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Original Contribution

Adaptation to hydrogen peroxide in *Saccharomyces cerevisiae*: The role of NADPH-generating systems and the SKN7 transcription factor

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Abstract

A total of 286 H_2O_2-sensitive *Saccharomyces cerevisiae* deletion mutants were screened to identify genes involved in cellular adaptation to H_2O_2 stress. *YAP1*, *SKN7*, *GAL11*, *RPE1*, *TKL1*, *IDP1*, *SLA1*, and *PET8* were important for adaptation to H_2O_2. The mutants were divisible into two groups based on their responses to a brief acute dose of H_2O_2 and to chronic exposure to H_2O_2. Transcription factors Yap1p, Skn7p, and Gal11p were important for both acute and chronic responses to H_2O_2. Yap1p and Skn7p were acting in concert for adaptation, which indicates that upregulation of antioxidant functions rather than generation of NADPH or glutathione is important for adaptation. Deletion of *GPX3* and *YBP1* involved in sensing H_2O_2 and activating Yap1p affected adaptation but to a lesser extent than *YAP1* deletion. NADPH generation was also required for adaptation. *RPE1, TKL1,* or *IDP1* deletants affected in NADPH production were chronically sensitive to H_2O_2 but resistant to an acute dose, and other mutants affected in NADPH generation tested were similarly affected in adaptation. These mutants overproduced reduced glutathione (GSH) but maintained normal cellular redox homeostasis. This overproduction of GSH was not regulated at transcription of the gene encoding γ-glutamylcysteine synthetase.
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Introduction

Aerobic organisms use oxygen for cellular respiration as a primary means to break down complex organic molecules for generation of energy. In the mitochondrion, most oxygen molecules are reduced to water, but about 1–5% undergoes incomplete reduction, forming reactive oxygen species (ROS), such as the hydroxyl radical ($OH^{\bullet}$), superoxide anion ($O_2^{\bullet -}$), and hydrogen peroxide (H_2O_2). These ROS induce DNA damage, protein oxidation, and lipid peroxidation. Organisms have therefore evolved various antioxidant defense systems to counter these detrimental effects. Oxidative stress arises when the balance and metabolism of these ROS are perturbed [1,2]. Oxidative stress has been of major interest in medical and pharmaceutical fields because it has been implicated in the pathogenesis of cancer, chronic gut inflammation, cardiovascular disease, arthritis, and aging [3,4].

Cells pretreated with a low sublethal dose of an oxidant such as H_2O_2, rapidly mount a transient protective response to a subsequent dose that would otherwise be lethal [5–7]. This adaptive response to oxidative stress occurs in prokaryotes, including *Escherichia coli* and *Salmonella typhimurium* [8], and eukaryotes, such as *Saccharomyces cerevisiae* [5–7] and mammals [9]. In microbial systems, complete adaptive responses require gene activation and de novo protein synthesis, but adaptation is not necessarily the overall transcriptional response of an organism to treatment with a ROS. In some cases, pretreatment with one oxidant results in cross-protection

Abbreviations: GSH, reduced glutathione; GSSG, oxidized glutathione; NADPH, reduced nicotinamide adenine dinucleotide phosphate; ROS, reactive oxygen species; ORFs, open ready frames.

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to another type of oxidant, but this response is not always reciprocal, indicating the existence of hierarchical network in this response [10].

The *E. coli* adaptive response to H_2O_2 is regulated by the OxyR transcription factor. OxyR exists either in an oxidized disulfide form or in a reduced dithiol form. Upon activation, H_2O_2 is sensed via direct oxidation of OxyR activating the transcription factor for production of antioxidant functions, such as *katG* (hydroperoxidase), *gorA* (glutathione reductase), and *grxA* (glutaredoxin). When redox balance is restored the oxidized OxyR is reduced by enzymatic reaction with glutaredoxin 1 (Grx1) in an autoregulated manner [11].

S. cerevisiae is an ideal eukaryotic model for study of the adaptive response to oxidative stress induced by H_2O_2 because it is genetically manipulable, its antioxidant defence systems are well characterized, the genome sequence of yeast is accessible [12], and availability of single-gene deletion mutants for all nonessential genes greatly facilitates the study of gene functions [13]. The complete adaptive response of *S. cerevisiae* cells to H_2O_2 involves de novo synthesis of proteins because cycloheximide abolishes some of the response [6]. Proteomic analyses have shown that many proteins are induced or repressed in response to H_2O_2 [14–16] although these studies have been done mainly at higher doses than those leading to maximal adaptation [5,6]. Those proteins that do change play roles in cellular antioxidant defence, heat shock, carbohydrate metabolism, translation, and protein degradation [14]. The oxidative-stress-induced transcription factor Yap1p is crucial for the maximal adaptive response to H_2O_2 stress [15]. However, the adaptive response is not completely abolished in a *yap1* mutant, indicating the involvement of other factors in adaptation.

