the deletion of the whole N-terminal region (the first 249 amino acids of the sequence) were created by CRISPR technology (Tif4631ΔN-ter, Tif4631ΔBOX1, Tif4631ΔBOX2 and Tif4631ΔRNA1) in a *tif4632Δ* background. Immunoblotting using α-TAP was performed to compare the protein levels in each mutant.

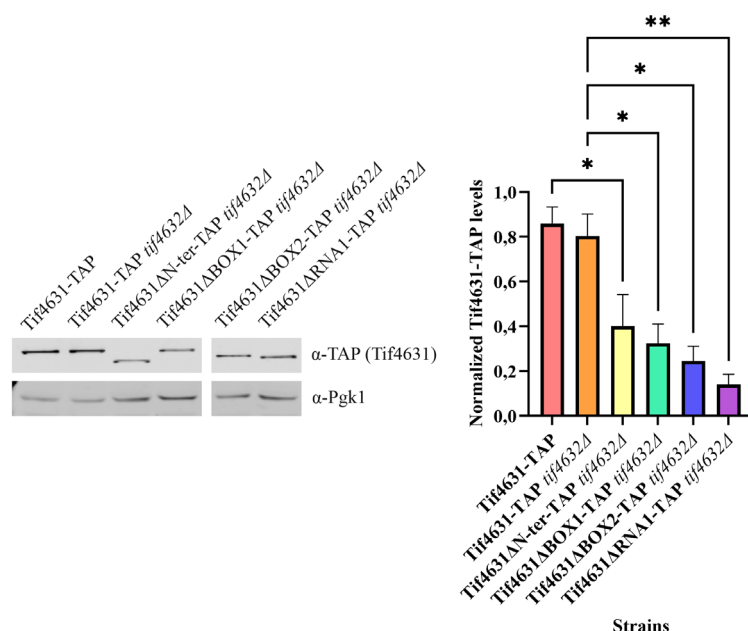


Figure 56. Deletions of the different regions in the N-terminal domain of Tif4631 affect protein levels. Immunoblotting for the detection of different versions of Tif4631-TAP with the different N-ter domain regions deleted in the *tif4632Δ* strain using the indicated antibodies. Pgk1 was also detected using the indicated antibody as a load control (left). Bar graphs that represent the ratio TAP/Pgk1 quantified by the Fiji software. For the normalization, a value of 1 was assigned to the band with the higher protein levels of each gel analyzed. Data represent the mean and the standard error (SE) of three biological replicates (right).

On the one hand, Tif4631-TAP levels were observed to be similar in the *tif4632Δ* mutant and the WT strain (**Figure 56**), which reinforces the idea that this mutation does not seem to affect significantly Tif4631. On the other hand, all the Tif4631 mutants, both the ones of the different N-terminal regions and the whole N-ter deletion mutant, present a statistically significant decrease in Tif4631 protein levels (**Figure 56**).

Deletion of the N-terminal domain of Tif4631 causes an increase in the duplication time under optimal conditions

Simultaneous deletion of *TIF4631* and *TIF4632* is lethal for the cell. While the *tif4632Δ* mutant does not present any growth phenotype, the absence of only Tif4631 partially impairs cell proliferation. Thus, it was suggested that the presence of Tif4631 is more relevant for growth than Tif4632 (Clarkson et al., 2010; Goyer et al., 1993). To study how the deletions of the Tif4631 BOX1, BOX2, RNA1 and N-terminal domains affect cell viability, mutant strains were compared to WT in growth assays.

As expected, the *tif4632Δ* growth curve is similar to the one of the WT strain, as well as their duplication times (**Figure 57**). Interestingly, only the Tif4631ΔN-ter mutant presents a growth defect (**Figure 57**).

Deletions of the BOX1 and RNA1 regions of the Tif4631 N-terminal domain affect cell growth after the diauxic shift

The experiments above were performed in cells growing at optimal conditions. However, the role of this protein in SG assembly occurs during the stress response (Chaves-Arquero et al., 2022). Therefore, a growth assay was carried out in which the mutants lacking the different N-terminal regions were grown for a week. The study focused on the diauxic shift, when the cell has already used all the glucose present in the medium and it must change its metabolism in order to respond to this lack of nutrients (Galdieri et al., 2010). Both PBs and SGs are present in yeast cells in the stationary phase. While PB assembly occurs prior to the cell entry in the stationary phase, SGs are not detected until the cells have already entered into the stationary phase (Shah et al., 2013).

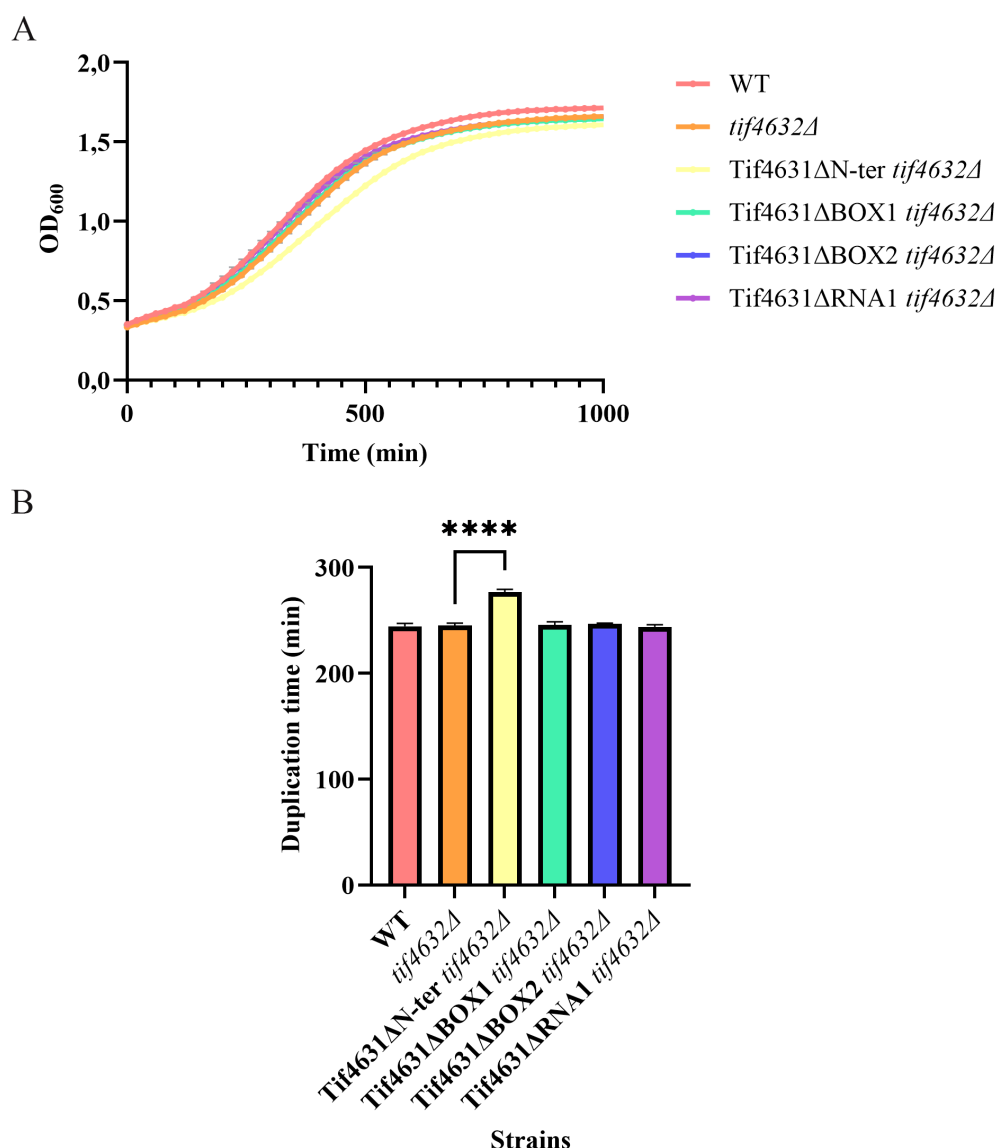


Figure 57. Deletion of the N-terminal domain of Tif4631 causes an increase in the duplication time under optimal conditions. (A) Growth curve of wild-type (WT), *tif4632Δ*, Tif4631ΔN-ter *tif4632Δ*, Tif4631ΔBOX1 *tif4632Δ*, Tif4631ΔBOX2 *tif4632Δ* and Tif4631ΔRNA1 *tif4632Δ* strains under optimal conditions. Data represent the mean and the standard error (SE) of three biological replicates. (B) Duplication time of wild-type (WT), *tif4632Δ*, Tif4631ΔN-ter *tif4632Δ*, Tif4631ΔBOX1 *tif4632Δ*, Tif4631ΔBOX2 *tif4632Δ* and Tif4631ΔRNA1 *tif4632Δ* strains under optimal conditions. Data represent the mean and the standard error (SE) of three biological replicates. **** p value < 0,001.

Thus, the study of cell growth in these conditions might shed light on the relevance of Tif4631 regions in the formation of these foci.

Interestingly, under these specific circumstances in which translation must be finetuned regulated, not only the deletion of the N-terminal domain but also the deletion of both the BOX1 and the RNA1 regions lead to a decrease in cell growth (Figure 58). In contrast, the deletion of the BOX2 region is similar to the one of the *tif4632Δ* mutant (Figure 58). All these evidences may suppose

that BOX1 and RNA1 regions are important for the Tif4631 function related to the diauxic shift and, likely, with the assembly of stress granules.

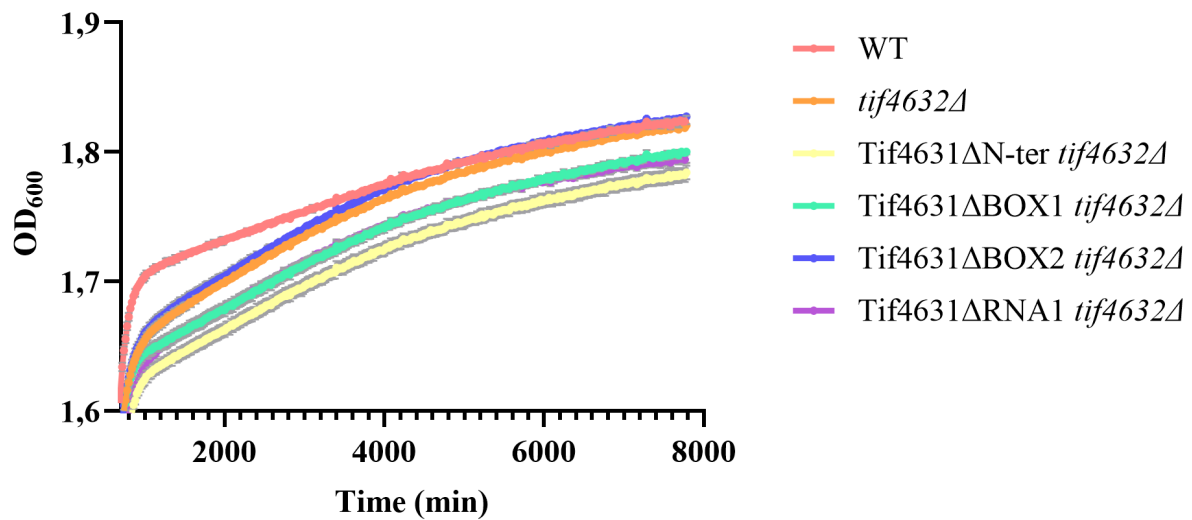


Figure 58. Deletions of the BOX1 and RNA1 regions of the Tif4631 N-terminal domain affect cell growth after the diauxic shift. Growth curve of wild-type (WT), *tif4632Δ*, Tif4631ΔN-ter *tif4632Δ*, Tif4631ΔBOX1 *tif4632Δ*, Tif4631ΔBOX2 *tif4632Δ* and Tif4631ΔRNA1 *tif4632Δ* strains after the diauxic shift. Data represent the mean and the standard error (SE) of three biological replicates.

DISCUSSION



The interconnection of the different levels that comprise gene expression is essential for the regulation and the proper completion of the process. Sus1 subunit, for instance, belongs to both the H2B deubiquitination module of the SAGA complex and TREX-2 complex, so that it couples histone post-translational modifications, transcription and mRNA export (Galán & Rodríguez-Navarro, 2012; Soffers & Workman, 2020). Transcription termination and mRNA processing are also tightly interconnected, since the factors involved in mRNA maturation are also necessary for the end of the transcription process (Bentley, 2005; Mandel et al., 2006; Rodríguez-Molina et al., 2023). Besides, the interaction of RNA with RBPs also plays an important role in regulating RNA homeostasis. The RBPs present in yeast cells are numerous, as well as in other eukaryotic organisms, but the specific function of some of them still remains scarcely known (Gebauer et al., 2021; Glisovic et al., 2008; Hogan et al., 2008).

Mip6 is an RBP that regulates gene expression of certain stress-response genes; although other kinds of target genes were also described, such as trehalose biosynthesis genes or ribosomal protein genes (Martín-Expósito et al., 2019; Nuño Cabanes, 2024; Nuño-Cabanes et al., 2020). Nevertheless, the exact mechanism by which this protein is able to regulate mRNA levels of these genes is still unknown, even if it has been related to different steps of the gene expression process. Thus, in this work, we focus on Mip6 role in the regulation of the stress response, deepening in its crosstalk with transcription factors Msn2/4 and the HDAs Rpd3 and Hda1. Furthermore, mechanisms regulating Mip6 location were also studied, since it changes under certain circumstances, which suggests the significance of its location in Mip6 function.

Mip6 INTERACTS WITH BOTH THE TRANSCRIPTION AND THE mRNA DEGRADATION MACHINERY

Mip6 is an RBP that not only binds certain mRNAs depending on the circumstances, but it also regulates the levels of the transcripts it interacts with. When Mip6 is absent, stress response genes regulated by transcription factors Msn2/4 are overexpressed, as well as genes whose expression is mediated by Hsf1. Besides, there are other kinds of genes, like the ribosomal protein genes, which are underexpressed (Martín-Expósito et al., 2019; Nuño-Cabanes et al., 2020). However,



DISCUSSION

whether Mip6 role is more related to their transcription or their degradation is not well clarified yet.

Recent evidence seems to tip the scales in favor of Mip6 role in transcription. This factor physically and genetically interacts with the RNAPolIII subunit Rpb1 (**Figure 12**; Martín-Expósito et al., 2019) and the crosstalk of Mip6 with Msn2/4 and HDAs, factors that participate in transcription regulation, was described throughout this work (**Figure 19**, **Figure 20** and **Figure 25**). Besides, the lack of Mip6 alters RNAPolIII occupancy at the ORF of certain genes (Nuño Cabanes, 2024).

Nevertheless, Mip6 is also related to the mRNA degradation machinery. Genetic interaction of *MIP6* with proteins taking part in mRNA decay, such as the exonucleases Rrp6 and Xrn1, was observed. Furthermore, deletion of both *MIP6* and *RRP6* supposes a higher increment in Msn2/4-dependent transcripts levels compared with their corresponding single mutants. And, in addition, Xrn1 absence alters Mip6 location (Martín-Expósito et al., 2019). Thus, Mip6 may regulate the expression of stress response genes by participating in both their transcription and mRNA decay. Indeed, proteins that take part in the degradation process, including Dcp2, which colocalizes with Mip6 upon HS (Martín-Expósito et al., 2019), and Xrn1, also participate in transcription initiation and elongation. Dcp2 and Xrn1 shuttle between the cytoplasm and the nucleus and are able to interact with the chromatin and promote transcription (Haimovich et al., 2013). Therefore, like these proteins, Mip6 could present a dual role in regulating the stress response transcripts levels.

Besides, there are RNA binding proteins, such as Rpb4 and Rpb7, which participate in a process called mRNA imprinting. Both Rpb4 and Rpb7 are subunits of the RNAPolIII that can form a dissociable heterodimer and interact with mRNAs. This interaction is present throughout the life of the RNA and regulates its transcription, export, translation and decay (Garrido-Godino et al., 2021; Harel-Sharvit et al., 2010; Kumar et al., 2019). Thus, Mip6 may also participate in mRNA imprinting, binding to mRNAs and regulating the different gene expression processes they undergo.

Mip6 ROLE UNDER OPTIMAL CONDITIONS IS NOT ESSENTIAL FOR CELL VIABILITY

Mip6 preferentially binds to Msn2/4-dependent transcripts under optimal conditions, negatively regulating their expression (Martín-Expósito et al., 2019). Nevertheless, this protein is not essential for cell viability under optimal conditions and it does not genetically interact with Msn2/4 (**Figure 14**). Even if there are certain genes whose expression is altered in *msn2Δ* and *msn4Δ*, both strains are viable mutants under no stress conditions (Y. M. Lee et al., 2017; Mühlhofer et al., 2024). Although the transcription of *MSN4* is induced during the stress response, *MSN2* expression is constitutive (Gasch et al., 2000), but its location and, hence, function is well regulated. While the stress response is not triggered, Msn2 remains phosphorylated thanks to PKA and Snf1 and this mark prevents its import to the nucleus so that it cannot perform its function (De Wever et al., 2005; Görner et al., 2002; P. Lee et al., 2013). Therefore, the lack of genetic interaction with *MIP6* could be explained by the low significance of the function of Msn2/4 present under optimal conditions. Nonetheless, Mip6 lack does affect stress response transcripts metabolism even in the absence of any stress (Martín-Expósito et al., 2019), which indicates that, under optimal growth conditions, a mild increase of stress response mRNAs do not affect cell viability.

Mip6 CONTRIBUTION IN CELL SURVIVAL DEPENDS ON THE KIND OF STRESS AND THE TRANSCRIPTION FACTOR

Both Msn2 and Msn4 individual absences suppose a slowing down in cell growth upon HS (**Figure 15**). Interestingly, the *msn2Δ* mutant presents a tendency to grow slower than the *msn4Δ* strain (**Figure 15**). Even if they are functionally redundant, this redundancy is not complete and their contribution to gene expression may differ in certain genes and stress conditions (AkhavanAghdam et al., 2016; Estruch, 2000). However, when both Msn2 and Msn4 lack, cell growth upon HS is recovered up to the WT rate (**Figure 15**), which suggests the existence of an alternative mechanism activated or enhanced when both proteins are impaired. Apart from Msn2/4, there is another general stress response transcription factor, Hsf1. Even if their contributions are independent and they bind to different elements in gene promoters (Amorós & Estruch, 2001), they also share several of their target genes, since these genes present both STRE and HSE elements in their promoters (Boy-Marcotte et al., 1999; Ciccarelli et al., 2023).



DISCUSSION

Indeed, although both transcription factors present their specific set of target genes (chaperones for Hsf1 and metabolic genes related to the stress response for Msn2/4), there are some overlaps (Mühlhofer et al., 2024). Thus, the main player of this alternative pathway could be Hsf1.

However, although Mip6 regulates mRNA levels of the same target genes, *MIP6* deletion does not suppose any statistically significant difference in the cell growth rate either alone or combined with Msn2 and/or Msn4 absences. But there is a tendency for the *msn2Δ* strain to grow slightly slower than the *msn2Δmip6Δ* mutant (**Figure 15**). Thus, more replicates are needed, in order to have a stronger statistical power, or a different assay must be performed for checking the existence of a functional interaction between *MIP6* and *MSN2*. On the other hand, the growth rate of the *msn4Δ* and *msn4Δmip6Δ* are very similar (**Figure 15**), which indicates that either there is no genetic interaction between these two genes or they participate in the same pathway. Mip6 regulates Msn2 recruitment to the promoters of the genes it regulates (Nuño Cabanes, 2024); but its role in Msn4 recruitment is unknown, being interesting to study in order to deepen Msn4 and Mip6 crosstalk.

Strikingly, the growth pattern of the studied mutants slightly changes depending on the nature of stress. On the one hand, upon a severe HS, neither Msn2 nor Msn4 absences suppose any defect in cell growth in our assays, contrary to mild HS (**Figure 16**). The lack of Hsp12, an Msn2/4-regulated protein involved in the maintenance of membrane organization under stress conditions, provokes a defect growth when cells are exposed to a severe HS (Welker et al., 2010), which does not correlate with the phenotype observed in Msn2/4 absence. Nevertheless, Hsp12 expression is also regulated by transcription factor Hsf1 (MacIsaac et al., 2006; Yamamoto et al., 2005), hence, Hsf1 may be the main player in the HSR when cells face a severe HS.

