

Role of Oxidative Stress Response and Trehalose Accumulation in the Longevity of Fission Yeast

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Background: Glucose is the preferred carbon and energy source in most organisms and plays an active role in the regulation of many biological processes. However, an excess of glucose leads to such undesirable conditions as diabetes and age-related diseases. Since *Schizosaccharomyces pombe* homologous of many human genes, it offers several advantages for the investigation of the molecular mechanisms underlying human disease and aging studies. We have identified two glucose-repression-resistant mutants (*ird5* and *ird11*) of *S. pombe*.

Objectives: We aimed to investigate the possible relationship between lifespan extension and oxidative stress response induced by exposure to hydrogen peroxide alongside the trehalose accumulation level by using the two *S. pombe* mutants (i.e. *ird5* and *ird11*), which are repressed by glucose and are resistant to oxidative stress.

Materials and Methods: We employed trehalose accumulation measurement and colony-forming unit (CFU) counting using the *ird* mutants in exponential and stationary phases and compared them to the wild type grown in repressed, de-repressed, and stressed conditions to clarify the possible relationship between glucose signaling, oxidative stress response, and lifespan in *S. pombe*.

Results: The lifespan of the *ird5* mutant was significantly longer than that of either the *ird11* mutant or the wild type cells. Under repressed condition, the trehalose content was increased remarkably on the 3rd day of the study in the *ird11* mutant and the wild type. Under de-repressed condition, the level of intracellular trehalose was notably increased on the 3rd day in *ird11*. Under stressed condition, the trehalose level in *ird11* was increased on the 3rd day as a pattern similar to that observed in the wild type.

Conclusions: Our results demonstrated no significant correlation between the *ird5* lifespan and the trehalose concentration. Likewise, the correlation between lifespan extension, trehalose accumulation, and cellular resistance to hydrogen peroxide was not significant.

Keywords: Trehalose; Glucose; Repression; Fission Yeast

1. Background

Aging and lifespan extension have recently received considerable attention the world over. Earlier studies have reported that either caloric restriction or the inactivation of nutrient-dependent pathways is able to increase life extension in different eukaryotes. The extension of lifespan in various organisms is associated with increased resistance to oxidative stress (1). The findings of numerous studies using various models of organisms have provided indirect evidence for the hypothesis that reactive oxygen species (ROS) production and subsequent induction of ROS defense are essential contributors to longevity (2).

As in higher eukaryotes, the unicellular yeast, *Saccharomyces cerevisiae*, has been the most used yeast model in aging studies (3, 4). Yeast lifespan can be measured through two methods: replicative aging refers to the number of divisions a single mother cell undergoes before death, whereas chronological lifespan measures the viability of cultures at the stationary phase of the growth curve (5, 6). The fission yeast, *Schizosaccharomyces pombe*, has been

recently used as a model for chronological aging studies inasmuch as it is more similar to the last common ancestor of humans and fungi (7). Nutrient restriction increases resistance to oxidative stress, reduces macromolecular damage, and promotes lifespan in *S. pombe* (8-10). Roux et al. (11) revealed that the *S. pombe gpa2^{R176H}* mutant exhibits not only a short lifespan but also impaired mitochondrial regulation and high production of ROS. Zuin et al. (12) reported that calorie restriction favors oxidative metabolism, ROS production, and Sty1 MAP kinase activation and this stress pathway favors lifespan extension.

In *S. pombe*, glucose, which is a primary carbon source, is detected by G protein-coupled receptors and generates a signal via the cAMP-dependent protein kinase A (PKA) (13, 14). The glucose sensing and signaling pathways have also been found to be involved in metabolic adaptation and cellular response to diverse stress agents (15, 16). In *S. pombe*, the multistep phosphorelay system and the mitogen-activated protein kinase (MAPK) pathway govern the transcriptional regulation in response to oxidative stress,

which is generated by the accumulation of ROS (17). MAPK Sty1 plays an important role in the regulation of downstream targets through triggering two transcriptional activators, *Atf1* and *Pap1*, in oxidative stress response (18). These transcription factors activate or induce the expression of antioxidant genes such as *ctt1* (cytoplasmic catalase), *gpx1* (glutathione peroxidase), *ttr1* (thioredoxin reductase), *trx2* (thioredoxin), *ntp1⁺* (neutral trehalase), *pgr1* (glutathione reductase), and *sod1* (superoxide dismutase, SOD) (19).

