FEBS Letters 411 (1997) 48–52 FEBS 18723

High expression of the yeast syntaxin-related Vam3 protein suppresses the protein transport defects of a *pep12* null mutant

M. Götte, D. Gallwitz*

Max-Planck-Institute for Biophysical Chemistry, Department of Molecular Genetics, P.O. Box 2841, D-37018 Göttingen, Germany

Received 16 April 1997

Abstract The Pep12 protein of *Saccharomyces cerevisiae* is a member of the syntaxin family thought to function as target membrane receptor (t-SNARE) for vesicular intermediates travelling between the Golgi apparatus and the vacuole. Exploiting the temperature-sensitive growth phenotype of *pep12* deletion strains, we identified *VAM3* as a multicopy suppressor. Vam3p is another syntaxin-related protein which on high expression restored vacuole acidification of *pep12* null mutants and effectively suppressed their sorting and maturation defects of vacuolar hydrolases. We conclude that Vam3p acts either as a bypass suppressor or by functionally replacing Pep12p at an endosomal, prevacuolar compartment.

Key words: Syntaxin; t-SNARE; Vacuole; Vesicular transport; Yeast

1. Introduction

The vacuole of the yeast *Saccharomyces cerevisiae* is the functional equivalent of the mammalian lysosome. Apart from functions in the storage of metabolites and the regulation of ion homeostasis in the cytosol, its most prominent role is the degradation of macromolecules [[1], for review]. A variety of hydrolases reside in the acidic lumen, most of them being synthesized as inactive precursors in the ER. After passing the early stages of the secretory pathway, soluble vacuolar hydrolases are actively sorted away from secretory proteins in a late Golgi compartment and delivered to the vacuole via intermediate endosome-like compartments [[2], for review]. Upon arrival in the vacuole, the precursor forms are typically activated by proteolytic processing [[3,4] for review].

Mutants defective in vacuole function have been isolated through screening for both biochemical and morphological phenotypes: *pep* mutants [5] were isolated due to their property of missorting the vacuolar hydrolase proteinase A (PrA) which is also required for activation of the proteinases B (PrB) and C (PrC = carboxypeptidase Y, CPY); *vps* mutants [6,7] are characterized by the secretion of the Golgi-modified precursor form of CPY, and *vam* mutants [8] exhibit an altered vacuolar morphology, such as fragmentation.

Interestingly, in some of these mutants members of gene families are affected which have been identified as general components of vesicular transport [9,10]. For example, the small GTPases Ypt51p and Ypt7p have also been isolated as Vps21p and Vam4p, respectively [11–14]. Recently, the yeast Pep12 protein has been shown to act in a vesicular transport step between the Golgi apparatus and the vacuole [15]. Pep12p is a transmembrane protein of 288 amino acids which exhibits 25% identity and 56% similarity to rat syntaxin 6 [16]. It is thought to act as a so-called t-SNARE (target membrane soluble NSF attachment protein receptor; NSF = *N*-ethyl-maleimide-sensitive fusion protein) in the docking of transport vesicles at a still ill-defined, prevacuolar (endosomal) compartment [15,17]. Cells carrying a disruption of the *PEP12* gene are characterized by a single enlarged, acidification-defective vacuole, the accumulation of 50 nm vesicles and maturation and sorting defects of several vacuolar hydrolases [15]. Furthermore, *pep12* deletion strains exhibit a growth defect at 38°C. We exploited this phenotype to screen for multicopy suppressors of the deletion of *PEP12*. Multicopy suppressor screening previously led to the identification of many components of the vesicular transport machineries, such as t-SNAREs, v-SNAREs (vesicle membrane SNARE) and *SEC1*-homologues [18–21].

We identified the *VAM3* gene as a potent multicopy suppressor of the deletion of *PEP12*. According to its primary sequence, the Vam3 protein is a member of the syntaxin family [22]. Our results suggest that Vam3p acts as a t-SNARE either replacing Pep12p or activating an alternative, Pep12p-independent pathway.

