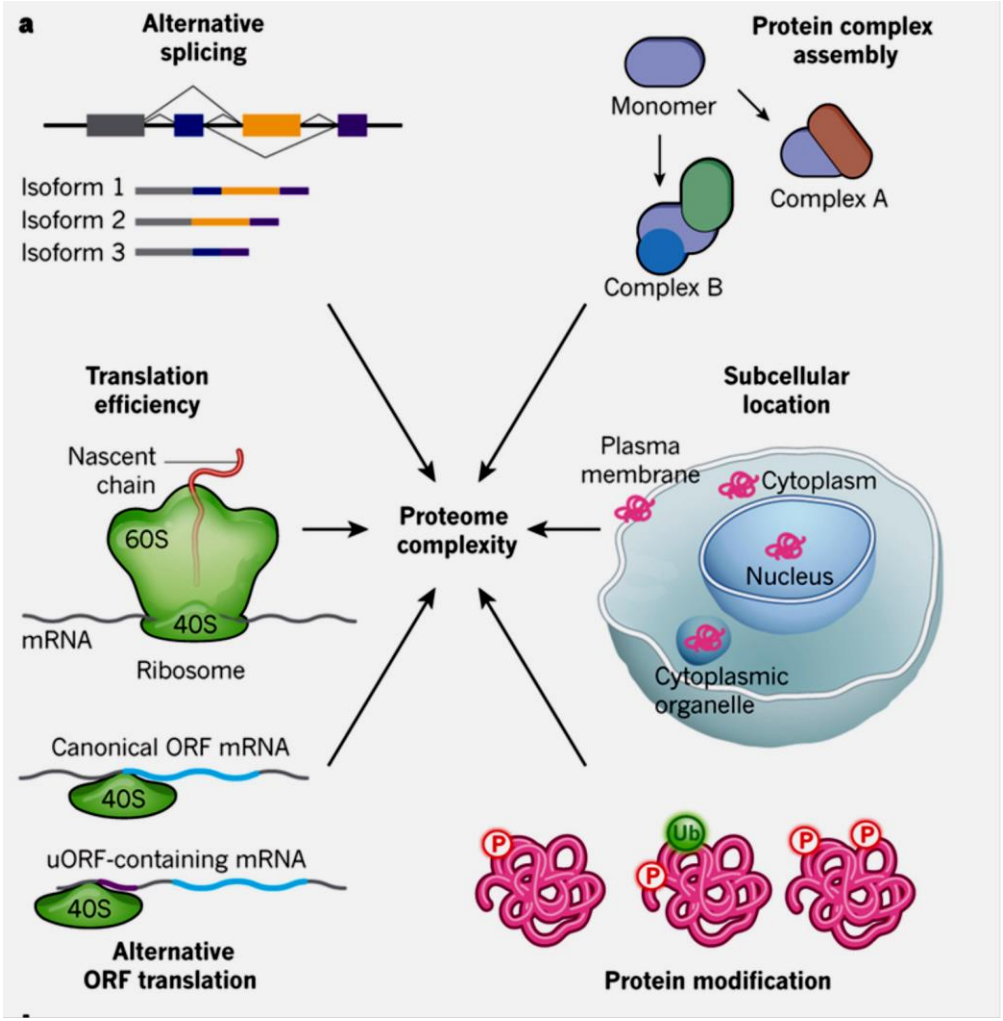
Lecture 9 Regulation of protein function

I. Protein complexity
II. Control of protein activity
III. Control of protein steady-state level
IV. Ubiquitination and protein degradation in the proteasome
V. Protein misfolding and human diseases

I. Protein complexity in a single cell

- The cellular proteome is exceedingly complex. Of the 20,000 or so protein-coding genes of the human genome, a typical cell transcribes about 10,000 genes, which have a cumulative copy number of 10^9–10^11 protein molecules per cell.

Protein complexity



II. Regulation of protein function

- 1. Intrinsic activity of protein
- 2. Change in location and concentration of protein in cells
- 3. Steady-state level of protein

1. Control of protein intrinsic activity

- Noncovalent binding
- Covalent bonding
  - phosphorylation/dephosphorylation
  - ubiquitination/deubiquitination
  - Sumolation, Neddylation,
  - acetylation, methylation
  - palmitylation on Cys, etc
- Protease cleavage

Many
Are reversible

Irreversible

Take tumor suppressor TP53 as an example

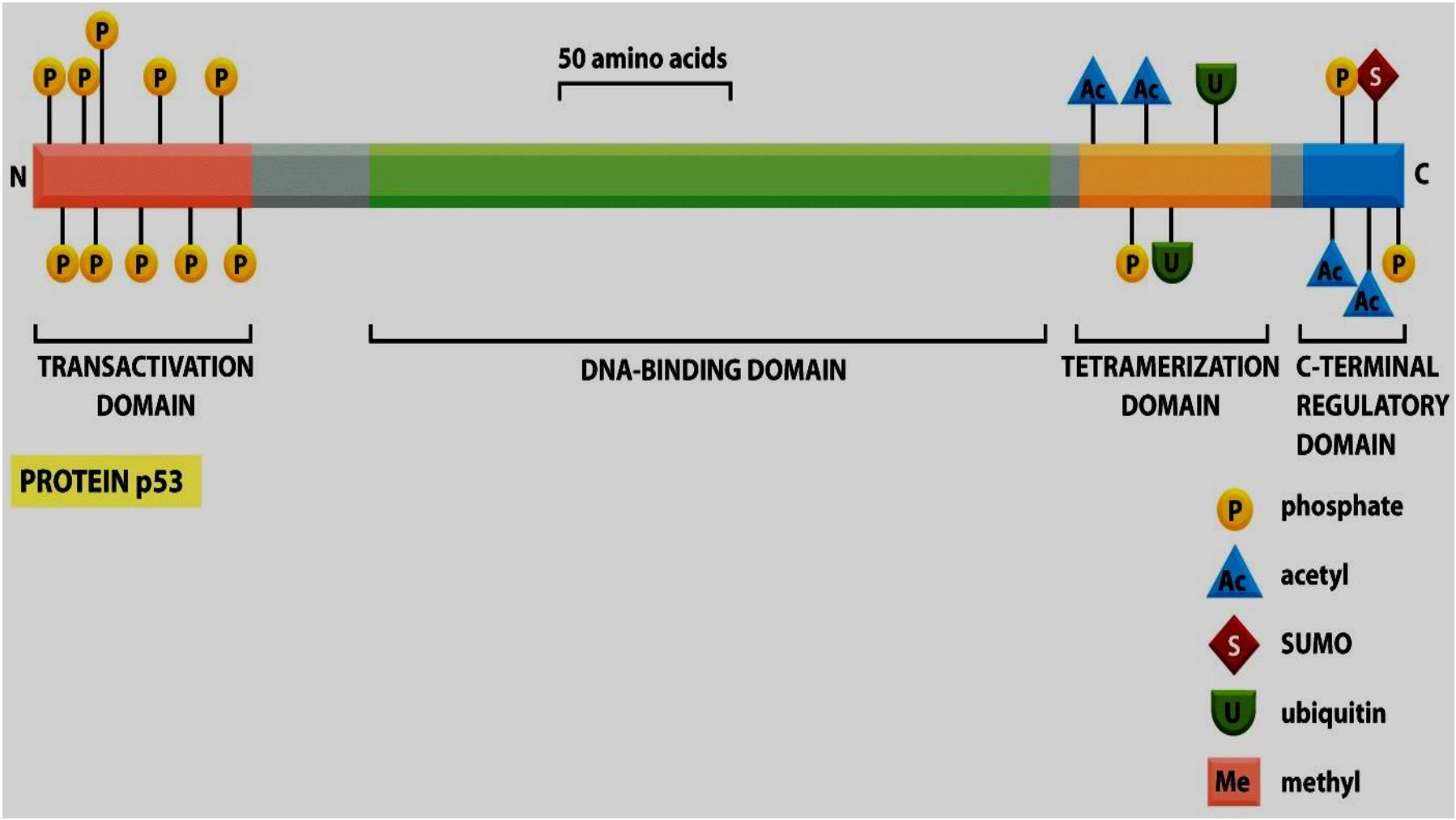


Table 3–3 Some Molecules Covalently Attached to Proteins Regulate Protein Function

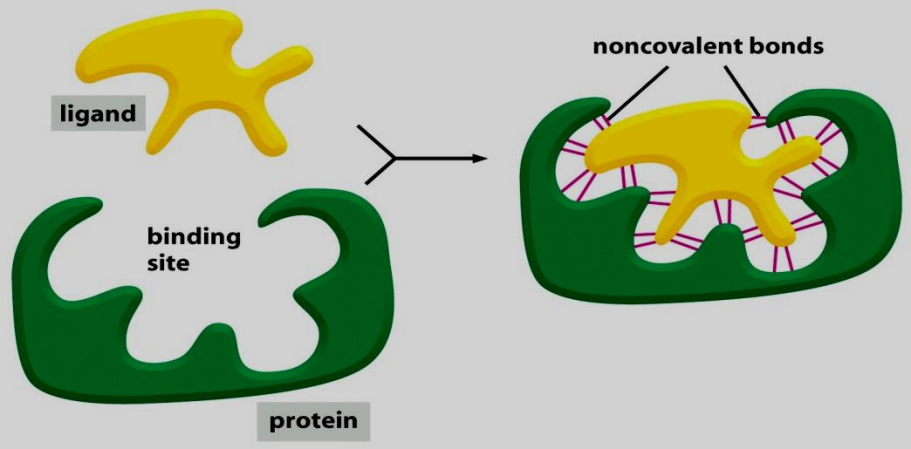
MODIFYING GROUP	SOME PROMINENT FUNCTIONS
Phosphate on Ser, Thr, or Tyr	Drives the assembly of a protein into larger complexes (see Figure 15–19).
Methyl on Lys	Helps to creates histone code in chromatin through forming either mono-, di-, or tri-methyl lysine (see Figure 4–38).
Acetyl on Lys	Helps to creates histone code in chromatin (see Figure 4–38).
Palmityl group on Cys	This fatty acid addition drives protein association with membranes (see Figure 10–20).
N-acetylglucosamine on Ser or Thr	Controls enzyme activity and gene expression in glucose homeostasis.
Ubiquitin on Lys	Monoubiquitin addition regulates the transport of membrane proteins in vesicles (see Figure 13–58).
	A polyubiquitin chain targets a protein for degradation (see Figure 3–79).

Ubiquitin is a 76 amino acid polypeptide; there are at least 10 other ubiquitin-related proteins, such as SUMO, that modify proteins in similar ways.

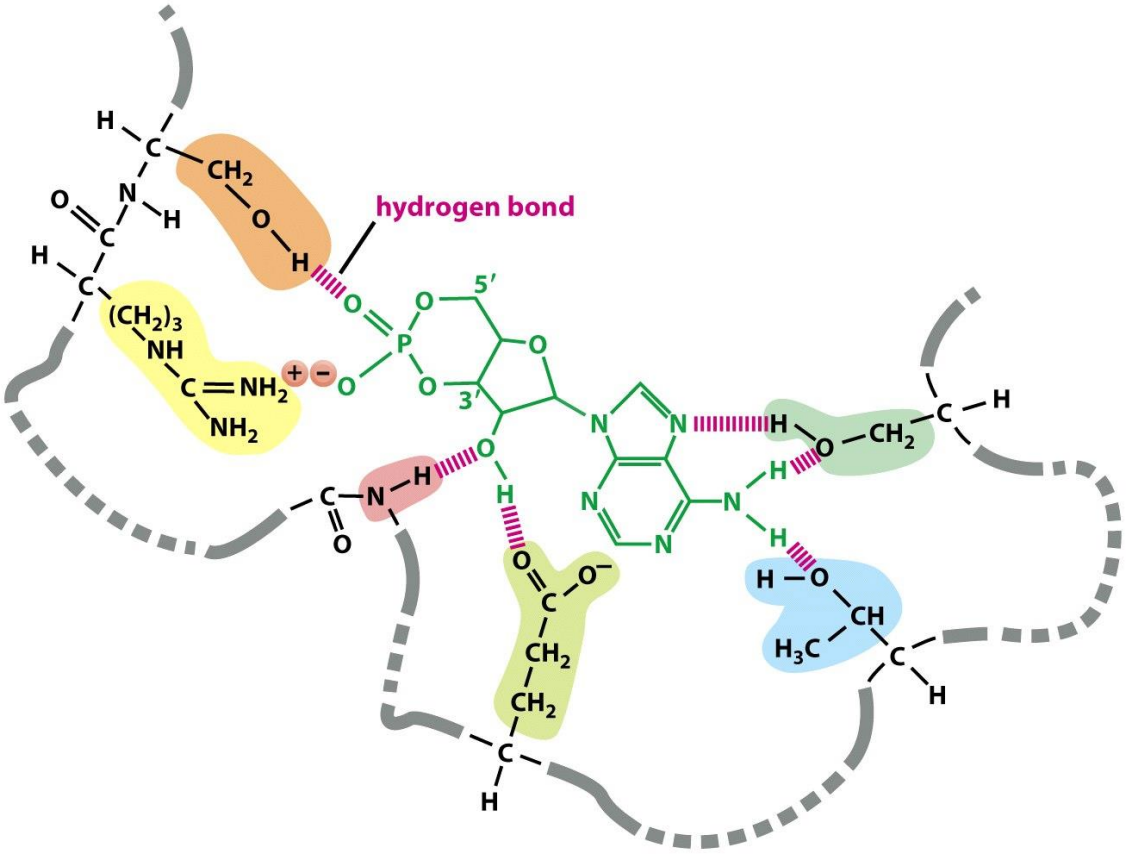
Protein binding

1. All proteins function by binding to other molecules, Which are called “ligands” , latin meaning “to bind”.
2. All bindings have specificity and different strength, which are determined by:

electrostatic attraction
hydrogen bond
van der Waals interaction
hydrophobic force

