BME 42-620 Engineering Molecular Cell Biology

Lecture 19:

Protein Degradation

Control of Gene Expression

Cell Signaling

Chapters 6, 7, & 15

Comments on Report for Project Assignment 2

- Please place all files to be submitted in one file directory with an identifiable name. A flash drive will be provided in class to collect the files.

- The follow components should be included
  - Required calculation results
  - Description of main ideas of your implementation
  - Any specific details you feel are important
  - <u>Instructions on how to run your code</u>
  - <u>General description of the files submitted</u>

Outline

- Ribosome structure and antibiotics design
- Chaperones
- Protein degradation

- Overview of gene expression control
- Noise in gene expression
- Overview of cell signaling

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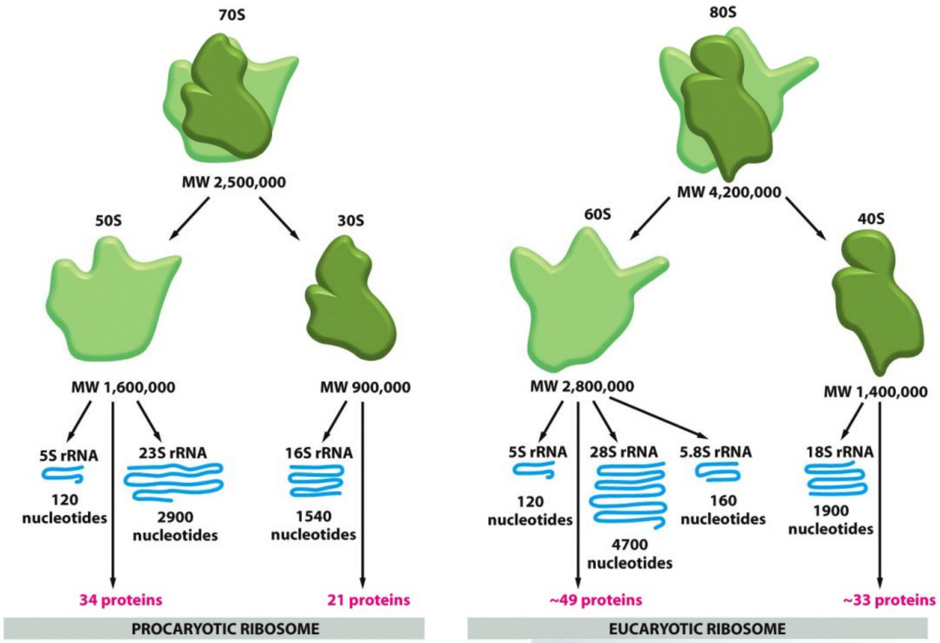
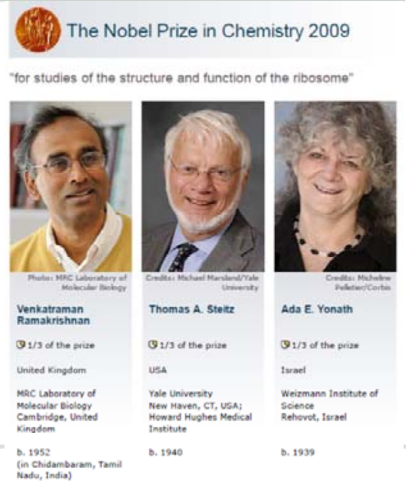
Ribosome Structure

- Main function: base pairing mRNA codon with tRNA anticodons to synthesize the polypeptide chain.
- Overall, more than 50 ribosomal proteins and rRNAs.
- Structure well conserved between bacteria and eukaryotes.
- Small subunit & large subunit first synthesized in the nucleus.





The Nobel Prize in Chemistry 2009

"for studies of the structure and function of the ribosome"

Photo: MRC Laboratory of Molecular Biology

Venkatraman Ramakrishnan

1/3 of the prize

United Kingdom

MRC Laboratory of Molecular Biology
Cambridge, United Kingdom

b. 1952
(in Chidambaram, Tamil Nadu, India)

Credits: Michael Marsland/Yale University

Thomas A. Steitz

1/3 of the prize

USA

Yale University
New Haven, CT, USA;
Howard Hughes Medical Institute

b. 1940

Credits: Micheline Pelletier/Corbis

Ada E. Yonath

1/3 of the prize

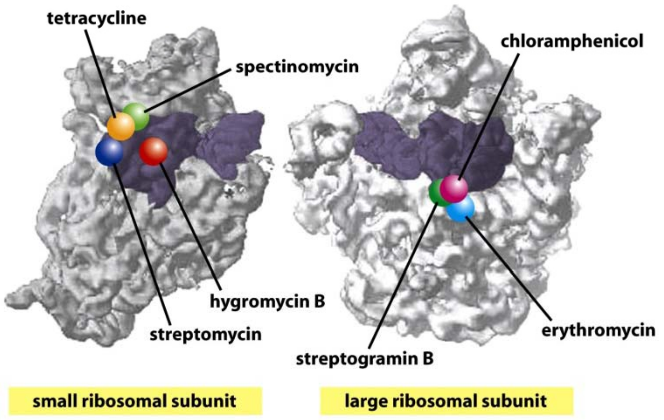
Israel

Weizmann Institute of Science
Rehovot, Israel

b. 1939

Ribosome & Antibiotics

- A strategy of designing antibiotics is to preferentially block bacterial protein synthesis.
  - Fungus (fungi): eukaryotic organisms that include yeasts, molds, and mushrooms.



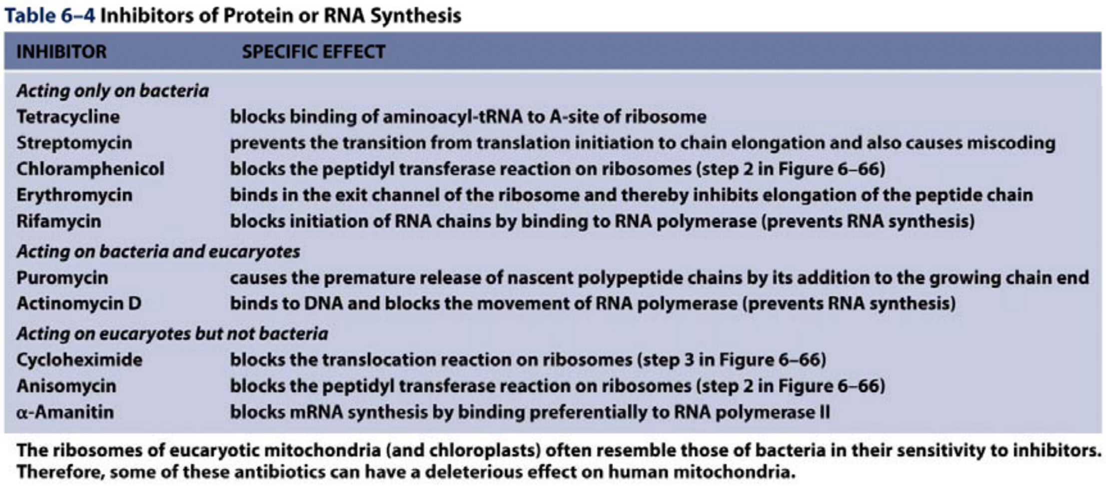
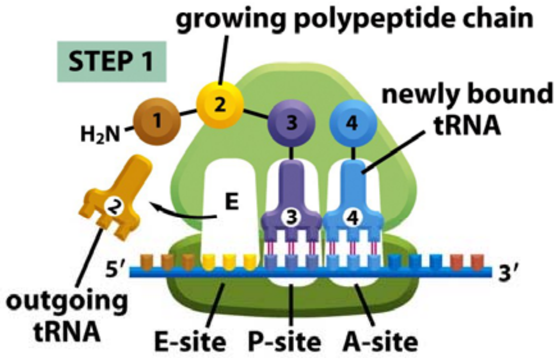


Table 6–4 Inhibitors of Protein or RNA Synthesis

INHIBITOR	SPECIFIC EFFECT
Acting only on bacteria	
Tetracycline	blocks binding of aminoacyl-tRNA to A-site of ribosome
Streptomycin	prevents the transition from translation initiation to chain elongation and also causes miscoding
Chloramphenicol	blocks the peptidyl transferase reaction on ribosomes (step 2 in Figure 6–66)
Erythromycin	binds in the exit channel of the ribosome and thereby inhibits elongation of the peptide chain
Rifamycin	blocks initiation of RNA chains by binding to RNA polymerase (prevents RNA synthesis)
Acting on bacteria and eucaryotes	
Puromycin	causes the premature release of nascent polypeptide chains by its addition to the growing chain end
Actinomycin D	binds to DNA and blocks the movement of RNA polymerase (prevents RNA synthesis)
Acting on eucaryotes but not bacteria	
Cycloheximide	blocks the translocation reaction on ribosomes (step 3 in Figure 6–66)
Anisomycin	blocks the peptidyl transferase reaction on ribosomes (step 2 in Figure 6–66)
α-Amanitin	blocks mRNA synthesis by binding preferentially to RNA polymerase II

The ribosomes of eucaryotic mitochondria (and chloroplasts) often resemble those of bacteria in their sensitivity to inhibitors. Therefore, some of these antibiotics can have a deleterious effect on human mitochondria.



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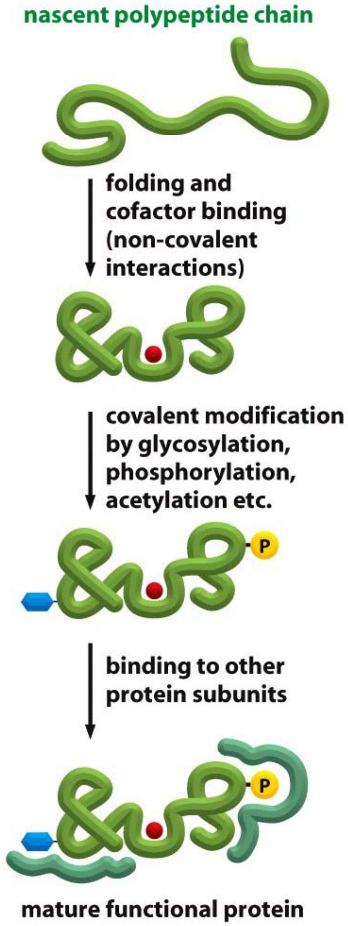
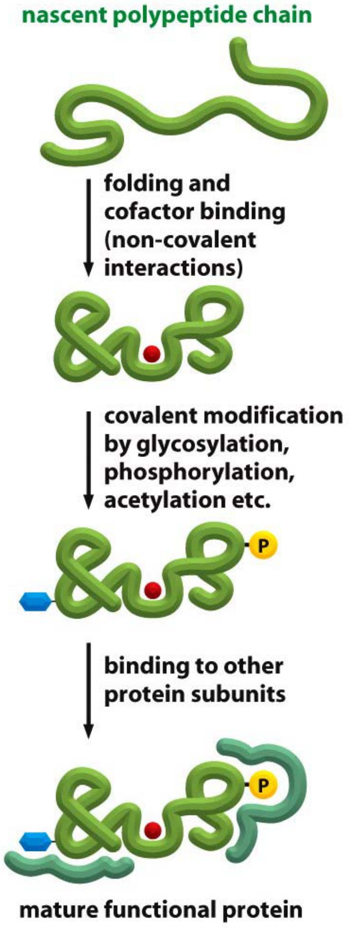
From Nascent Polypeptide Chain to Functional Protein

- Several additional steps are required to convert a nascent polypeptide chain into a functional protein.
- Some of these steps require noncovalent bond formation.
- Others require covalent modifications.



