

Yeast Sequencing Reports

Cloning and Characterization of the *Hansenula polymorpha* Homologue of the *Saccharomyces cerevisiae* *PMR1* gene

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A gene homologous to *Saccharomyces cerevisiae* *PMR1* has been cloned in the methylotrophic yeast *Hansenula polymorpha*. The partial DNA fragment of the *H. polymorpha* homologue was initially obtained by a polymerase chain reaction and used to isolate the entire gene which encodes a protein of 918 amino acids. The putative gene product contains all ten of the conserved regions observed in P-type ATPases. The cloned gene product exhibits 60.3% amino acid identity to the *S. cerevisiae* *PMR1* gene product and complemented the growth defect of a *S. cerevisiae* *pmr1* null mutant in the EGTA-containing medium. The results demonstrate that the *H. polymorpha* gene encodes the functional homologue of the *S. cerevisiae* *PMR1* gene product, a P-type Ca^{2+} -ATPase. The DNA sequence of the *H. polymorpha* homologue has been submitted to GenBank with the Accession Number U92083. © 1998 John Wiley & Sons, Ltd.

KEY WORDS — yeast; *PMR1*; *Hansenula polymorpha*; Ca^{2+} -ATPase

INTRODUCTION

A wide variety of heterologous proteins have been expressed in yeast for secretory production, offering certain advantages over intracellular production, such as ease of product recovery and correct processing of proteins (Romanos *et al.*, 1992). However, despite high levels of transcription and translation of heterologous genetic information introduced into yeast, often only a fraction of the expressed protein is secreted from the yeast cell. As an approach to overcome this limitation, mutant strains with an increased secretion capacity have been developed. One example of that kind is the *ssc1* mutant of *Saccharomyces cerevisiae* that has

been isolated as a mutant strain with the increased secretion of bovine prochymosin (Smith *et al.*, 1985). Interestingly, *SSC1* was later shown to be identical to *PMR1*, a gene coding for a P-type Ca^{2+} -ATPase, suggesting that the increased secretion resulted from the defect of the Ca^{2+} pump in the secretory pathway (Rudolph *et al.*, 1989). Intensive genetic and biochemical studies have provided evidence that Pmr1p is a Ca^{2+} -transporting ATPase in the Golgi of *S. cerevisiae* (Antebi and Fink, 1992; Sorin *et al.*, 1997). Due to the properties of Pmr1p distinct from sarco/endoplasmic reticulum and plasma membrane Ca^{2+} pumps, it has been suggested that Pmr1p represents a new class of P-type Ca^{2+} -ATPase that plays an important role in the secretory pathway (Sorin *et al.*, 1997). Mutation in the *PMR1* gene has also been shown to increase the secretion of

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some other heterologous proteins, and thus the *pmr1* mutant strains have been considered as suitable hosts for the secretory production of various recombinant proteins that are poorly secreted by the wild type (Turner *et al.*, 1991; Harmsen *et al.*, 1993, 1996; Ramjee *et al.*, 1996).

The methylotrophic yeast, *Hansenula polymorpha*, has been recently developed as an efficient host for the production of foreign proteins (Viale *et al.*, 1992; Hollenberg and Gellissen, 1997). This yeast, along with another methylotrophic yeast, *Pichia pastoris*, has intrinsic features that make it superior to *S. cerevisiae* as a host system: higher promoter strength and ease of growth to high cell density on the inexpensive substrate methanol. As a preliminary step in developing the *pmr1* mutant strain of *H. polymorpha*, we isolated and characterized a gene homologous to *S. cerevisiae* *PMR1* from the methylotrophic yeast *H. polymorpha*.

MATERIALS AND METHODS

Yeast strains and media

The *H. polymorpha* strain used in this study was DL-1 (ATCC 26012). The *pmr1* null strain of *S. cerevisiae* was constructed by transforming the strain L3262a (*MATa ura3-52 leu2-3,112 his4-34*) with the linear DNA fragment bearing the *S. cerevisiae* *PMR1* gene disrupted with the *LEU2* gene. A 3.7 kb DNA fragment containing the whole *S. cerevisiae* *PMR1* gene was obtained by polymerase chain reaction (PCR) and the 2.1 kb *AocI/XbaI* fragment from the *PMR1* gene was deleted and replaced by the *LEU2* gene. All standard media for general cultivation of yeast were prepared as outlined by Sherman *et al.* (1986) and synthetic Ca^{2+} -deficient media were prepared as previously described (Rudolph *et al.*, 1989).

