Mai1p is essential for maturation of proaminopeptidase I but not for autophagy

Henning Barth, Khuyen Meiling-Wesse, Ulrike D. Epple, Michael Thumm*

University of Stuttgart, Institute of Biochemistry, Pfaffenwaldring 55, 70569 Stuttgart, Germany

Received 6 December 2001; revised 27 December 2001; accepted 27 December 2001

First published online 17 January 2002

Edited by Horst Feldmann

Abstract We here identify Mai1p, a homologue of the autophagy protein Aut10p, as a novel component essential for proaminopeptidase I (proAPI) maturation under non-starvation conditions. In *mai1*Δ cells mature vacuolar proteinases are detectable and vacuolar acidification is normal. In *mai1*Δ cells autophagy occurs, though at a somewhat reduced level. This is indicated by proAPI maturation during starvation and accumulation of autophagic bodies during starvation with phenylmethylsulfonyl fluoride. Homozygous diploid *mai1*Δ cells sporulate, but with a slightly reduced frequency. Biologically active Ha-tagged Mai1p, chromosomally expressed under its native promoter, is at least in part peripherally membrane-associated. In indirect immunofluorescence it localizes to the vacuolar membrane or structures nearby. In some cells Ha-tagged Mai1p appears concentrated at regions adjacent to the nucleus. © 2002 Published by Elsevier Science B.V. on behalf of the Federation of European Biochemical Societies.

Key words: Autophagy; Cytoplasm to vacuole targeting; Aminopeptidase I

1. Introduction

Aminopeptidase I, a vacuolar enzyme, is synthesized as a cytosolic proform, and proteolytically matured upon arrival in the vacuole. In contrast to other vacuolar proteinases proaminopeptidase I (proAPI) does not enter the classical route via the endoplasmic reticulum and Golgi to the vacuole [1]. In non-starved cells proAPI instead uses the cytoplasm to vacuole targeting (cvt) pathway and in cells starved for nitrogen it is targeted to the vacuole via autophagy. For reviews see [2,3].

The cvt pathway starts with the formation of 150 nm diameter double membrane-layered vesicles (cvt vesicles), which specifically enclose proAPI, but exclude cytosol [4]. The outer membrane layer of these cvt vesicles fuses with the vacuolar membrane and a still membrane-enclosed cvt body is released to the vacuolar lumen. After disintegration of this vesicle, proAPI is matured by proteinase B. In starved cells autophagy is induced and proAPI is targeted via this otherwise unspecific process to the vacuole. Autophagy and the cvt pathway are mechanistically very similar and share most of their molecular components [5,6]. Even though during autophagy significantly larger ~300 nm transport vesicles (autophagosomes) are formed, which unspecifically enclose parts of the cytosol.

Beside the extensive mechanistical and genetic overlap between autophagy and the cvt pathway a number of gene products such as Tlg2p [7], Vps45p [7], Vac8p [8] and Cvt19p [9,10] have been identified. These are only required for the cvt pathway but not for autophagy. On the other hand, gene products such as Apg17p are only needed for autophagy [11].

The molecular principles of autophagy have interested us for many years. Based on our initial EMS mutagenesis screen [12] we identified eight autophagy (*AUT*) genes. Since identification of mutated genes by complementation with a genomic library is time consuming, we started a reverse genetics approach using the ~5000 yeast deletion strains, generated in the world wide 'Yeast Deletion Project'. We screened these strains for defects to survive nutrient limitation, a phenotype of autophagy mutants, using a simple colony-based phenotype. On starvation plates containing the red dye phloxine B [13] starvation sensitive colonies turn red, due to the staining of dead cells.

This screen identified ~1300 strains, which we further analyzed in immunoblots for defects in proAPI maturation. We thus identified *AUT8* and *AUT10*, as novel autophagy genes, which both also affect the cvt pathway [14,15]. Interestingly, Aut10p shares significant homologies with Ypl100wp and Ygr223wp, two *Saccharomyces cerevisiae* proteins of unknown function. Here we identify the essential function of Ypl100wp (Mai1p) for proAPI maturation under non-starvation conditions. In *mai1*Δ cells mature vacuolar proteinase B and carboxypeptidase Y are detectable and quinacrine accumulation indicated normal vacuolar acidification. Interestingly, Mai1p is not essential for autophagy. In *mai1*Δ cells starved for nitrogen, proAPI is matured and in the presence of the proteinase B inhibitor phenylmethylsulfonyl fluoride (PMSF) autophagic bodies accumulated in the vacuole. Indirect immunofluorescence localized a biologically active Ha-tagged Mai1p expressed from the chromosome under its endogenous promoter to parts of the vacuolar membrane or structures closely attached. Most interestingly, Mai1p appeared concentrated in regions of the vacuolar membrane adjacent to the nucleus in some cells.

2. Materials and methods

2.1. Strains, media, antibodies and reagents

Standard media were used [16]. Starvation medium contained 1% potassium acetate. Antibodies: anti-Ha: clone 16B12 (BabCo, USA); anti-3-phosphoglycerate kinase, anti-carboxypeptidase Y (Molecular

*Corresponding author. Fax: (49)-711-6854392.
E-mail address: thumm@po.uni-stuttgart.de (M. Thumm).