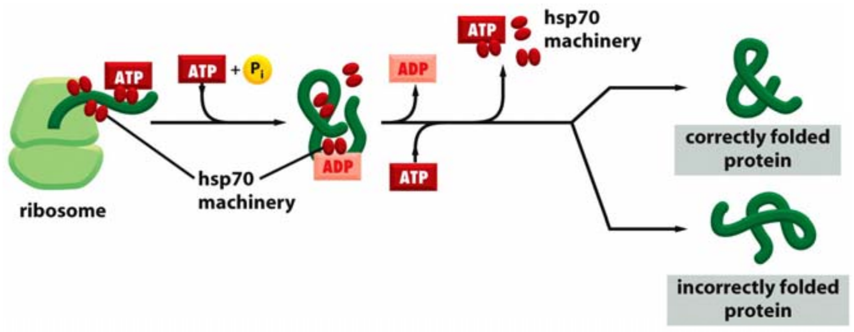
Chaperone-Assisted Protein Folding (I)

- Chaperones can prevent premature aggregation of hydrophobic regions.
- Chaperones can assist folding.
- Many chaperones are heat-shock proteins.
- There are multiple families of chaperones
  - Hsp60, Hsp70, Hsp90, Hsp40
  - High affinity for exposed hydrophobic domains.

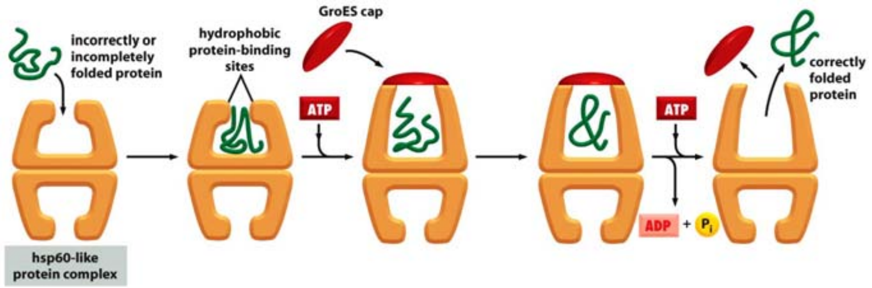


Chaperons-Assisted Protein Folding (II)

- Hsp70 chaperons
  - Most prevalent. Act early in the synthesis of protein.
  - Binds and releases peptides with hydrophobic residues.



- Hsp60 chaperons
  - Assist folding of synthesized peptides.
  - Different names
    - Hsp60 in mitochondria;
    - GroEL/GroES in bacteria



- Exposed hydrophobic regions are important signals for quality control.

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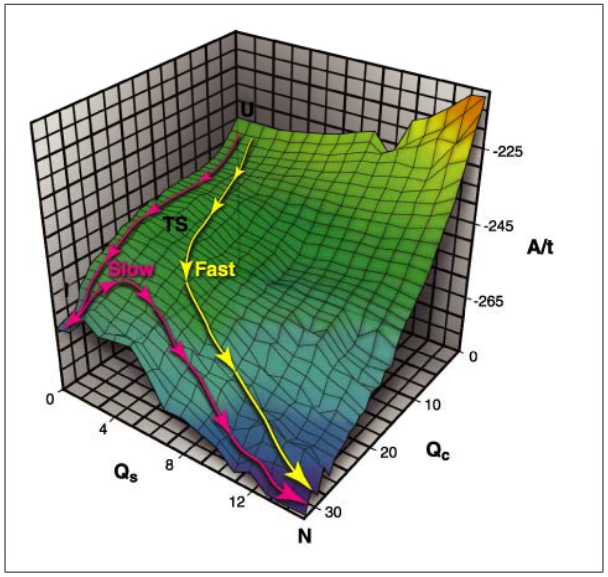
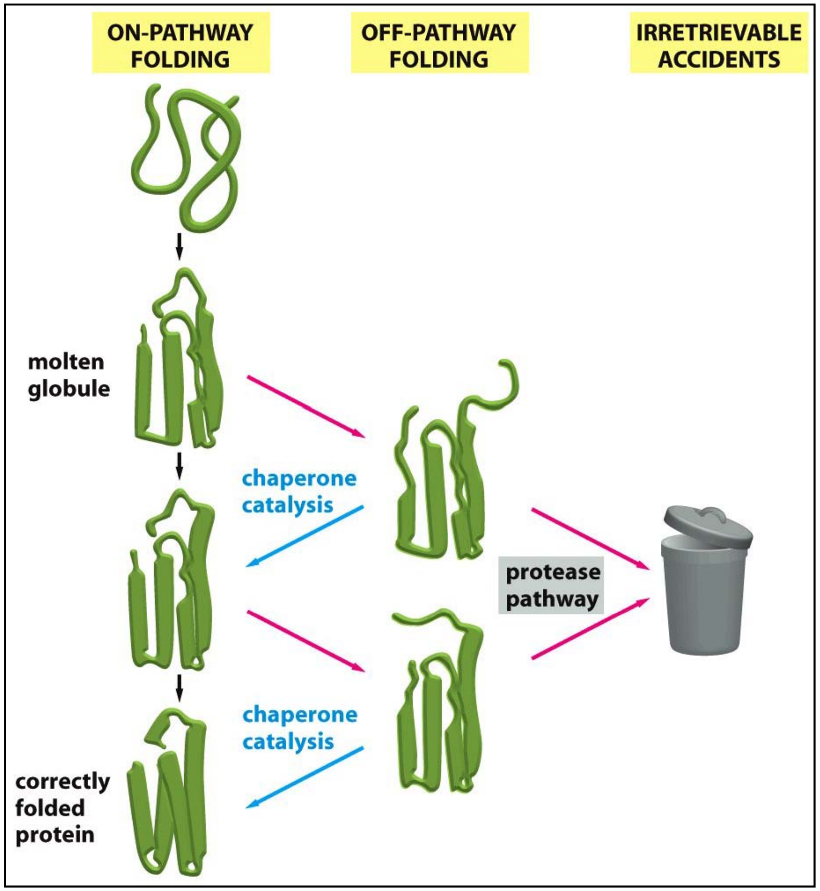
Different Outcomes of Newly Synthesize Proteins





ON-PATHWAY FOLDING

OFF-PATHWAY FOLDING

IRRETRIEVABLE ACCIDENTS

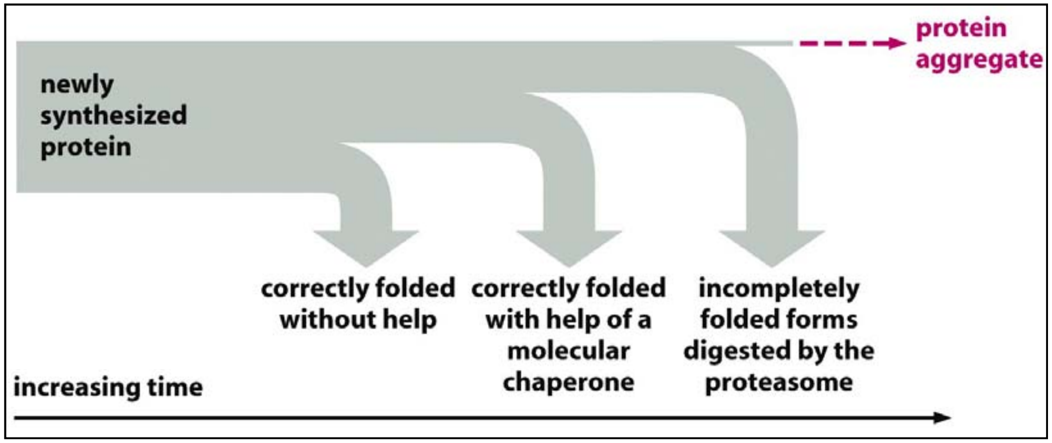
molten globule

chaperone catalysis

chaperone catalysis

protease pathway

correctly folded protein



newly synthesized protein

protein aggregate

correctly folded without help

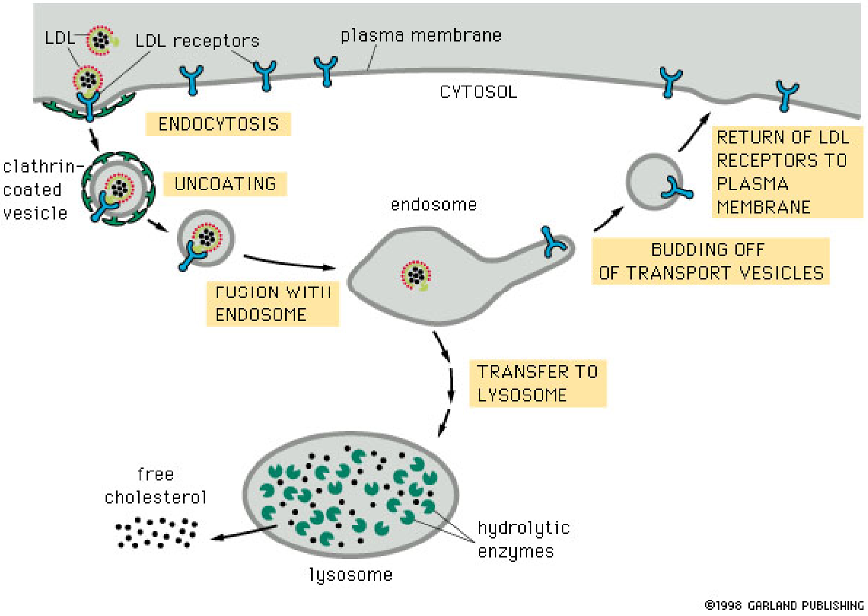
correctly folded with help of a molecular chaperone

