REGULATION BY PEPTIDE BOND CLEAVAGE

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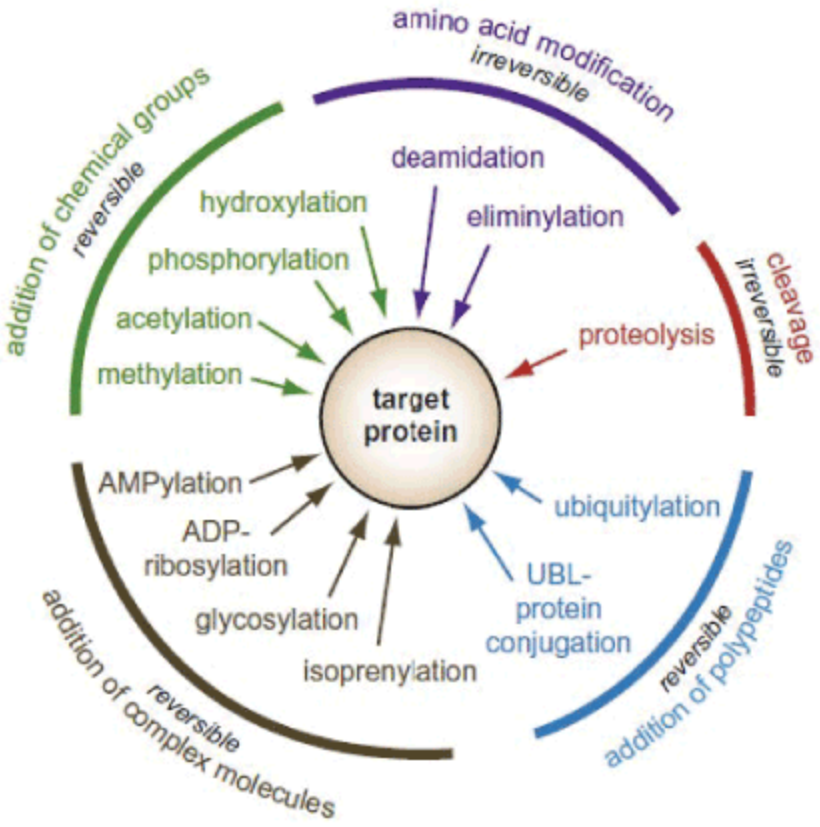
SUSTC

Post-translational modification

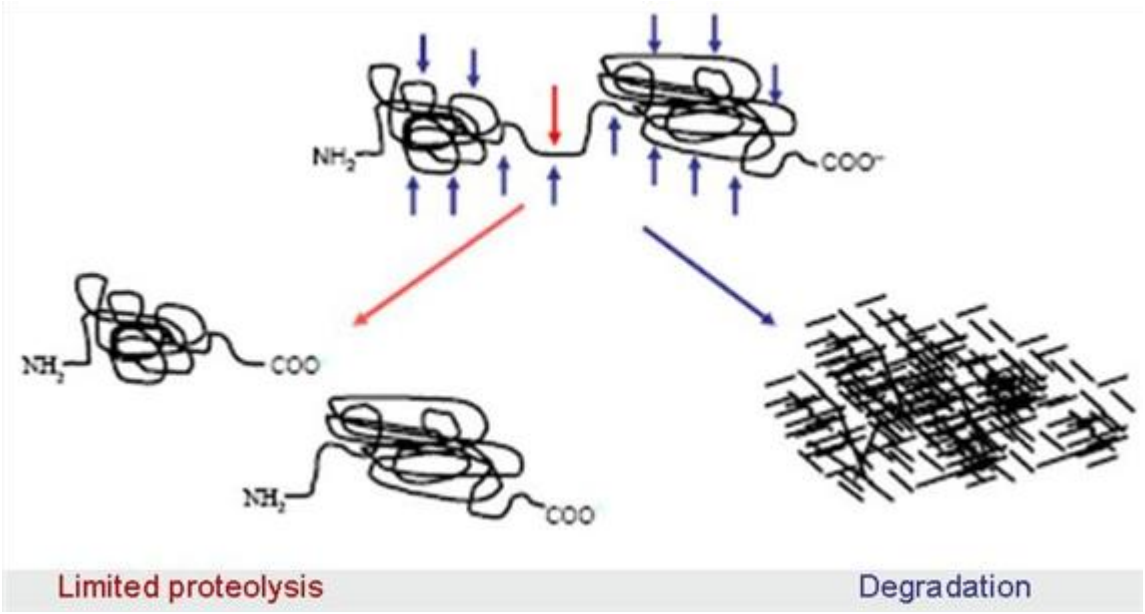


PTM and peptide bond cleavage

- Proteolysis
- Intein splicing
- Targeted degradation
  - Proteasome
  - Ubiquitination
  - SUMOylation

