BIO 230

Lecture 7:

Regulation of the Proteome

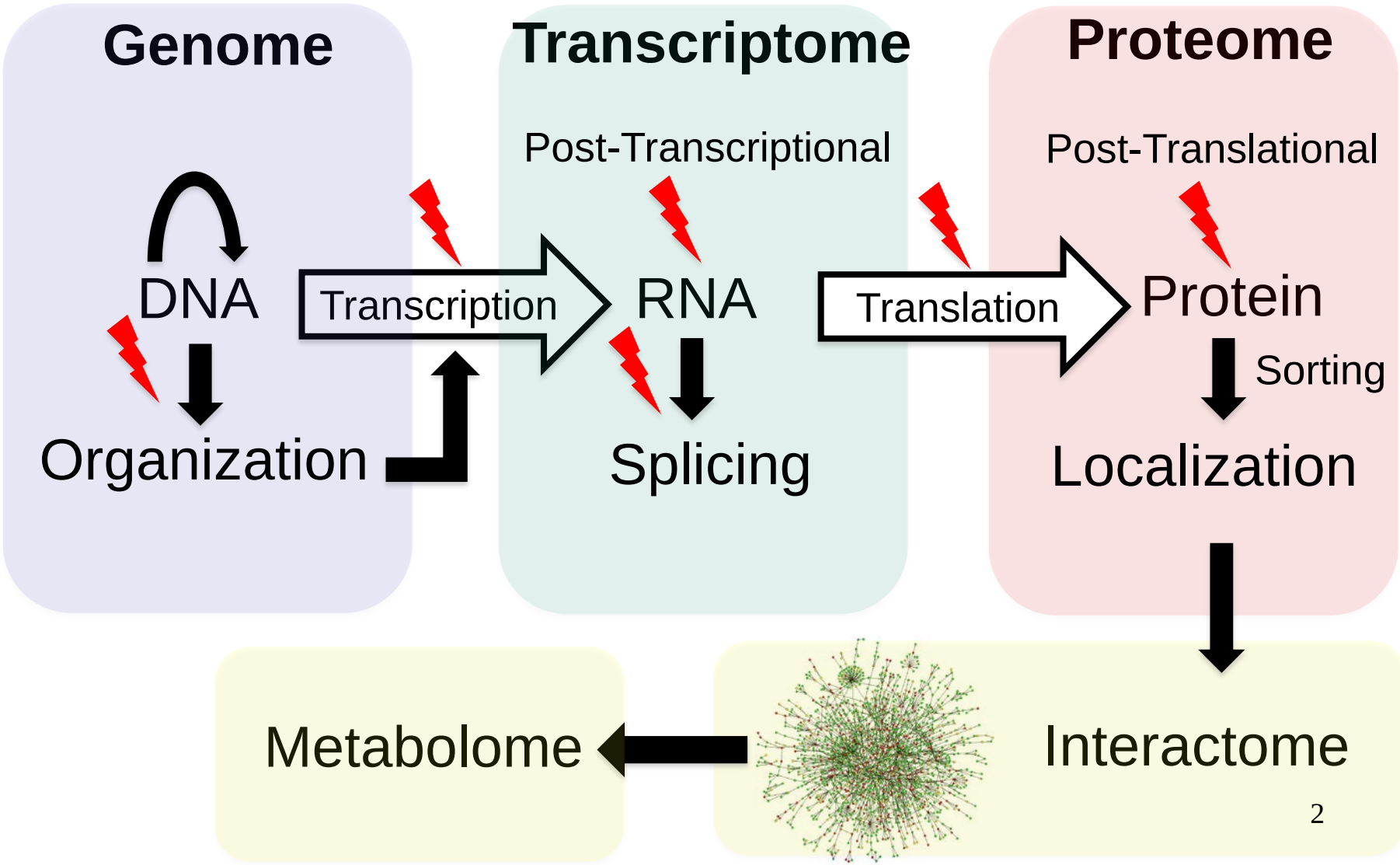
1) Translational Regulation

2) Post-translational Regulation

Readings (Alberts *et al.*, custom text)

15-29; 119-123; 82-91

Regulation of Genome Expression



Regulation of the Proteome

Gel electrophoresis makes proteins migrate by size and charges.

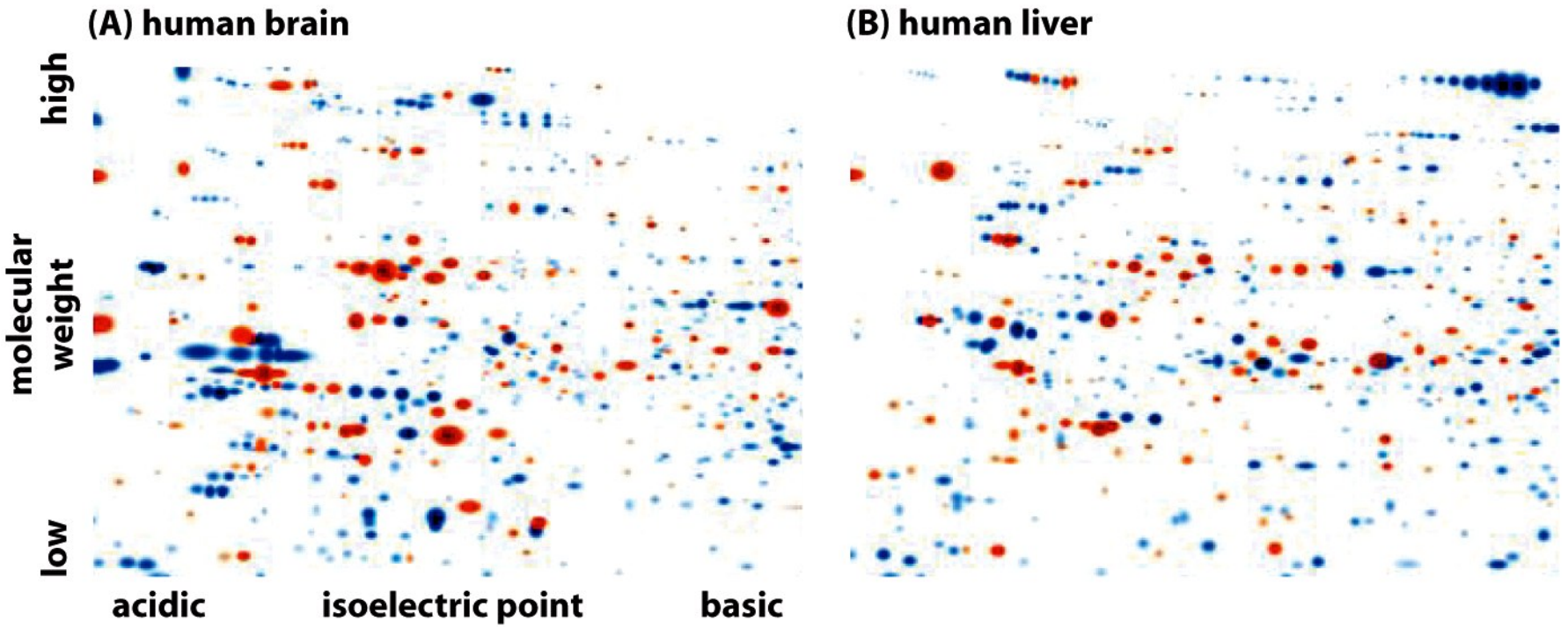


Figure 7-4 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Differences in the proteins expressed by two human tissues

Red: common to both Blue: Tissue specific

Translational Regulation

Both prokaryotes and eukaryotes use translational control mechanisms to regulate protein expression often in response to stressful situations such as low nutrients, infection, or environmental stresses (eg. temperature)

Prokaryotes

- mRNAs have a six nucleotide Shine-Dalgarno (SD) sequence upstream of AUG start codon i.e. ATG in the genome as start codon
- correctly positions AUG in the ribosome and provides translational control mechanisms

accessibility to SD plays important role in prokaryotic cells

Translational Regulation

Prokaryotes

Mechanism 1: RNA binding protein ● blocks access to SD sequence

SD sequence

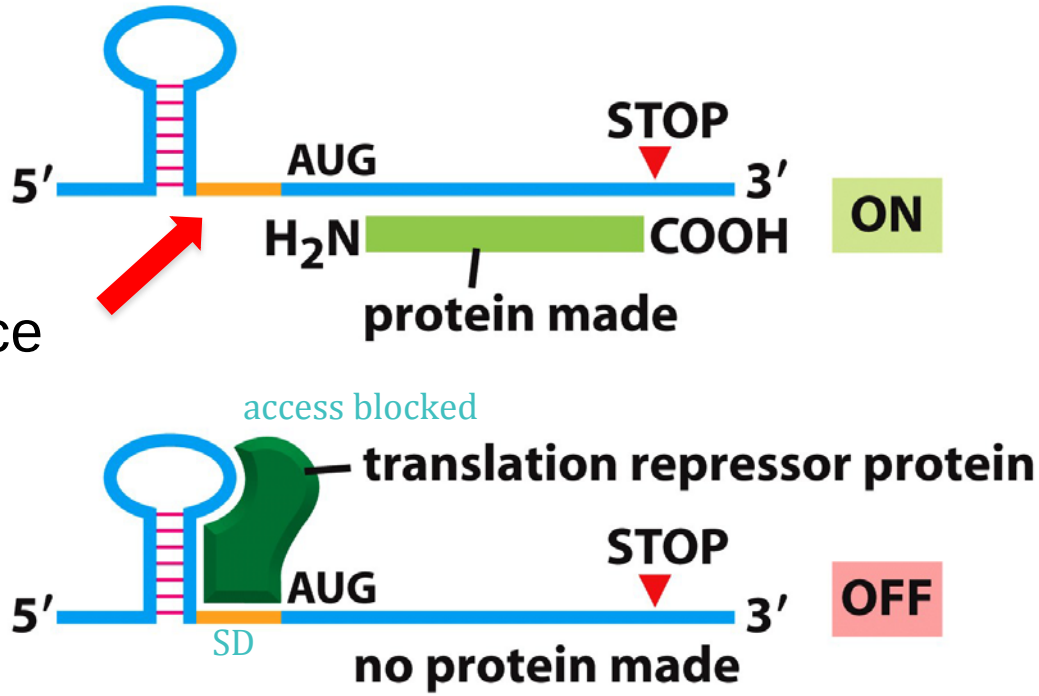


Figure 7-106a Molecular Biology of the Cell 5/e (© Garland Science 2008)

Translational Regulation

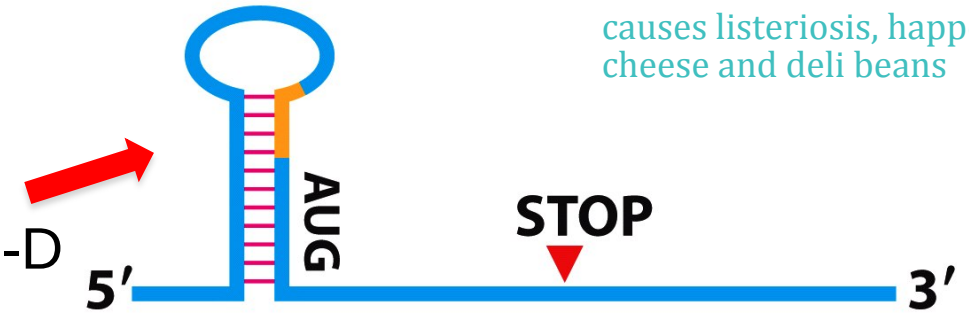
Prokaryotes

Mechanism 2: Temperature regulated RNA structures

eg. virulence genes of human pathogen *Listeria monocytogenes*

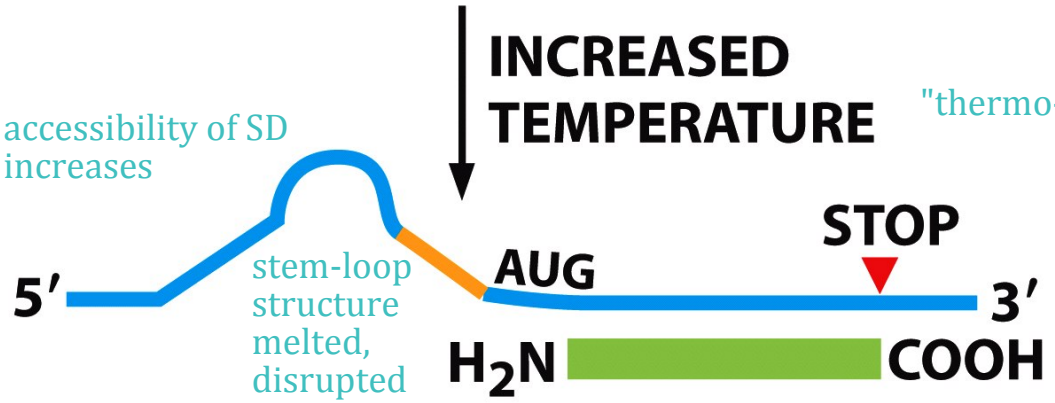
causes listeriosis, happens in improper kept cheese and deli beans

stem-loop RNA structure blocks S-D



OFF

room temperature, lower than 37 C found in human body



ON

Figure 7-106b Molecular Biology of the Cell 5/e (© Garland Science 2008)

Temperature triggers immune evasion by *Neisseria meningitidis* (found in your nasal, can infect you once you get influenza)

Edmund Loh[1]*, Elisabeth Kugelberg[2]*, Alexander Tracy[1], Qian Zhang[2], Bridget Gollan[2], Helen Ewles[2], Ronald Chalmers[3], Vladimir Pelicic[2] & Christoph M. Tang[1,2]

[1]Sir William Dunn School of Pathology, University of Oxford, Sir Parks Road, Oxford OX1 3RE, UK. [2]Centre for Molecular Microbiology and Infection, Imperial College London, London SW7 2AZ, UK. [3]School of Biomedical Sciences, University of Nottingham, Nottingham NG7 2NR, UK.
*These authors contributed equally to this work.

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When you get a fever, Neisseria meningitidis uses the leviated temperature utilize the opportunity to upregulate RNA transcription.
You can get meningitis because the pathogen has thermo-sensor.