incompletely folded forms digested by the proteasome

increasing time

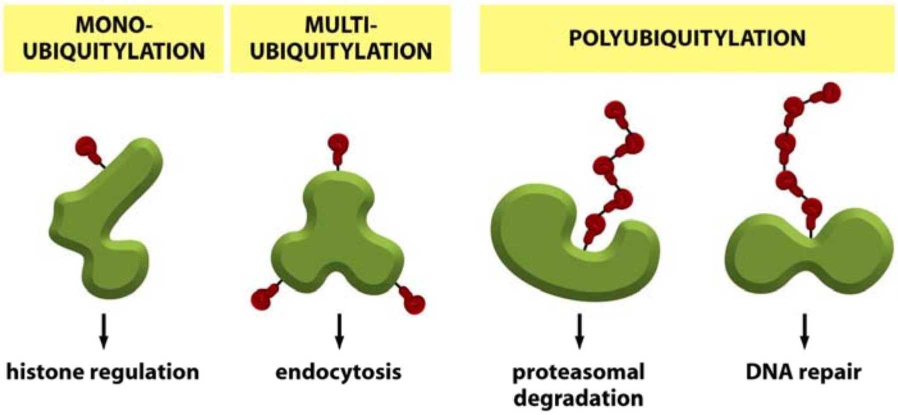
Protein Degradation Pathways

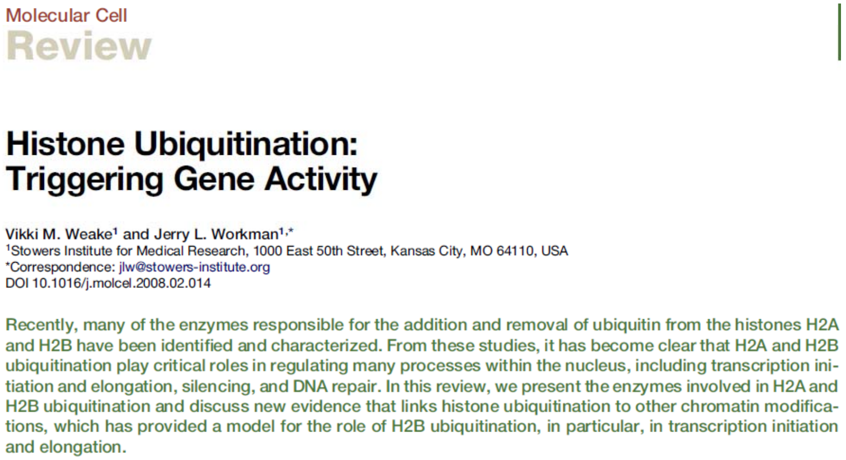
- Approximately 1/3 of newly synthesized proteins are degraded.
- Lysosome degrades proteins and lipids taken in by endocytosis.
- Proteasome is present in both nucleus and cytoplasm.
- Proteasome degrades both cytoplasmic and nuclear proteins after they are marked through conjugation with ubiquitin.



Different Ubiquitylation Related Pathways

- A ubiquitin contains 76 amino acids.
- Ubiquitin conjugation requires a elaborate system of hundres of protein factors.




Molecular Cell
Review

Histone Ubiquitination: Triggering Gene Activity

Vikki M. Weake[1] and Jerry L. Workman[1,*]
[1]Stowers Institute for Medical Research, 1000 East 50th Street, Kansas City, MO 64110, USA
*Correspondence: jlw@stowers-institute.org
DOI 10.1016/j.molcel.2008.02.014

Recently, many of the enzymes responsible for the addition and removal of ubiquitin from the histones H2A and H2B have been identified and characterized. From these studies, it has become clear that H2A and H2B ubiquitination play critical roles in regulating many processes within the nucleus, including transcription initiation and elongation, silencing, and DNA repair. In this review, we present the enzymes involved in H2A and H2B ubiquitination and discuss new evidence that links histone ubiquitination to other chromatin modifications, which has provided a model for the role of H2B ubiquitination, in particular, in transcription initiation and elongation.

Weake & Workman, Molecular Cell, 29:653:663, 2008

References on Protein Misfolding Response

- M. Schroder, RJ Kaufman, The mammalian unfolded protein response, *Ann. Rev. Biochem*. 24:739-789,2005.

- R. Sitia, I. Braakman, Quality control in the ER protein factory, *Nature*, 426:891-894, 2003.

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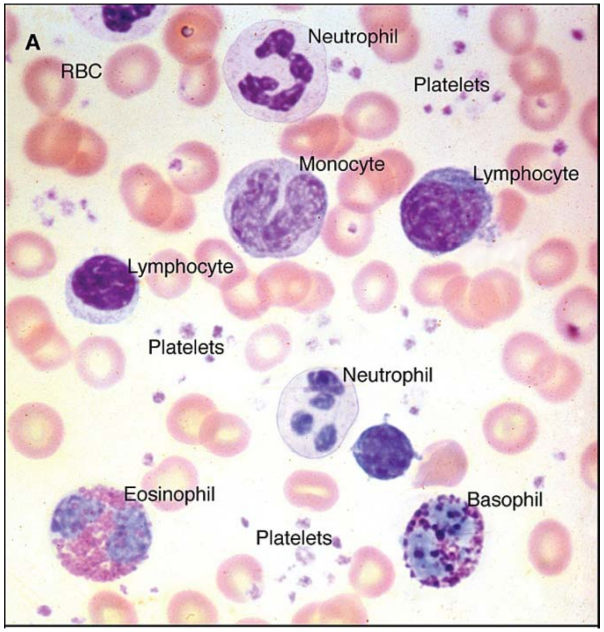
Differential Gene Expression in Different Cells

- The DNA of a cell encodes all the RNA and proteins required for its construction.

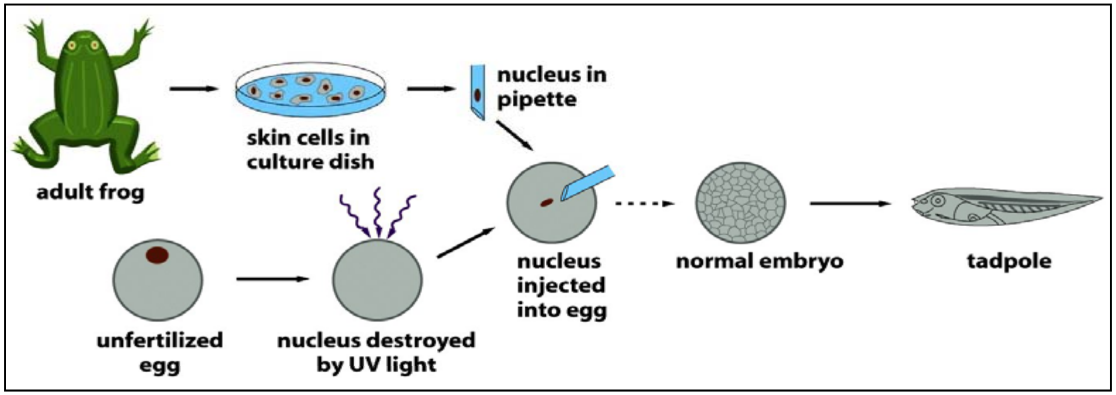
- Different cell types of a multicellular organism contain the same DNA.

- The expression of the same DNA differs dramatically in different cell types.

