Genome-Based Functional Analysis of the Met4p-Mediated Sulfur Regulatory Network in the Methylotrophic Yeast *Hansenula polymorpha*

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Many organisms possess a complicate regulatory circuit that controls the expression of various sets of enzymes involved in acquisition and assimilation of sulfur. As a methylotrophic yeast, which requires glutathione (GSH) for oxidation and detoxification of toxic methanol oxidation intermediates, *Hansenula polymorpha* is considered as a good model system to study the metabolism and functions of GSH in the response to stress generated by heavy metal and sulfur starvation. To obtain comprehensive overview on the sulfur regulatory networks in this thermotolerant methylotrophic yeast, we isolated a *H. polymorpha MET4* homologue (*HpMET4*), encoding a bZIP protein, and analyzed the effect of *HpMET4* deletion on sulfur metabolism, GSH biosynthesis and cadmium (Cd) resistance. Despite of low overall identity to *Saccharomyces cerevisiae MET4* (21%), the *H. polymorpha* mutant strain of *HpMET4* (*Δhpmet4*) showed a growth defect in the minimal medium containing only inorganic sulfur sources and hypersensitivity to Cd as does the *S. cerevisiae met4* mutant (*Δscmet4*) strain. However, the growth of *Δhpmet4* was dependent on the supplementation of GSH or cysteine, contrary to the methionine auxotrophic phenotype of *Δscmet4*. Transcriptome analysis revealed that compared to the wild type, the *Δhpmet4* strain exhibited more dramatic changes of gene expression under sulfur limitation and Cd exposure conditions, indicating the essential roles of *HpMET4* for cell homeostasis in both stress conditions. Moreover, the GSH biosynthesis analysis using ^{35}S-Met and ^{35}S-Cys labeling strongly suggested that *H. polymorpha* might lack the reverse transsulfuration pathway, a route for the conversion of methionine to cysteine, and thus could not use methionine efficiently as the sole sulfur source, differently from *S. cerevisiae*. The putative sulfur pathway of *H. polymorpha* was reconstructed based on the combined analysis of amino acid sequence alignment from genome information and transcriptome profile data. Overall, we showed that the sulfur metabolism pathway and its regulation by HpMet4p in *H. polymorpha* retains a similar design but has a significant diversity in the nature of the components and the regulation mechanism, distinctive among other yeast species.

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