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## Signalling Oxidative Stress in *Saccharomyces cerevisiae*

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### 1. Introduction

Oxidative stress occurs in the natural environment with exposure to aerobic conditions and UV light. Reactive oxygen species (ROS) are also the consequence of normal cellular metabolism. Aerobic organisms sense redox perturbations and develop several different adaptive mechanisms in order to acquire survival capacity (Zheng & Storz, 2000).

The mitochondrial respiratory chain is the major ROS source of reactive oxygen species. ROS can damage a wide range of molecules, including nucleic acids, proteins and lipids. The accumulation of oxidised proteins, DNA damage and the increased production of ROS, concomitant with a depletion of antioxidant defences, seem to be key factors in aging and cell death.

Mitochondrial oxidation appears to be a major cause of signalling to different pathways; however it is still unclear which one of the inflammatory or the apoptotic signals plays a more relevant role in the mitochondrial generation of ROS. Starvation can increase ROS steady-state concentration and autophagy. Hydrogen peroxide appears to be the major oxidant in these conditions, and would oxidise specific cysteines in autophagoc genes leading to the increase in the autophagosome formation. However it is unknown how the signal is transduced to specific targets (Reviewed in Finkel, 2011).

Mitogen activated protein kinases (MAPK) are required in all the eukaryotic cells to properly activate responses in order to allow cells to respond to the different external stresses. The finality of this is to assure cell survival (Wagner & Nebreda, 2009). Several stimuli, included oxidative stress are signalled to phosphorylate certain MAPK thus activating their kinase activity to phosphorylate specific substrates (Shiozaki & Russell, 1995; Nguyen et al., 2000).

The eukaryotic microorganism *Saccharomyces cerevisiae* serves as a model system to study the signal transduction pathways involved in the response to oxidative stress. Thus, TOR,

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RAS and CWI pathways are the best characterised routes known to play a relevant role in transducing the oxidative signal in budding yeast.

## 2. Sensing oxidative stress

Mitochondria are believed to be the major factories of ROS as a byproduct of the respiratory metabolism. However recent studies indicate that ROS produced in mitochondria are signalling molecules capable to activate proteins such as the stress c-Jun N terminal kinase (Finkel, 2011). The activation of the kinase occurs because ROS in fact, inactivate a cysteine dependent phosphatase that regulates c-Jun (Kamata, 2005). The major source of ROS in mitochondrial respiratory chain are complexes I and III. Therefore, mitochondria are ROS generators and consequently act as sensors/transmitters of oxidative stress in eukaryotic cells.

In budding yeast, actin appears to be another possible candidate for sensing oxidative stress (discussed in part 6.2 below)

Transmembrane proteins act also as sensors of a number of stimuli. These proteins can function as detectors of environmental changes and also as a potential transmission molecules, to perhaps downstream signal transduction pathways. Rajavel et al. (1999) identified Mtl1 protein, a Mid2 homologue with a role in cell integrity signalling in vegetative growth. Mtl1 is an element of the cell integrity pathway, overexpression of *MTL1* suppress defects in Rho1 function (Sekiya-Kawasaki et al., 2002). In the context of oxidative stress, the most likely candidate for being a transmembrane protein sensor is Mtl1 protein. Vilella et al. (2005) showed that Mtl1 is a cell surface sensor for oxidative stress. Mtl1 functions as oxidative stress sensor since its function is necessary to survive in response to oxidants and to transmit the oxidative signal to the downstream elements of the cell integrity pathway (Vilella et al., 2005; Petkova et al., 2010a). Mtl1 is a transmembrane protein and localises to the cell periphery in all the stress conditions tested (submitted), supporting the hypothesis that Mtl1 acts as a cell-wall sensor, specifically as an oxidative stress sensor, given that *mtl1* mutant cells are only sensitive to oxidative conditions and nutritional starvation (both conditions generate ROS production in the cells). Whether Mtl1 detects extracellular or/and intracellular oxidative stress is still unknown.

## 3. Transducing oxidative stress

### 3.1 CWI, TOR and RAS pathways

Signal transduction pathways function as transmitters of environmental stimuli to specific substrates. In budding yeast characteristic routes involved in this processes are CWI, TOR and RAS.

Protein kinase C (PKC) is a protein with a role in oxidative stress response (for a review, Nitti et al., 2008).

In human cells, PKC is involved in the protection against oxidative stress in the heart. The knowledge of this signalling is essential to the development of drugs to treat stroke and cardiac arrhythmias (Barnett et al., 2007). Another important feature of the activation of the PKC signal transduction pathway is its role in aging, as reported by Battaini & Pascale

(2005). Importantly, Pascale et al. (2007) exposed in a review how alterations of the PKC cascade may have implications in physiological and pathological brain aging, such as Alzheimer's disease.