Moreover, *msn2Δmsn4Δmip6Δ* is the only mutant that presents a clear growth defect under severe HS (**Figure 16**). This result does not only indicate a genetic interaction of *MSN2*, *MSN4* and *MIP6* upon severe HS, but it also suggests the participation of these proteins in different cell survival mechanisms. Mip6 regulates the expression of several Hsf1-dependent genes, since different subsets of Hsf1-regulated transcripts were either overexpressed or underexpressed in the *mip6Δ*

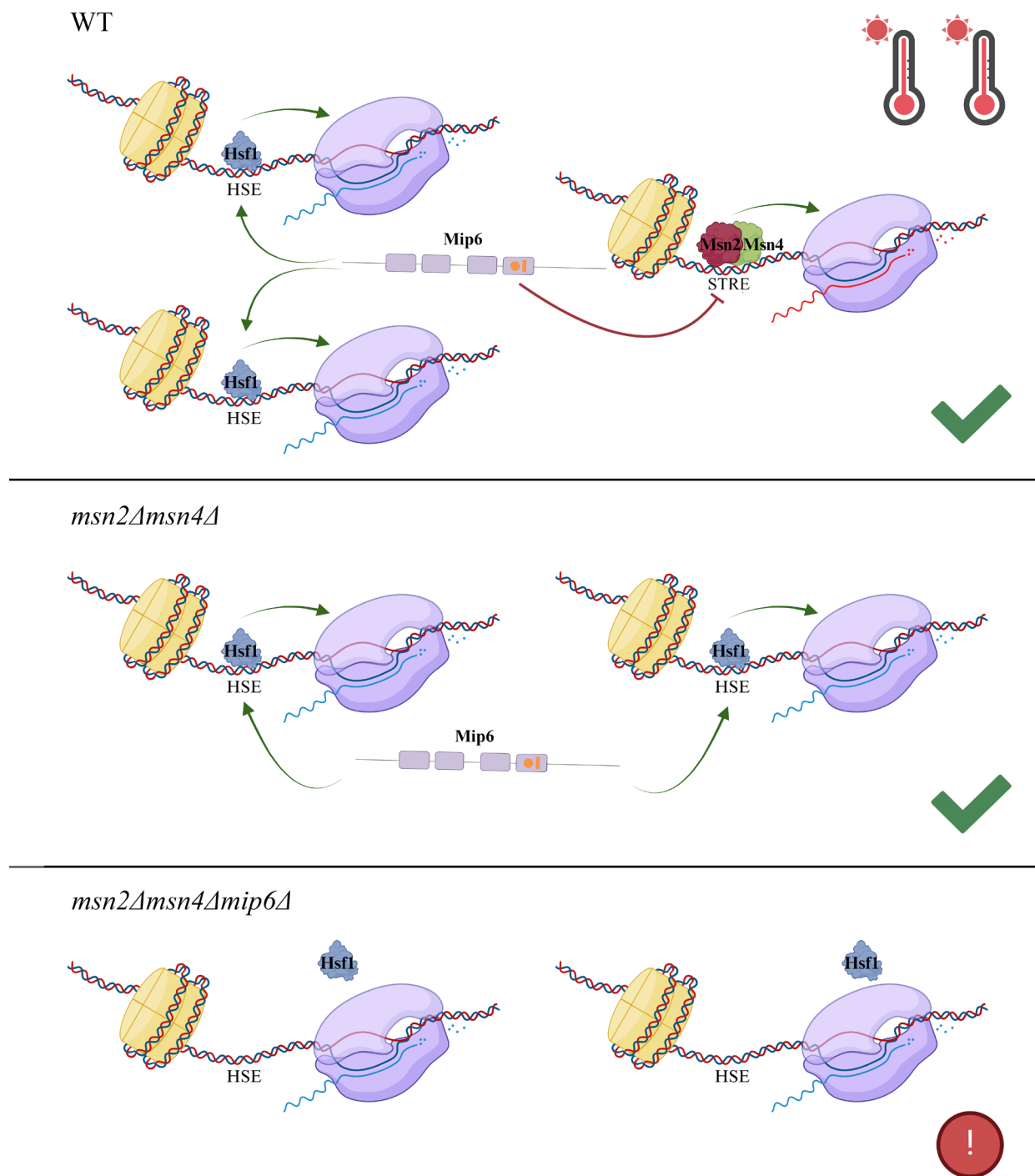


Figure 59. Upon severe heat stress, Mip6 may act as a positive regulator of transcription factor Hsf1. Mip6 regulates the expression of stress response genes controlled by both Hsf1 and Msn2/4, since their transcript levels are altered in the absence of Mip6. Upon severe heat stress, the lack of Msn2/4 does not affect cell survival, since an alternative mechanism, probably regulated by Hsf1, supplies this absence. However, when the cells also lack Mip6, Hsf1 is not properly recruited to the genes it regulates, affecting cell survival. Created with BioRender.com.

mutant under both optimal and HS conditions (Nuño-Cabanes et al., 2020) and, in addition, Hsf1 recruitment to the promoters of the genes it regulates is also altered when Mip6 is absent (Nuño



Cabanes, 2024). Besides, Hsf1 was described to be essential for cell adaptation to prolonged heat stress (Mühlhofer et al., 2024). Therefore, Mip6 may act as an Hsf1 modulator during extended exposure to severe HS, which would explain why the lack of both Mip6 and Msn2/4 affects cell survival more than the corresponding single mutants (**Figure 59**).

On the other hand, after an oxidative shock, differences in the growth rate of the studied mutants are observed. The lack of Msn2 leads to the slowing down of the growth rate, which is not observed when the protein absent is Msn4 (**Figure 17**). Besides, the *msn2Δmsn4Δ* double mutant recovers the WT growth rate, which suggests, as in a mild HS, the activation of an alternative pathway that properly triggers the stress response, probably with Hsf1 as the main transcription factor. However, the combination of these mutations with *MIP6* deletion leads again to a growth defect, which indicates that Mip6 could be also an Hsf1 positive regulator. Surprisingly, Mip6 lack partially recovers the growth problem observed in the *msn2Δ* mutant. Therefore, Mip6 may also be acting as a negative regulator of the Hsf1 alternative pathway. When Mip6 is absent, Hsf1-regulated genes present a different phenotype depending on the subset of genes, being both overexpressed or underexpressed (Nuño-Cabanes et al., 2020). This dual regulatory property would explain why Mip6 could act as both an activator and an inhibitor of the Hsf1 function (**Figure 60**).

In conclusion, Mip6 could act as a modulator of both Msn2/4 and Hsf1 responsive genes; however, its contribution would depend not only on the transcription factor, but also on the nature of the stress. Mip6 would negatively regulate Msn2/4-dependent genes (Martín-Expósito et al., 2019), while it would either positively or negatively regulate the ones dependent on Hsf1 (Nuño-Cabanes et al., 2020). Nevertheless, more experiments must be performed in order to deepen the mechanism by which Mip6 mediates the function of these transcription factors.

IS Mip6 A NEGATIVE REGULATOR OF HDAC Rpd3?

Mip6 role in regulating histone acetylation levels seems to be more related to deacetylation than with the HATs, since *MIP6* genetically interacts with the HDAs Rpd3 and Hda1 under both optimal and stress conditions, but no genetic interaction with *GCN5* was observed (**Figure 25**).

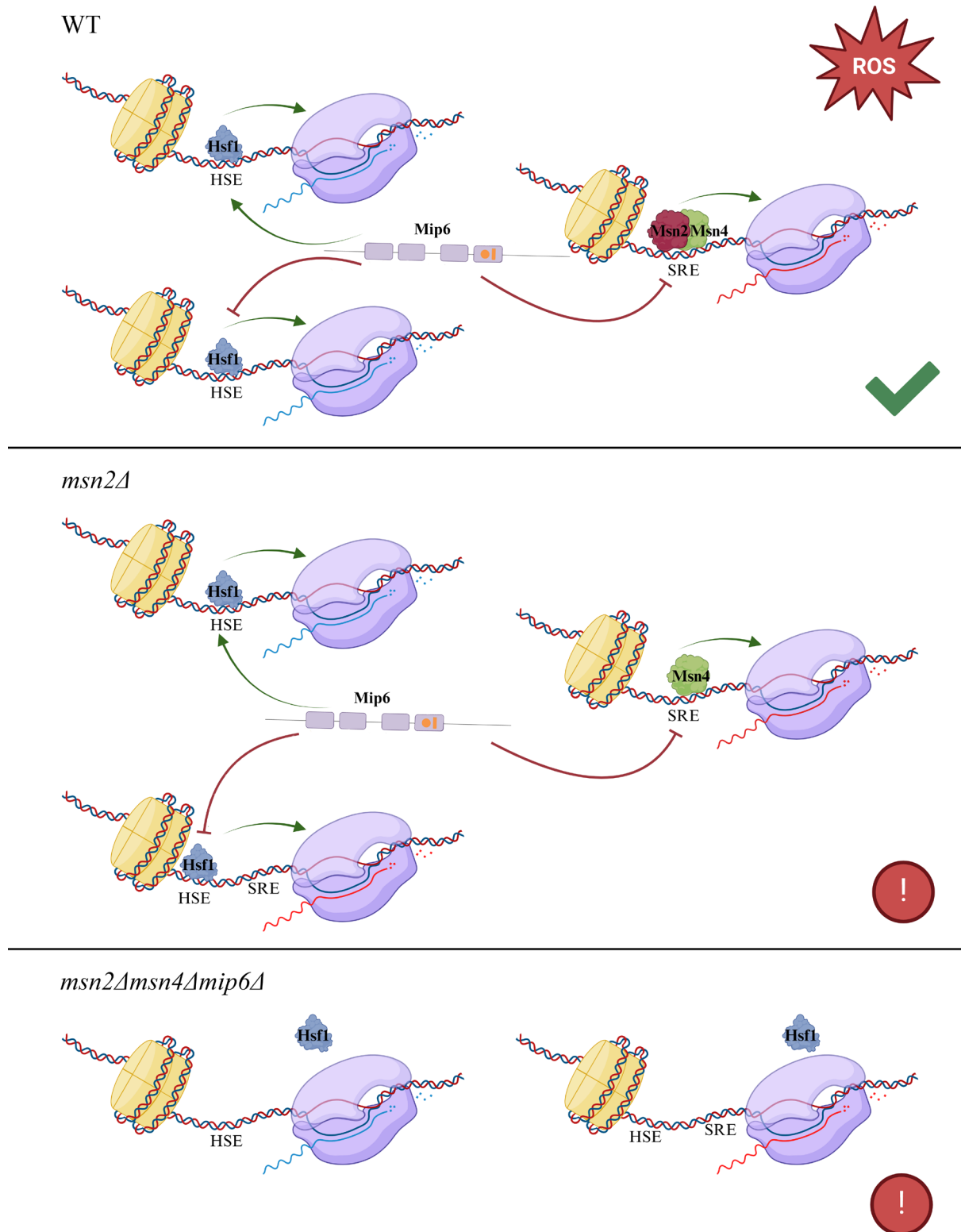


Figure 60. Mip6 acts as both a positive and a negative regulator of the stress response upon oxidative stress. Mip6 participates in the homeostasis of stress response genes, regulating the levels of their transcripts. However, its contributions depend on the transcription factor, repressing the Msn2/4-dependent genes and either repressing or activating the Hsf1-dependent ones. In the absence of Msn2, Msn4 by itself is not enough to properly trigger the OxSR and Mip6 is repressing the backup pathway by inhibiting Hsf1 recruitment to the promoter of the genes. However, when both Msn2 and Msn4 lack, a Mip6-regulated alternative pathway is activated, so that when the three proteins are absent, cell survival is affected. Thus, Mip6 would act as both an activator and a repressor of the backup mechanism depending on the circumstances. Created with BioRender.com.



However, Gcn5 was copurified with Mip6 in a TAP purification assay (our unpublished data), so a less significant function together with HATs does not have to be discarded. The lack of only one of the studied HDAs does not affect cell viability upon any kind of the studied stresses (**Figure 25**). This could indicate that the function of the different HDAs is redundant, so that the absence of only one of them does not drastically affect cell survival.

Nevertheless, in the *rpd3Δmip6Δ* and *hda1Δmip6Δ* double mutants cell growth rate is affected under optimal conditions and HS. This result suggests a Mip6 role in histone deacetylation, probably regulating both Rpd3 and Hda1 function. Thus, the alteration of the function of both HDAs would explain the observed phenotypes. Mip6 physically interacts with Rpd3 under both optimal and HS (**Figure 20**) and the different Rpd3 complexes regulate the same target genes as Mip6 (Hsf1- and Msn2/4-dependent and RPG; Kremer & Gross, 2009; Robyr et al., 2002; Ruiz-Roig et al., 2010; Sadeh et al., 2011; Martín-Expósito et al., 2019; Nuño-Cabanes et al., 2020). Besides, *HSP12* and *CTT1* mRNA levels decrease in the *rpd3Δmip6Δ* strain, just like in *rpd3Δ* and contrary to the phenotype of the *mip6Δ* single mutant (**Figure 26A y B**). This suggests that Mip6 and Rpd3 may regulate the expression of these genes, participating in the same pathway, being the presence of Rpd3 essential for Mip6 function. Thus, Mip6 may act as a Rpd3 negative regulator (**Figure 61**). Mip6 regulates the recruitment of both Msn2 and Hsf1 to the promoters of the genes they regulate (Nuño Cabanes, 2024), hence it may also participate in the regulation of Rpd3 recruitment to the genes. Mip6 might avoid Rpd3 recruitment under optimal conditions and restrict it upon heat stress. However, more experiments are necessary for deepening the crosstalk between Rpd3 and Mip6, including the study of Mip6 possible role in Rpd3 recruitment.

Regarding OxS, the *hda1Δmip6Δ* mutant is the only one with a clear growth defect (**Figure 25**). Thus, this genetic interaction suggests that Mip6 would also be participating in HDA in the OxSR, but in a different pathway than Hda1. Mip6 interaction in OxS may be focused on Rpd3 regulation. Whether this protein interacts with Rpd3 regardless of the complex it belongs to or Mip6 only associates with a specific complex is not clarified yet; however, a crosstalk of Mip6 with the Rpd3 μ complex may explain this result. Rpd3 μ gains importance during osmotic and

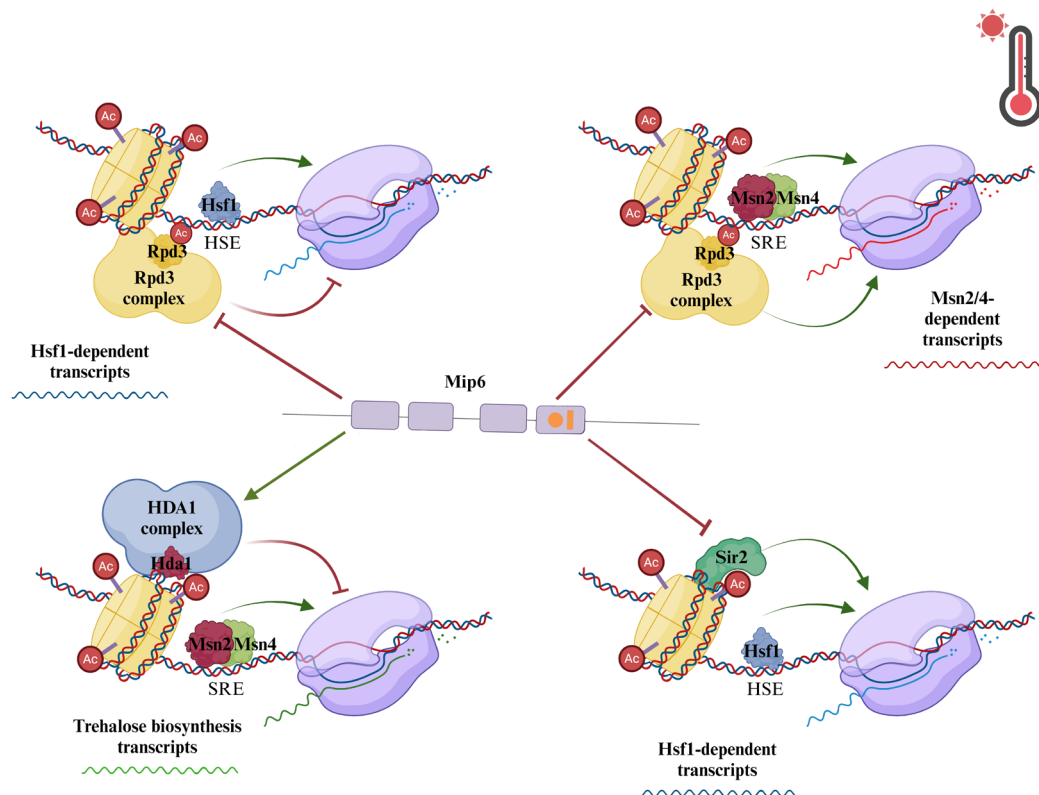


Figure 61. Mip6 participates in the regulation of histone deacetylation. Mip6 may act as a negative regulator of Rpd3 complexes, hence regulating the expression of Hsf1- and Msn2/4-dependent genes upon heat stress. Besides, this protein also interacts with other HDA proteins, such as Hda1 and Sir2, regulating the trehalose biosynthesis genes and Hsf1-dependent genes, respectively. Created with BioRender.com.

oxidative stress and, in addition, Rpd3 is recruited to the promoters of stress response genes independently of the other Rpd3 μ subunits (Baker et al., 2013; McDaniel & Strahl, 2013). It would be interesting to study a Mip6 possible participation in this recruitment, as well as Mip6 physical interaction with the other Rpd3 complexes subunits in order to deepen the crosstalk between these two proteins.

Mip6 and Hda1 genetic interaction under both optimal and stress conditions (Figure 25) may also suggest a possible role of Mip6 regulating Hda1; nevertheless, the results are not representative yet, since neither *HSP12* nor *CTT1* mRNA levels are not altered in the *hda1 Δ* mutant (Figure 26C). However, recent evidence indicates a role of Hda1 in the regulation of the trehalose biosynthesis genes, since its absence leads to transcription activation (Yu et al., 2021), the same as in *mip6 Δ* (Martín Expósito, 2019). Therefore, Mip6 might be collaborating with Hda1 in the repression of trehalose metabolism (Figure 61). So, in order to check this, we will focus our future research on the study of the crosstalk of Mip6 and Hda1 in modulating trehalose genes.



Furthermore, Rpd3 and Hda1 are not the only HDA with a described genetic interaction with *MIP6*, since the *sir2Δmip6Δ* strain also exacerbates the defect growth observed in its corresponding single mutant (Nuño Cabanes, 2024). Sir2 is another HDA involved in gene repression and chromatin silencing (Blander & Guarente, 2004; Sauty et al., 2021). Besides, Sir2 has a role in the connection of the unfolded protein response and the HSR, by activating Hsf1 (Weindling & Bar-Nun, 2015). Thus, Mip6 interaction with Sir2 might explain its dual role by both activating or repressing Hsf1-regulated genes (**Figure 61**), since Rpd3 acts as a repressor and Sir2 functions as an activator of these genes (Kremer & Gross, 2009; Weindling & Bar-Nun, 2015). Nonetheless, the crosstalk between these two proteins must be further studied in order to confirm this theory.

YEAST CELLS PRESENT A TRANSCRIPTIONAL MEMORY MECHANISM TO RESPOND TO HEAT STRESS EXPOSURE

Transcriptional memory is a mechanism present in several organisms that allows them to respond faster to a specific stimulus when they have already been exposed to it and this quick response may be inherited even up to 14 generations later (Brickner et al., 2007; D'Urso et al., 2016; Light et al., 2013; Sump & Brickner, 2022). Heat stress transcriptional memory has been already described in other eukaryotic organisms, such as *Arabidopsis* (Ohama et al., 2017); however, studies in yeast have been focused on the expression of *GAL* and *INO1* genes up to date (D'Urso et al., 2016; Sood et al., 2017).

A different behavior in gene expression upon HS is observed in cells depending on whether they have been exposed before to a high temperature or not (**Figure 27** and **Figure 28**), which indicates that yeast cells also present a heat stress transcriptional memory. Two thirds of the yeast genome is modified in a second exposure to the stress; nonetheless, three different expression patterns could be identified among the altered genes.

