Yeast vacuolar aminopeptidase yscI

Isolation and regulation of the APE1 (LAP4) structural gene*

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Received 12 October 1989

The structural gene, APE1, (LAP4), for the vacuolar aminopeptidase I of *Saccharomyces cerevisiae* was cloned with the aid of a staining technique which permitted monitoring of aminopeptidase activity in yeast colonies. Genetic linkage data demonstrate that integrated copies of the cloned gene map to the APE1 locus. The nucleotide sequence of the cloned gene was determined. The open reading frame of APE1 consists of 514 codons and, therefore, encodes a larger protein (MW 57003) than the reported mature aminopeptidase yscI (MW 44800), suggesting that proteolytic processing must occur. A 1.75-kb mRNA, which is made in substantial amounts only when yeast cells have exhausted the glucose supply, was identified.

Aminopeptidase; Peptidase, vacuolar; Gene cloning; Nucleotide sequence; (*Saccharomyces cerevisiae*, Yeast)

1. INTRODUCTION

The vacuole of *Saccharomyces cerevisiae* contains several proteases that are synthesized as higher molecular weight inactive precursors (see [1,2] for reviews).

One of the vacuolar hydrolases is the aminopeptidase yscI [3], (also called LAPIV, for nomenclature see [4]). It is a soluble metallo-exopeptidase of about MW 640000 made up from a single type of subunit with MW 53000, that contains about 12% of conjugated carbohydrate and 0.02% Zn^{2+} and having a complex quaternary structure [5,6]. A putative precursor to aminopeptidase yscI of MW 61000 has been detected [7]. The expression of aminopeptidase yscI activity is known to depend upon the levels and function of proteinase yscA, the product of the PEP4 gene [8–11]. Activity of aminopeptidase yscI increases as the cells approach the stationary growth phase, a phenomenon thought to reflect a release from carbon catabolite re-

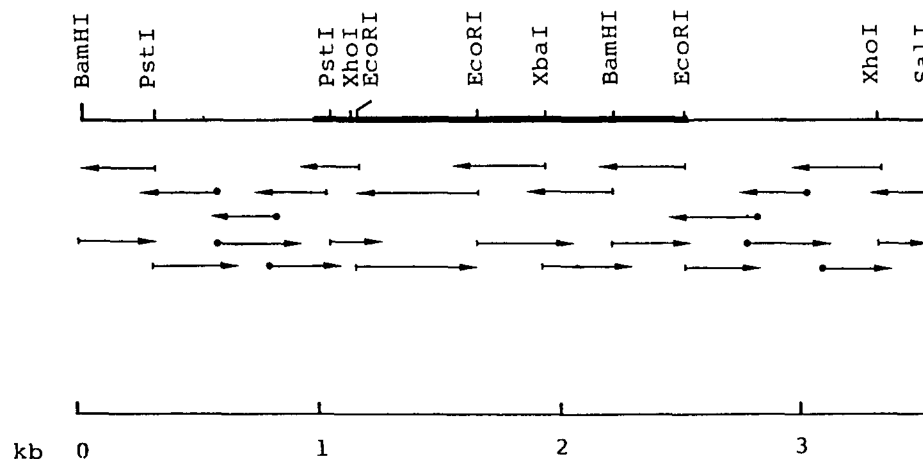

Fig.1. Restriction map and sequencing strategy for the APE1 gene subcloned into M13 vectors. The arrows indicate the strands sequenced, while dots indicate that priming was carried out with 17-base synthetic oligonucleotides; 100% of the sequence was determined from both strands. The thick line indicates the protein coding region.

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* Part of this work was presented at the 14th International Conference on Yeast Genetics and Molecular Biology (Espoo, Finland, August 7–13, 1988).

