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PTF1 encodes an essential protein in *Saccharomyces cerevisiae*, which shows strong homology with a new putative family of PPIases

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Abstract Complementation of a temperature sensitive mutant of the yeast *Saccharomyces cerevisiae* resulted in the isolation of *PTF1* (processing/termination factor 1), an essential gene encoding a putative 3′-end processing or transcription termination factor of pre-mRNAs. Ptf1p shows significant homology to a newly discovered family of PPIases. This family is characterized by its insensitivity to immunosuppressive drugs and the lack of homology with cyclophilins and FK-506 binding proteins [Rahfeld et al. (1994) FEBS Lett. 352, 180–184]. Should Ptf1p display PPIase activity, it would be the first characterized, eukaryotic member of this putative family, which is essential for growth.

Key words: Peptidyl-prolyl *cis/trans* isomerase; Nucleotide sequence; Sequence homology; *Saccharomyces cerevisiae*

1. Introduction

Peptidyl-prolyl *cis/trans* isomerases (PPIases, EC 5.2.1.8) are enzymes which catalyze the *cis/trans* isomerization of the peptidyl-prolyl bonds in oligopeptides and are thought to accelerate slow steps in protein folding and trafficking [1–3]. PPIases are ubiquitous and have been found in bacteria, fungi, mammals and plants. Until very recently, the PPIases were divided into two different classes of unrelated protein families [4] according to their sensitivities towards immunosuppressive drugs: (i) the cyclophilins, which are inhibited by cyclosporin A and (ii) the FK-506 binding proteins (FKBPs), which are inhibited by FK-506 and rapamycin. The members of each family are characterized by conserved amino acid cores but these are not related to each other [5]. Cyclophilins and FKBPs bind tightly to the immunosuppressive drugs with a time-dependent loss of enzymatic activity.

Recently, Rahfeld et al. [6,7] discovered Parvulin, a member of a third family of PPIases in *Escherichia coli*. Parvulin has neither protein sequence homology to the two known PPIase-families nor is it inhibited by the immunosuppressive drugs. A database search with the protein sequence of Parvulin showed highly significant similarity with a number of prokaryotic and two eukaryotic protein sequences [8]. The matches with the prokaryotic sequences correspond to well characterized proteins. Those with the eukaryotic sequences correspond to two uncharacterized putative proteins, (i) a previously unrecognized open reading frame from *Dictyostelium discoideum* and (ii) a human expressed sequence tag (EST). In this study we describe the gene PTF1 from *Saccharomyces cerevisiae*, the 5′ and 3′ mapping of the corresponding mRNA and sequence comparison. Computer database searches with Ptf1p and multiple alignments revealed proteins which display homology to the newly described *E. coli* protein, Parvulin and to the conserved motifs of the members of this putative PPIase family.

2. Experimental

2.1. Strains

Escherichia coli XL 1 Blue (*recA1 endA1 gyrA96 thi-1 hsdR17 supE44 relA1 lac* [F′*proAB lacIqZ*ΔM15 Tn10(*tet*)] [9] (Stratagene) was used for cloning procedures. *Escherichia coli* BL21 (DE3) [F^- *ompT* r_B^- m_B^- *(DE3)*] [10] (Novagen) was used for expression of Ptf1p.

Yeast mRNA was isolated from DH484 cells (*MAT*a *ade2-1 leu2-3 leu2-112 can1-100 trp5-48 ura4-11 lys1-1*).

2.2. DNA analysis

Deletions on both sides of the 2.7 kb *Xba*I subfragment were performed using the *Exo*III/Mung Bean deletion method [11]. DNA-sequencing of the 2.7 kb *Xba*I subfragment, its deletions and the RACE-PCR products was performed by the chain termination method of Sanger et al. [12] as modified for supercoiled plasmids [13].

2.3. RNA analysis

Yeast total RNA was prepared by the hot phenol method as described by Köhrer and Domdey [14]. Poly(A)$^+$ RNA was isolated with an mRNA purification kit purchased from Pharmacia. For Northern blot analysis, 1.5 μg of glyoxal-treated poly(A)$^+$ RNA was separated on a 1.5% agarose gel and transferred [15] to a Hybond N nylon membrane (Amersham). The oligonucleotides JH22 and JH23 served as hybridization probes at position −20 to −39 and at position 85 to 114, respectively (see Fig. 1B).

