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Oxidative Stress Responses of the Yeast *Saccharomyces cerevisiae*

DEREK J. JAMIESON*

Department of Biological Sciences, Heriot-Watt University, Riccarton, Edinburgh EH14 4AS, U.K.

All aerobically growing organisms suffer exposure to oxidative stress, caused by partially reduced forms of molecular oxygen, known as reactive oxygen species (ROS). These are highly reactive and capable of damaging cellular constituents such as DNA, lipids and proteins. Consequently, cells from many different organisms have evolved mechanisms to protect their components against ROS. This review concentrates on the oxidant defence systems of the budding yeast *Saccharomyces cerevisiae*, which appears to have a number of inducible adaptive stress responses to oxidants, such as H_2O_2, superoxide anion and lipid peroxidation products. The oxidative stress responses appear to be regulated, at least in part, at the level of transcription and there is considerable overlap between them and many diverse stress responses, allowing the yeast cell to integrate its response towards environmental stress. © 1998 John Wiley & Sons, Ltd.

KEY WORDS — *Saccharomyces cerevisiae*; oxidative stress; stress response; signal transduction

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INTRODUCTION

Oxygen is a highly reactive molecule and can be partially reduced to form a number of chemically-reactive agents, known as reactive oxygen species (ROS), such as the hydroxyl radical (HO^-). These forms of oxygen are highly damaging towards cellular constituents, including DNA, lipids and proteins.[135,150] In addition to these highly reactive molecules, both H_2O_2 and superoxide anions (O_2^-) are often also referred to as ROS as they can lead to production of more reactive species, particularly in the presence of metal ions. ROS are generated endogenously in many cells as a consequence of metabolic processes. ROS can also be formed by exposure of cells to either ionizing radiation, redox-cycling chemicals present in the environment or by exposure to heavy metals.[1,9] Through these mechanisms, all aerobically growing organisms are continuously exposed to reactive oxidants and oxidative stress occurs when the concentration of these oxidants increases beyond the antioxidant buffering capacity of the cell. Given the ubiquitous nature of ROS, it is hardly surprising that most (if not all) organisms have evolved the means to protect their cellular components against reactive oxidants. This review focuses on the antioxidant defence systems of the budding yeast *Saccharomyces cerevisiae*.

*Correspondence to: D. J. Jamieson, Department of Biological Sciences, Heriot-Watt University, Riccarton, Edinburgh EH14 4AS, UK.

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OXIDANT DEFENCE SYSTEMS

Cells possess both enzymatic and non-enzymatic defence systems to protect their cellular constituents and maintain cellular redox state. Non-enzymatic defence systems typically consist of small molecules which are soluble in either an

aqueous or, in some instances, a lipid environment. They act in general as radical scavengers, being oxidized by ROS and thereby removing oxidants from solution.

Non-enzymatic defence systems

Glutathione Perhaps the best-known example of a non-enzymatic defence system is glutathione (GSH), a tripeptide γ-L-glutamyl-L-cystinylglycine. GSH acts as a radical scavenger with the redox-active sulphydryl group reacting with oxidants to produce reduced glutathione (GSSG). Glutathione is possibly the most abundant redox scavenging molecule in cells,[98] consequently, its role in maintaining cellular redox state is important.

The genes involved in GSH biosynthesis have been identified in *S. cerevisiae* (*GSH1* and *GSH2*, encoding γ-glutamylcysteine synthetase and glutathione synthetase respectively) and *gsh1* and *gsh2* mutants have been isolated in yeast.[73,115,116] As is the case for *E. coli*, yeast *gsh1* and *gsh2* mutants are still viable, although they have a slower growth rate and a longer lag phase than wild-type cells, they also show a defect in sporulation.[115,130] Mutants deficient in *gsh1* are absolutely dependent upon exogenous GSH for growth in minimal media, while *gsh2* mutants can grow in unsupplemented minimal media, albeit less well than their wild-type counterparts.[49,133] In addition, *gsh1* mutants display a petite phenotype, being unable to grow on non-fermentable carbon sources such as glycerol. Moreover, the wild-type *GSH1* gene was initially isolated as a high copy assessor of a mutation in a gene encoding a mitochondrial protein (*pet5155*^{ts}), suggesting that glutathione plays an important role in protecting the mitochondrion from oxidants produced as a result of respiration.[92]

A number of studies have shown that GSH is an important antioxidant molecule in yeast. Glutathione-deficient mutants have been shown to be hypersensitive to H_2O_2, plumbagin and menadione (superoxide anion generators), as well as other compounds such as cadmium and methylglyoxyl.[64,73,133] Despite being hypersensitive to oxidants, GSH-deficient mutants are still able to induce an adaptive stress response to both H_2O_2 and menadione, suggesting that GSH is not important as a sensor molecule in these stress responses.[133] In addition to being a radical scavenger, GSH can also be conjugated to toxic electrophiles and the conjugate excreted into the vacuole, presumably via the recently identified GSH-conjugate pump Ycf1p.[86] Evidence has been presented for the formation and transport of both GSH-Cd and -menadione conjugates.[86,153] Interestingly, γ-glutamylcysteine has been shown to be able to partially substitute for GSH, when used as a medium supplement.[49] However, it is clear that GSH has one or more additional functions for which γ-glutamylcysteine cannot substitute, as *gsh2* mutants still require GSH for wild-type rates of growth.[49] In *S. cerevisiae*, GSH can also be used as a source of nitrogen and sulphur under starvation conditions.[36,102]