The cell-wall integrity pathway in budding yeast involves a protein kinase (MAPKs) cascade which participates in sensing and transmitting several extracellular signals and stresses, including: cell-wall, osmotic, mating and nutritional stress (for a review see Heinisch et al., 1999; Levin, 2005), oxidative (Vilella et al., 2005) and pH (Serrano et al., 2006) stresses. The PKC1-MAPK pathway is integrated by several cell-wall proteins that are putative cell-membrane receptors of different stimuli, they are: the Wsc1-Wsc4 family, Mid2 and Mtl1. They transmit signals to Rom2 which activates the G protein Rho1 which in its turn activates the kinase Pkc1 (this protein has high degree of homology with other isoforms of PKC in eukaryotic cells). Pkc1 activates a MAP kinase module: Bck1 (which is the MAPKKK) phosphorylates the redundant MAPKKs Mkk1 and Mkk2 and together they both activate Slt2, the last kinase member of the pathway. There are two downstream events which correlate to Slt2 activation: transcriptional activity driven by Rlm1 and Swi6 phosphorylation (Heinish et al., 1999; Levin, 2005). Swi6 is one output of Slt2 activity. Swi6 is phosphorylated by Slt2 via the CWI (Sidorova et al., 1995; Madden et al., 1997). However when the external input is oxidative stress, *SWI6* acts as a sensor through the oxidation of its Cys-404 to a sulfenic residue affecting the cell capability to arrest the cell cycle in G1 (Chiu et al., 2011).

The upper elements of the cell integrity pathway are involved in the organisation of the actin cytoskeleton under different conditions, including cell-wall and nutritional stresses (Helliwell et al., 1998; Delley & Hall, 1999; Torres et al., 2002), oxidative stress (Vilella et al., 2005) and pH (Motizuki et al., 2008) among others.

Exposure of *rom2* to oxidising agent results in diminished Slt2/Mpk1 phosphorylation (Vilella et al., 2005). Pkc1 is also required but the MAP kinase module, downstream of Pkc1, seems to be dispensable for this mechanism. Pkc1 overexpression confers cells with more resistance to oxidising agents. It has been demonstrated that upon oxidative stress Pkc1 translocates to the cell periphery. However, Pkc1 transmits the signal to Slt2/Mpk1 if cells have intact secretory machinery (Vilella et al., 2005)

The Pkc1 pathway is also related to the TOR pathway. Budding yeast have two different TOR genes: *TOR1* and *TOR2*, which share 67% sequence identity and are partly redundant in function (Helliwell et al., 1994). Loewith et al. (2002) purified and identified the components of two distinct TOR complexes, TORC1 and TORC2. TORC1 modulates translation initiation, inhibits protein turnover and represses the transcription of genes related to nutrient starvation. Early studies in *Saccharomyces cerevisiae* indicated that TOR has at least two functions: one regulated by the TORC1 complex which is sensitive to rapamycin and the other which is driven by the TORC2 complex and closely related to the organisation of the actin cytoskeleton and independent of rapamycin inhibition (Loewith et al., 2002; and for reviews Wullschleger et al., 2006; Inoki et al., 2005; Inoki & Guan, 2006). Tor2 functions in both complexes while Tor1 only participates in the TORC1 complex. The unique Tor2 function is related to Pkc1 and the organisation of the actin cytoskeleton (Helliwell et al., 1998). However, the rapamycin-insensitive Tor2-unique function has not been described in other eukaryotic model systems (Crespo and Hall, 2002). Rapamycin also induces depolarisation of the actin cytoskeleton

through Sit4 and Tap42, two downstream elements of the TORC1 complex (Torres et al., 2002). TOR function controls a variety of cellular activities. In a global sense TOR inhibits transcription of stress-responsive elements, the nitrogen pathway, starvation-genes, and genes involved in the retrograde response. In this regulation there is a general mechanism: the sequestration of the transcription factors: Msn2/Msn2, Gln3 (Beck & Hall, 1999) and Rtg1/Rtg3 (Crespo et al., 2002; Dlova et al., 2004) in the cytoplasm.

Mitochondrial retrograde signalling (RTG) is a pathway of communication from mitochondria to the nucleus under normal and pathophysiological conditions. The best understood of this pathway is in the budding yeast *Saccharomyces cerevisiae*. It involves multiple factors that sense and transmit mitochondrial signals to induce changes in nuclear gene expression. These changes lead to a reconfiguration of metabolism to accommodate cells to defects in mitochondria that provoke abnormal ROS production. RTG is linked to aging, chronological life span, mitochondrial DNA maintenance, TOR signalling, and nutrient sensing pathways, and is conserved in other fungal species (Liu & Butow, 2006). Lst8 is an integral component of TOR kinase complex. It negatively regulates the RTG pathway at the level of Rtg2. The critical regulatory step of the RTG pathway is the dynamic interaction between Rtg2 and Mks1. The prototypical target of the RTG pathway is *CIT2* (encoding a peroxisomal isoform of citrate synthase, which enables cells to utilize two carbon compounds, such as acetate and ethanol, as sole carbon sources) under the control of Rtg3/Rtg1 heterodimer (Liao et al., 1991).

Tor function also regulates ribosomal protein expression in response to environmental conditions via PKA. This regulation involves the Forkhead factor, *FHL1*, and two cofactors: *IFH1* and *CRF1* (Martin et al., 2004). Tor controls ribosomal gene transcription by maintaining *CRF1* in the cytoplasm, then upon Tor inhibition, *CRF1* translocates to the nucleus and inhibits ribosomal expression, though it is probable that other target transcription factors and different regulatory mechanisms are also involved in this signalling.

The Msn2/Msn4 transcription factor binds and activates genes containing the stress response element (STRE: CCCCT) in response to a wide variety of stresses, including nutritional, osmotic, acidic and oxidative stress (Martínez-Pastor et al., 1996; Schmitt & McEntee, 1996; Beck & Hall, 1999; Hasan et al., 2002). The Ras-cAMP-PKA pathway also negatively regulates Msn2/Msn4 nuclear localisation (Martínez-Pastor et al., 1996; Boy-Marcotte et al., 1998; Görner et al., 1998).

