PNAS Research Article. This is a contributed (Track-III) paper.

Section: *Biochemistry*

September 6, 2021

Supporting Information (SI):

Aminopeptidases trim Xaa-Pro proteins, initiating their degradation by the Pro/N-degron pathway

Shun-Jia Chen[a,1], Leehyeon Kim[b,1], Hyun Kyu Song[b], and Alexander Varshavsky[a,*]

[a]Division of Biology and Biological Engineering, California Institute of Technology, Pasadena, CA 91125.

[b]Department of Life Sciences, Korea University, Seoul 02841, South Korea.

*Contributing coauthor/NAS member. Email: avarsh@caltech.edu

[1]These authors contributed equally to this work.

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SI Materials and Methods

Antibodies and Other Reagents:

"Complete Protease Inhibitor Cocktail" tablets and tetracycline (Tc) hydrochloride were from Roche and Sigma, respectively. Zymolase (Zymo research, E1005), β-Mercaptoethanol (Sigma) and QIAprep Spin Miniprep Kit (Qiagen) were used for yeast genomic DNA purification. Antibodies to the following antigens were used for immunoblotting: anti-flag M2 monoclonal antibody (Sigma, F1804), anti-c-Myc-9E10 monoclonal antibody (Sigma, M5546), anti-hemagglutinin (ha) monoclonal antibody (Sigma, H6908). Secondary antibodies for immunoblotting were IRDye-conjugated goat anti-mouse 800CW (Li-Cor, C60405-05) or anti-rabbit 680RD (Li-Cor, C51104-08). Fluorescence patterns were detected and quantified using Odyssey 9120 (Li-Cor, Lincoln, NE). Restriction endonucleases and T4 DNA ligase were from New England Biolabs.

Yeast Strains, Media, and Genetic Techniques:

S. cerevisiae strains used in this study are cited in Table S1. Standard techniques (1, 2) were employed for strain construction and transformation. *S. cerevisiae* media were YPD medium (1% yeast extract, 2% peptone, 2% glucose; only most relevant components are cited); SD medium (0.17% yeast nitrogen base, 0.5% ammonium sulfate, 2% glucose); SE medium (0.17% yeast nitrogen base, 0.5% ammonium sulfate, 2% ethanol); and synthetic complete (SC) medium (0.17% yeast nitrogen base, 0.5% ammonium sulfate, 2% glucose), plus a mixture of compounds required by a given auxotrophic strain. The alternative carbon sources, in either liquid or plate media, were 2% ethanol or 2% glucose. Several BY4741-based *S. cerevisiae* strains (Table S1) were used for tetracycline (Tc)-based promoter reference technique (PRT)-chase degradation assays. Yeast strains containing epitope-tagged genes (Table S1) were used for observing expression and degradation of specific endogenous proteins.

Construction of Plasmids:

The *Escherichia coli* strains DH5α, STBL2 and BL21(DE3) (Invitrogen) (Table S1) were used for cloning and maintaining plasmids, and for expressing *S. cerevisiae* $Fra1_{His8}$ protein. Phusion High-Fidelity DNA polymerase and Q5 Hot Start High-Fidelity DNA polymerase (New England Biolabs) were used for PCR. Plasmids and PCR primers used in this study are described in Tables 2 and 3, respectively.

Plasmids pCSJ121 and pCSJ122 expressed (M)**SP**-$Pck1_{3f}$ and (M)**SS**-$Pck1_{3f}$ in *S. cerevisiae* from a P_{TDH3}-based promoter (SI Appendix, Fig. 2*C* and Table S2). They were constructed as described previously (3).

To construct pCSJ1067, which expressed C-terminally (Ct) triple-flagged (M)**AP**-Aro10 in *S. cerevisiae* from the P_{TDH3}-based promoter, an Aro10 encoding DNA fragment was amplified by PCR from *S. cerevisiae* genomic DNA using primers CSJ1178/CSJ1179 (Table S3). The amplified DNA fragment was digested with *Asc*I/*Bam*HI and ligated into *Asc*I/*Bam*HI-cut pCSJ125 downstream of its P_{TDH3} based promoter, yielding pCSJ1067.

Plasmid pCSJ1154 expressed the Ct-single-myc tagged $Fra1_{myc}$ in *S. cerevisiae* from a high copy pRS426-based plasmid bearing the P_{CUP1} promoter and T_{CYC1} terminator (Table S2). The P_{CUP1}

promoter DNA fragment was transferred from pBW261 plasmid using digestion with *Sac*I/*Bam*HI. The T_{CYC1} terminator DNA fragment was obtained from p406TDH3 (Addgene) using digestion with *Xho*I/*Kpn*I. The resulting plasmid, pCSJ105, was based on pRS426 and contained both P_{CUP1} and T_{CYC1}. The $Fra1_{myc}$-encoding DNA fragment was amplified by PCR from *S. cerevisiae* genomic DNA using the primers CSJ1265/CSJ1266 (Table S3). The amplified DNA fragment was digested with *Bgl*II/*Xho*I and ligated into *Bam*HI/*Xho*I-cut pCSJ105 downstream of its P_{CUP1} promoter, yielding pCSJ1154 (Table S2).