0014-5793/02/$22.00 © 2002 Published by Elsevier Science B.V. on behalf of the Federation of European Biochemical Societies.
PII: S0014-5793(02)02252-4

Probes, Leiden, The Netherlands); horseradish peroxidase (HRP)-conjugated goat anti-rabbit (Medac, Hamburg, Germany) and HRP-conjugated goat anti-mouse (Dianova, Hamburg, Germany); anti-proAPI [14].

Chemicals: zymolyase 100T (Seikagaku, Tokyo, Japan); PMSF (Sigma, Deisenhofen, Germany); oligonucleotides (MWG-Biotec, Ebersberg, Germany), other analytical grade chemicals were from Sigma or Merck (Darmstadt, Germany). For immunoblots we used the ECL detection kit (Amersham, Braunschweig, Germany).

YHB4 and YHB5, isogenic to WCG4a *Matα ura3 his3-11,15 leu2-3,112* [17], were generated as described below. Homozygous diploid strains isogenic to BY4743 (*MATa/MATα his3Δ1/his3Δ1 leu2Δ0/leu2Δ0 met15Δ0/MET15 LYS2/lys2Δ0 ura3Δ0/ura3Δ0*) were from Euroscarf, Frankfurt, Germany.

2.2. MAI1 chromosomal deletion

A PCR fragment with the kanamycin resistance gene flanked by *MAI1* sequences was generated using pl100w-1 (TTCCACTCCTTTG-GATTTGAAATAGACAGATAGAAAAGGATCAGCTGAAGCT-TCGTACGC), and pl100w-2 (CGTACAATATCTATTAAGATTA-TGAAAACTGCACATATGCAGCATAGGCCACTAGTGGATCT-G) and plasmid pUG6 [18]. Chromosomal replacement of *MAI1* with this fragment in WCG4a yielded YHB4. Gene replacement was confirmed by Southern analysis (not shown).

2.3. Probing vacuolar acidification

Cells were washed in 10 mM HEPES, 2% glucose pH 7.4, incubated in this buffer for 3–5 min with 200 μM quinacrine [19] and washed with buffer again. Cells were then viewed using a Zeiss Axioskop 2 plus fluorescence microscope.

2.4. Determination of sporulation frequency

Homozygous diploid cells grown to stationary phase were shifted to 1% K-acetate. After 3 and 6 days at room temperature the number of sporulated cells was counted and expressed as percentage of total cells. In the genetic background used (BY4743, Euroscarf, Frankfurt, Germany) large amounts of diades were often seen in addition to tetrads. Sporulated cells are counted as the sum of both.

2.5. Ha-tagging of Mai1p

YHB5 was made by chromosomal integration of a PCR fragment consisting of a triple Ha-tag and a *Schizosaccharomyces pombe HIS5* marker in WCG4a. The fragment was generated with plasmid p3xHA-HIS5 (S. Munroe, Cambridge, UK), and primers PL100w-HA-1 (ACCTGGTGAATGTGTGCT GACTAAAAATAA TAAAT-TTACACAT CATCATCATCATCATG GAGCAGGGGCGGG-TGC), and PL100w-HA-2 (CGTACAATATCTA TTAAG ATTAT GAA AACTGCACATA TGCAGAGGTCGACGGTATCGATA-AG). Transformants were selected on plates lacking histidine. Southern blotting confirmed correct gene replacement (not shown).

2.6. Indirect immunofluorescence

Immunofluorescence was done according to [20] with the following modifications. Cells were fixed for 2 h at room temperature by adding 3.5% formaldehyde and 1 M potassium phosphate pH 6.5 to a concentration of 100 mM. After washing three times with SP buffer (1.2 M sorbitol, 0.1 M KH_2PO_4 pH 6.5), the cells were resuspended in SP buffer containing 20 mM β-mercaptoethanol and 45 μg/ml zymolyase 100T and spheroplasted at 30°C for 30 min. After incubation with mouse anti-Ha antibody, Cy™3-conjugated goat anti-mouse IgG (Dianova, Hamburg, Germany) served as the secondary antibody. Cells were then covered with Citifluor containing DAPI (200 μl Citifluor+8 μl DAPI (1 μg/ml)) and visualized with a Zeiss Axioskop 2 plus with an Axiocam image system. Overlays were pseudo-colorized with Photoshop.

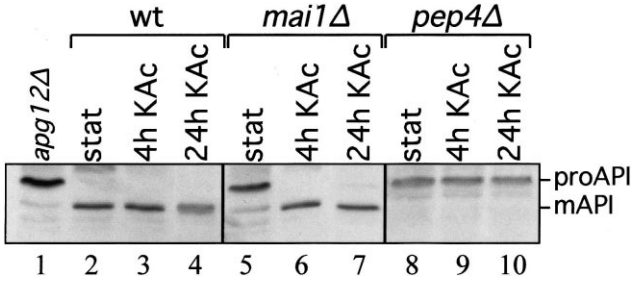


Fig. 1. ProAPI maturation is impaired in non-starved *mai1*Δ cells. Extracts of cells of the stationary phase (lanes 1, 2, 5, and 8), starved for 4 h (lanes 3, 6, and 9) or 24 h (lanes 4, 7, and 10) in 1% K-acetate were separated by SDS–PAGE, electroblotted on PVDF membrane and probed with antibodies against proAPI. *apg12*Δ cells, defective in autophagy, and *pep4*Δ cells, lacking vacuolar proteinase A, are included. wt: wild-type; proAPI: proaminopeptidase I; mAPI: mature aminopeptidase I.

