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Biochemistry

A gene encoding a putative tyrosine phosphatase suppresses lethality of an N-end rule-dependent mutant

(yeast/protein degradation/heat stress/ubiquitin)

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ABSTRACT **The N-end rule relates the *in vivo* half-life of a protein to the identity of its N-terminal residue. In the yeast *Saccharomyces cerevisiae*, mutational inactivation of the N-end rule pathway is neither lethal nor phenotypically conspicuous. We have used a "synthetic lethal" screen to isolate a mutant that requires the N-end rule pathway for viability. An extragenic suppressor of this mutation was cloned and found to encode a 750-residue protein with strong sequence similarities to protein phosphotyrosine phosphatases. This heat-inducible gene was named *PTP2*. Null *ptp2* mutants grow slowly, are hypersensitive to heat, and are viable in either the presence or absence of the N-end rule pathway. We discuss possible connections between dephosphorylation of phosphotyrosine in proteins and the N-end rule pathway of protein degradation.**

Enzymatic phosphorylation/dephosphorylation of tyrosine in proteins is central to a number of biological functions, from control of the cell cycle to the action of hormones and other effectors (1–4). This paper describes the isolation and analysis of a gene, named *PTP2*, that encodes a putative protein phosphotyrosine phosphatase (PTPase) of the yeast *Saccharomyces cerevisiae*.

We have isolated the *PTP2* gene while studying the N-end rule, a previously identified relationship between the *in vivo* half-life of a protein and the identity of its N-terminal residue (5). Distinct versions of the N-end rule operate in all organisms examined, from mammals to yeast and bacteria (5–9). The N-end rule is the manifestation of a degradation signal called the N-degron (10). The eukaryotic N-degron comprises two distinct determinants: a destabilizing N-terminal residue and an internal lysine residue (or residues) (7, 11). The latter is the site of attachment of a multiubiquitin chain, whose formation follows recognition of an N-end rule substrate and is required for its degradation (12, 13).

In *Saccharomyces cerevisiae*, the recognition component of the N-end rule pathway is encoded by the *UBR1* gene (14). The 225-kDa Ubr1 protein, called N-recognin [also known as E3α or the type 1, 2 E3 protein (15)], selects potential proteolytic substrates by binding to their destabilizing N-terminal residues (14, 15). A *ubr1*Δ mutant is viable but unable to degrade N-end rule substrates (14). Although the absence of the N-end rule pathway results in a slight retardation of growth and a subtle sporulation defect, the viability of the *ubr1*Δ mutant and its wild-type sensitivity to a variety of metabolic and physical stresses indicate nonessentiality of this pathway (14). Thus, cell viability may not depend on the degradation of natural N-end rule substrates. It is also possible that cell viability or stress-specific functions may, in fact, require a down-regulation of certain N-end rule substrates. To be consistent with the above experimental constraints (14), this down-regulation must be achievable not only through proteolysis via the N-end rule pathway but by some independent means as well—for instance, by proteolysis via a different degradation signal or through an enzymatic modification of the same substrate. A precedent for multiple degradation signals in a protein is the naturally short-lived yeast Matα2 repressor, which contains two degradation signals, neither of which is an N-degron (16, 17).

In a test of these and related ideas about functions of the N-end rule, we have used a "synthetic lethal" screen (18) to isolate a mutant that requires the N-end rule pathway for viability. An extragenic suppressor of this mutation was isolated and found to encode a putative PTPase, named Ptp2, the second known PTPase in *Saccharomyces cerevisiae*. The first PTPase gene, *PTP1*, was isolated through its homologies to *PTP* genes of other organisms (19). We consider the properties of *PTP2** and mechanisms that may underlie a connection between the N-end rule and dephosphorylation of phosphotyrosine in proteins.

MATERIALS AND METHODS

Strains, Media, and Genetic Techniques. Table 1 lists *Saccharomyces cerevisiae* strains produced in this work. Rich [yeast extract/peptone/dextrose (YPD)] and minimal synthetic yeast media were prepared as described (21). Synthetic complete medium (SC) is minimal synthetic yeast medium containing uracil, adenine, arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, and tryptophan (21). FOA plates contained SC and 0.2% 5-fluoroorotic acid (FOA) (PCR Research Chemicals, Gainesville, FL) (22). Yeast mating, sporulation, and tetrad analyses were carried out as described (21). Yeast were transformed using the method of Dohmen *et al.* (23).

