Emerging Role of SUMO Modifications in Response to Heat Shock

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Burke Group Literature Seminar
12.14.13
System-Wide Changes to SUMO Modifications in Response to Heat Shock

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(Published 26 May 2009; Volume 2 Issue 72 ra24)
Ubiquitination Post-Translationally Modifies Proteins

SUMO is a Member of the Ubiquitin-Like Protein Modifier (Ubl) Family

Table 1 | Known and putative UBLs and their activating and conjugating enzymes

<table>
<thead>
<tr>
<th>UBL*</th>
<th>Identity with ubiquitin (%)</th>
<th>E1 (UBL-activating enzyme)*</th>
<th>E2 (UBL-conjugating enzyme)*</th>
<th>Comments on UBL</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Known UBLs</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ubiquitin</td>
<td>100</td>
<td>Uba1 (UBA6)</td>
<td>Many</td>
<td>Precursors encoded by multiple genes</td>
</tr>
<tr>
<td>Rub1 (NEDD8)</td>
<td>55</td>
<td>Uba3–Ula1 heterodimer</td>
<td>Ubc12</td>
<td>Substrates are cullins and p53</td>
</tr>
<tr>
<td>FUB1 (also known as MNSF-β or FAU)</td>
<td>38</td>
<td>NI</td>
<td>NI</td>
<td>Derived from a ribosomal-protein precursor</td>
</tr>
<tr>
<td>FAT10</td>
<td>32 and 40†</td>
<td>UBA6</td>
<td>NI</td>
<td>Contains a β-grasp fold</td>
</tr>
<tr>
<td><strong>Known UBLs cont.</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Iso1</strong></td>
<td>32 and 37†</td>
<td>UBEIL</td>
<td>UBC10</td>
<td>Production induced by type I interferons</td>
</tr>
<tr>
<td>Smt3 (SUMO1, SUMO2, SUMO3)</td>
<td>18</td>
<td>Uba2–Aos1 heterodimer</td>
<td>Ubc9</td>
<td>SUMO encoded by 3–4 genes in vertebrates, depending on the species</td>
</tr>
<tr>
<td>Atg8</td>
<td>ND</td>
<td>Atg7</td>
<td>Atg3</td>
<td>Three known isoforms in humans</td>
</tr>
<tr>
<td>Atg12</td>
<td>ND</td>
<td>Atg7</td>
<td>Atg10</td>
<td>~20% identical to Atg8</td>
</tr>
<tr>
<td>Urm1</td>
<td>ND</td>
<td>Uba4</td>
<td>NI</td>
<td>Related to the small sulphur-carrying proteins MoaD and ThiS</td>
</tr>
<tr>
<td><strong>Putative UBLs</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BUBL1, BUBL2</td>
<td>Variable (up to 80%)</td>
<td>NI</td>
<td>NI</td>
<td>Putative autoprocessed proteins in ciliates</td>
</tr>
<tr>
<td>UBL-1</td>
<td>40</td>
<td>NI</td>
<td>NI</td>
<td>A precursor to ribosomal proteins in nematodes</td>
</tr>
<tr>
<td>SF3A120</td>
<td>30</td>
<td>NI</td>
<td>NI</td>
<td>UBL domain at C terminus No data about conjugation</td>
</tr>
<tr>
<td>Oligoadenylate synthetase</td>
<td>30 and 42†</td>
<td>NI</td>
<td>NI</td>
<td>UBL domain at C terminus No data about conjugation</td>
</tr>
</tbody>
</table>

ND, not detectable by standard BLAST searches. NI, not identified. *UBLs are listed as the yeast (Saccharomyces cerevisiae) symbol if the UBL is present in yeast, otherwise vertebrate symbols are listed. Known vertebrate orthologues with symbols that differ from yeast proteins are listed in parentheses. For E1s and E2s, yeast symbols are listed if the protein is found in yeast. (UBA6 has a much more limited phylogenetic distribution than Uba1.) †The identities listed are for each of two ubiquitin-related domains.

SUMO is Structurally Similar to Ubiquitin

• 20% sequence identity and structural similarity with ubiquitin
• Covalent modification at lysine residues of target protein
• Mammalian cells:
  • SUMO-2 and SUMO-3 (97% sequence identity)
  • SUMO-1 (50% sequence identity)
• Heat shock induces modification by SUMO-2 and SUMO-3
• SUMO K.D. and K.O.
  • Fatal in mammalian cells
  • Embryonic lethality in mice
  • Cell cycle arrest in yeast

The Prevalence of Protein SUMOylation in the Cell

- Global SUMOylation increase:
  - Heat shock
  - Osmotic stress
  - Hibernation
- Nucleus:
  - Transcription factors
  - Chromatin organization
  - Replication and repair
- Modulates ion channel activity
- Leads to internalization of membrane proteins
- Affects mitochondrial fission and fusion

