

Induction of *DAN/TIR* yeast cell wall mannoprotein genes in response to high hydrostatic pressure and low temperature

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Abstract Global transcriptional profiles of *Saccharomyces cerevisiae* were studied following changes in growth conditions to high hydrostatic pressure and low temperature. These profiles were quantitatively very similar, encompassing 561 co-upregulated genes and 161 co-downregulated genes. In particular, expression of the *DAN/TIR* cell wall mannoprotein genes, which are generally expressed under hypoxia, were markedly upregulated by high pressure and low temperature, suggesting the overlapping regulatory networks of transcription. In support of the role of mannoproteins in cell wall integrity, cells acquired resistance against treatment with SDS, Zymolyase and lethal levels of high pressure when preincubated under high pressure and low temperature.

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1. Introduction

Hydrostatic pressure and temperature are key parameters that influence the rate constant of any chemical reaction and have influenced the distribution of organisms on the earth. Approximately, 70% of the earth is composed of oceans with an average depth of 3800 m. Accordingly, the majority of the earth is a high pressure, cold environment in terms of area occupied. There is growing interest in understanding and applications of deep-sea microbes under these extreme conditions that have a significant impact on the greater part of life. However, there are practical limitations in manipulating their genome and functional analysis as well as maintaining their viability in laboratory conditions. Consequently, the fundamental understanding of the effects of high hydrostatic pressure on living cells remains fragmentary. To illustrate a more explicit picture of the adaptation to high-pressure environments, systematic investigative approaches are desired.

We have been analyzing the effects of high hydrostatic pressure on living cells using the yeast *Saccharomyces cerevisiae* as a model organism. One of our notable findings is that the uptake of tryptophan is the limiting factor on growth under pressure conditions of 15–25 MPa (approximately 150–

250 kg/cm²) [1]. Experimental wild-type strains usually have several nutrient-auxotrophic markers such as *trp1*, *leu2*, *lys2*, *his3*, *ade2* and *ura3*. Regardless of other forms of auxotrophy, *trp1* cells exhibit marked growth defects at 15–25 MPa due to reduced tryptophan uptake activity under pressure, while tryptophan prototrophic strains are capable of growth under the same conditions [1]. The effect of high pressure is analogous to that of low temperature on yeast cell growth. *trp1* cells also exhibit growth defects at 10–15 °C and atmospheric pressure (for simplicity, 0.1 MPa is used throughout to denote unpressurization), while tryptophan prototrophic strains do not [1]. Any factor that leads to increased tryptophan availability enables *trp1* cells to grow at both high pressure and low temperature, e.g., overexpression of tryptophan permease Tat1 or Tat2 [1–3], mutations in Rsp5 ubiquitin ligase [2], or disruption of the ubiquitin-specific protease genes [4].

In this study, global transcriptional profiles were studied in a tryptophan prototrophic *S. cerevisiae* strain following changes in growth conditions to high hydrostatic pressure and low temperature. To avoid confusing the large-scale effects observed in this study with the previously described stress response to lethal levels of high pressure (e.g., 150 or 200 MPa for 30 or 60 min) [5,6], the present investigation focused on the adaptation allowing yeast cells to develop a defense system prior to being subjected to lethal levels of high pressure and low temperature rather than as a consequence of cellular injury. The expression of a subset of *DAN/TIR* cell wall mannoprotein genes, which is known to be induced under hypoxia [7–9] or low temperature [10–12], was also induced under high pressure. The role of the *DAN/TIR* cell wall mannoproteins in adaptation and tolerance to high pressure was therefore investigated. This study highlights the possibility that a reduction in membrane fluidity caused by hypoxia, high pressure or low temperature could trigger *DAN/TIR* gene expression.

