How Polyubiquitin Chains are Made and Unmade

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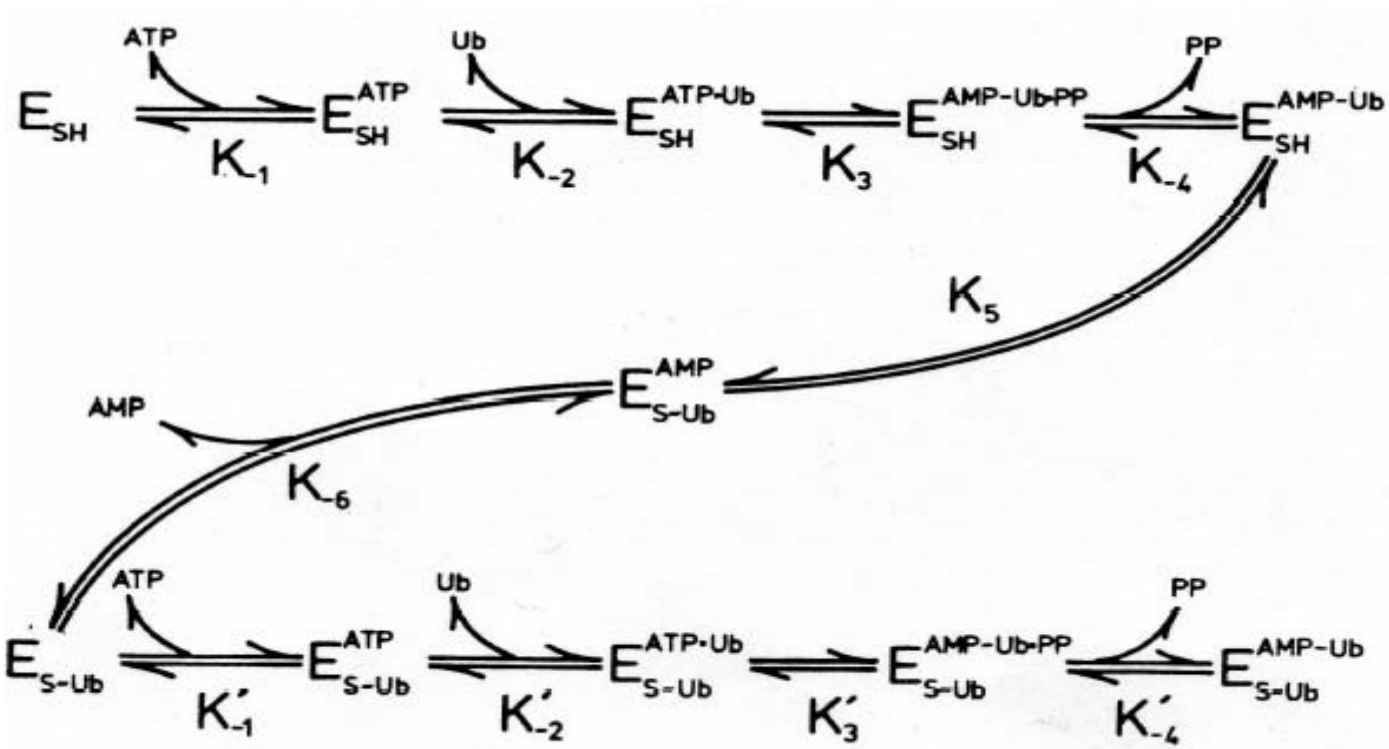
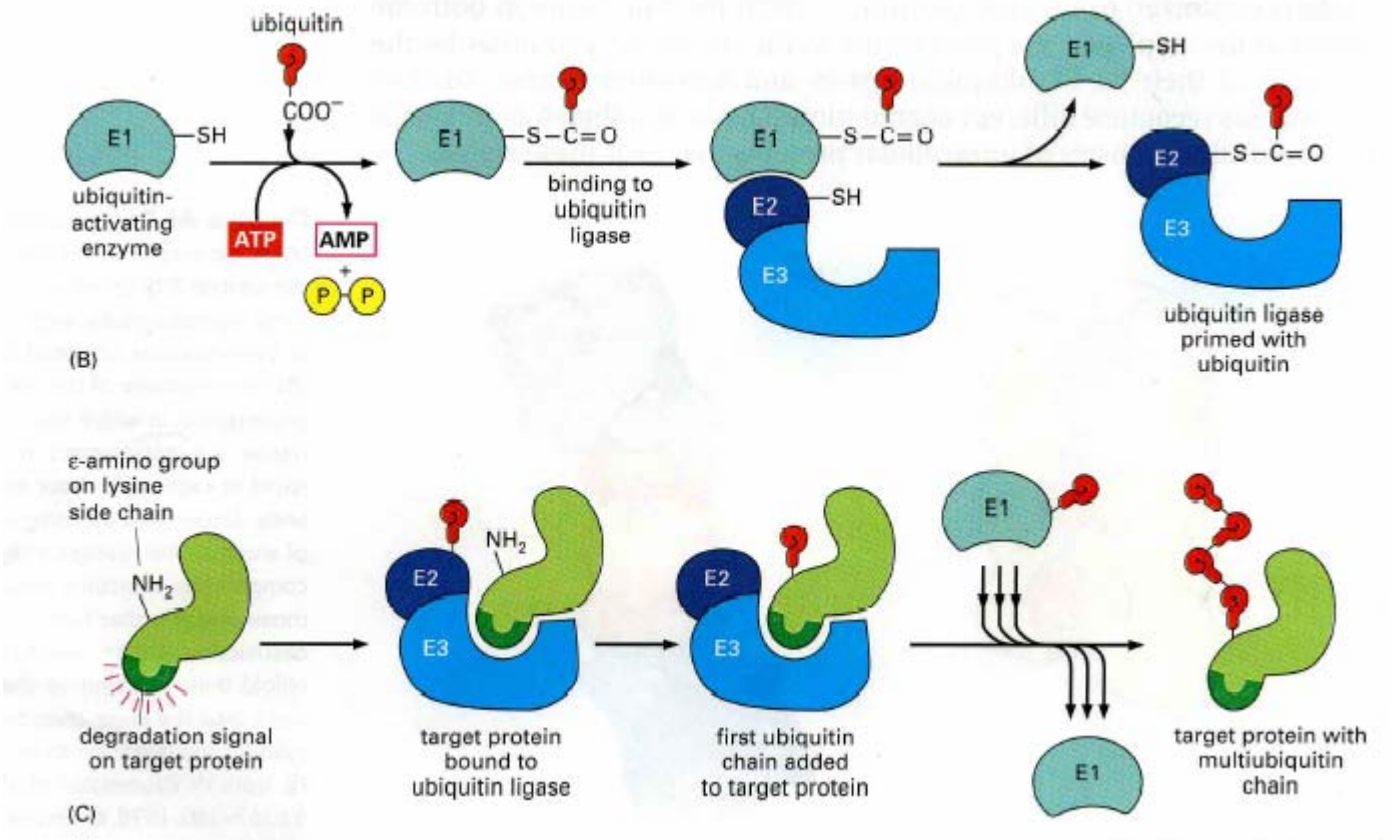
Intermediates in Ub Activation by E1