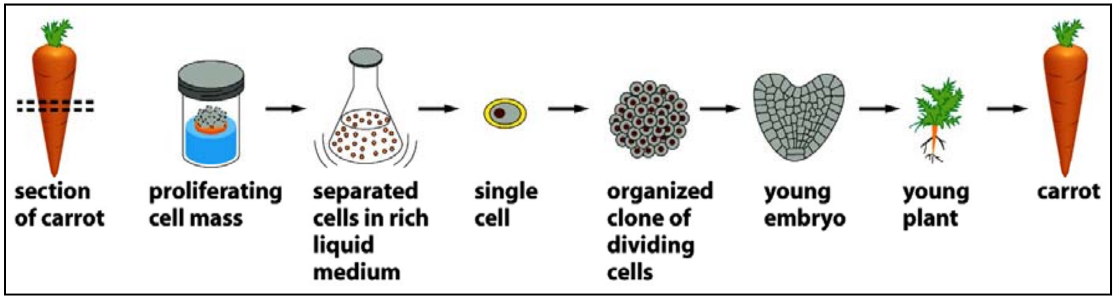


Same DNA in Different Cell of A Multicellular Organism

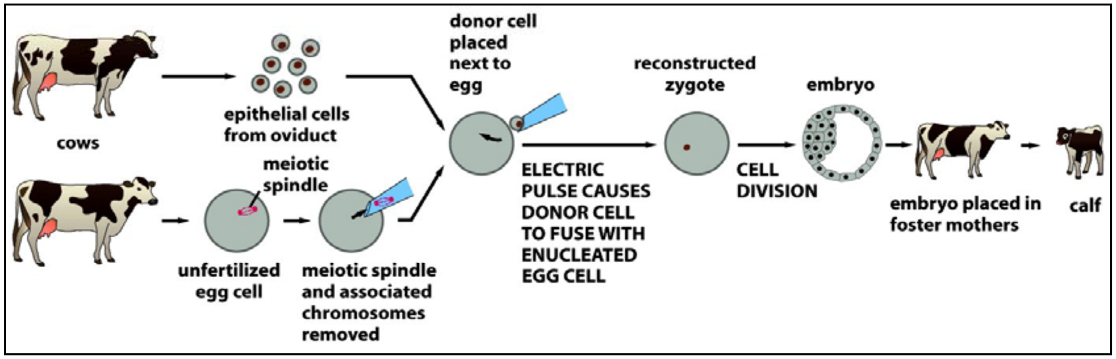
Vertebrates



Plants

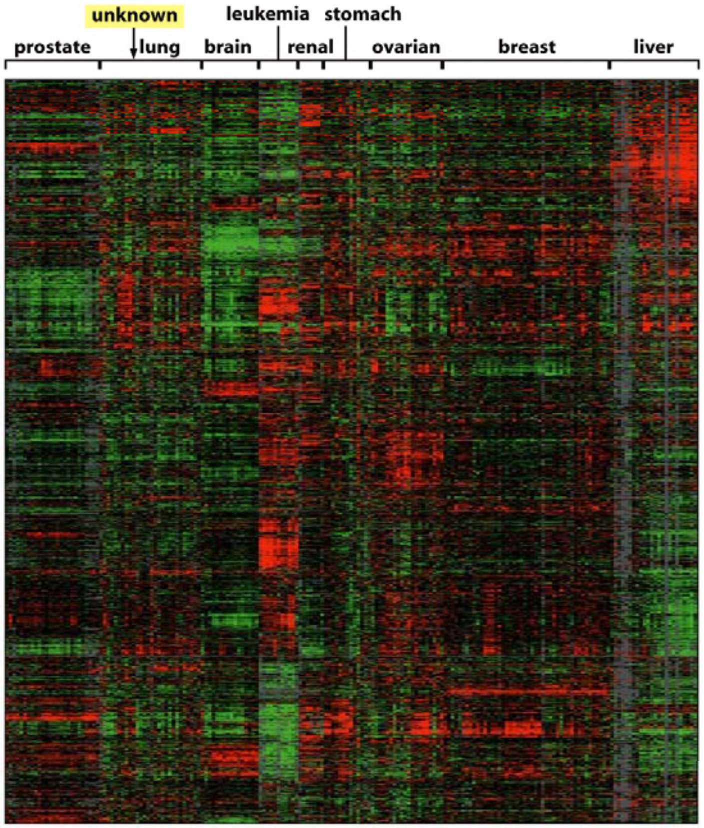


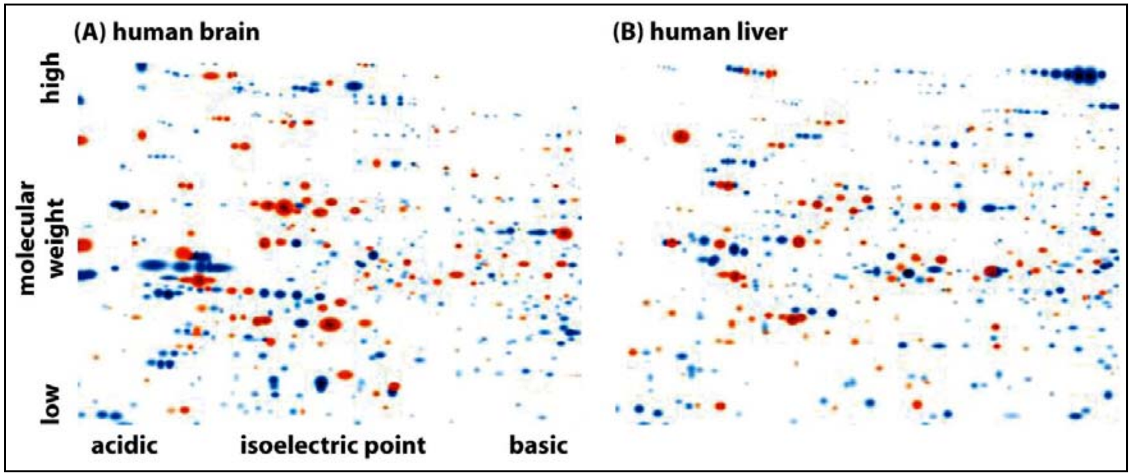
Mammals



Control of Gene Expression

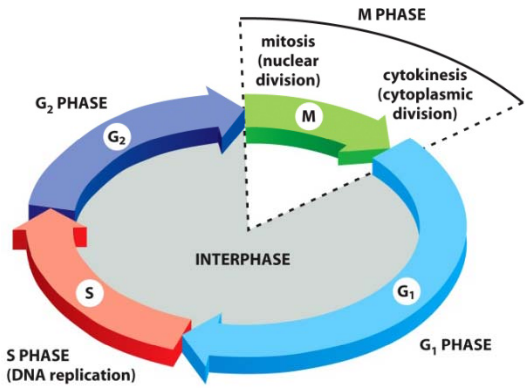
- Different mRNA levels of 1800 genes in 142 different human tumor cell lines.
- Different proteins expressed in human brain and liver.
- Different levels of same proteins in human brain and liver.

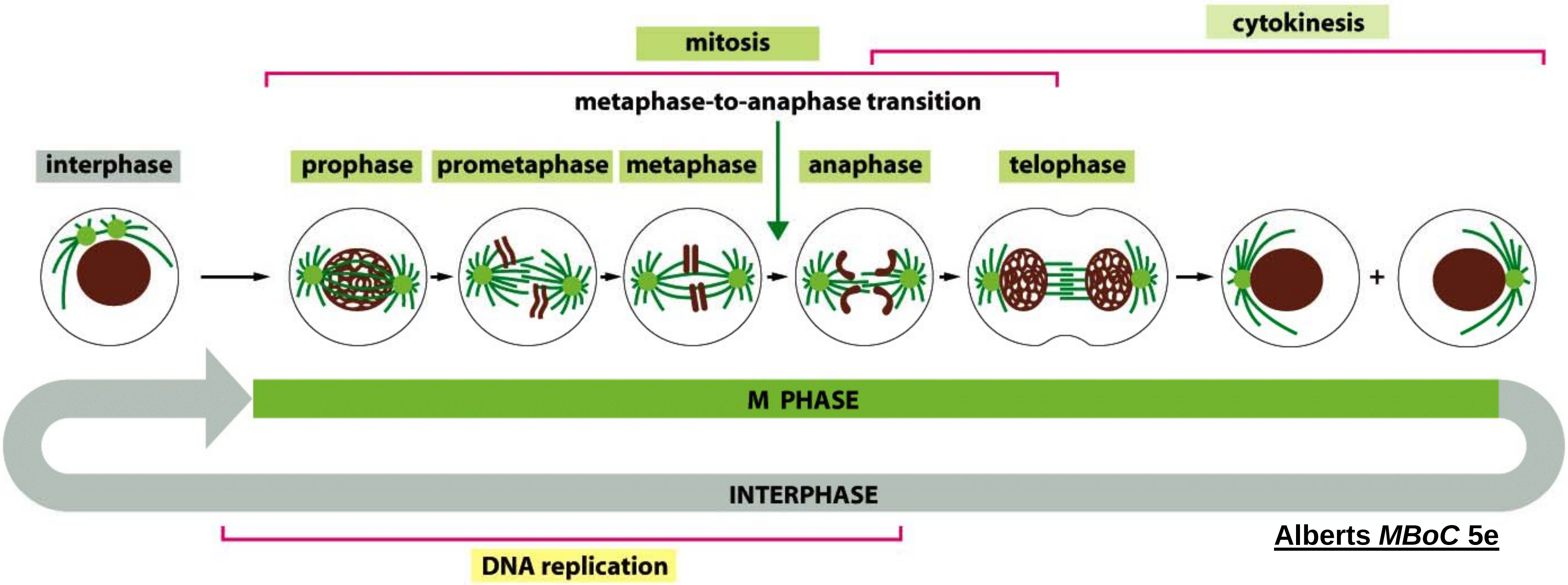




Internal Signals Change Gene Expression

- Different expression profiles in different phases of the cell cycle.





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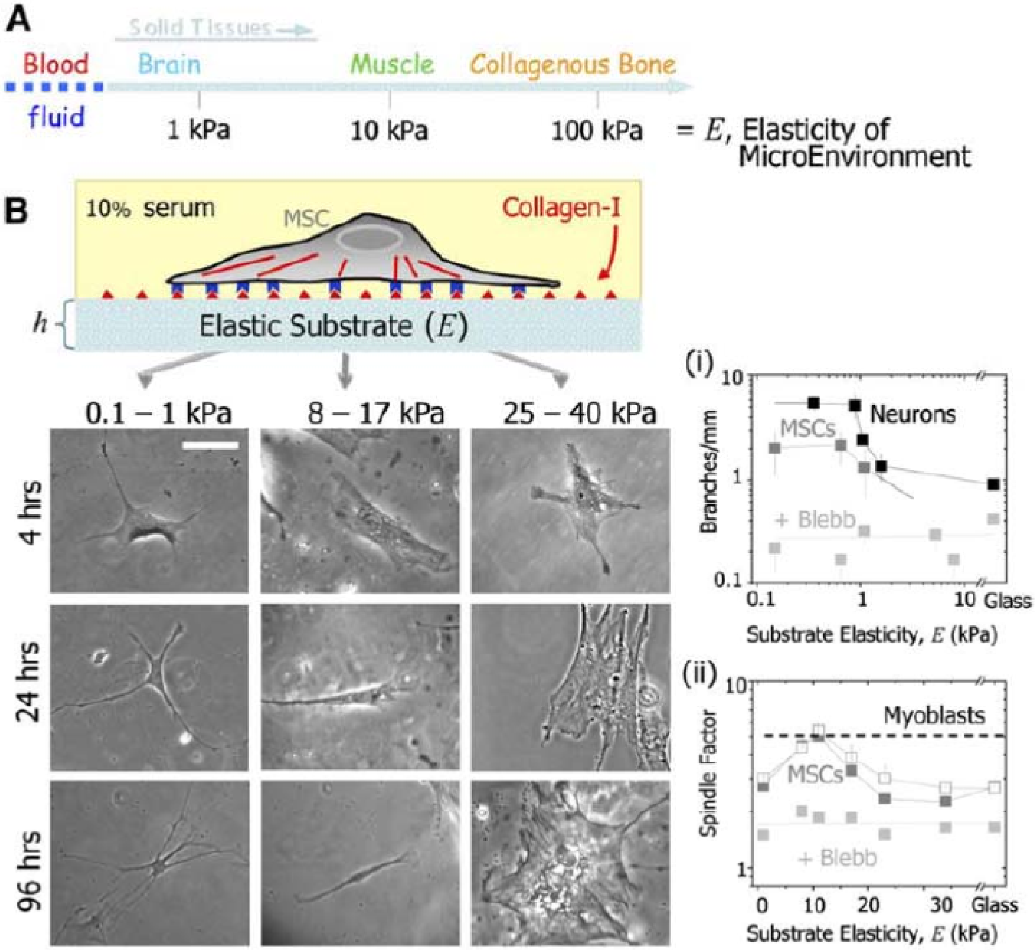
External Signals Can Change Gene Expression

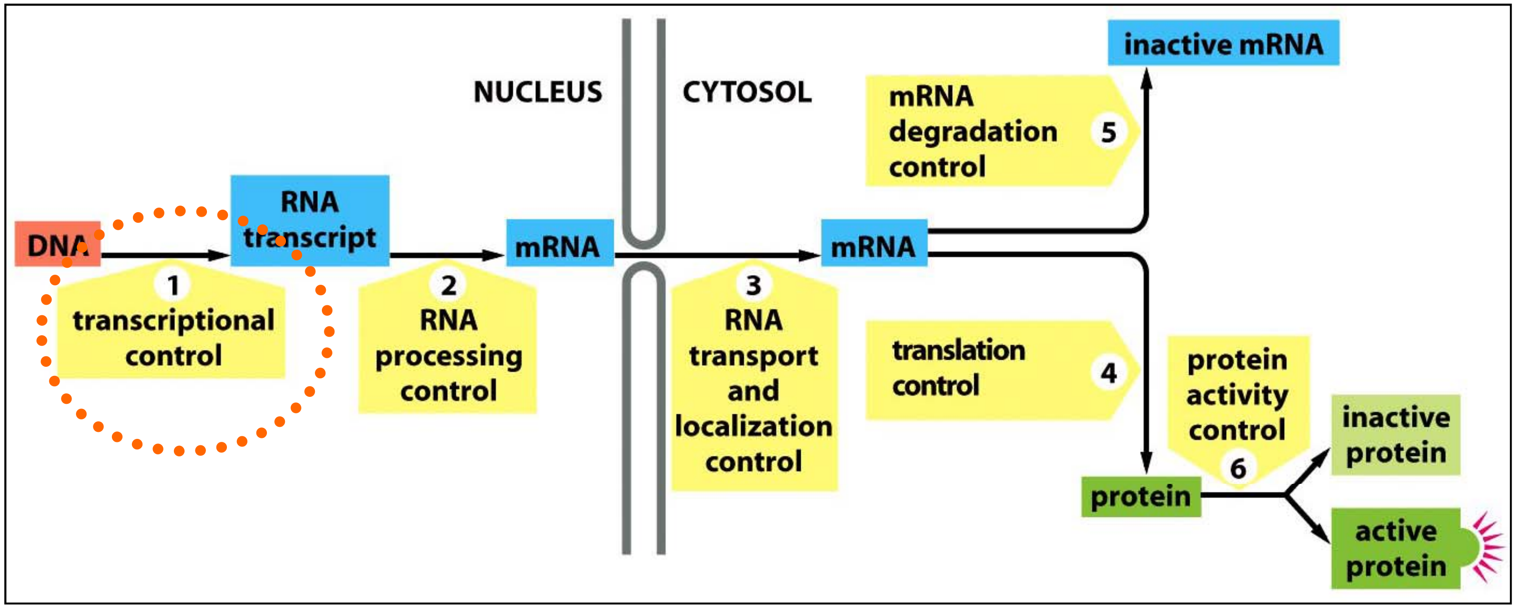
- Mesenchymal stem cells differentiate into different cell types under different substrate stiffness.