Southern and Northern Hybridization. Genomic DNA of *Saccharomyces cerevisiae* was isolated and used for Southern hybridizations with ^{32}P-labeled DNA probes as described (24). For Northern hybridization, total RNA was isolated (24) from either exponentially growing (in YPD at 30°C) or heat-stressed (30 min at 39°C) cultures of the strain YPH500 (25). The isolated RNA was electrophoresed in formaldehyde-containing agarose gels (24), blotted onto GeneScreen in 25 mM sodium phosphate, pH 6.5, and hybridized (24) with the ^{32}P-labeled, 698-base-pair (bp) *Pst* I fragment of *PTP2* (probe 3 in Fig. 2*A*).

RESULTS AND DISCUSSION

Isolation of a *sln1* Mutant. To isolate *sln* mutants (synthetic lethals of N-end rule), defined as mutants that require the N-end rule pathway (specifically, the *UBR1* gene) for viability, we have used the FOA-based counterselection technique (18, 22). In this screen, yeast cells lacking chromosomal

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Abbreviations: PTPase, protein phosphotyrosine phosphatase; *sln*, synthetic lethal of N-end rule; FOA, 5-fluoroorotic acid; ORF, open reading frame.

*The sequence of the *PTP2* gene reported in this paper has been deposited in the GenBank data base (accession no. M82872).

Table 1. *Saccharomyces cerevisiae* strains produced in this work

Strain	Genotype
IOY1*	*MATa PTP2 sln1-1 ubr1-Δ1::LEU2*(pUBR1) *trp1-1 ura3-52 his3-Δ200 leu2-3,112 lys2-801 gal*
IMY21†	*MATa*/MATα *ptp2-Δ1::HIS3/PTP2 UBR1/UBR1 trp1-1/trp1-1 ura3-52/ura3-52 his3-Δ200/his3-Δ200 leu2-3,112/leu2-3,112 lys2-801/lys2-801 gal/gal*
IMY31‡	*MATa*/*MATα ptp2-Δ1::HIS3/PTP2 ubr1-Δ1::LEU2/ubr1-Δ1::LEU2 trp1-1/trp1-1 ura3-52/ura3-52 his3-Δ200/his3-Δ200 leu2-3,112/leu2-3,112 lys2-801/lys2-801 gal/gal*
IMY21a§	*MATa ptp2-Δ1::HIS3 UBR1 trp1-1 ura3-52 his3-Δ200 leu2-3,112 lys2-801 gal*
IMY21c§	*MATa PTP2 UBR1 trp1-1 ura3-52 his3-Δ200 leu2-3,112 lys2-801 gal*
IMY51¶	*MATα PTP2::HIS3 ubr1-Δ1::LEU2 trp1-1 ura3-52 his3-Δ200 leu2-3,112 lys2-801 gal*
IMY61¶	*MATα PTP2::HIS3 ubr1-Δ1::LEU2 trp1-1 ura3-52 his3-Δ200 leu2-3,112 lys2-801 gal*

*Mutant (*sln1*) that requires *UBR1* (carried in pUBR1) for viability.
†Diploid heterozygous for the *ptp2* deletion, derived from strain DF5 (20).
‡Diploid heterozygous for the *ptp2* deletion, derived from strain BBY53 (14).
§His^+ (*ptp2::HIS3*) and His^- (*PTP2*) segregants derived from a single meiotic tetrad of strain IMY21.
¶Strains constructed for linkage analysis. Strain IMY51 has *PTP2* and *HIS3* oriented in the same direction, whereas in strain IMY61 these genes are oriented in opposite directions.

copies of both *URA3* and a nonessential gene of interest and carrying both of these genes on a single plasmid are mutagenized and then tested for growth on plates containing FOA and uracil (FOA plates; see *Materials and Methods*). Because FOA selects against *URA3*-expressing cells (22), the class of mutants that grow without but not with FOA should include mutants that require the plasmid-borne, *URA3*-linked gene of interest for viability (18).

