14

Signalling Oxidative Stress in *Saccharomyces cerevisiae*

Maria Angeles de la Torre-Ruiz[1*], Luis Serrano[2], Mima I. Petkova[1] and Nuria Pujol-Carrion[1]
[1]*Dept. Ciències Mèdiques Bàsiques-IRBLleida, Faculty of Medicine, University of Lleida*
[2]*Dept. Producció Vegetal & Ciència Forestal, ETSEA, University of Lleida, Spain*

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 autophagyc 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 phosporylate 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,

* Corresponding Author

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/transmissors 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* supress 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 oxidatives 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 transmissors of environmental estimuli 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 arrithmias (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 imput 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; Dilova 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 respiratiory 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

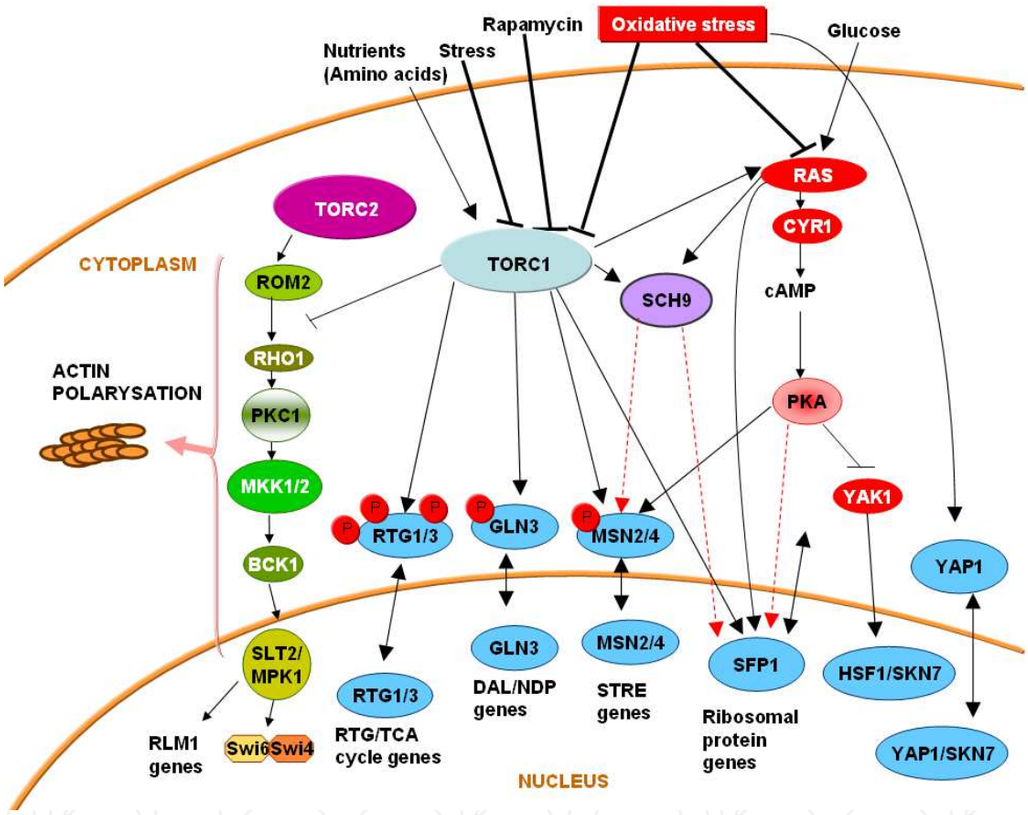


Fig. 1. Oxidative response signalling network in *Saccharomyces cerevisiae*. Oxidative stress negatively regulates TORC1 and RAS activities. TORC1 responds to nutrient availability and is inhibited by rapamycin. This complex when activated promotes the cytoplasm sequestration of specific transcription 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 allatoin; 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 rapamycine treatment (Krause & Gray, 2002, Torres et al., 2002). Moreover, rapamycine 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 Phospatases 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 theorethical 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 oxisided 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 citoplasm 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 dimmers. 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-methoxypsolaren 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. Haghnazari & 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 Slt2 mutants present synthetic lethality with Rad9 mutants, suggesting a connection between CWI elements and DNA-damage checkpoint genes yet unknown.

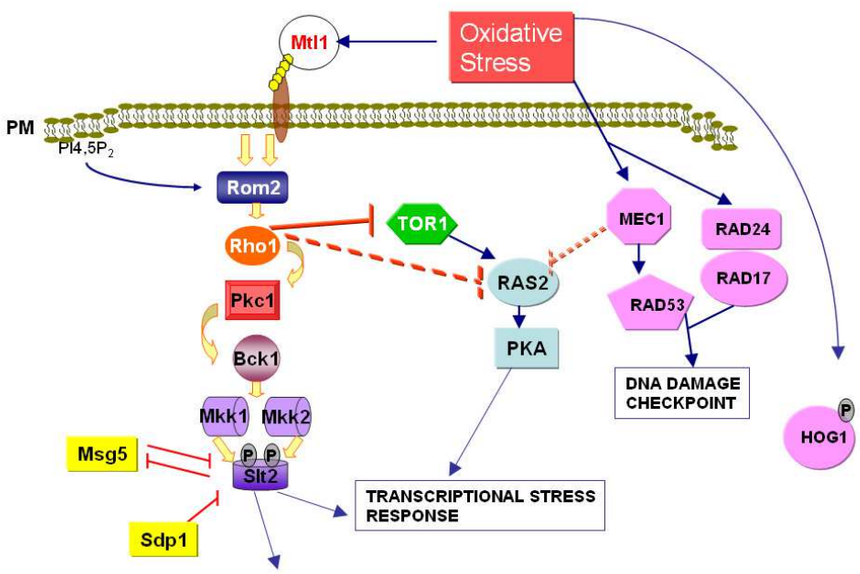


Fig. 2. Schematic diagram of the cross-talk between signal transduction pathways and DNA damage checkpoint genes, in budding yeast. Mtl1 senses oxidative stress and transmit the signal to the downstream elements of the CWI pathway. Rho1 signals to TOR1 and/or RAS2 to induce transcriptional responses 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 anemina, demonstrated that these cells posses 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.

