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Ask yeast how to burn your fats: Lessons learned from the metabolic adaptation to salt stress

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Abstract Here we review and update the recent advances in the metabolic control during the adaptive response of budding yeast to hyperosmotic and salt stress, which is one of the best understood signaling events at the molecular level. This environmental stress can be easily applied and hence has been exploited in the past to generate an impressively detailed and comprehensive model of cellular adaptation. It is clear now that this stress modulates a great number of different physiological functions of the cell, which altogether contribute to cellular survival and adaptation. Primary defense mechanisms are the massive induction of stress tolerance genes in the nucleus, the activation of cation transport at the plasma membrane or the production and intracellular accumulation of osmolytes. At the same time and in a coordinated manner, the cell shuts down the expression of housekeeping genes, delays the progression of the cell cycle, inhibits genomic replication and modulates translation efficiency in order to optimize the response and to avoid cellular damage. To this fascinating interplay of cellular functions directly regulated by the stress we have to add yet another layer of control, which is physiologically relevant for stress tolerance. Salt stress induces an immediate metabolic readjustment, which includes the up-regulation of peroxisomal biomass and activity in a coordinated manner with the reinforcement of mitochondrial respiratory metabolism. Our recent findings are consistent with a model where salt stress triggers a metabolic shift from fermentation to respiration fueled by the enhanced peroxisomal oxidation of fatty acids. We discuss here the regulatory details of this stress-induced metabolic shift and its possible roles in the context of the previously known adaptive functions.

Keywords salt stress, *Saccharomyces cerevisiae*, high osmolarity glycerol pathway, peroxisome, mitochondria, integrated stress adaptation, metabolic switch.

Budding yeast is an attractive and powerful model for the investigation of abiotic stress responses (Ho and Gasch 2015). Important environmental challenges include temperature, pH, oxidative, xenobiotic and osmotic stresses. Hyperosmotic or salt stress has been an exceptionally fruitful line of abiotic stress investigation, and particular aspects of the cellular adaptation induced by it have been revealed with great molecular resolution (Saito and Posas 2012). The simple addition of cell permeable salts such as NaCl or less permeable osmotically active compounds such as sorbitol provoke a critical stress to the yeast cell mainly because of an immediate loss of water from the cell and the accumulation of potentially harmful cations in the cell interior (Hohmann et al. 2007; Hohmann 2015). In the absence of an efficient and timely response, salt stress will lead to cell death. As one can expect, the cellular response to this stress has many different layers (Figure 1) and despite the intensive investigation in this field over the past decades we anticipate to still see many novel insights leading to an integrative view of the yeast osmostress response.

The main signaling pathway operating upon osmotic stress has been discovered over 20 years ago with the identification of the stress activated MAP kinase Hog1, which represents the downstream effector of the High Osmolarity Glycerol (HOG) pathway (Brewster and Gustin 2014). Since then, it has been shown that the regulatory functions of activated Hog1 are surprisingly complex (de Nadal et al. 2002; Saito and Posas 2012). Thus the HOG pathway is the central, although not the exclusive signaling route, which orchestrates a cellular rescue program upon osmotic and salinity challenge. One prominent function of Hog1 takes place in the nucleus, where it coordinates a complex change in the global gene expression program of the cell (Martinez-Montanes et al. 2010; de Nadal and Posas 2015). Many stress-responsive genes, which are normally expressed at very low rates or even actively repressed, are transiently induced. Activated Hog1 promotes this burst of stimulated gene expression in an integrative manner, as it directly participates in the early events of chromatin remodeling and PIC formation (Alepuz et al. 2001; Proft et al. 2001; Proft and Struhl 2002; Alepuz et al. 2003; de Nadal et al. 2003; De Nadal et al. 2004; Mas et al. 2009; Ruiz-Roig et al. 2012), in the transcriptional elongation process (Proft et al. 2006), in mRNA processing, stability and export (Molin et al. 2009; Romero-Santacreu et al. 2009; Regot et al. 2013), and in the process of translation (Bilsland-Marchesan et al. 2000; Teige et al. 2001; Warringer et al. 2010). In a global view, RNA polymerase complexes are transiently reallocated from housekeeping to stress-responsive gene promoters in the yeast genome (Cook and O'Shea 2012; Nadal-Ribelles et al. 2012). This allows the preferential expression of genes involved in stress adaptation, while the expression of genes involved in cell growth and proliferation is shut down at the same time. Surprisingly, this profound reprogramming of gene expression is not essential for the tolerance of acute osmotic stress, as has been shown in engineered yeast cells with a membrane-sequestered Hog1 kinase unable to induce gene expression upon stress (Westfall et al. 2008). However, the reinforced expression of stress response genes might be beneficial for the long-term tolerance to osmotic stress (Berry and Gasch 2008; Mettetal et al. 2008) and indeed it has been shown that the transcriptional response gradually declines after repeated exposure to salt stress (Rienzo et al. 2015). In this respect it is important to note that the main osmolyte system of yeast, the biosynthesis of glycerol, is also placed under a strong transcriptional control. Intracellular glycerol accumulation is essential for yeast cells to adapt to hyperosmolarity and salt stress (Hohmann et al. 2007). Many layers of control have been identified in the past, the majority of them again depend on the HOG pathway. The expression of glycerol biosynthesis structural genes is heavily induced upon stress

