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Complementation of *Saccharomyces cerevisiae* acid phosphatase mutation by a genomic sequence from the yeast *Yarrowia lipolytica* identifies a new phosphatase

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Summary. A *Yarrowia lipolytica* gene library was constructed in vector YRp7 and transformed into a *Saccharomyces cerevisiae* strain lacking both major acid phosphatase activities. A 2.18 kb genomic sequence restoring the ability to hydrolyze α-naphthyl phosphate was isolated. Its sequencing revealed an ORF encoding 358 amino acids without significant homology with any known phosphatase. A putative signal peptide and several possible sites for N-glycosylation were identified. Phosphate-regulated expression of the cloned gene was observed in *Y. lipolytica*. Disruption data favoured the hypothesis that it might encode a minor phosphatase species.

Key words: Acid phosphatase – Heterologous complementation – *Yarrowia lipolytica*

Introduction

Acid phosphatases [orthophosphoric monoester phosphohydrolases (acid optimum) EC 3.1.3.2: APases] have been reported in many yeast species, e.g., *Candida albicans* (Odds and Hierholzer 1973), *Kluyveromyces lactis* (Altikrete et al. 1984), *Rhodotorula glutinis* (Trimble et al. 1981), *Saccharomyces cerevisiae* (Tonino and Stein-Parvé 1963), *Saccharomyces fragilis* (Weimberg and Orton 1966), *Schizosaccharomyces pombe* (Dibenedetto 1972), *Yarrowia lipolytica* (Ogrydziak et al. 1982), and *Zygosaccharomyces rouxii*, formerly *Saccharomyces mellis* (Weimberg and Orton 1964).

Yeast APases are cell surface glycoproteins which are active on a broad substrate spectrum. Their role is most probably to scavenge phosphorus for growing cells (Heredia et al. 1963; Günther and Kattner 1968). They hydrolyze external phosphate esters, unable to cross the plasmic membrane, and so provide inorganic phosphate which is transported into the cells by a permease (Roomans and Borst-Pauwels 1979). Most of them are subject to phosphate negative control. In *S. cerevisiae*, this regulation involves a rather complex, but well-documented, gene control system (Oshima 1982).

APases from *S. cerevisiae* and *S. pombe* have been extensively studied. A family of at least three genes sharing considerable homology encodes phosphate-repressible and constitutive APases from *S. cerevisiae* (Rogers et al. 1982). The *PHO5* and *PHO11* genes encode, respectively, a major and a minor species of phosphate-repressible APases, whereas *PHO3* is the structural gene for a constitutive species. A phosphate-repressible and a constitutive APase have been identified in *S. pombe* too. They are encoded by the *PHO1* and *PHO4* genes respectively. No significant homology was found with their *S. cerevisiae* counterparts.

Recently, an APase was purified from the yeast *Y. lipolytica* (Lopez and Dominguez 1988) and its enzymatic properties were studied (Moran et al. 1989). As expected, it is a glycoprotein exported to the cell surface and subject to phosphate control. There was no indication for the existence of a constitutive species.

We isolated a *Y. lipolytica* genomic sequence able to restore APase activity in a *S. cerevisiae* pho$^-$ mutant. Biochemical and genetical evidence indicated that it carried the structural gene for an as yet unknown phosphate-repressible APase species.

Materials and methods

Media, strains and plasmids. The yeast high-phosphate media employed were complete YPD medium and minimal YNB-glucose medium, as described by Sherman et al. (1986). For de-repression of APase activity, YPD medium was phosphate-depleted (Low P_i-YPD) according to Rubin (1974) and YNB-glucose was prepared with only 30 mg (LP_{30}) or 10 mg (LP_{10}) KH_2PO_4/l. Asparagin (2 g/l) and glutamate (1 g/l) were nitrogen sources for *S. cerevisiae* and *Y. lipolytica* respectively. When necessary, amino-acids and bases were added at 100 mg/l. LB and 2 × TY media for *E. coli* were prepared as described in Miller (1972) and supplemented with ampicillin (50 mg/l), tetracyclin (10 mg/l), or both, when required. Strains and plasmids are listed in Table 1.

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Table 1. Strains and plasmids used