While many studies have shown that menadione can lead to the production of superoxide anions, via redox-cycling there is still some doubt as to the precise mode of toxicity of menadione. Cu/ZnSOD-deficient yeast mutants are hypersensitive to menadione, implying that at least part of its toxicity is via the production of superoxide anions.[67] However, a recent report suggests that menadione may also be rendered toxic by conjugation with GSH.[153] In common with higher eukaryotes, exposure of exponentially growing yeast cells to oxidants for short periods of time does not lead to marked alterations in the levels of total GSH.[64,133] However, Zadinski *et al.* have found significant depletion of GSH levels after treatment of stationary phase yeast cultures with menadione, whereas the same group found only limited depletion in response to a number of agents, including H_2O_2.[35,153]

Phytochelatins In many fungi and plants, phytochelatins play an analogous role to GSH. Phytochelatins have the structure (γ-glutamylcysteine)n-glycine and have been isolated from the fission yeast *Schizosaccharomyces pombe*, but as yet they have not been shown to be present in *S. cerevisiae*.[15]

Polyamines In addition to glutathione, amino acid-derived polyamines have also been implicated in protecting yeast against oxidant stress. Indeed, both spermine and spermidine have been found to be essential for aerobic growth of *S. cerevisiae* and an *spe2* null mutant was found to be hypersensitive to oxygen.[3,4]

Ascorbic acid Ascorbic acid has been known for some time to be an important antioxidant in higher eukaryotes, particularly in plants. In yeasts, and especially in *S. cerevisiae*, the position is less clear.

There are a few reports claiming to have found ascorbic acid in *S. cerevisiae*,[56,113] although the levels appear low. However, erythroascorbic acid has been identified in both *Candida albicans* and *S. cerevisiae*.[60,72] Given that the properties of erythroascorbic acid are similar to those of ascorbic acid itself, it seems likely that this compound will have antioxidant properties, though its precise role and importance awaits further investigation.

Lipid-soluble antioxidants Although, to date, little is known about lipid-soluble antioxidant molecules in *S. cerevisiae*, it has been observed that the membrane lipid composition of yeast is important in conferring resistance to oxidative stress, with cells containing membranes with a higher level of saturated fatty acids being more resistant than those with a higher level of polyunsaturated fatty acids.[59,131]

Trehalose A number of studies have implicated the disaccharide trehalose as being important for the stress tolerance of yeast. However, several investigators have cast some doubt on the role of trehalose as an antioxidant, as trehalose levels in several yeast strains did not correlate well with the level of resistance to H_2O_2.[85] Moreover, H_2O_2 exposure did not induce expression of a number of genes involved in glycogen and trehalose biosynthesis.[118] However, it does seem likely that a certain threshold level of trehalose is vital for resistance to environmental stresses, including oxidative stress. Moreover, the baking strains used by Lewis *et al*.[85] all possessed higher levels of trehalose compared to laboratory strains and also demonstrated a greater degree of H_2O_2 resistance.

Metallothioneins There is now overwhelming support for a direct link between metal ions and oxidant resistance/sensitivity. Mutations in a number of genes give rise to resistance/hypersensitivity towards toxic levels of both metal ions and oxidants. This close link seems to make biological sense, given the role of metal ions such as Cu^+ and Fe^{2+} on the production of oxidants, and results in a coordinated response to both metal and oxidant stress. Metallothioneins are a class of small cysteine-rich proteins with antioxidant properties and have the capacity to bind a number of different metal ions.[52] Metallothioneins are particularly important in countering the toxicity of metals such as copper. Yeast metallothioneins are encoded by the *CUP1* and *CRS5* genes and have been shown to play a role in protecting yeast cells against oxidants, as the oxidant-sensitive phenotype of strains lacking the Cu/ZnSod can be complemented by the over-expression of either yeast or human metallothionein.[21,137] Also, expression of the *CUP1* gene is inducible following exposure towards menadione and is important in conferring menadione resistance.[90]

Metal ion homeostasis The link between oxidant stress resistance and metal ion homeostasis was strengthened with the finding that, by altering metal ion homeostasis, it was possible to suppress the oxidant hypersensitivity of Cu/ZnSOD mutants. Mutations in two genes, *BSD1* and *BSD2*, have been shown to be able to suppress the defects of a Cu/ZnSOD mutant.[88] Further work on these mutants demonstrated that Bsd1p was identical to Pmr1p, a P-type ATPase transporter protein found in the Golgi and playing an important role in manganese and Ca^{2+} homeostasis,[82] while the *BSD2* gene was shown to encode a 37·5 kDa protein found in the endoplasmic reticulum.[89] As with Bsd1p, the Bsd2 protein is involved in regulating metal ion homeostasis and has been shown to regulate metal ion transport systems.[91] A high copy number suppressor of the defect of a *sod1*, *2* double mutant, the *ATX1* gene, was demonstrated to participate in metal ion homeostasis, particularly that of copper.[87] Moreover, *atx1* null mutants were found to be hypersensitive to both H_2O_2 and superoxide anion generators.[87]

Flavohaemoglobin Another metallo-protein with a possible role in oxidant stress protection and/or sensing is the yeast flavohaemoglobin (Yhb1p).[154] Mutants deficient in Yhb1p are slightly sensitive to oxidants such as diamide, and expression of the gene is induced in the presence of oxygen.[154] Nevertheless, the exact physiological role of this protein in conferring oxidant resistance remains unknown.