First, 2288 of the genes with differential expression are genes repressed upon heat stress (**Figure 29**). However, in a second heat exposure, they present lower repression, either because they already present higher expression levels under optimal conditions (cluster 2) or because, even if their optimal mRNA levels are similar in both kinds of samples, their reduction upon heat shock

softens with memory (cluster 4). This weakened repression during the second exposure could be a consequence of a faster adaptation to heat stress. Nevertheless, higher levels of these transcripts do not have to indicate a higher amount of protein, since most of the genes repressed under stress conditions remain “sequestered” in both SGs and PBs during HSR (Grousl et al., 2022; Guzikowski et al., 2019; Ivanov et al., 2019).

Among the genes activated upon heat stress, two different subsets can be observed (**Figure 29**). On the one hand, there are 497 genes whose expression is activated faster and stronger when the cells have already been exposed to heat stress (cluster 3), following the expression pattern that corresponds with the traditional idea of transcriptional memory (Pascual-Ahuir et al., 2020; Sump & Brickner, 2022). Genes that participate in mitochondrial translation are enriched as GO in this group (**Table 11**).

On the other hand, 1738 of the studied transcripts do not reach the same activated levels in the second exposure compared to the first one (cluster 1). One of the possible explanations of this phenotype could be that the 60min recovery phase at 30°C has not been enough for the complete recovery of the cell. Then, even if the transcripts levels before the heat shock are similar in both kinds of samples, cells are still affected after the first heat exposure and their second response is compromised. However, there is a second possible explanation which would implicate transcriptional memory rather than an expression problem. *HSP12* mRNA levels follow this pattern. But, regarding its protein levels, Hsp12 was described to be upregulated after the 60min recovery phase (Nuño Cabanes, 2024). This indicates that, even an hour after heat exposure, cells still maintain the levels of stress response proteins high. Thus, when they are exposed to a second heat shock, the response could be faster, because they do not have to wait for those proteins to be expressed. This result would also explain why their transcripts levels do not need to reach the same levels as in the first exposure. Besides, recent evidence indicates that transcription regulation is not the only mechanism that controls transcriptional memory, since the mRNA degradation machinery also takes part in the process (Li et al., 2023). Therefore, the other steps



DISCUSSION

comprising gene expression, such as protein degradation, could also be involved in the regulation of the transcriptional memory.

Mip6 seems to take part in transcriptional memory, since there are 580 genes that “lose” their memory when *MIP6* is deleted and 588 genes that also show a differential expression in the *mip6Δ* mutant (**Figure 28**). Strikingly, among these genes, we found genes related to sucrose and oligosaccharide metabolism, being *Msn2/4* the main regulator of metabolic genes during HS (Mühlhofer et al., 2024). In fact, *MSN4* is one of the genes differentially expressed in *mip6Δ*. Besides, transcripts coding for Rpd3C subunits also seem to present their transcriptional memory altered only in the mutant. Thus, here we found another functional link of Mip6 with both *Msn2/4* and HDAC Rpd3.

Moreover, even if in the four represented clusters, gene behavior is similar in both WT and *mip6Δ* strains, there are specific sets of genes that show some differences by heatmap (Tejada-Colón et al., under revision). Furthermore, representing these data in Sankey plots, some differences in changes in gene expression over time are also observed between both strains (Tejada-Colón et al., under revision). While 1706 genes in no memory samples and 2119 genes in the memory ones showed no induction or repression after 15min of stress in the WT, this behavior is observed in only 1615 genes in the no memory samples and 1886 genes in the memory ones in the *mip6Δ*. Whereas in the *mip6Δ* mutant, there are more genes that show a strong activation or repression after 15min of stress (876 genes in the no memory samples, compared with 772 in the WT and 797 genes in the memory samples, compared with 637 in the WT). A similar effect was also observed when comparing the time 0 with time 20 min. Thus, these results reinforce the idea of Mip6 participation in fine-tuning their mRNA levels.

MAY Mip6 REGULATE THE EXPRESSION OF THE EARLY MEIOTIC GENES?

Mip6 participates in meiosis together with its paralogue protein Pes4. They regulate the expression of a certain subset of genes that are transcribed in the meiotic prophase, but they are not translated until late meiosis; Mip6 and Pes4 are able to protect these transcripts and avoid their degradation before they can be translated (Jin et al., 2017). Mip6 absence does not affect sporulation by itself,

but the *mip6 Δ pes4 Δ* mutant does present a sporulation defect, which suggests that, in this context, both proteins have a redundant function (Jin et al., 2017). However, two considerations have to be kept in mind. First, all these experiments were carried out with cell synchronization by controlling Ndt80 expression. Thus, cells were blocked after the pairing and the segregation of homologous chromosomes, when the role of this meiotic factor starts, so that all the cells were in the same meiotic stage to perform the experiment. This means that any slowing down of the process that happens before this point cannot be detected. And second, the strains used in the work belong to the SK1 background, which is extensively used in sporulation studies due to the fact that these cells sporulate fast and synchronously (Jin et al., 2017; Kane & Roth, 1974; Rimal et al., 2020). Nevertheless, this phenotype is because these strains overpass some of the meiotic checkpoints, in which the process stops to check if the previous stages have been properly completed. Thus, in case the protein of study could take part in one of those overpassed checkpoints, the use of the SK1+W303 background may be interesting. This background was created by the cross of an SK1 cell with a W303 one and presents a faster sporulation process than the W303 strains while maintaining the corresponding checkpoints (Cooper, 1997; Hochwagen & Amon, 2006; Kim & Strich, 2016; Law et al., 2014; Mallory et al., 2007, 2012).

Furthermore, histone deacetylases, and specifically Rpd3, play an important role in the regulation of the meiotic process. Rpd3 participates in the chromatin reorganization triggered in order to start meiosis (Kadosh & Struhl, 1998; Yukawa et al., 2009), and it also regulates by deacetylation Ume6, a protein that acts as both an activator and a repressor of the EMG expression (Goldmark et al., 2000; Mallory & Strich, 2003; Strich et al., 1994; Law et al., 2014; Mallory et al., 2012).

Therefore, since Mip6 seems to function as an Rpd3 negative regulator (**Figure 20**, **Figure 25** and **Figure 26**), it may also participate in the regulation of the EMG expression. Indeed, even if Mip6 is a very low expressed protein, not being detected either by Western blot or by confocal microscopy (Ghaemmamghami et al., 2003; Martín-Expósito et al., 2019), its transcripts levels rise upon meiosis induction in a similar profile to meiosis general transcription factor Ndt80 (Borde et al., 2009). Whether this increase in mRNA levels correlates with a higher expression



of Mip6 protein would be interesting to study, mainly because Mip6-GFP can be observed by confocal microscopy during the late stages of sporulation (Jin et al., 2017). Thus, this rise in Mip6 expression in these circumstances suggests a significant role of this protein in the sporulation process.

For this reason, one of the objectives of this thesis was to study Mip6 role in the regulation of the early meiotic genes. Thus, as part of my Thesis work, I generated mutant strains of Mip6 and Pes4, as well as, Mip6-GFP were created in both SK1 and SK1+W303 backgrounds. Besides, during a research stay in the laboratory of Dr. Randy Stritch, I could learn the techniques and methodology necessary for cell sporulation, useful for the study of meiosis, since in the group where this thesis was carried out, we had always worked with mitotic growth. Therefore, these techniques were tuned in our lab, so that a study of Mip6 function in EMG expression can be followed by my colleagues in the near future.

THERE IS A NUCLEAR EXPORT SIGNAL IN THE RRM4 OF Mip6

Mip6 shuttles between the nucleus and the cytoplasm partially mediated by its interaction with Mex67 (**Figure 62**; Martín-Expósito et al., 2019). Interestingly, the mutation of the Mip6 W₄₄₂ residue does not suppose a nuclear retention that important than the one observed in both Mex67 Δ UBA and Mip6 Δ RRM4 strains (**Figure 33**; Martín-Expósito et al., 2019). This phenotype suggests that Mip6 physical interaction with Mex67 may not be that significant for the protein transport and other circumstances, such as a defect in mRNA general export, might play a more relevant role. In any case, neither Mex67 inactivation nor the deletion of its UBA domain, the one that directly interacts with Mip6, block the total export of Mip6 (Martín-Expósito et al., 2019). Thus, at least another Mex67-independent Mip6 export mechanism must regulate the transport.

Mip6 presents a class 2 hydrophobic isoleucine spacing NES sequence in its RRM4 domain (Kosugi et al., 2008; Martín Expósito, 2019). The functionality of this sequence as a Mip6 export mechanism aroused doubts because point mutations in the NES affect the Mip6-Mex67 interaction (**Figure 30**). However, throughout this work, the NES sequence in the Mip6 RRM4 has been

proven to function as an export pathway by itself (**Figure 62**), being able to transport the strong SV40-TAg-NLS from the nucleus to the cytoplasm under both optimal and stress conditions (**Figure 39**). Besides, the combination of the Mip6W₄₄₂A mutation and point mutations in the NES sequence provokes a higher nuclear accumulation than only affecting the Mip6-Mex67 interaction (**Figure 33**), which confirms them as two different Mip6 export pathways. In addition, these results would explain the higher amount of nuclear retention when the RRM4 of Mip6 is deleted, since in this domain both the W₄₄₂ residue and the NES sequence are present (Martín-Expósito et al., 2019).

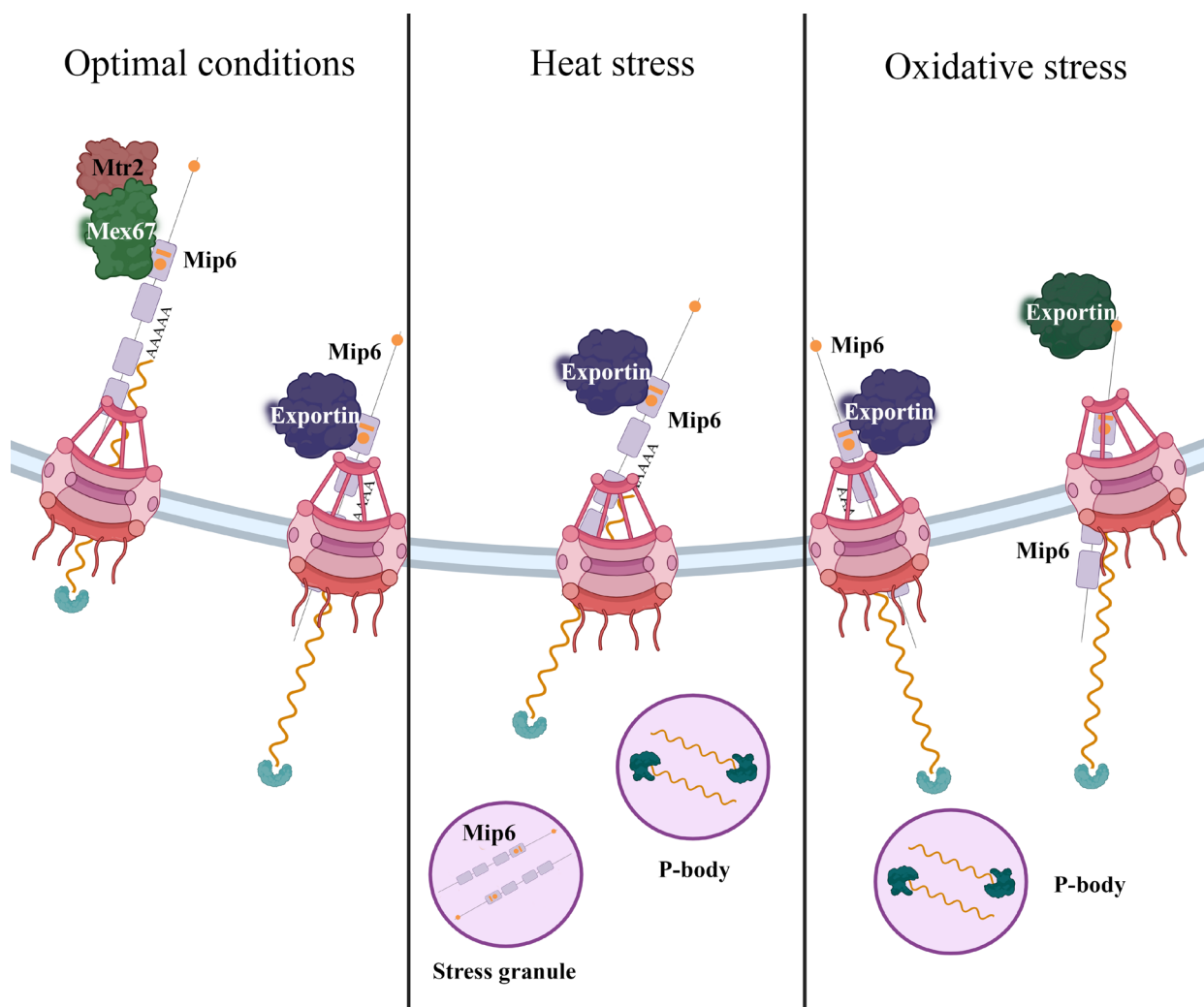


Figure 62. Mip6 shuttles from the nucleus to the cytoplasm through different export mechanisms. Mip6 export pathways depend on the growth conditions of the cells. During optimal growth, there are at least two different Mip6 transport pathways, one mediated by the export factor Mex67 and another one due to the NES sequence present in its RRM4. Upon heat stress, Mip6 export seems to be less significant, since the protein is accumulated in stress granules and the impairing of both mechanisms does not suppose any Mip6 location change. However, during the OxSR, Mip6 does not accumulate in granules and their transport from the nucleus to the cytoplasm gains importance again. In this case, apart from the mechanism already described, Mip6 export is also regulated by an NES sequence present in its CTD. Created with BioRender.com.



Under H_2O_2 -induce OxS, the Mip6 localization phenotype is very similar (**Figure 37**), which indicates that the NES sequence is a Mip6 export signal also under these conditions (**Figure 62**). Besides, no Mip6 accumulation in stress granules is observed (**Figure 37**). Even if stress granules are formed in a great variety of stress conditions, such as sodium azide-induced OxS, the foci observed in the cell cytoplasm during H_2O_2 -induce OxS correspond to p-bodies (Buchan et al., 2008, 2011; Wallace et al., 2015). Mip6 colocalizes with both stress granules and p-bodies components (Martín-Expósito et al., 2019). However, stress granule formation is dependent on p-bodies and proteins of both kinds of foci colocalize during the first steps of SGs formation (Buchan et al., 2008). Therefore, there is a possibility that Mip6 is a component only in SGs and the closeness spot to PBs was because of this dependence, which would explain why there is no Mip6 accumulation in granules under H_2O_2 -induced OxS, when only PBs are present (**Figure 62**).

Surprisingly, even if the NES sequence is also functional upon HS (**Figure 39**), there is no remarkable Mip6 nuclear retention under these conditions (**Figure 35**). A possible explanation would be that the NES sequence may be less accessible during the HSR, since the transport of most proteins is regulated by differences in the accessibility of the karyopherins to the signal by means of post-translational modifications (Ashida et al., 2022; Nardozzi et al., 2010; Wendler & Enenkel, 2019). Further work is required to fully understand the coordination of the different Mip6 structural elements to regulate its cellular functions.

Four cysteine residues are found surrounding the NES sequence (**Figure 45**). The formation of disulfide bonds between Cys residues is often used as a sensor of oxidative stress and they are key players in the triggering of the OxSR, as in the case of Yap1 activation, the master regulator of the OxSR (Delaunay et al., 2002). Therefore, the proximity of these residues to the NES sequence makes them a perfect mechanism to regulate its accessibility. This would also explain the absence of Mip6 nuclear accumulation in the NES mutants upon HS, since the HSR under aerobic conditions also leads to the imbalance of the ROS equilibrium (Davidson & Schiestl, 2001). The mutation of one of these Cys residues does not change Mip6 location either under optimal conditions or upon OxS (**Figure 47**). However, under these conditions, the NES is functional as

shown in our experiments (**Figure 33** and **Figure 37**). During the HSR, Mip6C₄₅₀A-GFP nuclear accumulation was not observed either (**Figure 47**). Nevertheless, if this mutation affects the NES protection so that the sequence would be more accessible, point mutations in the NES would be also necessary in order to observe a Mip6 retention phenotype. Thus, this experiment must be performed in order to confirm these Cys residues as a regulatory mechanism of the NES.

Strikingly, the Mip6C₄₅₀A mutation leads to a higher Mip6 presence in the cytoplasm (**Figure 47**), which may mean a higher Mip6 accumulation in stress granules, even if no difference in the number of granules was observed (**Figure 48**). This would indicate that this residue might be important for Mip6 location in these foci. Indeed, in the Mip6 Δ RRM4-GFP mutant, Mip6 accumulation in stress granules also seems to be less prominent (Martín Expósito, 2019), suggesting the importance of this region for Mip6 location in these foci too. Besides, point mutations in the RRM4 domain of Mip6 paralogous protein Pes4 lead to a higher Pes4 presence in cytoplasmic granules upon HS (**Figure 44**). This result would be another evidence pointing to the importance of these regions in the presence of both Mip6 and Pes4 in SGs.

On the other hand, it is not clear if Msn5 mediates Mip6 export, since we were unable to demonstrate that they interact in our conditions (**Figure 31**). Crm1/Xpo1 is a major protein export factor, interacting with NES signals of hundreds of proteins and mediating their transport from the nucleus to the cytoplasm (Fornerod et al., 1997; Ossareh-Nazari et al., 1997; Stewart, 2019). Nevertheless, this exportin was already discarded as a regulator of Mip6 export because its inactivation does not change Mip6 location (Martín Expósito, 2019). However, the *xpo1-1* strain is a thermosensitive Crm1 mutant, hence Crm1 inactivation was performed upon HS, and no difference in the location of Mip6 was observed when the NES sequence is affected in these conditions (**Figure 35**). Therefore, a more interesting and enlightening experiment would be to check if the inactivation of Crm1 affects the NES ability to export a construct bearing a strong NLS, since this ability is still present under HS (**Figure 39**).



Mip6 EXPORT IS DEPENDENT ON ITS INTERACTION WITH mRNAs

Since Mip6 and Msn5 do not seem to copurify in our hands, the incremented presence of Mip6 nuclear pool may be due to an indirect effect. Msn5 also regulates the Msn2/4 transport from the nucleus to the cytoplasm, its absence leading not only to the block of the export of these transcription factors, but it also provokes the decrease in Msn2 levels (Durchschlag et al., 2004; Görner et al., 2002; Sunnåker et al., 2013). Moreover, when transcription is affected, such as in the *rpb1-1* mutant, Mip6 export is also impaired (Martín-Expósito et al., 2019), even if the Mip6-Mex67 interaction is still present (**Figure 13**), which suggests that what blocks Mip6 transport in both scenarios is the general decrease in the mRNA levels. Indeed, the individual deletions of both RRM1 and RRM2 regions, the ones that present more affinity with the RNA, provoke a huge Mip6 accumulation in the nucleus (Martín Expósito, 2019). Therefore, Mip6 interaction with the transcripts through all its RRMs seems to be essential for its transport from the nucleus to the cytoplasm.

IS THE PUTATIVE NES IN THE Mip6 CTD A FUNCTIONAL EXPORT SIGNAL?

Apart from the studied Mip6 export mechanism, at least a third transport pathway must exist, since in the Mip6_{I₄₂₇R;W₄₄₂A} and Mip6_{I₄₂₇R;W₄₄₂A} double mutants there is still a cytoplasmic pool of Mip6 (**Figure 33**). Indeed, a second NES signal was predicted in Mip6 CTD. However, its deletion does not increase Mip6 presence in the nucleus under optimal or stress conditions (**Figure 51A**, **Figure 53A** and **Figure 54A**).

Nevertheless, the putative NES by itself is able to export the strong SV40-TAg-NLS upon OxS (**Figure 54B**). Thus, it could be a functional Mip6 NES during the OxSR. The accessibility of the importins/exportins to the NES/NLS sequences of their cargo proteins is regulated by different mechanisms. Msn2 import to the nucleus is regulated by kinases PKA and Snf1 (De Wever et al., 2005; Görner et al., 2002; P. Lee et al., 2013). Phosphorylation also may serve as an activation signal; such as for the NLS sequence of the protein STAT1, which is exposed due to a conformational change of the protein triggered by its phosphorylation (McBride et al., 2002), or for the classical NLS of the SV40, whose recognition by importin α 1 is enhanced also thanks to a phosphorylation mark (Xiao et al., 1996). Equally, the NES sequence in Mip6 CTD would



be regulated, either by phosphorylation or by other mechanisms, so that the signal would be active under OxS. Nevertheless, this export mechanism does not seem to be significant for Mip6 location or, at least, can be easily supplied, since the deletion of the signal in the same conditions does not suppose the block of the protein export (**Figure 54A**).