The Sequence and Distribution of Enzyme Intermediates
(Haas & Rose, 1982)



SCHEME 1

How a protein may become polyubiquinate?



(Alberts, The Cell, p. 360)

Test of E1 as a Continuous Ub Source

E1.AMP-Ub*.S-Ub* (PULSE)

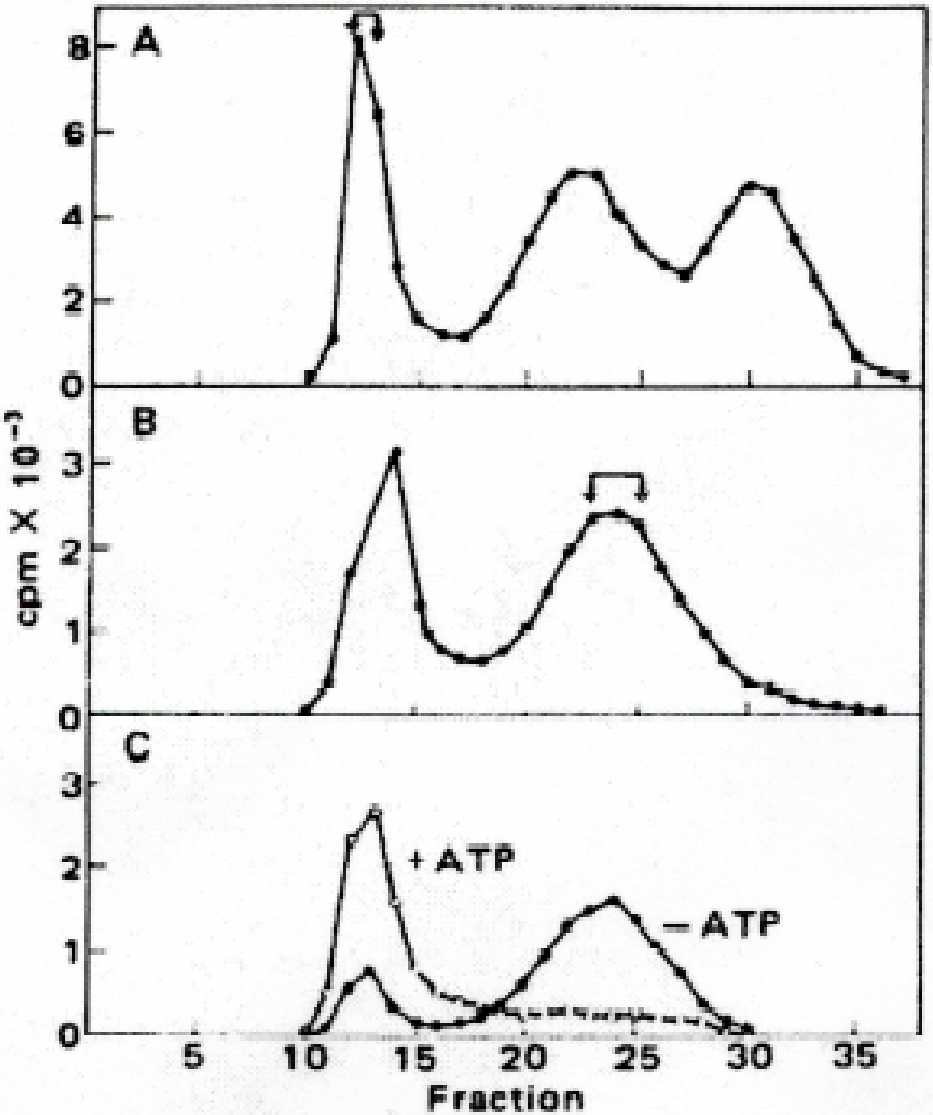
⇩

E2.E3.Protein + ATP+Ub (CHASE)

⇩

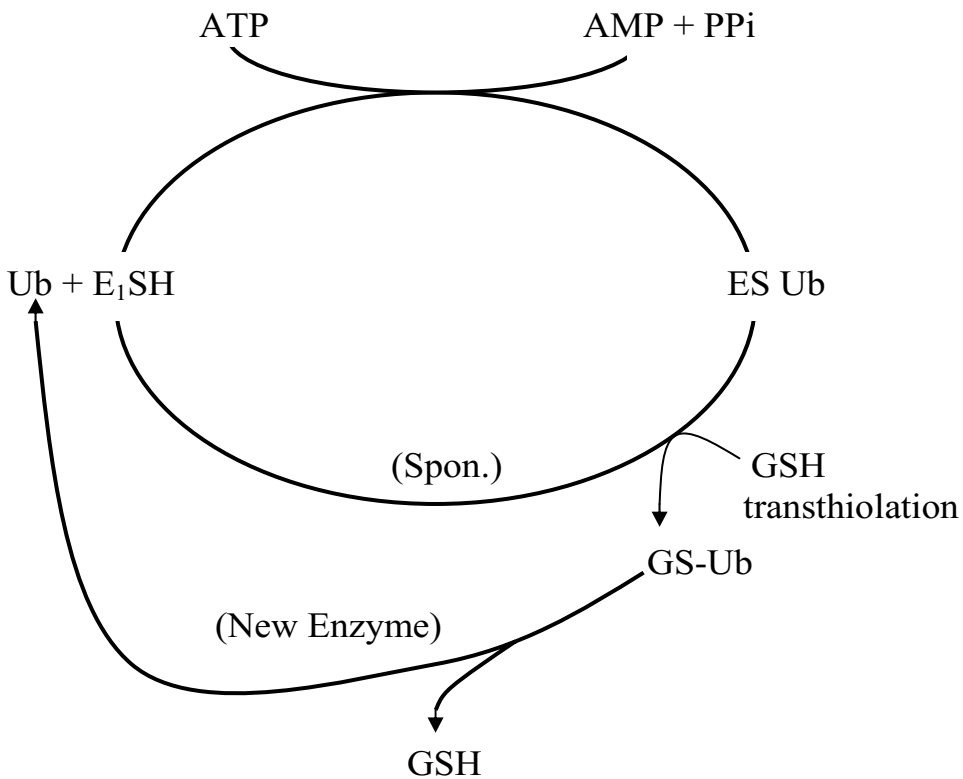
Protein-Ub-Ub*-Ub* if E1AMP-Ub→ES-Ub is fast

