

Buněčná signalizace

doc. Mgr. Vítězslav Bryja, Ph.D.

SIGNALIZACE

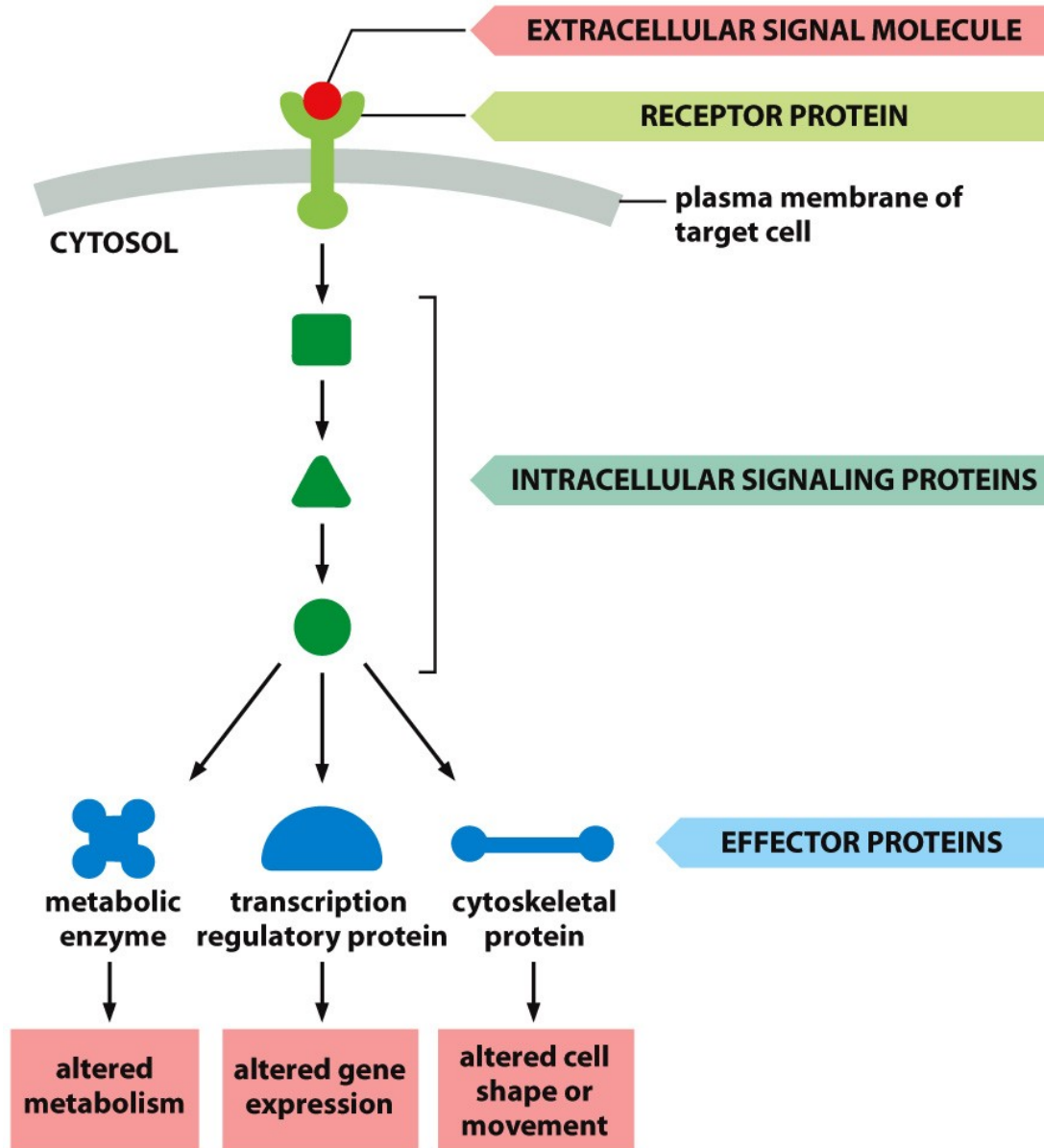


Figure 15-1 Molecular Biology of the Cell 6e (© Garland Science 2015)

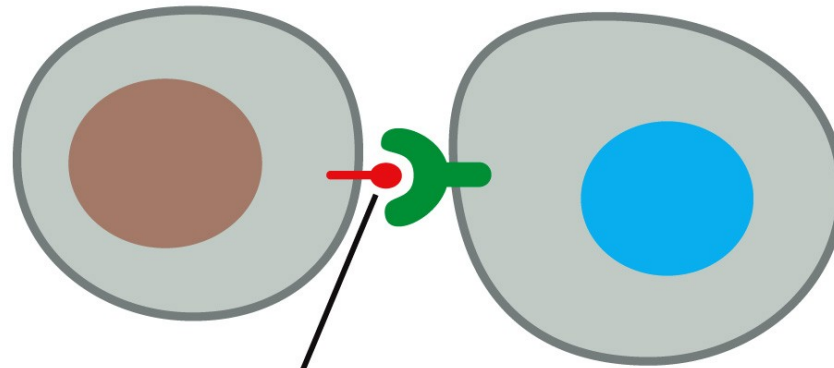
Členění podle různých parametrů

- Dle vztahu buňky vysílající a přijímající signál
 - závislé na kontaktu (contact-dependent)
 - parakrinní
 - synaptické
 - endokrinní

CONTACT-DEPENDENT

signaling cell

target cell



membrane-bound signal molecule

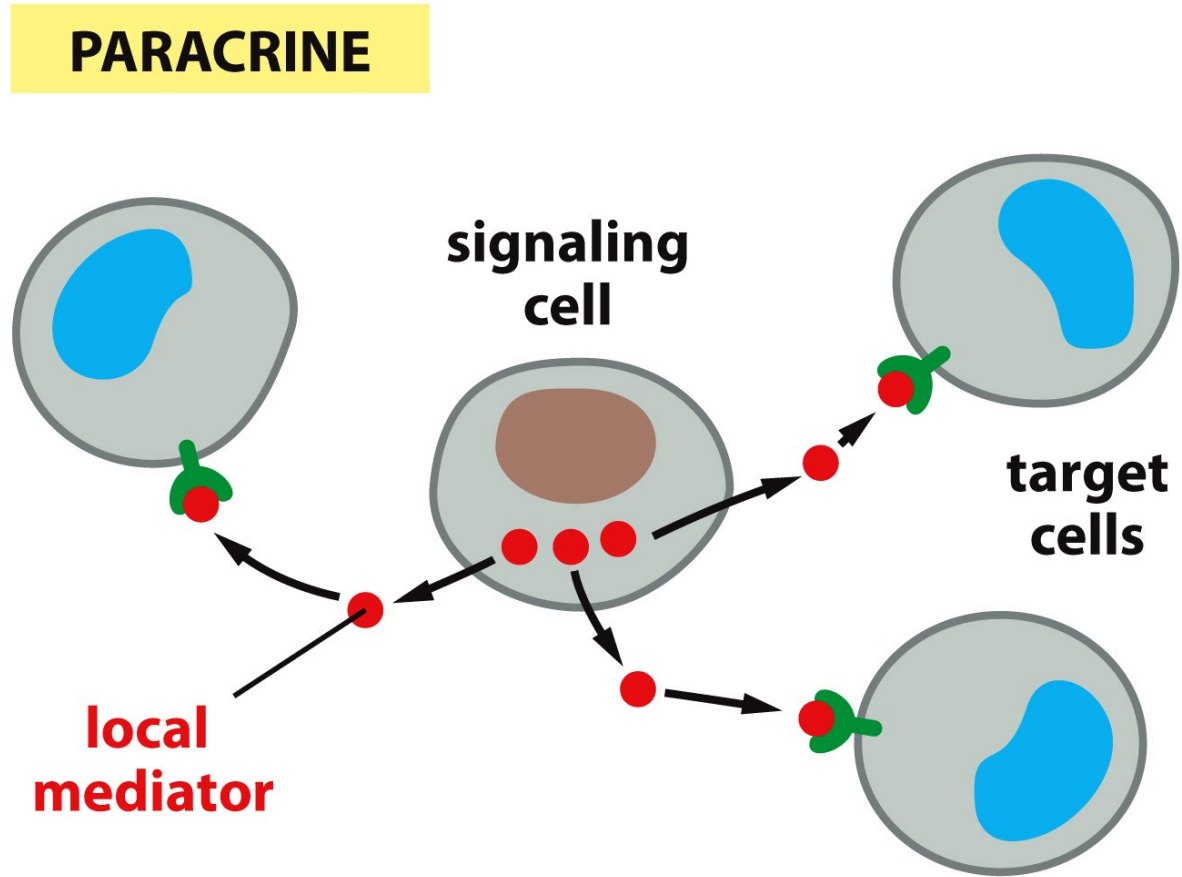


Figure 15-2b Molecular Biology of the Cell 6e (© Garland Science 2015)