RAS/cAMP pathway is activated when exposed of an optimal carbon source (Broach et al., 1990; Thevelein, 1994). In budding yeast there are two RAS proteins Ras1 and Ras2, both of them are GTPases that signal to the protein kinase PKA and cAMP production (Broach 1991). In optimal growth conditions RAS/cAMP pathway is activated and repress the function of the general stress transcription factor Msn2/Msn4 (Martínez-Pastor et al., 1996; Boy-Marcotte et al., 1998; Görner et al., 2002). On the contrary, nutrient starvation and oxidative stress conditions (Petkova et al., 2010a) are concomitant with RAS/cAMP repression.

*SCH9* encodes for a protein kinase involved in life span regulation. Sch9 activates respiratory metabolism in quiescent phase thus provoking increase in ROS concentration, this effect induces a decrease in life span and increases DNA damage (Madia et al., 2009). Sch9 negatively regulates PKA activity (Zhang et al., 2011). To extend life span it is necessary to reduce TOR activity leading to a decrease of mitochondrial activity, but it is necessary to signal to Sch9 downregulation (Pan & Shadel, 2009).



In recent years several studies there have been published that demonstrate the relationship that exists between the TOR and cAMP-PKA pathways. Schmelzle et al. (2004) suggested that the RAS/cAMP pathway could be a novel TOR effector branch. More recently, Chen & Powers (2006) have demonstrated that the TOR and PKA-cAMP pathways coregulate different biosynthetic pathways which control the expression of genes involved in fermentation and aerobic respiration. Both the TOR and cAMP-PKA pathways regulate the expression of genes needed to overcome the diauxic and stationary phases (Cardenas et al., 1999; Garreau et al., 2000), and in whose regulation Msn2/Msn4 transcriptional activity has been reported to be essential (Powers et al., 2006) (Figure 1). Stationary is a phase prone to

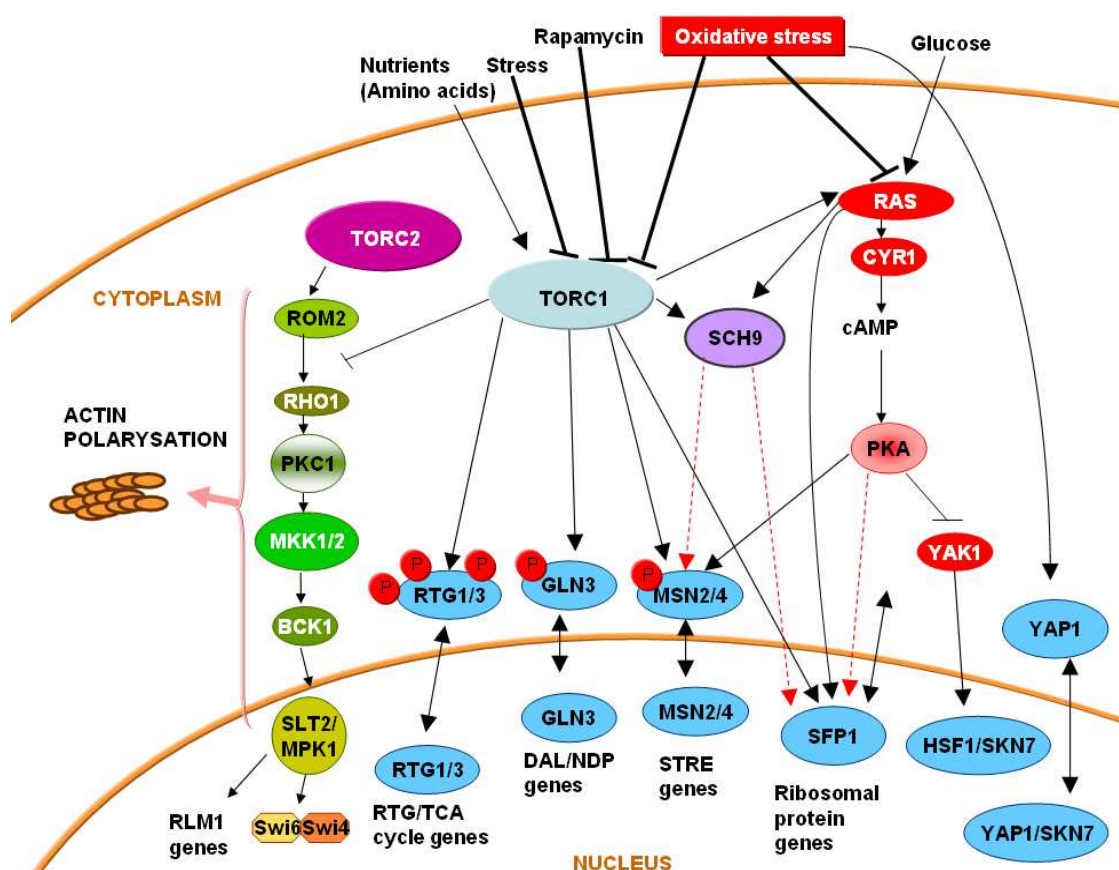


Fig. 1. Oxidative response signalling network in *Saccharomyces cerevisiae*. Oxidative stress negatively regulates TORC1 and RAS activities. TORC1 responds to nutrient availability and is inhibited by rapamycin. This complex when activated promotes the cytoplasm sequestration of specific transcription factors depicted in the figure. TORC1 and RAS/cAMP pathways activate Sfp1 transcription factor inducing ribosomal gene expression. Both TORC1 and RAS converge in SCH9. RAS signals to PKA kinase that inhibits both YAK1 kinase and MSN2/MSN4 by promoting MSN2/MSN4 cytoplasm sequestration. YAK1 in its turn activates SKN7/HSF1 transcription factor that is required for the oxidative response. For this response TORC1 signals to RAS activation. TORC1 inhibits the CWI activity. However, TORC2 complex signals to cytoplasm elements of the CWI pathway to organise actin cytoskeleton. Red circles containing letters depict phosphorylated amino acid residues. CWI, cell wall integrity; STRE, stress-responsive element; DAL, degradation of urea and allantoin; NDP, nitrogen discrimination pathway; RTG, retrograde pathway.

