RNA POLYMERASE II-ASSOCIATED PROTEINS AND EPIGENETIC MODIFICATIONS INVOLVED IN EARLY MEIOSIS EVENTS

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ABSTRACT

Saccharomyces cerevisiae is widely used as a eukaryotic model because of its fast growth and high degree of conservation with regard to mammals. The most common mode of vegetative growth in *S. cerevisiae* is asexual reproduction by budding. However, under high-stress nutrient starvation, diploid cells are induced to undergo meiosis and sporulation forming four haploid cells. These events are highly regulated to ensure cell survival. In these lines, specific patterns of gene expression are crucial to guarantee the correct induction of meiosis.

One of the most important steps during gene expression is the synthesis of all mRNAs carried out by the RNA polymerase II (RNAPII), which is a multiprotein complex that forms the core of the transcription machinery. In addition to RNAPII, a wide range of transcription factors and protein complexes are required for targeting RNAPII to upstream gene promoters and begin transcription.

One of them is the polymerase associated factor complex (PAF1c). PAF1c is formed by 5 subunits and it is required for the monoubiquitination of histone H2B and the methylation of H3K4, two of the most important epigenetic modifications in histones. Interestingly, PAF1c is not only important for transcription during vegetative growth, but mutations in some subunits indicate it to be also important in the formation of double-stranded breaks (DSBs) of meiotic chromosomes, an important event that occurs in prophase I.

The aim of this project is to do a bibliographical study of the moonlighting functions of proteins involved in transcription, which have been recently discovered to participate in early meiotic events as well as some epigenetic modifications implied in this process. An *in silico* interactome between PAF1c and a protein involved in H2B ubiquitination and H3K4 methylation, Mog1, is constructed to establish some connections between regulation and meiosis. Last, the study of these proteins and complexes in yeast is useful because human orthologs can be found overexpressed in some cancers, like parathyroid carcinome (PC) and other development pathologies.

KEY WORDS

Meiosis; yeast; PAF1 complex; histones; epigenetics; RNAPII; Mog1

Título	Proteínas asociadas a la ARN polimerasa II y modificaciones epigenéticas involucradas en eventos tempranos de la meiosis
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RESUMEN

Saccharomyces cerevisiae es ampliamente utilizada como modelo eucariota debido a su rápido crecimiento y alto grado de conservación con respecto a los mamíferos. El modo de crecimiento vegetativo más común en *S. cerevisiae* es la reproducción asexual por gemación. Sin embargo, bajo condiciones de estrés por falta de nutrientes, las células diploides son inducidas a sufrir meiosis y esporulación formando cuatro células haploides. Estos eventos están altamente regulados para garantizar la supervivencia celular, siendo los patrones específicos de expresión génica cruciales para la inducción correcta de la meiosis.

Uno de los pasos más importantes durante la expresión génica es la síntesis de todos los ARNm realizada por la ARN polimerasa II (RNAPII), complejo multiproteico que forma el núcleo de la maquinaria de transcripción. Además de la RNAPII, se requiere una amplia gama de factores de transcripción y complejos de proteínas para dirigir la RNAPII a los promotores génicos y comenzar la transcripción.

Uno de ellos es el complejo de factores asociados a la polimerasa (PAF1c). PAF1c está formado por 5 subunidades y es requerido para la monoubiquitinación de la histona H2B y la metilación de H3K4, dos de las modificaciones epigenéticas más importantes en las histonas. Curiosamente, PAF1c no solo es importante para la transcripción durante el crecimiento vegetativo, sino que mutaciones en algunas subunidades indican que también es importante en la formación de rupturas bicatenarias (DSB) de cromosomas meióticos, un evento importante que ocurre en la profase I.

El objetivo de este proyecto es hacer un estudio bibliográfico de las funciones de las proteínas involucradas en la transcripción, que recientemente se ha descubierto que participan en eventos meióticos tempranos, así como algunas modificaciones epigenéticas implicadas en este proceso. Se construye un interactoma *in silico* entre PAF1c y una proteína implicada en la ubiquitinación de H2B y la metilación de H3K4, Mog1, para establecer algunas conexiones entre la regulación y la meiosis. Por último, el estudio de estas proteínas y complejos en la levadura resulta útil porque los ortólogos humanos se pueden sobreexpresar en algunos tipos de cáncer, como el carcinoma paratiroideo (PC) y otros trastornos del desarrollo.

PALABRAS CLAVE

Meiosis; levadura; complejo PAF1; histonas; epigenética; RNAPII; Mog1

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Y sí haremos, pues estamos
en mundo tan singular
que el vivir sólo es soñar;
y la experiencia me enseña,
que el hombre que vive, sueña
lo que es, hasta despertar.

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LIST OF ABBREVIATIONS

DNA	deoxyribonucleic acid
RNA	ribonucleic acid
RNAPII	RNA polymerase II
mRNA	messenger RNA
TF	transcription factor
PIC	preinitiation complex
CTD	carboxy terminal domain
TBP	TATA binding protein
MED	Mediator
Ser	serine
CPF	cleavage and polyadenylation machinery
NPC	nuclear pore complex
SAGA	Spt-Ada-Gcn5-acetyltransferase
PAF1c	polymerase associated factor complex
FACT	facilitates chromatin transcription
DSB	double-strand break
SPB	spindle pole body
COMPASS	complex of proteins associated with Set1
HR	homologous recombination
RME1	repressor of meiosis I
NDR	nucleosome-depleted regions
TSS	transcription start site
MRX	Mre11-Rad50-Xrs2 complex
RMM	Rec114-Mer2-Mei4 complex
FISH	fluorescence *in situ* hybridization
GFP	green fluorescent protein
DAPI	4′,6-diamidino-2-phenylindole
WT	wild type
DUBm	deubiquitinating module
SGA	synthetic genetic array
PTM	post-translational modification
HMT	histone methyltransferase
m6A	N6-methyladenosine
5mC	5-methylcytosine
GO	gene onthology
LGL	large graph layout
PC	parathyroid carcinoma
HPT-JT	hyperparathyroidism-jaw tumour syndrome
MEN	multiple endocrine neoplasia
FIHP	familial isolated primary hyperparathyroidism
AML	acute myeloid leukemia
PD2	pancreatic differentiation 2

1. Introduction

Human molecular and genetic complexity increase the need of other models to be studied. *Saccharomyces cerevisiae*, also called budding yeast, is a small, single-celled member of the kingdom of fungi, closely related to animals. There are some properties that make them favourite as a model organism (Nielsen, 2019):

- Ease to grow in a simple nutrient medium.
- Rapid division like bacteria, either with a vegetative (by cell division), or sexual reproduction (by mitosis or meiosis).
- Tough cell wall and possession of mitochondria.
- Small genome sequenced in 1996 (first eukaryote sequenced).
- High degree of conservation between human and yeast processes.

The relevance of studying fundamental questions in yeast is noted by Nobel Prize winners. Among others, Oshumi discovered in 2016 some mechanisms of autophagy and Hartwell, Nurse and Hunt described in 2001 key regulators of the cell cycle (Hohmann, 2016). All of them won the Nobel Prize using yeasts. Understanding and clarifying some molecular mechanisms allows a better study in human diseases like cancer, promoting new advances in drug technologies and immunotherapies (Ferreira et al., 2019).

The main aim of this work is to review the novel role of the PAF1c during meiotic recombination, but first, a summary of its role in transcription is required.

1.1. An overview of yeast transcription

Each individual has its own instructions for living, growing and reproducing. These instructions are written in genes, which are organized into very long DNA molecules. As there is a lot of information, DNA molecules are packaged into chromosomes. *S. cerevisiae* contains 6,275 genes organized in 16 chromosomes. In eukaryotes, DNA is also packed around a core histone octamer (containing two copies of histones H2A, H2B, H3 and H4) called nucleosome (Kornberg, 1974).

Instructions are written using four letters or bases: adenine (A), thymine (T), guanine (G), and cytosine (C). DNA must be first converted to RNA for translating these instructions into proteins to effect different cellular actions. This conversion process is called transcription.

In order to initiate transcription, the transcriptional machinery must be able to access the DNA strand, which implies the assembly and disassembly of the nucleosomes controlled by the modification of histones. It is increasingly evident that transcription factors partake in different activities to facilitate the release of nucleosomes. These modifications consist on acetylation, phosphorylation, methylation, and ubiquitination (Gerber & Shilatifard, 2003; Kouzarides, 2007).

The basic mechanism of transcription by RNA polymerases is similar in all cells. However, yeast have three different polymerases, which are different in structure and function. RNAPI transcribes rRNA, RNAPII transcribes mRNA and some snRNAs and RNAPIII transcribes tRNAs and some other snRNAs (Mason et al., 2016). A first overview about mRNA transcription and some transcription factors that help RNA polymerase II (RNAPII) will help further reading.

1.1.1. RNA polymerase II (RNAPII)

Transcription is divided in 3 main phases (Figure 1): initiation, elongation, and termination (Buratowski, 2009). Let us introduce some important characters.

Transcription factors (TFs) interact with RNAPII to form a preinitiation complex (PIC) in the C-terminal domain (CTD). These transcription factors are the TATA binding protein (TBP), TFIIA, -B, -D, -E, -F, -H and Mediator (MED) (Hantsche & Cramer, 2017).

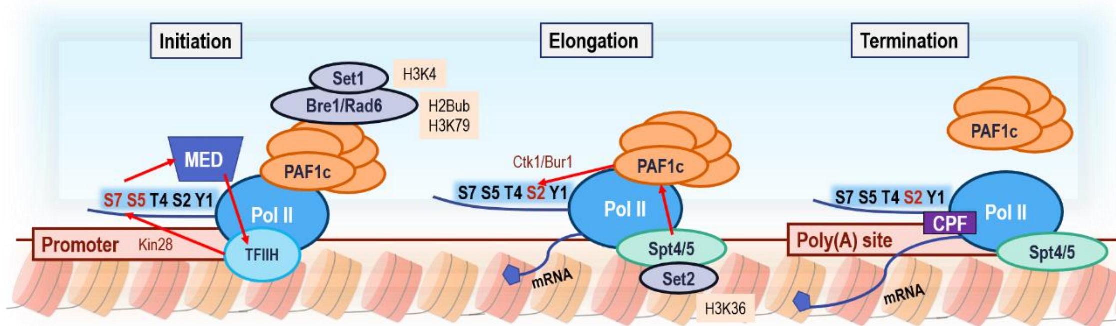


Figure 1 ***Transcription diagram***. It is divided in three stages in which CTD amino acid sequence (Y_1-S_2-P_3-T_4-S_5-P_6-S_7) is modified by kinases, specially serine 5 and 2 (in red) during initiation and elongation phases. Some subunits of RNAPII-associated complexes participate in histone modification, remarked in orange boxes. PAF1C is attached to RNAPII till the end of termination phase.