The 5′ end of *PTF1* was mapped by primer extension with AMV reverse transcriptase [16] . 1 μg of poly(A)$^+$ RNA was used to prime reverse transcription with the 5′-radiolabeled oligonucleotide JH23 (Fig. 1B) for cDNA synthesis. The cDNA was analyzed on a 6% denaturing polyacrylamide sequencing gel together with Sanger dideoxy reactions on plasmid DNA using the same primers.

In order to create a *PTF1*-antisense RNA, the 2.7 kb *Xba*I fragment was cloned into pBluescript II KS$^-$, with the T3 promoter downstream of *PTF1*. From the resulting construct, the 3′ half of *PTF1* and the following downstream region was deleted by digestion with *Eco*RV and *Hin*cII followed by self-ligation. In this way, also the *Acc*I site in the polylinker of the vector was destroyed. The remaining sequence ends in the middle of *PTF1*, upstream of the T3 promoter. The resulting plasmid was linearized at the *Acc*I site in the 3′ terminal region of the open reading frame (ORF X). In vitro RNA synthesis was performed as described by Melton et al. [17]. The radiolabeled transcription product was purified by electrophoresis on a denaturing 6% polyacrylamide gel. Approximately 80,000 cpm of the excised and eluted RNA was hybridized to 1 μg of yeast poly(A)$^+$ RNA and digested with an RNase A/T1 mixture [18] . The RNase A/T1 resistant fragment was then analyzed by electrophoresis on a denaturing 6% polyacrylamide gel.

Reverse transcription and amplification of specific polyadenylated mRNA by RACE-PCR was done by the method of Frohman et al. [19] with modifications according to Heidmann et al. [20].

2.4. Production of recombinant Ptf1p

The coding region of *PTF1* was amplified by PCR, [21] and inserted in pET15a, an *E. coli* expression vector (Novagen). The expressed protein contains an oligohistidine tag at its Nterminus, followed by a thrombin recognition site.

E. coli BL21(DE3) (Novagen) cells harboring the expression plasmid

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were grown at room temperature up to an OD_{600} of 0.6 and then induced with 4 mM IPTG at 37°C for another 4 h. Cells were spun down and resuspended in 20 ml lysis buffer containing 20 mM Tris-HCl pH 7.4, 10% glycerol (vol/vol), 0.5 M NaCl, 1 mM AEBSF (4-(2-aminoethyl)-benzenesulfonyl fluoride hydrochlorine, ICN), 5 mM DTT, 20 μg/ml leupeptin (ICN), 10 μg/ml aprotinin (ICN), 2 μg/ml pepstatin A (ICN), 20 μg/ml benzamidine (Sigma), 2 μg/ml TPCK (*N*-Tosyl-L-phenylalanine chloromethyl ketone, Sigma). Cells were lysed by sonification for 3 × 5 min (Branson Sonifier) and centrifuged for 30 min at 4°C at 40,000 × *g*. Proteins in the supernatant were precipitated with 50% ammonium sulfate and pelleted at 40,000 × *g*. The supernatant containing Ptf1p was dialyzed 2 × 2 h against 1 l of buffer BC400 containing 20% glycerol, 20 mM Tris-HCl pH 7.3, 400 mM KCl and the protease inhibitors AEBSF (0.1 mM),

Benzamidine (20 μg/ml) and TPCK (2 μg/ml). The protein suspension was adjusted to 1 mM imidazole and loaded directly on a BC400 equilibrated nickel-nitriloacetic acid-agarose column [22] (Qiagen), and washed overnight with buffer BC400. Ptf1p was eluted with a linear gradient from 1–100 mM imidazole in buffer BC400. 5 mM DTT was added to each of the collected fractions, prior to shock freezing in liquid nitrogen. SDS-PAGE was done in 10% polyacrylamide gels with tricine buffer as described [23].