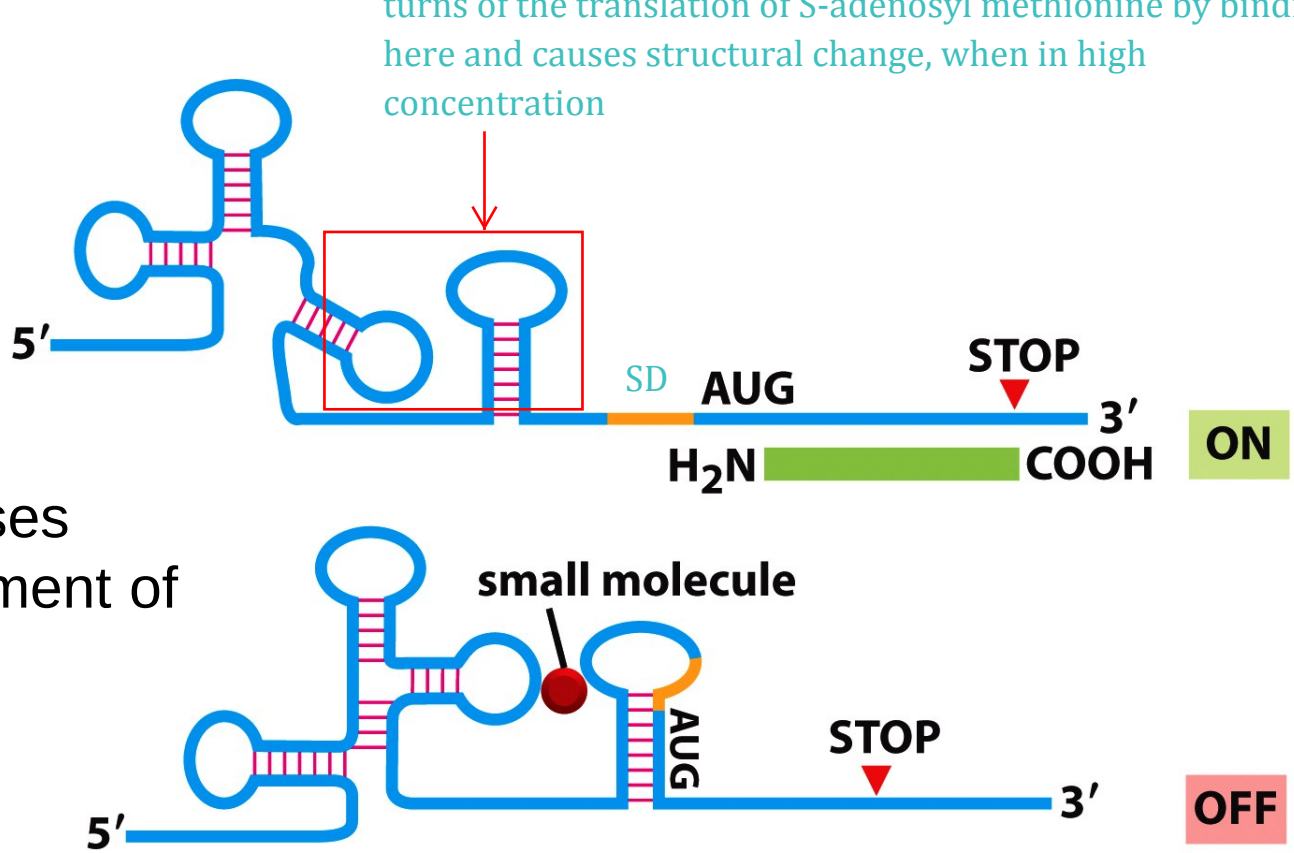
Neisseria meningitidis **has several strategies to evade complement-mediated killing, and these contribute to its ability to cause septicaemic disease and meningitis. However, the meningococcus is primarily an obligate commensal of the human nasopharynx, and it is unclear why the bacterium has evolved exquisite mechanisms to avoid host immunity. Here we demonstrate that mechanisms of meningococcal immune evasion and resistance against complement increase in response to an increase in ambient temperature. We have identified three independent RNA thermosensors located in the 5′ untranslated regions of genes necessary for capsule biosynthesis, the expression of factor H binding protein, and sialylation of lipopolysaccharide, which are essential for meningococcal resistance against immune killing[1,2]. Therefore increased temperature (which occurs during inflammation) acts as a 'danger signal' for the meningococcus, enhancing its defence against human immune killing. Infection with viral pathogens, such as influenza, leads to inflammation in the nasopharynx with an increased temperature and recruitment of immune effectors[3,4]. Thermoregulation of immune defence could offer an adaptive advantage to the meningococcus during co-infection with other pathogens, and promote the emergence of virulence in an otherwise commensal bacterium.**

other pathogens use this mechanism as well to cope with host environment

Translational Regulation

Prokaryotes

Mechanism 3: Riboswitch
eg. S-adenosyl methionine



Small molecule causes structural rearrangement of RNA blocking SD

Figure 7-106c Molecular Biology of the Cell 5/e (© Garland Science 2008)

Translational Regulation

Prokaryotes

Mechanism 4: Antisense RNA

eg. iron storage proteins

when there's low iron in the cell:

when there's a lot of iron in the cell:

Figure 7-106d Molecular Biology of the Cell 5/e (© Garland Science 2008)

Antisense RNA produced elsewhere in the genome base-pairs with mRNA and blocks SD to prevent translation of bacterial RNA

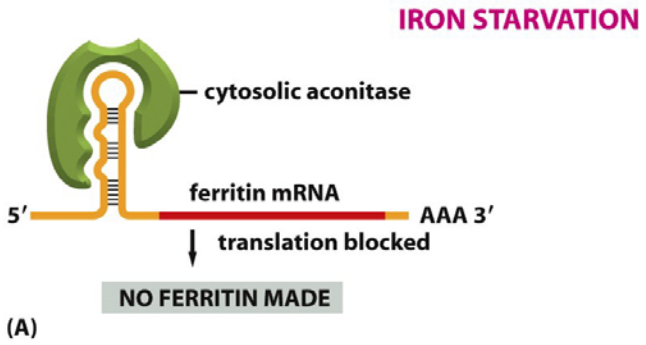
Translational Regulation

Eukaryotes

- No shine-Dalgarno sequences but similar mechanisms as prokaryotes

– translational repressors can bind near initiator AUG and inhibit translation (eg. aconitase) (cytosolic aconitase, regualted by iron)

acts like a repressor



expose AUG start codon translation can start now

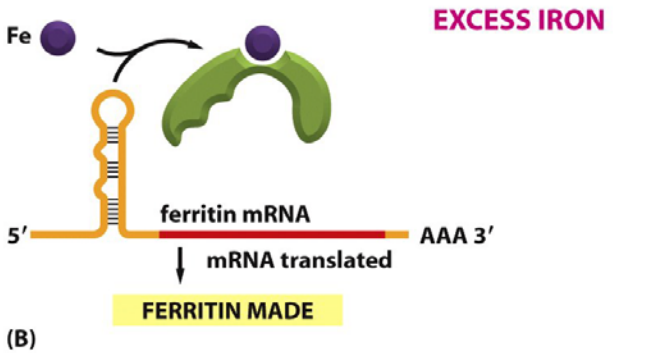


Figure 7-111 Molecular Biology of the Cell 5/e (© Garland Science 2008)

iron storage protein, sucks out iron when the cell doesn't need it, then release the excess when the cell needs it. Translational regulation.

Ferritin- binds iron and releases it in a controlled manner

– not needed when iron is low

aconitase binds to the ferritin RNA near the start site blocks translation

- translated when iron is in excess
- aconitase binds iron
- conformational change
- ferritin RNA released

(recall aconitase also regulates transferrin receptor)

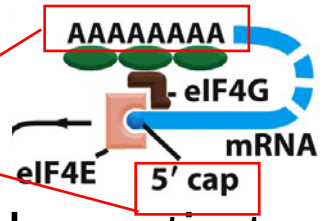
Translational Regulation

Eukaryotes

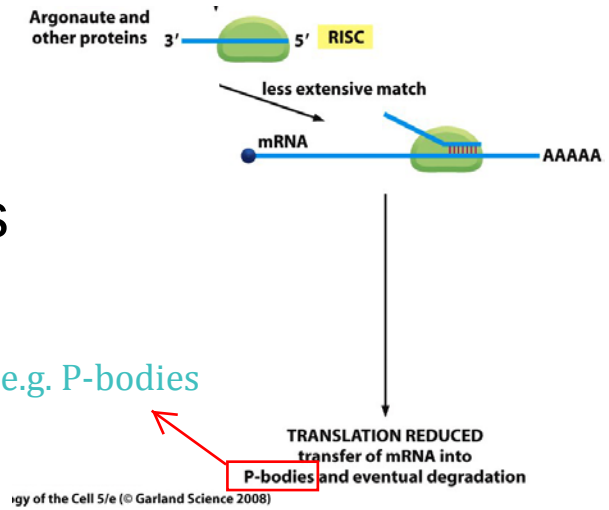
not just interacting with AUG start codon

-repressor proteins can also interfere with
- 5' cap and 3' poly-A tail interactions required for efficient translation

eukaryotic example



- small RNA molecules can also regulate eukaryotic translation (miRNAs), but different mechanism than in prokaryotes



- also other eukaryotic specific mechanisms (eg. eIF2)

Translational Regulation

Eukaryotic specific translational regulation

1)Regulation of eukaryotic initiation factors (eIFs)

other wise you will never get the initiator tRNA recruited to the ribosome

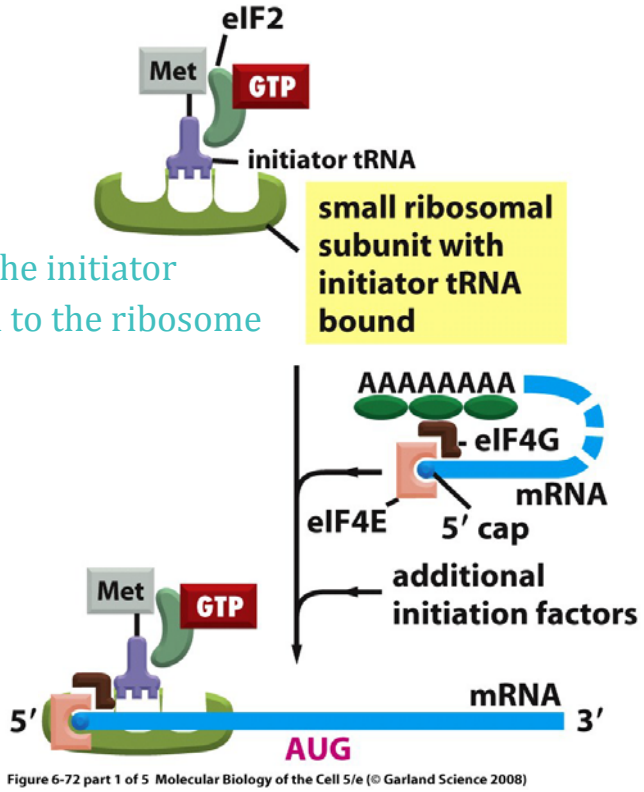


Figure 6-72 part 1 of 5 Molecular Biology of the Cell 5/e (© Garland Science 2008)

- eIF2 plays a crucial role in translation initiation

- eIF2 forms a complex with GTP and recruits initiator tRNA (methionyl) to small ribosomal subunit

- small ribosomal subunit binds 5’ end of mRNA and scans for AUG

Translational Regulation

Eukaryotic specific translational regulation

1)Regulation of eukaryotic initiation factors (eIFs)

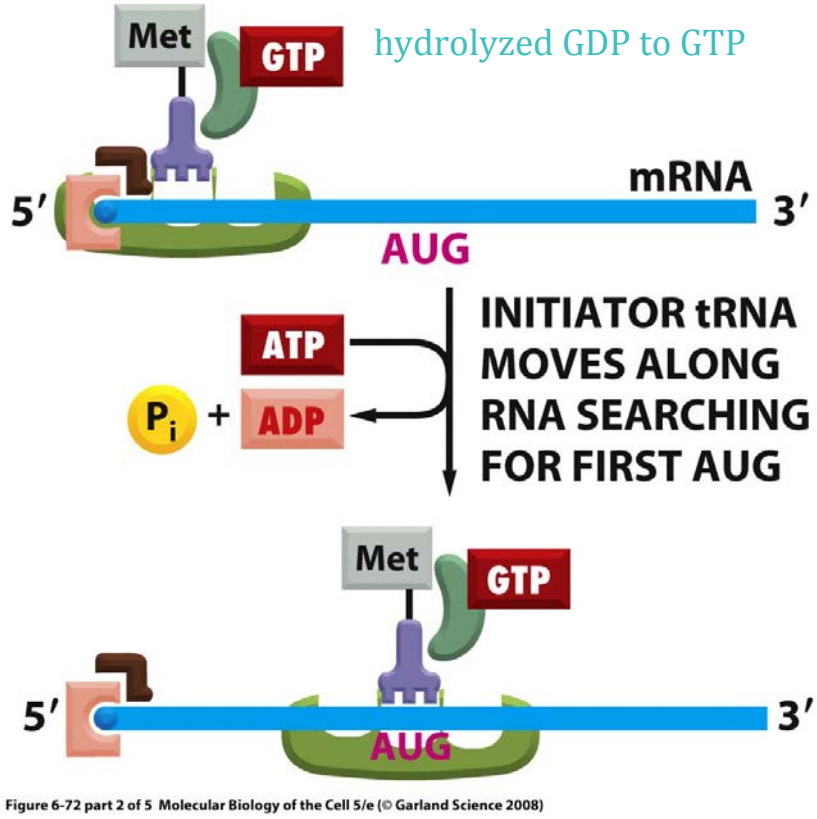


Figure 6-72 part 2 of 5 Molecular Biology of the Cell 5/e (© Garland Science 2008)

- when AUG is recognized eIF2 it hydrolyzes GTP to GDP

- GTP hydrolysis causes a ● conformational change in eIF2

- eIF2 bound to GDP is released from small ribosomal subunit

- eIF2 bound to GDP is ● inactive Must be reactivated- how?

Translational Regulation

Eukaryotic specific translational regulation

1)Regulation of eukaryotic initiation factors (eIFs)

-reactivation of eIF2 requires ● eIF2B which is a ● guanine nucleotide exchange factor (GEF) which exchanges GDP for GTP (on eIF2)

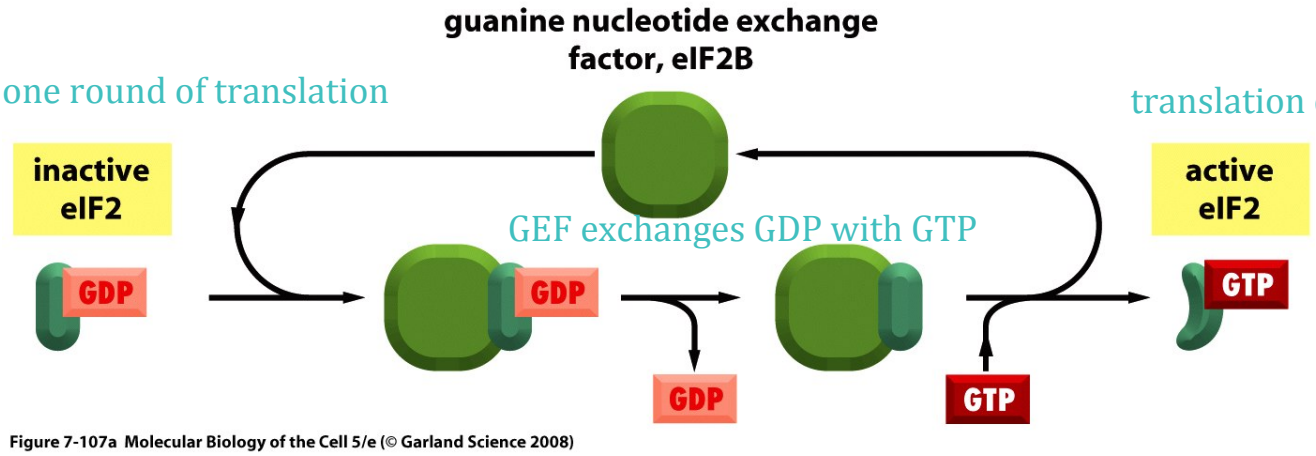


Figure 7-107a Molecular Biology of the Cell 5/e (© Garland Science 2008)

under stressful conditions or more dormant state, translation needs to be down-regulated.