Engler et al, *Cell*, 126:677, 2006.

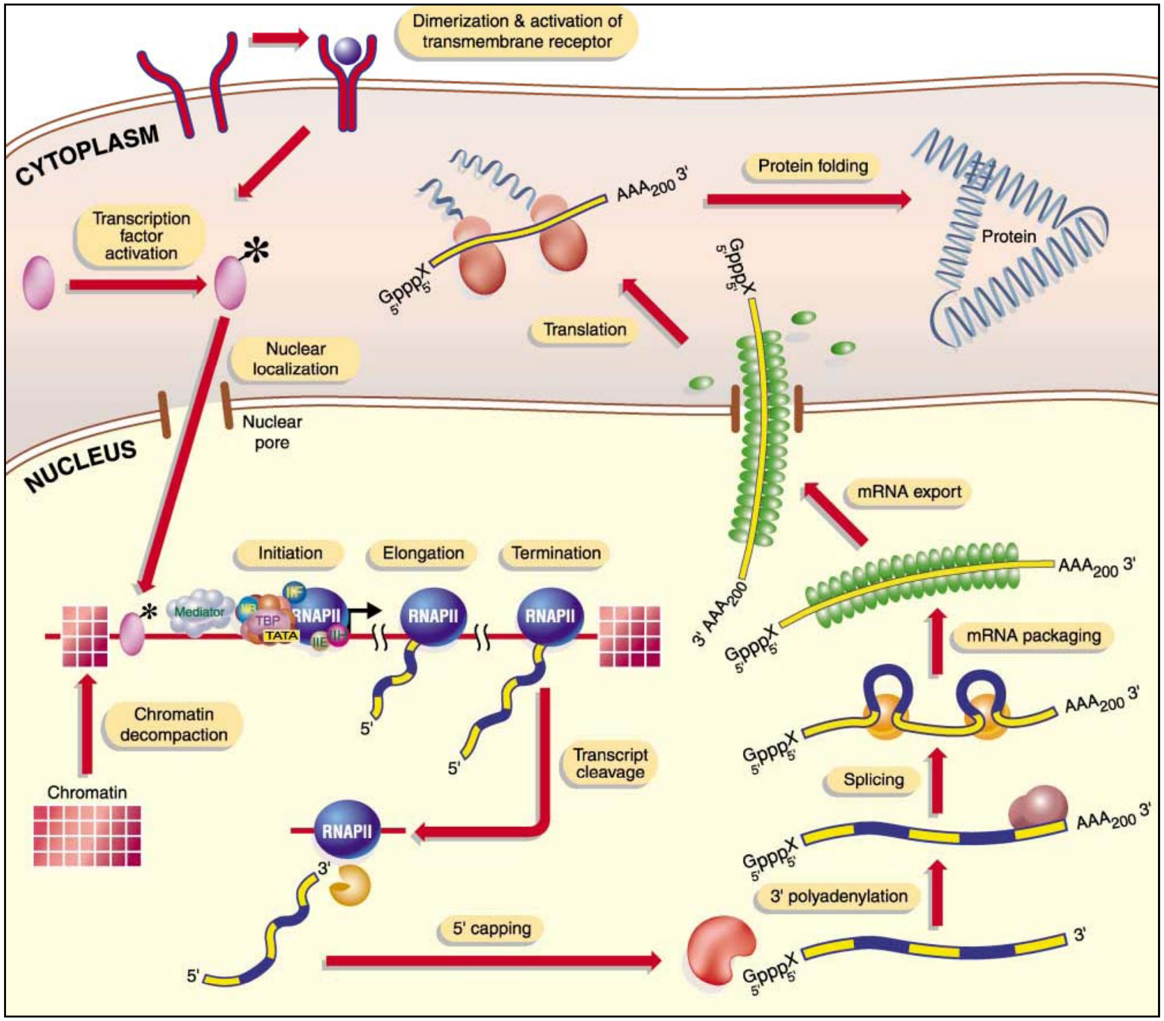
Regulation of Gene Expression at Different Levels

- Gene expression is regulated at different levels.
- For most genes, transcriptional control is the most important.



Traditional View of Eukaryotic Gene Expression

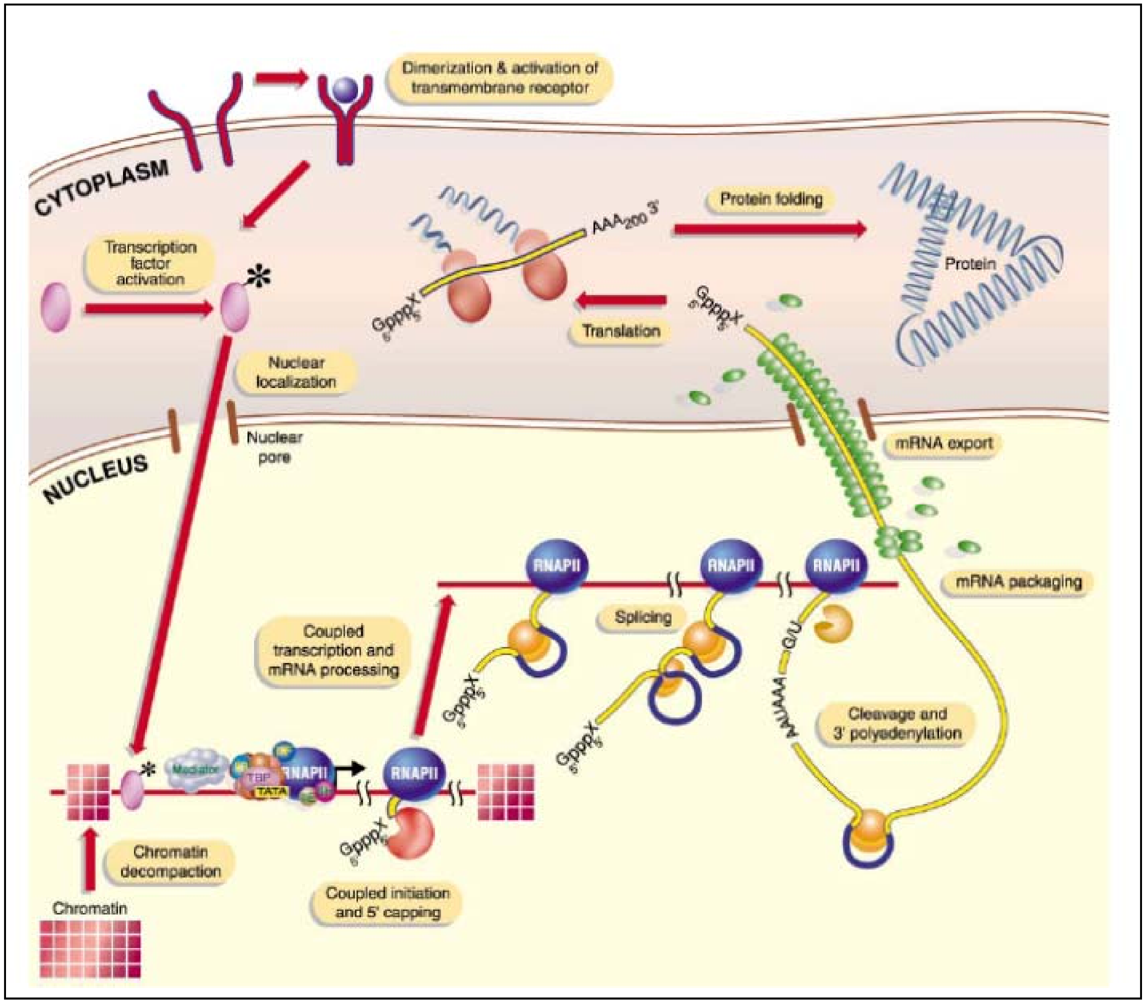
- Gene expression consists of multiple steps.
- Different steps are relatively independent of each other.



Orphanides & Reinberg, *Cell*, 108:439, 2002.

A Contemporary View of Gene Expression

- Gene expression is a continuous process that can be divided into different stages.
- Different stages are physically and functionally connected.



Orphanides & Reinberg, *Cell*, 108:439, 2002.

Quantitative Analysis and Modeling of Gene Expression

- Different classes of genes are regulated at different stages by different regulation mechanisms.

- Using simplified models:

  - bacterial cells provide convenience of manipulation but lacks advanced regulatory mechanisms of higher eukaryotic cells.

- Identification and analysis of modules.

- Synthetic approach.