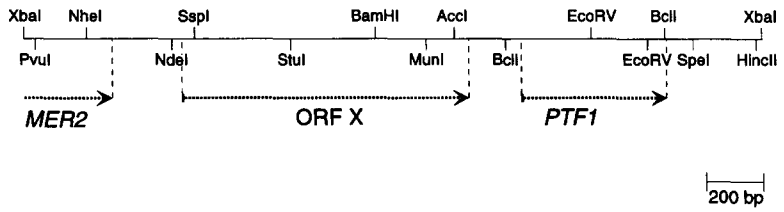
2.5. PPIase assay

The PPIase activity of recombinant Ptf1p was tested with a protease-coupled assay with chymotrypsin and the substrate Succinyl-Ala-Leu-Pro-Phe-4-nitroanilide according to Kofron et al. [24]. A Perkin-Elmer (λ16) UV/VIS spectrophotometer was used for monitoring the time course of the reaction. As positive controls human cyclophilin and human FKBP from Boehringer Mannheim were used.

2.6. Computer methods

Database searches and alignments were performed using the UWGCG (University of Wisconsin Genetics Computer Group) and

A



B

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-198 ggacaattccctcccacgtataaacacatacatatatattcttatattatataataatat -139
-138 cctatttattattacatcacctttcacgagcgggtatcatacctttttcccttcgatcta -79
                                                    JH22
 -78 agtagaaaaaggacctgaatgatcattgtggtacttattcccacgcataaccgacTACGG -19
                    +1
 -18 CACCACAACGACGCACCAATGCCATCTGACGTAGCATCGAGGACCGGGCTGCCAACCCCG 42
   1                    MetProSerAspValAlaSerArgThrGlyLeuProThrPro 14
                                                    JH23
  43 TGGACCGTCAGGTATAGTAAGTCCAAGAAAAGAGAGTATTTTTTCAATCCAGAGACGAAG 102
  15 TrpThrValArgTyrSerLysSerLysLysArgGluTyrPhePheAsnProGluThrLys 34
     JH23
 103 CACTCGCAATGGGAGGAGCCTGAGGGCACCAACAAGGACCAGCTACACAAGCACTTAAGA 162
  35 HisSerGlnTrpGluGluProGluGlyThrAsnLysAspGlnLeuHisLysHisLeuArg 54

 163 GACCATCCAGTGCGTGTAAGATGCCTGCACATTCTCATCAAGCACAAGGATTCAAGAAGA 222
  55 AspHisProValArgValArgCysLeuHisIleLeuIleLysHisLysAspSerArgArg 74

 223 CCCGCATCGCATAGATCCGAGAACATTACGATATCCAAGCAAGACGCTACGGACGAACTG 282
  75 ProAlaSerHisArgSerGluAsnIleThrIleSerLysGlnAspAlaThrAspGluLeu 94

 283 AAAACCTTAATCACGAGGTTGGATGACGACTCTAAGACGAACTCCTTCGAGGCCCTGGCT 342
  95 LysThrLeuIleThrArgLeuAspAspAspSerLysThrAsnSerPheGluAlaLeuAla 114

 343 AAAGAGAGATCAGATTGCTCTTCATACAAGCGAGGCGGCGACCTCGGCTGGTTCGGGAGA 402
 115 LysGluArgSerAspCysSerSerTyrLysArgGlyGlyAspLeuGlyTrpPheGlyArg 134

 403 GGCGAGATGCAGCCTAGCTTTGAAGACGCTGCCTTCCAGCTCAAGGTCGGCGAGGTAAGC 462
 135 GlyGluMetGlnProSerPheGluAspAlaAlaPheGlnLeuLysValGlyGluValSer 154

 463 GATATCGTTGAATCAGGAAGCGGTGTTCATGTGATCAAGCGGGTAGGTTAGGTTGGCATT 522
 155 AspIleValGluSerGlySerGlyValHisValIleLysArgValGly *           170

