12 Biochemical Signaling

Signal Transduction Is a Universal Property of Living Cells

- **signal transduction** = the conversion of information into a chemical change
  - signal represents *information* that is detected by specific receptors
  - conversion of the signal to a cellular response always involves a *chemical* process

P1 Principle 1 (1 of 5)

Cells respond to external signals through receptor-mediated processes that amplify the signal, integrate it with input from other receptors, transmit the signal to the appropriate effectors, and eventually end the response.

P2 Principle 2 (1 of 4)

There is a high degree of evolutionary conservation of signaling proteins and transduction mechanisms across the animal phyla. At least a billion years of evolution have passed since the plant and animal branches of the eukaryotes diverged, which is reflected in the differences in signaling mechanisms used in the two kingdoms. We focus here on the animal kingdom.

P3 Principle 3 (1 of 3)

In multicellular animals, GPCRs with seven transmembrane helices are the largest group of plasma membrane receptors. These *G P*rotein–*C*oupled *R*eceptors act through G proteins, which are turned on when they bind guanosine triphosphate (GTP). Animals have hundreds of different GPCRs, each able to respond to a specific signal.

P4 Principle 4 (1 of 5)

Plasma membrane receptors with an intracellular tyrosine kinase activity act through cascades of protein kinases to transduce signals about the metabolic state, including growth factors.

P5 Principle 5 (1 of 6)

Phosphorylation of intrinsically disordered regions of signaling proteins acts as a switch, toggling enzyme activity or creating binding sites for other molecules. Signal responses are integrated by multiprotein signaling complexes with modular domains that bind phosphorylated Tyr, Ser, or Thr residues.

P6 Principle 6 (1 of 5)

Ion channels gated by membrane potential or ligands are central to signaling in all cells, including bacteria, plants, and animals.

P7 Principle 7 (1 of 2)

Some hormone signals act inside the cell, not at the plasma membrane, forming complexes with specific receptor proteins that regulate gene expression.

P8 Principle 8 (1 of 2)

Cells receive extracellular signals that determine progress through the cell division cycle, or trigger cell death—processes regulated by phosphorylation and dephosphorylation of key regulatory proteins.

P9 Principle 9 (1 of 6)

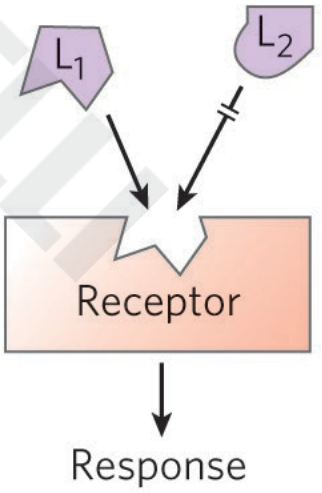
Defective signaling proteins or defective regulation of their synthesis and breakdown can disrupt cell cycle regulation and lead to tumor formation (cancer).

12.1 General Features of Signal Transduction

Signal Transductions Are Remarkably Specific

- **specificity** = achieved by precise molecular complementarity between the signal and receptor molecules
  - mediated by weak (noncovalent) forces

(a) Specificity
Signaling ligand fits binding site on its complementary receptor; other ligands do not fit.



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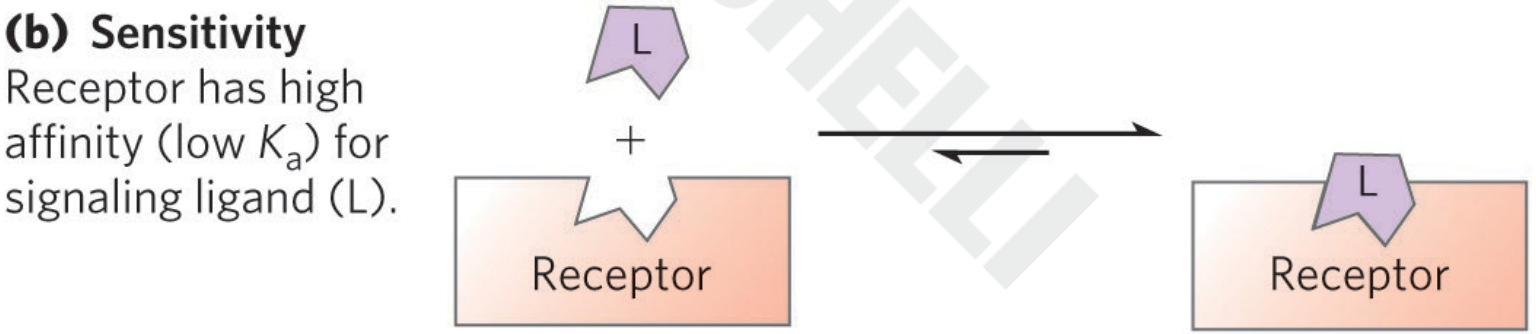
Signal-Transducing Systems Share Common Features

- **sensitivity** = results from the high affinity of signal receptors for their ligands
  - dissociation constant, K_d, is commonly < 10^{-7} M



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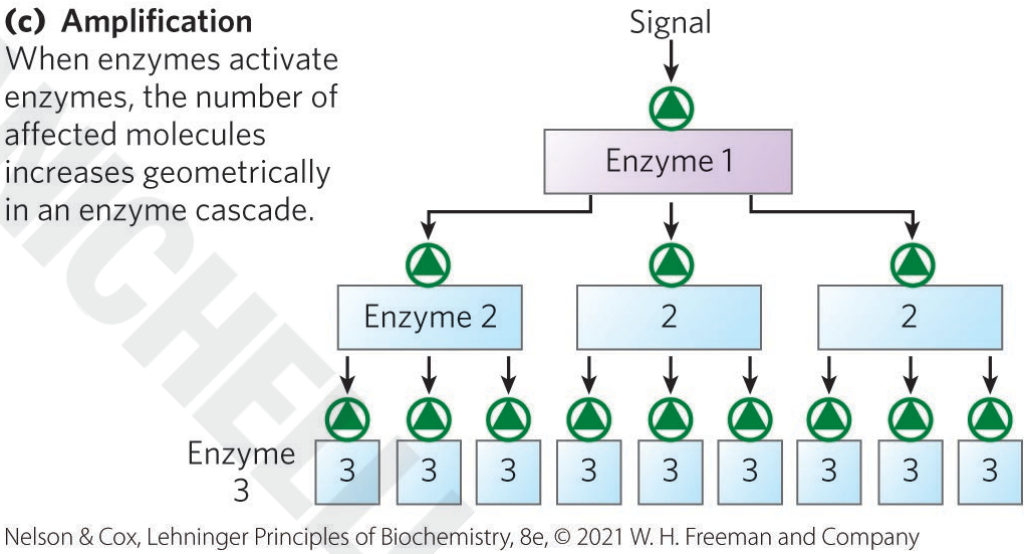
P1 Principle 1 (2 of 5)

Cells respond to external signals through receptor-mediated processes that amplify the signal, integrate it with input from other receptors, transmit the signal to the appropriate effectors, and eventually end the response.

Amplification and Enzyme Cascades

- **amplification** = results when an enzyme is activated by a signal receptor and, in turn, catalyzes the activation of many molecules of a second enzyme, and so on, in an **enzyme cascade**



P5 Principle 5 (2 of 6)

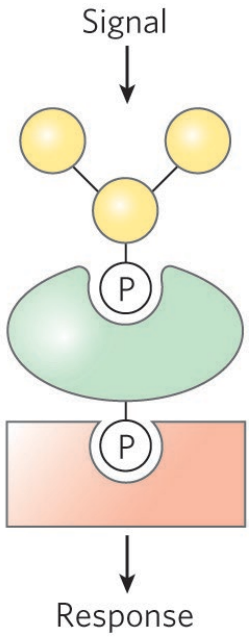
Phosphorylation of intrinsically disordered regions of signaling proteins acts as a switch, toggling enzyme activity or creating binding sites for other molecules. Signal responses are integrated by multiprotein signaling complexes with modular domains that bind phosphorylated Tyr, Ser, or Thr residues.

Interacting Signaling Proteins Are Modular

- **modular** = has multiple domains that recognize specific features
  - allows cells to mix and match a set of signaling molecules

- **scaffold proteins** = nonenzymatic proteins that bring together enzymes that interact in cascades

(d) Modularity
Proteins with multivalent affinities form diverse signaling complexes from interchangeable parts. Phosphorylation provides reversible points of interaction.



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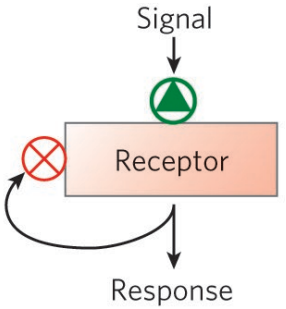
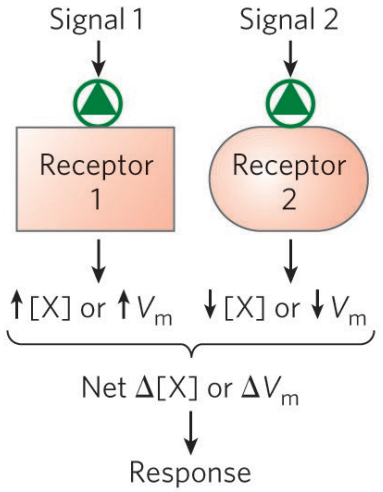
Desensitization and Integration of Receptor Systems

- **desensitized** = no longer responsive to a signal
  - occurs when a signal is present continuously
- **integration** = the ability of the system to receive multiple signals and produce a unified response

(e) Desensitization/Adaptation
Receptor activation triggers a feedback circuit that shuts off the receptor or removes it from the cell surface.



(f) Integration
When two signals have opposite effects on a metabolic characteristic such as the concentration of a second messenger X, or the membrane potential V_m, the regulatory outcome results from the integrated input from both receptors.



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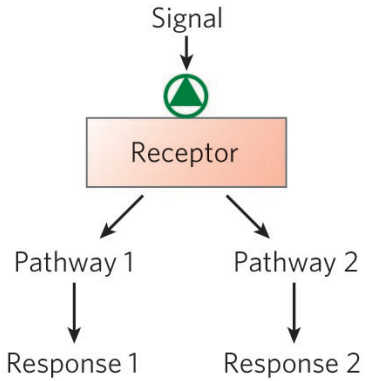
Signaling Pathways Are Often Divergent and Provide A Localized Response

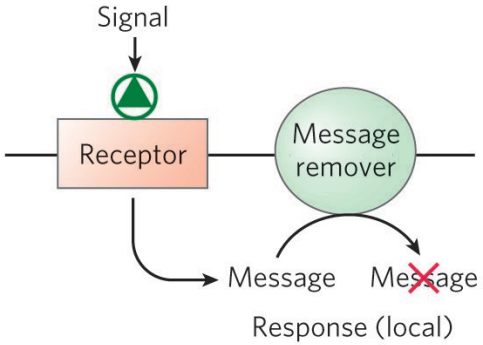
- **divergent** = branched rather than linear
  - occurs when a signal is present continuously

- **response localization** = cells confine signaling system components to a structure to regulate processes locally

(g) Divergence
When a receptor is activated by a signal, it activates two or more pathways with different end effects.



(h) Localized response
When the enzyme that destroys an intracellular message is clustered with the message producer, the message is degraded before it can diffuse to distant points, so the response is only local and brief.



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P5 Principle 5 (3 of 6)

Phosphorylation of intrinsically disordered regions of signaling proteins acts as a switch, toggling enzyme activity or creating binding sites for other molecules. Signal responses are integrated by multiprotein signaling complexes with modular domains that bind phosphorylated Tyr, Ser, or Thr residues.

Cells Respond to Thousands of Different Biological Signals

Table 12-1 Some Signals to Which Cells Respond	
Antigens	Mechanical touch
Cell surface glycoproteins, oligosaccharides	Microbial, insect pathogens
Developmental signals	Neurotransmitters
Extracellular matrix components	Nutrients
Growth factors	Odorants
Hormones	Pheromones
Hypoxia	Tastants
Light	

The Basic Protein Components of Signal Transduction Machinery

Table 12-2 Some Conserved Elements of Animal Signaling Systems
Plasma membrane receptors with 7 transmembrane (7tm) helices
G proteins that bind GTP or GDP and interface with membrane receptors
Membrane enzymes with cyclic nucleotides as substrates or products
Protein kinases that phosphorylate GPCR receptors
Membrane protein tyrosine kinases
Cyclic nucleotide-dependent protein kinases
Ca^{2+}-binding proteins
Ca^{2+}-dependent protein kinases
Protein kinases that are activated during cell division
Nonenzymic protein scaffolds that bring modules together

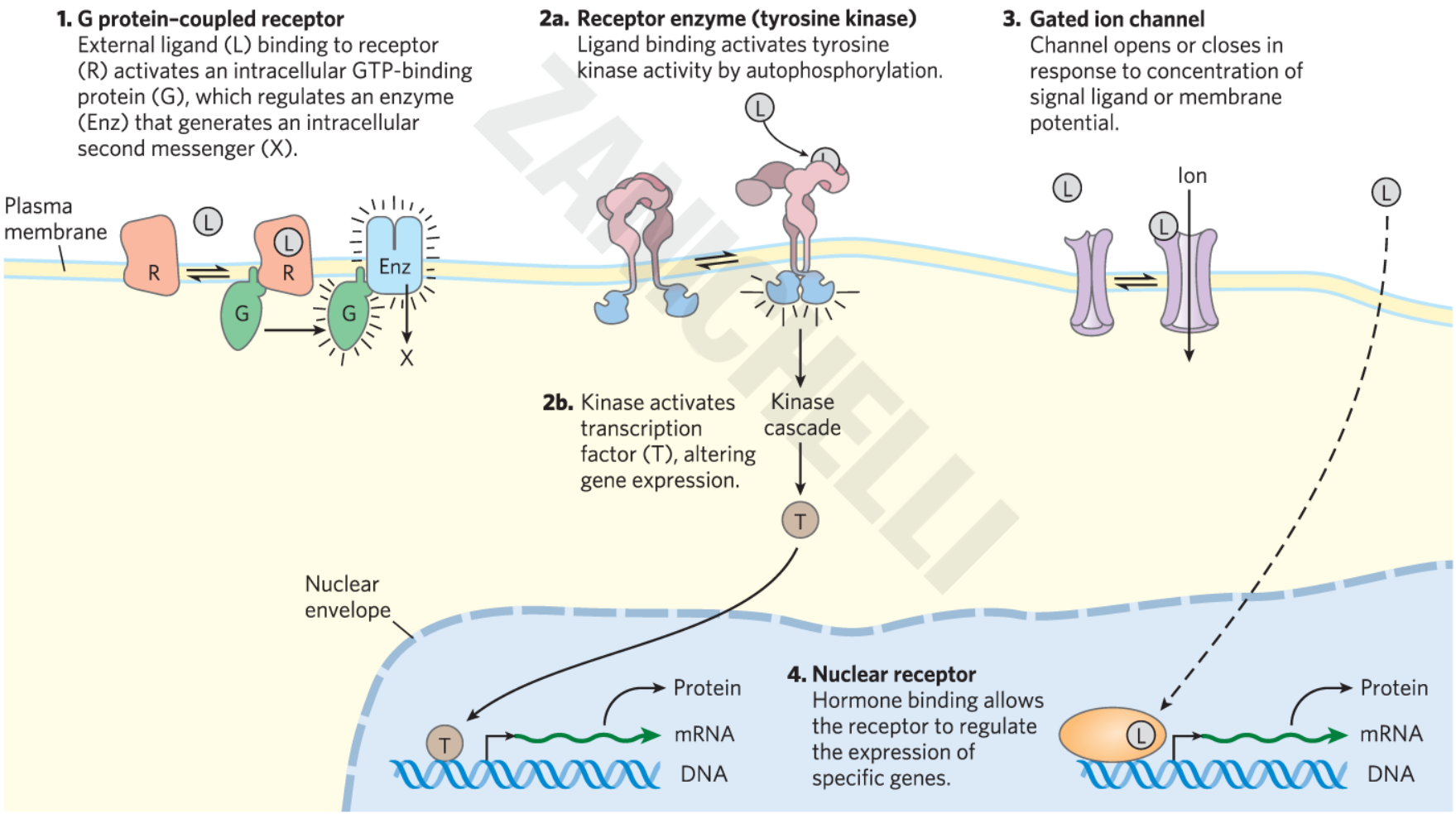
P1 Principle 1 (3 of 5)

Cells respond to external signals through receptor-mediated processes that amplify the signal, integrate it with input from other receptors, transmit the signal to the appropriate effectors, and eventually end the response.

The General Process of Signal Transduction in Animals Is Universal

- general features of signal transduction:
  - a signal (ligand) interacts with a receptor
  - the activated receptor interacts with cellular machinery to produce a second signal or a change in protein activity
  - cellular metabolic activity changes
  - the transduction event ends

Four General Types of Signal Transducers



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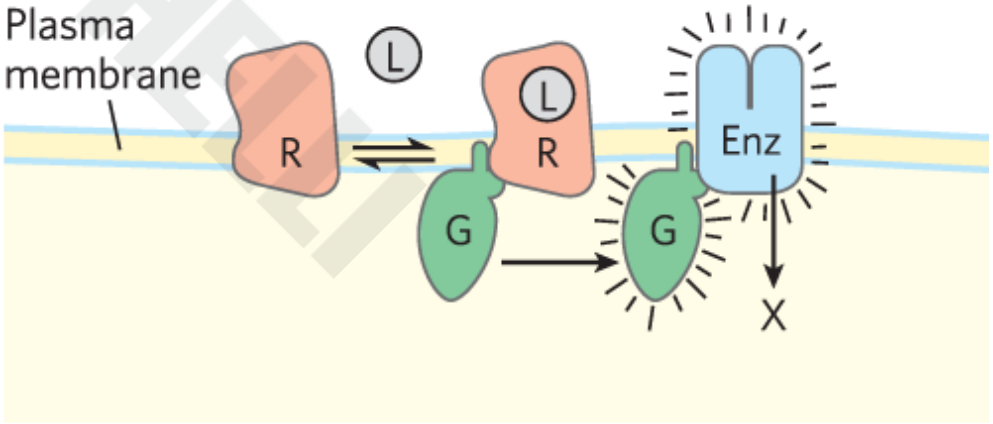
12.2 G Protein-Coupled Receptors and Second Messengers

G Protein-Coupled Receptors (GPCRs)

- **G protein-coupled receptors (GPCRs)** = receptors that act through a member of the **guanosine nucleotide-binding protein (G protein)** family

1. G protein–coupled receptor
External ligand (L) binding to receptor (R) activates an intracellular GTP-binding protein (G), which regulates an enzyme (Enz) that generates an intracellular second messenger (X).



P3 Principle 3 (2 of 3)

In multicellular animals, GPCRs with seven transmembrane helices are the largest group of plasma membrane receptors. These *G P*rotein–*C*oupled *R*eceptors act through G proteins, which are turned on when they bind guanosine triphosphate (GTP). Animals have hundreds of different GPCRs, each able to respond to a specific signal.

Components of Signal Transduction Through GPCRs

- three essential components:
  - a plasma membrane receptor with seven transmembrane helical segments
  - a G protein that cycles between active (GTP-bound) and inactive (GDP-bound) forms
  - an effector enzyme (or ion channel) in the plasma membrane that is regulated by the activated G protein

First and Second Messengers

- "first messenger" = extracellular signal that activates a receptor from the outside of the cell

- **second messenger** = low MW metabolite or inorganic ion that changes in concentration due to the effector enzyme
  - functions to activate or inhibit downstream targets

The β-Adrenergic Receptor System Acts through the Second Messenger cAMP

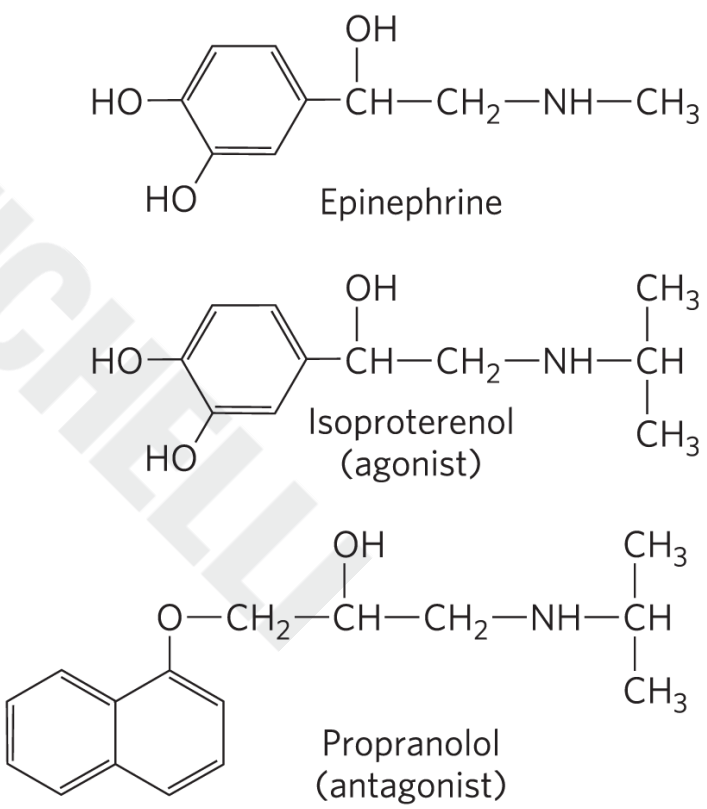
- **adrenergic receptors** = protein receptors in the plasma membrane that bind epinephrine
  - four general types: α_1, α_2, β_1, β_2

- **β-adrenergic receptors** = applies to both β_1 and β_2 subtypes

Agonists and Antagonists

- **agonist** = molecule that binds a receptor and produces the effects of the natural ligand

- **antagonist** = analog that binds the receptor and blocks the effects of the agonist, including the natural ligand



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P3 Principle 3 (3 of 3)

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The β-Adrenergic Receptor Is a GPCR

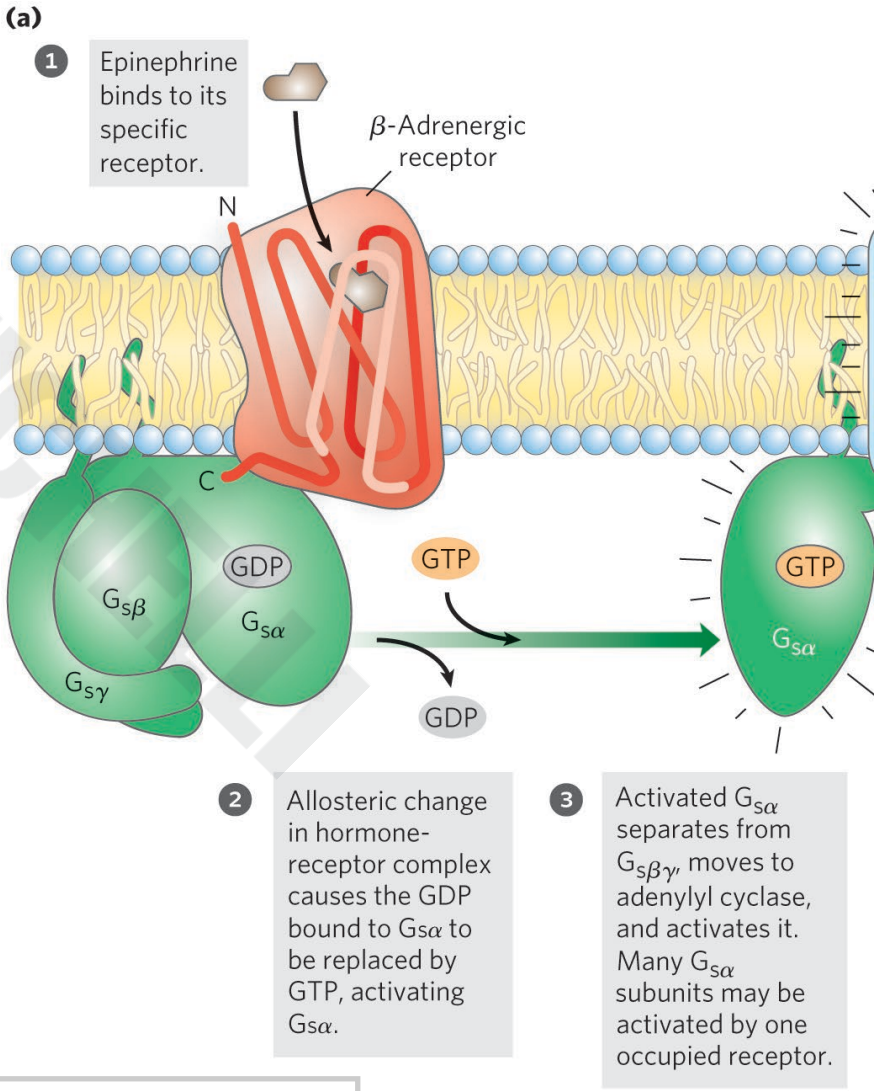
- GPCRs **(seven-transmembrane (7tm)** or **heptahelical receptors)** span the membrane seven times
  - interact with heterotrimeric G proteins