Nucleic acid manipulations

General DNA manipulations were performed as described by Sambrook *et al.* (1989) and total yeast DNA was isolated according to the method of Holm *et al.* (1986). The DNA probes were labelled with digoxigenin using DIG labelling kits (Boehringer Mannheim).

DNA sequencing and analysis

DNA sequencing was done with double-stranded templates on an ABI Model 373A automatic DNA sequencer (model 373A; Applied Biosystems). Comparison of deduced amino

acid sequence was made by FASTA searches of GenBank, EMBL, and SWISS-PROT databases. The DNA sequence of the *H. polymorpha* *PMR1* gene presented in this paper has been submitted to GenBank with the accession number U92083.

RESULTS AND DISCUSSION

Cloning of the *H. polymorpha* *PMR1* gene

Recently we have successfully cloned the *Yarrowia lipolytica* *PMR1* gene using the degenerated primers (Park *et al.*, 1998), which were designed based on the amino acid sequences of the conserved regions a (amino acids 134–140) and j (amino acids 724–730) in the *S. cerevisiae* *Pmr1* protein (Rudolph *et al.*, 1989) and the rat homologue (Günteski-Hamblin *et al.*, 1992). In order to clone a *PMR1* homologue in *H. polymorpha*, a PCR experiment was carried out using the same primers and a single PCR product of 1.8 kb was amplified from the genomic DNA of *H. polymorpha* DL-1. DNA sequencing of the PCR product revealed a high sequence homology to the *S. cerevisiae* *PMR1* gene, suggesting that it is a partial fragment of the *H. polymorpha* *PMR1* gene. The PCR product was used as a probe in Southern blot analysis of *H. polymorpha* chromosomal DNA, which produced a strong hybridization signal at the 6.0 kb *EcoRI* fragment, at the 6.5 kb *BamHI* fragment, and at the 8.0 kb *HindIII* fragment (Figure 1). To clone the full-length *H. polymorpha* *PMR1* gene, the *EcoRI* fragments of 5–6 kb and the *BamHI* fragments of 6–7 kb of *H. polymorpha* chromosomal DNA were gel-eluted and ligated into the *EcoRI* site and the *BamHI* site of the bacterial plasmid pBluescript (Stratagene), respectively. From the constructed libraries, positive clones hybridized with the PCR product were isolated and partially sequenced. A 6.0 kb *EcoRI* fragment was found to contain the whole coding region of the *H. polymorpha* *PMR1* gene and the nucleotide sequence of the 3.5 kb *BglII-PstI* DNA fragment was determined (Figure 2).

Sequence analysis of *Hansenula polymorpha* *PMR1*

The amino acid sequence deduced from the DNA sequence of *H. polymorpha* *PMR1* revealed that the coding region contains 2754 bp which code for a protein of 918 amino acids with a molecular weight of 100,266 daltons and a pI of 5.4 (Figure 3). The putative gene product has all

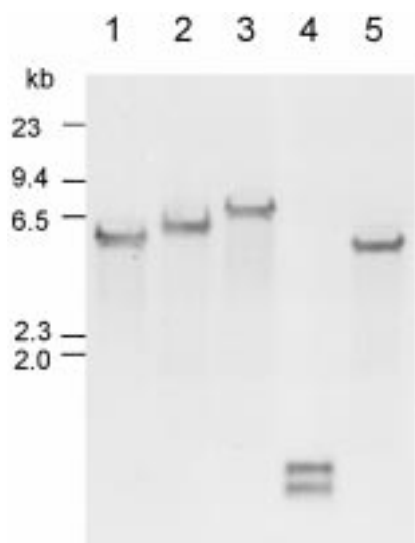


Figure 1. Southern hybridization analysis of *H. polymorpha* chromosome DNA. 6 µg of chromosome DNA from *H. polymorpha* DL-1 was digested with *Eco*RI (lane 1), *Bam*HI (lane 2), *Hind*III (lane 3), *Sal*I (lane 4), and *Bam*HI/*Hind*III (lane 5), fractionated in 0.8% agarose gel, and then hybridized with the digoxigenin-labelled 1.8 kb DNA fragment of the *H. polymorpha* *PMR1* gene obtained by the polymerase chain reaction.

