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RESEARCH NOTE

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^{1}H-NMR Analysis of Metabolic Changes Induced by Snf1/AMP-Activated Protein Kinase During Environmental Stress Responses

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ABSTRACT

AMP-activated protein kinase sucrose non-fermenting 1 (Snf1) is a representative regulator of energy status that maintains cellular energy homeostasis. In addition, Snf1 is involved in the mediation of environmental stress such as salt stress. Snf1 regulates metabolic enzymes such as acetyl-CoA carboxylase, indicating a possible role for Snf1 in metabolic regulation. In this article, we performed nuclear magnetic resonance (NMR) spectroscopy to profile the metabolic changes induced by Snf1 under environmental stress. According to our NMR data, we suggest that Snf1 plays a role in regulating cellular concentrations of a variety of metabolites during environmental stress responses.

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Since yeast mainly uses glucose as a carbon source, sensing of glucose levels is important for mediating yeast energy metabolism. Sucrose non-fermenting 1 (Snf1)/AMP-activated protein kinase is a representative controller of energy status that maintains cellular energy homeostasis [1–3]. Snf1 is a regulatory kinase that is highly conserved in eukaryotic cells and plays a critical role in regulating a variety of activators and repressors required for energy balance mechanism [1–3]. In addition, Snf1 is involved in the mediation of environmental stimuli such as salt stress [4–11].

Snf1 phosphorylates and regulates metabolic enzymes such as acetyl-CoA carboxylase, suggesting a crucial role for Snf1 in metabolic control [12]. To date, there have not been any reports describing the metabolic changes induced by Snf1 during environmental stress responses. Nuclear magnetic resonance (NMR) spectroscopy is a useful technique for structure elucidation due to its various two-dimensional measurements, which makes NMR an ideal tool for metabolic analysis [13,14]. Here, we report that Snf1 regulates metabolic changes in response to environmental stresses in *Saccharomyces cerevisiae*.

We investigated whether Snf1 is involved in responses to multiple stresses such as salt, heat, and energy (potassium cyanide (KCN), a specific inhibitor of cytochrome c oxidase for ATP synthesis). Consistent with previous reports [9,11], the Δ*snf1* mutant showed increased sensitivity to salt and heat stresses (Supplementary Figure S1). In addition, our result revealed that Snf1 is required for a proper response against KCN stress (Supplementary Figure S1). Snf1 functions as a key sensor of energy status for the maintenance of cellular energy homeostasis [2,3,9]. Therefore, our work indicates that Snf1 might play a role in energy regulation as a protective mechanism under various stress conditions.

To explore changes in yeast stress responses from a metabolic perspective, we profiled the metabolites in yeast cells using ^{1}H-NMR spectroscopy (Figure 1 and Supplementary Table S1). ^{1}H-NMR spectroscopy and raw data processing were essentially performed as described previously [15]. Chemical shifts of signals were assigned to the metabolites in the area of amino acids, organic acids, carbohydrates, and nucleotide derivatives. On the basis of the ^{1}H-NMR spectra, we identified 36 metabolites in the whole cell extracts with chemical shifts and coupling patterns (Figure 1 and Supplementary Table S1). The main variations are summarized in the form of a heatmap shown in Figure 2(A) and Supplementary Table S2. ^{1}H-NMR spectra of wild-type and Δ*snf1* exhibited differentiated resonance spectra under various stress conditions. Partial least squares-discriminant analysis (PLS-DA), a supervised

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Supplemental data for this article can be accessed here.