The haploid *ubr1*Δ strain BBY47 was transformed with pUBR1, a *UBR1*-containing, *URA3*, *CEN4*-based plasmid (14). Cells from a culture in exponential phase were mutagenized with ethyl methanesulfonate (21) to a viability of ≈33% and plated onto SC(-Ura) plates; the resulting colonies were then replica-plated onto FOA plates. Colonies that grew on SC(-Ura) but not on FOA plates were retested for lack of growth on FOA plates. At this stage, 53 FOA-sensitive candidates for *sln* mutants were identified among $\approx 2 \times 10^4$ colonies screened. These isolates were tested further; only one candidate, *sln1*, consistently passed the additional tests (see Fig. 1 and its legend). In the *UBR1* background, the (recessive) *sln1* mutation is viable but confers a small (slowly growing) colony phenotype (Fig. 1*B*); *sln1 UBR1* cells also grew ≈2-fold more slowly than wild-type cells in liquid (YPD) medium (data not shown).

Isolation of a *sln1*-Complementing Gene. The strain IOY1 [*sln1 ubr1*Δ(pUBR1)] (Table 1) was transformed with a *Saccharomyces cerevisiae* genomic DNA library carried in the *TRP1*, *ARS1*-based vector YRp7 (28). Approximately 2×10^4 transformants were selected on SC(-Trp) plates, and replica-plated onto FOA(-Trp) plates. Complementation of *sln1* by a gene in a *TRP1*-based library should make IOY1 cells FOA-resistant by allowing the loss of the *URA3*-containing pUBR1. Ninety FOA-resistant colonies were chosen for subculturing, followed by isolation of the plasmid DNA, its amplification in *Escherichia coli*, and analysis by restriction mapping. A large fraction of the FOA-resistant transformants was found to carry *UBR1* in the *TRP1*-containing vector of the library, most likely the result of either a recombinational transfer of *UBR1* from the *URA3*-based pUBR1 or the presence of *UBR1* in the original DNA library. However, one plasmid, pM42, while lacking *UBR1*, consistently conferred FOA resistance when transformed into IOY1. This property of the ≈6.7-kilobase (kb) insert in pM42 was retained upon its subcloning into YCplac22 (29), a *TRP1*, *CEN4*-based vector (Fig. 2*A* and data not shown). Several fragments of the ≈6.7-kb insert did not complement the *sln1* mutation. Therefore, a portion of the insert was sequenced at random, and a 2.25-kb open reading frame (ORF) was identified (Figs. 2*A* and 3). This information was used to produce an ≈3.7-kb *Pvu* II fragment (Fig. 2*A*) containing the entire ORF, which was subcloned into YCplac22, yielding the *sln1*-complementing plasmid pHSe.

The protein encoded by the cloned ORF (Fig. 3) has strong sequence similarities to known PTPases (see below). This protein was named Ptp2 (see Introduction).

***PTP2* Is an Extragenic Suppressor of *sln1* and a Nonessential Gene.** Lethality of the *sln1 ubr1* mutant could be complemented by *PTP2* carried on either a high-copy (2μ-based) or low-copy (CEN-based) vector (data not shown). A null allele of *PTP2* in which ≈86% of the *PTP2*-coding sequence was substituted by the *HIS3* gene (Fig. 2*B*), was used to replace the wild-type *PTP2* in diploid *his3*Δ strains (34). Sporulation

