BME 42-620 Engineering Molecular Cell Biology

Lecture 22:

Cell Signaling (III)

Cell Cycle (I)

Chapter 15

Comments on Reading Assignment 5 (I)

GENERAL NATURE OF THE GENETIC CODE FOR PROTEINS

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"I assume that this means the FC0 is the original mutation that causes the frameshift and there is a second mutation that occurs at 18 different places that "revert" this frameshift (that is why they are called suppressors). But the third paragraph in page 2 said that "in all cases the suppressor was a non-leaky r. That is, it gave an r plague on B and would not grow on K. This is the phenotype shown by a complete deletion." If these 18 has already reverted the effect of mutation on FC0, then why are they still showing up as non-leaky r?"

<u>Leaky mutation: growth on K, somewhat different plaque-type on B.</u>

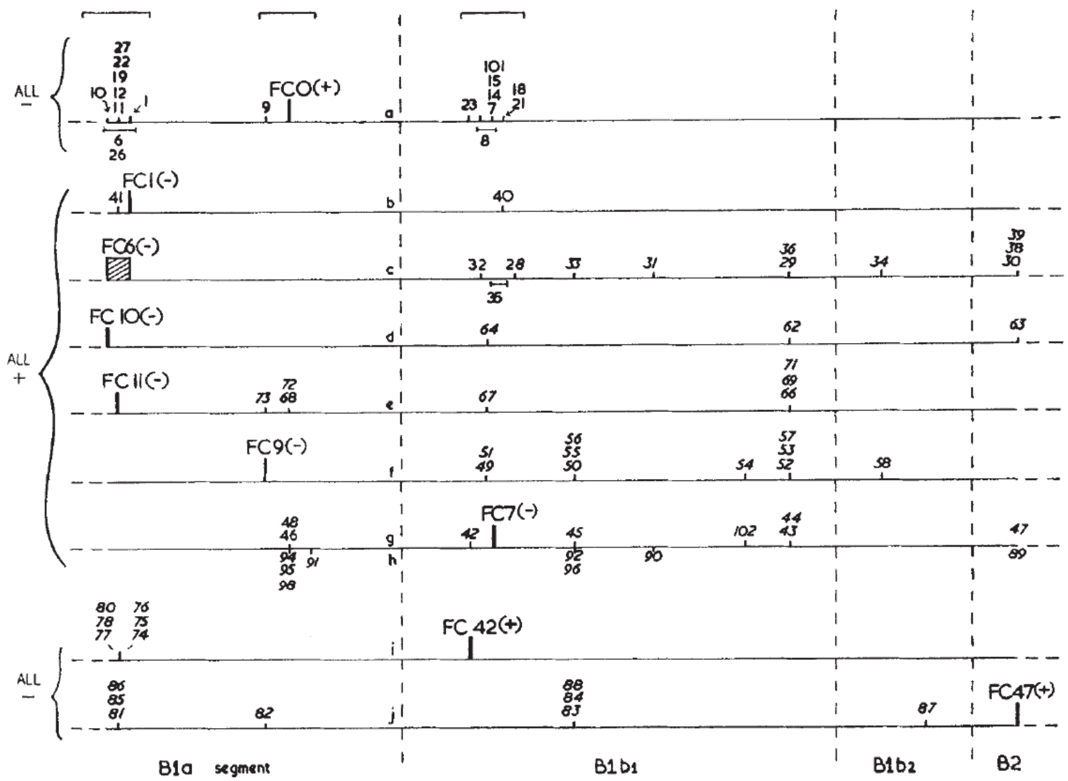

Fig. 2. A tentative map—only very roughly to scale—of the left-hand end of the *B* cistron, showing the position of the *FC* family of mutants. The order of sites within the regions covered by brackets (at the top of the figure) is not known. Mutants in italics have only been located approximately. Each line represents the suppressors picked up from one mutant, namely, that marked on the line in bold figures

Comments on Reading Assignment 5 (II)

More over, our symbols + and – must not be taken to mean literally the addition or subtraction of a single base.

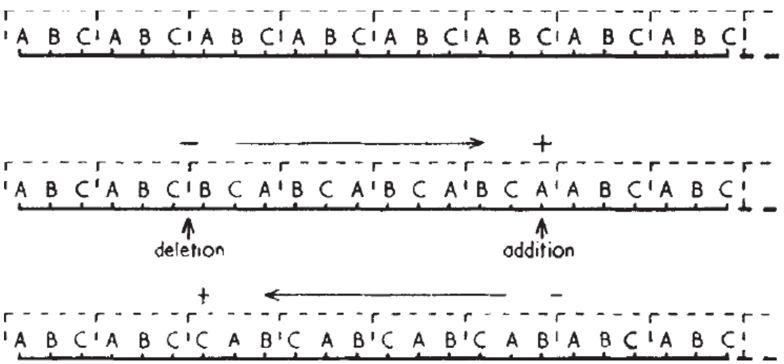

Table 1. Double Mutants having the r Phenotype

− With −	+ With +	
FC (1 + 21)	*FC* (0 + 58)	*FC* (40 + 57)
FC (23 + 21)	*FC* (0 + 38)	*FC* (40 + 58)
FC (1 + 23)	*FC* (0 + 40)	*FC* (40 + 55)
FC (1 + 9)	*FC* (0 + 55)	*FC* (40 + 54)
	FC (0 + 54)	*FC* (40 + 38)

Table 3. Triple Mutants having a Wild or Pseudo-wild Phenotype

FC (0 + 40 + 38)
FC (0 + 40 + 58)
FC (0 + 40 + 57)
FC (0 + 40 + 54)
FC (0 + 40 + 55)
FC (1 + 21 + 23)

Comments on Reading Assignment 5 (III)

"Of course, now that we know the answer, it all seems so completely obvious that no one nowadays remembers just how puzzling the problem seemed then. If by chance you do not know the answer, I ask you to pause a moment and reflect on what the answer might be. There is no need, at this stage, to bother about the details of the chemistry. It is the principle of the idea that matters.

The problem was not made easier by the fact that many of the properties of proteins and genes just outlined were not known for certain. <u>All of them plausible and most of them seemed very probable</u> but, as in most problems near the frontiers of research, there were always nagging doubts that one or more of these assumptions might be dangerously misleading. <u>In research the front line is almost always in a fog.</u>"

- Francis Crick, "What mad pursuit"

Outline

- Second messengers
- Overview of cell cycle
- Different phases of cell cycle
- Overview of checkpoints
- Cyclin and cyclin-dependent kinases

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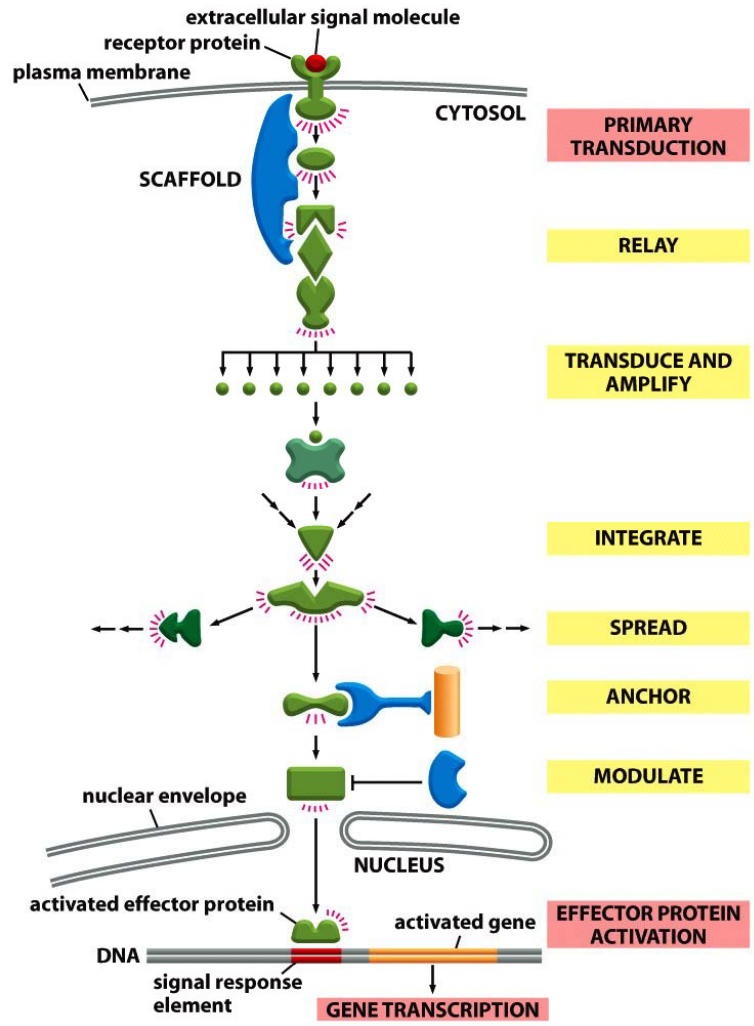
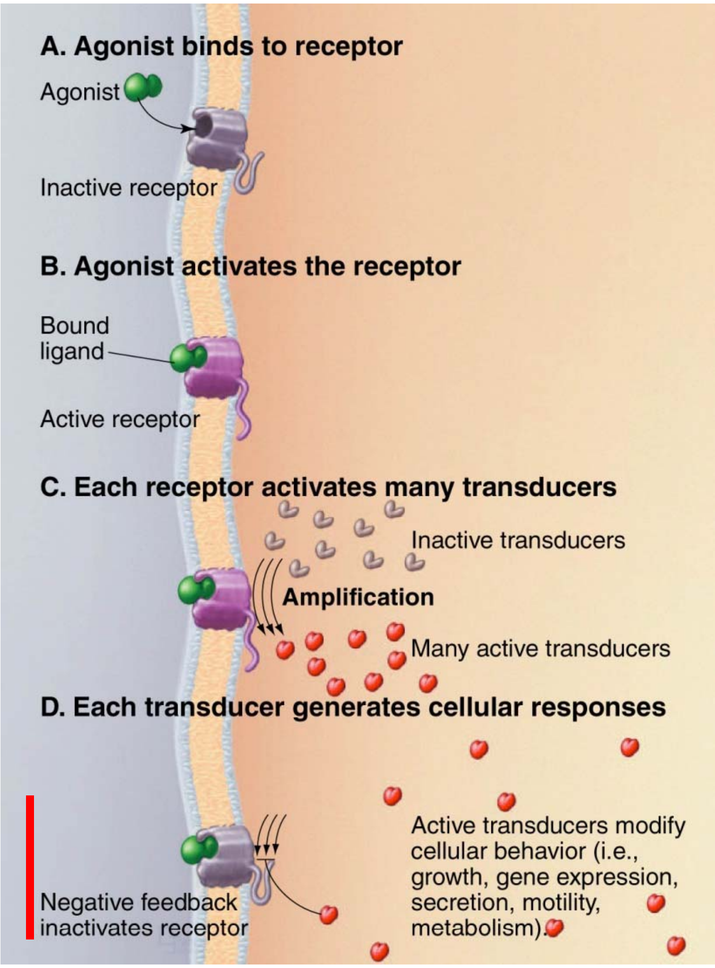
Overview of Cell Signaling

- Cascade of signaling events
  - ↓Receptors
  - ↓Signal transducers
  - ↓Effector proteins
- Relay, integration, and distribution of signals require transducers.
- Signaling pathways regulate nearly all cellular functions.