REGIONS OF THE Tif4631 N-TERMINAL DOMAIN WOULD BE IMPORTANT FOR ITS FUNCTION IN SG FORMATION

BOX1 region of Tif4631 shows amyloid-like properties (Chaves-Arquero et al., 2022), which makes the protein prone to self-assembly and aggregation and would facilitate its aggregation with other proteins in the SG formation. Indeed, the deletion of these regions, as well as the whole N-terminal domain, affects protein stability (**Figure 56**), which would indicate the significance of these regions for Tif4631. However, the experiment was carried out under optimal conditions, where no SGs are formed. Thus, whether this phenotype is present upon a stress must be checked to conclude. In any case, the deletion of these regions does alter protein survival during the diauxic shift, when the cell has already used all the glucose present in the medium and it must change its metabolism in order to respond to this lack of nutrients (Galdieri et al., 2010). The BOX1 and RNA1 mutations lead to a decrease in cell growth, while the deletion of the BOX2 region presents a growth curve similar to the Tif4631 WT version (**Figure 58**). Although this study is just the beginning and more experiments must be carried out in order to confirm the involvement of these regions in the SG assembly, these evidences may suppose that BOX1 and RNA1 regions would be important for the Tif4631 function related to the diauxic shift and, likely, with the formation of SGs.

CONCLUSIONS





Based on the results obtained throughout this thesis, the main conclusions that can be drawn are listed below.

1. Mip6 physically interacts with the RNAPolIII subunit Rpb1, suggesting its participation in transcription.
2. Mip6 participates in the regulation of the stress response; however, its contribution depends on the nature of the stress and the transcription activator.
3. Mip6 physically and genetically interacts with histone deacetylase Rpd3. Besides, their participation in stress response transcripts levels regulation are opposites, which suggests that Mip6 would act as an Rpd3 negative regulator.
4. Yeast cells present a transcriptional memory mechanism to respond to heat stress exposure. Mip6 absence affects the transcriptional memory of a subset of genes, which indicates Mip6 participation in the transcriptional memory regulation of these genes.
5. The NES sequence in the RRM4 region of Mip6 is a functional export signal that regulates its transport from the nucleus to the cytoplasm under both optimal and stress conditions. Its accessibility and, hence, Mip6 export may be regulated by cysteine residues that surround the NES sequence.
6. The sequence homologous to the Mip6 RRM4 NES in Pes4 is not a functional export signal. However, its mutation leads to an alteration in Pes4 accumulation in cytoplasmic granules.
7. Mip6 CTD presents a NES that is functional upon H₂O₂-induced oxidative stress.
8. RNA1 and BOX1 regions in the N-terminal domain of Tif4631 affect protein levels and cell growth during the diauxic shift, which suggests the importance of these regions in Tif4631 function during the diauxic shift.

BIBLIOGRAPHY





- Afonina, Z. A., & Shirokov, V. A. (2018). Three-Dimensional Organization of Polyribosomes—A Modern Approach. *Biochemistry. Biokhimiia*, 83(Suppl 1), S48-S55. <https://doi.org/10.1134/S0006297918140055>
- Ahn, S.-G., & Thiele, D. J. (2003). Redox regulation of mammalian heat shock factor 1 is essential for Hsp gene activation and protection from stress. *Genes & Development*, 17(4), 516-528. <https://doi.org/10.1101/gad.1044503>
- AkhavanAghdam, Z., Sinha, J., Tabbaa, O. P., & Hao, N. (2016). Dynamic control of gene regulatory logic by seemingly redundant transcription factors. *eLife*, 5, e18458. <https://doi.org/10.7554/eLife.18458>
- Alber, F., Dokudovskaya, S., Veenhoff, L. M., Zhang, W., Kipper, J., Devos, D., Suprpto, A., Karni-Schmidt, O., Williams, R., Chait, B. T., Sali, A., & Rout, M. P. (2007). The molecular architecture of the nuclear pore complex. *Nature*, 450(7170), 695-701. <https://doi.org/10.1038/nature06405>
- Alepuz, P. M., Matheos, D., Cunningham, K. W., & Estruch, F. (1999). The *Saccharomyces cerevisiae* RanGTP-Binding Protein Msn5p Is Involved in Different Signal Transduction Pathways. *Genetics*, 153(3), 1219-1231. <https://doi.org/10.1093/genetics/153.3.1219>
- Alkalaeva, E. Z., Pisarev, A. V., Frolova, L. Y., Kisselev, L. L., & Pestova, T. V. (2006). In vitro reconstitution of eukaryotic translation reveals cooperativity between release factors eRF1 and eRF3. *Cell*, 125(6), 1125-1136. <https://doi.org/10.1016/j.cell.2006.04.035>
- Allfrey, V. G., Faulkner, R., & Mirsky, A. E. (1964). Acetylation and methylation of histones and their possible role in the regulation of rna synthesis*. *Proceedings of the National Academy of Sciences*, 51(5), 786-794. <https://doi.org/10.1073/pnas.51.5.786>
- Amorós, M., & Estruch, F. (2001). Hsf1p and Msn2/4p cooperate in the expression of *Saccharomyces cerevisiae* genes HSP26 and HSP104 in a gene- and stress type-dependent manner. *Molecular Microbiology*, 39(6), 1523-1532. <https://doi.org/10.1046/j.1365-2958.2001.02339.x>
- Anderson, P., & Kedersha, N. (2002). Stressful initiations. *Journal of Cell Science*, 115(16), 3227-3234. <https://doi.org/10.1242/jcs.115.16.3227>
- Ashida, S., Morita, R., Shigeta, Y., & Harada, R. (2022). Phosphorylation in the accessory domain of yeast histone chaperone protein 1 exposes the nuclear export signal sequence. *Proteins*, 90(2), 317-321. <https://doi.org/10.1002/prot.26240>
- Baker Brachmann, C., Davies, A., Cost, G. J., Caputo, E., Li, J., Hieter, P., & Boeke, J. D. (1998). Designer deletion strains derived from *Saccharomyces cerevisiae* S288C: A useful set of strains and plasmids for PCR-mediated gene disruption and other applications. *Yeast*, 14(2), 115-132. [https://doi.org/10.1002/\(SICI\)1097-0061\(19980130\)14:2<115::AID-YEA204>3.0.CO;2-2](https://doi.org/10.1002/(SICI)1097-0061(19980130)14:2<115::AID-YEA204>3.0.CO;2-2)
- Baker, L. A., Ueberheide, B. M., Dewell, S., Chait, B. T., Zheng, D., & Allis, C. D. (2013). The Yeast Snt2 Protein Coordinates the Transcriptional Response to Hydrogen Peroxide-Mediated Oxidative Stress. *Molecular and Cellular Biology*, 33(19), 3735-3748. <https://doi.org/10.1128/MCB.00025-13>
- Bakhrat, A., Baranes-Bachar, K., Reshef, D., Voloshin, O., Krichevsky, O., & Raveh, D. (2008). Nuclear export of Ho endonuclease of yeast via Msn5. *Current Genetics*, 54(5), 271-281. <https://doi.org/10.1007/s00294-008-0216-8>
- Balasubramanian, R., Pray-Grant, M. G., Selleck, W., Grant, P. A., & Tan, S. (2002). Role of the Ada2 and Ada3 Transcriptional Coactivators in Histone Acetylation*. *Journal of Biological Chemistry*, 277(10), 7989-7995. <https://doi.org/10.1074/jbc.M110849200>
- Bannister, A. J., & Kouzarides, T. (2011). Regulation of chromatin by histone modifications. *Cell Research*, 21(3), 381-395. <https://doi.org/10.1038/cr.2011.22>
- Battaglia, S., Lidschreiber, M., Baejen, C., Torkler, P., Vos, S. M., & Cramer, P. (2017). RNA-dependent chromatin association of transcription elongation factors and Pol II CTD kinases. *eLife*, 6, e25637. <https://doi.org/10.7554/eLife.25637>
- Beck, T., & Hall, M. N. (1999). The TOR signalling pathway controls nuclear localization of nutrient-regulated transcription factors. *Nature*, 402(6762), 689-692. <https://doi.org/10.1038/45287>
- Bentley, D. L. (2005). Rules of engagement: Co-transcriptional recruitment of pre-mRNA processing factors. *Current Opinion in Cell Biology*, 17(3), 251-256. <https://doi.org/10.1016/j.ceb.2005.04.006>
- Beringer, M., & Rodnina, M. V. (2007). The ribosomal peptidyl transferase. *Molecular Cell*, 26(3), 311-321. <https://doi.org/10.1016/j.molcel.2007.03.015>



- Bi, X. (2014). Heterochromatin structure: Lessons from the budding yeast. *IUBMB Life*, 66(10), 657-666. <https://doi.org/10.1002/iub.1322>
- Blanchet, S., & Ranjan, N. (2022). Translation Phases in Eukaryotes. En K.-D. Entian (Ed.), *Ribosome Biogenesis* (Vol. 2533, pp. 217-228). Springer US. https://doi.org/10.1007/978-1-0716-2501-9_13
- Blander, G., & Guarente, L. (2004). The Sir2 family of protein deacetylases. *Annual Review of Biochemistry*, 73, 417-435. <https://doi.org/10.1146/annurev.biochem.73.011303.073651>
- Boisnard, S., Lagniel, G., Garmendia-Torres, C., Molin, M., Boy-Marcotte, E., Jacquet, M., Toledano, M. B., Labarre, J., & Chédin, S. (2009). H₂O₂ activates the nuclear localization of Msn2 and Maf1 through thioredoxins in *Saccharomyces cerevisiae*. *Eukaryotic Cell*, 8(9), 1429-1438. <https://doi.org/10.1128/EC.00106-09>
- Bolognesi, B., Lorenzo Gotor, N., Dhar, R., Cirillo, D., Baldrighi, M., Tartaglia, G. G., & Lehner, B. (2016). A Concentration-Dependent Liquid Phase Separation Can Cause Toxicity upon Increased Protein Expression. *Cell Reports*, 16(1), 222-231. <https://doi.org/10.1016/j.celrep.2016.05.076>
- Borde, V., Robine, N., Lin, W., Bonfils, S., Géli, V., & Nicolas, A. (2009). Histone H3 lysine 4 trimethylation marks meiotic recombination initiation sites. *The EMBO Journal*, 28(2), 99-111. <https://doi.org/10.1038/emboj.2008.257>
- Börner, G. V., Hochwagen, A., & MacQueen, A. J. (2023). Meiosis in budding yeast. *Genetics*, 225(2), iyad125. <https://doi.org/10.1093/genetics/iyad125>
- Boy-Marcotte, E., Lagniel, G., Perrot, M., Bussereau, F., Boudsocq, A., Jacquet, M., & Labarre, J. (1999). The heat shock response in yeast: Differential regulations and contributions of the Msn2p/Msn4p and Hsf1p regulons. *Molecular Microbiology*, 33(2), 274-283. <https://doi.org/10.1046/j.1365-2958.1999.01467.x>
- Braun, K. A., & Young, E. T. (2014). Coupling mRNA synthesis and decay. *Molecular and Cellular Biology*, 34(22), 4078-4087. <https://doi.org/10.1128/MCB.00535-14>
- Bregues, M., & Parker, R. (2007). Accumulation of Polyadenylated mRNA, Pab1p, eIF4E, and eIF4G with P-Bodies in *Saccharomyces cerevisiae*. *Molecular Biology of the Cell*, 18(7), 2592-2602. <https://doi.org/10.1091/mbc.E06-12-1149>
- Brickner, D. G., Cajigas, I., Fondufe-Mittendorf, Y., Ahmed, S., Lee, P.-C., Widom, J., & Brickner, J. H. (2007). H2A.Z-Mediated Localization of Genes at the Nuclear Periphery Confers Epigenetic Memory of Previous Transcriptional State. *PLoS Biology*, 5(4), e81. <https://doi.org/10.1371/journal.pbio.0050081>
- Brito Querido, J., Sokabe, M., Kraatz, S., Gordiyenko, Y., Skehel, J. M., Fraser, C. S., & Ramakrishnan, V. (2020). Structure of a human 48S translational initiation complex. *Science (New York, N.Y.)*, 369(6508), 1220-1227. <https://doi.org/10.1126/science.aba4904>
- Buchan, J. R., Muhlrad, D., & Parker, R. (2008). P bodies promote stress granule assembly in *Saccharomyces cerevisiae*. *The Journal of Cell Biology*, 183(3), 441-455. <https://doi.org/10.1083/jcb.200807043>
- Buchan, J. R., Yoon, J.-H., & Parker, R. (2011). Stress-specific composition, assembly and kinetics of stress granules in *Saccharomyces cerevisiae*. *Journal of Cell Science*, 124(2), 228-239. <https://doi.org/10.1242/jcs.078444>
- Bullard, J. H., Purdom, E., Hansen, K. D., & Dudoit, S. (2010). Evaluation of statistical methods for normalization and differential expression in mRNA-Seq experiments. *BMC Bioinformatics*, 11, 94. <https://doi.org/10.1186/1471-2105-11-94>
- Cabal, G. G., Genovesio, A., Rodriguez-Navarro, S., Zimmer, C., Gadal, O., Lesne, A., Buc, H., Feuerbach-Fournier, F., Olivo-Marin, J.-C., Hurt, E. C., & Nehrbass, U. (2006). SAGA interacting factors confine sub-diffusion of transcribed genes to the nuclear envelope. *Nature*, 441(7094), 770-773. <https://doi.org/10.1038/nature04752>
- Carrozza, M. J., Florens, L., Swanson, S. K., Shia, W.-J., Anderson, S., Yates, J., Washburn, M. P., & Workman, J. L. (2005). Stable incorporation of sequence specific repressors Ash1 and Ume6 into the Rpd3L complex. *Biochimica et Biophysica Acta (BBA) - Gene Structure and Expression*, 1731(2), 77-87. <https://doi.org/10.1016/j.bbaexp.2005.09.005>
- Carrozza, M. J., Li, B., Florens, L., Suganuma, T., Swanson, S. K., Lee, K. K., Shia, W.-J., Anderson, S., Yates, J., Washburn, M. P., & Workman, J. L. (2005). Histone H3 Methylation by Set2 Directs Deacetylation of Coding Regions by Rpd3S to Suppress Spurious Intragenic Transcription. *Cell*, 123(4), 581-592. <https://doi.org/10.1016/j.cell.2005.10.023>
- Causton, H. C., Ren, B., Koh, S. S., Harbison, C. T., Kanin, E., Jennings, E. G., Lee, T. I., True, H. L., Lander, E. S., & Young, R. A. (2001). Remodeling of Yeast Genome Expression in Response to Environmental Changes. *Molecular Biology of the Cell*, 12(2), 323-337.



- Chaves-Arquero, B., Martínez-Lumbreras, S., Sibille, N., Camero, S., Bernadó, P., Jiménez, M. Á., Zorrilla, S., & Pérez-Cañadillas, J. M. (2022). eIF4G1 N-terminal intrinsically disordered domain is a multi-docking station for RNA, Pab1, Pub1, and self-assembly. *Frontiers in Molecular Biosciences*, 9, 986121. <https://doi.org/10.3389/fmolb.2022.986121>
- Chen, H., & Pugh, B. F. (2021). What do Transcription Factors Interact With? *Journal of Molecular Biology*, 433(14), 166883. <https://doi.org/10.1016/j.jmb.2021.166883>
- Chen, X.-F., Kuryan, B., Kitada, T., Tran, N., Li, J.-Y., Kurdastani, S., Grunstein, M., Li, B., & Carey, M. (2012). The Rpd3 Core Complex is a Chromatin Stabilization Module. *Current biology : CB*, 22(1), 56-63. <https://doi.org/10.1016/j.cub.2011.11.042>
- Chou, K. Y., Lee, J.-Y., Kim, K.-B., Kim, E., Lee, H.-S., & Ryu, H.-Y. (2023). Histone modification in *Saccharomyces cerevisiae*: A review of the current status. *Computational and Structural Biotechnology Journal*, 21, 1843-1850. <https://doi.org/10.1016/j.csbj.2023.02.037>
- Chowdhary, S., Kainth, A. S., Paracha, S., Gross, D. S., & Pincus, D. (2022). Inducible transcriptional condensates drive 3D genome reorganization in the heat shock response. *Molecular Cell*, 82(22), 4386-4399.e7. <https://doi.org/10.1016/j.molcel.2022.10.013>
- Christie, M., Chang, C.-W., Róna, G., Smith, K. M., Stewart, A. G., Takeda, A. A. S., Fontes, M. R. M., Stewart, M., Vértessy, B. G., Forwood, J. K., & Kobe, B. (2016). Structural Biology and Regulation of Protein Import into the Nucleus. *Journal of Molecular Biology*, 428(10 Pt A), 2060-2090. <https://doi.org/10.1016/j.jmb.2015.10.023>
- Chu, S., DeRisi, J., Eisen, M., Mulholland, J., Botstein, D., Brown, P. O., & Herskowitz, I. (1998). The Transcriptional Program of Sporulation in Budding Yeast. *Science*, 282(5389), 699-705. <https://doi.org/10.1126/science.282.5389.699>
- Chu, S., & Herskowitz, I. (1998). Gametogenesis in yeast is regulated by a transcriptional cascade dependent on Ndt80. *Molecular Cell*, 1(5), 685-696. [https://doi.org/10.1016/s1097-2765\(00\)80068-4](https://doi.org/10.1016/s1097-2765(00)80068-4)
- Ciccarelli, M., Masser, A. E., Kaimal, J. M., Planells, J., & Andréasson, C. (2023). Genetic inactivation of essential HSF1 reveals an isolated transcriptional stress response selectively induced by protein misfolding. *Molecular Biology of the Cell*, 34(10), ar101. <https://doi.org/10.1091/mbc.E23-05-0153>
- Cieniewicz, A. M., Moreland, L., Ringel, A. E., Mackintosh, S. G., Raman, A., Gilbert, T. M., Wolberger, C., Tackett, A. J., & Taverna, S. D. (2014). The Bromodomain of Gcn5 Regulates Site Specificity of Lysine Acetylation on Histone H3*. *Molecular & Cellular Proteomics*, 13(11), 2896-2910. <https://doi.org/10.1074/mcp.M114.038174>
- Clancy, M. J., Shambaugh, M. E., Timppte, C. S., & Bokar, J. A. (2002). Induction of sporulation in *Saccharomyces cerevisiae* leads to the formation of N6-methyladenosine in mRNA: A potential mechanism for the activity of the IME4 gene. *Nucleic Acids Research*, 30(20), 4509-4518. <https://doi.org/10.1093/nar/gkf573>
- Clarkson, B. K., Gilbert, W. V., & Doudna, J. A. (2010). Functional overlap between eIF4G isoforms in *Saccharomyces cerevisiae*. *PloS One*, 5(2), e9114. <https://doi.org/10.1371/journal.pone.0009114>
- Coleman, S. T., Epping, E. A., Steggerda, S. M., & Moye-Rowley, W. S. (1999). Yap1p activates gene transcription in an oxidant-specific fashion. *Molecular and Cellular Biology*, 19(12), 8302-8313. <https://doi.org/10.1128/MCB.19.12.8302>
- Collart, M. A., & Oliviero, S. (2001). Preparation of yeast RNA. *Current Protocols in Molecular Biology*, Chapter 13, Unit13.12. <https://doi.org/10.1002/0471142727.mb1312s23>
- Collet, J.-F., D'Souza, J. C., Jakob, U., & Bardwell, J. C. A. (2003). Thioredoxin 2, an oxidative stress-induced protein, contains a high affinity zinc binding site. *The Journal of Biological Chemistry*, 278(46), 45325-45332. <https://doi.org/10.1074/jbc.M307818200>
- Concordet, J.-P., & Haeussler, M. (2018). CRISPOR: Intuitive guide selection for CRISPR/Cas9 genome editing experiments and screens. *Nucleic Acids Research*, 46(W1), W242-W245. <https://doi.org/10.1093/nar/gky354>
- Conesa, A., Nueda, M. J., Ferrer, A., & Talón, M. (2006). maSigPro: A method to identify significantly differential expression profiles in time-course microarray experiments. *Bioinformatics (Oxford, England)*, 22(9), Article 9. <https://doi.org/10.1093/bioinformatics/btl056>
- Cooper, K. F. (1997a). Stress and developmental regulation of the yeast C-type cyclin Ume3p (Srb11p/Ssn8p). *The EMBO Journal*, 16(15), 4665-4675. <https://doi.org/10.1093/emboj/16.15.4665>