The nucleotide sequence(s) presented here has (have) been submitted to the EMBL/GenBank database under the accession number Y07522

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 -282  AAAAAATAATATGGATAGTTTTCCGAAAA

 -253  AAAACTAGGGGTGATGCTGTTTTTGCACCACTACTACTGTAAGATAGACTATGAAAGCAAACGACAGTAAGAAAGAATAGGGTTAAGTGAAATAGTTGACACACCCCTTAATCGTTTCA

 -134  TTAGATAATAAAAATTCGTTTATAAGCAGTTGACTTCACAGAACAAGACATACATTGTATAGAGCCTGACCTGTAATCTTAGTGCAATTGTAGAAACCTGCACAACCAACAAAATTAAA

  -15  ACAAGAAAAAAAAGA ATG GAG GAA CAA CGT GAA ATA CTG GAA CAA TTG AAG AAA ACT CTG CAG ATG CTA ACT GTA GAG CCA TCT AAA AAT AAC
                       MET Glu Glu Gln Arg Glu Ile Leu Glu Gln Leu Lys Lys Thr Leu Gln MET Leu Thr Val Glu Pro Ser Lys Asn Asn

   79  CAA ATC GCC AAC GAA GAA AAG GAA AAG AAA GAA AAT GAA AAT TCG TGG TGC ATC CTC GAG CAC AAT TAT GAG GAT ATT GCA CAG GAA TTC
   27  Gln Ile Ala Asn Glu Glu Lys Glu Lys Lys Glu Asn Glu Asn Ser Trp Cys Ile Leu Glu His Asn Tyr Glu Asp Ile Ala Gln Glu Phe

                                   *
  169  ATT GAT TTC ATT TAC AAG AAC CCT ACC ACT TAC CAT GTA GTA TCA TTT TTC GCG GAG CTG TTA GAT AAG CAT AAC TTC AAA TAC TTG AGC
   57  Ile Asp Phe Ile Tyr Lys Asn Pro Thr Thr Tyr His Val Val Ser Phe Phe Ala Glu Leu Leu Asp Lys His Asn Phe Lys Tyr Leu Ser

                                                                               *           *
  259  GAG AAA TCC AAT TGG CAG GAC TCC ATT GGC GAA GAT GGT GGG AAA TTC TAC ACT ATA AGA AAT GGA ACT AAC CTA TCT GCC TTT ATC CTG
   87  Glu Lys Ser Asn Trp Gln Asp Ser Ile Gly Glu Asp Gly Gly Lys Phe Tyr Thr Ile Arg Asn Gly Thr Asn Leu Ser Ala Phe Ile Leu

  349  GGC AAA AAC TGG AGA GCC GAA AAG GGT GTC GGT GTC ATT GGA TCT CAT GTG GAC GCT TTG ACG GTC AAA TTG AAG CCC GTC TCC TTT AAA
  117  Gly Lys Asn Trp Arg Ala Glu Lys Gly Val Gly Val Ile Gly Ser His Val Asp Ala Leu Thr Val Lys Leu Lys Pro Val Ser Phe Lys

  439  GAC ACA GCG GAA GGT TAC GGA AGA ATT GCT GTT GCT CCC TAT GGA GGT ACA CTG AAT GAA TTG TGG CTA GAC AGA GAC CTA GGT ATT GGT
  147  Asp Thr Ala Glu Gly Tyr Gly Arg Ile Ala Val Ala Pro Tyr Gly Gly Thr Leu Asn Glu Leu Trp Leu Asp Arg Asp Leu Gly Ile Gly

  529  GGT CGC CTT CTT TAC AAG AAG AAG GGC ACT AAC GAA ATT AAA AGC GCC TTG GTT GAT TCT ACA CCC CTA CCT GTT TGT CGA ATT CCT TCC
  177  Gly Arg Leu Leu Tyr Lys Lys Lys Gly Thr Asn Glu Ile Lys Ser Ala Leu Val Asp Ser Thr Pro Leu Pro Val Cys Arg Ile Pro Ser

  619  TTG GCT CCC CAT TTC GGT AAA CCT GCT GAA GGC CCA TTT GAT AAA GAG GAC CAA ACT ATC CCG GTC ATC GGC TTC CCC TCT CCG GAT GAG
  207  Leu Ala Pro His Phe Gly Lys Pro Ala Glu Gly Pro Phe Asp Lys Glu Asp Gln Thr Ile Pro Val Ile Gly Phe Pro Ser Pro Asp Glu