Thioredoxin Thioredoxin is a small sulphydryl-rich protein which can be used as a reductant for thioredoxin peroxidase and for ribonucleotide reductase. However, the precise physiological functions of thioredoxin are not clearly understood. *S. cerevisiae* possesses two genes encoding thioredoxin proteins. *TRX1* and *TRX2*.[109] Deletion of either or both genes is not a lethal event for *S. cerevisiae*. Although the *TRX2* deletion is not lethal, it does render cells

hypersensitive to H_2O_2 but, paradoxically, resistant to diamide.[177,111] Deletion of both the *TRX1* and *TRX2* genes results in an extension of S-phase of the cell cycle and a marked increase in the level of oxidized glutathione.[109,111] Moreover, evidence has been presented that suggests that thioredoxin (and thioredoxin reductase) deficient mutations stimulate the transcription of certain cell cycle-regulated genes, in particular those transcribed mainly at the G_1/S boundary.[93] Interestingly, H_2O_2 has been found to arrest the cell cycle in G1 and G2, with the Rad9 check point protein being implicated in oxidant stress protection.[34] Yeast *rad9* and *cdc28* mutants were found to be hypersensitive to H_2O_2, although *rad9* mutants were not hypersensitive to the superoxide anion generator menadione.[34]

Glutaredoxin In common with most organisms, *S. cerevisiae* also possesses glutaredoxins, a class of small proteins with an active site containing two redox sensitive cysteines.[38] These proteins are thought to act in much the same way as thioredoxins; indeed, it is likely that glutaredoxin can act as a source of electrons for ribonucleotide reductase in yeast.[111] More recently, *S. cerevisiae* has been shown to have two glutaredoxin genes (*GRX1* and *GRX2*).[94] Intriguingly, despite the relatively high degree of similarity between the two proteins, it appears that they perform different roles, with *GRX1* and *GRX2* protecting the cell against H_2O_2 and superoxide anions, respectively.[94] Thus, in common with thioredoxins, glutaredoxins are required for resistance to oxidative stress.

Enzymatic defence systems

Cellular antioxidant defences also include several enzymes which are capable of removing oxygen radicals and their products and/or repairing the damage caused by oxidative stress.

Catalase Catalase catalyses the breakdown of H_2O_2 to O_2 and H_2O. *S. cerevisiae* has two such enzymes, catalase A and catalase T, encoded by the *CTA1* and *CTT1* genes, respectively.[16,54] Catalase A is located in the peroxisome and the main physiological role of this enzyme appears to be to remove H_2O_2 produced by fatty acid β-oxidation.The physiological role of the cytosolic catalase T protein is less clear; *CTT1* gene expression is, however, regulated by oxidative and osmotic stress and by starvation.[124] Yeast strains deficient in both Cta1p and Ctt1p are hypersensitive to H_2O_2 in stationary phase and both single and double catalase mutants are unable to mount an adaptive stress response to H_2O_2.[65] Thus, although the catalase genes are only moderately inducible by H_2O_2, both catalases are clearly important for resistance towards H_2O_2.

Superoxide dismutase Yeast cells, in common with other eukaryotes, possess two intracellular superoxide dismutases (SOD), the mitochondrially-located (MnSod (encoded by the *SOD2* gene) and the cytoplasmically-located Cu/ZnSod (encoded by the *SOD1* gene).[5,140] Superoxide dismutases disproportionate superoxide anion to H_2O_2 and O_2. The Cu/ZnSod appears to be the major enzyme involved in removing superoxide anions from the cytoplasm and possibly also the peroxisome,[45,67] while the physiological role of the MnSod appears to be to protect the mitochondria from superoxides generated during respiration and exposure to ethanol, and seems to play little role in countering the toxicity of superoxide anions generated by exogenously added redox cycling compounds during fermentative growth.[20,43,67] There is also some evidence for a role of the Cu/ZnSOD in protecting cells against respiration derived superoxide anions.[2] For example, *sod1* null mutants fail to grow on media containing lactate as the sole carbon source (a phenotype typical of respiratory-deficient cells).[45] Inhibition of respiration reverses the negative effect of *sod* mutations on yeast long term viability.[93] It is now also evident that the Cu/ZnSOD can play a role in buffering the intracellular copper concentration, although this function appears to be unrelated to its role in protection against oxidative stress.[22]

Pentose phosphate pathway enzymes Glucose 6-phosphate dehydrogenase (*ZWF1*), transketolase (*TKL1*) and ribulose 5-phosphate epimerase (*RPE1*) are all enzymes involved in the pentose phosphate metabolic pathway, and as such are crucial for the production of cellular reducing power in the form of NADPH. The enzymes glutathione reductase and thioredoxin reductase (see below) both require NADPH as a reductant to reduce oxidized glutathione (GSSG) and thioredoxin. Therefore, given that GSH and thioredoxin are important antioxidants, it is perhaps not surprising that mutations in the *ZWF1*, *TKL1* and *RPE1* genes, negatively affecting the pentose phosphate pathway, render the cells hypersensitive to oxidants such as H_2O_2.[68,79,114,129]

Glutathione reductase Crucial to the role of glutathione as an antioxidant is the maintenance of a high reduced–oxidized ratio inside the cell. The enzyme glutathione reductase is primarily responsible for the reduction of oxidized glutathione and maintenance of the GSH/GSSG ratio in cells. The gene encoding glutathione reductase in *S. cerevisiae* (*GLR1*) has been identified and null mutants, although viable, accumulate an excess of oxidized glutathione and are hypersensitive to oxidants.[18,47] Mutations in the *GLR1* gene were identified during a synthetic lethal screen, designed to isolate thioredoxin-dependent mutants, suggesting that thioredoxin may also help to maintain the intracellular GSH/GSSG ratio.[111]

Glutathione peroxidase As mentioned above, GSH is an important antioxidant and, while GSH can react directly with radicals and electrophiles, it can also act as a source of electrons for glutathione peroxidase. The enzyme glutathione peroxidase catalyses the reduction of hydroperoxides, using GSH as a reductant. yeast glutathione peroxidase activity towards both H_2O_2 and organic hydroperoxides has been demonstrated; interestingly, both these activities could be induced by a shift from anaerobic to aerobic growth conditions.[37] A gene has been isolated that, when present on a high copy number vector, increases resistance towards *tert*-butyl hydroperoxide and H_2O_2, and gives rise to increased levels of GSH-dependent peroxidase activity.[63] this gene (*OSR1*) has been shown to be alleleic to a gene (*ZRC1*, encoding a putative membrane protein) which, when present in high copy number, gives rise to increased resistance to cadmium.[70,75] Intriguingly, the *osr1* null mutation resulted in a lowering of cellular GSH levels, raising the possibility that this protein may regulate GSH biosynthesis,[75] although how Zrc1p regulates GSH levels and GSH-peroxidase activity is not yet known.