Trehalose (α , α -1,1-diglucose) is a storage disaccharide and is present in particularly high concentrations in resting and stressed yeast cells (20). Trehalose 6-phosphate (T6P) is synthesized in *S. pombe* from glucose 6-phosphate and UDP-glucose by T6P synthetase, encoded by the *tps1⁺* gene (21), and converted to trehalose by T6P phosphatase, encoded by the *tpp1⁺* gene (22). The breakdown of trehalose to glucose is catalyzed by the enzyme neutral trehalase, encoded by the *ntp1⁺* gene (23). Generally in yeast, the regulation of trehalose synthesis and breakdown is done by cAMP-dependent phosphorylation mechanisms (24). It has been reported that the *ntp1⁺* expression is regulated by the pathway of protein kinase cascade activated under osmotic or oxidative stress (23, 25) or by the binding of the transcription factors to elements such as cAMP-response element under thermal stress (26). In the present study, *ird5* and *ird11* were used to evaluate whether or not trehalose contributes to survival under moderate oxidative stress.

2. Objectives

In a previous study, we identified two glucose-repression-resistant mutants, namely *ird5* and *ird11* (27). In the *ird11* mutant, the oxidative stress response is affected by glucose signaling in a manner different from that caused by glucose deprivation (28). The *ird5* mutant has a lower caloric intake owing to reduced glucose consumption efficiency (29). The inefficient glucose uptake in *ird5* might be a cause of increased oxidative stress response. Accordingly, in the present study, through trehalose accumulation measurement and colony-forming unit (CFU) counting, we compared the *ird* mutants and the wild type to clarify the possible relationship between glucose signaling, oxidative stress response, and lifespan in *S. pombe*.

3. Materials and Methods

3.1. Yeast Strains and Media

In this study, the wild type of *S. pombe* *Lindner liquefaciens* (972 h⁻¹) and its relative invertase mutants (i.e. *ird5* and *ird11*), which are resistant to glucose suppression (27), were used. The selective medium consisted of 0.5% yeast extract and 3% sucrose and 400 μ g/mL 2-Deoxy-D-glucose (2-DOG) was developed for the *ird* mutants. The strains were cultured in the YE medium containing 3% glucose (repressed condition) and 0.5% glucose (glucose

starvation condition) and the YE medium containing 0.1% glucose plus 3% glycerol (de-repressed condition). All the chemicals were provided by Sigma-Aldrich, Germany.

3.2. Induction of Oxidative Stress

The exponentially growing *S. pombe* cells (wild type and *ird5* and *ird11*) under repressed conditions were split into two tubes. In the experimental group, 2 mM hydrogen peroxide (H₂O₂) (Sigma-Aldrich, Germany) was added to the medium, resulting in a mild level of oxidative stress in the *S. pombe* cells (30). The control group was not exposed to oxidative stress. After one hour, both experimental and control cells were removed by centrifugation and washed with sterile distilled water so that their trehalose content could be measured.

3.3. Lifespan Analysis

Chronological lifespan analyses were done on the wild type and the *ird* mutant cells, grown under repressed and de-repressed conditions for 21 days. Usually one day (the exponential phase) after starting the cultures, and this time point was designated as day 0, measurements were started and continued via sampling at 2-day intervals (3rd, 5th, ..., 21st days of the study).