 523 TGCTTCTCCACCTCACCTTTTTATGTTCTTATTCCCTCTTGTATCCATTCCGTCCACACT 582
 583 GAACCCATGAAAAGAAAAAAAATTAACTAGTCACATAGTTAAATGGCGCTGTTCTTCGCT 642
 643 GCGAGCGGAACCGGGCCTGAAAAAAAATTTTCTCTTTTCCTCTCCAGATGACTCAGTAAT 702
                                                    ↓         ↓
 703 ATAGTAGTATATTATATAATGTAACAAAAGAAGAGACTGAAGTTTTATTATGCAttgtag 762
 763 cgcctgtaatctttagtaacggattcttgtatttttttgtaaacagccaagaaaaaagta 822
 823 gagatgggcttgttaacgaaagttgctacatctaga                         858
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Fig. 1. (A) Restriction map of the 2.7 kb *XbaI* subfragment that complemented the temperature sensitive mutants (see text). The arrows below mark the open reading frames. (B) Nucleotide sequence of *PTF1* and flanking regions. The predicted protein sequence is depicted below. The DNA sequence which is shown, begins behind the stop codon of the unassigned open reading frame, ORF X (position −198). The reading frame of *PTF1* starts at position +1 of the nucleotide sequence and ends at position 513. The transcription initiation site is marked by a horizontal arrow; the two polyadenylation sites are marked by vertical arrows. An asterisk denotes the stop codon. The oligonucleotide sequences used as probes for Northern blot and primer extension analyses are underlined; the arrowheads indicate their polarity. Transcribed nucleotides are shown in upper letters.

PIR (Protein Identification Resource) program packages. The FASTA algorithm [25] was used for similarity searches. Multiple sequence alignments were generated using PILEUP and PRETTY. The comparisons in Table 1 were performed with the GAP program.

3. Results and discussion

PTF1 (processing/termination factor 1) was isolated in a genetic screen by virtue of its ability to rescue temperature sensitive yeast mutants, showing read-through transcripts of an *ADH1* 3′-end formation sequence at the non-permissive temperature. The aim of this screen was to find *trans*-acting factors involved in mRNA 3′-end processing of the yeast *S. cerevisiae* (unpublished results).

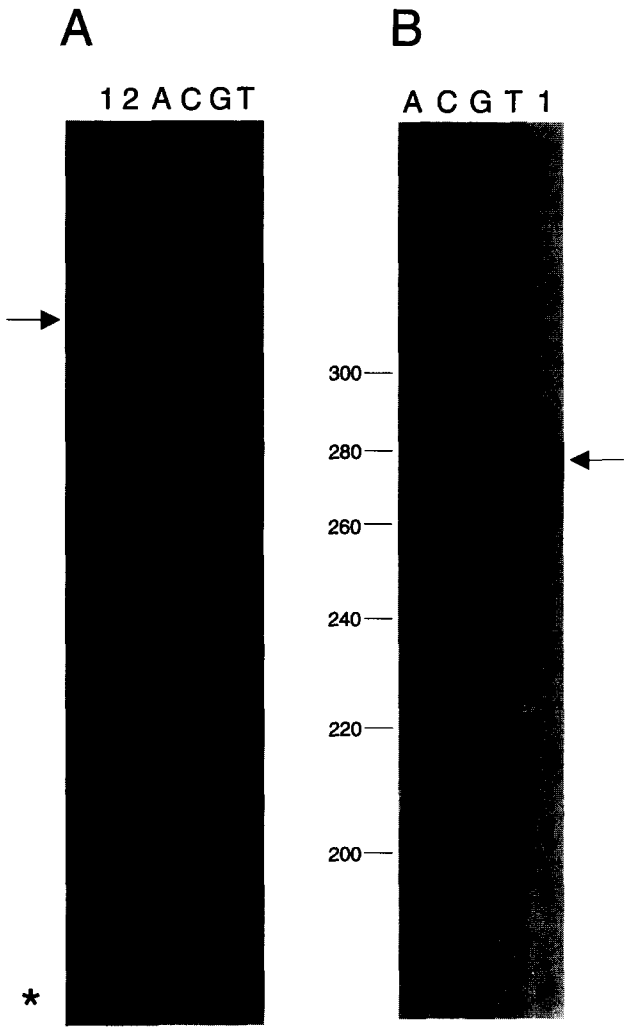

Fig. 2. Mapping of the transcription start site of *PTF1*. (A) Primer extension analysis of the *PTF1* transcript using oligonucleotide JH23 as primer (see Fig. 1B) and poly(A)$^+$ RNA as template. The extension products of RNAs isolated from two different yeast DH484 cultures are shown (arrow in lane 1 and 2). The DNA sequence in the adjacent lanes was generated with the same primer using the 2.7 kb *Xba*I DNA fragment as template. The asterisk denotes the position of the labeled oligonucleotide JH23. (B) RNase A/T1 protection assay of *PTF1* mRNA. Yeast poly(A)$^+$ RNA was hybridized with a radiolabeled antisense transcript of the 5′ region of *PTF1* (position −235 to 253). The RNase A/T1 resistant fragment (276 nucleotides) is indicated by an arrow. A DNA sequencing ladder was used as molecular weight marker (lanes A, C, G, T).