Proteolysis

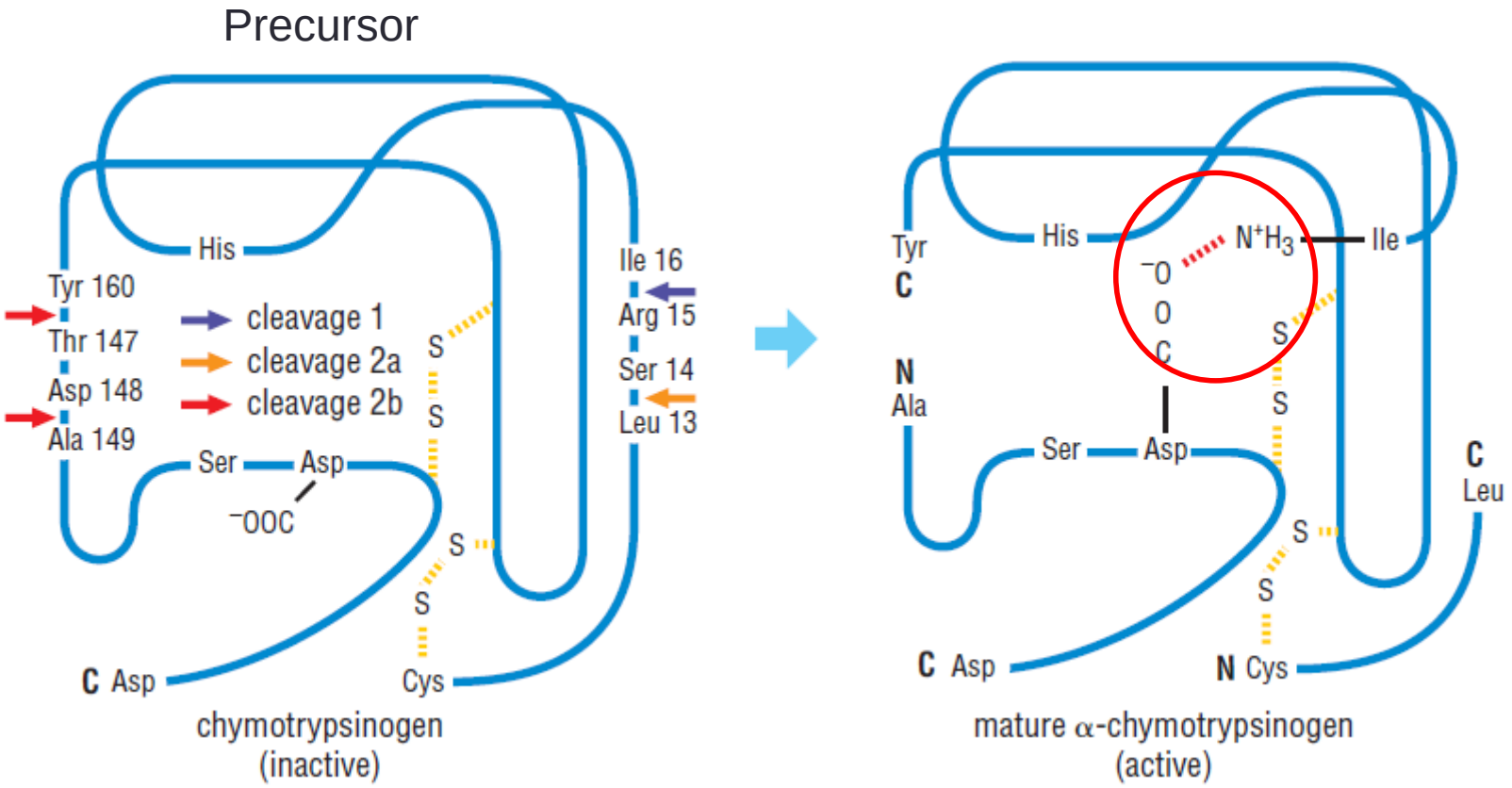
- Polypeptide chains are degraded to small peptides or individual amino acids
- Limited proteolysis



Limited proteolysis

- Many proteins are post-translationally modified by limited proteolytic digestion that produces an active form from their inactive or marginally active precursors
- Limited proteolysis involves the cleavage of a target protein at no more than a few specific sites usually by a specific protease
- The resulting cleaved protein can have one of two fates:
  - The fragments remain associated covalently (e.g. disulfidelinked) or noncovalently
  - The fragments dissociate to give two or more different polypeptides, each of which may have a completely separate fate and function
- Biological consequences of limited proteolysis
  - Activating enzymes
  - Producing hormones