Thioredoxin peroxidase and thioredoxin reductase Yeast possesses an abundant thioredoxin peroxidase (Tsa), which is similar to bacterial alkyl hydroperoxidases.[13] Thioredoxin peroxidase levels are increased about two-fold in response to growth in the presence of 95% O_2 or thiol-containing agents such as mercaptoethanol.[71] Thioredoxin peroxidase reduces both H_2O_2 and alkyl hydroperoxides, in conjunction with thioredoxin reductase, thioredoxin and NADPH.[12,13,81,112]

Methionine reductase It has been proposed recently that methionine residues exposed on the surface of proteins can act an antioxidants to protect the active sites of proteins.[84] Methionine residues on the surface of an enzyme would be oxidized to methionine sulphoxide, effectively removing the oxidant and protecting the active site of the protein. The enzyme methionine sulphoxide reductase would then reverse the process. While this has yet to be tested *in vivo*, yeast possess a peptide–methionine sulphoxide reductase. Moreover, yeast deleted for the *MRS* gene grow considerably less well in the presence of oxidants.[106]

Apn1 endonuclease In view of the well-documented ability of reactive oxidants to damage DNA, it is not surprising that several yeast DNA repair associated activities are important in conferring resistance to oxidants. Yeast mutants deficient in the *APN1* gene, which encodes the principal apurinic/apyrimidinic endonuclease, are hypersensitive to oxidants.[123] Also, several oxidant-sensitive mutants isolated recently have been shown to have phenotypes similar to those expected for mutations in DNA repair-associated genes.[122]

INDUCIBLE ADAPTIVE RESPONSES TOWARDS OXIDATIVE STRESS

While yeast possess a degree of constitutive resistance towards oxidants, several laboratories have demonstrated that treatment of aerobic exponential phase yeast cultures with sub-lethal levels of oxidants can lead to the transient induction of increased protection against subsequent exposure to normally lethal levels of oxidants.[17,25,32,66] Using the protein synthesis inhibitor cycloheximide it has been shown that protection requires new protein synthesis, and is presumably the result of *de novo* synthesis of protective enzymes.[17,32] Initially, *S. cerevisiae* was shown to possess at least two distinct adaptive stress responses to oxidants: one induced by H_2O_2 and the other by exposure to compounds such as menadione, which produce a flux of superoxide anions in the cell.[66,67] The response to H_2O_2 appeared to be distinct from that induced by menadione, on the basis of cross-protection experiments.[66] Furthermore, different polypeptides appeared to be synthesized in response to exposure to two oxidants, H_2O_2 and

menadione,[67] although there was clearly some overlap between the two responses.[67] More recently, adaptive stress responses to malandialdehyde, and possibly also towards linoleic acid hydroperoxide, have been identified.[30,139] These stress responses have been less extensively studied compared to the H_2O_2 and superoxide stress responses.

Much recent work has understandably concentrated on the transcriptional regulation of specific genes encoding antioxidant function. These studies have revealed that the adaptive stress response towards H_2O_2 is not composed of a single regulon, but rather a complex network of several different regulons. For example, several genes (e.g. *TRX2*) appear to be regulated directly by the transcription factor Yap1, whereas others, such as *SSA1*, are regulated by Yap1 indirectly.[132] Recently, another Yap1-dependent gene, *GSH1*, was found to be inducible by H_2O_2, but only when cells were grown in the presence of certain amino acids, whereas H_2O_2 induction of both *TRX2* and *SSA1* was much less dependent upon the presence of amino acids in the growth media.[134] It is also clear that some, although not all, STRE-regulated genes are inducible by H_2O_2. The *CTT1* gene is modestly inducible by H_2O_2 via STRE elements in its promoter.[98] Thus, the oxidant adaptive stress responses, as measured by increased protection against oxidants, are complex.

REGULATION OF GENE EXPRESSION BY OXIDANTS

A number of studies have presented evidence for much of the regulation of the oxidant stress responses in *S. cerevisiae* to be at the level of transcription.[17,67,83,132,134] Regulation of gene expression by oxidative stress in yeast appears to be more complex than that observed in prokaryotes. There is also considerable overlap between the oxidative stress responses and other stress responses; including starvation, heat-shock, osmotic shock and resistance to heavy metals such as cadmium. A number of transcription factors have been identified and demonstrated to play some role in regulating gene expression in response to oxidants.

bZip transcription factors

Several bZip-type transcription factors with some sequence similarity to mammalian transcription factors, such as c-Jun, and characteristic basic DNA-binding and dimerization domains, have been identified in *S. cerevisiae*.[31] Three of these proteins, Yap1, Yap2 and Gcn4, have been extensively studied and all play roles in protecting yeast cells against stress. In addition, several other Yap-like proteins of unknown function have been identified by the yeast genome sequencing project.[31,39,104]

