

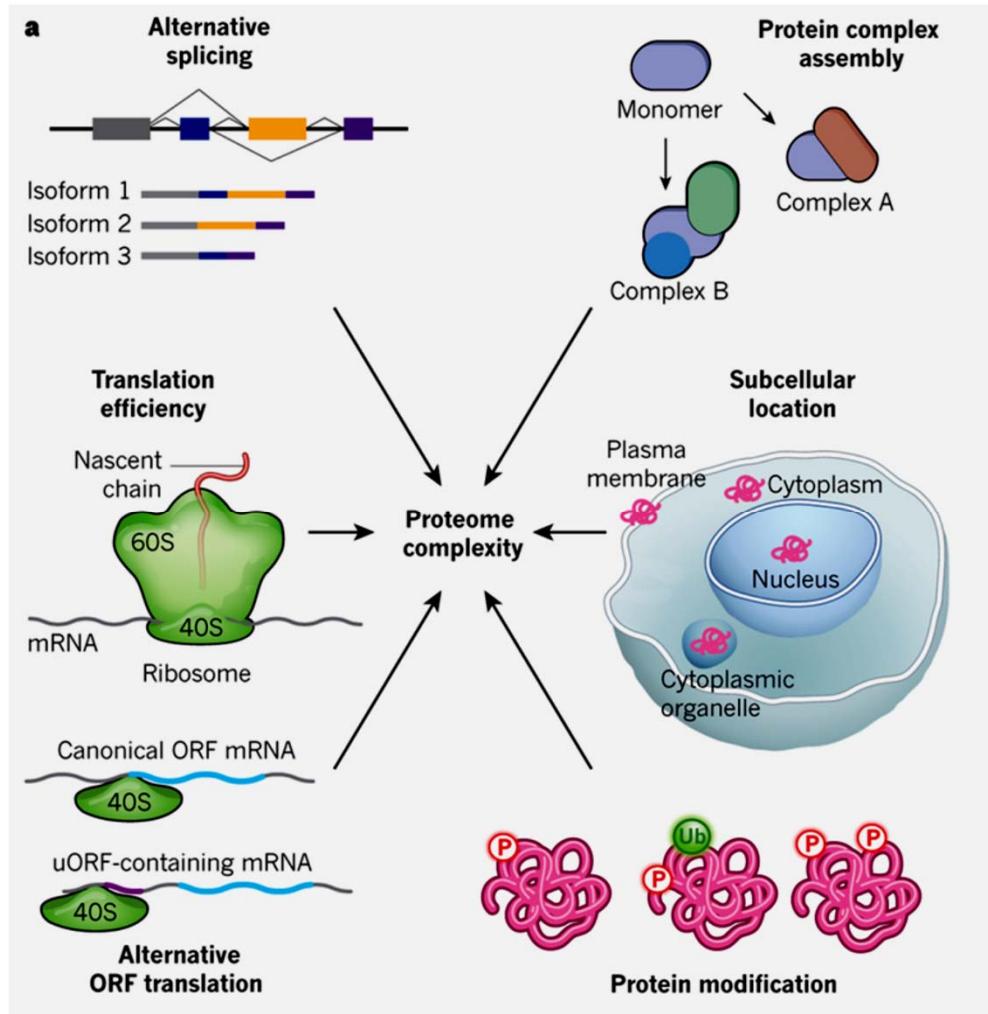
Regulation of protein function

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

I. Protein complexity in a single cell

- The cellular proteome is exceedingly complex:
 - Of the about **20,000** or so protein-coding genes of the human genome, a typical cell transcribes only about **10,000** genes, which have a cumulative copy number of **1-100 billion** protein molecules per cell.

1. Controls/modulation of protein activity



- Alternative splicing
- Translation efficiency
- Alternative translation of the open reading frame (ORF)
- Assembly into protein complexes
- Subcellular localization
- Protein modifications

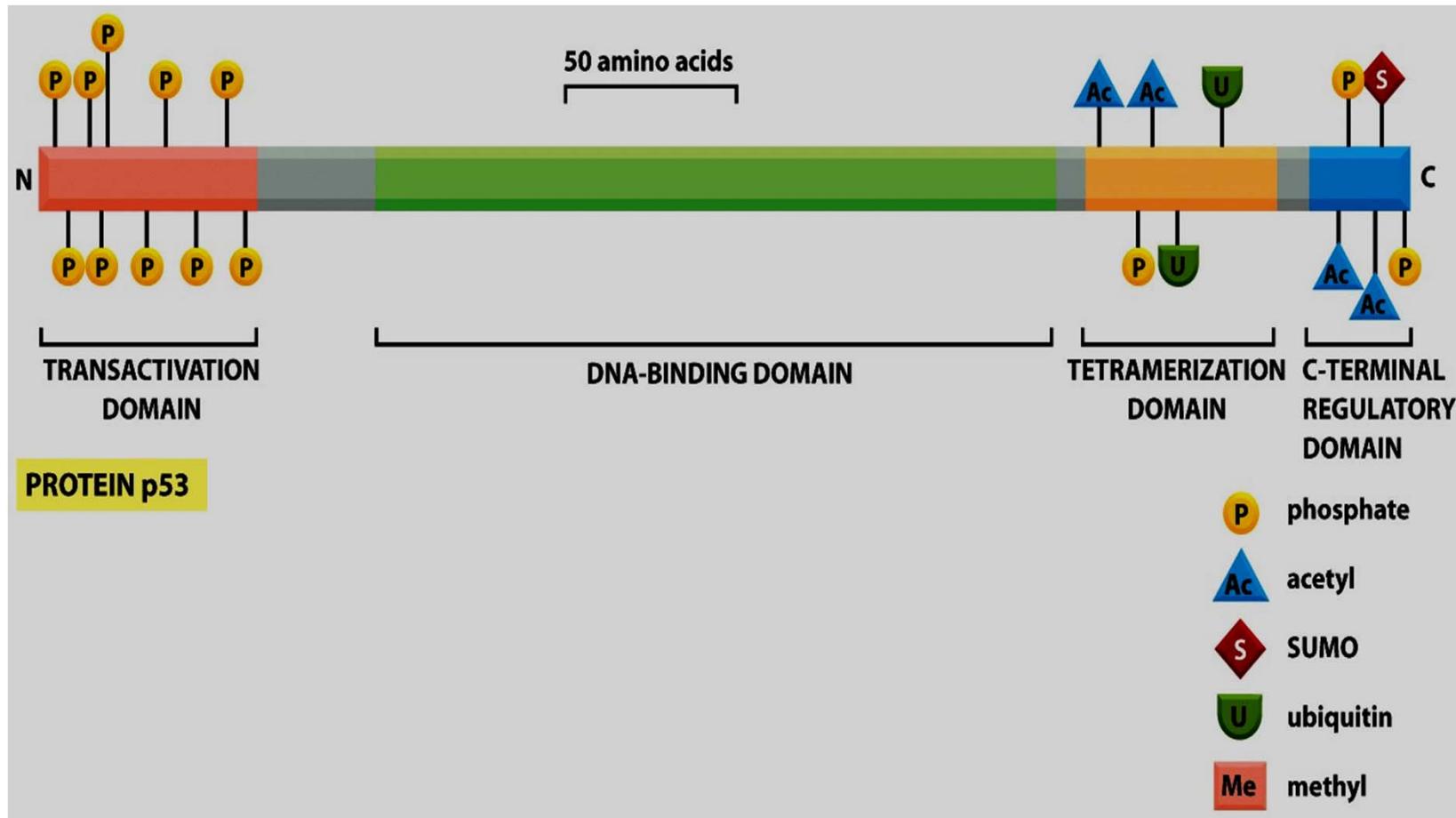
1. Factors that control overall protein activity

1. Regulation of **intrinsic** activity of proteins
2. Change of protein **concentration**
3. Change of the **localization** of proteins
within a cell
4. The **steady-state level** of proteins

1. Control of intrinsic protein activity

- Noncovalent binding
 - protein-protein interactions
 - assembly into protein complexes
 - Covalent bonding
 - phosphorylation/dephosphorylation
 - ubiquitination/deubiquitination
 - sumoylation (addition of small ubiquitin-like modifiers)
 - neddylation (addition of the “ubiquitin-like” protein NEDD8)
 - acetylation, methylation
 - palmitoylation on Cys, etc
 - Protease cleavage ←————— Irreversible
- 
- Many
are reversible

Example: modifications of the tumor suppressor TP53



Wow, that is impressive!

Molecular modifiers of protein function

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). |

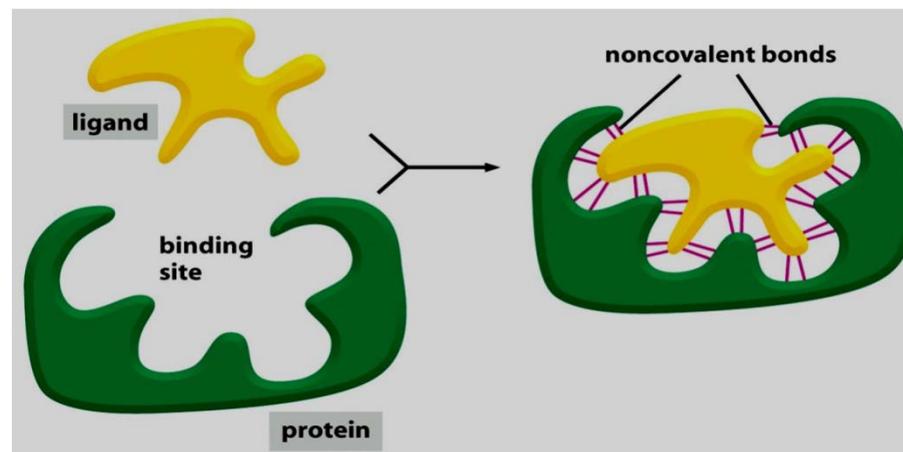
Ubiquitin is a 76 amino acid polypeptide; there are at least 10 other ubiquitin-related proteins, such as SUMO, that modify proteins in similar ways.

Protein function requires binding to other molecules

1. All proteins function by binding to other molecules, which are called “ligands”, Latin: *ligare* means “to bind”.
2. All bindings have specificity and different strength.

This is determined by:

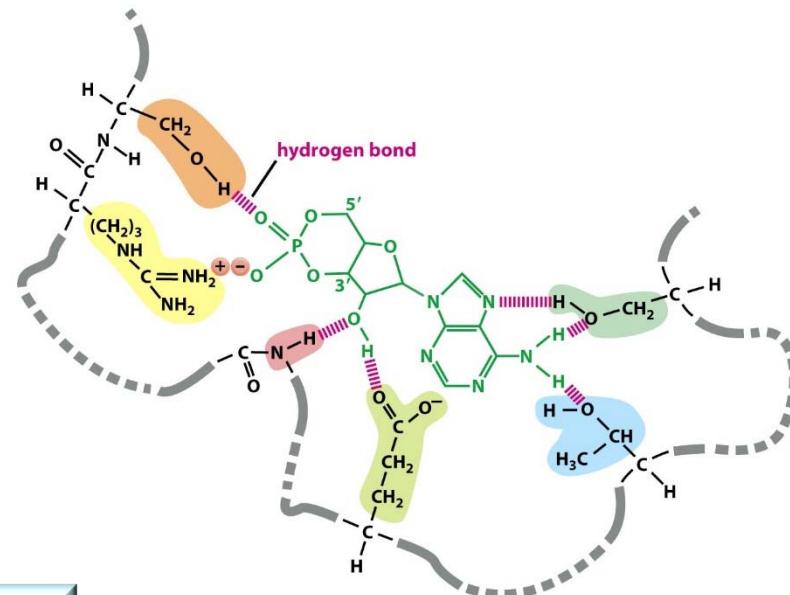
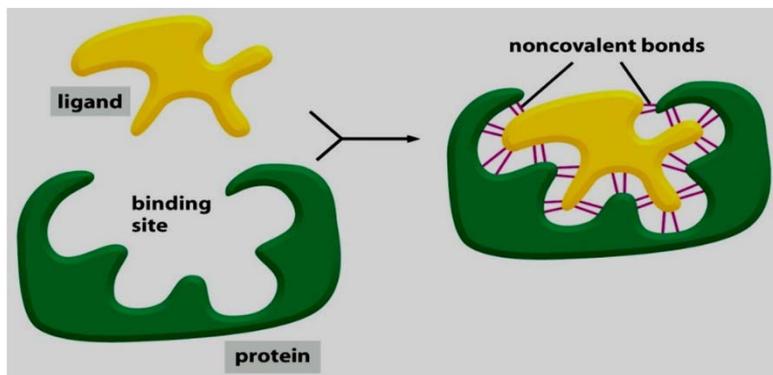
- electrostatic attraction
- hydrogen bonds
- van der Waals interaction
- hydrophobic force



Many weak noncovalent bonds result in a tight molecular interaction.