2. Materials and methods

2.1. Strains, growth conditions and genetic methods

The following yeast strains were used in this study: MSUC-3D: *Mat***a** *ura3 leu2 his3 trp1 lys2* (this laboratory); YMG7: MSUC-3D *pep12::kanMX4* (this study); SEY6210: *Mat***a** *suc2-D9 ura3-52 leu2-3,113 his3-D200 trp1-D901 lys2-801* [6]; YMG5: SEY6210 *pep12::kanMX4* (this study); YMG8: SEY6210 *vam3::LEU2* (this study). Manipulations of *E. coli* and DNA were performed according to standard procedures [23]. Yeast strains were grown in 1% yeast extract (Gibco, Eggenstein), 2% peptone 140 (Gibco, Eggenstein), 2% glucose (YEPD), or in synthetic glucose medium (SD) supplemented as necessary [24]. Solid media were prepared by adding 2% agar (Gibco, Eggenstein). Lithium acetate transformation of yeast cells was performed as previously described [25].

2.2. Construction of recombinant plasmids

To clone the *PEP12* gene, a fragment encompassing codons 14 to 189 was amplified using standard PCR techniques. The PCR fragment was digoxygenin-labelled using the DIG-labelling kit (Boehringer Mannheim) and used to screen approximately 19000 *E. coli* clones of a YEp13-based genomic yeast library as described previously [18]. Plasmids were isolated from two candidate colonies and the presence of *PEP12* was verified by Southern blotting and DNA-sequencing using the Sequenase 2.0 kit (USB, Braunschweig). A 1245 bp *ClaI/NsiI–PEP12* fragment was subcloned into *ClaI/PstI*-cut pBluescript II KS+ (Stratagene, Heidelberg) to create pMG1. A 1290 bp *XhoI/EagI* fragment of pMG1 was subcloned into the respective polylinker sites of the shuttle vectors pRS316 [26] and pRS326 [27] to create pMG13 and pMG6, respectively. The *VAM3* gene was cloned as follows. Utilizing the *XhoI*-polylinker site of the library vector pTSS25, a

*Corresponding author. Fax: (49) 551-201-1718.
E-mail: dgallwi1@gwdg.de

PII S0014-5793(97)00575-9

1462 bp *Xho*I(*Taq*I)/*Eag*I fragment of *VAM3* representing base pairs 518 930 to 520 392 of chromosome XV was cloned into *Xho*I/*Eag*I-cut pRS326 and pRS316 to create pMG49 and pMG56, respectively. The plasmid pMG48 (pBS-VAM3) was constructed by subcloning the 1768 bp *Avr*II/*Nsi*I *VAM3* fragment of the original suppressor plasmid pTSS1 into *Pst*I/*Xba*I-cut pBluescript II KS+. The *SED5* gene, cloned from a genomic library, was inserted into pRS326 as a 4.2 kb *Xba*I fragment. All plasmid constructions were controlled by sequencing.

2.3. Gene disruptions

The *PEP12* gene was deleted using the LFH–PCR disruption technique as described by Wach [28]. The oligonucleotides MG56 5′-AATTAACCCTCACTAAGGG-3′ and MG57 5′-CCCTTTAGTGAGGGTTAATTCATCTCAACACAATTATTG-3′ were used to amplify the 5′-megaprimer, whereas MG58 5′-GCCCTATAGTGAGTCGTATTACGGCTTCAGACGAGCTAAGG-3′ and MG59 5′-GTAATACGACTCACTATAGGGC-3′ were used to amplify the 3′-megaprimer under standard PCR conditions. Single-step gene disruption of the chromosomal *VAM3* gene was done with a linear DNA fragment. Part of the protein-coding region of the gene was removed and replaced by the yeast marker gene *LEU2* as follows. From pMG48, a blunt-ended 928 bp *Stu*I/*Bst*EII fragment containing more than 90% of the protein-coding region of *VAM3* (−166 to +792) was removed and replaced by *LEU2* on a 2.2 kb *Hpa*I fragment. The *vam3* deletion strain YMG8 was constructed by transforming the haploid strain SEY6210 with a 2.7 kb *Not*I(*Avr*II)/*Dra*I *vam3::LEU2* fragment which contained 140 bp and 280 bp of sequence extending 5′ and 3′ from the deletion end points. In all cases, correct integration was verified by Southern blot analysis using the ECL system (Amersham Buchler, Braunschweig).

2.4. Radiolabelling and immunoprecipitation

Pulse-chase experiments were performed exactly as described previously by Tsukada and Gallwitz [29] using the anti-CPY- and anti-alkaline phosphatase (ALP) antisera described by Benli et al. [30].

2.5. Vital staining

Quinacrine staining of yeast vacuoles was essentially performed as described in Weisman et al. [31]. Staining with CDCFDA was done exactly as described by Roberts et al. [32].