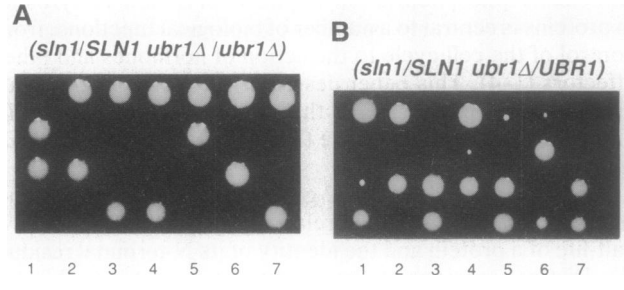

FIG. 1. Haploid segregants of diploid strains heterozygous at *SLN1*. (*A*) Strain IOY1 [*sln1 ubr1*Δ(pUBR1)] (Table 1) was mated to the congenic *SLN1 ubr1*Δ strain BBY46 (14). The resulting diploids were picked by using a micromanipulator (21) and allowed to grow on YPD plates and then plated on FOA plates to select diploids (*sln1/SLN1 ubr1*Δ/*ubr1*Δ) that had lost the *URA3*-based pUBR1. Tetrad analysis of these diploids yielded 2:2 segregation of viable and inviable spores. (*B*) Conditions were the same as in *A*, but the cross was to strain BBY48 (*SLN1 UBR1*) (14). The resulting (pUBR1-free) diploid (*sln1/SLN1 ubr1*Δ/*UBR1*) yielded the segregation patterns of viable and inviable spores of 3:1 and 2:2. The 4:0 segregation pattern was also observed (data not shown). The small-colony segregants were invariably *UBR1*, a property expected of a *UBR1*-requiring mutant [*UBR1* was scored as Leu^-; *LEU2* had been used to mark the *ubr1*Δ allele (14)]. The original *sln1* mutant was identified among 53 FOA-sensitive (*sln*) candidates by tests that included replacement of *URA3*, *CEN4*-based pUBR1 plasmid with pSOB35, which carries *UBR1* in the *TRP1*, 2μ-based vector YEplac112 (14). A *UBR1*-requiring, pSOB35-containing mutant should be able to lose pUBR1 without losing viability and, therefore, should grow on FOA plates. This test eliminated seven *sln* candidates. The rest were mated either to the congenic *ubr1*Δ strain BBY46 or to the congenic *UBR1* strain BBY48 (14), both of which contained wild-type (*SLN*) versions of the sought genes. The resulting diploids were cured of pUBR1 on FOA plates. The sporulation of a diploid (*sln/SLN ubr1*Δ/*ubr1*Δ) strain should produce a 2:2 segregation of viable (*SLN ubr1*Δ) and inviable (*sln ubr1*Δ) spores. For unlinked loci, the sporulation of a diploid (*sln/SLN ubr1*Δ/*UBR1*) should produce tetrads in which the segregation patterns of viable and inviable spores are 3:1 in approximately two-thirds of the tetrads, 2:2 in approximately one-sixth of the tetrads, and 4:0 in the remaining one-sixth (21, 26, 27). Only one *sln* candidate, named *sln1*, consistently passed these and related tests.

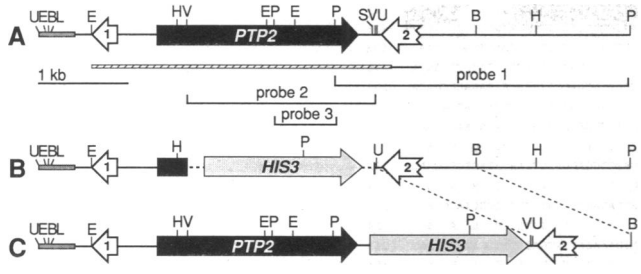

FIG. 2. *PTP2*-based constructs. (*A*) Restriction map of the *Saccharomyces cerevisiae PTP2* locus within plasmid pHS6.7. The *PTP2* ORF (see Fig. 3) is indicated by a filled arrow. The ≈6.7-kb *Sal* I/*Hin*dIII fragment of pM42, the original plasmid that complemented the FOA sensitivity of IOY1, was subcloned into YCplac22 (29), yielding pHS6.7. Portions of the insert sequenced by using standard methods (24) on either both or one strand are indicated, respectively, by hatched bar and attached line. Vector sequence, encompassing portions of the *lacZ* gene (from YCplac22; ref. 29) and the *tet* gene (from YRp7; ref. 28), is indicated by a stippled bar at left. A portion of an ORF 5′ to *PTP2* and a partially sequenced ORF 3′ to *PTP2* are indicated by open arrows. The complete ORF1 is the gene *RET1*, which encodes the second largest subunit of RNA polymerase III (30). The sequenced portion of ORF2 is 42% identical [and 60% similar (31)] to the amino acid sequence of *E. coli* nicotinic acid phosphoribosyltransferase (32), suggesting that the complete ORF2 is the previously unidentified gene for a yeast counterpart of the *E. coli* enzyme. B, *Bam*HI; E, *Eco*RI; H, *Hpa* I; L, *Sal* I; P, *Pst* I; V, *Eco*RV; S, *Sna*BI; U, *Pvu* II. (*B*) *ptp2*Δ::*HIS3* allele. The ≈4.8-kb *Bam*HI fragment of pHS6.7 (see *A*) was subcloned into the *Bam*HI site of pUC19 (24), yielding pHS4.85. The 1.8-kb *HIS3*-containing *Bam*HI fragment of YEp6 (33) was filled in by using Klenow polymerase I and ligated to the *Eco*RV-cut pHS4.85, yielding plasmid pPTP2Δ. Dashed line indicates the 2.1-kb *Eco*RV fragment, the deletion of which left intact the first 311 bp (filled rectangle) of the 2.25-kb ORF of *PTP2*. The ≈4.4-kb *Bam*HI fragment from pPTP2Δ was used for deletion/disruption of *PTP2*, with DNA probe 1 (see *A*) used to confirm it (34). (*C*) *PTP2*::*HIS3* allele. pHS4.85 (see *B*) was cut at the *Sna*BI site downstream from *PTP2* (see *A*), and the 1.8-kb, *HIS3*-containing fragment (see *B*) was inserted in both orientations, yielding pPTP2L1 and pPTP2L2, in which *PTP2* and *HIS3* were oriented in either the same or opposite directions, respectively (only the former arrangement is shown). The ≈6.6-kb *Bam*HI fragment from either of these plasmids was transformed into BBY46 (14). Southern hybridization analyses of His^+ transformants [strains IMY51 and IMY61 (Table 1)], using DNA probe 2 (see *A*), confirmed the expected insertions of *HIS3* (in either orientation) downstream of the *PTP2* gene (data not shown).