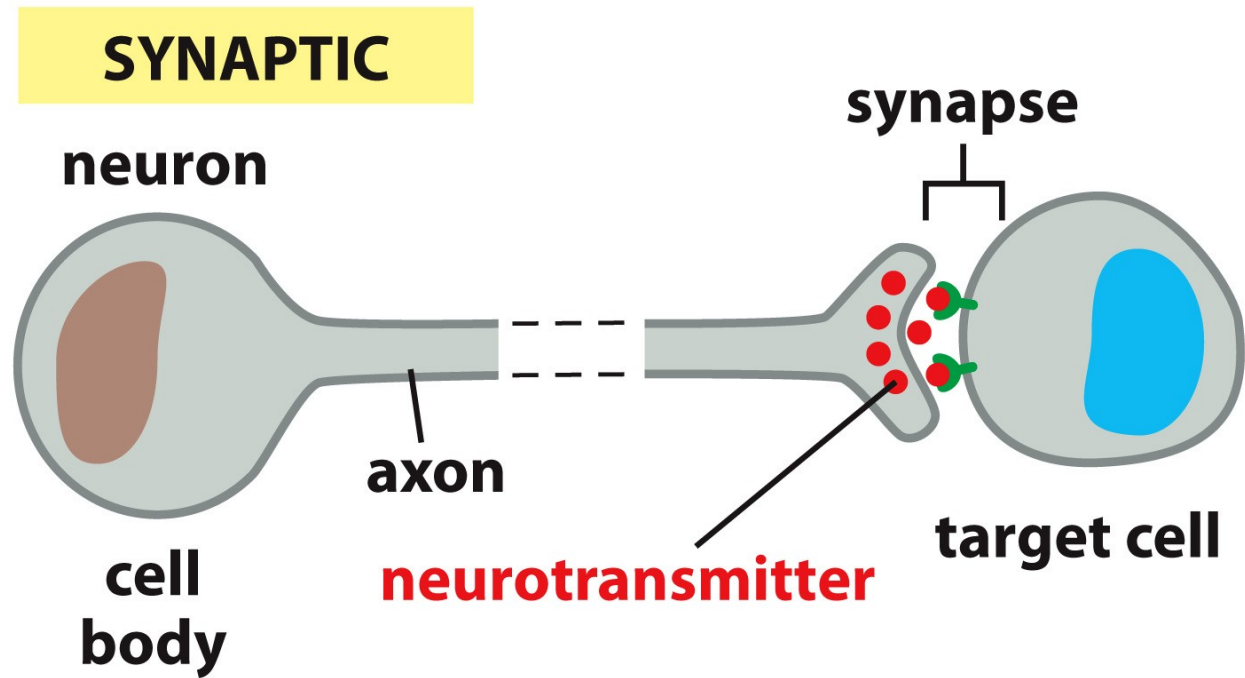


Figure 15-2c Molecular Biology of the Cell 6e (© Garland Science 2015)

ENDOCRINE

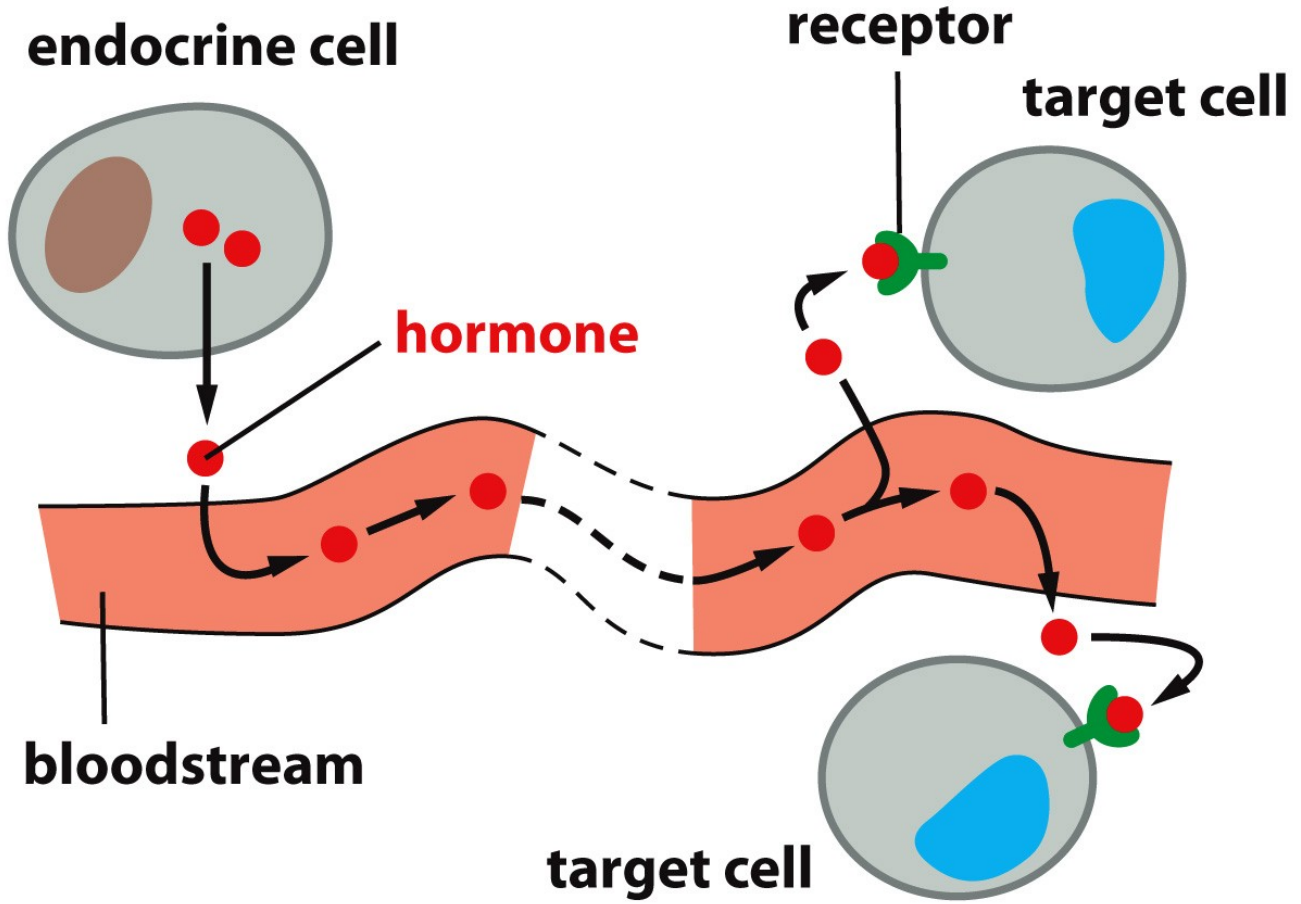


Figure 15-2d Molecular Biology of the Cell 6e (© Garland Science 2015)