Table 1
Comparison of the 3′ terminal region of Ptf1p with the homologous sequences of the putative members of the new PPIase family

	% Identity	% Similarity	S.D.
PTF1 S.c.	100	100	48.3
PrsA B.s.	49.40	63.85	14.9
ORF EST H.s.	49.18	67.21	13.3
CBF2 C.j.	44.32	59.09	12.1
Parvulin E.c.	39.76	54.22	11.4
Nifm A.v.	31.25	52.08	10.5
Sur A (2) E.c.	37.63	55.91	9.2
PrtM L.l.	30.95	52.38	9.2
Nifm A.c.	29.17	51.04	8.4
Nifm K.p.	29.67	53.85	6.6
Sur A (1) E.c.	27.37	49.47	6.6
ORF B.s.	37.04	59.26	6.3
ORF D.d.	35.30	47.06	4.7

S.D. = standard deviation; the accession numbers and the species names are as given in Fig. 3. The comparisons were performed with the GAP program with the Randomizations = 50 parameter. This parameter reports the average alignment score and standard deviation from 50 randomized alignments in which the second sequence is repeatedly shuffled, maintaining the length and composition of the original sequence, and then aligned to the first sequence.

Complementation of the temperature sensitivity of two independent mutants with different genomic yeast libraries resulted in the cloning and isolation of two DNA fragments of 8 and 10 kb, respectively, which were shown to be identical in a Southern blot (data not shown). A complementing 2.7 kb *Xba*I subfragment was sequenced using the *Exo*III/Mung Bean deletion method (Fig. 1). This fragment contained the 3′ terminal region of *MER2* followed by two open reading frames. By performing complementation analyses with the deletion clones used for sequencing, we could unambiguously assign the third open reading frame (*PTF1*) as the gene responsible for rescue of the temperature sensitive phenotype (Fig. 1A and data not shown). The complete nucleotide sequence is shown in Fig. 1B. Searching the protein sequence database Swiss-Prot, with the FASTA program, we found 97.6% identity in a 127 amino acids overlap with the 5′ coding region of ESS1, an essential yeast gene previously described by Hanes et al. [26].

The region of identity between Ptf1p and Ess1p was found to be restricted to the central part of the protein, due to several discrepancies in the DNA sequence derived from the same gene. One difference was a frameshift in the coding sequence, resulting in a different amino acid sequence in the now 18 residues longer C-terminal region of the protein.

Initial evidence that the corresponding transcript was shorter on its 5′ site than previously implicated [26] came from a Northern blot analysis (data not shown), in which a radiolabeled oligonucleotide complementary to the predicted translation start [26] failed to hybridize (oligonucleotide JH22, see Fig. 1B). However, another radiolabeled oligonucleotide, which was located 104 nucleotides downstream, yielded a signal (oligonucleotide JH23, Fig. 1B). The 5′-end of the mRNA was mapped both by primer extension and RNase A/T1 protection analyses to a position downstream of the published translation initiation site (Fig. 2). As a consequence, the coding region starts with the second methionine codon of the published *ESS1* sequence. The 3′-end of the mRNA was mapped by RACE-PCR. This experiment revealed two polyadenylation sites, at positions 749 and 756 of the nucleotide sequence, 236 and 243 nucleotides down-