of the heterozygous diploids (*ptp2*Δ::*HIS3*/*PTP2 ubr1*Δ/*ubr1*Δ) and (*ptp2*Δ::*HIS3*/*PTP2 UBR1*/*UBR1*) yielded tetrads with four viable spores in which the His^+ (*ptp2*Δ) marker segregated 2:2, indicating that *PTP2* is not essential for either spore germination or vegetative growth.

Nonessentiality of *PTP2* in either *UBR1* or *ubr1*Δ backgrounds strongly suggested that *PTP2* is distinct from the *SLN1* gene, whose recessive mutation is lethal in the absence of *UBR1* (see above). To test this hypothesis directly, the *HIS3* gene was inserted immediately downstream of *PTP2* in the *ubr1*Δ strain BBY46 (14) (Fig. 2*C*), and the resulting strain was mated to strain IOY1 [*sln1 ubr1*Δ(pUBR1)]. The diploid obtained was sporulated and subjected to tetrad analysis. If the site of the *sln1* mutation, detectable by following FOA sensitivity of the [*sln1 ubr1*Δ(pUBR1)] segregants, is distinct from the *PTP2* locus (marked by the *HIS3* gene), ≈50% of the (slowly growing) FOA-sensitive segregants would be expected to be His^+ (21, 26, 27). Conversely, almost none of the FOA-sensitive segregants should be His^+ were *PTP2* and *SLN1* one and the same gene. Of the 12 small-colony, FOA-sensitive haploid segregants examined, 5 were His^+ (data not shown), indicating that *PTP2* and *SLN1* are distinct genes.

***PTP2* Encodes a Putative PTPase.** The 2.25-kb ORF of *PTP2* encodes a 750-residue protein with a calculated molecular mass of 85,814 Da and a calculated pI of 9.4 (Fig. 3). The codon adaptation index (36) of *PTP2* is 0.138, characteristic of weakly expressed yeast genes. Computer-aided comparisons (37) of the predicted amino acid sequence of Ptp2 to sequences in data bases revealed strong similarities to PTPases (Fig. 4). Genes encoding either putative PTPases or proteins whose PTPase activity could be demonstrated directly have been identified in mammals, *Styelae plicata* (a protochordate), *Drosophila melanogaster*, *Schizosaccharomyces pombe* (38), *Saccharomyces cerevisiae* (19), *Yersinia pestis* (a bacterium), and in vaccinia virus (see Fig. 4 and its legend; also refs. 1 and 2). PTPase activity has yet to be demonstrated for either the *Schizosaccharomyces pombe* pyp1 protein (38) or the *Saccharomyces cerevisiae* Ptp2 protein of the present work.

Similarities to known PTPases are confined to the C-terminal half of the 750-residue Ptp2; its N-terminal region (residues 1 to ≈400) lacks significant similarities to sequences in data bases (Fig. 4 and data not shown). Ptp2 and other apparently cytosolic PTPases each contain a single "PTPase domain"; some of them also contain large, mutually nonhomologous N-terminal regions (38, 43, 44).

While this paper was being written, we learned that P. James, E. A. Craig (University of Wisconsin, Madison), and B. D. Hall (University of Washington, Seattle) (personal communication; see also ref. 30), as well as K. Guan, R. J. Deschenes, and J. Dixon (University of Michigan, Ann Arbor, MI) (personal communication) have independently isolated the *PTP2* gene.