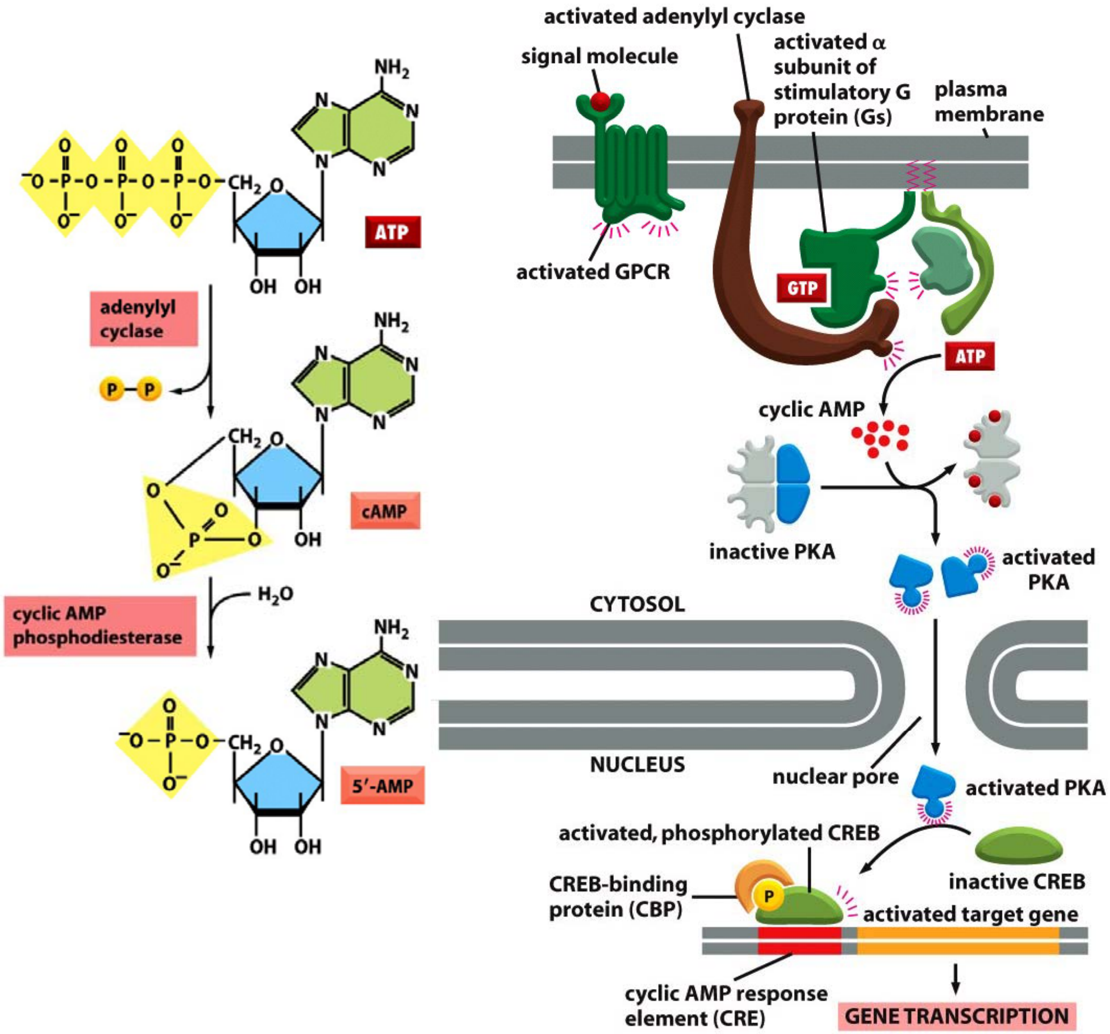
Transducers in Signaling

- Signaling proteins
  - Kinases
  - Phosphatases
  - GTPases
  - Adapters
- Second messengers
  - cAMP, cGMP
  - Lipids
  - Calcium
  - NO (nitrogen monoxide)



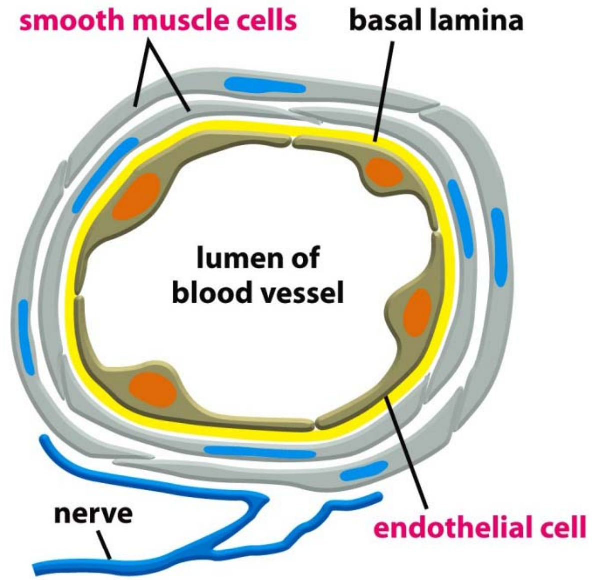
Example: Regulation of cAMP by G Proteins

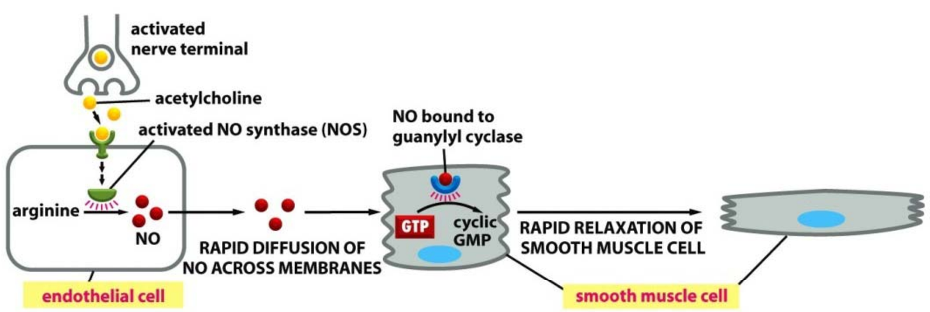
- Cyclic AMP is synthesized from ATP by adenylyl cyclase.
- Cyclic AMP is degraded by cAMP phosphodiesterases through hydrolysis.



Intracellular Receptor: Guanylyl Cyclase Receptors

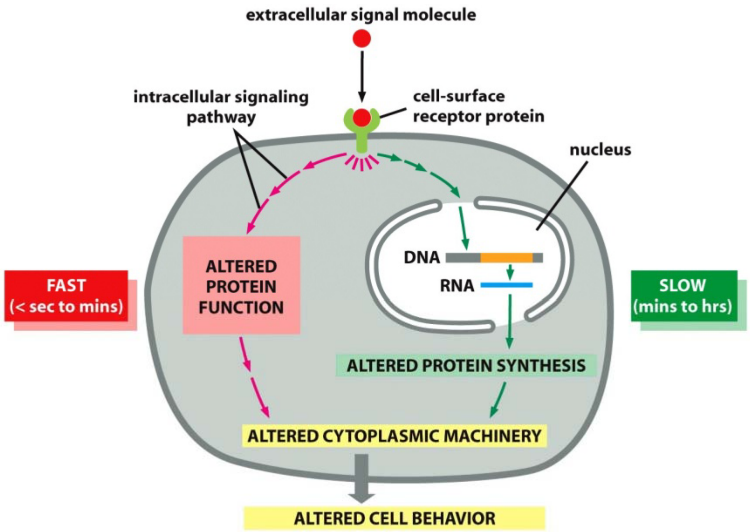
- Soluble guanylyl cyclase is a mammalian NO/CO sensor.
- NO signaling is critical to many physiological processes involving cardiovascular and neuronal systems.
- Related drugs work by blocking the breakdown of cGMP.





Overview of Second Messengers (I)

- Main types of second messengers
  - Cyclic nucleotides: cAMP, cGMP
  - Calcium
  - Lipids
  - Nitric oxide



- Most second messengers are small molecules.