- Cooper, K. F. (1997b). Stress and developmental regulation of the yeast C-type cyclin Ume3p (Srb11p/Ssn8p). *The EMBO Journal*, 16(15), Article 15. <https://doi.org/10.1093/emboj/16.15.4665>
- Cooper, K. F., & Strich, R. (2002). Saccharomyces cerevisiae C-type cyclin Ume3p/Srb11p is required for efficient induction and execution of meiotic development. *Eukaryotic Cell*, 1(1), 66-74. <https://doi.org/10.1128/EC.01.1.66-74.2002>
- Cooper, K. F., & Strich, R. (2011). Meiotic control of the APC/C: Similarities & differences from mitosis. *Cell Division*, 6, 16. <https://doi.org/10.1186/1747-1028-6-16>
- Creamer, T. J., Darby, M. M., Jamonnak, N., Schaughency, P., Hao, H., Wheelan, S. J., & Corden, J. L. (2011). Transcriptome-Wide Binding Sites for Components of the Saccharomyces cerevisiae Non-Poly(A) Termination Pathway: Nrd1, Nab3, and Sen1. *PLoS Genetics*, 7(10), e1002329. <https://doi.org/10.1371/journal.pgen.1002329>
- Davidson, J. F., & Schiestl, R. H. (2001). Mitochondrial Respiratory Electron Carriers Are Involved in Oxidative Stress during Heat Stress in Saccharomyces cerevisiae. *Molecular and Cellular Biology*, 21(24), 8483-8489. <https://doi.org/10.1128/MCB.21.24.8483-8489.2001>
- Davidson, L., Francis, L., Eaton, J. D., & West, S. (2020). Integrator-Dependent and Allosteric/Intrinsic Mechanisms Ensure Efficient Termination of snRNA Transcription. *Cell Reports*, 33(4), 108319. <https://doi.org/10.1016/j.celrep.2020.108319>
- de Jonge, W. J., O'Duibhir, E., Lijnzaad, P., van Leenen, D., Groot Koerkamp, M. J., Kemmeren, P., & Holstege, F. C. (2017). Molecular mechanisms that distinguish TFIID housekeeping from regulatable SAGA promoters. *The EMBO Journal*, 36(3), 274-290. <https://doi.org/10.15252/emboj.201695621>
- De Wever, V., Reiter, W., Ballarini, A., Ammerer, G., & Brocard, C. (2005). A dual role for PP1 in shaping the Msn2-dependent transcriptional response to glucose starvation. *The EMBO Journal*, 24(23), 4115-4123. <https://doi.org/10.1038/sj.emboj.7600871>
- Dea, A., & Pincus, D. (2024). The heat shock response as a condensate cascade. *Journal of Molecular Biology*, 168642. <https://doi.org/10.1016/j.jmb.2024.168642>
- Delaunay, A., Pflieger, D., Barrault, M. B., Vinh, J., & Toledano, M. B. (2002). A thiol peroxidase is an H₂O₂ receptor and redox-transducer in gene activation. *Cell*, 111(4), 471-481. [https://doi.org/10.1016/s0092-8674\(02\)01048-6](https://doi.org/10.1016/s0092-8674(02)01048-6)
- DeVit, M. J., & Johnston, M. (1999). The nuclear exportin Msn5 is required for nuclear export of the Mig1 glucose repressor of Saccharomyces cerevisiae. *Current Biology: CB*, 9(21), 1231-1241. [https://doi.org/10.1016/s0960-9822\(99\)80503-x](https://doi.org/10.1016/s0960-9822(99)80503-x)
- Ding, Y., Liu, N., Virlouvet, L., Riethoven, J.-J., Fromm, M., & Avramova, Z. (2013). Four distinct types of dehydration stress memory genes in Arabidopsis thaliana. *BMC Plant Biology*, 13, 229. <https://doi.org/10.1186/1471-2229-13-229>
- Donczew, R., Warfield, L., Pacheco, D., Erijman, A., & Hahn, S. (2020). Two roles for the yeast transcription coactivator SAGA and a set of genes redundantly regulated by TFIID and SAGA. *eLife*, 9, e50109. <https://doi.org/10.7554/eLife.50109>
- Durant, M., Mucelli, X., & Huang, L. S. (2024). Meiotic Cytokinesis in Saccharomyces cerevisiae: Spores That Just Need Closure. *Journal of Fungi*, 10(2), 132. <https://doi.org/10.3390/jof10020132>
- Durchschlag, E., Reiter, W., Ammerer, G., & Schüller, C. (2004). Nuclear localization destabilizes the stress-regulated transcription factor Msn2. *The Journal of Biological Chemistry*, 279(53), 55425-55432. <https://doi.org/10.1074/jbc.M407264200>
- D'Urso, A., Takahashi, Y., Xiong, B., Marone, J., Coukos, R., Randise-Hinchliff, C., Wang, J.-P., Shilatifard, A., & Brickner, J. H. (2016). Set1/COMPASS and Mediator are repurposed to promote epigenetic transcriptional memory. *eLife*, 5, e16691. <https://doi.org/10.7554/eLife.16691>
- Eaton, J. D., & West, S. (2020). Termination of Transcription by RNA Polymerase II: BOOM! *Trends in Genetics: TIG*, 36(9), 664-675. <https://doi.org/10.1016/j.tig.2020.05.008>
- Engel, C., Neyer, S., & Cramer, P. (2018). Distinct Mechanisms of Transcription Initiation by RNA Polymerases I and II. *Annual Review of Biophysics*, 47(1), 425-446. <https://doi.org/10.1146/annurev-biophys-070317-033058>
- Estruch, F. (2000). Stress-controlled transcription factors, stress-induced genes and stress tolerance in budding yeast. *FEMS Microbiology Reviews*, 24(4), 469-486. <https://doi.org/10.1111/j.1574-6976.2000.tb00551.x>



- Fabrizio, P., Garvis, S., & Palladino, F. (2019). Histone Methylation and Memory of Environmental Stress. *Cells*, 8(4), 339. <https://doi.org/10.3390/cells8040339>
- Fernandez-Martinez, J., Kim, S. J., Shi, Y., Upla, P., Pellarin, R., Gagnon, M., Chemmama, I. E., Wang, J., Nudelman, I., Zhang, W., Williams, R., Rice, W. J., Stokes, D. L., Zenklusen, D., Chait, B. T., Sali, A., & Rout, M. P. (2016). Structure and Function of the Nuclear Pore Complex Cytoplasmic mRNA Export Platform. *Cell*, 167(5), 1215-1228.e25. <https://doi.org/10.1016/j.cell.2016.10.028>
- Fornerod, M., Ohno, M., Yoshida, M., & Mattaj, I. W. (1997). CRM1 Is an Export Receptor for Leucine-Rich Nuclear Export Signals. *Cell*, 90(6), 1051-1060. [https://doi.org/10.1016/S0092-8674\(00\)80371-2](https://doi.org/10.1016/S0092-8674(00)80371-2)
- Fox, M. J., & Mosley, A. L. (2016). Rrp6: Integrated roles in nuclear RNA metabolism and transcription termination. *Wiley interdisciplinary reviews. RNA*, 7(1), 91-104. <https://doi.org/10.1002/wrna.1317>
- Galán, A., & Rodríguez-Navarro, S. (2012). Sus1/ENY2: A multitasking protein in eukaryotic gene expression. *Critical Reviews in Biochemistry and Molecular Biology*, 47(6), 556-568. <https://doi.org/10.3109/10409238.2012.730498>
- Galdieri, L., Mehrotra, S., Yu, S., & Vancura, A. (2010). Transcriptional Regulation in Yeast during Diauxic Shift and Stationary Phase. *OMICS : a Journal of Integrative Biology*, 14(6), 629-638. <https://doi.org/10.1089/omi.2010.0069>
- Gao, J., Qin, Y., & Schimenti, J. C. (2024). Gene regulation during meiosis. *Trends in Genetics: TIG*, 40(4), 326-336. <https://doi.org/10.1016/j.tig.2023.12.006>
- Gardner, J. M., & Jaspersen, S. L. (2014). Manipulating the Yeast Genome: Deletion, Mutation, and Tagging by PCR. En J. S. Smith & D. J. Burke (Eds.), *Yeast Genetics: Methods and Protocols* (pp. 45-78). Springer. https://doi.org/10.1007/978-1-4939-1363-3_5
- Garrido-Godino, A. I., Gupta, I., Gutiérrez-Santiago, F., Martínez-Padilla, A. B., Alekseenko, A., Steinmetz, L. M., Pérez-Ortín, J. E., Pelechano, V., & Navarro, F. (2021). Rpb4 and Puf3 imprint and post-transcriptionally control the stability of a common set of mRNAs in yeast. *RNA Biology*, 18(8), 1206-1220. <https://doi.org/10.1080/15476286.2020.1839229>
- Gasch, A. P., Spellman, P. T., Kao, C. M., Carmel-Harel, O., Eisen, M. B., Storz, G., Botstein, D., & Brown, P. O. (2000). Genomic expression programs in the response of yeast cells to environmental changes. *Molecular Biology of the Cell*, 11(12), 4241-4257. <https://doi.org/10.1091/mbc.11.12.4241>
- Gavade, J. N., Puccia, C. M., Herod, S. G., Trinidad, J. C., Berchowitz, L. E., & Lacefield, S. (2022). Identification of 14-3-3 proteins, Polo kinase, and RNA-binding protein Pes4 as key regulators of meiotic commitment in budding yeast. *Current Biology: CB*, 32(7), 1534-1547.e9. <https://doi.org/10.1016/j.cub.2022.02.022>
- Gebauer, F., Schwarzl, T., Valcárcel, J., & Hentze, M. W. (2021). RNA-binding proteins in human genetic disease. *Nature Reviews Genetics*, 22(3), 185-198. <https://doi.org/10.1038/s41576-020-00302-y>
- Ghaemmaghami, S., Huh, W.-K., Bower, K., Howson, R. W., Belle, A., Dephoure, N., O'Shea, E. K., & Weissman, J. S. (2003). Global analysis of protein expression in yeast. *Nature*, 425(6959), 737-741. <https://doi.org/10.1038/nature02046>
- Gialitakis, M., Arampatzi, P., Makatounakis, T., & Papamatheakis, J. (2010). Gamma Interferon-Dependent Transcriptional Memory via Relocalization of a Gene Locus to PML Nuclear Bodies. *Molecular and Cellular Biology*, 30(8), 2046-2056. <https://doi.org/10.1128/MCB.00906-09>
- Gietz, R. D., & Schiestl, R. H. (2007). High-efficiency yeast transformation using the LiAc/SS carrier DNA/PEG method. *Nature Protocols*, 2(1), 31-34. <https://doi.org/10.1038/nprot.2007.13>
- Girbig, M., Misiaszek, A. D., & Müller, C. W. (2022). Structural insights into nuclear transcription by eukaryotic DNA-dependent RNA polymerases. *Nature Reviews Molecular Cell Biology*, 23(9), 603-622. <https://doi.org/10.1038/s41580-022-00476-9>
- Glisovic, T., Bachorik, J. L., Yong, J., & Dreyfuss, G. (2008). RNA-binding proteins and post-transcriptional gene regulation. *FEBS Letters*, 582(14), 1977-1986. <https://doi.org/10.1016/j.febslet.2008.03.004>
- Goldmark, J. P., Fazio, T. G., Estep, P. W., Church, G. M., & Tsukiyama, T. (2000). The Isw2 chromatin remodeling complex represses early meiotic genes upon recruitment by Ume6p. *Cell*, 103(3), 423-433. [https://doi.org/10.1016/S0092-8674\(00\)00134-3](https://doi.org/10.1016/S0092-8674(00)00134-3)
- Görner, W., Durchschlag, E., Wolf, J., Brown, E. L., Ammerer, G., Ruis, H., & Schüller, C. (2002). Acute glucose starvation activates the nuclear localization signal of a stress-specific yeast transcription factor. *The EMBO Journal*, 21(1-2), 135-144. <https://doi.org/10.1093/emboj/21.1.135>



- Goyer, C., Altmann, M., Lee, H. S., Blanc, A., Deshmukh, M., Woolford, J. L., Trachsel, H., & Sonenberg, N. (1993). TIF4631 and TIF4632: Two yeast genes encoding the high-molecular-weight subunits of the cap-binding protein complex (eukaryotic initiation factor 4F) contain an RNA recognition motif-like sequence and carry out an essential function. *Molecular and Cellular Biology*, 13(8), 4860-4874. <https://doi.org/10.1128/mcb.13.8.4860-4874.1993>
- Grant, P. A., Winston, F., & Berger, S. L. (2021). The biochemical and genetic discovery of the SAGA complex. *Biochimica et Biophysica Acta (BBA) - Gene Regulatory Mechanisms*, 1864(2), 194669. <https://doi.org/10.1016/j.bbagr.2020.194669>
- Green, M. R., Sambrook, J., & Sambrook, J. (2012). *Molecular cloning: A laboratory manual* (4th ed). Cold Spring Harbor Laboratory Press.
- Grousl, T., Vojtova, J., Hasek, J., & Vomastek, T. (2022). Yeast stress granules at a glance. *Yeast (Chichester, England)*, 39(4), 247-261. <https://doi.org/10.1002/yea.3681>
- Grüner, S., Weber, R., Peter, D., Chung, M.-Y., Igraja, C., Valkov, E., & Izaurralde, E. (2018). Structural motifs in eIF4G and 4E-BPs modulate their binding to eIF4E to regulate translation initiation in yeast. *Nucleic Acids Research*, 46(13), 6893-6908. <https://doi.org/10.1093/nar/gky542>
- Grunstein, M. (1997). Histone acetylation in chromatin structure and transcription. *Nature*, 389(6649), 349-352. <https://doi.org/10.1038/38664>
- Guzikowski, A. R., Chen, Y. S., & Zid, B. M. (2019). Stress-Induced mRNP Granules: Form and Function of P-bodies and Stress Granules. *Wiley interdisciplinary reviews. RNA*, 10(3), e1524. <https://doi.org/10.1002/wrna.1524>
- Gwizdek, C., Iglesias, N., Rodriguez, M. S., Ossareh-Nazari, B., Hobeika, M., Divita, G., Stutz, F., & Dargemont, C. (2006). Ubiquitin-associated domain of Mex67 synchronizes recruitment of the mRNA export machinery with transcription. *Proceedings of the National Academy of Sciences of the United States of America*, 103(44), 16376-16381. <https://doi.org/10.1073/pnas.0607941103>
- Ha, S. D., Ham, S., Kim, M. Y., Kim, J. H., Jang, I., Lee, B. B., Lee, M. K., Hwang, J.-T., Roh, T.-Y., & Kim, T. (2019). Transcription-dependent targeting of Hda1C to hyperactive genes mediates H4-specific deacetylation in yeast. *Nature Communications*, 10, 4270. <https://doi.org/10.1038/s41467-019-12077-w>
- Hahn, S. (2004). Structure and mechanism of the RNA Polymerase II transcription machinery. *Nature structural & molecular biology*, 11(5), 394-403. <https://doi.org/10.1038/nsmb763>
- Haimovich, G., Medina, D. A., Causse, S. Z., Garber, M., Millán-Zambrano, G., Barkai, O., Chávez, S., Pérez-Ortín, J. E., Darzacq, X., & Choder, M. (2013). Gene Expression Is Circular: Factors for mRNA Degradation Also Foster mRNA Synthesis. *Cell*, 153(5), 1000-1011. <https://doi.org/10.1016/j.cell.2013.05.012>
- Halliwell, B. (2006). Reactive species and antioxidants. Redox biology is a fundamental theme of aerobic life. *Plant Physiology*, 141(2), 312-322. <https://doi.org/10.1104/pp.106.077073>
- Harel-Sharvit, L., Eldad, N., Haimovich, G., Barkai, O., Duek, L., & Choder, M. (2010). RNA polymerase II subunits link transcription and mRNA decay to translation. *Cell*, 143(4), 552-563. <https://doi.org/10.1016/j.cell.2010.10.033>
- Harris, A., & Ünal, E. (2023). The transcriptional regulator Ume6 is a major driver of early gene expression during gametogenesis. *Genetics*, 225(2), iyad123. <https://doi.org/10.1093/genetics/iyad123>
- Harshman, K. D., Moye-Rowley, W. S., & Parker, C. S. (1988). Transcriptional activation by the SV40 AP-1 recognition element in yeast is mediated by a factor similar to AP-1 that is distinct from GCN4. *Cell*, 53(2), 321-330. [https://doi.org/10.1016/0092-8674\(88\)90393-5](https://doi.org/10.1016/0092-8674(88)90393-5)
- Hill, C. H., Boreikaitė, V., Kumar, A., Casañal, A., Kubík, P., Degliesposti, G., Maslen, S., Mariani, A., von Loeffelholz, O., Girbig, M., Skehel, M., & Passmore, L. A. (2019). Activation of the Endonuclease that Defines mRNA 3' Ends Requires Incorporation into an 8-Subunit Core Cleavage and Polyadenylation Factor Complex. *Molecular Cell*, 73(6), 1217-1231.e11. <https://doi.org/10.1016/j.molcel.2018.12.023>
- Hillers, K. J., Jantsch, V., Martinez-Perez, E., & Yanowitz, J. L. (2017). Meiosis. *WormBook: The Online Review of C. Elegans Biology*, 2017, 1-43. <https://doi.org/10.1895/wormbook.1.178.1>
- Hobeika, M., Brockmann, C., Gruessing, F., Neuhaus, D., Divita, G., Stewart, M., & Dargemont, C. (2009). Structural Requirements for the Ubiquitin-associated Domain of the mRNA Export Factor Mex67 to Bind Its Specific Targets, the Transcription Elongation THO Complex Component Hpr1 and Nucleoporin FXFG Repeats. *The Journal of Biological Chemistry*, 284(26), 17575-17583. <https://doi.org/10.1074/jbc.M109.004374>