  709  GAA GGT AAT GAA CCT CCC ACG GAT GAT GAA AAG AAA TCG CCC TTA TTT GGC AAA CAC TGC ATC CAC CTG TTA AGG TAC GTT GCC AAA TTA
  237  Glu Gly Asn Glu Pro Pro Thr Asp Asp Glu Lys Lys Ser Pro Leu Phe Gly Lys His Cys Ile His Leu Leu Arg Tyr Val Ala Lys Leu

  799  GCC GGT GTG GAA GTG TCC GAA TTG ATT CAA ATG GAT TTA GAC TTA TTC GAT GTG CAA AAG GGT ACC ATT GGA GGT ATC GGT AAA CAC TTC
  267  Ala Gly Val Glu Val Ser Glu Leu Ile Gln MET Asp Leu Asp Leu Phe Asp Val Gln Lys Gly Thr Ile Gly Gly Ile Gly Lys His Phe

  889  CTT TTT GCA CCA CGT CTA GAT GAC AGG TTG TGT AGT TTC GCA GCA ATG ATT GCT TTG ATT TGC TAC GCT AAG GAT GTT GAT ACC GAA GAA
  297  Leu Phe Ala Pro Arg Leu Asp Asp Arg Leu Cys Ser Phe Ala Ala MET Ile Ala Leu Ile Cys Tyr Ala Lys Asp Val Asp Thr Glu Glu

  979  TCA GAG CTA TTC TCT ACT GTC ACT TTG TAT GAT AAT GAA GAA ATC GGA TCG TTG ACA AGA CAA GGC GCA AAA GGT GGC TTG TTG GAG TCA
  327  Ser Glu Leu Phe Ser Thr Val Thr Leu Tyr Asp Asn Glu Glu Ile Gly Ser Leu Thr Arg Gln Gly Ala Lys Gly Gly Leu Leu Glu Ser

 1069  GTG GTG GAA CGC AGT TCT TCT GCA TTC ACT AAG AAA GCG GTC GAT TTG CAT ACG GTT TGG GCT AAT TCC ATC ATC TTG TCC GCA GAC GTC
  357  Val Val Glu Arg Ser Ser Ser Ala Phe Thr Lys Lys Ala Val Asp Leu His Thr Val Trp Ala Asn Ser Ile Ile Leu Ser Ala Asp Val

 1159  AAC CAC CTC TAC AAC CCA AAC TTT CCT GAA GTC TAT TTG AAG AAT CAT TTT CCA GTG CCT AAT GTC GGA ATC ACT TTA TCA CTG GAT CCT
  387  Asn His Leu Tyr Asn Pro Asn Phe Pro Glu Val Tyr Leu Lys Asn His Phe Pro Val Pro Asn Val Gly Ile Thr Leu Ser Leu Asp Pro

 1249  AAC GGT CAT ATG GCC ACA GAT GTC GTA GGA ACT GCC CTA GTA GAA GAA TTA GCA CGC CGC AAT GGA GAC AAA GTG CAA TAT TTC CAA ATC
  417  Asn Gly His MET Ala Thr Asp Val Val Gly Thr Ala Leu Val Glu Glu Leu Ala Arg Arg Asn Gly Asp Lys Val Gln Tyr Phe Gln Ile

              *
 1339  AAA AAC AAT TCA AGA TCA GGT GGT ACT ATC GGC CCA TCA TTG GCT TCT CAA ACA GGT GCT CGT ACC ATA GAC CTG GGA ATT GCA CAG TTG
  447  Lys Asn Asn Ser Arg Ser Gly Gly Thr Ile Gly Pro Ser Leu Ala Ser Gln Thr Gly Ala Arg Thr Ile Asp Leu Gly Ile Ala Gln Leu