The Yap1 protein was purified on the basis of its ability to bind to the same sequence as the mammalian Ap-1 (Jun/Fos) transcription factor *in vitro* and *in vivo*.[53,108] Several laboratories isolated the *YAP1* gene by its ability when present in high copy number to render yeast resistant to a wide variety of toxic agents.[51,61,127] The role of Yap1p in the regulation of enzymes which protect against oxidants was first suggested when *yap1* mutants of *S. cerevisiae* were found to be hypersensitive to oxidants.[127] Mutants deficient in Yap1p have reduced activities of several enzymes with antioxidant activity such as superoxide dismutase, glucose-6-phosphate dehydrogenase, and glutathione reductase.[127] The adaptive response to H_2O_2 was also severely affected by *yap1* mutants, showing that Yap1p affects the transcription of genes involved in mediating this response.[132] However, the *yap1* mutant still retained a small but reproducible H_2O_2-adaptive stress response implicating additional factors in this response. The presence of the *YAP1* gene in high copy number also resulted in modest increases in total glutathione levels and in the activities of the above mentioned antioxidant enzymes.

Putative Yap1p-binding sites have been located in the 5′ promoter sequence of a number of yeast genes encoding antioxidant activities including the *SOD1*, *TWF*, *TRX2*, *GLR1* and *GSH1* genes.[18,51,77,127,152] The expression of both the *GLR1* and *GSH1* genes has been shown to be Yap1-dependent and oxidant-inducible.[47,132,152] Moreover, the H_2O_2 and diamide-mediated increase in expression of the *TRX2*, *GSH1* and *GLR1* genes is also Yap1p-dependent.[47,77,132] It was originally suggested that the binding of Yap1p to the *TRX2* promoter is induced by oxidation of pre-existing protein; however, this now seems not to be the case.[105] It is almost certainly of some significance that, although the basal level of transcription of the *TRX2* gene is severely diminished by a *yap1* mutation, the residual level of *TRX2* transcription is still inducible by H_2O_2, suggesting that additional factor(s) is involved in mediating

TRX2 induction in response to H_2O_2. Indeed, one possible additional factor may be the product of the *SKN7* gene.[79,80,105]

More recently, evidence has appeared that suggests that the nuclear localisation of the Yap1p protein is enhanced in response to diamide stress.[78] In this report, the authors point to a cysteine-rich region of the Yap1p carboxy terminal region as being involved in the stress-induced nuclear localization process. The situation with respect to H_2O_2-mediated oxidative stress appears even more complicated, as some mutants with high nuclear Yap1p levels are still hypersensitive to H_2O_2. Further evidence of the Yap1 protein responding differently to H_2O_2 and diamide stress has been obtained by a number of different laboratories.[134,144] Intriguingly, the same cysteine-rich region identified by Kuge *et al*. as being responsible for the nuclear localization of Yap1p was shown to be important for Yap1p-mediated regulation of a reporter gene.[144] This region of the Yap1 protein consists of three cysteine–glutamate–serine repeats; these workers found that mutations in some of these repeats had different effects with respect to the Yap1 protein's ability to induce transcription in response to H_2O_2 or diamide, or complement the hypersensitivity of a *yap1* null mutant.[144] A region of the Yap1 protein amino-terminal to the cysteine-rich region was also found to be important for the H_2O_2-mediated regulation by the Yap1 protein.[144] Thus, the Yap1 protein appears to have a diamide-responsive region at the carboxy terminus, while H_2O_2-responsiveness is more complex, requiring both the cysteine-rich domain and a region nearer the amino-terminal end of the protein.

The role of the Yap1 protein in regulating oxidant-inducible genes is further complicated by the finding that in some instances its role is indirect and may occur through separate stress response pathways. For example, induction of the *TPS2* (trehalose phosphate phosphatase) gene by heat shock requires the *YAP1* gene product; this induction is mediated through an STRE element and not a Yap1-binding site.[40] However, a more recent study could not demonstrate induction of the *TPS2* gene.[148] Gounalaki and Thireos[40] also suggest that regulation of *TPS2* by Yap1p is modulated via the Ras/cAMP kinase signal transduction pathway, since *TPS2* is expressed constitutively in a *cdc25* (protein kinase A) mutant.

The *YAP2* gene was identified by its ability, when in high copy number, to confer resistance to cadmium and by the hypersensitivity of *yap2* null mutants to oxidants.[7,58,151] The role of the *YAP2* gene in oxidant-dependent gene regulation is still unclear, as no target genes for Yap2p have yet been identified. However, Yap2p clearly plays a role in regulating the H_2O_2-adaptive stress response, since induction of this adaptive stress response was diminished in a *yap2* null mutant.[132] The existence of several b-Zip transcription factors that play a role in the H_2O_2-adaptive stress response raised the possibility that Yap1 and Yap2 could act as dimers similar to mammalian Jun and Fos proteins. However, the phenotypes of the *yap1* and *yap2* mutants, although similar, are not identical and several Yap1p-dependent genes were found to be Yap2p-independent.[132] These findings are inconsistent with the formation of a Yap1p–Yap2p heterodimer.

The Gcn4 protein was originally identified as being responsible for regulating the expression of many amino-acid biosynthetic genes.[57] The binding site for Gcn4p is very similar to that of Yap1p, and recently a role for Gcn4p in controlling, at least partially, the response of yeast towards UV-light has been proposed.[28] The Gcn4 protein has, however, not been directly implicated in regulating gene expression in response to oxidants, although, given that UV light can result in the production of reactive oxygen species, one could envision that it may play some role.