Plasmid pCSJ1226 contains the Nt-truncated Fra1 DNA fragment in pBluescript SK(+). This Ct-Fra1 DNA fragment (1347bp to 2247bp) was produced by digesting pCSJ1154 with *Cla*I/*Xho*I, and was ligated into *Cla*I/*Xho*I-cut pBluescript SK(+). This pCSJ1226 plasmid was used for site-directed mutagenesis, yielding DNA fragments that encoded specific Ct-fragments of three active-site mutants of Fra1 (see the main text). Plasmid pCSJ1227 expresses the fragment of Fra1(E660A) mutant . This plasmid was generated from pCSJ1226 using site-directed mutagenesis and primers CSJ1372/CSJ1373 (Table S3). The plasmid pCSJ1228, which expresses the otherwise identical fragment of Fra1(H531A), was constructed from pCSJ1226 using site-directed mutagenesis and primers CSJ1374/CSJ1375 (Table 3). pCSJ1229 expresses the otherwise identical fragment of Fra1(H531A, E660A) double mutant. pCSJ1229 was produced from pCSJ1228 using primers CSJ1372/CSJ1373 (Table S3).

Plasmids that expressed full-length (Ct-myc-tagged) Fra1 mutants were produced using the pCSJ1154 plasmid. Plasmid pCSJ1230 expresses full-length Fra1(E660A)-myc protein. DNA fragment encoding Ct-Fra1(E660A) fragment was produced from pCSJ1227 by digestion with *Cla*I/*Xho*I. That DNA fragment was ligated into *Cla*I/*Xho*I-cut pCSJ1154, yielding pCSJ1230 (Table S2). Analogous procedures were used to produce pCSJ1231 (it expresses Fra1(H531A)-myc) and pCSJ1232 (it expresses Fra1(H531A, E660A)-myc), using different DNA fragments (described above) from *Cla*I/*Xho*I-digested pCSJ1228 or pCSJ1229 plasmid, followed by cloning into *Cla*I/*Xho*I-cut pCSJ1154.

FRA1 gene was amplified by PCR using *S. cerevisiae* genomic DNA (strain BY4741; Table S1) as a template and primer pairs LK075/LK076 (Table S3). The amplified DNA fragment was digested with *Nde*I/*Xho*I and ligated into *Nde*I/*Xho*I-cut pETDuet-1 plasmid (Novagen), yielding pLK103 (Table S2), which encoded $Fra1_{His8}$.

Other plasmids, encoding analogous constructs (Table S2), were constructed similarly to the plasmids described above. Additional details of plasmid construction are available upon request. All final constructs were verified by DNA sequencing.

Tetracycline (Tc)-Based PRT-Chase Assays and Immunoblotting:
Designs of Tc/PRT-chase constructs are summarized in *SI Appendix*, Fig. S2*C*. Tc/PRT-chases were carried out similarly to the previously described cycloheximide (CHX)-chase assays with *S. cerevisiae* (3-6), but used low copy pJO629-based plasmids expressing a C-terminally (Ct) $flag_3$-tagged test protein and the long-lived, also tagged ($_fDHFR_{ha}$) reference protein from a pair of identical (modified) P_{TDH3} promoters, in a setting in which the synthesis of both proteins could be selectively extinguished by the addition of tetracycline (Tc) (SI Appendix, Fig. S2*C*).

S. cerevisiae carrying a Tc/PRT-based plasmid expressing a protein of interest (in addition to DHFR reference) was grown to A_{600} of 1.0-1.5 at 30°C in SC media whose exact composition was appropriate for a plasmid(s) carried by the yeast strain. Tc was then added to the medium, to the final concentration of 0.5 mM. At the beginning of a Tc/PRT-chase, a volume of cell suspension corresponding to 1 ml at A_{600} of 1.0 was withdrawn and cells were collected by centrifugation. The same volumes were withdrawn at different times of a chase. To compensate for Tc decay during longer chases, additional Tc was added to the medium (to 0.25 mM) at 2 h of chase. The pellet was resuspended in 0.8 ml of 0.2 M NaOH and incubated at room temperature (RT) for 5 min, followed by centrifugation at 11,200*g* for 1 min. The pellet was resuspended in 50 μl of HU buffer (8 M urea, 5% SDS, 1 mM EDTA, 0.1 M dithiothreitol (DTT), 0.005% bromophenol blue, 0.2 M Tris-HCl, pH 6.8) containing 1x-protease inhibitor cocktail (Roche), and heated for 10 min at 70°C. After centrifugation for 5 min at 11,200*g*, 15 μl of supernatant was subjected to SDS-4-10% PAGE, followed by immunoblotting (IB) as described previously (5, 7), using anti-ha (1:2,000) and anti-flag (1:2,000) antibodies as well as a secondary antibody (or antibodies) and quantification of resulting (green and/or red) fluorescence patterns using Odyssey 9120 (Li-Cor), its software, and manufacturer's manual.

In settings where $Fra1_{myc}$ was overexpressed from the P_{CUP1} promoter (Fig. 4, *C* and *D*), $CuSO_4$ (from 0.2 M stock solution in water) was added to the medium of all initial samples to the final concentration of 0.2 mM 2 h before Tc addition.

In some experiments (Fig. 3*A*), cells were incubated in ethanol-containing SE medium for 20 h before the transfer to glucose-containing medium. Specifically, cells were collected by centrifugation at 11,200*g* for 2min, washed once in pre-warmed SE medium, then resuspended in SE to A_{600} of 0.5 and grew in SE for 20 h at 30°C. Thereafter cells were harvested by centrifugation at 11,200*g* for 2 min and resuspended in fresh pre-warmed SC to A_{600} of 0.5, followed by the addition of Tc to the final concentration of 0.5 mM. At indicated times, a sample of cell suspension corresponding to A_{600} of 1.0 was withdrawn for processing by the procedures described above.