3.4. Extraction and Assay of Trehalose

This study was performed under three conditions: repressed; de-repressed; and stressed conditions. From each group cells, samples were collected on the 1st (the exponential phase), 3rd, and 5th (stationary phase) days of the study, and trehalose accumulation was measured for each case. Trehalose was extracted and assayed as is described by Parrou and Francois (31). The amount of the trehalose contents of the samples was measured by treatment with trehalase (Sigma-Aldrich, Germany). The amount of the generated glucose was determined enzymatically via the glucose oxidase-peroxidase system (GOD-POD assay) using a commercial kit (Fluitest®-GLU, Biocon, Germany). The data are given as mean values \pm standard deviation (SD) with 'n' denoting the number of experiments. Statistical comparisons were made using the one-way analysis of variance (ANOVA) module of GraphPad Prism 5. The differences in the mean values were considered significant when $P < 0.05$.

4. Results

4.1. Lifespan of *S. pombe* Cells under Repressed and De-Repressed Conditions

Of the strains under study, *ird5* showed the most longevity in both repressed and de-repressed conditions. The *ird11* mutant had a lower lifespan extension than the wild type under repressed condition, while it had a higher lifespan extension than the wild type under de-repressed condition. On the other hand, the lifespan extension of *ird11*

grown in de-repressed condition was similar to that of *ird5* grown in repressed or de-repressed condition (Figure 1).

4.2. Trehalose Accumulation of *S. pombe* Cells under Repressed, De-Repressed, and Stressed Conditions

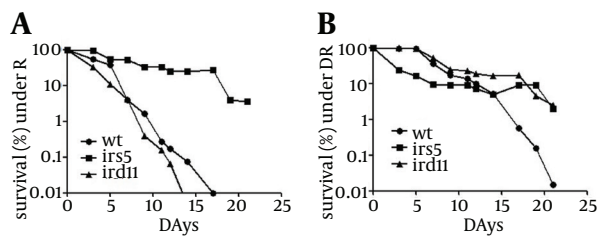
To comprehend the relationship between trehalose concentrations in the cells grown in repressed, de-repressed, and stressed conditions, the level of trehalose was estimated in the wild type and the mutant cells. The intracellular trehalose concentrations during the period of growth under repressed, de-repressed, and stressed conditions relative to the wild type and mutant cells are shown in Figure 2 A, Figure 2 B, and Figure 2 C, respectively. The results obtained from trehalose content measurement in the three conditions were evaluated in two ways. First, each group was evaluated individually and the trehalose contents of the groups on the 3rd and 5th days of the study were compared with those of the 1st day. Second, the trehalose content of each group was compared with that of the wild type.

When compared independently, as is depicted in Figure 2 A, except for *ird5* under repressed condition, the trehalose content was increased remarkably on the 3rd day of the study. However, a comparison with the wild type showed a sharp decrease in the trehalose level in the *ird* mutant. As is evident from Figure 2 B, under de-repressed condition, the level of intracellular trehalose was notably increased in the stationary phase (3rd day), while no significant changes were detected in either *ird5* or in *ird11*. Under stressed condition (Figure 2 C), during the stationary phase (3rd day), the trehalose level in *ird11* was increased as a pattern similar to that observed in the wild type; nevertheless, only trace amounts of alteration were seen in *ird5*. Finally, concerning the wild type during the stationary growth phase, a dramatic decrease was observed in the trehalose content in *ird5* (3rd and 5th days) and *ird11* (3rd day).