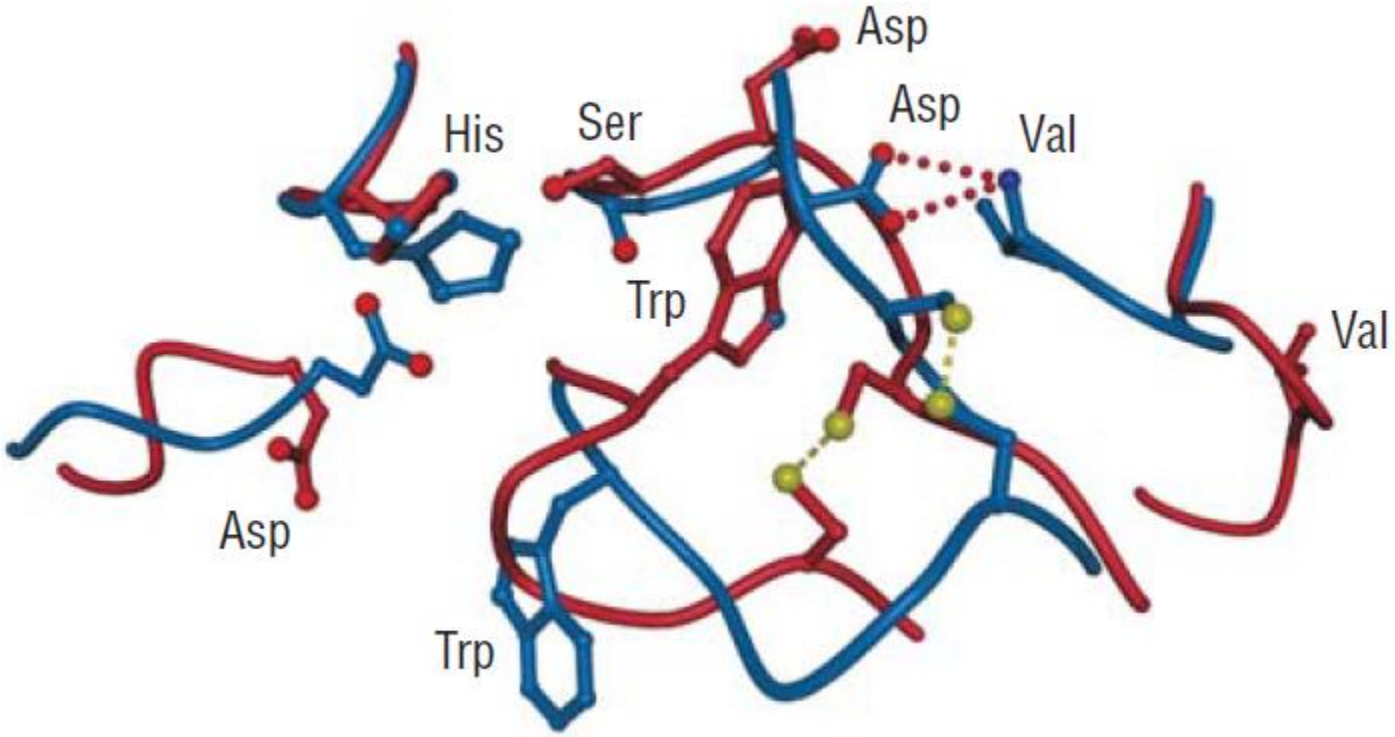
Activation of chymotrypsinogen



The requirement of activation for protease precursor prevent the improper activation

- Chymotrypsinogen has the serine protease fold with the exception that the active-site region lacks the proper configuration of main chain and catalytic side chains for both catalysis and substrate recognition
- It adopt a “spring-loaded” form: the existence of a covalent bond between Arg15 and Ile16 and a set of noncovalent interactions between these residues and their neighbors prevent it from rearranging into the correct conformation for catalysis
- Specific proteolytic cleavage between Arg15 and Ile16 by the protease trypsin releases the constraint