N-Terminal (Edman-Based) Sequencing of Isolated SP-Pck1$_{3f}$ and AP-Aro10$_{3f}$:
(M)**SP**-$Pck1_{3f}$ and (M)**AP**-$Aro10_{3f}$ were expressed from their endogenous genes in two *S. cerevisiae* strains that were a gift from the laboratory of Dr. C. S. Hwang (Table S1). The above strains, expressing, respectively, (M)**SP**-$Pck1_{3f}$ and (M)**AP**-$Aro10_{3f}$, were grown in YPD at 30°C and 250 rpm to A_{600} of ~1. Cells were pelleted by centrifugation at 5,000*g* for 10 min at 4°C. A pellet was resuspended in 1×PBS buffer containing 1 mM phenylmethyl sulfonyl fluoride (PMSF) and EDTA-free protease inhibitor cocktail (Roche). A high shear fluid processor (LM20 Microfluidizer, International Microfluidics Corp.) was used (at 27,000 psi) to disrupt resuspended cells. The sample was clarified by centrifugation at 35,000*g* for 1 h at 4°C. Each protein (**SP**-$Pck1_{3f}$ and **AP**-$Aro10_{3f}$) was purified from the supernatants by flag-tag-affinity chromatography (Sigma, Anti-FLAG M2 Affinity Gel, A2220). The gel was washed with 1×PBS at least 5 times, and protein was eluted with flag peptide (Sigma) at 0.2 mg/ml. Each protein was subjected to SDS-PAGE and transferred to the PVDF membrane using iBlot2 (Invitrogen). The membrane was soaked in fresh distilled water for 5 min, stained with Ponseau S, and was further washed with distilled water until the target protein band was visible. The membrane containing the band was cut out, followed by N-terminal sequencing by the Edman method (8). The

procedure involved PTH-C18 column chromatogram (Applied Biosystems, API492 protein sequencer). For each experiment, ~20 µg of protein were used. By analyzing the relative retention times and peak areas of the phenylthiohydantoin (PTH)-derivatized standard and target protein's amino acids, the peak height in every Edman cycle for each of the two proteins was quantified through comparisons to amounts of standard amino acids.

Expression and Purification of *S. cerevisiae* Fra1$_{His8}$ Aminopeptidase:
The pLK103 plasmid (see above), expressing *S. cerevisiae* Fra1$_{His8}$, was transformed into competent *E. coli* BL21(DE3). Overexpression of Fra1$_{His8}$ was induced (at A_{600} of 0.9-1.2) by adding isopropyl-β-D-thiogalactoside (IPTG) to the final concentration of 0.5 mM and further incubation at 18°C for 20 h at 150 rpm. Cells were pelleted by centrifugation at 6,300*g* for 30 min at 4°C. The pellet was resuspended in buffer A [0.2 M NaCl, 1 mM TCEP (Tris(2-carboxyethyl)phosphine hydrochloride), 50 mM Tris-HCl, pH 8.0], and cells were disrupted by sonication in the presence of 1 mM PMSF and EDTA-free protease inhibitor cocktail and then clarified by centrifugation at 35,000 g for 1 h. The supernatant was manually filtered, using a syringe filter (0.2 µm, S6534-FMOSK, Sartorius). The filtered supernatant was loaded onto Ni-NTA Superflow (Qiagen) resin and Fra1$_{His8}$ was eluted with buffer B (0.16 M NaCl, 1mM TCEP, 0.2 M imidazole, 50 mM Tris-HCl, pH 8.0).

In Vitro Assay for Fra1 Aminopeptidase Activity:
Aminopeptidase activity of Fra1$_{His8}$ was assayed using synthetic peptides that included **AP**VTIEK, **VP**VTIEK, **SP**SKMNA, and **PPP**TAQF. Before the assay, a sample of purified Fra1$_{His8}$ (450 µg of protein per ml, in buffer B; see above) was freshly diluted 10-fold with cold 1×PBS and incubated with 1 mM $MnCl_2$ (added from 1 M stock solution of $MnCl_2$) for 10 min at 37°C (9). After addition of a peptide in 1×PBS, each reaction sample, in the final volume of 0.1 ml, contained a peptide at 0.6 mM and Fra1$_{His8}$ at 0.2 µM. Reactions were carried out at 37°C using Eppendorf thermomixer. Reaction in each withdrawn sample was stopped at intervals indicated in Fig. 2 by heating at 90°C for 10 min. Samples of 10 µl were injected into HPLC column (Kinetex C18, 5 µm, 250 × 4.6 mm, Phenomenex). HPLC was performed at the flow rate of 0.9 ml/min using Agilent-1290 at the Korea Basic Science Institute (Seoul Center). Water containing 0.1% (v/v) trifluoroacetic acid (TFA) (solvent A) and 0.1% (v/v) TFA in acetonitrile (solvent B) were used as mobile phases. The samples were eluted with a linear gradient of solvent B concentration, from 5 to 15 % over 20 min. Retention times for the pairs of substrate and product were as follows: **AP**VTIEK (17.3 min), **P**VTIEK (16.4 min); **SP**SKMNA (10.6 min), **P**SKMNA (10.0 min). For **VP**VTIEK and **PPP**TAQF peptides, the elution was done by a linear gradient of solvent B, from 5 to 50% over 20 min. Retention times: **VP**VTIEK (8.8 min), **P**VTIEK (8.1 min); **PPP**TAQF (10.1 min), **PP**TAQF (9.9 min), and **P**TAQF (9.6 min). All peptides were detected spectrophotometrically at 214 nm. The activity of Fra1$_{His8}$ was quantified by measuring a decrease of the area of substrate peptide peak versus an increase of the area of product peak. The measurements were converted into changes in the levels of product by comparing the areas of peaks of reference peptides (of known concentration) with areas of peaks of Fra1 products.

