The ABC-Transporter Ste6 mediates the secretion of the mating pheromone a-factor in the yeast *Saccharomyces cerevisiae*. A Mat**a** *ste6* deletion strain produces mature a-factor, but is not able to secrete the peptide.

Ste6 is an extremely short-lived protein. We are interested in analyzing the mechanisms underlying the fast turnover of Ste6 by isolating Ste6-stabilizing mutants. Since it is difficult to directly assay the stabilization of Ste6 we fused it with LacZ as a reporter protein. The fusion protein behaves like wildtype Ste6:

- it is fully functional
- it fractionates with wildtype Ste6 (Fig. 1)
- it accumulates at the plasma membrane in an endocytois mutant (not shown)
- it is stabilized in a *pep4* mutant (Fig. 2B)

After EMS mutagenesis about 30 mutants with increased β-galactosidase activity could be identified in a lacZ-filter test. The five mutants with the highest Ste6-LacZ activity were characterized further (Fig. 2). It could be demonstrated by pulse chase experiments, that wildtype Ste6 was stabilized in these mutants (Fig. 3). A screen with a cDNA library and backcross experiments prooved, that *PEP4* was affected in mutant #81 (Fig. 4). The identification of a function which has already been shown to be involved in the degradation of Ste6 prooved the efficiency of the screen. Other mutants, in which Ste6 is even more stabilized than in *pep4* cells, and in which the mutation has no effect in the proteolytic function of the vacuole, are currently being characterized.

Co-fractionation of Ste6 and Ste6-LacZ

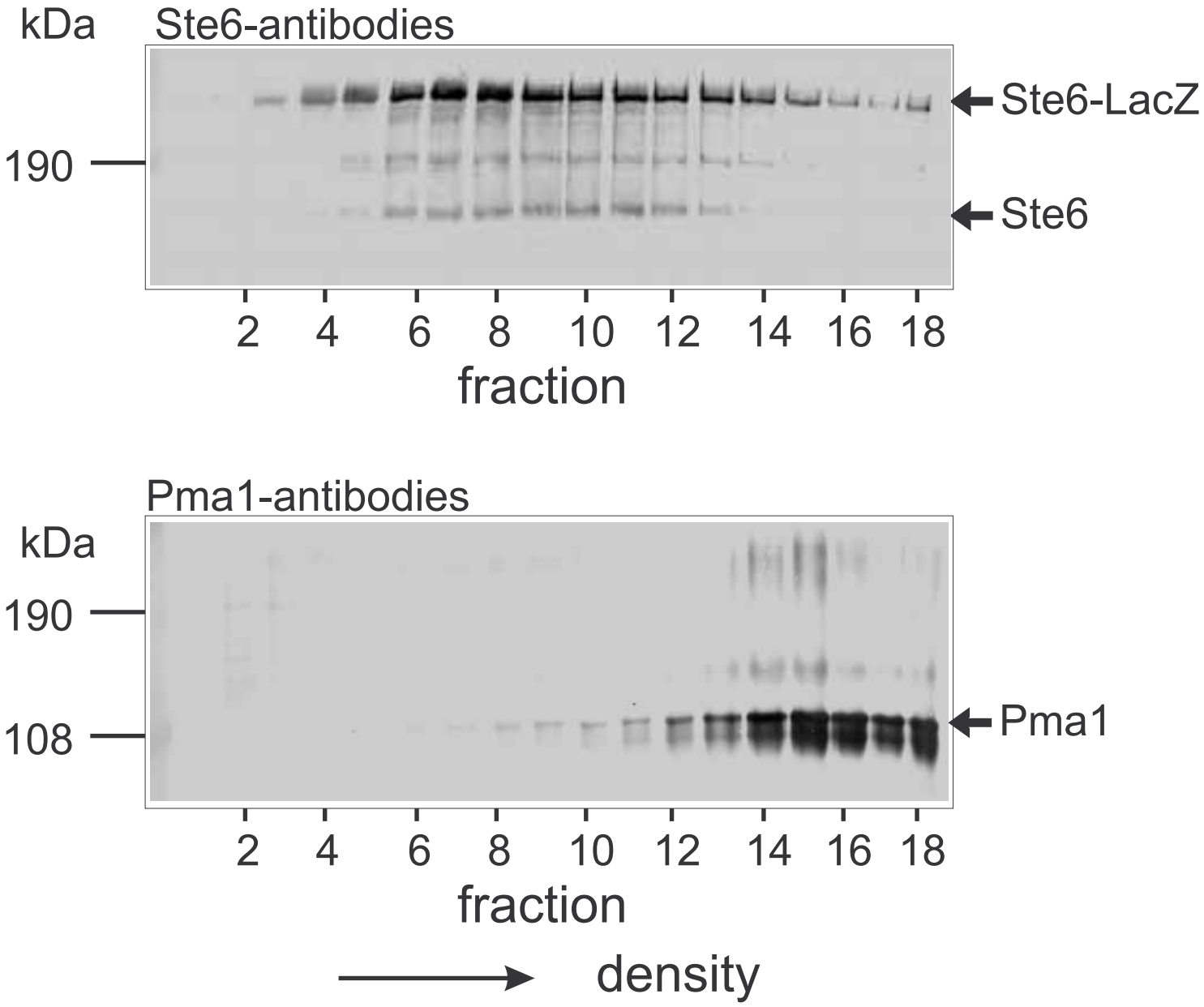
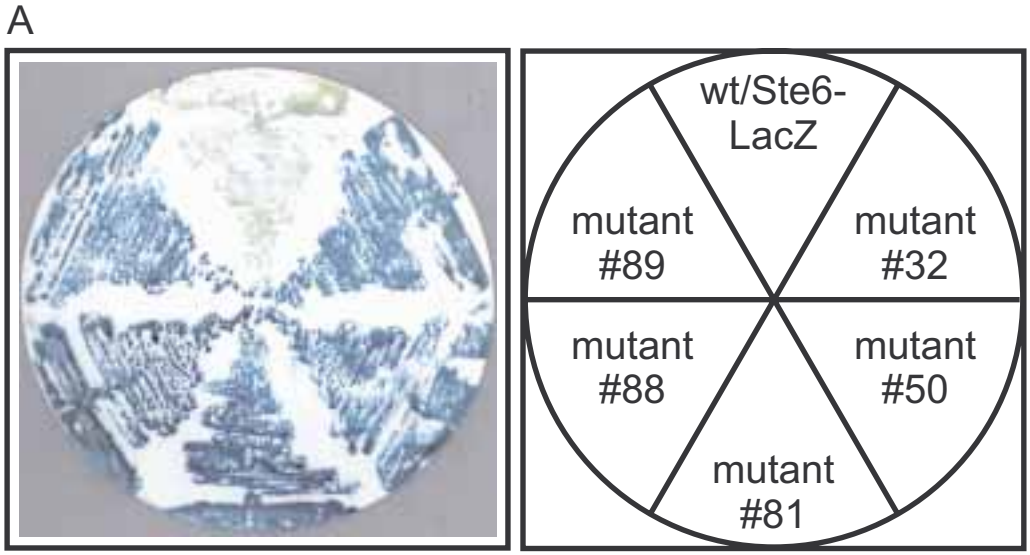