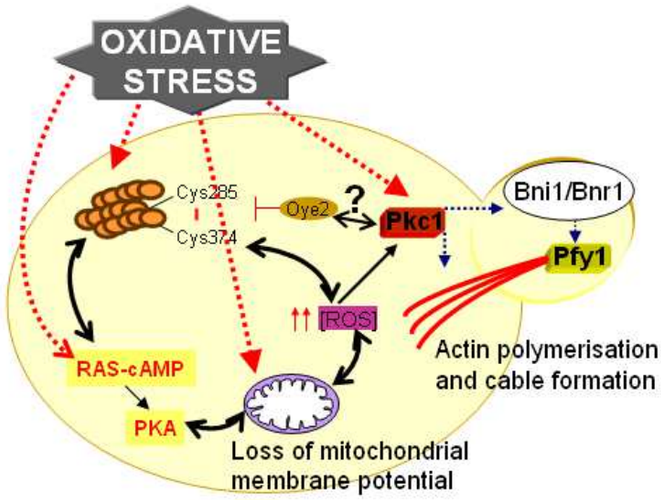


Fig. 3. Diagram of the possible oxidative stress signalling related to mitochondrial function and actin dynamics. Oxidative stress reduces actin dynamics and oxidises actin molecule in the residues depicted in the Figure. Reduced actin dynamics activate RAS/cAMP pathway. Increase of RAS activity activates 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.

8. References

Alao, J.P. & Sunnerhagen, P. (2008). Rad3 and Sty1 function in *Schizosaccharomyces pombe*: an integrated response to DNA damage and environmental stress?. *Molecular Microbiology*, Vol.68, No.2, (April 2008), pp. 246-254, ISSN 1365-2958

Alarco, A. M. & Raymond, M. (1999). The bZip transcription factor Cap1p is involved in multidrug resistance and oxidative stress response in *Candida albicans*. *Journal of Bacteriology*, Vol.181, No.3, (February 1999), pp. 700–708, ISSN 0021-9193

Alexander, A. & Walker, C.L. (2010). Differential localization of ATM is correlated with activation of distinct downstream signaling pathways. *Cell Cycle,* Vol.9, No.18, (September 2010), pp. 3685-3686, ISSN 1551-4005

Alonso-Monge, R., Navarro-García, F., Román, E., Negredo, A.I., Eisman, B., Nombela, C. & Pla, J. (2003). The Hog1 mitogen-activated protein kinase is essential in the oxidative stress response and chlamydospore formation in *Candida albicans*. *Eukaryotic Cell*, Vol.2, No.2, (April 2003), pp. 351-361, ISSN 1535-9778

Bandyopadhyay, S., Mehta, M., Kuo, D., Sung, M.K., Chuang, R., Jaehnig, E.J., Bodenmiller, B., Licon, K., Copeland, W., Shales, M., Fiedler, D., Dutkowski, J., Guénolé, A., van Attikum, H., Shokat, K.M., Kolodner, R.D., Huh, W.K., Aebersold, R., Keogh, M.C., Krogan, N.J. & Ideker, T. (2010). Rewiring of Genetic Networks in Response to DNA Damage. *Science*, Vol.330, No.6009, (December 2010), pp. 1385–1389 , ISSN 0036-8075

Barnett, M.E., Madgwick, D.K. and Takemoto, D.J. (2007). Protein kinase C as a stress sensor. *Cellular Signalling*, Vol.19, No.9, (September 2007), pp. 1820-1829, ISSN 0898-6568

Battaini, F. & Pascale, A. (2005). Protein kinase C signal transduction regulation in physiological and pathological aging. *Annals of the New York Academy of Sciences,* Vol.1057, (December 2005), pp. 177-192, ISSN 0077-8923

Beck, T. & Hall, M. (1999). The TOR signalling pathway controls nuclear localization of nutrient-regulated transcription factors. *Nature*, Vol.402, No.6762, (December 1999), pp. 689-692, ISSN 0028-0836

Bencsath, F.A., Shartava, A., Monteiro, C.A. & Goodman, S.R. (1996). Identification of the disulfide-linked peptide in irreversibly sickled cell beta-actin. *Biochemistry,* Vol.35, No.14, (April 1996), pp. 4403-4408, ISSN 0006-2960

Boy-Marcotte, E., Perrot, M., Bussereau, F., Boucherie, H. & Jacquet, M. (1998). Msn2p and Msn4p control a large number of genes induced at the diauxic transition which are repressed by cyclic AMP in *Saccharomyces cerevisiae*. *Journal of Bacteriology*, Vol.180, No.5, (March 1998), pp. 1044-1052, ISSN 0021-9193

Broach, J. R. & Deschenes, R. J. (1990). The function of ras genes in *Saccharomyces cerevisiae*. *Advances in Cancer Research*, Vol.54, pp. 79–139, ISSN 0065-230X

Broach, J. R. (1991). RAS genes in *Saccharomyces cerevisiae*: signal transduction in search of a pathway. *Trends in Genetics*, Vol.7, No.1, (January 1991), pp. 28–33, ISSN 0168-9525

Brombacher, K., Fischer, B. B., Rüfenach, K. and Eggen, R. I. L. (2006). The role of Yap1p and Skn7p-mediated oxidative stress response in the defence of *Saccharomyces cerevisiae* against singlet oxygen. *Yeast*, Vol.23, No.10, (July 2006), pp. 741-750, ISSN 0749-503X

