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Supplemental Materials for Research Article in *Microbial Cell*:

Formyl-methionine as a degradation signal at the N-termini of bacterial proteins

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These Supplemental Materials contain:

Figure S1 and its legend.

Tables S1 and S2.

Supplemental References.

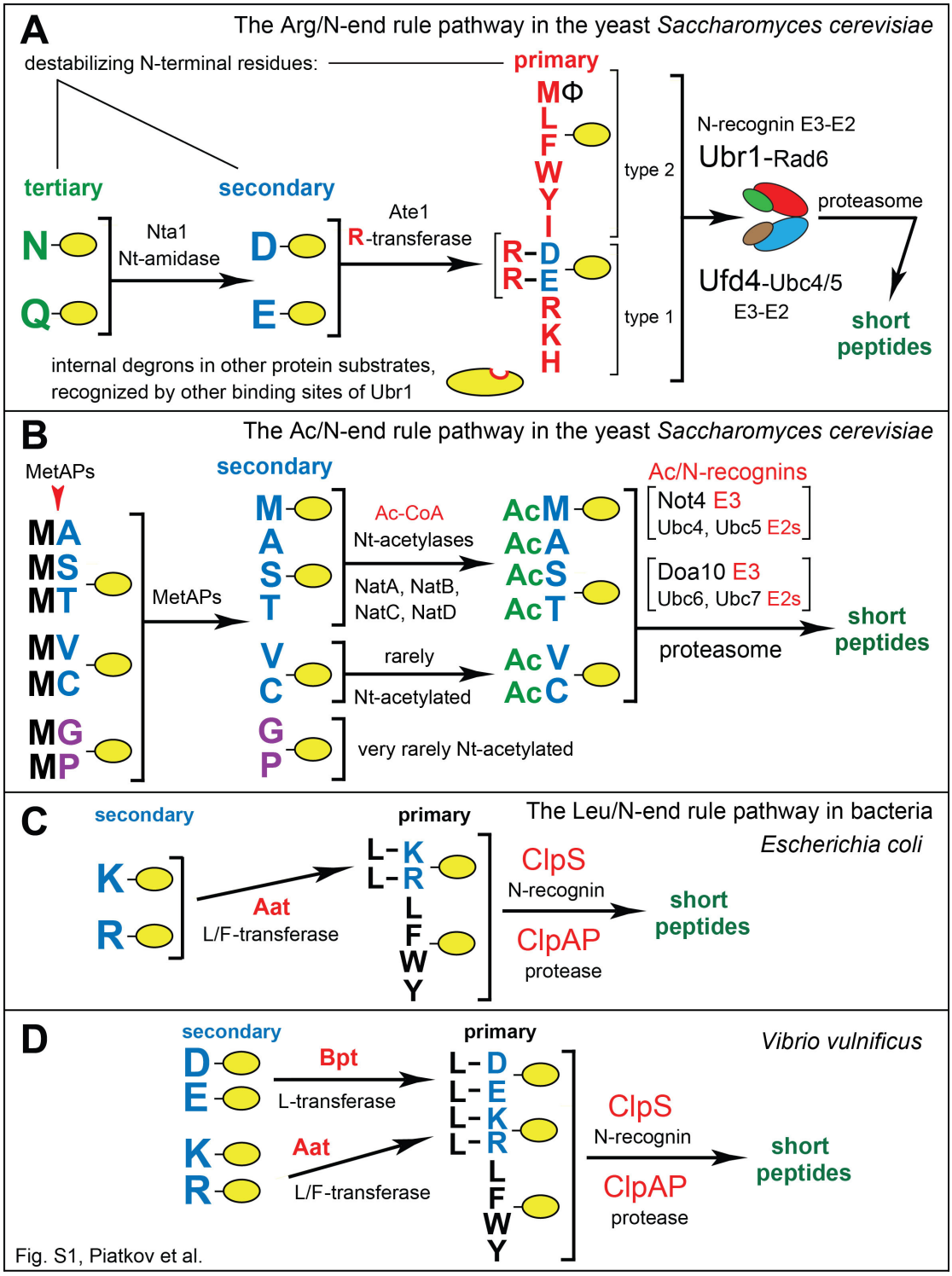

Figure S1. The Arg/N-End Rule Pathway and the Ac/N-End Rule Pathway. N-terminal residues are indicated by single-letter abbreviations. A yellow oval denotes the rest of a protein substrate.

The N-end rule pathway recognizes proteins containing N-terminal degradation signals called N-degrons, polyubiquitylates these proteins and thereby causes their degradation by the 26S proteasome. Recognition components of the N-end rule pathway are called N-recognins. Eukaryotic N-recognins are E3 ubiquitin (Ub) ligases that can target N-degrons. The main determinant of an N-degron is a destabilizing N-terminal residue of a protein. In eukaryotes, the N-end rule pathway consists of two branches, described in panels **A** and **B**.

(A) The Arg/N-end rule pathway in *S. cerevisiae* [1-5]. The prefix "Arg" in the pathway's name refers to Nt-arginylation of N-end rule substrates. The Arg/N-end rule pathway targets specific unacetylated N-terminal residues. It is mediated by the Ubr1 N-recognin, a 225 kDa RING-type E3 Ub ligase and a part of the targeting complex containing the Ubr1-Rad6 and Ufd4-Ubc4/5 holoenzymes. The Ubr1 (N-recognin) component of this complex recognizes (binds to) the "primary" destabilizing N-terminal residues Arg, Lys, His, Leu, Phe, Tyr, Trp and Ile, as well as the unmodified N-terminal Met residue, if Met is followed by a bulky hydrophobic (Φ) residue [5]. The terms "secondary" and "tertiary" refer to the indicated enzymatic modifications of specific N-terminal residues. N-terminal Cys can be arginylated by the Ate1 arginyltransferase (R-transferase) only after the oxidation of Cys to Cys-sulfinate or Cys-sulfonate, in reactions that involve nitric oxide (NO) and oxygen [2-4, 6, 7]. Regulated oxidation of N-terminal Cys takes place in multicellular eukaryotes but not in fungi such as *S. cerevisiae*, which apparently do not produce NO under normal conditions.