- **heterotrimeric G proteins** = conserved family of signaling proteins with three subunits: *α*, *β*, and *γ*
  - *α* subunit is the binding site for GDP or GTP

The β-Adrenergic Pathway

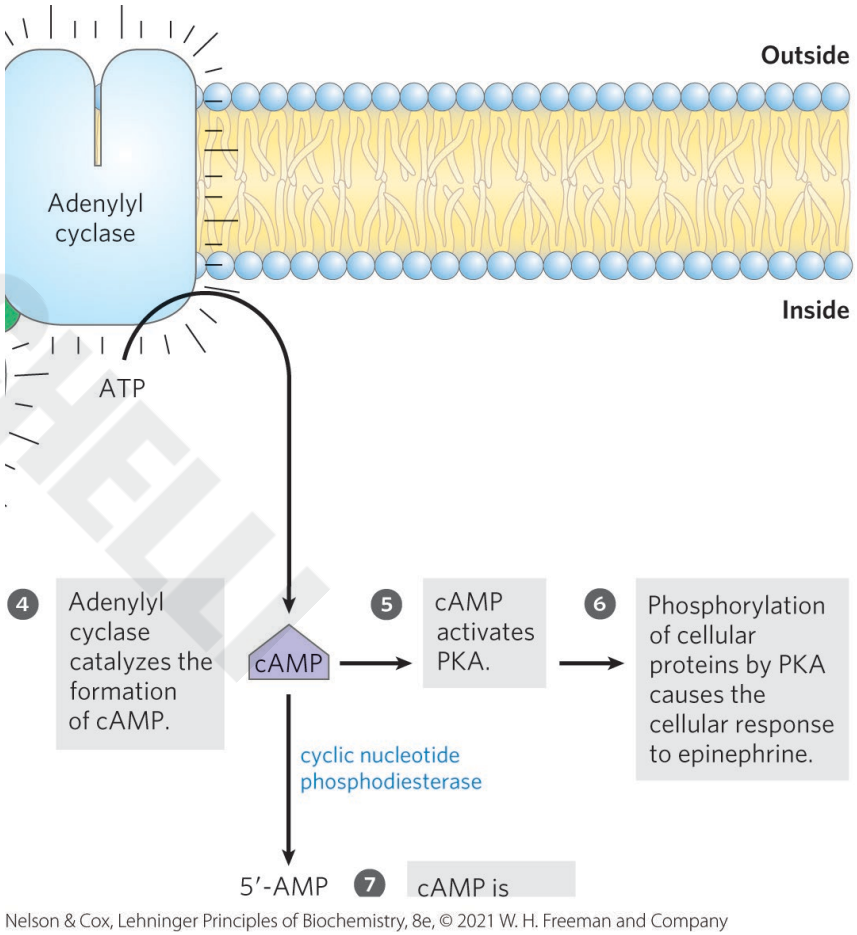
- hormone-bound GPCR acts as a **guanosine nucleotide–exchange factor (GEF)**

- because the G protein stimulates its effector, it is referred to as a **stimulatory G protein (G_s)**



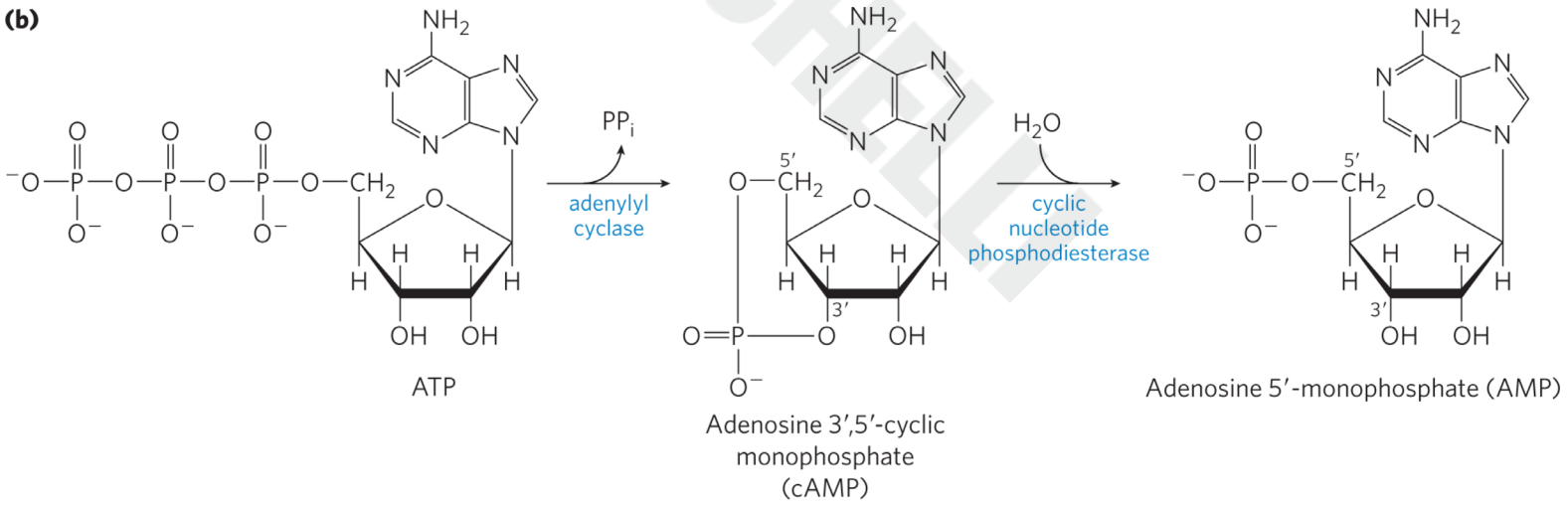
The β-Adrenergic Pathway, Continued

- **adenylyl cyclase** = integral protein in the plasma membrane that catalyzes the synthesis of cAMP from ATP when associated with active $G_{s\alpha}$



The Synthesis and Hydrolysis of cAMP

- cAMP activates PKA
- degradation of cAMP reverses the activation of PKA



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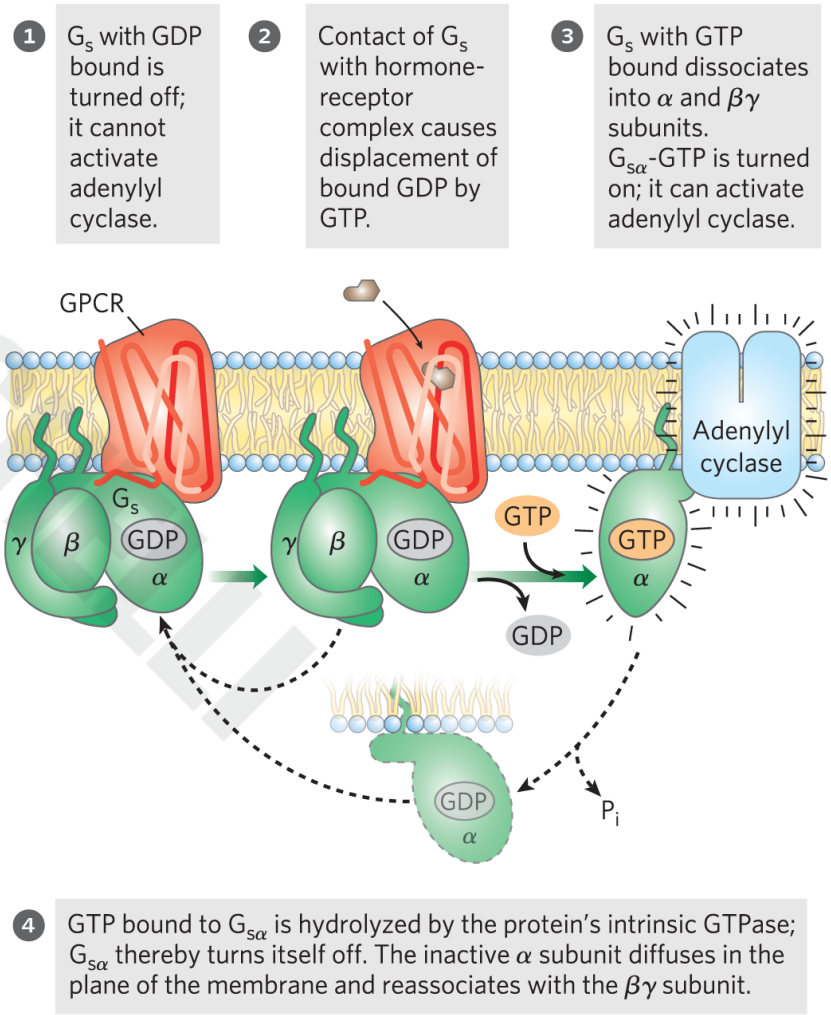
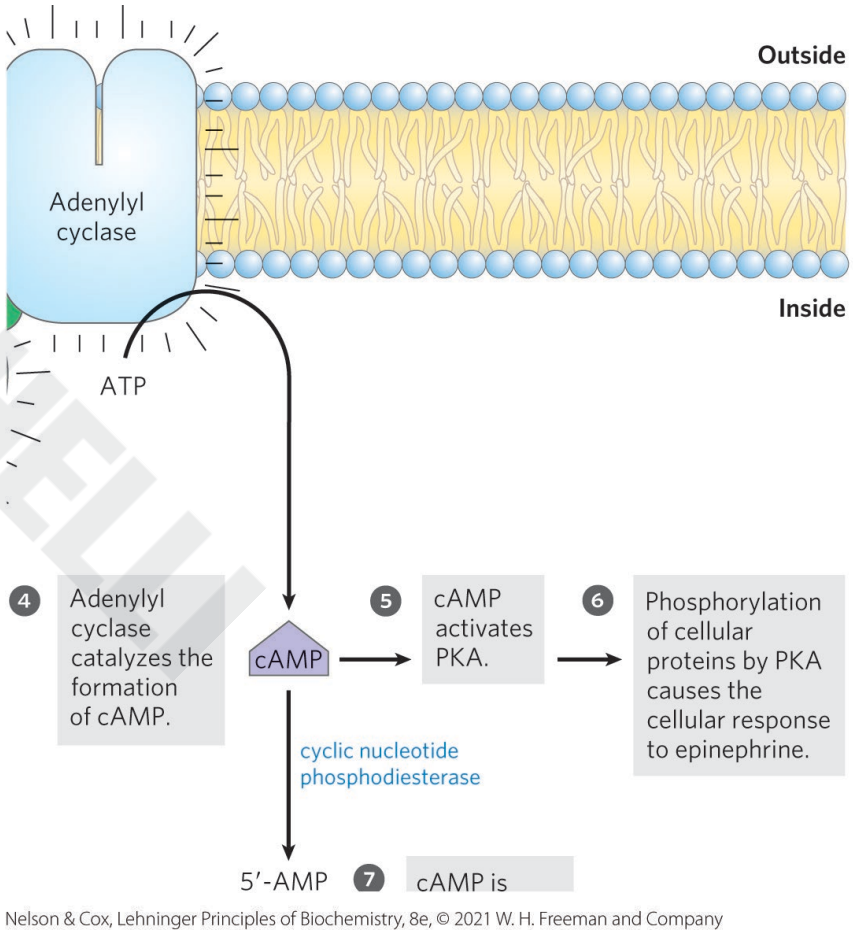
The GTPase Switch

- $G_{s\alpha}$ has intrinsic GTPase activity that switches $G_{s\alpha}$ to its inactive form by converting its bound GTP to GDP



Cyclic AMP Activates Protein Kinase A

- **cAMP–dependent protein kinase (protein kinase A** or **PKA)** = activated by cyclic AMP
  - catalyzes the phosphorylation of specific Ser or Thr residues of target proteins



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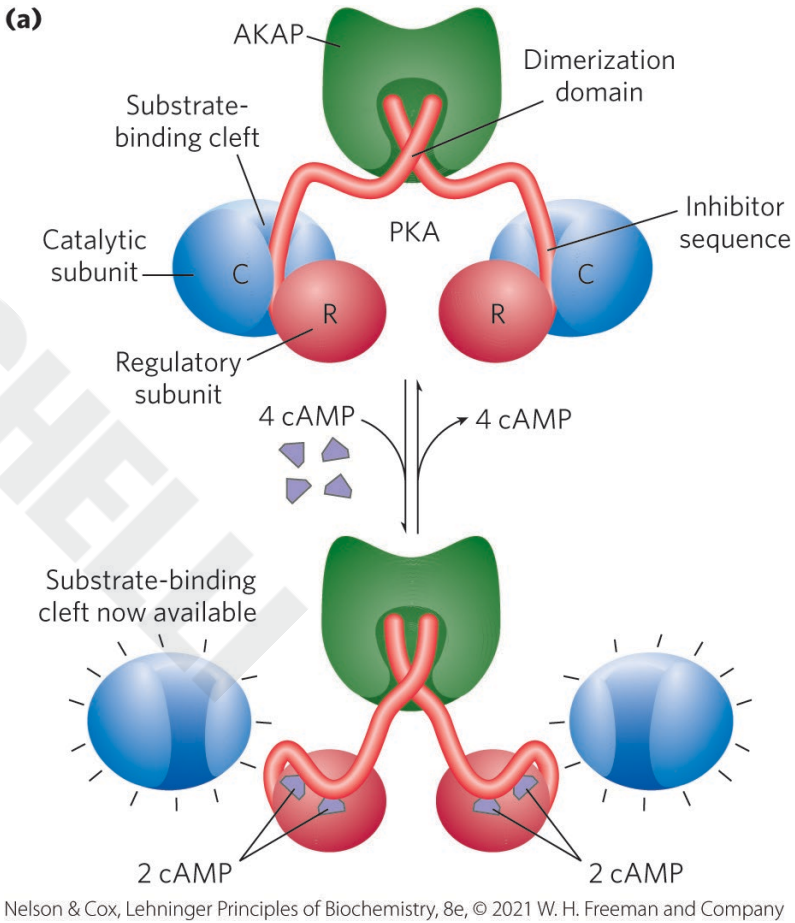
Activation of cAMP-Dependent Protein Kinase (PKA)

- R_2C_2 complex is catalytically inactive because the autoinhibitory domain of each R subunit occupies the substrate-binding cleft of each C subunit

- cyclic AMP is an allosteric activator of PKA
  - binding of cAMP yields two active C subunits



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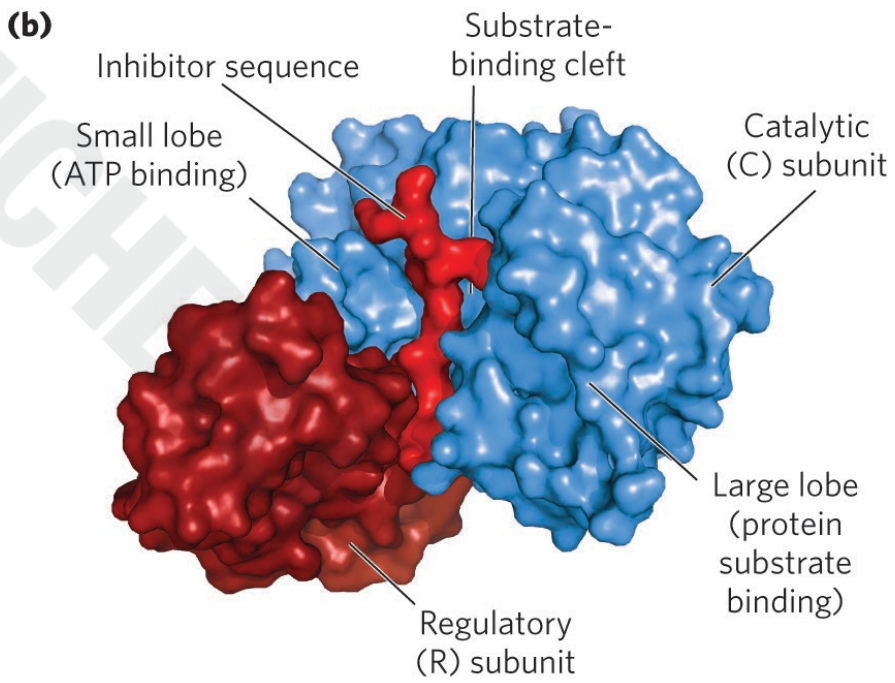
P5 Principle 5 (4 of 6)

Phosphorylation of intrinsically disordered regions of signaling proteins acts as a switch, toggling enzyme activity or creating binding sites for other molecules. Signal responses are integrated by multiprotein signaling complexes with modular domains that bind phosphorylated Tyr, Ser, or Thr residues.

Displacement of an Autoinhibitory Domain Mediates Allosteric Activation of Many Protein Kinases

- structure of the substrate-binding cleft in PKA is the prototype of all known protein kinases



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Consensus Sequences

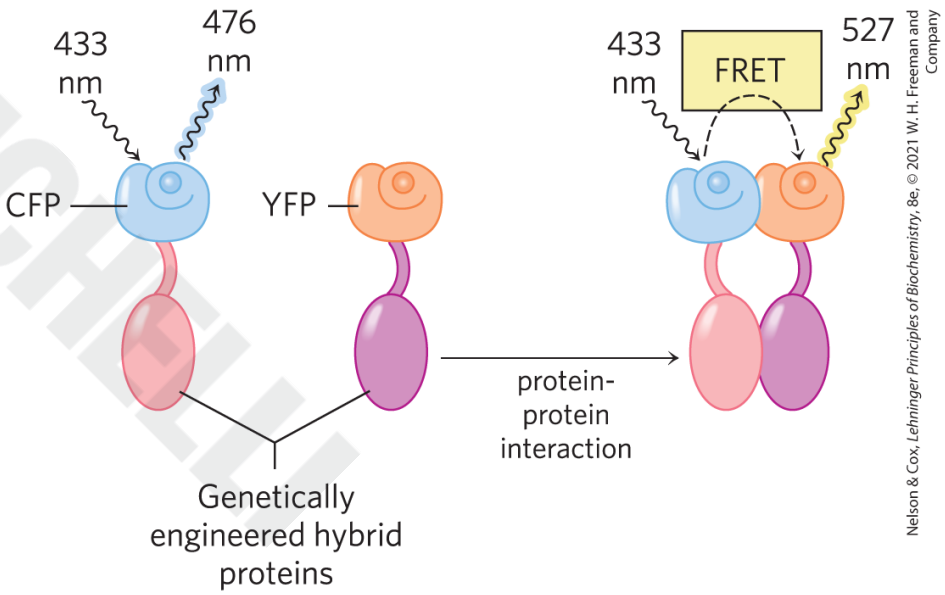
- **consensus sequences** = commonly contain the target for phosphorylation (Ser or Thr) by protein kinases

Table 6-10 Consensus Recognition Sequences for a Few Protein Kinases

Protein kinase	Consensus sequence and phosphorylated residue
Protein kinase A	-x-R-[RK]-x-[**ST**]-B-
Protein kinase G	-x-R-[RK]-x-[**ST**]-X-
Protein kinase C	-[RK](2)-x-[**ST**]-B-[RK](2)-
Protein kinase B	R-x-x-R-x-[**ST**]-x-ψ-
Ca^{2+}/calmodulin kinase I	-B-x-R-x(2)-[**ST**]-x(3)-B-
Ca^{2+}/calmodulin kinase II	-B-x-[RK]-x(2)-[**ST**]-x(2)-
Myosin light chain kinase (smooth muscle)	-K(2)-R-x(2)-**S**-x-B(2)-
Phosphorylase *b* kinase	*-K-R-K-Q-I-**S**-V-R-*
Extracellular signal-regulated kinase (ERK)	-P-x-[**ST**]-P(2)
Cyclin-dependent protein kinase (cdc2)	-x-[**ST**]-P-x-[KR]-
Casein kinase I	-[SpTp]-x(2)-[**ST**]-B
Casein kinase II	-x-[**ST**]-x(2)-[ED]-x-
β-Adrenergic receptor kinase	-[DE](*n*)-[**ST**]-x(3)
Rhodopsin kinase	-x(2)-[**ST**]-E(*n*)-vABL-[YLV]-**Y**-X_{1-3}-[PF]-
Epidermal growth factor (EGF) receptor kinase	*-E(4)-**Y**-F-E-L-V-*

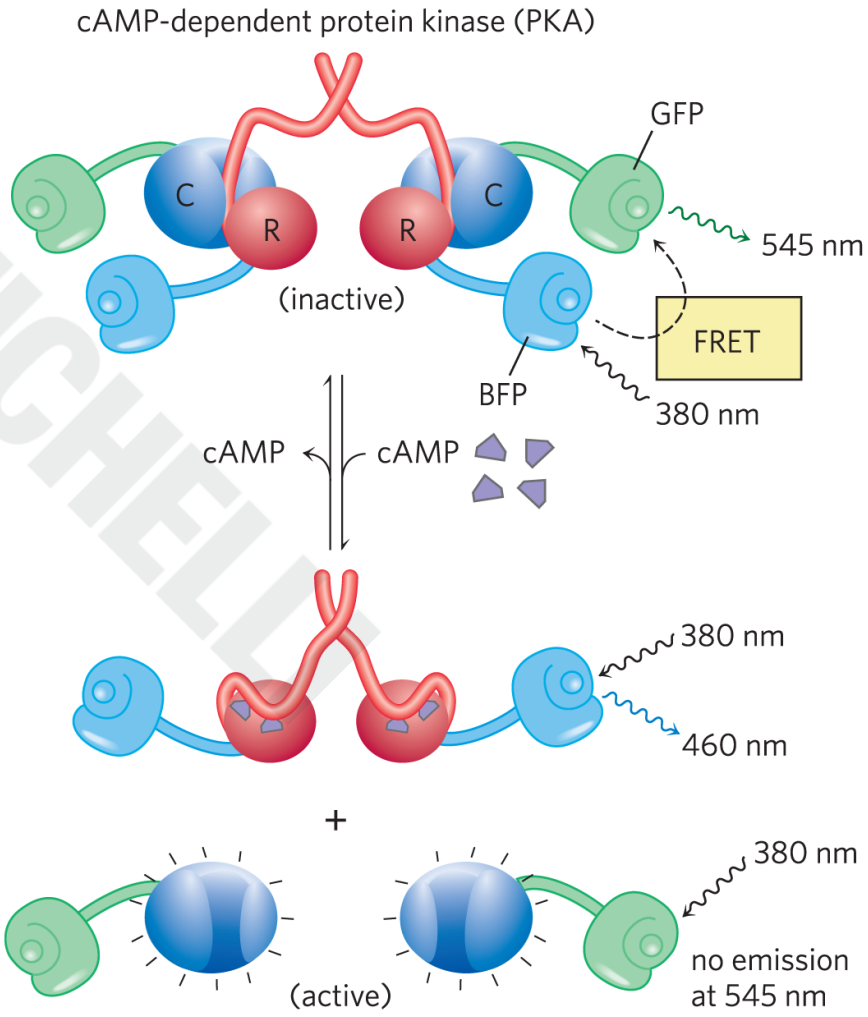
Fluorescence Resonance Energy Transfer (FRET)

- **fluorescence resonance energy transfer (FRET)** = measures the nonradiative transfer of energy between fluorescent probes attached to each protein
  - used to determine if two proteins interact and where in the cell they interact



Measuring [cAMP] with FRET

- when [cAMP] is low, R and C subunits of PKA are associated and FRET is exhibited
- when [cAMP] rises, R and C subunits of PKA dissociate and FRET ceases



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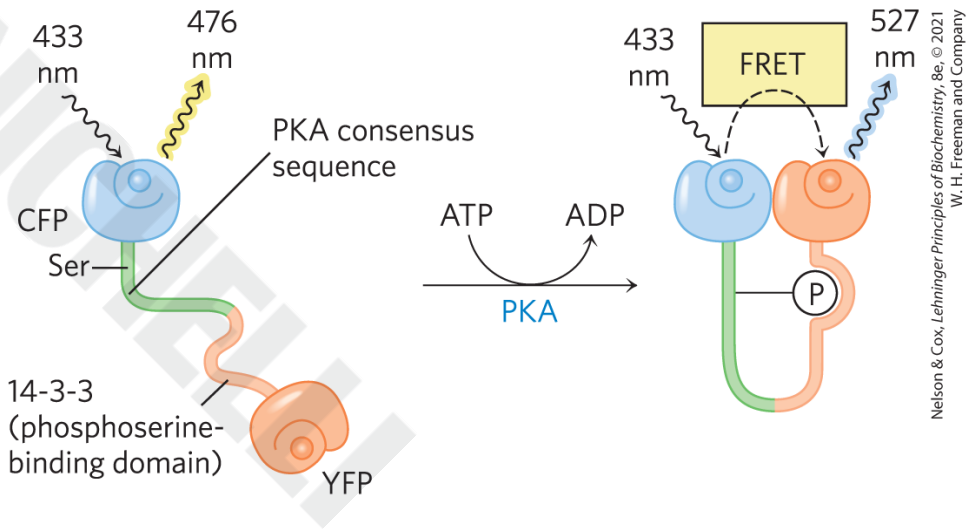
Measuring PKA Activity with FRET

- when PKA is inactive, the Ser residue is not phosphorylated, 14-3-3 has no affinity for the Ser residue, and FRET is not observed

- when PKA is active in the cell, the Ser residue is phosphorylated, 14-3-3 binds the Ser residue, and FRET is observed

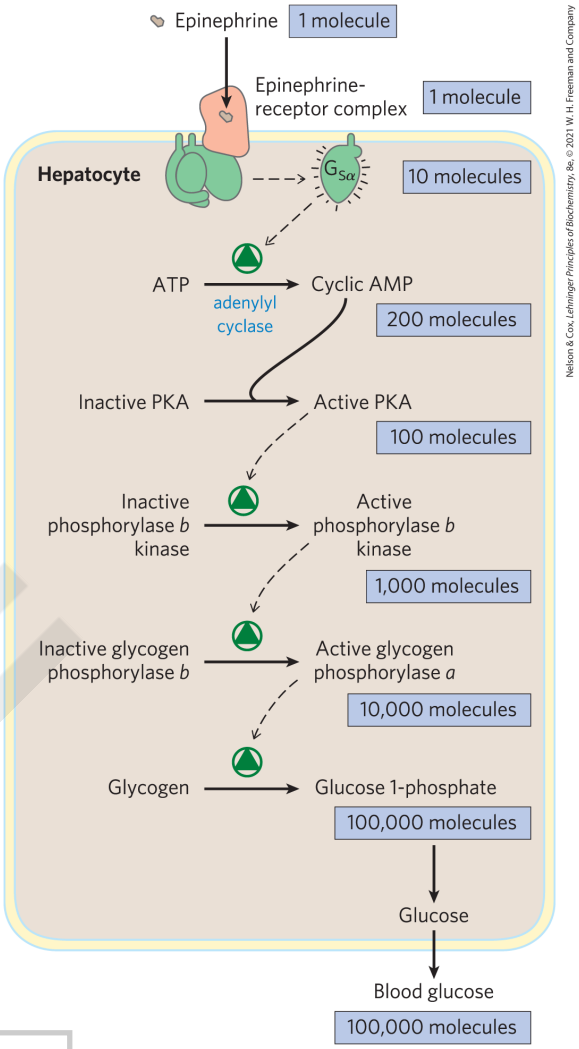


P1 Principle 1 (4 of 5)

Cells respond to external signals through receptor-mediated processes that amplify the signal, integrate it with input from other receptors, transmit the signal to the appropriate effectors, and eventually end the response.