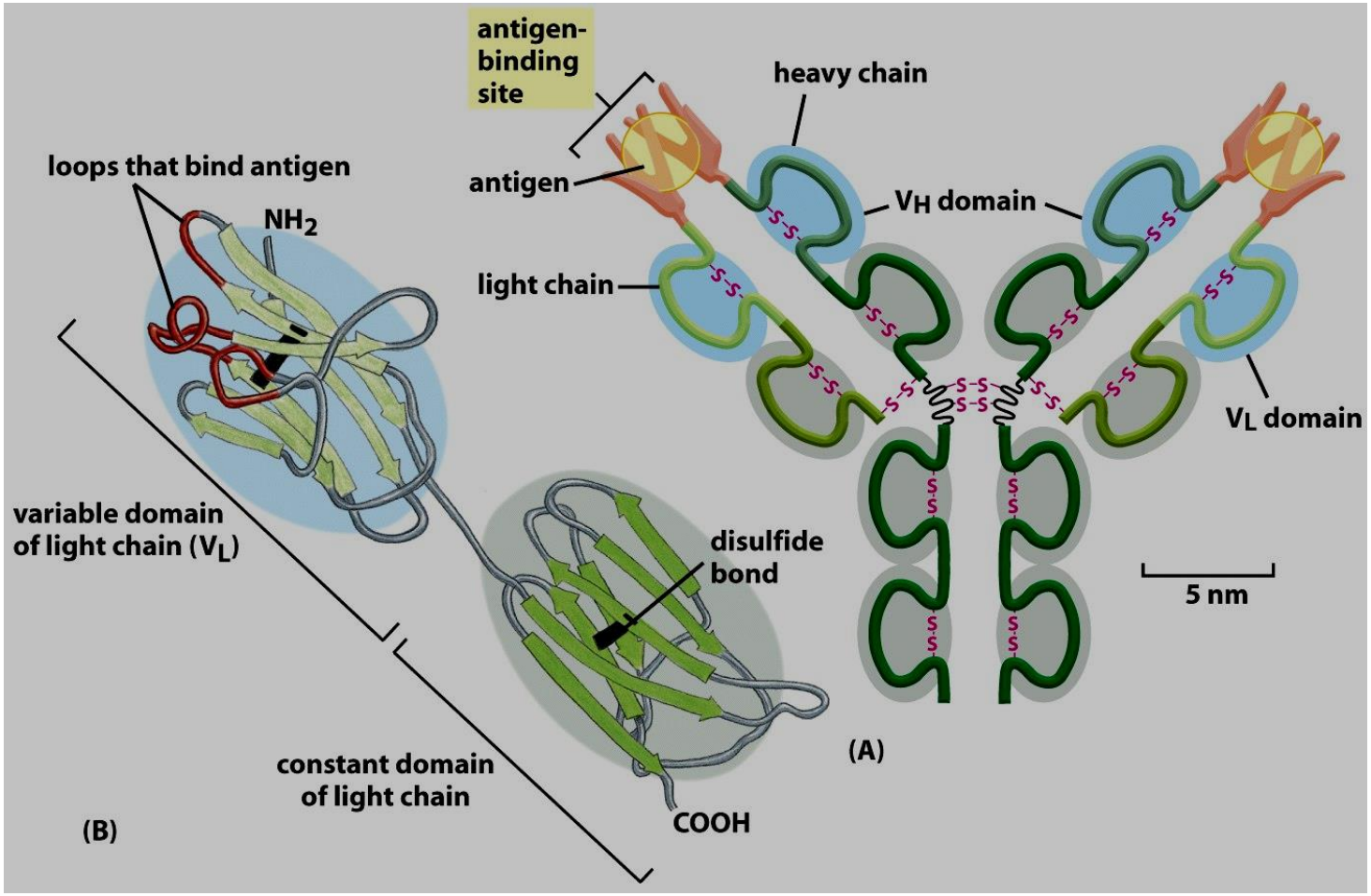


Close-up view of cAMP binding site in a protein



Mutations in these Key binding sites will disrupt the specific binding of protein with its ligand

Antibody binding sites are especially versatile



Non-covalent binding

- Graded allosteric changes

  such Oxygen versus hemoglobin

- Non-graded allosteric changes: turn on or off

  Calcium versus calmodulin

  GTP/GDP versus Ras
  - **Ras/GTP--- “ ON” state**
  - **Ras/GDP--- “ OFF” state**

Ras activity can be regulated by GTP/GDP binding

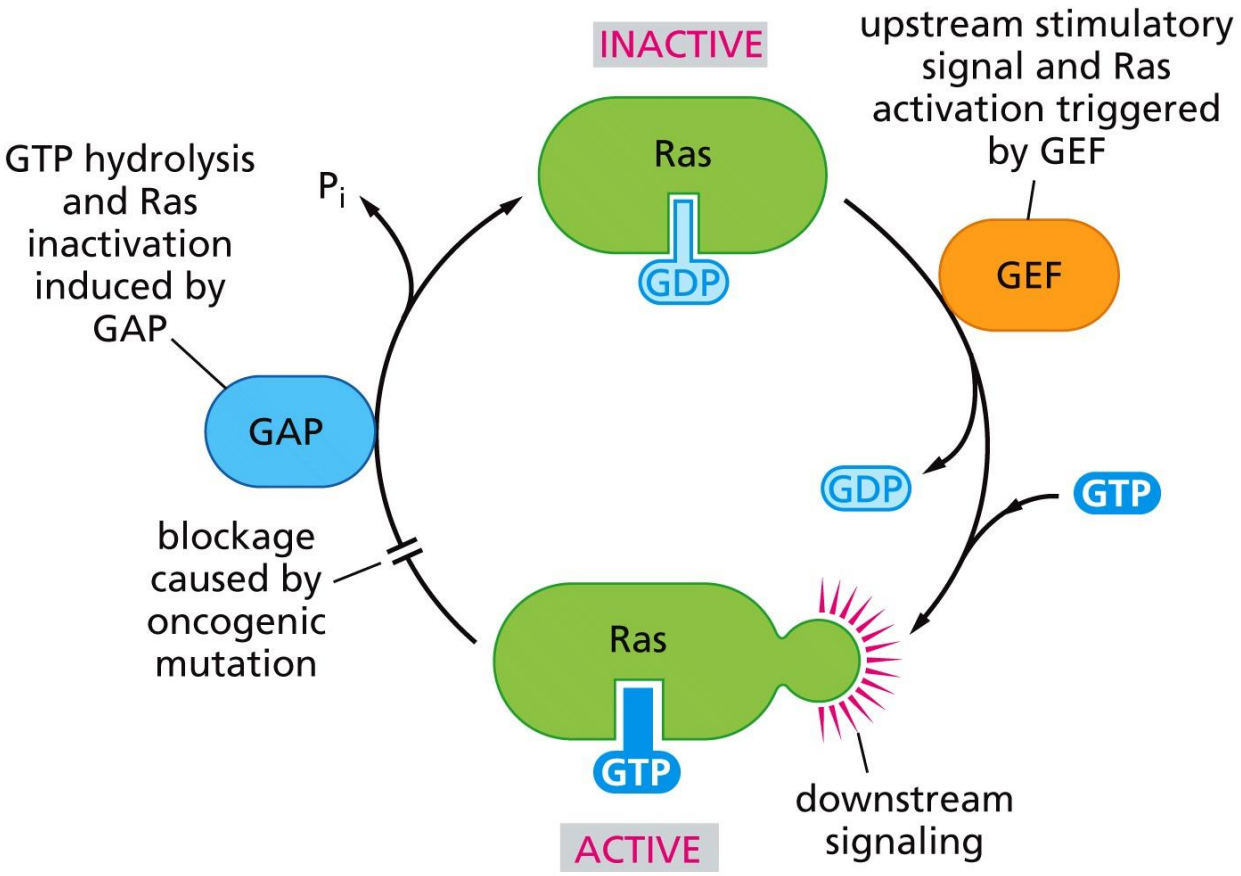


Figure 5.30 The Biology of Cancer (© Garland Science 2014)

How is Src activated?

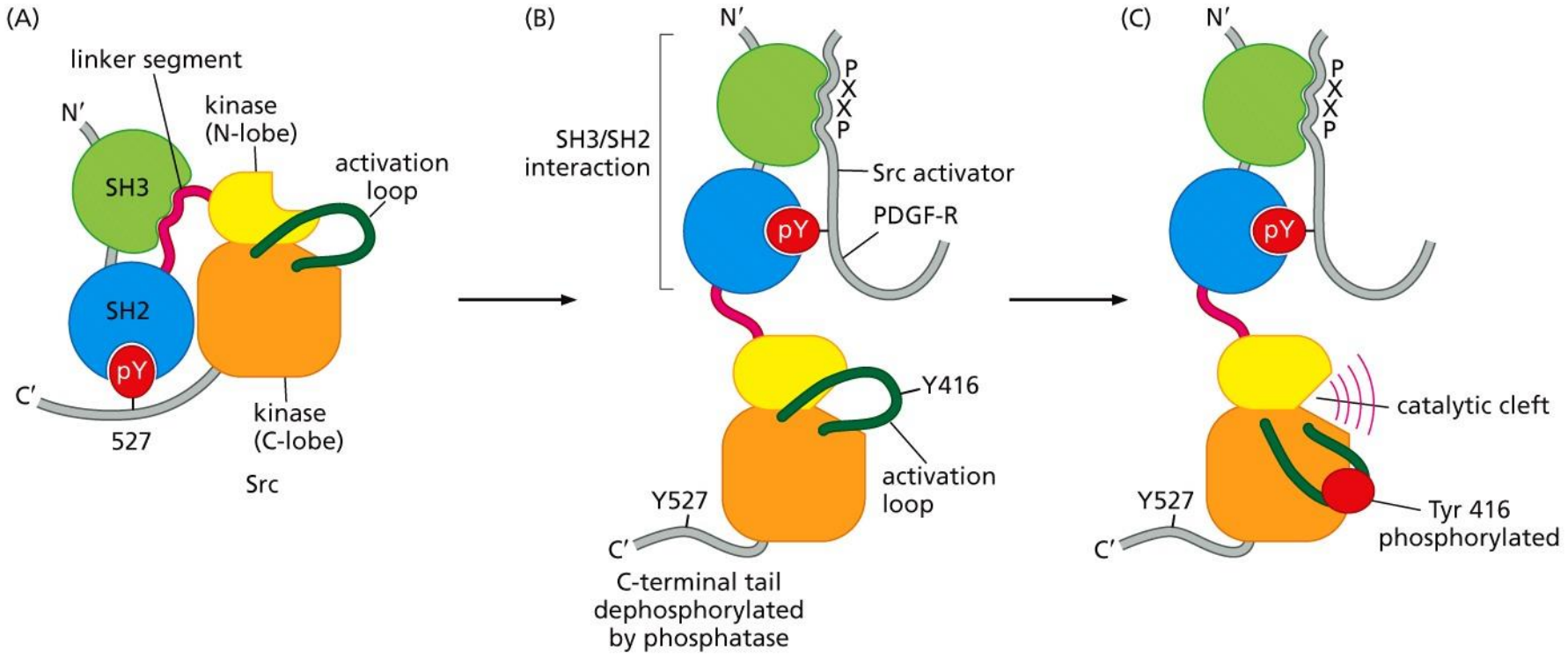
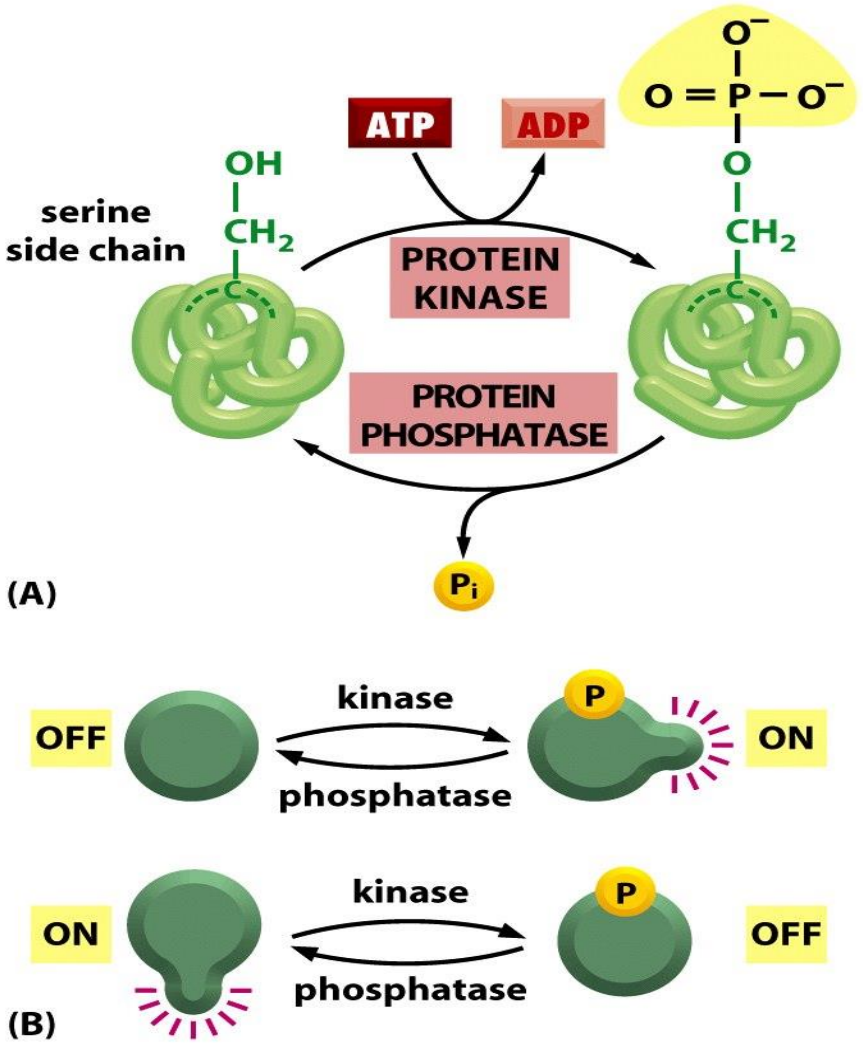


