

Abstract

Mechanism of fusion of intracellular membranes in eukaryotic cells involves several protein families including soluble *N*-ethylmaleimide-sensitive-factor attachment protein receptor (SNARE) proteins and Sec1/Munc-18 related proteins (SM proteins). It is known that the transport is evolutionary conserved from yeast to man. Therefore for facilitating of the research, we can use simple eukaryotes *Saccharomyces cerevisiae*. Mammalian SNARE protein syntaxin 16 has a yeast homologue Tlg2p which is used in this study as a model for studying affects of phosphorylation to the syntaxin 16 function. Also their binding partners, SM proteins mVps45p (mammalian) and yeast Vps45p are homologous.

Phosphorylation of SNARE proteins is known as a possible way of regulation of membrane fusion. Abolishment of one of the putative phosphorylation sites in Tlg2p protein, serine 90 leads to dominant effects on the exocytic and endocytic pathways. The work presented in this study shows some phenotypes of mutants based on this phosphorylation site of protein Tlg2p. Those mutants are S90A (cannot be phosphorylated) and S90D (phosphomimetic – acid carboxyl group mimics phosphate group). It was revealed that the phosphorylation of Tlg2p protein at serine 90 or the mutation Tlg2p-S90D may play some role in protecting Tlg2p protein from non-Pep4-dependent degradation, possibly by the proteasome. In the absence of *PEP4*, when the vacuolar degradation is prevented, the effect of the proteasome can be seen more clearly. Protein Pep4p is a vacuolar protease in yeast responsible for degradation of many yeast proteins. Other results also suggest that phosphorylation of Ser90 on Tlg2p or the S90D phosphomimetic mutation may stabilise the interaction between Tlg2p protein and Vps45p protein, and therefore stabilise the levels of Tlg2p in cell. It was also shown that phosphorylation has no impact on trafficking of carboxypeptidase Y phenotype and salt/osmotic stress sensitivity phenotype.

We also showed that it is possible to prepare in vitro hybrid SNARE complex containing mammalian protein syntaxin 16 and yeast proteins Tlg1p, Vti1p and Snc2p and that this complex is able to bind similar amounts of Vps45p protein as yeast SNARE complex with Tlg2p protein as a syntaxin part. These findings may be very usefull for future research of syntaxin 16 and the trafficking in mammalian cells. (In English)

Key words: Membrane fusion, SNARE, Syntaxins, Tlg2p, Sec1p/Munc18, Vps45p, Endocytosis