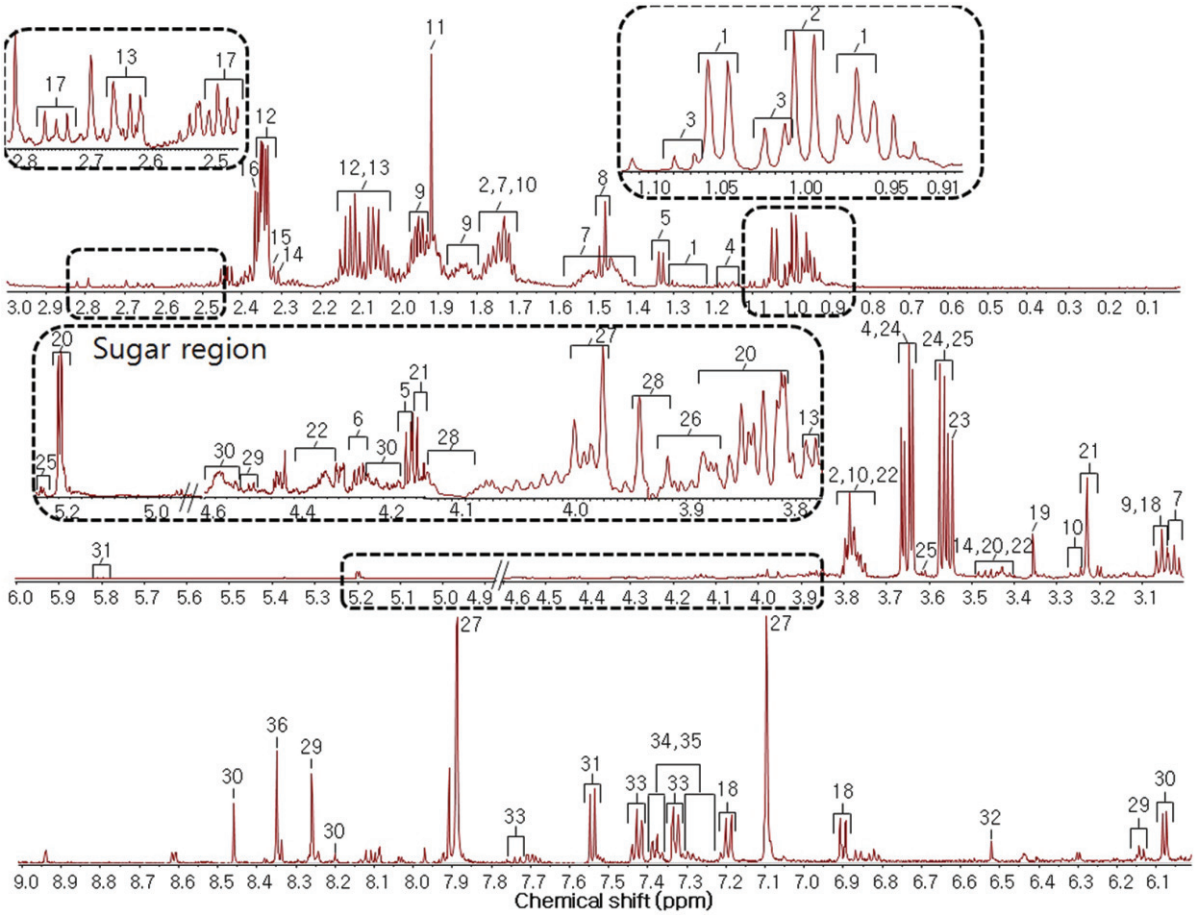

Figure 1. ^{1}H-NMR spectra of the metabolites of *S. cerevisiae*. ^{1}H-NMR spectroscopy and raw data processing were essentially performed as described previously [15].