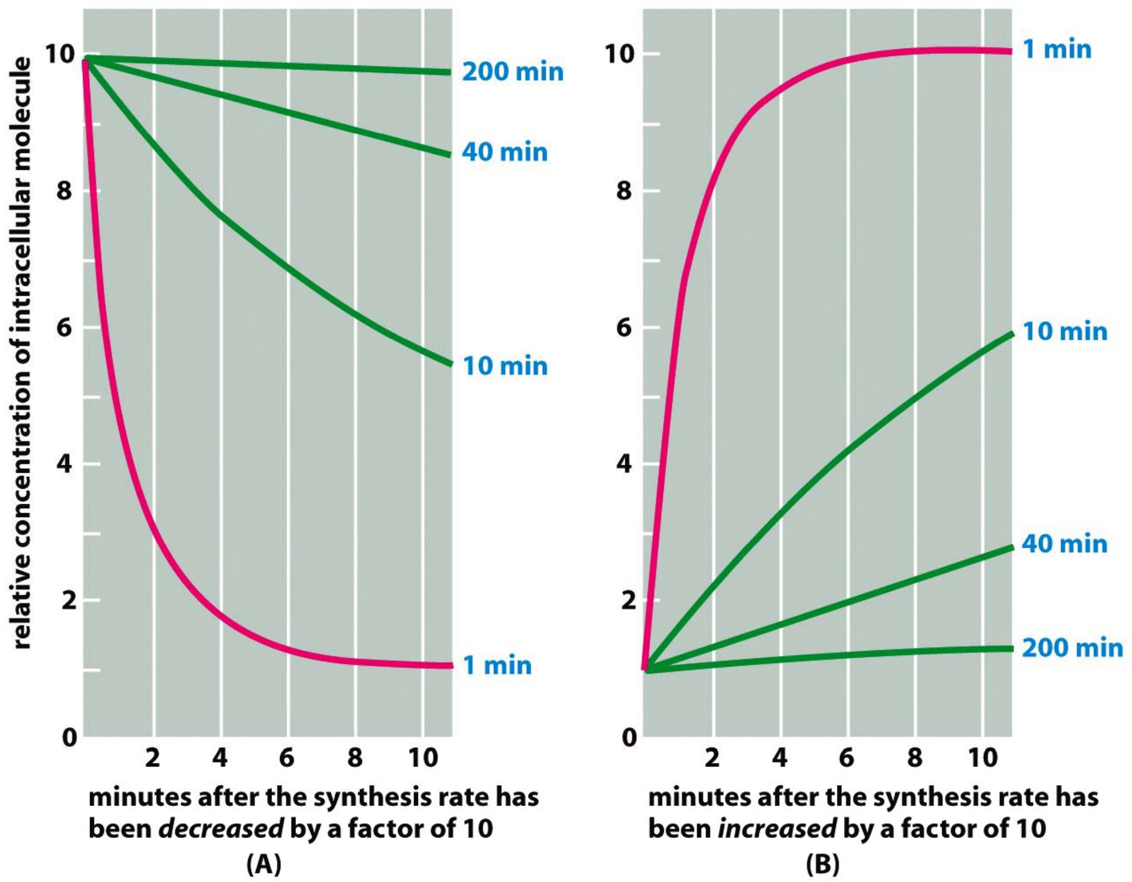
- Information encoded by local concentrations.

- Advantages
  - Range (e.g. broadcasting)
  - Response speed (up to ms)

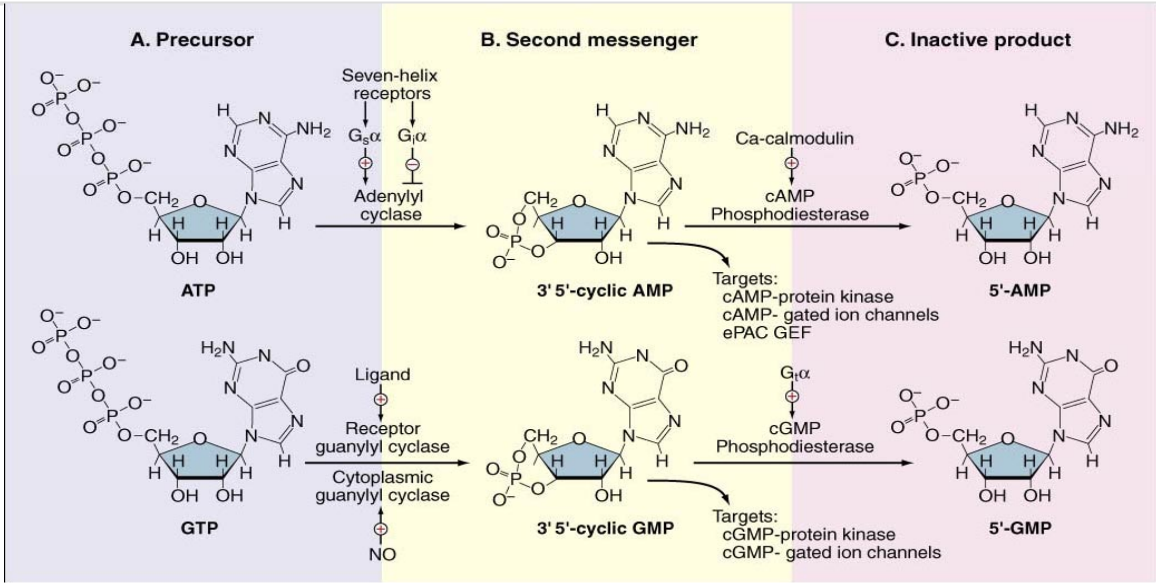
- Second messengers are interrelated.

Overview of Second Messengers (II)

- Production (source)
- Localization
- Target
- Degradation (sink)



Cyclic Nucleotide (I)



- Producer:
  - cAMP $\rightarrow$ adenylyl cyclase
  - cGMP $\rightarrow$ guanylyl cyclase

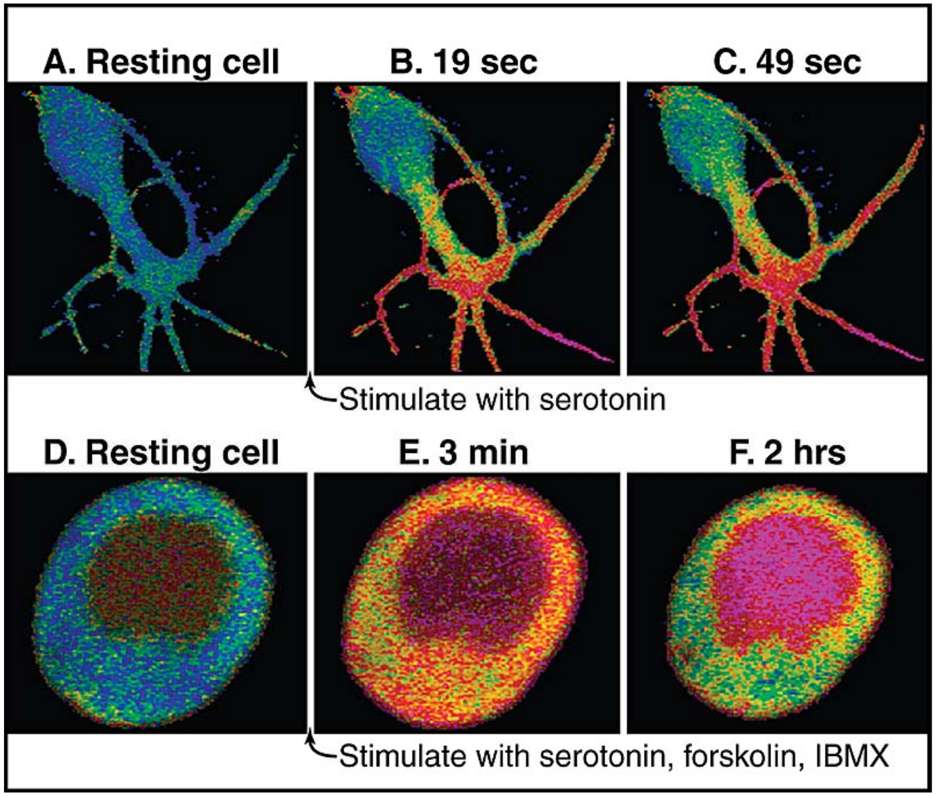
- Degrader:
  - cAMP phosphodiesterase
  - cGMP phospoodiesterase

cAMP (I)

- Diffuse rapidly through cytoplasm as in free solution
- May be modulated locally (through upstream G-proteins)
- Concentration in resting cell ~10^{-8}M
- Can amplify signal by 100-fold on time scale of seconds.
- Targets:
    - kinase
    - cyclic nucleotide-gated ion channels
    - Exchange factors for small GTPases (Rap1, Rap2)

cAMP (II)

- Serotonin is an activator of adenylyl cyclase.
- Forskolin is another activator of adenylyl cyclase.
- IBMX is an inhibitor of cAMP phosphodiesterase.
- Signal→ fluorescently labeled PKA (protein kinase A)

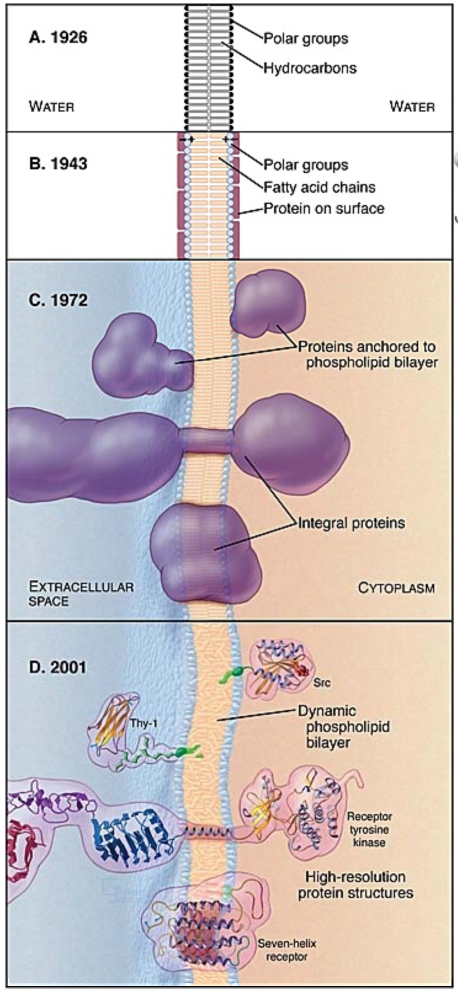


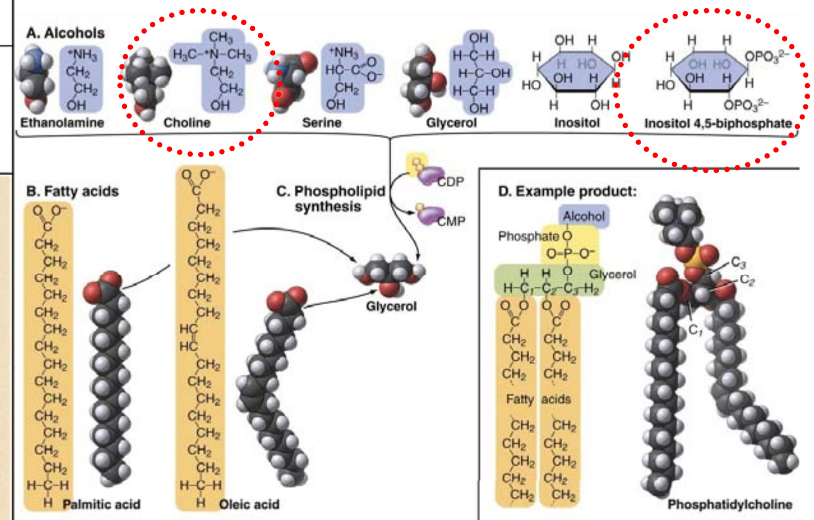
Cell Membrane

- There are many types of membrane lipids.