Bryan, B.A., Knapp, G.S., Bowen, L.M. & Polymenis, M. (2004). The UV Response in *Saccharomyces cerevisiae* Involves the Mitogen-Activated Protein Kinase Slt2p. *Current Microbiology*, Vol.49, No.1, (July 2004), pp. 32-34, ISSN 0343-8651

Bulavin, D.V., Higashimoto, Y., Popoff, I.J., Gaarde, W.A., Basrur, V., Potapova, O., Appella, E. & Fornace, A.J. Jr. (2001). Initiation of a G2/M checkpoint after ultraviolet radiation requires p38 kinase. *Nature*, Vol.411, No.6833, (May 2001), pp. 102–107, ISSN 0028-0836

Cardenas, M.E., Cutler, N.S., Lorenz, M.C., Di Como, C.J. and Heitman, J. (1999). The TOR signaling cascade regulates gene expression in response to nutrients. *Genes and Development*, Vol.13, No.24, (December 1999), pp. 3271-3279, ISSN 0890-9369

Chen, J.C.Y. and Powers, T. (2006). Coordinate regulation of multiple and distinct biosynthetic pathways by TOR and PKA kinases in *S. cerevisiae*. *Current Genetics*, Vol.49, No.5, (May 2006), pp. 281-293, ISSN 0172-8083

Chiu, J., Tactacan, C.M., Tan, S.X., Lin, R.C., Wouters, M.A. & Dawes, I.W. (2011). Cell cycle sensing of oxidative stress in *Saccharomyces cerevisiae* by oxidation of a specific cysteine residue in the transcription factor Swi6p. *The Journal of Biological Chemistry*, Vol.286, No.7, (February 2011), pp. 5204-5214, ISSN 1083-351X

Crespo, J.L. & Hall, M.N. (2002). Elucidating TOR signaling and rapamycin action: lessons from *Saccharomyces cerevisiae. Microbiol. Mol. Biol. Rev.* Vol. 66, No. 44 (December 2002), pp. 579-591, ISSN 0005-3678

Crespo, J.L., Powers, T., Fowler, B. and Hall, M.N. (2002). The TOR-controlled transcription activators *GLN3, RTG1*, and *RTG3* are regulated in response to intracellular levels of glutamine. *Proceedings of the National Academy of Sciences of the United States of America*, Vol.99, No.10, (May 2002), pp. 6784-6789, ISSN 0027-8424

Dall'Acqua, F. & Martelli, P. (1991). Photosensitizing action of furocoumarins on membrane components and consequent intracellular events. *Journal of Photochemistry and Photobiology. B, Biology,* Vol.8, No.3, (February 1991), pp. 235–254, ISSN 1011-1344

Dalle-Donne, I., Giustarini, D., Rossi, R., Colombo, R. and Milzani, A. (2003). Reversible S-glutathionylation of Cys 374 regulates actin filament formation by inducing structural changes in the actin molecule. *Free Radical Biology and Medicine,* Vol.34, No.1, (January 2003), pp. 23-32, ISSN 0891-5849

Dalle-Donne, I., Rossi, R., Milzani, A., Di Simplicio, P. and Colombo, R. (2001). The actin cytoskeleton response to oxidants: from small heat shock protein phosphorylation to changes in the redox state of actin itself. *Free Radical Biology and Medicine*, Vol.31, No.12, (December 2001), pp. 1624-1632, ISSN 0891-5849

Dardalhon, M., Agoutin, B., Watzinger, M. & Averbeck, D. (2009) . Slt2 (Mpk1) MAP kinase is involved in the response of *Saccharomyces cerevisiae* to 8-methoxypsoralen plus UVA. *Journal of Photochemistry and Photobiology B: Biology*, Vol.95, No.3, (June 2009), pp. 148–155, ISSN 1873-2682

Day, A.M. and Veal, E.A. (2010). Hydrogen peroxide-sensitive cysteines in the Sty1 MAPK regulate the transcriptional response to oxidative stress. *The Journal of Biological Chemistry*, Vol.285, No.10, (March 2010), pp. 7505-7516, ISSN 1083-351X

Degols, G. & Russell, P. (1997). Discrete roles of the Spc1 kinase and the Atf1 transcription factor in the UV response of *Schizosaccharomyces pombe*. *Molecular and Cellular Biology*, Vol.17, No.6, (June 1997), pp. 3356-3363, ISSN 0270-7306

Delley, P.A. & Hall, M.N. (1999). Cell wall stress depolarizes cell growth via hyperactivation of *RHO1*. *The Journal of Cell Biology*, Vol.147, No.1, (October 1999), pp. 163-174, ISSN 0021-9525

Devary, Y., Rosette, C., DiDonato, J.A. & Karin, M. (1993). NF-kappa B activation by ultraviolet light not dependent on a nuclear signal. *Science*. Vol.261, No.5127, (September 1993), pp. 1442-1445, ISSN 0036-8075

Dilova, I., Aronova, S., Chen, J.C.Y. & Powers, T. (2004). Tor signaling and nutrient-based signals converge on Mks1p phosphorylation to regulate expression of Rtg1.Rtg3p-dependent target genes. *The Journal of Biological Chemistry*, Vol.279, No.45, (November 2004), pp. 46527-46535, ISSN 0021-9258

Engelberg, D., Klein, C., Martinetto, H., Struhl, K. & Karin, M. (1994). The UV response involving the Ras signaling pathway and AP-1 transcription factors is conserved between yeast and mammals. *Cell*, Vol.77, No.3, (May 1994), pp. 381–390, ISSN 0092-8674