Figure 6.11 The Biology of Cancer (© Garland Science 2014)

Kinases and phosphotases are most common to regulate activity

Three amino acids
Can be modified by
Phosphorylation:

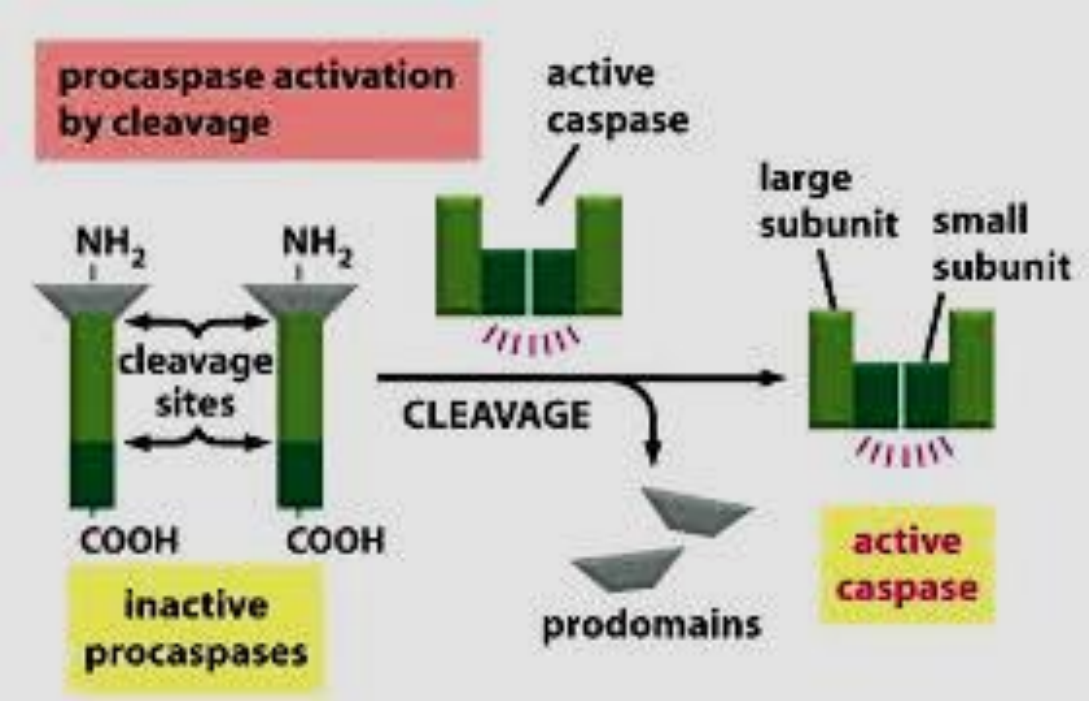
1. Tyrosine
2. Serine
3. Threonine



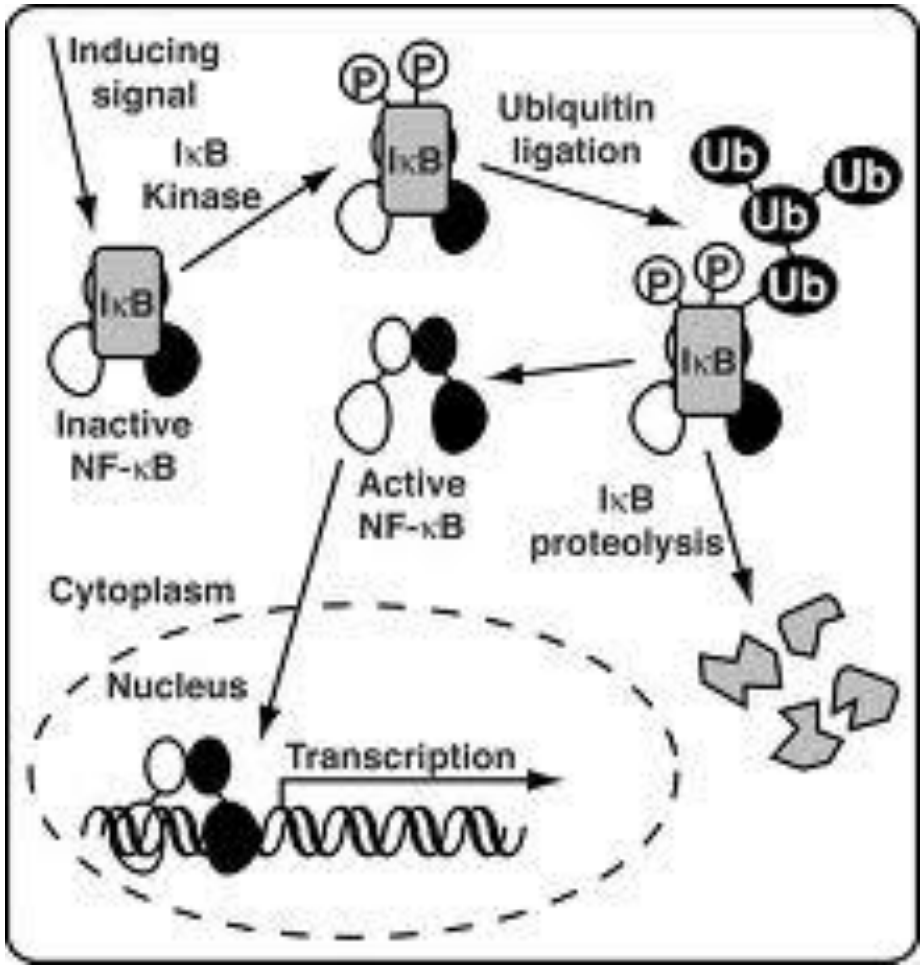
Protein cleavage (irreversible)

- Pro-insulin
- Zymogen: trypsinogen
- pro-caspases

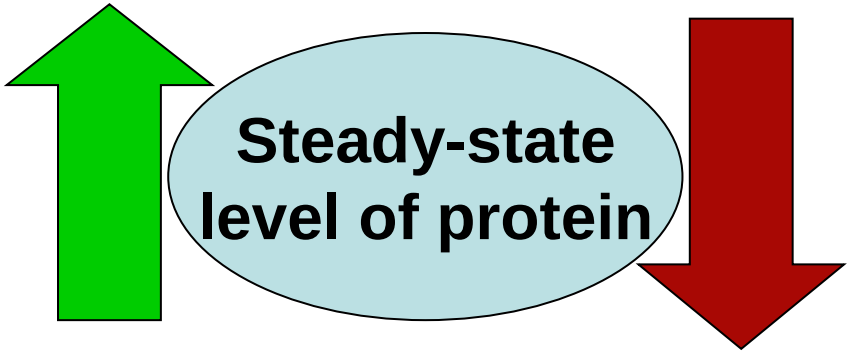
Example for protein cleavage



2. Control of protein localization and local concentration



3. Steady state level of protein



new protein synthesis Old protein degradation

Different proteins have different half lives:

- ♥ Short half life protein: cell cycle protein
- ♥ Long half life protein: house keeping genes such as tubulin, actin

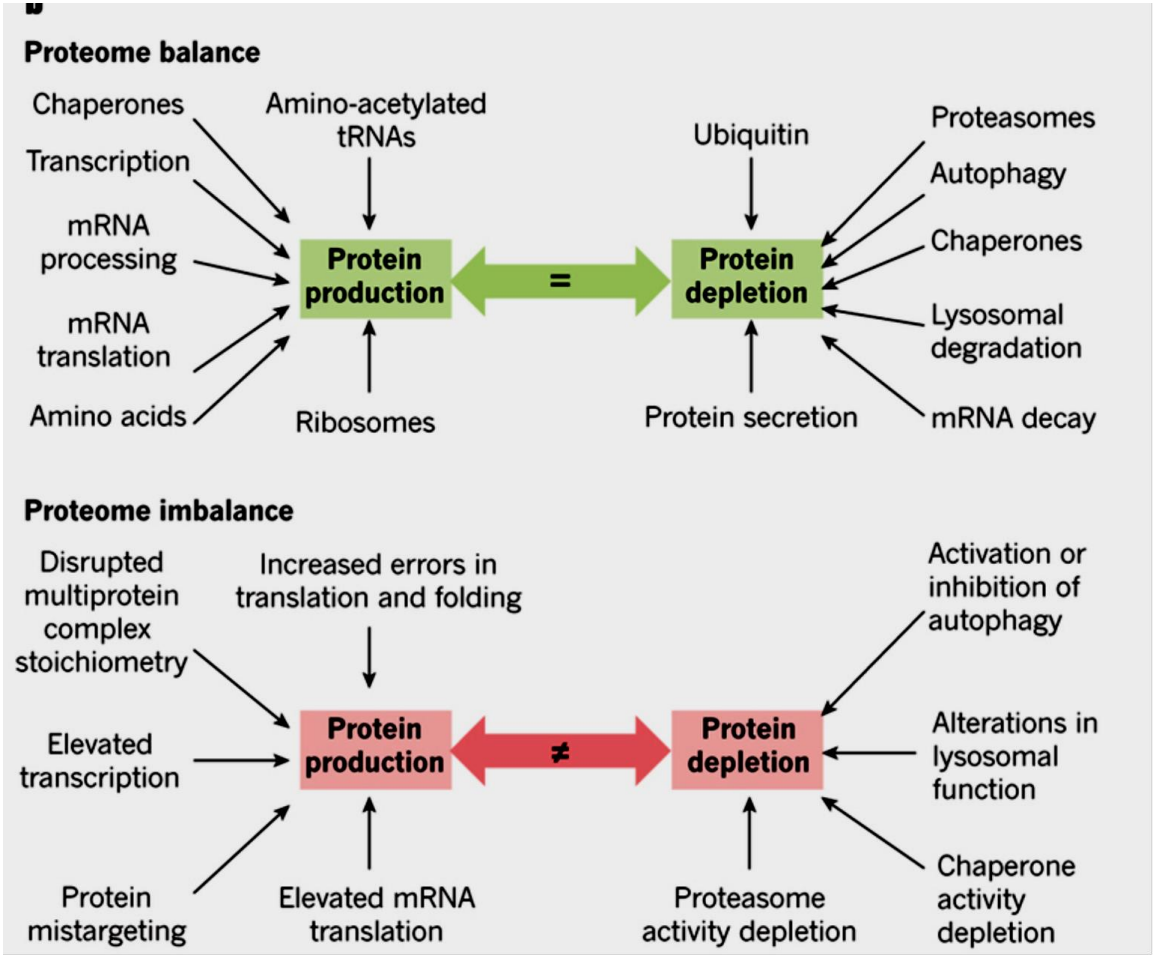
- Protein levels can be controlled at four levels :

