



Title	Functional analysis of PP2C protein phosphatase genes in response to environmental stresses in <i>Saccharomyces cerevisiae</i>
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Abstract of Thesis

Title: Functional analysis of PP2C protein phosphatase genes in response to environmental stresses in *Saccharomyces cerevisiae*

(出芽酵母のストレス応答におけるプロテインホスファターゼPP2Cの機能ゲノム科学)

Name Dilruba Sharmin

Chapter 1: General Introduction

Saccharomyces cerevisiae (budding yeast) is one of the best characterized organisms for understanding and engineering eukaryotic cell function beside its industrial importance. Yeast cells have evolved to be exceptionally proficient at surviving sudden changes in their external environment. This response includes ~900 genes whose expression was stereotypically changed during large variety of environmental stresses including temperature shocks, hyper- or hypoosmotic shock, amino acid starvation, and nitrogen source depletion. During stresses, cells respond to an external molecule that transmits signals inside the cell which is known as signal transduction. Organisms from prokaryote to human develop a number of parallel pathways to avoid improper activation or deactivation of the signaling cascades through regulatory mechanisms ranging from transcriptional to post-translational.

Reversible protein phosphorylation was considered the main posttranslational modification involving protein kinases (PKases) and protein phosphatases (PPases) that phosphorylate and dephosphorylates protein, respectively. There are so many studies on PKases were done before. By contrast, knowledge on PPases is very limited even in *S. cerevisiae*. In eukaryotes, >99% phosphorylation occurs in Ser and Thr residues of proteins and the role of Ser/Thr phosphatases in elimination of phosphates from these proteins is very important. In this study we focused on a certain class of Ser/Thr phosphatases that is protein phosphatase 2C (PP2C). This study will focus on the characterization of PP2C genes in environmental stress response and their regulatory mechanisms.

Chapter 2: Effects of deletion of different PP2C protein phosphatase genes on stress responses in *S. cerevisiae*

S. cerevisiae has 40 PPases, including seven protein phosphatase 2C (PP2C PPase) genes (*PTC1-PTC7*). However, the precise functions remain poorly understood. To elucidate their cellular functions and to identify those that are redundant, we constructed 127 strains with deletions of all possible combinations of the seven PP2C PPase genes. All 127 disruptants were viable under nutrient rich conditions, demonstrating that none of the combinations induced synthetic lethality under this condition. However, several combinations exhibited novel phenotypes, e.g., the $\Delta ptc5\Delta ptc7$ double disruptant and the $\Delta ptc2\Delta ptc3\Delta ptc5\Delta ptc7$ quadruple disruptant exhibited low (13°C) and high (37°C) temperature sensitive growth, respectively. Interestingly, the septuple disruptant $\Delta ptc1\Delta ptc2\Delta ptc3\Delta ptc4\Delta ptc5\Delta ptc6\Delta ptc7$ showed an essentially normal growth phenotype at 37°C. The $\Delta ptc2\Delta ptc3\Delta ptc5\Delta ptc7$ quadruple disruptant was sensitive to LiCl (0.4 M). Two double disruptants $\Delta ptc1\Delta ptc2$ and $\Delta ptc1\Delta ptc4$ displayed slow growth and $\Delta ptc1\Delta ptc2\Delta ptc4$ could not grow on medium containing 1.5 M NaCl. The $\Delta ptc1\Delta ptc6$ double disruptant showed increased sensitivity to caffeine, congo red (CR) and calcoflour white (CFW) compared to each single deletion strain. Our observations indicate that *S. cerevisiae* PP2C PPases have redundant role in responses to environmental stresses. These disruptants also provide a means for exploring the molecular mechanisms of redundant *PTC* gene functions under defined conditions.

Chapter 3: Type 2C protein phosphatase Ptc6 participates in activation of the Slt2-mediated cell wall integrity pathway in *S.cerevisiae*

We sought to determine the cellular role of *PTC6* in *S. cerevisiae* with disruption of *PTC1* genes. We found that cells with $\Delta ptc6$ disruption were tolerant to the cell wall-damaging agents CR and CFW; however, cells with simultaneous disruption of *PTC1* and *PTC6* were very sensitive to these agents. Thus, simultaneous disruption of *PTC1* and *PTC6* gave a synergistic response to cell wall damaging agents. The level of phosphorylated Slt2 increased significantly after CR treatment in $\Delta ptc1$ cells and more so in $\Delta ptc1\Delta ptc6$ cells; therefore, additional deletion of *PTC6* enhanced Slt2 phosphorylation in the $\Delta ptc1$ disruptant. The level of transcription of *KDX1* upon exposure to CR increased to a greater extent in the $\Delta ptc1\Delta ptc6$ double disruptant than the $\Delta ptc1$ and $\Delta ptc6$ single disruptants. The $\Delta ptc1\Delta ptc6$ double disruptant cells showed normal vacuole formation under standard growth conditions, but fragmented vacuoles were present in the presence of CR or CFW. These observations suggest that *S. cerevisiae PTC6* participates in the negative regulation of Slt2 phosphorylation and vacuole morphogenesis under cell wall stress conditions.