(B) The Ac/N-end rule pathway in *S. cerevisiae* [3, 5, 8-10]. This pathway recognizes substrates through their N^{α}-terminally acetylated (Nt-acetylated) residues. The corresponding degradation signals and E3 Ub ligases are called Ac/N-degrons and Ac/N-recognins, respectively. Red arrow on the left indicates the removal of N-terminal Met by Met-aminopeptidases (MetAPs). N-terminal Met is retained if a residue at position 2 is larger than Val [11-13]. The term "secondary" refers to the requirement for a modification (Nt-acetylation) of a destabilizing N-terminal residue before a protein can be recognized by an Ac/N-recognin.

(C) and **(D)** The bacterial Leu/N-end rule pathway, in *Escherichia coli* **(C)** and in *Vibrio vulnificus* **(D)** [14-32]. The Aat L/F-transferase conjugates (largely) Leu to N-terminal Arg or Lys. N-end rule substrates bearing primary (bulky hydrophobic) destabilizing N-terminal residues are recognized by the ClpS N-recognin and are delivered for degradation to the ClpAP protease. In *V. vulnificus*, the Leu/N-end rule pathway contains both the Aat L/F-transferase and the Bpt L-transferase. As a result, N-terminal Asp and Glu, which are not destabilizing residues in *E. coli*, function as secondary destabilizing residues in *V. vulnificus* [16].

Regulated degradation of proteins or their natural fragments by the N-end rule pathway mediates a broad range of biological functions (refs. [2-5, 8-10, 31] and refs therein).

Table S1. Bacterial strains used in this study.

***E. coli* strains**	**Genotype**	**Source or Ref.**
MG1655	*F- λ- rph-1*	[33]
MG-D	*F- λ- rph-1* Δ(def-fmt)::cm *ΔgloB*::frt	Gift from D. Mazel
DH5α	*φ80 lacZΔM15 endA1 recA1 gyrA96 thi-1 hsdR17* (r_k^- , m_k^-) *relA1 supE44 deoR Δ(lacZYA–argF)U169*	Promega
CAG12184	*λ^- rph-1 tolC210::Tn10 (tet)*	[34]
KPS73	*λ- rph-1 tolC210::Tn10 (tet) Δfmt ::Km*	This study
KPS74	*λ- rph-1 tolC210::Tn10 (tet) Δdef-Δfmt ::Km*	This study

Table S2. Plasmids used in this study.

Plasmid name	Description	Source or Ref.
pJT184	Cm^R; pACYC184-based plasmid with 2.8-kb BamHI/SalI insert containing *S. cerevisiae* Ubp1.	[14]
pBADET	Ap^R; pBAD2-based plasmid containing arabinose-inducible promoter and expanded multiple cloning site.	Gift from V. Ksenzenko
pACYC177	Ap^R, Km^R; low copy vector containing p15A repl. origin.	[35, 36]
pKD46	Ap^R; ts replication (repA101ts); encodes lambda Red genes (*exo, bet, gam*); native terminator (tL3) after *exo* gene; arabinose-inducible promoter for expression (P*ara*); encodes *araC* for repression of the Para promoter. This plasmid was used for chromosomal deletions of specific genes.	[37]
pKD3	Ap^R, Cm^R; R6K origin-based plasmid containing *cat*-cassette flanked by FRT sites. This plasmid was used as a template in PCR to create gene-specific *cat*-cassettes.	[37]
pCH178	Ap^R; p314CUP1-based plasmid expressing Ub-CK-e^K-ha-Ura3.	[9]
pKP249	Ap^R; pBADET-based plasmid expressing $P1^{T2}$-e^K-ha-Ura3 from P*ara* promoter.	This study
pKP250	Ap^R; pBADET-based plasmid expressing $P1^{T2D}$-e^K-ha-Ura3 from P*ara* promoter.	This study
pKP251	Ap^R; pBADET-based plasmid expressing Ub-$P1^{T2}$-e^K-ha-Ura3 from P*ara* promoter.	This study
pKP252	Ap^R; pBADET-based plasmid expressing Ub-$P1^{T2D}$-e^K-ha-Ura3 from P*ara* promoter.	This study
pKP257	Ap^R; pACYC177-based plasmid expressing $P1^{T2}$-e^K-ha-Ura3 from P_{KmR} promoter.	This study
pKP258	Ap^R; pACYC177-based plasmid	This study

	expressing $P1^{T2D}$-e^{K}-ha-Ura3 from P_{KmR} promoter.	
pKP286	Ap^{R}; pBADET-based plasmid expressing PpiB-8his-flag from P*ara* promoter.	This study
pKP287	Ap^{R}; pBADET-based plasmid expressing $PpiB^{V2D}$-8his-flag from P*ara* promoter.	This study
pKP335	Ap^{R}; pBADET-based plasmid expressing PpiB-8his-flag-Ub-PpiB-8his-flag from P*ara* promoter	This study
pKP458	Ap^{R}; pBADET-based plasmid expressing ^{MVTV}PpiB-8his-flag and ^{MDDD}PpiB-8his-flag-Ub from two identical and independent P*ara* promoters.	This study
pKP459	Ap^{R}; pBADET-based plasmid expressing ^{MVTV}PpiB-8his-flag and ^{MYFY}PpiB-8his-flag-Ub from two identical and independent P*ara* promoters.	This study

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