Activation of plasminogen

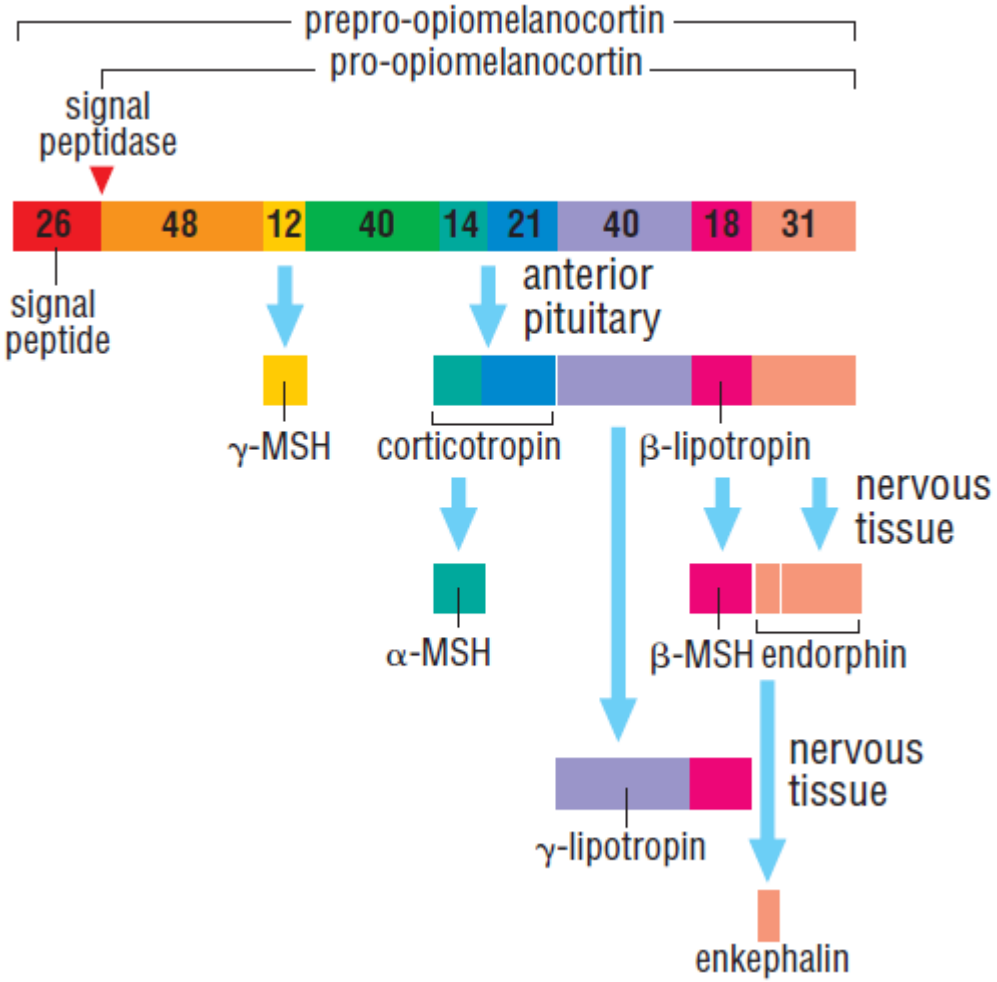


Comparison of the active sites of plasminogen and plasmin

- In chymotrypsinogen, most of the catalytic residues are already in the active configuration, but in plasminogen the entire active site is distorted
- This distortion is caused by a tryptophan residue that blocks the substrate-binding pocket and pushes the catalytic residues away from their correct orientations
- Cleavage in plasminogen creates a new positively charged amino-terminal amino group that interacts with the central aspartic acid residue, causing the peptide to rearrange into the correct configuration
- At the same time, the tryptophan swings out of the active site, unblocking the substrate-binding pocket and allowing the catalytic residues to switch to the correct configuration for catalysis

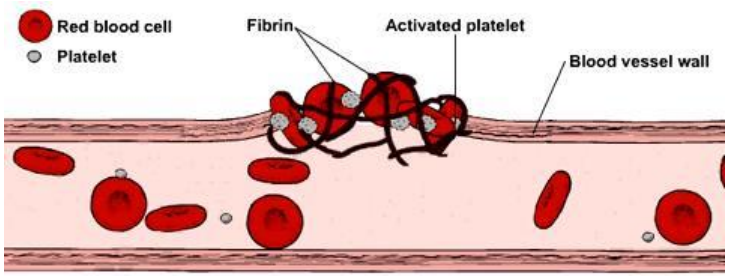
Multihormone precursor pro-opiomelanocortin

- Polypeptide hormones are synthesized in “prepro” form, with a signal (pre) sequence and additional (pro) sequences that are cleaved out during maturation
- A single precursor sequence may contain two or more hormones, each released by additional cleavages
- Pro-opiomelanocortin contains sequences for hormones having distinct target tissues and physiological activities
- Pro-opiomelanocortin is cleaved at different sites in different cell types so that they produce different spectra of hormones derived from the single precursor
- Each of the cleaving enzymes is specific for a particular sequence in the precursor protein, by virtue either of its active-site structure or of the compartment in which it encounters its substrate