- eIF2 reactivation is regulated by ● phosphorylation

Translational Regulation

Eukaryotic specific translational regulation

1)Regulation of eukaryotic initiation factors (eIFs)

- phosphorylated eIF2 sequesters eIF2B as an inactive complex

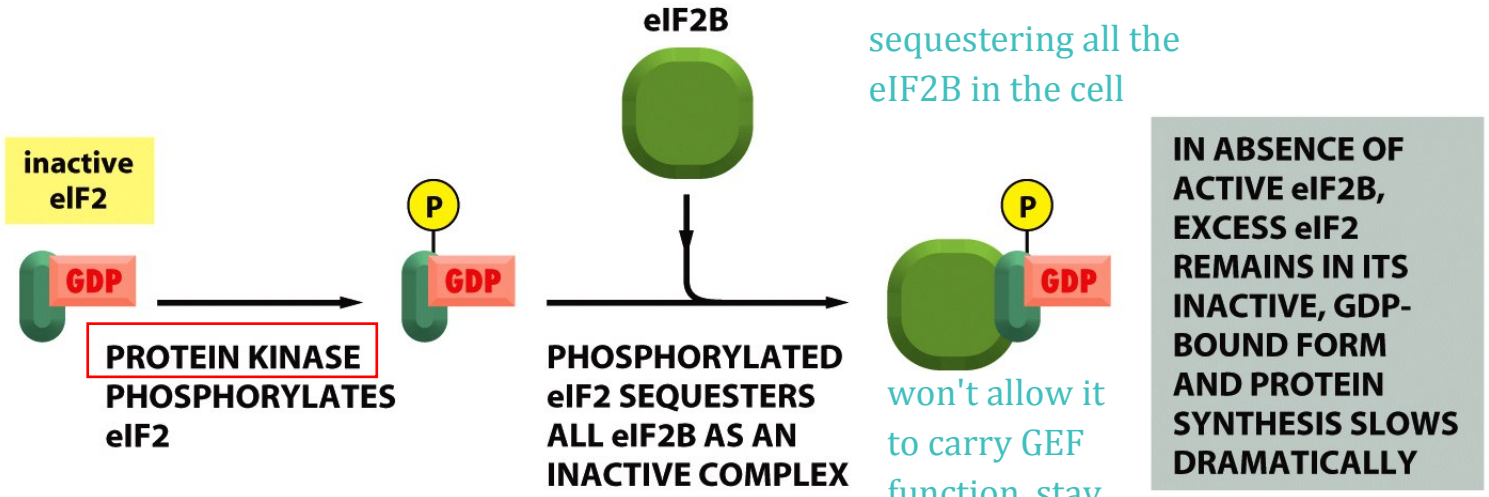


Figure 7-107b Molecular Biology of the Cell 5/e (© Garland Science 2008)

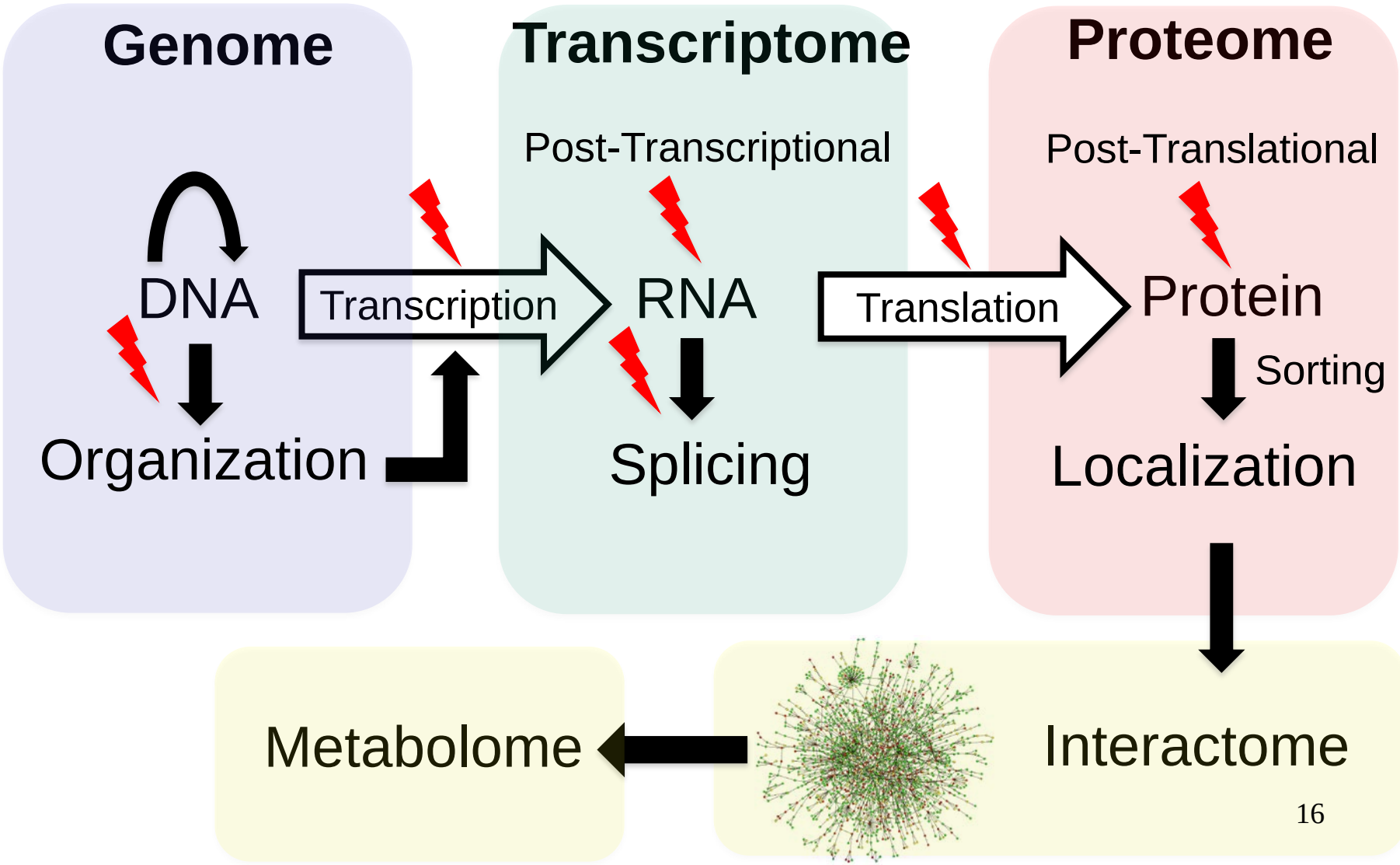
sequestering all the eIF2B in the cell

won't allow it to carry GEF function, stay inactive

- since there is more eIF2 than eIF2B in cells, all eIF2B is sequestered and translation is dramatically reduced
- not all mRNAs are equally affected by eIF2 phosphorylation

(won't get into details)

Regulation of Genome Expression



Post-Translational Regulation

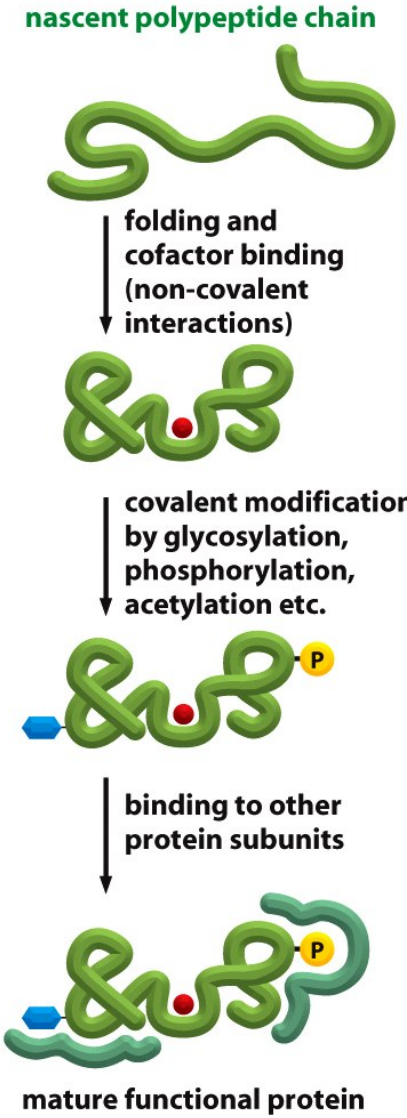
Protein Regulation

Proteins undergo a number of steps in order to become functional:

1) proteins must fold properly to adopt their 3D structure

2) proteins are covalently modified with chemical groups (eg. sugars, phosphate)

3) proteins interact with other proteins and small molecules (cofactors) required for protein function



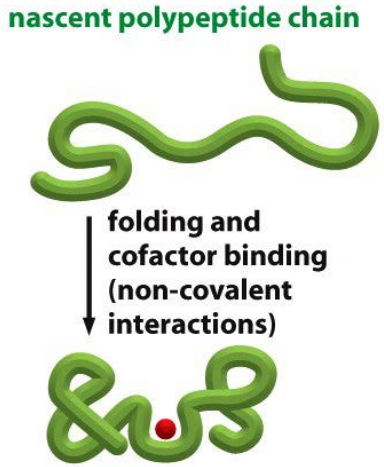
primary AAs will adopt secondary structure (beta strand or alpha helix), fold into tertriary structure to let the protein perform function

may turn it on/off

Figure 6-82 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Post-Translational Regulation

Protein Regulation- Protein Folding



- hydrophobic amino acids are buried in the ● interior core (ie. not surface exposed)

hydrophobic AAs will interact via non-covalently and adopt energy-favored 3D structure

- some proteins, folding begins as they ● emerge from ribosome : some completely folded after synthesis

The process of folding happens very fast.
Evolution selected them this way.

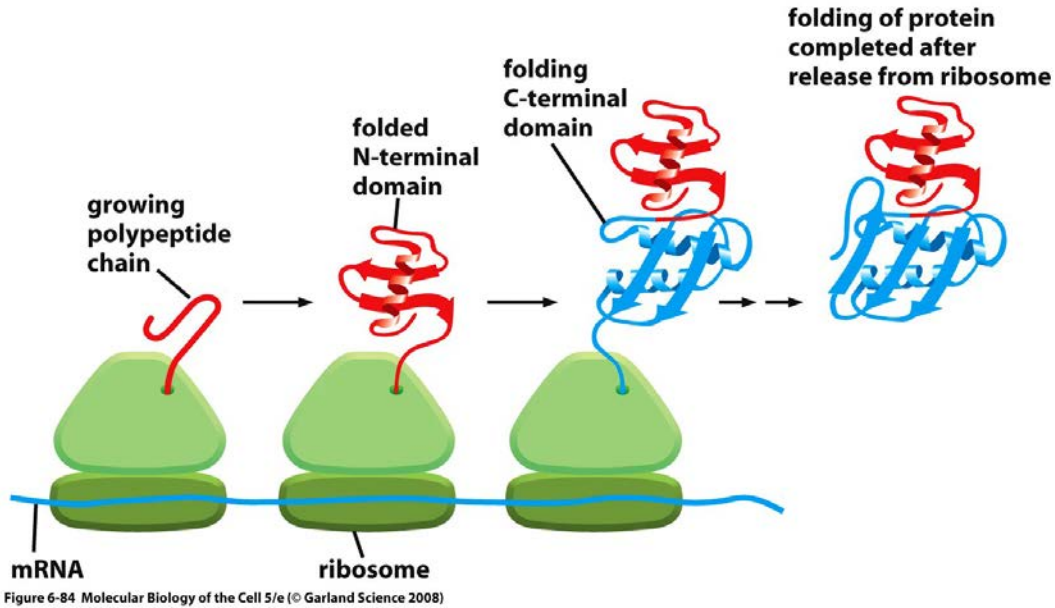


Figure 6-84 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Post-Translational Regulation

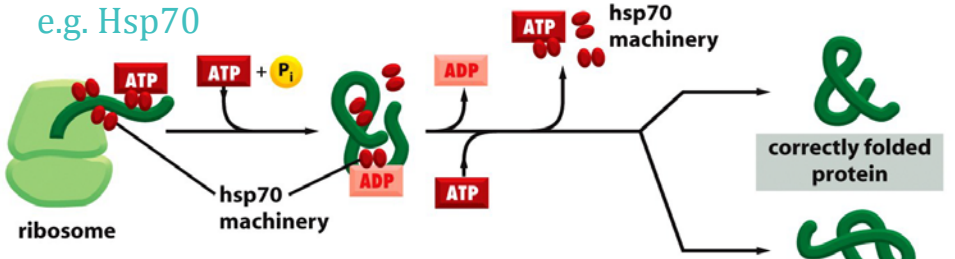
Protein Regulation- Protein Folding

-most proteins require a special class of proteins called ● chaperones for proper folding

-many chaperones are called ● heat-shock proteins (Hsp) since they are synthesized to high amounts by cells at elevated temperatures

deal with accidental denaturation & unfolding

e.g. Hsp70

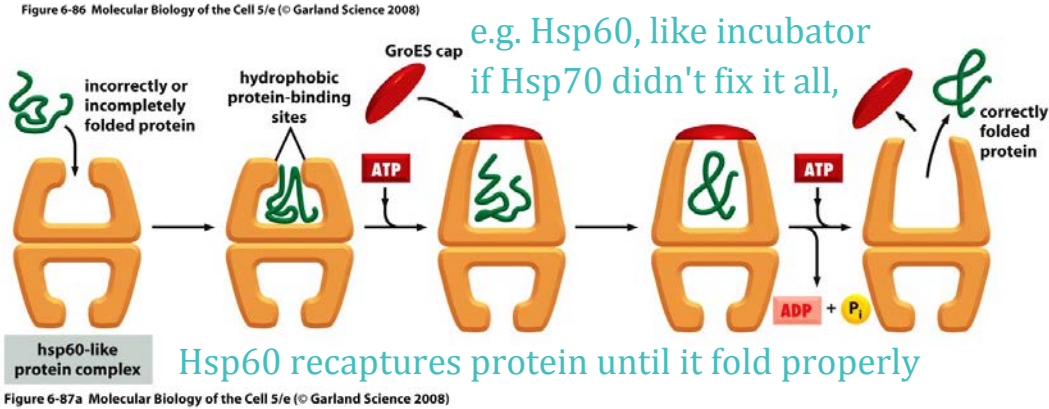


acts very early on soon when the protein emerges out of ribosome

Hsp70 and Hsp60 (chaperonin) assisted protein folding:

-Both interact with exposed ● hydrophobic residues of misfolded proteins

-Both use energy from ● ATP hydrolysis to promote proper folding



e.g. Hsp60, like incubator if Hsp70 didn't fix it all,

Hsp60 recaptures protein until it fold properly

Post-Translational Regulation

Protein Regulation- Protein Folding

e.g. Huntington's, Alzheimer's, Parkinson's

- improperly folded proteins can aggregate and become toxic to cells
- misfolded proteins are the cause of many inherited human diseases
- the process is closely monitored by a protein degrading apparatus called the proteasome (the protein garbage can)

-exposed hydrophobic residues mark protein for degradation by the proteasome; competes with chaperones for misfolded proteins

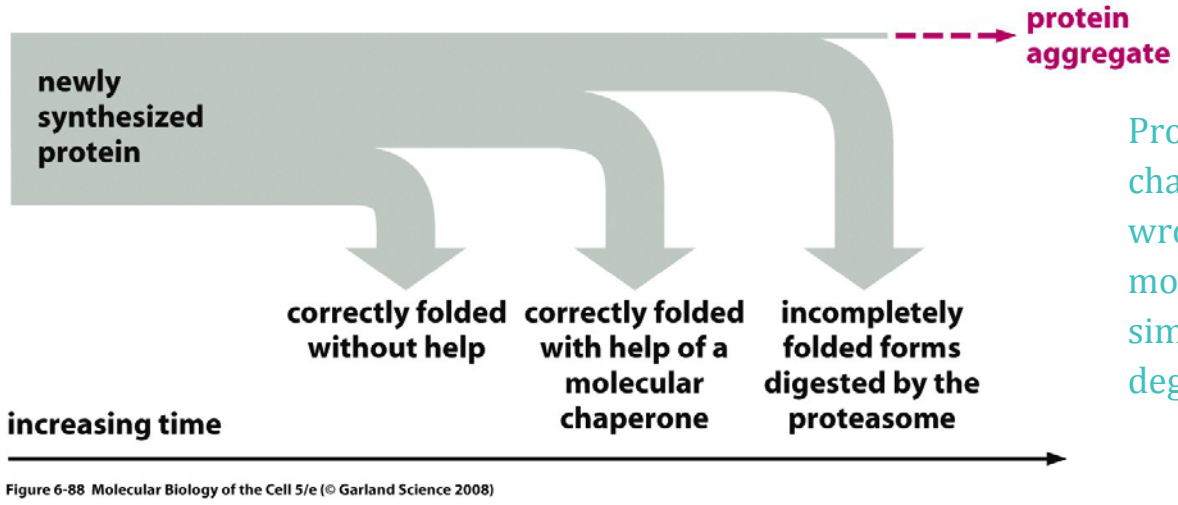


Figure 6-88 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Proteasome can't wait for chaperones to give the wrongly folded protein more chances anymore, so it simply digests it and degrades it.

- longer time to fold, more chance of being degraded

Post-Translational Regulation

Protein Regulation- Protein Destruction

- the proteasome is an abundant protein complex found in the
- cytosol and nucleus (~1% of cellular protein)
- hollow cylinder with cap at each end and active site in core, protects cellular proteins from degradation
-proteasome acts on proteins that have been marked for destruction by the addition of a small protein tag called ubiquitin

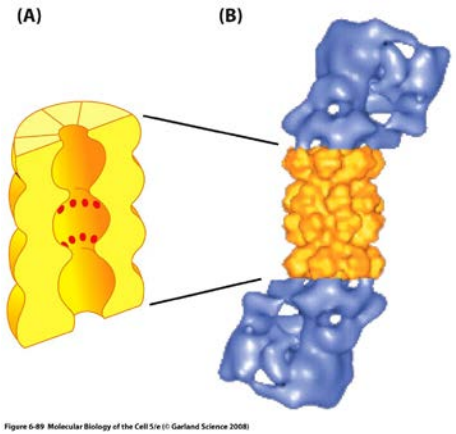


Figure 6-89 Molecular Biology of the Cell 5/e (© Garland Science 2008)

the red dots inside are active sites

polyubiquitin: a chain made out of ubiquitins

The whole chain gets swallowed.

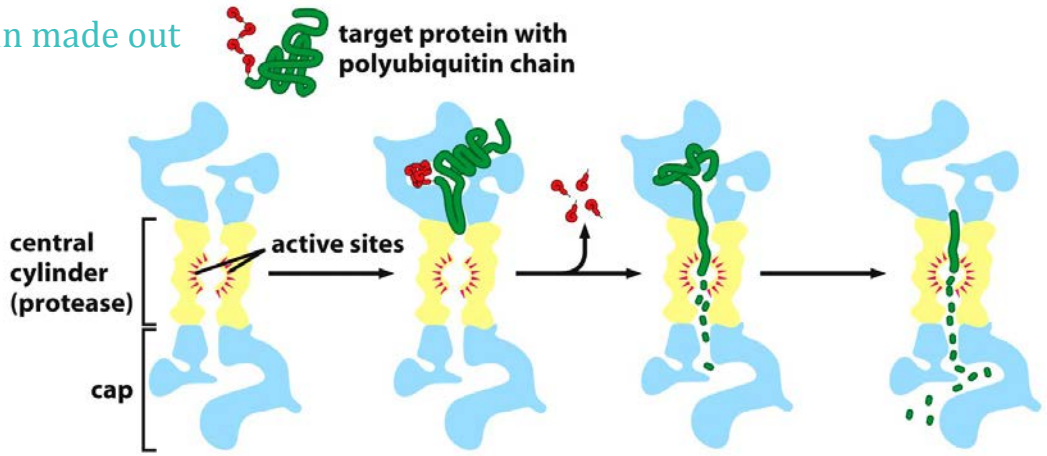


Figure 6-90 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Post-Translational Regulation

Protein Regulation- Protein Destruction

-ubiquitin is added to proteins by a ubiquitin-conjugating system made up of three enzymes

- E1: an ATP-dependent ● ubiquitin-activating enzyme creates an activated E1-bound ubiquitin

-E2: ● ubiquitin-conjugating enzyme accepts ubiquitin from E1 and exists as a complex with E3 ● selects substrates

- E2:E3 complex is called ● ubiquitin ligase transfers the proteins that are destined to be degraded

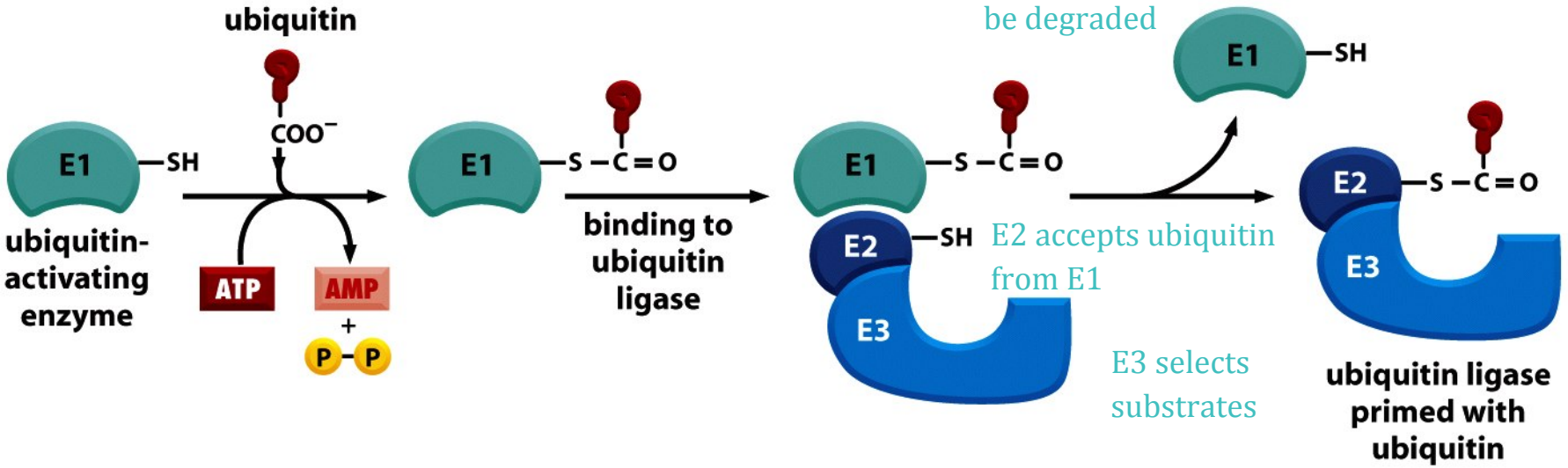


Figure 6-92b Molecular Biology of the Cell 5/e (© Garland Science 2008)

Post-Translational Regulation

Protein Regulation- Protein Destruction

- E3 binds to specific degradation sequences in substrates
- Ubiquitin molecule is added to lysine residue of target protein
- Process is repeated to form a polyubiquitin chain

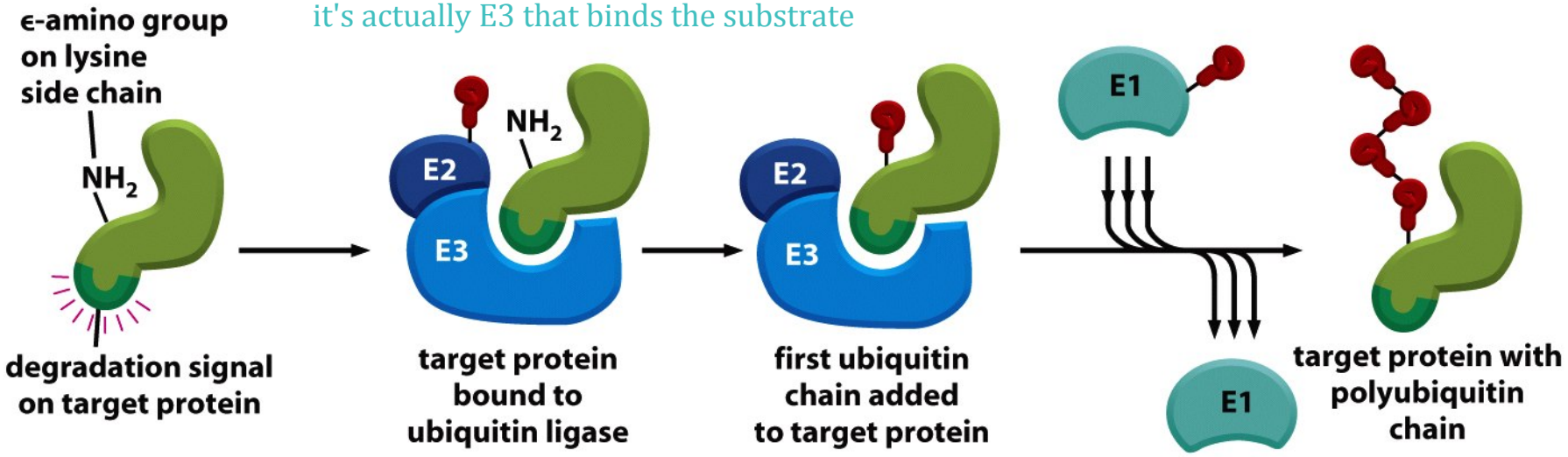


Figure 6-92c Molecular Biology of the Cell 5/e (© Garland Science 2008)

-polyubiquitin chain is recognized by specific receptor in the proteasome

Post-Translational Regulation

Protein Regulation-

- Ubiquitin modifications can have other functions
- Depends on
  - number of ubiquitin molecules and type of
  - linkage

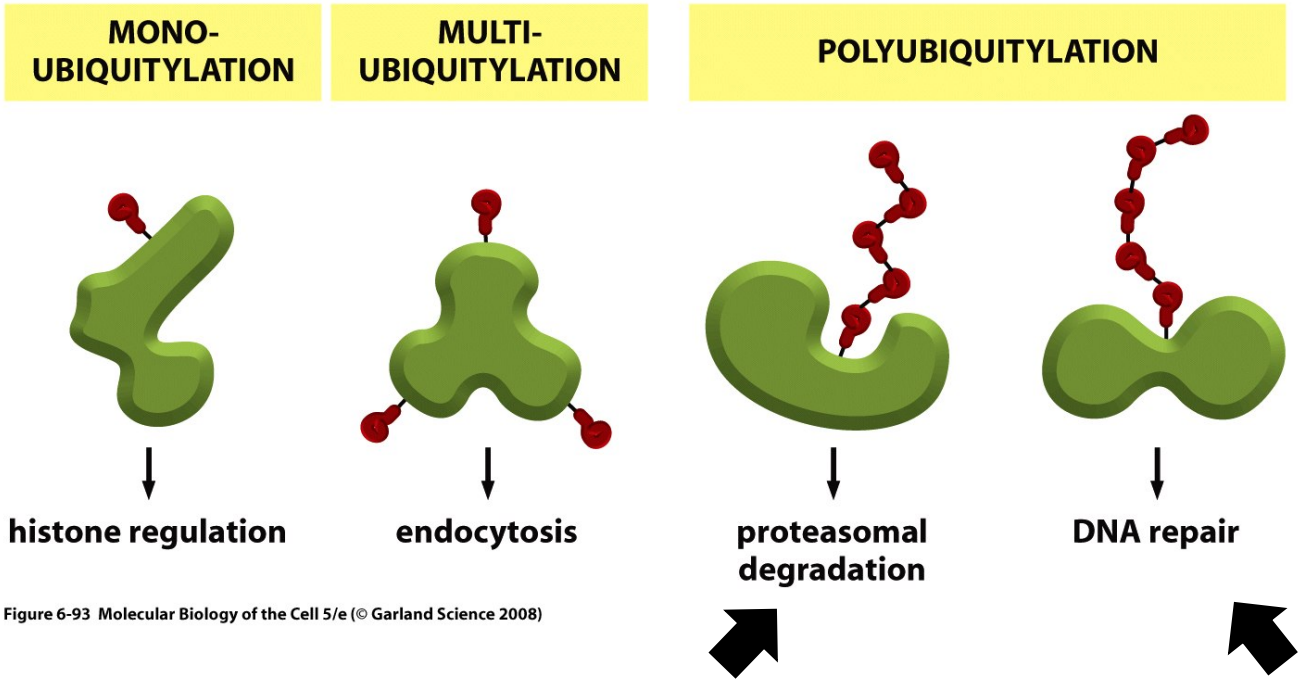


Figure 6-93 Molecular Biology of the Cell 5/e (© Garland Science 2008)

The difference in the linkages between these two polyubiquitin to the protein gives out different signals.