mRNA transcription
translation
post-transcriptional

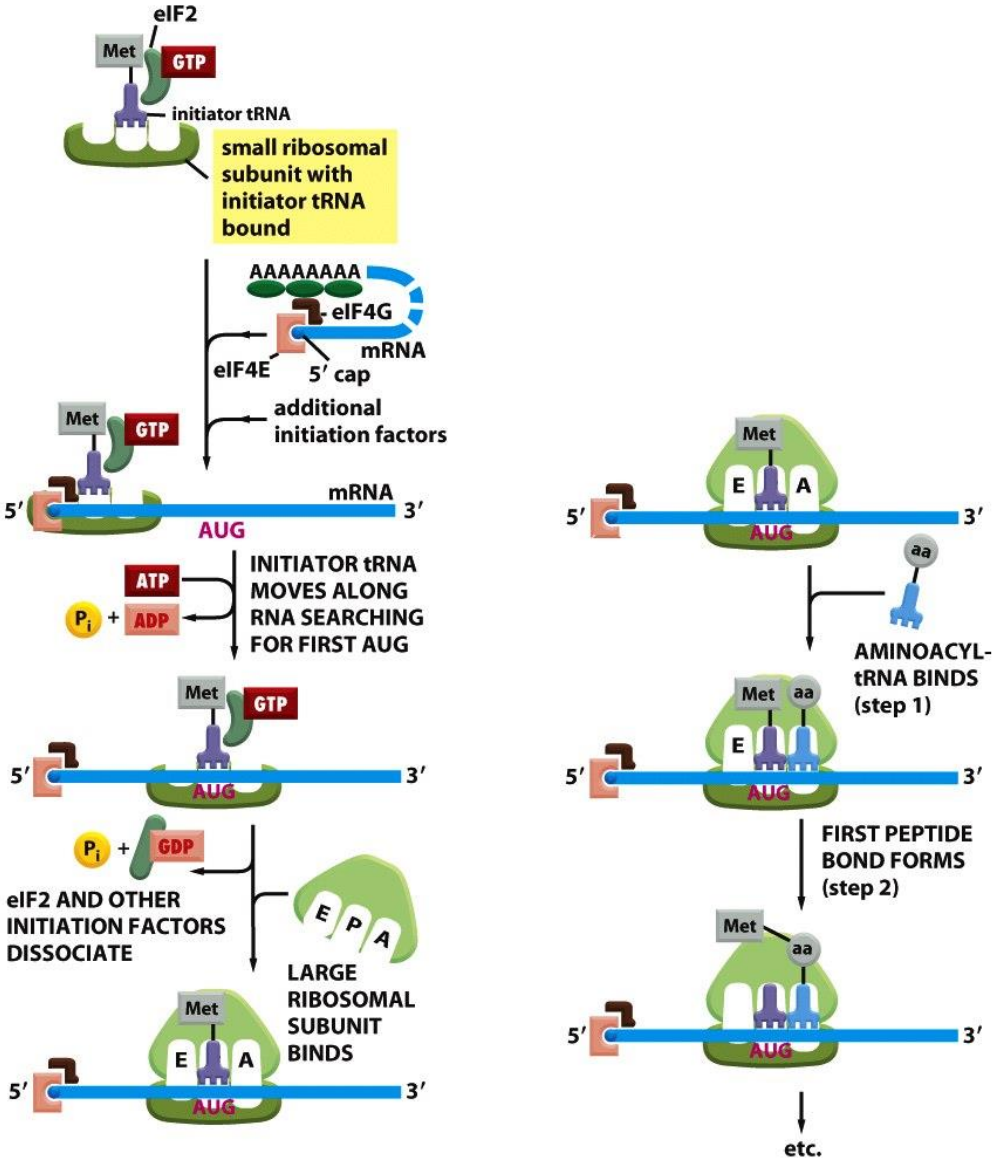
} Protein synthesis

post-translation ← Protein degradation

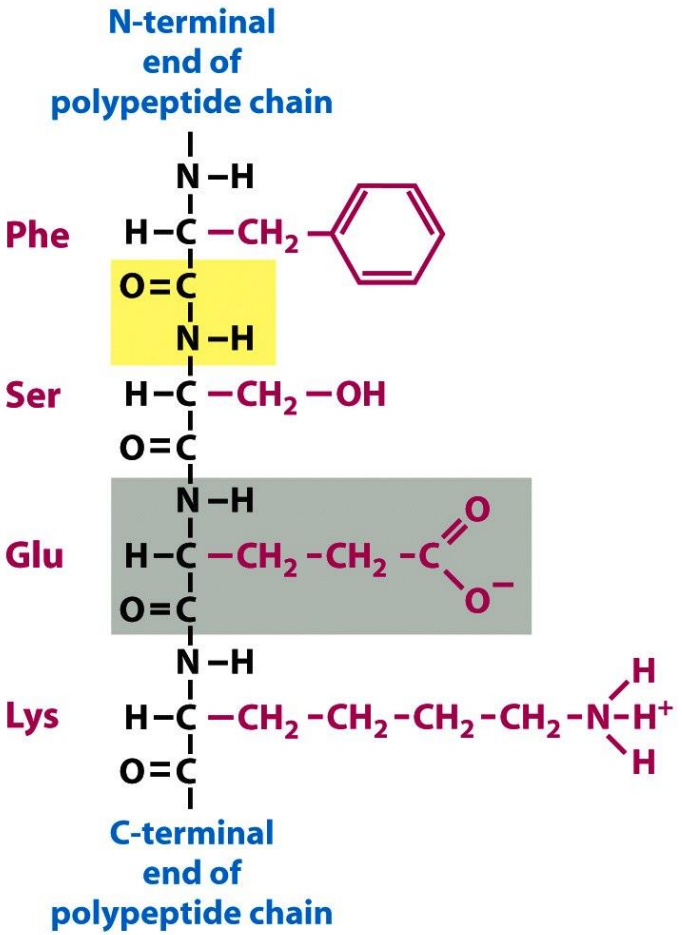
Balancing between protein production and protein depletion



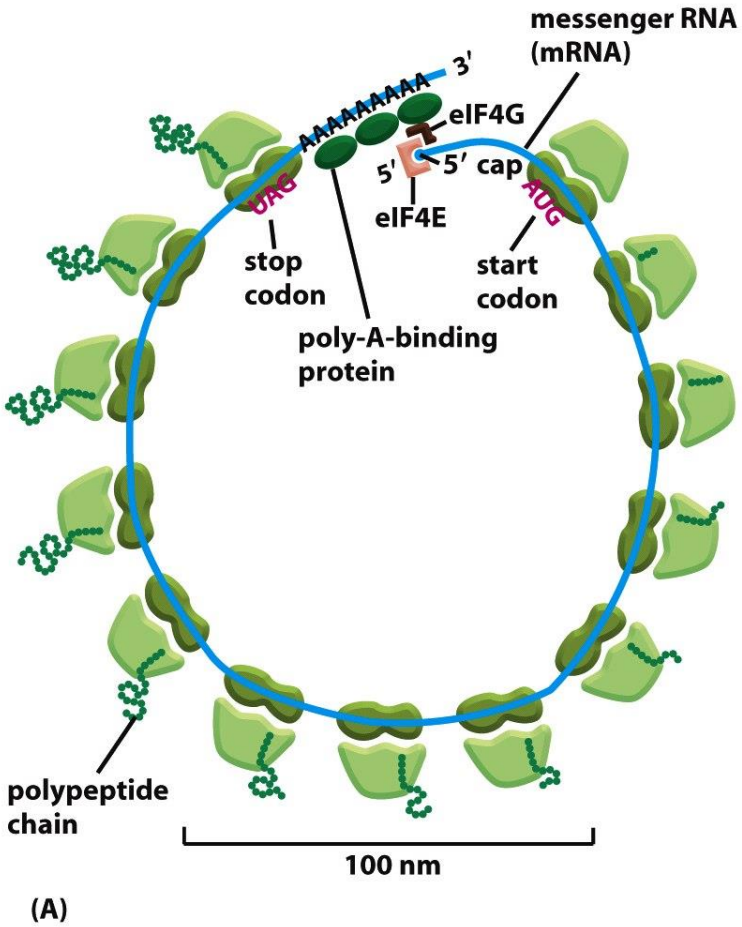
A brief look at protein translation



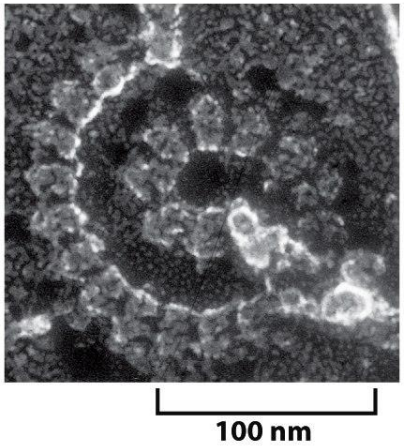
Peptide bond between amino acids



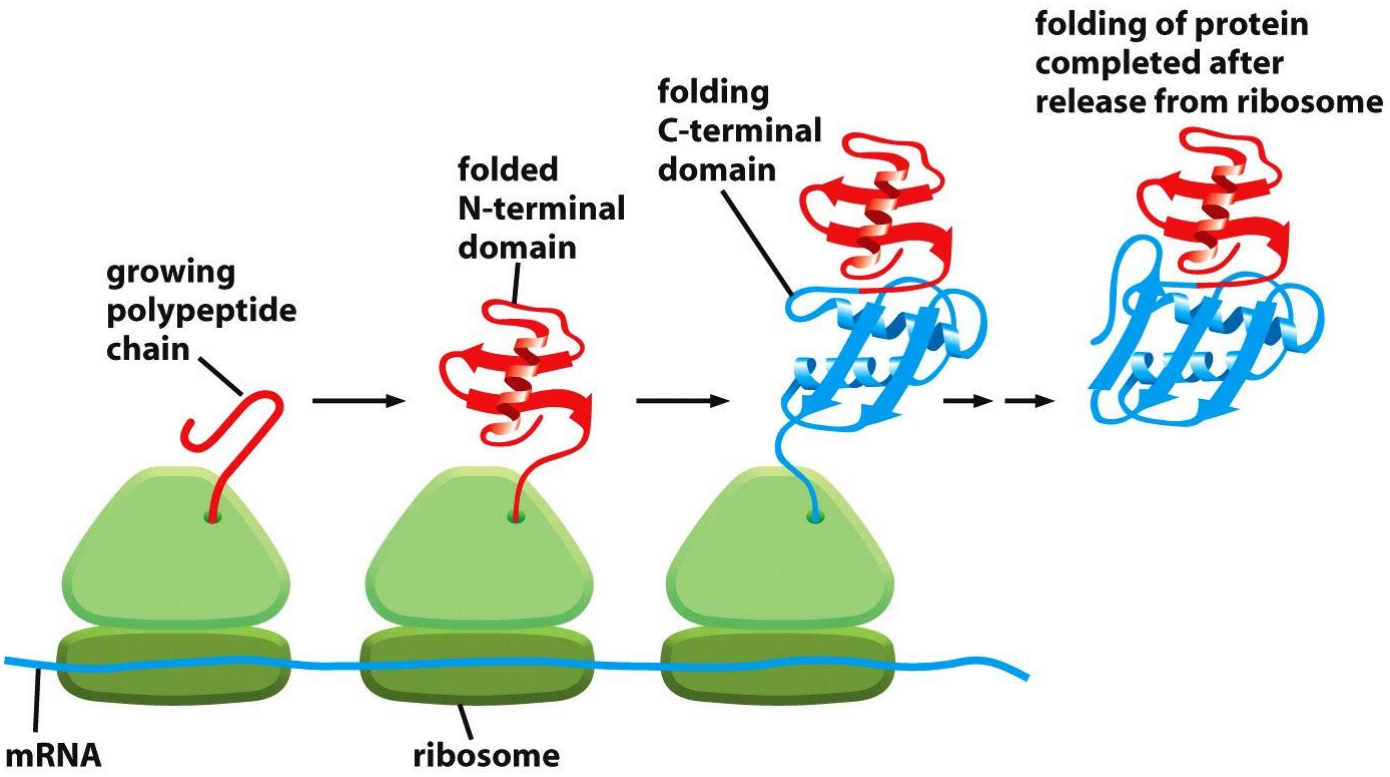
Proteins are usually synthesized efficiently on one mRNA--- formation of polyribosomes