Epinephrine Cascade

- the cascade amplifies the hormonal signal by orders of magnitude
  - accounts for the low concentration of epinephrine required for activity
- the signal leads to intracellular changes within fractions of a second



P1 Principle 1 (5 of 5)

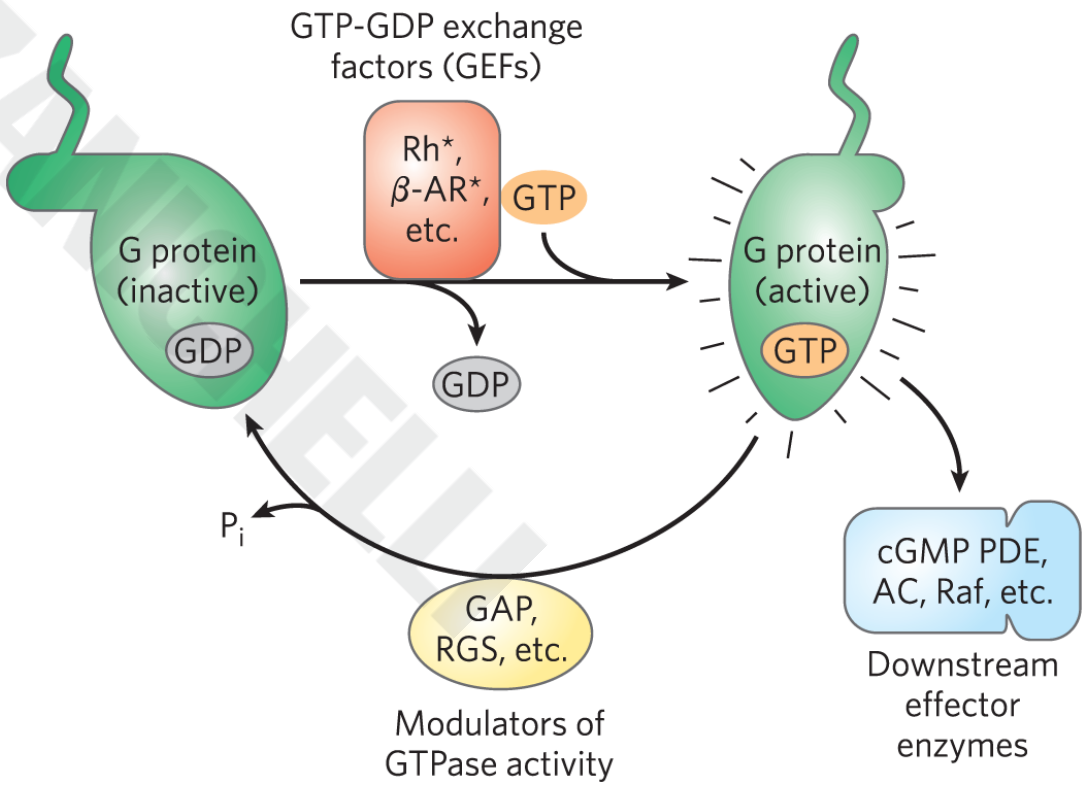
Cells respond to external signals through receptor-mediated processes that amplify the signal, integrate it with input from other receptors, transmit the signal to the appropriate effectors, and eventually end the response.

Several Mechanisms Cause Termination of the β-Adrenergic Response

- methods of termination:
  - epinephrine concentration drops below the K_d for its receptor
  - the GTPase activity of the G protein hydrolyzes the GTP bound to the G_α subunit
  - cAMP is hydrolyzed to 5′ AMP by **cyclic nucleotide phosphodiesterase**

GTPase Activator Proteins (GAPs)

- **GTPase activator proteins (GAPs) =** strongly stimulate GTPase activity



Phosphoprotein Phosphatases

- **phosphoprotein phosphatase** = enzyme that hydrolyzes phosphorylated Tyr, Ser, or Thr residues, releasing inorganic phosphate (P_i)
  - reverses the metabolic effects that result from PKA activity

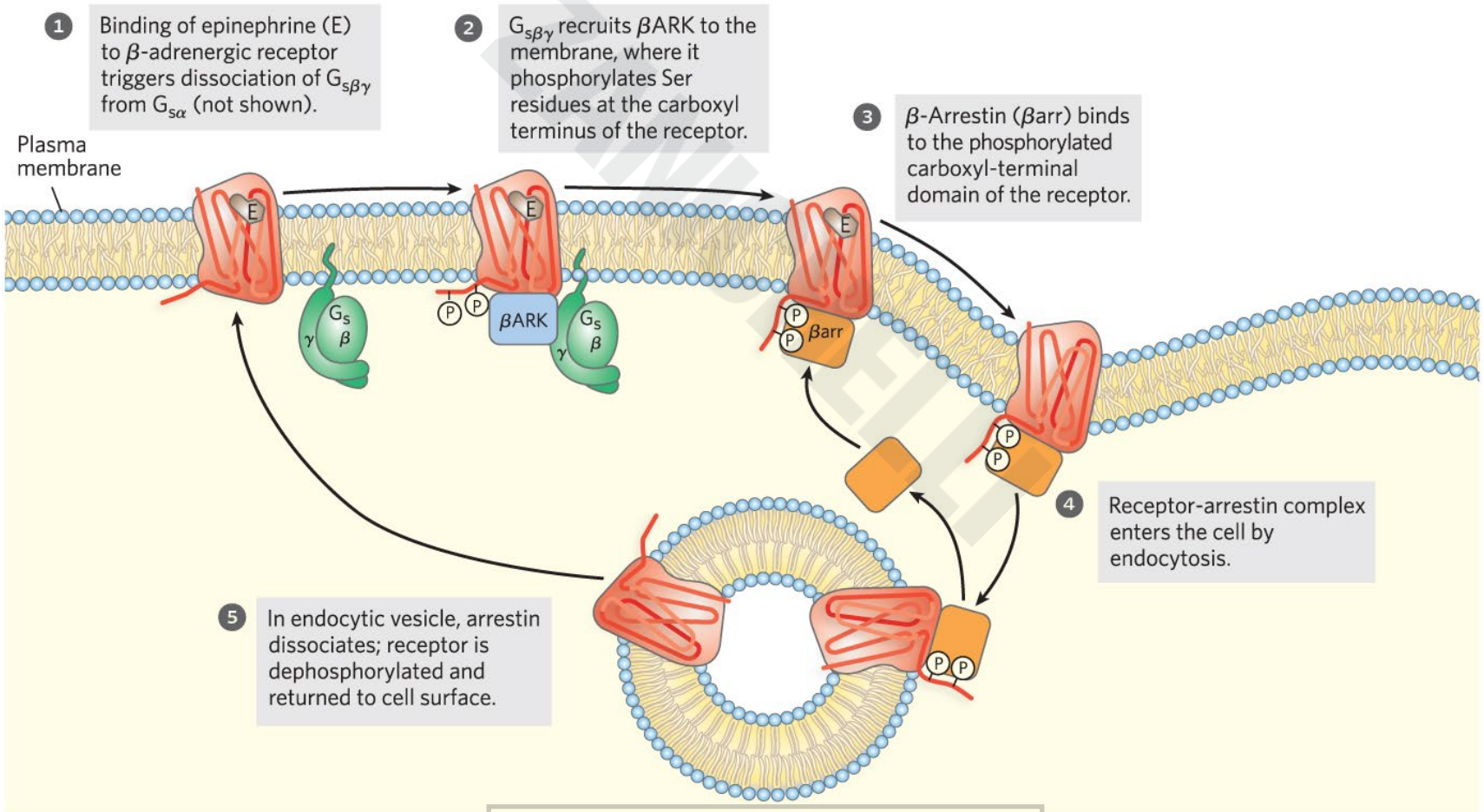
The β-Adrenergic Receptor is Desensitized by Phosphorylation and by Association with Arrestin

- desensitization decreases the response while the signal persists

- **β-adrenergic receptor kinase (βARK)** = phosphorylates several Ser residues near the receptor's carboxyl terminus when epinephrine is bound to the receptor

- **β-arrestin** (**βarr** or arrestin 2) = protein that binds to the receptor following receptor phosphorylation and blocks receptor sites that interact with the G protein

Desensitization of the β-Adrenergic Receptor in the Presence of Epinephrine



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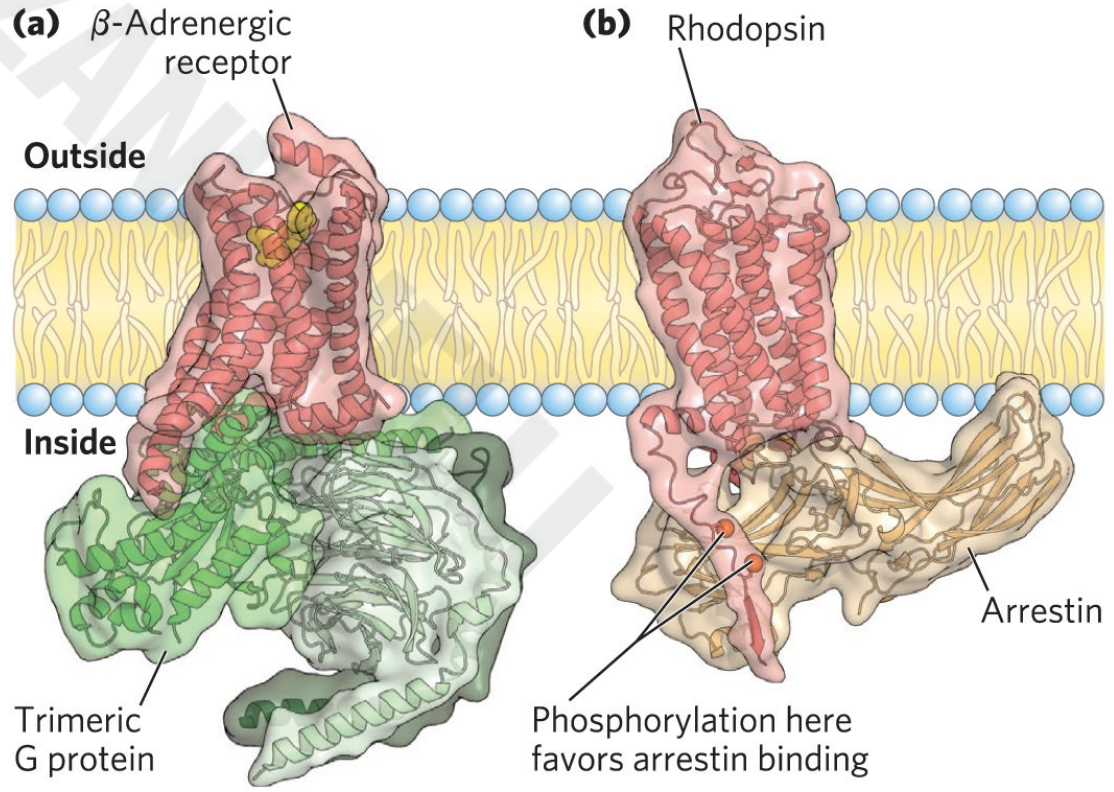
Mutual Exclusion of Trimeric G Protein and Arrestin in Their Interaction with a GPCR

- binding of arrestin blocks binding and further activation of the G protein and ends the response to the initial signal



G Protein-Coupled Receptor Kinases (GRKs)

- **G protein-coupled receptor kinases (GRKs)** = protein family that phosphorylates GPCRs on their carboxyl-terminal cytoplasmic domains and plays a role similar to that of *β*ARK in receptor desensitization and resensitization

P2 Principle 2 (2 of 4)

There is a high degree of evolutionary conservation of signaling proteins and transduction mechanisms across the animal phyla. At least a billion years of evolution have passed since the plant and animal branches of the eukaryotes diverged, which is reflected in the differences in signaling mechanisms used in the two kingdoms. We focus here on the animal kingdom.

Cyclic AMP Acts as a Second Messenger for Many Regulatory Molecules

- acts by changing intracellular [cAMP] and thus the activity of PKA

Table 12-3 Some Signals That Use cAMP as Second Messenger

Corticotropin (ACTH)
Corticotropin-releasing hormone (CRH)
Dopamine [D_1, D_2]
Epinephrine (β-adrenergic)
Follicle-stimulating hormone (FSH)
Glucagon
Histamine [H_2]
Luteinizing hormone (LH)
Melanocyte-stimulating hormone (MSH)
Odorants (many)
Parathyroid hormone
Prostaglandins E_1, E_2 (PGE_1, PGE_2)
Serotonin [5-HT_1, 5-HT_4]
Somatostatin
Tastants (sweet, bitter)
Thyroid-stimulating hormone (TSH)

cAMP Response Element Binding Protein (CREB)

- in some cells, the catalytic subunit of PKA can also move into the nucleus, where it phosphorylates cAMP response element binding protein (CREB)

- **cAMP response element binding protein (CREB)** = alters the expression of specific genes regulated by cAMP

Some Hormones Inhibit Adenylyl Cyclase

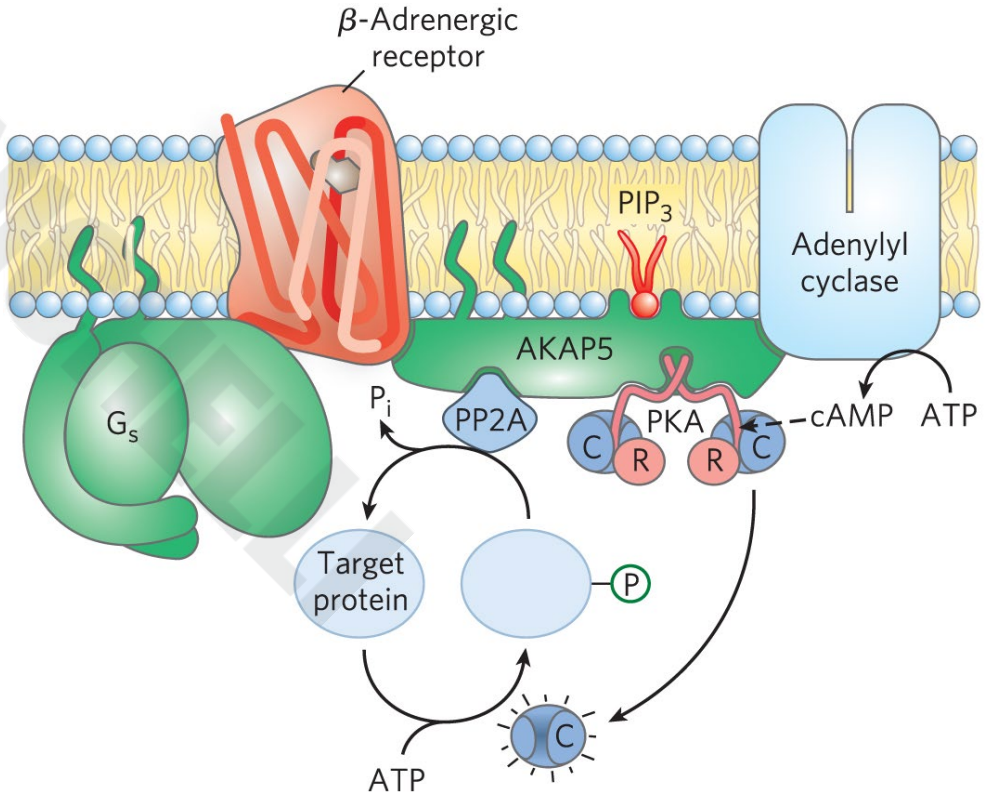
- **inhibitory G protein (G_i)** = inhibits adenylyl cyclase and lowers [cAMP]
  - activated by the binding of somatostatin to its receptor
  - structurally homologous to G_s

- extracellular signals have different effects on different tissues or cell types, depending on:
  - the type of receptor in the tissue
  - the type of G protein (G_s or G_i) coupled to the receptor
  - the set of PKA target enzymes in the cell

Adaptor Proteins Confine Signaling to a Specific Region of the Cell

- **adaptor proteins** = noncatalytic proteins that hold together other protein molecules that function in concert

- **AKAPs (A kinase anchoring proteins)** = have multiple, distinct protein-binding domains



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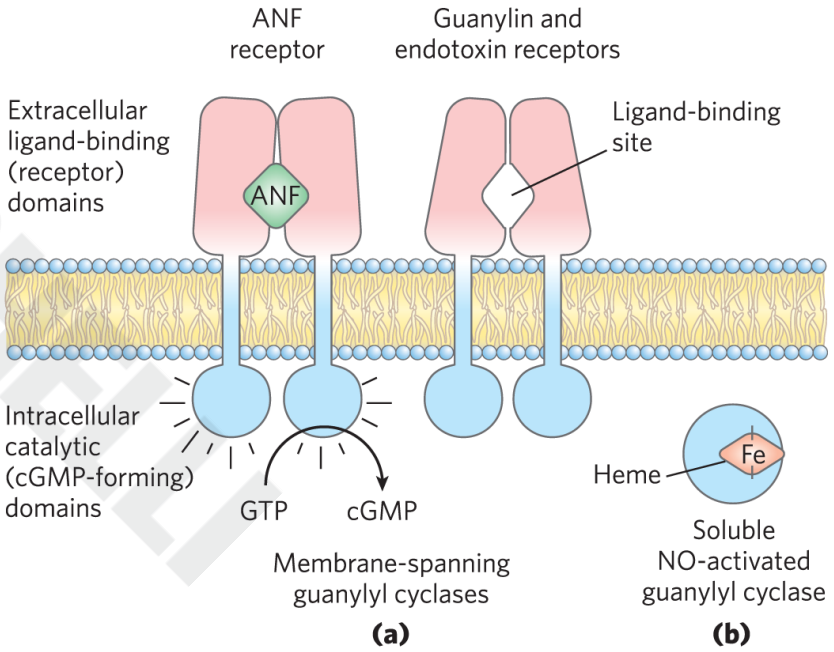
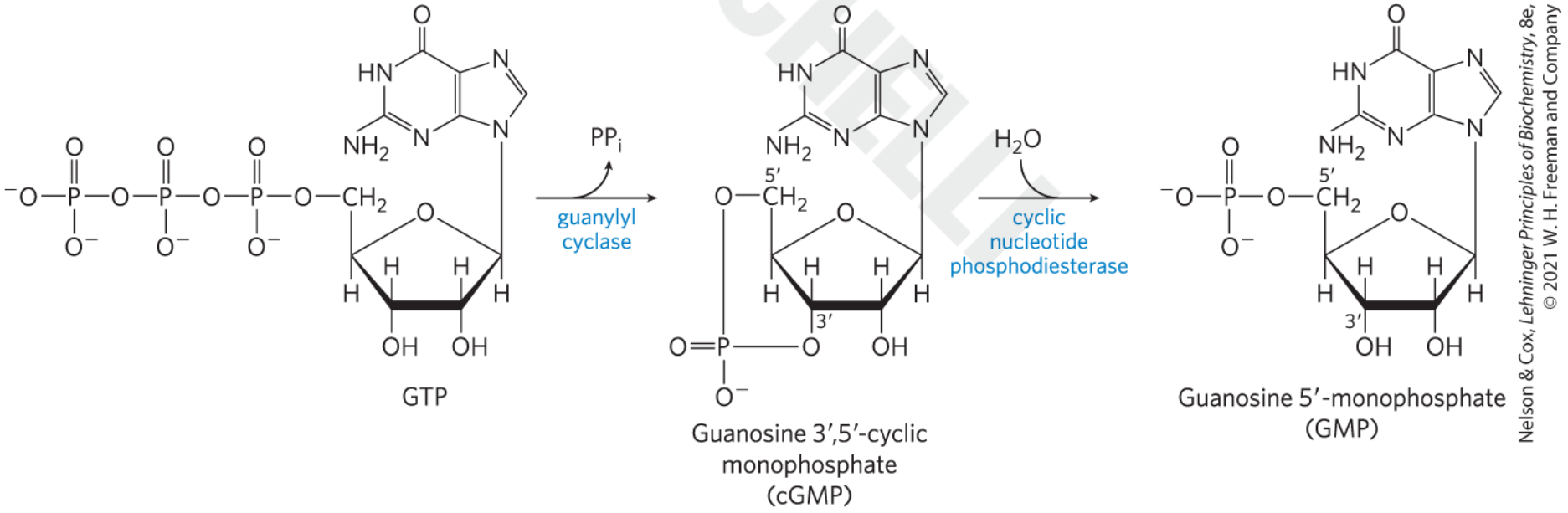
cGMP Activates a cGMP-Dependent Protein Kinase (PKG)

- guanylyl cyclase = converts GTP to cGMP when activated
- **guanosine 3′,5′-cyclic monophosphate (cGMP)** = second messenger
- **cGMP-dependent protein kinase** = **protein kinase G (PKG)** = mediates many of the actions of cGMP



The Synthesis and Hydrolysis of cGMP

- cGMP activates PKG
- degradation of cGMP reverses the activation of PKG



Atrial Natriuretic Factor (ANF)

- **atrial natriuretic factor (ANF)** = peptide hormone that activates guanylyl cyclase in the kidney
  - released by cardiac atrium cells when blood volume increases

- rise in [cGMP] triggers increased renal excretion of Na^+ and consequently of water
  - reduces blood volume

