

## Processing and maturation of carboxypeptidase Y and alkaline phosphatase in *Schizosaccharomyces pombe*

Hiroyuki Mukaiyama · Tomoko Iwaki · Alimjan Idiris · Kaoru Takegawa

Received: 6 September 2010 / Revised: 7 November 2010 / Accepted: 25 November 2010 / Published online: 14 December 2010  
© Springer-Verlag 2010

**Abstract** *Schizosaccharomyces pombe* carboxypeptidase Y (CPY) is synthesized as a zymogen and transported into the vacuole where maturation and activation occurs. The 110-kDa *S. pombe* CPY precursor is processed twice and finally converted to a mature form consisting of polypeptides of approximately 19 and 32 kDa linked by a single disulfide bond. In *Saccharomyces cerevisiae*, maturation of CPY occurs mostly through the activity of vacuolar aspartyl protease Pep4p, whereas a Pep4p homolog has not been found in the *S. pombe* genome database. Based on analysis of protease-deficient mutants, we found that *S. pombe* CPY was not able to be processed or activated in *isp6Δpsp3Δ* double disruptants. Both Isp6p and Psp3p are subtilase-type serine proteases with related sequences. Moreover, alkaline

phosphatase of *S. pombe* was found to be localized at the vacuolar membrane and was also unprocessed in *isp6Δpsp3Δ* double disruptants. Vacuolar localization of GFP-fused Isp6p and Psp3p was determined by fluorescence microscopy. These results suggest that the two serine proteases Isp6p and Psp3p are functional in the vacuole and are involved in proteolytic processing of vacuolar proteins.

**Keywords** *Schizosaccharomyces pombe* · CPY · Alkaline phosphatase · Isp6 · Psp3

### Introduction

The vacuole of the yeast *Saccharomyces cerevisiae* has been shown to contain a multitude of hydrolytic enzymes and consequently has been proposed to be the lysosome of yeast cells (Matile and Wiemken 1967; Martinoia et al. 1979). The biosynthesis and function of a variety of vacuolar proteinases has been studied (Mechler et al. 1982; Jones and Cavanagh 1984; Achstetter and Wolf 1985). *S. cerevisiae* carboxypeptidase Y (CPY) is one of the best characterized vacuolar proteins, and its biosynthesis and transport into the vacuole has been studied in detail (Klionsky and Emr 1990). *S. cerevisiae* CPY is synthesized as a high molecular weight precursor which is translocated into the ER, where it is core-glycosylated to generate the so-called p1 precursor form. It next traverses the Golgi complex, where its oligosaccharides are elongated to generate precursor p2CPY. In the Golgi apparatus, a *S. cerevisiae* CPY-specific sorting signal is recognized which leads to formation of a receptor–ligand complex (Klionsky and Emr 1990). The receptor has been

H. Mukaiyama · T. Iwaki · A. Idiris  
ASPEX Division, Research Center, Asahi Glass Co., Ltd.,  
1150 Hazawa-cho,  
Kanagawa 221-8755, Japan

H. Mukaiyama · K. Takegawa  
Department of Bioscience & Biotechnology,  
Faculty of Agriculture, Kyushu University,  
Hakozaki 6-10-1,  
Fukuoka 812-8581, Japan

H. Mukaiyama · T. Iwaki · K. Takegawa  
Department of Life Sciences, Faculty of Agriculture,  
Kagawa University,  
Miki-cho, Kagawa 761-0795, Japan

K. Takegawa (✉)  
Laboratory of Applied Microbiology, Department of Bioscience  
& Biotechnology, Faculty of Agriculture, Kyushu University,  
Hakozaki 6-10-1,  
Fukuoka 812-8581, Japan  
e-mail: takegawa@agr.kyushu-u.ac.jp