Close-up view of cAMP binding site in a protein

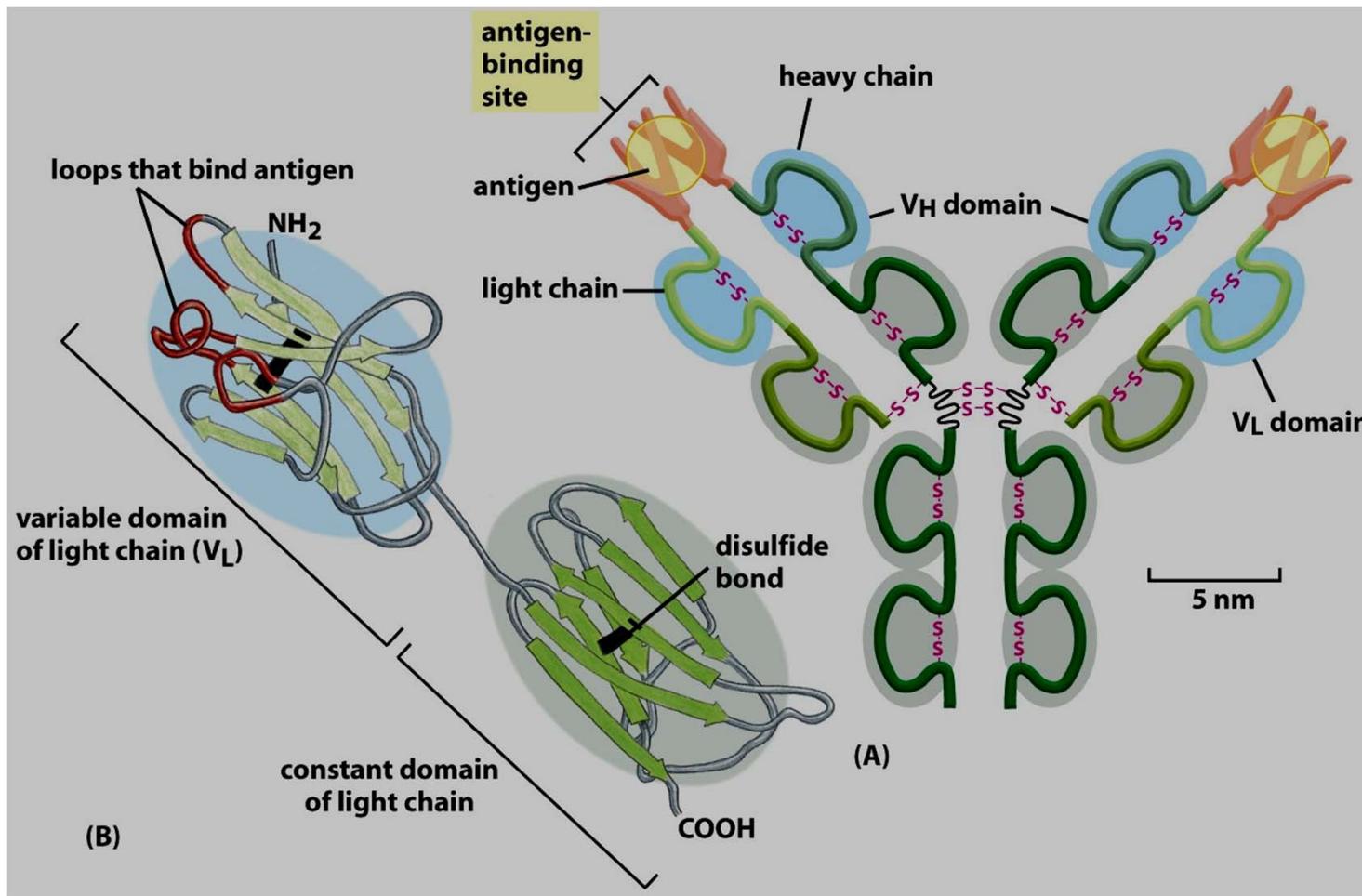
Many weak noncovalent bonds result in a tight molecular interaction



It is the protein surface that allows for specific interaction; that is why the structure of a protein is so important for its function!!!

Mutations in these key binding sites will disrupt ligand binding

Antigen binding sites of antibodies are especially versatile...



Variation of only the loops in the antigen binding site (sequence/length) results in the diversity of binding sites. This allows to recognize a specific interactor amongst billions of molecules

Modes of modulation by noncovalent binding

Modulations can be gradual or nongradual:

- Graded allosteric changes
 - oxygen versus hemoglobin
- Non-graded allosteric changes: turn on or switch off
 - calcium versus calmodulin
 - GTP/GDP versus Ras



Ras activity is regulated by GTP/GDP binding

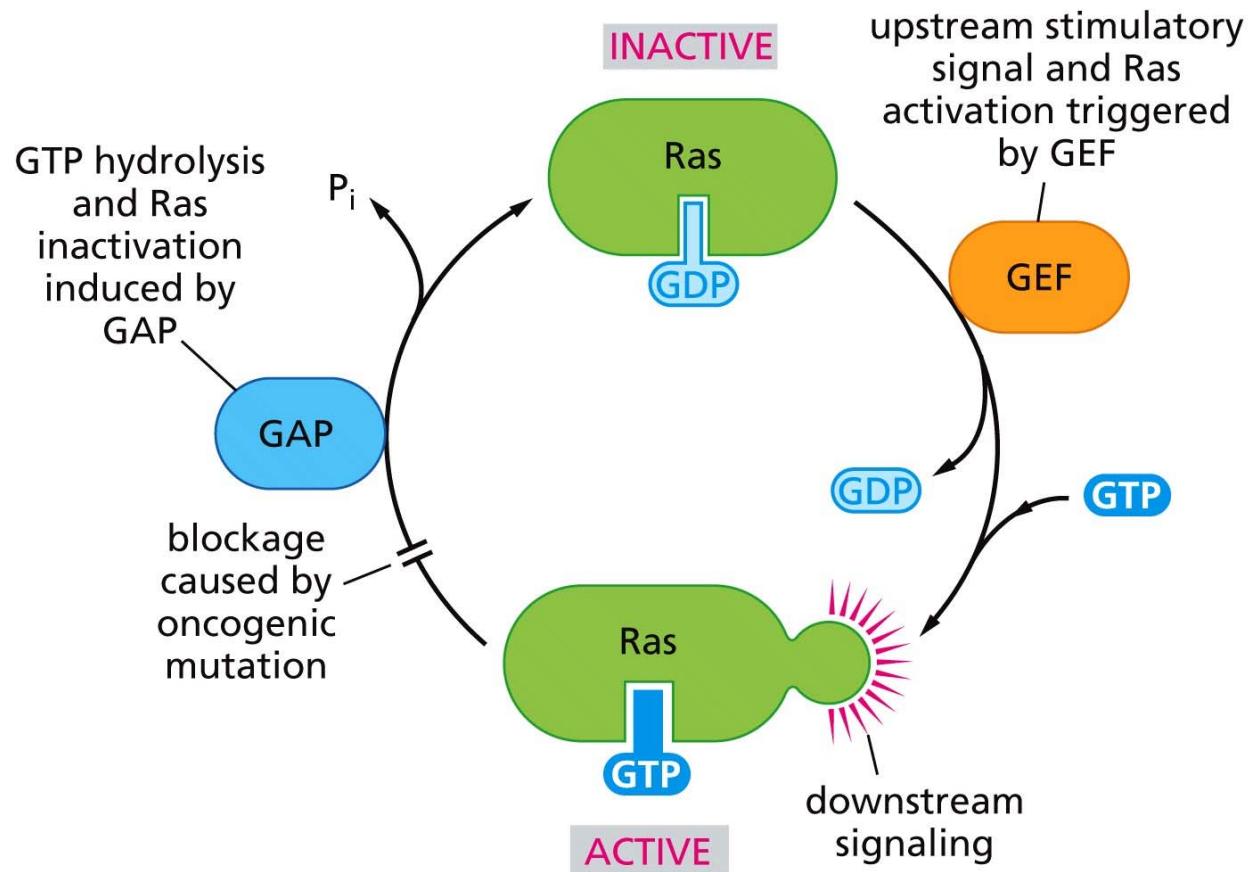
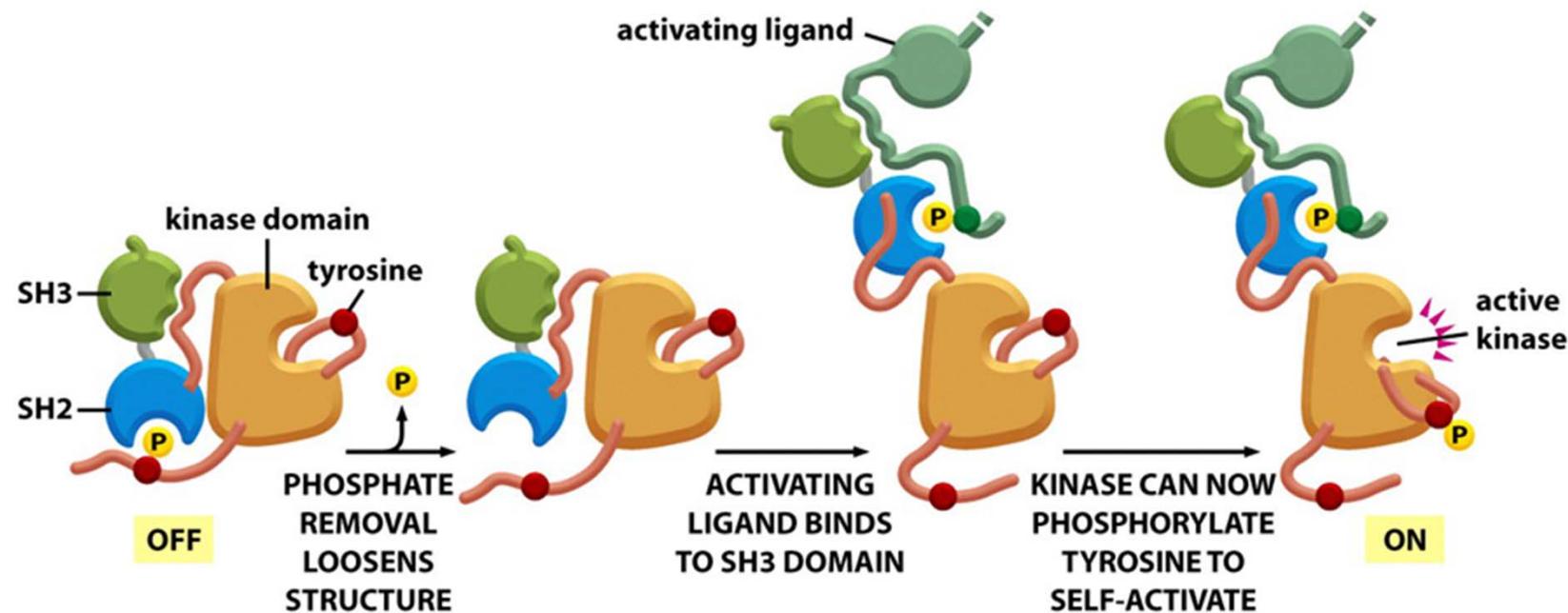


Figure 5.30 The Biology of Cancer (© Garland Science 2014)

Activation of the proto-oncogene tyrosine-protein kinase Src



OFF: phosphorylation of tyrosine (Y) 527 (inhibiting phosphotyrosine)

ON: phosphorylation of tyrosine (Y) 416 (activating phosphotyrosine)