- Hochwagen, A., & Amon, A. (2006). Checking your breaks: Surveillance mechanisms of meiotic recombination. *Current Biology: CB*, 16(6), R217-228. <https://doi.org/10.1016/j.cub.2006.03.009>
- Hofmann, S., Kedersha, N., Anderson, P., & Ivanov, P. (2021). Molecular mechanisms of stress granule assembly and disassembly. *Biochimica Et Biophysica Acta. Molecular Cell Research*, 1868(1), 118876. <https://doi.org/10.1016/j.bbamcr.2020.118876>
- Hogan, D. J., Riordan, D. P., Gerber, A. P., Herschlag, D., & Brown, P. O. (2008). Diverse RNA-Binding Proteins Interact with Functionally Related Sets of RNAs, Suggesting an Extensive Regulatory System. *PLoS Biology*, 6(10), e255. <https://doi.org/10.1371/journal.pbio.0060255>
- Hongay, C. F., Grisafi, P. L., Galitski, T., & Fink, G. R. (2006). Antisense transcription controls cell fate in *Saccharomyces cerevisiae*. *Cell*, 127(4), 735-745. <https://doi.org/10.1016/j.cell.2006.09.038>
- Horvathova, I., Voigt, F., Kotrys, A. V., Zhan, Y., Artus-Revel, C. G., Eglinger, J., Stadler, M. B., Giorgetti, L., & Chao, J. A. (2017). The Dynamics of mRNA Turnover Revealed by Single-Molecule Imaging in Single Cells. *Molecular Cell*, 68(3), 615-625.e9. <https://doi.org/10.1016/j.molcel.2017.09.030>
- Hoyle, N. P., Castelli, L. M., Campbell, S. G., Holmes, L. E. A., & Ashe, M. P. (2007). Stress-dependent relocalization of translationally primed mRNPs to cytoplasmic granules that are kinetically and spatially distinct from P-bodies. *The Journal of Cell Biology*, 179(1), 65-74. <https://doi.org/10.1083/jcb.200707010>
- Hu, Z., Wang, Y., Yu, L., Mahanty, S. K., Mendoza, N., & Elion, E. A. (2016). Mapping regions in Ste5 that support Msn5-dependent and -independent nuclear export. *Biochemistry and Cell Biology = Biochimie Et Biologie Cellulaire*, 94(2), 109-128. <https://doi.org/10.1139/bcb-2015-0101>
- Hubstenberger, A., Courel, M., Bénard, M., Souquere, S., Ernoult-Lange, M., Chouaib, R., Yi, Z., Morlot, J.-B., Munier, A., Fradet, M., Daunesse, M., Bertrand, E., Pierron, G., Mozziconacci, J., Kress, M., & Weil, D. (2017). P-Body Purification Reveals the Condensation of Repressed mRNA Regulons. *Molecular Cell*, 68(1), 144-157.e5. <https://doi.org/10.1016/j.molcel.2017.09.003>
- Iglesias, N., Tutucci, E., Gwizdek, C., Vinciguerra, P., Von Dach, E., Corbett, A. H., Dargemont, C., & Stutz, F. (2010). Ubiquitin-mediated mRNP dynamics and surveillance prior to budding yeast mRNA export. *Genes & Development*, 24(17), 1927-1938. <https://doi.org/10.1101/gad.583310>
- Ivanov, P., Kedersha, N., & Anderson, P. (2019). Stress Granules and Processing Bodies in Translational Control. *Cold Spring Harbor Perspectives in Biology*, 11(5), a032813. <https://doi.org/10.1101/cshperspect.a032813>
- Jacobs, R. Q., & Schneider, D. A. (2024). Transcription elongation mechanisms of RNA polymerases I, II, and III and their therapeutic implications. *The Journal of Biological Chemistry*, 300(3), 105737. <https://doi.org/10.1016/j.jbc.2024.105737>
- Jain, N. K., & Roy, I. (2009). Effect of trehalose on protein structure. *Protein Science: A Publication of the Protein Society*, 18(1), 24-36. <https://doi.org/10.1002/pro.3>
- Jain, S., Wheeler, J. R., Walters, R. W., Agrawal, A., Barsic, A., & Parker, R. (2016). ATPase-Modulated Stress Granules Contain a Diverse Proteome and Substructure. *Cell*, 164(3), 487-498. <https://doi.org/10.1016/j.cell.2015.12.038>
- Janke, C., Magiera, M. M., Rathfelder, N., Taxis, C., Reber, S., Maekawa, H., Moreno-Borchart, A., Doenges, G., Schwob, E., Schiebel, E., & Knop, M. (2004). A versatile toolbox for PCR-based tagging of yeast genes: New fluorescent proteins, more markers and promoter substitution cassettes. *Yeast (Chichester, England)*, 21(11), 947-962. <https://doi.org/10.1002/yea.1142>
- Jin, L., & Neiman, A. M. (2016). Post-transcriptional regulation in budding yeast meiosis. *Current Genetics*, 62(2), 313-315. <https://doi.org/10.1007/s00294-015-0546-2>
- Jin, L., Zhang, K., Sternglanz, R., & Neiman, A. M. (2017a). Predicted RNA Binding Proteins Pes4 and Mip6 Regulate mRNA Levels, Translation, and Localization during Sporulation in Budding Yeast. *Molecular and Cellular Biology*, 37(9), Article 9. <https://doi.org/10.1128/MCB.00408-16>
- Jin, L., Zhang, K., Sternglanz, R., & Neiman, A. M. (2017b). Predicted RNA Binding Proteins Pes4 and Mip6 Regulate mRNA Levels, Translation, and Localization during Sporulation in Budding Yeast. *Molecular and Cellular Biology*, 37(9), e00408-16. <https://doi.org/10.1128/MCB.00408-16>
- Kadosh, D., & Struhl, K. (1998). Histone deacetylase activity of Rpd3 is important for transcriptional repression in vivo. *Genes & Development*, 12(6), 797-805.
- Kane, S. M., & Roth, R. (1974a). *Carbohydrate Metabolism During Ascospore Development in Yeast*. <https://doi.org/10.1128/jb.118.1.8-14.1974>



- Kane, S. M., & Roth, R. (1974b). *Carbohydrate Metabolism During Ascospore Development in Yeast*. <https://doi.org/10.1128/jb.118.1.8-14.1974>
- Katahira, J., Strässer, K., Podtelejnikov, A., Mann, M., Jung, J. U., & Hurt, E. (1999). The Mex67p-mediated nuclear mRNA export pathway is conserved from yeast to human. *The EMBO Journal*, 18(9), 2593-2609. <https://doi.org/10.1093/emboj/18.9.2593>
- Kato, K., Yamamoto, Y., & Izawa, S. (2011). Severe ethanol stress induces assembly of stress granules in *Saccharomyces cerevisiae*. *Yeast (Chichester, England)*, 28(5), 339-347. <https://doi.org/10.1002/yea.1842>
- Kedersha, N., Chen, S., Gilks, N., Li, W., Miller, I. J., Stahl, J., & Anderson, P. (2002). Evidence that ternary complex (eIF2-GTP-tRNA(i)(Met))-deficient preinitiation complexes are core constituents of mammalian stress granules. *Molecular Biology of the Cell*, 13(1), 195-210. <https://doi.org/10.1091/mbc.01-05-0221>
- Keogh, M.-C., Kim, J.-A., Downey, M., Fillingham, J., Chowdhury, D., Harrison, J. C., Onishi, M., Datta, N., Galicia, S., Emili, A., Lieberman, J., Shen, X., Buratowski, S., Haber, J. E., Durocher, D., Greenblatt, J. F., & Krogan, N. J. (2006). A phosphatase complex that dephosphorylates γ H2AX regulates DNA damage checkpoint recovery. *Nature*, 439(7075), Article 7075. <https://doi.org/10.1038/nature04384>
- Keogh, M.-C., Kurdastani, S. K., Morris, S. A., Ahn, S. H., Podolny, V., Collins, S. R., Schuldiner, M., Chin, K., Punna, T., Thompson, N. J., Boone, C., Emili, A., Weissman, J. S., Hughes, T. R., Strahl, B. D., Grunstein, M., Greenblatt, J. F., Buratowski, S., & Krogan, N. J. (2005). Cotranscriptional set2 methylation of histone H3 lysine 36 recruits a repressive Rpd3 complex. *Cell*, 123(4), 593-605. <https://doi.org/10.1016/j.cell.2005.10.025>
- Khatte, H., Vorländer, M. K., & Müller, C. W. (2017). RNA polymerase I and III: Similar yet unique. *Current Opinion in Structural Biology*, 47, 88-94. <https://doi.org/10.1016/j.sbi.2017.05.008>
- Kiledjian, M. (2018). Eukaryotic RNA 5'-end NAD⁺ Capping and deNADding. *Trends in cell biology*, 28(6), 454-464. <https://doi.org/10.1016/j.tcb.2018.02.005>
- Kim, S. J., Fernandez-Martinez, J., Nudelman, I., Shi, Y., Zhang, W., Raveh, B., Herricks, T., Slaughter, B. D., Hogan, J., Upla, P., Chemmama, I. E., Pellarin, R., Echeverria, I., Shivaraju, M., Chaudhury, A. S., Wang, J., Williams, R., Unruh, J. R., Greenberg, C. H., ... Rout, M. P. (2018). Integrative Structure and Functional Anatomy of a Nuclear Pore Complex. *Nature*, 555(7697), 475-482. <https://doi.org/10.1038/nature26003>
- Kim, S. J., & Strich, R. (2016). Rpl22 is required for IME1 mRNA translation and meiotic induction in *S. cerevisiae*. *Cell Division*, 11, 10. <https://doi.org/10.1186/s13008-016-0024-3>
- Knutson, B. A., & Hahn, S. (2011). Domains of Tral Important for Activator Recruitment and Transcription Coactivator Functions of SAGA and NuA4 Complexes. *Molecular and Cellular Biology*, 31(4), 818-831. <https://doi.org/10.1128/MCB.00687-10>
- Koch, B., Mitterer, V., Niederhauser, J., Stanborough, T., Murat, G., Rechberger, G., Bergler, H., Kressler, D., & Pertschy, B. (2012a). Yar1 Protects the Ribosomal Protein Rps3 from Aggregation. *Journal of Biological Chemistry*, 287(26), 21806-21815. <https://doi.org/10.1074/jbc.M112.365791>
- Koch, B., Mitterer, V., Niederhauser, J., Stanborough, T., Murat, G., Rechberger, G., Bergler, H., Kressler, D., & Pertschy, B. (2012b). Yar1 Protects the Ribosomal Protein Rps3 from Aggregation. *Journal of Biological Chemistry*, 287(26), Article 26. <https://doi.org/10.1074/jbc.M112.365791>
- Kosugi, S., Hasebe, M., Tomita, M., & Yanagawa, H. (2008). Nuclear Export Signal Consensus Sequences Defined Using a Localization-Based Yeast Selection System. *Traffic*, 9(12), 2053-2062. <https://doi.org/10.1111/j.1600-0854.2008.00825.x>
- Kouzarides, T. (2007). Chromatin Modifications and Their Function. *Cell*, 128(4), 693-705. <https://doi.org/10.1016/j.cell.2007.02.005>
- Kremer, S. B., & Gross, D. S. (2009). SAGA and Rpd3 chromatin modification complexes dynamically regulate heat shock gene structure and expression. *The Journal of Biological Chemistry*, 284(47), 32914-32931. <https://doi.org/10.1074/jbc.M109.058610>
- Krogan, N. J., Dover, J., Wood, A., Schneider, J., Heidt, J., Boateng, M. A., Dean, K., Ryan, O. W., Golshani, A., Johnston, M., Greenblatt, J. F., & Shilatifard, A. (2003). The Paf1 Complex Is Required for Histone H3 Methylation by COMPASS and Dot1p: Linking Transcriptional Elongation to Histone Methylation. *Molecular Cell*, 11(3), 721-729. [https://doi.org/10.1016/S1097-2765\(03\)00091-1](https://doi.org/10.1016/S1097-2765(03)00091-1)
- Küenzi, M. T., Tingle, M. A., & Halvorson, H. O. (1974). Sporulation of *Saccharomyces cerevisiae* in the Absence of a Functional Mitochondrial Genome. *Journal of Bacteriology*, 117(1), 80-88.



- Kumar, D., Varshney, S., Sengupta, S., & Sharma, N. (2019). A comparative study of the proteome regulated by the Rpb4 and Rpb7 subunits of RNA polymerase II in fission yeast. *Journal of Proteomics*, 199, 77-88. <https://doi.org/10.1016/j.jprot.2019.03.007>
- Kundu, S., & Peterson, C. L. (2010). Dominant Role for Signal Transduction in the Transcriptional Memory of Yeast GAL Genes. *Molecular and Cellular Biology*, 30(10), 2330-2340. <https://doi.org/10.1128/MCB.01675-09>
- Kurdistani, S. K., & Grunstein, M. (2003). Histone acetylation and deacetylation in yeast. *Nature Reviews Molecular Cell Biology*, 4(4), 276-284. <https://doi.org/10.1038/nrm1075>
- Kushnirov, V. V. (2000). Rapid and reliable protein extraction from yeast. *Yeast (Chichester, England)*, 16(9), 857-860. [https://doi.org/10.1002/1097-0061\(20000630\)16:9<857::AID-YEA561>3.0.CO;2-B](https://doi.org/10.1002/1097-0061(20000630)16:9<857::AID-YEA561>3.0.CO;2-B)
- la Cour, T., Gupta, R., Rapacki, K., Skriver, K., Poulsen, F. M., & Brunak, S. (2003). NESbase version 1.0: A database of nuclear export signals. *Nucleic Acids Research*, 31(1), 393-396. <https://doi.org/10.1093/nar/gkg101>
- Lane, S., & Kauppi, L. (2019). Meiotic spindle assembly checkpoint and aneuploidy in males versus females. *Cellular and Molecular Life Sciences: CMLS*, 76(6), 1135-1150. <https://doi.org/10.1007/s00018-018-2986-6>
- Lapointe, C. P., Grosely, R., Sokabe, M., Alvarado, C., Wang, J., Montabana, E., Villa, N., Shin, B.-S., Dever, T. E., Fraser, C. S., Fernández, I. S., & Puglisi, J. D. (2022). eIF5B and eIF1A reorient initiator tRNA to allow ribosomal subunit joining. *Nature*, 607(7917), 185-190. <https://doi.org/10.1038/s41586-022-04858-z>
- Laughery, M. F., Hunter, T., Brown, A., Hoopes, J., Ostbye, T., Shumaker, T., & Wyrick, J. J. (2015). New vectors for simple and streamlined CRISPR-Cas9 genome editing in *Saccharomyces cerevisiae*: Vectors for simple CRISPR-Cas9 genome editing in yeast. *Yeast*, 32(12), 711-720. <https://doi.org/10.1002/yea.3098>
- Law, M. J., Mallory, M. J., Dunbrack, R. L., & Strich, R. (2014). Acetylation of the Transcriptional Repressor Ume6p Allows Efficient Promoter Release and Timely Induction of the Meiotic Transient Transcription Program in Yeast. *Molecular and Cellular Biology*, 34(4), 631-642. <https://doi.org/10.1128/MCB.00256-13>
- Lee, B. B., Choi, A., Kim, J. H., Jun, Y., Woo, H., Ha, S. D., Yoon, C. Y., Hwang, J.-T., Steinmetz, L., Buratowski, S., Lee, S., Kim, H. Y., & Kim, T. (2018). Rpd3L HDAC links H3K4me3 to transcriptional repression memory. *Nucleic Acids Research*, 46(16), 8261-8274. <https://doi.org/10.1093/nar/gky573>
- Lee, M. K., & Kim, T. (2020). Histone H4-Specific Deacetylation at Active Coding Regions by Hda1C. *Molecules and Cells*, 43(10), 841-847. <https://doi.org/10.14348/molcells.2020.0141>
- Lee, P., Cho, B.-R., Joo, H.-S., & Hahn, J.-S. (2008). Yeast Yak1 kinase, a bridge between PKA and stress-responsive transcription factors, Hsf1 and Msn2/Msn4. *Molecular Microbiology*, 70(4), 882-895. <https://doi.org/10.1111/j.1365-2958.2008.06450.x>
- Lee, P., Kim, M. S., Paik, S.-M., Choi, S.-H., Cho, B.-R., & Hahn, J.-S. (2013). Rim15-dependent activation of Hsf1 and Msn2/4 transcription factors by direct phosphorylation in *Saccharomyces cerevisiae*. *FEBS Letters*, 587(22), 3648-3655. <https://doi.org/10.1016/j.febslet.2013.10.004>
- Lee, Y. M., Kim, E., An, J., Lee, Y., Choi, E., Choi, W., Moon, E., & Kim, W. (2017). Dissection of the HOG pathway activated by hydrogen peroxide in *accharomyces cerevisiae*. *Environmental Microbiology*, 19(2), 584-597. <https://doi.org/10.1111/1462-2920.13499>
- Lee-Soety, J. Y., Resch, G., Rimal, A., Johnson, E. S., Benway, J., & Winter, E. (2024). The MAPK homolog, Smk1, promotes assembly of the glucan layer of the spore wall in *S. cerevisiae*. *Yeast (Chichester, England)*. <https://doi.org/10.1002/yea.3967>
- Li, B., Zeis, P., Zhang, Y., Alekseenko, A., Fürst, E., Sanchez, Y. P., Lin, G., Tekkedil, M. M., Piazza, I., Steinmetz, L. M., & Pelechano, V. (2023). Differential regulation of mRNA stability modulates transcriptional memory and facilitates environmental adaptation. *Nature Communications*, 14(1), 910. <https://doi.org/10.1038/s41467-023-36586-x>
- Light, W. H., Freaney, J., Sood, V., Thompson, A., D'Urso, A., Horvath, C. M., & Brickner, J. H. (2013). A Conserved Role for Human Nup98 in Altering Chromatin Structure and Promoting Epigenetic Transcriptional Memory. *PLoS Biology*, 11(3), e1001524. <https://doi.org/10.1371/journal.pbio.1001524>
- Liguori, I., Russo, G., Curcio, F., Bulli, G., Aran, L., Della-Morte, D., Gargiulo, G., Testa, G., Cacciatore, F., Bonaduce, D., & Abete, P. (2018). Oxidative stress, aging, and diseases. *Clinical Interventions in Aging*, 13, 757-772. <https://doi.org/10.2147/CIA.S158513>
- Lin, A., Du, Y., & Xiao, W. (2020). Yeast chromatin remodeling complexes and their roles in transcription. *Current Genetics*, 66(4), 657-670. <https://doi.org/10.1007/s00294-020-01072-0>



- Lin, D. H., & Hoelz, A. (2019). The Structure of the Nuclear Pore Complex (An Update). *Annual review of biochemistry*, 88, 725-783. <https://doi.org/10.1146/annurev-biochem-062917-011901>
- Liu, N., Ding, Y., Fromm, M., & Avramova, Z. (2014). Different gene-specific mechanisms determine the «revised-response» memory transcription patterns of a subset of *A. thaliana* dehydration stress responding genes. *Nucleic Acids Research*, 42(9), 5556-5566. <https://doi.org/10.1093/nar/gku220>
- Livak, K. J., & Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods (San Diego, Calif.)*, 25(4), 402-408. <https://doi.org/10.1006/meth.2001.1262>
- Llácer, J. L., Hussain, T., Marler, L., Aitken, C. E., Thakur, A., Lorsch, J. R., Hinnebusch, A. G., & Ramakrishnan, V. (2015). Conformational Differences between Open and Closed States of the Eukaryotic Translation Initiation Complex. *Molecular Cell*, 59(3), 399-412. <https://doi.org/10.1016/j.molcel.2015.06.033>
- MacIsaac, K. D., Wang, T., Gordon, D. B., Gifford, D. K., Stormo, G. D., & Fraenkel, E. (2006). An improved map of conserved regulatory sites for *Saccharomyces cerevisiae*. *BMC Bioinformatics*, 7, 113. <https://doi.org/10.1186/1471-2105-7-113>
- Madeira, F., Madhusoodanan, N., Lee, J., Eusebi, A., Niewielska, A., Tivey, A. R. N., Lopez, R., & Butcher, S. (2024). The EMBL-EBI Job Dispatcher sequence analysis tools framework in 2024. *Nucleic Acids Research*, gkae241. <https://doi.org/10.1093/nar/gkae241>
- Malathi, K., Xiao, Y., & Mitchell, A. P. (1997). Interaction of yeast repressor-activator protein Ume6p with glycogen synthase kinase 3 homolog Rim11p. *Molecular and Cellular Biology*, 17(12), 7230-7236. <https://doi.org/10.1128/MCB.17.12.7230>
- Mallory, M. J., Cooper, K. F., & Strich, R. (2007). Meiosis-specific destruction of the Ume6p repressor by the Cdc20-directed APC/C. *Molecular Cell*, 27(6), 951-961. <https://doi.org/10.1016/j.molcel.2007.08.019>
- Mallory, M. J., Law, M. J., Sterner, D. E., Berger, S. L., & Strich, R. (2012). Gcn5p-dependent acetylation induces degradation of the meiotic transcriptional repressor Ume6p. *Molecular Biology of the Cell*, 23(9), 1609-1617. <https://doi.org/10.1091/mbc.E11-06-0536>
- Mallory, M. J., & Strich, R. (2003). Ume1p represses meiotic gene transcription in *Saccharomyces cerevisiae* through interaction with the histone deacetylase Rpd3p. *The Journal of Biological Chemistry*, 278(45), 44727-44734. <https://doi.org/10.1074/jbc.M308632200>
- Mandel, C. R., Kaneko, S., Zhang, H., Gebauer, D., Vethantham, V., Manley, J. L., & Tong, L. (2006). Polyadenylation factor CPSF-73 is the pre-mRNA 3'-end processing endonuclease. *Nature*, 444(7121), 10.1038/nature05363. <https://doi.org/10.1038/nature05363>
- Mans, R., Van Rossum, H. M., Wijsman, M., Backx, A., Kuijpers, N. G. A., Van Den Broek, M., Daran-Lapujade, P., Pronk, J. T., Van Maris, A. J. A., & Daran, J.-M. G. (2015). CRISPR/Cas9: A molecular Swiss army knife for simultaneous introduction of multiple genetic modifications in *Saccharomyces cerevisiae*. *FEMS Yeast Research*, 15(2). <https://doi.org/10.1093/femsyr/fov004>
- Mans, R., Wijsman, M., Daran-Lapujade, P., & Daran, J.-M. (2018). A protocol for introduction of multiple genetic modifications in *Saccharomyces cerevisiae* using CRISPR/Cas9. *FEMS Yeast Research*, 18(7), foy063. <https://doi.org/10.1093/femsyr/foy063>
- Martin, B. J. E., Brind'Amour, J., Kuzmin, A., Jensen, K. N., Liu, Z. C., Lorincz, M., & Howe, L. J. (2021). Transcription shapes genome-wide histone acetylation patterns. *Nature Communications*, 12(1), 210. <https://doi.org/10.1038/s41467-020-20543-z>
- Martín Expósito, M. (2019a). *Functional characterization of the yeast RNA binding protein Mip6*. Universitat de València.
- Martín Expósito, M. (2019b). *Functional characterization of the yeast RNA binding protein Mip6*. Universitat de València.
- Martín-Expósito, M., Gas, M., Mohamad, N., Nuño-Cabanes, C., Tejada-Colón, A., Pascual-García, P., De La Fuente, L., Chaves-Arquero, B., Merran, J., Corden, J., Conesa, A., Pérez-Cañadillas, J. M., Bravo, J., & Rodríguez-Navarro, S. (2019a). Mip6 binds directly to the Mex67 UBA domain to maintain low levels of Msn2/4 stress-dependent mRNAs. *EMBO Reports*, 20(12), Article 12. <https://doi.org/10.15252/embr.201947964>
- Martín-Expósito, M., Gas, M., Mohamad, N., Nuño-Cabanes, C., Tejada-Colón, A., Pascual-García, P., De La Fuente, L., Chaves-Arquero, B., Merran, J., Corden, J., Conesa, A., Pérez-Cañadillas, J. M., Bravo, J., & Rodríguez-