Členění podle různých parametrů

- Typy receptorů
 - povrchové receptory
 - intracelulární receptory

CELL-SURFACE RECEPTORS

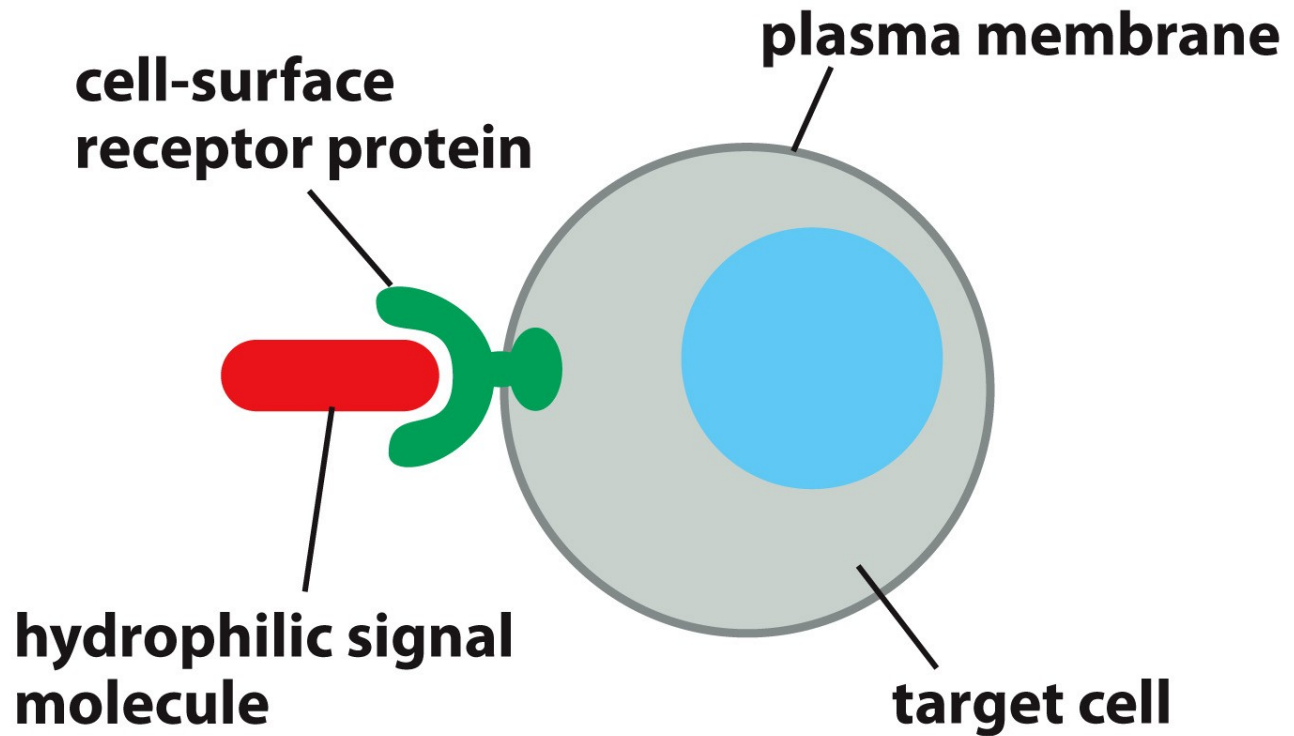


Figure 15-3a Molecular Biology of the Cell 6e (© Garland Science 2015)

INTRACELLULAR RECEPTORS

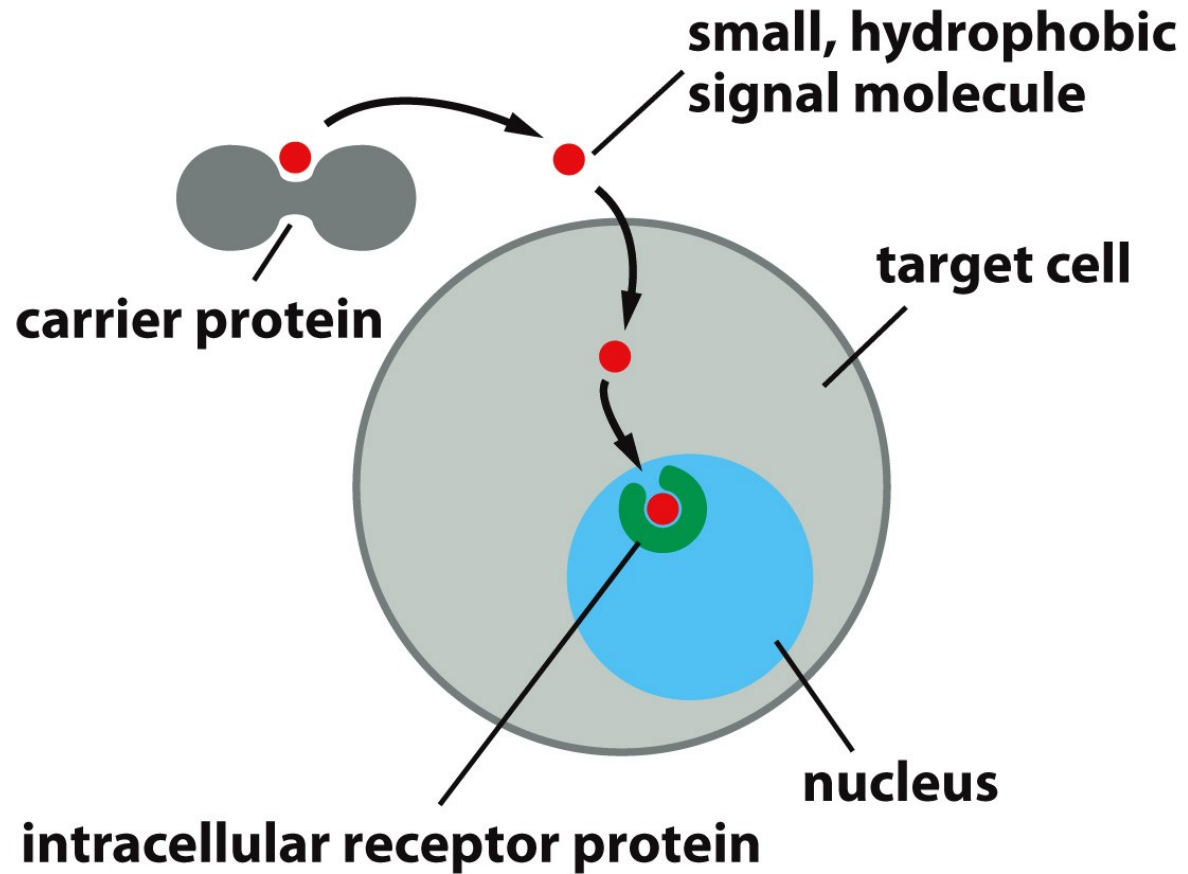


Figure 15-3b Molecular Biology of the Cell 6e (© Garland Science 2015)

Členění podle různých parametrů

➔ Dle důsledků

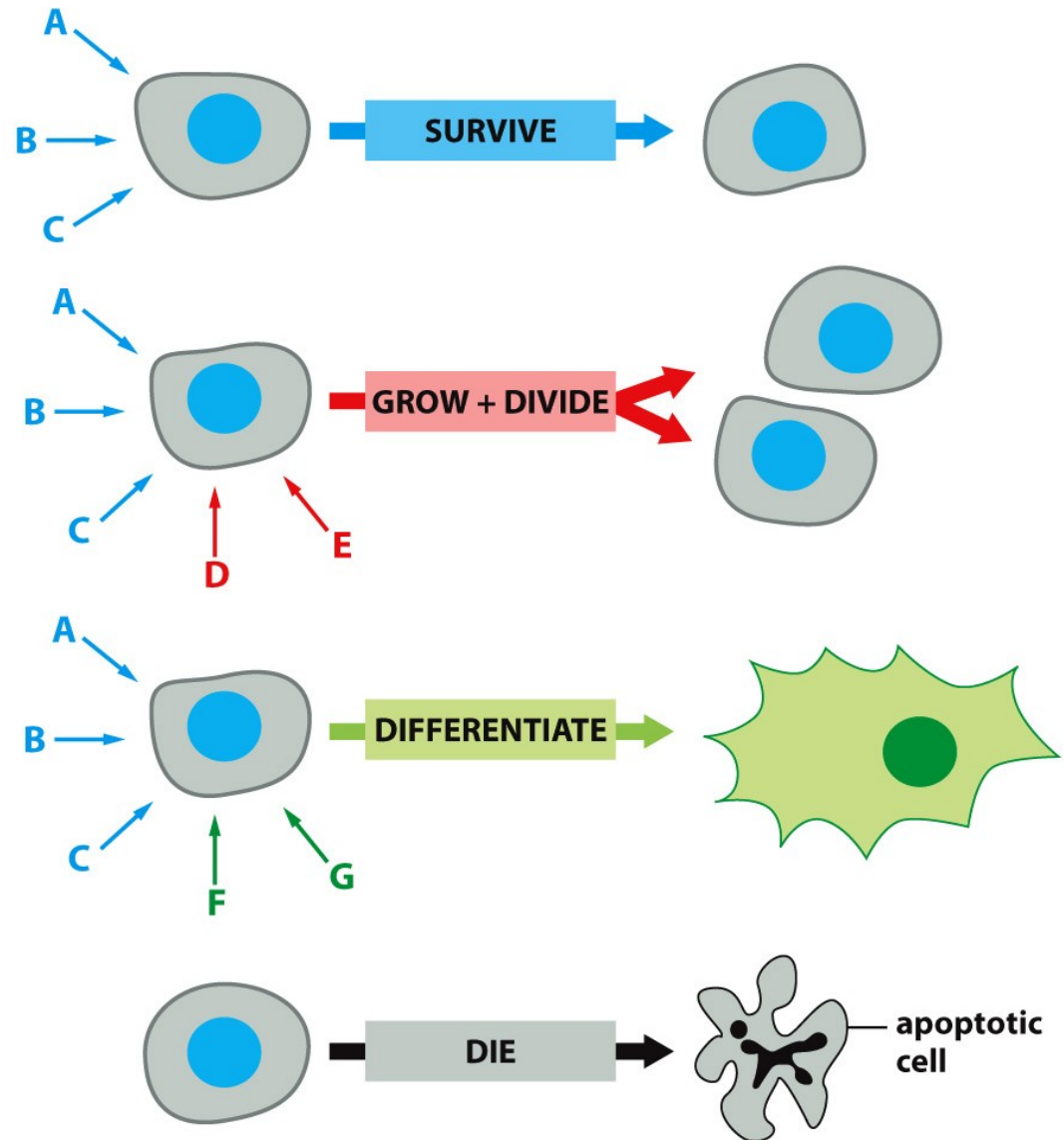
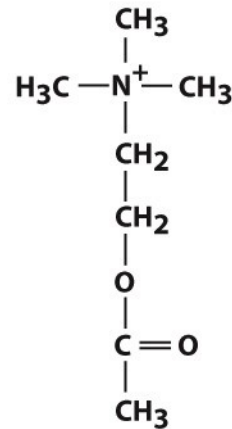


