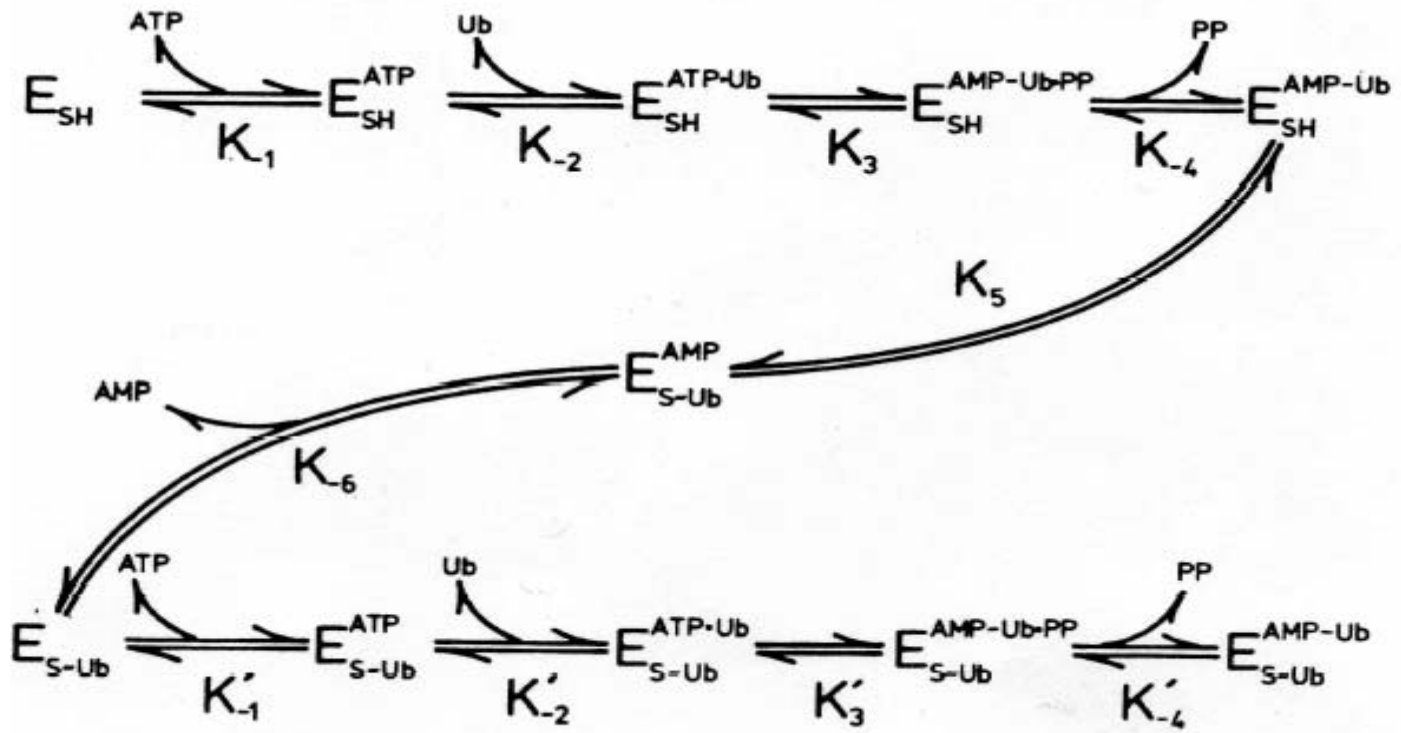


# *How Polyubiquitin Chains are Made and Unmade*

*Dr. Irwin A. Rose  
University of California, Irvine*

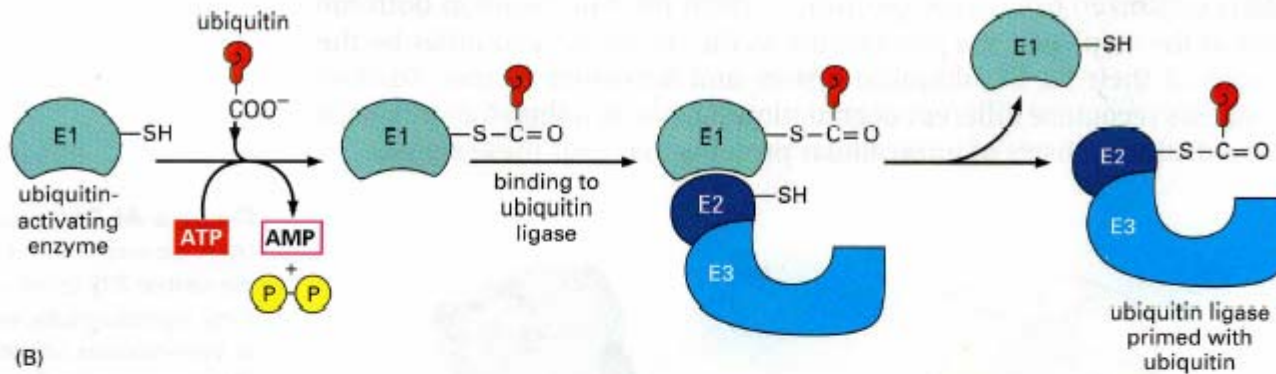
# Intermediates in Ub Activation by E1

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