The availability of the complete yeast genome sequence has led to the identification of open reading frames encoding additional bZip transcription factors (Yap3, YHL009c; Yap4 YOR028c; Yap5 YIR018w; Yap6 YDR259c; Yap7 YOL028c; Yap8 YPR199c). An analysis of the possible functions of these transcription factors has been performed.[31] The resulting information (although by no means complete) suggests that all of these transcription factors have distinct physiological roles (possibly involved in stress responses) with considerable overlap between them.

Copper-binding transcription factors

The Ace1 protein is a copper-binding protein possessing a 'fist'-like cysteine-rich domain.[36] Ace1p regulates the expression of some genes encoding activities involved in copper homeostasis, including *CUP1*, which encodes metallothionein, a small cysteine-rich protein found in all eukaryotes.[138,144] while metallothionein is an antioxidant, Ace1p also regulates the expression of *SOD1* and

in vitro footprinting experiments showed that Ace1p can bind to the *SOD1* promoter.[11,44] However, it is evident that transcription factors other than Ace1p are important in determining the level of *SOD1* expression, as there is still significant *SOD1* expression in an *ace1* null mutant.[45] At present, the contribution of Ace1p towards resistance to oxidants is not certain. Another copper-containing transcription factor, Mac1p, was identified as a protein with significant sequence similarity, in both the DNA binding domain and cysteine-rich copper-containing 'copper fist' domains, to the Ace1p and Amt1p transcription factors of *S. cerevisiae* and *Candida glabrata*, respectively.[69] The Mac1 protein was reported to regulate the transcription of genes involved in the reduction of iron and copper ions, as well as to mediate the H_2O_2-induction of the *CTT1* gene.[69] The regulation of *CTT1* transcription by Mac1p appears complex, however, since gain-of-function *MAC1* mutants fail to increase *CTT1* expression despite *mac1* null mutants preventing the H_2O_2-mediated induction of *CTT1* mRNA, and being hypersensitive to H_2O_2. It is possible that the role of Mac1p is indirect, particularly as there is no physical evidence for Mac1p binding to the *CTT1* promoter. The presence of bound copper in the Mac1p and Ace1p proteins suggests that these transcription factors may be redox-active and therefore may play a role in the cell's ability to sense oxidants.

Zinc-finger transcription factors

The Hap1 protein was shown to regulate the expression of both the *CYC1*(iso-1-cytochrome) and *CYC7*(iso-2-cytochrome *c*) genes in response to oxygen and heme.[155] Hap1p has subsequently been found to be involved in the regulation of a variety of genes encoding hemeo-proteins, such as *CTT1* and *CTA1* (the cytosolic and peroxisomal catalases) and components of the mitochondrial respiratory chain, as well as *SOD2* (mitochondrial manganese superoxide dismutase).[46,149] The expression of several enzymes involved in protection against oxidants is therefore regulated by Hap1p, However, *hap1* mutants still possessed an inducible H_2O_2-adaptive stress response;[17] it therefore seems likely that the adaptive oxidative stress responses are not wholly dependent upon heme-containing proteins and that heme is not a sensor of oxidative stress.

In addition to Hap1p, the Hap2, Hap3 and Hap4 proteins are also involved in controlling gene expression in response to heme. They have also been shown to be required for the heme-independent expression of a number of genes[23] while, more recently, these proteins have been implicated in the stationary phase-dependent regulation of the *SOD2* gene.[33] At present, the requirement for these general transcription factors in regulating the expression of other genes involved in antioxidant functions is not known.

The expressions of several yeast genes have been shown to be inducible by a wide variety of stresses, ranging from heat-shock, osmotic stress, DNA damage and hydrogen peroxide.[40,74,98] These genes, including *CTT1*, *DDR2*, *HSP12*, *TPS2*, *GSY2* and *GPH1*, were all found to possess multiple copies of an element called the stress responsive element (STRE). The STRE element has ben most extensively characterized for the *CTT1* gene and contains the sequence AGGGG.[98] In a recent search of the yeast genome, a number of yeast genes which may potentially be regulated by STRE-elements have been identified,[107] although one must be cautious about the exact number of genes so regulated, as it is clear that STRE-mediated regulation is to some extent promoter context-dependent.[148]

Two zinc-finger proteins encoded by the *MSN2* and *MSN4* genes were recently shown to be required for STRE-dependent induction and are STRE-binding proteins. These two genes were initially identified as multicopy suppressors of the invertase defect observed for *snf1* mutants.[29] Strains carrying disruptions of both *MSN2* and *MSN4* showed higher sensitivity to several different stresses, including carbon source starvation, heat shock, and severe osmotic and oxidative stresses. Also, the *MSN2* and *MSN4* genes are required for the STRE-mediated activation of *CTT1*, *DDR2* and *HSP12*.[99,126] The full-length Msn2 protein and the DNA-binding domain of Msn4p have been shown to specifically bind the STRE-element *in vitro*.

Despite the Msn2 and Msn4 proteins regulating expression of (amongst others) the *CTT1* gene, it is evident that mutations in these genes do not abolish H_2O_2-regulation of gene expression.[99] Therefore, there have to be additional transcription factors which recognize the STRE-element and regulate expression in response to H_2O_2. The nature and identity of these proteins is presently unknown. Intriguingly, in common with the Yap1 protein, the cellular localization of the Msn2 protein was found to be regulated;

in response to stress the protein reversibly accumulates in the nucleus.[41]