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                    1                                                  50
Nifm A.v.    (147) HILVtinEdf pentr..... ......eaar trietilkrl rgkperFaeq
Nifm A.c.    (148) HILVtinEdf pentr..... ......eaar trieailkrl rgkperFaeq
Nifm K.p.    (130) HlLltvdndr .......... ......eavh qrilglyrqi nasrdaFapL
Cbf2 C.j.    (137) HILVatekea kd........ ......iine lkglkgkeld ak....FseL
PrtM L.l.    (150) HILtsdeDta kqv....... .......... .......isd laagkdFamL
ORF B.s.     (160) HIvVkdeEea .......... .......rev lkelk..... ..ggssFeav
SurA (2) E.c.(288) HILlkpspim tde....... .......qar vkleqiaadi ksgkttFaaa
ORF D.d.       (?) .......... .......... .......... .......... ..........
SurA (1) E.c.(177) HILIplpEnp tsd....... .....qvnea esqaraivdq arngadFgkL
PTF1 S.c.     (63) HILIkhkDsr rpashrseni tiskqdatde lktlitrldd dsktnsFeaL
PrsA B.s.    (140) HILVadk... .......... .......... .ktaeevekk lkkgekFedL
Parvulin E.c.  (8) HILVkeekl. .......... .......... ...aldlleq ikngadFgkL
ORF EST H.s.   (?) .......... .......... .......... .......... ......Fxsp
       Consensus  Hilv---e-- ---------- ---------- ---------- ------F--l

                    51                                                 100
Nifm A.v.          AmkhS.ECpt AmqGGlLGe. vvp.GtLyPe LdaclFqMar GELSp.vleS
Nifm A.c.          AakhS.ECpt AmqGGlLGe. vvp.GtLyPe LdaclFqMaq GqLSp.vleS
Nifm K.p.          AqrhS.hCpS AleeGrLGW. isr.GlLyPq LetAlFsLae naLSl.PIaS
Cbf2 C.j.          AKekSiDpgS knqGGELGW. fdqs.tMvkp FtDAaFaLKn GtitttPVkt
PrtM L.l.          AKtdSiDtat kdnGGkisFe lnnk.tLdat FkDAaYkLKn GDytqtPVkv
ORF B.s.           AaerStDryt spyGGDLGFv teasdnipsa YiEeaktLKe dEwSqePIkv
SurA (2) E.c.      AKefSqDpgS AnqGGDLGW. .atpdiFdPa FrDAltrLnk GqMSa.PVhS
ORF D.d.           .......gdp rqrGGDLGW. .apatnyvqp FaEAvtkLKk GqLvdkPVqt
SurA (1) E.c.      AiahSaDq.q AlnGGqMGW. .griqeLpgi FaqAlstaKk GDivg.PIrS
PTF1 S.c.          AKerS.DCsS ykrGGDLGW. fgr.GeMqPs FeDAaFqLKv GEvSd.iVeS
PrsA B.s.          AKeyS.tdsS AskGGDLGW. fakeGqMdet FskAaFkLKt GEvSd.PVkt
Parvulin E.c.      AKkhS.iCpS gkrGGDLGe. f.rqGqMvPa FdkvvFscpv lEptg.Plht
ORF EST H.s.       AsqfS.DCsS AkarGDLGa. fsk.GqMqkp FeDpwFarrt GEMSg.tVft
       Consensus   Ak--S-dc-s a--gGdlgw- ----g-l-p- f-da-f-lk- gels--pv-s