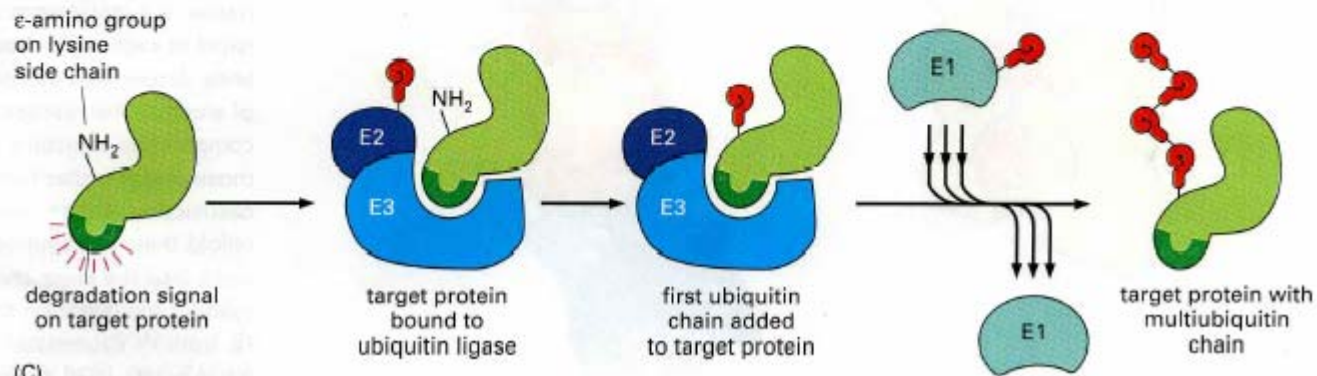


SCHEME 1

# How a protein may become polyubiquitinated?



(B)

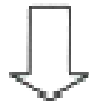


(C)

(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)