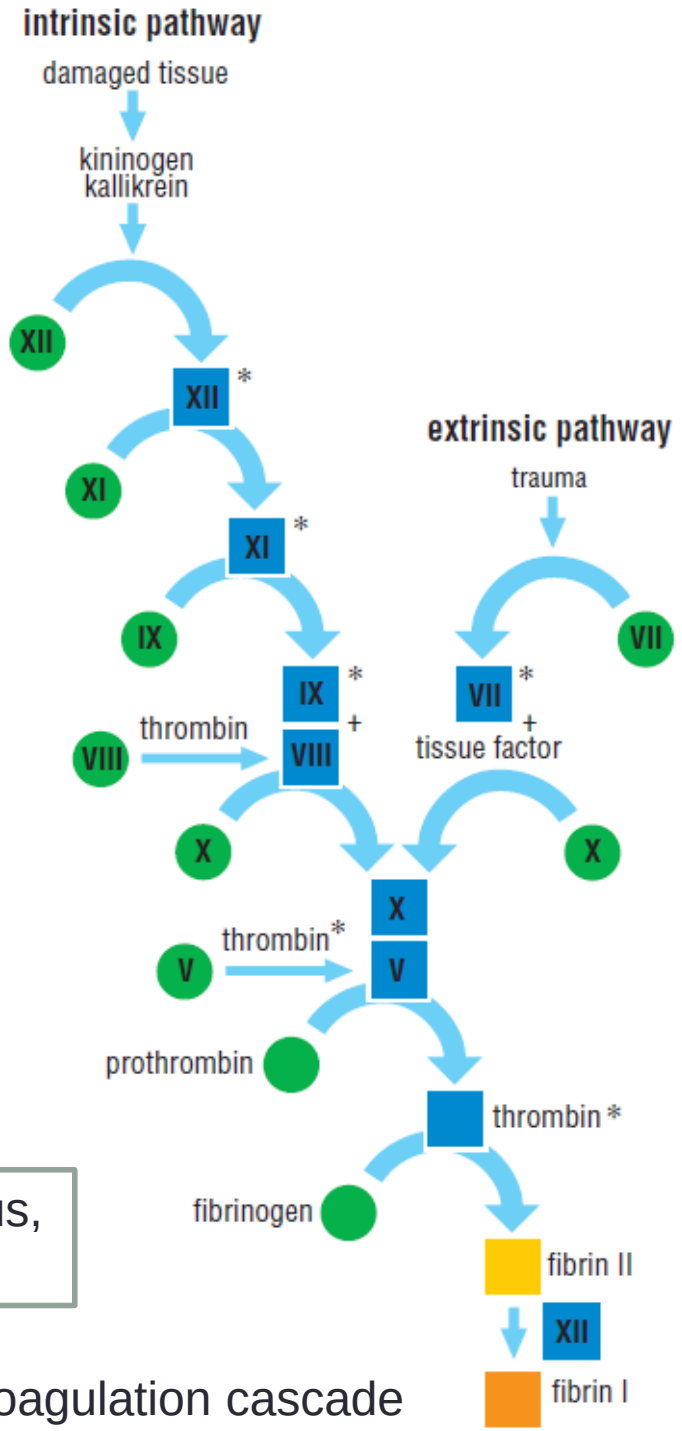


Limited proteolysis can produce polypeptides with new functions

Proteolytic cascade

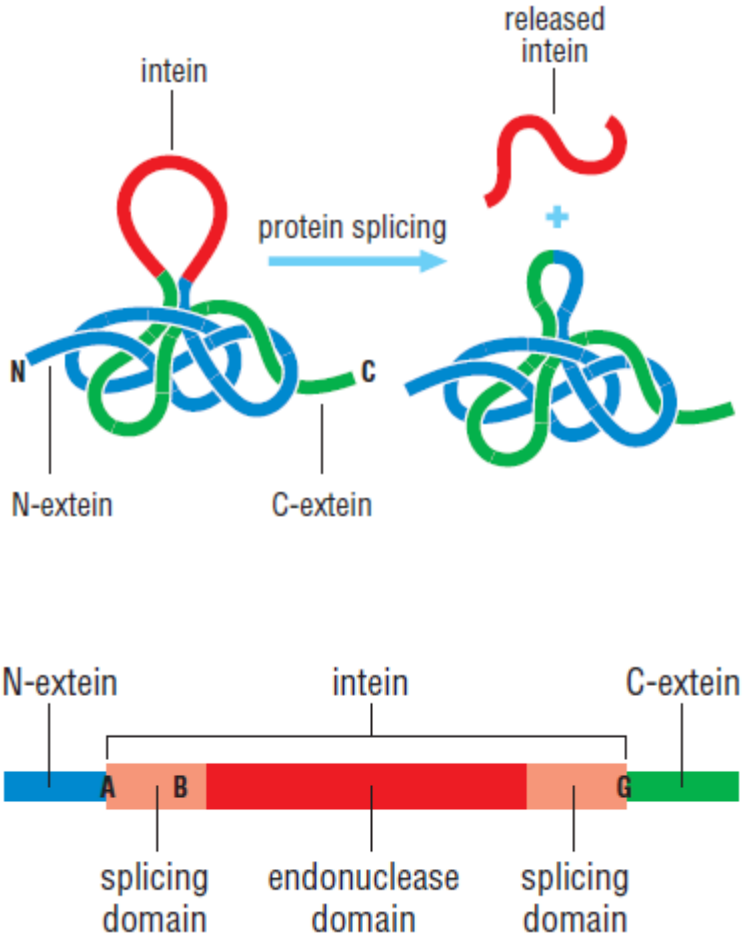


Limited proteolysis can provide for enormous, extremely rapid amplification of a signal



The blood coagulation cascade

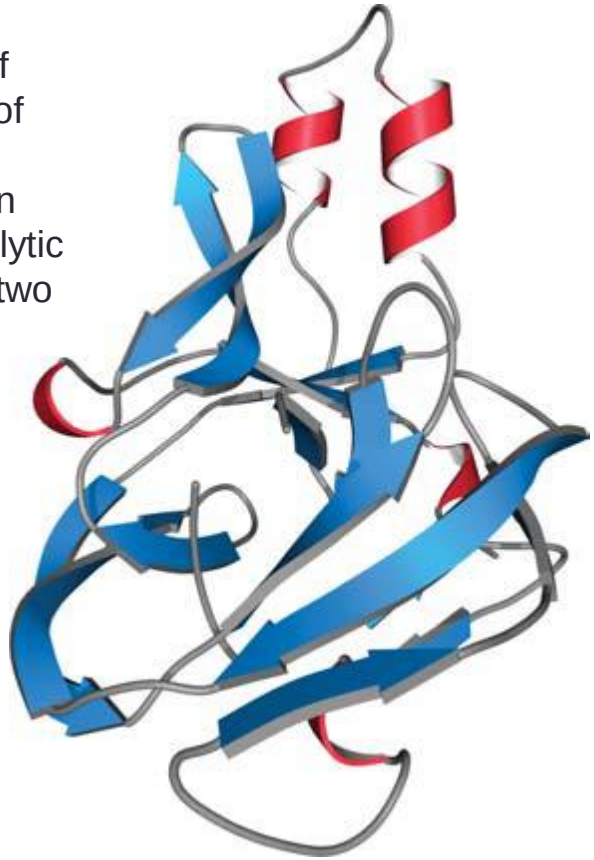
Protein splicing: autoproteolysis by Inteins