Fig. 1: Sucrose gradients were used to fractionate crude cell extracts. The gradient fractions were assayed for the presence of Ste6, Ste6-LacZ and Pma1 (a plasma membrane protein) by Western Blotting. As shown previously, Ste6 does not co-fractionate with the plasma membrane.

Accumulation of Ste6-lacZ in selected mutants



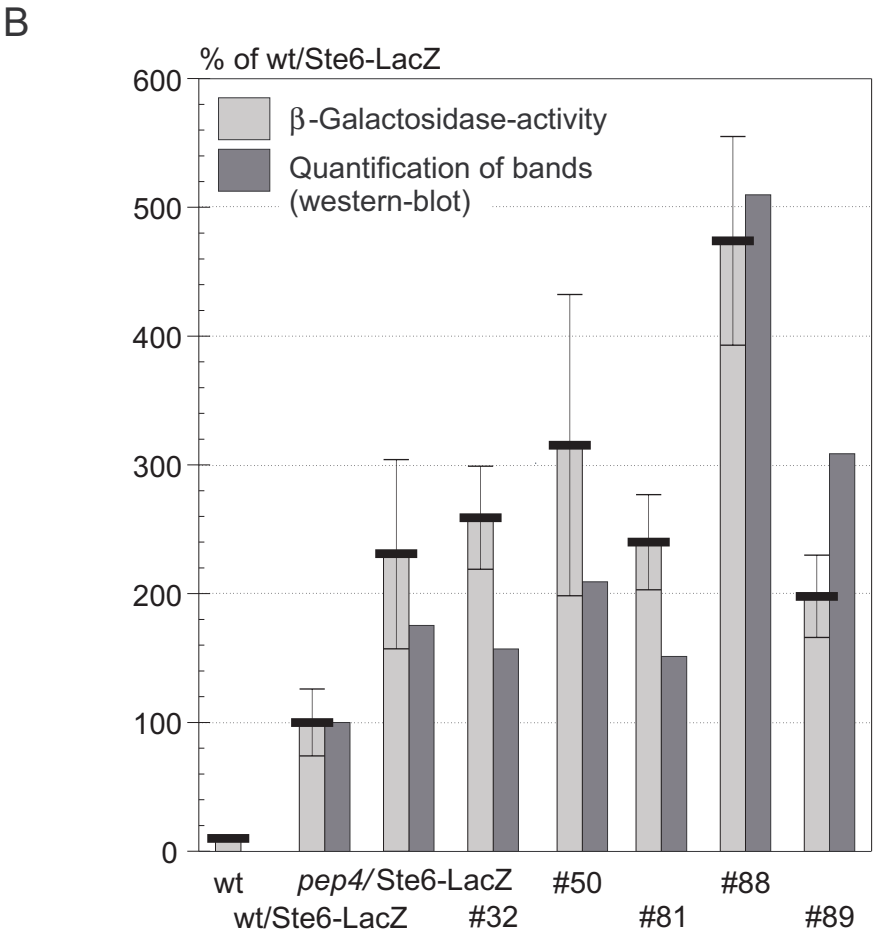


Fig. 2: (A) Semi-quantitative measurement of β-galactosidase activity with a LacZ-filter test. (B) Comparison between quantitative enzyme tests (light grey) and quantification of signals in an immunoblot experiment (dark grey).

