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Adaptation to oxidative stress induced by polyunsaturated fatty acids in yeast

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ABSTRACT

To create a conditional system for molecular analysis of effects of polyunsaturated fatty acids (PUFA) on cellular physiology, we have constructed a strain of yeast (*Saccharomyces cerevisiae*) that functionally expresses, under defined conditions, the Δ12 desaturase gene from the tropical rubber tree, *Hevea brasiliensis*. This strain produces up to 15% PUFA, exclusively under inducing conditions resulting in production of 4-hydroxy-2-nonenal, one of the major end products of *n*−6 polyunsaturated fatty acid peroxidation. The PUFA-producing yeast was initially more sensitive to oxidative stress than the wild-type strain. However, over extended time of cultivation it became more resistant to hydrogen peroxide indicating adaptation to endogenous oxidative stress caused by the presence of PUFA. Indeed, PUFA-producing strain showed an increased concentration of endogenous ROS, while initially increased hydrogen peroxide sensitivity was followed by an increase in catalase activity and adaptation to oxidative stress. The deletion mutants constructed to be defective in the catalase activity lost the ability to adapt to oxidative stress. These data demonstrate that the cellular synthesis of PUFA induces endogenous oxidative stress which is overcome by cellular adaptation based on the catalase activity.

1. Introduction

Appearance of oxygen in the atmosphere leads to the evolution of highly efficient cellular energy generation systems, but also introduced the major problem of oxygen toxicity. Molecular oxygen is highly reactive and its partial reduction generates numerous chemically active agents, termed reactive oxygen species (ROS). Although most of the ROS are radical species like hydroxyl and superoxide radical, there are also non-radical species like hydrogen peroxide. ROS are highly damaging to all biological molecules, including DNA, proteins [1–4] and lipids [1,5]. Polyunsaturated fatty acids that are esterified in membrane or storage lipids are subject to ROS-induced peroxidation and may yield cytotoxic aldehydes, like 4-hydroxy-2-nonenal (HNE), malondialdehyde (MDA) and acrolein [[1]. The membrane damaging properties of peroxidized fatty acids and their reactive byproducts are believed to be associated with numerous chronic and acute diseases like cancer, cardiovascular diseases, neurodegenerative disorders, Down's syndrome, as well as with ageing [6–9].

Under normal physiological conditions, ROS are produced in low amounts as a result of active aerobic metabolism. Main source of ROS is leakage of electrons from the respiratory chain and by microsomal metabolism, giving rise to superoxide anion [10], which may be catabolized by superoxide dismutase to hydrogen peroxide (H_2O_2), which can be converted to the highly reactive and cell damaging hydroxyl radical via the metal-ion catalyzed Fenton reaction [11]. Therefore, cells have evolved efficient enzymatic and non-enzymatic defense mechanisms to deal with potential pro-oxidants and oxidative damage [12–14]. Non-enzymatic mechanisms involve small hydrophobic or hydrophilic molecules that act as radical scavengers, such as glutathione [14–16]. Enzymatic defense systems are more complex and may include cascades of reactions leading to complete detoxification of certain ROS and/or their byproducts. For instance, superoxide dismutase (SOD) disproportionates superoxide radicals to oxygen and hydrogen peroxide, which is further catabolized by catalase to water and molecular oxygen [14]. These mechanisms provide a certain degree of constitutive resistance to oxidative stress, however, it was also demonstrated that ROS may cause distinct adaptive responses. In addition, ROS may induce cross-adaptation, whereby pre-treatment of cells with one ROS increases resistance to another [15].

Saccharomyces cerevisiae is a well-defined unicellular and facultative aerob eukaryotic organism and an excellent model for studying molecular mechanisms of oxidative stress responses. Yeast does not synthesize polyunsaturated fatty acids (PUFAs) due to the lack of enzymes capable of introducing more than a single double bond into its fatty acids [16]. Thus, fatty acids of the yeast cells are more resistant to oxidative attack than those of mammalian cells. However, yeast is well capable of taking up and incorporating exogenous saturated and (poly)unsaturated fatty acids, which normally do not occur in its lipids [17,18], and they are highly sensitive to exogenous long-chain PUFA peroxides [19].

