Reasearch Highlights

Urm1, a sulfur carrier par excellence – ubiquitin-like urmylation is coupled to sulfur transfer and cysteine persulfidation

Posttranslational protein modifications are essential for life. Cysteine persulfidation is a reversible modification with emerging roles in biological processes like aging. The ancient protein Urm1 might function in directed persulfidation of specific redox-active cysteines.

Since its discovery in 2000 by Nobel Prize laureate Professor OHSUMI, the small eukaryotic protein Urm1 (ubiquitin related modifier 1) has remained an enigma in the life sciences. Although Urm1 shares high sequence similarity with prokaryotic sulfur carrier proteins (SCP), it was initially found to conjugate to target proteins posttranslationally. Such function, however, was only known from the eukaryotic protein ubiquitin and ubiquitin-like proteins (UBL) to which Urm1 was then assigned. Continuous studies revealed that Urm1 has a second function. As a matter of fact, it plays a key role as an SCP in the thiomodification of tRNA anticodons. In general, anticodon modifications are necessary for proper mRNA translation and protein synthesis, and malfunctions associate with human diseases, including cancer and neurodegeneration. Whereas the latter tRNA thiolation function of Urm1 had been established, the molecular mechanisms of its attachment to target proteins remained largely unknown.

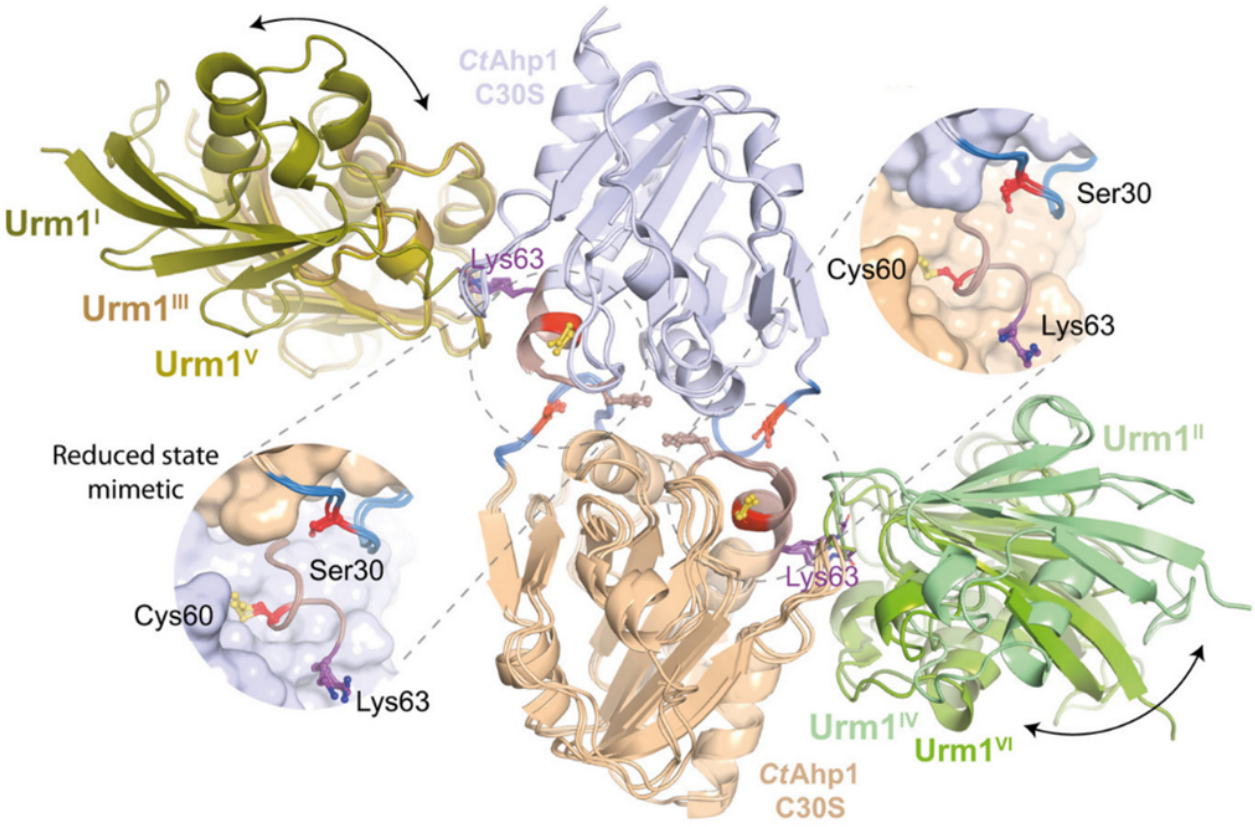

Fig. 1: Structure of the urmylated Ahp1 complex.