2.6. SDS-PAGE and Western blotting

SDS-PAGE [33] and Western blotting [34] were performed as described previously. For steady-state Western blotting, yeast extracts were prepared by alkaline lysis followed by TCA precipitation as described [30]. The monoclonal antibody specific for proteinase A was a kind gift from Akihiko Nakano (Tokyo). Polyclonal antisera specific for CPY and ALP have been described previously [30]. Secondary horseradish-peroxidase labelled anti-mouse or anti-rabbit IgG antibodies were from Amersham Buchler, Braunschweig. The ECL-system (Amersham Buchler, Braunschweig) was used for signal detection after Western blotting.

2.7. Reagents

Except stated otherwise, all reagents were from Sigma (Deisenhofen, Germany).

3. Results

In the course of our investigations on interacting partners for the small GTPases Ypt51p [12] and Ypt7p [13], we observed a weak genetic interaction of *YPT51* and *PEP12* (M. Götte, J.-S. Yoo, D. Gallwitz, unpublished). To identify additional components of the vesicular transport pathway from the Golgi apparatus to the vacuole, we searched for multicopy suppressors utilizing the temperature-sensitive phenotype of *pep12* null mutants. The *pep12* deletion strain YMG7 was transformed with a pRS326-based yeast genomic library [27]. Transformants on selective plates (-Ura) were incubated for 1 day at 30°C and thereafter at 38°C for 2 days. Of approximately 50 000 transformants, 58 were able to grow

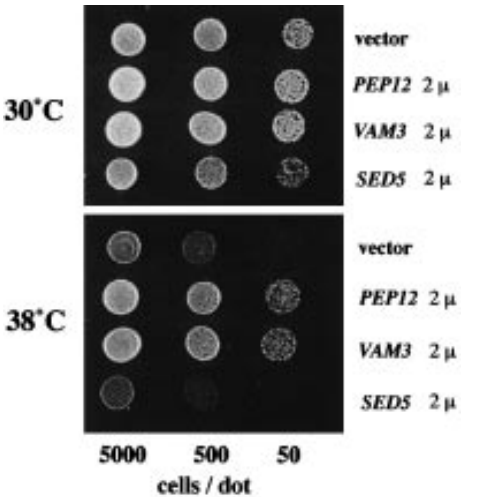

Fig. 1. Suppression of the temperature-sensitive growth defect of a *pep12* null mutant. 5 μl of logarithmic growing YMG5 (*pep12*$^-$) cells transformed with the plasmids indicated were spotted onto SD-Ura agar plates at concentrations of 1000, 100, and 10 cells/μl and incubated at 30°C (upper panel) or 38°C (lower panel) for 3 days, respectively. vector = pRS326; 2μ = multicopy plasmid.

at 38°C. Plasmids were isolated and amplified in *E. coli*. Plasmids exhibiting different restriction patterns were retransformed into YMG7 and retested for suppressor activity. Among these plasmids, 14 inserts contained the authentic *PEP12* gene. Two plasmids (pTSS1/pTSS25) contained overlapping DNA fragments of 6.6 kb and 8.0 kb with only one of the several open reading frames (ORF) (YOR106w) being present in both. This ORF is identical with *VAM3* (GenBank accession #U57827), which encodes a syntaxin-related protein of 283 amino acids. To confirm that the suppressing activity could be ascribed to the *VAM3* gene, the gene was subcloned into pRS326 and retested for suppression of the growth defect of the *pep12* null mutants YMG5 and YMG7 at 38°C. As can be seen in Fig. 1, high expression of *VAM3* conferred resistance to elevated temperatures in YMG5. Even expression from a centromeric plasmid relieved the temperature sensitivity as compared to cells transformed with the empty vector (data not shown). In contrast, high expression of *SED5*, which encodes a structurally related t-SNARE acting in ER-to-Golgi transport [35] did not restore growth of YMG5 cells at the non-permissive temperature. The same effects were observed in YMG7 (results not shown). As many studies on *vps* mutants have been carried out in the genetic background of YMG5, this strain was chosen for further analyses.