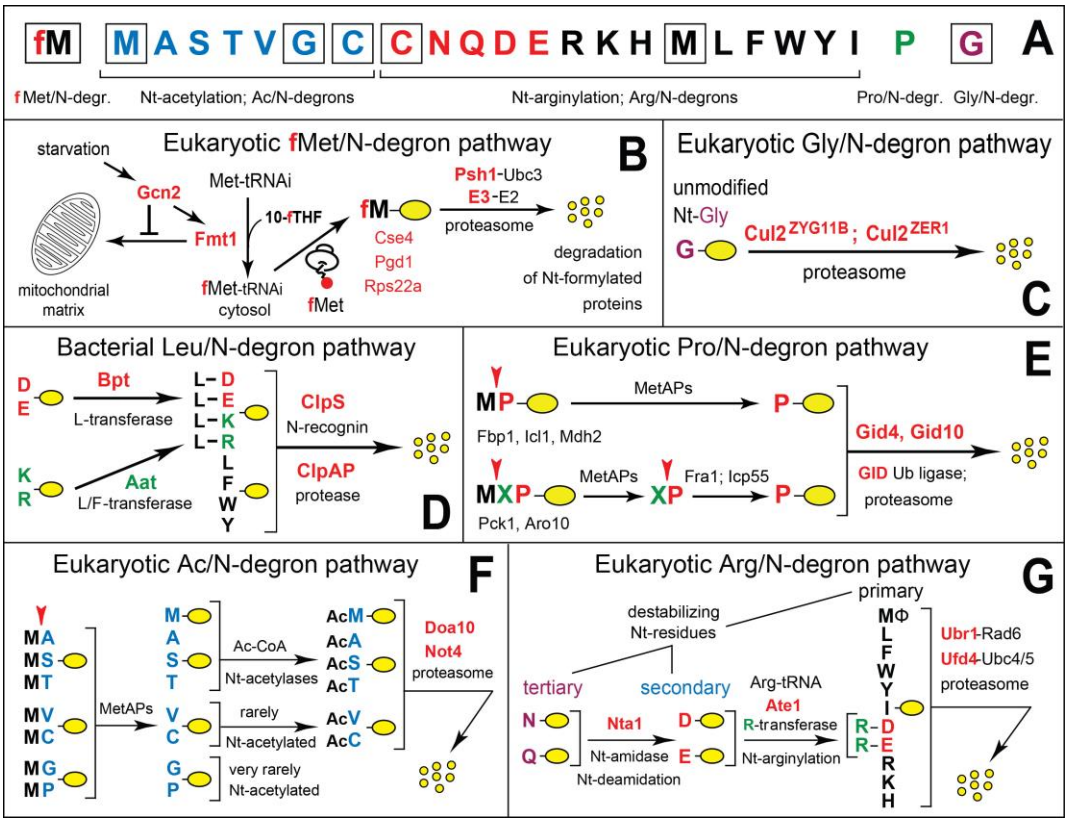

Fig. S1. N-degron pathways. (***A***) 20 amino acids of the genetic code. Amino acids cited more than once are marked by squares. Nt-Met is cited thrice: it can be targeted by the Ac/N-degron pathway (as Ac-Met); by the Arg/N-degron pathway (as Nt-Met-Φ motif, with Φ denoting a bulky hydrophobic residue); and by the fMet/N-degron pathway (as Nt-formylated fMet). Nt-Cys is cited twice: it can be targeted by the Ac/N-degron pathway (as Nt-acetylated Cys) and by the Arg/N-degron pathway (as oxidized Nt-Cys*, but not in unstressed *S. cerevisiae*). Nt-Gly is cited twice: it can be targeted by the Gly/N-degron pathway (as unmodified Nt-Gly), and by the Ac/N-degron pathway (as Nt-acetylated Gly). (***B***) Eukaryotic fMet/N-degron pathway. (***C***) Eukaryotic Gly/N-degron pathway. (***D***) Bacterial Leu/N-degron pathway. (***E***) Eukaryotic Pro/N-degron pathway, with the mention of aminopeptidases that were identified, in the present study, as pathway's components. See the main Fig. 5 for a more detailed diagram of this GID-based proteolytic system. (***F***) Eukaryotic Ac/N-degron pathway. (***G***) Eukaryotic Arg/N-degron pathway. See (10-13) and references therein for details.