During initiation, the Mediator complex binds to the CTD to assemble all the TFs and bring the RNAPII. A component of TFIIH phosphorylates the CTD on its fifth serine (Ser5P) and once the PIC is ready, the Mediator dissociates from RNAPII (Robinson et al., 2016). Set1, a methyltransferase Ser5p-binding protein required for histone H3K4 methylation and to maintain H2B ubiquitination, is also important in transcription initiation.

Some of the molecular events during transcription elongation include: i) the participation of the factors Spt4/Spt5; ii) the phosphorylation of CTD serine in position 2 (Ser2P) by the Ctk1/Bur1 kinase. In the elongation phase there is a higher concentration of Ser2P instead of Ser5P as in initiation (Joo et al., 2019), which indicates the elongation start and iii) H3K36 methylation by Set2, a Ser2P-binding protein.

During the termination phase, the cleavage and polyadenylation machinery (CPF) participates in late processes. These events are 3′ polyadenylation, 5′ capping and splicing, where pre-mRNA becomes a mature mRNA.

The mature mRNA will be exported from nucleus to cytoplasm through the nuclear pore complex (NPC) thanks to several linkers like Sus1 (part of SAGA complex) and TREX-2. These protein complexes coordinate transcription with mRNA export (Rodríguez-Navarro & Hurt, 2011).

1.1.2. Polymerase-associated factor 1 complex (PAF1c)

Having reviewed shortly some important aspects about transcription, let us focus on the polymerase-associated factor complex 1 or PAF1c. This complex is involved in RNA synthesis from the beginning to the end and in the regulation of some epigenetic modifications (van Oss et al., 2017).

PAF1c has five subunits in budding yeast: Paf1, Ctr9, Cdc73, Leo1 and Rtf1 (represented in Figure 2). Some higher eukaryotes like humans also contain an additional subunit: Ski8/Wdr61.

This complex was firstly discovered in budding yeast 25 years ago (Stolinski et al., 1997; Wade et al., 1996). Later it was characterised in humans while studying parafibromin gene, 27% identical to Cdc73 in its C-terminal region and co-purifying with human homologues Paf1, Ctr9 and Leo1 (Jaehning, 2010).

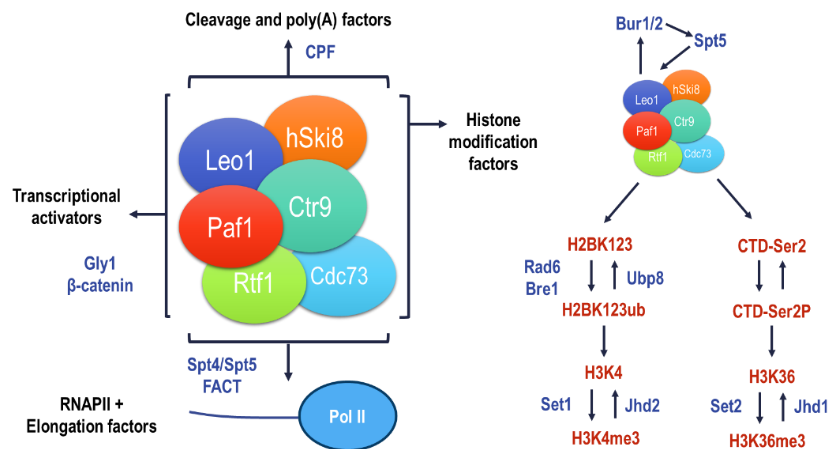


Figure 2 ***PAF1c functions***. On the left, PAF1c interactions with transcriptional activation, histone modification, elongation and termination factors. On the right, histone and CTD modification-dependent of Paf1C. Human Ski8 subunit (hSki8) is included. Adapted from Jaehning, 2010.

PAF1c interacts with Spt4/Spt5, particularly, the Rtf1 subunit binds to Spt5. It also interacts with the FACT (FAcilitates Chromatin Transcription) complex, which facilitates chromatin transcription and Ctk1, which is in charge of Ser2 phosphorylation

(van Oss et al., 2017). Near the poly(A) site, PAF1c detaches from the RNAPII and Spt4/Spt5 continue until termination (Jaehning, 2010).

PAF1c is involved in chromatin modifications such as H2B ubiquitination and methylation of H3K4, H3K79 and H3K36 (Table 1). This function is better detailed in Section 5.

1.2. An overview of yeast reproduction

It has been talked that each individual has instructions for reproducing. On a molecular scale, a cell divides to produce two daughter cells. To carry out cell division, firstly chromosomes must duplicate themselves to then distribute their information properly to the two daughter cells. In other words, a diploid cell will divide into two haploid cells.

Yeasts can reproduce by either asexual or sexual reproductive cycles. The most common way is asexual reproduction by budding, where a haploid cell is formed. Under high-stress nutrient starvation, diploid cells would die. This is the reason why diploid cells are induced to undergo meiosis and sporulation.

1.2.1. Mating-type

In *S. cerevisiae*, haploid cells are determined by the mating-type (MAT) locus at chromosome III by two different alleles: *a* and *α*.

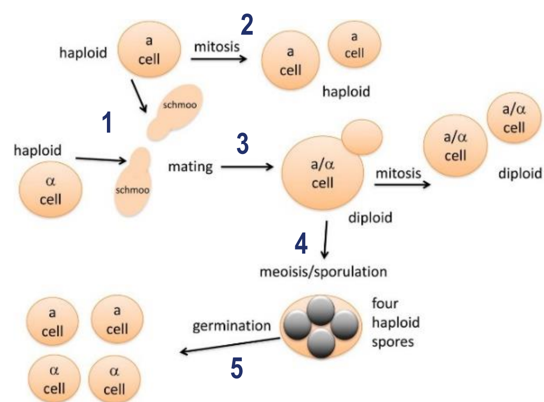


Figure 3 Simplified life cycle diagram of a budding yeast. Modified from Duina et al., 2014.

Budding yeast have acquired the capacity to convert some cells in a colony from one haploid mating type to the other, a process called homothallism. Diploid cells cannot mate but can reproduce mitotically or can undergo meiosis to produce haploid spores (Hanson & Wolfe, 2017).

To sum up, an *a* cell only mates with an *α* cell, and vice versa (Figure 3.1.). This interaction, called "shmoo" (because the shape remembers to a cartoon creature) results in G1-phase of mitotic proliferation (Figure 3.2.) (Merlini et al., 2013) or in meiosis and sporulation (Figure 3.3-4.) if there is nutrient starvation. In general, the structure which contains spores (ascus) will have four haploid spores (two *α* and two a, Figure 3.5.).

1.2.2. Meiosis and sporulation

Meiosis is the division of a mother cell to produce two daughter cells (Figure 4) that contain only half of the genetic material. It consists of two successive divisions. Some of the important milestones are described below.

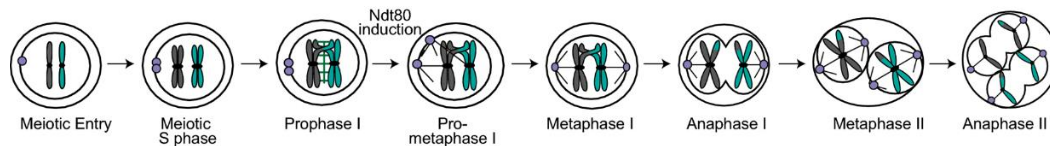


***Figure 4* Early meiotic phases representation**. From *Ballew & Lacefield, 2019*.

During prophase I of the first meiosis, homologous chromosomes interact thanks to the formation of a protein structured known as the synaptonemal complex. Genetic recombination takes place on it, stimulating double-strand breaks (DSBs). The physical place where homologous chromosomes have exchanged their information is called chiasma, and they are held together thanks to cohesins (Petronczki et al., 2003).

Due to the recombination complexity, prophase I is subdivided in 5 phases: leptotene, zygotene, pachytene, diplotene and diakinesis. In metaphase I, the pair of chromosomes align at the equator, pulling them by spindle pole body (SPB) in anaphase I. In telophase I the resulting cells have half the number of chromosomes. The second meiosis occurs like mitosis (with the same phases) without further DNA replication, where the sister chromatids are pulled apart and segregated to produce haploid cells (Alberts et al., 2008).

Yeast meiotic cells have an interphase period which is like mitosis, with three subphases: G1 (growth phase), S (DNA synthesis) and G2 (second growth phase). Between meiosis I and meiosis II there are some remarkable differences between budding yeast, and the fission yeast, *Schizosaccharomyces pombe*. Fission yeast interphase has all three phases, but budding yeast only have two of the: G1 and S phase. As the name says, *S. cerevisiae* divides by budding and *S. pombe* divides by forming partition (Alberts et al., 2008).

As specified above, cell division implies sporulation resulting in the production of four haploid nuclei. This work is focused on *S. cerevisiae* but comparison with the meiotic program of fission yeast shows a high orthology between meiotic core genes (Mata et al., 2002). The *S. cerevisiae* SK1 strain is a rapid and synchronously sporulating diploid used to study both sporulation and meiosis (Kane & Roth, 1974).

Sporulation could be divided in three major phases, as shown in Figure 5 (Neiman, 2011). In the early phase, the cell exits from the mitotic cycle in G1 and enters into the premeiotic S phase because of a stress, generally nitrogen starvation. DNA is duplicated and homologous recombination (HR) takes place, characteristic of meiotic prophase events. In the middle phase the most important cytological events occur to

form four haploid nuclei such as the modification and duplication of SPB to form the four membrane compartments, named prospore membranes. In the late phase the prospore membrane is closed. The compaction of chromatin and organelle regeneration is carried out inside the ascus.

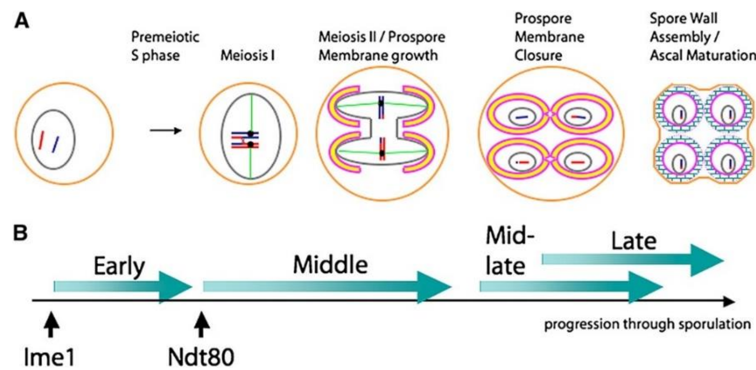


***Figure 5* Meiosis and sporulation phases across the time**. (A) Blue and red homologous chromosomes are inside of the grey nuclear envelope. Orange mother cell plasma membrane will convert to ascal membrane. In green, spindle microtubules that pull homologous chromosomes. Prospore formation is drawn in pink. (B) Ime1 and Ndt80 induce early and middle meiotic phases. Figure from Neiman, 2011.