generate ROS species given that yeast are committed to a respiratory metabolism. The cell integrity pathway is also required for viability in quiescence since Slt2 phosphorylation is necessary for cells to survive in stationary phase and upon rapamycin treatment (Krause & Gray, 2002, Torres et al., 2002). Moreover, rapamycin treatment induces depolarisation of the actin cytoskeleton in a cell-integrity pathway-dependent way (Torres et al., 2002) involving the participation of Wsc1 transmembrane proteins.

### 3.2 Phosphatases as regulatory elements

Although oxidative stress is able to induce MAP kinase cascades, reversal of MAPK activation requires the transcriptional induction of specialised cysteine-based phosphatases that mediate MAPK dephosphorylation. In some occasions, oxidative stress inactivates phosphatases by thiol modification leading to abnormal MAPK upregulation. Recently, Fox et al. (2007) described a mechanism by which the stress inducible MAPK phosphatase Sdp1 acquired enhanced catalytic activity under oxidative conditions. Sdp1 uses an intramolecular disulphide bridge and an invariant histidine side chain to recognise a tyrosine-phosphorylated MAPK substrate in oxidant conditions. The disulphide bridge seems to be essential in order to reach a maximum activity. It is well known that yeast develop several strategies to recognize and adapt to oxidative stress. Reversible formation of disulphide bonds is a major way to regulate oxidative stress response in prokaryotic and eukaryotic microorganisms as well as higher eukaryotes (Lushchak, 2011). This must be one of the most efficient mechanisms in the oxidative context given that it is conserved in regulatory proteins, such as the phosphatase Sdp1. The reverse oxidation seems to be a rapid and effective activating mechanism to regulate stress responsive MAPK proteins.

### 3.3 ROS detoxifier proteins acting as signalling regulatory molecules

The elements that form part in signalling pathways that are involved in the response to oxidative stress are susceptible to be oxidised and consequently to be impaired in their functions. Therefore, molecules that repair oxidised proteins are likely to be associated to signalling proteins.

In mammals, there are repairing molecules that interplay in the oxidative stress mechanism. These are: thioredoxins, glutaredoxins, peroxiredoxins and other enzymes with an important role on tumourgenesis and oxidative damage resistance. In yeast some peroxiredoxins have been described to play a role in the regulation of the expression of specific stress genes (Ross et al., 2000).

Watson et al. (1999) have demonstrated the interaction between thioredoxins (proteins that reduce oxidised proteins) and PKC. Kahlos et al. (2003) also demonstrated the functional interaction between oxidoreductases and PKC (from endothelial pulmonary cells). In particular, these authors determined that these oxydoreductases were able to reduce disulphide bonds formed in the PKC protein as a consequence of nitric oxide treatment. PICOT protein, is a PKC interacting protein that negatively regulates its function (Witte et al., 2000).

Another form to activate and therefore also regulate MAPK upon oxidative stress has been described in *Schizosaccharomyces pombe* (Day & Veal 2010). These authors demonstrated that

redox state regulation of cysteines in Sty1 is needed for the hydrogen-peroxide induced increment of Aft1 mRNA levels. This leads to the transcriptional activation of specific genes and the subsequent augment in the oxidative stress survival (Degols & Russell, 1997).

Also in *S. pombe* Veal et al. (2004) showed the existence of an interesting mechanism by which a peroxiredoxin acts a redox sensor specifically required to activate Sty1. Sty1 is a MAPK responsive to all the stresses in *S. pombe*. High concentrations of hydrogen peroxide promote Sty1 activation by the peroxiredoxine, whereas at low concentrations of the oxidising agent this peroxiredoxin also regulates Pap1 nuclear accumulation (Veal et al., 2007). All these studies show that hydrogen peroxide is capable of oxidising specific kinases, and that certain molecules involved in oxidative repair, such as peroxiredoxins, are required in order to achieve a correct signalling response.

Grx3 and Grx4, two monothiol glutaredoxins of *S. cerevisiae*, regulate Aft1 nuclear localisation and negatively regulate its function. PICOT thioredoxin has a high degree of sequence homology with both Grx3 and Grx4 proteins of *S. cerevisiae*. The absence of both proteins makes the cells sensitive to hydrogen peroxide. In the absence of both Grx3 and Grx4 there is a constitutive oxidative stress induced, in part, by the deregulation of iron homeostasis (Pujol-Carrion et al., 2006). There are some other reports demonstrating similar regulations of other protein kinases. Therefore, there is important to isolate and characterise proteins involved in regulating the redox state of protein kinases in order to maintain an oxidatory equilibrium which allows correct cellular function. It will also be interesting to elucidate the degree of conservation of these proteins and their regulatory functions in evolution.