Farah, M. E., and Amberg, D. C. (2007). Conserved actin cysteine residues are oxidative stress sensors that can regulate cell death in yeast. *Molecular Biology of the Cell*, Vol.18, No.4, (April 2007), pp. 1359-1365, ISSN 1059-1524

Farah, M.E., Sirotkin, V., Haarer, B., Kakhniashvili, D. & Amberg, D.C. (2011). Diverse protective roles of the actin cytoskeleton during oxidative stress. *Cytoskeleton (Hoboken, N.J.)*, Vol.68, No.6, (June 2011), pp. 340-354, ISSN 1949-3592

Finkel, T. (2011). Signal transduction by mitochondrial oxidants. *The Journal of Biological Chemistry*. (Epub ahead of print)

Fox, G.C., Shafiq, M., Briggs, D.C., Knowles, P.P., Collister, M., Didmon, M.J., Makrantoni, V., Dickinson, R.J., Hanrahan, S., Totty, N., Stark, M.J., Keyse, S.M. & McDonald,

N.Q. (2007). Redox-mediated substrate recognition by Sdp1 defines a new group of tyrosine phosphatases. *Nature*, Vol.447, No.7143, (May 2007), pp. 487-492, ISSN 1476-4687

Garreau, H., Hasan, R.N., Renault, G., Estruch, F., Boy-Marcotte, E. & Jacquet, M. (2000). Hyperphosphorylation of Msn2p and Msn4p in response to heat shock and the diauxic shift is inhibited by cAMP in *Saccharomyces cerevisiae*. *Microbiology*, Vol.146, No.9, (September 2000), pp. 2113-2120, ISSN 1350-0872

Görner, W., Durchschlag, E., Martínez-Pastor, M.T., Estruch, F., Ammerer, G., Halmilton B., Ruis, H. & Schüller, C. (1998). Nuclear localization of the C2H2 zinc finger protein Msn2p is regulated by stress and protein kinase A activity. *Genes and Development,* Vol.12, No.4, (February 1998), pp. 586-597, ISSN 0890-9369

Görner, W., Durchschlag, E., Wolf, J., Brown, E. L., Ammerer, G., Ruis, H. & Schüller, C. (2002). Acute glucose starvation activates the nuclear localization signal of a stress-specific yeast transcription factor. *The EMBO journal*, Vol.21, No. 1-2, (January 2002), pp. 135–144, ISSN 0261-4189

Gourlay, C.W. & Ayscough, K.R. (2005a). Identification of an upstream regulatory pathway controlling actin-mediated apoptosis in yeast. *Journal of Cell Science,*. Vol.118, No. 10, (May 2005), pp. 2119-2132, ISSN 0021-9533

Gourlay, C.W. & Ayscough, K.R. (2005b). A role for actin in aging and apoptosis. *Biochemical Society Transactions,* Vol.33, No. 6, (December 2005), pp. 1260-1264, ISSN 0300-5127

Gourlay, C.W. & Ayscough, K.R. (2006). Actin-induced hyperactivation of the Ras signaling pathway leads to apoptosis in *Saccharomyces cerevisiae*. *Molecular and Cellular Biology,* Vol.26, No. 17, (September 2006), pp. 6487-6501, ISSN 0270-7306

Haarer, B. K. and Amberg, D. C. (2004). Old yellow enzyme protects the actin cytoskeletonfrom oxidative stress. *Molecular Biology of the Cell*, Vol.15, No. 10, (October 2004), pp. 4522-4531, ISSN 1059-1524

Haarer, B. K., Lillie, S. H., Adams, A. E. M., Magdolen, V., Bandlow, W. and Brown, S. S. (1990). Purification of profilin from *Saccharomyces cerevisiae* and analysis of profilin-deficient cells. *The Journal of Cell Biology*, Vol.110, No. 1, (January 1990), pp. 104-114, ISSN 0021-9525

Haghnazari, E. & Heyer, W.D. (2004). The Hog1 MAP kinase pathway and the Mec1 DNA damage checkpoint pathway independently control the cellular responses to hydrogen peroxide. *DNA Repair*, Vol.3, No.7, (July 2004), pp. 769-776, ISSN 1568-7864

Hasan, R., Leroy, C., Isnard, A.D., Labarre, J., Boy-Marcotte, E. & Toledano, M.B. (2002). The control of the yeast H2O2 response by the Msn2/4 transcription factors. *Molecular Microbiology*, Vol.45, No.1, (July 2002), pp. 233-241, ISSN 0950-382X

He, X. J. & Fassier, J. S. (2005). Identification of novel Yap1p and Skn7p binding sites involved in the oxidative stress response of *Saccharomyces cerevisiae*. *Molecular Microbiology*, Vol.58, No.5, (December 2005), pp. 1454-1467, ISSN 0950-382X

He, X.J., Mulford, K.E. & Fassler, J.S. (2009). Oxidative stress function of the *Saccharomyces cerevisiae* Skn7 receiver domain. *Eukaryotic cell*, Vol. 8, No.5, (May 2009), pp. 768-778, ISSN 1535-9786

Heinisch, J.J., Lorberg, A., Schmitz, H.P. & Jacoby, J.J. (1999). The protein kinase C-mediated MAP kinase pathway involved in the maintenance of cellular integrity in

Saccharomyces cerevisiae. *Molecular Microbiology*, Vol.32, No.4, (May 1999), pp. 671-680, ISSN 0950-382X

Helliwell, S. B., Wagner, P., Kunz, J., Deuter-Reinhard, M., Henriquez, R. & Hall, M.N. (1994). *TOR1* and *TOR2* are structurally and functionally similar but not identical phosphatidylinositol kinase homologues in yeast. *Molecular Biology of the Cell*, Vol.5, No.1, (January 1994), pp. 105-118, ISSN 1059-1524