Guanylin and Nitric Oxide

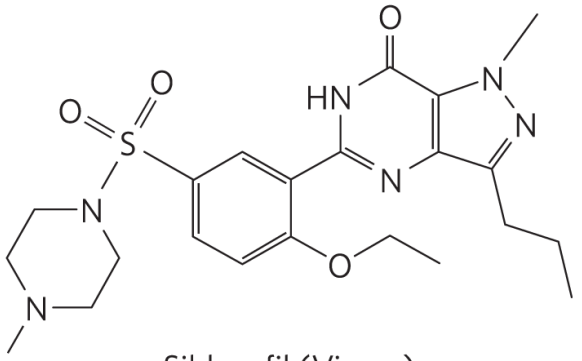
- **guanylin** = peptide that activates guanylyl cyclase in the plasma membrane of intestine epithelial cells
  - regulates Cl^- secretion

- **NO synthase** = produces nitric oxide from arginine

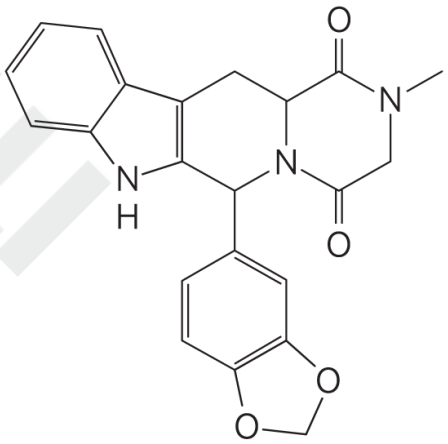
- nitric oxide = activates a soluble cytosolic guanylyl cyclase with an associated heme group
  - relieves **angina pectoris** (pain caused by constriction of a heart deprived of O_2)

Erectile Dysfunction Drugs Inhibit cGMP PDE

- the erectile dysfunction drugs Viagra and Cialis inhibit the cGMP phosphodiesterase (cGMP PDE) in the blood vessels of the penis
  - cause [cGMP] to remain elevated



Sildenafil (Viagra)



Tadalafil (Cialis)

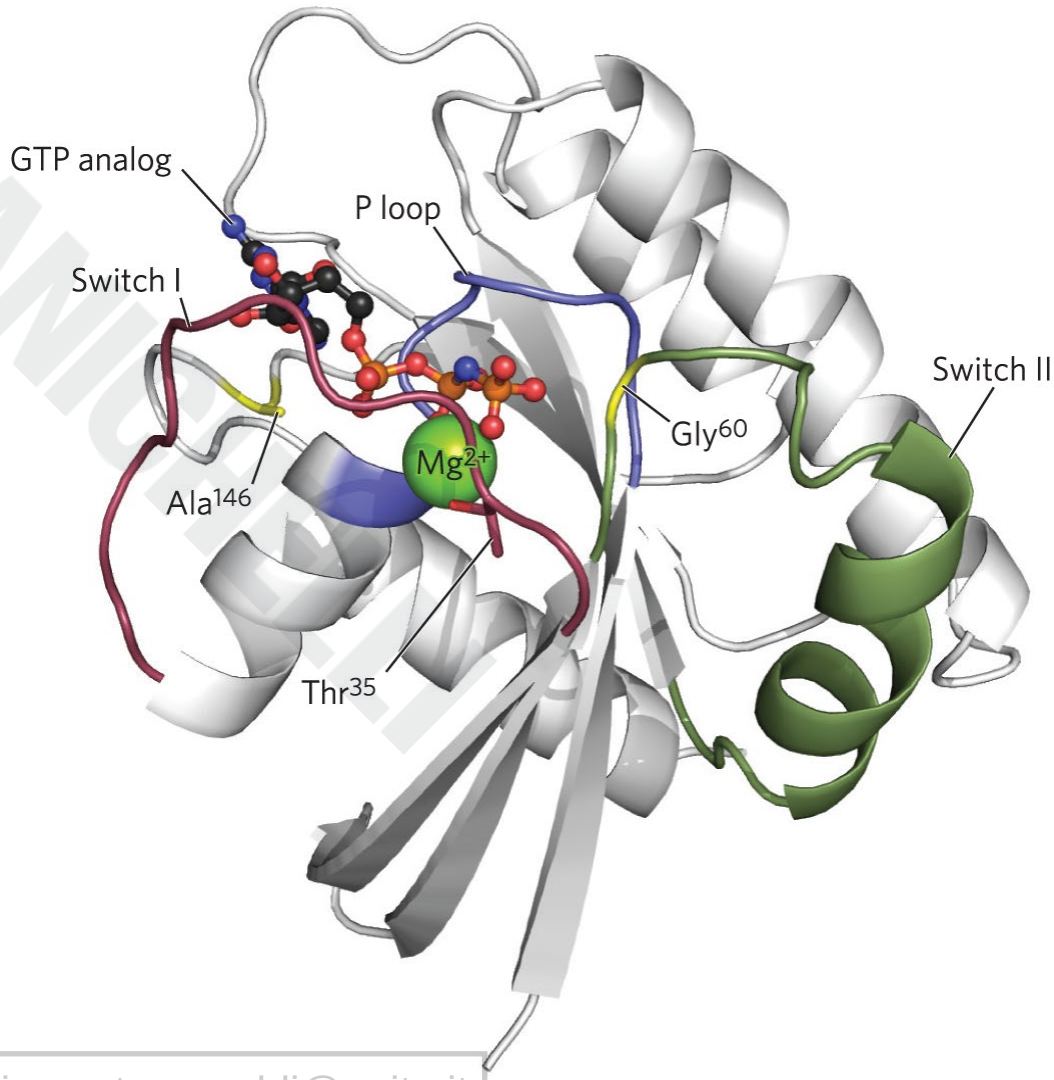
G Proteins Act as Self-Limiting Switches in Many Processes

- play roles in cellular processes, including:
  - sensory perception
  - signaling for cell division
  - growth and differentiation
  - intracellular movements of proteins and membrane vesicles
  - protein synthesis

Ras, the G-Protein Prototype

- **Ras** = a ~20 kDa minimal signaling unit

- in the nucleotide-binding site, Ala^{146} hydrogen bonds to the guanine oxygen, allowing GTP, but not ATP, to bind



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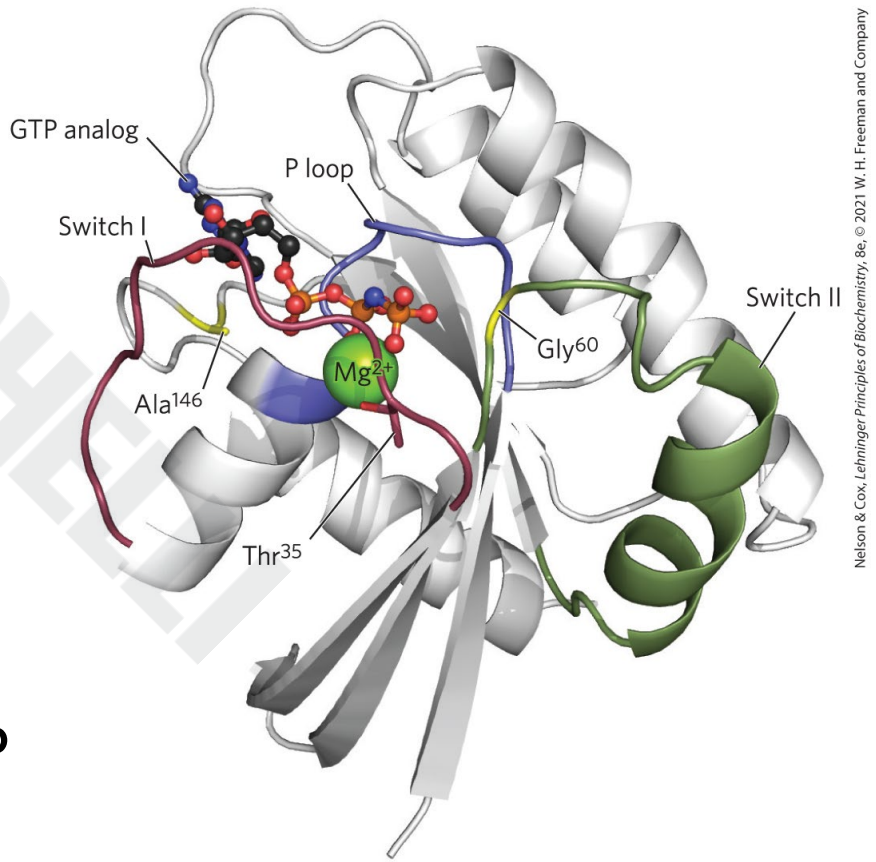
Regions of Ras

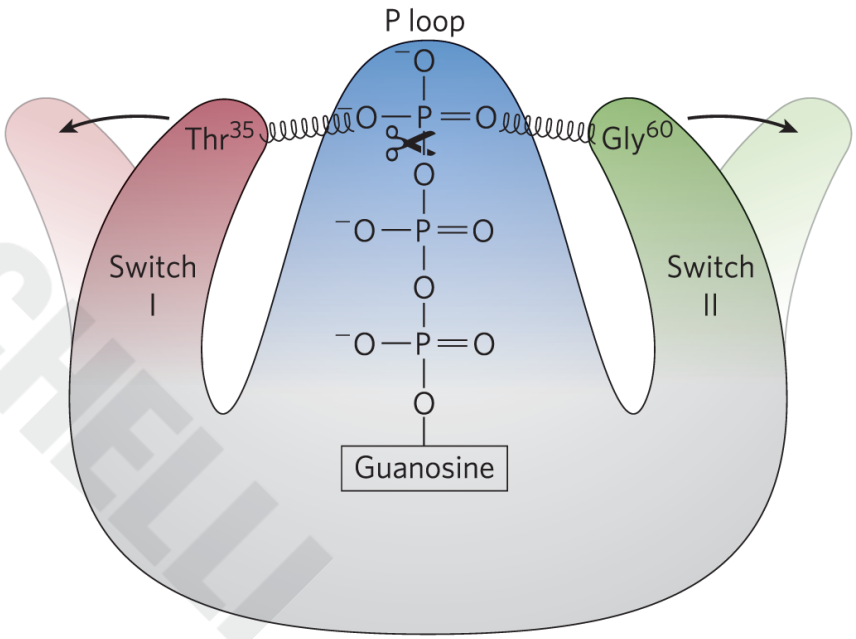
- **switch I** and **switch II** = regions that are exposed when GTP is bound
  - Thr^{35} and Gly^{60} hydrogen bond with the oxygens of the *γ* phosphate of GTP

- **P loop** (*p*hosphate-binding) = contains a Lys residue that binds the *γ* phosphate of GTP



GTP Hydrolysis Flips the Switches in Ras

- when GTP is hydrolyzed, the loss of hydrogen bonds allows the switch I and switch II regions to relax into a buried conformation



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GTPase Activator Proteins (GAPs) and Regulators of G-Protein Signaling (RGSs)

- **GTPase activator proteins (GAPs)** = increase the GTPase activity of G proteins
  - called **regulators of G-protein signaling (RGSs)** in the case of heterotrimeric G proteins

Guanosine Nucleotide-Exchange Factors (GEFs)

- **guanosine nucleotide-exchange factors (GEFs)** = catalyze the exchange of bound GDP with GTP
  - example: the *β*-adrenergic receptor

P9 Principle 9 (2 of 6)

Defective signaling proteins or defective regulation of their synthesis and breakdown can disrupt cell cycle regulation and lead to tumor formation (cancer).

Defects in G Proteins Are Implicated in Human Cancers

- a mutation in Ras eliminates its GTPase activity, causing the protein to remain constantly active
  - occurs in ~25% of human cancers

- mutations in the tumor suppressor gene *NF1* (which encodes a GAP) cause Ras to stay active for an extended period

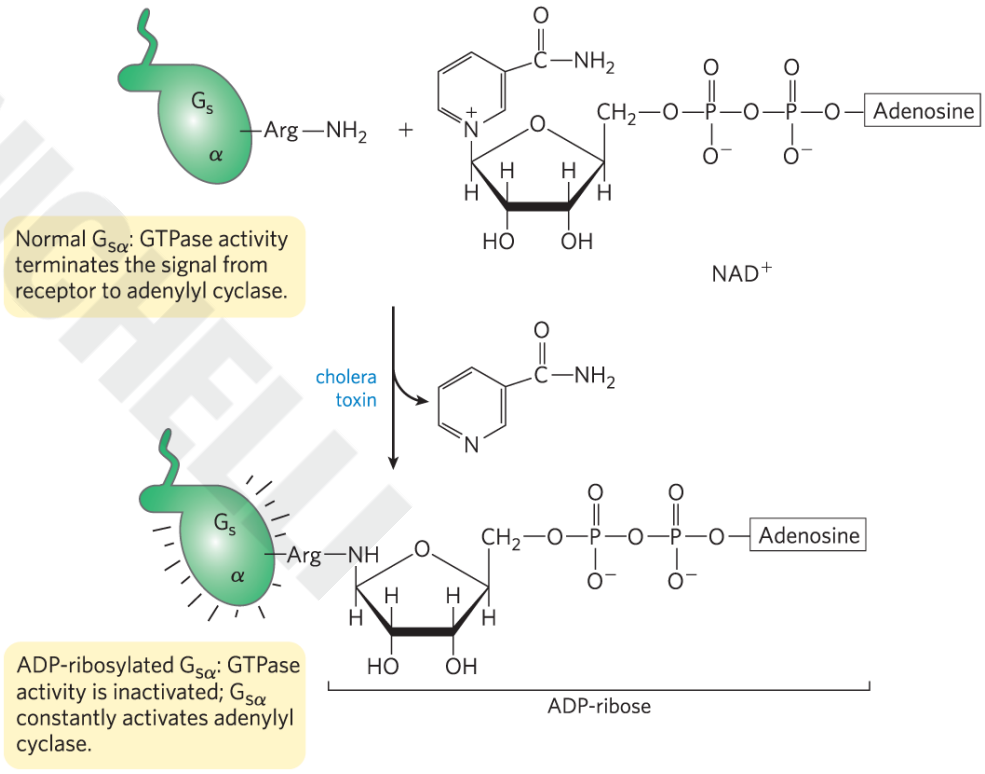
“Activating” and “Inactivating” Mutations in G_{α}

- “activating” mutations = lead to a continuously elevated [cAMP]
  - found in ~40% of adenomas

- “inactivating” mutations = cause individuals to be unresponsive to hormones that act through cAMP

Cholera Toxin Blocks the GTPase Activity of G_s

- **cholera toxin** = enzyme that catalyzes transfer of the ADP-ribose moiety of NAD^+ to an Arg residue of $G_{s\alpha}$
  - secreted by *Vibrio cholerae*
  - causes massive water loss due to chronically active PKA causing an efflux of NaCl



Diacylglycerol, Inositol Triphosphate, and Ca^{2+} Have Related Roles as Second Messengers

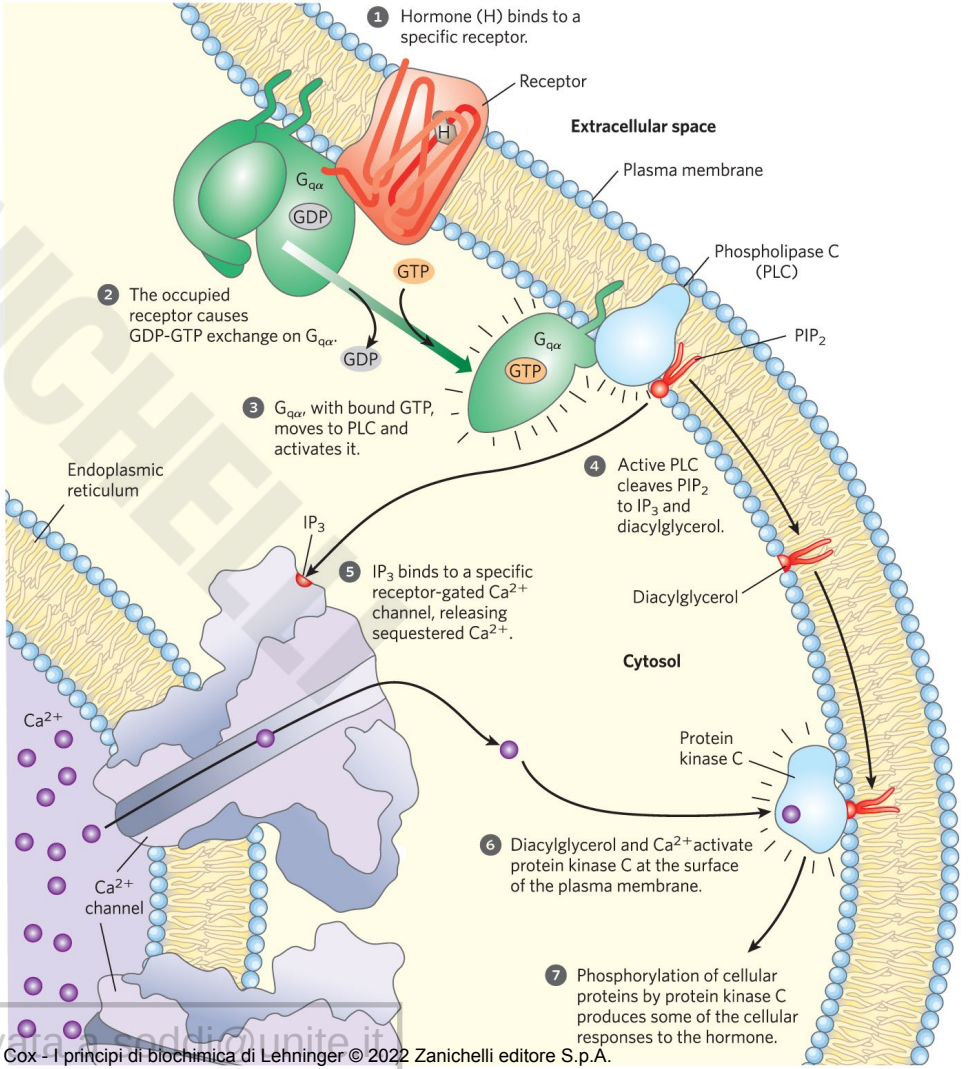
- **phospholipase C (PLC)** = catalyzes cleavage of the membrane phospholipid phosphatidylinositol 4,5-bisphosphate, or PIP_2

Table 12-4 Some Signals that Act through Phospholipase C, IP_3, and Ca^{2+}		
Acetylcholine [muscarinic M^1]	Gastrin-releasing peptide	Platelet-derived growth factor (PDGF)
α_1-Adrenergic agonists	Glutamate	Serotonin [$5\text{-}HT_2$]
Angiogenin	Gonadotropin-releasing hormone (GRH)	Thyrotropin-releasing hormone (TRH)
Angiotensin II	Histamine [H_1]	Vasopressin
ATP [P_{2x}, P_{2y}]	Light (*Drosophila*)	
Auxin	Oxytocin	

The Production of Diacylglycerol and Inositol 1,4,5-Triphosphate (IP_3)

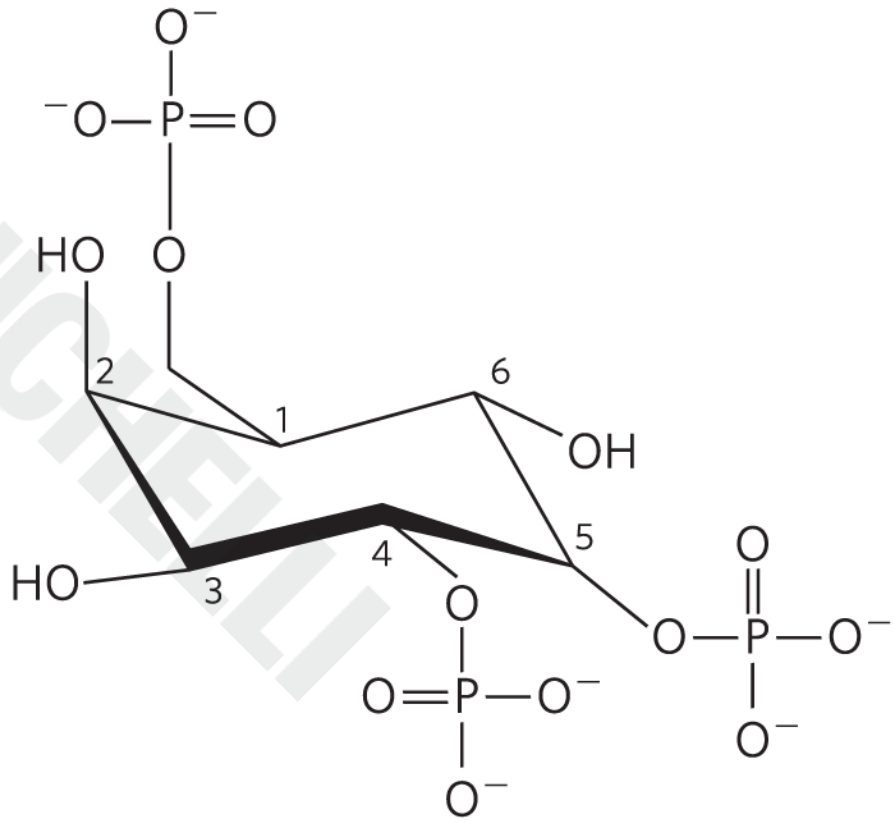
- **G_q** = associated trimeric G protein that activates the PIP_2-specific PLC when an agonist binds its specific receptor

- **diacylglycerol** and **inositol 1,4,5-triphosphate (IP_3) =** potent second messengers



IP_3 Opens the IP_3-Gated Ca^{2+} Channel

- **IP_3-gated Ca^{2+} channel** = receptor-gated channel in the endoplasmic reticulum that opens to release sequestered Ca^{2+} into the cytosol

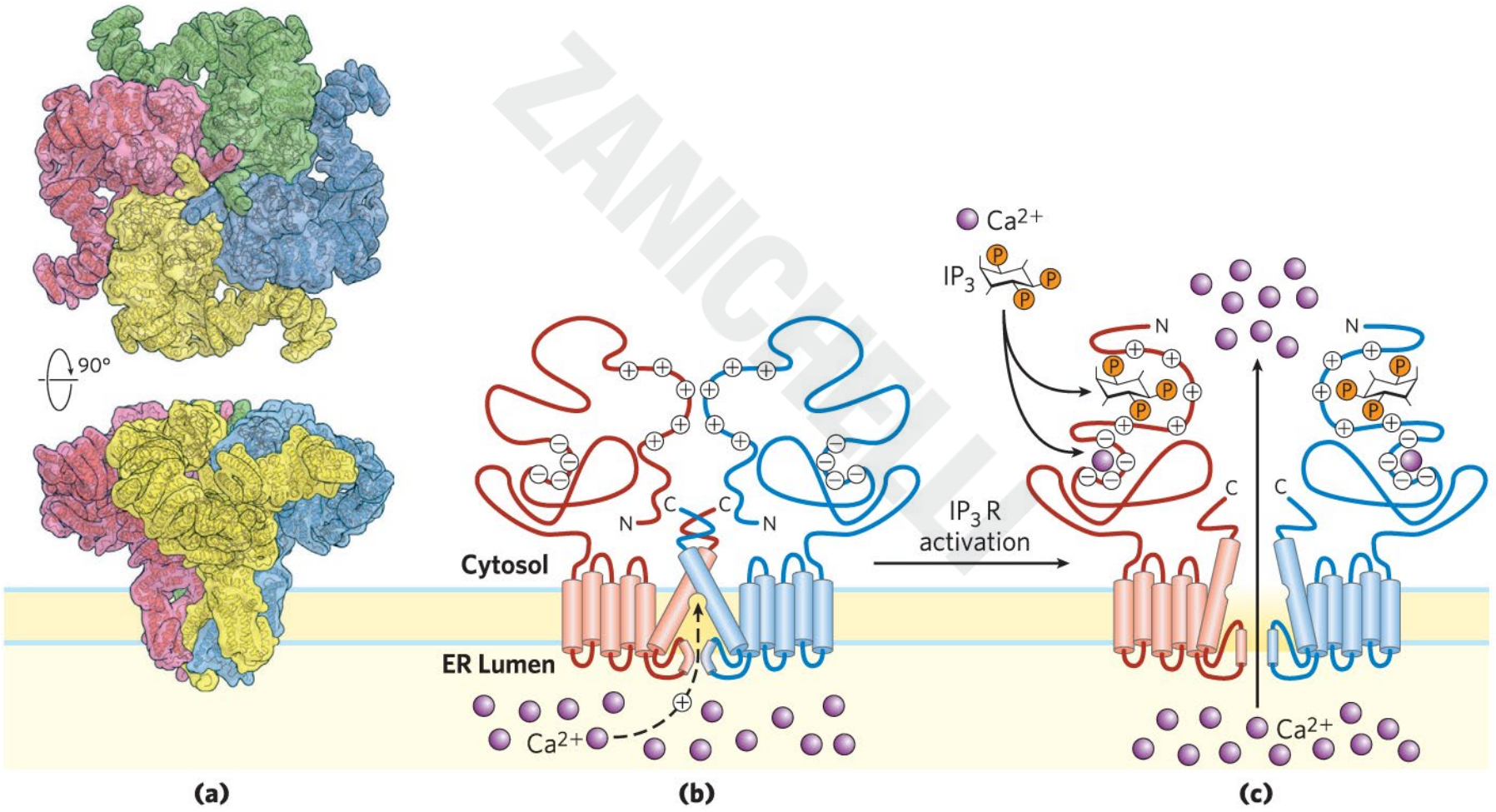


Inositol 1,4,5-trisphosphate (IP_3)

Elevated [Ca^{2+}] and Diacylglycerol Activate Protein Kinase C (PKC)

- **protein kinase C (PKC)** = binds and phosphorylates proteins that contain a PKC consensus sequence
  - several known isozymes exist

Proposed Mechanism of Action of the IP_3-Gated Ca^{2+} Channel



P6 Principle 6 (2 of 5)

Ion channels gated by membrane potential or ligands are central to signaling in all cells, including bacteria, plants, and animals.