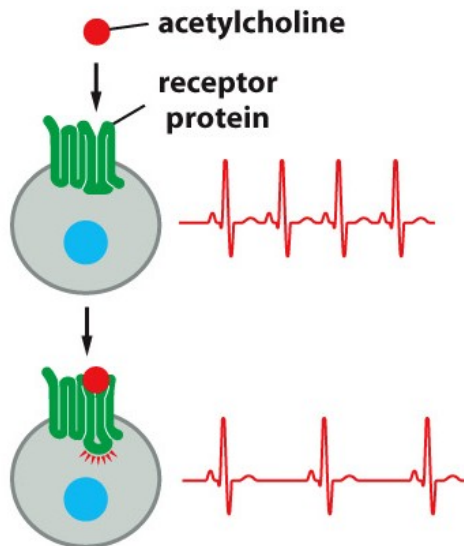
Figure 15-4 Molecular Biology of the Cell 6e (© Garland Science 2015)

- ➔ Stejná signální molekula může působit různě na různé buněčné typy

(A) acetylcholine

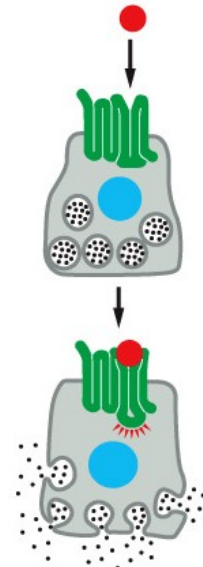


(B) heart pacemaker cell



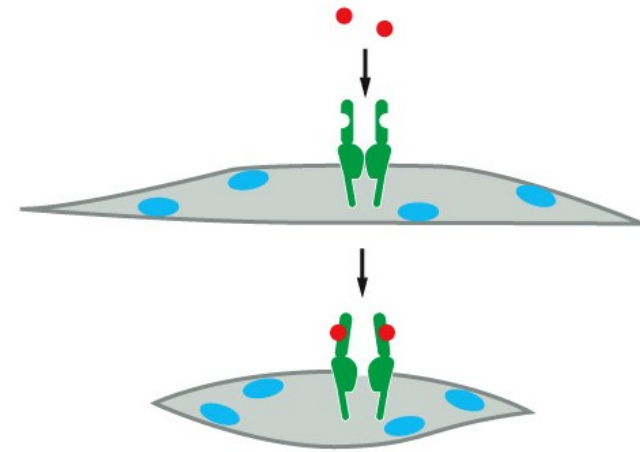
DECREASED RATE OF FIRING

(C) salivary gland cell



SECRETION

(D) skeletal muscle cell



CONTRACTION

Členění podle různých parametrů

Podle typu membránových receptorů

- Receptory typu iontových kanálů
- Receptory spřažené s G-proteiny
- Receptory spřažené s enzymy/enzymatickou funkcí

Základní typy membránových receptorů

- Receptory typu iontových kanálů

ION-CHANNEL-COUPLED RECEPTORS

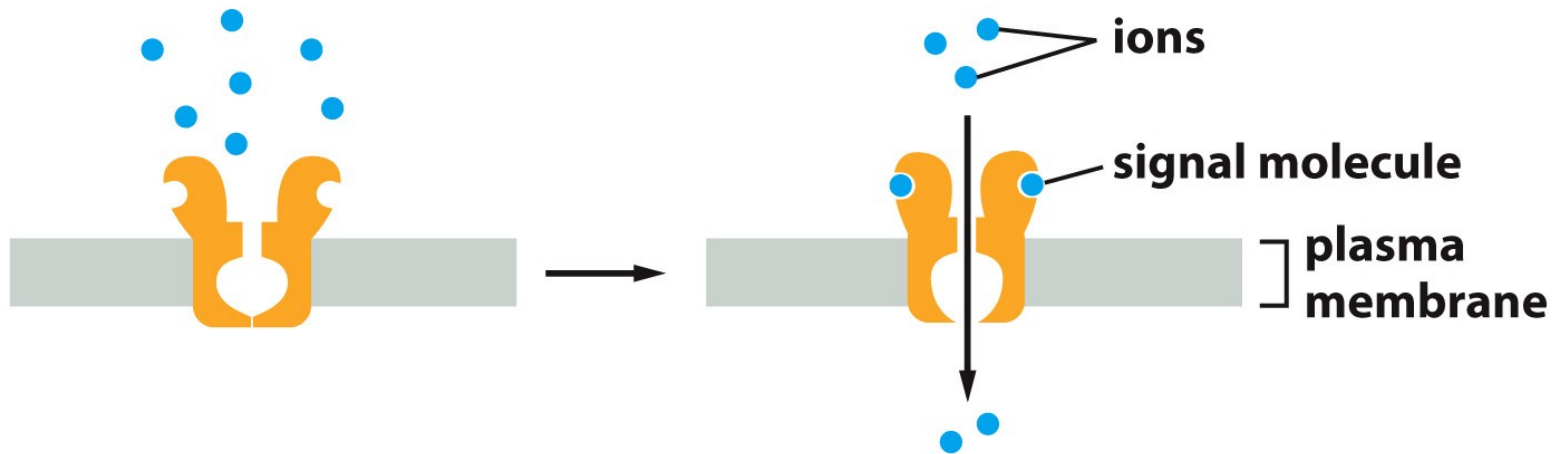


Figure 15-6a Molecular Biology of the Cell 6e (© Garland Science 2015)

Základní typy membránových receptorů

➤ Receptory spřažené s G-proteiny

G-PROTEIN-COUPLED RECEPTORS

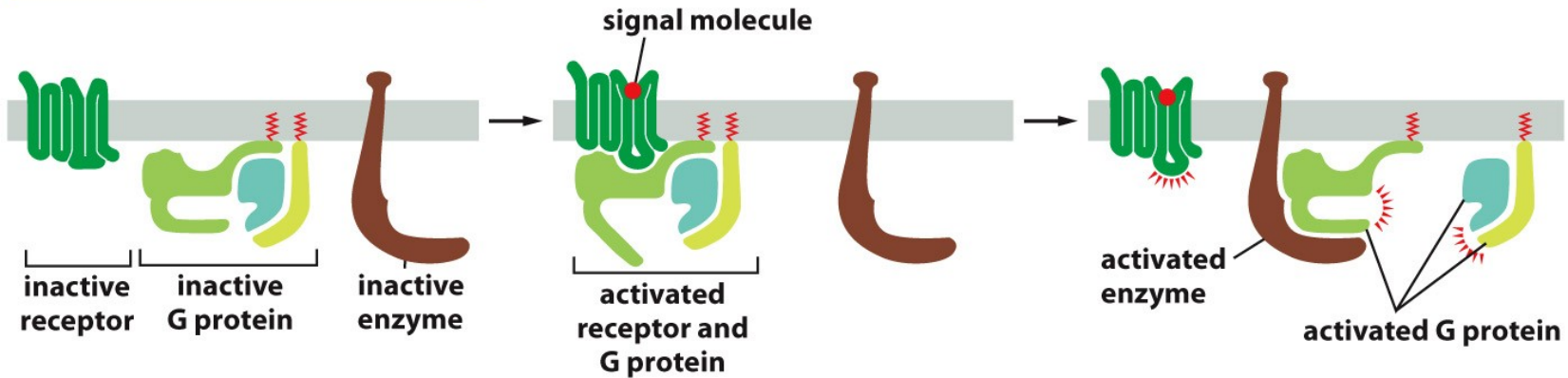


Figure 15-6b Molecular Biology of the Cell 6e (© Garland Science 2015)