Due to a malfunction in the assembly of two subunits of the vacuolar ATPase [36], *pep12* mutants exhibit a defect in vacuole acidification [37] which can be monitored using the basic fluorescent dye quinacrine [31]. As can be seen in Fig. 2b, quinacrine did not stain the vacuole of *pep12* mutant cells transformed with the empty vector. In contrast, expression from the multicopy plasmid of both the *PEP12* and the *VAM3* gene led to stained vacuoles indicating restoration of vacuole acidification (Fig. 2d,f). While expression of *PEP12* from a centromeric plasmid allowed vacuole staining, low expression of *VAM3* from a single copy plasmid was not sufficient to restore vacuole acidification (results not shown). Occasionally, a few weakly stained cells could be observed.

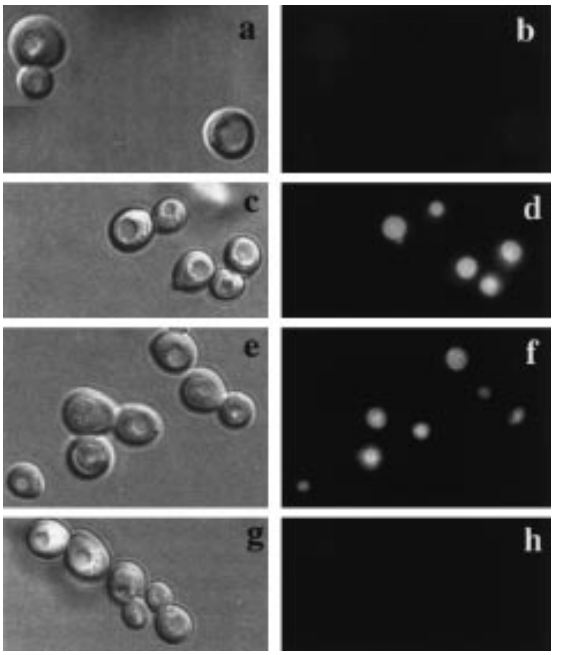

Fig. 2. Suppression of the vacuolar acidification defect of a *pep12* null mutant. The vacuoles of YMG5 (*pep12*$^-$) cells transformed with pRS326 (a,b), pRS326-PEP12 (c,d), pRS326-VAM3 (e,f) or pRS326-SED5 (g,h) were stained with quinacrine as described in Section 2 and viewed by Nomarski (a,c,e) or fluorescence (b,d,f) microscopy, respectively.

Importantly, high expression of *SED5* could not suppress the vacuole acidification defect of YMG5 (Fig. 2h).

All *vps* and *pep* mutants are defective in vacuolar protein maturation and they missort the soluble vacuolar hydrolases proteinase A (PrA) and CPY [5–7]. As shown in steady-state Western blots (Fig. 3), the mature forms of PrA (42 kDa) and CPY (61 kDa) appeared to be absent from *pep12* null mutant cells but they were readily detectable in the *pep12*$^-$ strains transformed with either pRS326-PEP12 or pRS326-VAM3. In the case of PrA, the immature ER- and Golgi-modified forms (48 and 44 kDa species) could be seen in *pep12*$^-$ cells. They disappeared completely in *PEP12*-expressing cells and, to a large extent, on high expression of *VAM3*. In contrast, high expression of *SED5* had no effect on the processing of both CPY and ALP (results not shown).

We next followed the maturation kinetics and delivery to the vacuole of soluble CPY and the membrane-bound alkaline phosphatase (ALP). Following a 15-min pulse with Tran^{35}S-label and a 30-min chase with unlabelled amino acids, the maturation of newly synthesized CPY and ALP was severely inhibited in spheroplasts derived from *pep12*$^-$ cells, and a significant portion of CPY was missorted and secreted in its Golgi-modified p2 precursor form (Fig. 4). The maturation of ALP appeared to be less affected. Most importantly, the maturation and sorting defects were effectively suppressed on high expression of *VAM3*.

To further characterize the genetic interaction of *VAM3* and *PEP12*, we investigated the effect of high expression of the *PEP12* gene in the *vam3* deletion strain YMG8. Deletion of the *vam3* gene resulted in phenotypes previously described for the original *vam3* mutants [8]: while the steady-state levels of the mature forms of PrA and CPY were comparable to those of wild-type cells (results not shown), the most prominent phenotype was a highly fragmented vacuole (Fig. 5a,b). High expression of the *PEP12* gene in YMG8 cells resulted in a partial suppression of this phenotype. Although still fragmented, the vacuoles of *vam3* cells transformed with pRS326-PEP12 (Fig. 5e,f) appeared larger than the vacuoles of control cells (Fig. 5a,b). However, the single large vacuoles of YMG8 transformed with pRS316-VAM3 (Fig. 5c,d) were not seen.