- Phosphoglycerides are the main constituents of membrane bilayers.

- There are more than 100 major phos-phoglycerides based on different fatty acids and head groups



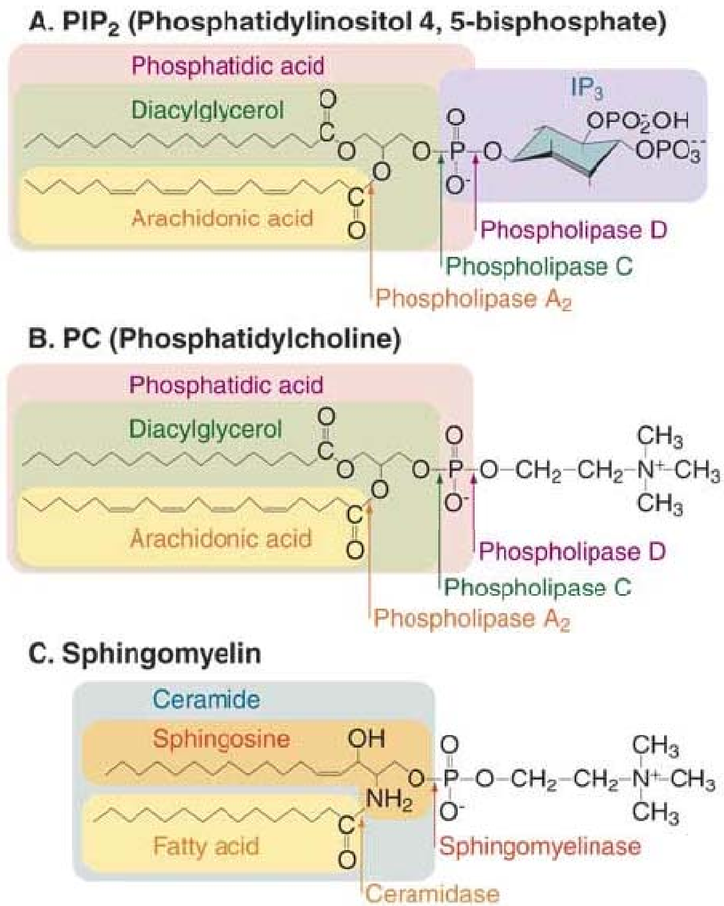


Lipid-Derived Second Messengers (I)

- Three classes of enzymes produce most lipid-derived second messengers.
  - Phospholipases
  - Lipid kinases
  - Lipid phosphotases

- Localization: membrane, cytoplasm, extracellular environment

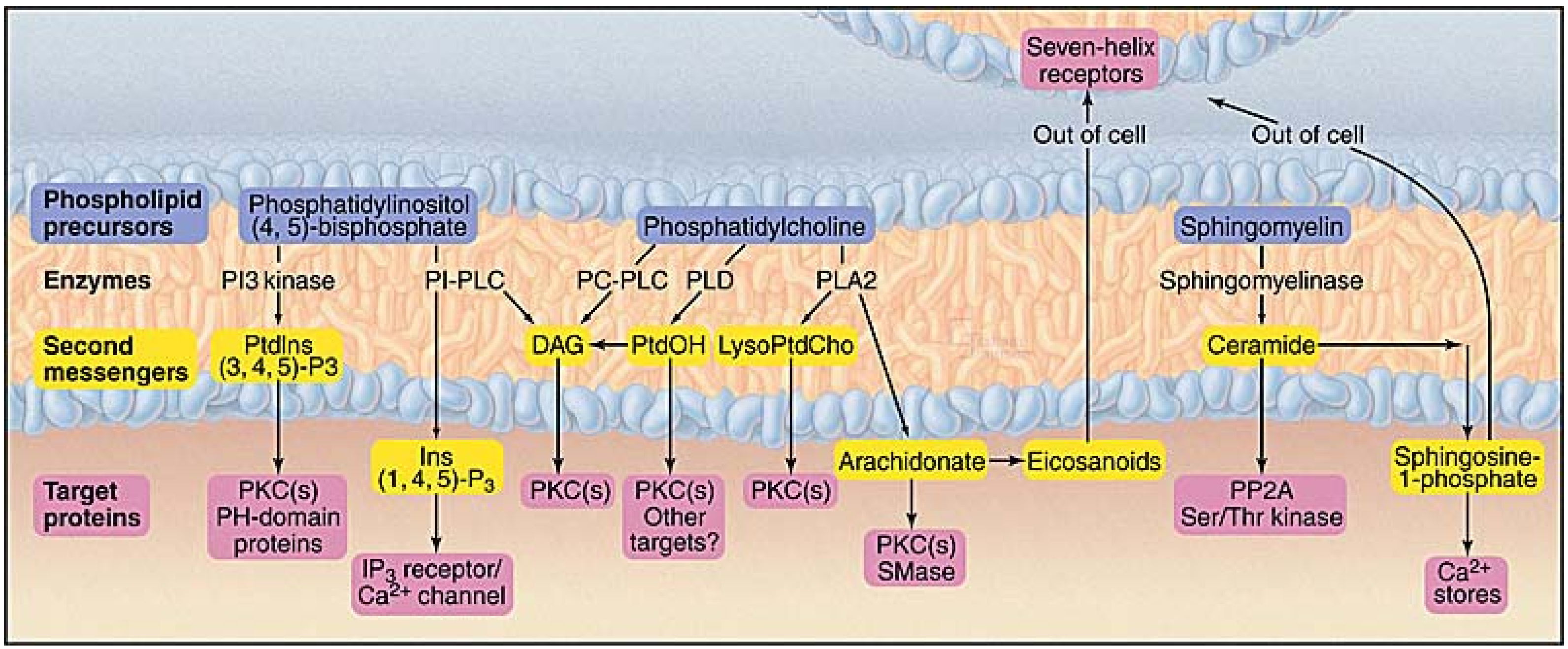
- A great variety of membrane lipids are produced as chemical signals.



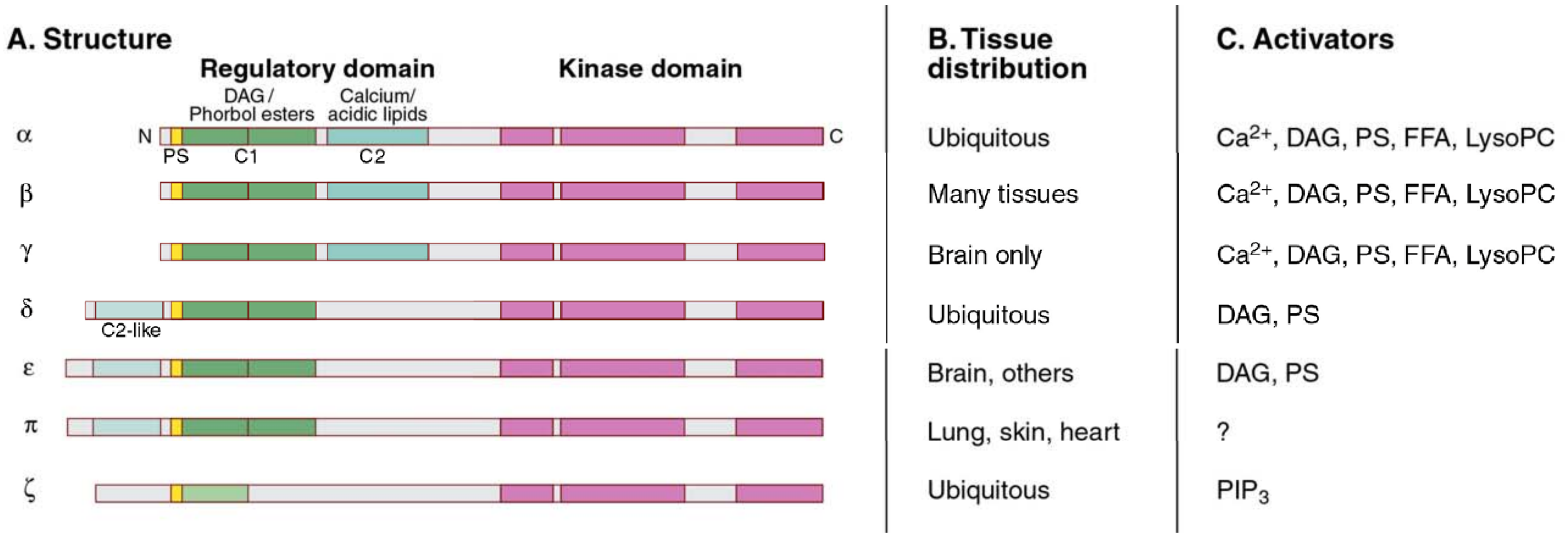
Targets of Lipid Second Messengers (II)

Targets

- PKC
- GPCR
- Ca^{2+} stores
- PH-domains, IP_3 receptor



Protein kinase C (PKC)



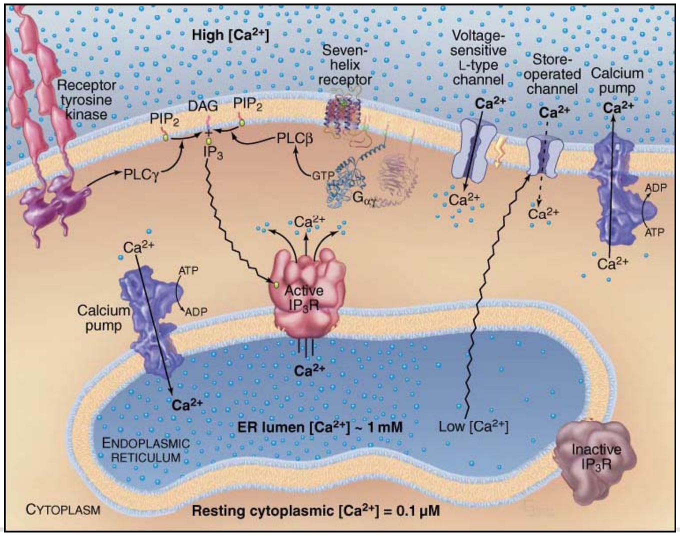
- PKC is an important substrate to lipid second messengers.