- Navarro, S. (2019b). Mip6 binds directly to the Mex67 UBA domain to maintain low levels of Msn2/4 stress-dependent mRNAs. *EMBO Reports*, 20(12), e47964. <https://doi.org/10.15252/embr.201947964>
- Martínez-Pastor, M. T., Marchler, G., Schüller, C., Marchler-Bauer, A., Ruis, H., & Estruch, F. (1996). The *Saccharomyces cerevisiae* zinc finger proteins Msn2p and Msn4p are required for transcriptional induction through the stress response element (STRE). *The EMBO Journal*, 15(9), 2227-2235.
- Masser, A. E., Kang, W., Roy, J., Mohanakrishnan Kaimal, J., Quintana-Cordero, J., Friedländer, M. R., & Andréasson, C. (2019). Cytoplasmic protein misfolding titrates Hsp70 to activate nuclear Hsf1. *eLife*, 8, e47791. <https://doi.org/10.7554/eLife.47791>
- Matsuo, Y., & Inada, T. (2023). Co-Translational Quality Control Induced by Translational Arrest. *Biomolecules*, 13(2), 317. <https://doi.org/10.3390/biom13020317>
- Mattenberger, F., Sabater-Muñoz, B., Hallsworth, J. E., & Fares, M. A. (2017). Glycerol stress in *Saccharomyces cerevisiae*: Cellular responses and evolved adaptations. *Environmental Microbiology*, 19(3), 990-1007. <https://doi.org/10.1111/1462-2920.13603>
- McBride, K. M., Banninger, G., McDonald, C., & Reich, N. C. (2002). Regulated nuclear import of the STAT1 transcription factor by direct binding of importin-alpha. *The EMBO Journal*, 21(7), 1754-1763. <https://doi.org/10.1093/emboj/21.7.1754>
- McDaniel, S. L., & Strahl, B. D. (2013). Stress-Free with Rpd3: A Unique Chromatin Complex Mediates the Response to Oxidative Stress. *Molecular and Cellular Biology*, 33(19), 3726-3727. <https://doi.org/10.1128/MCB.01000-13>
- Meinel, D. M., Burkert-Kautzsch, C., Kieser, A., O'Duibhir, E., Siebert, M., Mayer, A., Cramer, P., Söding, J., Holstege, F. C. P., & Sträßer, K. (2013). Recruitment of TREX to the Transcription Machinery by Its Direct Binding to the Phospho-CTD of RNA Polymerase II. *PLoS Genetics*, 9(11), e1003914. <https://doi.org/10.1371/journal.pgen.1003914>
- Miller, J. H. (1972). *Experiments in Molecular Genetics*. Cold Spring Harbor Laboratory.
- Mitchell, A. P., & Herskowitz, I. (1986). Activation of meiosis and sporulation by repression of the RME1 product in yeast. *Nature*, 319(6056), 738-742. <https://doi.org/10.1038/319738a0>
- Morano, K. A., Grant, C. M., & Moye-Rowley, W. S. (2012). The Response to Heat Shock and Oxidative Stress in *Saccharomyces cerevisiae*. *Genetics*, 190(4), 1157-1195. <https://doi.org/10.1534/genetics.111.128033>
- Mühlhofer, M., Offensperger, F., Reschke, S., Wallmann, G., Csaba, G., Berchtold, E., Riedl, M., Blum, H., Haslbeck, M., Zimmer, R., & Buchner, J. (2024). Deletion of the transcription factors Hsf1, Msn2 and Msn4 in yeast uncovers transcriptional reprogramming in response to proteotoxic stress. *FEBS Letters*, 598(6), 635-657. <https://doi.org/10.1002/1873-3468.14821>
- Mustaev, A., Roberts, J., & Gottesman, M. (2017). Transcription elongation. *Transcription*, 8(3), 150-161. <https://doi.org/10.1080/21541264.2017.1289294>
- Nardozi, J. D., Lott, K., & Cingolani, G. (2010). Phosphorylation meets nuclear import: A review. *Cell Communication and Signaling : CCS*, 8, 32. <https://doi.org/10.1186/1478-811X-8-32>
- Nasmyth, K. (1993). Regulating the HO endonuclease in yeast. *Current Opinion in Genetics & Development*, 3(2), 286-294. [https://doi.org/10.1016/0959-437X\(93\)90036-O](https://doi.org/10.1016/0959-437X(93)90036-O)
- Neiman, A. M. (2011). Sporulation in the budding yeast *Saccharomyces cerevisiae*. *Genetics*, 189(3), 737-765. <https://doi.org/10.1534/genetics.111.127126>
- Neiman, A. M. (2024). Membrane and organelle rearrangement during ascospore formation in budding yeast. *Microbiology and Molecular Biology Reviews: MMBR*, e0001324. <https://doi.org/10.1128/mmbr.00013-24>
- Niño, C. A., Hérisant, L., Babour, A., & Dargemont, C. (2013). mRNA nuclear export in yeast. *Chemical Reviews*, 113(11), 8523-8545. <https://doi.org/10.1021/cr400002g>
- Nudler, E. (2012). RNA polymerase backtracking in gene regulation and genome instability. *Cell*, 149(7), 1438-1445. <https://doi.org/10.1016/j.cell.2012.06.003>
- Nueda, M. J., Ferrer, A., & Conesa, A. (2012). ARSyN: A method for the identification and removal of systematic noise in multifactorial time course microarray experiments. *Biostatistics (Oxford, England)*, 13(3), 553-566. <https://doi.org/10.1093/biostatistics/kxr042>
- Nuño Cabanes, C. (2024). *Study of the role of SAGA/SLIK complexes and Mip6 protein in gene expression regulation in eukaryotes*. Universidad Politécnica de Valencia.



- Nuño-Cabanes, C., Ugidos, M., Tarazona, S., Martín-Expósito, M., Ferrer, A., Rodríguez-Navarro, S., & Conesa, A. (2020). A multi-omics dataset of heat-shock response in the yeast RNA binding protein Mip6. *Scientific Data*, 7(1), 69. <https://doi.org/10.1038/s41597-020-0412-z>
- Ohama, N., Sato, H., Shinozaki, K., & Yamaguchi-Shinozaki, K. (2017). Transcriptional Regulatory Network of Plant Heat Stress Response. *Trends in Plant Science*, 22(1), 53–65. <https://doi.org/10.1016/j.tplants.2016.08.015>
- Oliete-Calvo, P., Serrano-Quílez, J., Nuño-Cabanes, C., Pérez-Martínez, M. E., Soares, L. M., Dichtl, B., Buratowski, S., Pérez-Ortín, J. E., & Rodríguez-Navarro, S. (2018). A role for Mog1 in H2Bub1 and H3K4me3 regulation affecting RNAPII transcription and mRNA export. *EMBO Reports*, 19(11), e45992. <https://doi.org/10.15252/embr.201845992>
- Ossareh-Nazari, B., Bachelierie, F., & Dargemont, C. (1997). Evidence for a Role of CRM1 in Signal-Mediated Nuclear Protein Export. *Science*, 278(5335), 141–144. <https://doi.org/10.1126/science.278.5335.141>
- Park, E.-H., Walker, S. E., Lee, J. M., Rothenburg, S., Lorsch, J. R., & Hinnebusch, A. G. (2011). Multiple elements in the eIF4G1 N-terminus promote assembly of eIF4G1•PABP mRNPs in vivo. *The EMBO Journal*, 30(2), 302–316. <https://doi.org/10.1038/emboj.2010.312>
- Park, E.-H., Zhang, F., Warringer, J., Sunnerhagen, P., & Hinnebusch, A. G. (2011). Depletion of eIF4G from yeast cells narrows the range of translational efficiencies genome-wide. *BMC Genomics*, 12, 68. <https://doi.org/10.1186/1471-2164-12-68>
- Parker, R., & Sheth, U. (2007). P bodies and the control of mRNA translation and degradation. *Molecular Cell*, 25(5), 635–646. <https://doi.org/10.1016/j.molcel.2007.02.011>
- Pascual-Ahuir, A., Fita-Torró, J., & Proft, M. (2020). Capturing and Understanding the Dynamics and Heterogeneity of Gene Expression in the Living Cell. *International Journal of Molecular Sciences*, 21(21), 8278. <https://doi.org/10.3390/ijms21218278>
- Pascual-Garcia, P., Debo, B., Aleman, J. R., Talamas, J. A., Lan, Y., Nguyen, N. H., Won, K. J., & Capelson, M. (2017). Metazoan Nuclear Pores Provide a Scaffold for Poised Genes and Mediate Induced Enhancer-Promoter Contacts. *Molecular cell*, 66(1), 63–76.e6. <https://doi.org/10.1016/j.molcel.2017.02.020>
- Pascual-Garcia, P., Little, S. C., & Capelson, M. (2022). Nup98-dependent transcriptional memory is established independently of transcription. *eLife*, 11, e63404. <https://doi.org/10.7554/eLife.63404>
- Peng, G., & Hopper, J. E. (2002). Gene activation by interaction of an inhibitor with a cytoplasmic signaling protein. *Proceedings of the National Academy of Sciences of the United States of America*, 99(13), 8548–8553. <https://doi.org/10.1073/pnas.142100099>
- Pérez-Martínez, M. E., Benet, M., Alepuz, P., & Tordera, V. (2019). Nut1/Hos1 and Sas2/Rpd3 control the H3 acetylation of two different sets of osmotic stress-induced genes. *Epigenetics*, 15(3), 251–271. <https://doi.org/10.1080/15592294.2019.1664229>
- Piekarska, I., Rytka, J., & Rempola, B. (2010). Regulation of sporulation in the yeast *Saccharomyces cerevisiae*. *Acta Biochimica Polonica*, 57(3). https://doi.org/10.18388/abp.2010_2401
- Pinskaya, M., Gourvennec, S., & Morillon, A. (2009). H3 lysine 4 di- and tri-methylation deposited by cryptic transcription attenuates promoter activation. *The EMBO Journal*, 28(12), 1697–1707. <https://doi.org/10.1038/emboj.2009.108>
- Pisareva, V. P., Pisarev, A. V., Hellen, C. U. T., Rodnina, M. V., & Pestova, T. V. (2006). Kinetic analysis of interaction of eukaryotic release factor 3 with guanine nucleotides. *The Journal of Biological Chemistry*, 281(52), 40224–40235. <https://doi.org/10.1074/jbc.M607461200>
- Porrua, O., & Libri, D. (2013). A bacterial-like mechanism for transcription termination by the Sen1p helicase in budding yeast. *Nature Structural & Molecular Biology*, 20(7), 884–891. <https://doi.org/10.1038/nsmb.2592>
- Puig, O., Caspary, F., Rigaut, G., Rutz, B., Bouveret, E., Bragado-Nilsson, E., Wilm, M., & Séraphin, B. (2001). The Tandem Affinity Purification (TAP) Method: A General Procedure of Protein Complex Purification. *Methods*, 24(3), 218–229. <https://doi.org/10.1006/meth.2001.1183>
- Quilis, I., & Igual, J. C. (2012). Molecular basis of the functional distinction between Cln1 and Cln2 cyclins. *Cell Cycle (Georgetown, Tex.)*, 11(16), 3117–3131. <https://doi.org/10.4161/cc.21505>
- Randise-Hinchliff, C., Coukos, R., Sood, V., Sumner, M. C., Zdraljevic, S., Meldi Sholl, L., Garvey Brickner, D., Ahmed, S., Watchmaker, L., & Brickner, J. H. (2016). Strategies to regulate transcription factor-mediated gene positioning and interchromosomal clustering at the nuclear periphery. *The Journal of Cell Biology*, 212(6), 633–646. <https://doi.org/10.1083/jcb.201508068>



- Ren, Y., Schmiede, P., & Blobel, G. (2017). Structural and biochemical analyses of the DEAD-box ATPase Sub2 in association with THO or Yra1. *eLife*, 6, e20070. <https://doi.org/10.7554/eLife.20070>
- Riggs, C. L., Kedersha, N., Ivanov, P., & Anderson, P. (2020). Mammalian stress granules and P bodies at a glance. *Journal of Cell Science*, 133(16), jcs242487. <https://doi.org/10.1242/jcs.242487>
- Rimal, A., Kamdar, Z. P., Tio, C. W., & Winter, E. (2020). Isc10, an Inhibitor That Links the Anaphase-Promoting Complex to a Meiosis-Specific Mitogen-Activated Protein Kinase. *Molecular and Cellular Biology*, 40(16), e00097-20. <https://doi.org/10.1128/MCB.00097-20>
- Ringel, A. E., Cieniewicz, A. M., Taverna, S. D., & Wolberger, C. (2015). Nucleosome competition reveals processive acetylation by the SAGA HAT module. *Proceedings of the National Academy of Sciences*, 112(40), E5461-E5470. <https://doi.org/10.1073/pnas.1508449112>
- Robyr, D., Suka, Y., Xenarios, I., Kurdastani, S. K., Wang, A., Suka, N., & Grunstein, M. (2002). Microarray deacetylation maps determine genome-wide functions for yeast histone deacetylases. *Cell*, 109(4), 437-446. [https://doi.org/10.1016/s0092-8674\(02\)00746-8](https://doi.org/10.1016/s0092-8674(02)00746-8)
- Rodríguez-Molina, J. B., West, S., & Passmore, L. A. (2023). Knowing when to stop: Transcription termination on protein-coding genes by eukaryotic RNAPII. *Molecular Cell*, 83(3), 404-415. <https://doi.org/10.1016/j.molcel.2022.12.021>
- Rodríguez-Navarro, S., Fischer, T., Luo, M.-J., Antúnez, O., Brettschneider, S., Lechner, J., Pérez-Ortín, J. E., Reed, R., & Hurt, E. (2004). Sus1, a functional component of the SAGA histone acetylase complex and the nuclear pore-associated mRNA export machinery. *Cell*, 116(1), 75-86. [https://doi.org/10.1016/s0092-8674\(03\)01025-0](https://doi.org/10.1016/s0092-8674(03)01025-0)
- Rowlands, H., Shaban, K., Cheng, A., Foster, B., & Yankulov, K. (2019). Dysfunctional CAF-I reveals its role in cell cycle progression and differential regulation of gene silencing. *Cell Cycle*, 18(22), 3223-3236. <https://doi.org/10.1080/15384101.2019.1673100>
- Ruiz-Roig, C., Viéitez, C., Posas, F., & De Nadal, E. (2010). The Rpd3L HDAC complex is essential for the heat stress response in yeast. *Molecular Microbiology*, 76(4), 1049-1062. <https://doi.org/10.1111/j.1365-2958.2010.07167.x>
- Sadeh, A., Movshovich, N., Volokh, M., Gheber, L., & Aharoni, A. (2011). Fine-tuning of the Msn2/4-mediated yeast stress responses as revealed by systematic deletion of Msn2/4 partners. *Molecular Biology of the Cell*, 22(17), 3127. <https://doi.org/10.1091/mbc.E10-12-1007>
- Sagee, S., Sherman, A., Shenhar, G., Robzyk, K., Ben-Doy, N., Simchen, G., & Kassir, Y. (1998). Multiple and distinct activation and repression sequences mediate the regulated transcription of IME1, a transcriptional activator of meiosis-specific genes in *Saccharomyces cerevisiae*. *Molecular and Cellular Biology*, 18(4), 1985-1995. <https://doi.org/10.1128/MCB.18.4.1985>
- Sambrook, J., & Russell, D. W. (2006). Preparation of denaturing polyacrylamide gels. *CSH Protocols*, 2006(1), pdb.prot3793. <https://doi.org/10.1101/pdb.prot3793>
- Santiago, A. M., Gonçalves, D. L., & Morano, K. A. (2020). Mechanisms of sensing and response to proteotoxic stress. *Experimental Cell Research*, 395(2), 112240. <https://doi.org/10.1016/j.yexcr.2020.112240>
- Santos-Rosa, H., Moreno, H., Simos, G., Segref, A., Fahrenkrog, B., Panté, N., & Hurt, E. (1998). Nuclear mRNA export requires complex formation between Mex67p and Mtr2p at the nuclear pores. *Molecular and Cellular Biology*, 18(11), 6826-6838. <https://doi.org/10.1128/MCB.18.11.6826>
- Sauty, S. M., Shaban, K., & Yankulov, K. (2021). Gene repression in *S. cerevisiae*-looking beyond Sir-dependent gene silencing. *Current Genetics*, 67(1), 3-17. <https://doi.org/10.1007/s00294-020-01114-7>
- Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, S., Rueden, C., Saalfeld, S., Schmid, B., Tinevez, J.-Y., White, D. J., Hartenstein, V., Eliceiri, K., Tomancak, P., & Cardona, A. (2012). Fiji: An open-source platform for biological-image analysis. *Nature Methods*, 9(7), 676-682. <https://doi.org/10.1038/nmeth.2019>
- Schulz, D., Schwalb, B., Kiesel, A., Baejen, C., Torkler, P., Gagneur, J., Soeding, J., & Cramer, P. (2013). Transcriptome surveillance by selective termination of noncoding RNA synthesis. *Cell*, 155(5), 1075-1087. <https://doi.org/10.1016/j.cell.2013.10.024>
- Segref, A., Sharma, K., Doye, V., Hellwig, A., Huber, J., Lührmann, R., & Hurt, E. (1997). Mex67p, a novel factor for nuclear mRNA export, binds to both poly(A)⁺ RNA and nuclear pores. *The EMBO Journal*, 16(11), 3256-3271. <https://doi.org/10.1093/emboj/16.11.3256>