ten (a–j) of the conserved regions observed in the P-type ATPase family (Serrano, 1988). In particular, region c shows the conserved T₁₉₉-G-E₂₀₁ that is thought to function in removing the phosphate group from the phosphorylated enzyme intermediate. Region f contains an aspartic acid residue, which is the phosphorylation site of P-type ATPases, at D₃₅₉KTGTG₃₆₄. Regions g and i display the ATP-binding sites remarkably similar

to those found in the family of P-type ATPases. Besides these conserved regions, the cloned gene product has a hydrophobic region near the carboxyl terminus, which shows a significant similarity to the transmembrane regions found in Ca²⁺-ATPases. Furthermore, near the amino terminus lies a stretch of 26 amino acids closely related to the Ca²⁺-binding site present in a variety of Ca²⁺-binding/regulatory proteins and *S. cerevisiae* Pmr1p (Rudolph *et al.*, 1989). The presence of these characteristic regions suggests that the cloned gene product is a P-type Ca²⁺-ATPase.

Comparison of the amino acid sequences with other P-type Ca²⁺-ATPases showed that there is the highest sequence identity (60.3% identity) between the Pmr1 proteins of *S. cerevisiae* and *H. polymorpha* (Figure 4). The relatively high sequence similarity (57.7% and 42.9% identities, respectively) was obtained with the *Y. lipolytica* Pmr1p (Park *et al.*, 1998) and the rat Ca²⁺-ATPase encoded by RS10-31 (Günteski-Hamblin *et al.*, 1992). In contrast, the relatively low sequence similarity (25.6% and 31% identities, respectively) was observed to the rat plasma membrane Ca²⁺-ATPase, PMCA3a (Greeb and Shull, 1989) and to the human sarco/endoplasmic reticulum Ca²⁺-ATPase, SERCA1 (Zhang *et al.*, 1995). The comparison analysis suggests that the *H. polymorpha* *PMR1* gene product belongs to a third class of Ca²⁺-ATPase, secretory pathway Ca²⁺-ATPases (Sorin *et al.*, 1997).

The *H. polymorpha* *PMR1* gene has a GC content of 53% and exhibits the codon usage bias for G/C containing triplets. The strong bias of codon usage has been observed in the highly

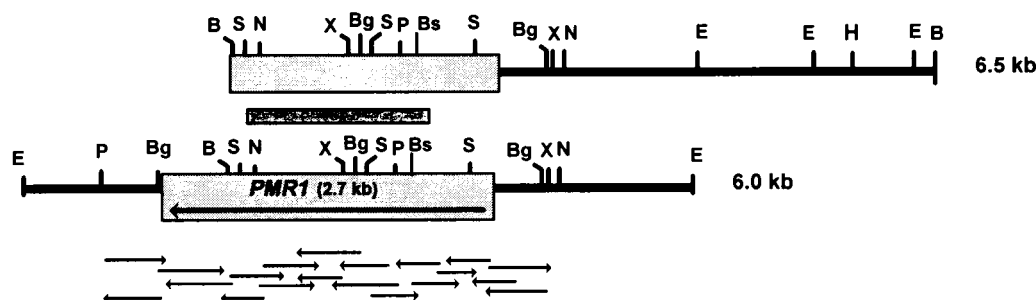


Figure 2. Partial restriction map of the *H. polymorpha* *PMR1* gene. The 6.5 kb *Bam*HI DNA fragment containing the partial coding region of *PMR1* gene and the 6.0 kb *Eco*RI DNA fragment containing the whole *PMR1* coding region (2.7 kb) are shown with the partial restriction enzyme sites (B = *Bam*HI, Bg = *Bgl*II, E = *Eco*RI, H = *Hind*III, N = *Nco*I, P = *Pst*I, S = *Sal*I, X = *Xho*I). The 1.8 kb PCR fragment is indicated as a hatched box. Sequencing strategy of the 3.5 kb *Bgl*II/*Pst*I fragment is indicated by arrows.

Figure 3. Nucleotide sequence of the *H. polymorpha* *PMR1* gene and its predicted amino acid sequences in single-letter code. The amino acid sequences corresponding to the regions a-j conserved in all P-type ATPase are underlined. The putative Ca^{2+} binding site in the N-terminus is marked with a dashed underline. Asterisks mark the transmembrane segments 5 and 6 identified by hydrophobicity analysis (Kyte and Doolittle, 1982; Rudolph *et al.*, 1989).