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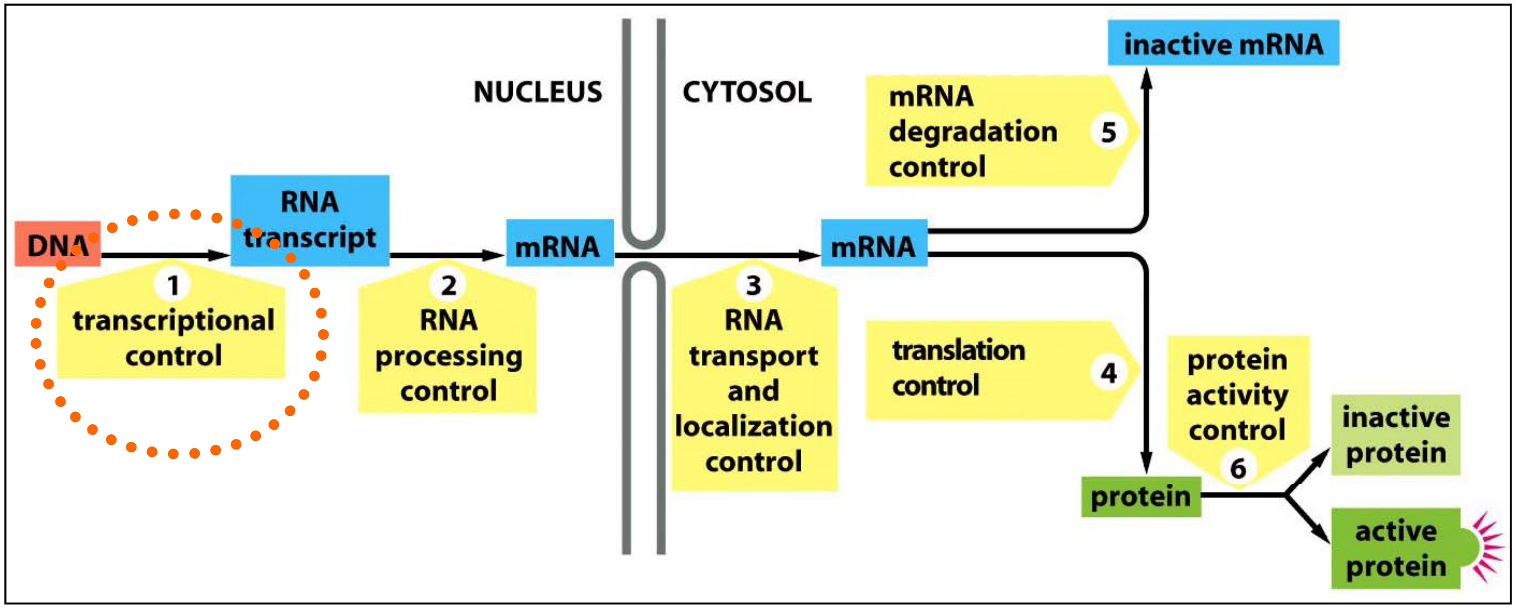
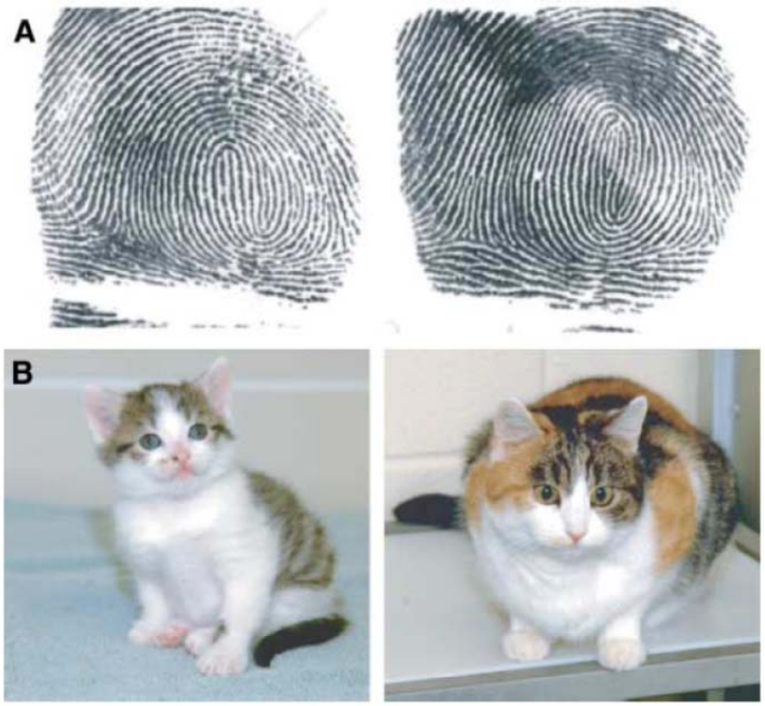


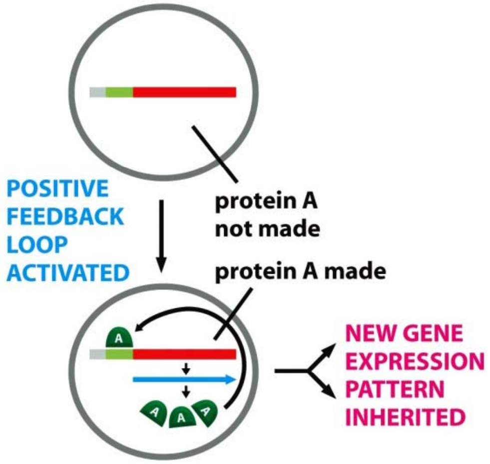
Noise in Gene Expression

- Identical twin humans and animal clones differ in appearance and behavior.
- Cells with the same genes, the same environment display variations in form and behavior due to the stochastic nature of biochemical reactions.

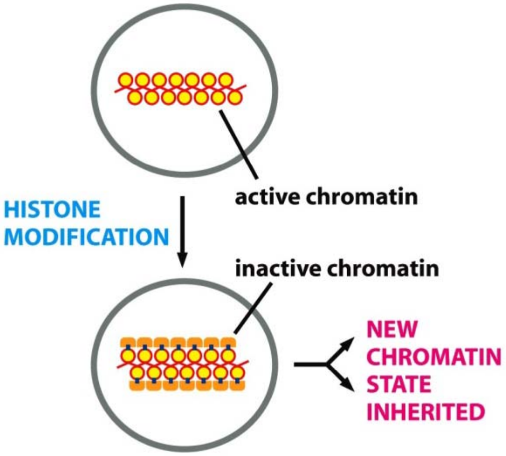


Raser & O'Shea, *Science*, 309:2010, 2005.

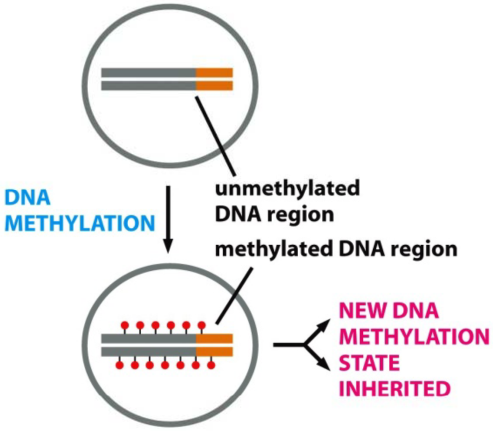
Epigenetic Inheritance of Gene Expression Control



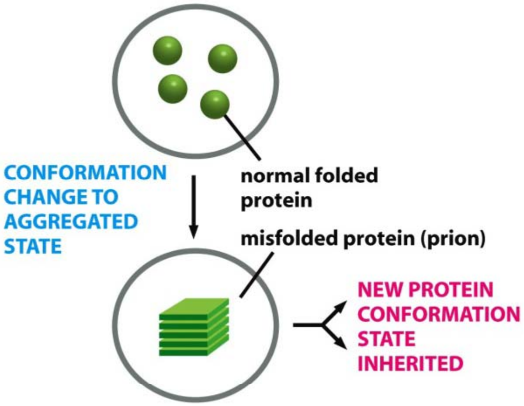
POSITIVE FEEDBACK



HISTONE MODIFICATION



DNA METHYLATION



PROTEIN AGGREGATION STATE

References

- Raser JM, O'Shea EK, Noise in gene expression: origin, consequences, and control, *Science*. 2005, 309:2010-2013.

- Vilar JM, Guet CC, Leibler S, Modeling network dynamics: the lac operon, as case study, *J. Cell Biology*, 2003. 161:471-476.

- Uri Alon, Network motifs: theory and experimental approaches, *Nature Reviews Genetics*. 2007, 8, 450-461.

- http://www.elowitz.caltech.edu/index.html

- http://www.weizmann.ac.il/mcb/UriAlon/

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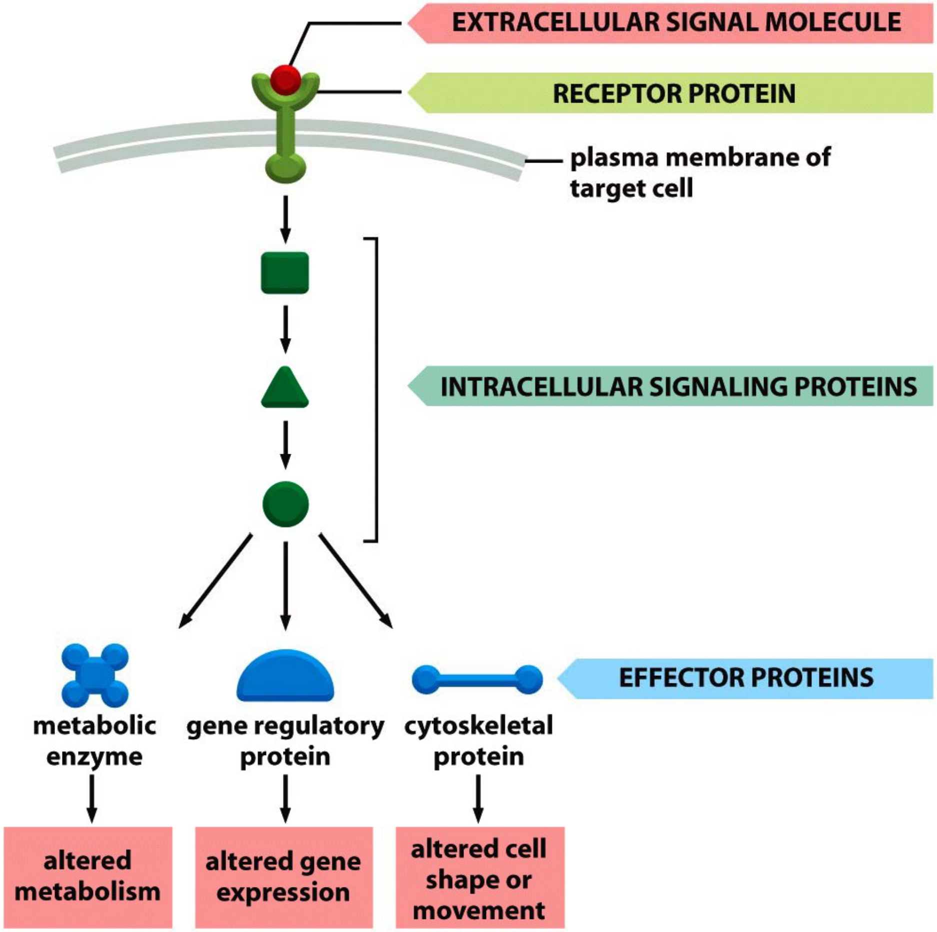
Overview of Cell Signaling

- Sources of extracellular signal
  - \- Non-cellular environment
  - \- Cellular environment (cell-cell communication)
  - \- Hundreds of types of signals
- Cells signaling
  - \- Stimulus sensing; communication
  - \- Information processing; decision making
- ↓Receptors
  ↓Signaling proteins
  ↓Effector proteins
- Signaling pathways regulate nearly all cellular functions.



