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SHORT COMMUNICATION

Genes Controlling 2-deoxyglucose Induced Lysis and Formation of Reactive Oxygen Species in *Schizosaccharomyces pombe*

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Abstract

Schizosaccharomyces pombe cells of strains each carrying a deletion of one of the genes *snf5*, *ypa1*, *pho7* and *pas1* and of a strain overexpressing gene *odr1*, have been previously shown to grow in presence of the toxic glucose analogue 2-deoxyglucose (2-DG). Here we report that these genes control 2-DG induced lysis and are, with the exception of *odr1*, also involved in control of formation of reactive oxygen species (ROS) upon exposure of cells to H_2O_2. Lysis of deletion strains, but not of strain overexpressing *odr1*, is dependent on glucose concentration of the medium whereas ROS formation is glucose independent.

Key words: 2-deoxyglucose, fission yeast, glucose signaling, lysis, ROS formation

Reactive oxygen species (ROS) are harmful by-products of basic cellular metabolism in aerobic organisms and are mainly known to be formed under oxidative stress (Apel and Hirt, 2004). They act as signalling molecules and are involved in many different biological processes in various organisms including mammalian cells and yeast (Apel and Hirt, 2004; Ikner and Shiozaki, 2005; Herrero *et al*., 2008; Ray *et al*., 2012; de la Torre-Ruiz *et al*., 2015). One of the agents which induces oxidative stress and ROS formation in higher cells is 2-deoxyglucose (2-DG). It is a glucose analogue, which, in yeast and mammalian cells is phosphorylated to toxic 2-DG-6-phosphate, which in turn interferes with many processes including glycolysis, protein glycosylation, cell wall synthesis, growth and others (Brown, 1962; Farkas *et al*., 1969; Biely *et al*., 1971; Krátký *et al*., 1975; O'Donnell *et al*., 2015). Knowledge of mode of action of 2-DG is still poorly understood but is, however, of special interest because it exhibits anticancer activity (Pelicano *et al*., 2006).

Budding yeast *Saccharomyces cerevisiae* and fission yeast *Schizosaccharomyces pombe* are widely used model systems for eukaryotic cells with fission yeast in many aspects closer related to higher organisms than *S. cerevisiae* (Hoffman *et al*., 2015). 2-DG has several effects on yeast cells including inhibition of growth and induction of lysis (Johnson, 1968). To identify genes involved in the control of 2-DG action in fission yeast, we recently identified, by screening a haploid deletion library, four genes (*snf5*, *ypa1*, *pho7*, and *pas1*) which when deleted, grow on plates containing the toxic 2-DG. By transforming cells with a wild type gene library we identified, in addition, a gene *odr1* which when overexpressed also exhibits similar resistance to 2-DG as the deletion strains (Vishwanatha *et al*., 2016).

In this study we show that these genes are involved in the control of 2-DG induced cell lysis, and in the control of ROS formation induced by the oxidative stress inducing agent H_20_2.

In a first series of experiments we grew cells of the 2-DG resistant strains and the control strains given in the Table I in liquid minimal medium (MM) in the

Table I
List of 2-DG resistant and control strains.

Strain name	Genotype	Plasmid
wild type	*972 h⁻*	
pREP4X	*ura4D18 h⁻*	*pRep4X*
pODR1	*ura4 D18 h⁻*	*pODR1*
parent	*ade6 M210 ura4 D18 leu1 h⁺*	
snf5Δ	*ade6 M210 ura4 D18 leu1 snf5::KanMX h⁺*	
ypa1Δ	*ade6 M210 ura4 D18 leu1 ypa1::KanMX h⁺*	
pho7Δ	*ade6 M210 ura4 D18 leu1 pho7::KanMX h⁺*	
pas1Δ	*ade6 M210 ura4 D18 leu1 pas1::KanMX h⁺*	