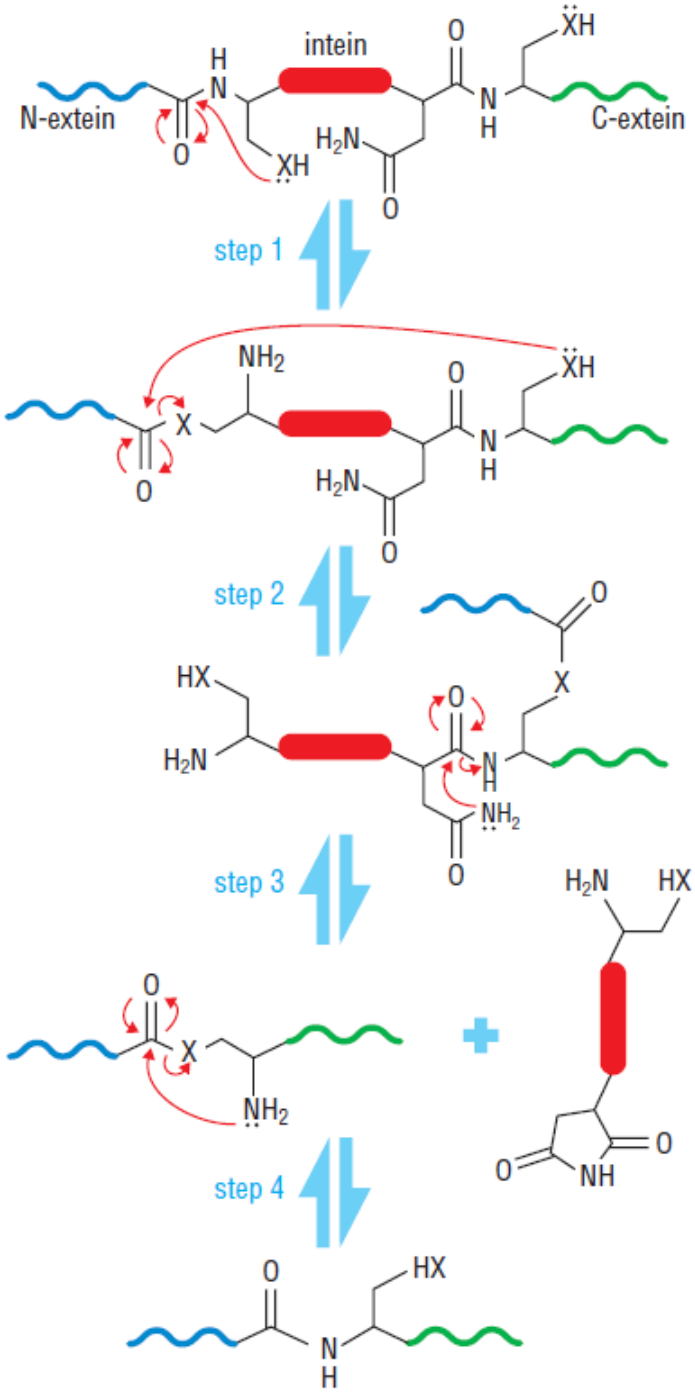
- Some proteins contain self-excising inteins
  - Intein is an internal domain that is subsequently cleaved out from the protein to form an independent protein
  - Prokaryotes and lower eukaryotes
- Protein splicing
  - Analogous to RNA splicing
  - The process generates two functional proteins from one polypeptide chain
  - Self-catalyzed
- Most inteins are involved in DNA replication and repair

Protein splicing mechanism

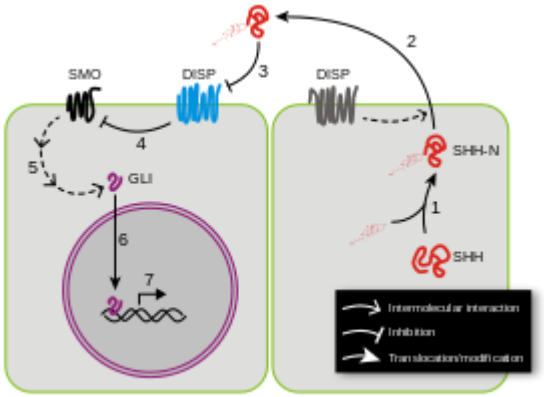
- Inteins are characterized by a few short signature sequence motifs
- The signature residues have specific roles in the structure of the intein and the mechanism of protein splicing
- The intein structure contains an unusual beta fold with the catalytic splice junctions at the ends of two adjacent beta strands



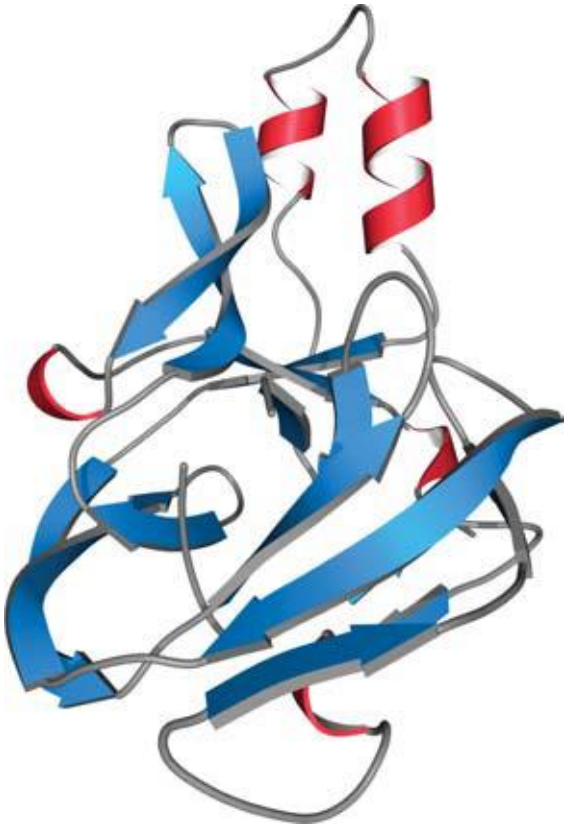
A intein, gyrase A subunit of *Mycobacterium xenopi*