Linkage through Lys48 of ubiquitin

Linkage through Lys63 of ubiquitin

Post-Translational Regulation

Protein Regulation- Regulated Destruction

- the destruction of a protein by the proteasome can be regulated
- they are more stable under certain conditions – How?
  - Activation of a ubiquitin ligase

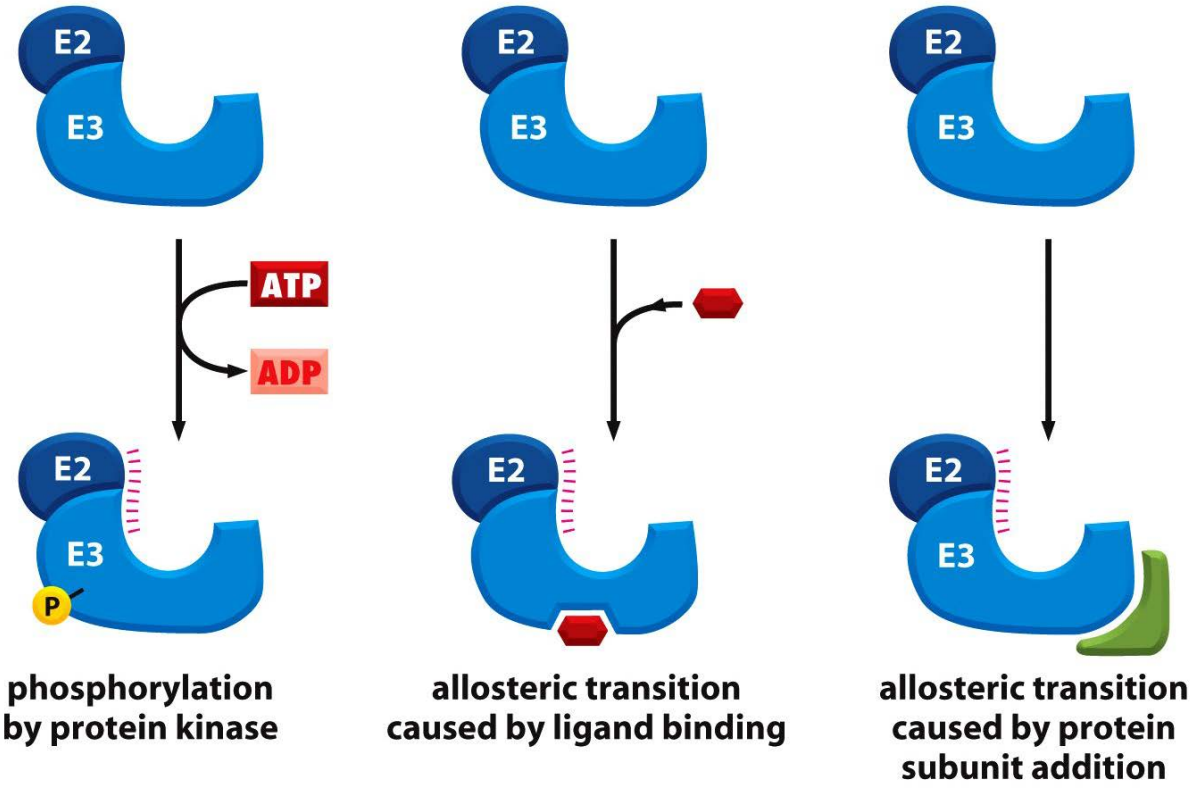


Figure 6-94a Molecular Biology of the Cell 5/e (© Garland Science 2008)

Post-Translational Regulation

Protein Regulation- Regulated Destruction

- Activation of a degradation signal The substrate is being regulated.

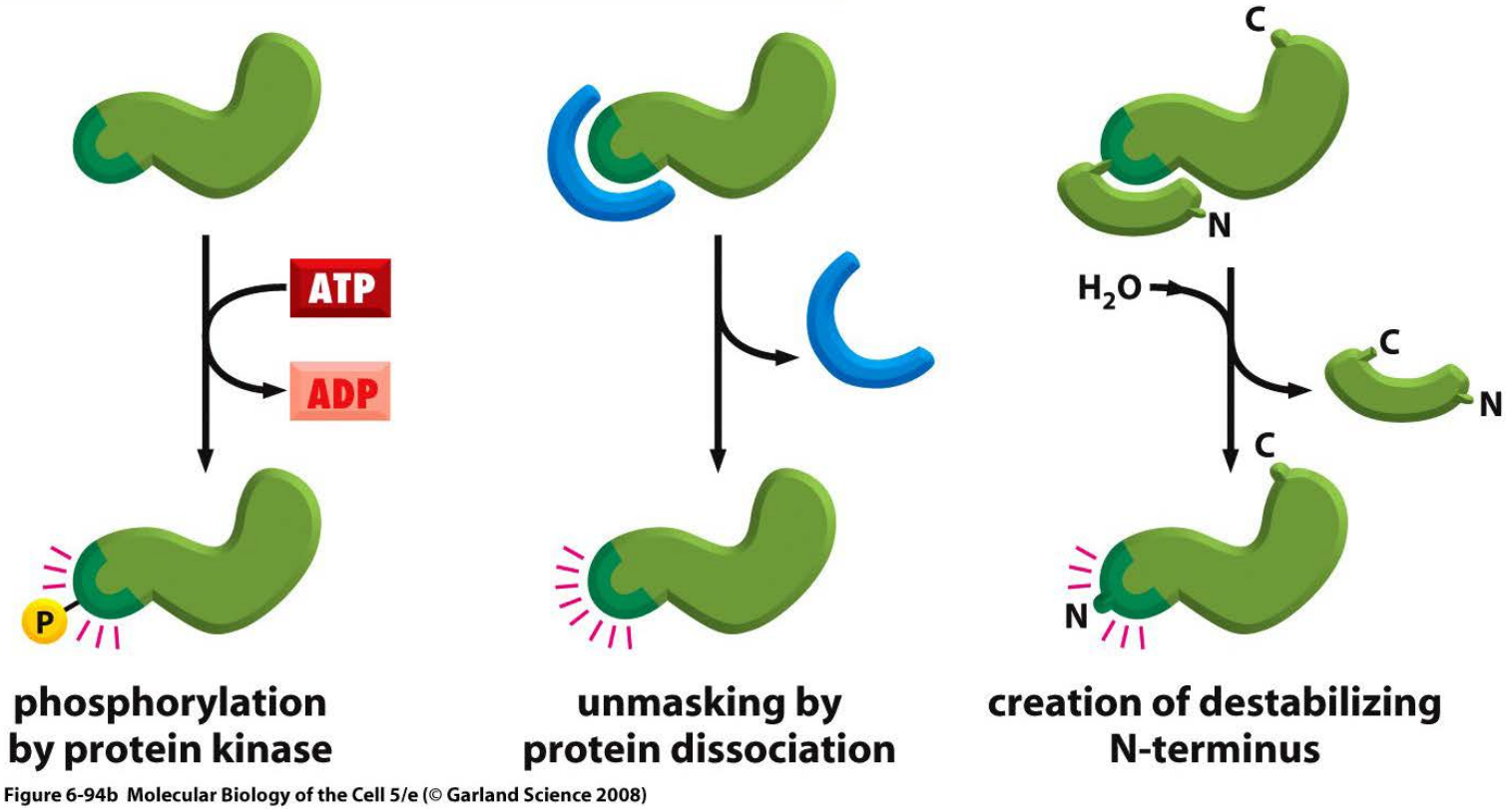


Figure 6-94b Molecular Biology of the Cell 5/e (© Garland Science 2008)

- Note how covalent modifications and protein associations can regulate protein function will be covered in next lecture

Clicker Question

Question: Treatment of cells with _____, should result in _____.

involved in protein folding

if no one helps folding the protein that needs help, everything will be recognized as wrongly folded and degraded by proteasome

A) HSP inhibitors; a decrease in protein degradation.

B) An iron chelator (removes excess cellular iron); a decrease in ferritin transcription not related

C) miRNA complementary to eIF2B; increase in transcription (GEF)

D) Bortezomib (a proteasome inhibitor); accumulation of polyubiquitinated proteins. a small molecule that inhibits proteasome

BIO 230

Lecture 8:

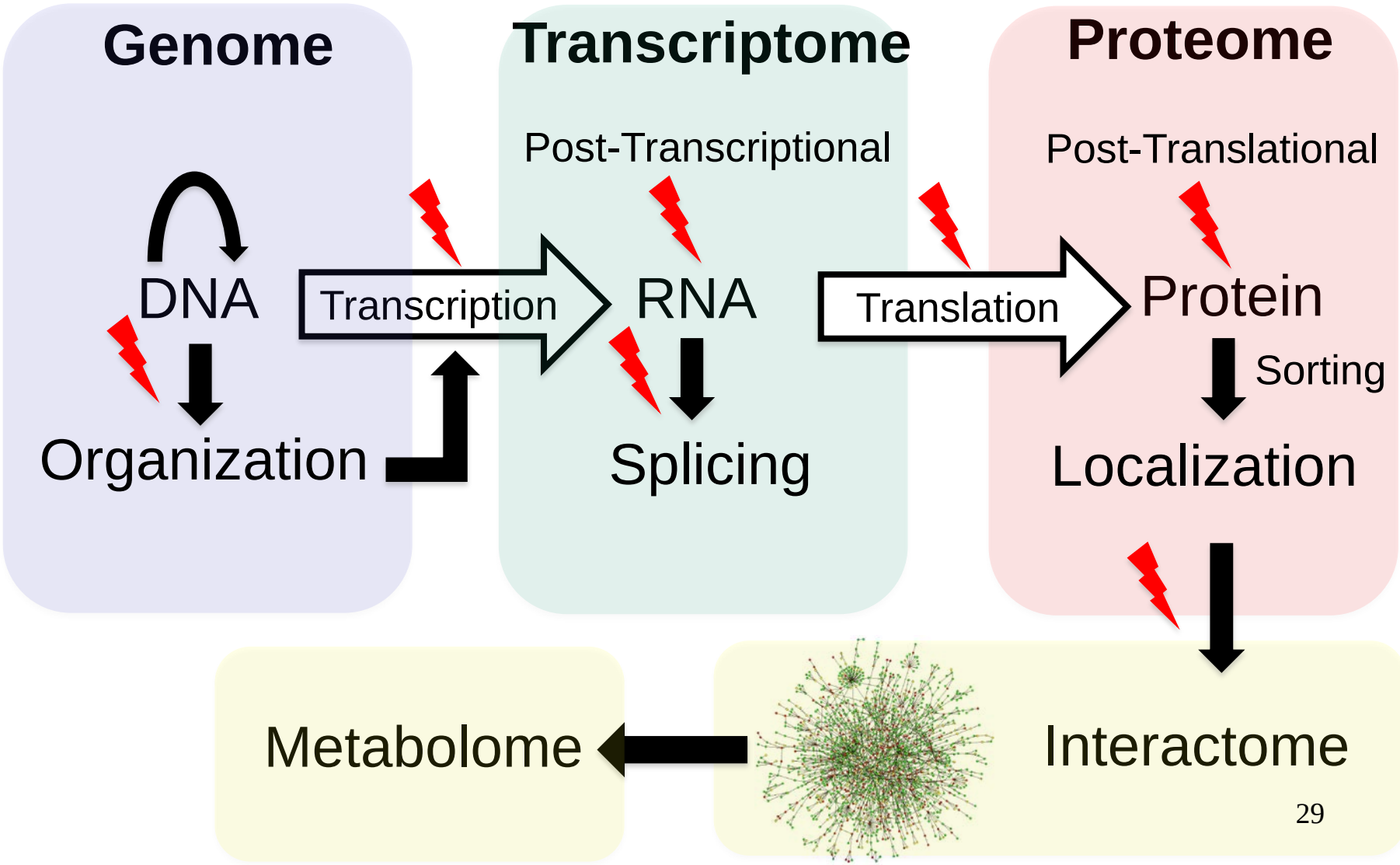
Regulation of the Proteome

1) Post-translational Regulation

Readings (Alberts *et al.*, custom text)

29-33;107-108; 226-228

Regulation of Genome Expression



Post-Translational Regulation

Protein Regulation

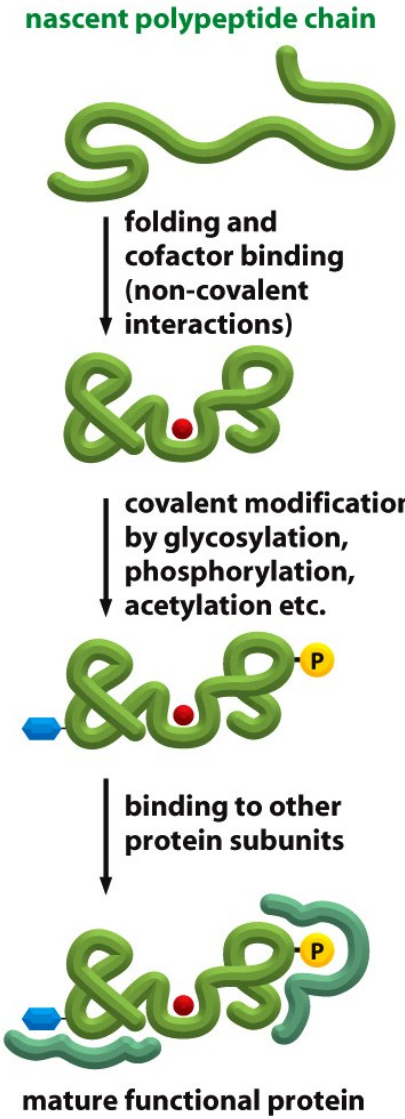
Proteins undergo a number of steps in order to become functional:

1) proteins must fold properly to adopt their 3D structure

2) proteins are covalently modified with chemical groups (eg. sugars, phosphate)

even ubiquitin binds covalently

3) proteins interact with other proteins and small molecules (cofactors)



Most protein actually need chaperones, can't really fold by themselves.

Aggregation of wrongly folded proteins is detrimental to the cells.

Ubiquitin conjugation system can tags the wrong proteins for proteasome to get rid of them

Figure 6-82 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Post-Translational Regulation

Covalent Post-Translational Modifications of Proteins

Phosphorylation e.g. eIF2/B

acetylation activates; methylation represses, e.g. histone. The generalization is not always true though.

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).