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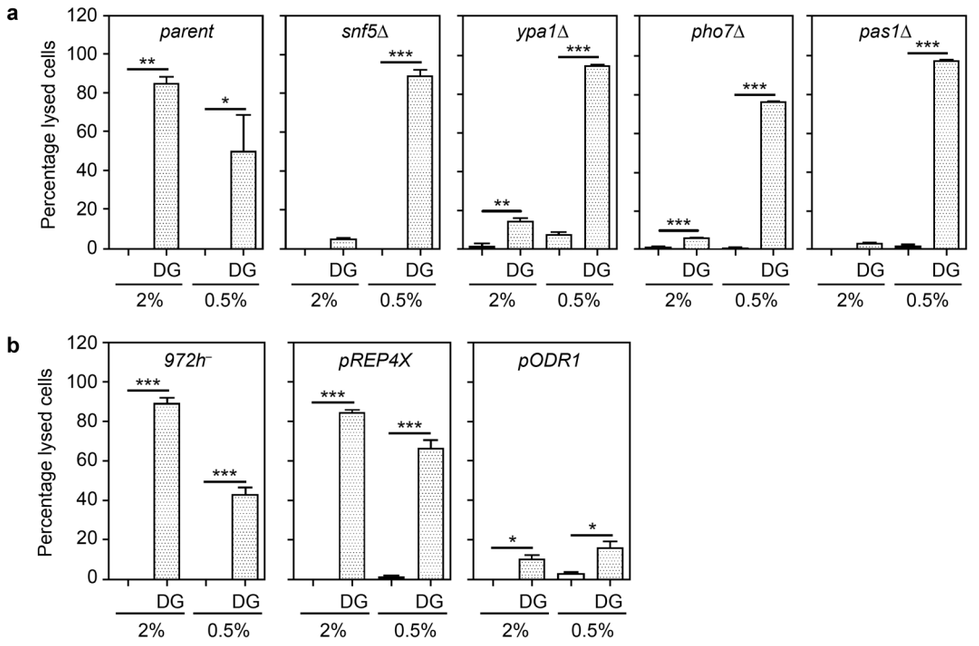

Fig. 1. Microscopic examination and quantification of lysis of 2-DG resistant strains.

Cells of the deletion strains *snf5*Δ, *ypa1*Δ, *pho7*Δ and *pas1*Δ (a) and the *odr1* overexpressing strain *pODR1* (b) were pregrown in MM (Vishwanatha *et al*., 2016) containing the supplements leucine, adenine and uracil (50 µg/ml) to log phase (OD_{600} 0.5–0.8), pelleted and resuspended in the same medium containing 2% and 0.5% glucose with or without 2-DG (0.25 mg/ml) (abbreviated as DG in figure) (≥ 99% pure, Sigma – Aldrich, USA) and grown for 4 hrs at 30°. Cells were concentrated and stained with Evans Blue. Cell lysis was examined under a bright field microscope with cells immobilized on concanavalinA (conA) coated coverslips. The cell number was counted and the proportion of lysed cells was expressed as percentage of the total cells counted. Statistical analyses were performed using GraphPad Prism 5® (GraphPad Software Inc., La Jolla, CA, USA) and statistical significance was determined either using one-way ANOVA, followed by Bonferroni post hoc test or by paired t test as applicable. Significance was accepted at $P < 0.05$ (*), $P < 0.01$ (**), and $P < 0.001$ (***). The pairs of data sets analysed for significance are marked by lines. The mean ± SEM is plotted. Asterisks represents the degree of significance of the differences between pairs of data sets analysed.

presence and absence of 2-DG and examined the cells microscopically.

Knowing that glucose can modify effects of 2-DG (McCartney *et al*., 2014; Vishwanatha *et al*., 2016), we tested cells at high (2%) and low (0.5%) glucose concentrations. As shown in Fig. 1 cells of the control strains lyse in the presence of 2-DG as reported previously (Megnet, 1965; Johnson, 1968). Lysis is more efficient in cells grown in a medium containing high glucose than cells grown in the presence of low glucose. Cells of the four deletion strains lyse in the presence of 2-DG when grown at the low glucose concentration but not so at the high concentration. This indicates that the four genes are involved in the control of 2-DG induced lysis and that this control is glucose dependent. Independent of the glucose concentration, cells of the strain containing the overexpressed gene *odr1* are not lysed by 2-DG.