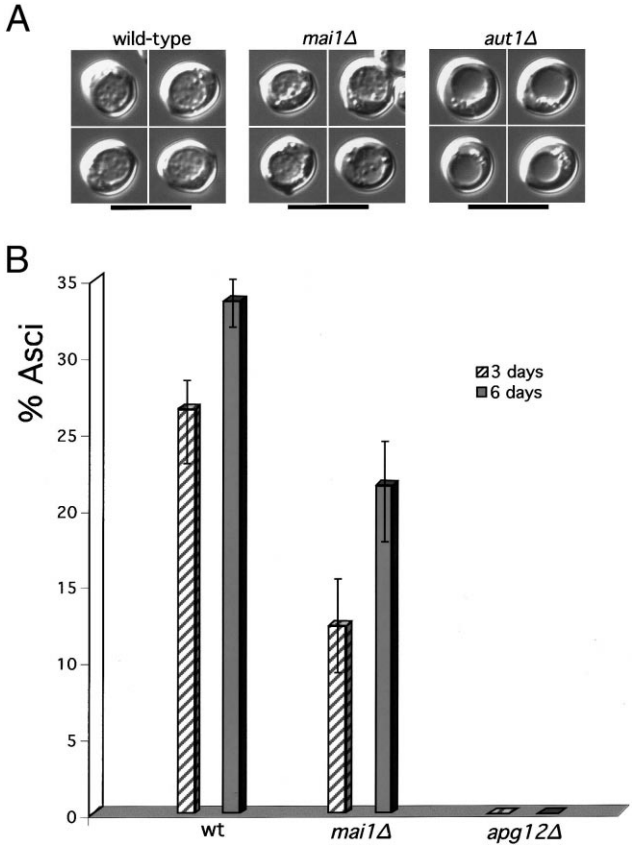


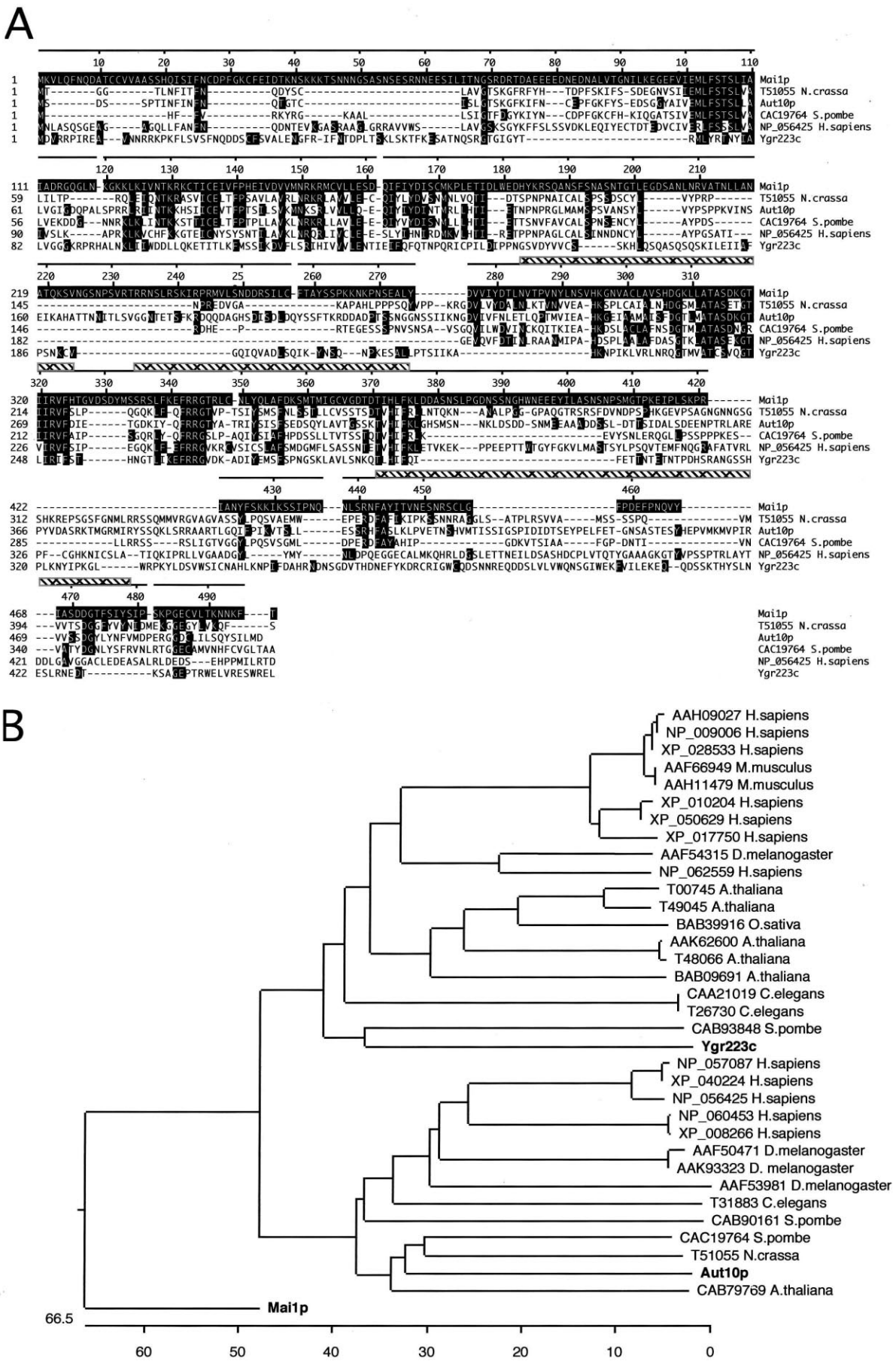
Fig. 2. Mai1p is not essential for autophagy. A: Nomarski optics visualizes autophagic bodies accumulating in the vacuole of *mai1*Δ cells starved for 4 h in 1% K-acetate in the presence of the proteinase B inhibitor PMSF. *aut1*Δ cells are defective in autophagy. Bar: 10 μm. B: Homozygous diploid *mai1*Δ cells can sporulate. Stationary grown cells were incubated in 1% K-acetate and the number of sporulated cells (both diades and tetrads, see Section 2.4) was counted after 3 and 6 days and expressed as percentage of total cells. Cells defective in autophagy (*apg12*Δ) are included. The average of four independent measurements is shown.