Actin is a target for oxidative stress. Actin cytoskeleton is of enormous relevance in *S. cerevisiae*. It is responsible for all the morphogenetic processes, stress responses, organelles delivery etc. There are two potential cysteines more susceptible for being oxidised. Moreover, other regulatory molecules such as Grx3 and Grx4 regulate actin function in oxidative conditions (Pujol-Carrion et al., 2010). Grx3 and Grx4 are two putative glutaredoxins but there have not been described any enzymatic property related to that putative function. They are involved in the regulation of Aft1 a transcription factor required for the correct iron homeostasis (Pujol-Carrion et al., 2006; Ojeda et al., 2006). Grx4 is required for the maintenance of cable structure. Grx3 plays a redundant role in conjunction with Grx4. Both Grx3 and Grx4 have two theoretical domains, one Trx domain close to the C terminus and a Glutaredoxin domain. Both glutaredoxins through their Trx domains are required to repolarise the actin cytoskeleton in oxidative conditions and also for survival in oxidative stress conditions. Interestingly, Grx4 plays a more direct role in the defence against oxidative stress since Grx4 overproduction increases cell survival when cells are exposed to oxidants (Pujol-Carrion et al., 2010).

#### 4. Transcriptional regulation

Different transcription factors regulate the adaptive response to oxidative stress conditions: the general stress response is mediated by the Msn2/Msn4 transcription factor, whereas specific responses are mediated by Yap1, Skn7 and Hsf1. Msn2/Msn4 nuclear localisation and activity are regulated by both TORC1 and PKA (detailed formerly). For the induction of many antioxidant genes, Skn7 and Yap1 act cooperatively upon oxidative stress (Lee et al.,



1999; Brombacher et al., 2006; He et al., 2005). The contribution of Skn7 to the oxidative stress response does not occur through any of the cysteines of the protein. In addition, SKN7 phosphorylation does not seem to be required in the oxidative stress response (Morgan et al., 1997). It has been proposed a model in which Skn7, when located in the nucleus, cooperates with oxidised Yap1. The association of Yap1 with Skn7 is a prerequisite for Skn7 phosphorylation and the activation of oxidative stress response genes (He et al., 2009). Skn7 interacts also with Hsf1 and both cooperate to induce heat shock genes specifically in response to oxidative stress (Raitt et al., 2000). Hsf1, like Msn2/Msn4, is negatively regulated by PKA via Yak1 kinase. Sfp1 is a transcriptional factor that induces ribosomal gene expression when located in the nucleus. It is positively regulated by either TOR or RAS activities. In response to oxidative stress and DNA damage Sfp1 translocates to the cytoplasm with the consequent adaptive downregulation of ribosomal gene expression (Marion et al., 2004) (Figure 1).

In the pathogen yeast *Candida albicans* there has been characterised a gene *CAP1*, which is homologue to the transcription factor Yap1 and has a role in oxidative stress resistance (Alarco et al., 1999). In *C. albicans* Hog1 pathway is also required for a correct oxidative stress response though through a different pathway than that used by *CAP1* gene (Alonso-Monge et al., 2003).

## 5. Signal crosstalk events in the oxidative transduction

Mtl1 is a transmembrane cell-wall protein required for cell survival upon oxidative conditions (Vilella et al., 2005). Mtl1 belongs to the CWI pathway and its essential function is to inhibit Tor1 and Ras2 function in conditions of oxidative stress and nutrient depletion. This signal is transduced through Rom2 and Rho1 (both elements of the CWI pathway), however, the rest of the downstream components of the mentioned pathway are dispensable for this essential function (Petkova et al., 2010a) (Figure 2). Consequently, upon oxidative stress both Tor1 and Ras2 functions must be transiently repressed. Downstream outputs of this signal are transcriptional induction mediated by Msn2/Msn4 and ribosomal gene repression, probably due in part to the regulation of Sfp1. In this study the authors propose two possible models: a) the oxidative signal from Mtl1 flows to Tor1 and Ras2 independently and converge in a common pathway; b) this signal flows to Tor1 and then to inhibit Ras2 for the regulation of Msn2/Msn4 activity and the downregulation of ribosomal gene expression. There is another crosstalk involving CWI elements, TOR and RAS, In this case the signal flows from RAS2 and TOR1 inactivation to induce the phosphorylation of Slt2 after treatment with hydrogen hydroxide and upon glucose starvation. Interestingly this backwards signal occurs in the absence of Mtl1 protein (Petkova et al., 2010b).

## 6. Cellular functions affected by oxidative stress

### 6.1 Cell cycle oxidation and DNA damage

There exist a wide variety of DNA damaging agents. They provoke different DNA lesions (reviewed in Sage & Harrison, 2011). UV light is a well known DNA damaging agent inducing the formation of thymidin dimers. DNA damaging agents activate a number of checkpoint genes (Lowndes & Murguia, 2000) in order to transiently block cell cycle progression and simultaneously to activate (transcriptional and/or postraductional) genes

that repair possible DNA lesions. UV response is characterised in yeast (Engelberg 1994). Oxidative stress is also known to provoke a wide number of genetic anomalies leading to genome instability cancer and inflammatory diseases. DNA damage checkpoints protect genome integrity (Latif et al., 2001). The DNA damage checkpoint is activated in response to oxidative stress (Leroy et al., 2001).