Meiosis and sporulation are highly regulated processes. Mating-type regulation is mediated by repressor of meiosis I (RME1). If RME1 is deleted, diploid cells can undergo meiosis and produce viable spores (Haber, 2012).

Ime1 is the regulator of the sporulation process, which is induced by glucose or nitrogen starvation. Activation of Ime1 leads the induction of "early" genes required for the entry into premeiotic S phase. The second important checkpoint is carried out by Ndt80, which controls the "middle" genes' expression and spore construction (Jin & Neiman, 2016). Let us focus on two processes that occur in early meiosis: homologous recombination and the spindle pole body formation.

1.2.3. Homologous recombination

Meiotic recombination takes place during prophase I between homologous chromosomes. This mechanism generates genetic diversity within species. In *S. cerevisiae*, to initiate homologous recombination, programmed DSBs are formed by Spo11, a topoisomerase-like protein (Neale & Keeney, 2006).

These breaks are not random and approximately 175 DSBs are formed in budding yeast (Zelkowski et al., 2019), but, rather, tend to concentrate in recombination hotspots. Recombinant sites are in nucleosome-depleted regions (NDRs) near gene promoters, where levels of H3K4 trimethylation are constitutively high as well as in GC rich zones. Set1, in addition to participate in H3K4 methylation and in meiotic S phase, it is also required in DSB formation by marking hotspots (Sollier et al., 2004). Spp1 subunit within the COMPASS (complex of proteins associated with Set1) play an important role in DSB

formation (Imai et al., 2017). Furthermore, DSBs do not take place near the centromeres, blocked by the kinetochore protein complex Ctf19 (Zelkowski et al., 2019).

NDRs are not directly associated with DSB activity, so chromatin accessibility is not the main condition. In the same way, Spo11-binding site it is not directly connected to DSBs. So, how can hotspots be identified and how do DSBs occur? This discrepancy is explained by the "tethered-loop axis model". Meiotic chromosomes are formed by chromatin loops attached to proteinaceous axis. The axial cores of the homologous chromosomes form the synaptonemal complex during zygotene phase. Spp1 binds to (or tethers, that is the reason of the name of tethered-loop axis model) some proteins to the loop to form a DSB (Cooper et al., 2016).

Some of these proteins are the MRX complex (Mre11-Rad50-Xrs2), the RMM complex (Rec114-Mer2-Mei4), Ski8, Rec102 and Rec104. Spp1 directly interacts with Mer2 from the RMM complex bringing Spo11 to the NDR (Figure 6). It is important to remark that Mer2 is phosphorylated by some cyclin-dependent kinases to recruit other proteins (de Massy, 2013).

Some meiosis-specific chromosomal proteins are localized in the synaptonemal complex. For instance, Hop1 participates in DSBs and in spindle detachment in conjunction with Red1. Mer2 recruitment needs the presence of Hop1, and Hop1/Red1 together switch on the Mek1/Mre4 complex, which controls the Rec8 cohesin and the Rad51 recombinase activation (Panizza et al., 2011).

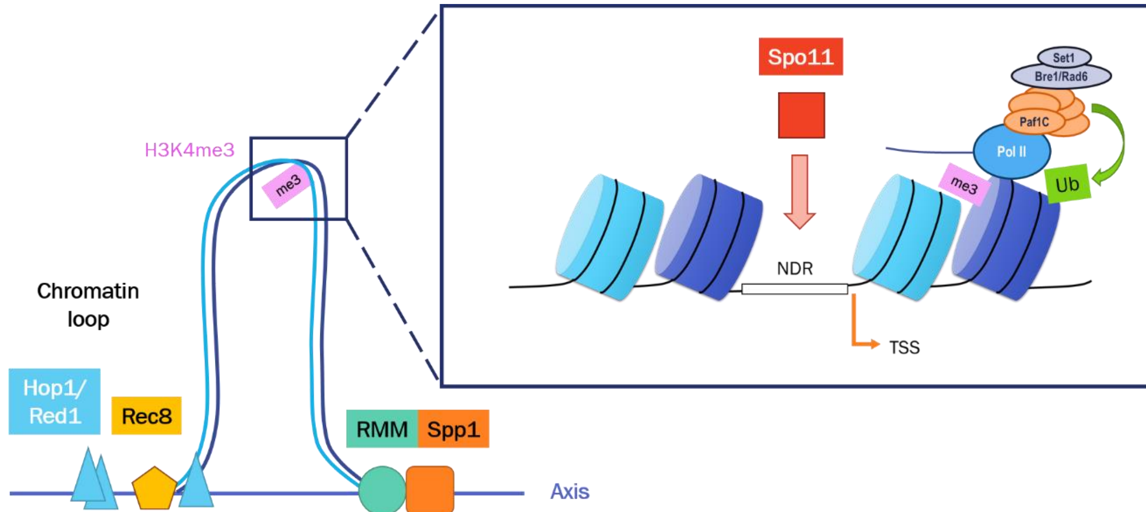


Figure 6 ***Tethered-loop axis model***. Ubiquitination mark is carried by Bre1/Rad6 helped with PAF1C. Consequently, H3K4me3 is deposited by Set1 (PRDM9 mice ortholog). As a result of H2Bub and H3K4me3 cross-talk, chromatin loop is attracted to Spp1, getting closer chromatin loop and chromatin axis. Spo11 is recruited and binded to NDR, located around the transcription start site (TSS) and giving rise to DSBs.

Once Spo11 has cut DNA, Rad51 and Dmc1 are attached to this short ssDNA. The Rad51 recombinase facilitates the search of homologous regions and is required for the normal progression through meiosis. In agreement, the deletion of Rad51 affects mating-type switching and sporulation, (Haber, 2012). Dmc1 is expressed only during

meiosis to facilitate strand invasion during recombination (Neale & Keeney, 2006). Dmc1 has a critical role in meiosis in some sexual organisms, but its molecular functions and how and why it differs from Rad51 are not well understood (Brooks Crickard et al., 2018).

After DSB formation, the repair of strands can result in either a reciprocal exchange of chromosomes, called crossover or in no exchange, named as non-crossover (Keeney et al., 2014). In budding yeast, from the average of DSBs, only half result in crossovers (Zelkowski et al., 2019). Rad51 and Dmc1 help ssDNA to look for duplex partner invading the homologue strand. A Holliday junction structure is arranged, processed by Holliday-resolvases (like Exo1) producing the crossovers. Dmc1 needs the turn on of Red1, Hop1 and Mek1/Mre4 (Hong et al., 2019). All the recombination process is detailed in Figure 7.

Another checkpoint is overseen by Ndt80. This protein that controls the entry into middle-phase during sporulation, is inhibited during recombination process by Spo11. When recombination has reached to its end, Spo11 is inhibited giving way to Ndt80 expression (Figure 5).

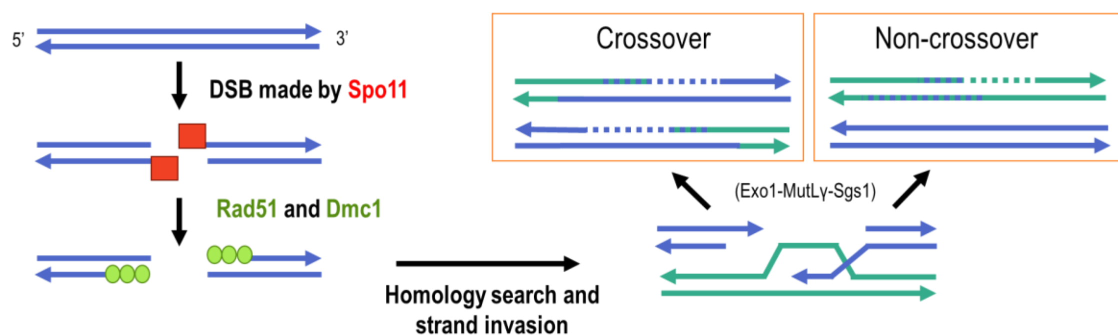


Figure 7 ***Homologous recombination.***

1.2.4. Spindle pole body structure

Once the chromosome recombination process has been successful, Ndt80 expression implies the entrance into middle phase. In this phase, cytological events to divide into 4 haploid cells are predominant (Neiman, 2011). It is essential to recognize some of the important components of its architecture to understand how it could interact with some proteins involved in meiosis and transcription on *in silico* analysis.

The spindle pole body (SPB) is the sole microtubule organizing centre in budding and fission yeast, embedded in the nuclear envelope throughout the life cycle. Its architecture is equivalent to animal cells' centrosome. In meiosis, two divisions occur, so the SPB duplicates twice: at the beginning of meiosis I and at the beginning of meiosis

II, without DNA duplication. In meiosis I, the two formed SPBs are similar to mitotic SPBs (Neiman, 2011).

The SPB in meiosis I is made up of 18 components. Among them, Spc72, Cnm67, Spc42 and Spc110 are part of the SPB core (Cavanaugh & Jaspersen, 2017). Spc72 acts as a receptor of γ-tubulin which disappears in meiosis II by proteolysis to form the meiosis II outer plaque (Neiman, 2011).

A relationship between SPB and DSB formation has been considered (Villoria et al., 2017). After DSB generation, there is an activation of Cdc14, triggering some cyclin-dependent kinases, phosphorylating Spc110. This study suggests that Spc110 phosphorylation stimulates the interaction of DSBs with the SPB to promote an efficient DNA repair by homologous recombination.

2. Objectives

In 2018, the *Gene Expression and RNA Metabolism Laboratory* at IBV-CSIC published that the double mutant *rtf1Δ* (from PAF1c) and *mog1Δ* hindered cells growth and blocked mRNA. In mRNA fluorescence *in situ* hybridization (FISH) experiments, some spots appeared in this double mutant, hypothesising that these spots could correspond to spindle pole bodies (SPBs). In light of these results and considering that Mog1 interacts physically with some COMPASS subunits, like Spp1, which are implied in DSBs formation, it could exist some relationship between PAF1c and Mog1.

