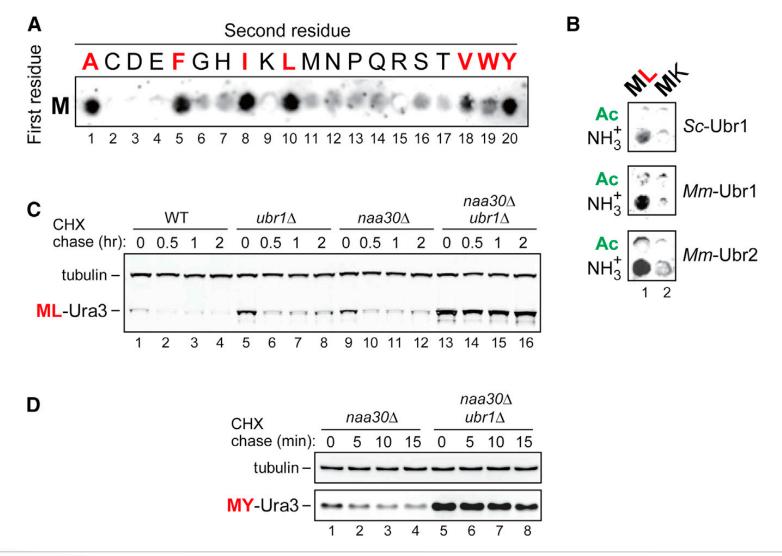
# The N-Terminal Methionine of Cellular Proteins as a Degradation Signal

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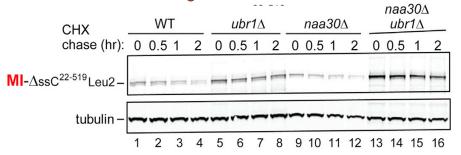
Figure 1. Specific Binding of Ubr1 to Unacetylated N-Terminal Methionine Followed by a Hydrophobic Residue



#### **Rule Pathway** naa30∆ В naa30∆ Α $naa30\Delta$ $ubr1\Delta$ CHX MK ML CHX chase (min): 0 5 10 15 0 5 10 15 chase (min): 0 15 30 60 0 15 30 60 tubulin tubulin -MI-Ura3-MZ-Ura3-3 1 2 4 5 6 7 8 2 3 5 6 78 1 4 **C**<sub>100</sub> Е D 100 MI-Ura3 MK-Ura3 Arg-Ala: Leu-Ala: Ubr1: naa30∆ ubr1∆ cells naa30∆ cells % remaining % remaining + (ML-GST)<sup>Ub</sup>n kDa 10 10 100 75 -55 -40 -35 -Ξ MI-Ura3 ML-Ura3 $\exists$ naa30∆ cells naa30∆ cells -ML-GST 25 -1 5 10 15 60 0 0 30 chase (min) 2 3 5 chase (min) 1 4 F BRR RING UBR AI Ubr1 1 1 1 450 / Т 900 1350 1800 1950 1 type-2 type-1 site site kDa roatters NNL 651 40 -35 -25 -GST G н (SS1 input input st. 1-1950: 98-518: 🕶 1-717: 209-717: 1-310: 🗩 209-1140: = 1 2 3 1 2 3 2 1

Figure 2. Unacetylated N-Terminal Methionine as an N-Degron of the Arg/N-End Rule Pathway

### Figure 3. Misfolded Proteins Containing Met-Based N-Degrons



WT cells В С MI MK WT  $ubr1\Delta$ CHX CHX chase (min): 0 15 30 60 0 15 30 60 chase (min): 0 15 30 60 0 15 30 60 tubulintubulin  $MI-\Delta^{22-58}$ Ura3 – **MZ-**Δ<sup>22-58</sup>Ura3-2 3 4 5 78 2 3 4 5 6 7 8 6 1 1 Е D 100 100 **MI-** $\Delta^{22-58}$ Ura3 in: ➡ naa30∆ ubr1∆ ■ ubr1∆ cells % remaining ▲ ubr1∆ % remaining 10 10 **MI-** $\Delta^{22-58}$ Ura3 in: WT cells WΤ **×** naa30∆ 1 1 0.5 1 60 0 2 0 30

chase (hr)

chase (min)