(Albertyn et al. 1994; Ansell et al. 1997). Additionally, a controlled accumulation of glycerol inside the cell is achieved by the regulation of the glycerol facilitator Fps1 and the glycerol uptake system Stl1 (Tamas et al. 1999; Ferreira et al. 2005). Glycolytic flux regulation during the use of glycolytic intermediates for osmolyte productions appears to be another adaptive function of the HOG pathway upon stress (Petelenz-Kurdziel et al. 2013). Thus, glycerol accumulation is the critical cell volume control system employed by yeast cells upon salinity challenge (Hohmann 2015). Moreover, an intracellular excess of Na^+ under these conditions might be reduced by the direct stimulation of cation transporters at the plasma and vacuolar membranes (Proft and Struhl 2004; Li et al. 2012). Of note, the ability to reinstall normal ionic strength and osmolarity in the cytosol is important for an efficient transcriptional response (Proft and Struhl 2004; Vanacloig-Pedros et al. 2015). Hence cellular osmolyte control and nuclear genomic reprogramming mutually control each other upon salt stress.

Other adaptive mechanisms have been identified whose function is to arrest the cell cycle in order to optimally dedicate the cells resources towards stress adaptation and to avoid damage upon salt stress. The Hog1 kinase, once activated by stress, is the molecule in charge of stopping cell cycle progression in different stages (Saito and Posas 2012). This rapid and transient cell cycle arrest is physiologically relevant for the adaptation to osmotic stress, since mutants with a defect in this process are stress sensitive (Escote et al. 2004; Clotet et al. 2006; Duch et al. 2013b). Hence, sophisticated mechanisms exist in yeast, which assure proper stress adaptation before entering again the cell cycle (Clotet and Posas 2007; Sole et al. 2015). Hog1 targets both components of the basal cell cycle machinery and the expression of cyclins. Related with this controlled cell cycle delay, osmotic stress via the Hog1 kinase shuts down genomic replication in order to avoid conflicts between competing DNA and RNA polymerases (Duch et al. 2013a). In this case, Hog1 targets the DNA replication complex in order to avoid genomic instability caused by simultaneously active transcription and replication upon stress (Duch et al. 2013b).

Our recent work has revealed yet another level of adaptation, which occurs in yeast cells upon salt challenge. In this case we focused at the metabolic balance between fermentation and respiration and found that salt stress induces an immediate shift towards respiration by the induction of mitochondrial biomass and specific mitochondrial components (Pastor et al. 2009). Now we have found that salt stress also activates peroxisomal biogenesis and the oxidation of fatty acids via β-oxidation (Manzanares-Estreder et al. 2017), which altogether suggests a model where a successful salt stress adaptation depends on a transient metabolic shift towards the oxidative catabolism of internal fatty acids at peroxisomes and mitochondria (Figure 2). The first indication of this stress-regulated metabolic shift came from the observation that many mutants in mitochondrial structural components were salt sensitive (Pastor et al. 2009). Moreover, the Hog1 and the nutrient regulated Snf1 protein kinases are involved in the activated expression of genes encoding mitochondrial functions upon salt shock. One function of mitochondrial reinforcement upon stress seemed to be the counteraction of intracellular reactive oxygen species (ROS), since mitochondria defective mutants over-accumulated ROS, which was reverted by exogenous addition of antioxidants (Pastor et al. 2009). Furthermore, a proteomic analysis of salt adapted mitochondria revealed the accumulation of antioxidant functions (Martinez-Pastor et al. 2010). In our recent study, we further explore the implication of peroxisomes in yeast salt tolerance. Peroxisomal activity is important for the successful adaptation to high salinity, which becomes even more evident upon sugar limitation. The HOG pathway controls the transcriptional up-regulation of numerous genes involved in the activation