With the aim of understanding the molecular events which occur during meiosis with the participation of the transcriptional complex PAF1, several approaches are taken:

A first approach would have consisted on studying functional interaction between PAF1c with meiotic SPB formation as well as with mRNA export. Experiments carried out would have been:

- Strain construction, by creating single mutants of PAF1, CDC73, RTF1, LEO1, CTR9 and MOG1 and double mutants between each PAF1c subunit and MOG1, all performed in a SK1 strain, used to study sporulation and meiosis.
- Once the set of mutants are constructed, some phenotypic assays as mRNA FISH and experiments to measure growth under different conditions will be carried out.
- Fusion protein construction with green fluorescence protein (GFP) and some subunits of the SPB complex, like Spc110 and Cnm67 expressing in the mutants. GFP fluorescence microscopy assays will be used to localise the different proteins.

Despite covid-19 situation and the impossibility to do the experiments, objectives were redirected to:

1. Accomplish a bibliographical review of proteins involved in transcription, focusing on PAF1c that have been recently linked with early meiotic events and epigenetics.
2. Construct an *in silico* interactome between PAF1c and Mog1, a protein involved in histone modifications to figure out some connections between epigenetics and meiosis.
3. Review human diseases and other development pathologies caused by overexpression of PAF1c subunits or misregulation of some epigenetic marks.

3. Materials and methods

To review the literature about transcription and meiosis in yeast, as well as some experiments related to the main topic, the following databases have been used to search for journals and articles:

- Google Scholar.
- PubMed from the National Center for Biotechnology Information.

The essential review articles are Jaehning, 2010 and van Oss et al., 2017 to be versed in transcription and the PAF1c and Neiman, 2011 to understand the molecular mechanisms about yeast sporulation and meiosis.

To study the main implications of PAF1c and meiotic recombination, data from spore viability experiments have been obtained from Gothwal et al., 2016 and Zhang et al., 2020. These two scientific papers just like Oliete-Calvo et al., 2018 are the basis of this project.

In order to carry out the *in silico* analysis between PAF1c and Mog1 interaction, databases employed to download information have been:

- Biological General Repository for Interaction Datasets (BioGRID).
- Osprey: Network Visualization System software from BioGRID.
- TheCellMap.org.

All data have been processed with Rstudio. In the case of interaction plots, the Igraph package from RStudio has been used.

4. PAF1c implication in meiotic recombination

An interesting point to study is how PAF1c in addition to regulate transcription is important during early meiotic events. Few studies have been published about PAF1c and its relationship with meiosis, but the main conclusions are:

- Rtf1 and Cdc73 subunits from PAF1c are functionally connected to early meiotic events.
- Rtf1 works upstream from Set1 but they are independent of each other although both are necessary for meiosis.
- Rtf1 and Set1 are necessary for DSB formation.
- Rtf1 interacts genetically with the RMM complex, and *rtf1Δ* mutants exhibit meiotic alterations.

Through the next section I will highlight the main experimental evidences supporting the role of PAF1c components in meiotic recombination.

4.1. Spore viability experiments

A spore viability experiment can be made by dissecting a certain number of spore tetrads and determining if the spore is alive, giving us information about how necessary a gene is in meiosis and sporulation by deleting it. In this case, a set of mutants is constructed with the five PAF1c subunits.

Rtf1 and *Cdc73* mutants have a significantly reduced spore viability (75.3 and 87.9%, respectively) against the wild-type (97.8%), shown in Figure 8. Set1, which plays an important role in meiosis (Sollier et al., 2004) has also a reduced percentage (89.5%).

To check if mutations are epistatic (this means that a mutation of gene "a" is dependent of gene "b" mutation) double mutants are constructed (*rtf1 set1* and *cdc73 set1*). Spore viability decreased for the *rtf1 set1* mutant (77.5%) but increased for *cdc73 set1* (90.2%) indicating that Set1 and/or Rtf1 are necessary for meiosis independently of methylation (Gothwal et al., 2016).

The RMM (Rec114-Mer2-Mei4) complex binds to chromosome axes near Spp1, which helps Mer2 bring Spo11 and start recombination (Figure 6). It has been recently studied that PAF1c also interacts with the RMM complex by regulating DSB formation (Zhang et al., 2020). *rtf1* mutants with both *Rec114-myc* and *Mer2-myc* spore viability (7.83 and 0.5%, respectively) suffered a strong decrease regarding the wild-type (97%). This indicates that Rtf1 subunit of PAF1c interacts genetically with Rec114 and Mer2.

Rec114-myc *rtf1* and Rec114-myc *set1* have different spore viabilities (7.8 and 76%, respectively). This states that Rtf1 and Set1 are independent, suggesting that there could be a novel mechanism involving Set1 for a proper chromosome segregation to ensure spore viability (Zhang et al., 2020).

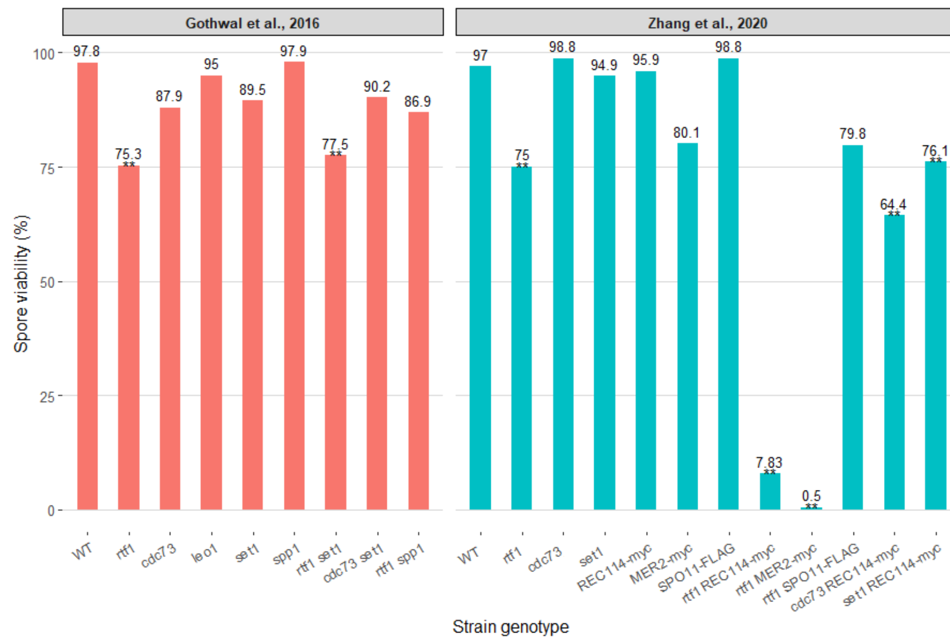


Figure 8 Spore viability experiments. In this bar plot both spore viability experiments carried by Shinohara's group are shown. Each bar indicates the percentage of spore viability. Those significant strain genotypes are marked with (**) below the percentage. Spores were incubated at 30ºC for 3 days. Both experiments are compared against its wild-type. Data from Gothwal et al., 2016 and Zhang et al., 2020.

Furthermore, the Cdc73 subunit shows a similar interaction with Rec114-myc but Spo11 maintained spore viability, indicating that Rtf1 does not show any kind of interaction with Spo11 (Zhang et al., 2020).

4.2. Kinetics of meiosis experiments

DAPI (4',6-diamidino-2-phenylindole) staining, which binds to DNA regions rich in adenine and thymine, can be used to study the first meiotic cell division. Since Rtf1 and Set1 are vital in sporulation and Rtf1 interacts with the RMM subcomplex, the next question is: if these subunits are mutated, how is meiosis timing altered?

As explained in Section 1.2.2., *set1* deletion causes an important meiosis delay. Figure 9A, shows the delay in completing meiosis for the *rtf1Δ* mutant which is 2 hours shorter than the one observed for the *set1Δ* mutant. Double mutant kinetics is similar to single *rtf1Δ* mutant, proffering that Rtf1 works upstream of Set1 (Gothwal et al., 2016).

Rad51 binds to the ssDNA of DSB ends for homologous strand exchange as described in Figure 7. Meiotic chromosomes with an *rtf1* mutation show a delay in Rad51 assembly (Figure 9B), observing less Rad51 foci stained with DAPI. These results propose that PAF1c, specially its Rtf1 subunit, is indispensable for DSB formation. This make sense since Rtf1 is critical in H3K4 methylation, as detailed in the next section.

Concurrently, both *rtf1 Rec114-myc* and *rtf1 Mer2-myc* showed a large reduction in Rad51 foci, restating that Rtf1 participates in DSB formation and the RMM complex is affected by mutations in PAF1c (Zhang et al., 2020).

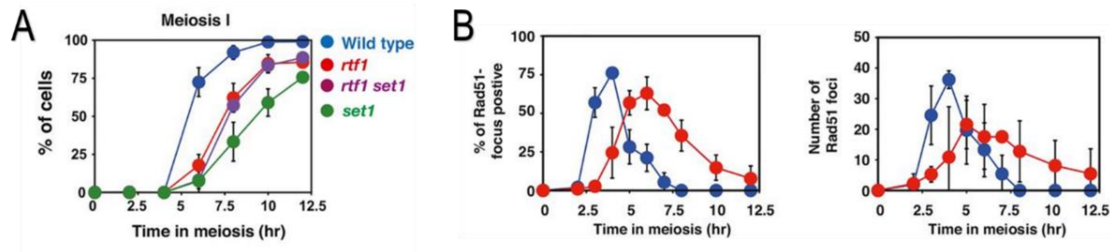


***Figure 9* Kinetics of first meiotic cell division and Rad51-focus**. (A) Meiosis I analysed by DAPI staining. 100 cells were counted for each point and each plotted value represents the mean ± SD from three independent time courses, in hours (hr). (B) A focus-positive cell was defined as a cell with more than five foci. 100 nuclei were counted for each time point and each plotted value represents the mean ± SEM from three independent time courses, in hours (hr). Blue circles represent wild type and red circles *rtf1* mutant. From Gothwal et al., 2016.

4.3. Other RNAPII-associated complexes involved in meiosis

High-throughput analysis in *S. cerevisiae* has allowed screenings for protein-protein and protein complex interactions, linking meiotic events with transcription. Sgf73 from the SAGA complex and Soh1 from the Mediator complex are proposed to participate in some of them (Jordan et al., 2007).