Helliwell, S.B., Schmidt, A., Ohya, Y. and Hall, M.N. (1998). The Rho1 effector Pkc1, but not Bni1, mediates signalling from Tor2 to the actin cytoskeleton. *Current Biology*, Vol.8, No.22, (November 1998), pp. 1211-1214, ISSN 0960-9822

Inoki, K. and Guan, K.L. (2006). Complexity of the TOR signaling network. *Trends in Cell Biology*, Vol.16, No.4, (April 2006), pp. 206-212, ISSN 0962-8924

Inoki, K., Ouyang, H., Li, Y. and Guan, K.L. (2005). Signaling by target of rapamycin proteins in cell growth control. *Microbiology and Molecular Biology Reviews*, Vol.69, No.1, (March 2005), pp. 79-100, ISSN 1092-2172

Kahlos, K., Zhang, J., Block, E.R. & Patel, J.M. (2003). Thioredoxin restores nitric oxide-induced inhibition of protein kinase C activity in lung endothelial cells. *Molecular and Cellular Biochemistry*, Vol.254, No.1-2, (December 2003), pp. 47-54, ISSN 0300-8177

Kamata, H., Honda, S., Maeda, S., Chang, L., Hirata, H. & Karin, M. (2005). Reactive oxygen species promote TNFalpha-induced death and sustained JNK activation by inhibiting MAP kinase phosphatases. *Cell,* Vol.120, No.5, (March 2005), pp. 649-661, ISSN 0092-8674

Krause, S.A. & Gray, J.V. (2002). The protein kinase C pathway is required for viability in quiescence in *Saccharomyces cerevisiae*. *Current Biology*, Vol.12, No.7, (April 2002), pp. 588-593, ISSN 0960-9822

Latif, C., Harvey, S.H. & O'Connell, M.J. (2001). Ensuring the stability of the genome: DNA damage checkpoints. *TheScientificWorldJournal,* Vol.1, (November 2001), pp. 684-702, ISSN 1537-744X

Leadsham, J.E. & Gourlay, C.W. (2008). Cytoskeletal induced apoptosis in yeast. *Biochimica et Biophysica Acta,* Vol.1783, No.7, (July 2008), pp. 1406-1412, ISSN 0006-3002

Leadsham, J.E., Kotiadis, V.N., Tarrant, D.J. & Gourlay, C.W. (2010). Apoptosis and the yeast actin cytoskeleton. *Cell Death and Differentiation*, Vol.17, No.5, (May 2010), pp. 754-762, ISSN 1476-5403

Leadsham, J.E., Miller, K., Ayscough, K.R., Colombo, S., Martegani, E., Sudbery, P. & Gourlay, C.W. (2009). Whi2p links nutritional sensing to actin-dependent Ras-cAMP-PKA regulation and apoptosis in yeast. *Journal of Cell Science*, Vol.122, No.5, (March 2009), pp. 706-715, ISSN 0021-9533

Lee, J., Godon, C., Lagniel, G., Spector, D., Garin, J., Labarre, J. & Toledano, M.B. (1999). Yap1 and Skn7 control two specialized oxidative stress response regulons in yeast. *The Journal of Biological Chemistry*, Vol.274, No.23, (June 1999), pp. 16040-16046, ISSN 0021-9258

Leroy, C., Mann, C. & Marsolier M.C. (2001). Silent repair accounts for cell cycle specificity in the signaling of oxidative DNA lesions. *The EMBO Journal*, Vol.20, No.11, (June 2001), pp. 2896-2906, ISSN 0261-4189

Levin, D.E. (2005). Cell wall integrity signaling in *Saccharomyces cerevisiae*. *Microbiology and Molecular Biology Reviews,* Vol.69, No.2, (June 2005), pp. 262-291, ISSN 1092-2172

Liao, X., Small, W.C., Srere, P.A. and Butow, R.A. (1991). Intramitochondrial functions regulate nonmitochondrial citrate synthase (CIT2) expression in *Saccharomyces cerevisiae*. *Molecular and Cellular Biology*, Vol.11, No.1, (January 1991), pp. 38-46, ISSN 0270-7306

Liu, Z. & Butow, R.A. (2006). Mitochondrial retrograde signaling. *Annual Review of Genetics,* Vol.40, pp. 159-185, ISSN 0066-4197

Loewith, R., Jacinto, E., Wullschleger, S., Lorberg, A., Crespo, J.L., Bonenfant, D., Oppliger, W., Jenoe, P. & Hall, M.N. (2002). Two TOR complexes, only one of which is rapamycin sensitive, have distinct roles in cell growth control. *Molecular Cell*, Vol.10, No.3, (September 2002), pp. 457-468, ISSN 1097-2765

Lowndes, N.F. & Murguia, J.R. (2000). Sensing and responding to DNA damage. *Current Opinion in Genetics & Development,* Vol.10, No.1, (February 2000), pp. 17-25, ISSN 0959-437X

Lushchak, V.I. (2011). Adaptive response to oxidative stress: Bacteria, fungi, plants and animals. *Comp. Biochem. Physiol. C. Toxicol. Pharmacol,* Vol. 153, No. 2, (March 2011), pp. 175-190, ISSN 1532-0456

Madden, K., Sheu, Y.J., Baetz, K., Andrews, B. & Snyder, M. (1997). SBF cell cycle regulator as a target of the yeast PKC-MAP kinase pathway. *Science,* Vol.275, No.5307, (March 1997), pp. 1781-1784, ISSN 0036-8075

Madia, F., Wei, M., Yuan, V., Hu, J., Gatazo, C., Pham, P., Goodman, M.F. & Longo, V. (2009). Oncogene homologue Sch9 promotes age-dependent mutations by a superoxide and Rev1/Polzeta-dependent mechanism. *The Journal of Cell Biology*, Vol.186, No.4, (August 2009), pp. 509-523, ISSN 1540-8140