Cycloheximide freezes ribosomes
On a single mRNA



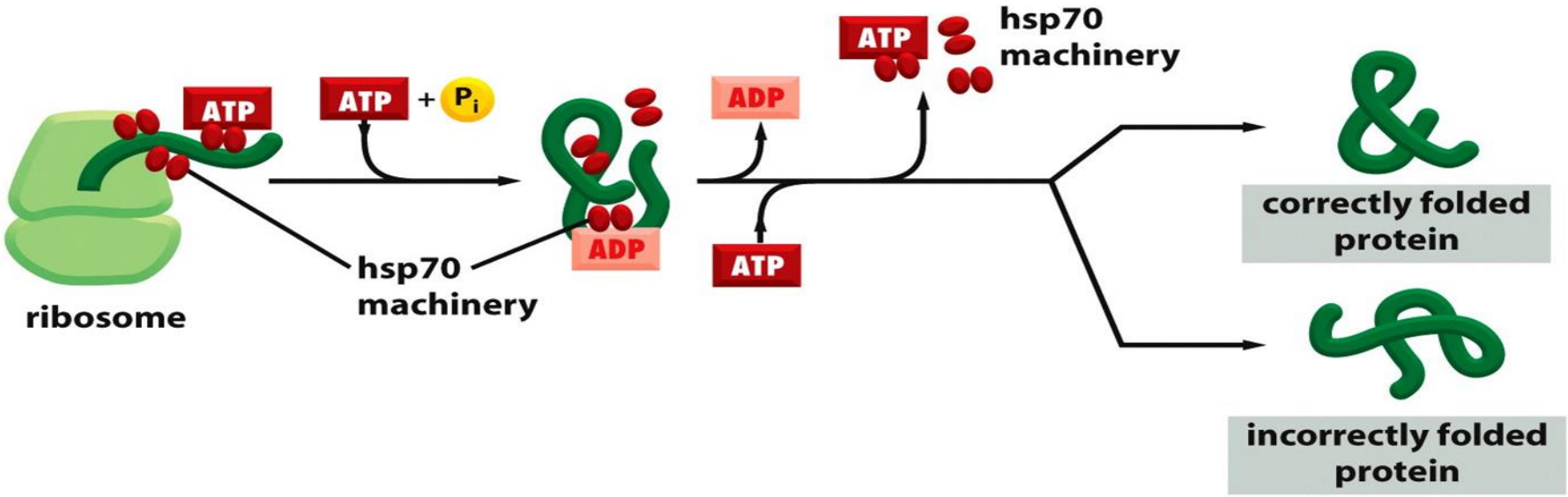
Co-translational protein folding



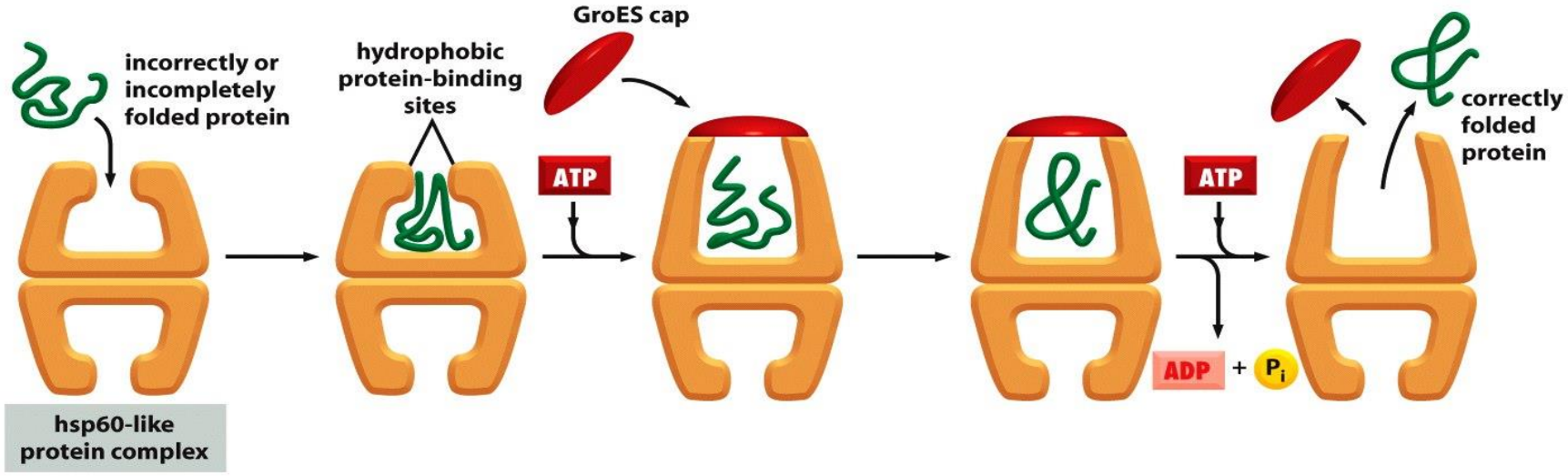
Molecular chaperons

- Many of them are heat shock proteins (Hsp), such as Hsp60, Hsp70.
- Heat shock proteins are rapidly induced by heat shock, meaning in higher temperature, more protein are misfolded, cells need to produce molecular chaperons to help these proteins refolds
- How to identify a misfolded protein? Recognize the exposed hydrophobic region and facilitate the correct folding with the expenditure of energy.

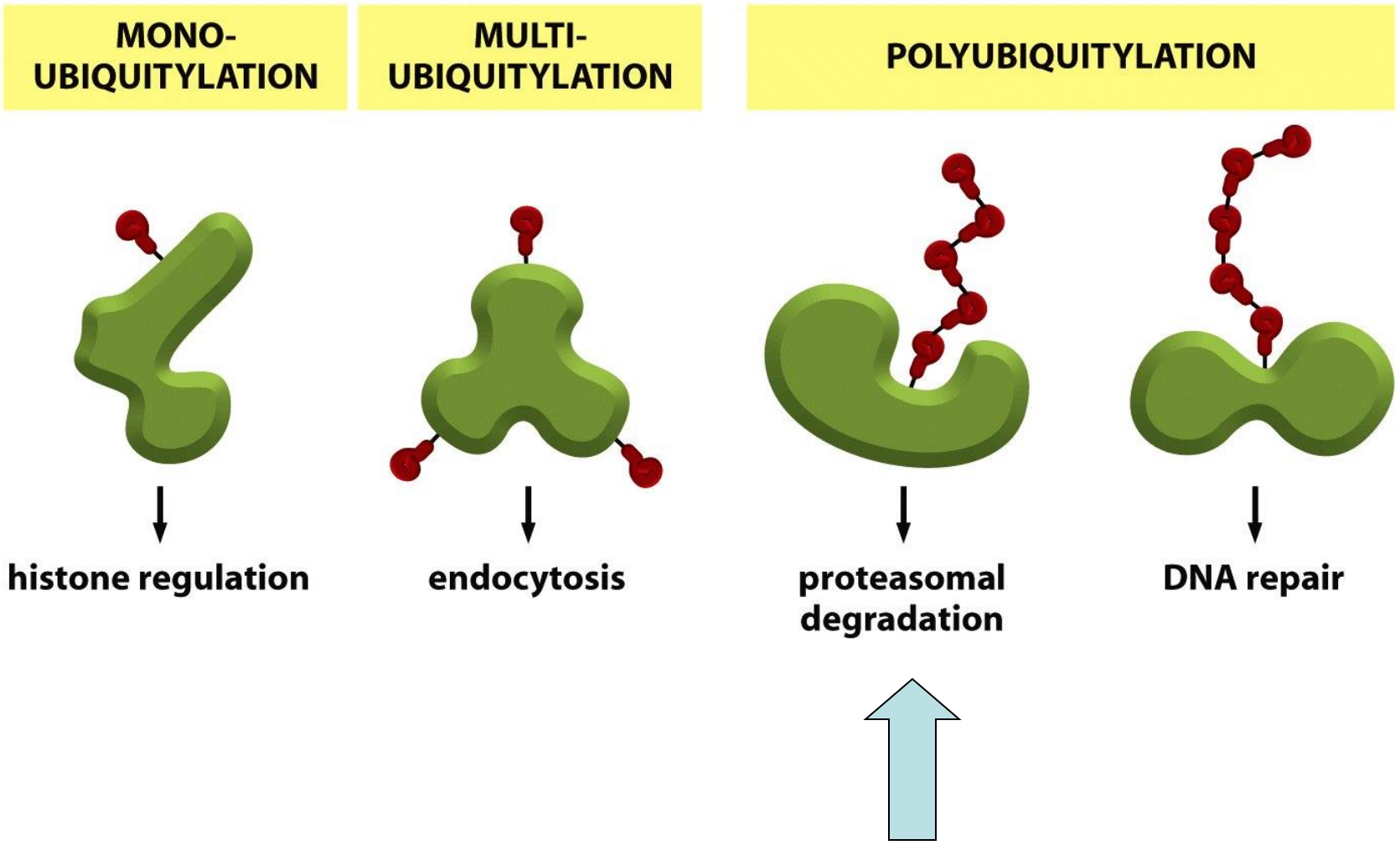
Hsp70 acts first to help fold partially translated protein



Hsp60 later helps to fold a complete protein



When a protein misfolds…



Two major ways to degrade proteins

- Lysosome (hydrolytic enzymes, pH~5)

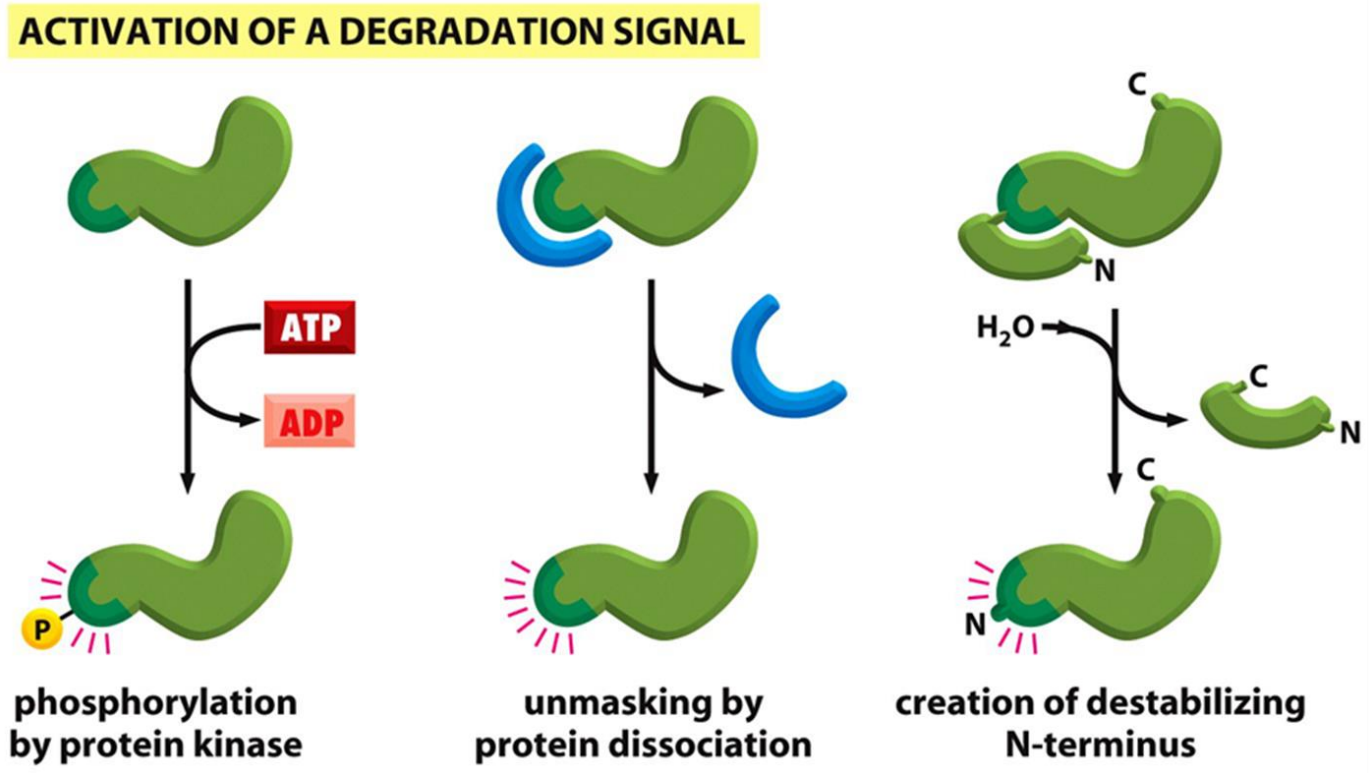
  primarily for aged or damaged organelles

- Proteasome pathway (large molecular machinery)

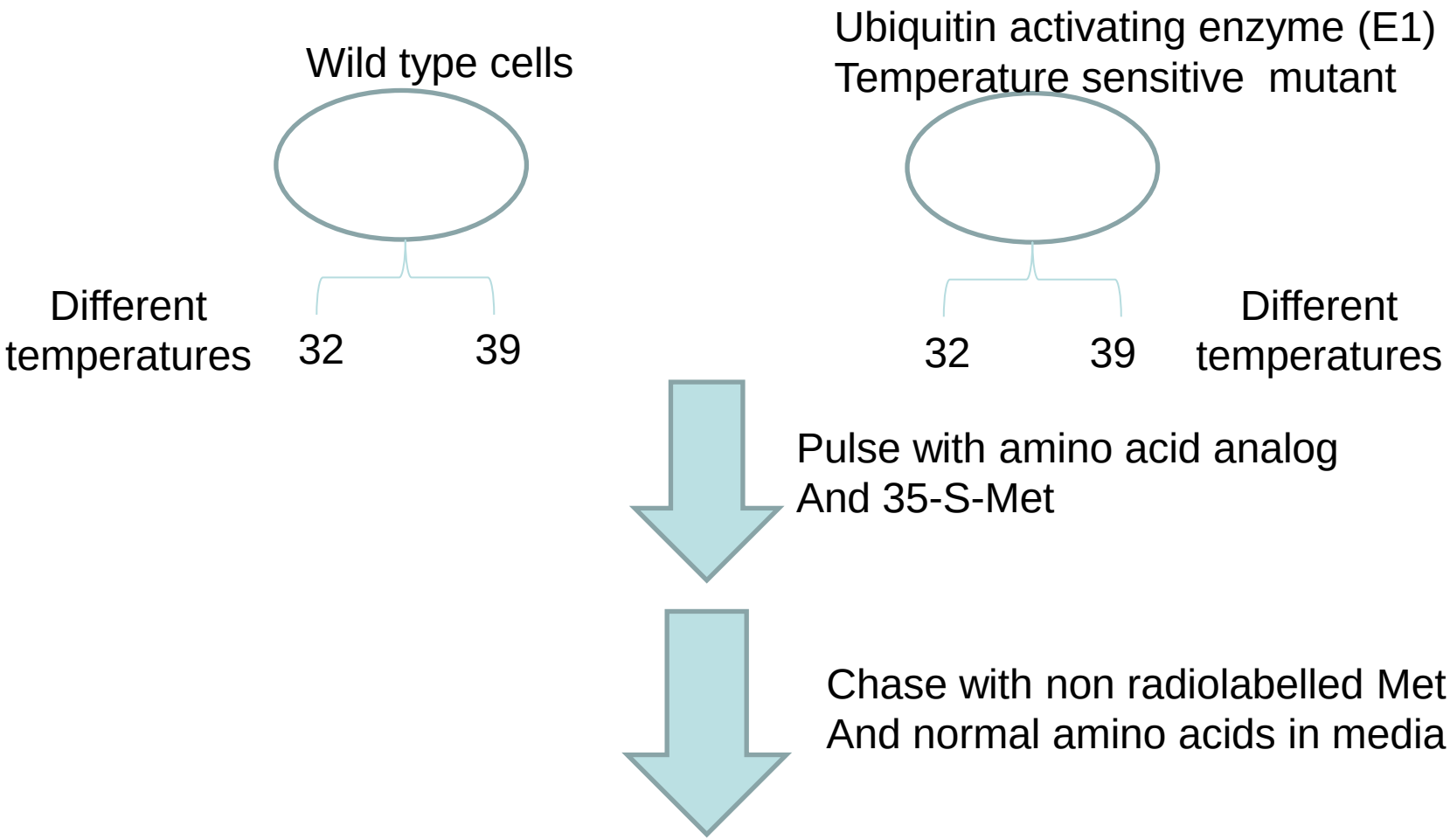
  Take up to 90% of all protein degradation in mammalian cells