N-linked or spare gene-linked

(won't get into too much details)

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.

Table 3-3 Molecular Biology of the Cell 5/e (© Garland Science 2008)

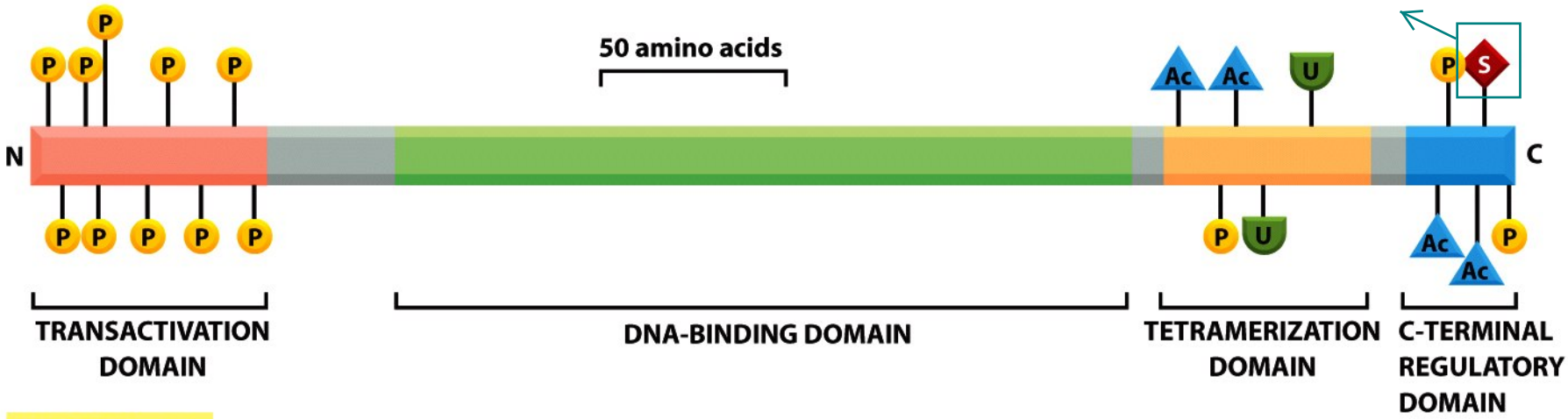
Ubiquitin on Lysine: depends on the linkage, it can repairs or remodel.

Post-Translational Regulation

Covalent Post-Translational Modifications of Proteins

- Multiple modifications can occur on the same protein

SUMO is one of the ubiquitins on human cells



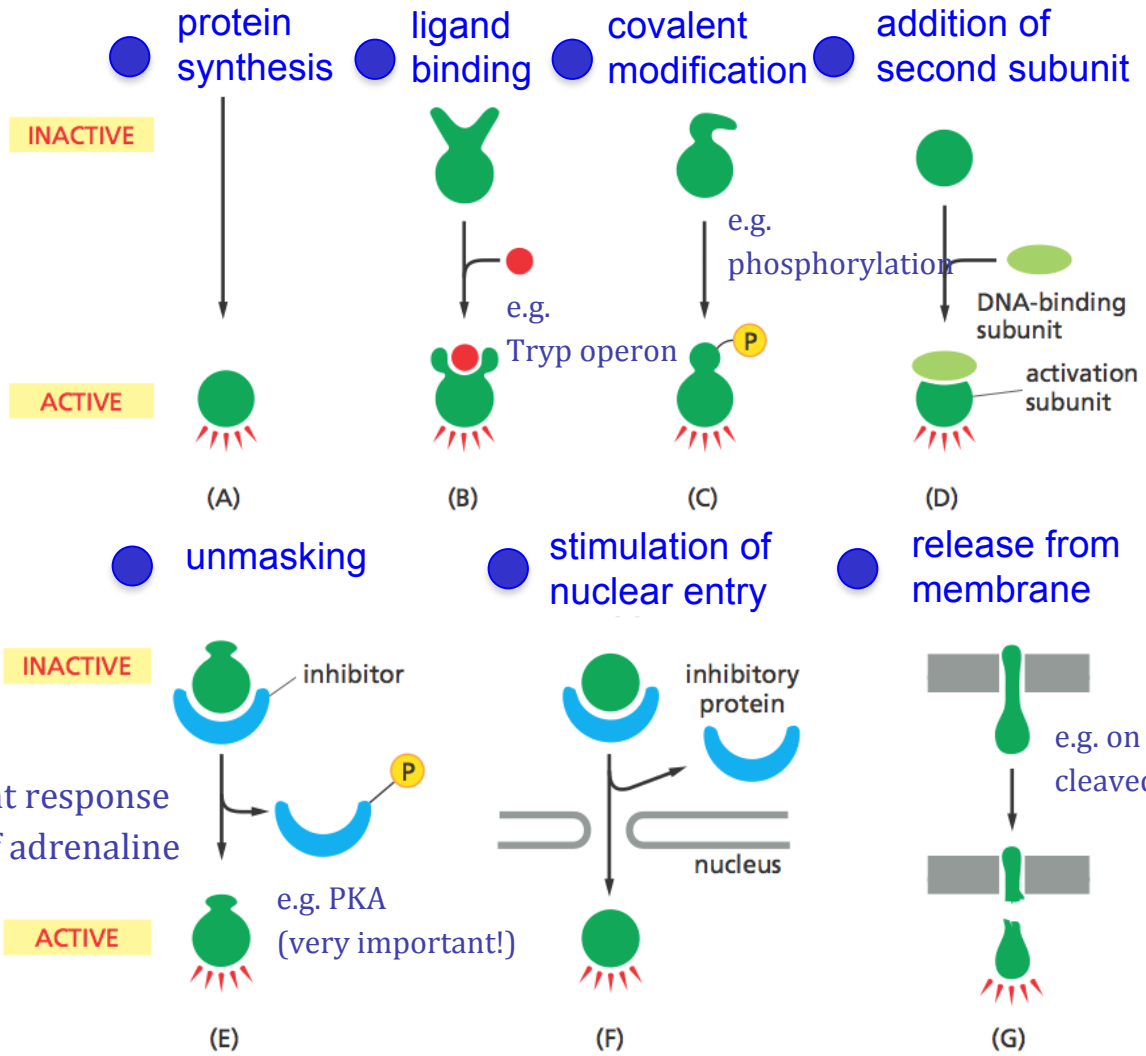
These proteins are very important for regulating transcription.

People who only inherit half p53 than normal tend to get cancer/tumor at early age;
HPV can inactivate p53 and lead to cervical cancer.
p53 is the most studied famous protein in cancer research.

Figure 3-81a Molecular Biology of the Cell 5/e (© Garland Science 2008)

Post-Translational Regulation

Post-translational regulation of gene regulatory proteins



Why is a gene expressed in one cell type but not another?
- chromatin structure of the cell
- gene regulatory proteins in the cell

PKA:
- fright-or-flight response
- production of adrenaline

Regulation of Gene Expression by PKA

Example: Protein Kinase A (PKA)

- Numerous extracellular stimuli result in increased levels of the small molecule :
  - cyclic AMP (cAMP)
- activation of protein kinase A (PKA)
- Inactive state: normally kept inactive
  - two regulatory subunits
  - two catalytic subunits

signaling pathway of producing adrenaline in fright-or-flight response

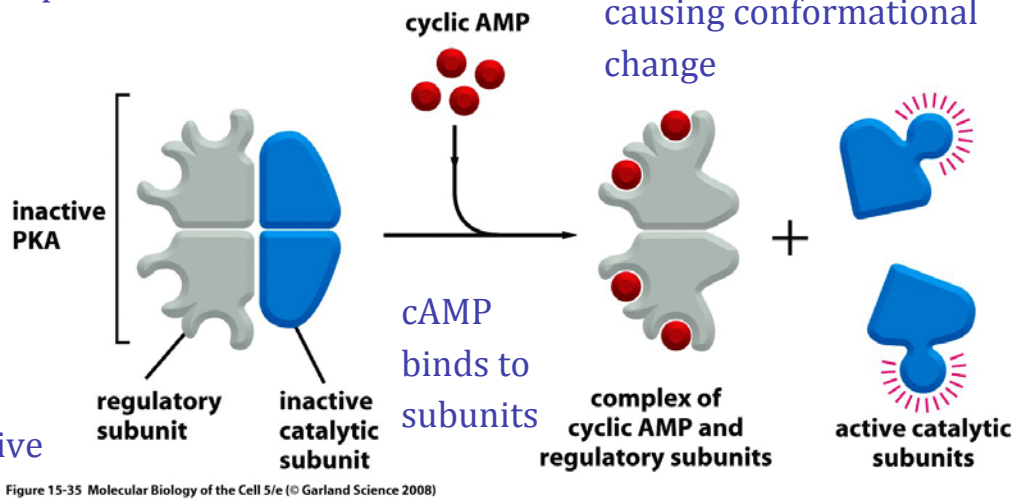


Figure 15-35 Molecular Biology of the Cell 5/e (© Garland Science 2008)

no activation, no working PKA

Binding of cAMP to regulatory subunits causes a

- conformational change and release of active catalytic subunits

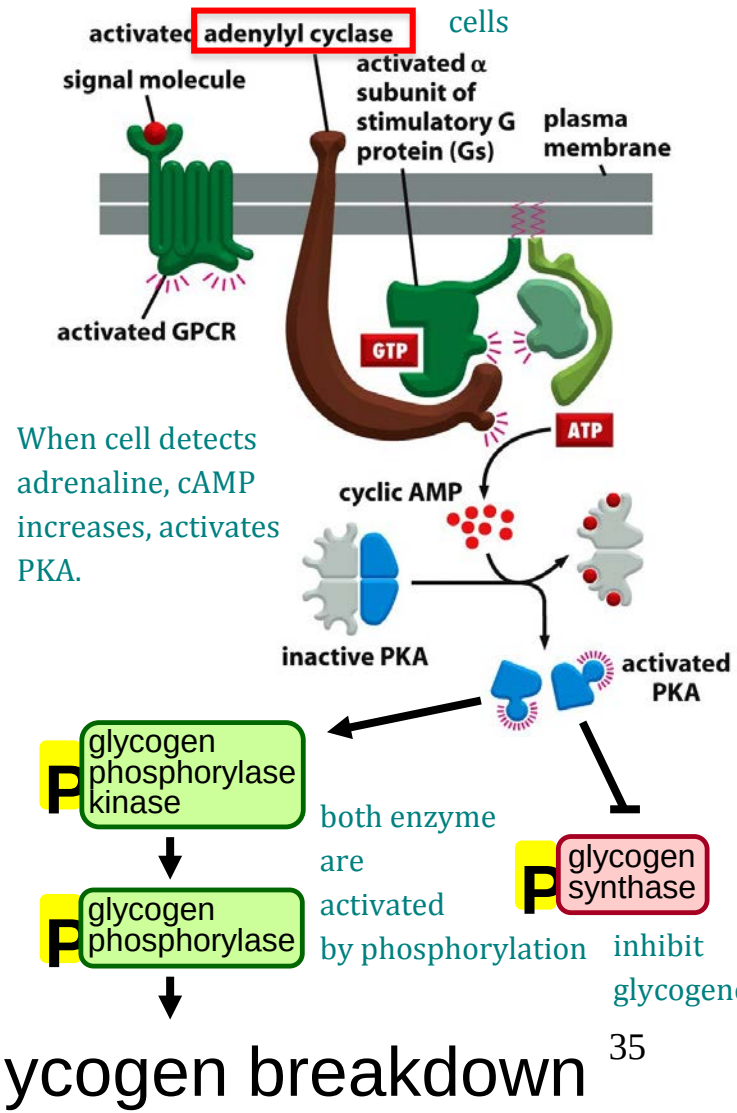
PKA Substrates Include Enzymes involved in Glycogen Metabolism in Skeletal Muscle and Liver

- ligand = adrenaline (epinephrine)
- response ➢ to promote glucose release

only one way is going at a time

- activated PKA has 2 effects:
  - ➢ promote breakdown of glycogen (glycogenolysis)
  - ➢ inhibit glycogen synthesis (glycogenesis)
- glycogen is broken down into glucose-1-phosphate (which is converted to glucose-6-phosphate → glycolytic pathway)

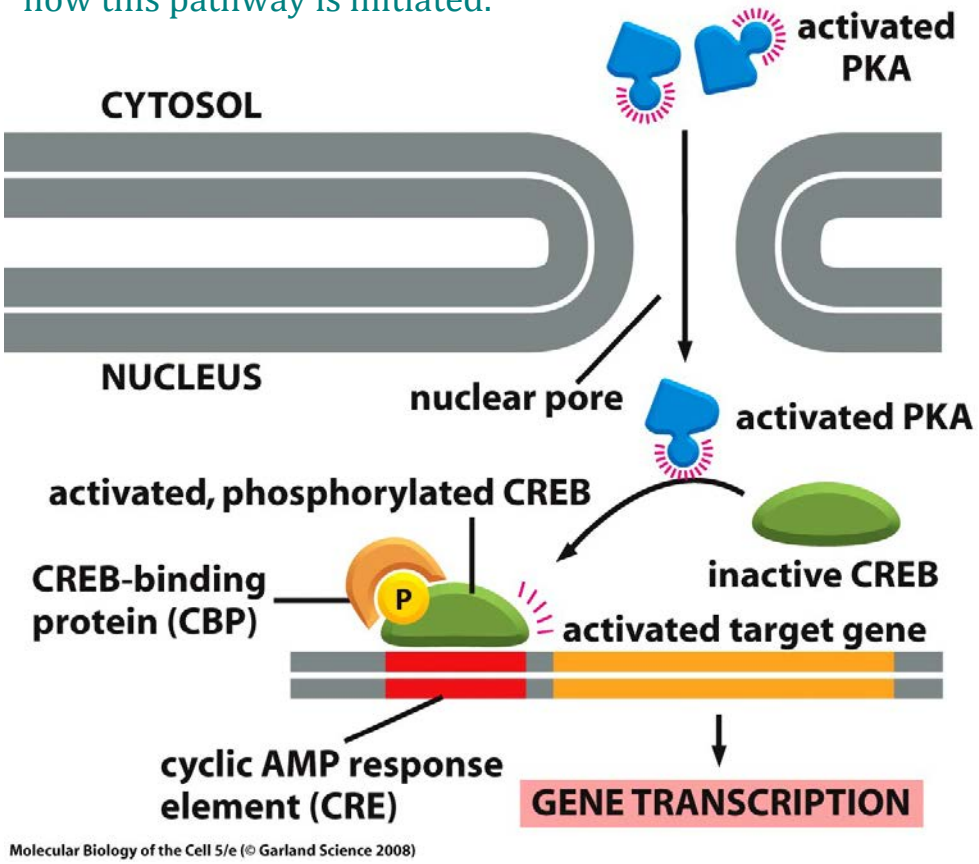
mainly in liver and muscle cells



Regulation of Gene Expression by PKA

- Inactive PKA is located in the
  - cytosol
- Activated PKA catalytic subunits translocate to the
  - nucleus
- PKA catalytic subunits
  - phosphorylate specific substrate proteins
  - activation of target genes with **c**AMP **R**esponsive **E**lements (CRE)

The second part of the course will focus more on how this pathway is initiated.