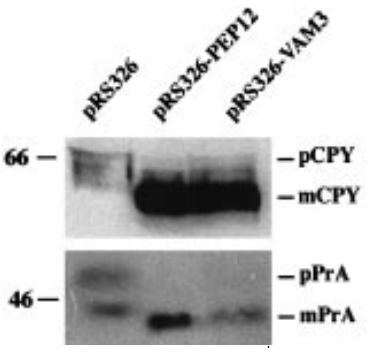

Fig. 3. Processing of soluble vacuolar enzymes in YMG5-transformants under steady-state conditions. Cell extracts of YMG5 transformed with the plasmids indicated were subject to SDS-PAGE and Western blotting with anti-CPY (upper panel) and anti-PrA-antibodies (lower panel) as described in Section 2. The migration positions (in kDa) of molecular mass markers are indicated on the left margin. The migration positions of the precursor (p) and mature (m) forms of CPY and PrA are indicated on the right margin. pPrA indicates the position of the largest (52 kDa) PrA precursor.

4. Discussion

Pep12p has been suggested by Becherer et al. [15] to act in transport of Golgi-derived vesicles carrying soluble vacuolar enzymes to a late endosomal ('prevacuolar') compartment and it was shown that the membrane-bound ALP might be transported via a Pep12p-independent route. There is evidence that the transport pathways of vacuolar hydrolases and of endocytosed α-factor merge [13,38–40] and it is well possible that some or all of the vacuolar proteins that pass the Golgi ap-

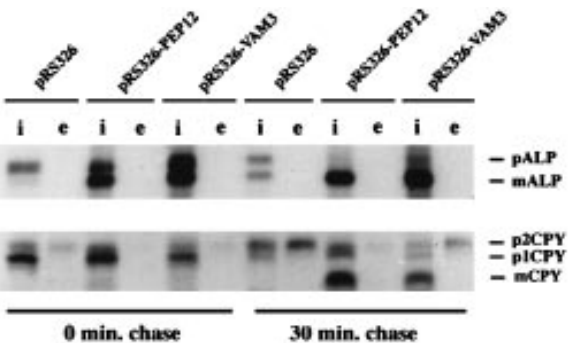

Fig. 4. Rescue from transport defects of newly synthesized vacuolar hydrolases in *pep12* null mutant cells. Spheroplasts of YMG5 cells transformed with either pRS326, pRS326-PEP12 or pPR326-VAM3 were subjected to a pulse-chase with Tran^{35}S-label. Zero or 30 min after initiating the chase, anti-ALP (upper panel) or anti-CPY (lower panel) antiserum was used for immunoprecipitation. i, internal fraction; e, secreted fraction. The migration positions of the precursor (p) and mature (m) forms of ALP and CPY (in kDa) are indicated to the right. p1CPY, ER-modified form of CPY; p2CPY, Golgi-modified form of CPY.

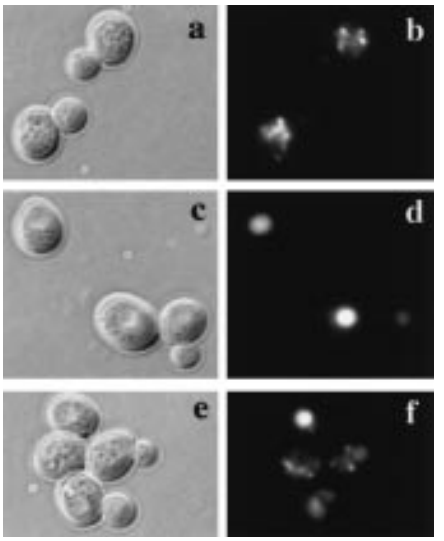

Fig. 5. Partial suppression of vacuolar fragmentation of a *vam3* null mutant. The vacuoles of YMG8 (*vam3*$^-$) cells transformed with pRS326 (a,b), pRS326-PEP12 (c,d) or pRS316-VAM3 (e,f) were stained with CDCFDA as described in Section 2 and viewed by Nomarski (a,c,e) or fluorescence (b,d,f) microscopy.

paratus travel via early and late endosome-like organelles [13]. Different endosomal compartments appear to exist in yeast [41,42]. According to the SNARE hypothesis [17], distinct vesicular and target membrane receptors would be required for consecutive steps of the Golgi-to-vacuole transport. As *pep12* and *vam3* mutants display phenotypic alterations [15,8] that resemble those of *ypt51* and *ypt7* mutants, respectively [11–13], the presumptive t-SNAREs are likely to function at different steps of the vacuolar protein transport pathway(s).