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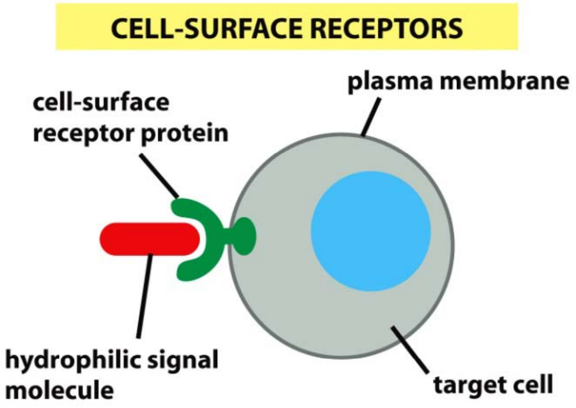
Membrane & Intracellular Receptors

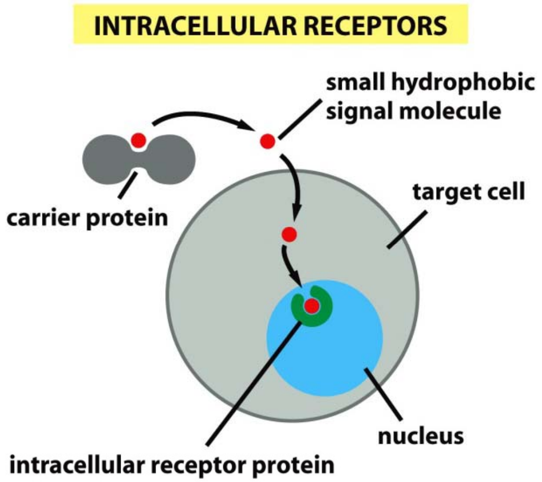
- Receptors bind signaling molecules (ligands)

- Receptors are highly sensitive and specific.
  - Typical signal molecule concentration $<10^{-8}$ M
  - More than 1500 human genes encode receptors

- Most receptors are at the cell surface.

- Some receptors are intracellular (e.g. light, gas receptors).





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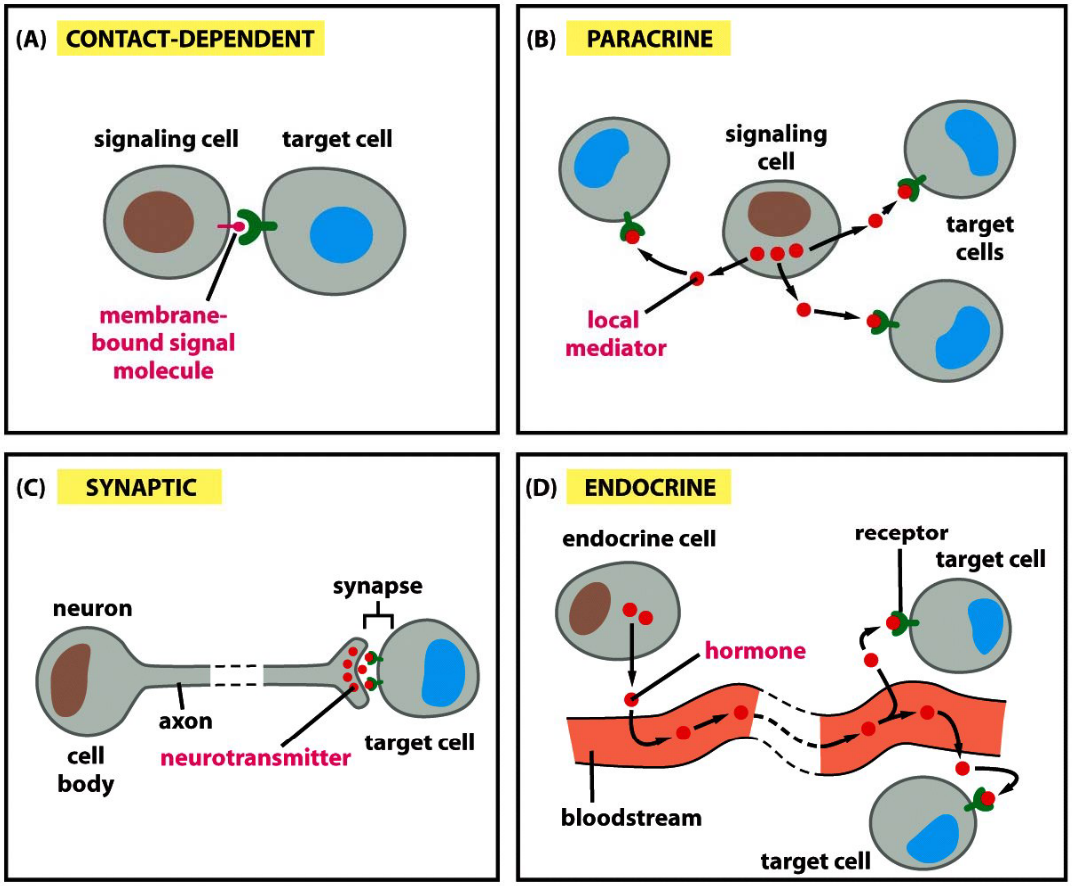
General Principles of Signaling (I)

- Four forms of intercellular signaling

- Paracrine signaling acts locally over different types of cells.

- Autocrine signaling acts locally over the same types of cells including themselves.

- Endocrine signaling acts over long distance.



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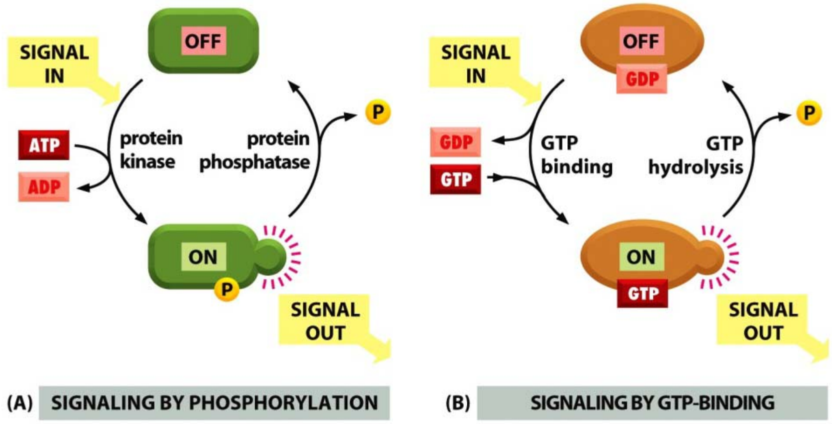
General Principles of Signaling (II)

- Many signaling proteins act as molecular switches
- Two ways to activate/deactivate signaling proteins
- Human genomes encodes ~520 kinases and ~150 phosphatases
- Two main types of kinases
    - tyrosine kinase
    - serine/threonine kinase
- Two types of GTP-binding proteins
    - Trimeric G proteins
    - Monomeric GTPases



(A) SIGNALING BY PHOSPHORYLATION

(B) SIGNALING BY GTP-BINDING

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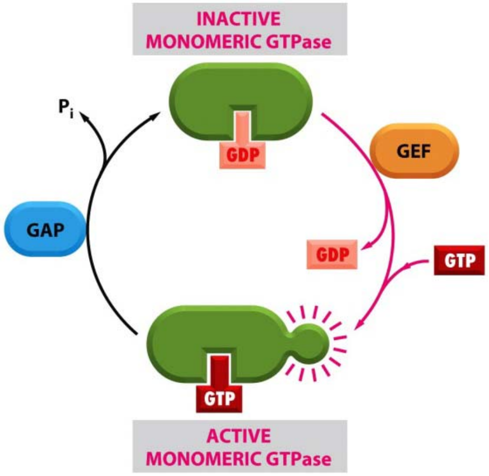
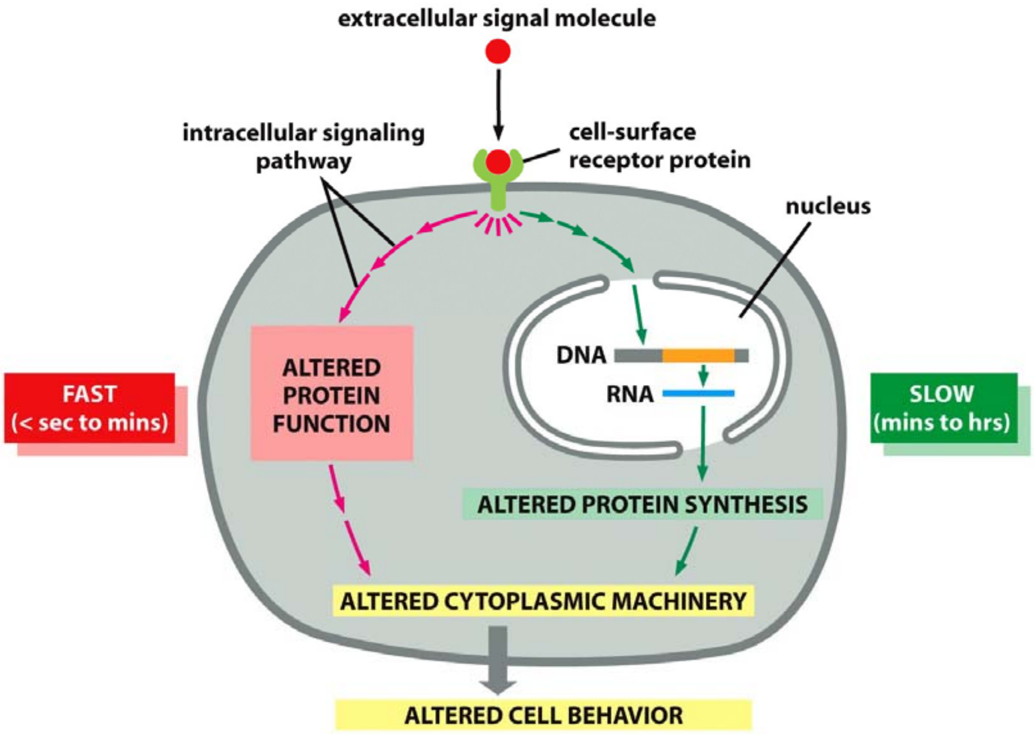


General Principles of Signaling (III)

- Different pathways have different rates of response.

- Pathways involve gene expression regulation are usually slow.