Three distinct domains: SH2, SH3, kinase domain

Activation of the proto-oncogene tyrosine-protein kinase Src

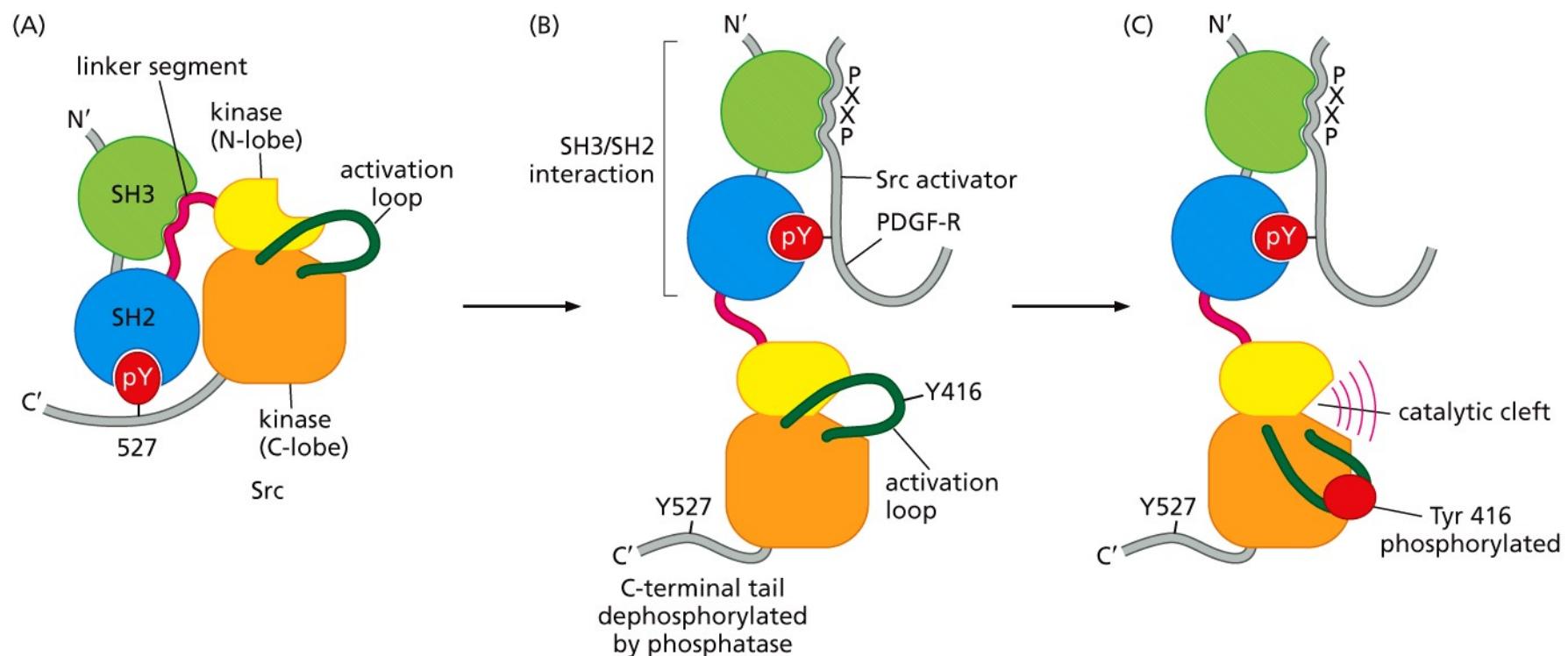
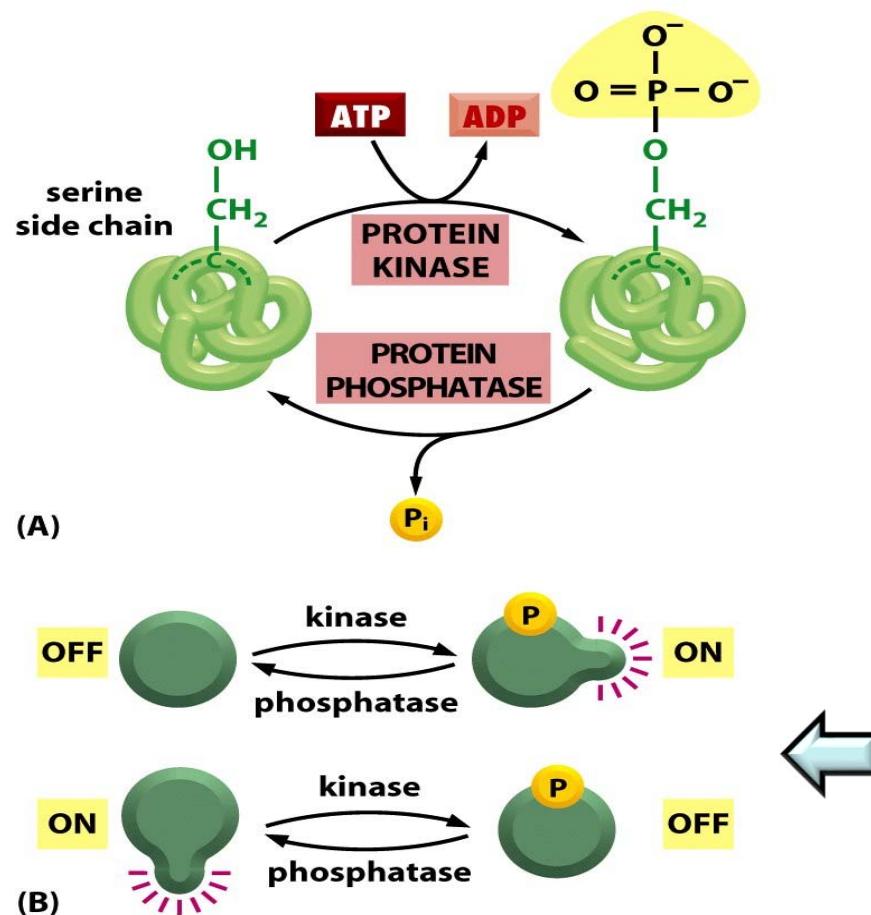


Figure 6.11 The Biology of Cancer (© Garland Science 2014)

Kinases and phosphatases are common regulators of activity

Phosphorylation and dephosphorylation is reversible, allowing to switch the protein “ON” and “OFF”



Three amino acids can be modified by phosphorylation:

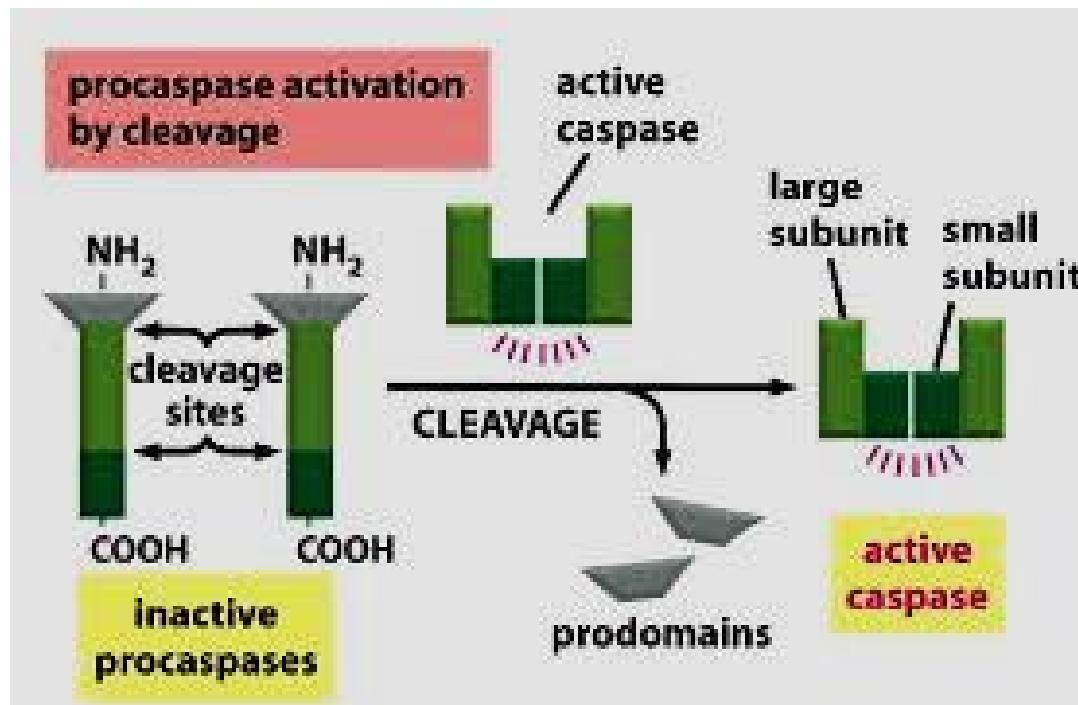
- Tyrosine
- Serine
- Threonine

Phosphorylation can either **activate or deactivate** a protein function.
The effect is specific for the respective protein !!!

Protein cleavage: irreversible modification

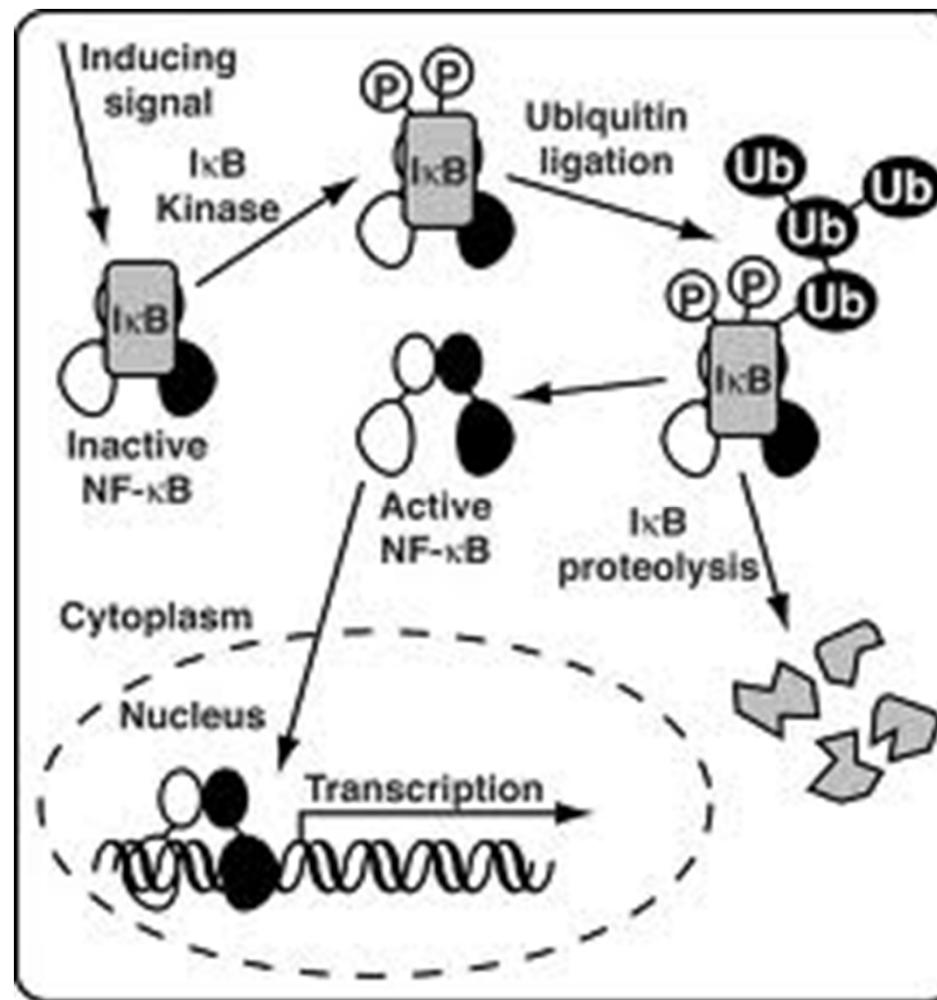
- peptide hormones
 - proinsulin
- zymogens (inactive proenzymes):
 - trypsinogen → trypsin (active)
 - procaspases → caspases (active)
- fibrous proteins:
 - procollagen → collagen

Example for protein cleavage: procaspase

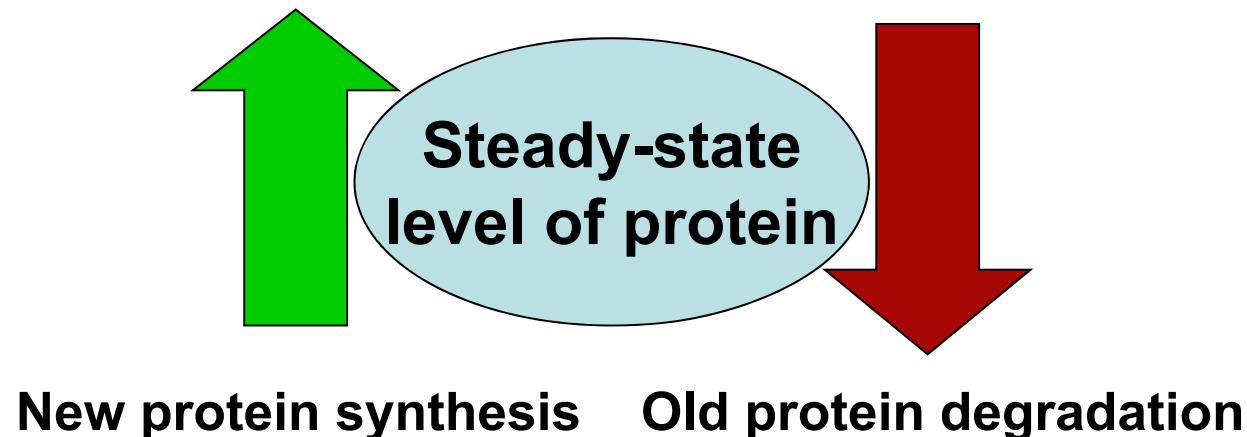


2. Control of protein localization and local concentration

The transcription factor NF- κ B : from the cytosol into the nucleus



3. Steady state level of proteins



Different proteins have different half live times:

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

Control of the protein level

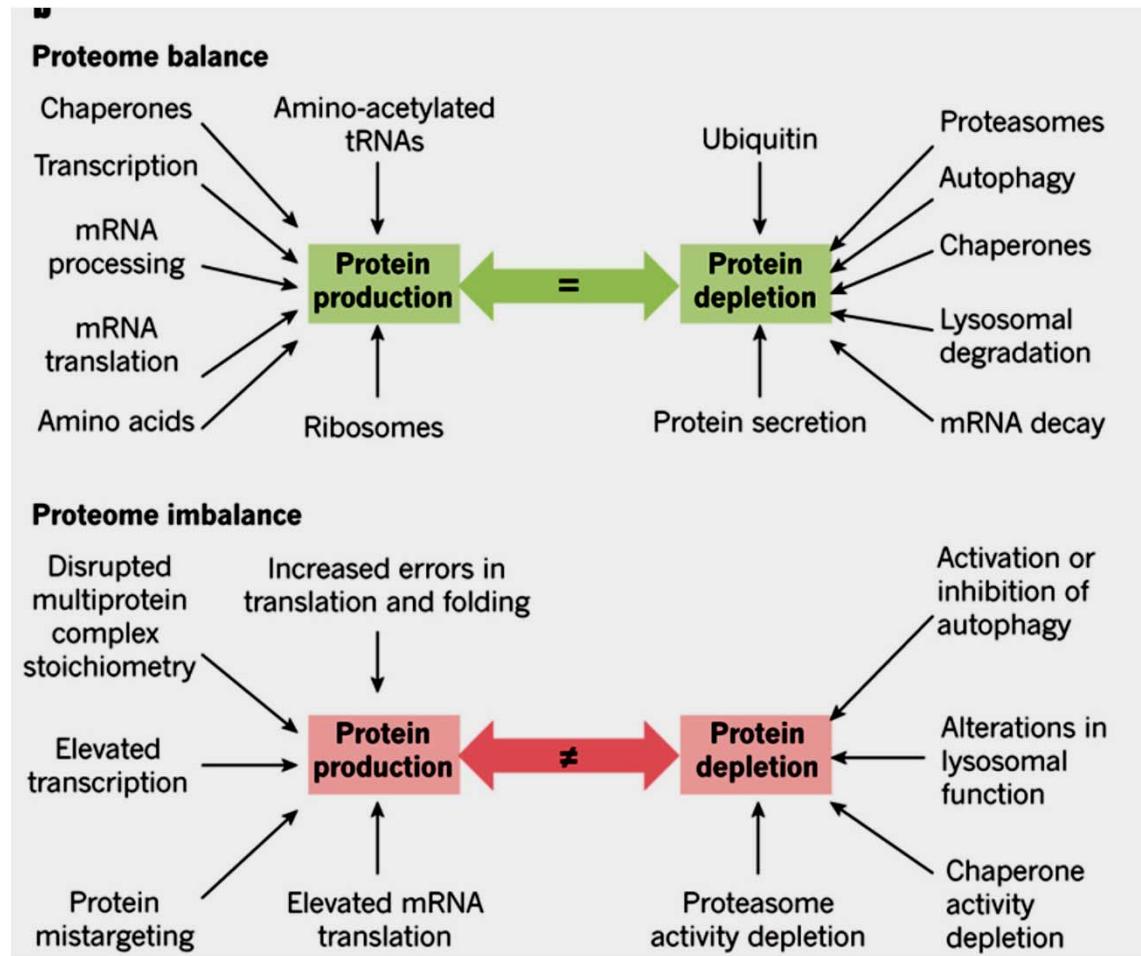
Protein levels can be controlled at four levels :

- mRNA transcription
- translation
- post-transcriptional

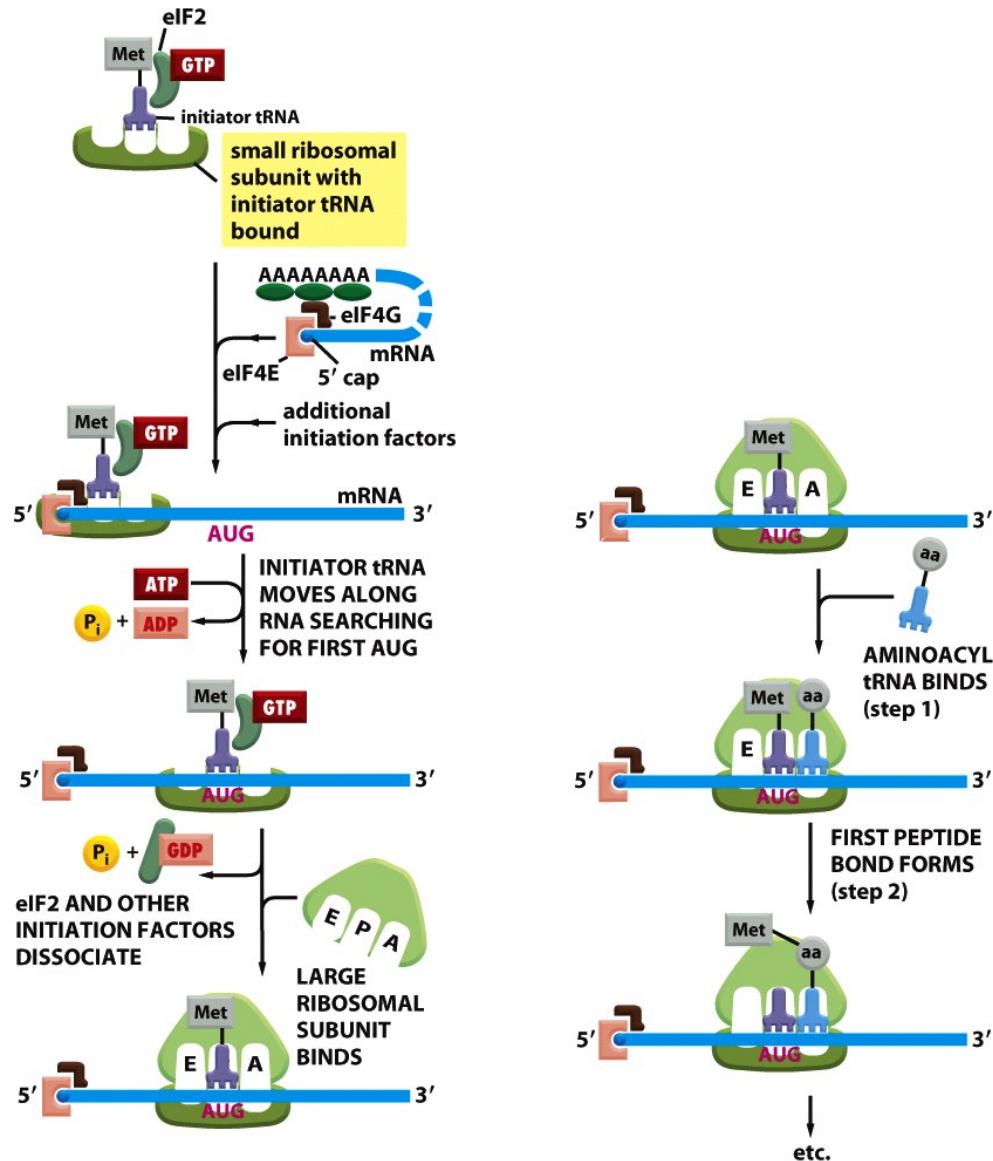
Protein **synthesis**

post-translation ← Protein **degradation**

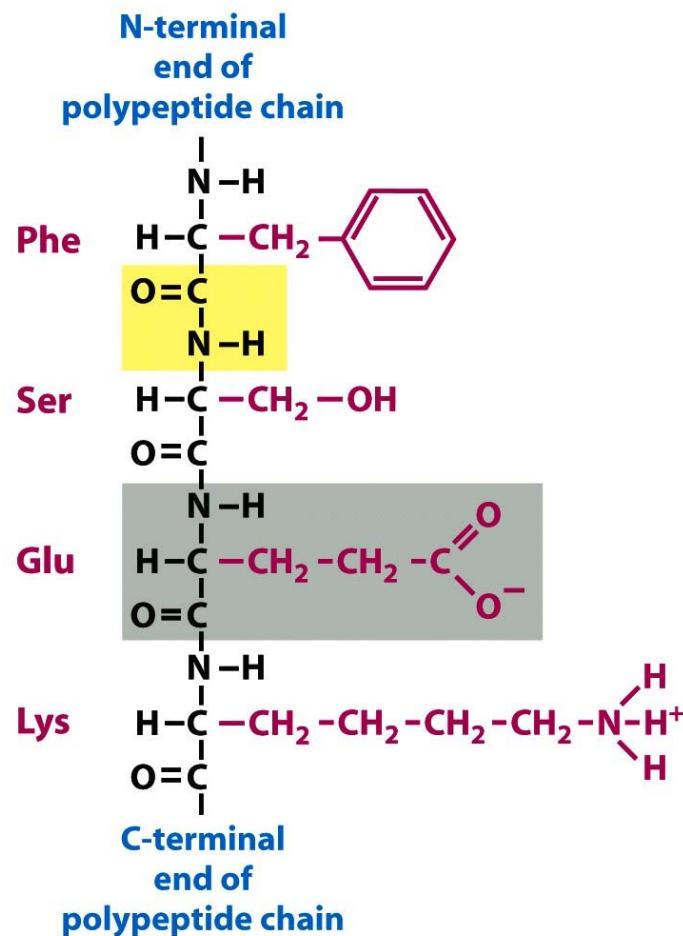
Balancing between protein production and protein depletion



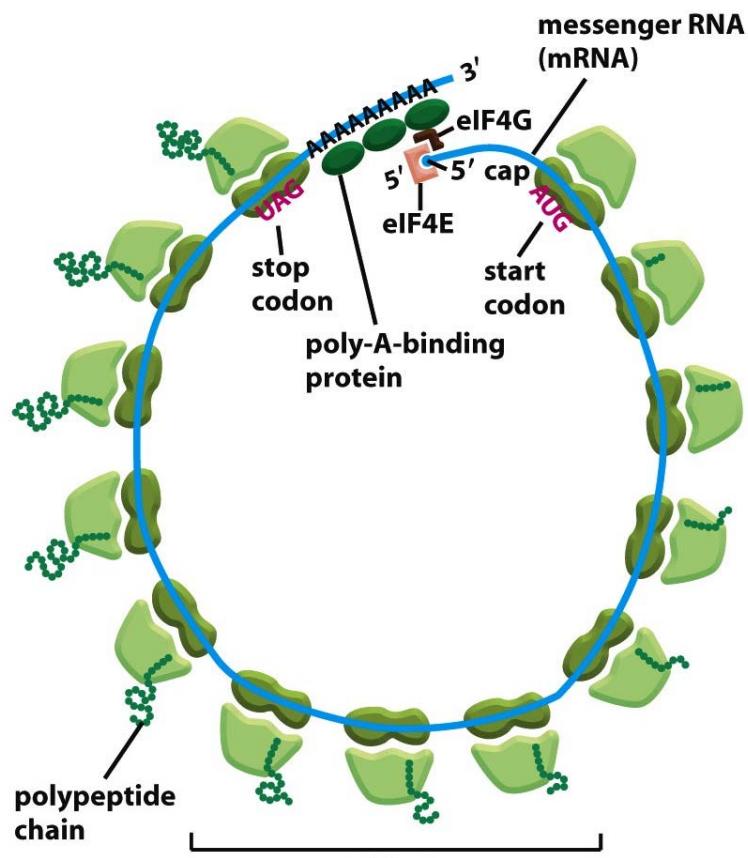
A brief look at protein translation



Peptide bond between amino acids

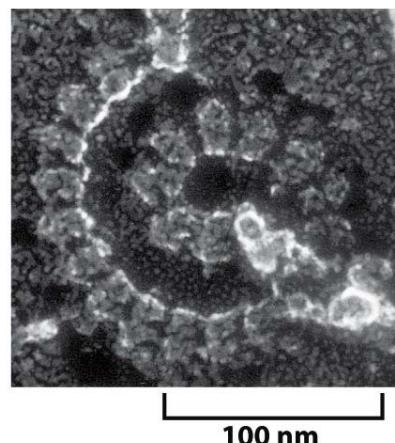


Proteins are usually synthesized efficiently on one mRNA--- formation of polyribosomes



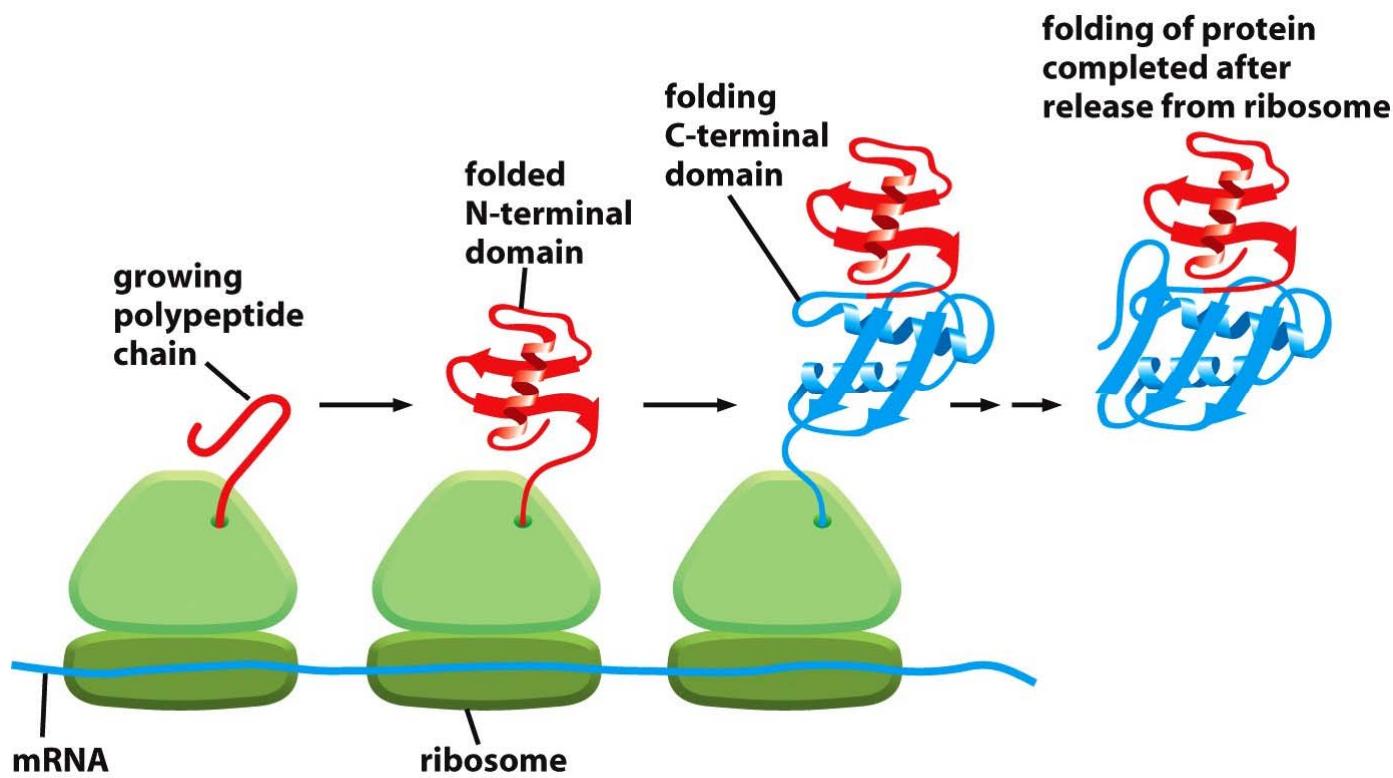
(A)