Front view of the $CtAhp1_{C30S}$-$CtUrm1_{C55S}$ complex structure in cartoon representation. All three entities in the asymmetric unit are superimposed on Ahp1 and the various Urm1 molecules are labeled. The position of Lys63 (violet) is highlighted and labeled. Insets: Close-up of the active sites highlighting the position of the redox-active cysteines (Cys60 and Ser30 in red).

Interestingly, SCP as well as UBL share a common three-dimensional fold, the β-grasp fold and are activated in a similar manner to fulfill their functions. Activation of Urm1 is special as it requires sulfur-transfer onto its C-terminal carboxyl group (-COOH), a step thought to be typically restricted to prokaryotic SCP. The resulting thiocarboxylate (-COSH) in Urm1 and all SCP is essential for subsequent thiolation reactions including Urm1-dependent tRNA thiomodification (see above).

Protein modification cascades of ubiquitin and UBL typically involve specific enzymes called E1, E2 and E3. Urm1 conjugation (aka urmylation) is best studied with yeast peroxiredoxin Ahp1, an antioxidant enzyme reducing organic hydroperoxides via redox-active cysteine residues. However, no E2 or E3 enzymes are known so far for Ahp1 urmylation to occur and neither the role nor the fate of the sulfur within the unique -COSH moiety (see above) have been identified during or upon urmylation.

In our recent collaboration, which was jointly published in *EMBO J* (2022, **41**: e111318) by members of the laboratories of Professor Sebastian LEIDEL (University of Bern, Switzerland), Professor Raffael SCHAFFARATH (University of Kassel) and Dr hab. Sebastian GLATT (University of Krakow, Poland), we found that the conjugation of Urm1 is accompanied by the transfer of sulfur to cysteine residues in the target proteins also known as cysteine persulfidation (-S-SH). The data emphasize that both functions of Urm1, i.e., protein urmylation & tRNA thiolation, do involve sulfur transfer onto biomolecules reminiscent of the thiolation functions of prokaryotic SCP (see above). We further showed that thiocarboxylated Urm1 can conjugate to Ahp1 *in vitro* under conditions of mild oxidative stress and importantly, without any requirement for E2 or E3 enzymes (see above). This urmylation reaction depends on the -COSH moiety of Urm1 and a conserved redox-active cysteine in Ahp1. Ultimately, Urm1 is preferably linked to lysine residues in Ahp1 by an isopeptide bond or to a lesser extent to serine and threonine residues by ester bonds.