2. Materials and methods

2.1. Yeast strain and culture conditions

The wild-type *S. cerevisiae* strain BY4742 (*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0*; Invitrogen, Carlsbad, CA, USA) was grown in synthetic complete medium with some modifications (SC, 0.67% yeast extract nitrogen base w/o amino acids, adenine sulfate 20 mg/L, uracil 20 mg/L, tryptophan 40 mg/L, histidine-HCl 20 mg/L, leucine 90 mg/L, lysine-HCl 30 mg/L, arginine-HCl 20 mg/L, methionine 20 mg/L, tyrosine 30 mg/L, isoleucine 30 mg/L, phenylalanine 50 mg/L, glutamic acid 100 mg/L, aspartic acid 100 mg/L, threonine 200 mg/L, serine 400 mg/L, 2% D-glucose) [2]. Cell growth was examined at 0.1 MPa and 24 °C (control), 25 MPa and 24 °C (high pressure) or 0.1 MPa and 15 °C (low temperature) as described previously [1,2].

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2.2. DNA microarray analysis

Cells were grown until cell density of $1\text{--}2 \times 10^7$ cells/ml at 0.1 MPa and 24 °C had been reached with a volume of 50 ml in a 100-ml conical flask. Total RNA was prepared from the cells for the control sample as described previously [13] using the hot phenol method [14]. Cells were also incubated at 25 MPa and 24 °C or 0.1 MPa and 15 °C for 5 h, followed by total RNA preparation. To average accidental errors in total RNA preparation, equal amounts of total RNA purified from three independent cultures under each condition were mixed and the mixture was used for the experiments. Subsequent procedures including the isolation of poly A⁺-RNA, sample labeling and hybridization for DNA microarrays were performed by NimbleGen Systems Inc. (Madison, WI, USA) and GeneFrontier Inc. (Tokyo, Japan). Fifteen perfectly matching 60-mer probes for individual genes were used for hybridization. Because this analysis yields results with a high confidence level, a 1.5-fold difference between multiple samples generally corresponds to the level of significance ($P < 0.05$) obtained from hybridization signals from 15 perfectly matching probes for each gene. The large-scale DNA microarray data was deposited with Gene Expression Omnibus (<http://www.ncbi.nlm.nih.gov/geo/>). The accession number is GSE9136.

2.3. Reverse-transcriptase polymerase chain reaction

To confirm the DNA microarray analysis, transcription levels of some genes were analyzed with the reverse-transcriptase polymerase chain reaction (RT-PCR) using a One Step RNA PCR Kit (TaKaRa Bio Inc., Otsu, Japan) and relevant oligonucleotide primers. Total RNA was prepared from cells cultured independent of those for the DNA microarray analysis.

2.4. Resistance of cells against SDS, Zymolyase and a lethal level of high pressure

Cells were incubated at 0.1 MPa and 24 °C, 25 MPa and 24 °C or 0.1 MPa and 15 °C in SC medium for 5 h to yield a cell density of 1×10^7 cells/ml at the end of each culture. Subsequently, the cells were exposed to 0.05% SDS in water or 500 µg/ml of Zymolyase 100 T (Seikagaku Corporation, Tokyo, Japan) in 100 mM of potassium-phosphate buffer (pH 6.5) for 1 h, or exposed to a lethal level of high pressure of 125 MPa at 24 °C in water for 1 h. After these treatments, the cells were spread on YPD agar plates, followed by incubation for 2 days at 24–30 °C. The survival rate was expressed as colony-forming units (CFUs) relative to no treatment.

3. Results

3.1. Global transcriptional profiles during growth at high pressure and low temperature

When exposed to pressure of 25 MPa at 24 °C, the cells showed a reduced growth rate but a normal growth curve with an extended doubling time from 2.0 h to 3.7 h (Fig. 1). When exposed to low temperature of 15 °C at 0.1 MPa, the cells also showed a reduced growth rate with a doubling time of 5.2 h (Fig. 1). There was no reduction in cell viability upon exposure to the pressure and temperature for at least 20 h (data not shown). Global transcriptional analysis was employed with total RNA samples prepared from cells at 0 and 5 h after the change in growth conditions to high pressure or low temperature.