  Mostly ubiquitin (76aa polypeptide) mediated, on Lysine, polyubiquintination is called “ kiss of death”

Protein phosphorylation can provide signals for protein ubiquitination

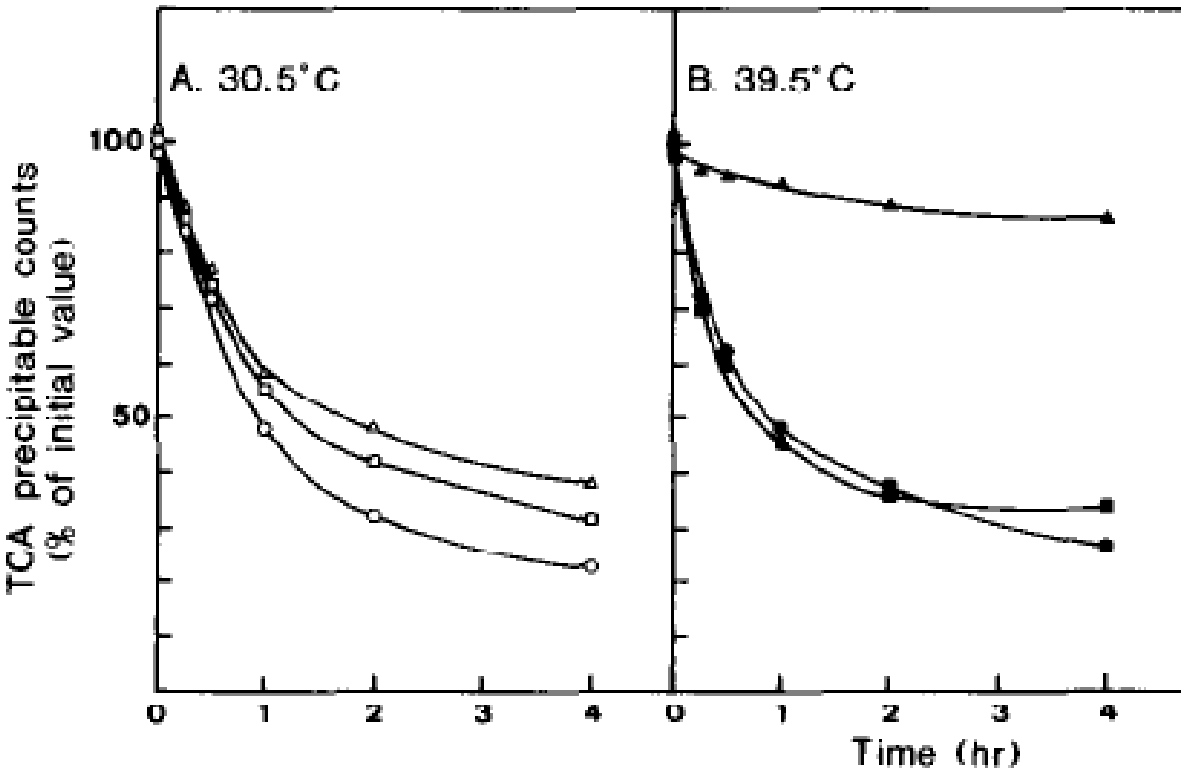


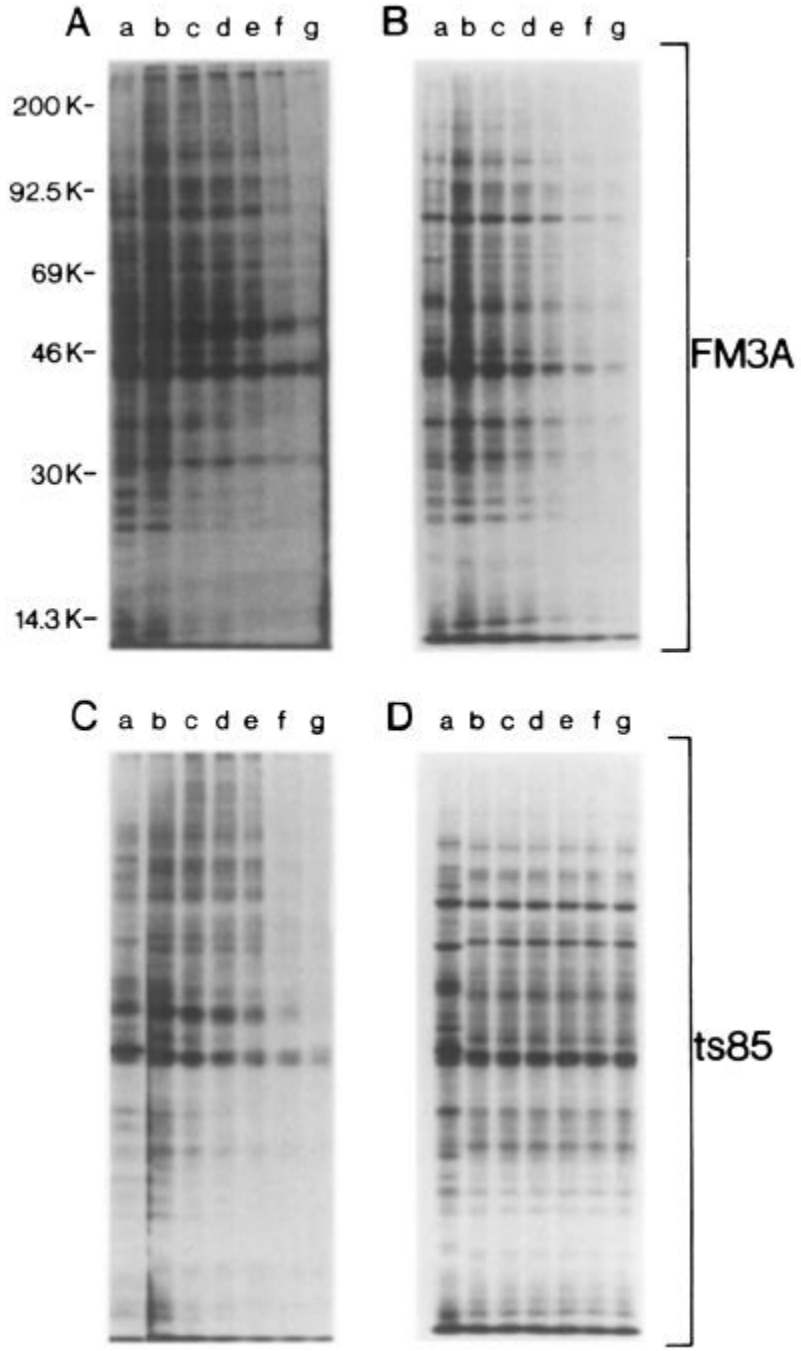
IV. Ubiquitination dependent protein degradation in cells



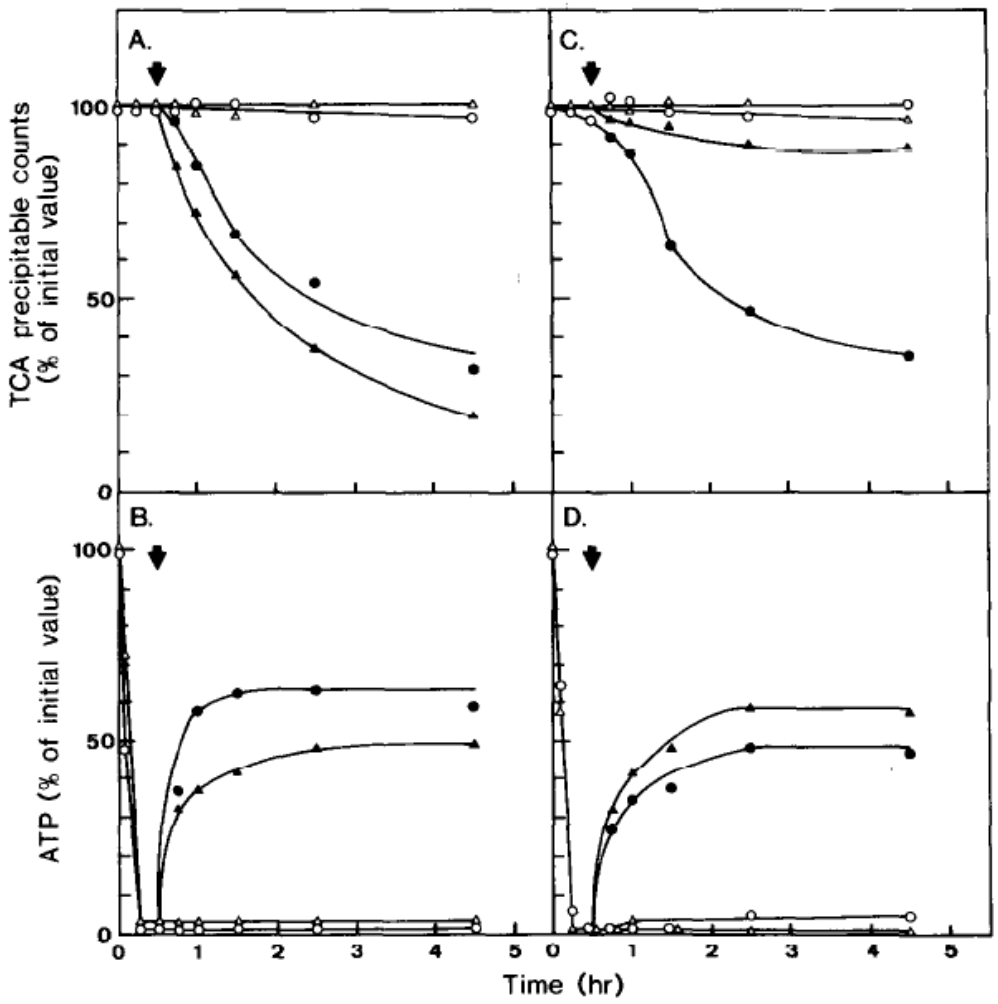
Alex Varshavsky
Cell, 1984

Here is what they observed for the experimental results




A a b c d e f g
B a b c d e f g
200K-
92.5K-
69K-
46K-
30K-
14.3K-
FM3A
C a b c d e f g
D a b c d e f g
ts85

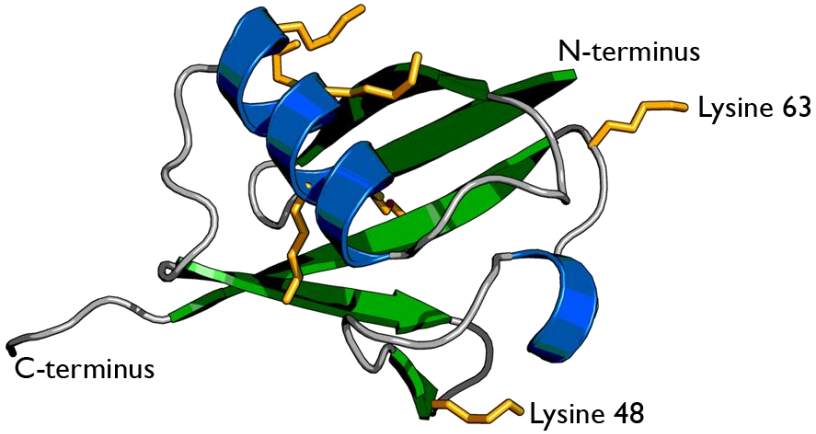
Open symbols: NaCN treatment all the time;
Closed symbols: NaCN treatment for first 30 min.