Additional transcription factors

Several additional yeast transcription factors that are important for the yeast response to oxidative stress. There have been suggestions that oxidants and antioxidants may modulate the activity of the heat-shock factors in eukaryotic cells.The Thiele laboratory has recently shown that *CUP1* induction by the superoxide anion generating compound, menadione, requires the HSE element and Hsf1p.[90] Also, it was observed that the Hsf1 protein was phosphorylated in response to oxidative stress,[90] while heat-induced cell death in *S. cerevisiae* involves oxidative stress.[24] The *SKN7* gene was identified as a high-copy suppressor of a mutation affecting cell wall β-glucan assembly. Regions of the Skn7 protein show similarity to the Hsf1p DNA-binding domain as well as to the prokaryotic receiver units in two-component response regulators.[10] Recently, Krems *et al.* have found that *skn7*(*pos9*) mutants displayed a similar degree of sensitivity to H_2O_2 to strains with mutations in *yap1* and showed reduced H_2O_2 induction of *TRX2*[80] while Morgan *et al.* demonstrated that the Skn7 protein can bind the *TRX2* promoter *in vitro* and may well do so as part of a complex containing Yap1p.[105]

A search for bleomycin-sensitive mutants resulted in the identification of the *IMP2* gene. Mutants deficient in Imp2p are hypersensitive to bleomycin, H_2O_2 and certain metal ions.[100,101] Although the Imp2 protein appears to lack a DNA-binding domain, it can activate transcription when fused to one.[100] The precise role of this protein in mediating oxidant resistance is still unclear, although it may well do so by affecting metal ion homeostasis.[101]

All of the above-mentioned transcription factors act as activators. To date, there is only one example of a transcriptional repressor implicated in the response towards oxidative stress. The *XBP1* gene was identified on the basis of homology with the DNA-binding domain of the Swi4. The Xbp1 protein has been shown to be a transcriptional repressor, whose own expression is regulated by various environmental stresses, including oxidative stress.[98] The 72 kDa Xbp1 protein is a member of the Swi1p/Mbp1p family of transcription factors, containing a conserved helix–loop–helix motif responsible for DNA binding. The *XBP1* promoter contains five STRE-, one HSE- (heat-shock element) and one ARE- (Yap1p binding site) element. It is not certain which of these elements is responsible for the observed regulation of *XBP1*. However, from the expression pattern it seems likely that the regulation is mediated via the STRE-elements. At present, no definite target genes for Xbp1 have been identified, although Mai and Breeden[97] present a list of genes containing putative Xbp1p-binding sites. It is known that in response to oxidative stress the levels of some proteins decreases, although whether this is due to transcriptional repression or increased protein degradation is not known.[67] It therefore seems a likely possibility that the Xbp1 protein is responsible for the observed reduction in protein levels, via repression of gene expression. It will be interesting to identify Xbp1p target genes and to determine why reduction in their expression is important for resistance to stress.

RELATIONSHIP BETWEEN THE OXIDATIVE STRESS RESPONSES AND OTHER ENVIRONMENTAL STRESS RESPONSES

Adaptive stress responses to cold-shock, heat-shock, ethanol exposure and osmotic stress have also been characterized.[19,76,96,141] Are these stress responses all distinct from one another or are they all manifestations of a global general stress response? Much work with bacteria and yeast has shown that many (if not all) of the different stress responses are indeed distinct, although there is significant overlap between many of the responses. In yeast, while it is clear that there are genetically distinct oxidant and other stress responses, the STRE-regulon may well constitute a general stress response, serving to regulate the expression of genes whose products are required for protection against most (if not all) environmental stresses.

Heat-shock response

Perhaps the best characterized adaptive stress response is the heat-shock response.[120] Cross-protection experiments with yeast showed that heat-shock was able to confer protection against stress caused by H_2O_2, superoxide anion and linoleic acid hydroperoxide.[30,66] The mechanism of resistance of heat-shocked cells to oxidants is not known; however, transcription of the *CTT1* gene is elevated by heat-shock, via STRE elements.[147]

Therefore, the combination of increased catalase activity, the heat-shock-protein-encoding genes and other STRE-regulated genes may explain the observed resistance. In support of this idea, yeast lacking Cta1, Ctt1, Cu/ZnSOD, MnSOD and cytochrome *c* peroxidase were found to be less thermotolerant than wild-type cells, while over-expression of these genes conferred a degree of protection towards lethal heat-shock.[24] Furthermore, induction of *CUP1* expression by menadione requires Hsf1p.[90]

Post-diauxic shift and stationary phase

There is some debate about the exact nature and definition of stationary phase, which is beyond the scope of this review. For simplicity, I will discuss the post-diauxic shift point with stationary phase. This should not be taken to mean that a single mechanism operates to regulate gene expression and oxidant resistance at these points in the growth of batch cultures of yeast. When yeast cells growing in batch culture reach late exponential phase or stationary phase they become remarkably resistant to a number of diverse stresses, including high temperatures and oxidants (both H_2O_2 and superoxide anion).[66,125] Is this stationary phase response related to the adaptive stress responses observed at the early exponential phase of growth? Experiments suggested that this is not the case, as the degree of observed resistance in stationary phase is higher than that induced by the exponential phase adaptive stress responses. Also, the fact that stationary phase cells become cross-resistant to a vast array of different stresses suggests a different mechanism.[66] A similar phenomenon has been observed with heat-shock-induced thermotolerance, where it was noted that the degree of resistance during stationary phase was considerably longer-lived than the rather transient nature of that induced by heat-shock during exponential growth.[142]