To investigate in a conditional system the effects of PUFA (peroxidation) on cellular physiology, we have constructed a yeast strain that functionally expresses a Δ12 desaturase gene from the tropical

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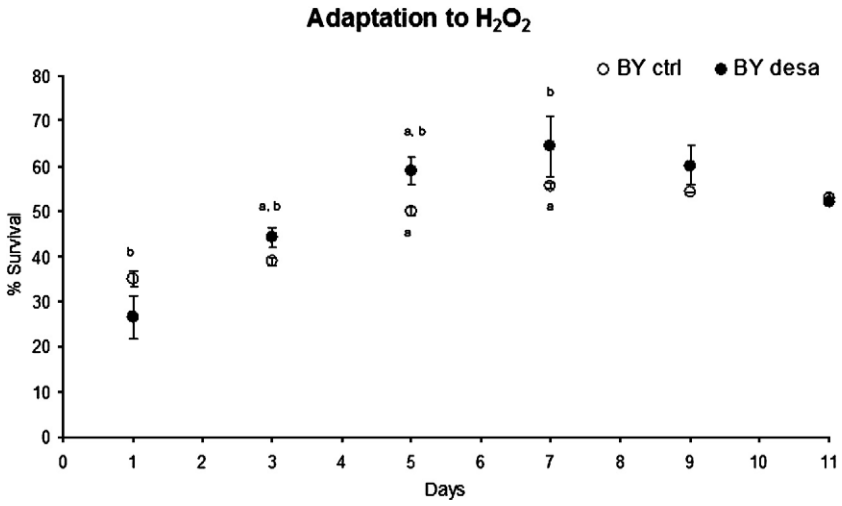

Fig. 1. Continuous desaturase expression leads to adaptation to oxidative stress. Wild-type (BYctrl; black circles) and Δ12 desaturase-expressing (BYdesa; open circles) strains were grown in standard defined galactose medium by re-inoculation into fresh medium after every 24 h for 11 consecutive days. Aliquots were taken out and cells were incubated with 5 mM hydrogen peroxide for 4 h. After treatment, cells were diluted and plated in triplicate onto YEPD medium to monitor cell viability. Percentage survival is expressed relative to the untreated controls. [a]Significantly different if compared to the growth of the same strain on the previous day ($p<0.05$). [b]Significantly different if compared to the wild type at the same time point ($p<0.05$).

rubber tree, *Hevea brasiliensis*. Only under inducing conditions, this strain produces up to 15% PUFA, mostly 9Z,12Z-C18:2 (linoleic acid) but also 9Z,12Z-C16:2, defining the enzyme as a Δ12 desaturase [20]. In the transgenic strain, the presence of these polyunsaturated fatty acids significantly increases the sensitivity against oxidative stress, induced by the addition of hydrogen peroxide. Oxidative stress and loss of viability of the strain are accompanied by formation of 4-hydroxy-2-nonenal, one of the end products of n−6 polyunsaturated (e.g. linoleic) fatty acid peroxidation [20]. Although the spectrum of lipid peroxidation products generated in this yeast strain does not resemble the major lipid peroxidation products in mammalian cells under similar oxidative stress conditions, the yeast system provides powerful genetic and biochemical means to track the deleterious effects of lipid peroxidation as well as the cellular antioxidant defense mechanisms [19].

While short-term expression of the desaturase rendered the cells more sensitive to H_2O_2 stress, extended expression and PUFA production resulted in development of increased resistance to this pro-oxidant. This observation indicated some level of adaptation to endogenous oxidative stress caused by the presence of PUFA. Indeed, induced PUFA production increased levels of endogenous ROS, and was correlated with an increase in catalase activity that is required for hydrogen peroxide detoxification. Deletion mutants defective in peroxisomal catalase lost the ability to adapt to oxidative stress. These data demonstrate the importance of catalase in the process of adaptation to endogenous oxidative stress caused by the presence of PUFA in yeast.