Α

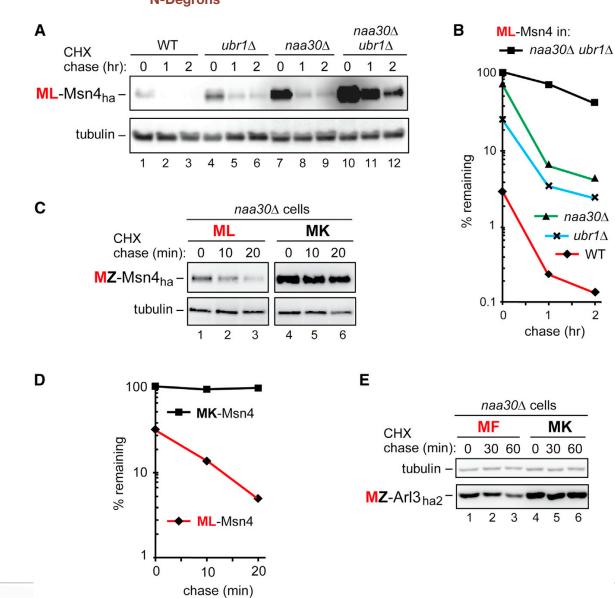
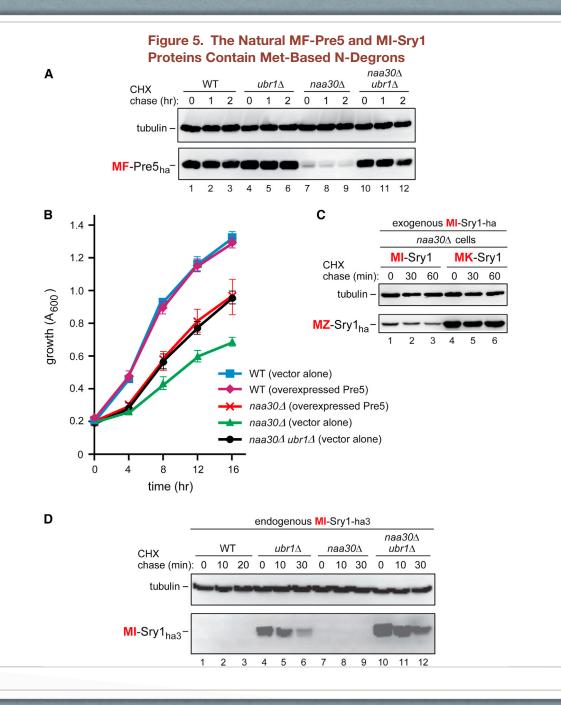


Figure 4. The Natural ML-Msn4 and MF-Arl3 Proteins Contain Met-Based N-Degrons



#### Figure 6. Complementary Specificities of the Arg/N-End Rule Pathway and the Ac/ N-End Rule Pathway

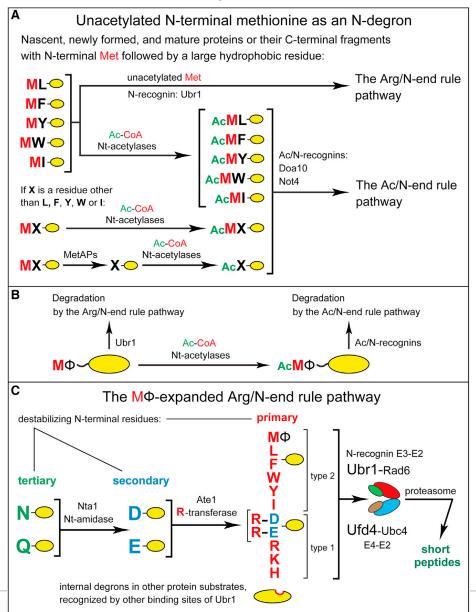


Figure 7. Conditionality of Ac/N-Degrons and Protein Remodeling by the N-End Rule Pathway

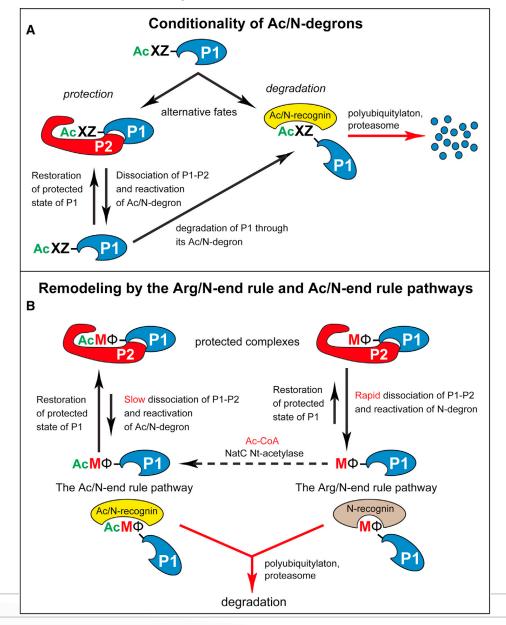


Figure S1. The Ac/N-End Rule Pathway and the Arg/N-End Rule Pathway, Refers to Figures 1, 2, 3, 4, 5, 6, and 7