2.7. Membrane association of Mai1-Hap

Stationary cells spheroplasted with oxalyticase were hypotonically lysed as described [9]. Aliquots were then incubated with equal volumes of buffer, 5 M urea, 0.2 M Na_2CO_3 (pH 11.2), 2 M K-acetate or

→

Fig. 3. Mai1p shows homologies with the *S. cerevisiae* proteins Aut10p and Ygr223cp and ~30 proteins from different species. A: Clustal alignment [24] shows that homologies are not restricted to the putative WD-40 repeats of Mai1p (hatched boxes). Residues identical with Mai1p are shaded. B: Phylogenetic analysis (using Clustal) groups the homologues in subfamilies, interestingly Mai1p is the sole member of its subfamily.


A
B
AAH09027 H.sapiens
NP_009006 H.sapiens
XP_028533 H.sapiens
AAF66949 M.musculus
AAH11479 M.musculus
XP_010204 H.sapiens
XP_050629 H.sapiens
XP_017750 H.sapiens
AAF54315 D.melanogaster
NP_062559 H.sapiens
T00745 A.thaliana
T49045 A.thaliana
BAB39916 O.sativa
AAK62600 A.thaliana
T48066 A.thaliana
BAB09691 A.thaliana
CAA21019 C.elegans
T26730 C.elegans
CAB93848 S.pombe
Ygr223c
NP_057087 H.sapiens
XP_040224 H.sapiens
NP_056425 H.sapiens
NP_060453 H.sapiens
XP_008266 H.sapiens
AAF50471 D.melanogaster
AAK93323 D. melanogaster
AAF53981 D.melanogaster
T31883 C.elegans
CAB90161 S.pombe
CAC19764 S.pombe
T51055 N.crassa
Aut10p
CAB79769 A.thaliana
Mai1p
66.5
60
50
40
30
20
10
0

2% Triton X-100, respectively. Centrifugation for 1 h at 100 000 × *g* then yielded supernatant and pellet fractions.

3. Results

3.1. ProAPI maturation is impaired in non-starved *mai1Δ* (*ypl100wΔ*) cells

We screened the ~5000 yeast deletion strains of the 'Yeast Deletion Project' for strains with a reduced ability to survive nitrogen starvation. This phenotype, typical for autophagy mutants and to a significantly less extent also for strains defective in the cvt pathway, was monitored on starvation plates containing the red dye phloxine B. Since more than 1300 deletion strains were positive, we further analyzed in immunoblots the proAPI maturation in non-starved cultures of these cells. We here identify *ypl100wΔ* cells in this screen. Due to the defect in maturation of proaminopeptidase I we termed this gene *MAI1*.

For further analysis we chromosomally deleted *ypl100w* (*MAI1*) in our wild-type WCG4a (see Section 2.2) and confirmed gene replacement by Southern analysis (not shown). ProAPI is transported to the vacuole via autophagy in starving cells and via the cvt pathway in non-starved cells. As expected in our background a significant defect in proAPI maturation is also seen in *mai1Δ* cells under non-starvation conditions (Fig. 1, lane 5). This shows the requirement of Mai1p for proAPI maturation under conditions where the cvt pathway is active. However, shifting the cells to nitrogen-free starvation medium for 4 and 24 h, respectively, rescued this maturation defect (Fig. 1, lanes 6 and 7), suggesting the occurrence of starvation-induced autophagy.

3.2. Mai1p is not essential for autophagy

In the vacuolar lumen of wild-type cells autophagic bodies are rapidly broken down, dependent on vacuolar proteinase B [21]. Addition of the proteinase B inhibitor PMSF to starving wild-type cells therefore leads to the accumulation of autophagic bodies in the vacuole [21]. Since autophagic bodies are visible in Nomarski optics, this is an easy way to monitor autophagy (Fig. 2A). Consistent with proAPI maturation during starvation, autophagic bodies accumulate in *mai1Δ* cells under these conditions (Fig. 2A). A slightly reduced number of autophagic bodies per vacuole indicates the occurrence of autophagy, even if at a slightly reduced level compared to wild-type cells. Autophagy mutants are unable to undergo the cell differentiation process of sporulation [22]. We also used this phenotype to assess the autophagic capacity of homozygous diploid *mai1Δ* cells. These cells sporulate, however the sporulation frequency (see Section 2.4), determined in an average of four independent experiments, only reached ~65% of the wild-type level (Fig. 2B). This further supports that autophagy occurs in *mai1Δ* cells, but at a somewhat reduced level.