***PTP2* Is Inducible by Heat and Is Required for Wild-type Growth and Aspects of Thermotolerance.** Northern hybridization analysis of total yeast RNA from cultures in exponential growth at 30°C and after a heat stress at 39°C for 30 min showed a strong heat induction of the ≈2.7-kb PTP2 transcript (Fig. 5*A*). In contrast to most heat-inducible genes, which have promoters containing specific binding sites for the *HSF*-encoded heat stress transcription factor, Hsf (45), the 5′ flanking region of *PTP2* (Fig. 3) lacks obvious Hsf-binding sites. However, it does contain a 41-bp sequence (boxed in Fig. 3) that is 67% identical to a distinct, apparently Hsf-independent promoter element in the heat-inducible *Saccharomyces cerevisiae* gene *DDRA2* (35).

Both *PTP2* and congenic *ptp2*Δ cells grew at similar rates in liquid (YPD) cultures. They formed colonies with comparable plating efficiencies ≈60 hr after plating on YPD and incubation at 23°C, except that *ptp2*Δ colonies were smaller (Fig. 5*B*). This difference was greatly amplified, however, upon exposure to a heat stress (39°C, 18 hr) before transfer to 23°C: the heat-treated wild-type (*PTP2*) cells formed visible colonies 54 hr after the return to 23°C, whereas the identically treated *ptp2*Δ cells had not formed visible colonies (Fig. 5*B*). After a further 46 hr at 23°C, some of the *ptp2*Δ cells recovered to yield ≈4-fold fewer colonies than the identically treated wild-type cells (Fig. 5*B* and data not shown).

On the Functions of *PTP2*, *SLN1*, and the N-End rule. The *sln1* mutation is lethal in a *ubr1*Δ but not in a *UBR1* genetic background, suggesting that the viability of the *sln1* mutant requires the N-end rule pathway. We have found an extragenic suppressor of *sln1* that is distinct from *UBR1* and encodes a putative PTPase. This gene, named *PTP2*, is the second *PTP* gene identified in *Saccharomyces cerevisiae* (see Introduction).

One model that accounts for our results involves a short-lived protein X (or a set of proteins) whose periodic or constitutive down-regulation by degradation is essential for cell viability. Protein X is postulated to have the following properties. (*i*) It contains two degradation signals, one of which is the N-degron (7, 11), while the other is targeted by a different proteolytic pathway. A precedent for a naturally short-lived protein containing two distinct degrons is provided by the yeast Matα2 repressor (see Introduction). (*ii*) Either the