Cycloheximide freezes ribosomes
On a single mRNA



(B)

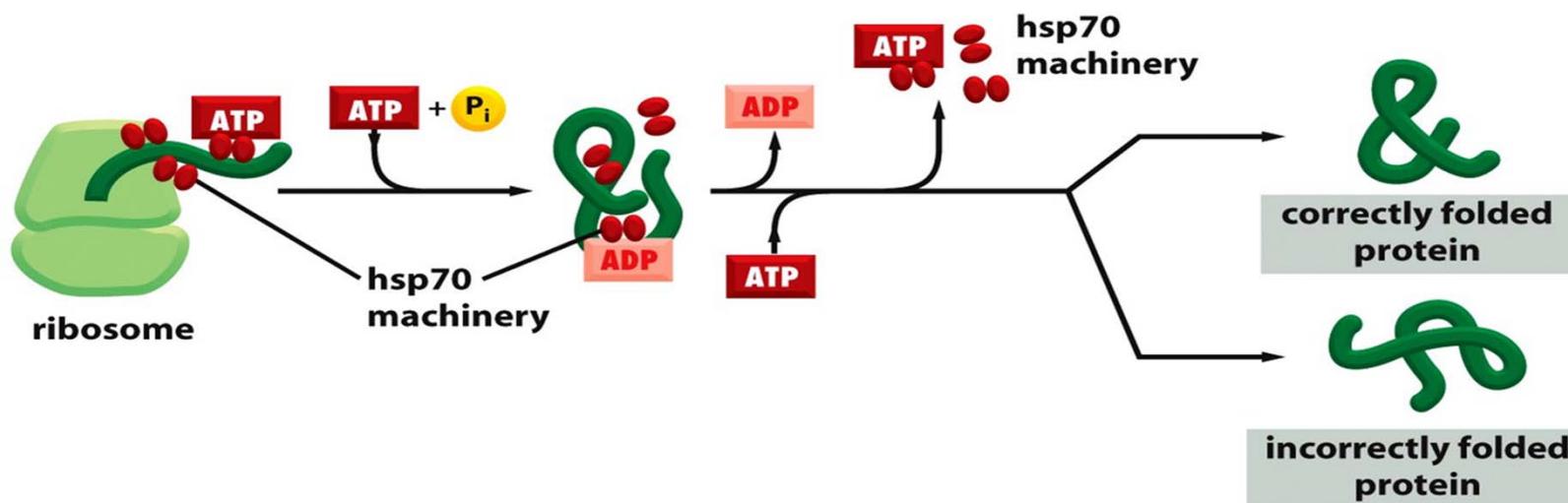
Co-translational protein folding



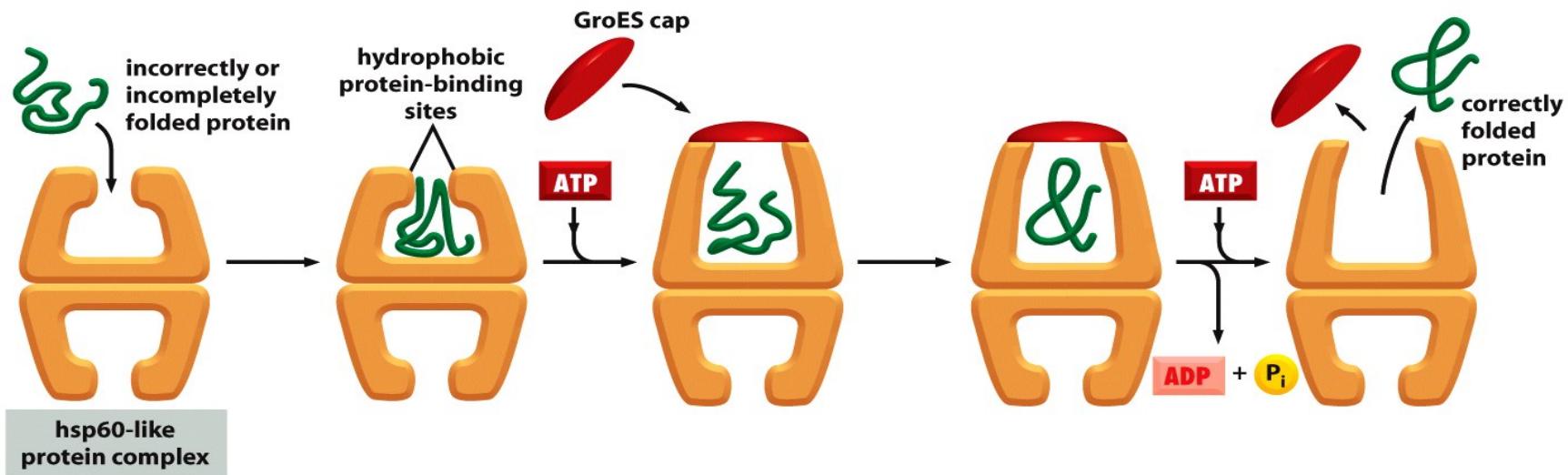
Molecular chaperons assist folding

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

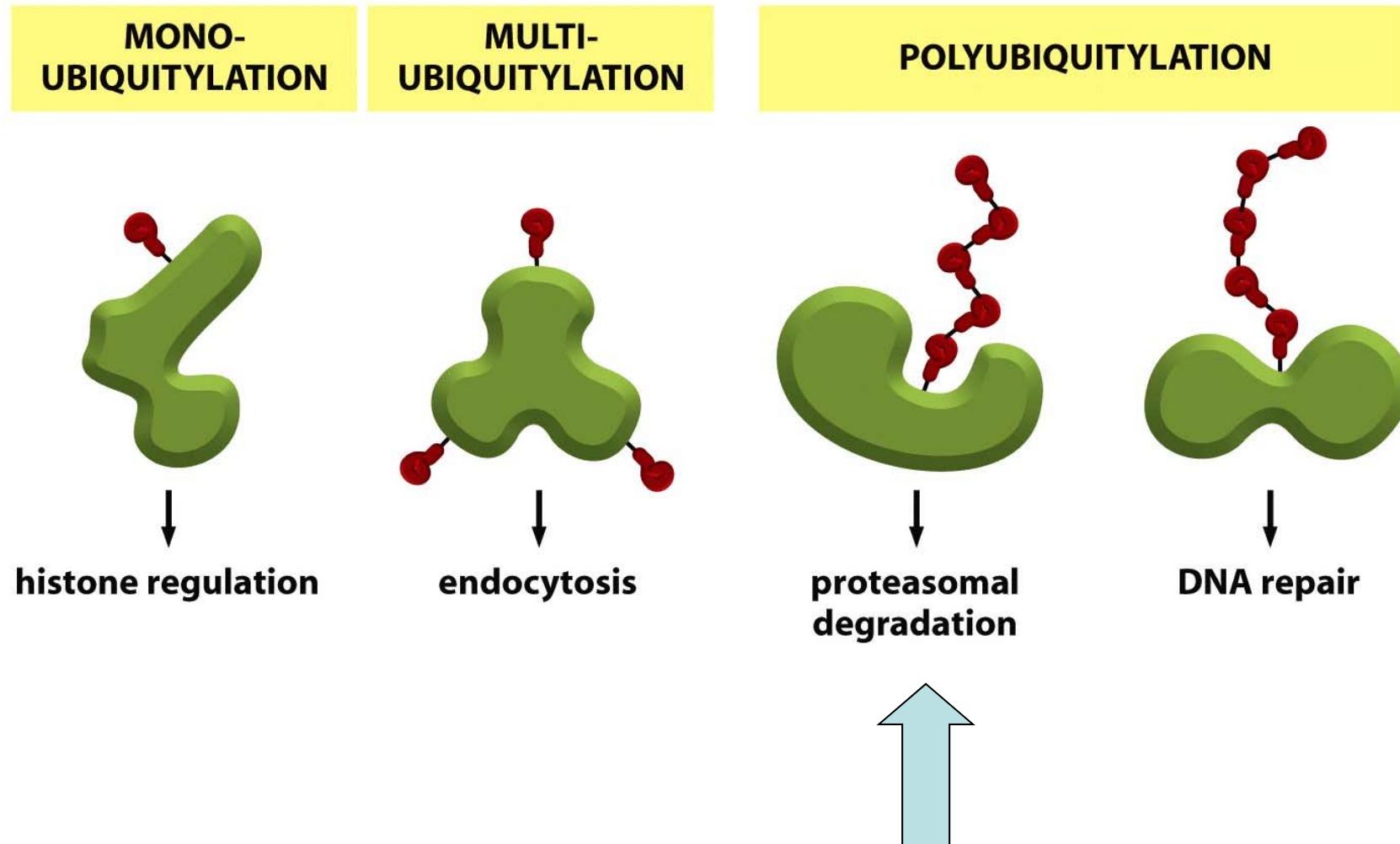
Hsp70 acts first to help folding of partially translated proteins



Hsp60 helps later to completely fold a protein



When a protein misfolds...

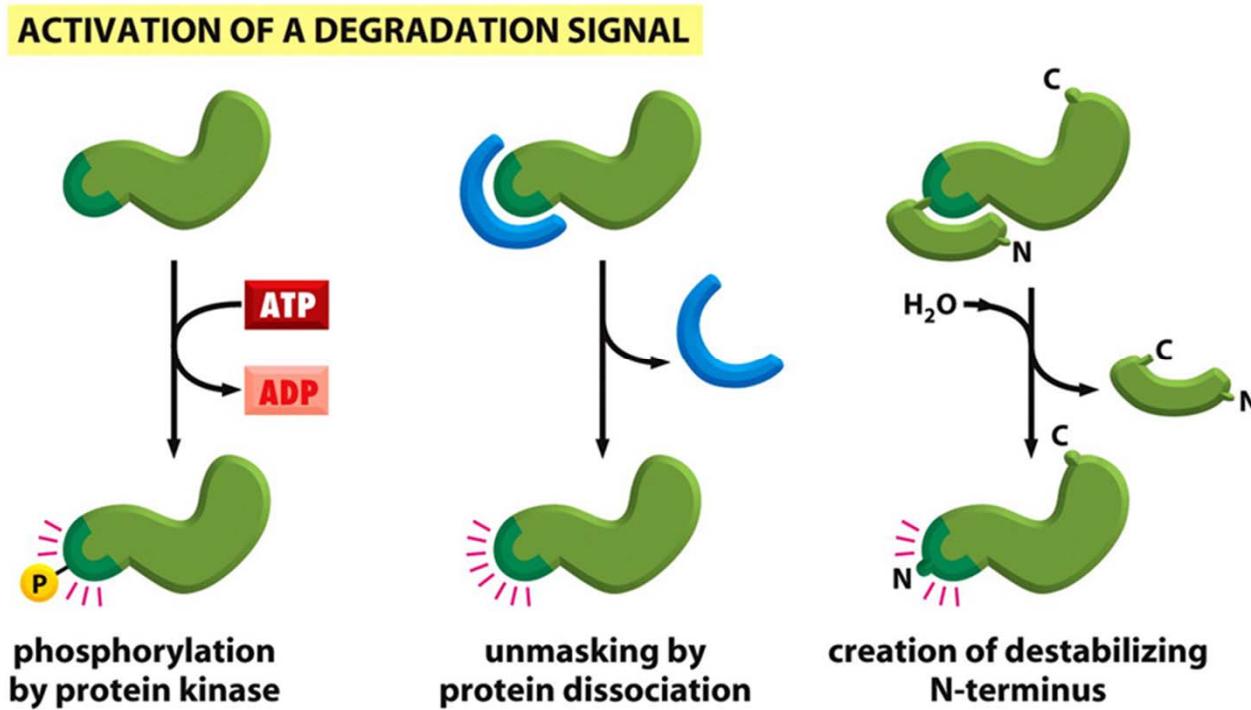


Two major ways to degrade proteins

- Lysosomal/vacuolar degradation (hydrolytic enzymes, pH~5)
 - primarily for aged or damaged organelles
- Proteasome pathway (large molecular machinery)
 - Take up to 90% of all protein degradation in mammals
 - Mostly ubiquitin (76aa polypeptide)-mediated,
occurs on lysine residues, polyubiquitination is called
“kiss of death”