5. Discussion

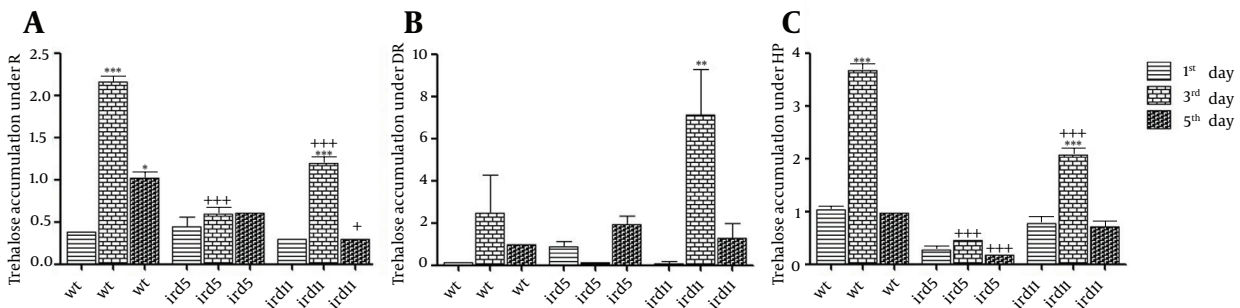
The PKA activated by glucose starvation and stress-activated MAPK pathways regulate the transcription of downstream genes via protein-DNA interactions at UAS1 and UAS2 of the *fbp1* gene (32). The existence of cross-talking between Sty1p and Pka1p regulates not only glucose repression and oxidative stress response pathways but also trehalose accumulation. Paredes et al. (25) reported that the expression of the *ntp1⁺* and *tps1⁺* genes in *S. pombe* is partially regulated by the Sty1p kinase under salt-induced osmotic stress and conditions of slight oxidative stress and is fully dependent on this kinase under severe oxidative stress. Another study showed that cell viability may depend on capacity to rapidly degrade the trehalose that is accumulated during oxidative stress. The authors indicated that glutathione reductase can be inhibited by trehalose in a dose-dependent manner. On the other hand, Sanchez-Fresneda et al. (33) reported that the stress-induced trehalose accumulation is Hog1-independent in *Candida albicans*.

Figure 1. Lifespan of the *S. pombe* Wild Type and *ird* Mutants



A, Grown in repressed condition (YE-3% glucose); B, Grown in de-repressed condition (YE-0.1% glucose plus 3% glycerol); (wt: wild type, R: repressed, DR: de-repressed condition).

Figure 2. Trehalose Accumulation of the *S. pombe* Wild Type and *ird* Mutants



A, Grown in repressed condition (YE-3% glucose); B, Grown in repressed condition (YE-0.1% glucose plus 3% glycerol); C, Grown in stressed condition (2 mM H₂O₂, one hour). The trehalose contents are expressed as mg glucose per 0.2 g of wet mass of the cells. Statistical significance was evaluated by one-way ANOVA ($P < 0.05-0.001$). (wt: wild type, R: repressed, DR: de-repressed, HP: oxidative stress conditions). *, **, ***: Each group was evaluated individually and the trehalose contents of the groups on the 3rd and 5th days of the study were compared with those of the 1st day. +, ++, +++: Trehalose content of each group is compared with that of the wild type.

The extension of lifespan in *S. pombe* is associated with both calorie restriction and increased resistance to oxidative stress (10-12). Chiming in with a study done on *S. cerevisiae* by Mahmud et al. (34), our results suggest that lifespan extension in *S. pombe* may not be associated with oxidative stress resistance and trehalose accumulation. The oxidative stress-resistant mutant *ird11*, which is affected by glucose signaling in a manner different from that caused by glucose deprivation (28), had a lower lifespan extension than the wild type under repressed condition and had a higher lifespan extension than the wild type under de-repressed condition. Indeed, the lifespan extension of *ird11* grown in de-repressed condition was similar to that of *ird5* grown in repressed or de-repressed condition.

We suggest that the longevity of the *ird5* mutant grown in both repressed and de-repressed conditions might be, in part, in consequence of glucose depletion condition caused by a reduced glucose consumption rate in these cells compared to the *ird11* mutant and the wild type. Because, contrary to *ird11*, there is an adaptive response to oxidative stress in *ird5*, caused by glucose sensing/signaling which emerges in glucose starvation (29). In light of the results of the present study, it seems that lifespan extension is mostly related to glucose sensing/signaling rather than oxidative stress response and trehalose accumulation.

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Authors' Contributions

Bedia Palabiyik: 1, Hypothesis; 2, Methodology; 3, Assessment of result and preparation of manuscript; 4. Contribution to lab study; Farinaz Jafari Ghods: 1, Lab study; 2, preparation of manuscript.

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