Marion, R.M., Regev, A., Segal, E., Barash, Y., Koller, D., Friedman, N. & O'Shea, E.K. (2004). Sfp1 is a stress- and nutrient-sensitive regulator of ribosomal protein gene expression. *Proceedings of the National Academy of Sciences of the United States of America*, Vol. 101, No.40, (October 2004), pp. 14315-14322, ISSN 0027-8424

Martin, D.E., Soulard, A. & Hall, M.N. (2004). TOR regulates ribosomal protein gene expression via PKA and the Forkhead transcription factor FHL1. *Cell*, Vol.119, No.7, (December 2004), pp. 969-979, ISSN 0092-8674

Martínez-Pastor, M.T., Marchler, G.C.S., Marchler-Bauer, A., Ruis, H. & Estruch, F. (1996). The *Saccharomyces cerevisiae* zinc finger proteins Msn2p and Msn4p are required for transcriptional induction through the stress response element (STRE). *The EMBO Journal*, Vol.15, No.9, (May 1996), pp. 2227-2235, ISSN 0261-4189

Morgan, B.A., Banks, G.R., Toone, W.M., Raitt, D.C., Kuge, S. & Johnston, L.H. (1997). The Skn7 response regulator controls gene expression in the oxidative stress response of the budding yeast *Saccharomyces cerevisiae*. *The EMBO Journal,* Vol.16, No.5, (March 1997), pp. 1035-1044, ISSN 0261-4189

Motizuki, M., Yokota, S. & Tsurugi, K. (2008). Effect of low pH on organization of the actin cytoskeleton in *Saccharomyces cerevisiae*. *Biochimica et Biophysica Acta*, Vol.1780, No.2, (February 2008), pp. 179-184, ISSN 0006-3002

Nguyen, A.N., Lee, A., Place, W. & Shiozaki, K. (2000). Multistep phosphorelay proteins transmit oxidative stress signals to the fission yeast stress-activated protein kinase. *Molecular Biology of the Cell*, Vol.11, No.4, (April 2000), pp. 1169-1181, ISSN 1059-1524

Nitti, M., Pronzato, M.A., Marinari, U.M. & Domenicotti, C. (2008). PKC signaling in oxidative hepatic damage. *Mol. Aspects Med.* Vol. 29, No. 1-2, (February-April 2008), pp. 36-42, ISSN 0098-2997

Odat, O., Matta, S., Khalil, H., Kampranis, S. C., Pfau, R., Tsichlis, P. N. & Makris, A. M. (2007). Old yellow enzymes, highly homologous FMN oxidoreductases with modulating roles in oxidative stress and programmed cell death in yeast. *The Journal of Biological Chemistry*, Vol.282, No.49, (December 2007), pp. 36010-36023, ISSN 0021-9258

Ojeda, L., Keller, G., Muhlenhoff, U., Rutherford, J.C., Lill, R. & Winge, D.R. (2006). Role of glutaredoxin-3 and glutaredoxin-4 in the iron regulation of the Aft1 transcriptional activator in *Saccharomyces cerevisiae*. *The Journal of Biological Chemistry*, Vol. 281, No.26, (June 2006), pp. 17661-17669, ISSN 0021-9258

Pan, Y. & Shadel, G.S. (2009). Extension of chronological life span by reduced TOR signaling requires down-regulation of Sch9p and involves increased mitochondrial OXPHOS complex density. *Aging,* Vol.1, No.1, (January 2009), pp. 131-145, ISSN 1945-4589

Pascale, A., Amadio, M., Govoni, S. & Battaini, F. (2007). The aging brain, a key target for the future: the protein kinase C involvement. *Pharmacological research*, Vol.55, No.6, (June 2007), pp. 560-569, ISSN 1043-6618

Petkova, M.I., Pujol-Carrion, N., Arroyo, J., García-Cantalejo, J. & de la Torre-Ruiz, M.A. (2010a). Mtl1 is required to activate general stress response through Tor1 and Ras2 inhibition under conditions of glucose starvation and oxidative stress. *The Journal of Biological Chemistry*, Vol.285, No.25, (June 2010), pp. 19521-19531, ISSN 1083-351X

Petkova, M.I., Pujol-Carrion, N., de la Torre-Ruiz, M.A. (2010b). Signal flow between CWI/TOR and CWI/RAS in budding yeast under conditions of oxidative stress and glucose starvation. *Communicative & Integrative Biology*, Vol.3, No.6, (November 2010), pp. 555-557, ISSN 1942-0889

Pujol-Carrion, N. & de la Torre-Ruiz, M.A. (2010). Glutaredoxins Grx4 and Grx3 of *Saccharomyces cerevisiae* play a role in actin dynamics through their Trx domains, which contributes to oxidative stress resistance. *Applied and Environmental Microbiology*, Vol.76, No.23, (December 2010), pp. 7826-7835, ISSN 1098-5336

Pujol-Carrion, N., Belli, G., Herrero, E., Nogues, A. & de la Torre-Ruiz, M.A. (2006). Glutaredoxins Grx3 and Grx4 regulate nuclear localisation of Aft1 and the oxidative stress response in *Saccharomyces cerevisiae*. *Journal of Cell Science*, Vol.119, No.21, (November 2006), pp. 4554-4564, ISSN 0021-9533

Queralt, E and Igual, J.C. (2005). Functional connection between the Clb5 cyclin, the protein kinase C pathway and the Swi4 transcription factor in *Saccharomyces cerevisiae*. *Genetics*, Vol.171, No.4, (December 2005), pp. 1485–1498, ISSN 0016-6731

