Abstract

Oxidative stress can lead to the reprogramming of tRNA modifications, which has a ripple effect on protein synthesis that governs the cells' ability to survive. To refine translation and optimize protein-synthesis, the introduction of sulfur-based chemical modifications (thiolation) of tRNA bases are essential. Thiolation levels of tRNA change dynamically, which influences how cells respond to fluctuating environmental conditions, in addition to regulating protein homeostasis.

The Ubiquitin-related modifier 1 (Urm1) pathway is responsible for tRNA thiolation in eukaryotes. Several studies have shown that alteration in the Urm1 pathway enzymes can lead to severe human diseases. The same pathway is essential for cancer cells to adapt to oxidative stress during their progression and metastasis, even when exposed to therapy.

Urm1, which plays a key role as a sulfur carrier protein (SCP) in 2-thiolation of wobble base position (U_{34}), also functions as a Ubiquitin-like (UBL) protein. Ubiquitin-like protein activator 4 (Uba4) initiates a two-step process by adenylating and then thiocarboxylating the C-terminus of Urm1. This activated form of Urm1, known as Urm1-SH, can serve as a sulfur donor for specific tRNA thiolases or take part in ubiquitin-like conjugation reactions. Urm1 has been observed to conjugate to target proteins in response to oxidative stress and moreover, enzymes of the Urm1 pathway themselves were found to be targets of "urmylation". However, the mechanism of attachment and how Urm1's SCP properties may influence its conjugation remain unclear. Uba4 has been instrumental in activating Urm1, yet the structural and mechanistic aspects of its enzymatic reaction remain mysterious. To comprehend the divergence between UBLs and SCPs, it is essential to gain molecular insight into this process.

In this thesis, I report the crystal structure of the trapped Uba4-Urm1 complex and show how the two domains of Uba4 are responsible for the recognition, binding, and thiocarboxylation of Urm1 and its C-terminus. I show how the reaction cycle is greatly enhanced by the communication between Uba4's catalytic domains and identify a vital redox mechanism that allows Uba4 to prevent itself from being conjugated with its own product, namely activated Urm1-SH. Furthermore, my work on Urm1 also provide insights into the evolutionary aspects of thioester formation between all eukaryotic E1 enzymes and their respective UBLs. In addition, I have reconstituted the covalent attachment of Urm1 to target proteins *in vitro*. In contrast to other known ubiquitin-like proteins, the attachment of Urm1 to different cellular target proteins does not involve E2 conjugation enzymes or E3 ligases. Urm1 can conjugate to lysine, serine, and threonine residues of the target proteins under oxidative stress conditions. I determined the crystal structures of the yeast peroxiredoxin Ahp1 before and after Urm1 attachment. The most surprising result of my thesis revealed that Urm1 can transfer a sulfur atom to the target protein during the conjugation reaction, leading to the persulfidation of a cysteine residue in the target protein.

In summary, my research reconceptualizes the Uba4-Urm1 system as a central evolutionary bridge between the numerous UBL modifications found in eukaryotes and prokaryotic SCPs, while also revealing its essential role in safeguarding proteins during mild oxidative stress.

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