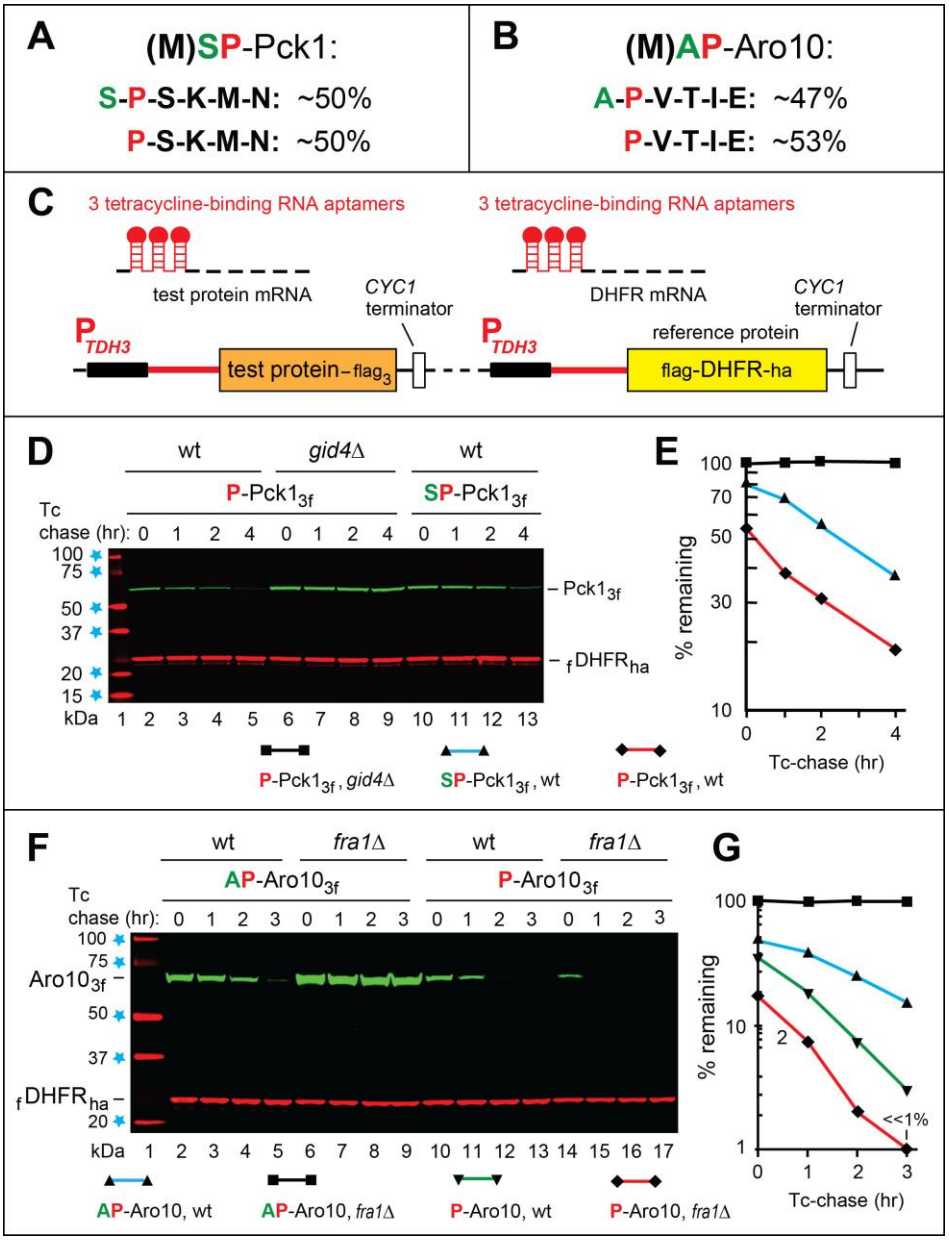

Fig. S2. N-terminal sequencing of proteins by Edman degradation, and promoter reference technique (PRT)-chases of (M)SP-Pck1$_{3f}$, (M)AP-Aro10$_{3f}$, and their derivatives. (***A***) Nt-sequencing, using Edman degradation, of (M)SP-Pck1$_{3f}$ isolated from *S. cerevisiae* that expressed this protein from the endogenous *PCK1* gene and its native promoter (see Materials and Methods, and the main text). (***B***) Same as in ***A*** but with (M)AP-Aro10$_{3f}$. (***C***) The promoter reference technique (PRT) (3, 6, 14). In this method, a long-lived protein (dihydrofolate reductase (DHFR)) and a test protein are expressed from two identical P_{TDH3} promoters containing additional DNA elements (14). In an mRNA, these elements form 5'-aptamers that can bind to tetracycline (Tc) upon its uptake by a cell. The binding of Tc halts translation of just two genes, and thereby allows chase-degradation assays that avoid the use of global translation inhibitors. DHFR reference increases the accuracy of Tc/PRT, since relative levels of a test protein can be measured as its ratios to the reference (14). (***D***) Lane 1, kDa markers. Lanes 2-5,

Tc/PRT-chase (see ***C***) of (engineered) **P**-Pck1$_{3f}$ for 0-3 hrs at 30°C in wild-type (wt) cells. Lanes 6-9, same as 2-5 but in *gid4Δ* cells. Lanes 10-13, same as 2-5 but with **SP**-Pck1$_{3f}$.
(***E***) Quantification of data in ***D***. (***F***) Lane 1, kDa markers. Lanes 2-5, Tc/PRT-chase of **AP**-Aro10$_{3f}$ at 30°C in wt cells. Lanes 6-9, same as 2-5 but in *fra1Δ* cells. Lanes 10-13, same as 2-5 but with (engineered) **P**-Aro10$_{3f}$. Lanes 14-17, same as 6-9 but in *fra1Δ* cells.
(***G***) Quantification of data in ***F***. Re the absence of standard-deviation bars in ***E*** and **G**: see a detailed explanation in the legend to the main Fig. 1.