Region a		Region b	
QEYRSEKSLEALNKLVP	LAH--LTR	VMAS	TLVPGDLVHFQVGDRI
QEYRSEKSLEALNKLVP	AECH--LMR	VLA	STLVPGDLVHFRI
QEYRSEKSLEALNKLVP	PEAH--LIR	VLA	STLVPGDLVEFSVGDRI
QEYRSEKSLEELSKLVP	PECH--CVR	TLA	RDLVPGD TVCLSVGDRV
QERNAENAEALKEYE	PEMGK--VYR	IKA	RDIVPGDIVEVAVGDKVP
NDWSKEKQFRGLQSR	IEQEKF	VPVA	ALVVGDIAQVKYGDLL
			PADGVL
Region c		Region d	
IDESNLTGETNPISK		FMG	TLVRDGHGSGIVIATS-HKTALGG
IDESNLTGENEPVHK		YM	GTLVRDGNGTGIVVGTG-SHTAFGA
IDESNLTGETTPVTK		YM	GTLVKEGHGKGIVVGTG-TNTSFGA
IDESSLTGETTPCSK		FM	GTLVRCGKAKGIVIGTG-ENSEFGE
VDQSILTGESVSVIK		FS	GTNIAAGKALGIVATTG-VGTEIGK
IDESSLTGESDHVRK		ME	GSGRMVVTAVGVNSQTGIIFTLLGA
Region e		Region f	
FQISVCLAVAAIPEGLPI	IVA	MAK	QRAIVKKLP
FQISVSLAVAAIPEGLPI	IVT	MAK	RKAIVRRLLP
FTIGVSLAVAAIPEGLPI	IVT	MSR	QKAIVRKLP
FTISVSLAVAAIPEGLPI	IVT	MV	KKRAIVKKLP
FKIAVALAVAAIPEGLPA	VIT	MAK	KNAIVRSLP
FIIGVTVLVAVPEGLPLA	VIT	MM	KDNNLVRHLDACETMGNATAICS
			DKTGTLT
			AKGAI
			VKGAF
			VKGAG
			MKGAY
			VKGAP
			SKGAS
Region h			
FCGLMGMNDPPRPQVSQSIASLIRGGVHVIMITGDSSEVTATNIARKIGM			
FTGLIGMNDPPRPNVKFAIEQLLQGGVHIIMITGDSSENTAVNIAKQIGI			
FAGLMGLYDPPRPDPVRAIRRLTTGGVRVVMITGDSAAATALSIGRRIGM			
LLGLVGIIDPPRTGVKEAVTTLIASGVSIKMITGDSQETAIAIASRLGL			
FVGVMGLDPPRKEVTGSIQLCRDAGIRVIMITGDNKGTAIAICRRIGI			
CIAVVGIEDPVRPEVPEAIRKCQRAGITVRMITGDNINTARAIAAKCGI			
Region i			
FARTTPEHK-----VSIVRALQMRGDIVAMTGDGVNDAPALKLADIGIAM			
FARATPEHK-----LNIVRALRKRGDVVAMTGDGVNDAPALKLSDIGVSM			
FARTSPEDK-----MKIVKGFQRRGDVVAMTGDGVNDAPALKLADIGIAM			
FYRASPRHK-----MKIIKSLQNGSVVAMTGDGVNDAVALKAADIGVAM			
FARVEPSHK-----SKIVEYLQSYDEITAMTGDGVNDAPALKKAEIGIAM			
LARSSPTDKHTLVKGIIDSTTGEQRQVAVTGDGTNDGPALKKADVGFM			
Region j			
NGTDVAKEAADMVLTDDDFSTILHAIREGKGI	<i>H. polymorpha</i> Pmrlp	(100%)	
IGTDVAKEASDMVLTDDDFSTILTAIEEGKGI	<i>S. cerevisiae</i> Pmrlp	(60.3%)	
GGTDVAKEAADMILTDDDFATILSAIEEGKGI	<i>Y. lipolytica</i> Pmrlp	(57.7%)	
TGTDVCKEADMILVDDDFQTIMSIEEGKGI	RS10-31	(42.9%)	
CGTAVAKTASEMVLADDNFSTIVAAVEEGRAI	SERCA1	(31%)	
AGTDVAKEASDIILTDDNFSTIVKAVMWGRNV	PMCA3a	(25.6%)	

Figure 4. Amino acid sequence alignments of the *H. polymorpha* PMR1 gene product with the conserved domains (regions a-j) of P-type Ca^{2+} -ATPases. RS10-31, Ca^{2+} -ATPase of rat secretory pathway; SERCA1, Ca^{2+} -ATPase of human sarco/endoplasmic reticulum; PMCA3a, Ca^{2+} -ATPase of rat plasma membrane. Values in parentheses indicate the percentage of identical amino acids after sequence alignment to the *H. polymorpha* Pmrlp.