3.3. Homologues and features of Mai1p

MAI1 encodes a 496 amino acid protein with a calculated molecular mass of 55 kDa. No putative transmembrane domains are predicted. Search for motives identified three putative WD40 repeats from amino acids 284–325, 335–376, and 442–479, respectively (Fig. 3A). Mai1p is not classified as a typical WD repeat protein, however, since WD repeat proteins contain at least four repeats [23]. WD repeat proteins,

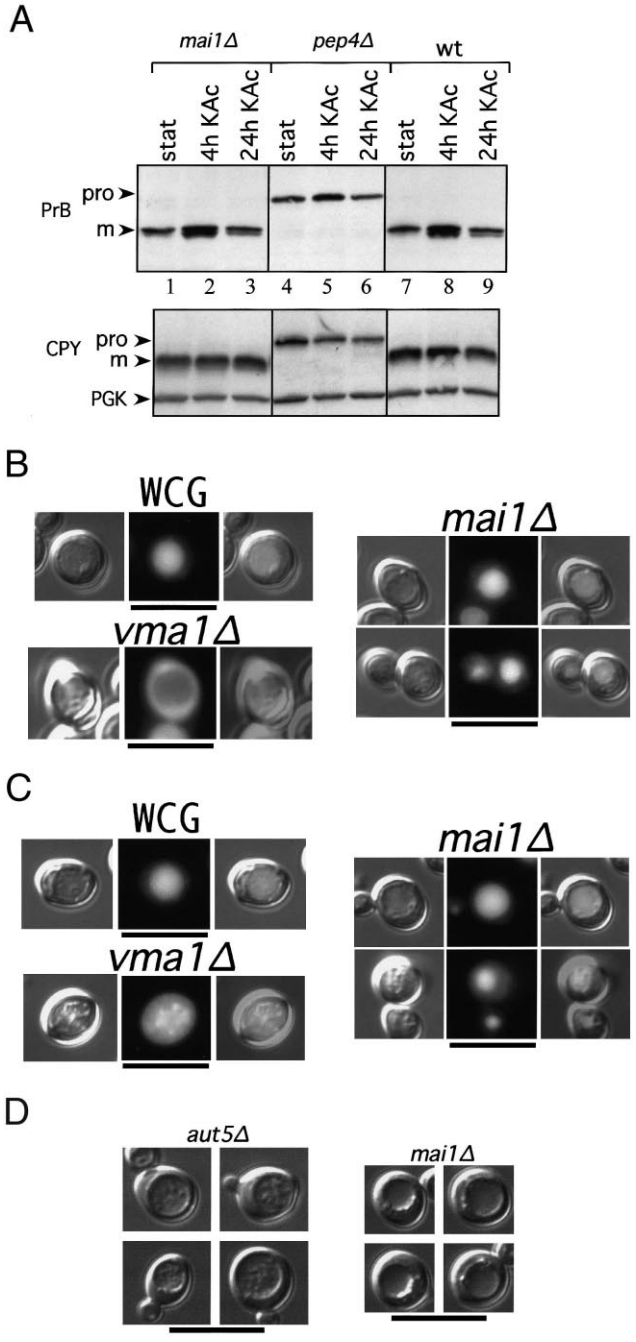


Fig. 4. A: Mature vacuolar proteinase B and carboxypeptidase Y are detectable in *mai1Δ* cells. Crude extracts of cells grown to the stationary growth phase (lanes 1, 4, and 7) starved for 4 h (lanes 2, 5, and 8) or 24 h (lanes 3, 6, and 9) in 1% K-acetate were subjected to SDS–PAGE, transferred to PVDF membrane and probed with antibodies against proteinase B (PrB) (upper panel), or carboxypeptidase Y (CPY) and 3-phosphoglycerate kinase (PGK) (lower panel). pro: proform; m: mature. B,C: Accumulation of the fluorescent dye quinacrine in the vacuole of *mai1Δ* cells indicates normal vacuolar acidification. Cells grown to the stationary growth phase (B) or starved for 4 h in 1% K-acetate (C) were stained with quinacrine (see Section 2.3) and visualized with Nomarski (left) and fluorescence microscopy (middle). An overlay of Nomarski and fluorescence is on the right. *vma1Δ* cells are defective in vacuolar acidification. WCG: wild-type. Bar: 10 μm. D: *mai1Δ* cells are able to lyse autophagic bodies inside their vacuoles. *aut5Δ* [20] cells are defective in lysing autophagic bodies. Cells were starved for 4 h in 1% K-acetate and visualized with Nomarski optics. Bar: 10 μm.

a well studied member is the Gβ subunit of heterotrimeric G-proteins, are thought to act as a scaffold for interactions with other proteins. Mai1p has over 30 homologues in various species like *Neurospora crassa*, *S. pombe*, *Caenorhabditis elegans*, *Homo sapiens*, *Drosophila melanogaster* and *Arabidopsis thaliana* (Fig. 3A,B). Mai1p is a homologue to Aut10p (19% identity) and Ygr223cp (14% identity) from *S. cerevisiae*. Phylogenetic analysis using Clustal [24] suggests the existence of three subfamilies, each containing one of the yeast homologues. Most interestingly, Mai1p is the only representative of its subfamily (Fig. 3B).