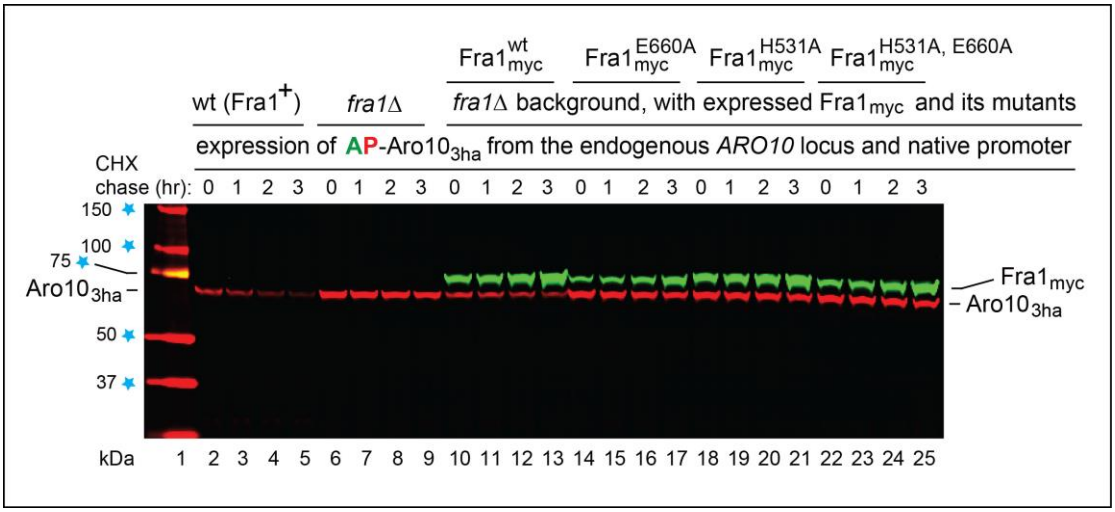

Fig. S3. Destabilization of (M)AP-Aro10$_{3f}$ in *fra1Δ* cells by expression of wild-type Fra1 but not of its active-site mutants. Lane 1, kDa markers. Lanes 2-5, cycloheximide (CHX)-chase of AP-Aro10$_{3f}$ for 0-3 hrs at 30°C in wt, Fra1^{+} cells. Lanes 6-9, same as 2-5 but in *fra1Δ* cells. Lanes 10-13, same as 6-9 but in the presence of wt Fra1$_{myc}$. Lanes 14-17, same as 6-9 but in the presence of mutant $Fra1^{H531A}_{myc}$. Lanes 18-21, same as 6-9 but in the presence of mutant $Fra1^{E660A}_{myc}$. Lanes 22-25, same as 6-9 but in the presence of double-mutant $Fra1^{H531A,E660A}_{myc}$. The bands of AP-Aro10$_{3f}$ and Fra1$_{myc}$ are indicated on the right. See the main text for additional details.