Chapter 4: General discussion and conclusion

This work describes how different PP2C protein phosphatase genes participate in stress signaling in *S. cerevisiae*. One of the main purposes of this work was to discover functional redundancy among PTC PPases. With this purpose we discussed here the effects of deletion of different PP2C protein phosphatase genes in response to various environmental stresses. Systematic phenotype testing under various led us to discover several new phenotypes and several combinations of PP2C genes were found to be functionally redundant in spite that total protein sequence comparison gives very low similarity (4-29%) (i.e., Ptc5 and Ptc7 having 10% similarity could show redundant role in cold sensitivity). Also PTC PPases localized in different cellular compartments are able to perform same function. For example, simultaneous deletion of *PTC1* and *PTC6* increased Slt2 phosphorylation in the presence of cell wall damaging agents in spite that Ptc1 is localized in cytoplasm and nucleus whereas the localization of Ptc6 is mitochondria. Finally, since several new functions of PTC PPases are reported for the first time in this study, it can be said that although PTC PPases are mostly involved in stress signaling pathways but still they might possess unknown function that enhance or inhibit cellular regulation by other PPases irrespective of their structural similarity and cellular localization.

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論文審査の結果の要旨			
<p>A key mechanism of signal transduction in eukaryotes is reversible protein phosphorylation mediated through protein kinases and protein phosphatases (PPases). Modulation of signal transduction by this means regulates many biological processes. <i>Saccharomyces cerevisiae</i> has 40 PPases, including seven protein phosphatase 2C (PP2C PPase) genes (<i>PTC1-PTC7</i>). However, the precise functions remain poorly understood. To elucidate their cellular functions and to identify those that are redundant, we constructed 127 strains with deletions of all possible combinations of the seven PP2C PPase genes. All 127 disruptants were viable under nutrient rich conditions, demonstrating that none of the combinations induced synthetic lethality under these conditions. However, several combinations exhibited novel phenotypes, e.g., the $\Delta ptc5\Delta ptc7$ double disruptant and the $\Delta ptc2\Delta ptc3\Delta ptc5\Delta ptc7$ quadruple disruptant exhibited low and high temperature sensitive growth, respectively. Interestingly, the septuple disruptant $\Delta ptc1\Delta ptc2\Delta ptc3\Delta ptc4\Delta ptc5\Delta ptc6\Delta ptc7$ showed an essentially normal growth phenotype at high temperature. The $\Delta ptc2\Delta ptc3\Delta ptc5\Delta ptc7$ quadruple disruptant was sensitive to LiCl. Two double disruptants $\Delta ptc1\Delta ptc2$ and $\Delta ptc1\Delta ptc4$ displayed slow growth and $\Delta ptc1\Delta ptc2\Delta ptc4$ could not grow on medium containing NaCl. The $\Delta ptc1\Delta ptc6$ double disruptant showed increased sensitivity to caffeine, congo red and calcofluor white compared to each single deletion. Our observations indicate that <i>S. cerevisiae</i> PP2C PPases have a shared and important role in responses to environmental stresses. These disruptants also provide a means for exploring the molecular mechanisms of redundant <i>PTC</i> gene functions under defined conditions.</p>			
<p>Reversible protein phosphorylation is an important aspect of signal transduction that regulates many biological processes in eukaryotic cells. Here, we sought to determine the cellular role of <i>PTC6</i> in <i>S. cerevisiae</i> with disruption of <i>PTC</i> genes. We found that cells with $\Delta ptc6$ disruption were tolerant to the cell wall-damaging agents Congo red (CR) and calcofluor white (CFW); however, cells with simultaneous disruption of <i>PTC1</i> and <i>PTC6</i> were very sensitive to these agents. Thus, simultaneous disruption of <i>PTC1</i> and <i>PTC6</i> gave a synergistic response to cell wall damaging agents. The level of phosphorylated Slt2 increased significantly after CR treatment in $\Delta ptc1$ cells and more so in $\Delta ptc1\Delta ptc6$ cells; therefore, deletion of <i>PTC6</i> enhanced Slt2 phosphorylation in the $\Delta ptc1$ disruptant. The level of transcription of <i>KDX1</i> upon exposure to CR increased to a greater extent in</p>			

the $\Delta ptc1\Delta ptc6$ double disruptant than the $\Delta ptc1$ single disruptant. The $\Delta ptc1\Delta ptc6$ double disruptant cells showed normal vacuole formation under standard growth conditions, but fragmented vacuoles were present in the presence of CR or CFW. Our analyses indicate that *S. cerevisiae* *PTC6* participates in the negative regulation of Slt2 phosphorylation and vacuole morphogenesis under cell wall stress conditions.

Sharmin described the effect of *PP2C* disruption at various combinations on multilevel environmental stresses in *S. cerevisiae*. This is the first report she obtained is that *PTC6* participates in the negative regulation of Slt2 phosphorylation and vacuole morphogenesis under cell wall stress conditions. Although the exact function of *PTC6* in Slt2 CWI pathway is still unknown, it was assumed that *PTC6* might not be the main phosphatase of Slt2 rather it enhances the function of other phosphatases that regulates Slt2 phosphorylation. It is known that *PTC1* is involved in the negative regulation of Slt2 phosphorylation under cell wall stresses and the transcription of several cell wall related genes i.e., *KDX1*, *CRH1* *SED1* is increased in $\Delta ptc1$ deletion cells. It was assumed that the $\Delta ptc6$ deletion cells will also show increased transcription of these cell wall related genes. Her results showed that $\Delta ptc6$ deletion has synergistic effects on both Slt2 phosphorylation and transcription of only *KDX1*. The abnormal vacuole morphology of $\Delta ptc1$ under standard growth and cell wall stresses was also observed previously but additional disruption of *PTC6* could rescue the fragmented vacuole in $\Delta ptc1$ cells at least under standard growth. In conclusion, the involvement of *PTC6* in CWI pathway will be useful for further investigation of its precise function in the same pathway and also other stress signaling pathways in *S. cerevisiae*. Judging from these achievements, this dissertation deserves the degree of Doctor of Engineering.