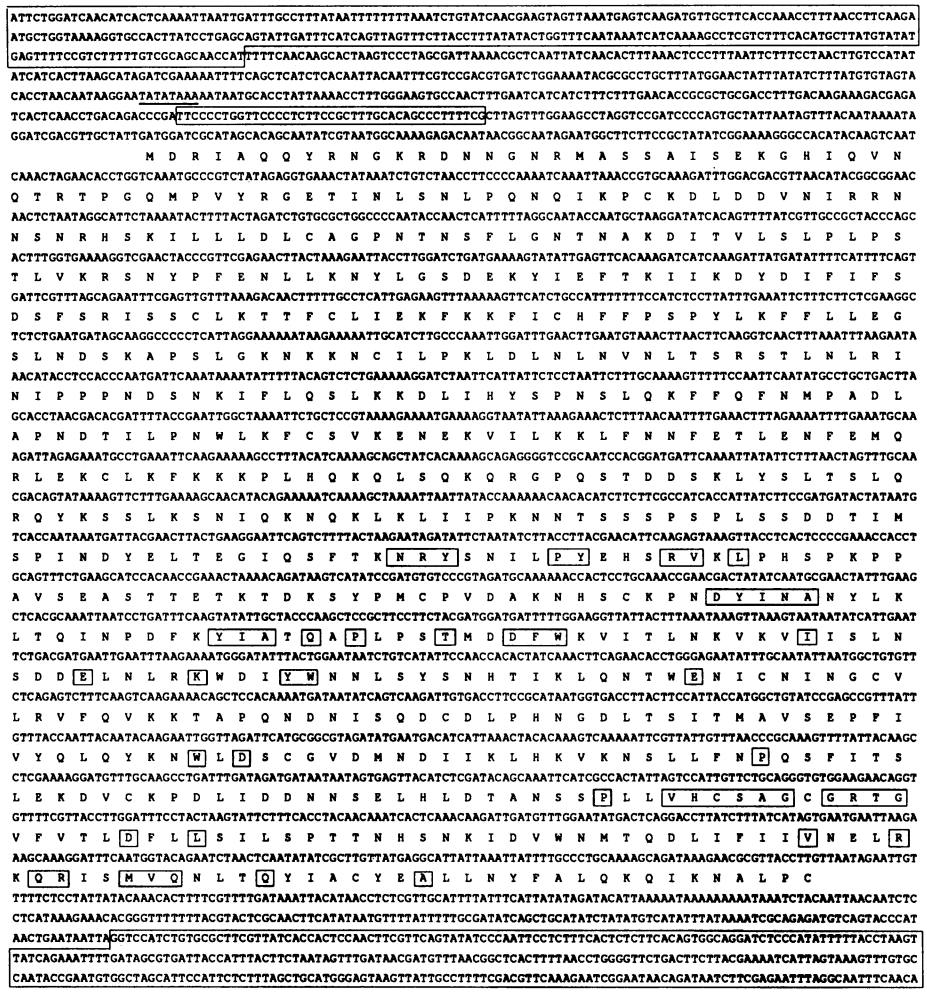

FIG. 3. Nucleotide sequence of the *Saccharomyces cerevisiae PTP2* gene and deduced amino acid sequence of the Ptp2 protein. Boxed regions upstream and downstream of *PTP2* are portions of the other, divergently oriented ORFs (ORF1 and ORF2; see Fig. 2*A* and its legend). A match to the TATA box consensus at positions −243 to −236 is underlined. A boxed 41-bp region (−118 to −78) is 67% identical to a sequence upstream of the *Saccharomyces cerevisiae DDRA2* gene that confers heat inducibility (35). Position of the start (ATG) codon in *PTP2* was inferred so as to yield the longest ORF. Boxes within the ORF show identities between Ptp2 and other PTPases (see Fig. 4).

N-degron alone or the second degron alone is sufficient for maintaining, at least in part, the essential aspects of metabolic instability of protein X. (*iii*) The second degron is regulated (activated or inhibited) by an overexpression of *PTP2*.

This model is consistent with our results; it predicts that a *sln1 UBR1* mutant would be viable but possibly impaired, whereas a *sln1 ubr1*Δ mutant would be inviable, as observed. It also predicts that lethality of the *sln1* mutant in the *ubr1*Δ