of fatty acids from internal lipid stores, their internalization and β-oxidation in peroxisomes and their conversion into acetyl-carnitine (Manzanares-Estreder et al. 2017). The transcription factor in charge of this switch is Adr1, which was previously known to be regulated during the transition from fermentation to respiration via the Snf1 kinase (Ratnakumar and Young 2010). As a consequence, salt stress triggers the rapid induction of the cells β-oxidation activity and a failure in this process makes cells more vulnerable to this stress condition. Peroxisomal adaptation to salt stress is also microscopically evident, since the number of the organelle quickly increases upon exposure to high salinity. The dynamin related GTPases Dnm1 and Vps1 are involved in this stress-stimulated process, which depends on retrograde signaling but not the HOG pathway (Manzanares-Estreder et al. 2017). Overall, a yeast cell which actively combats a salinity insult, roughly doubles the number of peroxisomes attached to the mitochondrial network, and peroxisomal activity is actually needed to induce mitochondrial respiration under these conditions. This metabolic effort, which can be described as a transient diet change from sugar fermentation to the oxidative respiration of internal fats, is needed for salt adaptation. A very recent unbiased fitness survey using the yeast knockout collection under continuous osmotic stress indeed identified peroxisomal (as well as mitochondrial) functions as one of the most enriched functional category among the sensitive strains (Gonzalez et al. 2016). This underscores the physiological importance of peroxisomal metabolism upon salt stress.

The potential reasons why a yeast cell is forced to switch from sugar fermentation to oxidative degradation of its internally stored fats during salt stress might actually be numerous and in any case are illustrative for the importance of metabolic regulation in order to efficiently combat abiotic stresses (Figure 3). It has been known for a long time that even moderate NaCl concentrations strongly interfere with the uptake of sugars in yeast cultures (Wei et al. 1982). Thus the transporter activity of sugar permeases might be directly inhibited under high salinity stress. This would explain that osmotic stress very strongly induces the expression of several sugar uptake systems located at the plasma membrane as a plausible compensatory mechanism (Posas et al. 2000; Rep et al. 2000). Furthermore the rapid induction of glycerol biosynthesis might lead to a decrease in the metabolic flux in the lower glycolytic pathway. This might cause a metabolic bottleneck for the cell despite the fact that during the transient growth arrest upon salt stress glycolytic intermediates are not used for biomass production (Petelenz-Kurdziel et al. 2013). The massive glycerol biosynthesis could additionally lower the glucose uptake capacity by yet another mechanism, the formation of the toxic byproduct methylglyoxal. This intermediate is known to accumulate in yeast cells upon osmostress and hence triggers the induction of detoxifying enzymes such as Glo1, Gre2 or Gre3 (Aguilera and Prieto 2001; Rep et al. 2001; Maeta et al. 2005). But despite the induced detoxification, methylglyoxal might still trigger inhibitory effects in the cell upon stress, and one of the toxicity mechanisms has been recently revealed as the inhibition of sugar permeases (Yoshida et al. 2012; Roy et al. 2016). The importance of a high glycolytic flux for osmoadaptation is further highlighted by studies using alternative carbon sources. Osmotolerance is lower upon growth on the less fermentable carbon source galactose accompanied by a reduced accumulation of glycerol (Vanacloig-Pedros et al. 2015). Moreover, in the absence of a fermentable carbon source, yeast cells do no longer accumulate glycerol, and the adjustment of osmotic balance is further delayed (Babazadeh et al. 2017).