Sgf73 is part of the DUB module in the SAGA (Spt-Ada-Gcn5-acetyltransferase) complex that participates in the deubiquitination of H2B together with Ubp8, Sgf11 and Sus1 (Rodríguez-Navarro, 2009). It is a conserved protein which is involved in human spinocerebellar ataxia type 7 (human ortholog ATXN7) by associating with microtubules. Deletions in *SGF73* cause a lower induction of *IME1*, causing a slower duplication of DNA in first meiotic division and ineffective nuclear divisions. On the other hand, Sgf73 may not participate in DSB formation (Jordan et al., 2007).

Soh1 (Med31) is a component of the Mediator complex (Linder & Gustafsson, 2004). Synthetic genetic arrays (SGA) analyses (explained in detail in Section 6) show that Soh1 interacts with Set2, as well as with PAF1c subunits (Krogan et al., 2003). Comparing Soh1 deletion and wild type, a delay of meiosis and DSB formation was observed (Jordan et al., 2007). Med31 is the most conserved subunit in the Mediator complex. Phylogenetic studies show that this canonical Mediator subunit has a high homology among budding yeast, fission yeast, human and some protozoan. Studies in *Tetrahymena thermophila*, a ciliate protozoan used as a unicellular model eukaryote, reveal that Med31 localizes in early meiotic prophase and it is essential in developmental genes expression. Med31 could interfere with DNA rearrangements and replication during sexual reproduction, observed as extrusion bodies in meiotic nuclei (Garg et al., 2019).

5. Early meiotic events influenced by histone modifications

DNA is packaged with proteins called histones. Histone architecture is divided in the globular core and histone tails, which are subject to post-translational modifications (PTM). The most common covalent modifications are acetylation, methylation, phosphorylation and ubiquitination, depicted in Figure 10 (Craig et al., 2010). These changes which do not alter DNA sequence are referred to as "epigenetics".

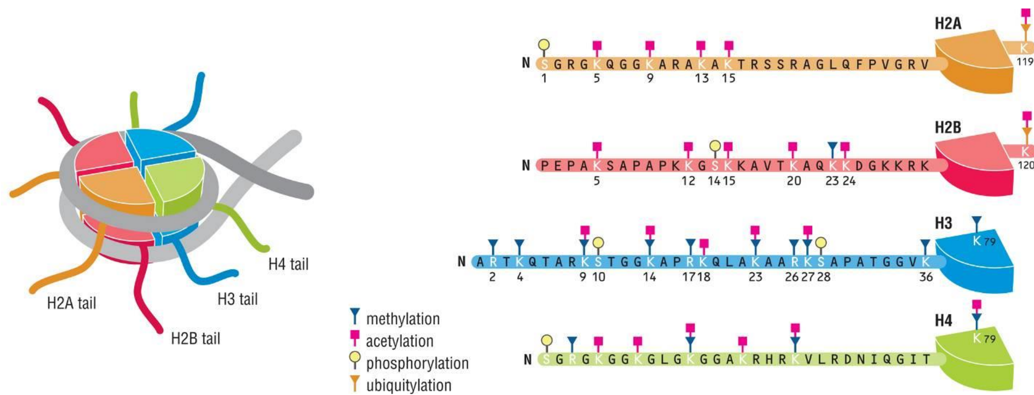


***Figure 10* Histone modifications diagram**. From Craig et al., 2010.

DSBs occur in DNA as explained above in specific places called nucleosome depleted regions (NDR) (Figure 6). The involvement of these PTMs in different molecular events during meiosis appears to be unquestionable, for it is one of the most precise ways to regulate the whole process (Luense et al., 2016). Turning our attention to yeast, it will be highlighted several PTMs that may have influence on early meiotic events (summarized on Table 1).

***Table 1* Histone modifications involved in meiosis**

Complex (Writer)	Histone	Modification
Rad6/Bre1	H2B	Ubiquitination
Set1/COMPASS	H3K4	Methylation
Set2	H3K36	Methylation
Dot1	H3K79	Methylation

5.1. H3 methylation

Histone H3 methylation is balanced between histone methyltransferases (HMTs) or "writers" and demethylases or "erasers". These methyl marks are recognised by

different effector proteins or "readers" (Hyun et al., 2017). Methylation was first reported by Alfred and Mirsky in the 1960s, but it was not until 40 years later that the first HMT was described with the catalytic SET domain (Rea et al., 2000).

Lysine (K) is the most common residue favourable to be methylated. There are three K methylation states: mono-, di- and trimethylation (me1, me2 and me3, respectively). These PTMs confer active or repressive transcription depending on their positions and methylation states. H3K4, H3K36 (represented in Figure 2 right) and H3K79 methylations are considered to mark active transcription (Black et al., 2012).

5.1.1. H3 modifications and meiosis

rtf1 mutants show undetectable levels of H3K4me1/me2/me3 and H3K79me3. This is because PAF1c subunits interact with Set1/COMPASS and Dot1 physical and genetically (Krogan et al., 2003), being essential for transcriptional elongation and chromatin methylation. Gothwal et al. confirmed this interaction in 2016 by Western blotting showing that mutations in *RTF1* (Figure 11) cause defective H3K4me1/me2/me3 and HK79me3 and they also reported a slight decrease in methylation in both H3 residues.

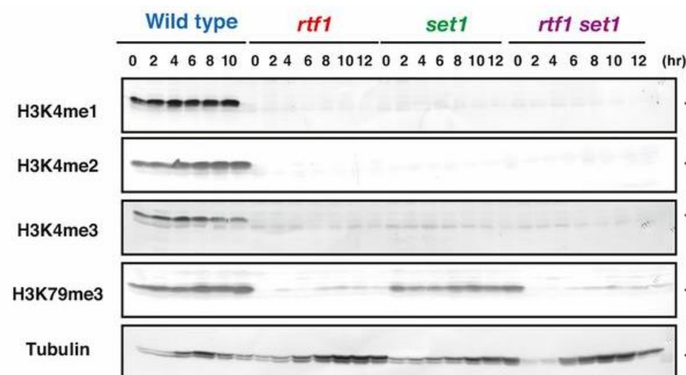


Figure 11 ***Histone methylation marks during meiosis***. Histone H3K4me1/me2/me3, H3K79me3 and α-tubulin as a loading control were verified by Western blotting. From Gothwal et al., 2016.

Diverse studies affirm that H3K4me is a fundamental tag for meiotic DSB formation, process that is quite conserved from yeast to human (Székvölgyi et al., 2015). As explained in the previous section, deletions in *SET1* severely reduce meiotic DSB levels at canonical hotspots, as it happens with point mutations in H3K4 (Acquaviva et al., 2013).

H3K4me marks are recognized by a constituent of the COMPASS complex, the Spp1 protein, which plays an important role in recombination initiation during meiosis. Spp1 binds to chromosome axes (Figure 6) and also interacts with the Mer2/RMM complex, paying an additional role in DSB generation (Sommermeyer et al., 2013). The

interaction between Spp1 and Mer2 enables their anchoring to DSB hotspots, which conducts DSB formation with dependence on Spo11. Some research (Adam et al., 2018) provides that Spp1 preserves H3K4 methylation levels independently of Set1 in three steps: first Spp1 methylates H3K4, second Spp1 maintains methylated levels without interaction of Set1 protecting it from demethylases (like Jhd2) and as last step Spp1 and Mer2 help the recruitment of Spo11.

A conserved proof that there is a relationship between H3K4me3 and meiotic-driven DSBs can be found in mammals, where the histone methylase Prdm9 leads DSBs to occur in DNA motifs recognized by its zinc finger domain (Diagouraga et al., 2018; Powers et al., 2016).

5.2. H2B monoubiquitination

The largest histone modification consists in the covalent attachment of a 76-amino acid protein, ubiquitin, to K side chains. Ubiquitin is attached to histones in a series of enzymatic steps and can be removed by a deubiquitinating enzyme (DUB). Histone ubiquitination plays roles in regulating different steps of transcription as well as of DNA repair (Craig et al., 2010).

It has been mentioned that in budding yeast, H3K4 methylation is carried out by Set1, H3K36 by Set2 and H3K79 by Dot1. Unexpectedly, H3K4 and H3K79 methylation depend on Rad6, an enzyme that ubiquitinates the histone H2B (Ng et al., 2003), and Bre1, an E3 ubiquitin ligase required for Rad6 function. This relationship between H3 methylation and H2B ubiquitination is known as "cross-talk" (represented in Figure 12) and its mechanism has been unknown but recently some studies have shed light to this riddle (Hsu et al., 2019; Worden et al., 2020). More details about histone modifications and their role in transcription can be read in Serrano-Quílez et al., 2020's review (Supplemental I)

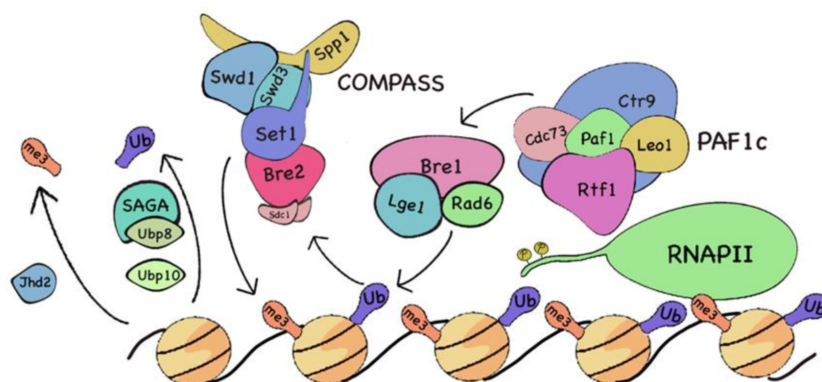


***Figure 12* Histone H3K4me & H2Bub cross-talk**. PAF1c recognizes Ser2P and Ser5P of the RNAPII CTD and promotes H2B ubiquitination by Bre1-Lge1-Rad6. This modification is recognized by the COMPASS complex, which trimethylates H3K4. Conversely, H2Bub is removed by the ubiquitin-proteases Ubp10 and Ubp8, the last belonging to the DUBm of SAGA. Trimethylation is eliminated by the Jhd2 demethylase. From Serrano-Quílez et al., 2020.

5.2.1. H2Bub and meiosis

Histone H2B monoubiquitination (H2Bub) on its lysine 123 is controlled by the E2-conjugating enzyme Rad6, the E3-ligase Bre1 and the regulatory cofactor Lge1 (Wood et al., 2003). H2Bub also requires the participation of other factors like PAF1c (Ng et al., 2003) and FACT.

In addition to its role in gene expression, H2B ubiquitination has also been related to DSBs formation. This epigenetic mark leads to chromatin relaxation, enabling the recruitment of several DSB repair factors to their accurate positions at hotspots (Xu et al., 2016). Likewise, Bre1 and Lge1 proteins have also been related to meiotic processes; *lge1Δ* and *bre1Δ* cells initiate meiotic DNA replication in the S-phase, later than the wild-type, and take much longer to complete it, presenting a reduced DSB formation as well (Jordan et al., 2007).