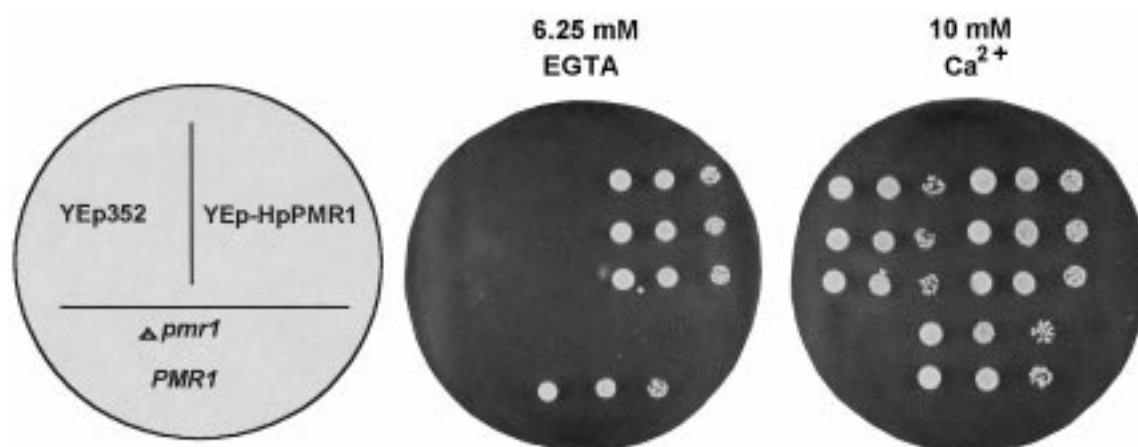


Figure 5. Functional complementation of *S. cerevisiae* *pmr1* mutation by *H. polymorpha* *PMR1*. The *pmr1* null strain of *S. cerevisiae* was transformed with the plasmid YEp352 and the plasmid YEp352-HpPMR1 containing the 6.0 kb *Eco*RI fragment of the *H. polymorpha* *PMR1* gene. Three individual transformants from each transformation were suspended in water and serially diluted. The suspensions were spotted in parallel on the synthetic Ca^{2+} -deficient media supplemented with 6.25 mM-EGTA or 10 mM- Ca^{2+} . The plates were incubated at 30°C for 2 days.

expressed genes of *H. polymorpha*, such as *MOX* and *DAS*, which have a high GC content (55.3% on average) in their coding regions (Janowicz *et al.*, 1985; Lederboer *et al.*, 1985). In contrast, the *H. polymorpha* *URA3* gene, which is thought to be expressed at a very low level, has a GC content of 43% and a balanced codon usage (Merkelbach *et al.*, 1993). It has been generally assumed that highly expressed genes are most biased, whereas barely expressed genes show a random codon usage pattern (Bennetzen and Hall, 1982). As observed in the *MOX* and *DAS* genes, some codons frequently used in *H. polymorpha* *PMR1* are ones rarely used in *S. cerevisiae*, including CTG (Leu), GTG (Val), TCG (Ser), ACG (Thr), CAG (Gln), and GAG (Glu). Similar to other genes of *H. polymorpha* (Janowicz *et al.*, 1985; Lederboer *et al.*, 1985; Bruinenberg *et al.*, 1989; Merkelbach *et al.*, 1993; Agaphonov *et al.*, 1994), TTA (Leu), CTA (Leu), ATA (Ile), GTA (Val), and TCA (Ser) are rare in the *H. polymorpha* *PMR1* gene. This feature of codon usage suggests that the codon preference is significantly different between the two yeast species.