Základní typy membránových receptorů

➤ Receptory spřažené s enzymy/enzymatickou funkcí

ENZYME-COUPLED RECEPTORS

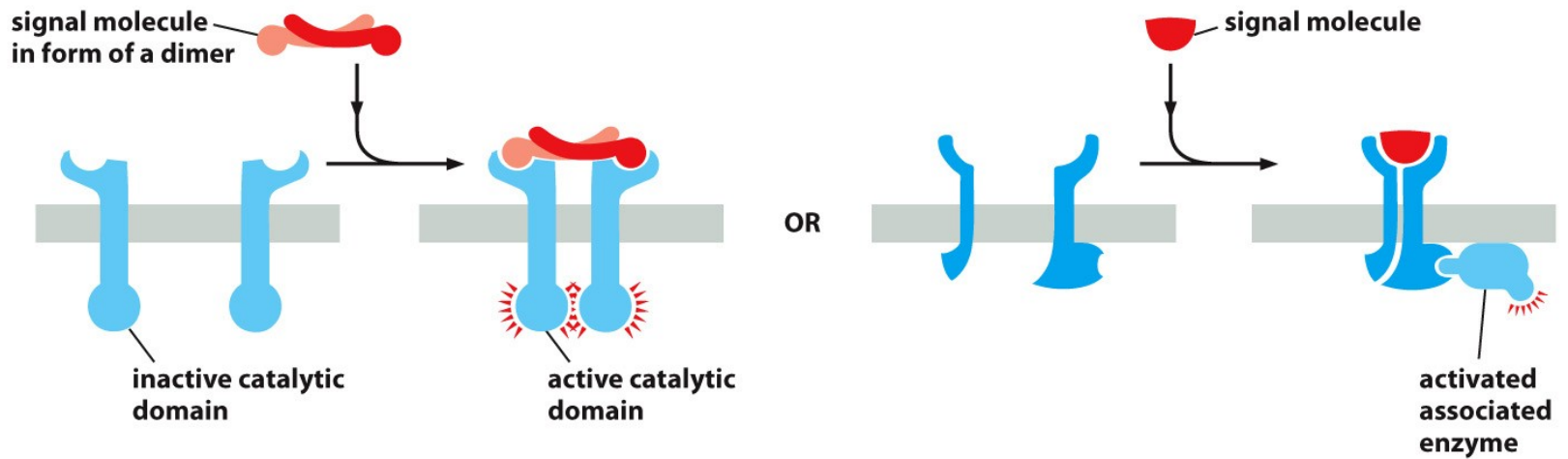


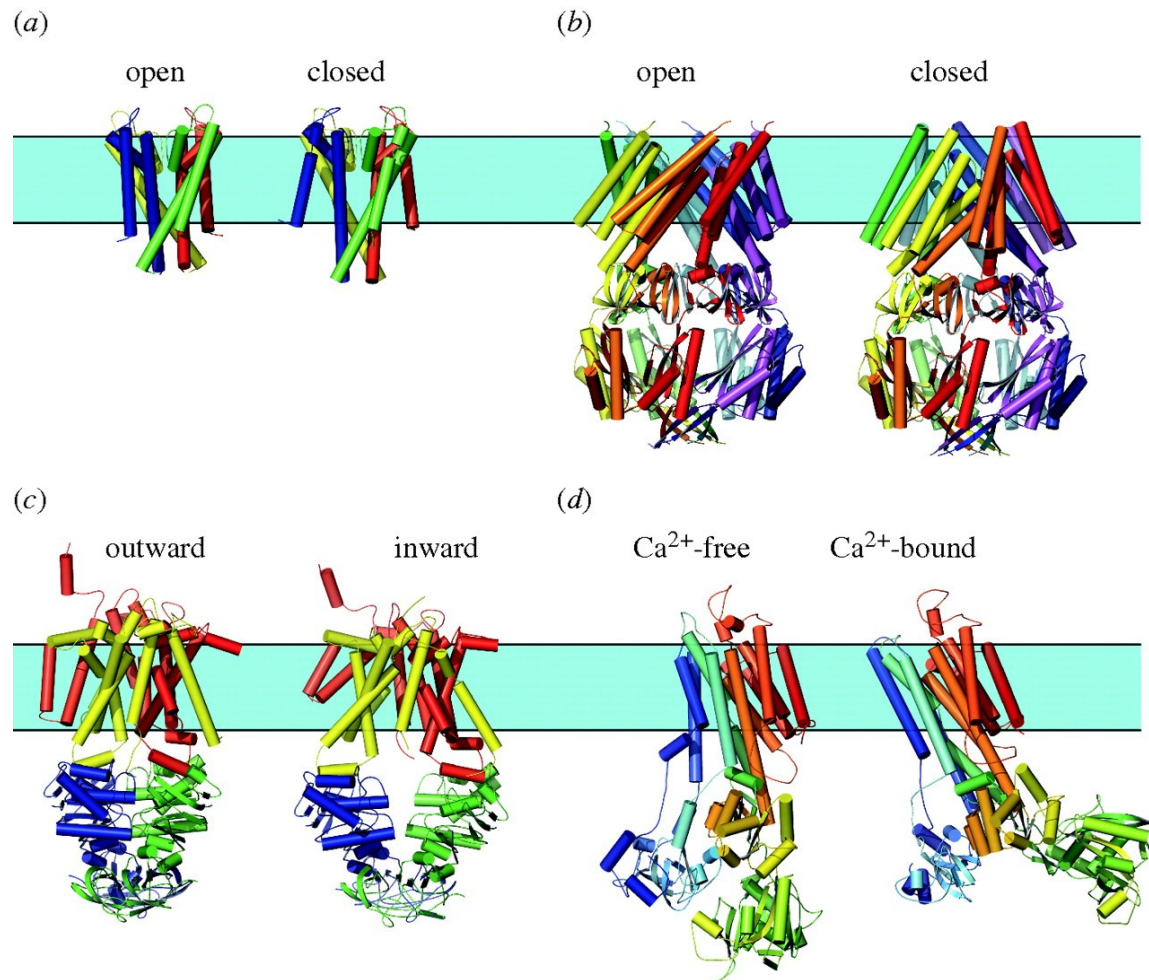
Figure 15-6c Molecular Biology of the Cell 6e (© Garland Science 2015)

Základní biochemické mechanismy buněčné signalizace

- 1. Změna proteinové konformace
- 2. Fosforylace – kovalentní přidání anorganického fosfátu (z ATP) na molekulu proteinu enzymem, který se jmenuje kináza
- 3. Nekovalentní záměna GDP za GTP

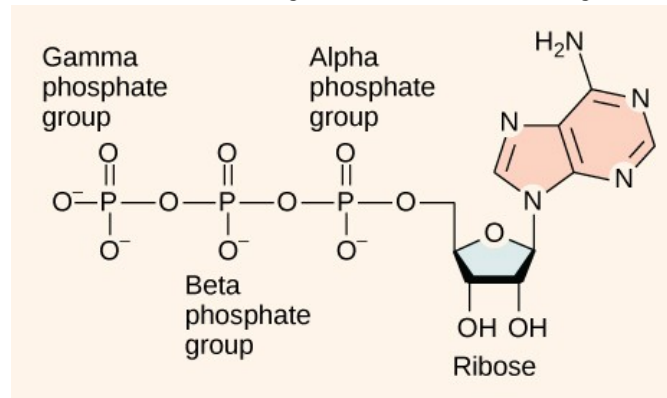
Základní biochemické mechanismy buněčné signalizace

➤ 1. Změna proteinové konformace

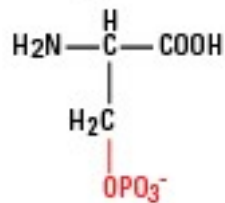


Základní biochemické mechanismy buněčné signalizace

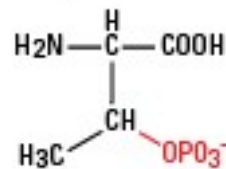
- 2. Fosforylace – kovalentní přidání anorganického fosfátu (z ATP) na molekulu tyrosinu nebo serinu/threoninu enzymem, který se jmenuje kináza