Since MAPKs are activated in response to several stresses, they are also involved in the UV and oxidative response. In particular JNKs and p38 (Engelberg, 1994; Rouse et al., 1994) are involved in these responses. Both kinases are conserved in yeast. Sty1 in *S pombe* and Hog1 in *S cerevisiae* have a high degree of similarity with JNK and p38 mammalian kinases and are known to be involved in the UV and oxidative stress responses (Haghnazari & Heyer, 2004; Alao & Sunnerhagen, 2008).

Among the possible MAP kinases involved in the UV response, Slt2 and Hog 1 are required for survival whereas Mlp1 and Fus3 are dispensable, (Brian et al, 2004). These authors propose that Slt2 is specifically required for survival in front of UV, based on the observation that *slt2* mutant is not significantly sensitive to MMS (Methyl methanesulfonate) treatment. They demonstrate that addition of sorbitol suppressed the requirement for Slt2, what suggests that probably UV is provoking damage at the level of the cell surface. 8-MOP 8-furocoumarin 8-methoxypsoralen is a chemical agent used in the treatment of psoriasis and other skin diseases. The combination of 8-MOP plus UVA, causes DNA double strand breaks, being Slt2 function required for survival (Dardalhon et al., 2009). At present it is unknown which is the precise role of Slt2 and the other members of the CWI pathway in these responses (Bryan et al., 2004). One possible explanation would be that 8-MOP+UV induces increase in ROS steady-state levels. Another interpretation is that oxygen reacts with 8-MOP and components of the cell membrane leading to lipid peroxidation. This would be the starting point to signal to the CWI (Dall'Acqua & Martelli, 1991; Zarebska, 2000; Dardalhon et al., 2009)

In a recent report, Bandyopadhyay et al. (2010) have used a new methodological approach called differential epistasis mapping. By doing so they have also found a genetic interaction between Slt2 and DNA repair genes. Moreover, MMS treatment induced Slt2 translocation to the nucleus and the transcriptional activation of ribonucleotide reductase genes.

There exist cross-talk between DNA damage and oxidative stress since the UV response in mammals is believed to be induced at the cell membrane through the peroxidation of lipids. On the contrary, alkylating agents might induce the UV response through the oxidation of SH free groups and subsequent glutathione pool depletion (Devary et al., 1993). In addition, the UV transcription response mediated by AP-1 in mammals induces the expression of genes required for the response to oxidative damage (van Dam et al., 1995). In conclusion, there exists some evidence that UV response helps to combat oxidative stress.

In mammals oxidative stress activates all the known MAPK. ERK activation promotes cell survival whereas JNK and p38 suppress apoptosis and induce cellular responses to stress (Runchel et al., 2011).

In mammals a crosstalk between DNA damage checkpoint genes and certain MAPK has been well described (Shafman et al., 1995; Bulavin et al., 2001). In an attempt to describe an equivalent crosstalk in yeast Haghnazari & Heyer (2004) analysed the possible relationship

between Hog1, a MAPK sensitive to oxidative stress and Rad53. These two proteins were selected based in the evidence that Hog1 becomes phosphorylated and is also required for cell survival in response to mild oxidative stress. Rad53 is a kinase required for cell cycle arrest upon DNA damage and as a consequence of that, upon an increase in ROS concentration. Haghazari & Heyer (2004) demonstrated that the oxidative response mediated by Hog1 is independent on that governed by Rad53, consequently there is not such a crosstalk in yeast at least until now. Sublethal oxidative stress signals to Rad53 phosphorylation dependently on Mec1 (a PIK-like kinase whose human homologue is ATR). This checkpoint induces a transient delay in S phase and is also dependent on the Rad17 and Rad24 checkpoint genes (Leroy et al., 2001) (Figure 2). In response to DNA damage, Mec1 also plays a role in the inhibition of mitosis by mediating the phosphorylation of Cdc20, consequently the degradation of Pds1 and Clb2 is abolished. It has also been suggested that Mec1 might phosphorylate PKA in this mechanism (Searle et al., 2004). Queralt & Igual (2005) reported that Pkc1 and Slr2 mutants present synthetic lethality with Rad9 mutants, suggesting a connection between CWI elements and DNA-damage checkpoint genes yet unknown.