3.4. Mature vacuolar proteinases are detectable in *mai1Δ* cells and vacuolar acidification is wild-type like

A prerequisite for maturation of proAPI is the presence of mature vacuolar proteinase B. We analyzed crude extracts of *mai1Δ* cells starved for nitrogen (Fig. 4A, lanes 2 and 3) and from the stationary growth phase (Fig. 4A, lane 1) in immunoblots and detected significant amounts of mature vacuolar proteinase B and carboxypeptidase Y.

Vacuolar disintegration of autophagic bodies further requires vacuolar acidification [25]. Vacuolar acidification is indicated by the accumulation of the fluorescent dye quinacrine [19] in the vacuole. In both starved and non-starved *mai1Δ* cells quinacrine accumulated in the vacuole suggesting normal vacuolar acidification (Fig. 4B,C).

In *mai1Δ* cells, starved for nitrogen without PMSF, no autophagic bodies are visible in the vacuole (Fig. 4D). The ability to break down autophagic bodies further confirms both the presence of mature vacuolar proteinases and normal vacuolar acidification.

3.5. Biologically active Ha-tagged Mai1p is recruited to the vacuolar membrane or structures nearby

To localize Mai1p, we generated a chromosomally integrated triple Ha-tagged Mai1p (Mai1-Hap) expressed from its native promoter. As detailed in Section 2.5, we chromosomally integrated a PCR fragment consisting of a triple Ha-tag and an auxotrophic marker at the 3′-terminus of the *MAI1* gene. Southern analysis confirmed correct integration (not shown). Chromosomally expressed Mai1-Hap was clearly detectable at the expected molecular mass of ~60 kDa in im-

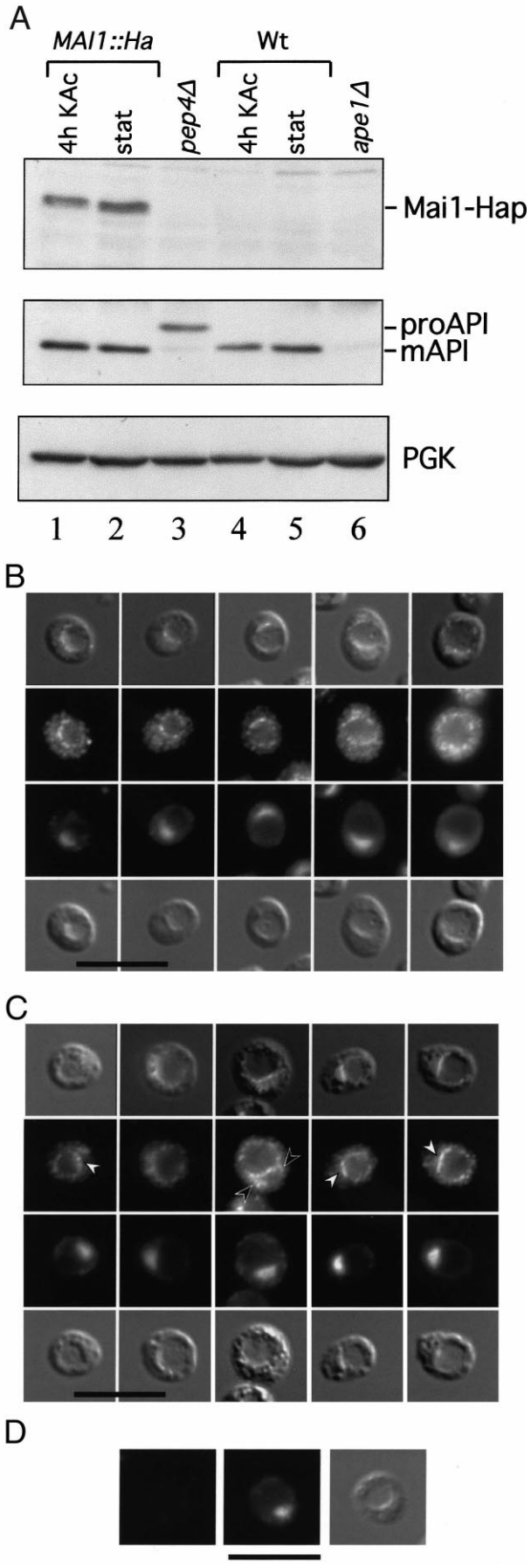


Fig. 5. A: A triple Ha-tagged Mai1p (Mai1-Hap) expressed from the chromosome under its native promoter is biologically active as indicated by proAPI maturation under non-starvation conditions. Extracts of cells grown to the stationary growth phase (lanes 2 and 5) or starved for 4 h in 1% K-acetate (lanes 1, 3, 4, and 6) were subjected to SDS–PAGE, electroblotted on PVDF membrane and probed with antibodies against Ha (upper panel), proAPI (middle panel) and 3-phosphoglycerate kinase (PGK) (lower panel). ProAPI: proaminopeptidase I; mAPI: mature aminopeptidase I. B,C: Indirect immunofluorescence microscopy of cells expressing under its endogenous promoter a triple Ha-tagged Mai1p from the chromosome. White arrowheads show regions adjacent to the nucleus where Mai1-Hap appears concentrated in some cells. Black arrowheads mark offshoots to the nucleus. Cells grown to the stationary growth phase (B) or starved for 4 h in 1% K-acetate (C) were fixed, spheroplasted and processed for indirect immunofluorescence with antibodies against Ha (see Section 2.6). From bottom to top: Nomarski, nuclear staining with DAPI, immunofluorescence and an overlay is shown. D: Wild-type cells lacking a Ha-tag. From left to right: immunofluorescence, nuclear staining with DAPI and Nomarski. Bar: 10 μm.