Oxidative stress in *S. pombe* is greatly affected by glucose and its signalling/sensing pathways (Palabiyik *et al*., 2012; 2013). Glucose starvation induces oxidative stress, activating a stress induced mitogen activated protein (MAP) kinase pathway resulting in an increased expression of Atf1-dependent stress response genes (Madrid *et al*., 2004; 2006; 2013; Kato *et al*., 2013). Glucose signalling mediated by PKA is also affected by glucose starvation (Gupta *et al*., 2011). Also, a 2-DG resistant mutant, *ird11* was reported to be defective in oxidative stress response (Suslu *et al*., 2011; Palabiyik *et al*., 2012). Knowing this and the fact that the 2-DG resistant mutants we identified are defective in glucose signalling (Vishwanatha *et al*., 2016), we examined whether cells of the deletion strains are also altered in ROS formation upon oxidative stress. As shown in many studies ROS formation can be visualized by staining cells with 2', 7' Dichlorofluorescein diacetate (DCFDA) which is converted in the cells to the fluorescent 2', 7' Dichlorofluorescein (DCF) (Wu and Yotnda, 2011). Since the control cells of the parent strain are lysing in the presence of 2-DG we could not directly test the effect of 2-DG on ROS formation. For this reason we cultivated cells in the presence of non-lethal concentration and absence of H_2O_2 which is well known to induce oxidative stress (Apel and Hirt, 2004; Roux *et al*., 2009). As shown in Fig. 2 H_2O_2 induces ROS formation in the wild type (*972 h*$^-$) and the control strains (pREP4X and parent). Its production is more prominent in cells grown at a low rather than at a high glucose concentration. In the deletion strains ROS formation