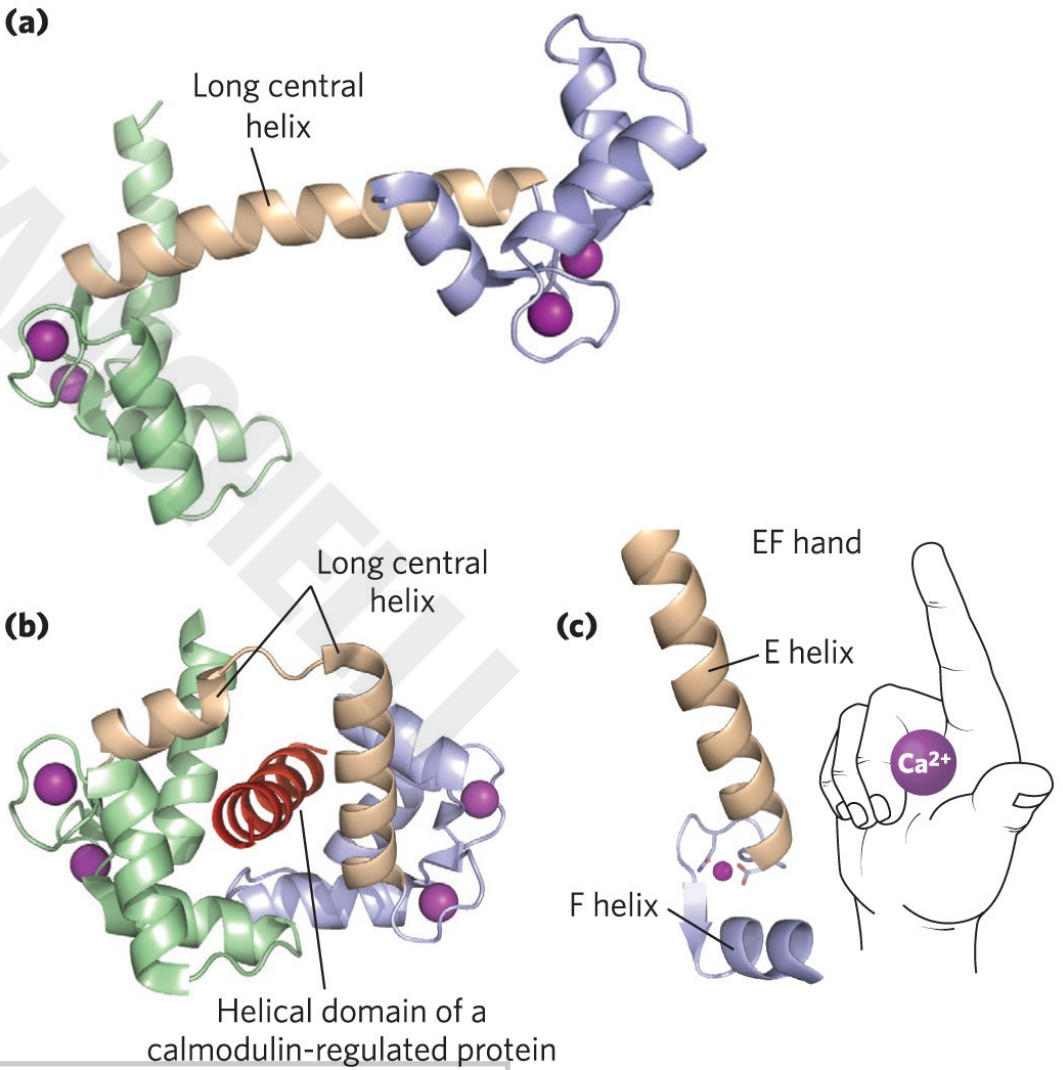
Calcium Is a Second Messenger That Is Limited in Space and Time

- in unstimulated cells, cytosolic [Ca^{2+}] is kept very low by Ca^{2+} pumps

- hormonal, neural, or other stimuli cause either:
  - an influx of Ca^{2+} into the cell through Ca^{2+} channel
  - the release of sequestered Ca^{2+} into the cytosol

Calmodulin (CaM)

- **calmodulin (CaM)** = acidic protein that undergoes a conformational changes when Ca^{2+} binds
  - associates with a variety of proteins and modulates their activities



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(b, c) PDB ID 1CDL, W. E. Meador et al., *Science* 257:1251, 1992.

Many Enzymes Are Modulated by Ca^{2+} Through Calmodulin

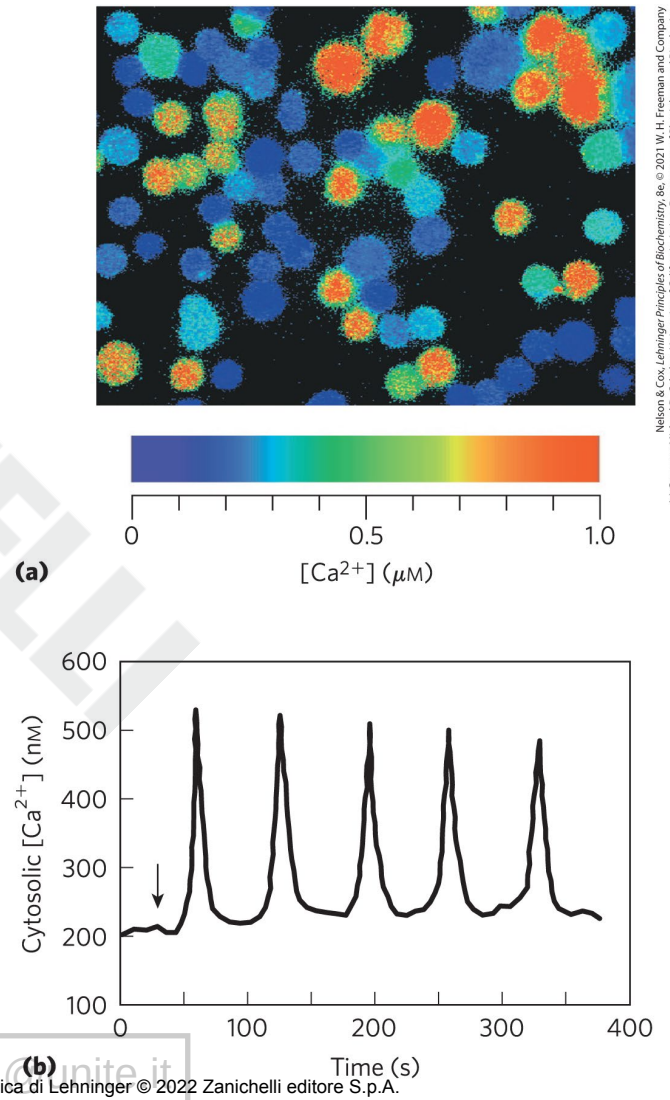
- **Ca^{2+}/calmodulin-dependent protein kinases (CaM kinases) =** phosphorylates target enzymes following activation by Ca^{2+}-bound calmodulin

- calmodulin is a regulatory subunit of phosphorylase *b* kinase of muscle, which is activated by Ca^{2+}

Table 12-5 Some Proteins Regulated by Ca^{2+} and Calmodulin
Adenylyl cyclase (brain)
Ca^{2+}/calmodulin-dependent protein kinases (CaM kinases I to IV)
Ca^{2+}-dependent Na^{+} channel (*Paramecium*)
Ca^{2+}-release channel of sarcoplasmic reticulum
Calcineurin (phosphoprotein phosphatase 2B)
cAMP phosphodiesterase
cAMP-gated olfactory channel
cGMP-gated Na^{+}, Ca^{2+} channels (rod and cone cells)
Glutamate decarboxylase
Myosin light-chain kinases
NAD^{+} kinase
Nitric oxide synthase
Phosphatidylinositol 2-kinase
Plasma membrane Ca^{2+} ATPase (Ca^{2+} pump)
RNA helicase (p68)

Extracellular Signals Trigger Oscillating Intracellular [Ca^{2+}] Levels

- mechanism underlying oscillations likely entails feedback regulation by Ca^{2+}



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(a) Courtesy Michael D. Cahalan, University of California, Irvine, Department of Physiology and Biophysics.

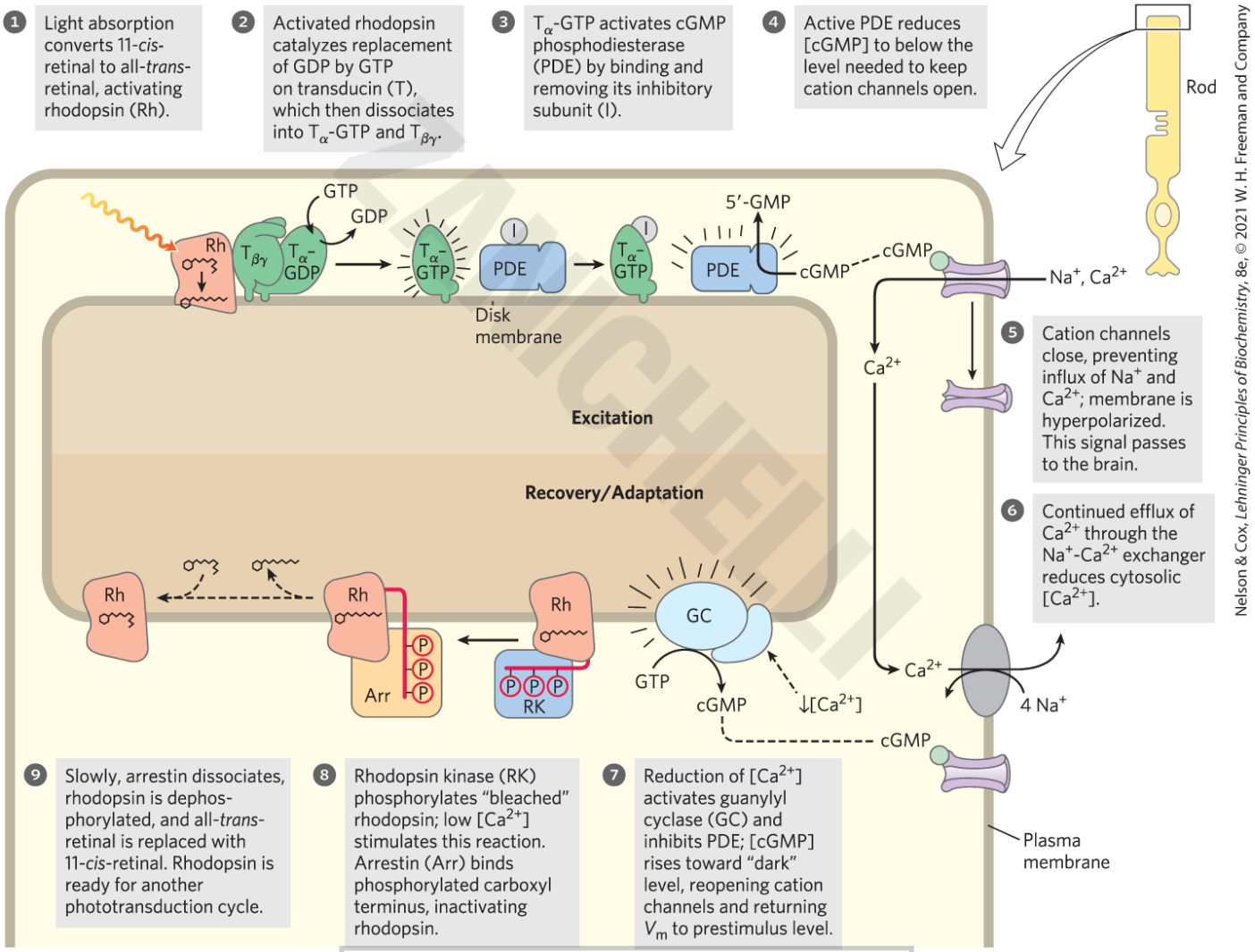
12.3 GPCRs in Vision, Olfaction, and Gustation

The Vertebrate Eye Uses Classic GPCR Mechanisms

- **rhodopsin** = a GPCR in the disk membranes of rod cells of the vertebrate eye

- **opsin** = the protein component of rhodopsin
  - covalently attached to the light-absorbing pigment (chromosphore) 11-*cis*-retinal

Photon Absorption by Rhodopsin



Rhodopsin Kinase and Arrestin 1

- **rhodopsin kinase** = phosphorylates Thr and Ser residues in the carboxyl-terminal domain of rhodopsin
  - functionally and structurally homologous to the *β*ARK that desensitizes the *β*-adrenergic receptor

- **arrestin 1** = binds the phosphorylated carboxyl-terminal domain of rhodopsin to prevent further interaction between activated rhodopsin and transducin

P5 Principle 5 (5 of 6)

Phosphorylation of intrinsically disordered regions of signaling proteins acts as a switch, toggling enzyme activity or creating binding sites for other molecules. Signal responses are integrated by multiprotein signaling complexes with modular domains that bind phosphorylated Tyr, Ser, or Thr residues.

Vertebrate Olfaction and Gustation Use Mechanisms Similar to the Visual System

- G_{olf} = G protein analogous to transducing and to G_s
- **receptor potential** = small depolarization produced by the influx of Na^+ and Ca^{2+} following the opening of cAMP-gated Na^+ and Ca^{2+} channels in the plasma membrane
  - neuron fires an action potential when the receptor potential is strong enough

Gustducin

- **gustducin** = heterotrimeric G protein coupled to GPCRs in taste sensory neurons
  - stimulates cAMP production by adenylyl cyclase

- the closing of K^+ channels in the plasma membrane following phosphorylation by PKA sends an electrical signal to the brain

All GPCR Systems Share Universal Features (1 of 2)

- GPCRs are encoded in vertebrates, *Drosophila, Caenorhabditis elegans*, *Saccharomyces*

- of the ~20,000 genes in the human genome, as many as 800 are encoded as GPCRs

All GPCR Systems Share Universal Features (2 of 2)

Amines	Peptides	Protein hormones	Prostanoids	Others
Acetylcholine (muscarinic)	Angiotensin	Follicle-stimulating hormone	Prostacyclin	Cannabinoids
Dopamine	Bombesin	Gonadotropin	Prostaglandin	Lysosphingolipids
Epinephrine	Bradykinin	Lutropin-choriogonadotropic hormone	Thromboxane	Melatonin
Histamine	Chemokine	Thyrotropin		Olfactory stimuli
Serotonin	Colecystokinin (CCK)			Opioids
	Endothelin			Rhodopsin
	Gonadotropin-releasing hormone			
	Interleukin-8			
	Melanocortin			
	Neuropeptide Y			
	Neurotensin			
	Orexin			
	Somatostatin			
	Tachykinin			
	Thyrotropin-releasing hormone			
	Vasopressin			

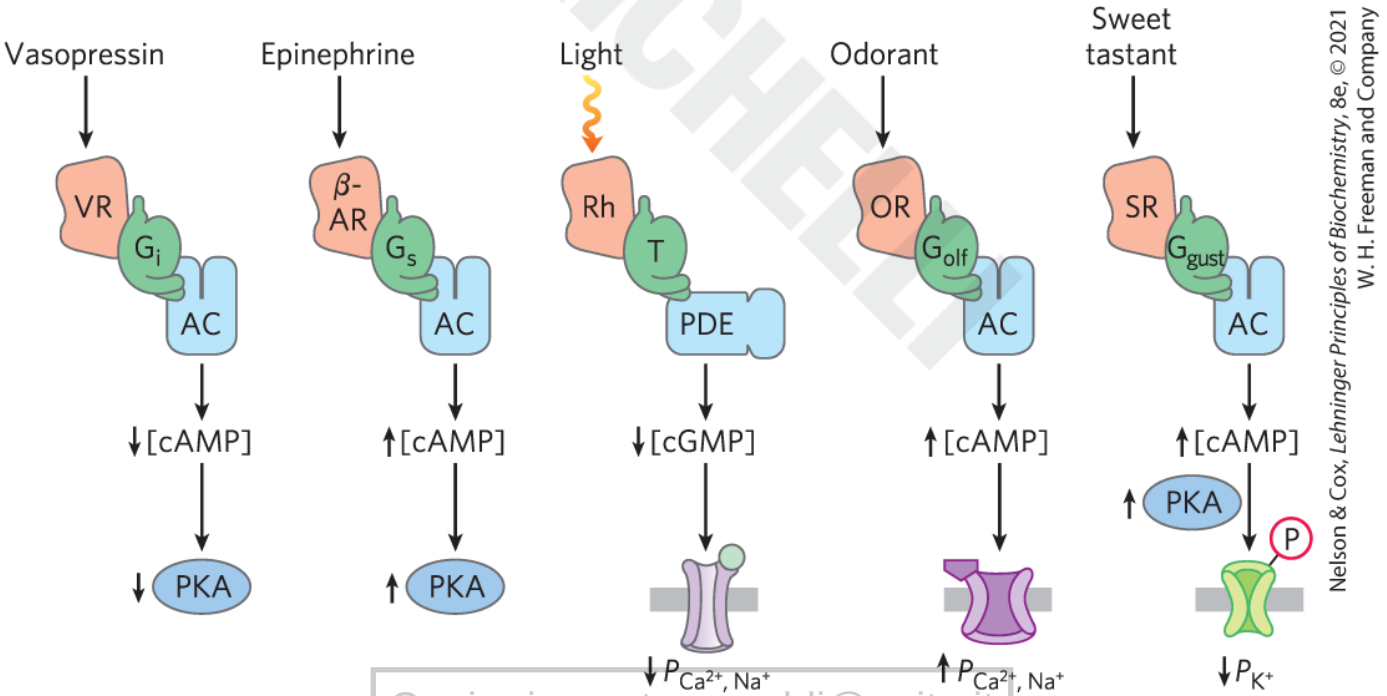
Table 12-6 Some Signals That Act through GPCRs

P2 Principle 2 (3 of 4)

There is a high degree of evolutionary conservation of signaling proteins and transduction mechanisms across the animal phyla. At least a billion years of evolution have passed since the plant and animal branches of the eukaryotes diverged, which is reflected in the differences in signaling mechanisms used in the two kingdoms. We focus here on the animal kingdom.

Common Features of Signaling Systems Detecting Hormones, Light, Smells, and Tastes

- GPCRs with seven transmembrane helices, G proteins with intrinsic GTPase activities, cyclic nucleotides, and protein kinases are central to signaling



12.4 Receptor Tyrosine Kinases

P4 Principle 4 (2 of 5)

Plasma membrane receptors with an intracellular tyrosine kinase activity act through cascades of protein kinases to transduce signals about the metabolic state, including growth factors.

Receptor Tyrosine Kinases (RTKs)

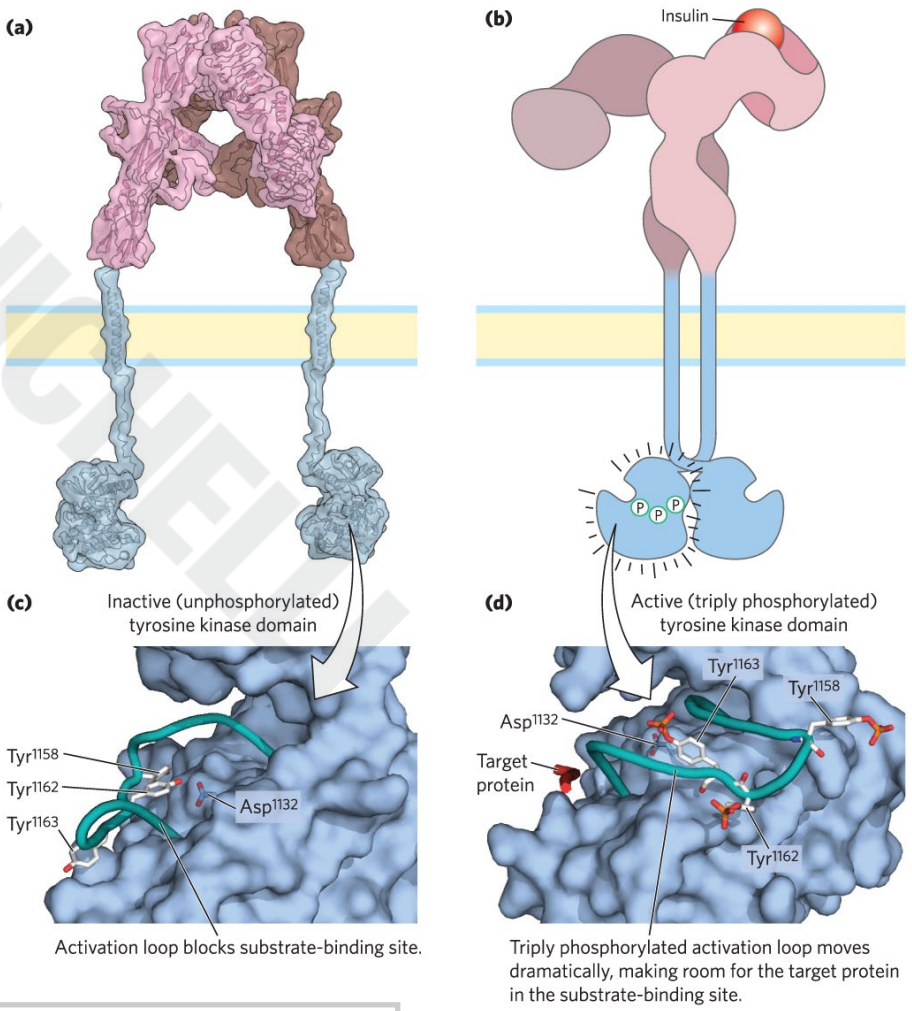
- **receptor tyrosine kinases (RTKs)** = family of plasma membrane receptors with protein kinase activity
  - have an extracellular ligand binding domain and a cytoplasmic Tyr kinase domain

Stimulation of the Insulin Receptor Initiates a Cascade of Protein Phosphorylation Reactions

- active insulin receptor protein (INSR) = a dimer of $\alpha\beta$ monomers
  - α subunits contain the insulin-binding domain
  - intracellular domains of the β subunits contain the protein kinase activity

Activation of Tyrosine Kinase by Autophosphorylation

- **autophosphorylation** = each β subunit phosphorylates three essential Tyr residues near the C-terminus of the other β subunit
  - opens the active site



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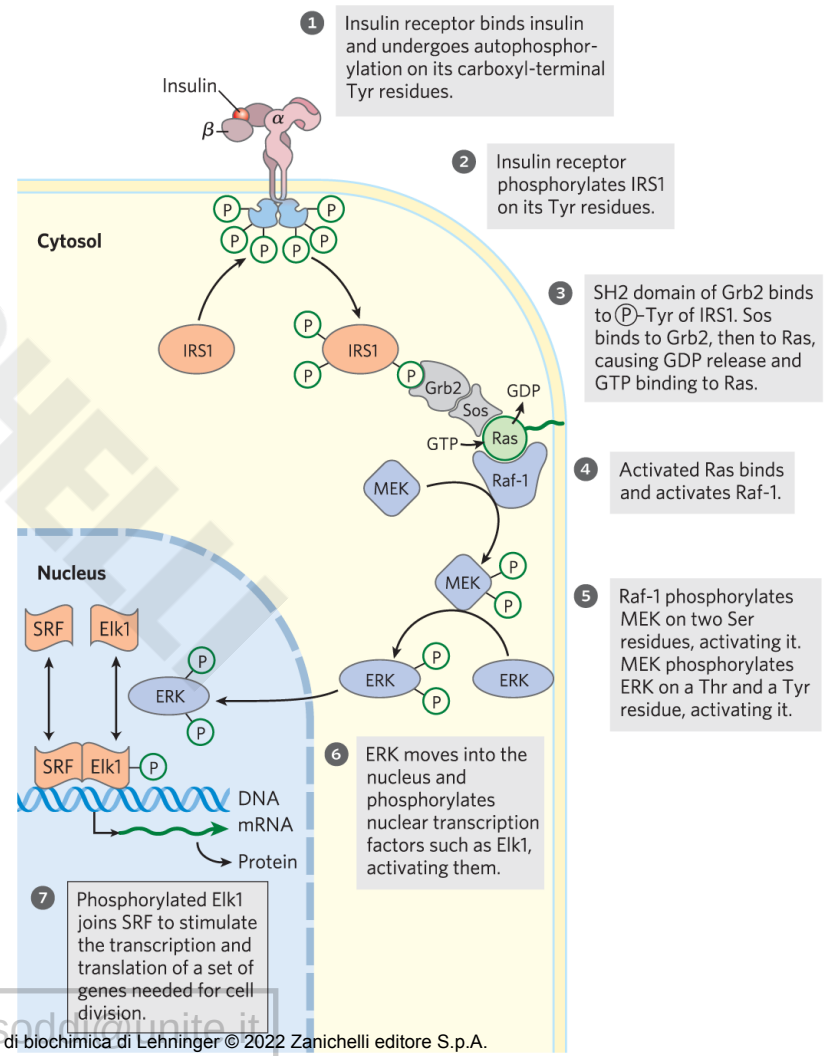
(d) PDB ID 1IR3, S. R. Hubbard, *EMBO J.* 16:5572, 1997.

