Correspondence: avarsh@caltech.edu (A.V.), cshwang@postech.ac.kr (C.-S.H.)
http://dx.doi.org/10.1016/j.cell.2013.11.031

Journal Club Presentation
January 14, 2014
Prashant Kota
Figure 1. Specific Binding of Ubr1 to Unacetylated N-Terminal Methionine Followed by a Hydrophobic Residue

A. Second residue

| First residue | A | C | D | E | F | G | H | I | K | L | M | N | P | Q | R | S | T | V | W | Y |
| M            |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |

B. Diagram showing AcNH₃⁺ Sc-Ubr1, AcNH₃⁺ Mm-Ubr1, and AcNH₃⁺ Mm-Ubr2.

C. CHX chase (hr):

<table>
<thead>
<tr>
<th>CHX chase (hr)</th>
<th>WT</th>
<th>ubr1Δ</th>
<th>nna30Δ</th>
<th>nna30Δ ubr1Δ</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0.5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
</tbody>
</table>

D. CHX chase (min):

<table>
<thead>
<tr>
<th>CHX chase (min)</th>
<th>nna30Δ</th>
<th>nna30Δ ubr1Δ</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>15</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Please cite this article in press as: Kim et al., The N-Terminal Methionine of Cellular Proteins as a Degradation Signal, Cell (2014), http://dx.doi.org/10.1016/j.cell.2013.11.031
Figure 2. Unacetylated N-Terminal Methionine as an N-Degron of the Arg/N-End Rule Pathway

A CHX chase (min): \(n_{aa30\Delta}\) urb1\(\Delta\) cells

B CHX chase (min): ML MK

C MI-Ura3

D ML-Ura3

E Arg-Ala: -- + + +
Leu-Ala: -- -- + + +
Ubr1: + + + + +

F Ubr1

G Input GST ML-GST

H Input GST ML-GST

I markers ML-GST

please cite this article in press as: Kim et al., The N-Terminal Methionine of Cellular Proteins as a Degradation Signal, Cell (2014), http://dx.doi.org/10.1016/j.cell.2013.11.031
would preclude their capture by Ubr1 while making them vulnerable to the Ac/N-end rule pathway. An unacetylated Met-protein to the Ac/N-end rule pathway. However, at least some molecules of this protein would be irreversibly Nt-acetylated before their targeting of these otherwise identical proteins. These partly random choices were determined largely by the fact that the cited proteins are Nt-acetylated in WT yeast. The dual-pathway circuit that is revealed by this understanding was more strongly but not entirely abolished in the Sry1 mutant, suggesting the presence of an internal degron as well. In a most telling pattern analogous to but even more striking than the results with engineered MZ-Ura3 proteins, the prechase level of ML-Msn4 was partially stabilized in double-mutant cells, indicating the presence of AcMet-proteins. These partly random choices were determined largely by the fact that the cited proteins are Nt-acetylated in WT yeast.

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

(A) The proteins were analyzed in CHX-chase assays with a low copy plasmid. In (A)–(E), the corresponding CHX-chase assays were performed. (B) CHX-chases with MI-ΔssC22-519Leu2, MI-Δ22-58Ura3, and MZ-Δ22-58Ura3 in WT and ubr1Δ cells. (C) CHX-chases with MI and MK in WT cells. (D) Quantification of data in (A). (E) Quantification of data in (B).
Figure 4. The Natural ML-Msn4 and MF-Arl3 Proteins Contain Met-Based N-Degrons

A

CHX chase (hr): 

<table>
<thead>
<tr>
<th></th>
<th>WT</th>
<th>ubr1Δ</th>
<th>naa30Δ</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

ML-Msn4ha-

<table>
<thead>
<tr>
<th></th>
<th>WT</th>
<th>ubr1Δ</th>
<th>naa30Δ</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

MZ-Msn4ha-

<table>
<thead>
<tr>
<th></th>
<th>ML</th>
<th>MK</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
</tbody>
</table>

C

CHX chase (min):

<table>
<thead>
<tr>
<th></th>
<th>ML</th>
<th>MK</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
</tbody>
</table>

D

% remaining

E

CHX chase (min):

<table>
<thead>
<tr>
<th></th>
<th>MF</th>
<th>MK</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>30</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>60</td>
<td>60</td>
<td>60</td>
</tr>
</tbody>
</table>

Please cite this article in press as: Kim et al., The N-Terminal Methionine of Cellular Proteins as a Degradation Signal, Cell (2014), http://dx.doi.org/10.1016/j.cell.2013.11.031

Elsevier Inc.
owing to its degradation by the Arg/N-end rule pathway. We now unacetylated and short-lived MF-Pre5 proteasomal subunit,