- Activated PKCs are involved in regulation of many cellular functions.

- There are many other lipid-derived signaling pathways associated with PC, PIP2 & spingomyelin.

Calcium Ion (I)

- Calcium ion is a versatile second messenger that regulates many cellular processes
- Ca^{2+} levels are controlled by release and removal from the cytoplasm.
  - Release through channels in ER, mitochondria, & cell membrane
  - Removal from cytoplasm through ATP-driven Ca^{2+} pumps



Calcium Ion (II)

- ATP driven Ca^{2+} pumps maintain a 10,000-fold concentration gradient across membranes.

- Resting level: 0.1uM

- Calcium ion enters cytoplasm in sparks through channels.

- Ca^{2+} signals work locally.

  - Diffusion of Ca^{2+} is very slow due to sequestering and many binding partners (300 uM).

  - Only 1% is allow to freely diffuse.

Calcium Ion (III)

- Range of diffusion
  - Without calmodulin: 0.1μm; half-time for free ion: 30μs
  - With calmodulin: 5 μm
- Removal of calcium ion by pumps
  - Pumps: transfer two ions per ATP hydrolysis
  - Activated until the cytoplasmic Ca^{2+} level falls to 0.1uM
- Removal of calcium ion by sequestering
  - Calsequestrin (mostly in muscle)
  - Calreticulin (ER)
- Intracellular calcium stores can be refilled using extracelluar Ca^{2+} (through the TRP channels)

Calcium Ion Channels

- Voltage-gated channels

- Ligand-gated channels
  - IP_3 receptors (generic physiological)
  - Ryanodine receptors (ER; muscle-related)

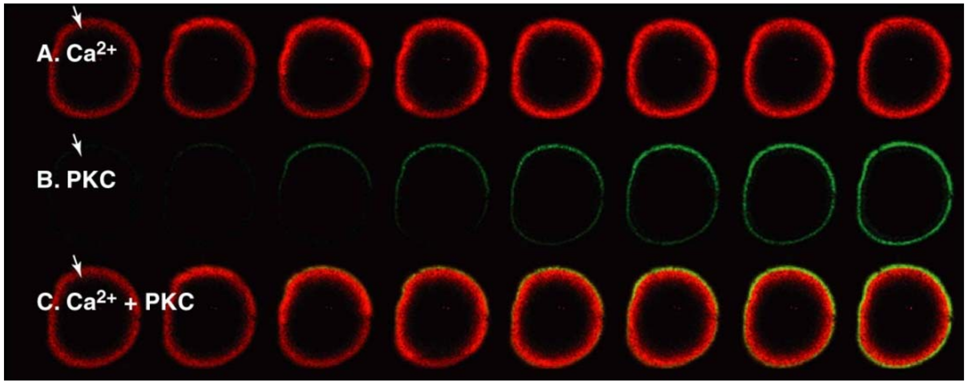
CA^{2+}-RELEASE CHANNELS

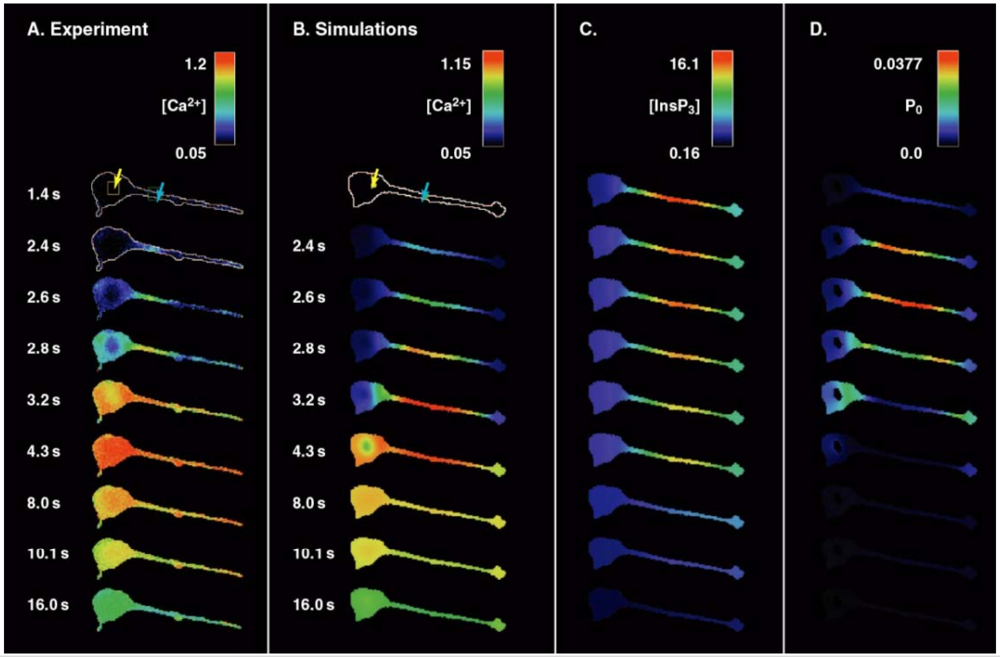
Type	Distribution	Control	Features
Plasma Membrane Ca^{2+} Channels			
ATP-activated channel	Smooth muscle	Extracellular ATP	
cAMP-activated channel	Sperm	Cytoplasmic cAMP	
L-type Ca^{2+}-channel	Skeletal and cardiac muscle, brain, other nonmuscle cells	Voltage	Excitation-contraction coupling, defective in muscular dysgenesis. High threshold, dihydropyridine (DHP)-sensitive, regulated by PKA
N-type Ca^{2+}-channel	Neurons, endocrine cells	Voltage	Neurotransmitter release, modulated by G-proteins. High threshold, conotoxin-sensitive
P-type Ca^{2+}-channel	Purkinje neurons	Voltage	Insensitive to dihydropyridine and conotoxin
T-type Ca^{2+}-channel		Voltage	Low threshold
Endoplasmic Reticulum Ca^{2+} Channels			
IP_3 receptors	Most cells including brain and smooth muscle	IP_3, Ca^{2+}	Heparin-sensitive
Type I ryanodine receptor	Skeletal muscle	DHP-receptor, Ca^{2+}	Ca^{2+} release stimulates contraction
Type II ryanodine receptor	Cardiac muscle, other cells	Ca^{2+}, cADP-ribose	Ca^{2+} release stimulates contraction
Type III ryanodine receptor	Smooth muscle, other cells	Ca^{2+}, cADP-ribose	Ca^{2+} release stimulates contraction

Calcium Dynamics

- Calcium dynamics is regulated in space and time

- Stimulation
  Upper panel: a needle prick
  Lower panel: bradykinin





Calcium Ion Targets

- Membrane proteins
- Enzymes
- Cytoskeletal proteins
- Calcium-binding proteins

Examples of Ca^{2+} Regulated Proteins

Protein	Binding Site	Function
First-Order Proteins That Bind Ca^{2+} Directly		
Membrane Proteins		
Annexins	Novel	Promote membrane interactions
Ca^{2+}-activated Cl^- channels	Novel	Participate in secretion
Ca^{2+}-activated K^+ channels	Novel	Control membrane excitability
IP_3 receptor	Novel	Ca^{2+}-release channel; activated and inhibited by Ca^{2+}
Ryanodine receptor	Novel	Ca^{2+}-release channel, activated and inhibited by Ca^{2+}
Synaptotagmin	Novel	A synaptic vesicle Ca^{2+} sensor
Enzymes		
Calmodulin-domain protein kinases	[a]EF hand	Plant protein kinases
Calpain	EF hand	Ca^{2+}-dependent protease
Protein kinase C, some isozymes	C2 domain	Multifunctional protein kinases activated by Ca^{2+}
Cytoskeletal Proteins		
α-Actinin (some isoforms)	EF hand	Actin filament cross-linking protein
Centrin/caltractin	EF hand	Ca^{2+}-sensitive contractile fibers
Gelsolin, villin	Novel	Actin filament severing and capping proteins
Molluscan myosin light chains	EF hand	Regulate muscle contraction; activated by Ca^{2+}
Troponin C	EF hand	Ca^{2+}-activated regulator striated muscle contraction
Calcium-Binding Proteins		
Calmodulin	EF hand	Ca^{2+}-activated regulator of many proteins
Calbindin-D28K	EF hand	Cytoplasmic Ca^{2+} buffer
Calretinin	EF hand	Activates guanylyl cyclase
Parvalbumin	EF hand	Cytoplasmic Ca^{2+} buffer
S100 proteins (18 human isoforms)	EF hand	Diverse regulatory functions; some isoforms may be secreted
S100 calbindin-D9K	EF hand	Cytoplasmic Ca^{2+} buffer
Recoverin	EF hand	Regulates visual phototransduction
Second-Order Proteins Activated by Ca^{2+}-Calmodulin		
Membrane Proteins		
Adenylyl cyclase (some isoforms)		Produces cAMP
Ca^{2+}-dependent Na^+ channels		Na^+ currents
cGMP-gated cation channels		Phototransduction
Plasma membrane Ca^{2+}-ATPase pumps	Clears cytoplasm of Ca^{2+}	
Enzymes		
Calcineurin		Protein phosphatase 2B
CaM kinase (several isozymes)		Multifunctional protein kinase
cAMP phosphodiesterase		Degrades cAMP
IP_3 kinase		Phosphorylates IP_3
Myosin light chain kinase		Activates smooth muscle and cytoplasmic myosin
NAD kinase		Phosphorylates NAD
Nitric oxide synthetase		Makes nitric oxide
Phosphorylase kinase		Phosphorylates phosphorylase
Cytoskeletal Proteins		
MARCKS		Actin filament cross-linking protein

[a]EF hand is the abbreviation for the Ca^{2+} binding site in calmodulin consisting of α-helices E and F.