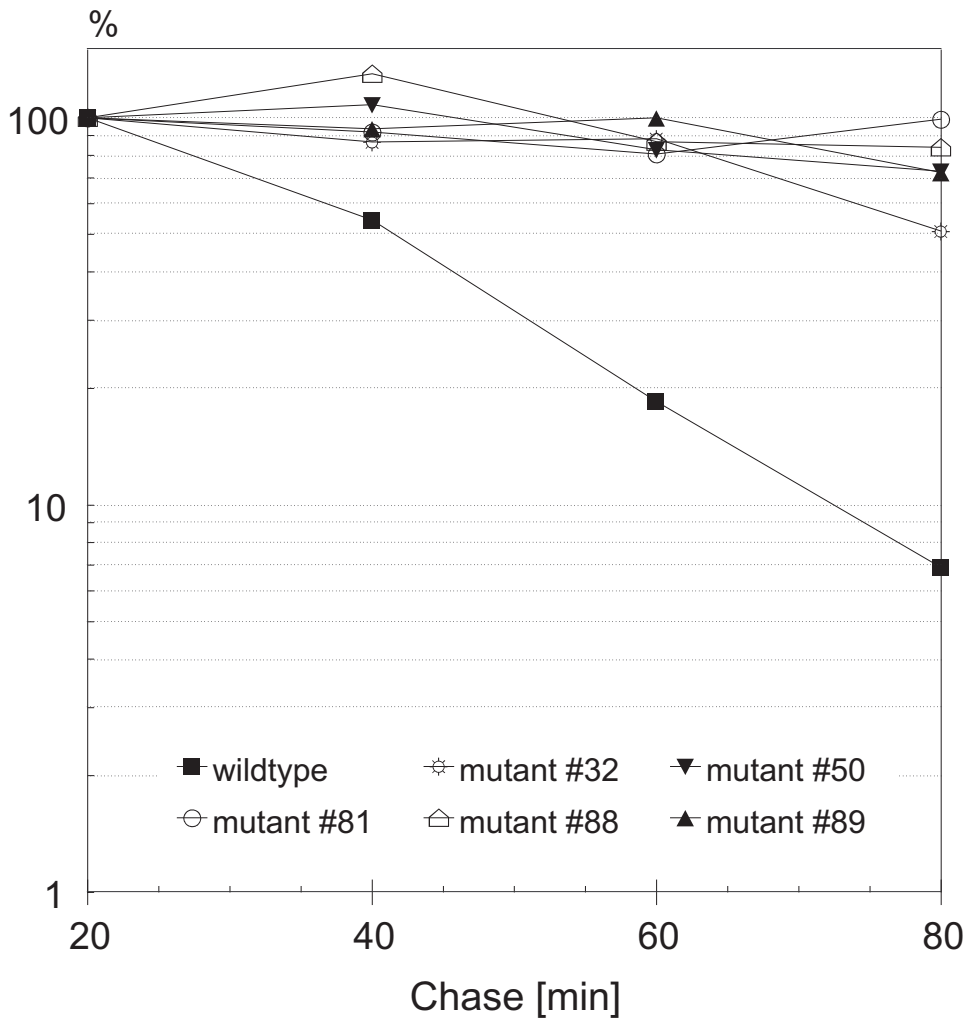


Fig. 3: Pulse-chase experiments with wildtype Ste6 in selected mutants. The mutant strains were transformed with a 2µ plasmid containing a *STE6*-gene. The proteins were labelled with radioactive methionin. Cells were harvested every 20 minutes after chase with an excess of unlabelled methionin. Ste6 was immunoprecipitated from cell extracts using Ste6-antibodies and detected by autoradiography.

The PEP4 gene is affected in mutant #81

A

(1) wt/Ste6-LacZ

(2) mutant #81

(3) mutant #81/2μ-PEP4

B

(1) wt x wt

(2) pep4 x wt

(3) pep4 x pep4

(4) mutant #81 x wt

(5) mutant #81 x pep4

Fig. 4: (A) We isolated a gene bank plasmid encoding Pep4 which reduces β-galactosidase activity in mutant #81 to wildtype level. (B) Backcross experiments with mutant strain #81. Strains were crossed as indicated and the LacZ activity of the resulting diploid strains was determined by a LacZ-filter test. A higher Ste6-LacZ level, resulting in a dark blue colour, was only detected in cross 3 (*pep4* x *pep4*) and in cross 5 (mutant #81 x *pep4*), indicating that mutant #81 is affected in *PEP4*.