2. Materials and methods

2.1. Yeast strains and cultivation conditions

The *S. cerevisiae* strains used in this study were wild-type BY4742 (*MATα his3Δ1 leu2Δ1 lys2Δ0 ura3Δ0*; Euroscarf), a catalase A deficient strain, cta1 (*MAT***a** *his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 YDR256c::KanMX4*; Euroscarf) and a catalase T deficient strain, ctt1 (*MAT***a** *his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 YGR088w::KanMX4*; Euroscarf). Cells were grown either in rich YEPD media (2% w/v glucose, 2% w/v Bacto peptone, 1% w/v yeast extract) or in minimal synthetic-defined (SD) media (1% w/v glucose or 1% w/v galactose, 0.17% Difco Yeast Nitrogen Base) supplemented with appropriate amino acids and bases: 0.004% adenine, 0.002% arginine, 0.002% histidine, 0.01% leucine, 0.003% lysine, 0.002% methionine, 0.01% threonine, and 0.002% tryptophan. Media were solidified by the addition of 2% (w/v) agar.

2.2. Transformation of wild-type and deletion mutant strains

The cDNA encoding fatty acid Δ12 desaturase from *H. brasiliensis* was cloned into the yeast episomal expression vector pYES2 (Invitrogen), containing the *Gal1/10* promoter and a *URA3* selection marker, as described previously [20]. Plasmids pYES2 and pYES2-desa1[21] were transformed into wild-type strain BY4742 and cta1 and ctt1 mutant strains by the lithium acetate method [21], and transformants were selected on media plates lacking uracil. Transformed strains were referred to as BYctrl, Ctactrl and Cttctrl for control strains harbouring the pYES2 plasmid without insert, and BYdesa, Ctadesa and Cttdesa for strains harbouring expression plasmid, pYES2-desa1 encoding the Δ12 fatty acid desaturase [20].

2.3. Adaptation and stress resistance analysis of PUFA-producing yeast strains

Wild-type and cta1 and ctt1 mutants transformed with pYES2 (empty plasmid) or pYES2-desa1 (Δ12 desaturase) were pre-grown overnight in SD medium lacking uracil and containing 1% glucose, washed and inoculated into SD medium without uracil and with 1% galactose, for induction of desaturase expression. To maintain a high level of desaturase expression cells were re-inoculated to an OD_{600}=0.1 every 24 h for 11 consecutive days, in the absence of stressor. Cell growth was monitored by OD_{600} measurement. Sensitivity of cells against hydrogen peroxide was assessed after harvesting cells at the indicated time points and dilution of the cell suspension to OD_{600}=1.0. Cells were treated with 5 mM H_2O_2 or 2 M NaCl for 4 h, aliquots diluted and plated in triplicate on YEPD plates and incubated at 30 °C. Viable cell counts (colony forming units) were scored after 3 days of growth.

2.4. Catalase activity analysis

Cells were grown in SD media without uracil, and desaturase expression was induced for 72 h in the presence of 1% galactose. Aliquots of the BYctrl and BYdesa cell cultures were collected and stored at −80 °C until further analysis. Cells were lysed mechanically with glass beads in phosphate buffer (60 mM K-PO_4, pH 7.4) and cell lysates cleared by centrifugation at 13,400 rpm for 15 min. Catalase activity was measured in 25 μl of the supernatant in phosphate buffer (60 mM, pH 7.4), after addition of 100 mM H_2O_2 as the substrate. The reaction was stopped by addition of 100 mM ammonium molybdate, and color development was measured spectroscopically in a plate reader at 450 nm [22]. Protein concentration was measured according to Lowry et al. [23], using bovine serum albumin as a standard.

2.5. Measurement of ROS production

Cellular ROS production was examined by using a nonfluorescent probe for intracellular ROS detection 2',7'-dichlorofluorescin diacetate (DCFH-DA, Fluka). This cell-permeable dye is widely used in both mammalian [24,25] and yeast cells [26,27] for sensitive and rapid quantitation of ROS in response to oxidative stress. Namely, DCFH-DA probe remains nonfluorescent inside the cell until the acetate groups are removed by intracellular esterases and oxidized by intracellular ROS to the fluorescent compound 2',7'-dichlorofluorescein (DCF) which can be detected as a measure for intracellular ROS [28]. After preincubation of yeast cells at an OD_{600}=1.0 in 1 ml phosphate-buffered saline (PBS) with 100 μM DCFH-DA at 28 °C for 60 min, the cell suspensions were treated with 5 mM H_2O_2 for 1 h and then washed and resuspended in 1 ml PBS. Fluorescence intensity was read with a Cary Eclipse Fluorescence Spectrophotometer (Varian) with excitation at 500 nm and emission detection at 530 nm.