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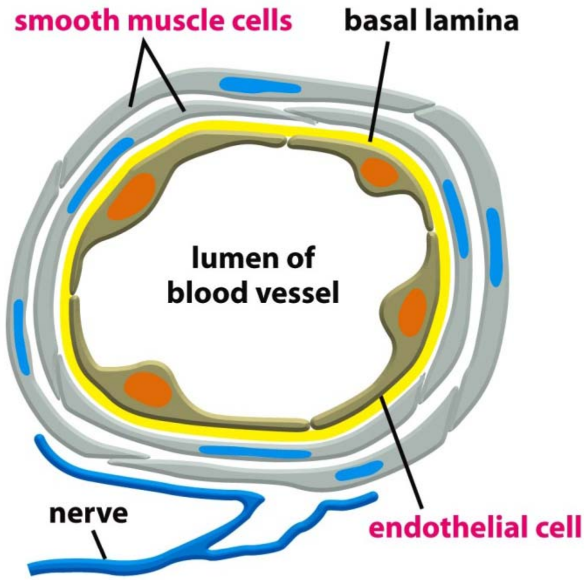
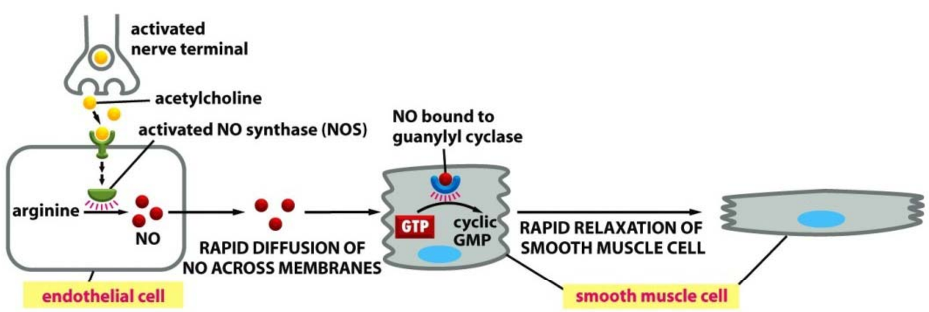
Nitric Oxide

- NO diffuses rapidly through membranes.
- NO reacts with oxygen and has a half-life of only a few seconds $\rightarrow$ must be produced continuously.
- Produced by nitric oxide synthases (NOS).
- Inactivated by binding to hemoglobin and then excreted.
- Main target: guanylyl cyclase (cGMP producer)
- Main physiological functions
  - Killing pathogens
  - Regulating blood flow and blood pressure

Nitric Oxide Activates Guanylyl Cyclase

- Soluble guanylyl cyclase is a mammalian NO/CO sensor.
- NO signaling is critical to many physiological processes involving cardiovascular and neuronal systems.
- Related drugs work by blocking the breakdown of cGMP.

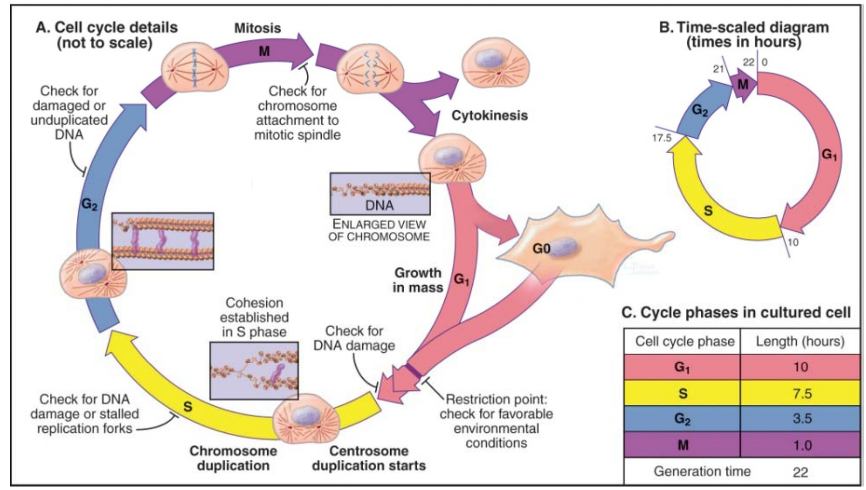


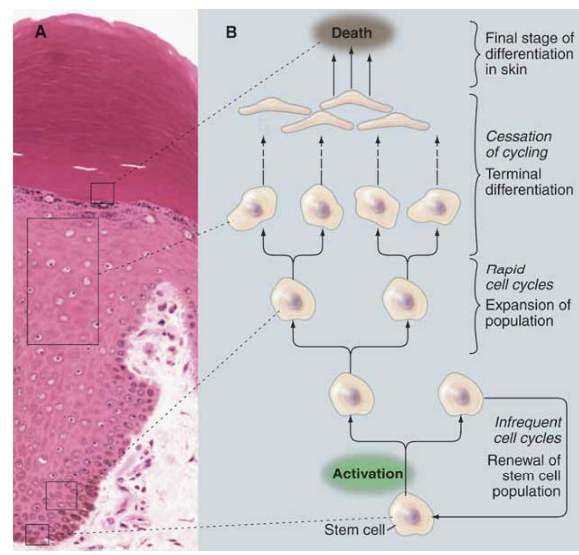


- Second messengers
- Overview of cell cycle
- Different phases of cell cycle
- Overview of checkpoints
- Cyclin and cyclin-dependent kinases

Overview of Cell Cycle (I)

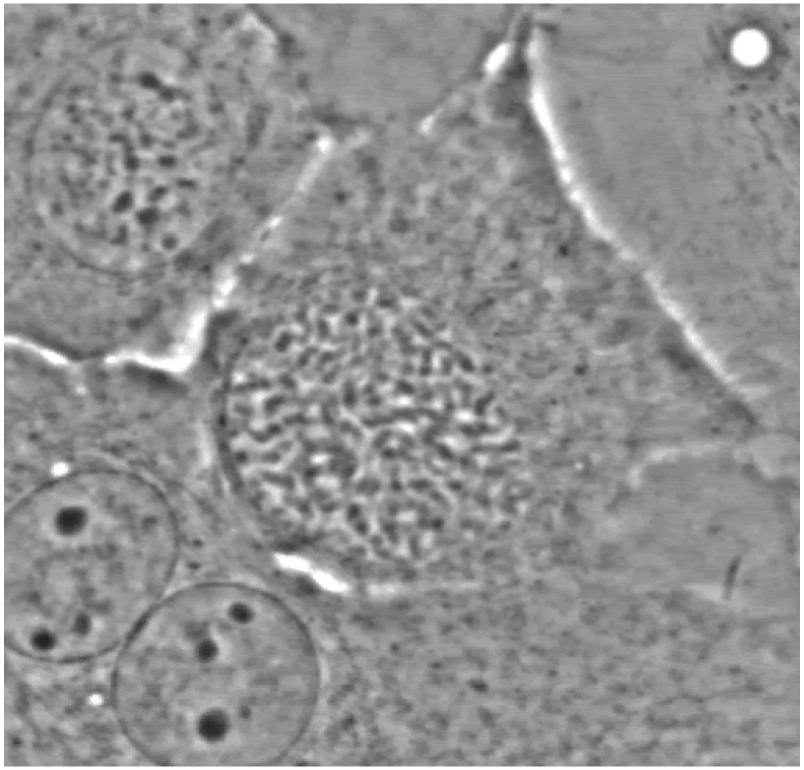
- The cell cycle is a series of events that leads to the replication and division of a cell.
- Two daughter cells inherit the same genetic information from the mother cell.
- Cell cycle must be tightly regulated.
- Basic mechanisms of cell cycle regulation are well conserved in eukaryotes.





Overview of Cell Cycle (II)

- Cell cycle is controlled by a series of biochemical switches (checkpoints).
- Additional layer of regulation ensures fidelity of cell division by responding to internal and external signals.
- In addition to replicating their genome, most cells replicate their other organelles and macromolecules.
- Growth in cell mass must be regulated too.