Taken together, we hypothesize that high salinity causes a nutritional stress in yeast cells, which is counteracted by different metabolic adjustments. The stress induced expression of the Mpc3 mitochondrial pyruvate carrier might indeed reflect the fact that

the intracellular levels of lower glycolytic intermediates decrease upon salt stress (Timon-Gomez et al. 2013). In this scenario, the switch from the low affinity Mpc1/2 to the high affinity Mpc1/3 pyruvate carrier might be necessary to sustain mitochondrial function upon salt stress (Bender et al. 2015). Stress-activated peroxisomal β-oxidation seems to be the alternative compensatory route upon high osmolarity, which fuels the mitochondrial respiration by additional import of Acetyl-CoA. Thus, salt stress and nutritional stress are tightly connected. Indeed, sugar depletion caused by salt stress might actually be the reason why the Snf1 protein kinase is activated upon these environmental conditions (Hong and Carlson 2007). As a consequence Snf1 has been found to participate in diverse regulatory steps upon salt stress, for example in the transcriptional activation of mitochondrial respiration components or ion transporters (Ye et al. 2006; Pastor et al. 2009). We have also to consider that salt stress could directly inhibit mitochondrial activity, which would induce compensatory mechanisms to alleviate the mitochondrial damage. Indeed, harsh conditions of osmotic stress induce programmed cell death via mitochondrial dysfunction in yeast (Silva et al. 2005). Additionally, activated Hog1 MAP kinase directly induces gene expression via the Rtg1/3 transcription factors (Ruiz-Roig et al. 2012), which respond to mitochondrial dysfunction within the retrograde pathway (Sekito et al. 2000). Interestingly, Hog1 is required, at least partially, for the induction of mitophagy, although at present it is unknown whether autophagic control mechanisms of the mitochondria are relevant for salt stress adaptation (Mao et al. 2011).

Taken together, salt and osmotic stress tolerance is intimately linked to metabolic readjustments, which have emerged in the past years and will probably be extended in the future. These insights might also be important to better evaluate our experiments done with a "simple" stimulus such as the addition of salt to a growing yeast culture, which causes profound metabolic changes in the cell. We anticipate that similar metabolic readjustments might also be important for the adaptation to other abiotic stresses beyond salt stress.

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Figure legends

Fig. 1 Summary of the main adaptive mechanisms in budding yeast upon salt and hyperosmotic stress. Global gene expression is transiently shifted from “housekeeping” genes towards the activated expression of “defense genes”. The major osmolyte produced upon osmotic stress is glycerol. Stimulation of membrane cation transport is responsible for ion homeostasis during salt stress. During the adaptive response, cells arrest the cell cycle in a regulated manner and inhibit genomic replication to avoid instability caused by simultaneous transcription and replication. Mitochondrial activity is reinforced during the stress adaptation, and the stimulation of peroxisomal β-oxidation activity is required to induce mitochondrial respiration and to efficiently adapt to salt stress. FA = fatty acid.

Fig. 2 Overview of the signaling events leading to peroxisomal activation upon salt stress. Genes encoding enzymes for fatty acid activation and peroxisomal β-oxidation are induced via the Adr1 transcription factor in a Hog1 dependent manner. Peroxisomal proliferation is stimulated upon salt stress depending on retrograde signaling components and the Vps1 and Dnm1 GTPases. Stimulated oxidation of fatty acids reinforces mitochondrial respiration through the import of Acetyl-CoA via the carnitine shuttle Crc1. The mechanisms of stimulated glycerol production via Hog1 and the transcription factor Hot1 upon salt stress are included because of its possible interference with intracellular pyruvate levels, which might be compensated by the induction of the high affinity pyruvate carrier via the Sko1 transcription factor.

Fig. 3 Summary of the possible physiological connections between salt and metabolic stress. The model predicts that high salinity causes a decrease in the glycolytic flux by direct inhibition of sugar transport, the deviation of glycolytic intermediates during glycerol biosynthesis and inhibition by toxic byproducts of osmolyte production. Compensatory mechanisms are the induction of the high affinity mitochondrial pyruvate carrier Mpc1/3, the induction of peroxisomal fatty acid β-oxidation activity to fuel mitochondrial respiration via alternative carbon sources and the activation of retrograde signaling in response to mitochondrial damage.