Regulation of Gene Expression by Insulin

- **insulin receptor substrate 1 (IRS1)** = becomes the point of nucleation for a complex of proteins that carry the message from the receptor to end targets

- **SH2** domain = binds phosphorylated Tyr residues in a protein partner



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Raf-1, MEK, and ERK Are Members of Larger Families

- ERK is in the **MAPK** family (*m*itogen-*a*ctivated *p*rotein *k*inases)
  - specific for Ser or Thr residues

- MEK is in the MAP kinase kinase (MAPKK) family
  - activates the MAP kinase
  - phosphorylates both Ser and Try residues

- Raf-1 is in the MAP kinase kinase kinase (MAPKKK) family
  - activates the MAP kinase kinase
  - specific for Ser or Thr residues

P4 Principle 4 (3 of 5)

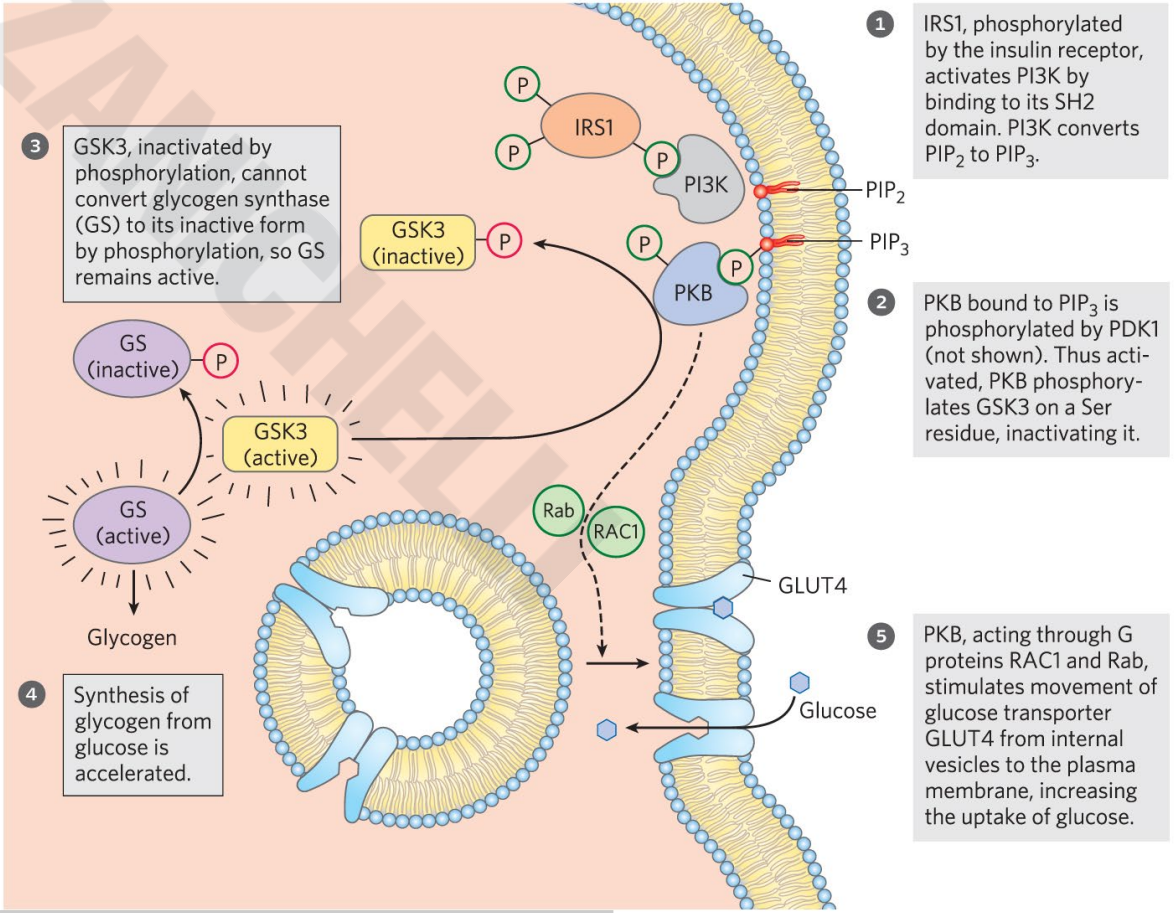
Plasma membrane receptors with an intracellular tyrosine kinase activity act through cascades of protein kinases to transduce signals about the metabolic state, including growth factors.

MAPK Cascades

- **MAPK cascades** = mediate signaling initiated by a variety of growth factors
  - amplify the initial signal by many orders of magnitude

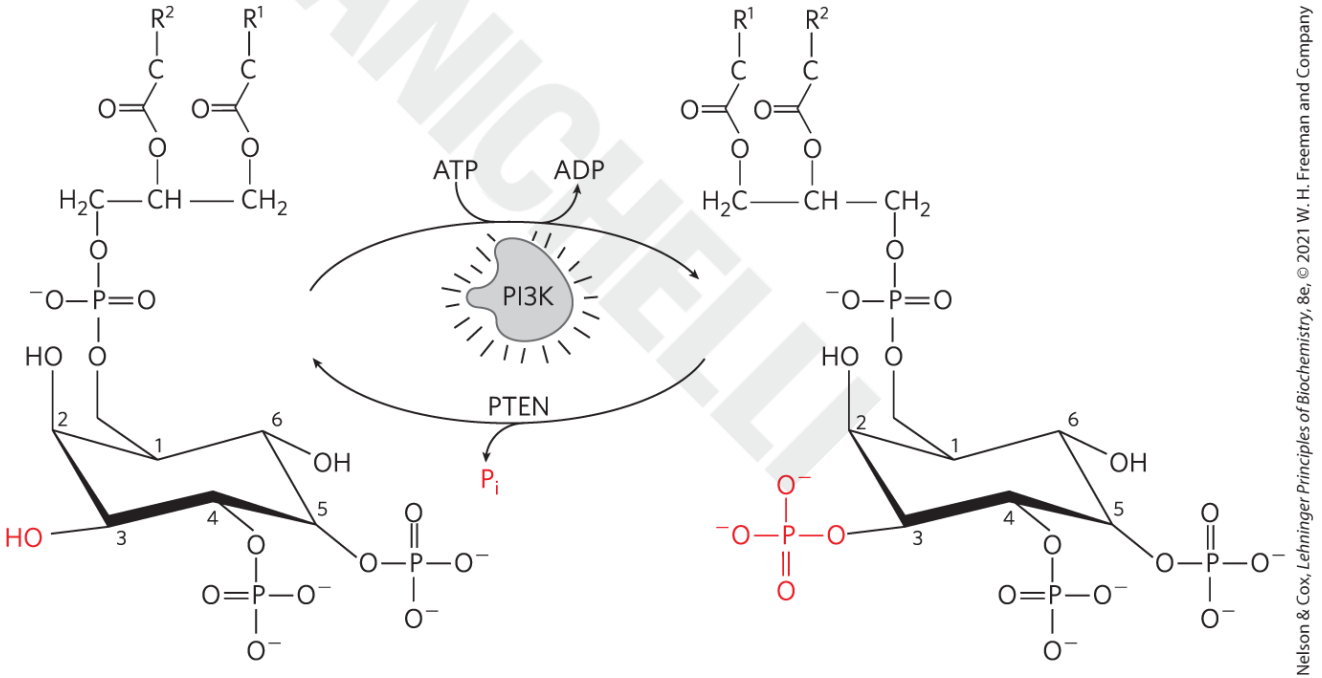
The Membrane Phospholipid PIP_3 Functions at a Branch in Insulin Signaling

- activation of PI3K by phosphorylated IRS1 initiates movement of GLUT4 to the plasma membrane and activation of glycogen synthase

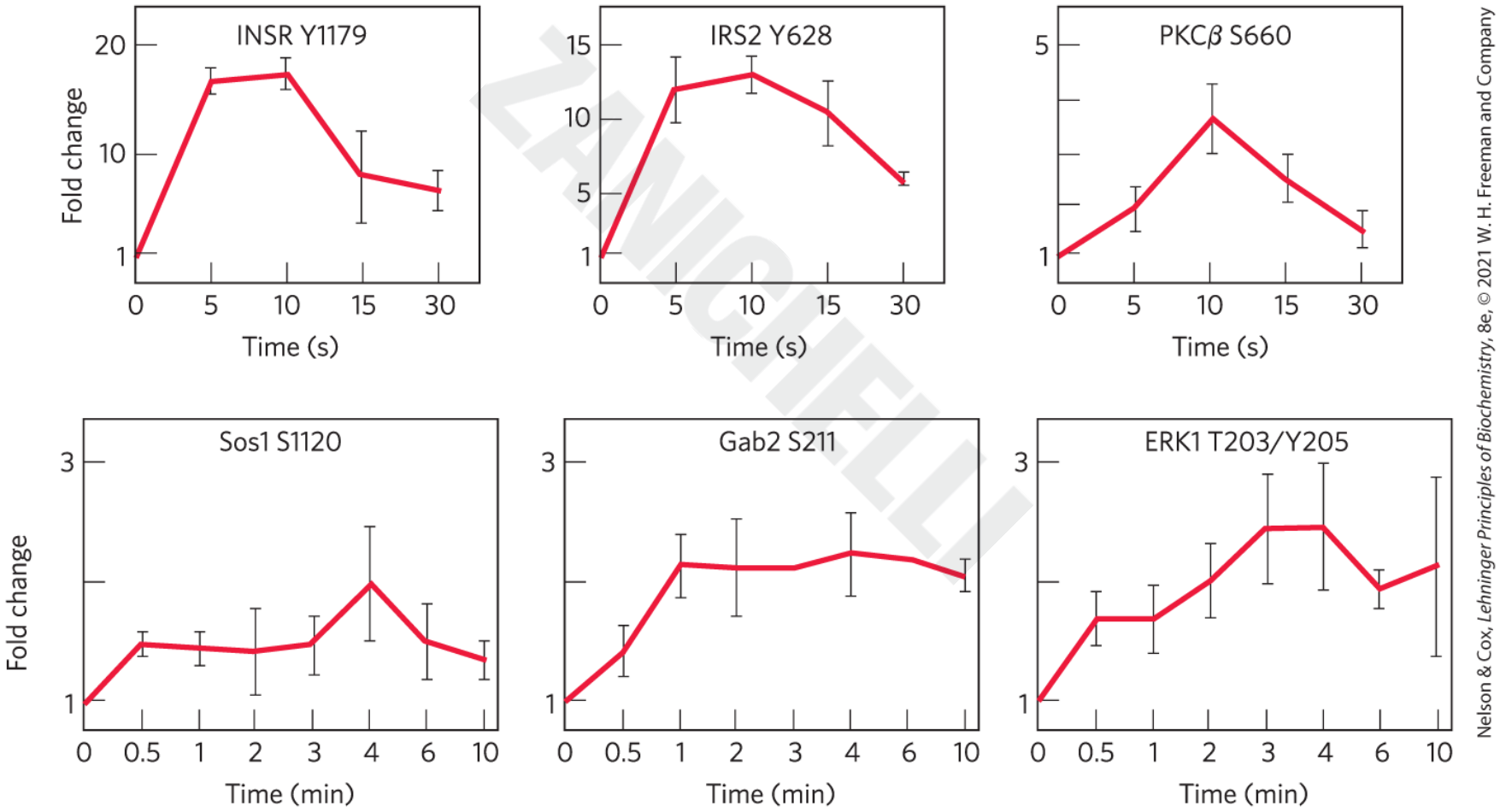


Regulation of PIP_3 Formation and Breakdown

- once activated, PI3K converts the membrane lipid PIP_2 to PIP_3 by the transfer of a phosphoryl group from ATP



Time Course of Phosphorylations Triggered by Insulin



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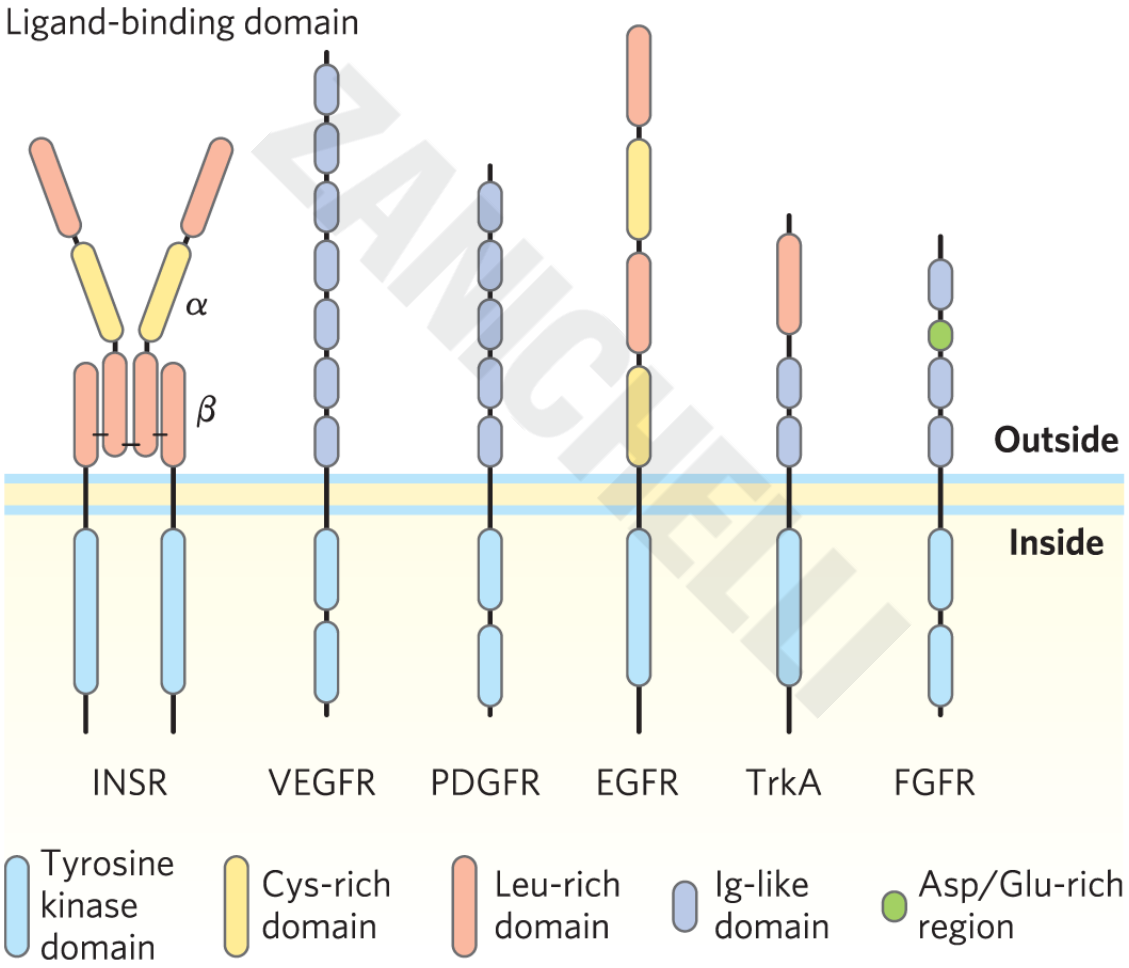
P4 Principle 4 (4 of 5)

Plasma membrane receptors with an intracellular tyrosine kinase activity act through cascades of protein kinases to transduce signals about the metabolic state, including growth factors.

The Insulin Receptor Is A Prototype for Receptor Tyrosine Kinases



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P2 Principle 2 (4 of 4)

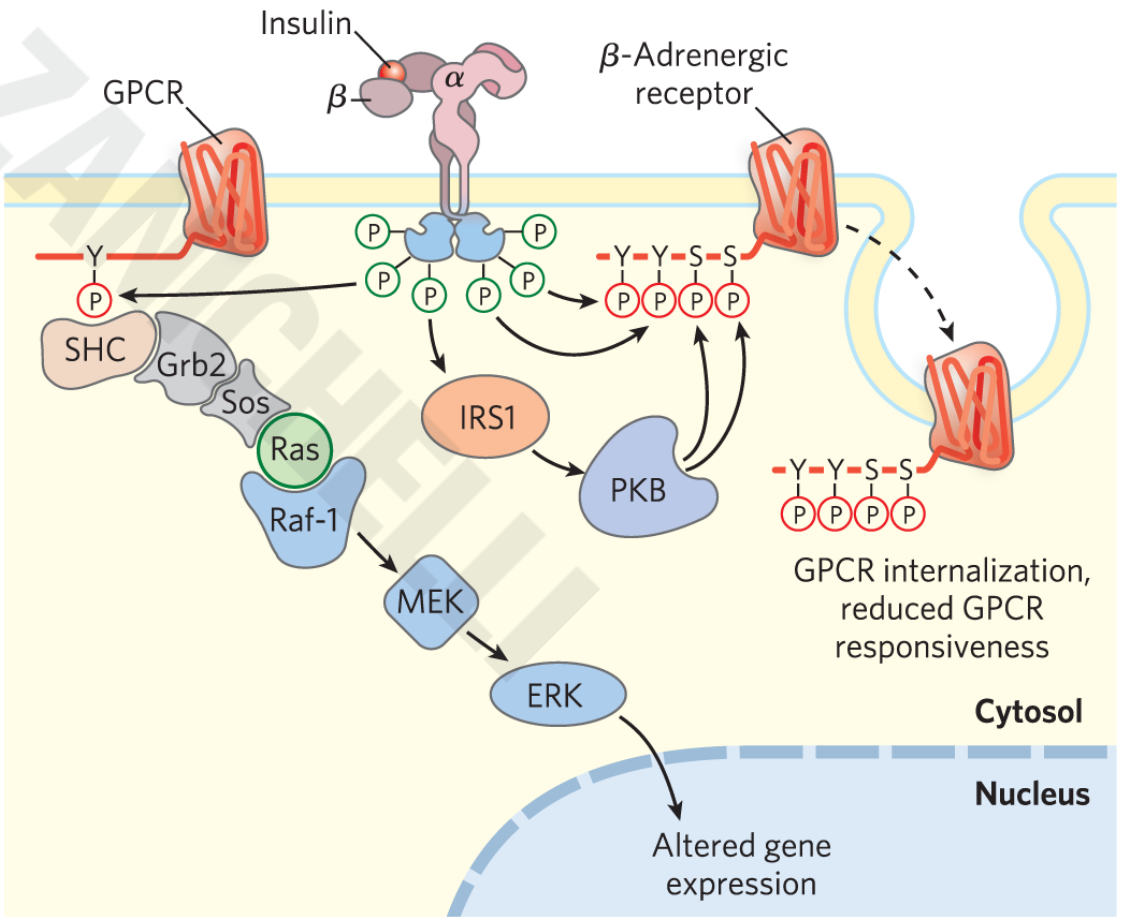
There is a high degree of evolutionary conservation of signaling proteins and transduction mechanisms across the animal phyla. At least a billion years of evolution have passed since the plant and animal branches of the eukaryotes diverged, which is reflected in the differences in signaling mechanisms used in the two kingdoms. We focus here on the animal kingdom.

Evolution of Insulin Receptor Signaling Machinery

- the regulatory machinery:
  - allows for amplification of the insulin signal
  - provides for the integration of signals from different receptors
  - can trigger two or more signaling pathways
  - involves closely related IRS proteins, each with its own characteristic tissue distribution and function

Cross Talk among Signaling Systems Is Common and Complex

- extensive interconnections among signaling pathways allow integration and fine-tuning of multiple hormonal effects



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12.5 Multivalent Adaptor Proteins and Membrane Rafts

P4 Principle 4 (5 of 5)

Plasma membrane receptors with an intracellular tyrosine kinase activity act through cascades of protein kinases to transduce signals about the metabolic state, including growth factors.