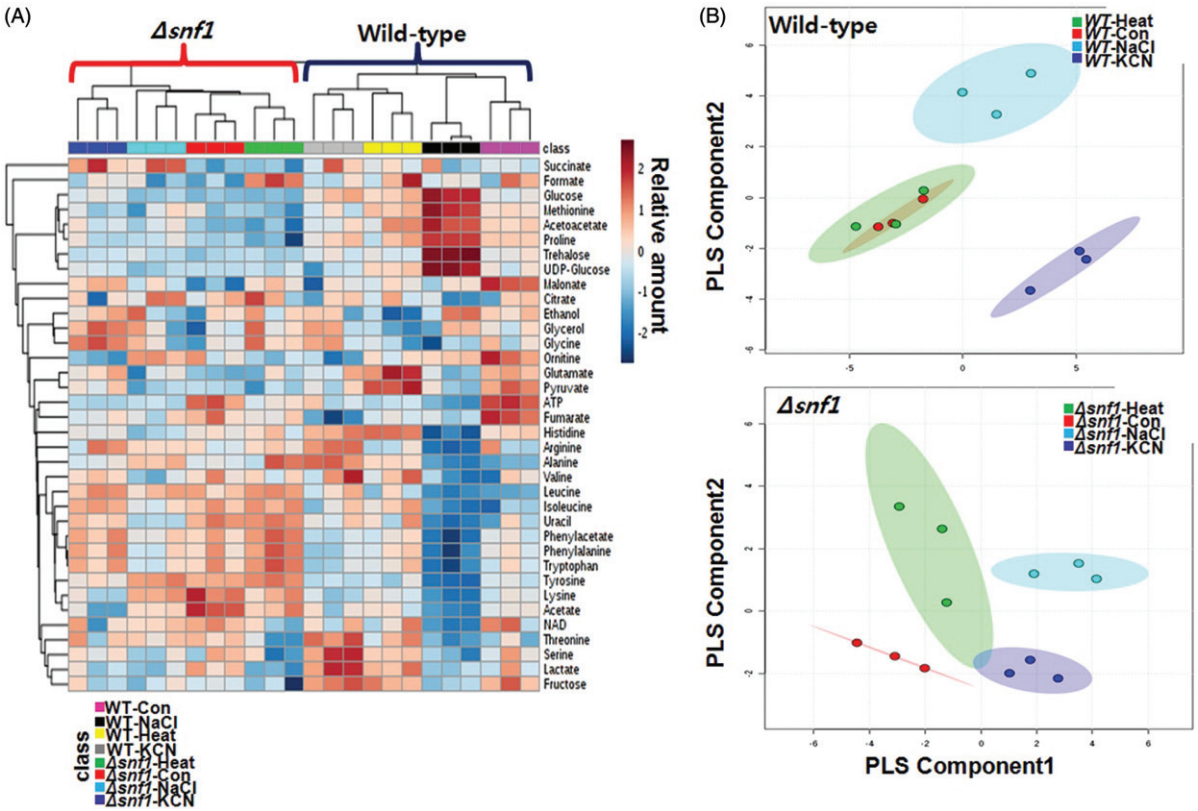

Figure 2. Heatmap of main metabolite variations (A) and PLS-DA score plot (B) in wild-type and Δ*snf1* under various stress conditions. The *S. cerevisiae* wild-type (W303-1A) and Δ*snf1* strains were grown on YPD medium. Yeast cells were treated with 0.8 M NaCl, 5 mM KCN, or 40 °C for 1 h, and then subjected to ^{1}H-NMR. Similar results were obtained from three independent experiments. ^{1}H-NMR spectroscopy and raw data processing were essentially performed as described previously [15].

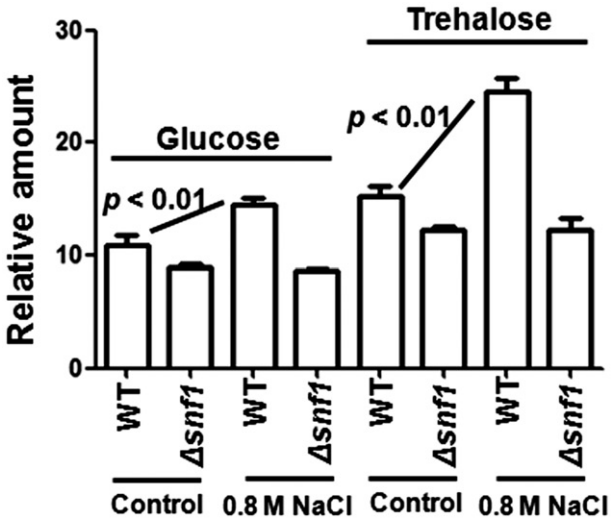

Figure 3. Snf1 regulates cellular concentrations of trehalose and glucose under salt stress. Yeast cells were treated with 0.8 M NaCl for 1 h, and then subjected to ^{1}H-NMR. Cellular concentrations of glucose and trehalose are differentially regulated in wild-type and Δ*snf1* under salt stress. Experimental values are the means of three independent experiments with standard deviation.

multivariate data analysis method, was used to investigate intrinsic variation in ^{1}H-NMR data. In the wild-type PLS-DA score plots, two principal components, PC1 and PC2, were calculated with the R^2Y and Q^2Y parameters of 0.95 and 0.77, and the Δ*snf1* mutant PLS-DA score plots were calculated with the R^2Y and Q^2Y parameters of 0.93 and 0.63, respectively (Figure 2(B)). As shown in Figure 2(B), the PLS-DA score plot of ^{1}H-NMR spectra showed a clear separation between wild-type and Δ*snf1* under various stress conditions.

Our ^{1}H-NMR data suggest that Snf1 plays a role in the regulation of metabolic changes induced by stressful environments in yeast (Figure 2). Especially, cellular concentrations of glucose and trehalose are increased in wild-type under salt stress, but not in Δ*snf1* (Figure 3). Trehalose functions as a representative osmolyte in order to cope with changes in osmotic pressure [16]. Meanwhile, maintenance of ATP balance is vital for all cells and the hydrolysis of ATP is the main energy source. In response to salt stress, a rapid increase in cellular ATP metabolism may reflect the higher energy demands required for salt stress tolerance [17,18]. Under normal condition, glucose production was slightly reduced in Δ*snf1* compared to wild-type (WT). In response to salt stress, glucose production was increased in WT, however it was not increased in Δ*snf1* at all (Figure 3). At present, we do not know whether Snf1 is involved in the mechanism of directly increasing glucose production in response to salt stress. Nevertheless, it can be assumed that Snf1 is related to glucose production under salt stress response. Our ^{1}H-NMR data will make a contribution to our understanding of metabolic changes induced by Snf1 during environmental stress in yeast.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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