Autocatalysis in Hedgehog protein

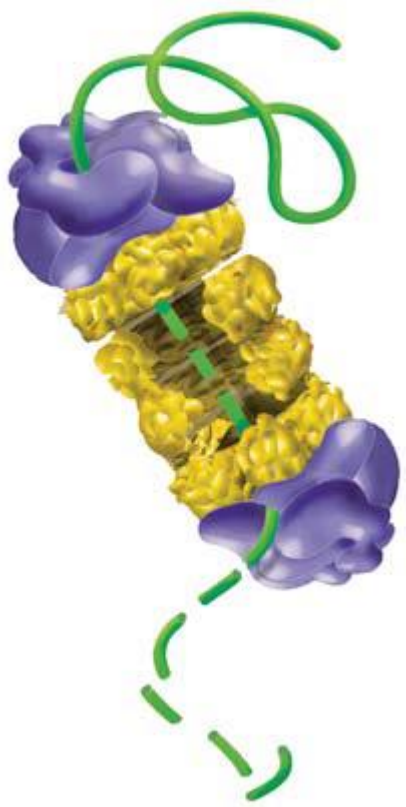


The Hedgehog C-terminal autoprocessing domain

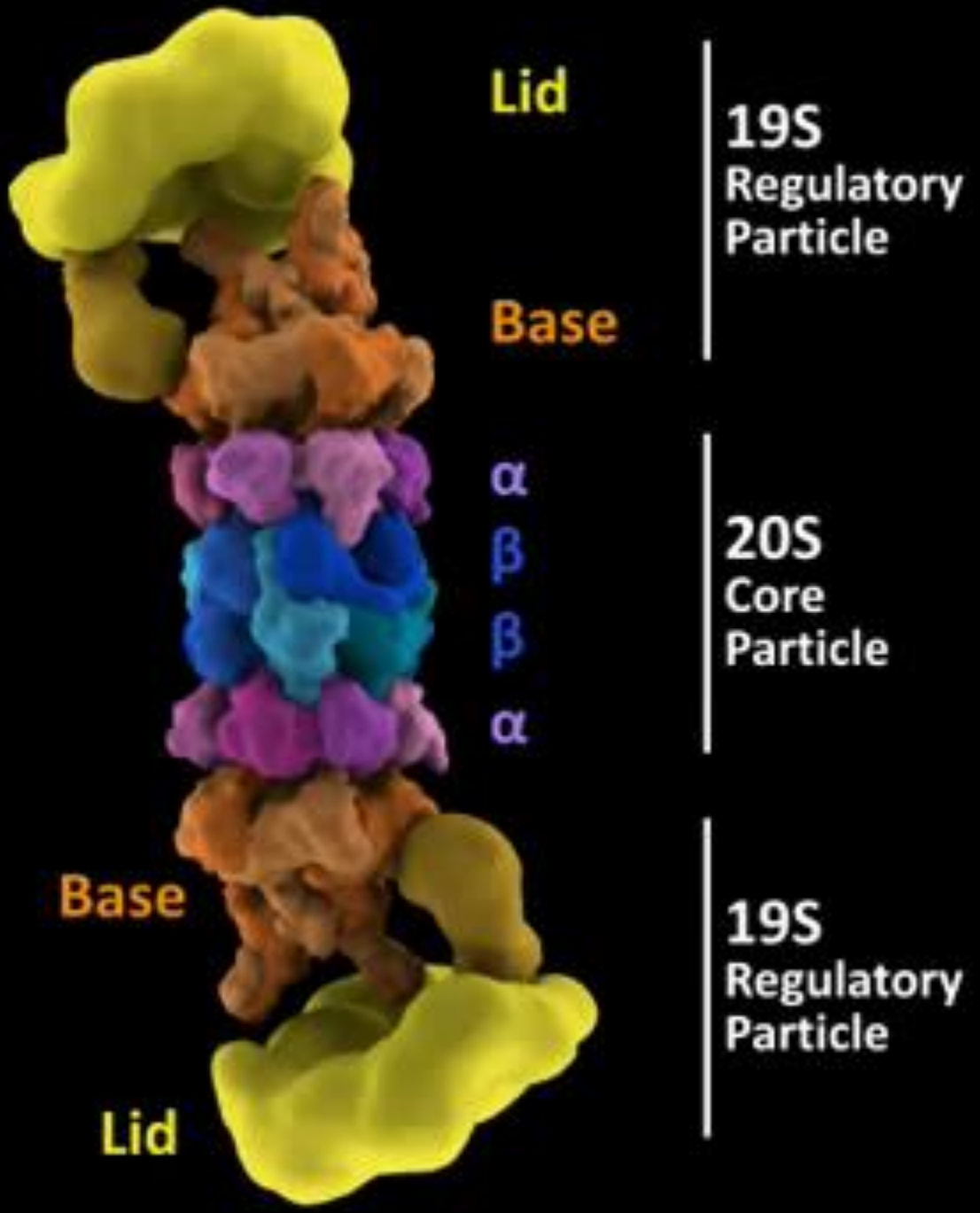


The mechanism of autocatalysis is similar for inteins from unicellular organisms and metazoan Hedgehog protein