Yap1p plays a critical role in the cellular responses to a range of oxidants and xenobiotics [18–20]. It is activated by H_2O_2 by disulfide bond formation [21] mediated via an H_2O_2 receptor, which is a glutathione peroxidase homologue (Gpx3/Orp1) with thioredoxin-dependent peroxidase activity [22]. Oxidized Yap1p is translocated from the cytosol to the nucleus, and the Gpx3p-mediated recruitment is dependent on the Yap1p-binding protein Ybp1p [23,24]. The transcriptional activity of Yap1p has been proposed to be determined by a balance between the oxidation to form disulfides in the transcription factor and their reduction by thioredoxin [25]. The main regulation of Yap1p results from altered export from the nucleus [26,27]; disulfide-induced structural changes in the nuclear export signal located in a cysteine-rich C-terminal domain inhibit binding to the nuclear export receptor Crm1p [28,29]. Recently, it has been shown in vitro that oxidation of the cysteines is a multistep process leading to formation of interdomain disulfides [29]. These lead to a form of Yap1p that is relatively resistant to reduction by thioredoxin. The authors propose that this provides a mechanism to extend the level and duration of transcription in response to H_2O_2 stress. There appear to be additional factors involved in activation of some promoters by Yap1p because transcription of *TRX2* encoding thioredoxin 2 depends on formation of interdomain disulfides in Yap1p and recruitment of the Rox3p mediator to the promoter [30]. On the other hand, expression of *GSH1*, encoding γ-glutamylcysteine synthetase, the key enzyme in glutathione synthesis, appears to depend mainly on nuclear accumulation of Yap1p and can be supported by a mutant form of Yap1p that does not activate *TRX2* [30].

Genome-wide screening identified 286 mutants sensitive to H_2O_2 [31]. Because similar screening to identify genes involved in the adaptive response to H_2O_2 stress would be difficult even with suitable robotics, we screened the 286 H_2O_2-sensitive mutants for those impaired in adaptation to H_2O_2 using a simple, semiquantitative spot-test method. Subsequent detailed analysis identified eight genes including three encoding transcription factors and three encoding proteins involved in production of NADPH. This prompted further testing of functions associated with the activation of Yap1p and NADPH generation in the cell.

Experimental procedures

Strains, plasmids, and media

The set of single gene deletion strains constructed in the homozygous diploid BY4743 (*MAT***a**/*MATα his3Δ1/his3Δ1 leu2Δ0/leu2Δ0 met15Δ0/MET15 LYS2/lys2Δ0 ura3Δ0/ura3Δ0*) was obtained from EUROSCARF http://www.uni-frankfurt.de/fb15/mikro/euroscarf. Plasmid pRS416 (Invitrogen, Carlsbad, CA, USA) was used to generate complementing plasmids. Centromeric plasmids pyDJ73 and pSC99, containing *GSH1::lacZ* and *TRX2::lacZ*, were, respectively, provided by Derek Jamieson and Scott Moye-Rowley. Yeast strains were grown in YEPD medium containing 1% yeast extract, 2% (w/v) peptone, and 2% (w/v) glucose or synthetic medium (SD) containing 0.17% yeast nitrogen base without amino acids and ammonium sulfate, 0.5% ammonium sulfate, 2% (w/v) D-glucose, and auxotrophic supplements. H_2O_2 agar plates were prepared 1 day before use by adding H_2O_2 to the desired concentration (0.25, 0.5, 0.75, 1.0, 1.25, 1.5, and 1.75 mM) to sterile synthetic medium agar at 55 °C. Normal synthetic medium agar plates were prepared as controls.

Systematic screening for mutants defective in adaptation to H_2O_2-induced stress on agar plates

H_2O_2-sensitive mutants identified previously [16] were screened for their ability to adapt to H_2O_2-induced oxidative stress by a spot-test method. Cells were grown to early stationary phase in synthetic medium in 96-well plates, reinoculated into fresh synthetic medium, and grown to exponential phase. Because the strains had different growth rates, the mutants were tested in batches of strains showing similar growth rates. At OD_{600} of ~0.5, they were treated with sublethal concentrations of H_2O_2 (0.05–0.75 mM) at 30 °C for 1 h in SD medium and spotted on SD medium agar containing various concentrations of H_2O_2 (0–1.75 mM) using a sterile 96-pin replicator, and the plates were incubated at 30 °C for 2 days. The adaptative phenotype of each mutant was scored by comparing growth of colonies obtained from the untreated culture with that from the treated culture with the wild-type used as a control. The *yap1* deletant was used as a negative control. Mutants with an

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