Designation	Relevant genotype or description	Source or reference
Strains		
E. coli		
HB101	*hsdR*$^-$, *hsdM*$^-$, *recA13*, *supE44*, *lacZ4*, *leuB6*, *proA2*, *thi1*, *Sm*^{R}	Boyer and Roulland-Dussoix (1969)
XL1-Blue	*recA1*, *endA1*, *gyrA96*, *thi*, *hsdR17* (*rk*-,*mk*+), *supE44*, *relA1*, *λ*-, *lac*-, [*F'*, *proAB*, *lacIq*ZDM15, Tn10 (*Tet*^{R})]	Stratagene
S. cerevisiae		
GRF-18	*Matα*, *his3-11*, *his3-15*, *leu2-3*, *leu2-112*, *can*^{R}	R. Haguenauer-Tsapis
AH220	*Mat a*, *his3-11*, *his3-15*, *leu2-3*, *leu2-112*, *trp1-1*, *pho3*, *pho5*	Silve et al. (1987)
IH23	*trp1* strain with *PHO5* gene replaced by *URA3*	R. Haguenauer-Tsapis
IH53	*trp1* strain with both *PHO3* and *PHO5* genes replaced by *URA3*	R. Haguenauer-Tsapis
Y. lipolytica		
W_{29} (ATCC 20460)	Wild-type strain, *Mat A*	Gaillardin et al. (1973)
JM12	*Mat B*, *leu2-35*, *lys5-12*, *ura3-18*	Nicaud et al. (1989)
Plasmids		
YRp7	*TRP1 ARS1* from *S. cerevisiae* at the *Eco*RI site of pBR322	Struhl et al. (1979)
pJDB207/PHO5	*S. cerevisiae PHO5* gene in pJDB207	Haguenauer-Tsapis and Hinnen (1984)
pMC1403	*lacZY* from *E. coli* in pBR322	Casadaban et al. (1980)
pINA62	*Y. lipolytica LEU2* gene in pBR322	Gaillardin and Ribet (1987)
pINA156	*Y. lipolytica URA3* gene in pUC13	Wing R.W. and Ogrydziak D.M. (unpublished)
p3L4/4	15 kb *Sau*3A I fragment from *Y. lipolytica* DNA at *Bam*HI site of YRp7	This work
p3L4/49	4.3 kb *Bam*HI-*Sau*3A I fragment from p3L4/4 insert at *Bam*HI site of YRp7	This work
p3L4/51	Disruption vector: 2 kb *Sal*I fragment of p3L4/49 replaced by 1.6 kb fragment from pINA156 carrying *Y. lipolytica URA3* gene	This work
p3L4/52	2.18 kb *Dra*I fragment of p3L4/49 insert at *Pvu*II site of YRp7	This work
p3L4/55	Disruption vector: p3L4/51 modified by elimination of its 0.75 kb *Pvu*II-*Dra*I fragment	This work
pMCYL	pMC1403 with *Y. lipolytica LEU2* gene at *Sal*I site and with *Bam*HI site destroyed by blunt-ending	This work
pMC-Hae	0.294 kb *Hae*III from pBSM25 (see after), carrying promoter sequences, at blunt-ended *Bam*HI site of pMC1403	This work
pMC-HaeYL	5.3 kb *Sal*I fragment from pINA62, carrying *Y. lipolytica LEU2* gene, at *Sal*I site of pMC-Hae	This work
p3L4/61	pMC-Hae with 0.145 kb *Eco*RI-*Eco*RV fragment replaced by 0.79 kb *Eco*RI-*Eco*RV fragment from pBSM25	This work
p3L4/61YL	p3L4/61 with *Y. lipolytica LEU2* gene at *Sal*I site	This work
Phagemids		
Bluescript(-)KS	*Amp*^{R}	Stratagene
pBSM9	2.18 kb *Dra*I fragment from p3L4/49 insert at *Eco*RV site of Bluescript(-)KS	This work
pBSM25	same as pBSM9, but insert in reverse orientation	This work
pBSM9Y	1.45 kb *Eco*RI fragment from YRp7 at *Eco*RI site of pBSM9	This work
pBSM25	1.45 kb *Eco*RI fragment from YRp7 at *Eco*RI site of pBSM25	This work

DNA transformation. *E. coli* transformation was carried out according to Dagert and Ehrlich (1979). The *Y. lipolytica* gene library was transformed into *S. cerevisiae* by the method of Hinnen et al. (1978). Other *S. cerevisiae* transformations were performed as in Ito et al. (1983). *Y. lipolytica* was transformed by the method of Davidow et al. (1985) as modified by Xuan et al. (1988).

DNA extraction. Plasmid DNA was extracted from *E. coli* by the boiling method of Holmes and Quigley (1981). For large scale preparation, DNA was banded twice on CsCl-ethidium bromide gradients. Plasmids were rescued from *S. cerevisiae* by *E. coli* transformation with yeast DNA extracted according to Holm et al. (1986) or Hoffman and Winston (1987). Yeast total DNA was routinely extracted by the method of Hoffman and Winston (1987), except for large scale preparation of very high molecular weight DNA, which was performed as in Gaillardin et al. (1985).

DNA techniques. Digestion with restriction endonucleases, de-phosphorylation, ligation, agarose gel electrophoresis, transfer to Pall Biodyne A or Amersham Hybond N nylon membranes, and hybridization with radioactive probes were all performed using standard techniques (Davis et al. 1980; Maniatis et al. 1982).

DNA sequencing. The fragment to sequence was cloned in both orientations into Blue script(-)KS (Stratagene). Nested deletions were generated by *Exo*III/Mung Bean treatment according to Stratagene instructions. After transformation into *E. coli* strain XL1-Blue, the two initial constructions and their deleted derivatives