 1429  TCC ATG CAC AGC ATC AGA GCT GCT ACA GGG TCC AAG GAT GTC GGA TTA GGT GTT AAG TTC TTC AAC GGA TTT TTC AAG CAC TGG AGA TCA
  477  Ser MET His Ser Ile Arg Ala Ala Thr Gly Ser Lys Asp Val Gly Leu Gly Val Lys Phe Phe Asn Gly Phe Phe Lys His Trp Arg Ser

 1519  GTC TAC GAT GAA TTC GGC GAG TTG TGA TTTTGCTACACTCTTTTTATTTTTTTTTGATTTCTGTTTCTTTATCCTTTTTCTAAAAATACTTTTATTTTGAATCAGGAAGA
  507  Val Tyr Asp Glu Phe Gly Glu Leu TER

 1629  AAAGAAAAATGTATAAAAAAAAAAATAAAACACCACAATCGTCAAGATATAAATATCTATTTATAAATAAAACATCAAAAATACCTAAACCCCAAACATTCTACTAGGAAAGAATATTA

 1748  ACAGTAACCACGAACTATAAAAAA
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Fig.2. Nucleotide and deduced amino acid sequences of aminopeptidase yscI gene. A TATA-like sequence, located 109 bases upstream of the initiation codon is underlined. Asterisks identify potential glycosylation sites.

pression [12,13]. We report here the cloning and sequencing of APE1, the structural gene for aminopeptidase yscI and provide evidence that at least part of the regulation is at the level of transcription.

2. MATERIALS AND METHODS

2.1. *Materials*

All the reagents used in recombinant DNA manipulations were obtained from Boehringer. Growth media were from Difco (USA).

Chemicals used in biochemical tests were purchased from either Sigma (USA) or Bachem (Switzerland).

2.2. *Strains and culture conditions*

Saccharomyces cerevisiae strain II-21 (Mat a, lap1, lap2, lap3, lap4, leu2-3,2-112) a segregant of strain 1189 (a generous gift from Dr Trumbly [9]), was grown on complete YPD or mineral medium and cell extracts were prepared as described [14]. Bacterial strain HB101, used to propagate plasmids, was grown in LB media supplemented with ampicillin [15].

2.3. *Recombinant DNA procedures*

All cloning manipulations were performed by using standard techniques [15]. The plasmid used to map the integrated copy of the cloned gene was a derivative of one of the original complementing plasmids pRC1, deleted for the *Bgl*II fragment that carries 2 μ sequences in the YEp13 parent plasmid. pRC1 was open with *Xba*I to direct the integration into the chromosome at the APE1 locus. Southern and Northern blot analyses were performed as described [15,16]. DNA sequencing was performed using the method of Sanger et al. [17] following the strategy shown in fig.1, by using single-strand forward and reverse primers of M13 or with 17-base oligonucleotides synthesized in a solid-phase Applied Biosystems DNA synthetizer, model 381A and Sequenase kit (United States Biochemical Corporation).

2.4. *Biochemical procedures*

Aminopeptidase activity in colonies or patches of yeast cells made permeable by chloroform treatment [14] was monitored by pouring a mixture prepared by adding 15 ml of 1.2% melted agar in 0.1 M sodium phosphate buffer, pH 7.4, to 6 ml of a solution containing 1.5 mg L-amino acid oxidase, 2.4 mg peroxidase, 32 mg L-leucylglycine amide and 600 μl *o*-dianisidine, made in the same buffer. Plates were incubated at 37°C for 2 h. Development of a brown color resulting from the oxidation of the *o*-dianisidine indicated aminopeptidase activity.