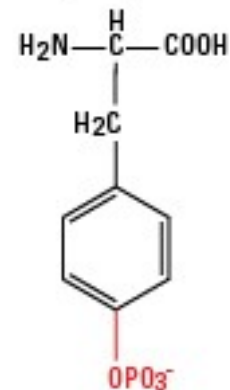
Phosphoserine



Phosphothreonine

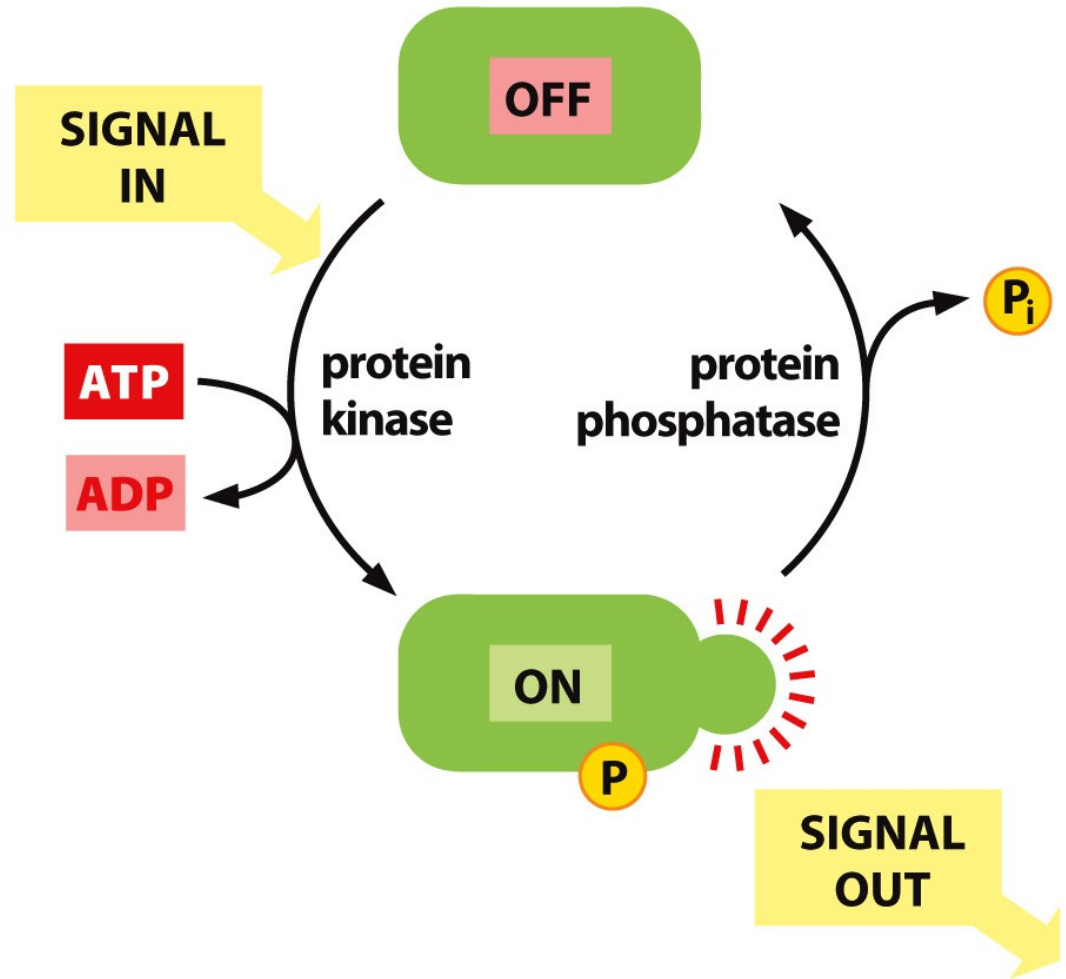


Phosphotyrosine



Základní mechanismy buněčné signalizace

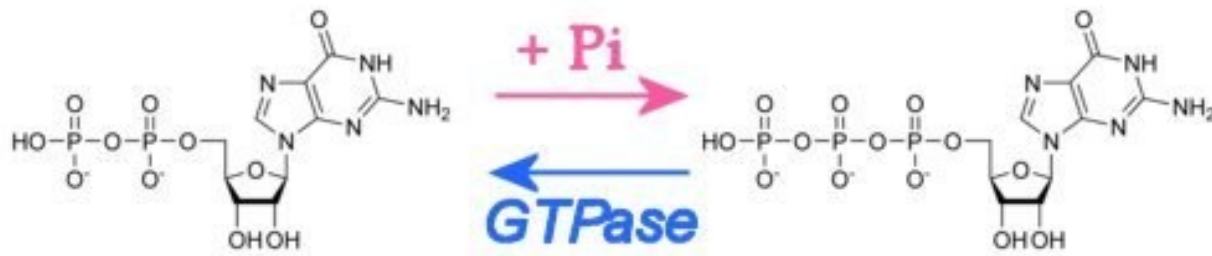
SIGNALIZACE



SIGNALING BY PHOSPHORYLATION

Základní biochemické mechanismy buněčné signalizace

- ▶ 3. Nekovalentní záměna GDP za GTP



Základní mechanismy buněčné signalizace

SIGNALIZACE

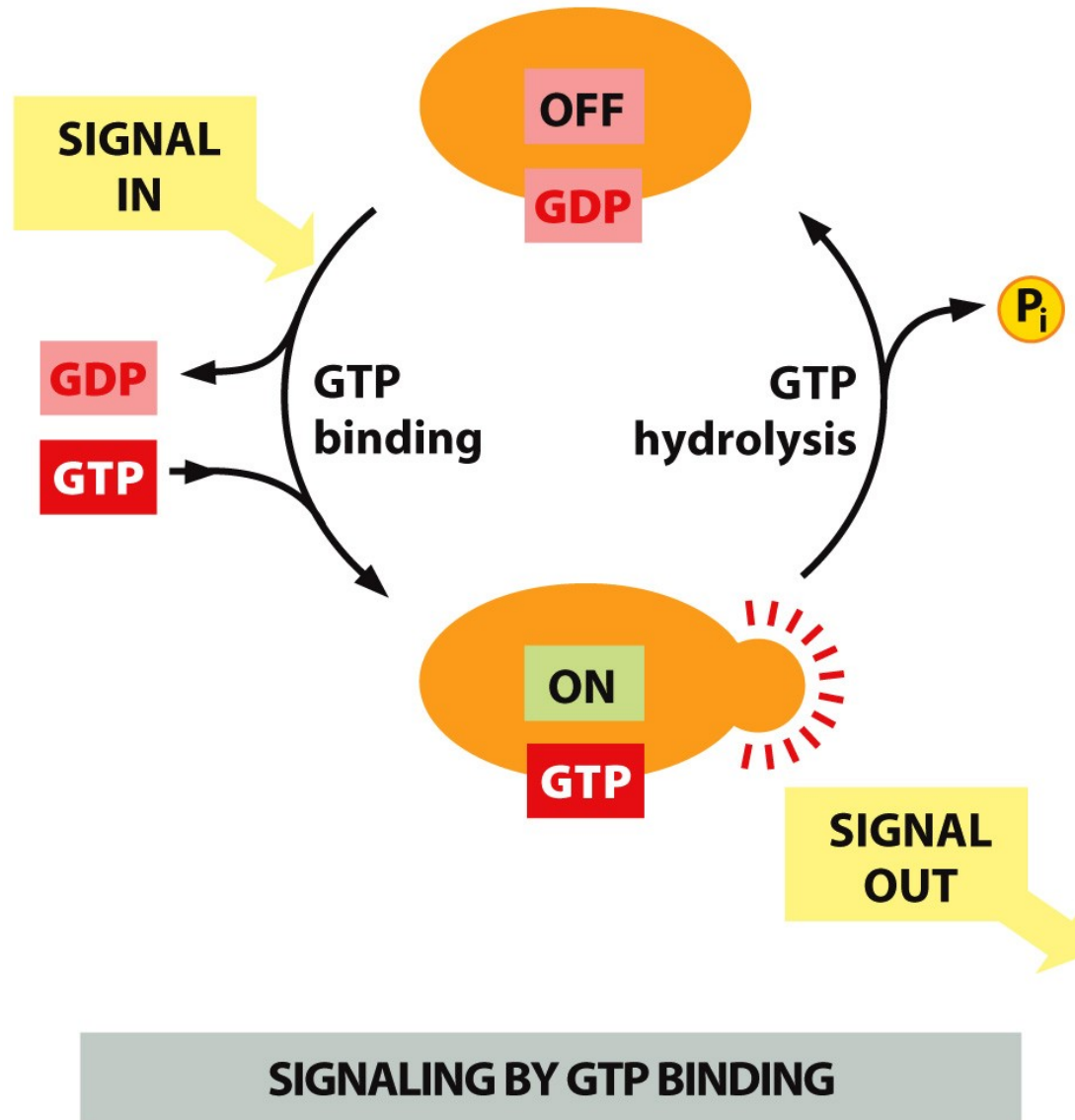


Figure 15-7b Molecular Biology of the Cell 6e (© Garland Science 2015)