How can the suppressor activity of *VAM3* in *pep12* deletion strains be envisaged? One possibility would be the activation by Vam3p of a putative bypass to the vacuole or of a Pep12p-independent transport route. A high Vam3p receptor density at an endosomal organelle distinct from the Pep12p compartment (or even at the vacuole) would thus allow docking of transport vesicles which under wild-type conditions would dock at the Pep12p-bearing compartment. Alternatively, Vam3p at high dosage might directly replace Pep12p at the endosomal/prevacuolar compartment where Pep12p is thought to reside. Carboxyl terminally anchored type II membrane proteins, such as v- and t-SNAREs, appear to reach their final destination via the secretory pathway [43–45]. It is therefore conceivable that if Vam3p would have to pass the Pep12 compartment on its way to a following organelle, perhaps the vacuole, a receptor density high enough for vesicle docking would be attained at this compartment. In either case one would have to assume that a putative v-SNARE normally pairing with Pep12p would also be able to recognize Vam3p, but most likely with lower affinity. We would like to point out that the suppression of Pep12p-lacking cells by high dosage of Vam3p appears to be specific as the structurally related Sed5 protein was unable to rescue *pep12*$^-$ cells and high levels of Pep12p did only marginally affect the phenotypic alterations seen in *vam3*$^-$ cells.

The phenotypic resemblance of *vam3* and *ypt7* mutants [8,13] together with the finding that the Ypt7 GTPase is required for vacuole/vacuole fusion [46] lends credence to the assumption that Vam3p acts as a t-SNARE at the vacuole.

Acknowledgements: We are indebted to Drs S.D. Emr and H.D. Schmitt for providing yeast strains. We thank Hans-Peter Geithe for DNA sequencing and synthesizing oligonucleotides and Rita Schmitz-Salue for technical assistance. This work was supported in part by grants to D.G. from the Deutsche Forschungsgemeinschaft. M.G. was supported by a Max-Planck Fellowship.