3. RESULTS AND DISCUSSION

Plasmids capable of complementing the lap4 mutation [9] were recovered from the YEp13 bank [18]. After transformation [19] of strain II-21, approximately 15 000 Leu + transformants were screened for aminopeptidase activity by using the plate assay described in section 2. Seven positive colonies showed overproduction of vacuolar aminopeptidase yscI when analyzed by non-denaturing polyacrylamide gel electrophoresis according to the method of Hirsch et al. [20] (data not shown). Three plasmids were recovered which shared the overlapping fragment whose restriction enzyme map and sequencing strategy are illustrated in fig.1. Fig.2 shows the complete DNA sequence and deduced protein sequence for aminopeptidase yscI. The first 5′ ATG which obeys Kozak's rule [21] was assigned at the initiation codon. An open reading frame of 1542 nucleotides encoding a polypeptide of 514 amino acids extends from this initiation codon to a stop codon TGA. The calculated MW of the encoded protein is 57 003. This polypeptide is larger than the purified aminopeptidase yscI reported by Metz and Röhm [5] having a MW of 44 800 calculated from amino acid analysis but agrees with the size of the putative precursor (MW 61 000) detected by Distel et al. [7]. These data together with the reported dependence of aminopeptidase yscI activity upon the function of the PEP4 gene [8,9] indicate a zymogenic synthesis of this vacuolar peptidase and subsequent processing by proteinase yscA.

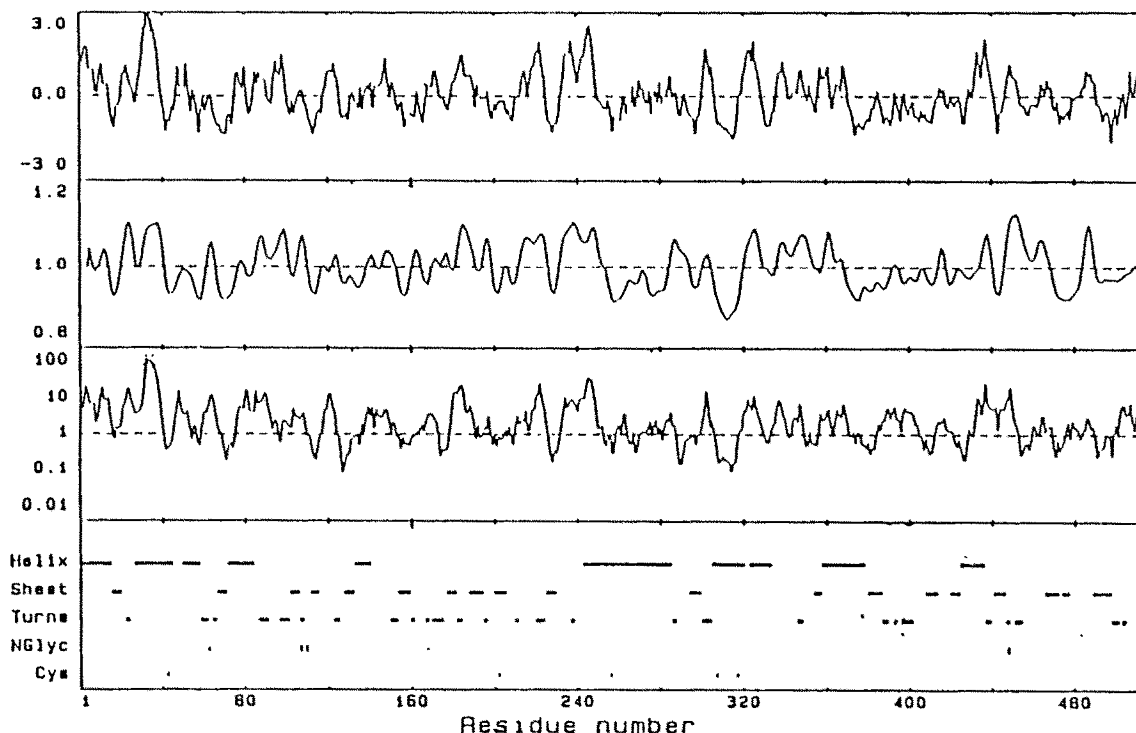

Fig.3. Protein structure analysis of the APE1 gene product. The methionine encoded by the initiation codon is numbered 1.