\textbf{DISCUSSION}

\textbf{Figure 5. The Natural MF-Pre5 and MI-Sry1 Proteins Contain Met-Based N-Degrons}

<table>
<thead>
<tr>
<th>A</th>
<th>CHX chase (hr):</th>
<th>WT</th>
<th>ubr1Δ</th>
<th>naa30Δ</th>
<th>ubr1Δ</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>tubulin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MF-Pre5\textsubscript{ha}\textsuperscript{+}</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
</tbody>
</table>

\textbf{B}

\textbf{C}

\textbf{D}

\textbf{Figures 1 C, 1D, 2A, 2C, and 5D, and Figures S1 D, 4, and 5.}

\textbf{Figures S1 S2, S4, and 7.}

\textbf{Figures 6 B).}

\textbf{Figures 6 B).}

\textbf{Figures 6 B).}

\textbf{Figures 6 B).}

\textbf{Figures 6 B).}
can be destroyed as well, because it contains a Met. AcMet can target an Nt-acetylated AcMet-
of their Nt-acetylation. Specifically, the Ac/N-end rule pathway makes possible the proteolysis-

Figures 1

...proteins. For this reason alone, the present advance has signifi-

Figures 6

pathway circuit is summarized in

Ubr1-dependent Arg/N-end rule pathway. The resulting dual-

This previously unknown class of N-degrons is recognized by the

Please cite this article in press as: Kim et al., The N-Terminal Methionine of Cellular Proteins as a Degradation Signal, Cell (2014), http://

...interval of N-degrons (V) and Ubr2 can recognize Met-

Figures 6

and is targeted by Ubr1 through this, previously unknown class

between the Arg/N-end rule and Ac/N-end rule

Figure 6. Complementary Specificities of

N-End Rule Pathway

the Arg/N-End Rule Pathway and the Ac/

N-End Rule Pathway

(A) Unacetylated N-terminal methionine as an N-degron

Nascent, newly formed, and mature proteins or their C-terminal fragments with N-terminal Met followed by a large hydrophobic residue:

if X is a residue other than L, F, Y, W or I:

MX

MetAPs

Ac-CoA

Ni-acetylases

AcMX

MX

Ac-CoA

Ni-acetylases

AcX

The Arg/N-end rule pathway

The Ac/N-end rule pathway

B Degradation by the Arg/N-end rule pathway

Degradation by the Ac/N-end rule pathway

MΦ

Ubr1

Ac-CoA

Ni-acetylases

AcMΦ

Ac/N-recognins

C The MΦ-expanded Arg/N-end rule pathway

destabilizing N-terminal residues: primary

tertiary secondary

MLF

WL

EI

Nta1

Nt-amidase

Ate1

R-transferase

N-recognin E3-E2

Ubr1-Rad6

proteasome

short peptides

internal degrons in other protein substrates, recognized by other binding sites of Ubr1
Cog1 goes up in a cell. The cause of Cog1 destabilization was endogenous Cog1 becoming short lived if the production of Ac/N-degrons levels. However, an overexpressed Cog1 is short lived. N-degrons, as has been explained, among other things, how the prevalence of Ac/N-degrons, but with the transient formyl moiety (instead of permanent acetyl moiety) at the N termini of nascent bacterial proteins. For example, the previously long-lived MF-Pre5 of S. cerevisiae by, in the absence of its Nt-acetylation is based on structural studies of Nt-acetylated proteins by Schulman, Shemorry et al., 2013. Nevertheless, Cog1 is long lived if expressed at normal (endogenously) can be consistent with the fact that most Nt-acetylated proteins are at least intermittently long lived in vivo. The targeting of Met replacement-mediated conversion of P1-P2 into a similar but more stable complex containing the Nt-acetylation state of the P2 protein. To maximize generality of this description, the Nt-acetylation state of the P2 protein was left unspecified, in contrast to the P1 subunit. Nt-acetylation of cellular proteins is largely cotranslational. The dashed arrow signifies the dissociation of Nt-acetylated (AcMet-P1) subunit of a P1-P2 complex upon its dissociation would allow the replacement of P1-P2 into a similar but more stable complex containing the Nt-acetylation state of the P2 protein. The Arg/N-end rule and Ac/N-end rule pathways of the COG complex, was shown to contain an Ac/N-degron. This understanding of the dynamics of Nt-acetylated proteins by the Arg/N-end rule pathway (see Figure S1) in conjunction with the initial discovery of the conditionality of Ac/N-degrons. This diagram summarizes the previously attained understanding of the Arg/N-end rule and Ac/N-end rule pathways. The Ac/N-end rule pathway and of Nt-degrons by, the subunit-selective degradation of the P1 subunit was left unspecified, in contrast to the P1 subunit. Nt-acetylation of cellular proteins is largely cotranslational. The dashed arrow signifies the dissociation of Nt-acetylated (AcMet-P1) subunit of a P1-P2 complex upon its dissociation would allow the replacement of P1-P2 into a similar but more stable complex containing the Nt-acetylation state of the P2 protein. The Arg/N-end rule and Ac/N-end rule pathways of the COG complex, was shown to contain an Ac/N-degron. This understanding of the dynamics of Nt-acetylated proteins by the Arg/N-end rule pathway (see Figure S1) in conjunction with the initial discovery of the conditionality of Ac/N-degrons. This diagram summarizes the previously attained understanding of the Arg/N-end rule and Ac/N-end rule pathways. The Ac/N-end rule pathway and of Nt-degrons by, the subunit-selective degradation of the P1 subunit was left unspecified, in contrast to the P1 subunit. Nt-acetylation of cellular proteins is largely cotranslational. The dashed arrow signifies the dissociation of Nt-acetylated (AcMet-P1) subunit of a P1-P2 complex upon its dissociation would allow the replacement of P1-P2 into a similar but more stable complex containing the Nt-acetylation state of the P2 protein.
Regulated degradation of proteins or their fragments by the N-end rule pathway mediates a strikingly broad range of biological functions, which are broad in their effects, ranging from cell death and differentiation to protein localization and degradation. The N-end rule pathway is comprised of a group of enzymes that target specific destabilizing N-terminal residues (N-degrons). These enzymes include Ac/N-recognins, which recognize and ubiquitinate N-terminal degradation signals, and the proteasome, which degrades ubiquitinated proteins.