As explained above, PAF1c component Rtf1 has been described as participating in the meiotic process. Rtf1 is important for the formation of DSBs (Gothwal et al., 2016), and this role is independent of the presence of Set1. Rtf1 directly interacts with Rad6 and stimulates H2Bub independently of transcription (Kim et al., 2009).

Rtf1 is recruited by Mog1, a nuclear Ran-binding protein. Deletions of *MOG1* result in low global levels of H2Bub and H3K4me3. In SGA, *MOG1* was confirmed that is genetically linked with *SUS1*. Sus1, a component of the SAGA DUBm and TREX-2 plays an important role in coupling transcription activation, H2Bub deubiquitination and mRNA export (Oliete-Calvo et al., 2018). PAF1c and Mog1 interactions could linked transcription, meiosis and mRNA export but some research needs to be performed.

Furthermore, in yeast H2B deubiquitination is performed by two proteases: Ubp10 and Ubp8. Interestingly, Ubp8 belongs to the SAGA complex, and in particular to its deubiquitination module (DUBm) (Ingvarsdottir et al., 2005; Köhler et al., 2006; Lee et al., 2005). Ubp8 from SAGA DUBm is involved in H2B and Cse4 deubiquitination. Cse4 is a centromeric, H3-like histone protein (vertebrates' centromere protein A or CENP-A orthologue) and its ubiquitination regulates its localization to centromeres, where the spindle pole body is attached to the chromosome (Agarwal et al., 2015).

Dysregulations of H2Bub modification in higher eukaryotes lead to important pathological events related with development, tumorigenesis, and deficiencies in cellular differentiation.

5.3. Other epigenetic modifications involved in meiosis

DNA and RNA are covalently modified post-synthesis as well as histones, like the classic DNA 5-methylcytosine (5mC) of CpG islands. Some modifications have been reported to control some meiotic genes. One that needs to be mentioned is the methylation of adenosine at the N6 position to form N6-methyladenosine (m6A), which is the most abundant base modification known in eukaryotic mRNA (Fu et al., 2014).

In *S. cerevisiae* a core RNA methyltransferase (MIS) complex has been identified, being comprised of: Ime4 (mammalian ortholog METTL3), Mum2 (orthologous to mammalian Wilm's-tumor-1-associated protein, WTAP) and Slz1 (Schwartz et al., 2013).

As introduced in Section 1.2.2., meiosis is regulated by Rme1. Rme1 represses Ime1, the transcriptional activator of meiosis. When Rme1 is highly expressed, meiotic mRNA methylation is needed. Ime4 m6A methyltransferase activity is required to reduce Rme1 expression, so, Ime4 is an upstream inhibitor of Rme1, supported by epistasis analyses (Bushkin et al., 2019). The downregulation of Rme1 enables meiotic entry relieving Ime1 repression and permitting DNA replication. In this case, the methylation of an adenosine controls meiotic entry.

Furthermore, it has been studied that an *IME4* deletion delays Ndt80 induction, composition of the synaptonemal complex and DSBs as described above.

6. *In silico* analysis of PAF1c-Mog1 interaction

As previously described, Mog1 is required to recruit Rad6, Bre1 and Rtf1 (this last one from PAF1c) to ubiquitinate H2B (Oliete-Calvo et al., 2018). Recent studies have correlated PAF1c and meiosis, so the *Gene Expression and RNA Metabolism Laboratory* at IBV-CSIC, is interested in studying if Mog1 and PAF1c interact with some early-meiotic proteins.

During this project, some proteins that are involved in early meiotic events as well as in histone modifications have been introduced (listed in Supplemental II). These proteins have been used to search a genetic network of Mog1, Paf1, Rtf1, Leo1, Cdc73 and Ctr9 in BioGRID (*TYERSLAB*, 2020).

As shown in Figure 13, Mog1 interacts with the Mediator subunit Soh1, which has been established that interacts with RNAPII, and consequently with PAF1c (Krogan et al., 2003). This graphical diagram must be accompanied with SGA genetic interaction experiments and some kind of quantification. For this purpose, there is a repository of genome-scale SGA experiments with *S. cerevisiae* called TheCellMap (*UNIVERSITY OF TORONTO AND UNIVERSITY OF MINNESOTA*, 2016).

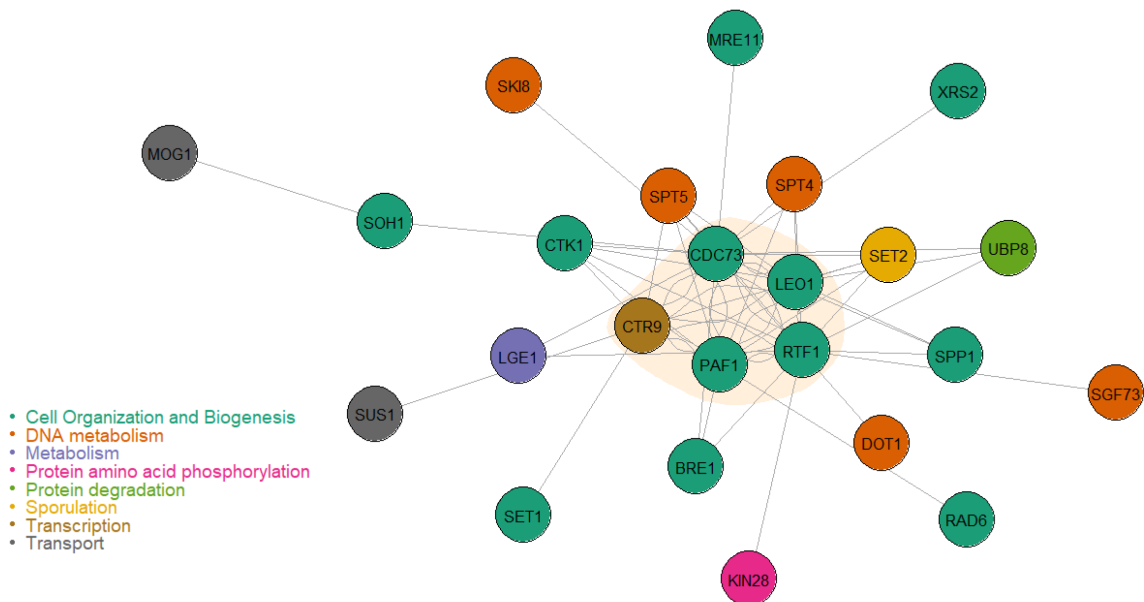


***Figure 13* Interaction network between PAF1c and Mog1.** All known physical or genetic interactions were downloaded from BioGRID database. Osprey software *(TYERSLAB, 2003)* was used to obtain the graphical representation by gene ontology (GO), legend shown in the bottom left of the figure. All data was managed and represented with the Igraph package *(NETWORK ANALYSIS SOFTWARE OPEN SOURCE, 2020)* from RStudio (*R FOUNDATION FOR STATISTICAL COMPUTING, 2020*). Large graph layout (LGL) was used to dynamically visualize. Orange blurred circle contains the five subunits of PAF1c.

SGA analysis combines arrays of either nonessential gene deletion mutants or conditional alleles of essential genes to enable high throughput construction of haploid yeast double mutants and quantitative analysis of genetic interaction.

An interaction is defined by deviation of a double-mutant organism's phenotype from the expected neutral phenotype (Mani et al., 2008). If this difference is very high, it is defined as a synergistic or synthetic interaction (synthetic lethality in the extreme case). Genetic interactions can be grouped into two categories: negative and positive (Costanzo et al., 2016). Negative genetic interactions describe double mutants that exhibit a more severe phenotype than expected, synthetic lethality in an extreme case; while positive genetic interactions describe double mutants exhibiting a less severe phenotype, like genes encoding members of the same nonessential protein complex or epistatic genes.

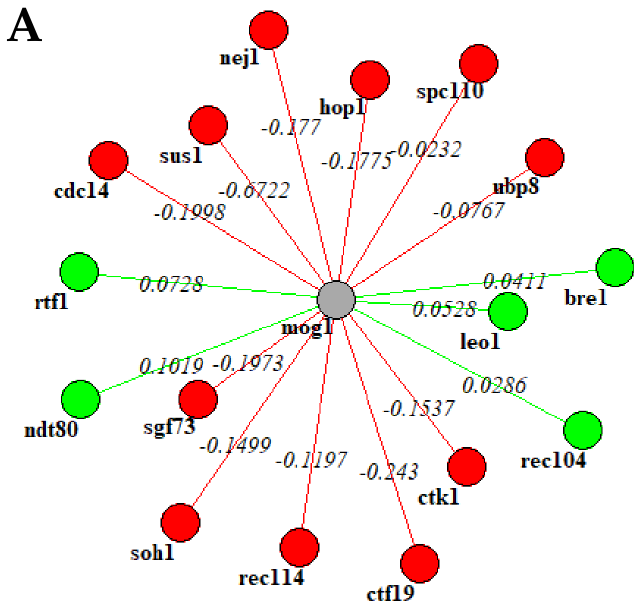
TheCellMap provided for a selected gene a list of genes extracted by SGA analysis with its SGA score. SGA score measures the extent to which a double mutant observed colony size deviates from the expected colony size from combining two mutations together. The magnitude of the SGA score is indicative of the strength of interaction. It is defined as a significant positive genetic interaction if SGA score > 0.08, and significant negative genetic interaction if SGA score < -0.08 (Usaj et al., 2017). It is important to remark that these data are obtained mostly from haploid cells growing under vegetative conditions, therefore new connections should be found experimentally when using the correct experimental conditions.

In Figure 14, SGA interaction network plots are shown for *MOG1* and the five genes encoding for subunits of PAF1c. It is important to remark that *MOG1* has a positive interaction with *RTF1* and *LEO1* genes, although it is not significant. It interacts with some elements of the spindle like *HOP1* (significant negative genetic interaction) and *SPC110*. It also interacts significantly with *NDT80*, involved in sporulation control and *CDC14*, which stimulates DSB formation.

Interestingly, in the case of PAF1c subunits there are some aspects to highlight. *PAF1* interacts non-significantly with only the centromeric protein gene *CSE4*. All the rest have in common the positive genetic interaction with the ubiquitin ligase *BRE1*. All of them except *CTR9* interacts with SPB-associated proteins.