The comparative results of transcriptional profiles revealed that the majority of genes were transcribed at similar levels between the normal growth condition and high pressure with a correlation coefficient (R^2) of 0.869 (Fig. 2A), and between the normal growth condition and low temperature with an R^2 of 0.875 (Fig. 2B). Notably, there was greater similarity in transcription levels between high pressure and low temperature with an R^2 of 0.942 (Fig. 2C). Of the 6337 genes, 561 were concurrently upregulated by high pressure and low temperature, while 161 were downregulated. Supplementary Tables

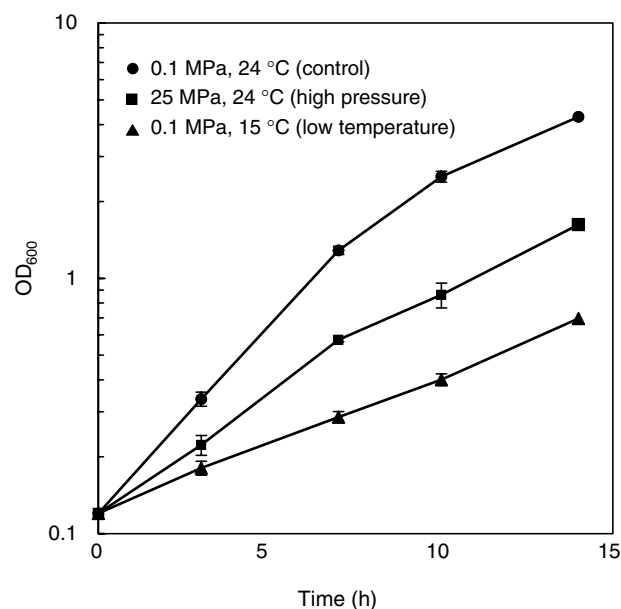


Fig. 1. Cell growth of *Saccharomyces cerevisiae* strain BY4742 under high pressure and low temperature. Cells were cultured under the conditions described in the text. The OD_{600nm} value was measured immediately after decompression at every time point of culture. The OD₆₀₀ values were measured after appropriate dilution. Data were represented as means $\pm$ S.D. ($n = 3$).

S1 and S2 represent genes upregulated under high pressure and low temperature, respectively. The remarkable similarity in transcriptional regulation implies that intracellular overlapping networks are involved in the adaptive responses to high pressure and low temperature (Table 1, S1 and S2). Iwahashi et al. [5], Fernandes et al. [6] and Iwahashi et al. [15] reported on the numbers of genes upregulated by high pressure. Although there are some overlaps in the genes identified here and in their reports, the number of differentially expressed genes is much larger. A direct comparison between these four studies is invalid because yeast strains, media and especially pressure conditions differed (180 MPa at 4 °C briefly [5]; 40 MPa at 4 °C for 16 h [5]; 200 MPa at room temperature for 30 min [6]; 30 MPa at 25 °C overnight [15]). There are also some overlaps in terms of low temperature-responsive genes identified here and by Sahara et al. (0.25 to 8 h after shifting to 10 °C) [16] and Schade et al. (10–120 min after shifting to 10 °C) [17] with greater differences.

One of the most striking but unexpected results was that the transcriptional profiles under high pressure and low temperature were quite similar with those observed under low oxygen levels (hypoxic or anaerobic condition) (Table 1). Particularly, mannoprotein genes involved in remodeling of the cell wall during anaerobiosis were dramatically up-regulated, which were *DAN1*, *TIR1*, *TIR2*, *TIR3*, *TIR4* and *TIP1* responsive to high pressure, while *DAN1*, *TIR1*, *TIR2* and *TIP1* responsive to low temperature (Table 2). These are referred to as the *DAN/TIR* genes comprising four *DAN* genes, four *TIR* genes and *TIP1* [11,12]. It has been also demonstrated that *TIP1*, *TIR1*, *TIR3* and *TIR4* are induced by low temperature but the *DAN* genes are not [11]. Most of genes upregulated under hypoxia [9] were not induced by high pressure and low temperature except for *DAN1*, *TIR1*, *TIR2*, *FET4* and *HEM13*. Kwast et al. also identified a number of anaerobic

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