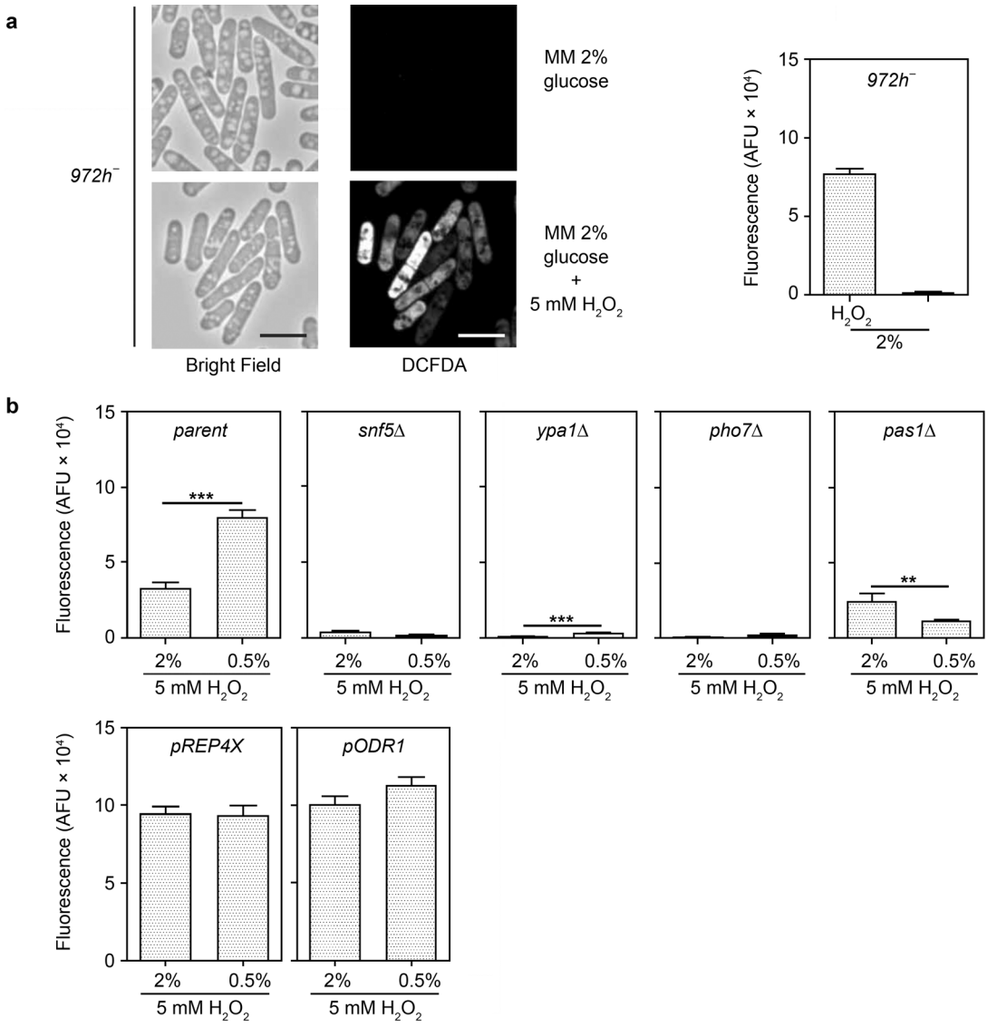

Fig. 2. Induction of ROS formation by H_2O_2 in 2-DG resistant *S. pombe* strains.

Cells were cultured in MM as descibed in Fig. 1 in the presence and absence of 5 mM H_20_2 (SD Fine Chemicals) for 4 hrs at 30°, pelleted, resuspended in 200 µl of Fluorobrite™ DMEM (Life Technologies, USA) containing 25 µM DCFDA (Sigma Aldrich, USA), immobilized on con A coated coverslips and examined microscopically for ROS formation. (a) A representative 5 second fluorescent exposure image after background correction along with bright field image of wild type *972h⁻* grown in the presence and absence of H_2O_2. Scale bars represent 10 µm. Corresponding quantification of fluoresence from the wild type is plotted as bar graph adjacent to the image. It was achieved by measuring fluoressence from at least 120 cells using Image J. Mean of integrated density values obtained after estimation was plotted as arbitrary fluorescence units (AFU). Statistical significance was determined as in Fig. 1 and the values are plotted as mean ± SEM. (b) Quantitated fluoresence achieved as given for (a) for the deletion strains and strain pODR1 along with control strains.

is drastically reduced both in cells grown at a high and a low glucose concentration indicating that the four genes *snf5*, *ypa1*, *pho7* and *pas1* are also responsible for ROS formation upon oxidative stress. Cells of strain pODR1 are unable to quench ROS formation. This may indicate that gene *odr1* is not involved in the control of ROS formation and may possibly have a function leading to the detoxification of 2-DG.

With these experiments we show that the genes *snf5*, *ypa1*, *pho7* and *pas1* are involved in 2-DG induced lysis and ROS formation upon oxidative stress. To our knowledge these functions of the genes have not yet been reported, either for yeast or for any other organism. Even though some biochemical functions of their gene products are known (Vishwanatha *et al*., 2016) we have no straightforward explanation how they control lysis and ROS formation. However these findings are of interest since 2-DG is known to induce oxidative stress in cancer cells (Coleman *et al*., 2008). Together with our previous result (Vishwanatha *et al*., 2016), they suggest interplay between oxidative stress controlling mechanisms and glucose signalling. For the genes *snf5*, *ypa1* and *pas1* human orthologues are known (van Slegtenhorst *et al*., 2005; Monahan *et al*., 2008; Goyal and Simanis, 2012; Vishwanatha *et al*., 2016). It remains to be seen whether deleting these orthologues

also inhibit ROS formation in cancer cells. The finding that gene *odr1* is preventing 2-DG induced lysis but does not prevent ROS formation is in accordance with our previous speculation that gene *odr1* might be 2-DG specific and may have a similar function as *S. cerevisiae* Dog1, which acts as a 2-DG-6 phosphate phosphatase (Randez-Gil *et al*., 1995; Vishwanatha *et al*., 2016).

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