- Second messengers
- Overview of cell cycle
- **Different phases of cell cycle**
- Overview of checkpoints
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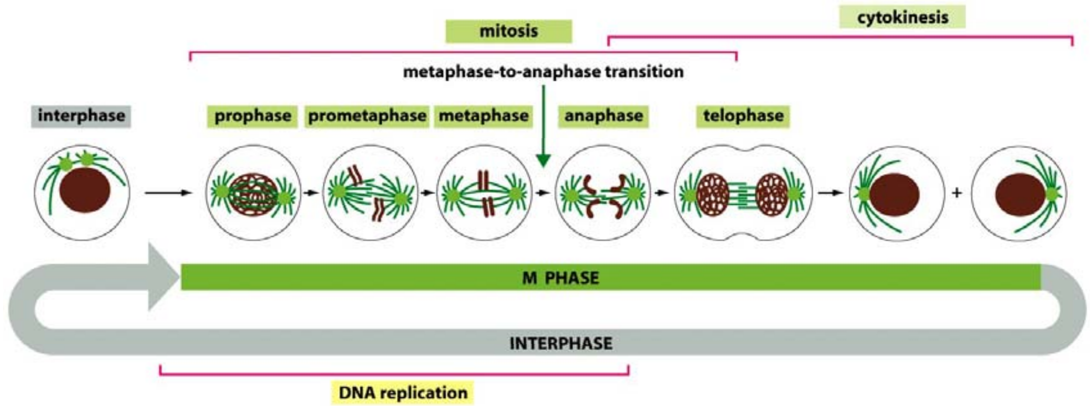
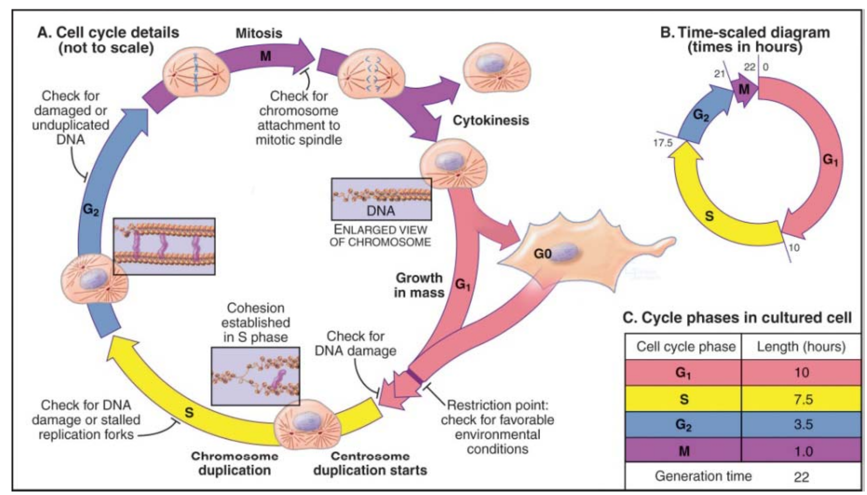
Phases of Cell Cycle (I)

- G_1 phase
  - Grow in cell mass
  - Genes required to activate cell cycle are turned off
  - Can be delayed
  - Can exit to G0



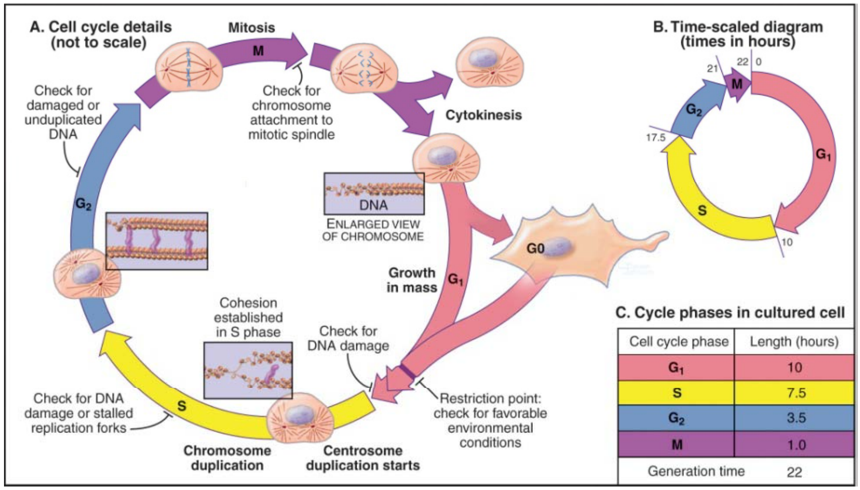
- G_0 and growth control
  - Cells no longer divide
  - Can exit from G_0
  - Cells are active in G_0



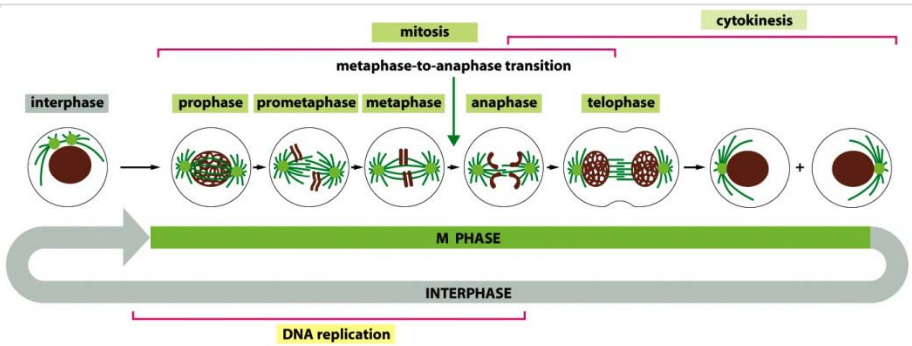
Phases of Cell Cycle (II)

- S phase
  - - Centrosome replication
  - - DNA replication
  - - Sister chromatids are connected by cohesin

- G_2 phase
  - - To check for unreplicated or damaged DNA
  - - To prepare for mitosis (e.g. accumulation of enzymes)



Phases of Cell Cycle (III)



- M phase
  - → prophase:
    - condensation of chromosomes; formation of two poles
  - → prometaphase
    - nuclear envelope breakdown; bipolar attachment at kinetochores
  - → metaphase
    - chromosomes aligned in the midzone
  - → anaphase
    - sister chromatids separated; moving to the two poles
  - → telophase
    - reformation of nuclear envelopes
  - → cytokinesis
    - formation of contractile ring; separation of two daughter cells

- Second messengers
- Overview of cell cycle
- Different phases of cell cycle
- **Overview of checkpoints**
- Cyclin and cyclin-dependent kinases

Checkpoints (I)

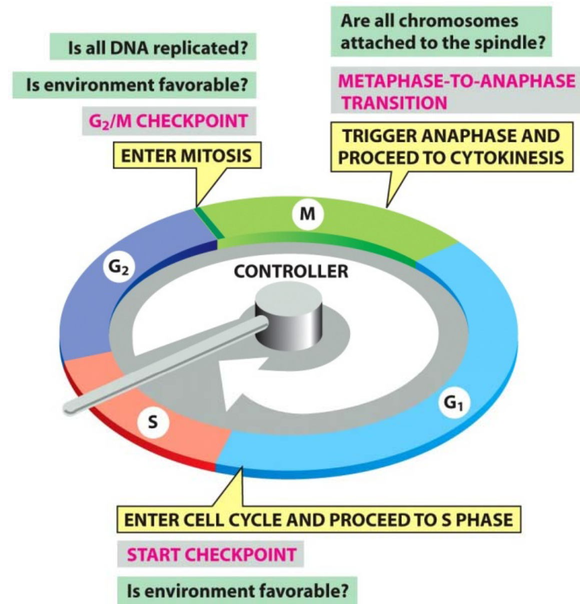
- Check points are biochemically implemented switches that control transition between cell-cycle stages

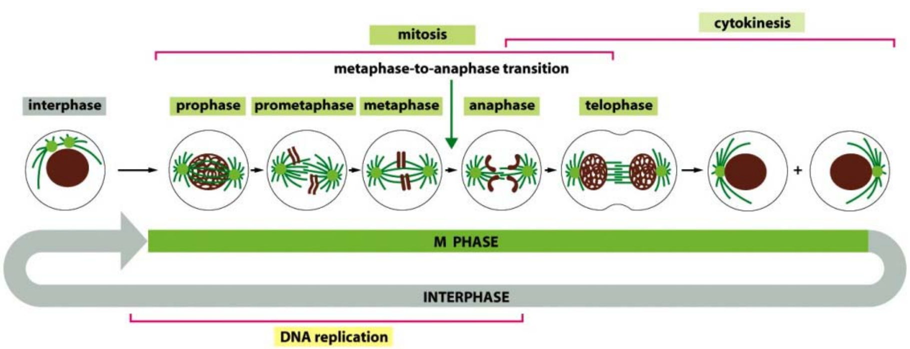
- Restriction point in G1 phase

- DNA damage checkpoint in G1, S, G2 phase

- DNA replication checkpoint

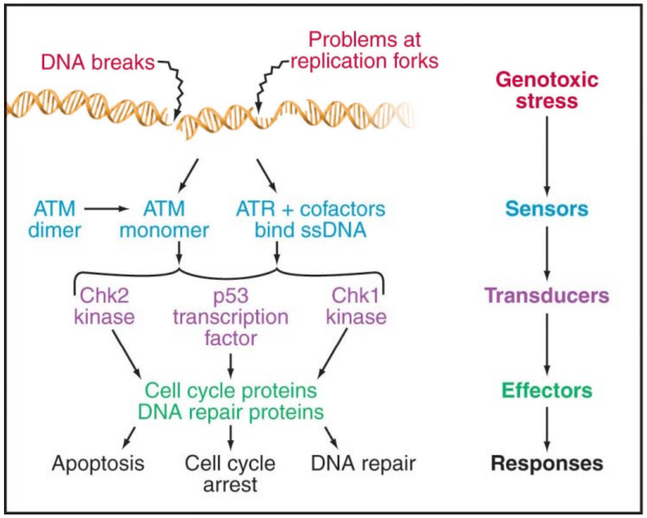
- Spindle assembly checkpoint





Checkpoints (II)

- DNA damage check point
- Sensing of DNA damage: ATM & ATR
- ATM & ATR activate Chk1& Chk2 and stabilizes p53.
- Cell-cycle progression is halted if DNA damage is detected.
- Cells can enter into several states
  - Cell death
  - Cell cycle arrest
  - Successful DNA repair; Resume cell cycle