Raitt, D.C., Johnson, A.L., Erkine, A.M., Makino, K., Morgan, B., Gross, D.S. & Jonston, L.H. (2000). The Skn7 response regulator of *Saccharomyces cerevisiae* interacts with Hsf1 in vivo and is required for the induction of heat shock genes by oxidative stress. *Molecular Biology of the Cell*, Vol.11, No.7, (December 2010), pp. 2335-2347, ISSN 1059-1524

Rajavel, M., Philip, B., Buehrer, B.M., Errede, B. & Levin, D.E. (1999). Mid2 is a putative sensor for cell integrity signaling in *Saccharomyces cerevisiae*. *Molecular and Cellular Biology*, Vol.19, No.6, (June 1999), pp. 3969-3976, ISSN 0270-7306

Ross, S.J., Findlay, V.J., Malakasi, P. & Morgan, B.A. (2000). Thioredoxin peroxidase is required for the transcriptional response to oxidative stress in budding yeast. *Molecular Biology of the Cell*, Vol.11, No.8, (August 2000), pp. 2631-2642, ISSN 1059-1524

Rouse, J., Cohen, P., Trigon, S., Morange, M., Alonso-Llamazares, A., Zamanillo, D., Hunt, T. & Nebreda, A.R. (1994). A novel kinase cascade triggered by stress and heat shock that stimulates MAPKAP kinase-2 and phosphorylation of the small heat shock proteins. *Cell*, Vol. 78, No.6, (September 1994), pp. 1027-1037, ISSN 0092-8674

Runchel, C., Matsuzawa, A. & Ichijo, H. (2011). Mitogen-activated protein kinases in mammalian oxidative stress responses. *Antioxidants & redox signaling*, Vol.15, No.1, (July 2011), pp. 205-218, ISSN 1557-7716

Sage, E. & Harrison, L. (2011). Clustered DNA lesion repair in eukaryotes: relevance to mutagenesis and cell survival. *Mutation research*, Vol.711, No.1-2, (June 2011), pp. 123-133, ISSN 0027-5107

Schmelzle, T., Beck, T., Martín, D.E. & Hall, M.N. (2004). Activation of the RAS/cyclic AMP pathway suppresses a TOR deficiency in yeast. *Molecular and Cellular Biology*, Vol.24, No.1, (January 2004), pp. 338-351, ISSN 0270-7306

Schmitt, A.P. & McEntee, K. (1996). Msn2p, a zinc finger DNA-binding protein, is the transcriptional activator of the multistress response in *Saccharomyces cerevisiae*. *Proceedings of the National Academy of Sciences of the United States of America*, Vol.93, No.12, (June 1996), pp. 5777-5782, ISSN 0027-8424

Schonbrun, M., Laor, D., López-Maury, L., Bähler, J., Kupiec, M. & Weisman, R. (2009). TOR complex 2 controls gene silencing, telomere length maintenance, and survival under DNA-damaging conditions. *Molecular and Cellular Biology*, Vol.29, No.16, (August 2009), pp. 4584-4594, ISSN 1098-5549

Searle, J.S. and Sanchez, Y. (2004). Stopped for repairs: A new role for nutrient sensing pathways?. *Cell Cycle*, Vol.3, No.7, (July 2004), pp. 865-868, ISSN 1551-4005

Sekiya-Kawasaki, M., Abe, M., Saka, A., Watanabe, D., Kono, K., Minemura-Asakawa, M., Ishihara, S., Watanabe, T. & Ohya, Y. (2002). Dissection of upstream regulatory components of the Rho1p effector, 1,3-beta-glucan synthase, in *Saccharomyces cerevisiae*. *Genetics,* Vol.162, No.2, (October 2002), pp. 663-676, ISSN 0016-6731

Serrano, R., Martín, H., Casamayor, A. & Ariño, J. (2006). Signaling alkaline pH stress in the yeast *Saccharomyces cerevisiae* through the Wsc1 cell surface sensor and the Slt2 MAPK pathway. *The Journal of Biological Chemistry*, Vol.281, No.52, (December 2006), pp. 39785-39795, ISSN 0021-9258

Shafman, T.D., Saleem, A., Kyriakis, J., Weichselbaum, R., Kharbanda, S. & Kufe, D.W. (1995). Defective induction of stress-activated protein kinase activity in ataxia-telangiectasia cells exposed to ionizing radiation. *Cancer Research*, Vol.55, No.15, (August 1995), pp. 3242-3245, ISSN 0008-5472

Shartava, A., Korn, W., Shah, A.K. & Goodman, S.R. (1997). Irreversibly sickled cell beta-actin: defective filament formation. *American Journal of Hematology*, Vol.55, No.2, (June 1997), pp. 97-103, ISSN 0361-8609

Shartava, A., Monteiro, C.A., Bencsath, F.A., Schneider, K., Chait, B.T., Gussio, R., Casoria-Scott, L.A., Shah, A.K., Heuerman, C.A. & Goodman, S.R. (1995). A posttranslational modification of beta-actin contributes to the slow dissociation of

the spectrin-protein 4.1-actin complex of irreversibly sickled cells. *The Journal of Cell Biology*, Vol.128, No.5, (March 1995), pp. 805-818, ISSN 0021-9525

Shiozaki, K. & Russell, P. (1995). Cell-cycle control linked to extracellular environment by MAP kinase pathway in fission yeast. *Nature*, Vol.378, No.6558, (December 1995), pp. 739-743, ISSN 0028-0836

Sidorova, J.M., Mikesell, G.E. & Breeden, L.L. (1995). Cell cycle-regulated phosphorylation of Swi6 controls its nuclear localization. *Molecular Biology of the Cell,* Vol.6, No.12, (December 1995), pp. 1641-1658, ISSN 1059-1524