Figure 1

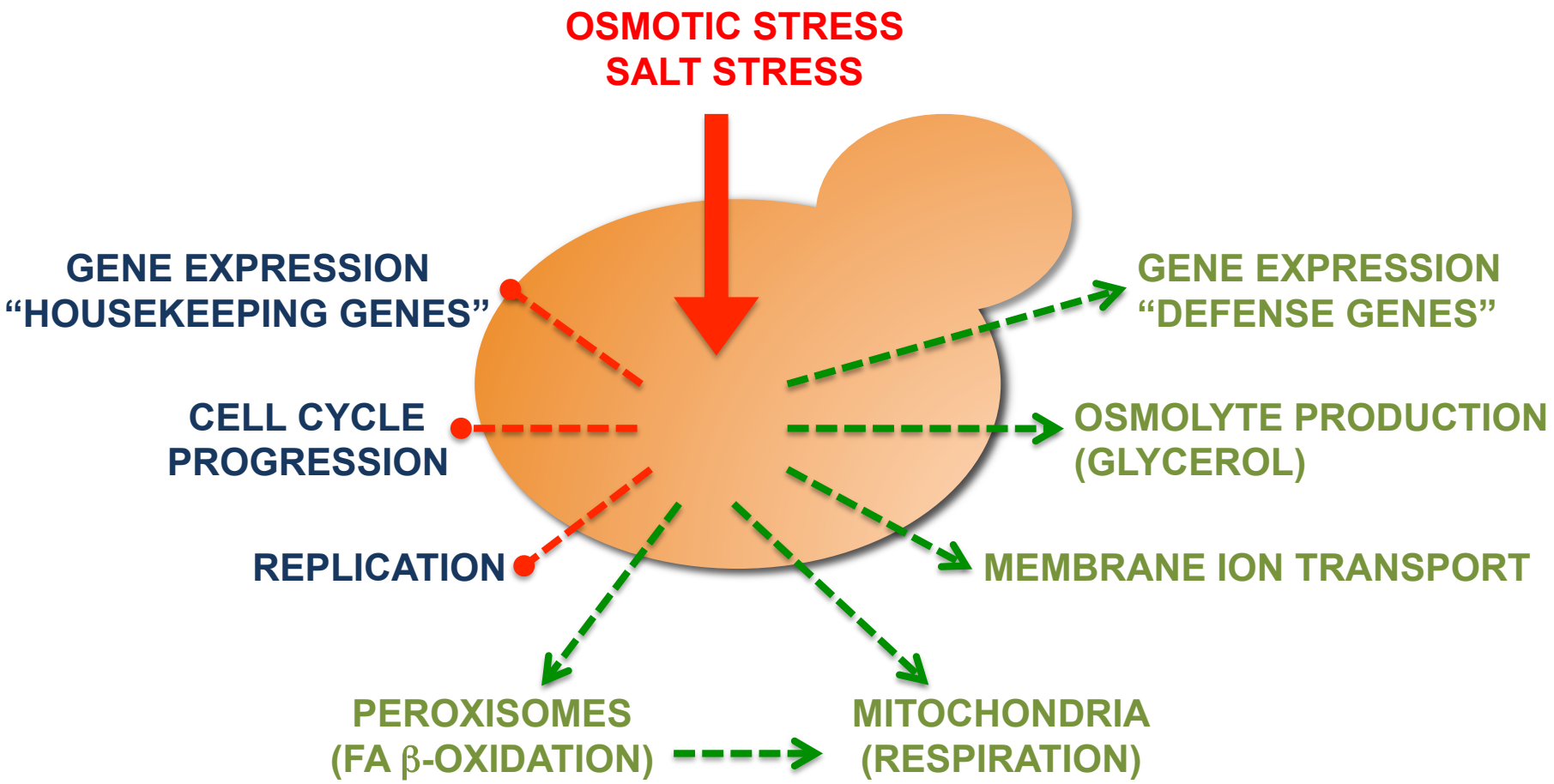

OSMOTIC STRESS
SALT STRESS
GENE EXPRESSION
“HOUSEKEEPING GENES”
CELL CYCLE
PROGRESSION
REPLICATION
GENE EXPRESSION
“DEFENSE GENES”
OSMOLYTE PRODUCTION
(GLYCEROL)
MEMBRANE ION TRANSPORT
PEROXISOMES
(FA β-OXIDATION)
MITOCHONDRIA
(RESPIRATION)