**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**

A. The Ac/N-end rule pathway

- **Ac/N-recognins**
  - Not4 E3
  - Ubc4, Ubc5 E2s

B. The Arg/N-end rule pathway (before Mφ expansion, in this study)

- **Destabilizing N-terminal residues**:
  - Nt-acetylated
  - Nt-deamidated

C. The Arg/N-end rule pathway (after Mφ expansion, in this study)

- **Destabilizing N-terminal residues**:
  - Nt-acetylated
  - Nt-arginylated

References:
- Hwang et al., 2010a; Hwang et al., 2011; Hwang and Varshavsky, 2008; Kim et al., 2013; Kitamura and Fujiwara, 2013; Kurosaka et al., 2012; Gibbs et al., 2011; Graciet et al., 2006; Graciet and Wellmer, 2010; Heck et al., 2010; Holman et al., 2009; Hu et al., 2005; Hu et al., 2008; Hwang et al., 2010b; Shemorry et al., 2013; Varshavsky, 2011; Wang et al., 2009; Wang et al., 2011; Xia et al., 2008; Yang et al., 2010; Zenker et al., 2005; Zhang et al., 2010b.
Figure S2. Substrate Specificities and Subunit Compositions of *S. cerevisiae* N°-Terminal Acetylases (Nt-acetylases), Refers to Figures 1, 2, 3, 4, 5, 6, and 7

(A) Substrate specificity of Nt-acetylases. ''Ac'' denotes the N\(^{\text{a}}\)-terminal acetyl moiety. The bulk of Nt-acetylases is associated with ribosomes (Gautschi et al., 2003; Polevoda et al., 2008). This compilation of *S. cerevisiae* Nt-acetylases and their specificities is derived from data in the literature (Arnesen, 2011; Arnesen et al., 2009; Bienvenut et al., 2012; Goetze et al., 2009; Helbig et al., 2010; Helsens et al., 2011; Hole et al., 2011; Mullen et al., 1989; Park and Szostak, 1992; Perrot et al., 2008; Polevoda et al., 2009b; Polevoda and Sherman, 2003; Starheim et al., 2012; Starheim et al., 2009; Van Damme et al., 2011; Van Damme et al., 2012) and references therein.

(B) Subunits of *S. cerevisiae* Nt-acetylases. This paper employs the revised nomenclature for specific subunits of Nt-acetylases (Polevoda et al., 2009a). Older names of these subunits are cited in parentheses.

### N-terminal acetylation in *Saccharomyces cerevisiae* A

| Met-Ala- | Ala- | Ac-Ala- |
| Met-Ser- | Ser- | Ac-Ser- |
| Met-Thr- | Thr- | Ac-Thr- |
| Met-Cys- | Cys- | Ac-Cys- |
| Met-Gly- | Gly- | Ac-Gly- |
| Met-Val- | Val- | Ac-Val- |
| Met-Pro- | Ser-Gly-... | Ac-Ser-Gly-... |

(a sequence motif in histones H2A and H4)

- Cys: rarely Nt-acetylated.
- Val: rarely Nt-acetylated.
- Pro: very rarely Nt-acetylated.
- Gly: very rarely Nt-acetylated; often N-myristoylated.