Takashi, R. (1979). Fluorescence energy transfer between subfragment-1 and actin points in the rigor complex of actosubfragment-1. *Biochemistry*, Vol.18, No.23, (November 1979), pp. 5164-5169, ISSN 0006-2960

Thevelein, J. M. (1994). Signal transduction in yeast. *Yeast*, Vol.10, No.13, (December 1994), pp. 1753–1790, ISSN 0749-503X

Torres, J., Di Como, C.J., Herrero, E. & de la Torre-Ruiz, M.A. (2002). Regulation of the cell integrity pathway by rapamycin-sensitive TOR function in budding yeast. *The Journal of biological chemistry*, Vol. 277, No.45, (November 2002), pp. 43495-43504, ISSN 0021-9258

van Dam, H., Wilhelm, D., Herr, I., Steffen, A., Herrlich, P. & Angel, P. (1995). ATF-2 is preferentially activated by stress-activated protein kinases to mediate c-jun induction in response to genotoxic agents. *The EMBO Journal*, Vol. 14, No.8, (April 1995), pp. 1798-1811, ISSN 0261-4189

Veal, E.A., Findlay, V.J., Day, A.M., Bozonet, S.M., Evans, J.M., Quinn, J. & Morgan, B.A. (2004). A 2-Cys peroxiredoxin regulates peroxide-induced oxidation and activation of a stress-activated MAP kinase. *Molecular Cell*, Vol. 15, No.1, (July 2004), pp. 129-139, ISSN 1097-2765

Veal, E.A., Day, A.M. & Morgan, B.A. (2007). Hydrogen peroxide sensing and signaling. Mol. Cell, Vol. 26, No. 1 (April 2007), pp. 1-14, ISSN: 1097-2765

Vilella, F., Herrero, E., Torres, J. & de la Torre-Ruiz, M.A. (2005). Pkc1 and the upstream elements of the cell integrity pathway in *Saccharomyces cerevisiae*, Rom2 and Mtl1, are required for cellular responses to oxidative stress. *The Journal of Biological Chemistry*, Vol. 280, No.10, (March 2005), pp. 9149-9159, ISSN 0021-9258

Wagner, E.F. and Nebreda, A.R. (2009). Signal integration by JNK and p38 MAPK pathways in cancer development. *Nature Reviews. Cancer*, Vol. 9, No.8, (March 2005), pp. 537-549, ISSN 1474-1768

Watson, J.A., Rumsby, M.G. & Wolowacz, R.G. (1999). Phage display identifies thioredoxin and superoxide dismutase as novel protein kinase C-interacting proteins: thioredoxin inhibits protein kinase C-mediated phosphorylation of histone. *The Biochemical journal*, Vol. 343, No.2, (October 1999), pp. 301-305, ISSN 0264-6021

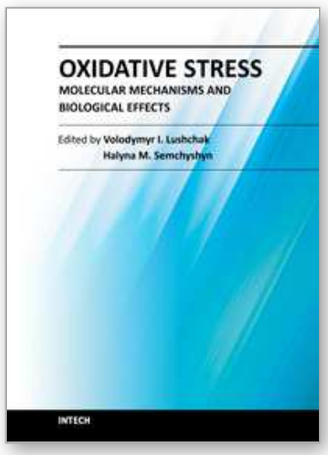
Witte, S., Villalba, M., Bi, K., Liu, Y., Isakov N. & Altman, A. (2000). Inhibition of the c-Jun N-terminal kinase/AP-1 and NF-kappaB pathways by PICOT, a novel protein kinase C-interacting protein with a thioredoxin homology domain. *The Journal of Biological Chemistry*, Vol. 275, No.3, (January 2000), pp. 1902-1909, ISSN 0021-9258

Wullschleger, S., Loewith, R. and Hall, M.N. (2006). TOR signaling in growth and metabolism. *Cell*, Vol. 124, No.3, (February 2006), pp. 471-484, ISSN 0092-8674

Zarebska, Z., Waszkowska, E., Caffieri, S. & Dall'Acqua, F. (2000). PUVA (psoralen + UVA) photochemotherapy: processes triggered in the cells. *Farmaco,* Vol. 55, No.8, (August 2000), pp. 515–520, ISSN 0014-827X

Zhang, A., Shen, Y., Gao, W. & Dong, J. (2011). Role of Sch9 in regulating Ras-cAMP signal pathway in *Saccharomyces cerevisiae*. *FEBS letters*, Vol. 585, No.19, (October 2011), pp. 3026-3032, ISSN 1873-3468

Zheng, M. & Storz, G. (2000). Redox sensing by prokaryotic transcription factors. *Biochemical pharmacology*, Vol. 59, No.1, (January 2000), pp. 1-6, ISSN 0006-2952



Oxidative Stress - Molecular Mechanisms and Biological Effects
Edited by Dr. Volodymyr Lushchak

ISBN 978-953-51-0554-1
Hard cover, 362 pages
Publisher InTech
Published online 25, April, 2012
Published in print edition April, 2012

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.

How to reference
In order to correctly reference this scholarly work, feel free to copy and paste the following:

Maria Angeles de la Torre-Ruiz, Luis Serrano, Mima I. Petkova and Nuria Pujol-Carrion (2012). Signalling Oxidative Stress in Saccharomyces cerevisiae, Oxidative Stress - Molecular Mechanisms and Biological Effects, Dr. Volodymyr Lushchak (Ed.), ISBN: 978-953-51-0554-1, InTech, Available from: http://www.intechopen.com/books/oxidative-stress-molecular-mechanisms-and-biological-effects/signalling-the-oxidative-stress-in-saccharomyces-cerevisiae

INTECH
open science | open minds

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

IntechOpen