Figure 2

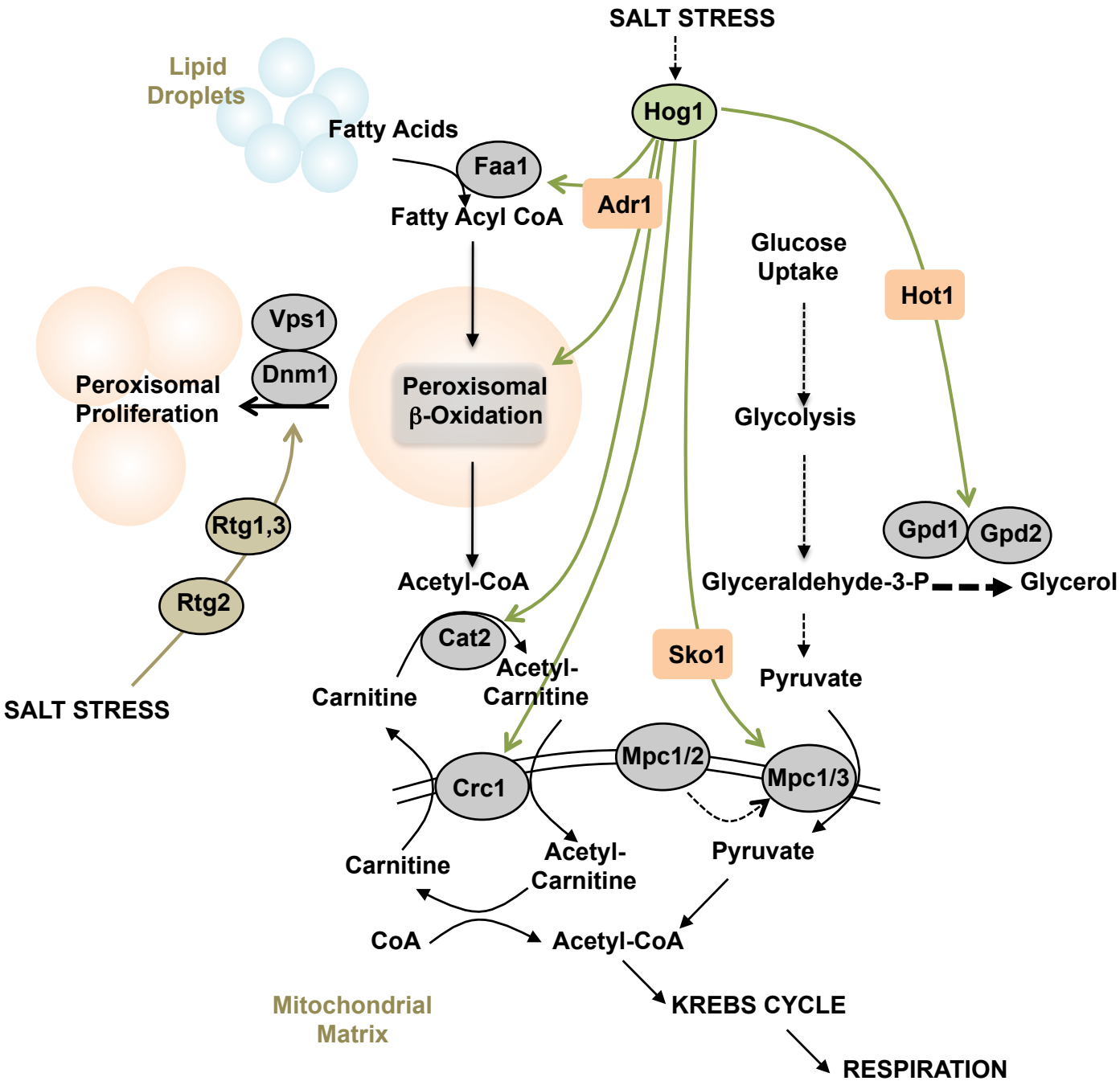
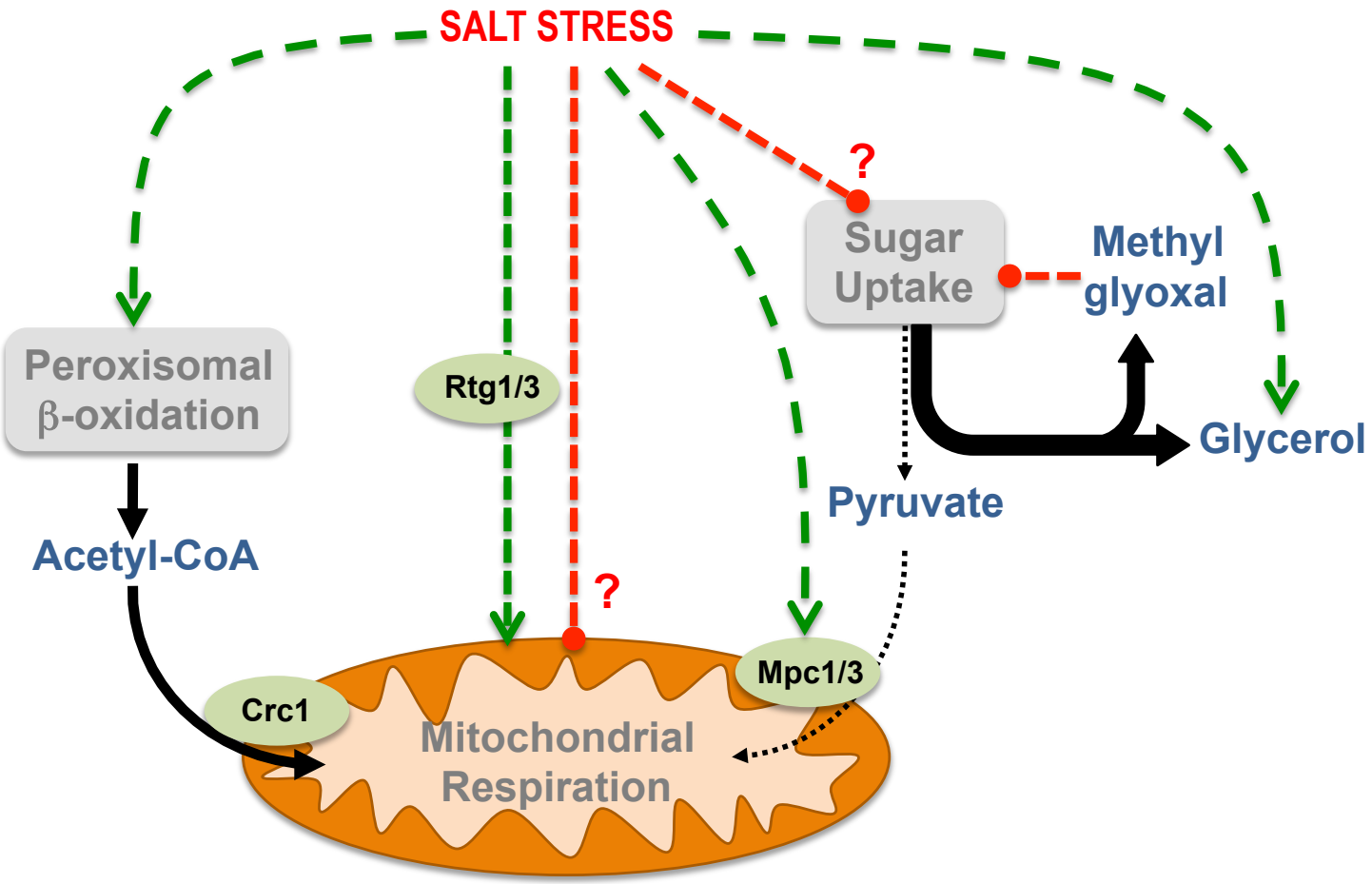

SALT STRESS
Lipid Droplets
Fatty Acids
Hog1
Faa1
Adr1
Fatty Acyl CoA
Glucose Uptake
Hot1
Vps1
Dnm1
Peroxisomal Proliferation
Peroxisomal β-Oxidation
Glycolysis
Rtg1,3
Gpd1
Gpd2
Acetyl-CoA
Glyceraldehyde-3-P
Glycerol
Rtg2
Cat2
Sko1
Acetyl-Carnitine
Pyruvate
SALT STRESS
Carnitine
Mpc1/2
Crc1
Mpc1/3
Carnitine
Acetyl-Carnitine
Pyruvate
CoA
Acetyl-CoA
Mitochondrial Matrix
KREBS CYCLE
RESPIRATION

Figure 3


SALT STRESS
?
Sugar Uptake
Methyl glyoxal
Peroxisomal β-oxidation
Rtg1/3
Glycerol
Pyruvate
Acetyl-CoA
?
Mpc1/3
Crc1
Mitochondrial Respiration