or Protein-Ub-Ub-Ub* if E3.Protein dissociates faster

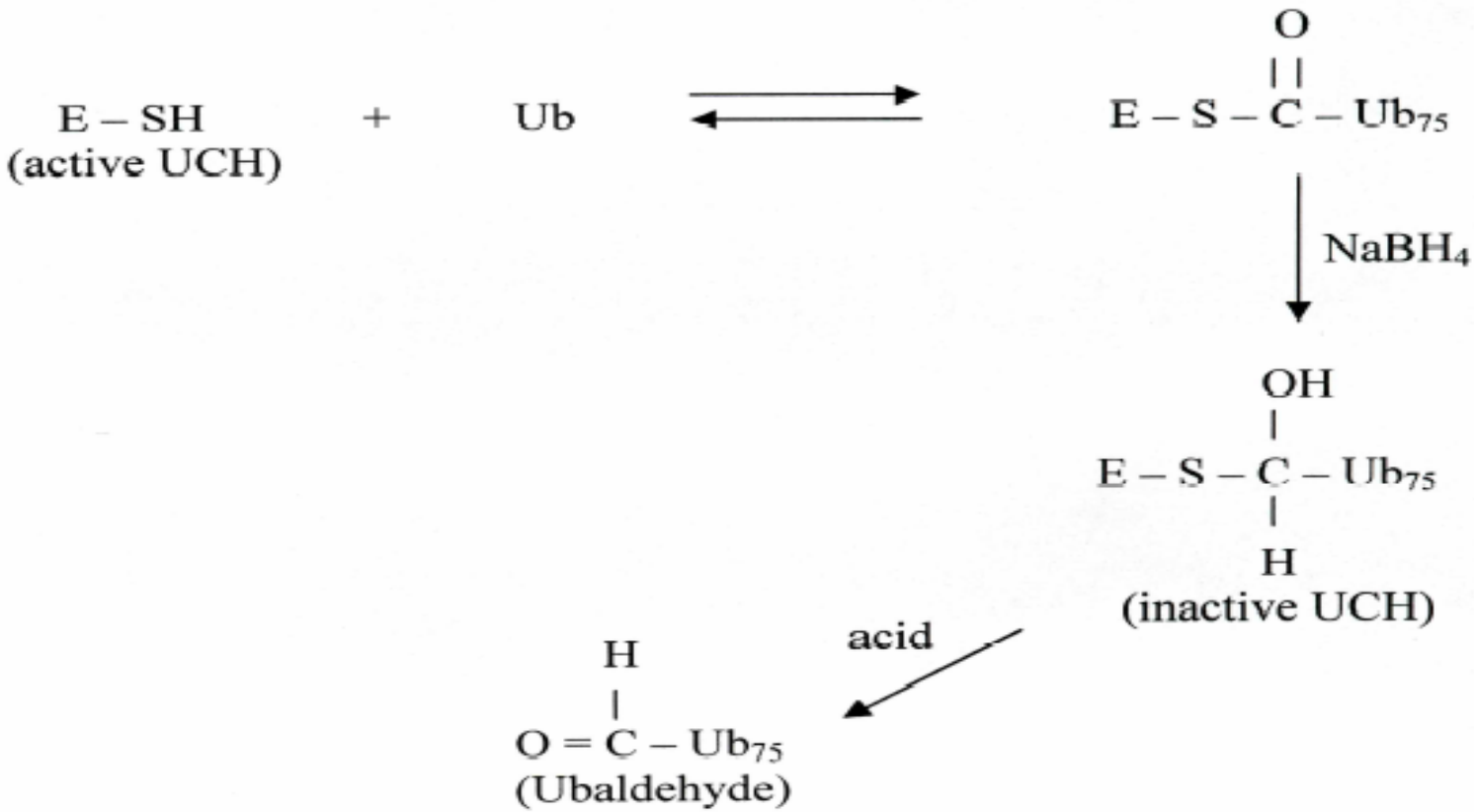


High mw conjugates formed with labeled Ub in reticulocyte extracts on G75 (A ⇄) are shown to breakdown to Ub if ATP is withdrawn as in B as shown by their regeneration when ATP is added to (B ⇄) (Hershko, et al, PNAS, 1980)

Ubiquitin Carboxy-terminal Hydrolase



Inactivation of UCH by $NaBH_4$



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1. Free Ubal + $NaBH_4$ ⟶ Ub_{75}-ethanolamine
2. Ubal binds UCH 1000x tighter than Ub
   (Pickart and Rose, 1986)

Q: Why is Ubal a good inhibitor?