                    101      111
Nifm A.v.          piGFHVlyce s  (49)    [F32055 PIR]
Nifm A.c.          piGFHVlfce s  (49)    [P23119 SWISS-PROT]
Nifm K.p.          elGWHllwce a  (45)    [S02510 PIR]
Cbf2 C.j.          nfGYHVIlke n  (45)    [X84703 PIR]
PrtM L.l.          tdGYeVIkmi n  (63)    [A32314 PIR]
ORF B.s.           snGYaIIqlk e  (50)    [D26185 GenBank
SurA (2) E.c.      sfGWHlIell d  (46)    [P21202 SWISS-PROT]
ORF D.d.           qfGWHVIqvd d  (39)    [X70280 GenBank]
SurA (1) E.c.      gvGFHIlkvn d  (156)   [P21202 SWISS-PROT]
PTF1 S.c.          gsGvHVIkrv g          [X85972 EMBL]
PrsA B.s.          qyGYHIIkkt e  (68)    [S15269 PIR]
Parvulin E.c.      qfGYHIIkvl y  (2)     [S45525 PIR]
ORF EST H.s.       dsGiHVIvrt e  (?)     [M86110 GenBank]
       Consensus   --Gyhvi--- -
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Fig. 3. Alignment of Ptf1p with potential members of a new family of PPIases. Ptf1p is presented in bold letters. Only the conserved region is shown. The bottom line shows the consensus sequence. The conserved residues in all the sequences are shown in upper case, while those conserved only in the majority of the sequences are presented in lower case. The amino acids of the aligned proteins that contribute to this consensus are written in upper case. The numbers of amino acids from this conserved area to the protein termini are indicated in parentheses at the beginning and at the end of each sequence. The accession number of the sequences and the sequence databases are set in brackets at the end of the sequences. SurA(1) and SurA(2) designate the amino-proximal and carboxy-proximal PPIase boxes of SurA, respectively. (A.v., *Azotobacter vinelandii*; A.c., *Azotobacter chroococcum*; K.p., *Klebsiella pneumoniae*; L.l., *Lactococcus lactis*; B.s., *Bacillus subtilis*; E.c., *Escherichia coli*; D.d., *Dictyostelium discoideum*; S.c., *Saccharomyces cerevisiae*; H.s., *Homo sapiens*, C.j., *Campylobacter jejuni*).

stream of the stop codon, respectively. In summary, *PTF1*, which has a transcript length of 772 or 779 nucleotides, without its poly(A)-tail, codes for a putative protein of 170 amino acids with a predicted molecular weight of 19,404 Da. The isoelectric point of the encoded protein was calculated to be 9.19. Expression of the *PTF1* gene in *E. coli* under control of the T7 promotor yielded a protein of the predicted size of 21,567 Da including an oligo histidine tag (Fig. 4 lane 1).

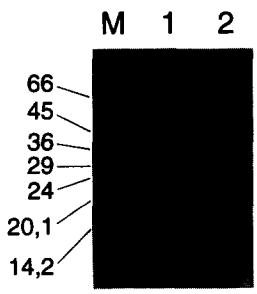

Fig. 4. SDS-PAGE of Ptf1p. Lane 1, recombinant Ptf1p; lane 2, Ptf1p after treatment with 1mg/ml chymotrypsin at 10°C for 20 s; lane M, molecular weight marker, sizes are indicated in kDa.

Computer similarity searches yielded a significant similarity with a newly described conserved domain of putative peptidyl-prolyl *cis/trans* isomerases (PPIases) [6–8] (Fig. 3). The most extensive similarity was found with *PrsA* from *B. subtilis*, followed closely by a human expressed sequence tag (EST), *CBF2*, a cell binding factor of *Campylobacter jejuni* and Parvulin from *E. coli* (Table 1). Except for CBF2, their conserved domains had already been shown to be very similar with each other [8]. It has been suggested, that these proteins belong to a third family of PPIases which has homology neither to FKBPs nor to cyclophilins. Hence Ptf1p could encode a new member of this group of isomerases. All PPIases catalyze the *cis-trans* isomerization of X-Pro peptide bonds and facilitate the refolding of denatured proteins in vivo. However, in a chymotrypsin-coupled spectrophotometric enzymatic assay, the PPIase activity of recombinant Ptf1p could not be demonstrated (data not shown).

This lack of PPIase enzymatic activity could be due to differ-

ent reasons, (i) Ptf1p is not a PPIase, despite sharing significant similarity with the new PPIase-family, (ii) the protein is inactive due to rapid degradation by chymotrypsin, or (iii) Ptf1p uses a different oligopeptide substrate than the one we used in our PPIase assay.

Indeed, we could demonstrate that Ptf1p is very sensitive to chymotrypsin treatment (Fig. 4, lane 2). Furthermore, the observed fact that Ptf1p is essential for growth and cannot be replaced by any other cellular component, might be a good indication for a rather stringent substrate specificity of Ptf1p. We can, however, only speculate how *PTF1* could be involved in transcription termination and/or 3′-end processing of pre-mRNAs. Since it was shown that ninaA, a *Drosophila* cyclophilin, builds a stable and highly specific complex with its target Rh1 [27], it is also conceivable that Ptf1p interacts in a similar way with one or more specific factors involved in transcription termination or 3′-end processing.

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