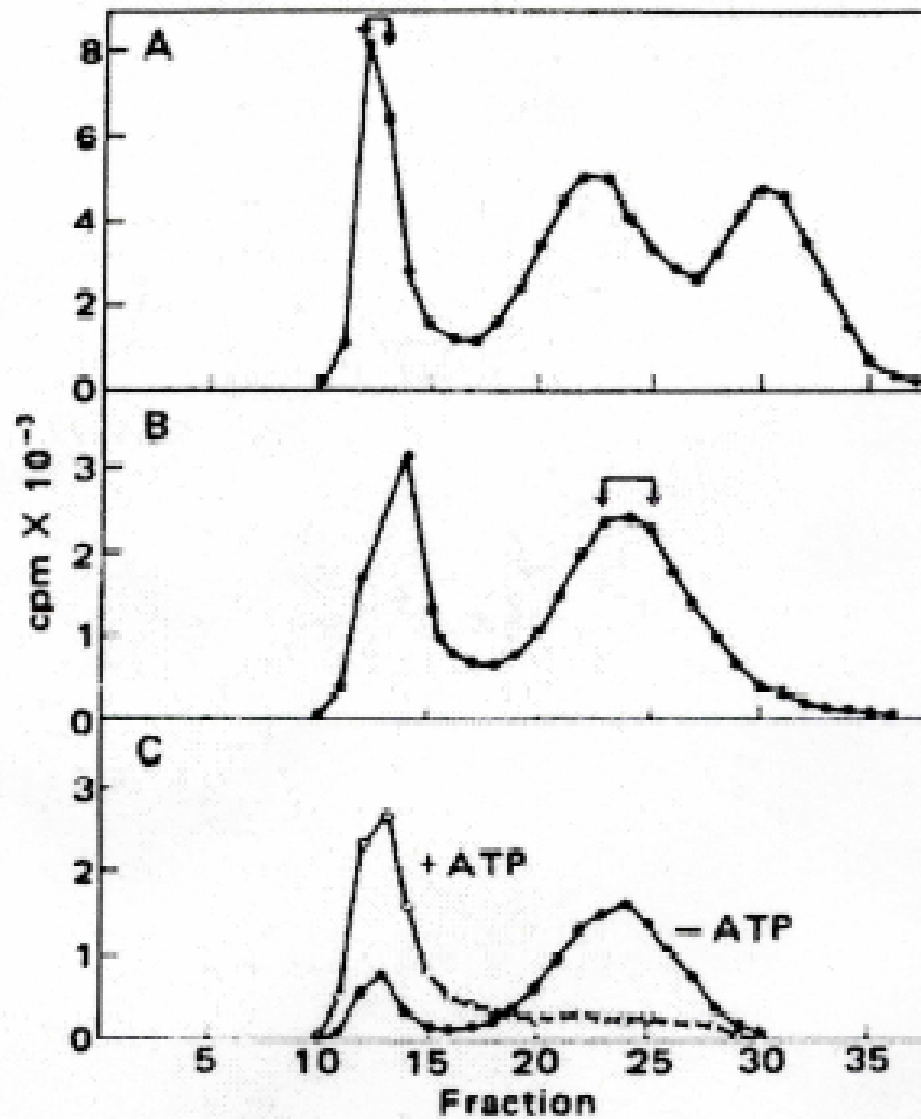


Protein-Ub-Ub\*-Ub\*

if E1AMP-Ub  $\rightarrow$  ES-Ub is fast

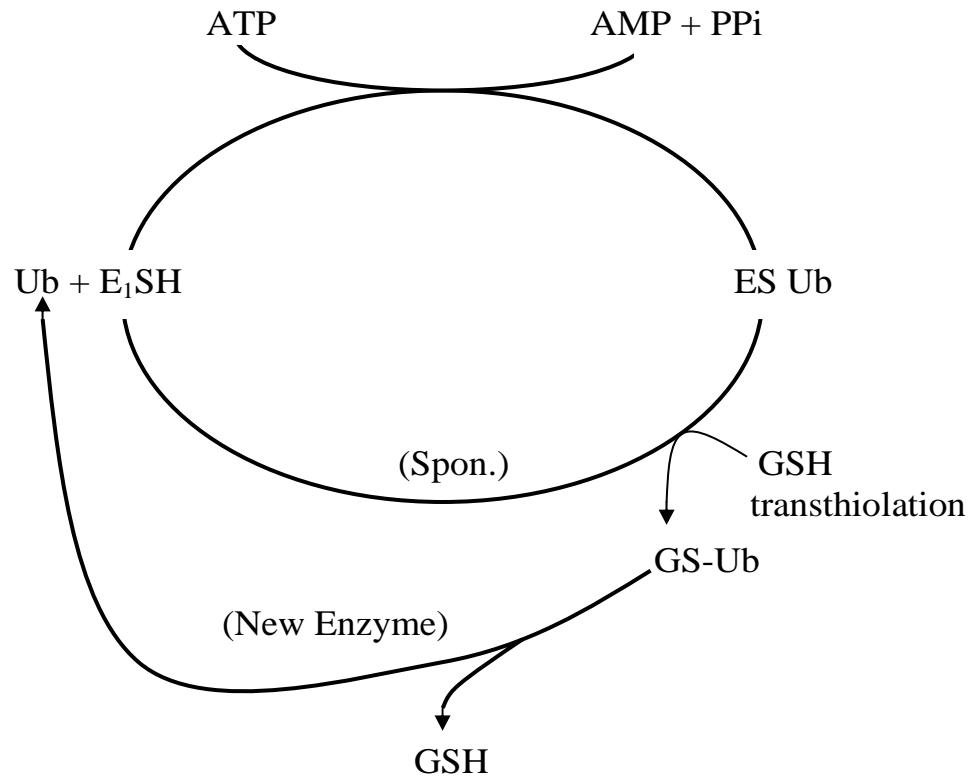
or Protein-Ub-Ub-Ub\*

if E3.Protein dissociates faster

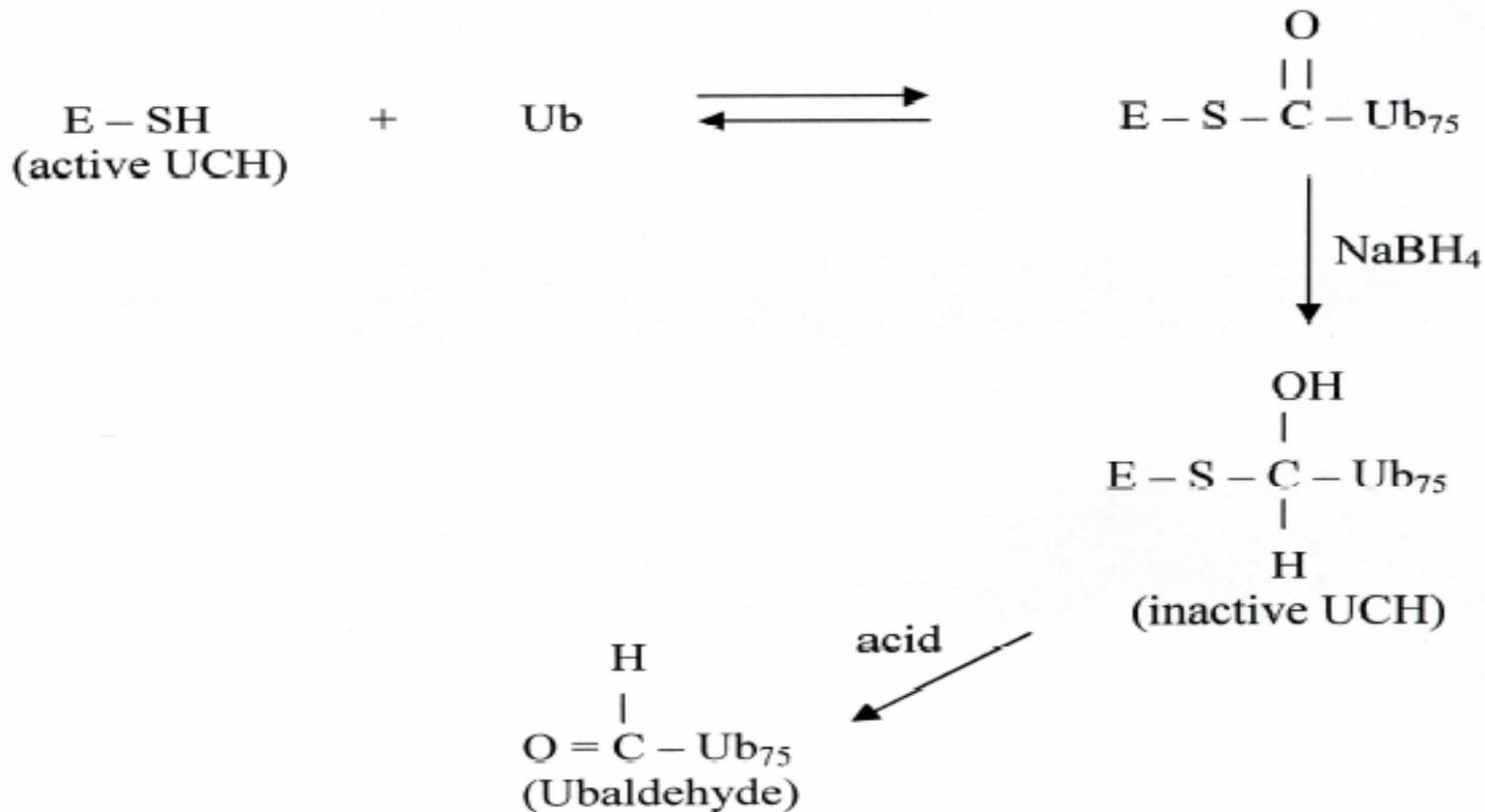


High mw conjugates formed with labeled Ub in reticulocyte extracts on G75 (A  $\nabla$ ) are shown to breakdown to Ub if ATP is withdrawn as in B as shown by their regeneration when ATP is added to (B  $\nabla$ ) (Hershko, et al, PNAS, 1980)

# Ubiquitin Carboxy-terminal Hydrolase



## Inactivation of UCH by NaBH<sub>4</sub>



- 
1. Free Ubal + NaBH<sub>4</sub> → Ub<sub>75</sub>-ethanolamine
  2. Ubal binds UCH 1000x tighter than Ub  
(Pickart and Rose, 1986)

# Q: Why is Ubal a good inhibitor?