Protein Ubiquitination

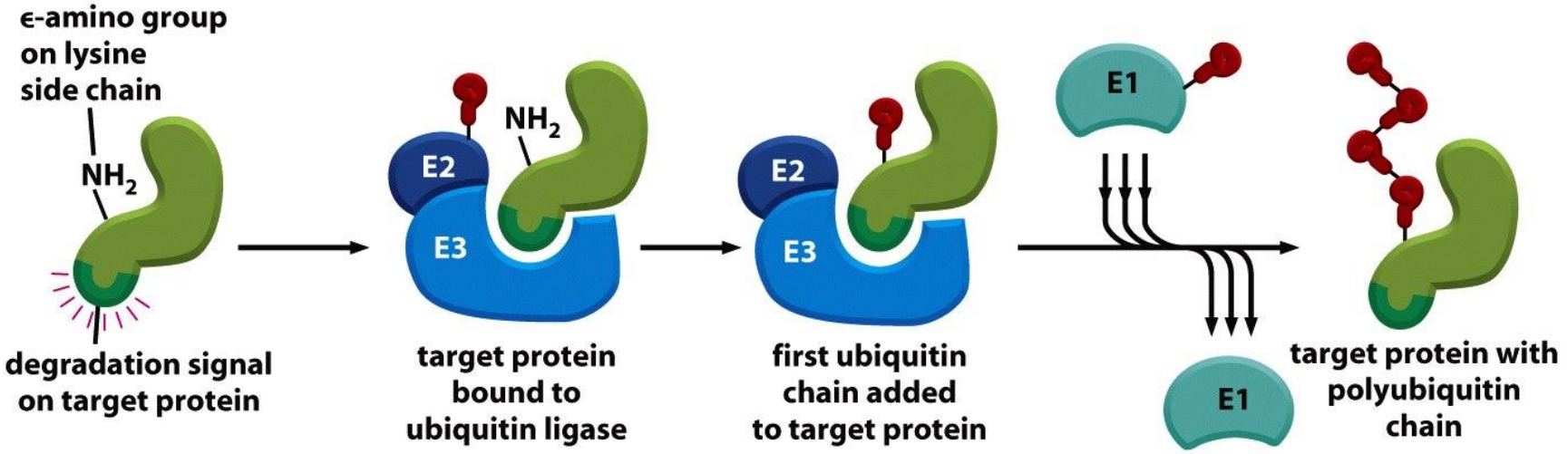
Ubiquitin

76aa regulatory protein

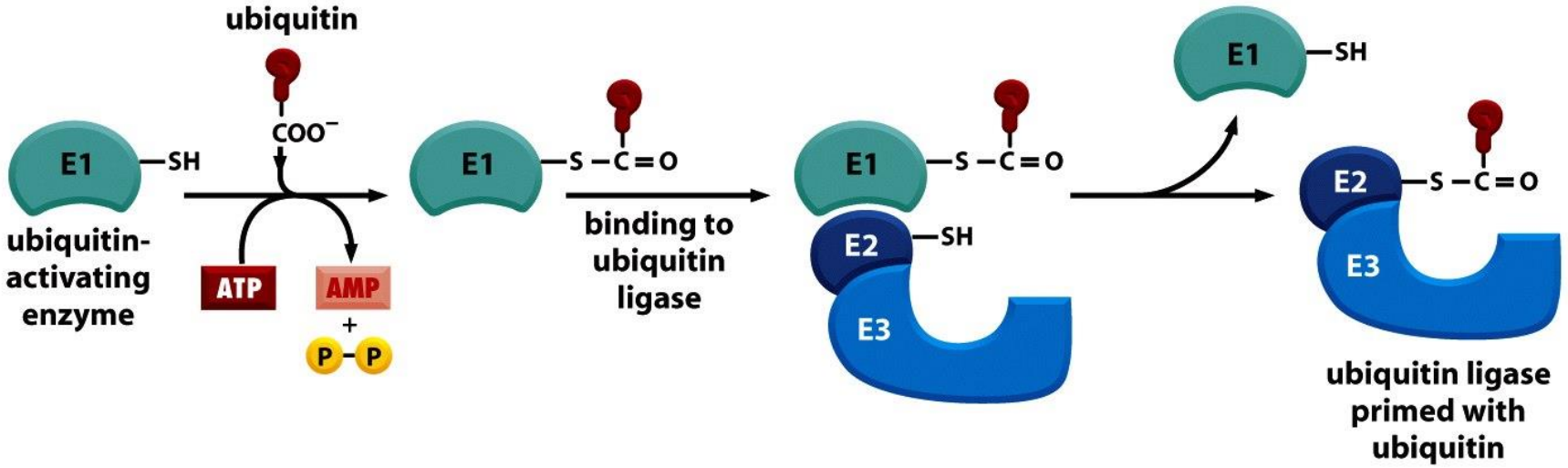


- Ubiquitin itself contains seven lysine residues, multiple molecules of ubiquitin can also become linked to each other to form polyubiquitin chains.
- The best understood function of ubiquitination is proteolysis, whereby lysine-48 (K48)-linked polyubiquitin chains allow recognition by the 26S proteasome.
- Protein can also be monoubiquitinated or polyubiquitinated through alternative (e.g., K63) linkages, and such modifications are thought to control protein activity or localization
- C-terminal glycine of ubiquitin becomes linked to primarily ε-amino of lysine residues in target proteins via an E1-E2-E3 cascade.

Ubiquitin ligase recognizes target proteins to be degrade, binds and add ubiquitins on its lysine through iso-peptide bond



Ubiquitin is added by three steps in succession(E1, E2, E3)



Ubiquitin: 76 aa peptide

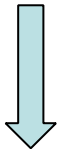
E1: ubiquitin-activating enzyme

E2: ubiquitin conjugating enzyme

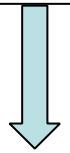
E3: ubiquitin ligase

Ubiquitination systems

One common E1 enzyme



~30 E2 enzymes in mammals

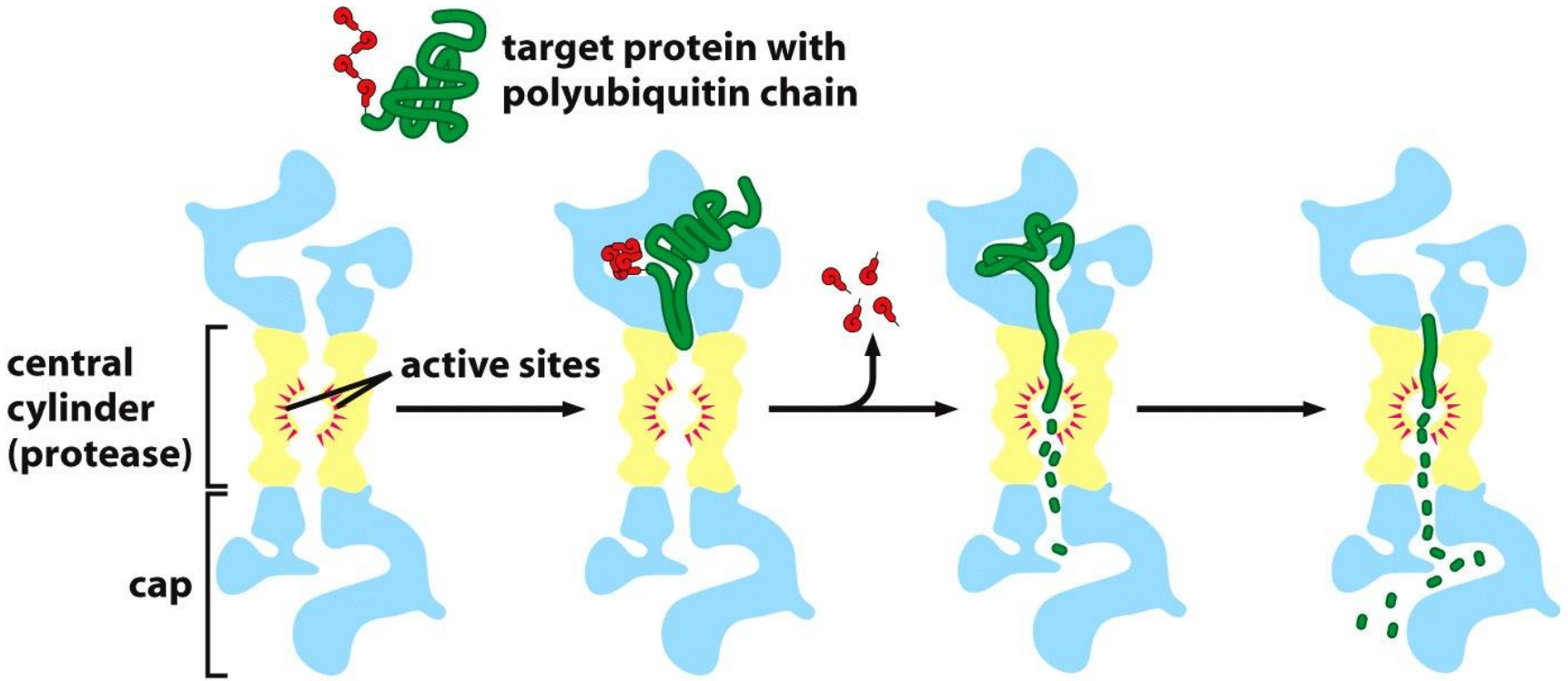


~hundreds of E3 enzymes

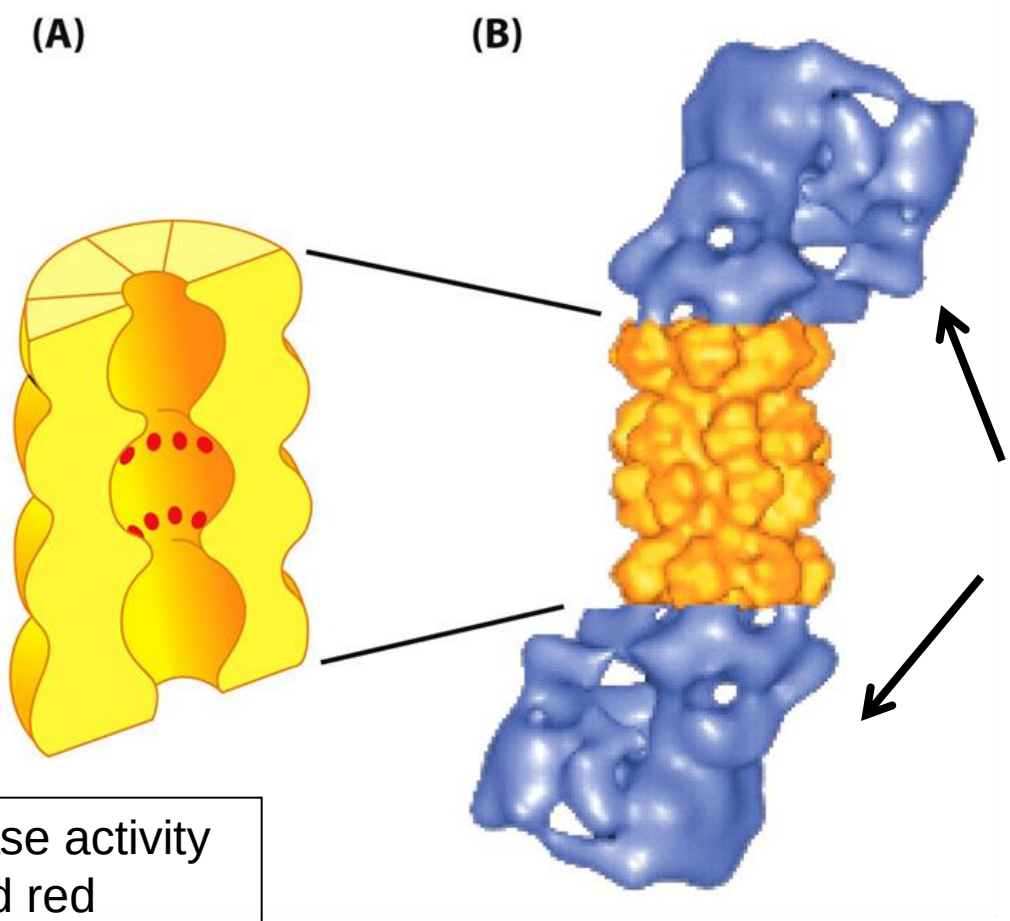
Different E3 recognizes distinct degradation signals and targets distinct subsets of protein for degradation

If I want to inhibit a specific ubiquitylation enzyme complex, would I choos an inhibitor which targets E1 ?