Základní mechanismy buněčné signalizace

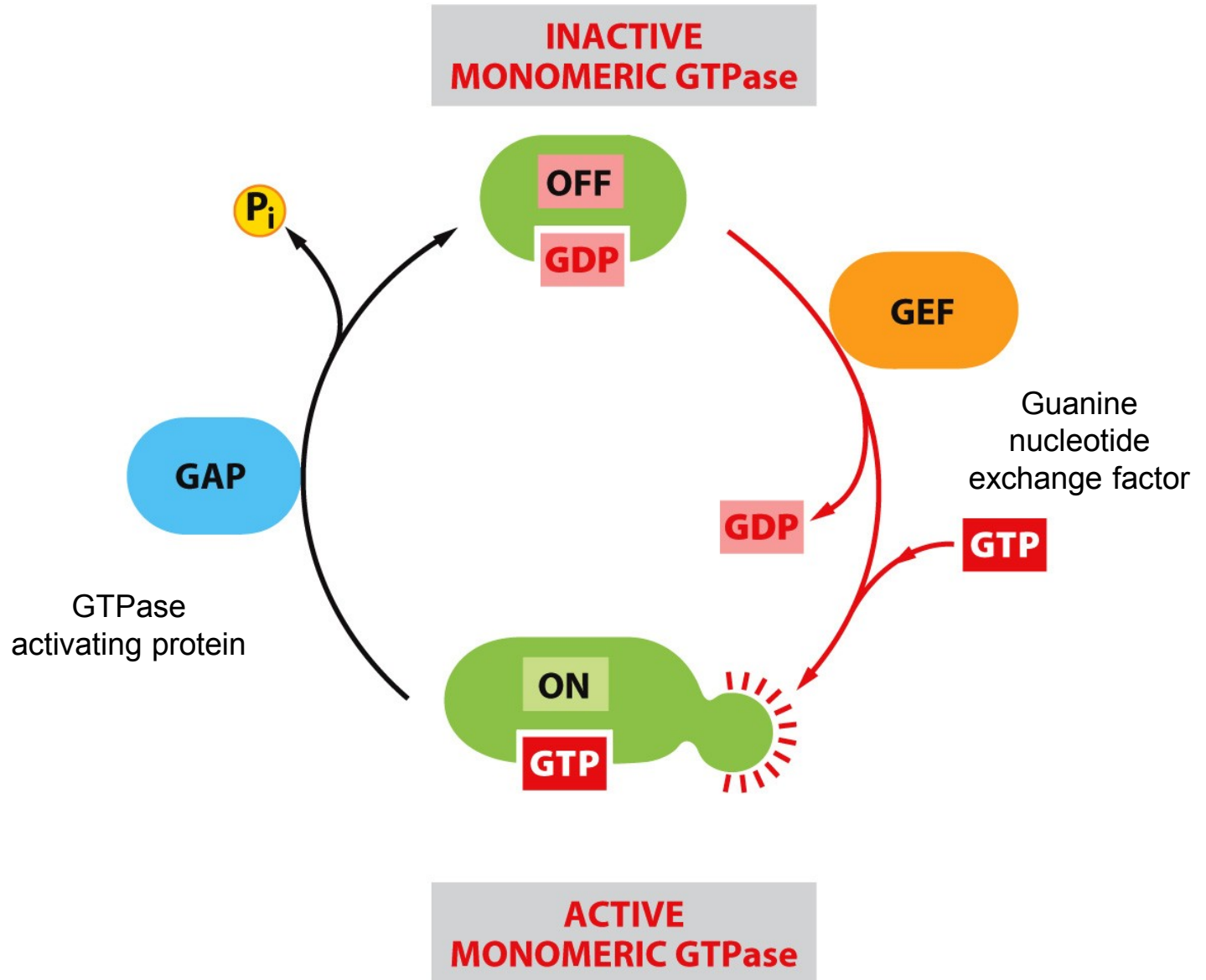


Figure 15-8 Molecular Biology of the Cell 6e (© Garland Science 2015)

Signální molekuly často tvoří velké uspořádané komplexy, které organizují efektivní přenos signálu

- Na cytoplazmatických „lešeních“ (scaffolding proteinech)
- Na receptorech
- Na membránových doménách

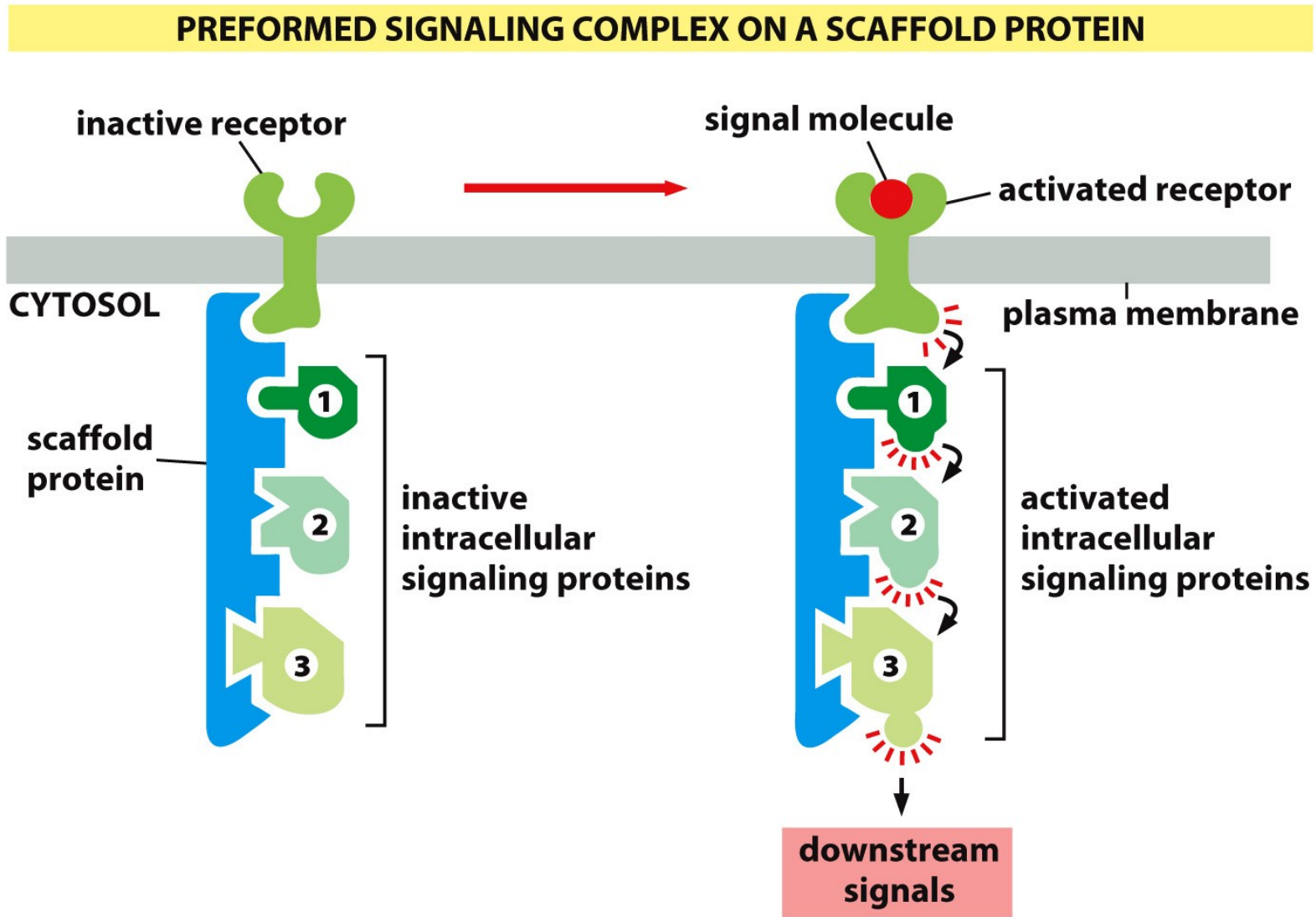


Figure 15-10a Molecular Biology of the Cell 6e (© Garland Science 2015)