According to Section 4.1., *rtf1* and *cdc73* mutants showed a decrease in spore viability experiments. Rtf1 interacts negatively with Set2, which main GO term is sporulation, as described in Figure 13. Cdc73 interacts with Ndt80, which regulates sporulation and early genes induction. According to spore viability experiments (Figure 8, Zhang et al., 2020), Rtf1 does not interact with Spo11. In Figure 14C, it is shown that Rtf1 has a negative interaction with Spo11, but its SGA score is not significant.

As PAF1c interaction experiments are so recent, it is quite probable that TheCellMap database has not been updated. It must consider that SGA scores data come from SGA experiments from Boone and Andreys' labs, in The Donnelly Centre for Cellular and Biomolecular Research at the University of Toronto.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hop1
spc110
sus1
cdc14
ubp8
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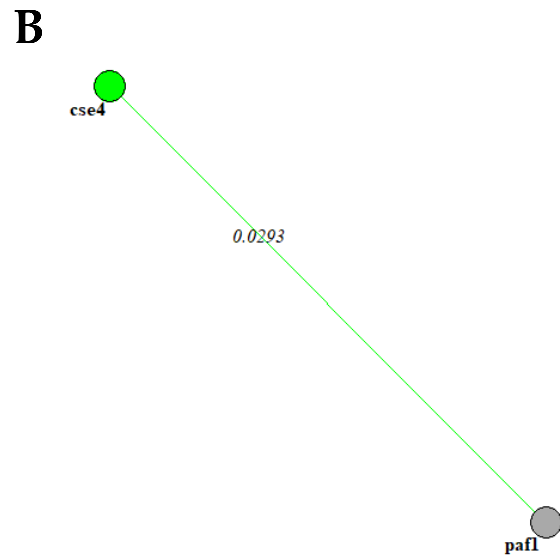
-0.1775
-0.0232
-0.6722
-0.1998
-0.0767
rtf1
0.0728
0.0411
bre1
0.0528
mog1
leo1
0.1019
-0.1973
0.0286
ndt80
sgf73
-0.1537
rec104
-0.1499
-0.1197
-0.243
ctk1
soh1
rec114
ctf19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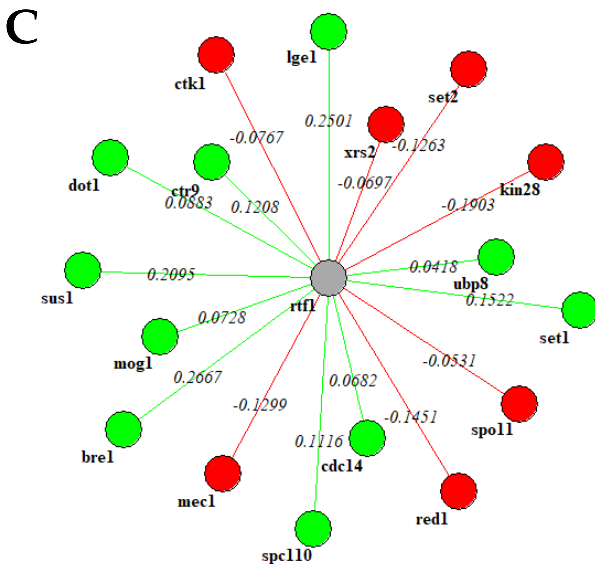
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ctk1
set2
0.2501
xrs2
-0.1263
-0.0767
dot1
ctr9
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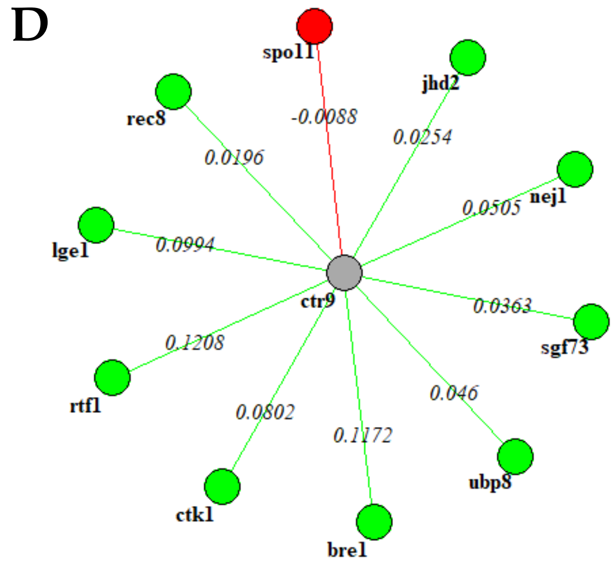
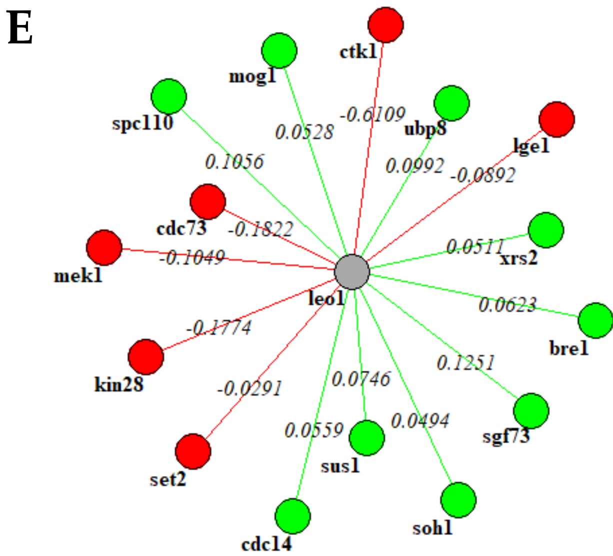
-0.0697
kin28
-0.1903
0.0418
ubp8
sus1
0.2095
rtf1
0.1522
set1
0.0728
mog1
0.2667
-0.0531
0.0682
-0.1299
-0.1451
spo11
bre1
0.1116
cdc14
mec1
red1
spc110


D
spo11
jhd2
rec8
-0.0088
0.0254
0.0196
nej1
0.0505
lge1
0.0994
ctr9
0.0363
sgf73
0.1208
rtf1
0.046
0.0802
0.1172
ubp8
ctk1
bre1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spc110
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lge1
0.1056
0.0992
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cdc73
-0.1822
mek1
-0.1049
0.0511
xrs2
leo1
0.0623
bre1
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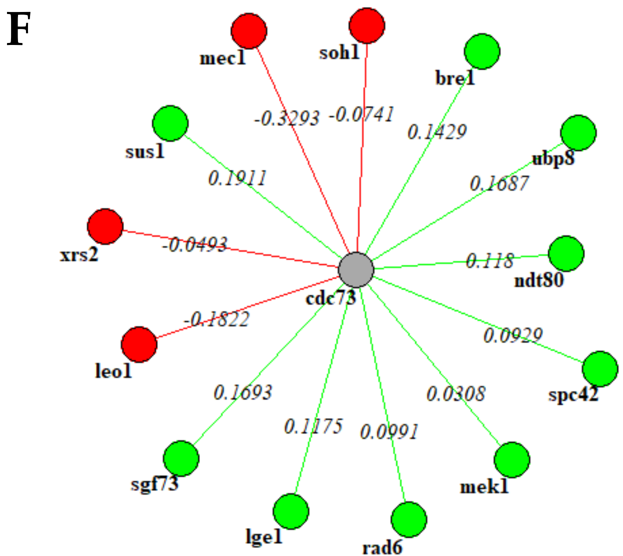
sus1
set2
soh1
cdc14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mec1
soh1
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0.1429
sus1
ubp8
0.1911
0.1687
xrs2
-0.0493
0.118
ndt80
cdc73
-0.1822
leo1
0.0929
spc42
0.1693
0.0308
0.1175
0.0991
sgf73
mek1
lge1
rad6

***Figure 14* Network visualization of SGA interactions** of (A) *MOG1*, (B) *PAF1*, (C) *RTF1*, (D) *CTR9*, (E) *LEO1* and (F) *CDC73* between genes listed in Supplemental II. Data was downloaded from *TheCellMap*. Positive SGA interactions are coloured in green while negative SGA interactions are in red. SGA scores are shown in the middle of the vertices. All data was managed and represented with Igraph package from RStudio.

7. Human diseases

It has been explained how some RNAPII-associated proteins and subunits participate in meiosis and in histone modifications in *S. cerevisiae*. Some of the protagonists introduced have their homologous in humans and their overexpression or misfunction can cause diseases.

In this last section will be mentioned diseases associated with PAF1c and with some proteins involved that participate in epigenetic modifications.

7.1. PAF1C associated diseases

PAF1c has been correlated with cancer and development (van Oss et al., 2017). In Table 2 some of the pathologies associated to PAF1c are mentioned.

***Table 2* Some of the main PAF1c subunits-associated diseases**

Subunit	Pathologies associated	Reference
Paf1	Pancreatic cancer	(Chaudhary et al., 2007)
	Ovarian cancer	(Sha et al., 2019)
	Oocyte development	(Karmakar et al., 2018)
	Leukemia	(Meeks & Shilatifard, 2017)
	Mitophagy	(Shu et al., 2020)
Cdc73	Parathyroid carcinoma	(Cardoso et al., 2017)
	Liver and breast carcinoma	(Chaudhary et al., 2007)
	Embryonic development	(Wang et al., 2008)
Leo1	Colorectal cancer	(Chaudhary et al., 2007)
	Leukemia	(Chong et al., 2019)
Ctr9	Lung cancer and leukemia	(Chaudhary et al., 2007)
Rtf1 (hSki8)	Pancreatic and colorectal cancer	(Chaudhary et al., 2007)
	B-cell lymphoma	(Chaudhary et al., 2007)

7.1.1. Paf1

Paf1/PD2 is overexpressed in differentiated pancreatic cancer cell lines. It is located in chromosome XIX, in the same region as the oncogene AKT2 involved in tumorigenesis and cell-cycle progression. The overexpression of both Paf1/PD2 and AKT2 and the same location in pancreatic cancer cells suggests a synergistic role in the development of an oncogenic phenotype (Moniaux et al., 2006). The Paf1/PD2 subunit interacts with CXXC

zinc finger protein 1 (CXXC1 or Cfp1, Spp1 yeast ortholog from the COMPASS complex) which binds to unmethylated CpG islands (Yang et al., 2020) upregulated in ovarian and pancreatic stem cells and in oocyte development (Sha et al., 2019).

An ATP-dependent chromatin remodelling protein is regulated by PD2/Paf1, important in maintaining open chromatin and the maintenance of stem cell self-renewal and pluripotency. In addition, a component of the human methyltransferase complex MLL1/Set1 interacts with PD2/Paf1, mediating self-renewal and reprogramming via stem cell core transcriptional network (Karmakar et al., 2018). MLL/Set1, from COMPASS complex, is responsible of H3K4me3, which has been reported to participate in leukemia and in epigenetic regulation (Meeks & Shilatifard, 2017).