Phosphorylation-Dependent Protein Interactions

- protein-protein interactions brought about by reversible phosphorylation create docking sites for other proteins

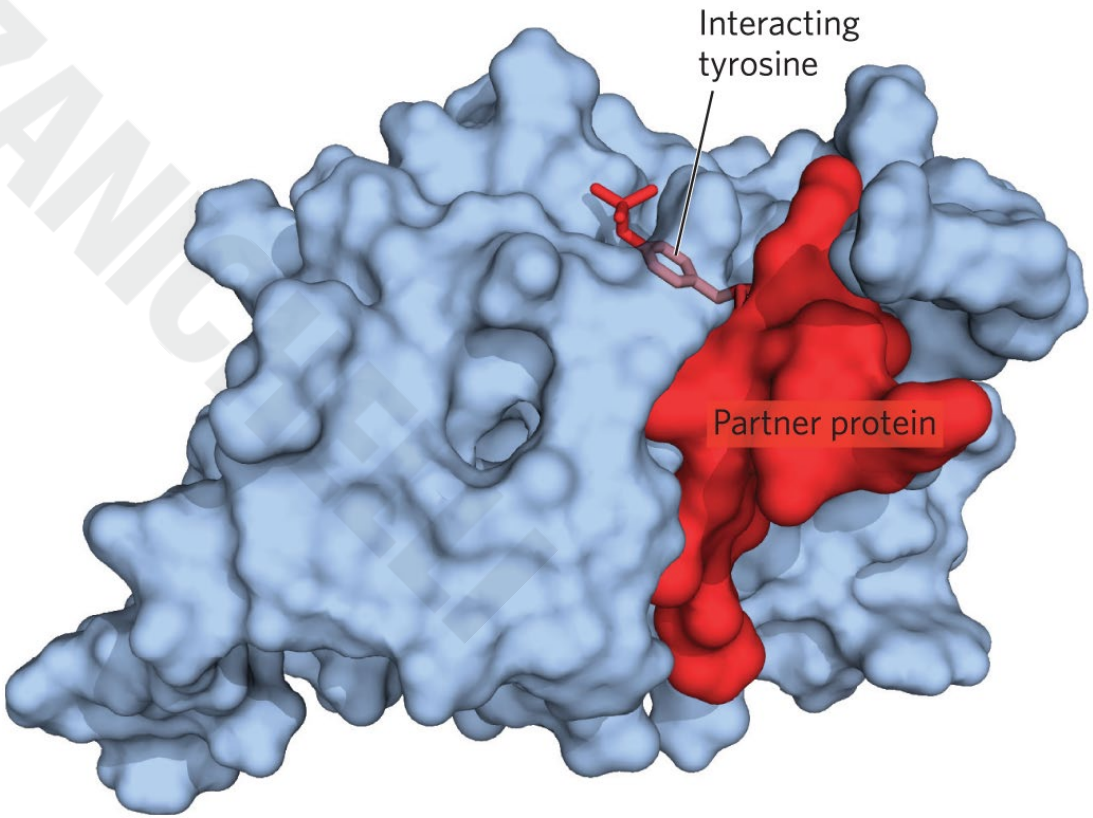
- many signaling proteins are *multivalent*
  - due to flexible intrinsically disordered regions (IDRs)

Protein Modules Bind Phosphorylated Tyr, Ser, or Thr Residues in Partner Proteins

- SH2 domains bind to proteins containing phosphorylated Tyr residues
- other domains bind phosphorylated Ser and phosphorylated Thr residues in various contexts
- SH3 and PH domains bind the membrane phospholipid PIP_3

Phosphotyrosine-Binding Domains (PTB Domains)

- phosphotyrosine-binding domains **(PTB domains)** = another binding partner for phosphorylated Tyr



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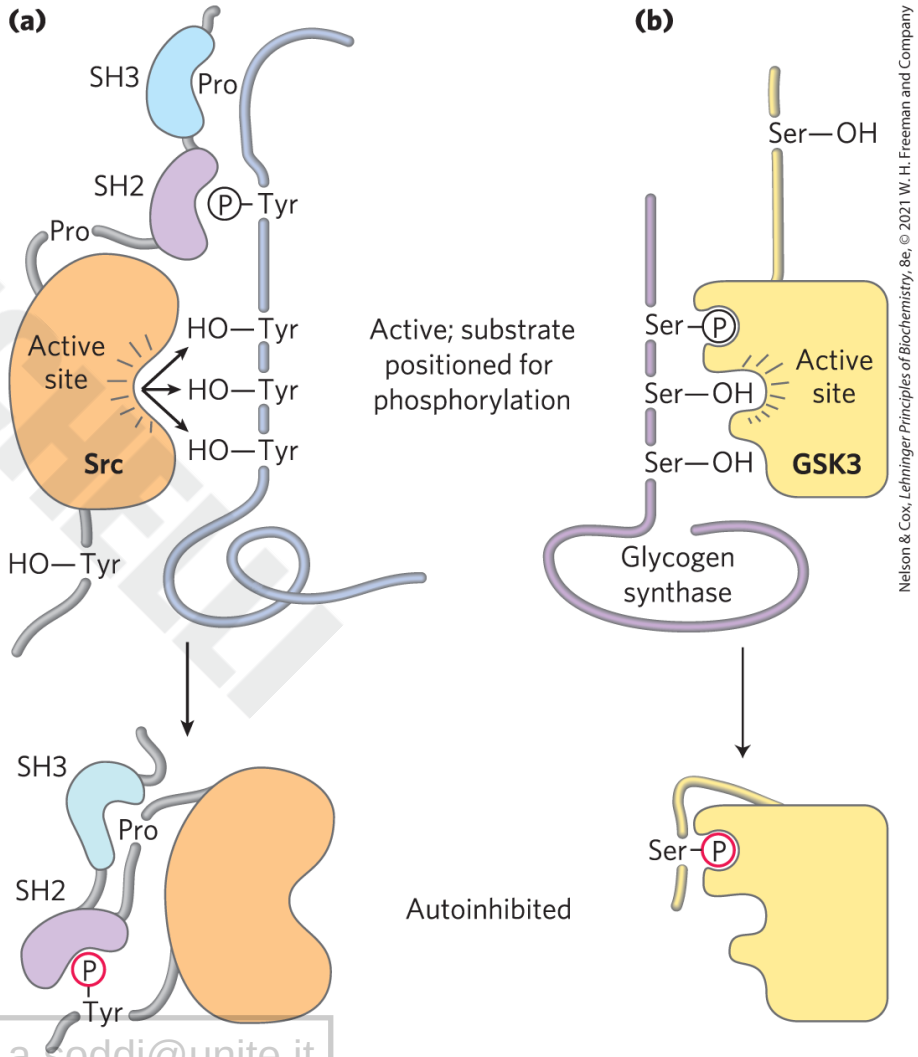
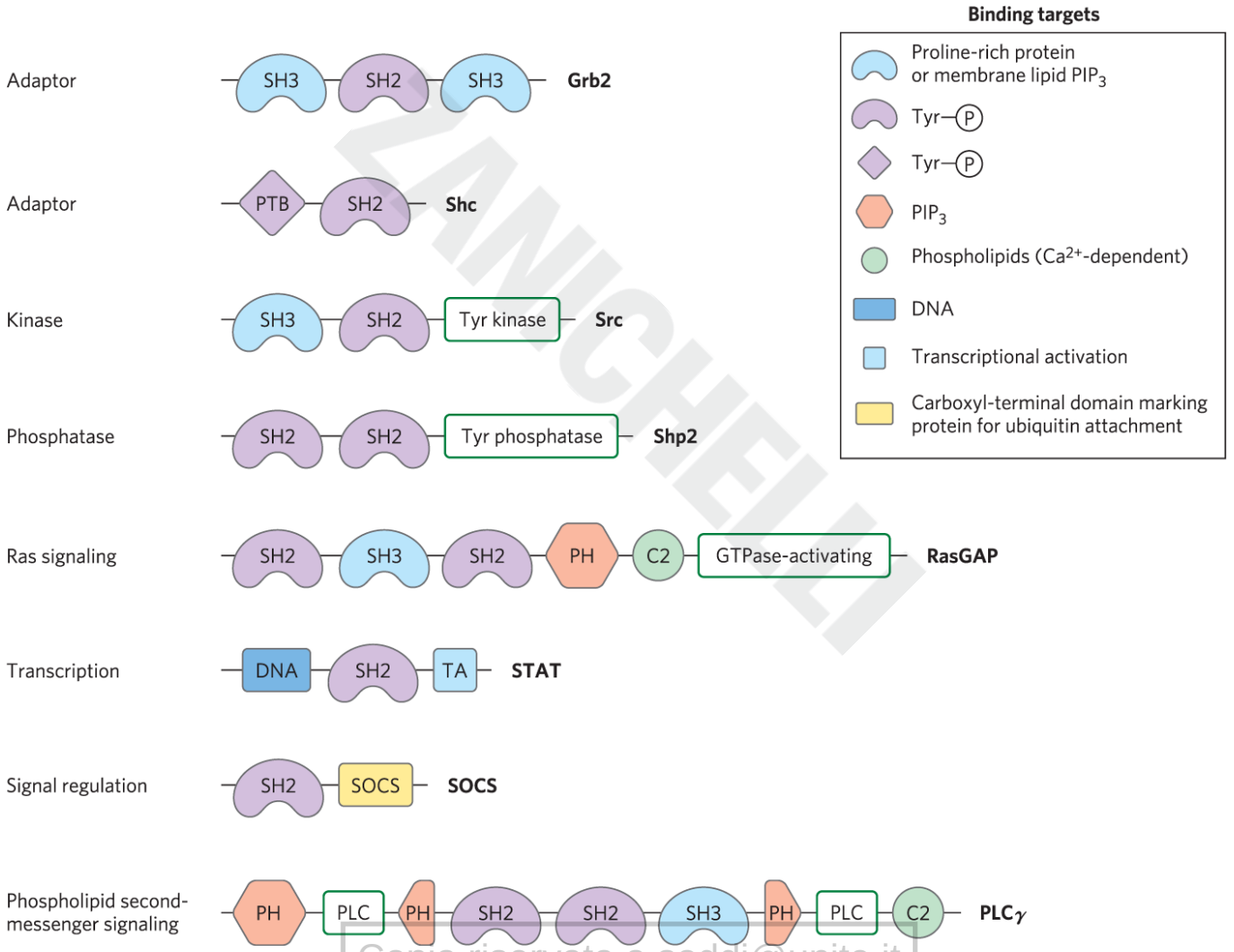
Mechanism of Autoinhibition of Src and GSK3

- removal of a phosphorylated Tyr residue turns on the Tyr kinase activity of Src
- dephosphorylation of a Ser residue in the autoinhibitory domain of GSK3 frees the enzyme



Some Binding Modules of Signaling Proteins



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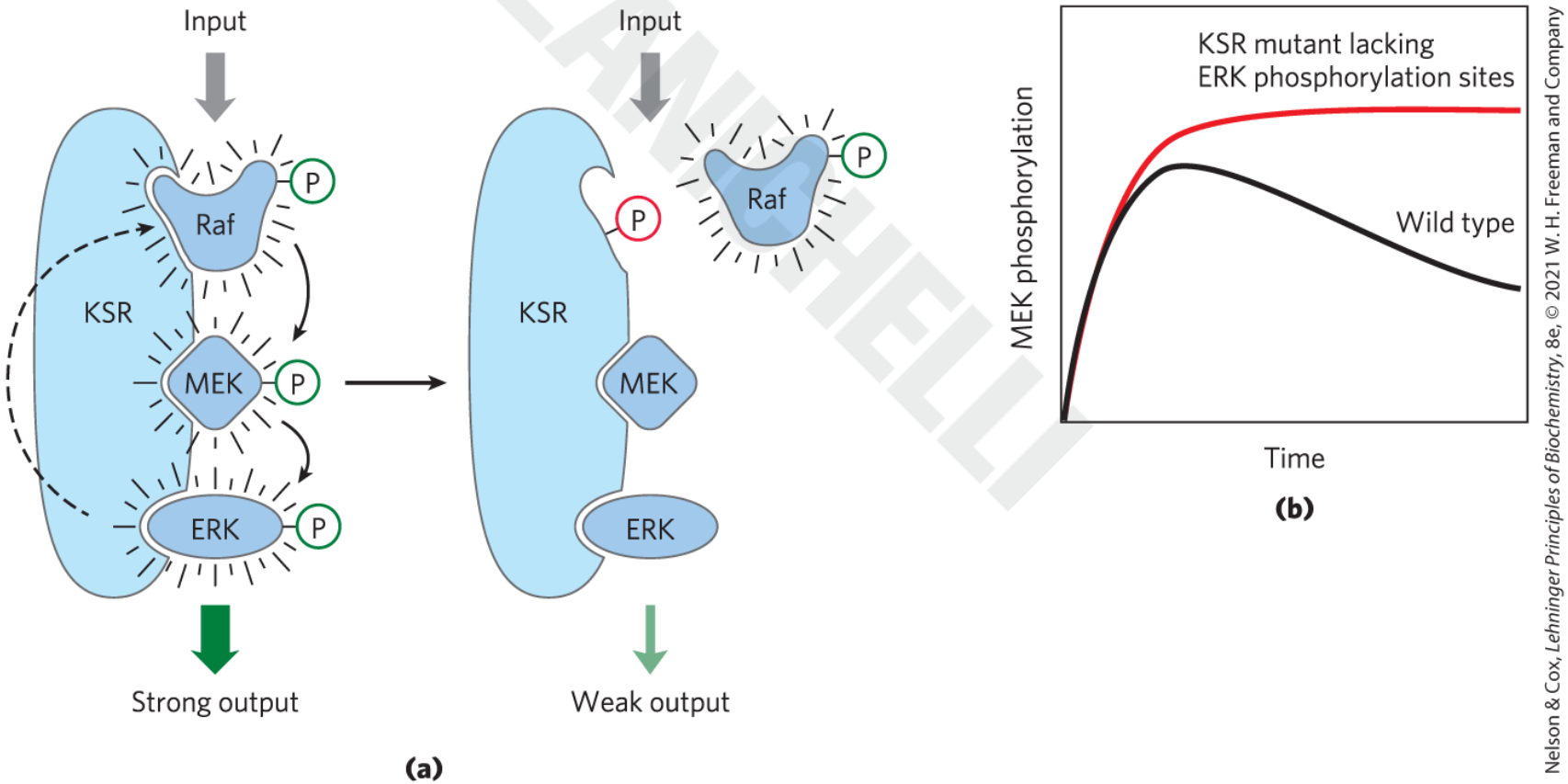
P5 Principle 5 (6 of 6)

Phosphorylation of intrinsically disordered regions of signaling proteins acts as a switch, toggling enzyme activity or creating binding sites for other molecules. Signal responses are integrated by multiprotein signaling complexes with modular domains that bind phosphorylated Tyr, Ser, or Thr residues.

Scaffolds with Multivalent Binding Capacities Assemble Large Multiprotein Complexes



Membrane Rafts and Caveolae Segregate Signaling Proteins

- membrane rafts = regions of the membrane bilayer enriched in sphingolipids, sterols, and proteins attached to the bilayer by GPI anchors
- caveolae = specialized membrane rafts
- segregating signaling proteins in rafts and caveolae:
  - raises their local concentrations
  - makes signaling more efficient

12.6 Gated Ion Channels

P6 Principle 6 (3 of 5)

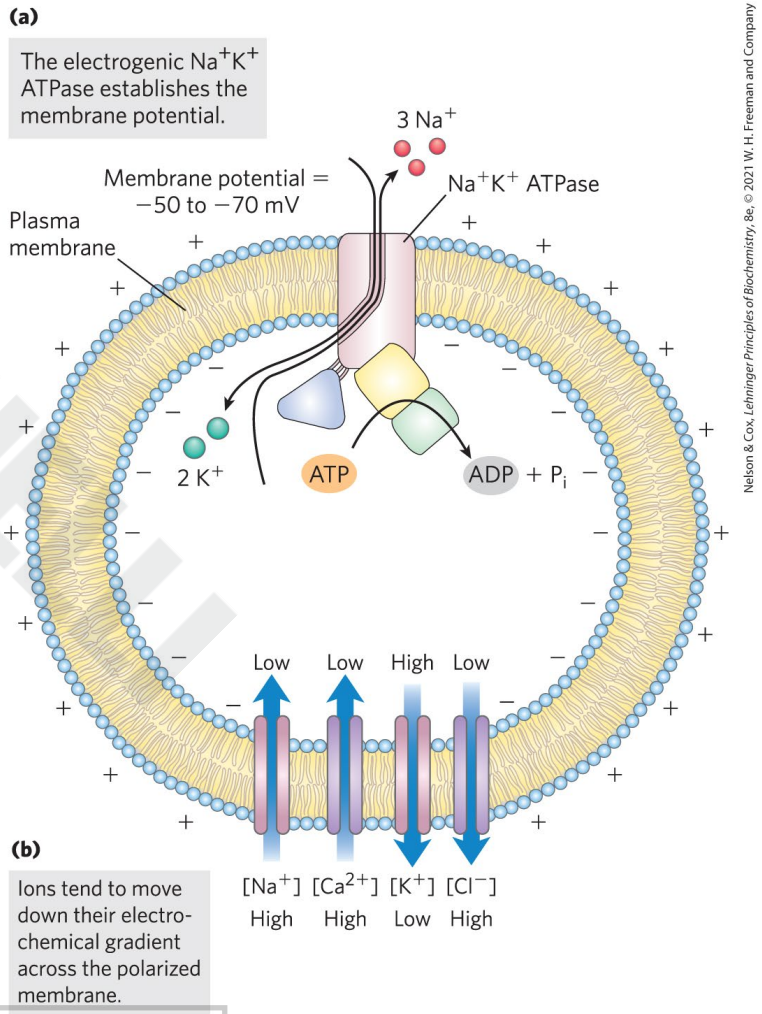
Ion channels gated by membrane potential or ligands are central to signaling in all cells, including bacteria, plants, and animals.

Ion Channels Underlie Rapid Electrical Signaling in Excitable Cells

- ion channels open when a specific ligand binds to its associated receptor or there is change in the transmembrane electrical potential (**voltage-gated ion channels**)

The Na^+K^+ ATPase Is Electrogenic

- electrochemical potential determines spontaneous ion flow across a polarized membrane
- when their respective channel opens,
  - Na^+ and Ca^{2+} flow inward
  - K^+ and Cl^- flow outward



P6 Principle 6 (4 of 5)

Ion channels gated by membrane potential or ligands are central to signaling in all cells, including bacteria, plants, and animals.

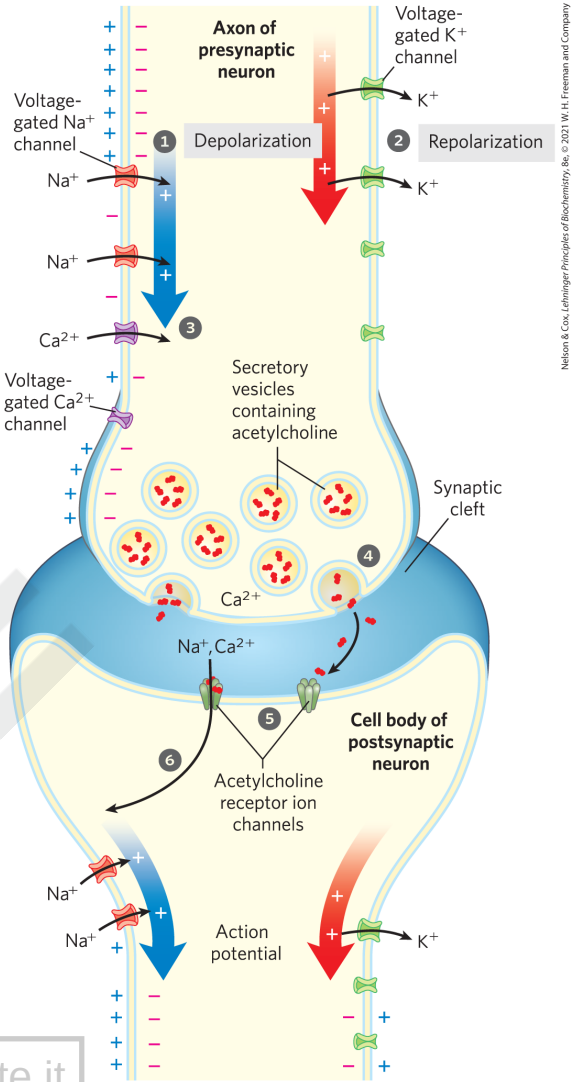
The Opening and Closing of Ion Channels Underlies Electrical Signaling

- Na^+, K^+, and Cl^- ion fluxes have essentially no effect on the concentrations of these ions

- membrane potential at a given time depends on the types and numbers of ion channels open at that instant
  - transient changes in membrane potential underlie electric signaling

Voltage-Gated Ion Channels Produce Neuronal Action Potentials

- **action potential** = wave of depolarization (Na^+ influx) followed by repolarization (K^+ efflux)
  - travels the length of a neuron from the cell body to the end of an axon
  - carried by the voltage-gated Na^+ and K^+ channels of neuronal membranes
  - arrival at the distal end of a presynaptic neuron triggers neurotransmitter release



P6 Principle 6 (5 of 5)

Ion channels gated by membrane potential or ligands are central to signaling in all cells, including bacteria, plants, and animals.

Neurons Have Receptor Channels That Respond to Different Neurotransmitters

- **ionotropic** receptors = receptors that are ion channels
- **metabotropic** receptors = receptors that generate a second messenger
- ligands (neurotransmitters) each binds to their specific receptors and triggers a change in V_m
  - the target cell's V_m is the sum of the effects of all ion-channel contributions

Toxin Target Ion Channels

- neurotoxins attack neuronal ion channels
  - fast-acting and deadly

- examples:
  - dendrotoxin (from the black mamba snake) affects voltage-gated K^+ channels
  - tetrodotoxin (produced by puffer fish) affects voltage-gated Na^+ channels
  - cobrotoxin (from cobras) affects acetylcholine receptor ion channels

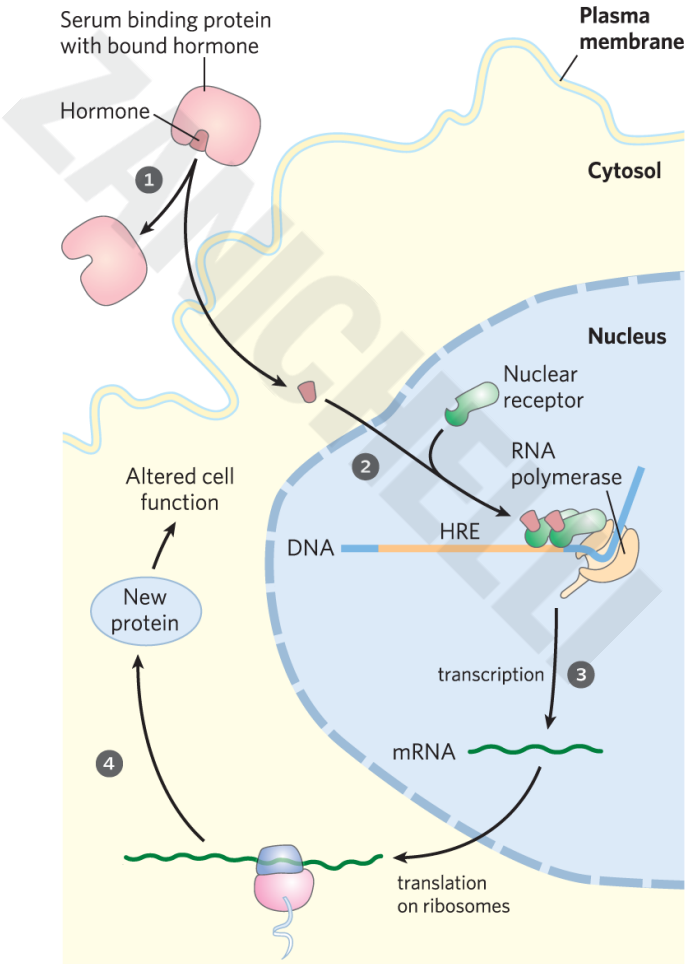
12.7 Regulation of Transcription by Nuclear Hormone Receptors

P7 Principle 7 (2 of 2)

Some hormone signals act inside the cell, not at the plasma membrane, forming complexes with specific receptor proteins that regulate gene expression.

Steroid Hormones Diffuse Into The Cell and Regulate Gene Expression

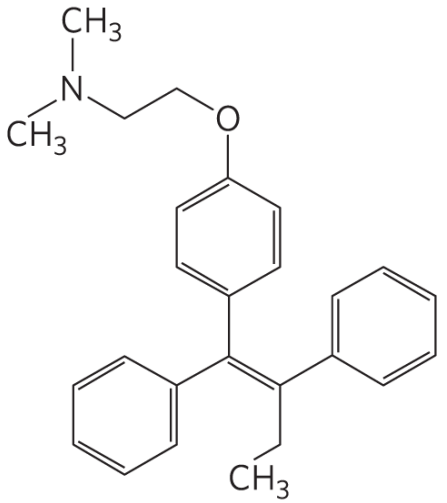
- **hormone response elements (HREs)** = specific regulatory sequences in DNA that interact with receptor proteins following hormone binding



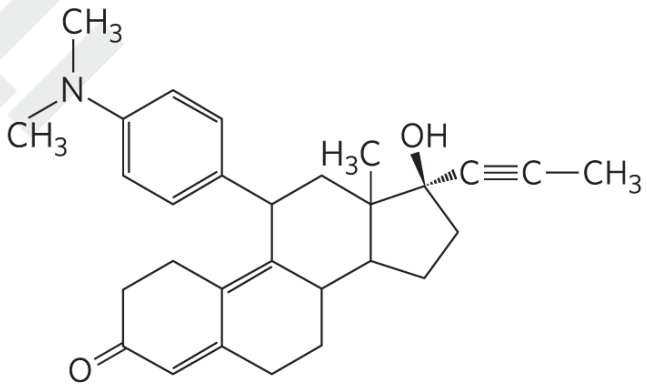
1. Hormone, carried to the target tissue on serum binding proteins, diffuses across the plasma membrane and binds to its specific receptor protein in the nucleus.
2. Hormone binding changes the conformation of the receptor; it forms homo- or hetero-dimers with other hormone-receptor complexes and binds to specific regulatory regions called hormone response elements (HREs) in the DNA adjacent to specific genes.
3. Receptor attracts coactivator or corepressor protein(s) and, with them, regulates transcription of the adjacent gene(s), increasing or decreasing the rate of mRNA formation.
4. Altered levels of the hormone-regulated gene product produce the cellular response to the hormone.