Poly-ubiquitinylated protein is then degraded by proteasome



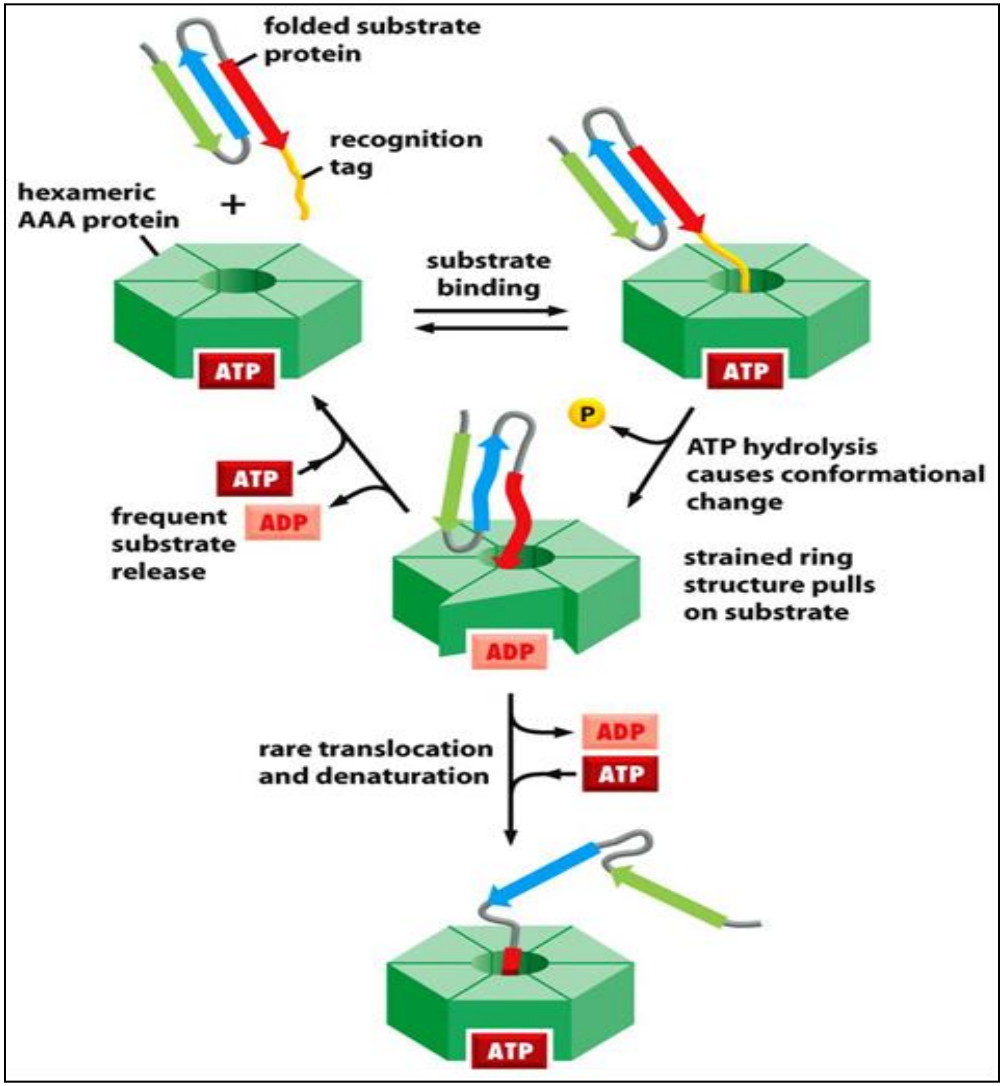
The 20S proteasome



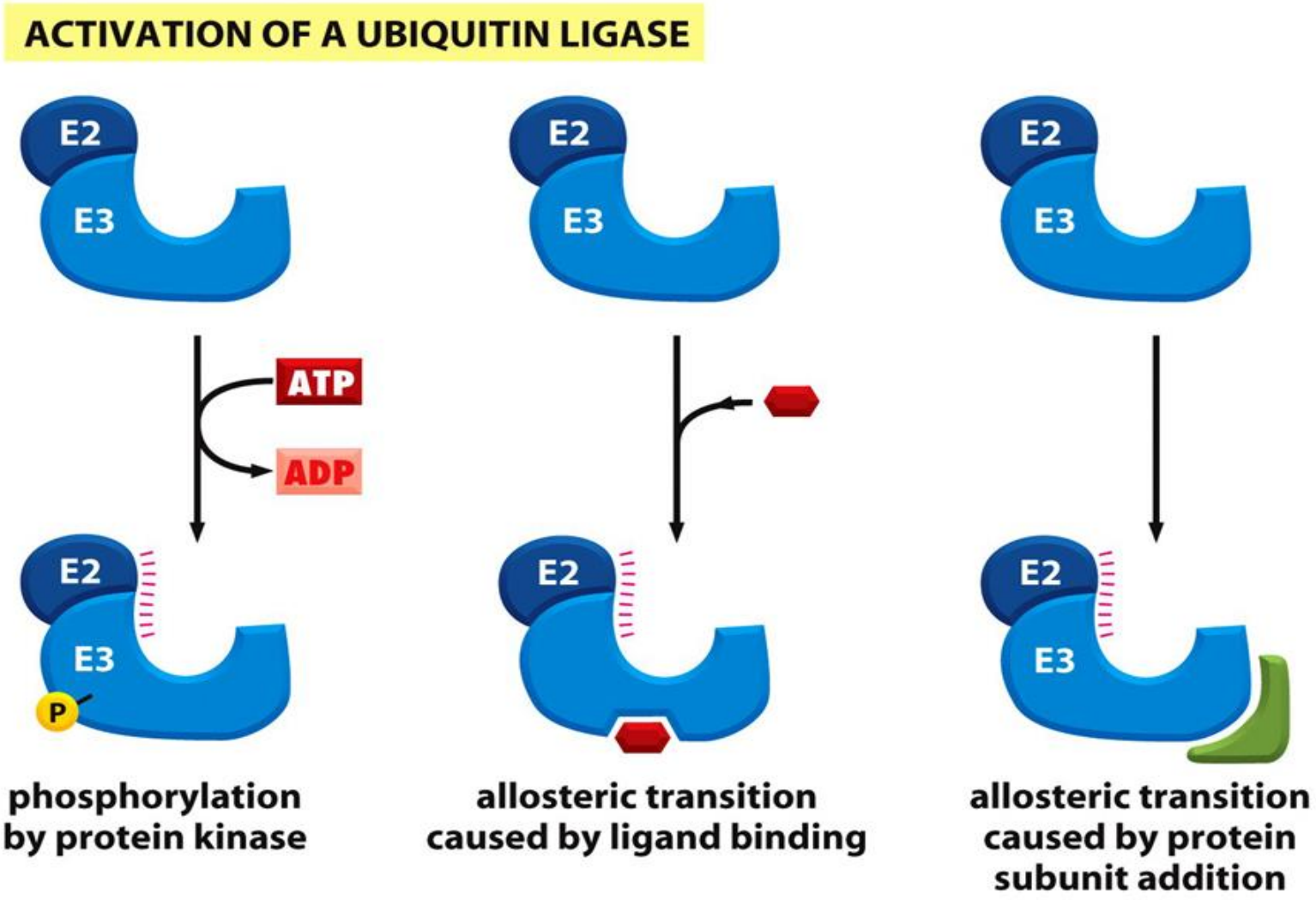
19S cap binds and unfolds substrates

20S cylinder with protease activity active sites are indicated red

19S cap--- a hexameric protein unfoldase



How to activate E3-ubiquitin ligase



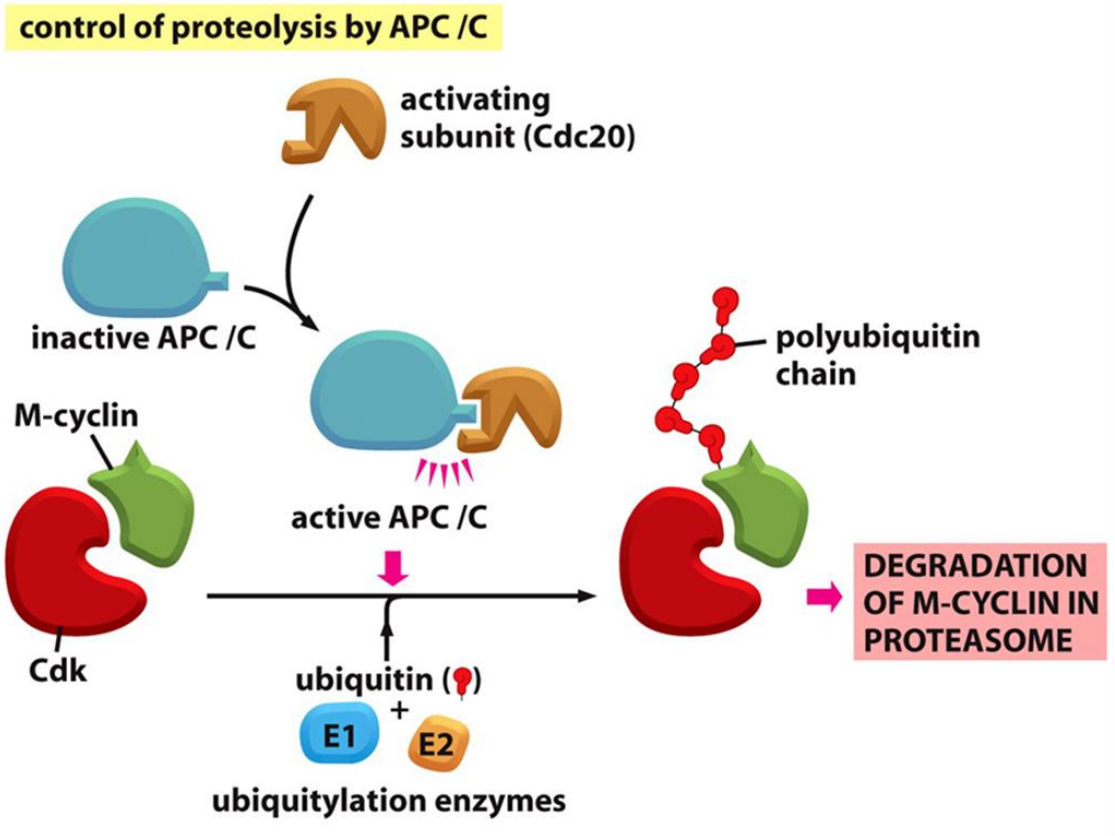
Not just misfolded protein…

Many proteins are degraded in a controllable manner:

e. g. Cell cycle proteins are controlled during mitosis by Anaphase-promoting complex (APC)---ubiquitin ligase

Metaphase to anaphase transition in controlled by proteolysis

- One Key player: anaphase-promoting-complex, or cyclosome (APC/C), a ubiquitin ligase



V. Protein misfolding causes human diseases

Protein aggregates primarily cause neurodegenerative diseases

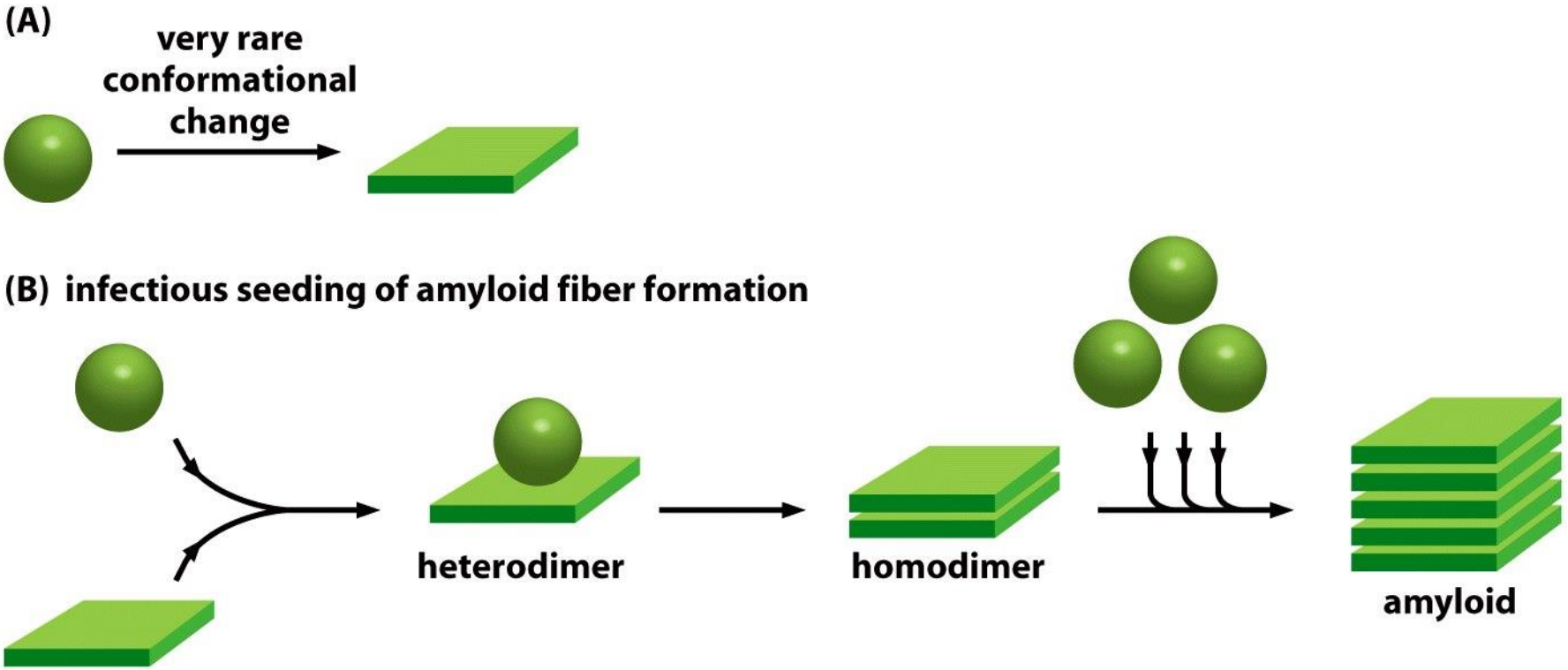
(朊病毒）

- Prion diseases such as scrapie in sheep, Creutzfeldt-Jacob disease , bovine spongiform encephalopathy (BSE)

- Protein aggregate, huntingtons's disease Alzheimer's disease.

Prion protein misfolds, in turn changes the next normal protein folding

--- It can spread from one organism to another



（淀粉样蛋白）

highly resistant to protein degradation