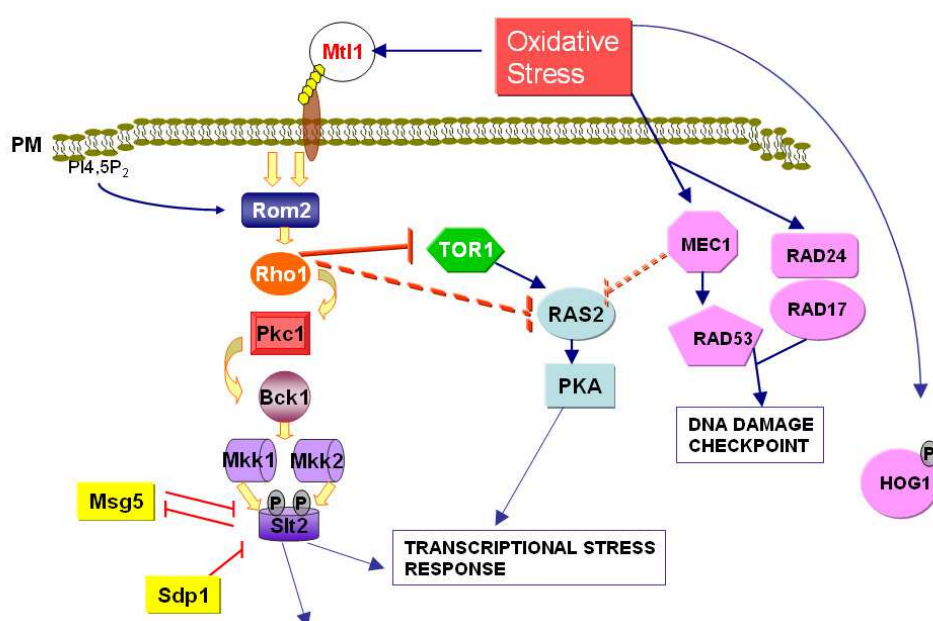


Fig. 2. Schematic diagram of the cross-talk between signal transduction pathways and DNA damage checkpoint genes, in budding yeast. Mtl1 senses oxidative stress and transmit the signal to the downstream elements of the CWI pathway. Rho1 signals to TOR1 and/or RAS2 to induce transcriptional responses that ensure cell survival. Mec1, Rad53, Rad24 and Rad17 are involved in the oxidative response by provoking a DNA-damage checkpoint in S phase. Oxidative stress induces Hog1 phosphorylation independently on Mec1 function. Discontinuous lanes represent signalling events that are very likely to occur.

In mammals there are two central PIK-like kinases whose role is sensing and signalling DNA damage. ATR and ATM (Mec1 and Tel1 in budding yeast, respectively). ATM plays a role in the response to high levels of ROS by repressing mTOR1 expression, although the mechanism by which this occurs is still unknown (Alexander & Walker, 2010). In fission yeast TORC2

complex mediates tolerance to DNA damage and the absence of *tor1* confers cells with more sensitivity to hydroxiurea and MMS, both of them DNA damaging agents. Upon the cell cycle arrest characteristic of the DNA damage checkpoint, *tor1* is required to dephosphorylate and reactivate Cdc2, what elicits the resumption of mitosis (Schonbrun et al., 2009).

## 6.2 Actin cytoskeleton organisation

The actin molecule is sensitive to oxidative stress (Dalle-Donne et al., 2001). Upon oxidative conditions the actin molecule can be oxidised and a disulphide bond can be formed between cysteins 284 and 373 (Dalle-Donne et al., 2001, 2003).

Cysteine is one amino acid prone to be oxidised when ROS levels increase. In actin Cysteine 374 is the most susceptible to oxidation, according to Takashi (1979). Investigations with erythrocytes from patients suffering sickle cell anemia, demonstrated that these cells possess actin molecules oxidised and forming intramolecular disulphide bonds between C284-C373 (Shartava et al., 1995, 1997; Bencsath et al., 1996). This modification correlates with a decrease in actin polymerisation rates. In yeast these cysteines are homologous to C285 and C374. Recently, Farah et al. (2011) in an elegant study, described the formation of oxidation induced actin bodies (OABs) upon oxidative stress by using budding yeast as a cellular model. These bodies resemble big patches and contain proteins and oxidised actin with intramolecular disulphide bonds between C285 and C374. These authors demonstrated that the formation of C285-374 responds to a protective mechanism against actin oxidation. OABs come from cortical patches. C285-374 are required for the adaptive response and recovery in front to oxidative damage. If actin again is a sensor for oxidative stress, it remains to be elucidated which are all the signalling outputs that govern the cellular responses to oxidative stress once actin oxidation starts the signalling process in the cells. Actin oxidation accelerates cell death in yeast (Dalle-Donne et al., 2001). Studies in eukaryotic model *S. cerevisiae* have allowed the identification of the oxidoreductase OYE2 (Old Yellow Enzyme 2) that is important to protect actin molecules from being oxidised in Cys285 and Cys374 (Haarer et al., 2004). A deletion in the OYE2 gene induces an increase in ROS steady-state levels and makes cells more sensitive to oxidation (Farah et al., 2007; Odat et al., 2007). Although Oye enzymes are placed in the signalling network that governs ROS, actin cytoskeleton and survival, it remains unknown at the molecular level which is the connection between any specific signal transduction pathway and Oye2 in response to the redox signal.

Vilella et al. (2005) describe a role for CWI pathway in connecting oxidative stress stimulus with the actin cytoskeleton. This study reveals that oxidative stress depolarises the actin cytoskeleton. None of the CWI elements is required to mediate this depolarisation, however Pkc1 is essential in order to restore the organisation of the actin cytoskeleton in oxidative conditions, concomitantly with an increase in cell viability (Vilella et al., 2005).

In a recent work (Pujol-Carrion et al., 2010) it has been demonstrated that actin polymerisation is a target of hydrogen peroxide. The authors develop an assay based on total protein extracts obtained from different strains of *S. cerevisiae*. These protein extracts are used as polymerisation seeds to study actin assembly. Actin filaments are detected by means of the technique of fluorescence recovery after photobleaching (FRAP). The rationale of this assay is that the association of small amounts of protein extracts with actin monomers



could enhance or even inhibit actin nucleation/polymerisation. If the activity of certain protein extracts could promote actin polymerisation, then small oligomers of actin will be created, acting as polymerisation precursors than can accelerate or increase the extent of actin polymerisation (Haarer et al., 1990). By means of this assay the authors demonstrate that Pkc1 plays an important role in promoting actin nucleation both under normal growth conditions and in response to treatment with hydrogen peroxide.