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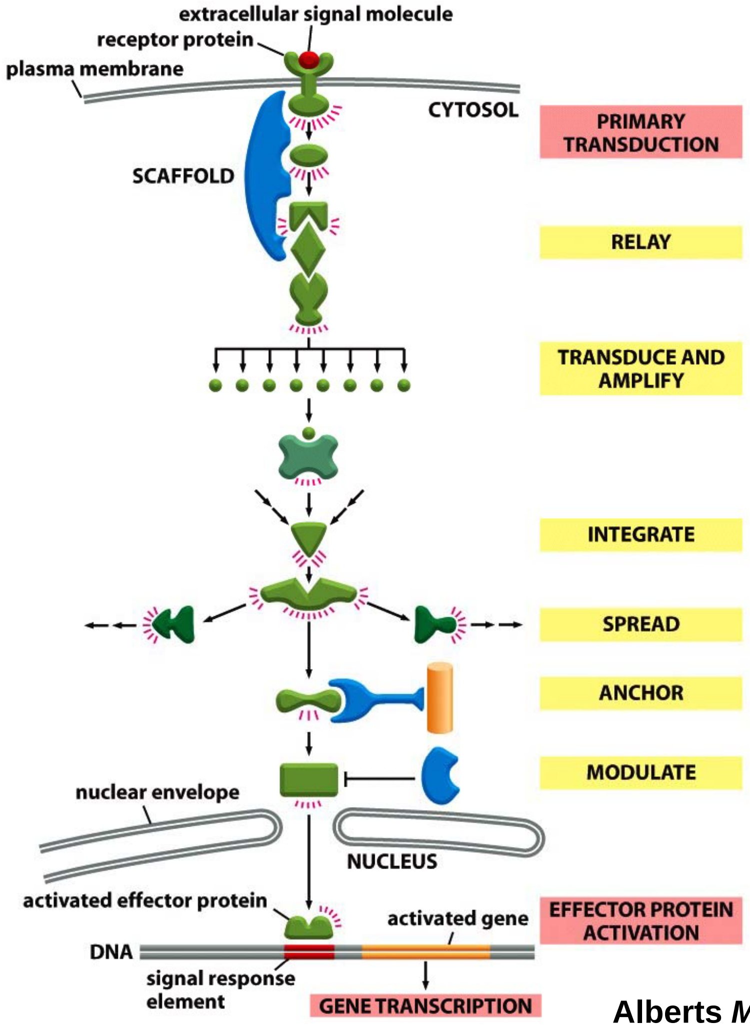
General Principles of Signaling (IV)

- A scaffold brings multiple signaling proteins together for faster and more efficient interactions.
- Signaling proteins may be anchored to a specific cellular structure.
- Activities of signaling proteins may be modulated by other signaling proteins.



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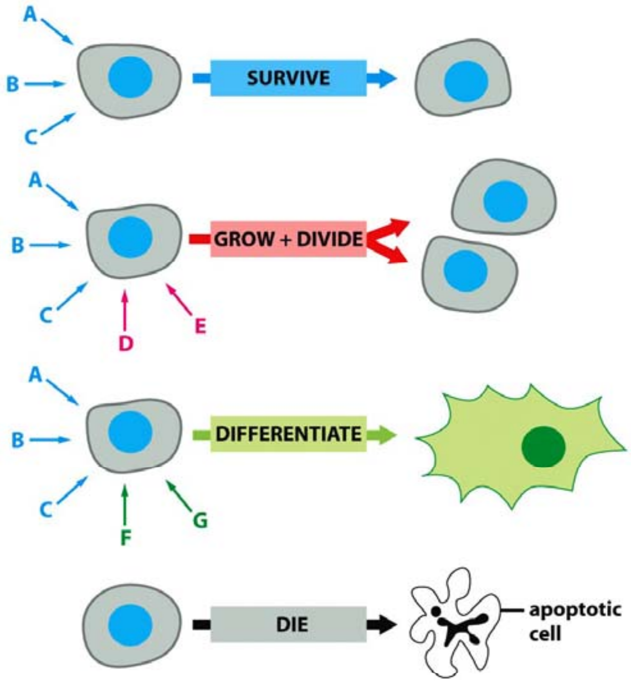
Specific Reponses of Cells to Signaling

- A cell in a multicellular organism may be exposed to hundreds of signals.
- A major challenge is to understand how the cells process such information and make decisions.

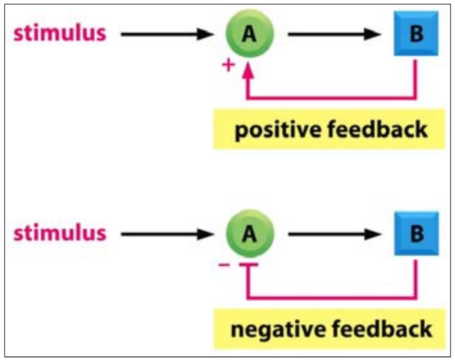


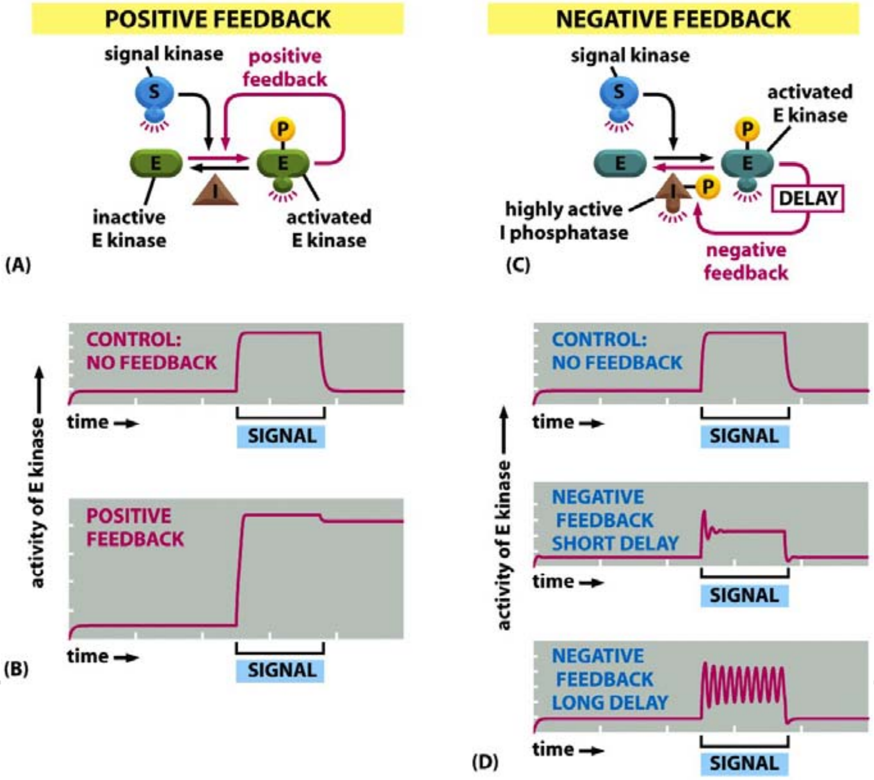
Feedback Loops in Signaling Networks

- Two types of feedback loops
  - Positive feedback
  - Negative feedback

- Positive feedback loop
  - Bistability

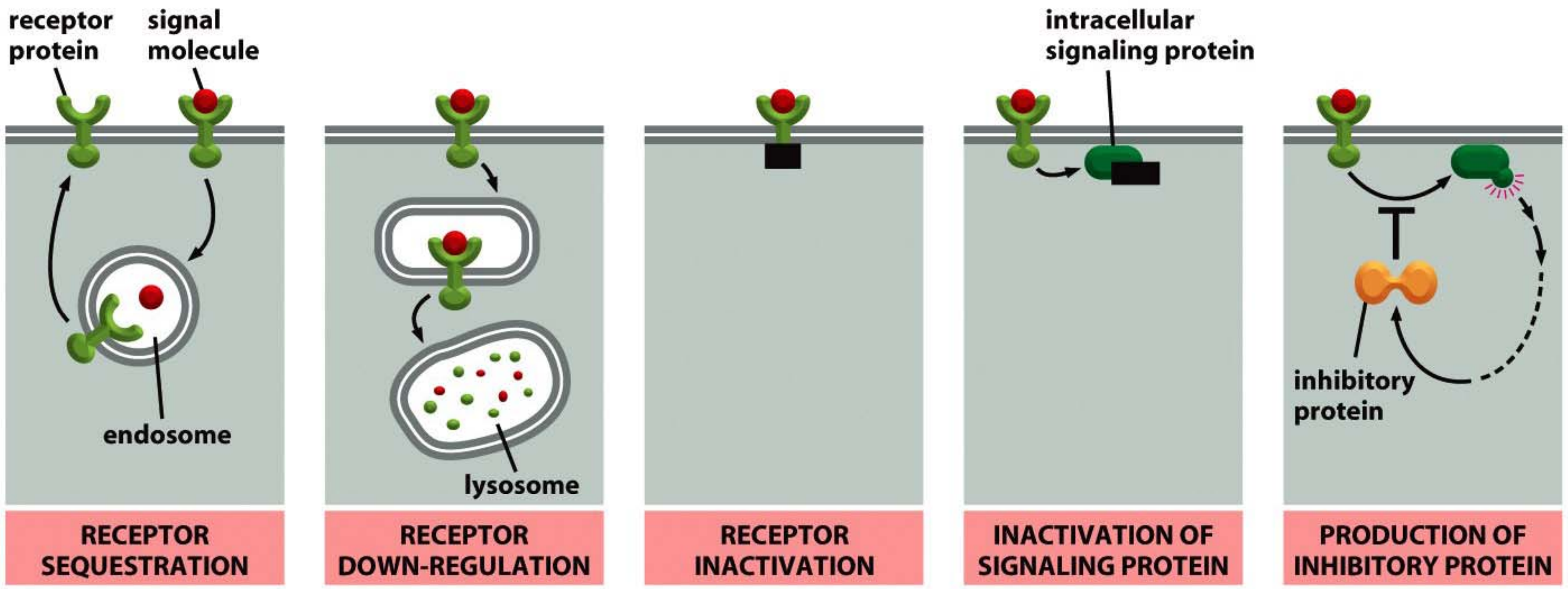
- Negative feedback loop
  - Robustness to noise





Adaptation of Sensitivity to Signaling

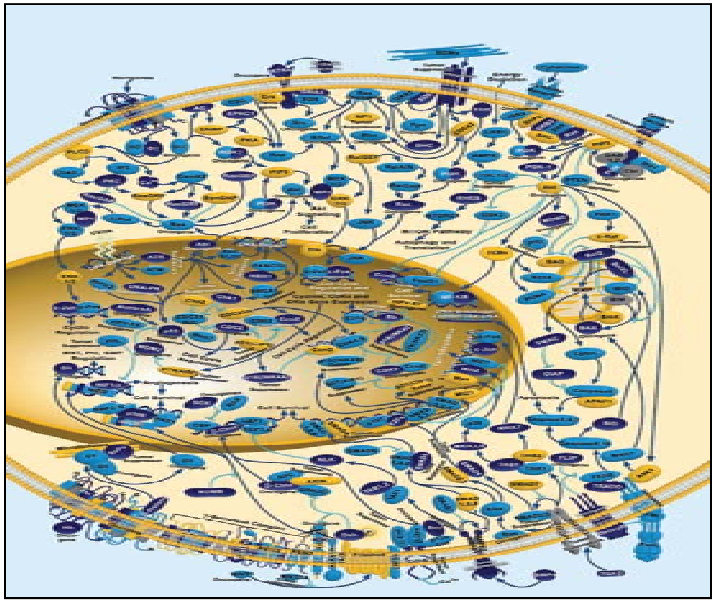
- Cells can adapt to external stimuli through sensitivity adjustment.



Challenges in Analyzing Signaling Pathways

- Hundreds of signaling pathways.
- Pathways frequently branch and converge.
- Positive and negative feedback loops are common.
- Outcomes of signaling pathways can be spatial and temporal dependent.
- Analysis typically uses graph models.

Human cancer pathways



References

- J. Hancock, Cell Signaling, 3rd ed., Oxford University Press, 2010.
- F. Marks et al, Cell Signal Processing, Garland Science, 2008.

Questions ?