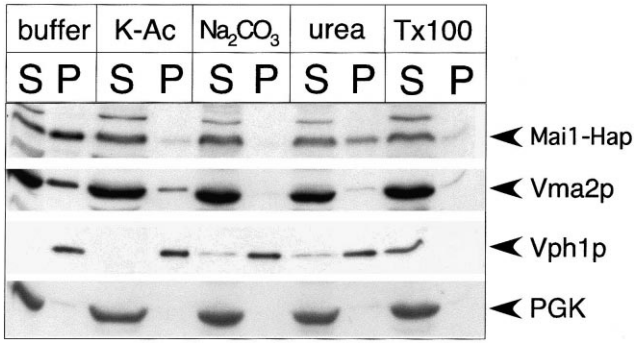


Fig. 6. Mai1-Hap is at least in part peripherally membrane-associated. Osmotically lysed spheroplasts of stationary cells expressing Mai1-Hap from the chromosome were treated with buffer, 1 M K-acetate, 0.1 M Na_2CO_3, 2.5 M urea and 1% Triton X-100. After centrifugation supernatant (S) and pellet (P) fractions were subjected to immunoblotting. Controls are: Vph1p (integral membrane protein); Vma2p (peripheral membrane protein); PGK (3-phosphoglycerate kinase) (soluble cytosolic protein).

munoblots using antibodies against Ha (Fig. 5A, lanes 1 and 2). Biological activity of the Ha-tagged Mai1p (Mai1-Hap) is indicated by proAPI maturation in non-starved cells (Fig. 5A, lane 2, middle panel).

Indirect immunofluorescence microscopy with an anti-Ha antibody detected Mai1-Hap in non-starved cells at punctate structures near or on the vacuolar membrane and in the cytosol (Fig. 5B). Cytosolic staining appeared somewhat granulated. In cells starved for 4 h in nitrogen-free medium (Fig. 5C) a significant staining of the vacuolar rim was also seen. The limited resolution of microscopy prevents to clearly distinguish whether the vacuolar membrane itself or structures nearby were stained. Compared to non-starved cells more Mai1-Hap seemed to be concentrated at the vacuolar surface. Most interestingly, Mai1-Hap appeared focused at regions of the vacuolar membrane adjacent to the nucleus in some cells (Fig. 5C, white arrowheads). Furthermore, in some cells, offshoots from the vacuolar membrane are seen in proximity of the nucleus (Fig. 5C, black arrowheads). Consistent with the localization, Mai1-Hap is at least in part peripherally membrane-associated (Fig. 6).

4. Discussion

Mai1p is a homologue to Aut10p and Ygr223cp of *S. cerevisiae* and ~30 proteins from yeast to humans. Phylogenetic analysis groups the homologues in three subfamilies (Fig. 3B), each containing one yeast homologue. Since Mai1p is the sole representative of his subfamily this might suggest a specific function of this protein for yeast. In a previous study we identified Aut10p as a component of both autophagy and the cvt pathway [15]. We now show that Mai1p is essential for proAPI maturation in non-starved cells, conditions where the cvt pathway is active (Fig. 1). However, Mai1p is not essential for starvation-induced autophagy (Figs. 1 and 2). Mai1p in this respect resembles Tlg2p, Vps45p, and Vac8p [7,8]. *tlg2*Δ, *vps45*Δ and *vac8*Δ cells are defective in the cvt pathway, but after starvation induction of autophagy proAPI is matured. It is presumed that these proteins affect a step specific for the cvt pathway, like for example formation of cvt vesicles or conversion between the cvt pathway and autophagy. Further extensive studies are necessary to clarify whether Mai1p plays a similar role in maturation of proAPI.

One might speculate about a similar function of Ygr223cp, the third yeast homologue. We also generated *ygr223c*Δ cells in our background and included these cells in our experiments (not shown). Negative results are difficult to communicate, but under the conditions used no obvious phenotype could be detected for *ygr223c*Δ cells. We therefore expect that Ygr223cp either has a redundant function or acts in another pathway.

This fits well with a most recent phenotypic analysis of these three open reading frames. Also in this study no specific phenotype was found for *ygr223c*Δ cells [26]. Based mainly on growth phenotypes on several media, the authors expect that the three proteins are not part of the same pathway, and speculate that Yfr021wp (Aut10p) and Ypl100wp (Mai1p) might be related to amino acid signalling pathways. Interestingly, a *mai1*Δ *aut10*Δ *ygr223c*Δ triple deletion strain did not grow on a specific acetate medium at 15°C, but the double deletion strains did [26]. This suggests that unless each of the proteins has a distinct function, there is some functional relationship between all three proteins.