- Serrano-Quílez, J., Roig-Soucase, S., & Rodríguez-Navarro, S. (2020). Sharing Marks: H3K4 Methylation and H2B Ubiquitination as Features of Meiotic Recombination and Transcription. *International Journal of Molecular Sciences*, 21(12), 4510. <https://doi.org/10.3390/ijms21124510>
- Seto, E., & Yoshida, M. (2014). Erasers of Histone Acetylation: The Histone Deacetylase Enzymes. *Cold Spring Harbor Perspectives in Biology*, 6(4), a018713. <https://doi.org/10.1101/cshperspect.a018713>
- Shah, K. H., Zhang, B., Ramachandran, V., & Herman, P. K. (2013). Processing Body and Stress Granule Assembly Occur by Independent and Differentially Regulated Pathways in *Saccharomyces cerevisiae*. *Genetics*, 193(1), 109-123. <https://doi.org/10.1534/genetics.112.146993>
- Shen, D., He, X., Weng, P., Liu, Y., & Wu, Z. (2022). A review of yeast: High cell-density culture, molecular mechanisms of stress response and tolerance during fermentation. *FEMS Yeast Research*, 22(1), foac050. <https://doi.org/10.1093/femsyr/foac050>
- Sikorski, R. S., & Hieter, P. (1989). *A System of Shuttle Vectors and Yeast Host Strains Designed for Efficient Manipulation of DNA in Saccharomyces cerevisiae*.
- Singh, N., Asalam, M., Ansari, M. O., Gerasimova, N. S., Studitsky, V. M., & Akhtar, M. S. (2022). Transcription by RNA polymerase II and the CTD-chromatin crosstalk. *Biochemical and Biophysical Research Communications*, 599, 81-86. <https://doi.org/10.1016/j.bbrc.2022.02.039>
- Siwek, W., Tehrani, S. S. H., Mata, J. F., & Jansen, L. E. T. (2020). Activation of Clustered IFN γ Target Genes Drives Cohesin-Controlled Transcriptional Memory. *Molecular Cell*, 80(3), 396-409.e6. <https://doi.org/10.1016/j.molcel.2020.10.005>
- Soffers, J. H. M., & Workman, J. L. (2020). The SAGA chromatin-modifying complex: The sum of its parts is greater than the whole. *Genes & Development*, 34(19-20), 1287-1303. <https://doi.org/10.1101/gad.341156.120>
- Soniati, M., & Chook, Y. M. (2015). Nuclear localization signals for four distinct karyopherin- β nuclear import systems. *Biochemical Journal*, 468(3), 353-362. <https://doi.org/10.1042/BJ20150368>
- Sood, V., Cajigas, I., D'Urso, A., Light, W. H., & Brickner, J. H. (2017). Epigenetic Transcriptional Memory of GAL Genes Depends on Growth in Glucose and the Tup1 Transcription Factor in *Saccharomyces cerevisiae*. *Genetics*, 206(4), 1895-1907. <https://doi.org/10.1534/genetics.117.201632>
- Sorger, P. K., & Nelson, H. C. (1989). Trimerization of a yeast transcriptional activator via a coiled-coil motif. *Cell*, 59(5), 807-813. [https://doi.org/10.1016/0092-8674\(89\)90604-1](https://doi.org/10.1016/0092-8674(89)90604-1)
- Sorger, P. K., & Pelham, H. R. (1987). Purification and characterization of a heat-shock element binding protein from yeast. *The EMBO Journal*, 6(10), 3035-3041. <https://doi.org/10.1002/j.1460-2075.1987.tb02609.x>
- Sou, I. F., Pryce, R. M., Tee, W.-W., & McClurg, U. L. (2021). Meiosis initiation: A story of two sexes in all creatures great and small. *The Biochemical Journal*, 478(20), 3791-3805. <https://doi.org/10.1042/BCJ20210412>
- Souquere, S., Mollet, S., Kress, M., Dautry, F., Pierron, G., & Weil, D. (2009). Unravelling the ultrastructure of stress granules and associated P-bodies in human cells. *Journal of Cell Science*, 122(Pt 20), 3619-3626. <https://doi.org/10.1242/jcs.054437>
- Squazzo, S. L., Costa, P. J., Lindstrom, D. L., Kumer, K. E., Simic, R., Jennings, J. L., Link, A. J., Arndt, K. M., & Hartzog, G. A. (2002). The Paf1 complex physically and functionally associates with transcription elongation factors in vivo. *The EMBO Journal*, 21(7), 1764-1774. <https://doi.org/10.1093/emboj/21.7.1764>
- Sterner, D. E., Grant, P. A., Roberts, S. M., Duggan, L. J., Belotserkovskaya, R., Pacella, L. A., Winston, F., Workman, J. L., & Berger, S. L. (1999). Functional Organization of the Yeast SAGA Complex: Distinct Components Involved in Structural Integrity, Nucleosome Acetylation, and TATA-Binding Protein Interaction. *Molecular and Cellular Biology*, 19(1), 86-98. <https://doi.org/10.1128/MCB.19.1.86>
- Stewart, M. (2019a). Polyadenylation and nuclear export of mRNAs. *The Journal of Biological Chemistry*, 294(9), 2977-2987. <https://doi.org/10.1074/jbc.REV118.005594>
- Stewart, M. (2019b). Structure and Function of the TREX-2 Complex. En J. R. Harris & J. Marles-Wright (Eds.), *Macromolecular Protein Complexes II: Structure and Function* (pp. 461-470). Springer International Publishing. https://doi.org/10.1007/978-3-030-28151-9_15
- Strässer, K., Bassler, J., & Hurt, E. (2000). Binding of the Mex67p/Mtr2p heterodimer to FXFG, GLFG, and FG repeat nucleoporins is essential for nuclear mRNA export. *The Journal of Cell Biology*, 150(4), 695-706. <https://doi.org/10.1083/jcb.150.4.695>



- Strässer, K., Masuda, S., Mason, P., Pfannstiel, J., Oppizzi, M., Rodriguez-Navarro, S., Rondón, A. G., Aguilera, A., Struhl, K., Reed, R., & Hurt, E. (2002). TREX is a conserved complex coupling transcription with messenger RNA export. *Nature*, 417(6886), 304-308. <https://doi.org/10.1038/nature746>
- Strich, R., Surosky, R. T., Steber, C., Dubois, E., Messenguy, F., & Esposito, R. E. (1994). UME6 is a key regulator of nitrogen repression and meiotic development. *Genes & Development*, 8(7), 796-810. <https://doi.org/10.1101/gad.8.7.796>
- Sugiyama, M., Akase, S.-P., Nakanishi, R., Horie, H., Kaneko, Y., & Harashima, S. (2014). Nuclear localization of Haa1, which is linked to its phosphorylation status, mediates lactic acid tolerance in *Saccharomyces cerevisiae*. *Applied and Environmental Microbiology*, 80(11), 3488-3495. <https://doi.org/10.1128/AEM.04241-13>
- Sump, B., & Brickner, J. (2022). Establishment and inheritance of epigenetic transcriptional memory. *Frontiers in Molecular Biosciences*, 9, 977653. <https://doi.org/10.3389/fmolb.2022.977653>
- Sunnåker, M., Zamora-Sillero, E., Dechant, R., Ludwig, C., Busetto, A. G., Wagner, A., & Stelling, J. (2013). Automatic generation of predictive dynamic models reveals nuclear phosphorylation as the key Msn2 control mechanism. *Science Signaling*, 6(277), ra41. <https://doi.org/10.1126/scisignal.2003621>
- Taberner, F. J., Quilis, I., & Igual, J. C. (2009). Spatial regulation of the Start repressor Whi5. *Cell Cycle*, 8(18), 3013-3022. <https://doi.org/10.4161/cc.8.18.9621>
- Taberner, F. J., Quilis, I., Sendra, J., Bañó, M. C., & Igual, J. C. (2012). Regulation of cell cycle transcription factor Swi5 by karyopherin Msn5. *Biochimica Et Biophysica Acta*, 1823(4), 959-970. <https://doi.org/10.1016/j.bbamcr.2012.02.009>
- Tarazona, S., Furió-Tarí, P., Turrà, D., Pietro, A. D., Nueda, M. J., Ferrer, A., & Conesa, A. (2015). Data quality aware analysis of differential expression in RNA-seq with NOISeq R/Bioc package. *Nucleic Acids Research*, 43(21), e140. <https://doi.org/10.1093/nar/gkv711>
- Tarun, S. Z., & Sachs, A. B. (1996). Association of the yeast poly(A) tail binding protein with translation initiation factor eIF-4G. *The EMBO Journal*, 15(24), 7168-7177.
- Teixeira, D., & Parker, R. (2007). Analysis of P-body assembly in *Saccharomyces cerevisiae*. *Molecular Biology of the Cell*, 18(6), 2274-2287. <https://doi.org/10.1091/mbc.e07-03-0199>
- Tejada-Colon, A., Serrano-Quilez, J., Nuno-Cabanes, C., & Rodríguez-Navarro, S. (under revision). *Decoding Transcriptional Memory in Yeast Heat Shock and the Functional Implication of the RNA Binding Protein Mip6* (p. 2024.09.12.612644). bioRxiv: <https://doi.org/10.1101/2024.09.12.612644>
- Terry, L. J., & Wente, S. R. (2009). Flexible Gates: Dynamic Topologies and Functions for FG Nucleoporins in Nucleocytoplasmic Transport. *Eukaryotic Cell*, 8(12), 1814-1827. <https://doi.org/10.1128/EC.00225-09>
- Tessarz, P., & Kouzarides, T. (2014). Histone core modifications regulating nucleosome structure and dynamics. *Nature Reviews. Molecular Cell Biology*, 15(11), 703-708. <https://doi.org/10.1038/nrm3890>
- Thomas, B. J., & Rothstein, R. (1989). Elevated recombination rates in transcriptionally active DNA. *Cell*, 56(4), 619-630. [https://doi.org/10.1016/0092-8674\(89\)90584-9](https://doi.org/10.1016/0092-8674(89)90584-9)
- Thomas, M. C., & Chiang, C.-M. (2006). The General Transcription Machinery and General Cofactors. *Critical Reviews in Biochemistry and Molecular Biology*, 41(3), 105-178. <https://doi.org/10.1080/10409230600648736>
- Tian, S., Curnutte, H. A., & Trcek, T. (2020). RNA Granules: A View from the RNA Perspective. *Molecules (Basel, Switzerland)*, 25(14), 3130. <https://doi.org/10.3390/molecules25143130>
- Timmers, H. Th. M. (2021). SAGA and TFIID: Friends of TBP drifting apart. *Biochimica et Biophysica Acta (BBA) - Gene Regulatory Mechanisms*, 1864(2), 194604. <https://doi.org/10.1016/j.bbagrm.2020.194604>
- Tran, K., & Green, E. (2019). Assessing Yeast Cell Survival Following Hydrogen Peroxide Exposure. *BIO-PROTOCOL*, 9(2). <https://doi.org/10.21769/BioProtoc.3149>
- Treco, D. A., & Winston, F. (2008). Growth and Manipulation of Yeast. *Current Protocols in Molecular Biology*, 82(1), 13.2.1-13.2.12. <https://doi.org/10.1002/0471142727.mb1302s82>
- Trueheart, J., Boeke, J. D., & Fink, G. R. (1987). Two genes required for cell fusion during yeast conjugation: Evidence for a pheromone-induced surface protein. *Molecular and Cellular Biology*, 7(7), 2316-2328.
- Ugidos Guerrero, M. (2023). *Statistical Methods Development for the Multiomic Systems Biology* [Tesis doctoral, Universitat Politècnica de València]. <https://doi.org/10.4995/Thesis/10251/193031>



- Valkov, E., Dean, J. C., Jani, D., Kuhlmann, S. I., & Stewart, M. (2012). Structural basis for the assembly and disassembly of mRNA nuclear export complexes. *Biochimica et Biophysica Acta (BBA) - Gene Regulatory Mechanisms*, 1819(6), 578-592. <https://doi.org/10.1016/j.bbagr.2012.02.017>
- van Leeuwen, W., & Rabouille, C. (2019). Cellular stress leads to the formation of membraneless stress assemblies in eukaryotic cells. *Traffic (Copenhagen, Denmark)*, 20(9), 623-638. <https://doi.org/10.1111/tra.12669>
- van Werven, F. J., Neuert, G., Hendrick, N., Lardenois, A., Buratowski, S., van Oudenaarden, A., Primig, M., & Amon, A. (2012). Transcription of two long noncoding RNAs mediates mating-type control of gametogenesis in budding yeast. *Cell*, 150(6), 1170-1181. <https://doi.org/10.1016/j.cell.2012.06.049>
- Vanden Broeck, A., & Klinge, S. (2024). Eukaryotic Ribosome Assembly. *Annual Review of Biochemistry*. <https://doi.org/10.1146/annurev-biochem-030222-113611>
- Wagner, E. J., & Carpenter, P. B. (2012). Understanding the language of Lys36 methylation at histone H3. *Nature reviews. Molecular cell biology*, 13(2), 115-126. <https://doi.org/10.1038/nrm3274>
- Wallace, E. W. J., Kear-Scott, J. L., Pilipenko, E. V., Schwartz, M. H., Laskowski, P. R., Rojek, A. E., Katanski, C. D., Riback, J. A., Dion, M. F., Franks, A. M., Airoidi, E. M., Pan, T., Budnik, B. A., & Drummond, D. A. (2015). Reversible, specific, active aggregates of endogenous proteins assemble upon heat stress. *Cell*, 162(6), 1286-1298. <https://doi.org/10.1016/j.cell.2015.08.041>
- Wang, C., Molodtsov, V., Firlar, E., Kaelber, J. T., Blaha, G., Su, M., & Ebright, R. H. (2020). Structural basis of transcription-translation coupling. *Science (New York, N.Y.)*, 369(6509), 1359-1365. <https://doi.org/10.1126/science.abb5317>
- Wang, H., Dienemann, C., Stützer, A., Urlaub, H., Cheung, A. C. M., & Cramer, P. (2020). Structure of the transcription coactivator SAGA. *Nature*, 577(7792), 717-720. <https://doi.org/10.1038/s41586-020-1933-5>
- Wang, Y., Gibney, P. A., West, J. D., & Morano, K. A. (2012). The yeast Hsp70 Ssa1 is a sensor for activation of the heat shock response by thiol-reactive compounds. *Molecular Biology of the Cell*, 23(17), 3290-3298. <https://doi.org/10.1091/mbc.E12-06-0447>
- Weidberg, H., Moretto, F., Spedale, G., Amon, A., & van Werven, F. J. (2016). Nutrient Control of Yeast Gametogenesis Is Mediated by TORC1, PKA and Energy Availability. *PLoS Genetics*, 12(6), e1006075. <https://doi.org/10.1371/journal.pgen.1006075>
- Weindling, E., & Bar-Nun, S. (2015). Sir2 links the unfolded protein response and the heat shock response in a stress response network. *Biochemical and Biophysical Research Communications*, 457(3), 473-478. <https://doi.org/10.1016/j.bbrc.2015.01.021>
- Welker, S., Rudolph, B., Frenzel, E., Hagn, F., Liebisch, G., Schmitz, G., Scheuring, J., Kerth, A., Blume, A., Weinkauff, S., Haslbeck, M., Kessler, H., & Buchner, J. (2010). Hsp12 is an intrinsically unstructured stress protein that folds upon membrane association and modulates membrane function. *Molecular Cell*, 39(4), 507-520. <https://doi.org/10.1016/j.molcel.2010.08.001>
- Wendler, P., & Enenkel, C. (2019). Nuclear Transport of Yeast Proteasomes. *Frontiers in Molecular Biosciences*, 6, 34. <https://doi.org/10.3389/fmolb.2019.00034>
- Wilkinson, M. E., Charenton, C., & Nagai, K. (2020). RNA Splicing by the Spliceosome. *Annual Review of Biochemistry*, 89, 359-388. <https://doi.org/10.1146/annurev-biochem-091719-064225>
- Winter, E. (2012). The Sum1/Ndt80 transcriptional switch and commitment to meiosis in *Saccharomyces cerevisiae*. *Microbiology and Molecular Biology Reviews: MMBR*, 76(1), 1-15. <https://doi.org/10.1128/MMBR.05010-11>
- Wu, J., Suka, N., Carlson, M., & Grunstein, M. (2001). TUP1 Utilizes Histone H3/H2B-Specific HDA1 Deacetylase to Repress Gene Activity in Yeast. *Molecular Cell*, 7(1), 117-126. [https://doi.org/10.1016/S1097-2765\(01\)00160-5](https://doi.org/10.1016/S1097-2765(01)00160-5)
- Xiao, C. Y., Hübner, S., Elliot, R. M., Caon, A., & Jans, D. A. (1996). A consensus cAMP-dependent protein kinase (PK-A) site in place of the CcN motif casein kinase II site simian virus 40 large T-antigen confers PK-A-mediated regulation of nuclear import. *The Journal of Biological Chemistry*, 271(11), 6451-6457. <https://doi.org/10.1074/jbc.271.11.6451>
- Xie, Y., & Ren, Y. (2019). Mechanisms of nuclear mRNA export: A structural perspective. *Traffic (Copenhagen, Denmark)*, 20(11), 829-840. <https://doi.org/10.1111/tra.12691>
- Xu, B., Liu, L., & Song, G. (2021). Functions and Regulation of Translation Elongation Factors. *Frontiers in Molecular Biosciences*, 8, 816398. <https://doi.org/10.3389/fmolb.2021.816398>



- Xu, D., Marquis, K., Pei, J., Fu, S.-C., Çağatay, T., Grishin, N. V., & Chook, Y. M. (2015). LocNES: A computational tool for locating classical NESs in CRM1 cargo proteins. *Bioinformatics (Oxford, England)*, 31(9), 1357-1365. <https://doi.org/10.1093/bioinformatics/btu826>
- Yamamoto, A., Mizukami, Y., & Sakurai, H. (2005). Identification of a novel class of target genes and a novel type of binding sequence of heat shock transcription factor in *Saccharomyces cerevisiae*. *The Journal of Biological Chemistry*, 280(12), 11911-11919. <https://doi.org/10.1074/jbc.M411256200>
- Yang, X.-J., & Seto, E. (2008). The Rpd3/Hda1 family of lysine deacetylases: From bacteria and yeast to mice and men. *Nature reviews. Molecular cell biology*, 9(3), 206-218. <https://doi.org/10.1038/nrm2346>
- Yeheskely-Hayon, D., Kotler, A., Stark, M., Hashimshony, T., Sagee, S., & Kassir, Y. (2013). The Roles of the Catalytic and Noncatalytic Activities of Rpd3L and Rpd3S in the Regulation of Gene Transcription in Yeast. *PLoS ONE*, 8(12), e85088. <https://doi.org/10.1371/journal.pone.0085088>
- Yella, V. R., & Bansal, M. (2017). DNA structural features of eukaryotic TATA-containing and TATA-less promoters. *FEBS Open Bio*, 7(3), 324-334. <https://doi.org/10.1002/2211-5463.12166>
- Yu, R., Cao, X., Sun, L., Zhu, J., Wasko, B. M., Liu, W., Crutcher, E., Liu, H., Jo, M. C., Qin, L., Kaeberlein, M., Han, Z., & Dang, W. (2021). Inactivating histone deacetylase HDA promotes longevity by mobilizing trehalose metabolism. *Nature Communications*, 12(1), 1981. <https://doi.org/10.1038/s41467-021-22257-2>
- Yukawa, M., Yo, K., Hasegawa, H., Ueno, M., & Tsuchiya, E. (2009). The Rpd3/HDAC complex is present at the URS1 cis-element with hyperacetylated histone H3. *Bioscience, Biotechnology, and Biochemistry*, 73(2), 378-384. <https://doi.org/10.1271/bbb.80621>
- Yusupova, G., & Yusupov, M. (2017). Crystal structure of eukaryotic ribosome and its complexes with inhibitors. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 372(1716), 20160184. <https://doi.org/10.1098/rstb.2016.0184>
- Zander, G., Hackmann, A., Bender, L., Becker, D., Lingner, T., Salinas, G., & Krebber, H. (2016). mRNA quality control is bypassed for immediate export of stress-responsive transcripts. *Nature*, 540(7634), 593-596. <https://doi.org/10.1038/nature20572>
- Zapater, M., Sohrmann, M., Peter, M., Posas, F., & De Nadal, E. (2007). Selective Requirement for SAGA in Hog1-Mediated Gene Expression Depending on the Severity of the External Osmostress Conditions. *Molecular and Cellular Biology*, 27(11), 3900-3910. <https://doi.org/10.1128/MCB.00089-07>
- Zhai, L., & Xiang, S. (2014). mRNA quality control at the 5' end. *Journal of Zhejiang University. Science. B*, 15(5), 438-443. <https://doi.org/10.1631/jzus.B1400070>
- Zhang, H., Rigo, F., & Martinson, H. G. (2015). Poly(A) Signal-Dependent Transcription Termination Occurs through a Conformational Change Mechanism that Does Not Require Cleavage at the Poly(A) Site. *Molecular Cell*, 59(3), 437-448. <https://doi.org/10.1016/j.molcel.2015.06.008>
- Zheng, X., Krakowiak, J., Patel, N., Beyzavi, A., Ezike, J., Khalil, A. S., & Pincus, D. (2016). Dynamic control of Hsf1 during heat shock by a chaperone switch and phosphorylation. *eLife*, 5, e18638. <https://doi.org/10.7554/eLife.18638>
- Žumer, K., Maier, K. C., Farnung, L., Jaeger, M. G., Rus, P., Winter, G., & Cramer, P. (2021). Two distinct mechanisms of RNA polymerase II elongation stimulation in vivo. *Molecular Cell*, 81(15), 3096-3109.e8. <https://doi.org/10.1016/j.molcel.2021.05.028>