Complementation of *S. cerevisiae* *pmr1* mutation by *H. polymorpha* *PMR1*

The null mutation of *S. cerevisiae* *PMR1* shows pleiotropic effects, including strongly reduced outer-chain glycosylation of the secretory proteins, rapid loss of cell viability in stationary phase

cultures, defective sporulation of homozygous mutant diploids, and Ca^{2+} -related growth defects (Rudolph *et al.*, 1989). All these defects were reversed by adding Ca^{2+} into the culture medium, supporting the proposed role of Pmr1p as a Ca^{2+} pump. To demonstrate that the *H. polymorpha* *PMR1* gene product is a functional homologue of *S. cerevisiae* Pmr1p, we transformed a *S. cerevisiae* *pmr1* null mutant with the plasmid YEp352-HpPMR1 containing the 6.0 kb *Eco*RI fragment of *H. polymorpha* *PMR1* in the YEp352 (Hill *et al.*, 1986) and examined the Ca^{2+} -related growth phenotype. The *Eco*RI fragment is big enough to contain the sequence elements such as promoter and terminator required for *H. polymorpha* *PMR1* to be expressed in yeast (Figure 2). As shown in Figure 5, the *pmr1* null mutant of *S. cerevisiae* could not grow on the EGTA-containing medium but grew as well as the wild-type strain in the Ca^{2+} -containing medium. When the plasmid YEp352-HpPMR1 was introduced into the null mutant, the transformants restored the growth in the EGTA-containing medium. A negative control, the plasmid YEp352 alone could not complement the growth defect of the *S. cerevisiae* null mutant. Along with the high degree of sequence identity to *S. cerevisiae* Pmr1p, the result of functional complementation demonstrates that the *H. polymorpha* gene cloned in this study indeed encodes a homologue of *S. cerevisiae* Pmr1p, P-type secretory pathway Ca^{2+} -ATPase.

Future research will be concentrated on the construction of *H. polymorpha* *pmr1*-defective mutants to investigate the effects of *pmr1* mutation on the secretion and processing of heterologous proteins in *H. polymorpha*, which is expected to be a useful host system for secretory expression.