It should be pointed out here that while this work was in progress a report on the cloning and sequencing of aminopeptidase yscI appeared [22]. Our data extend the 5′ region by 112 nucleotides and the 3′ region by 45 nucleotides. There are four differences inside the ORF at positions 233 (Ser instead of Thr), 323 (Asp instead of Asn), 328 (Glu instead of Asp) and 369 (Ala instead of Pro) from the ATG initiation codon. There is a considerable difference at position −10 from the start codon (AACAAGA instead of G) and also in the 3′ non-coding region.

A computer program for protein structure prediction [23] applied to the protein sequence encoded by APE1 (fig.3) reveals that the stretch of 16 residues from the initial Met can adopt an α-helical conformation, consistent with previous results [22], not typical of other yeast vacuolar proteins [10,11]. Also, the high percentage of α-helix in the molecule is remarkable.

Southern blot analysis was carried out to demonstrate that integration of the cloned gene occurred at the genomic locus corresponding to aminopeptidase yscI, previously mapped on chromosome XI by Trymbly and Bradley [9]. The results presented in fig.4 show that APE1 is a single copy gene and that the integration of the LEU2-marked gene occurred at the genomic locus corresponding to the cloned gene. A tetrad analysis of diploids resulting from crossing a strain (APE1::LEU2) with a strain (apel LEU2) showed that the genes conferring Ape+ and Leu+ phenotypes were completely linked.

An RNA blot analysis indicated that the APE1 gene encodes a 1.75-kb mRNA (fig.5). As aminopeptidase yscI is subjected to carbon catabolite repression [12,13], we have used mRNA blot analysis to determine whether any part of this regulation is transcriptional in nature. The results presented in fig.5 indicate that substantial

Fig.4. Southern blot analysis of yeast genomic DNA. (A) Genomic DNA was cut with *Xba*I, run on a 0.7% agarose gel, transferred to a nitrocellulose membrane and probed with a 2.2-kb *Xho*I APE1 probe (see fig.1). (B) The detailed restriction maps for the apel (2), and APE1::LEU2 (1) alleles are shown. The solid black box is the LEU2 gene in the APE1 substitution. ORF, open reading frame.

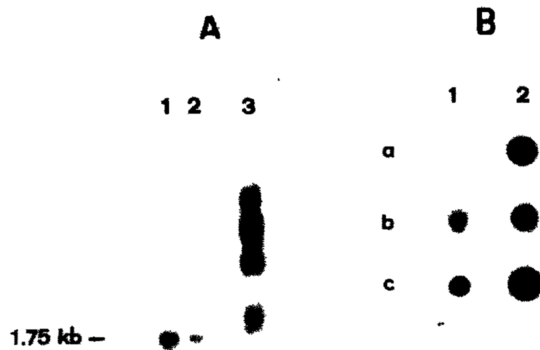

Fig.5. (A) Northern blot of yeast polyA m-RNA from a APE1 strain. RNA was prepared from stationary-phase grown cells (1: strain transformed with a multicopy plasmid containing the APE1 gene; 2: wild-type APE1 strain). Size standards in lane 3 are *E. coli* phage DNA digested with *Hin*dIII. The blot was hybridized with nick-translated DNA and the 0.3-kb *Eco*RI-*Xba*I APE1 fragment (fig.1). (B) Dot blot of yeast polyA m-RNA. RNA was prepared from logarithmic (a), diauxic (b) and stationary-phase grown cells (c) from a APE1 strain (1) and from a strain transformed with a multicopy plasmid containing the APE1 gene (2).

amounts of APE1 mRNA are not present until the supply of glucose is exhausted, confirming the suspected transcriptional regulation of the gene.

Acknowledgements: The authors are especially grateful to Prof. S. Gascón for encouragement and scientific advice, to Dr C. López-Otín for the synthesis of the oligonucleotides and to Dr R.S. Cármenes for doing the computer analysis of the protein. This work was supported in part by grant PBT87/0030 from the Comisión Interministerial de Ciencia y Tecnología of Spain. R.C. was a fellow of the Spanish Ministerio de Educación.

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