- Second messengers
- Overview of cell cycle
- Different phases of cell cycle
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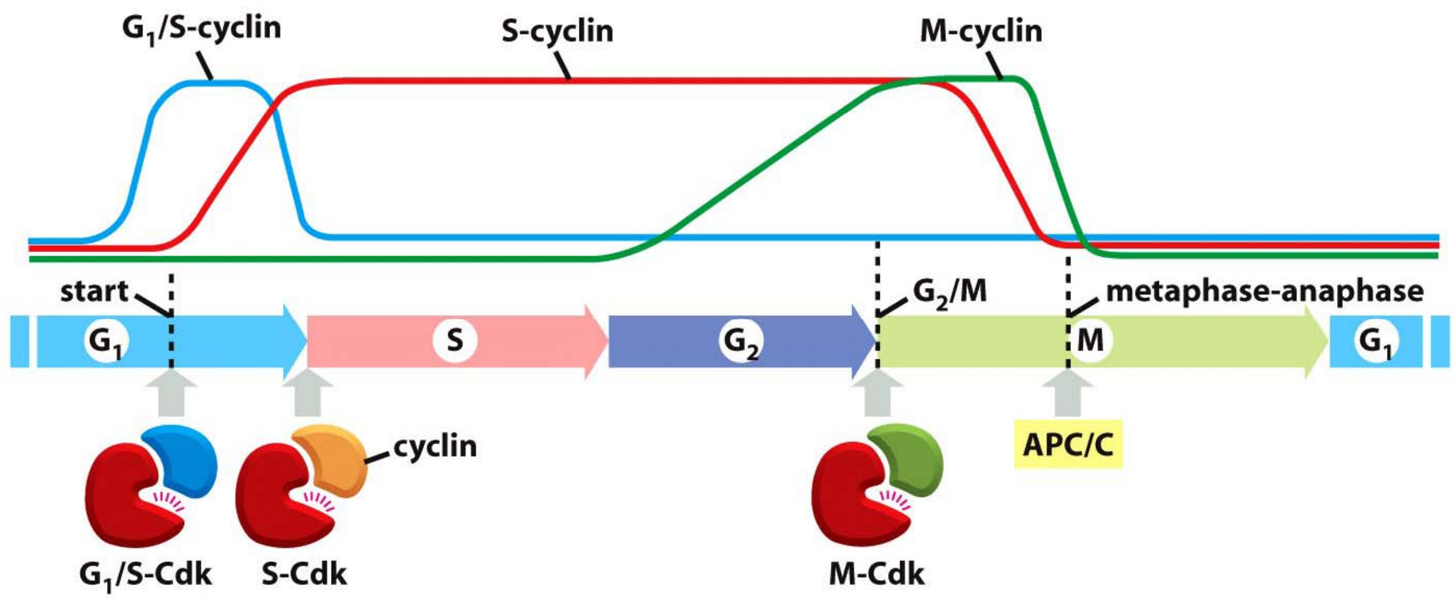
Cyclin and Cyclin-Dependent Kinase (I)

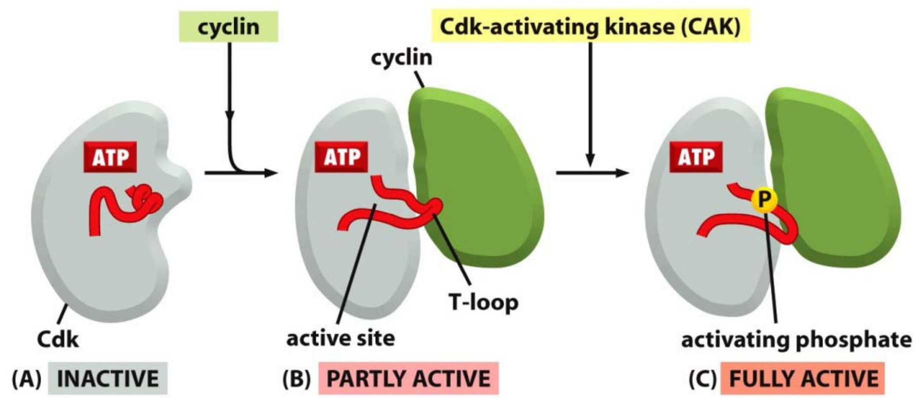
- Transition between different stages of the cell cycle are controlled by a network of kinases and phosphatases.

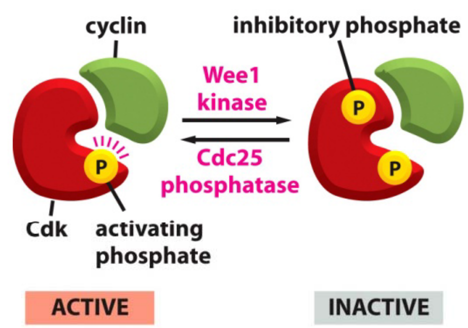
- Cyclin-dependent kinases play critical roles in regulating cell cycle.

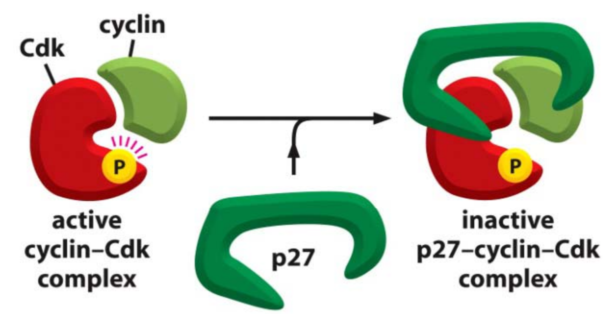


Cyclin and Cyclin-Dependent Kinase (II)

- CAK (cdk-activating kinase) actives cyclin-CDK complexes.
- Activated cyclin-CDK complexes can be inhibited by Cdk inhibitor proteins (CKI) or inhibitory phosphorylation.
- Redundant mechanisms used to ensure robustness and fidelity of cell-cycle control.







Cyclin and Cyclin-Dependent Kinase (III)

Table 17–1 The Major Cyclins and Cdks of Vertebrates and Budding Yeast

CYCLIN–CDK COMPLEX	VERTEBRATES		BUDDING YEAST	
	CYCLIN	CDK PARTNER	CYCLIN	CDK PARTNER
G_1-Cdk	cyclin D*	Cdk4, Cdk6	Cln3	Cdk1**
G_1/S-Cdk	cyclin E	Cdk2	Cln1, 2	Cdk1
S-Cdk	cyclin A	Cdk2, Cdk1**	Clb5, 6	Cdk1
M-Cdk	cyclin B	Cdk1	Clb1, 2, 3, 4	Cdk1

* There are three D cyclins in mammals (cyclins D1, D2, and D3).
** The original name of Cdk1 was Cdc2 in both vertebrates and fission yeast, and Cdc28 in budding yeast.

Protein Degradation in Cell Cycle (I)

- Protein degradation is a critical cell cycle regulatory mechanism.

- Exit from mitosis requires CDK inactivation

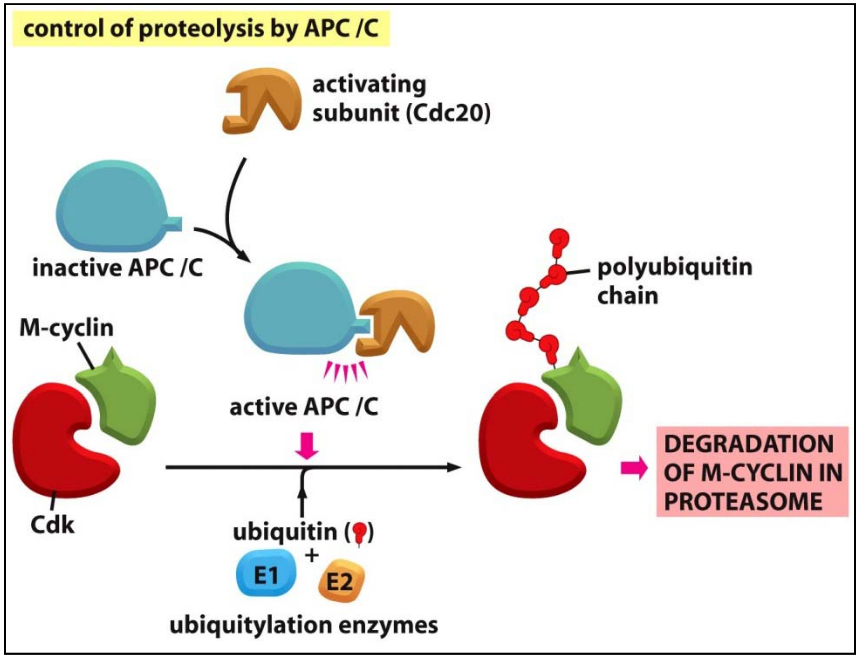
- This is achieved by degradation of cyclins and securin (regulator of sister chromatid separation).

- Destruction of cyclins inactivate CDKs.

- Degradation of cyclin is performed in proteasome and requires ubiquitin enzymes (E1, E2, E3).

Protein Degradation in Cell Cycle (II)

- Key regulator of cyclin degradation is APC/C (anaphase promoting complex/cyclosome).
- Two forms of APC/C
  - APC/C^{Cdc20}
  - APC/C^{Cdh1}
- APC/C^{Cdc20} is responsible for triggering metaphase-anaphase transition.



Summary of Cell Cycle Regulatory Proteins

Table 17–2 Summary of the Major Cell-Cycle Regulatory Proteins

GENERAL NAME	FUNCTIONS AND COMMENTS
Protein kinases and protein phosphatases that modify Cdks	
Cdk-activating kinase (CAK)	phosphorylates an activating site in Cdks
Wee1 kinase	phosphorylates inhibitory sites in Cdks; primarily involved in suppressing Cdk1 activity before mitosis
Cdc25 phosphatase	removes inhibitory phosphates from Cdks; three family members (Cdc25A, B, C) in mammals; primarily involved in controlling Cdk1 activation at the onset of mitosis
Cdk inhibitor proteins (CKIs)	
Sic1 (budding yeast)	suppresses Cdk1 activity in G_1; phosphorylation by Cdk1 at the end of G_1 triggers its destruction
p27 (mammals)	suppresses G_1/S-Cdk and S-Cdk activities in G_1; helps cells withdraw from cell cycle when they terminally differentiate; phosphorylation by Cdk2 triggers its ubiquitylation by SCF
p21 (mammals)	suppresses G_1/S-Cdk and S-Cdk activities following DNA damage
p16 (mammals)	suppresses G_1-Cdk activity in G_1; frequently inactivated in cancer
Ubiquitin ligases and their activators	
APC/C	catalyzes ubiquitylation of regulatory proteins involved primarily in exit from mitosis, including securin and S- and M-cyclins; regulated by association with activating subunits
Cdc20	APC/C-activating subunit in all cells; triggers initial activation of APC/C at metaphase-to-anaphase transition; stimulated by M-Cdk activity
Cdh1	APC/C-activating subunit that maintains APC/C activity after anaphase and throughout G_1; inhibited by Cdk activity
SCF	catalyzes ubiquitylation of regulatory proteins involved in G_1 control, including some CKIs (Sic1 in budding yeast, p27 in mammals); phosphorylation of target protein usually required for this activity

Questions ?