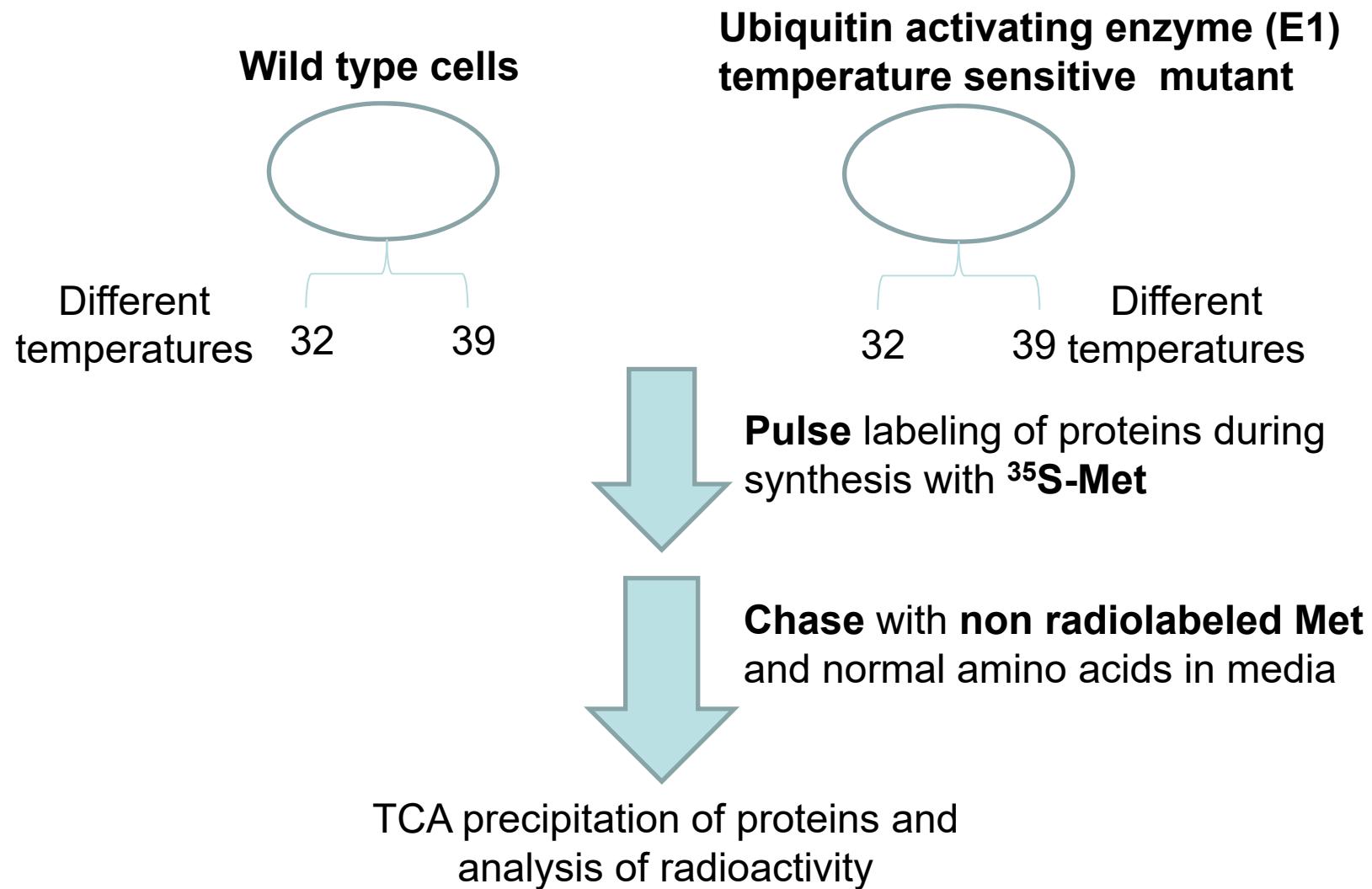
Protein phosphorylation can provide signals for protein ubiquitination

Different options to create an exposed degradation signal:



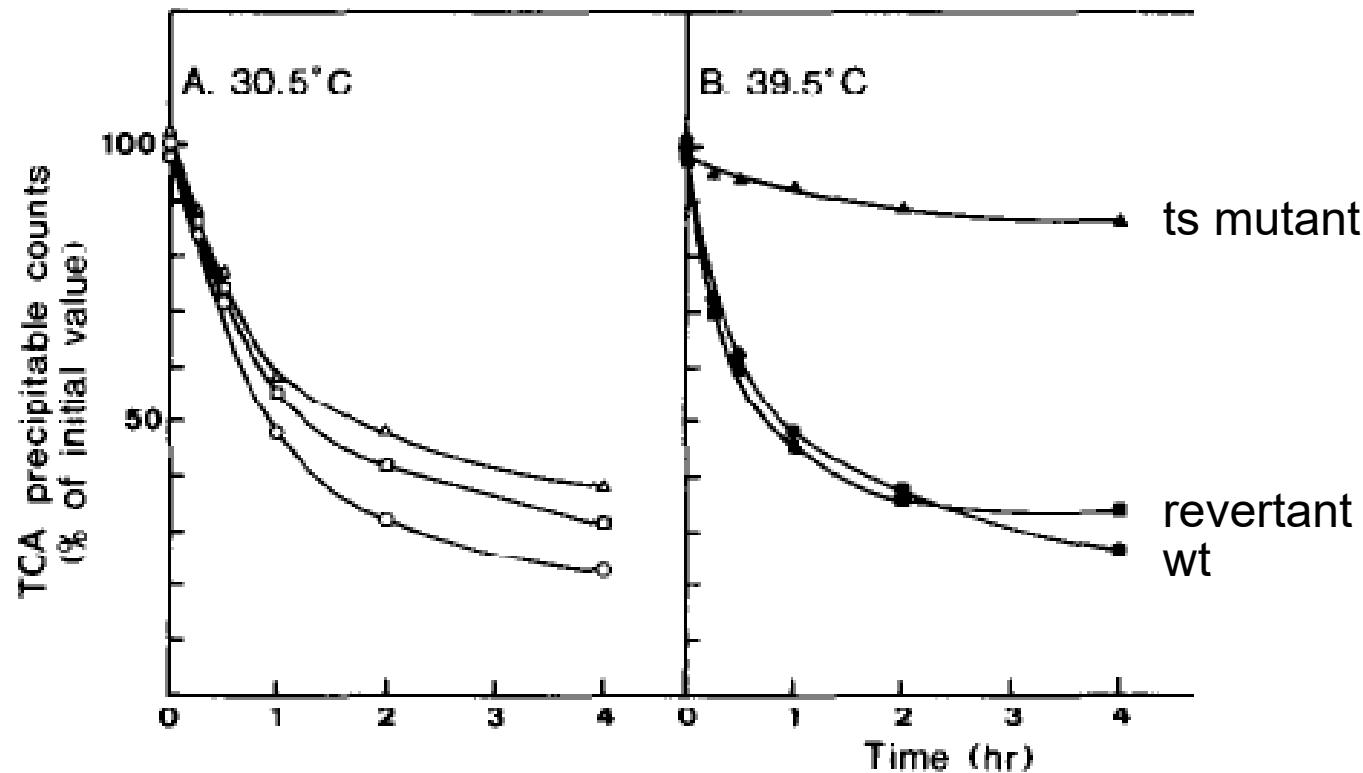
This signal binds a ubiquitin ligase, causing the addition of a polyubiquitin chain to a nearby lysine on the target protein.

IV. Ubiquitination-dependent protein degradation



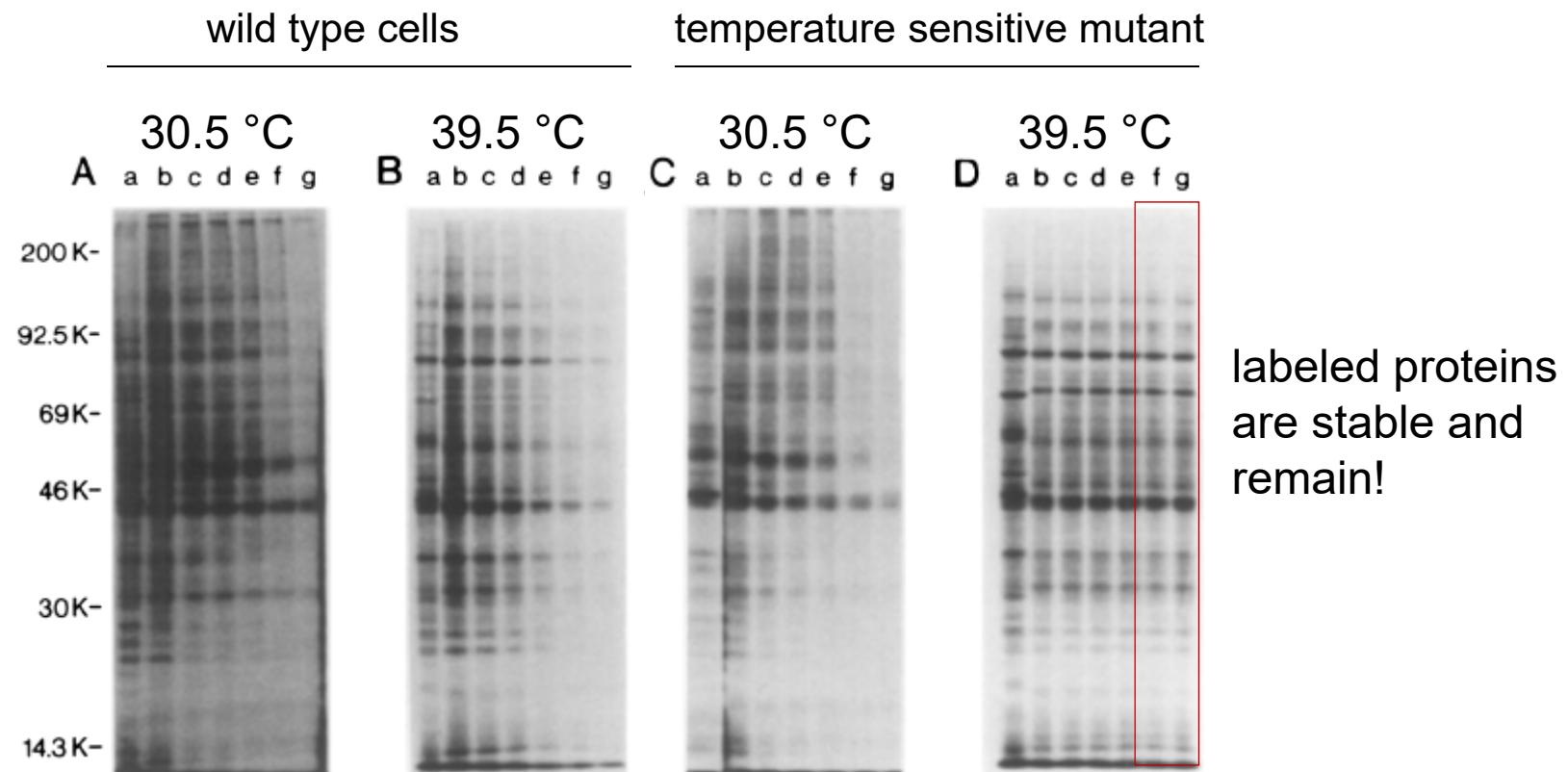
Alex Varshavsky, (1984) **Cell**

Here is what they observed:

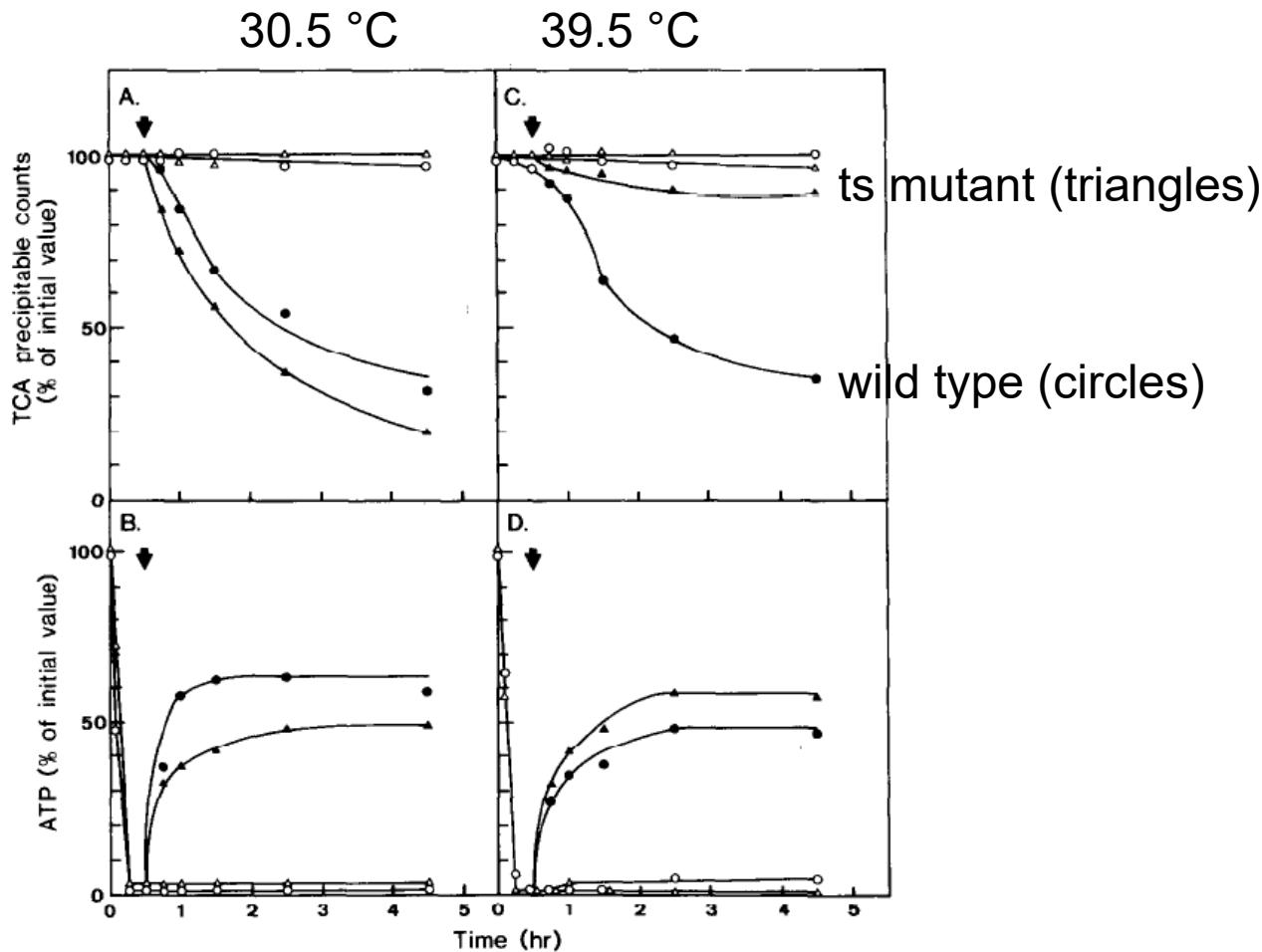


At the non-permissive temperature ($39.5\text{ }^{\circ}\text{C}$, the ts mutant fails to degrade the synthesized protein: enzyme function is required for the degradation

Here is what they observed:



The protein degradation depends on ATP



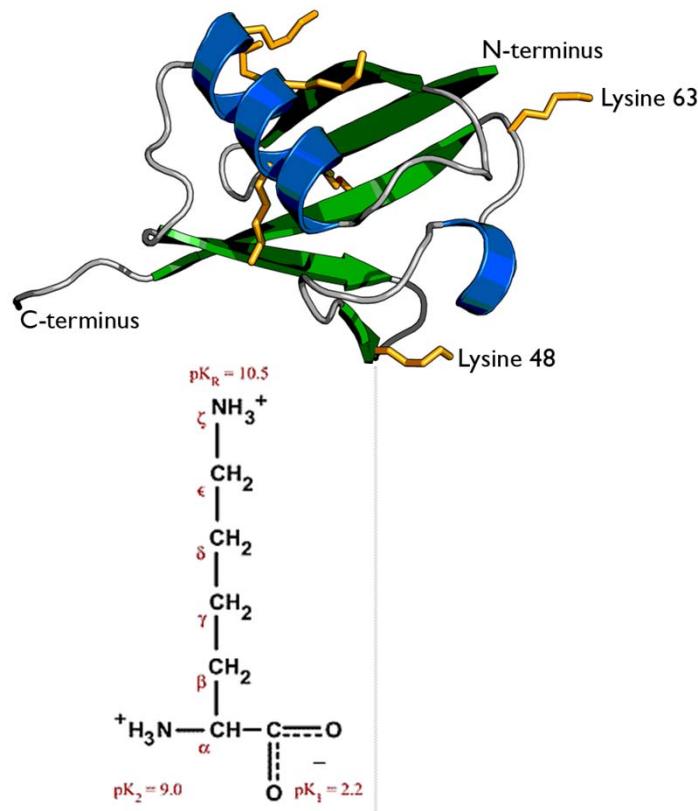
Open symbols: NaCN treatment all the time;

Closed symbols: NaCN treatment for the first 30 min only (washout)

Protein ubiquitination

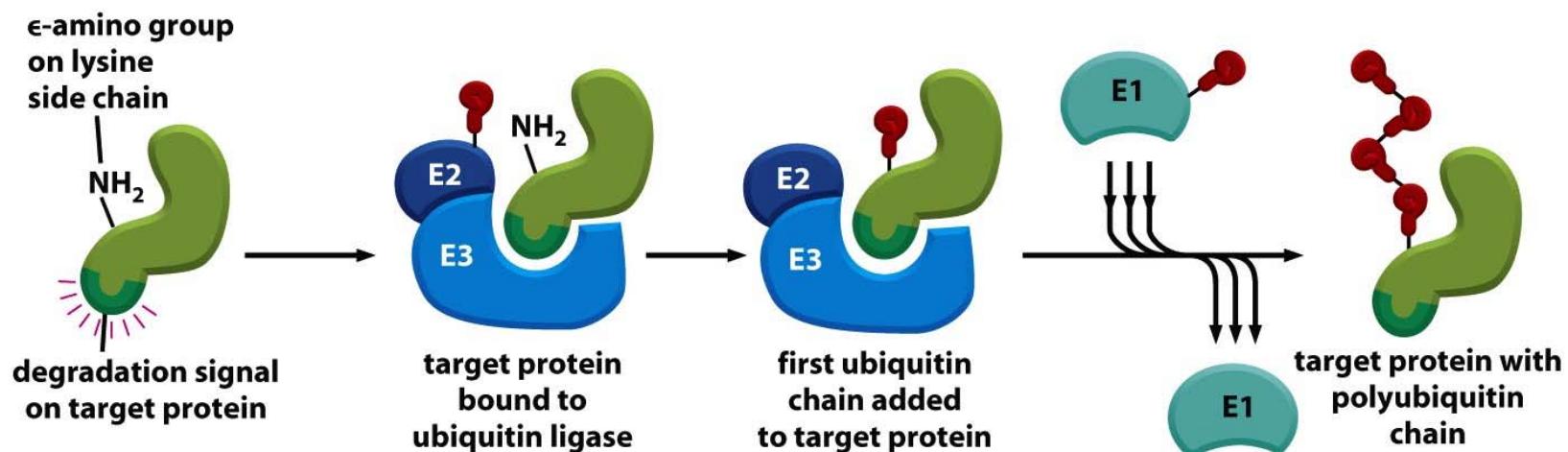
Ubiquitin

76aa regulatory protein

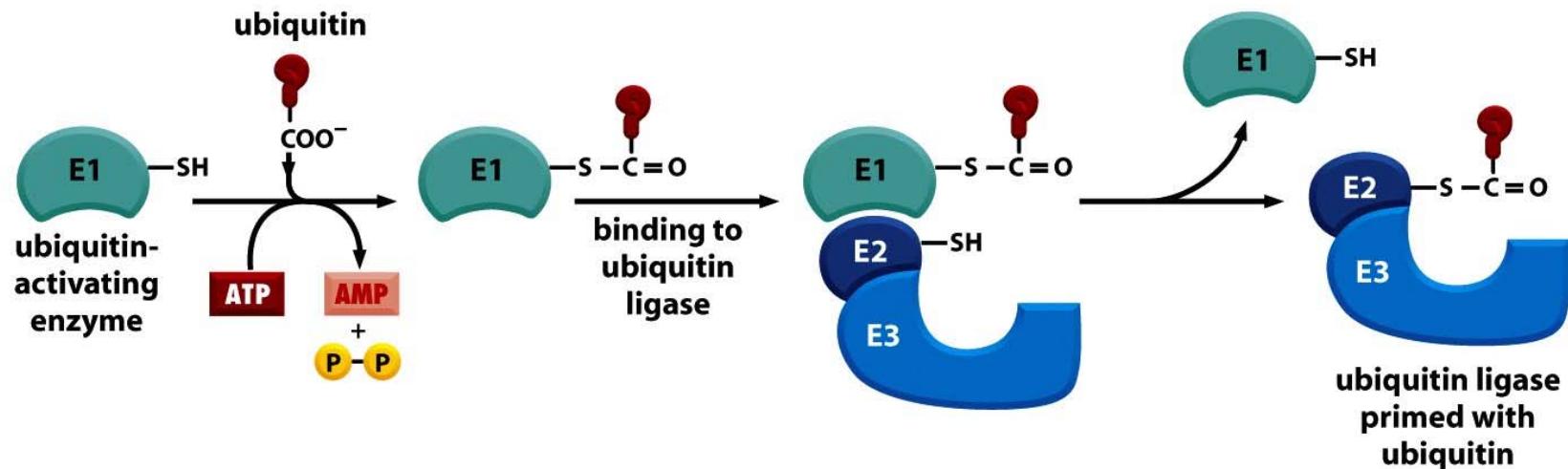


- Ubiquitin itself contains **seven lysine residues**, multiple molecules of ubiquitin can also become **linked to each other** to form **polyubiquitin chains**.
- The major function of ubiquitination is proteolysis, whereby **lysine-48 (K48)-linked polyubiquitin chains** allow **recognition by the 26S 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 (endocytosis)
- **C-terminal glycine of ubiquitin becomes linked to primarily ε-amino of lysine residues in target proteins via an E1-E2-E3 cascade.**

Ubiquitin ligases recognizes target proteins to be degraded, bind and add ubiquitin on its lysine through iso-peptide bond