For this part of the course, focus on types of regulation and how they influence transcription.

Regulation of Gene Expression by PKA

- activation of target genes with **c**AMP **R**esponsive **E**lements (CRE)
- activated PKA
  - phosphorylates CREB (**CRE B**inding protein)
- CREB recruits CBP (**C**REB **B**inding **P**rotein) — coactivator

coactivator: does not have its own binding domain, but helps with activation

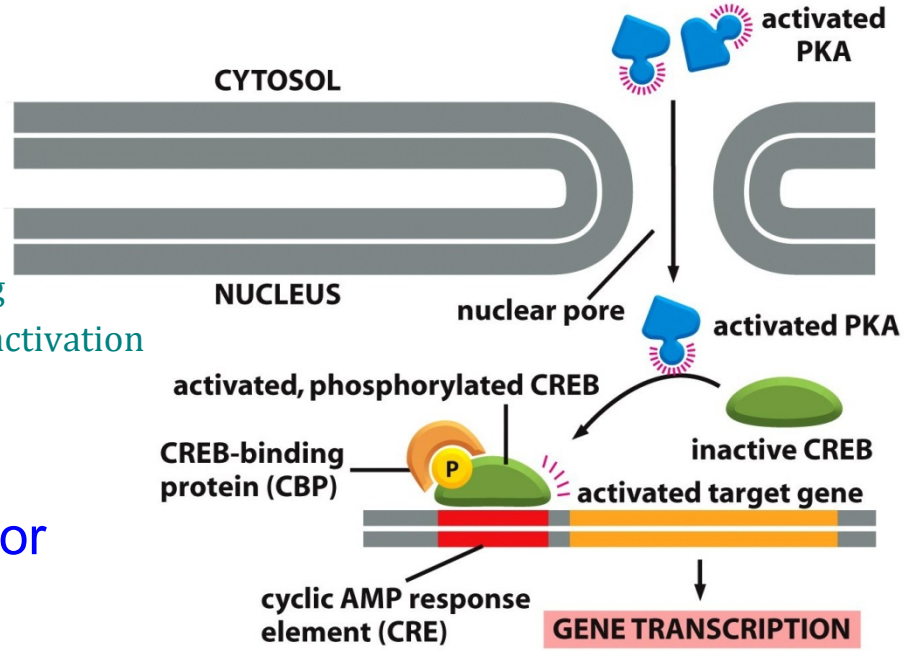


Figure 15-36 part 2 of 2 Molecular Biology of the Cell 5/e

➢ activate transcription of target genes

- e.g. liver: activation of the gene (glucose-6-phosphatase dephosphorylates glucose-6-P to glucose → released into blood)

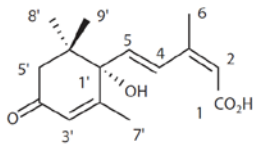
Now glucose can be uptaken by muscle cells to use for energy freely.

Glucose not phosphorylated can pass the cell membrane, but phosphorylated (G-6-P) can't pass membrane freely.

Regulation of Abscisic Acid (ABA) Signaling in Plants

Abscisic Acid - plant hormone

(ABA)



S-(+)-abscisic acid

Roles in:

Plant growth and development

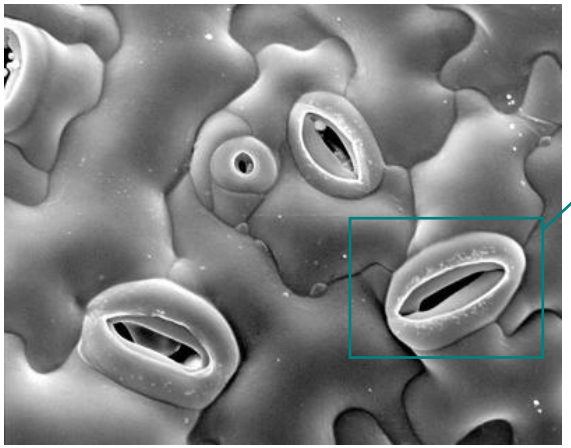
Regulating stomatal aperture

Adding abscisic acid will help plants regulate stomatas in drought stress and cope with wilting.

Responses to environmental stress (eg. drought)

Experiment was done in UofT.

Pore is the major exchange of gas between tissue and environment, regulating water loss.



guard cells regulate open/ close of pore

Regulation of Abscisic Acid (ABA) Signaling in Plants

Discovery of the Abscisic Acid Receptor – A UofT Link

Abscisic Acid Inhibits Type 2C Protein Phosphatases via the PYR/PYL Family of START Proteins

Sang-Youl Park,[1*] Pauline Fung,[2*] Noriyuki Nishimura,[4†] Davin R. Jensen,[8†] Hiroaki Fujii,[1] Yang Zhao,[2] Shelley Lumba,[2] Julia Santiago,[5] Americo Rodrigues,[5] Tsz-fung F. Chow,[2] Simon E. Alfred,[2] Dario Bonetta,[6] Ruth Finkelstein,[7] Nicholas J. Provart,[2,3] Darrell Desveaux,[2,3] Pedro L. Rodriguez,[5] Peter McCourt,[2] Jian-Kang Zhu,[1] Julian I. Schroeder,[4] Brian F. Volkman,[8] Sean R. Cutler[1,9,10,11‡]

Type 2C protein phosphatases (PP2Cs) are vitally involved in abscisic acid (ABA) signaling. Here, we show that a synthetic growth inhibitor called pyrabactin functions as a selective ABA agonist. Pyrabactin acts through *PYRABACTIN RESISTANCE 1* (*PYR1*), the founding member of a family of START proteins called PYR/PYLs, which are necessary for both pyrabactin and ABA signaling in vivo. We show that ABA binds to PYR1, which in turn binds to and inhibits PP2Cs. We conclude that PYR/PYLs are ABA receptors functioning at the apex of a negative regulatory pathway that controls ABA signaling by inhibiting PP2Cs. Our results illustrate the power of the chemical genetic approach for sidestepping genetic redundancy.

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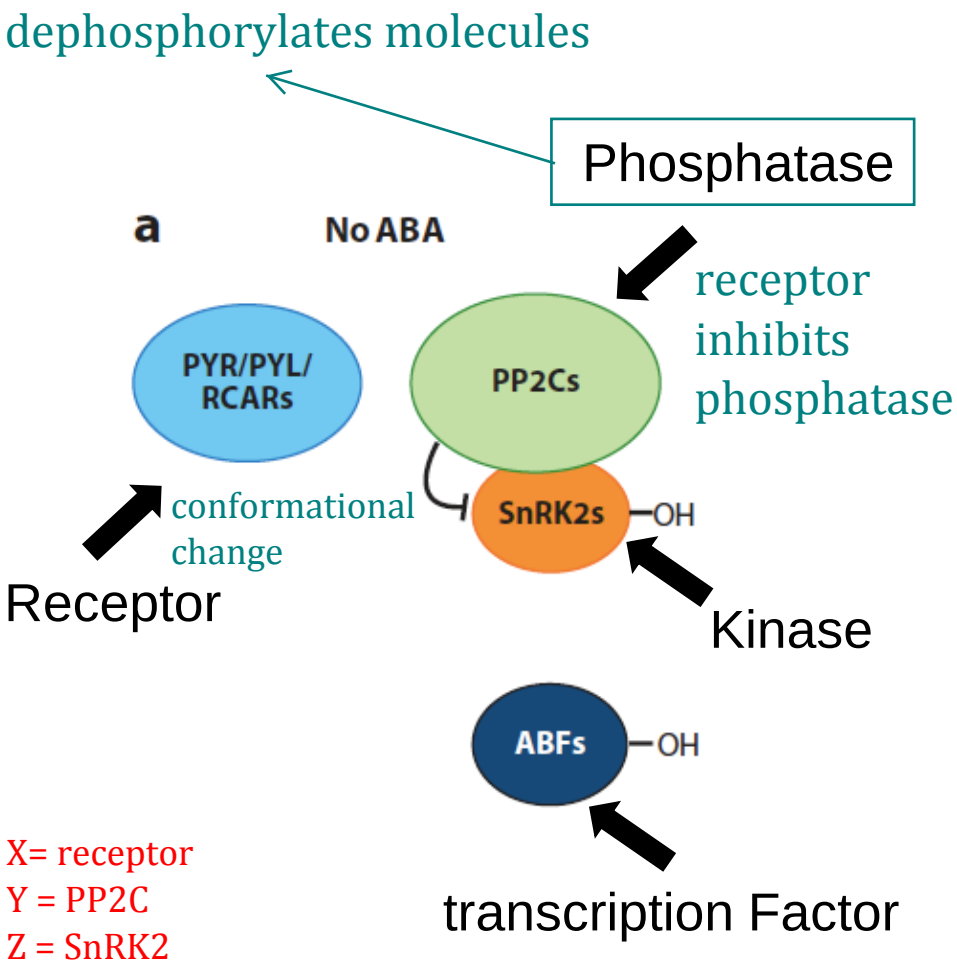
Regulation of Abscisic Acid (ABA) Signaling in Plants



X= receptor
Y = PP2C
Z = SnRK2

negative regulation on a negative regulator

X ⊣ Y ⊣ Z

In the absence of ABA

- PP2C phosphatases inhibit ● SnRK2 kinases

- SnRK2 kinases cannot activate ABF transcription factors

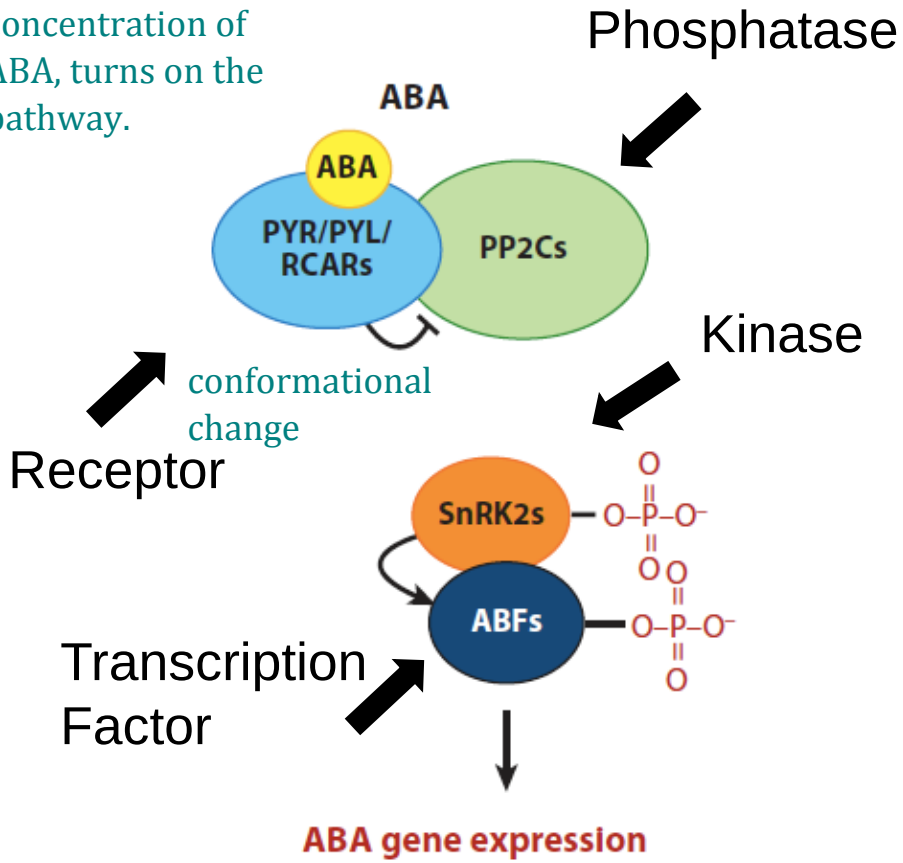
- ABF transcription factors are ● inactive

- ABA-responsive gene expression is ● off

These are all protein-protein interaction.

Regulation of Abscisic Acid (ABA) Signaling in Plants

Drought increases concentration of ABA, turns on the pathway.



Recall: X ⊣ Y ⊣ Z

In the presence of ABA

-ABA binds ABA receptor and induces a
- conformational change

- ABA receptor binds and inhibits PP2C phosphatases

- SnRK2 kinases - activate ABF transcription factors

- ABA-responsive gene expression is
- on

Post-Translational Regulation

Why Are Protein Interactions Important?

- Protein interactions lie at the heart of most biological processes

- Proteins interact with small molecules, nucleic acids and/or other proteins

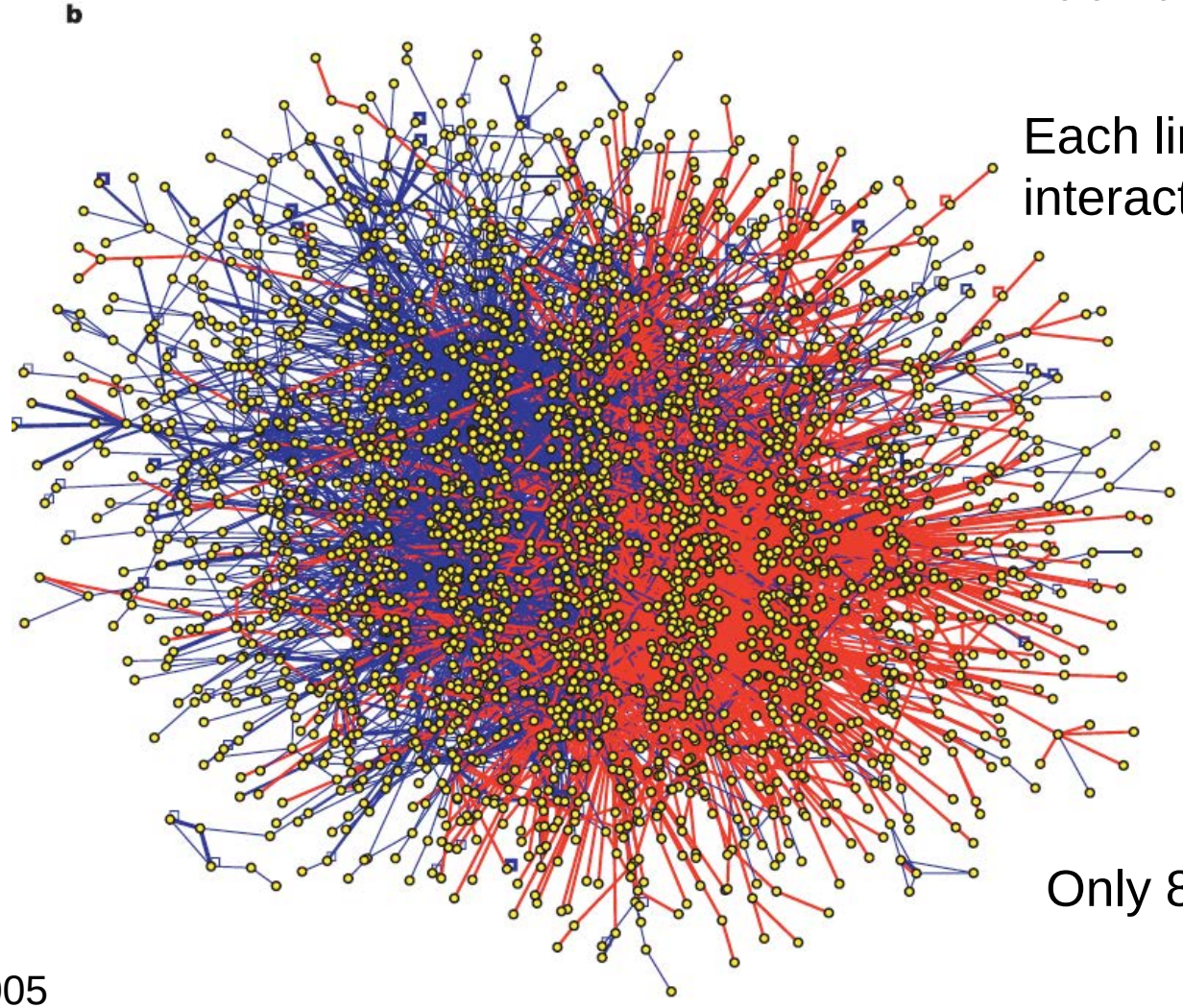
- Proteins usually function in large multi-protein complexes composed of ● static and ● transient interactions

- ● Interactome map is the complete collection of protein-protein interactions of an organism

tons of reactions; some are transient and hard to catch; some are across different cell types; some are only visible under certain stimulus.