Mai1p lacks obvious transmembrane domains. Indeed in indirect immunofluorescence a biologically active Ha-tagged Mai1p expressed at physiological levels is seen partly in the cytosol (Fig. 5B,C). However, a significant amount of Mai1-Hap is recruited to the vacuolar membrane or structures nearby (Fig. 5B,C). Interestingly, Mai1-Ha appeared focused in some cells on the vacuolar membrane adjacent to the nucleus (Fig. 5C, white arrowheads). Sometimes offshoots from the vacuolar surface to the nucleus are also visible (Fig. 5C, black arrowheads). Mai1-Hap is at least in part peripherally attached to a membrane (Fig. 6). It is tempting to speculate that this is due to interaction with still unknown membrane proteins. Identification of such putative interaction partners should give further insights in the function of Mai1p.

Acknowledgements: We thank the 'Yeast Deletion Project' labs and D.H. Wolf for support. This work was supported by DFG Grant Wo210/12-5 and the 'Fonds der Chemischen Industrie'.

References

[1] Klionsky, D.J., Cueva, R. and Yaver, D.S. (1992) J. Cell Biol. 119, 287–299.
[2] Klionsky, D.J. and Ohsumi, Y. (1999) Annu. Rev. Cell Dev. Biol. 15, 1–32.
[3] Thumm, M. (2000) Microsc. Res. Tech. 51, 563–572.
[4] Baba, M., Osumi, M., Scott, S.V., Klionsky, D.J. and Ohsumi, Y. (1997) J. Cell Biol. 139, 1687–1695.
[5] Harding, T.M., Hefner-Gravink, A., Thumm, M. and Klionsky, D.J. (1996) J. Biol. Chem. 271, 17621–17624.
[6] Scott, S.V., Hefner-Gravink, A., Morano, K.A., Noda, T., Ohsumi, Y. and Klionsky, D.J. (1996) Proc. Natl. Acad. Sci. USA 93, 12304–12308.
[7] Abeliovich, H., Darsow, T. and Emr, S.D. (1999) EMBO J. 18, 6005–6016.
[8] Scott, S.V., Nice, D.C., Nau, J.J., Weisman, L.S., Kamada, Y., Keizer-Gunnink, I., Funakoshi, T., Veenhuis, M., Ohsumi, Y. and Klionsky, D.J. (2000) J. Biol. Chem. 275, 25840–25849.
[9] Scott, S.V., Guan, J., Hutchins, M.U., Kim, J. and Klionsky, D.J. (2001) Mol. Cell 7, 1131–1141.
[10] Leber, R., Silles, E., Sandoval, I.V. and Mazon, M.J. (2001) J. Biol. Chem. 276, 29210–29217.
[11] Kamada, Y., Funakoshi, T., Shintani, T., Nagano, K., Ohsumi, M. and Ohsumi, Y. (2000) J. Cell Biol. 150, 1507–1513.

[12] Thumm, M., Egner, R., Koch, B., Schlumpberger, M., Straub, M., Veenhuis, M. and Wolf, D.H. (1994) FEBS Lett. 349, 275–280.
[13] Tsukada, M. and Ohsumi, Y. (1993) FEBS Lett. 333, 169–174.
[14] Barth, H. and Thumm, M. (2001) Gene 274, 151–156.
[15] Barth, H., Meiling-Wesse, K., Epple, U.D. and Thumm, M. (2001) FEBS Lett. 508, 23–28.
[16] Ausubel, F.M., Brent, R., Kingston, R.E. and Moore, D.D. (1987) Current Protocols in Molecular Biology, Greene Publishing Associates, New York.
[17] Suriapranata, I., Epple, U.D., Bernreuther, D., Bredschneider, M., Sovarasteanu, K. and Thumm, M. (2000) J. Cell Sci. 113, 4025–4033.
[18] Güldener, U., Heck, S., Fielder, T., Beinhauer, J. and Hegemann, J.H. (1996) Nucleic Acids Res. 24, 2519–2524.
[19] Roberts, C.J., Raymond, C.K., Yamahiro, C.T. and Stevens, T.H. (1991) Methods Enzymol. 194, 644–661.
[20] Epple, U.D., Suriapranata, I., Eskelinen, E.L. and Thumm, M. (2001) J. Bacteriol. 183, 5942–5955.
[21] Takeshige, K., Baba, M., Tsuboi, S., Noda, T. and Ohsumi, Y. (1992) J. Cell Biol. 119, 301–311.
[22] Straub, M., Bredschneider, M. and Thumm, M. (1997) J. Bacteriol. 179, 3875–3883.
[23] Smith, T.F., Gaitatzes, C., Saxena, K. and Neer, E.J. (1999) Trends Biochem. Sci. 24, 181–185.
[24] Higgins, D.G. and Sharp, P.M. (1989) Comput. Appl. Biosci. 5, 151–153.
[25] Nakamura, N., Matsuura, A., Wada, Y. and Ohsumi, Y. (1997) J. Biochem. (Tokyo) 121, 338–344.
[26] Georgakopoulos, T., Koutroubas, G., Vakonakis, I., Tzermia, M., Prokova, V., Voutsina, A. and Alexandraki, D. (2001) Yeast 18, 1155–1171.