### Subunit composition of *S. cerevisiae* Nt-acetylases: B

- **NatA**: Naa10 (Ard1); Naa15 (Nat1); Naa50 (Nat5).
- **NatB**: Naa20 (Nat3); Naa25 (Mdm20).
- **NatC**: Naa30 (Mak3); Naa35 (Mak10); Naa38 (Mak31).
- **NatD**: Naa40 (Nat4); other subunits?
Figure S3. $^{35}$S-Pulse-Chase and Cycloheximide-Chase Assays with ML-Ura3 and MI-Sry1, Refers to Figures 1 and 5

(A) $^{35}$S-pulse-chase with ML-eK-ha Ura3 (ML-Ura3) (see the main text), expressed through the cotranslational deubiquitylation of Ub-ML-Ura3, in \textit{naa30}Δ (lanes 1-4) and \textit{naa30}Δ ubr1Δ \textit{S. cerevisiae} (lanes 5-8).

(B) Quantification of data in A.

(C) Same as in A but with fewer time points (0, 0.5 and 1 hr) and with ML-Ura3 expressed directly, without the intermediacy of a Ub fusion.

(D) Quantification of data in C.

(E) Cycloheximide (CHX) chases with exogenous (expressed from a low copy plasmid and the P\textit{CUP1} promoter) MI-Sry1\textit{ha} in wild-type (WT) (lanes 1-3), ubr1Δ (lanes 4-6), \textit{naa30}Δ (lanes 7-9), and \textit{naa30}Δ ubr1Δ \textit{S. cerevisiae} (lanes 10-12). The upper panel shows immunoblot analyses of the same SDS-PAGE gel using an anti-tubulin antibody (loading controls).

(F) $^{35}$S-pulse-chase with directly expressed exogenous MI-Sry1\textit{ha} in \textit{naa30}Δ (lanes 1-4) and \textit{naa30}Δ ubr1Δ \textit{S. cerevisiae} (lanes 5-10).

(G) Quantification of data in F. In B-G, the assays were carried out at least three times and yielded results within 10% of the data shown.
Figure S4. Instability of the MF-Pre5 Proteasomal Subunit and Hypersensitivity of \textit{naa30}\textsuperscript{Δ} and \textit{naa30}\textsuperscript{Δ} \textit{ubr1}\textsuperscript{Δ} Cells to Multiple Stresses, Refers to Figures 2 and 5

(A) \textsuperscript{35}S-pulse-chase with MF-Pre5\textsubscript{ha} in \textit{naa30}\textsuperscript{Δ} (lanes 1–4) and \textit{naa30}\textsuperscript{Δ} \textit{ubr1}\textsuperscript{Δ} cells (lanes 5–8).

(B) Quantification of data in A. In A and B, the assays were carried out at least three times and yielded results within 10% of the data shown.

(C) RT-PCR analysis of the ML-Ura3 (\textit{Ub-ML-eK-ha-Ura3}) mRNA versus the actin \textit{ACT1} mRNA in \textit{ubr1}\textsuperscript{Δ} (lane 1) and \textit{naa30}\textsuperscript{Δ} \textit{ubr1}\textsuperscript{Δ} cells (lane 2).

(D) RT-PCR analysis of the exogenously expressed (from a low copy plasmid and the \textit{P\textsubscript{CUP1}} promoter) MI-SRY1 mRNA versus the actin \textit{ACT1} mRNA in \textit{naa30}\textsuperscript{Δ} (lane 1) and \textit{naa30}\textsuperscript{Δ} \textit{ubr1}\textsuperscript{Δ} cells (lane 2).

(E) Same as in D but for the endogenously expressed (from the \textit{SRY1} locus) MI-SRY1 mRNA.

(F) RT-PCR analysis of the \textit{MF-PRE5} mRNA versus the actin \textit{ACT1} mRNA in \textit{ubr1}\textsuperscript{Δ} (lane 1) and \textit{naa30}\textsuperscript{Δ} \textit{ubr1}\textsuperscript{Δ} cells (lane 2).

(G and H) Cell-growth assays with WT, \textit{ubr1}\textsuperscript{Δ}, \textit{naa30}\textsuperscript{Δ} and \textit{naa30}\textsuperscript{Δ} \textit{ubr1}\textsuperscript{Δ} \textit{S. cerevisiae} strains in a rich (YPD) medium that either lacked or contained canavanine (production of misfolded proteins); 2-azetidinecarboxylic acid (AZC) (production of misfolded proteins); 2% ethanol (EtOH) (perturbation of membranes and other structures); and Congo Red or Cacofluor White (their effects include impairments of cell-wall synthesis).
That's all Folks!