SUMOylation Modulates Protein Interactions

The Heat Shock Response

- Different organisms thrive in a wide range of temperatures (freezing to 113°C)
- Manifestations:
  - Protein unfolding
  - Cytoskeleton damage (improper localization of organelles)
  - Reduced ATP production (mitochondrial loss and ineffective ETC)
  - Reduced translation
  - Increased membrane permeability
  - Cell cycle arrest
- Heat shock activates transcription factor, heat shock factor 1 (HSF1)
- HSF1 is the “master regulator”
- Up-regulation of heat stress proteins (Hsps)

Motivation and Hypothesis

Motivation:
To elucidate the early signaling mechanisms, “thermometer” that promote the heat shock response and mechanisms involved in promoting global SUMOylation.

Hypothesis:
SUMO-2 acts as an early signaling molecule against heat shock cytotoxicity.
SUMO-2 and SUMO-3 are Indispensable for Survival after Heat Shock

Question: What is the biological relevance of SUMO-2 and SUMO-3 protein modification in response to heat shock?

- siRNA knockdown for 72 hrs. in U2OS cells (panel A).
- Heat shock for 30 min. at 45°C, image after 10-14 days (panel B).
- **Sevenfold reduction in survival of siRNA SUMO-2 and SUMO-3 K.D. group.**

Determination of SUMO-2 Targets and Effects of Heat Shock on Protein Conjugation State

Question: What are the protein targets of SUMO-2 after heat shock?

• HeLa cell line expressing SUMO-2 fused with a TAP tag
• TAP-SUMO-2 shows similar characteristics as that of SUMO-2
• TAP-SUMO-2 specifically labels target proteins

Determination of SUMO-2 Targets and Effects of Heat Shock on Protein Conjugation State

- Conducted stable isotope labeling of amino acids in cell culture (SILAC)
- Determine relative abundance of proteins
- After heat shock, Heat-shock transcription factor 1 (HSF1) showed increased SUMOylation!!

Triple SILAC Map Identifies 662 Putative Targets of SUMO-2

SUMOylation is a Dynamic Process in Response to Heat Shock

After heat shock, Western blot analysis shows an increase in proteins modified by SUMO-2 and a depletion of SUMO-2.

SUMOylation is a Dynamic Process in Response to Heat Shock

SUMOylation is reversible and proteins are deconjugated during the recovery phase.

Determination of SUMOylation Profile after Recovery from Heat Shock

• Overall increase in SUMOylation immediately after heat shock (5 min.)
• Some cases if SUMO-2 is lost during heat shock, did not regain after 2 hrs.
• Other cases, if SUMO-2 modification during heat shock, then subsequently removed.

Determination of SUMOylation Profile after Recovery from Heat Shock

Comparison with first SILAC experiment shows that differences in labeling are due to SUMO-2 modifications instead of changes in protein quantities.

Heat Shock Generates High Molecular Weight SUMO Conjugates

Observed majority of proteins with lower predicted molecular weight in slices 5 and 6.

Heat shock:
• Transcription
• Translation
• Apoptosis
• Cell cycle
• Protein folding and degradation
• DNA replication, recombination, and repair

Conclusions

• Through developing purification, SILAC proteomic, high-resolution mass spectrometry, and data analysis methods, the authors identified **574 novel targets of SUMO**
• Demonstrated *in vitro* that SUMO-2 and SUMO-3 are necessary for cell survival in response to heat shock
• SUMOylation is a dynamic and reversible process
• Significant overrepresentation of SUMO-2 target proteins involved in heat shock response
• Proposed that SUMO E3 ligase is hyperactivated in response to heat shock
Discussion

• Are both SUMO-2 and SUMO-3 necessary for proper cellular response to heat shock? Could either SUMO-2 or SUMO-3 functionally compensate for the other? Are SUMO-2 and SUMO-3 interchangeable?
• How can we discern what role SUMO-3 plays in the cell since it has 97% sequence identity to SUMO-2? What insight can we gain from these experiments?
• Given the large number of novel substrates of SUMO discovered in this paper, what experiments would you like to see done to demonstrate that SUMO-2 plays a direct role in regulating proteins involved in the heat shock response?
Discussion

• What can we learn from this paper for our own goals of understanding how AmB, hinokitiol, and other small molecules rescue cells at the molecular level?
• What key mechanisms are engaged to promote robust and vigorous restoration of cell growth in yeast and potentially human cells? At what level do cells engage these mechanisms i.e. mRNA or protein expression, enzyme activity, post-translational modifications?
Thank you! Any questions?