References

[1] Klionsky, D.J., Herman, P.K. and Emr, S.D. (1990) Microbiol. Rev. 54, 266–292.
[2] Riezman, H. (1993) Trends Cell Biol. 3, 273–277.
[3] Raymond, C.K., Roberts, C.J., Moore, K.E., Howald-Stevenson, I. and Stevens, T.H. (1992) Int. Rev. Cytol. 139, 59–120.
[4] Van den Hazel, H.B., Kielland-Brandt, M.C. and Winther, J.R. (1996) Yeast 12, 1–16.
[5] Jones, E.W. (1977) Genetics 85, 23–33.
[6] Robinson, J.S., Klionsky, D.J., Banta, L.M. and Emr, S.D. (1988) Mol. Cell. Biol. 8, 4936–4948.
[7] Rothman, J.H., Howald, I. and Stevens, T.H. (1989) EMBO J. 8, 2057–2065.
[8] Wada, Y., Ohsumi, Y. and Anraku, Y. (1992) J. Biol. Chem. 267, 18665–18670.
[9] Ferro-Novick, S. and Jahn, R. (1994) Nature 370, 191–193.
[10] Rothman, J.E. (1994) Nature 372, 55–63.
[11] Horazdovsky, B.F., Busch, G.R. and Emr, S.D. (1994) EMBO J. 13, 1297–1309.
[12] Singer-Krüger, B., Stenmark, H., Düsterhöft, A., Philippsen, P., Yoo, J.-S., Gallwitz, D. and Zerial, M. (1994) J. Cell Biol. 125, 283–298.
[13] Wichmann, H., Hengst, L. and Gallwitz, D. (1992) Cell 71, 1131–1142.
[14] Wada, Y., Ohsumi, Y., Kawai, E. and Ohsumi, M. (1996) Protoplasma 191, 126–135.
[15] Becherer, K.A., Rieder, S.E., Emr, S.D. and Jones, E.W. (1996) Mol. Biol. Cell 7, 579–594.
[16] Bock, J.B., Lin, R.C. and Scheller, R. (1996) J. Biol. Chem. 271, 17961–17965.
[17] Söllner, T., Whiteheart, S.W., Brunner, M., Erdjument-Bromage, H., Geromanos, S., Tempst, P. and Rothman, J.E. (1993) Nature 362, 318–324.
[18] Dascher, C., Ossig, R., Gallwitz, D. and Schmitt, H.D. (1991) Mol. Cell. Biol. 11, 872–885.
[19] Ossig, R., Dascher, C., Trepte, H.-H., Schmitt, H.D. and Gallwitz, D. (1991) Mol. Cell. Biol. 11, 2980–2993.
[20] Aalto, M.K., Ronne, H. and Keränen, S. (1993) EMBO J. 12, 4095–4104.
[21] Brennwald, P., Kearns, B., Champion, K., Keränen, S., Bankaitis, V. and Novick, P. (1994) Cell 79, 245–258.
[22] Bennett, M.K., Garcia-Arraras, J.E., Elferink, L.A., Peterson, K., Fleming, A.M., Hazuka, C.D. and Scheller, R.H. (1993) Cell 74, 863–873.
[23] J. Sambrook, E.F. Fritsch, T. Maniatis, Molecular Cloning: a Laboratory Manual, 2nd edn., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989.
[24] M.D. Rose, F. Winston, P. Hieter, Methods in Yeast Genetics. a Laboratory Course Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1990.
[25] Ito, H., Fukuda, Y., Murata, K. and Kimura, A. (1983) J. Bacteriol. 153, 163–168.
[26] Sikorski, R.S. and Hieter, P. (1989) Genetics 122, 19–28.
[27] Vollmer, P. and Gallwitz, D. (1995) Meth. Enzymol. 257, 118–128.
[28] Wach, A. (1996) Yeast 12, 259–265.
[29] Tsukada, M. and Gallwitz, D. (1996) J. Cell Sci. 109, 2471–2481.
[30] Benli, M., Döring, F., Robinson, D.G., Yang, X. and Gallwitz, D. (1996) EMBO J. 15, 6460–6475.
[31] Weisman, L.S., Bacallo, R. and Wickner, W. (1987) J. Cell Biol. 105, 1539–1547.
[32] Roberts, C.J., Raymond, C.K., Yamashiro, C.T. and Stevens, T.H. (1991) Meth. Enzymol. 194, 644–661.

[33] Laemmli, U.K. (1970) Nature 227, 680–685.
[34] Burnette, W.N. (1981) Anal. Biochem. 112, 195–203.
[35] Hardwick, K. and Pelham, H.R.B. (1992) J. Cell Biol. 119, 513–521.
[36] Rothman, J.H., Yamashiro, C.T., Raymond, C.K., Kane, P.M. and Stevens, T.H. (1989) J. Cell Biol. 109, 93–100.
[37] Preston, R.A., Murphy, R.F. and Jones, E.W. (1989) Proc. Natl. Acad. Sci. USA 86, 7027–7031.
[38] Schimmöller, F. and Riezman, H. (1993) J. Cell Sci. 106, 823–830.
[39] Vida, T.A., Huyer, G. and Emr, S.D. (1993) J. Cell Biol. 121, 1245–1256.
[40] Singer-Krüger, B., Stenmark, H. and Zerial, M. (1995) J. Cell Sci. 108, 3509–3521.
[41] Singer-Krüger, B., Frank, R., Crausaz, F. and Riezman, H. (1993) J. Biol. Chem. 268, 14376–14386.
[42] Hicke, L., Zanolari, B., Pypaert, M., Rohrer, J. and Riezman, H. (1997) Mol. Biol. Cell 8, 13–31.
[43] Kutay, U., Ahnert-Hilger, G., Hartmann, E., Wiedenmann, B. and Rapoport, T.A. (1995) EMBO J. 14, 217–223.
[44] Jäntti, J., Keränen, S., Tiokkanen, J., Kuismanen, E., Ehnholm, C., Söderlund, H. and Olkkonen, V.M. (1994) J. Cell Sci. 107, 3623–3633.
[45] Ossig, R., Laufer, W., Schmitt, H.D. and Gallwitz, D. (1995) EMBO J. 14, 3645–3653.
[46] Haas, A., Scheglmann, D., Lazar, T., Gallwitz, D. and Wickner, W. (1995) EMBO J. 14, 5258–5270.