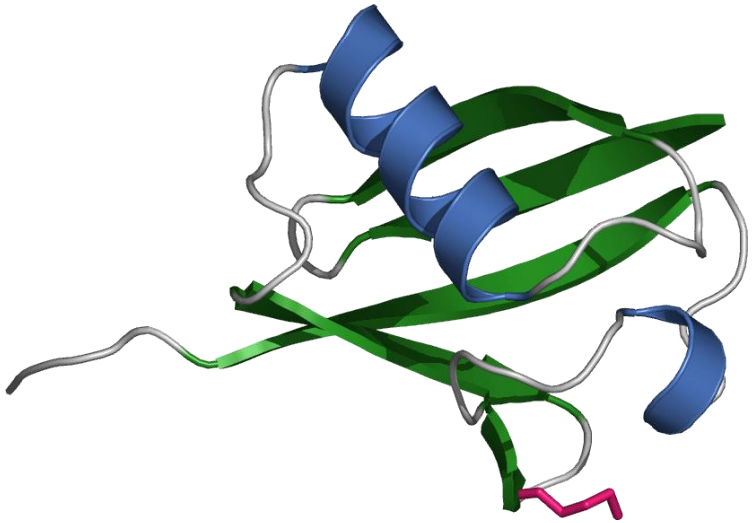
Regulation by degradation



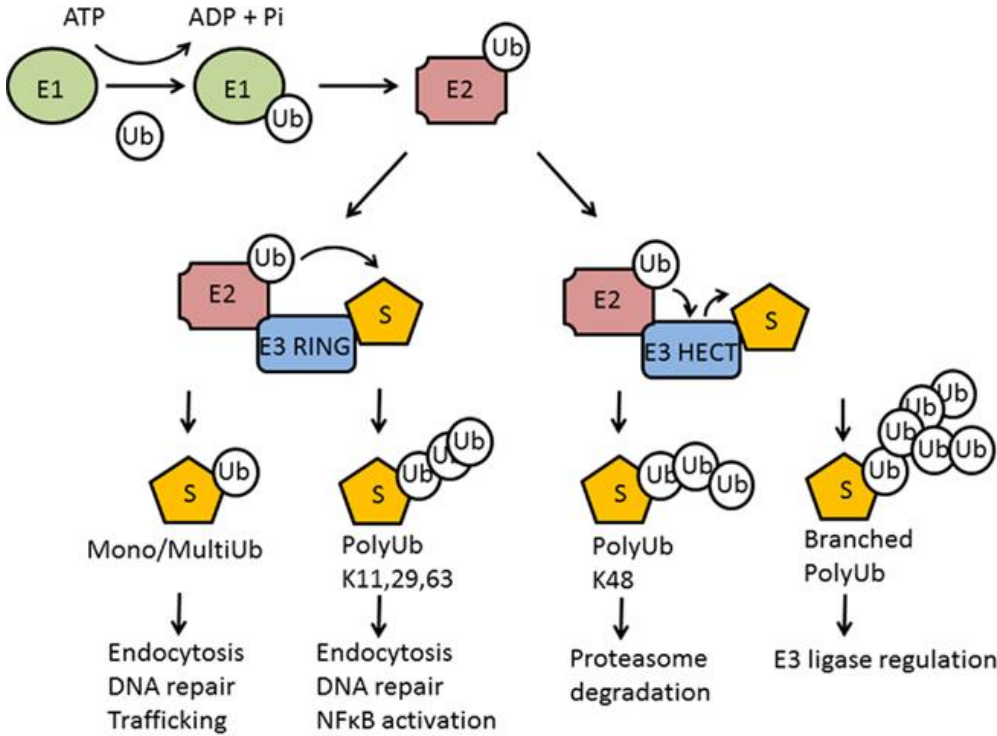
- Proteins not only carry signals that determine their location, they also carry signals that determine their lifetime
- Protein degradation in cells is accomplished by machinery that tags both misfolded and folded proteins for specific proteolysis
- The proteolytic machinery responsible for targeted protein degradation in cells is a giant multiprotein assembly called the proteasome

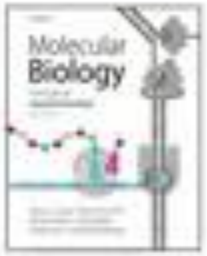

Lid
Base
α
β
β
α
Base
Lid
19S
Regulatory
Particle
20S
Core
Particle
19S
Regulatory
Particle

Ubiquitination



- Proteins carrying an appropriate signal for destruction are recognized by a tag
  - Ubiquitin, a small protein
- Ubiquitination is carried out by a multi-enzyme pathway that starts by recognition of an exposed lysine near the amino terminus of the target protein
- Ubiquitination system
  - E1: activating enzyme
  - E2: conjugating enzyme
  - E3: ligase





Molecular Biology: Principles of Genome Function

Second Edition

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Animation 11:
Ubiquitin

Animation produced by Connor Hendrich

SUMOylation

- SUMO (small ubiquitin-related modifier) is an ubiquitin-like protein
- The consensus sequence for sumoylation is yKXE (where y is a hydrophobic amino acid and X is any amino acid)
- Like ubiquitin, SUMO is attached to the amino group of lysine residues by specific SUMO-activating and -conjugating enzymes
- Attachment of SUMO to proteins has been shown to change their subcellular localization, transcriptional activity and stability