Tamoxifen and Mifepristone

- **tamoxifen** = estrogen antagonist used in the treatment of breast cancer
  - has little or no effect on gene expression
- **mifepristone (RU486)** = contraceptive
  - binds to the progesterone receptor and blocks hormone actions essential to implantation of the fertilized ovum



Tamoxifen



Mifepristone
(RU486)

12.8 Regulation of the Cell Cycle by Protein Kinases

P9 Principle 9 (3 of 6)

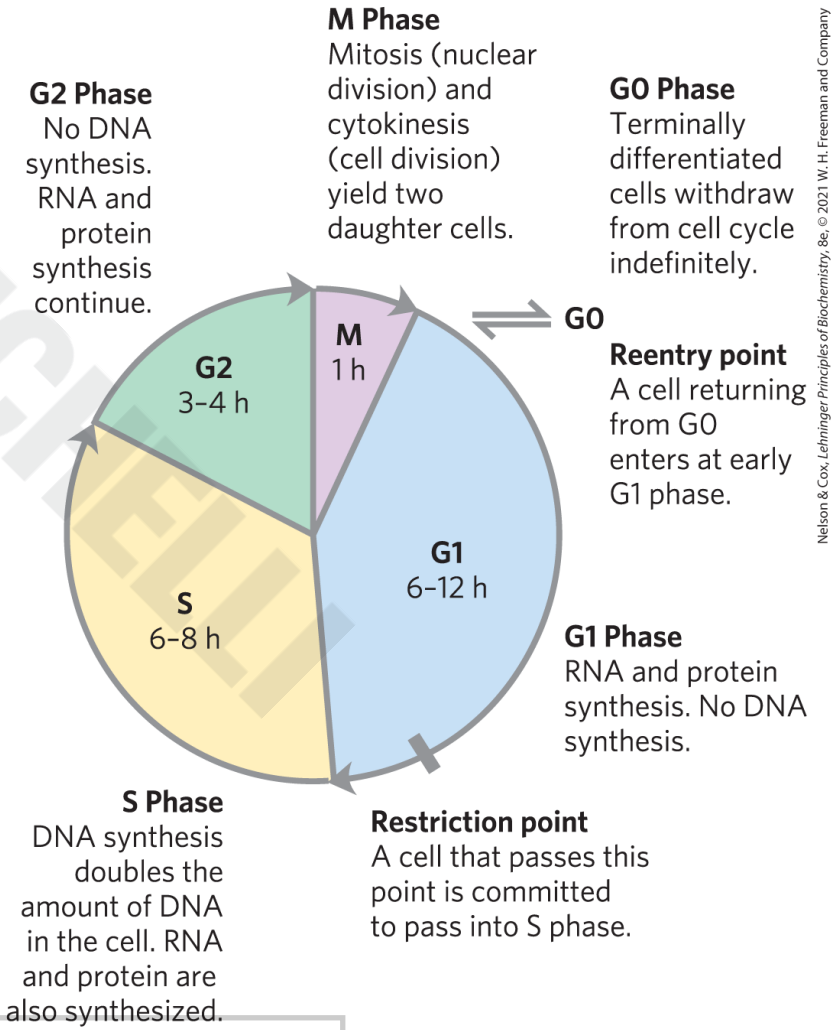
Defective signaling proteins or defective regulation of their synthesis and breakdown can disrupt cell cycle regulation and lead to tumor formation (cancer).

Cancer

- cancer occurs when:
  - the regulatory mechanisms that limit cell division are defective
  - cells undergo unregulated division

The Cell Cycle Has Four Stages

- after passing through the M phase and into G1, a cell either continues through another division or enters G0
- differentiated cells remain in the G0 phase

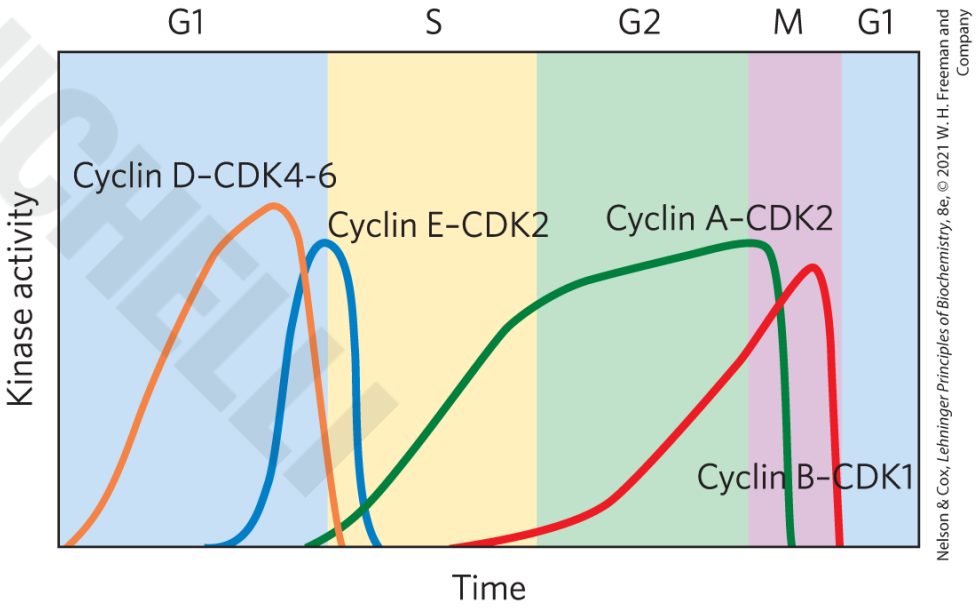


P8 Principle 8 (2 of 2)

Cells receive extracellular signals that determine progress through the cell division cycle, or trigger cell death—processes regulated by phosphorylation and dephosphorylation of key regulatory proteins.

Levels of Cyclin-Dependent Protein Kinases Oscillate

- a family of kinases controls the timing of the cell cycle
- **cyclin-dependent protein kinase (CDK)** = catalytic subunit of the kinases
- **cyclin** = regulatory subunit of the kinases



Oscillations Are the Result of Regulating CDK Activity

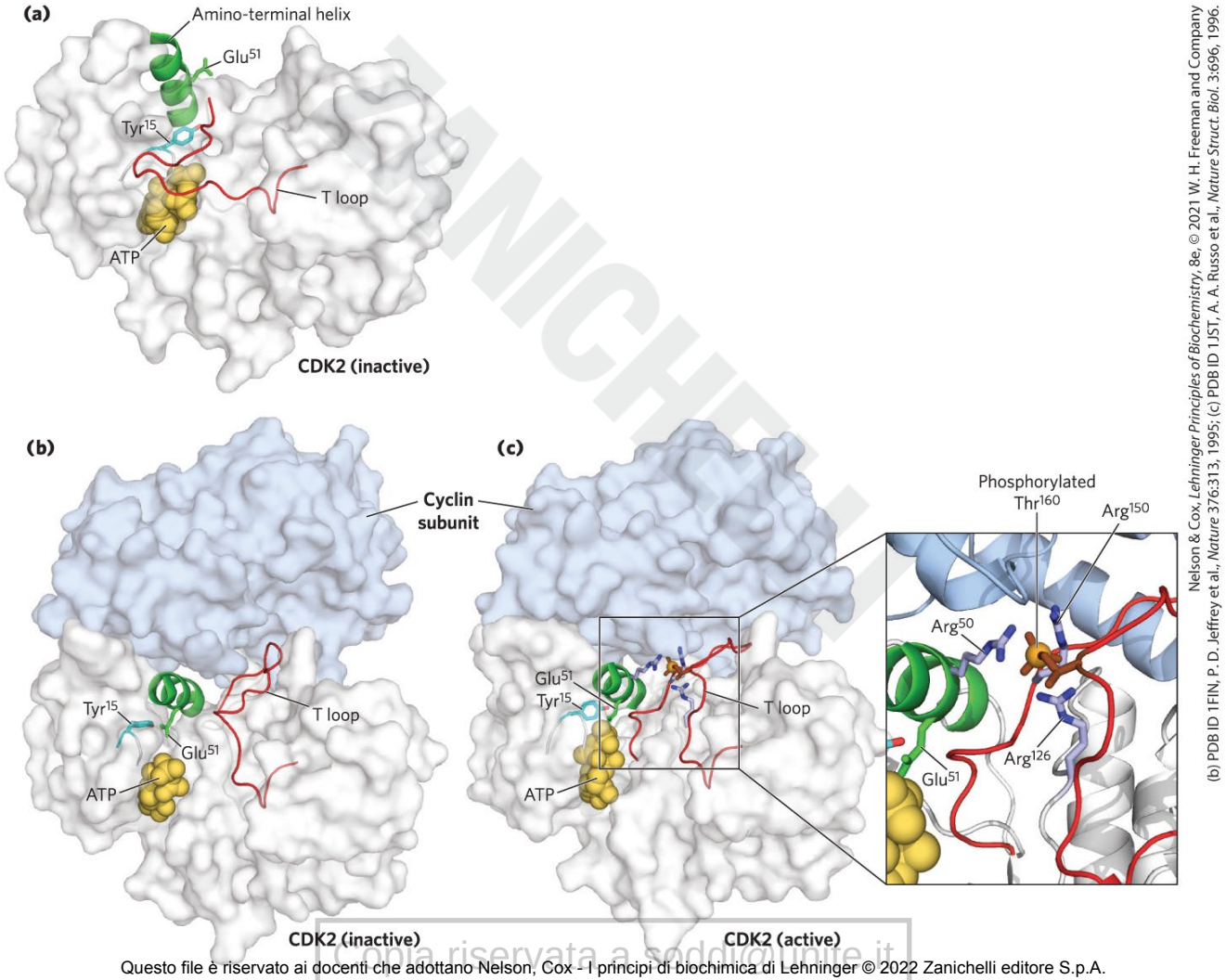
- oscillations are the result of:
  - phosphorylation or dephosphorylation of the CDK
  - controlled degradation of the cyclin subunit
  - periodic synthesis of CDKs and cyclins
  - the action of specific CDK-inhibiting proteins

CDKs Are Regulated by Phosphorylation, Cyclin Degradation, Growth Factors, and Specific Inhibitors

- phosphorylation of Thr^{160} of CDK2 opens the substrate-binding cleft in the kinase

- dephosphorylation of phosphorylated Tyr^{15} of CDK2 unblocks the ATP-binding site

Activation of Cyclin-Dependent Protein Kinases (CDKs)



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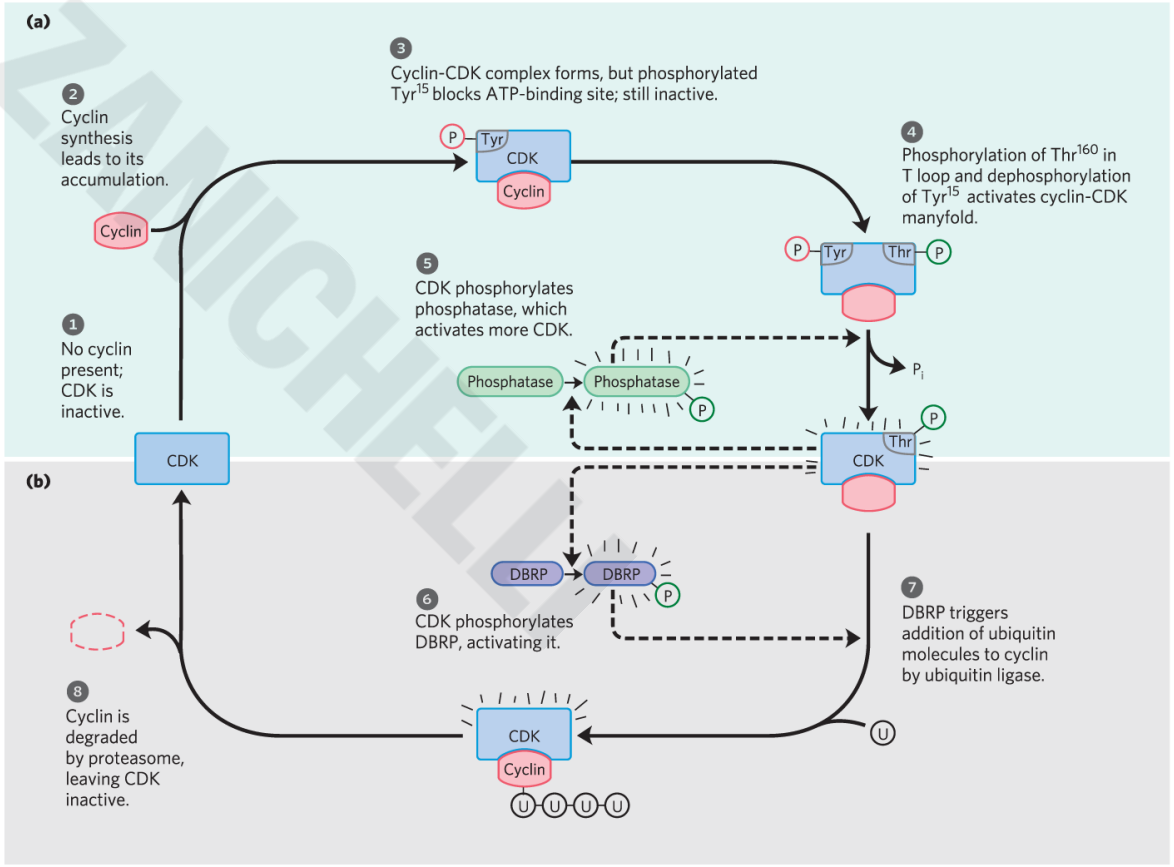
(b) PDB ID 1FIN, P. D. Jeffrey et al., *Nature* 376:313, 1995; (c) PDB ID 1JST, A. A. Russo et al., *Nature Struct. Biol.* 3:696, 1996.

Regulation of CDK by Phosphorylation and Proteolysis

- **ubiquitin** = regulatory protein that targets proteins for destruction
- **proteasomes** = proteolytic enzyme complexes



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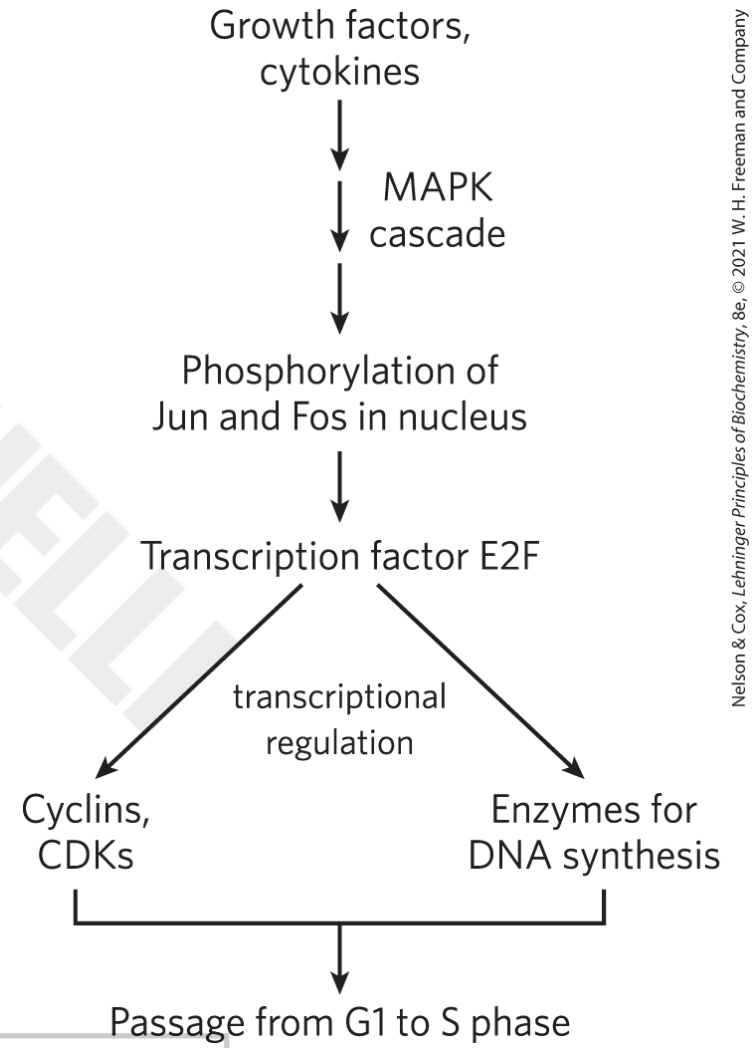
Regulation of Cell Division by Growth Factors and Inhibitors

- **growth factors** = extracellular signals that phosphorylate and activate nuclear transcription factors

- specific protein inhibitors bind to and inactivate specific CDKs
  - example: p21



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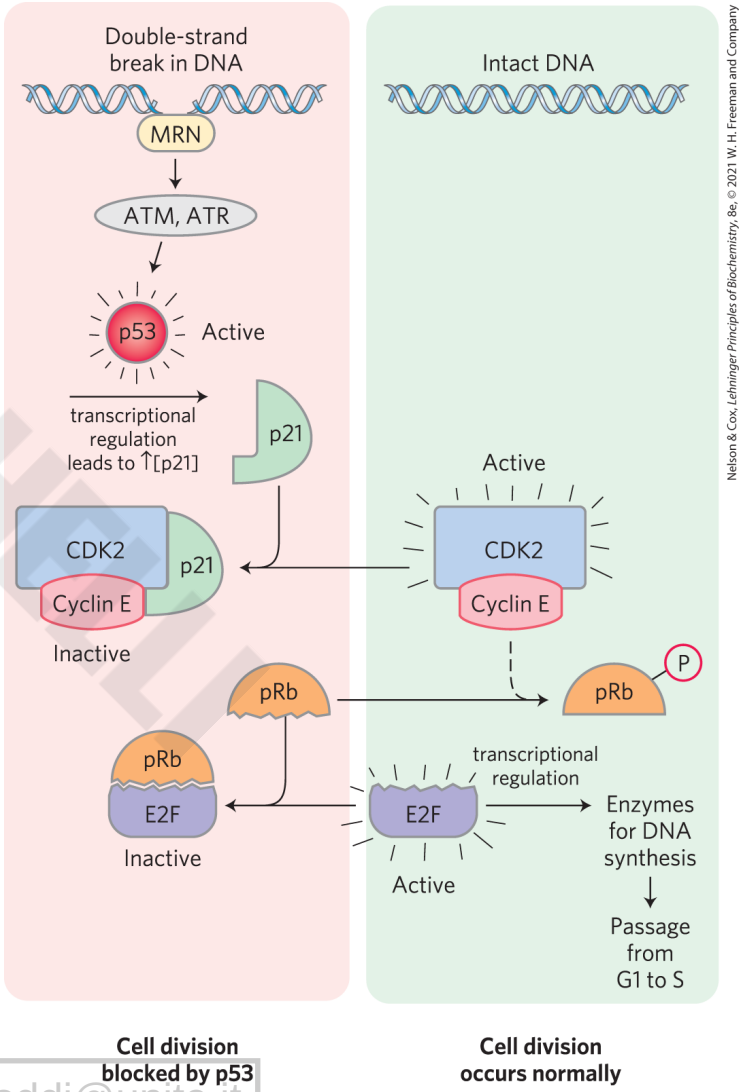
CDKs Regulate Cell Division by Phosphorylating Critical Proteins

- **lamin** = protein that makes up the intermediate filaments of the nuclear envelope
  - phosphorylation by a CDK causes depolymerization of the filaments

- actin and myosin = pinch a dividing cell into two parts during cytokinesis
  - phosphorylation of myosin by a CDK causes dissociation of myosin from actin filaments

Phosphorylation of pRb

- **retinoblastoma protein (pRb)** = binds the transcription factor E2F to arrest cell division in G1 when DNA damage is detected
  - phosphorylation by a CDK relieves the pRb-E2F blocking mechanism



12.9 Oncogenes, Tumor Suppressor Genes, and Programmed Cell Death

P9 Principle 9 (4 of 6)

Defective signaling proteins or defective regulation of their synthesis and breakdown can disrupt cell cycle regulation and lead to tumor formation (cancer).

Cell Division Is Regulated by Extracellular Growth Factors

- cell division is regulated by extracellular growth factors
  - causes a balance between the formation and destruction of cells

- defects in regulatory proteins disrupt this balance
  - can result in the formation of a tumor and cancer

P9 Principle 9 (5 of 6)

Defective signaling proteins or defective regulation of their synthesis and breakdown can disrupt cell cycle regulation and lead to tumor formation (cancer).

Oncogenes Are Mutant Forms of the Genes for Proteins That Regulate the Cell Cycle

- **oncogenes** = mutated versions of genes encoding signaling proteins involved in cell cycle regulation
  - lead to tumor formations
  - genetically dominant
  - may encode defective growth factors, receptors, G proteins, protein kinases, or nuclear regulators of transcription

- **proto-oncogenes** = genes in animal host cells that encode growth-regulating proteins

Defects in Certain Genes Remove Normal Restraints on Cell Division

- **tumor suppressor genes** = encode regulatory proteins that normally inhibit cell division
  - mutations in these genes are genetically recessive

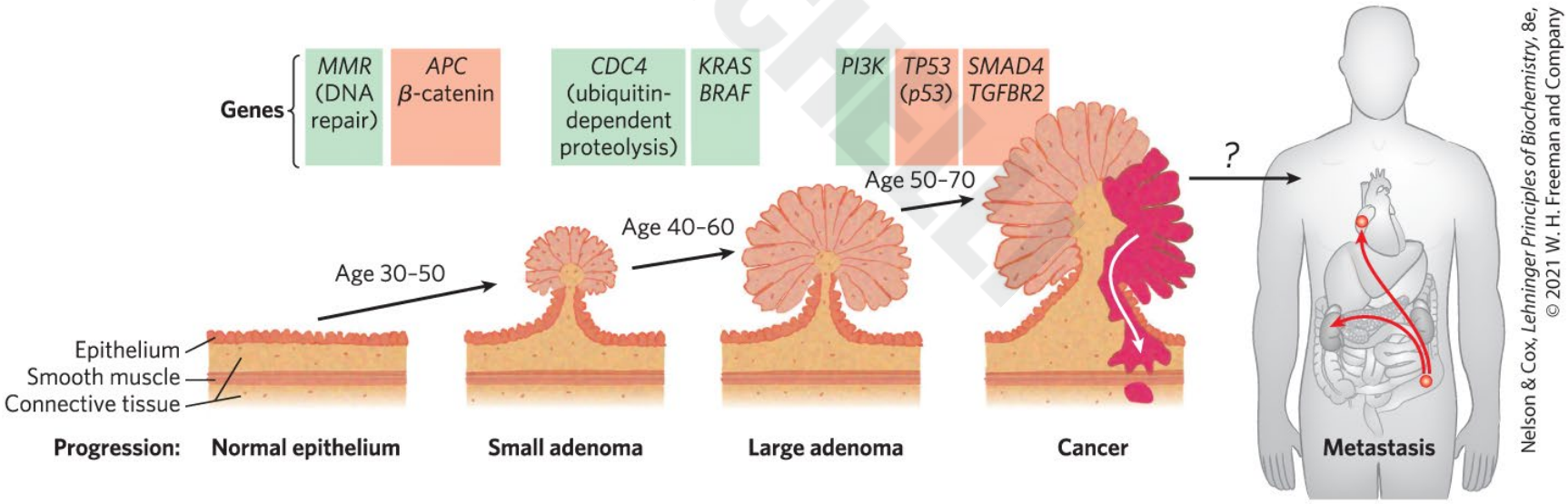
- mutations in both copies of the genes for pRb, p53, or p21 cause tumor formation

Stability Genes

- stability genes (caretaker genes) = encode proteins necessary for the repair of genetic damage
  - mutations cause other mutations to go unrepaired
  - can cause cancer when the unrepaired mutations are in proto-oncogenes and tumor suppressor genes

Multistep Transition from Normal Epithelial Cell to Colorectal Cancer

- in some (and perhaps all) cancers, the progression from a normal cell to a malignant tumor requires an accumulation of mutations



Driver and “Passenger” Mutations

- **driver mutations** = mutations that are the *cause* of unregulated cell division

- “passenger mutations” = mutations that occur randomly and do not confer a selective growth advantage

Apoptosis Is Programmed Cell Suicide

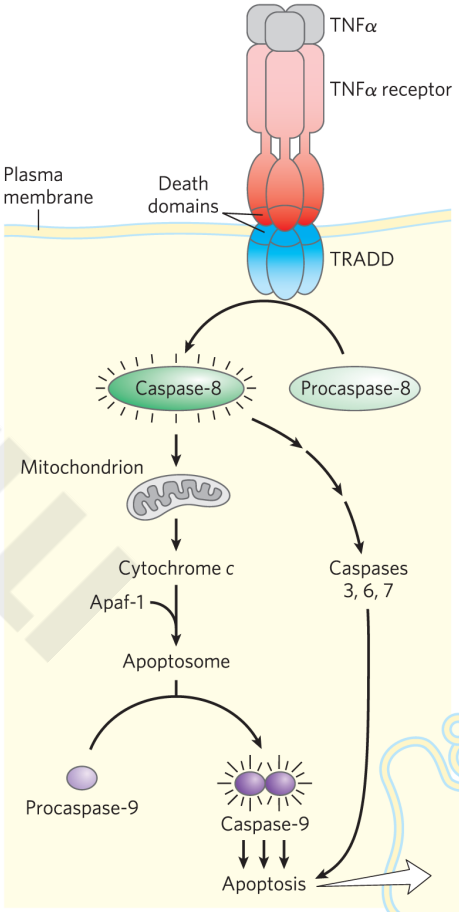
- **programmed cell death** = **apoptosis** = mechanism by which cells precisely control the time of their own death
  - triggered by irreparable DNA damage and severe stresses
  - occurs during normal embryo development

P9 Principle 9 (6 of 6)

Defective signaling proteins or defective regulation of their synthesis and breakdown can disrupt cell cycle regulation and lead to tumor formation (cancer).

Tumor Necrosis Factor (TNF) Can Trigger Apoptosis

- tumor necrosis factor (TNF) = produced by cells of the immune system
  - interacts with cells through specific TNF receptors
- "death domain" = carries the self-destruct signal through the membrane to cytosolic proteins



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