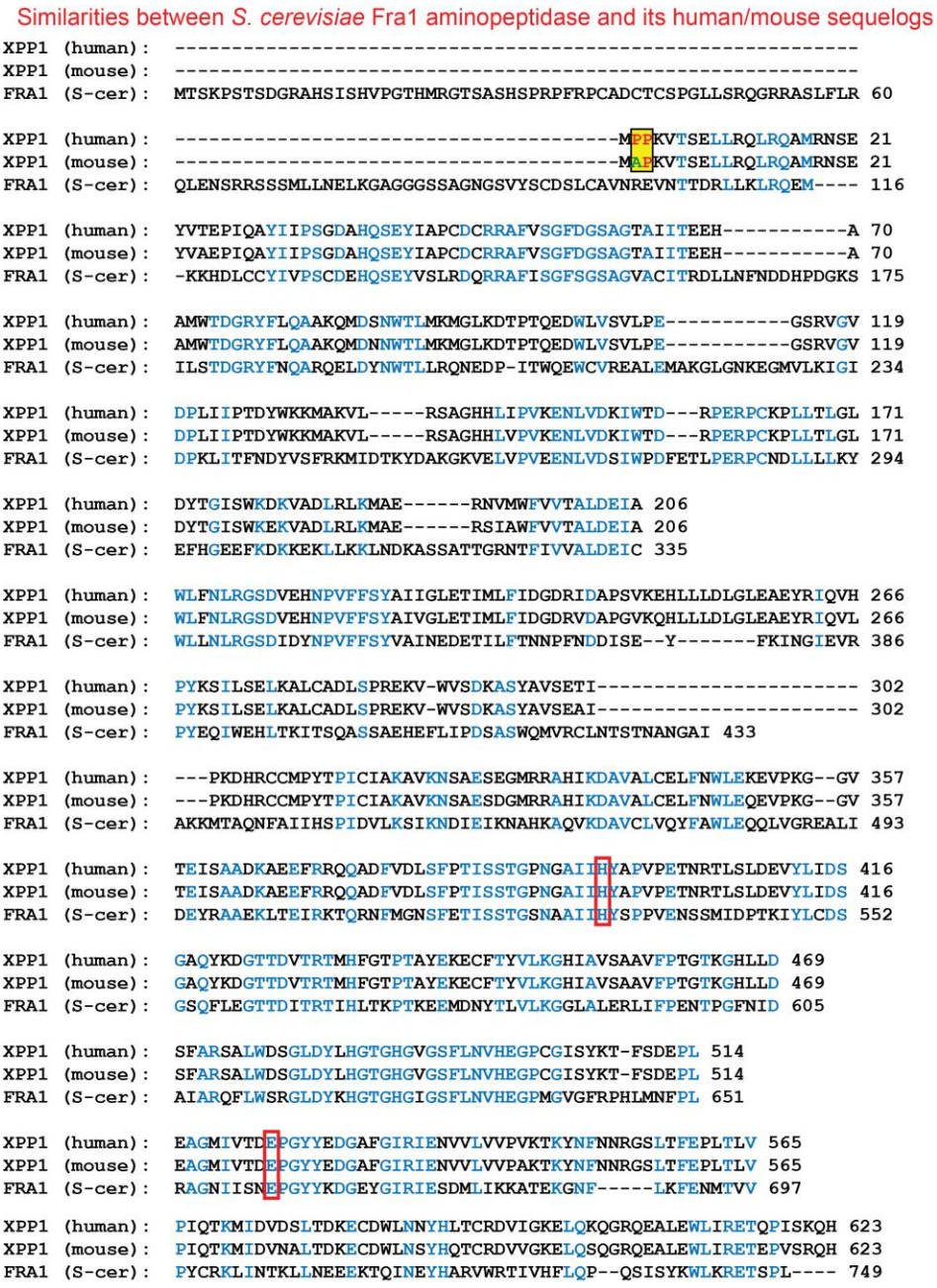

Fig. S4. Sequelogy (sequence similarity) (15) between the S. *cerevisiae* Fra1 aminopeptidase and its human/mouse counterparts. Residues that are identical in all three Fra1 sequelogs (15) are in blue. Red-frame rectangles denote active-site His-531 and Glu-660 of *S. cerevisiae* Fra1 that were mutated in the present study (*SI Appendix*, Fig. S3). In addition to extensive sequelogy between yeast and mammalian Fra1's, the human and mouse Xpp1 proteins (but not yeast Fra1) bear, respectively (after cotranslational removal of Nt-Met), the Nt-Pro-Pro (Nt-**PP**) and Nt-Ala-Pro (Nt-**AP**) Nt-sequences, which are marked by yellow rectangle. Thus, remarkably, the mammalian Fra1-type aminopeptidases are likely to be their own substrates, suggesting that the ability to "self-trim", yielding a single Nt-**P** residue, may regulate the levels of these aminopeptidases through their degradation by the Pro/N-degron pathway. Experiments to verify this hypothetical mechanism and function are under way.

Extended Data Table 1. *E. coli* and *S. cerevisiae* strains used in this study.

Strains	**Relevant genotypes**	**Sources**
E. coli strains:		
DH5a	*F^- Φ80lacZΔM15 Δ(lacZYA-argF) U169 recA1 endA1 hsdR17 (rK^-, mK^+) phoA supE44 λ^- thi-1 gyrA96 relA1*	Invitrogen
STBL2	*F^- endA1 glnV44 thi-1 recA1 gyrA96 relA1 Δ(lac-proAB) mcrA Δ(mcrBC-hsdRMS-mrr) λ^-*	Invitrogen
BL21 (DE3)	*F^- ompT $hsdS_B$ (r_B^-, m_B^-) gal dcm (DE3)*	Invitrogen
S. cerevisiae strains:		
BY4741	*MAT**a** his3-1 leu2-0 Met15-0 ura3-0*	Open Biosystems
BY3614	*GID2Δ::KanMX6* in BY4741	Open Biosystems
BY3244	*GID4Δ::KanMX6* in BY4741	Open Biosystems
CHY7027	*MAT**a** his3-1 leu2-0 Met15-0 ura3-0 Pck1-$(G)_6$-3xFLAG::HphMX4*	A gift from Dr. C. S. Hwang
CHY7029	*MAT**a** his3-1 leu2-0 Met15-0 ura3-0 Aro10-$(G)_6$-3xFLAG::HphMX4*	A gift from Dr. C. S. Hwang
yCSJ74	*MAT**a** his3-1 leu2-0 Met15-0 ura3-0 yfr006wΔ::KanMX6 fra1Δ::HphNT1*	This study
yCSJ87	*MAT**a** his3-1 leu2-0 Met15-0 ura3-0 fra1Δ::HphNT1*	This study
yCSJ90	*MAT**a** his3-1 leu2-0 Met15-0 ura3-0 fra1Δ::HphNT1 Aro10-SGSG-3xHA::KanMX6*	This study
yCSJ92	*MAT**a** his3-1 leu2-0 Met15-0 ura3-0 Fra1-$(G)_6$-3xFLAG::HphMX4 Aro10-SGSG-3xHA::KanMX6*	This study

Extended Data Table 2. Plasmids used in this study.

Plasmid	Description	Source or Reference
pFA6a-KanMX6	pFA6a-KanMX6	(*18*)
pFA6a-HphNT1	pFA6a-HphNT1	(*19*)
pFa6a-3xHa-KanMX6	pFa6a-3xHa-KanMX6	(*18*)
pFa6a-6xGly-3xFlag-HphMX4	pFa6a-6xGly-3xFlag-HphMX4	(*20*)
pETDuet-1	pETDuet-1	Novagen
p406TDH3	p406TDH3	Addgene
pBluescript SK(+)	pBluescript SK(+)	Lab collection
pBW261	P_{CUP1} in pRS313	Lab collection
pLK103	$Fra1_{His8}$ in pETDuet-1	This study
pCSJ105	P_{CUP1} -T_{CYC1} in pRS426	This study
pCSJ121	P_{TDH3}-Tc_3-MSP-$Pck1_{3FLAG}$-T_{CYC1} and P_{TDH3}-Tc_3-$_{FLAG}Dhfr_{HA}$-T_{CYC1} in pRS313	(*3*)
pCSJ122	P_{TDH3}-Tc_3-MSS-$Pck1_{3FLAG}$-T_{CYC1} and P_{TDH3}-Tc_3-$_{FLAG}Dhfr_{HA}$-T_{CYC1} in pRS313	(*3*)
pCSJ125	P_{TDH3}-Tc_3-MP-$Mdh2_{3FLAG}$-T_{CYC1} and P_{TDH3}-Tc_3-$_{FLAG}Dhfr_{HA}$-T_{CYC1} in pRS313	(*3*)
pCSJ1067	P_{TDH3}-Tc_3-MAP-$Aro10_{3FLAG}$-T_{CYC1} and P_{TDH3}-Tc_3-$_{FLAG}Dhfr_{HA}$-T_{CYC1} in pRS313	This study
pCSJ1105	P_{TDH3}-Tc_3-MAS-$Aro10_{3FLAG}$-T_{CYC1} and P_{TDH3}-Tc_3-$_{FLAG}Dhfr_{HA}$-T_{CYC1} in pRS313	This study
pCSJ1154	P_{CUP1}-Fra1-myc-T_{CYC1} in pRS426	This study
pCSJ1169	P_{TDH3}-Tc_3-MP-$Aro10_{3FLAG}$-T_{CYC1} and P_{TDH3}-Tc_3-$_{FLAG}Dhfr_{HA}$-T_{CYC1} in pRS313	This study
pCSJ1209	P_{TDH3}-Tc_3-MAPPP-$Aro10_{3FLAG}$-T_{CYC1} and P_{TDH3}-Tc_3-$_{FLAG}Dhfr_{HA}$-T_{CYC1} in pRS313	This study
pCSJ1226	C-terminal Fra1 fragment (450 to 749aa)$_{myc}$ in pBluescript SK(+)	This study
pCSJ1227	Site direct mutagenesis C-terminal Fra1 fragment (E660A)$_{myc}$ in pBluescript SK(+)	This study
pCSJ1228	Site direct mutagenesis C-terminal Fra1 fragment (H531A)$_{myc}$ in pBluescript SK(+)	This study
pCSJ1229	Double mutant C-terminal Fra1 fragment (H531A, E660A)$_{myc}$ in pBluescript SK(+)	This study
pCSJ1230	P_{CUP1}-Fra1 (E660A)-myc-T_{CYC1} in pRS426	This study
pCSJ1231	P_{CUP1}-Fra1 (H531A)-myc-T_{CYC1} in pRS426	This study