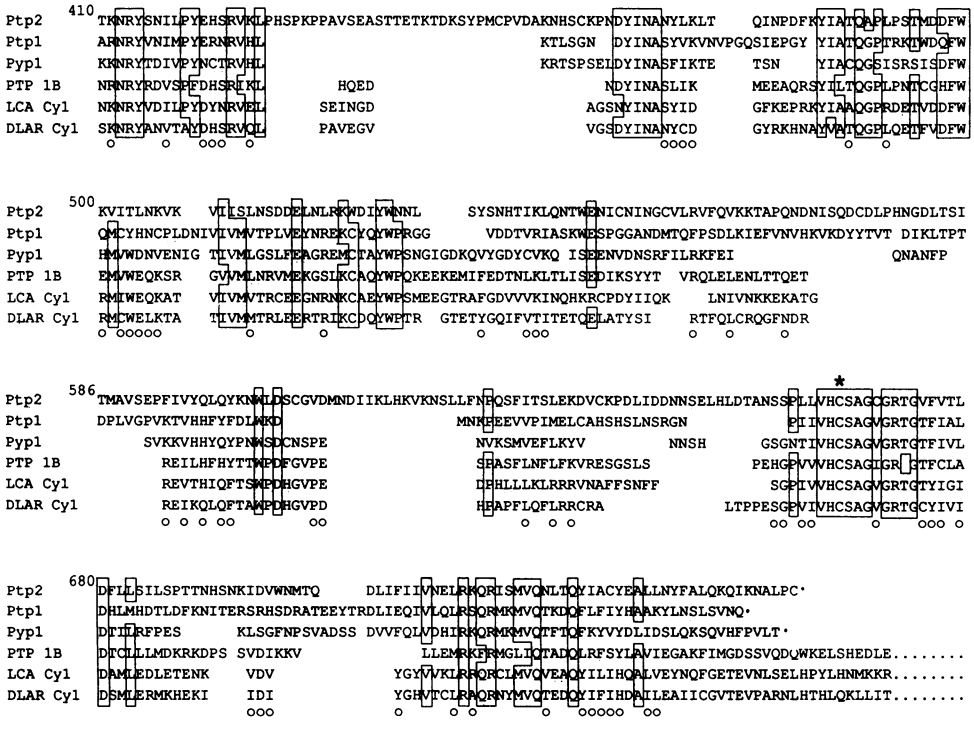

FIG. 4. Comparison of Ptp2 and PTPases. Identities among at least five of the compared sequences and conservative replacements (31) among at least four of the compared sequences are indicated, by vertical boxes and open circles below the sequences. Gaps were used to maximize alignment. The C-terminal half of the *Saccharomyces cerevisiae* Ptp2 protein shows significant similarities to Ptp1, a 335-residue PTPase of *Saccharomyces cerevisiae* (19), to a putative PTPase encoded by the *Schizosaccharomyces pombe pypl*$^{+}$ gene (38), to PTP 1B, a human placental PTPase (39, 40), to LCA (CD45) Cy1, a cytoplasmic PTPase domain of the human leukocyte antigen receptor (41), and to DLAR Cy1, a cytoplasmic domain of the *Drosophila* PTPase (42). An asterisk at Cys in the conserved sequence VHCSAG indicates the residue shown to be essential for the PTPase activities of LCA Cy1 and other PTPases (1, 42). The National Biomedical Research Foundation protein data base was searched using the FastA algorithm (37).

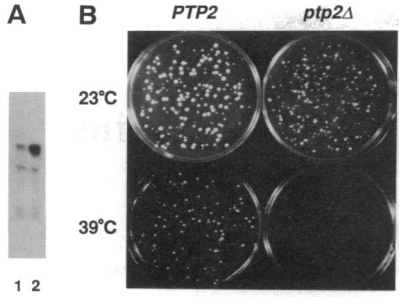

FIG. 5. Heat inducibility of *PTP2* and heat sensitivity of the *ptp2Δ* mutant. (*A*) Northern hybridization analysis, using DNA probe 3 (Fig. 2*A*), of total RNA from cultures of the *PTP2* strain YPH500 (25) that were growing exponentially at 30°C (lane 1) or were heated at 39°C for 30 min (lane 2) before RNA isolation. Equal amounts of total RNA were loaded onto a gel. (*B*) IMY21a (*ptp2Δ*) and IMY21c (*PTP2*) (Table 1) were tested for growth on solid YPD media either at 23°C or after a heat stress for 18 hr at 39°C followed by recovery at 23°C. Unlike the wild-type (*PTP2*) cells, the *ptp2Δ* cells formed no visible colonies 54 hr after the return to 23°C.

background would be complementable by either *UBR1* or an increased dosage of *PTP2*, as observed. In a related but distinct model, protein X can be down-regulated either through its degradation via the N-end rule pathway or through its functional inactivation by dephosphorylation, with an overexpressed Ptp2 being sufficient for the latter process. One possibility is that *SLN1* encodes a PTPase whose *in vivo* substrates partially overlap with those of the *PTP2*-encoded PTPase. Besides Ptp2, identified in the present work, *Saccharomyces cerevisiae* has at least one other PTPase, Ptp1 (ref. 19; see Introduction and Fig. 4), as well as a putative PTPase, Mih1 (46). Deletions of either *PTP1* (19) or *MIH1* (46) do not produce the pronounced slow-growth phenotype characteristic of the *sln1 UBR1* mutant, suggesting that *SLN1* is distinct from either of these genes. While the postulated regulation of a short-lived protein X by a PTPase is without an experimental precedent, it is made more likely by the known participation of PTPases in a variety of control circuits, including that of the cell cycle oscillator, which is driven in part by a regulated destruction of cyclins in their complexes with the p34 protein kinase (47, 48).

With one exception, no physiological substrates of the N-end rule pathway have been identified thus far in either bacteria or eukaryotes. The exception is the recent evidence that RNA polymerase of the Sindbis virus (a plus-stranded RNA virus) bears an N-terminal tyrosine, a destabilizing residue in the N-end rule, and is degraded by the N-end rule pathway (49).

Further analysis of the functional and mechanistic connections between the N-end rule, *SLN1*, and *PTP2* should advance the understanding of PTPases and is also likely to clarify the still hypothetical functions of the N-end rule (6–9, 11–14).

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