PAF1c also participates by regulating mitophagy (mitochondrial autophagy) with Spt4/Spt5's help (Shu et al., 2020).

7.1.2. Cdc73

Parafibromin is a component of hPAF1c and Cdc73 yeast homolog. It is encoded by the HRPT2 gene, associated with parathyroid carcinoma (PC). Furthermore, the HRPT2 locus is amplified in liver carcinoma and breast cancer (Chaudhary et al., 2007). PC includes the hyperparathyroidism-jaw tumour syndrome (HPT-JT), multiple endocrine neoplasia types 1 and 2 (MEN1 and MEN2), and familial isolated primary hyperparathyroidism (FIHP). Approximately 55% of reported germline CDC73 mutations are associated with HPT-JT. Of the remaining 45% of germline Cdc73 mutations, 21% are reported from patients with FIHP, 15% with sporadic PC, 6% with sporadic adenomas and 3% with sporadic ossifying fibromas of the jaw (Cardoso et al., 2017).

Parafibromin also has a role in embryonic development regulating genes involved in cell growth and survival (Tian et al., 2018) and in Wnt/β-catenin signalling pathway that controls cell fate and homeostasis (Cardoso et al., 2017; Wang et al., 2008).

7.1.3. Leo 1

The Leo1 subunit also interacts with β-catenin in the Wnt cascade. This subunit of PAF1C is amplified in colorectal cancer and bone malignant fibrous histiocytoma (Chaudhary et al., 2007). Aberrant activation of Wnt/β-catenin signalling pathway is also necessary for acute myeloid leukemia (AML). Recent studies show that Leo1 binds directly to β-catenin promoting its accumulation in the nucleus, triggering downstream target oncogenic genes (Chong et al., 2019).

7.1.4. Other PAF1c subunits

Ctr9 defects are associated with lung cancer and leukemia, as Paf1/PD2. The human Ski8 subunit of PAF1c (Rtf1 yeast homolog) plays a role in RNA surveillance and quality control during transcription. Ski8 also appears overexpressed in pancreatic tumours, colorectal cancer, and primary cutaneous B-cell lymphoma (Chaudhary et al., 2007).

7.2. Epigenetic proteins associated diseases

In Section 5 it has been discussed about histone methylation and ubiquitination, as well as other epigenetic modifications that affect DNA. We will now focus on Prdm9 methyltransferase and Mog1 proteins, and in m6A.

7.2.1. PRDM9

Recombination hotspots in mammals are triggered by PRDM9-mediated H3K4me3 (Diagouraga et al., 2018). Deficiencies in this gene are associated with male infertility and defective synapsis. Excess of Prdm9 has also been found to be related with acute lymphoblastic leukemia (Bhattacharyya et al., 2019; Paigen & Petkov, 2018). CXXC1 also interacts with PRDM9 in spermatocytes but it is not essential to form DSBs (Tian et al., 2018).

7.2.2. Mog1

Mog1 participates in nuclear export by binding to RanGTP in yeast. Human Mog1 is also a Ran-binding protein which participates in nuclear import, H2Bub, H3K4me3 and it also interacts with the cardiac sodium channel complex Nav1.5 (Kattygnarath et al., 2011). Brugada syndrome, a rare autosomal-dominant clinical entity is caused by mutations in this sodium channel.

Mog1 has been reported to participate in intracellular trafficking of Na^+ to the cell surface increasing cardiac sodium density and is one of the sources of Brugada syndrome (Duina et al., 2014; Yu et al., 2018).

7.2.3. m6A

It has been explained that the m6A modification controls meiosis entrance (Bushkin et al., 2019). Some physiological processes linked to N6-methyladenosine have been reviewed (Fu et al., 2014).

Some of the main implications of m6A rely on mutations within intron 1 of human fat mass and obesity-associated protein (FTO gene) associated with obesity, breast cancer and dopaminergic receptor levels decreasing. mRNAs involved in dopaminergic signalling pathways are hypermethylated, affecting neurones. It also affects development: human sperm development, *Drosophila melanogaster* oogenesis development and plant development (Meyer & Jaffrey, 2014).

8. Conclusions

The main purpose of my work has been to describe the players and mechanisms (mostly epigenetic) that are shared between molecular events taking place during meiosis with transcription in budding yeast, with special interest in PAF1 complex. To conclude with this project, some points are remarkable:

- Although transcription and meiotic recombination are two different biological processes, some players like PAF1c and Set1 act in both homologous recombination and transcription.
- The Rtf1 subunit seems to be the PAF1c subunit that is more implicated in sporulation and DSB formation according to several spore viability and kinetics experiments.
- PAF1c interacts with COMPASS complex which oversees histone methylation. It has been established that PAF1c and RMM interaction preserves H3K4 methylation and DSB formation. This broadens the role of PAF1c beyond transcription.
- Other RNAPII-associated proteins as Soh1 from Mediator complex or Sgf73 and Ubp8 from SAGA complex are present in early meiotic events like DNA replication and SPB attachment, respectively. Sgf73 and Ubp8 are part of DUBm participating in H2B deubiquitination, too.
- Other epigenetic modifications which alter mRNA like m6A have recently described that controls meiosis initiation.
- *In silico* SGA interaction analysis have revealed that Mog1 is related to Rtf1 and Leo1 subunits from PAF1c, as well as with some genes that encode sporulation control, DSB and SPB formation.

Many questions remain unanswered, for instance, (i) Are the molecular mechanisms behind each modification shared between molecular events? (ii) How can the histone readers discriminate between the distinct chromatin-based processes? (iii) Is there a set of specific factors that regulate the writers and the erasers only during meiotic recombination?

Studying how these proteins can interact between them in model systems, like *S. cerevisiae*, it is relevant because it could help to understand the molecular mechanisms of some human pathologies and diseases, expressly those who are related to cancer and development.

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Supplemental I

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Serrano-Quílez, J.; ***Roig-Soucase, S.****; Rodríguez-Navarro, S. Sharing Marks: H3K4 Methylation and H2B Ubiquitination as Features of Meiotic Recombination and Transcription.* Int. J. Mol. Sci. ***2020****,* 21*, 4510.*

Review

Sharing marks: H3K4 methylation and H2B ubiquitination as features of meiotic recombination and transcription

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Abstract: Meiosis is a specialized cell division that gives raise to four haploid gametes from a single diploid cell. During meiosis, homologous recombination is crucial to ensure genetic diversity and guarantee accurate chromosome segregation. Both the formation of programmed meiotic DNA double-strand breaks (DSBs) and their repair using homologous chromosomes are essential and highly regulated pathways. Similar to other processes that take place in the context of chromatin, histone posttranslational modifications (PTMs) constitute one of the major mechanisms to regulate meiotic recombination. In this review, we focus on specific PTMs occurring in histone tails as driving forces of different molecular events, including meiotic recombination and transcription. In particular, we concentrate on the influence of H3K4me3, H2BK123ub, and their corresponding molecular machineries that write, read, and erase these histone marks. The Spp1 subunit within the Complex of Proteins Associated with Set1 (COMPASS) is a critical regulator of H3K4me3-dependent meiotic DSB formation. On the other hand, the PAF1c (RNA polymerase II associated factor 1 complex) drives the ubiquitination of H2BK123 by Rad6-Bre1. We also discuss emerging evidence obtained by cryo-electron microscopy (EM) structure determination that has provided new insights into how the "cross-talk" between these two marks is accomplished.

Keywords: meiosis, recombination, DSB, transcription, COMPASS, histone, PAF1c, methylation, ubiquitination.

Supplemental II

Table S1 ***List of proteins analysed in interaction network plots.*** Each protein includes its name and a brief description. In red, Mog1 and the five subunits of PAF1c.

Name	Description
Bre1	E3 ubiquitin ligase that catalyze H2Bub
Bur1	Phosphorilates Ser2 CTD
Cdc14	Phosphorilates Spc110 and stimulates DSB
Cdc73	Component of the PAF1 complex
Cnm67	SPB component
Cse4	centromeric histone H3-like protein
Ctf19	Blocks DSB in centromeric regions
Ctk1	Phosphorilates Ser2 CTD
Ctr9	Component of the PAF1 complex
Ddc2	Recruits Mec1
Dmc1	Facilitates homologous region search
Dot1	Histone H3K79 methylation
Hop1	DSB formation and spindle detachment, in axis
Ime1	Regulates sporulation, induct early genes
Ime4	RNA methyltransferase, Ime1 inductor
Jhd2	De-methylase
Kin28	Phosphorilates Ser5 CTD
Leo1	Component of the PAF1 complex
Lge1	Regulatory cofactor Rad6/Bre1
Mec1	DNA damage response kinase. DSB checkpoint
Mei4	RMM complex, involved in DSB formation
Mek1	Control Rec8
Mer2	RMM complex, involved in DSB formation
Mog1	Ran-binding protein required for Rad6, Bre1 and Rtf1 recruitment to H2Bub
Mre11	MRX complex, involved in DSB formation
Mre4	Control Rec9
Ndt80	Regulates sporulation, induct middle genes
Nej1	Encodes non-homologous end-joining process
Paf1	Component of the PAF1 complex
Rad50	MRX complex, involved in DSB formation
Rad51	Facilitates homologous region search
Rad6	E2 ubiquitin ligase that catalyze H2Bub
Rec102	Required for meiotic DSB
Rec104	Required for meiotic DSB
Rec114	RMM complex, involved in DSB formation

Rec8	Cohesin
Red1	Component of the synaptonemal complex axial elements
Rme1	Repressor of meiosis I
Rtf1	Component of the PAF1 complex
Set1	From COMPASS required for H3K4me/H2Bub
Set2	Histone H3K36 methylation
Sgf73	From the SAGA complex, related with IME1
Ski8	Subunit of hPAF1c, in yeast is required for meiotic DSB
Soh1	Subunit of the RNA polymerase II Mediator complex
Spc110	SPB component
Spc42	SPB component
Spc72	SPB component
Spo11	Topoisomerase-like protein which programs DSB
Spp1	From COMPASS complex, opens the chromatin, interacts with Mer2
Spt4	Elongation control during transcription
Spt5	Elongation control during transcription
Sus1	From SAGA complex, related to mRNA export
Tel1	DNA damage response kinase. DSB checkpoint
Ubp8	Cse4 deubiquitination
Xrs2	MRX complex, involved in DSB formation