How Polyubiquitin Chains are Made and Unmade

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Intermediates in Ub Activation by E1

The Sequence and Distribution of Enzyme Intermediates
(Haas & Rose, 1982)
How a protein may become polyubiquinate?

(Alberts, The Cell, p. 360)
Test of E1 as a Continuous Ub Source

\[ E1\text{-AMP-}Ub^*, S=Ub^* \quad \text{(PULSE)} \]

\[ \downarrow \]

\[ E2.E3.\text{Protein} + \text{ATP} + \text{Ub} \quad \text{(CHASE)} \]

\[ \downarrow \]

\[ \text{Protein-}Ub\text{-}Ub^*\text{-}Ub^* \quad \text{if } E1\text{AMP-}Ub \rightarrow ES\text{-}Ub \text{ is fast} \]

or

\[ \text{Protein-}Ub\text{-}Ub\text{-}Ub^* \quad \text{if } E3.\text{Protein} \text{ dissociates faster} \]
High mw conjugates formed with labeled Ub in reticulocyte extracts on G75 (A ▼▼▼▼) are shown to breakdown to Ub if ATP is withdrawn as in B as shown by their regeneration when ATP is added to (B ▼▼▼▼) (Hershko, et al, PNAS, 1980)
Ubiquitin Carboxy-terminal Hydrolase

ATP

AMP + PPI

Ub + E₁SH

ES Ub

(Spon.)

GSH transthiolation

GS-Ub

(New Enzyme)

GSH
Inactivation of UCH by NaBH₄

\[ E - SH \quad (\text{active UCH}) \quad + \quad Ub \quad \xrightarrow{\text{acid}} \quad E - S - C - Ub_{75} \quad \text{(inactive UCH)} \]

\[ \xrightarrow{\text{NaBH}_4} \quad E - S - C - Ub_{75} \quad \xrightarrow{\text{OH}} \quad O = C - Ub_{75} \quad (\text{Ubaldehyde}) \]

1. Free Ubal + NaBH₄ → Ub₇₅-ethanolamine
2. Ubal binds UCH 1000x tighter than Ub

(Pickart and Rose, 1986)
Q: Why is Ubal a good inhibitor?