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学 位 論 文 題 名

A study on the function of the C-terminal half region of the Arf GTPase activating protein Gcs1p

(Arf GTPase activating protein Gcs1p のカルボキシル末端領域の機能についての研究)

GTP-binding proteins of the ADP-ribosylation factor (ARF) family are important regulators of intracellular vesicle traffic. These proteins are activated (GTP-bound form) by guanine nucleotide exchange factors (GEFs) and deactivated (GDP-bound form) by GTPase-activating proteins (ArfGAPs). In the yeast *S. cerevisiae*, there are four proteins with ArfGAP activity including Gcs1p, Glo3p, Age1p, and Age2p. Gcs1p is the homologue of mammalian ArfGAP1. ArfGAP1 has been shown to associate with the curved membranes through two central ALPS (Amphipathic Lipid Packing Sensor) motifs named ALPS1 and ALPS2. The ALPS motif is supposed to be unstructured in solution, but adsorbs preferentially onto highly curved membranes by folding into an amphipathic α -helix (AH) and insertion of its hydrophobic amino acids between loosely packed lipids. Previously it has been shown that Gcs1p functionally interacted with the phospholipid flippase Cdc50p-Drs2p in the early endosome-to-TGN retrieval pathway. Flippases regulate phospholipid asymmetry of membranes by translocating specific phospholipids from the exocytosomal leaflet to the cytoplasmic one. They are implicated in vesicle formation by generating membrane curvature through imbalance transport of phospholipids. Gcs1p also functions redundantly with two other ArfGAPs, Glo3p and Age2p in the Golgi-to-ER retrograde transport pathway and in transport from the TGN, respectively.

This study focuses on the function of the carboxyl-terminal region of Gcs1p. Although the existence of a region homologous to ALPS1 of ArfGAP1 has been reported in Gcs1p, there is no any region homologous to ALPS2 in this protein. However, hydrophobic cluster analysis suggested that there is another potential AH-forming region downstream of ALPS in Gcs1p. Using mutational analysis, the functional importance of ALPS and the predicted AH region was examined in the above vesicle transport pathways. Genetic interaction data showed that, the existence of ALPS, but not the predicted AH region, is inevitable for the Gcs1p function redundant with Glo3p. In contrast, either ALPS or the predicted AH region is sufficient for Gcs1p function redundant with Cdc50p-Drs2p or Age2p. The functional importance of ALPS and the predicted AH region in the early endosome-to-TGN pathway was further confirmed by electron microscopy, examining the endocytic recycling of Snc1p (an exocytic v-SNARE) and Chs3p (Chitin synthase III), and genetic interaction with *tlg2 Δ* (t-SNARE). Using flotation assay, the lipid binding property of ALPS and the predicted AH region was also examined. The data showed that the ALPS motif was the primary determinant in the recognition of membranes, while the predicted AH region was less important but had a compensatory activity to sense the small size liposomes. Like the ALPS motif,

the association of the predicted AH region with membranes seemed to be mediated through hydrophobic interactions. It was also found that the ALPS motif contributed to the physical interaction of Gcs1p with the SNAREs including Snc1p and Tlg1p, and that these interactions appeared to be mediated by hydrophobic effect.

These observations indicate that the lipid and protein binding sites overlap significantly. From the results of biochemical and in vivo experiments, the different behavior of Gcs1p in the pre and post-Golgi pathways might be explained in the following predicted model. In the beginning of vesicle formation, Gcs1p may associate with SNAREs via the ALPS motif and subsequently, Arf1p is recruited to the site of vesicle formation. During vesicle formation at the *cis*-Golgi, when the membrane is converted to the curved shape, the ALPS motif but not the predicted AH may preferentially shift Gcs1p from the SNAREs to the curved membrane, which therefore enables Gcs1p to promote GTP heterolysis by Arf1p. However, since the predicted AH region is a minor lipid interacting site, it may not interact with the *cis*-Golgi membranes. On the other hand, in the post-Golgi pathways, the predicted AH region may interact with the membranes, perhaps by the aid of flippases present at these membranes. Interestingly, in most of the experiments in which the early endosome-to-TGN pathway was examined, the predicted AH region was more important than ALPS. This region might be more adapted to the recognition of membrane curvature created by the action of flippases.

Taken together, this study revealed that Gcs1p contains a second ALPS like motif, which contributes together with ALPS to the function of this protein in different membrane trafficking pathways.