REFERENCES

- Agaphonov, M. O., Poznyakovski, A. I., Bogdanova, A. I. & Ter-Avanesyan, M. D. (1994). Isolation and characterization of the *LEU2* gene of *Hansenula polymorpha*. *Yeast* **10**, 509–513.
- Antebi, A. & Fink, G. R. (1992). The yeast Ca^{2+} -ATPase homologue, PMR1, is required for normal Golgi function and localizes in a novel Golgi-like distribution. *Mol. Biol. Cell* **3**, 633–654.
- Bennetzen, J. L. & Hall, B. D. (1982). Codon selection in yeast. *J. Biol. Chem.* **257**, 3026–3031.
- Bruinenberg, P. G., Evers, M., Waterham, H. R., Kuipers, J., Arnberg, A. C. & Ab, G. (1989). Cloning and sequencing of the peroxisomal amine oxidase gene from *Hansenula polymorpha*. *Biochim. Biophys. Acta* **1008**, 157–167.
- Greeb, J. & Shull, G. E. (1989). Molecular cloning of a third isoform of the calmodulin-sensitive plasma membrane Ca^{2+} -transporting ATPase that is expressed predominantly in brain and skeletal muscle. *J. Biol. Chem.* **264**, 18,569–18,576.
- Gunteski-Hamblin, A. M., Clarke, D. K. & Shull, G. E. (1992). Molecular cloning and tissue distribution of alternatively spliced mRNAs encoding possible mammalian homologues of the yeast secretory pathway calcium pump. *Biochemistry* **31**, 7600–7608.
- Harmsen, M. M., Langedijk, A. C., Tuinen, E. V., Geerse, R. H., Raue, H. A. & Maat, J. (1993). Effect of a *pmr1* disruption and different signal sequences on the intracellular processing and secretion of *Cyamopsis tetragonoloba* α -galactosidase by *Saccharomyces cerevisiae*. *Gene* **125**, 115–123.
- Harmsen, M. M., Bruyne, M. I., Raue, H. A. & Maat, J. (1996). Overexpression of binding protein and disruption of the *PMR1* gene synergistically stimulate secretion of bovine prochymosin but not plant thaumatin in yeast. *Appl. Microbiol. Biotechnol.* **46**, 365–370.
- Hill, J. E., Myers, A. M., Koerner, T. J. & Tzagoloff, A. (1986). Yeast/*E. coli* shuttle vectors with multiple unique restriction sites. *Yeast* **2**, 163–167.
- Holm, C., Meeks-Wagner, D. W., Fangman, W. L. & Botstein, D. (1986). A rapid efficient method for isolating DNA from yeast. *Gene* **42**, 169–173.
- Hollenberg, C. P. & Gellissen, G. (1997). Production of recombinant proteins by methylotrophic yeasts. *Curr. Opin. Biotechnol.* **7**, 525–530.
- Janowicz, Z. A., Eckart, M. R., Drewke, C., Roggenkamp, R. O. & Hollenberg, C. P. (1985). Cloning and characterization of the *DAS* gene encoding the major methanol assimilatory enzyme from the methylotrophic yeast *Hansenula polymorpha*. *Nucl. Acids Res.* **13**, 3043–3062.
- Kyte, J. & Doolittle, R. F. (1982). A simple method for displaying the hydropathic character of a protein. *J. Mol. Biol.* **157**, 105–132.
- Lederboer, A. M., Edens, L., Maat, J., *et al.* (1985). Molecular cloning and characterization of a gene coding for methanol oxidase in *Hansenula polymorpha*. *Nucl. Acids Res.* **13**, 3063–3081.
- Merkelbach, A., Godecke, S., Janowicz, Z. A. & Hollenberg, C. P. (1993). Cloning and sequencing of the *ura3* locus of the methylotrophic yeast *Hansenula polymorpha* and its use for the generation of a deletion by gene replacement. *Appl. Microbiol. Biotechnol.* **40**, 361–364.
- Park, C. S., Kim, J.-Y., Crispino, C., Chang, C. C. & Ryu, D. Y. (1998). Molecular cloning of *YIPMR1*, a *S. cerevisiae* *PMR1* homologue encoding a novel P-type secretory pathway Ca^{2+} -ATPase, in the yeast *Yarrowia lipolytica*. *Gene* **206**, 107–116.
- Ramjee, M. K., Petithory, J. R., McElver, J., Weber, S. C. & Kirsch, J. F. (1996). A novel yeast expression/secretion system for the recombinant plant thiol endoprotease propapain. *Protein Engng* **9**, 1055–1061.
- Romanos, M. A., Scorer, C. A. & Clare, J. J. (1992). Foreign gene expression in yeast: a review. *Yeast* **8**, 423–488.
- Rudolph, H. K., Antebi, A., Fink, G. R., *et al.* (1989). The yeast secretory pathway is perturbed by mutations in *PMR1*, a member of a Ca^{2+} -ATPase family. *Cell* **58**, 133–145.
- Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989). *Molecular Cloning: A Laboratory Manual*, 2nd edn. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
- Serrano, R. (1988). Structure and function of proton translocating ATPase in plasma membranes of plants and fungi. *Biochim. Biophys. Acta* **947**, 1–28.
- Sherman, F., Hicks, J. B. & Fink, G. R. (1986). *Methods in Yeast Genetics*. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
- Smith, R. A., Duncan, M. J. & Moir, D. T. (1985). Heterologous protein secretion from yeast. *Science* **229**, 1219–1224.
- Sorin, A., Rosas, G. & Rao, R. (1997). PMR1, a Ca^{2+} -ATPase in yeast Golgi, has properties distinct from sarco/endoplasmic reticulum and plasma membrane calcium pumps. *J. Biol. Chem.* **272**, 9895–9901.
- Turner, B. G., Avgerinos, G. C., Melnick, L. M. & Moir, D. T. (1991). Optimization of pro-urokinase secretion from recombinant *Saccharomyces cerevisiae*. *Biotechnol. Bioeng.* **37**, 869–875.
- Viale, R. A., Giuseppin, M. L. F., Van Eijk, H. M. J., Sudbery, P. E. & Verris, C. T. (1992). Development of a strain of *Hansenula polymorpha* for the efficient expression of guar α -galactosidase. *Yeast* **8**, 361–372.

- Zhang, Y., Fujii, J., Phillips, M. S., *et al.* (1995). Characterization of cDNA and genomic DNA encoding SERCA1, the Ca^{2+} -ATPase of human fast-twitch skeletal muscle sarcoplasmic reticulum, and its elimination as a candidate gene for Brody disease. *Genomics* **30**, 415–424.