

**Stony Brook University  
The Graduate School**

Doctoral Defense Announcement

**Abstract**

Insights into ubiquitin activation and transfer to E2  
from the structure of the Uba1-ubiquitin complex

By

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Covalent attachment of a small, highly conserved protein called ubiquitin is a predominant mechanism for regulating protein function in eukaryotes and its defective regulation is manifest in diseases that range from developmental abnormalities and autoimmunity to neurodegenerative diseases and cancer. Generally, ubiquitin and ubiquitin-like proteins (Ubls) are conjugated via their C termini to their targets by parallel, but specific, cascades involving three classes of enzymes known as E1, E2, and E3. E1 activating enzymes play key roles in the transfer cascades: Each E1 activates its cognate Ubl by first catalyzing a Ubl C-terminal adenylation, followed by formation of an E1~Ubl thioester intermediate, and ultimately generating a thioester-linked E2~Ubl product. The 2.7 Å resolution crystal structure of the complex between yeast Uba1, a 114 kDa monomeric E1 and ubiquitin shows modular nature of E1 enzymes with activities specified by individual domains. These domains pack together creating a large groove in the middle and Uba1 selectively recruits ubiquitin into the groove through a bipartite recognition mechanism, involving the acidic cleft that recognizes the positively charged ubiquitin C-terminal sequence through electrostatic interactions and specific contacts with side chains of the ubiquitin C terminus, and the hydrophobic surface on the adenylation domain that interacts with the canonical hydrophobic patch of ubiquitin defined by residues Leu8, Ile44, and Val70. Marked conformational changes in the C-terminal ubiquitin-fold domain (UFD), including movement of the linker connecting the domain to the rest of the enzyme, suggest a conformation-dependent mechanism for the activation and transthiolation functions of Uba1.

The *E. coli* MoeB and MoaD proteins are involved in molybdenum cofactor (Moco) biosynthesis, an evolutionarily conserved pathway. The MoeB- and E1-catalyzed reactions are mechanistically similar. Analogous to E1 enzymes, MoeB activates the C terminus of MoaD to form an acyl-adenylate. Subsequently, a sulfurtransferase converts the MoaD acyl-adenylate to a thiocarboxylate that acts as the sulfur donor during Moco biosynthesis. The crystal structures of the mutant MoeB-MoaD complex in ATP-bound and adenylate forms and ensuing biochemical assays provides a detailed mechanism for the adenylation reaction and serves as a model for all ubiquitin-like protein activation.

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