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



Ubiquitin: 76 aa peptide

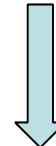
E1: ubiquitin-activating enzyme

E2: ubiquitin conjugating enzyme

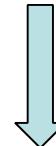
E3: ubiquitin ligase

Ubiquitination systems

One common E1 enzyme



~30 E2 enzymes in mammals

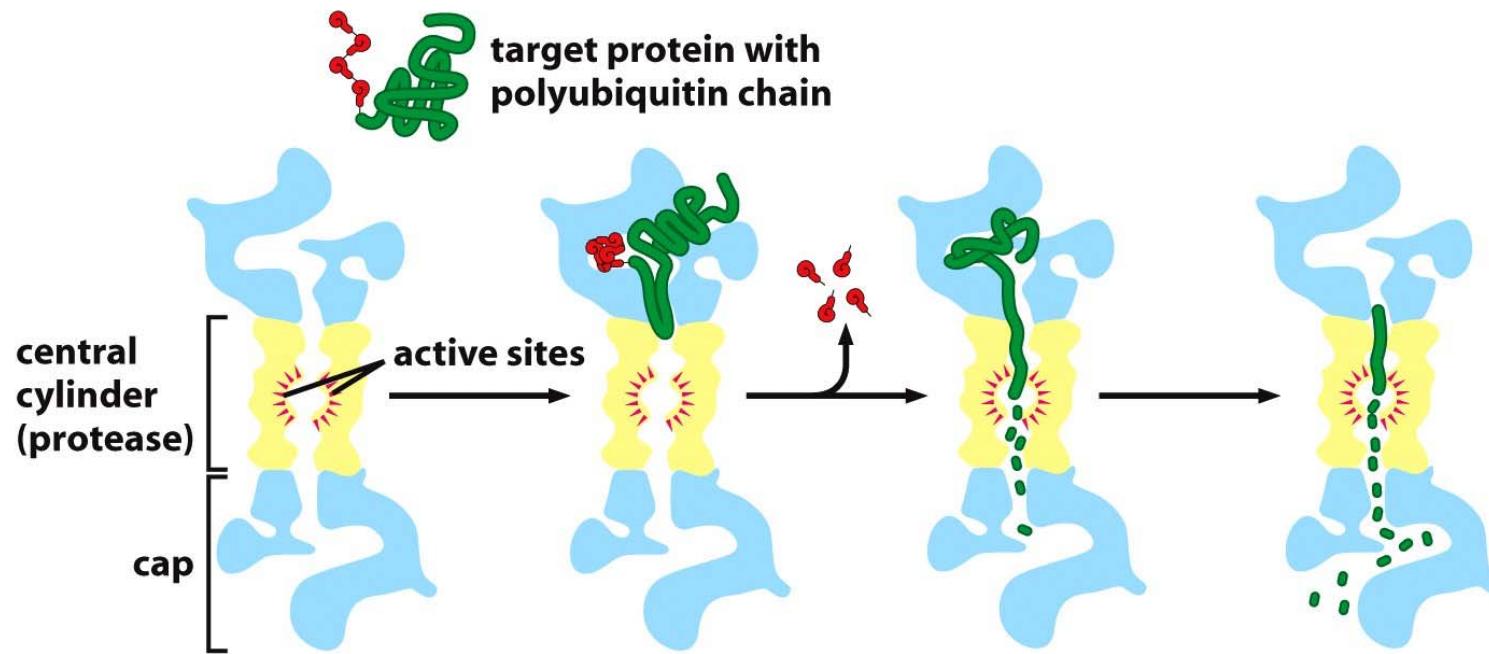


~hundreds of E3 enzymes

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

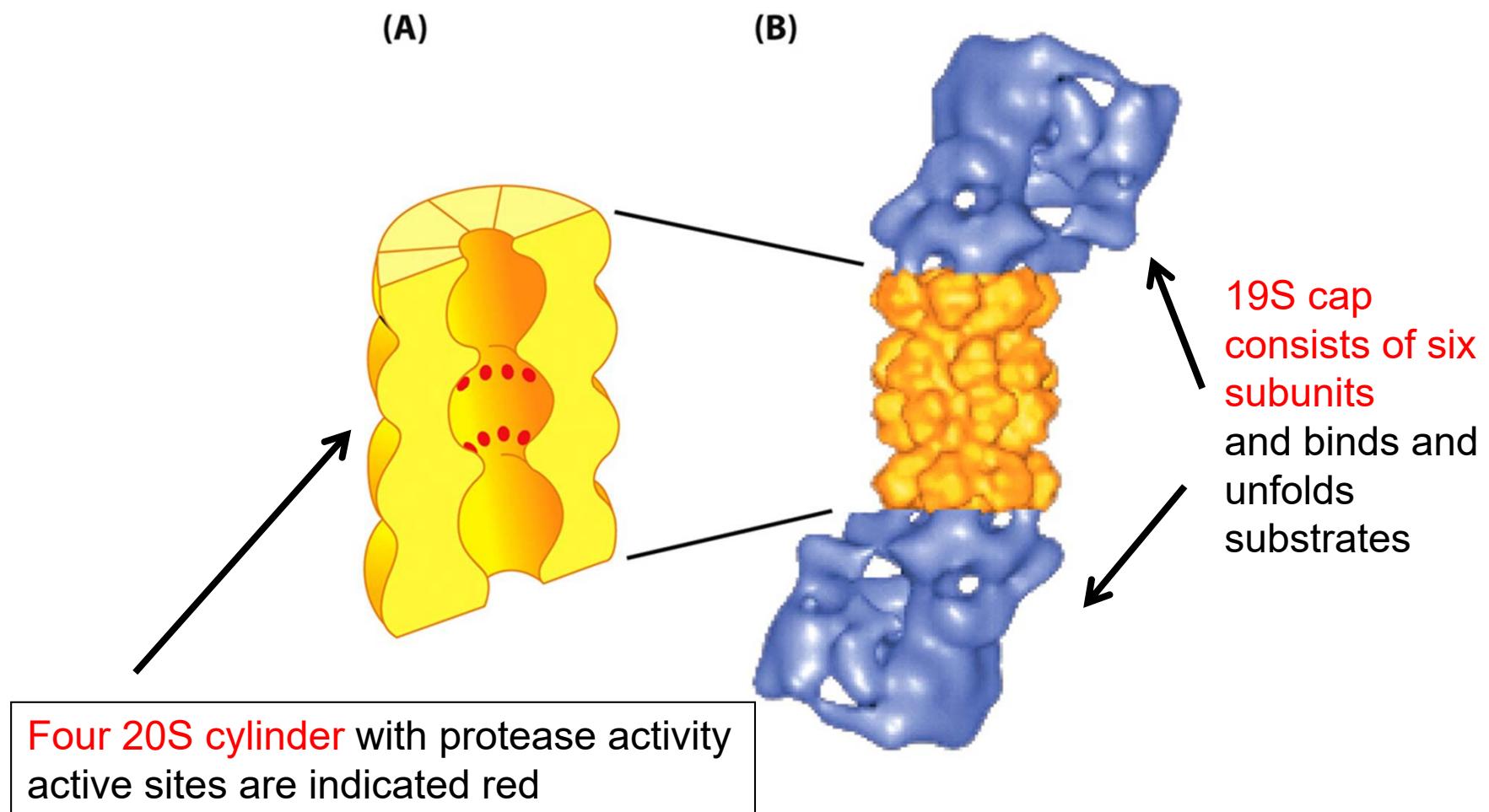
If I want to inhibit a specific ubiquitylation enzyme complex,
would I choose an inhibitor which targets E1 ?

Polyubiquitylated protein is then degraded by the proteasome in the cytosol

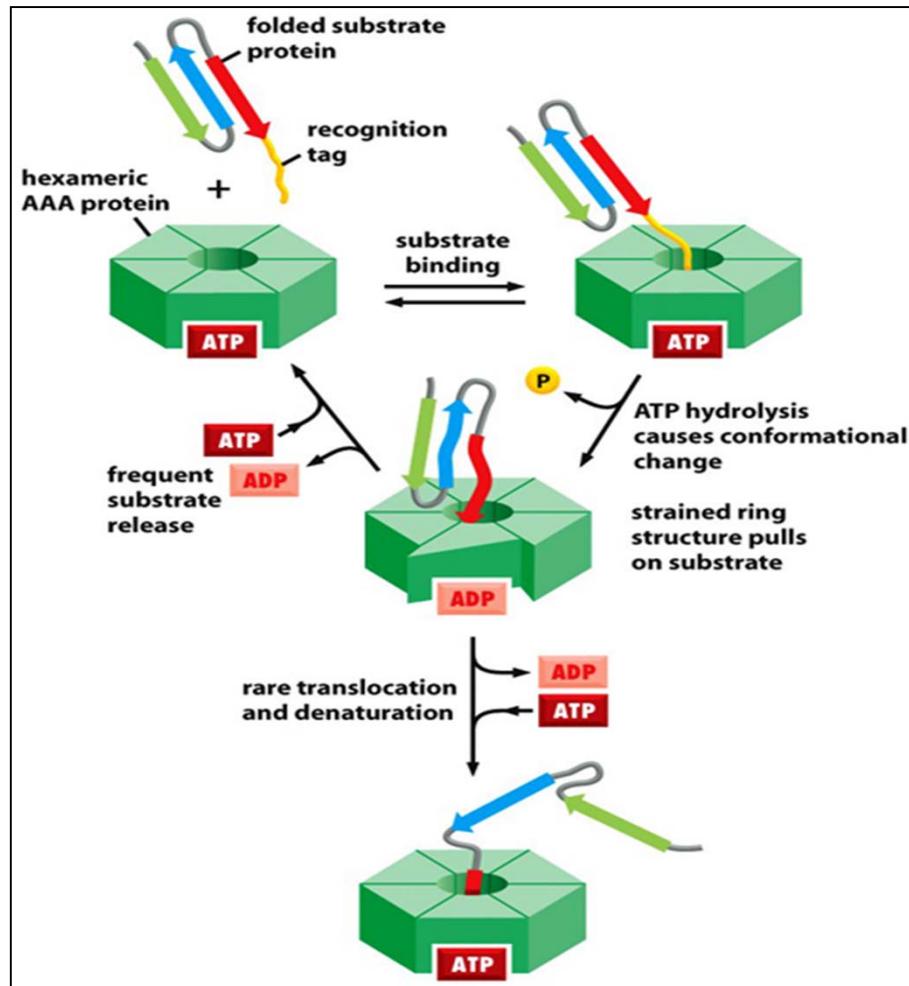


- **Translocation into the core** of the proteasome is mediated by a ring of **ATPases** that **unfold** the substrate **protein** (requires ATP)
- The **cap includes** a **ubiquitin receptor**, which holds a ubiquitylated protein in place while it begins to be **pulled into** the proteasome core, and a **ubiquitin hydrolase**, which **cleaves ubiquitin** from the doomed protein

The 20S proteasome



19S cap: a hexameric protein unfoldase



The **ATP-bound** form of a hexameric ring of AAA proteins **binds a folded substrate protein by its ubiquitin tag**.

A conformational change, driven by ATP hydrolysis, **pulls** the substrate into the central core and **strains the ring** structure.

At this point, the substrate protein can partially unfold and enter further into the pore or it can maintain its structure and partially withdraw.

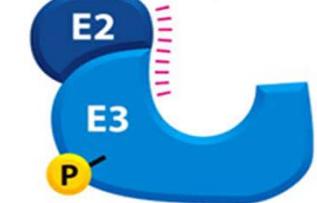
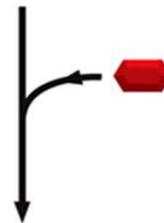
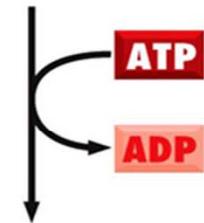
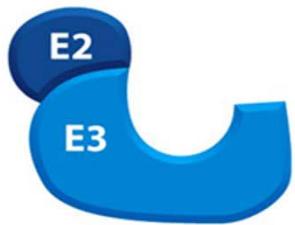
Very stable protein substrates may require hundreds of cycles of ATP hydrolysis and dissociation

AAA protein (**AT**Pases **A**ssociated with diverse cellular **A**ctivities)

How to activate E3-ubiquitin ligase?

Different options to activate an E3-ubiquitinligase:

ACTIVATION OF A UBIQUITIN LIGASE



phosphorylation
by protein kinase



allosteric transition
caused by ligand binding



allosteric transition
caused by protein
subunit addition

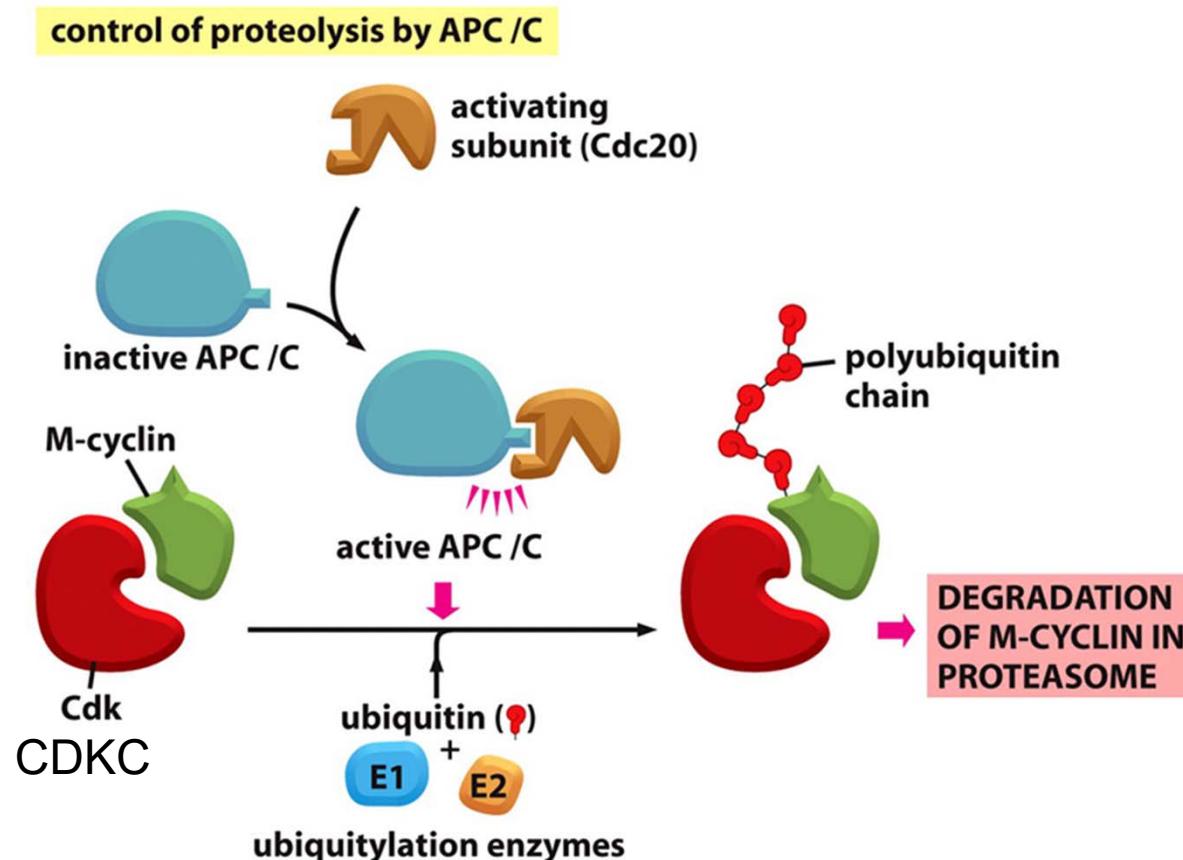
Degradation is not only for misfolded proteins...

Many proteins are degraded in a controllable manner:

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

Metaphase to anaphase transition is controlled by proteolysis

One Key player: anaphase-promoting-complex, aka the **cyclosome** (APC/C), a ubiquitin ligase that mediates inactivation of **CDKCs** (cyclin-dependent kinase complexes)



V. Protein misfolding causes human diseases

Protein aggregates primarily cause neurodegenerative diseases

- 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