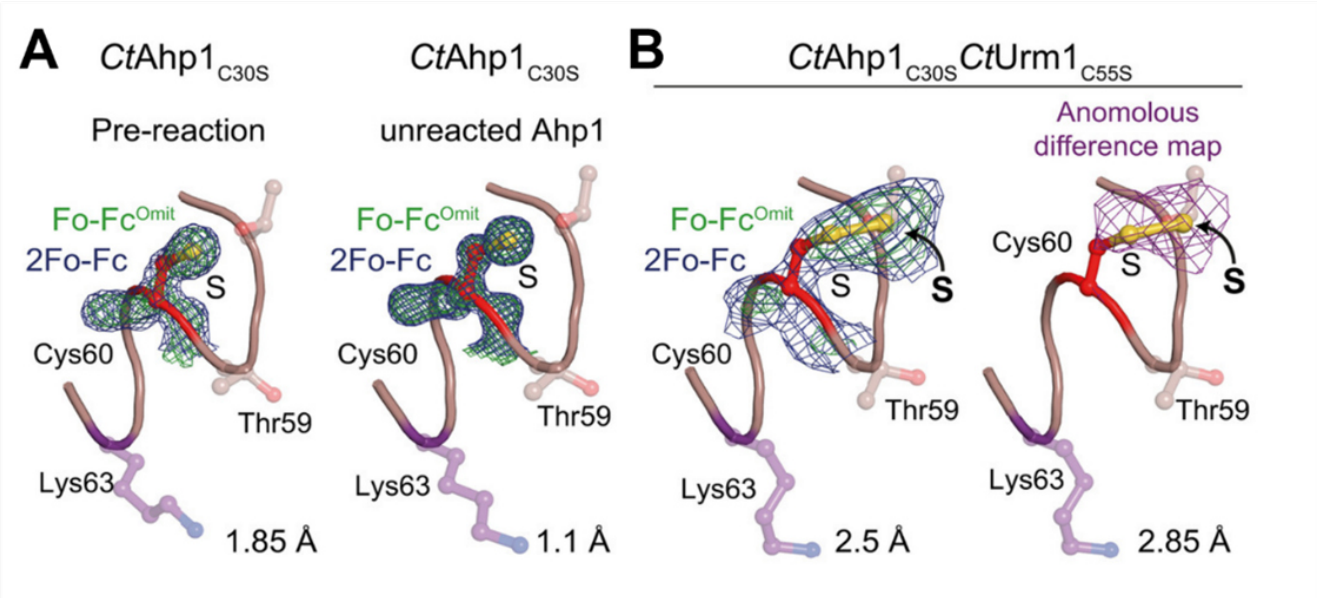

Fig. 2: Urm1 conjugation directly mediates cysteine persulfidation in vitro.

A. Structural close-up of the peroxidatic cysteine (Cys60) and Lys63 (violet) in CtAhp1$_{C30S}$ pre-conjugation (left) and unreacted Ahp1 from the conjugation reaction (right). The refined 2Fo-Fc density (blue) and the Fo-Fc omit map lacking the active site cysteine (green) is shown at comparable σ-levels. B. Same view as in A of the maps in the CtAhp1$_{C30S}$-CtUrm1$_{C55S}$ complex around Cys60 (red) at 3.5 σ (blue, left), 4 σ (green, left) and the anomalous difference Fourier map at 2 σ (violet, right). The respective resolution ranges of the maps are indicated.

Using protein sequences from *Chaetomium thermophilum*, a thermophilic fungus harboring highly stable proteins, we determined (*EMBO J*, 41: e111318) the structure of the urmylated Ahp1 complex by macromolecular crystallography (Fig. 1). The asymmetric unit contained three individual Ahp1-Urm1 dimers, which were all linked via the same isopeptide bond between the Lys63 sidechain of Ahp1 and the Urm1 C-terminus (Fig. 1). This conformational variation might result from the fact that Ahp1 and the attached Urm1 do not form an extensive interaction surface. Mass spectrometry analyses of the conjugation products revealed that additional lysine residues as well as serine and threonine residues can act as alternative attachment sites for Urm1.

Intriguingly, the sulfur atom from the Urm1 thiocarboxylate was not detectable at the site of linkage in our mass spectrometry analyses. Reanalysis of the crystallographic data showed a prominent density next to the sulfur atom of the cysteine redox active side chain in the crystal structure of the Ahp1-Urm1 complex, which was absent from individual Ahp1 structures alone (Fig. 2A). Calculation of anomalous difference Fourier maps confirmed that persulfidation of the Ahp1 cysteine side chain (-S-SH) only occurred during the conjugation with the Urm1 thiocarboxylate (Fig. 2B).

Our results reveal a novel and surprising link between ubiquitin-like conjugation of Urm1 and posttranslational cysteine persulfidation of its target proteins.

Further information

Ravichandran KE, Kaduhr L, Skupień-Rabian B, Shvetsova E, Sokołowski M, Krutyhołowa R, Kwasana D, Brachmann C, Lin S, Perez SG, Wilk P, Kösters M, Grudnik P, Jankowska U, Leidel SA, Schaffrath R, Glatt S (2022) E2/E3 independent ubiquitin-like conjugation by Urm1 is directly coupled to cysteine persulfidation. *EMBO J* **41**: e111318.
PMID: 36102610
doi: 10.15252/embj.2022111318

Research – Dr. hab Sebastian GLATT
https://glatt-lab.pl/research-2/
https://www.researchgate.net/profile/Sebastian-Glatt

Microbiology Division and Research – Professor SCHAFFRATH
http://www.uni-kassel.de/fb10/de/institute/biologie/fachgebiete/mikrobiologie
https://www.researchgate.net/profile/Raffael_Schaffrath

Dr. hab. Sebastian Glatt
phone: +48 12 664 - 6321
e-mail: sebastian.glatt@uj.edu.pl

Prof. Dr. Raffael Schaffrath
phone: +49 561 804 - 4175
e-mail: schaffrath@uni-kassel.de

Lars Kaduhr
phone: +49 561 804 - 4202
e-mail: kaduhr@uni-kassel.de

Keerthiraju Ethiraju Ravichandran
phone: +48 12 664 - 6322
e-mail: er.keerthiraju@gmail.com