Post-Translational Regulation

Human Interactome: Version 1



Each dot is a protein

● node

Each line is an interaction ● edge

These terms will show up in literature all the time.

Only 8000 proteins

Rual et al., 2005

Post-Translational Regulation

Protein Interactions

Large chunks of genomes is still unclear to us. The map gives you a organized way of summarizing the knowledge

Skp, Cullin, F-box containing complex

SCF E3 Ubiquitin Ligase

Skp / Cullin / F-box

Many times of F-box can be found in this substrate complex.

Scientists can find out which F-box is involved in cell cycle regulation or choosing methionine biosynthesis regulation, kinetochore complexes.

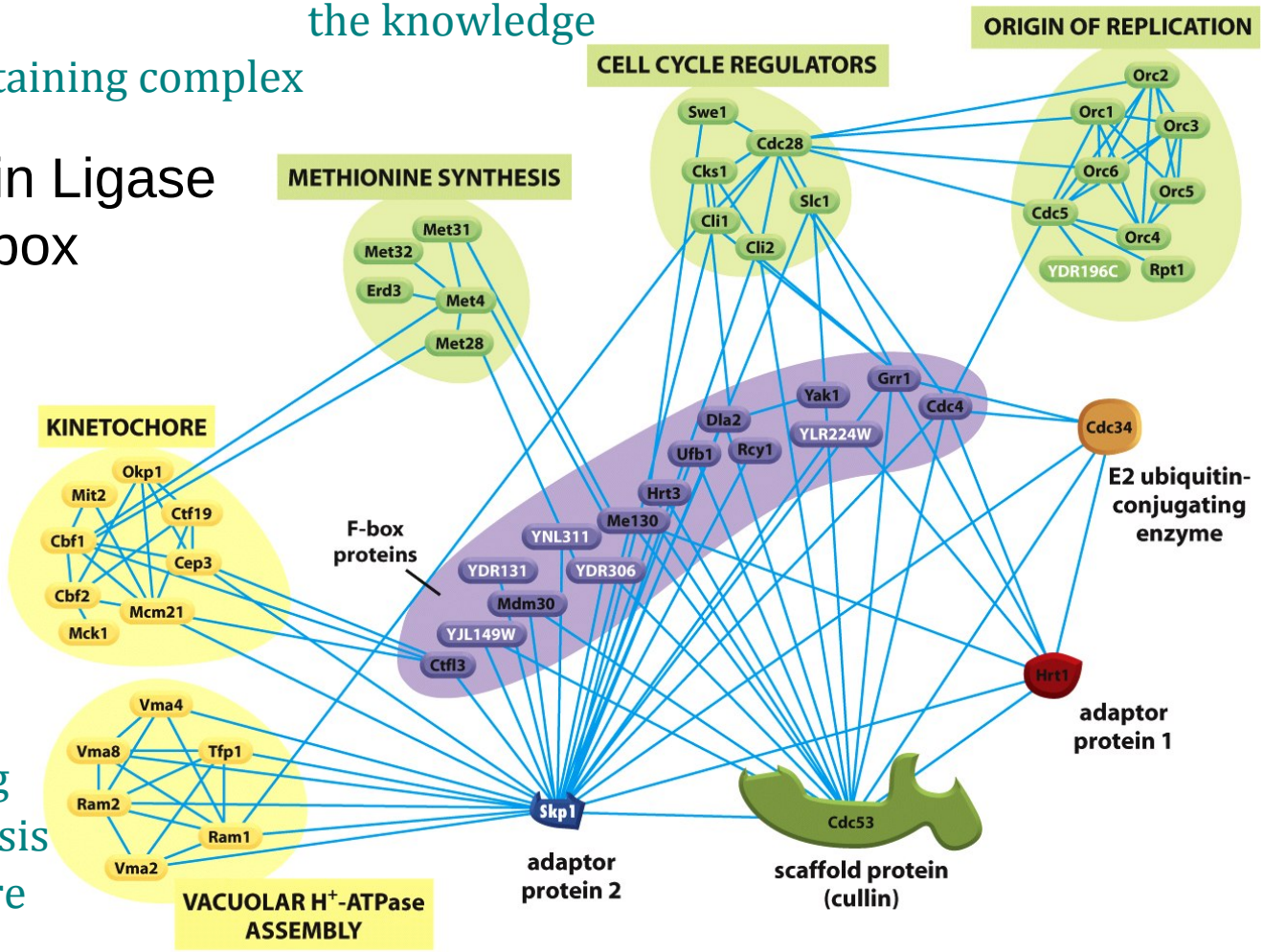


Figure 3-82 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Post-Translational Regulation

Protein Interactions: A UofT Link

Science is a very elaborate process. Many people are involved in one research, unlike only a few people getting Nobel Prize in the past. Research requires collaborative effort.

Interaction network containing conserved and essential protein complexes in *Escherichia coli*

Gareth Butland[1], José Manuel Peregrín-Alvarez[2], Joyce Li[1], Wehong Yang[1], Xiaochun Yang[1], Veronica Canadien[3], Andrei Starostine[1], Dawn Richards[3], Bryan Beattie[3], Nevan Krogan[1], Michael Davey[1], John Parkinson[2,4,5], Jack Greenblatt[1,3,5] & Andrew Emili[1,5]

[1]*Banting and Best Department of Medical Research, University of Toronto, 112 College Street, Toronto, Ontario M5G 1L6, Canada*
[2]*Hospital for Sick Children, 555 University Avenue, Toronto, Ontario M4K 1X8, Canada*
[3]*Affinium Pharmaceuticals, 100 University Avenue, Toronto, Ontario M5J 1V6, Canada*
[4]*Department of Biochemistry and* [5]*Department of Medical Genetics and Microbiology, University of Toronto, Medical Sciences Building, 1 King's College Circle, Toronto, Ontario M5S 1A8, Canada*

Proteins often function as components of multi-subunit complexes. Despite its long history as a model organism[1], no large-scale analysis of protein complexes in *Escherichia coli* has yet been reported. To this end, we have targeted DNA cassettes into the *E. coli* chromosome to create carboxy-terminal, affinity-tagged alleles of 1,000 open reading frames (~23% of the

letters to nature

Systematic identification of protein complexes in *Saccharomyces cerevisiae* by mass spectrometry

Yuen Ho*, Albrecht Gruhler*, Adrian Heilbut*, Gary D. Bader†‡, Lynda Moore*, Sally-Lin Adams*, Anna Millar*, Paul Taylor*, Keiryn Bennett*, Kelly Boutilier*, Lingyun Yang*, Cheryl Wolting*, Ian Donaldson*, Søren Schandorff*, Juanita Shewnarane*, Mai Vo*†, Joanne Taggart*†, Marilyn Goudreault*†, Brenda Muskat*, Cris Alfarano*, Danielle Dewar†, Zhen Lin†, Katerina Michalickova†‡, Andrew R. Willems†§, Holly Sassi†, Peter A. Nielsen*, Karina J. Rasmussen*, Jens R. Andersen*, Lene E. Johansen*, Lykke H. Hansen*, Hans Jespersen*, Alexandre Podtelejnikov*, Eva Nielsen*, Janne Crawford*, Vibeke Poulsen*, Birgitte D. Sørensen*, Jesper Matthiesen*, Ronald C. Hendrickson*, Frank Gleeson*, Tony Pawson†§, Michael F. Moran*, Daniel Durocher†§, Matthias Mann*, Christopher W. V. Hogue*†‡, Daniel Figeys* & Mike Tyers†§

** MDS Proteomics, 251 Attwell Drive, Toronto, Canada M9W 7H4, and Staermosegaardsvej 6, DK-5230 Odense M, Denmark*
† Programme in Molecular Biology and Cancer, Samuel Lunenfeld Research Institute, Mount Sinai Hospital, 600 University Avenue, Toronto, Canada, M5G 1X5
‡ Department of Biochemistry, University of Toronto, 1 Kings College Circle, Toronto, Canada M5S 1A8
§ Department of Medical Genetics and Microbiology, University of Toronto, 1 Kings College Circle, Toronto, Canada M5S 1A8

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ARTICLES

Global landscape of protein complexes in the yeast *Saccharomyces cerevisiae*

Nevan J. Krogan[1,2]*†, Gerard Cagney[1,3]*, Haiyuan Yu[4], Gouqing Zhong[1], Xinghua Guo[1], Alexandr Ignatchenko[1], Joyce Li[1], Shuye Pu[3], Nira Datta[1], Aaron P. Tikuisis[1], Thanuja Punna[1], José M. Peregrín-Alvarez[5], Michael Shales[1], Xin Zhang[1], Michael Davey[1], Mark D. Robinson[1], Alberto Paccanaro[4], James E. Bray[1], Anthony Sheung[1], Bryan Beattie[6], Dawn P. Richards[6], Veronica Canadien[6], Atanas Lalev[1], Frank Mena[6], Peter Wong[1], Andrei Starostine[1], Myra M. Canete[1], James Vlasblom[3], Samuel Wu[3], Chris Orsi[3], Sean R. Collins[7], Shamanta Chandran[1], Robin Haw[1], Jennifer J. Rilstone[1], Kiran Gandi[1], Natalie J. Thompson[1], Gabe Musso[1], Peter St Onge[1], Shaun Ghanny[1], Mandy H. Y. Lam[1,2], Gareth Butland[1], Amin M. Altaf-Ul[8], Shigehiko Kanaya[8], Ali Shilatifard[9], Erin O'Shea[10], Jonathan S. Weissman[7], C. James Ingles[1,2], Timothy R. Hughes[1,2], John Parkinson[5], Mark Gerstein[4], Shoshana J. Wodak[5], Andrew Emili[1,2] & Jack F. Greenblatt[1,2]

Identification of protein–protein interactions often provides insight into protein function, and many cellular processes are performed by stable protein complexes. We used tandem affinity purification to process 4,562 different tagged proteins of the yeast *Saccharomyces cerevisiae*. Each preparation was analysed by both matrix-assisted laser desorption/ionization-time of flight mass spectrometry and liquid chromatography tandem mass spectrometry to increase coverage and accuracy. Machine learning was used to integrate the mass spectrometry scores and assign probabilities to the protein–protein interactions. Among 4,087 different proteins identified with high confidence by mass spectrometry from 2,357 successful purifications, our core data set (median precision of 0.69) comprises 7,123 protein–protein interactions involving 2,708 proteins. A Markov clustering algorithm organized these interactions into 547 protein complexes averaging 4.9 subunits per complex, about half of them absent from the MIPS database, as well as 429 additional interactions between pairs of complexes. The data (all of which are available online) will help future studies on individual proteins as well as functional genomics and systems biology.

Protein interactions can provide insights into function: Guilt by Association

Guilt by Association provides insight of protein function, can generate hypothesis.

Post-Translational Regulation

Protein Interactions:

"Guilt by Association"

DNA Damage Response Network

letters to nature

Systematic identification of protein complexes in *Saccharomyces cerevisiae* by mass spectrometry

Yuen Ho*, Albrecht Gruhler*, Adrian Heilbut*, Gary D. Bader†‡, Lynda Moore*, Sally-Lin Adams*, Anna Millar*, Paul Taylor*, Keiryn Bennett*, Kelly Boutilier*, Lingyun Yang*, Cheryl Wolting*, Ian Donaldson*, Søren Schandorff*, Juanita Shewnarane*, Mai Vo*†, Joanne Taggart*†, Marilyn Goudreault*†, Brenda Muskat*, Cris Alfarano*, Danielle Dewar†, Zhen Lin†, Katerina Michalickova†‡, Andrew R. Willems†§, Holly Sassi†, Peter A. Nielsen*, Karina J. Rasmussen*, Jens R. Andersen*, Lene E. Johansen*, Lykke H. Hansen*, Hans Jespersen*, Alexandre Podtelejnikov*, Eva Nielsen*, Janne Crawford*, Vibeke Poulsen*, Birgitte D. Sørensen*, Jesper Matthiesen*, Ronald C. Hendrickson*, Frank Gleeson*, Tony Pawson†§, Michael F. Moran*, Daniel Durocher†§, Matthias Mann*, Christopher W. V. Hogue*†‡, Daniel Figeys* & Mike Tyers†§

* *MDS Proteomics, 251 Attwell Drive, Toronto, Canada M9W 7H4, and Staermosegaardsvej 6, DK-5230 Odense M, Denmark*
† *Programme in Molecular Biology and Cancer, Samuel Lunenfeld Research Institute, Mount Sinai Hospital, 600 University Avenue, Toronto, Canada, M5G 1X5*
‡ *Department of Biochemistry, University of Toronto, 1 Kings College Circle, Toronto, Canada M5S 1A8*
§ *Department of Medical Genetics and Microbiology, University of Toronto, 1 Kings College Circle, Toronto, Canada M5S 1A8*