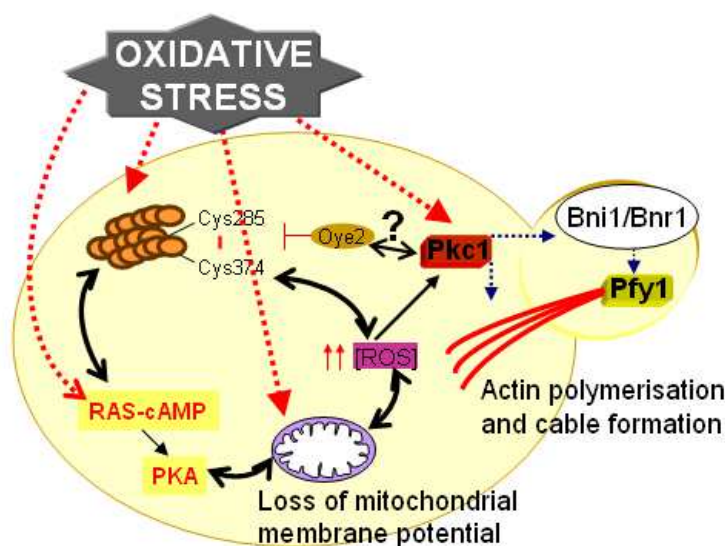


Fig. 3. Diagram of the possible oxidative stress signalling related to mitochondrial function and actin dynamics. Oxidative stress reduces actin dynamics and oxidises actin molecule in the residues depicted in the Figure. Reduced actin dynamics activate RAS/cAMP pathway. Increase of RAS activity activates mitochondrial function releasing high concentrations of ROS to cells. Oye2 repairs actin disulphide bonds and Pkc1 promotes actin dynamics and polymerisation. We have represented this signalling process as a circle of arrows because it is not clear which is the starting point of the cascade. ROS, reactive oxygen species.

Actin is a key element acting as a sensor for oxidative stress and nutritional status and a subsequent linker to ROS dependent mitochondrial release. According to this balance cells will be committed, or not, to cell death (Leadsham et al., 2010). A descent in the actin polymerising activity leads to the formation of actin aggregates associated to the accumulation of ROS in the cytosol (Gourlay & Ayscough, 2005a, 2006; Leadsham et al., 2009). This probably occurs because there is a tight connection between actin cytoskeleton and mitochondria, in addition, the Ras/cAMP pathway plays also an important role in this mechanism. Improper activation of Ras/cAMP leads to higher ROS mitochondrial production. Finally, several studies (Gourlay & Ayscough, 2005a, 2005b; Leadsham et al., 2008; Gourlay et al., 2006) propose the existence of a cross-talk between the actin cytoskeleton dynamics and Ras/cAMP that regulates mitochondrial function and ROS production. Actin is important for the correct distribution of mitochondria between the mother cell and the daughter, but certain proteins required for the remodelling of the cortical actin cytoskeleton induce ROS release from the mitochondria (Gourlay & Ayscough, 2005a, 2005b, 2006). In conclusion, abnormal mitochondrial function increments ROS intracellular levels; high ROS concentration affects actin dynamics and this upregulates the



Ras/cAMP pathway; finally this upregulation signals to the increase in ROS production from the mitochondria (Figure 3). However it not characterised, to date, which is the starting point in this interconnected signalling circle.

## 7. Conclusion

Oxidative stress provokes different types of damage to each of the components of all the cells. *Saccharomyces cerevisiae* is an optimal eukaryotic model to study signalling events related to this stress, given that the main cascades involved in oxidative stress are highly conserved in the evolution from yeast to men. There exist a number of studies demonstrating that several signal transduction pathways are relevant for this response, PKC, TOR and RAS are central molecules in all the organisms described. The connection between oxidative damage and DNA damage must be very tight. We know that there must be regulatory molecules in cells ensuring a perfect and tight connection between signalling pathways, responding to oxidative stress, and genes involved in DNA-damage checkpoints and DNA repair. We dispose of extended information in the literature regarding this matter, however the precise regulatory pattern that interconnects oxidative sensors, transducers and DNA damage is not totally characterised to date. Another point to be addressed in the future is the characterisation of the different oxidative stress sensors in each of the current cellular models. Actin, mitochondria, transmembrane proteins are good candidates. Future studies will be required to decipher all these questions.

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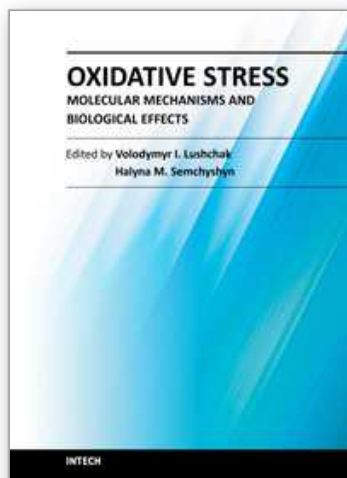
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## **Oxidative Stress - Molecular Mechanisms and Biological Effects**

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Since the discovery of free radicals in biological systems researchers have been highly interested in their interaction with biological molecules. Denoted in 1980, and due to fruitful results and ideas, oxidative stress is now appreciated by both basic and applied scientists as an enhanced steady state level of reactive oxygen species with wide range of biological effects. This book covers a wide range of aspects and issues related to the field of oxidative stress. The association between generation and elimination of reactive species and effects of oxidative stress are also addressed, as well as summaries of recent works on the signaling role of reactive species in eukaryotic organisms. The readers will gain an overview of our current understanding of homeostasis of reactive species and cellular processes they are involved in, as well as useful resources for further reading.

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