The resistance of yeast towards oxidants caused by the onset of stationary phase appears similar to that observed when yeast are starved for carbon.[66] Although the precise mechanism of resistance to oxidants generated by these conditions is not understood, several changes in the expression of genes encoding antioxidant activities take place. Many of these enzymes are also important for oxidant resistance during exponential growth. Mitochondrial biosynthesis increases and the transcription and synthesis of the mitochondrial respiratory chain is induced. Transcription of several of the heat-shock-protein-encoding genes, encoding Hsp150, Hsp84, Hsp70, Hsp26, Hsp12 and polyubiquitin, is also increased.[120] In post-diauxic-shift cultures, increased transcription of the *SSA3* gene is regulated by the post-diauxic-shift element (PDS).[6,145] In addition, some STRE-regulated genes respond positively to starvation and approaching stationary phase, including *SOD2* and *CTT1*.[33,146,149] Indeed, the STRE-binding proteins Msn2p and Msn4p have been found to regulate the synthesis of a variety of proteins at the diauxic shift point.[8] Both *yap1* and *yap2* null mutants show modest reductions in H_2O_2 resistance in stationary phase cultures.[132] Stationary phase cultures of glutathione-deficient mutants were also found to be hypersensitive to H_2O_2 and superoxide anion.[133] Moreover, Yap1p has been shown to be important for the stationary phase regulation of the *GLR1* gene.[47] Taken together, these results all point to an important role for glutathione in oxidant resistance in stationary phase.

Respiratory growth

Yeast can grow either fermentatively or by respiration. Respiring yeast cells are intrinsically more resistant to both H_2O_2 and superoxide anions, although the exact reason for this is still not clear.[66] In many respects, the levels of resistance towards oxidants observed in respiring yeast cells appear similar to those seen in stationary phase and starved cells.[66] Many, if not all, genes encoding respiratory functions are subject to glucose repression and are regulated by oxygen/heme availability mediated by the transcription factor Hap1p.[42] For example, the catalase genes are also under Hap1p transcriptional control, as is the *SOD2* gene.[46,119,124] The regulation of these genes by heme availability is perhaps not surprising, given the nature of the protein products and their proposed physiological roles. The catalases are haemoproteins, and MnSod is mitochondrially-located and responsible for countering the toxicity of superoxide anions produced from respiration.[43] Under respiring conditions, expression of *SOD2* and both catalase genes is elevated, as is cytochrome *c* peroxidase and the ubiquitin-encoding gene, *UB14*. Indeed, *ubi4* deletion mutants grown under respiring conditions are hypersensitive to H_2O_2.[14] This evidence suggests that there is an increase in anti-oxidant capacity in cells grown under these conditions.

The role of the mitochondria in the exponential phase adaptive oxidant stress responses has been investigated in yeast strains deficient in mitochondrial function. Strains lacking mitochondrial DNA (Rho^0) and strains with deletions in their mitochondrial DNA (Rho^-), were found to be more sensitive to H_2O_2, superoxide anion and bleomycin.[56,66] However, in both cases the adaptive stress responses were still inducible by these oxidants.[66] Nevertheless, respiratory deficient yeast were found to be hypersensitive to oxidants in stationary phase,[66] suggesting a requirement for some aspect of mitochondrial function for protection against oxidants under these conditions.

Yeast lacking specific components of the mitochondrial electron transport chain or blocked by the addition of inhibitors of electron transport were found to be hypersensitive to H_2O_2 and still capable of inducing an adaptive stress response to this oxidant.[50] To date, no satisfactory explanation for the dependence of oxidant resistance on respiratory function has been made. Grant *et al*. speculate that the H_2O_2 hypersensitivity of rho^- cells may be the result of one or more antioxidant activities having a requirement for energy.[50] Interestingly, rho^- cells were found to be more resistant to linoleic acid hydroperoxide than wild-type cells (although, paradoxically, functional mitochondria are required for adaptation to linoleic acid hydroperoxide).[30] A possible explanation for this apparent inconsistency is that rho^- cells are experiencing stress and are subject to lipid peroxidation and, as a result, induce an adaptive response to linoleic acid hydroperoxide.

SIGNAL TRANSDUCTION PATHWAYS

In contrast to the situation in fission yeast, the role of signal transduction pathways in the regulation of the response of *S. cerevisiae* towards oxidants is not well understood. The Ras signal transduction pathway (responsible for regulating intracellular cAMP levels) is clearly important. Many stress-inducible genes, including some that are regulated by oxidants, are responsive to cAMP levels. This regulatory response is mediated via both STRE and PDS elements; genes regulated in this manner include the *CTT1*, *SSA3*, *HSP26* and *HSP12* genes.[98,121,145] These genes are activated by decreased levels of intracellular cAMP. Stresses which lead to decreased intracellular cAMP levels include heat-shock, carbon and nitrogen starvation, and entry into stationary phase. Tolerance of *S. cerevisiae* towards freeze-thawing is also regulated by cAMP levels.[117] *cyr1* Mutants, which posses slow levels of cAMP due to a defective adenylate cyclase, are resistant to a large variety of stress conditions, including nutrient starvation, heat-shock and oxidants.[28,62,128] Moreover, a number of Msn2p/Msn4p-regulated proteins which are up-regulated following diauxic shift, respond to a reduction in cAMP levels.[8] The mechanism by which cAMP and protein kinase A regulates the activities of Yap1p, Msn2p and Msn4p is still not understood. For instance, does protein kinase A directly phosphorylate these proteins or is its role more indirect? In response to menadione exposure, Hsf1p is phosphorylated at several different sites, with phosphorylation of Hsf1p correlating with Hsf-mediated induction of *CUP1* expression.[90] Interestingly, the pattern of phosphorylation is different from that induced by heat-shock, suggesting that different kinases are involved. The identity of the kinase(s) and signal transduction pathway is not known.

CONCLUDING REMARKS

Our knowledge of the oxidative stress responses is increasingly rapidly and, in the next few years, major new insights into the regulatory networks and signal transduction pathways regulating and coordinating the stress regulons in *S. cerevisiae* will be made. Also, the identification and characterization of the regulatory proteins should allow investigators to address the question of the nature of the inducing signal(s).

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