pCSJ1232	P_{CUP1}-Fra1 (H531A, E660A)-myc-T_{CYC1} in pRS426	This study

Extended Data Table 3. PCR primers used in this study.

Primer	Sequence (5' to 3')
LK075	CCACATATGACTTCGAAACCATCCACCAGTGAC
LK076	GCTCTCGAGTAGTGGGCTTGTTTCTCTTTTCAG
OCH7331	ATCAAGACAGAGCCACACCAGATGTATTAGCCGCTGGTCCTCAATT CGAGTCGTACGCTGCAGGTCGAC
OCH7332	TCTTTTTTTTTTTTTTGGATTGAACATATCGAACGAACATGTTTCGTT TAATCGATGAATTCGAGCTCG
OCH7333	AACAGCTAAAGTGCATGGTTGAAGCAGCGGCACTTAAAAGAAATA AAAAATCGTACGCTGCAGGTCGAC
OCH7334	AAAACGAACAATTGGTAGCAGTGTTTTATAATTGCGCCCACAAGTT TCTAATCGATGAATTCGAGCTCG
CSJ217	CAGCGCGTTTATGATATGTTTG
CSJ218	CTCTTTTTCTCTTAAACTTTTG
CSJ219	CAAGTTACCAGATTTTCTGACTC
CSJ220	AATGATATTTTTGGGGGATTAC
CSJ1076	CATCAAAGCAGTCGGAGCAA
CSJ1077	ATGAAAGGGTGCGGTTGAAG
CSJ1178	ATATATGGCGCGCCATGGCACCTGTTACAATTGAAAAG
CSJ1179	ATATATGGATCCTTTTTTATTTCTTTTAAGTGCCGC
CSJ1207	ATATATGGCGCGCCATGGCATCTGTTACAATTGAAAAGTTC
CSJ1259	CGGAGACCTCAACAGAGCGAAACCAATAATTACAAACAAACGTAC GCTGCAGGTCGAC
CSJ1260	CTAACATTAATTGTATTGCGCCCCGCTCGCCTCGAAGTCAATCGATG AATTCGAGCTCG
CSJ1265	ATATATAGATCTGCGGCCGCATGACTTCGAAACCATCCAC
CSJ1266	ATATATCTCGAGTCACAAATCTTCTTCAGAAATCAACTTTTGTTCGT CGAC TAGTGGGCTTGTTTCTCTTTTC
CSJ1284	ATATATGGCGCGCCATGCCTGTTACAATTGAAAAGTTC
CSJ1312	GTGCATGGTTGAAGCAGCGGCACTTAAAAGAAATAAAAAATCAGG GTCTGGCTACCCATACGATGTTCCTGACTATG

CSJ1313	ACAATTGGTAGCAGTGTTTTATAATTGCGCCCACAAGTTTATCGATG AATTCGAGCTCG
CSJ1314	TATTTCATACAAGTGGCTGAAAAGAGAAACAAGCCCACTAGGGGGA GGCGGGGGTGGA
CSJ1315	ATACTAACATTAATTGTATTGCGCCCCGCTCGCCTCGAAGATCGATG AATTCGAGCTCG
CSJ1318	GGTCACATGCAGTTTCAATATCC GACTCCGGTTCGCAATTC
CSJ1325	CTGTTCGTGATTTCGCGTTTC
CSJ1326	GACTCCGGTTCGCAATTC
CSJ1326	GACTCCGGTTCGCAATTC
CSJ1344	ATATATGGCGCGCCATGGCACCTCCGCCTGTTACAATTGAAAAGTTC
CSJ1372	GTAACATAATAAGTAACGCTCCAGGTTATTATAAG
CSJ1373	CTTATAATAACCTGGAGCGTTACTTATTATGTTAC
CSJ1374	GTAACGCCGCCATCATAGCTTATTCGCCGCCCGTAG
CSJ1375	CTACGGGCGGCGAATAAGCTATGATGGCGGCGTTAC

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