

## Basic research towards improvement in the salt tolerance of yeasts

Tatsuya Maeda

Institute of Molecular and Cellular Biosciences, The University of Tokyo

Yeasts have been used for the production of traditional fermented high salt foods, including soy sauce, miso, and pickles. To maintain superior flavor and continue high production of these foods under high salt fermentation conditions, yeasts used for the fermentation must be highly salt tolerant. Yeasts achieve the salt tolerance by exerting various physiological responses to adapt to the stress under high salt conditions. In this study, among the signal transduction pathways activated by the salt stress to induce adaptive responses, we focused the Cpl1-Rim101 pathway, which responds to ion stress, and elucidated novel regulatory mechanisms of the pathway.

The Cpl1-Rim101 pathway responds ionic stress by proteolytically cleaving and thus activating a transcription factor, Rim101. Cpl1, a calpain-like protease implicated in the Rim101 cleavage, as well as Rim8, Rim9, Rim20, and Rim21 have been identified as constituents of this pathway. Mutants defective in any of these factors are not able to cleave Rim101, and thus are salt sensitive. In contrast, we found in this study that mutants defective in any of three proteins, Vps2, Vps4, and Vps24, cleaved Rim101 constitutively, even without ion stress. These three proteins are members of “the Type E Vps proteins”, whose function is required for the protein transport from the endosome to the vacuole. Our finding suggests tight linkage between the Cpl1-Rim101 pathway and endosome functions. We also determined the order of actions among these constituents in the pathway as follows.

Ion stress	Rim8• Rim9• Rim21	Vps2• Vps4• Vps24	Cpl1• Rim20
	Rim101 cleavage	Induction of salt tolerance	

In addition, we found that Rim101 was dephosphorylated in response to ion stress, by the Ca<sup>2+</sup>/calmodulin-dependent protein phosphatase, calcineurin, and that this dephosphorylation occurred independently to the proteolytic cleavage by Cpl1. These results suggest that Rim101 is under the complex control through these two catabolic regulations, the proteolytic cleavage and the dephosphorylation, to achieve effective responses to the ion stress.

The analysis of the salt stress-responsive signal transduction pathway elucidates an elaborate regulatory mechanism coordinating the salt stress responses and the cellular physiology. Based on the observation obtained in this study, we would like to clarify the molecular basis for the acquisition of the yeast salt tolerance, and to design highly salt tolerant yeast strains.