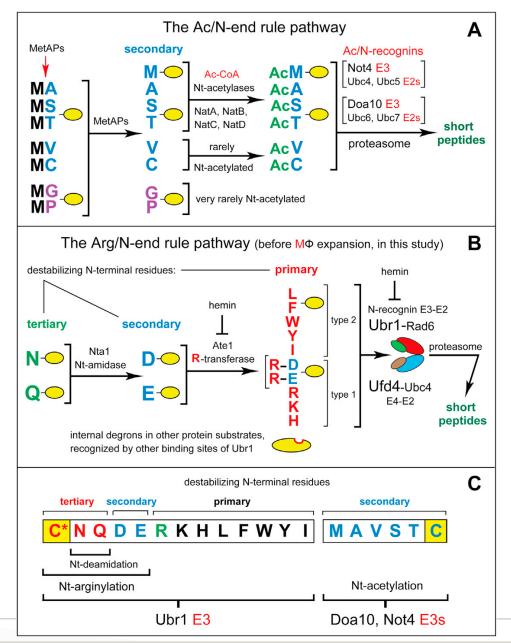
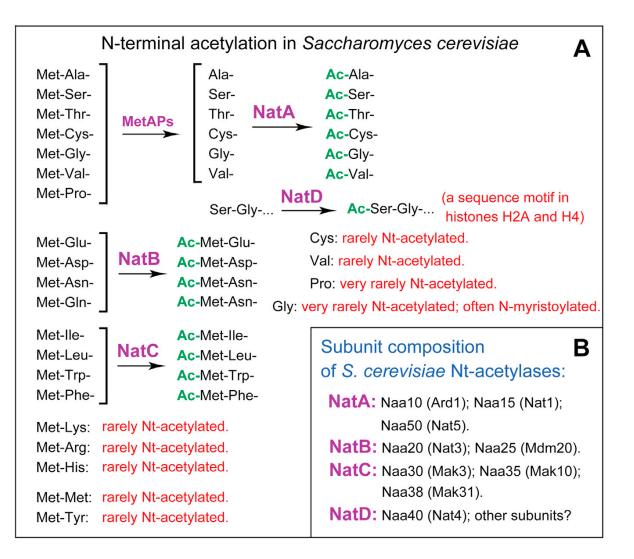


Figure S2. Substrate Specificities and Subunit Compositions of *S. cerevisiae*  $N^{\alpha}$ -Terminal Acetylases (Nt-acetylases), Refers to Figures 1, 2, 3, 4, 5, 6, and 7



## Figure S3. <sup>35</sup>S-Pulse-Chase and Cycloheximide-Chase Assays with ML-Ura3 and MI-Sry1, Refers to Figures 1 and 5

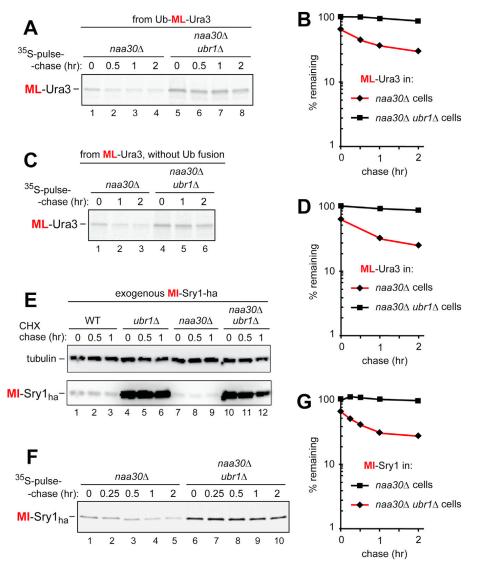


Figure S4. Instability of the MF-Pre5 Proteasomal Subunit and Hypersensitivity of  $naa30 \varDelta$  and  $naa30 \varDelta$  ubr1 $\varDelta$  Cells to Multiple Stresses, Refers to Figures 2 and 5