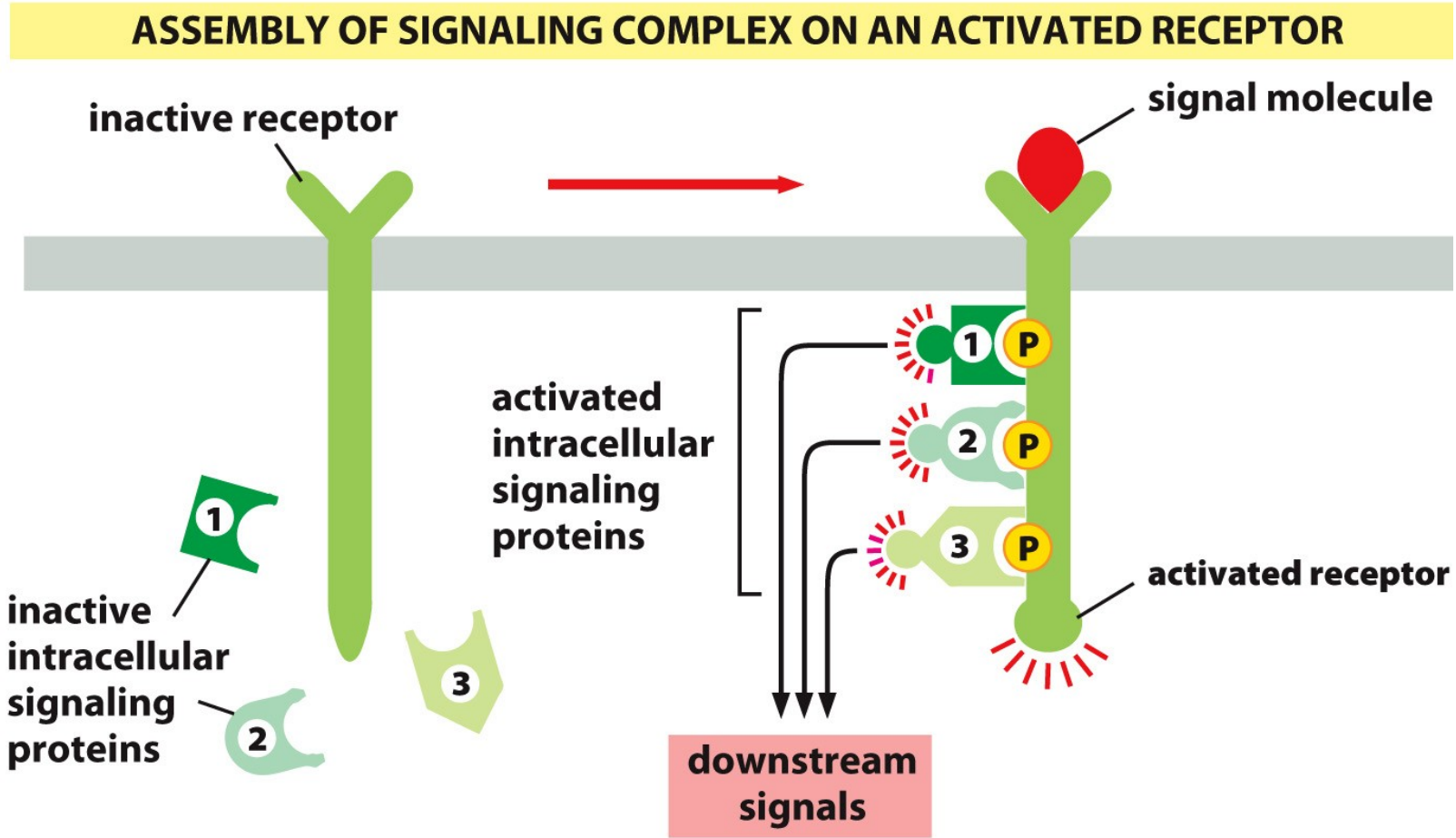


Figure 15-10b Molecular Biology of the Cell 6e (© Garland Science 2015)

ASSEMBLY OF SIGNALING COMPLEX ON PHOSPHOINOSITIDE DOCKING SITES

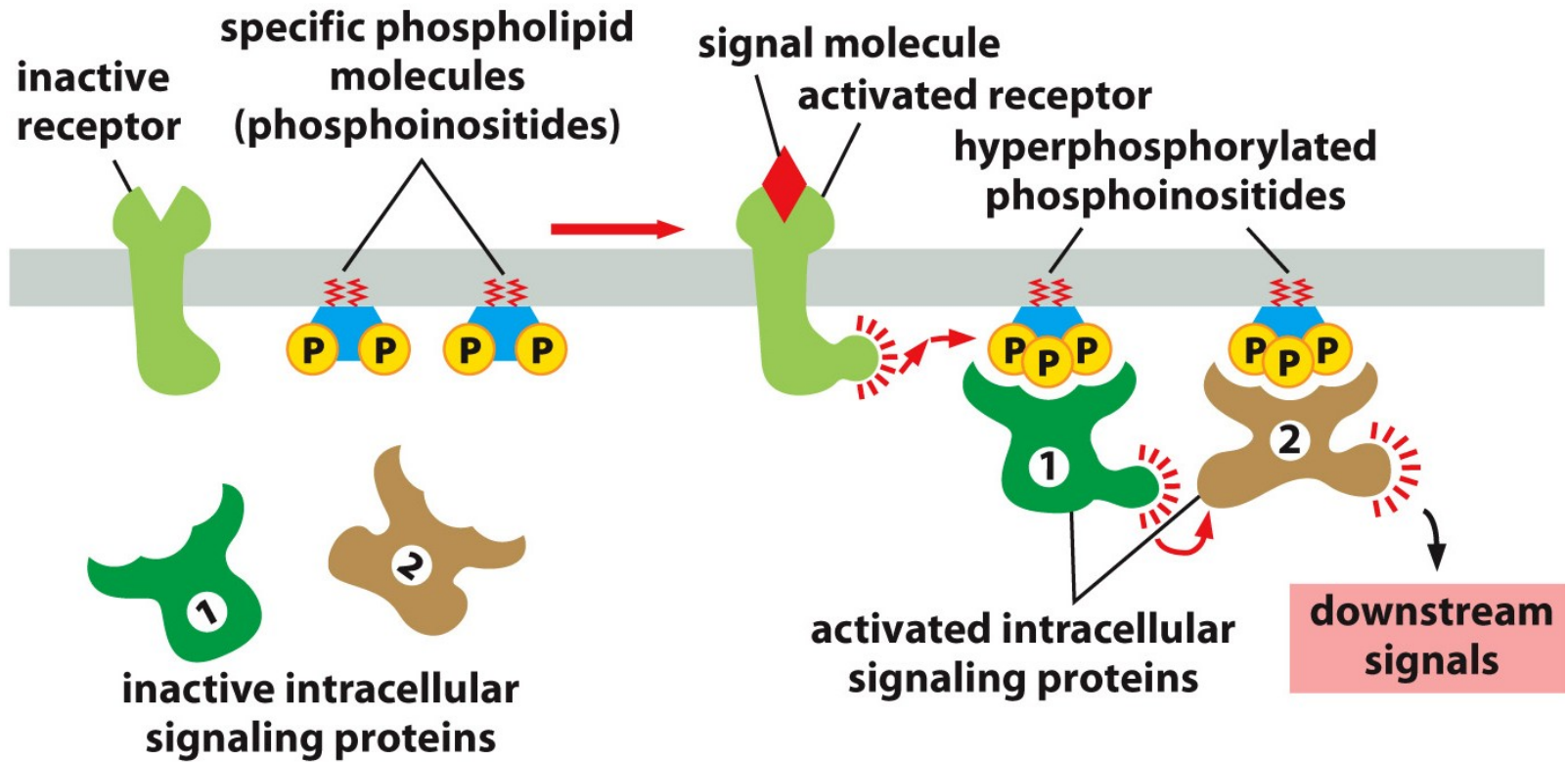


Figure 15-10c Molecular Biology of the Cell 6e (© Garland Science 2015)

Proteinové domény a organizace signálních komplexů

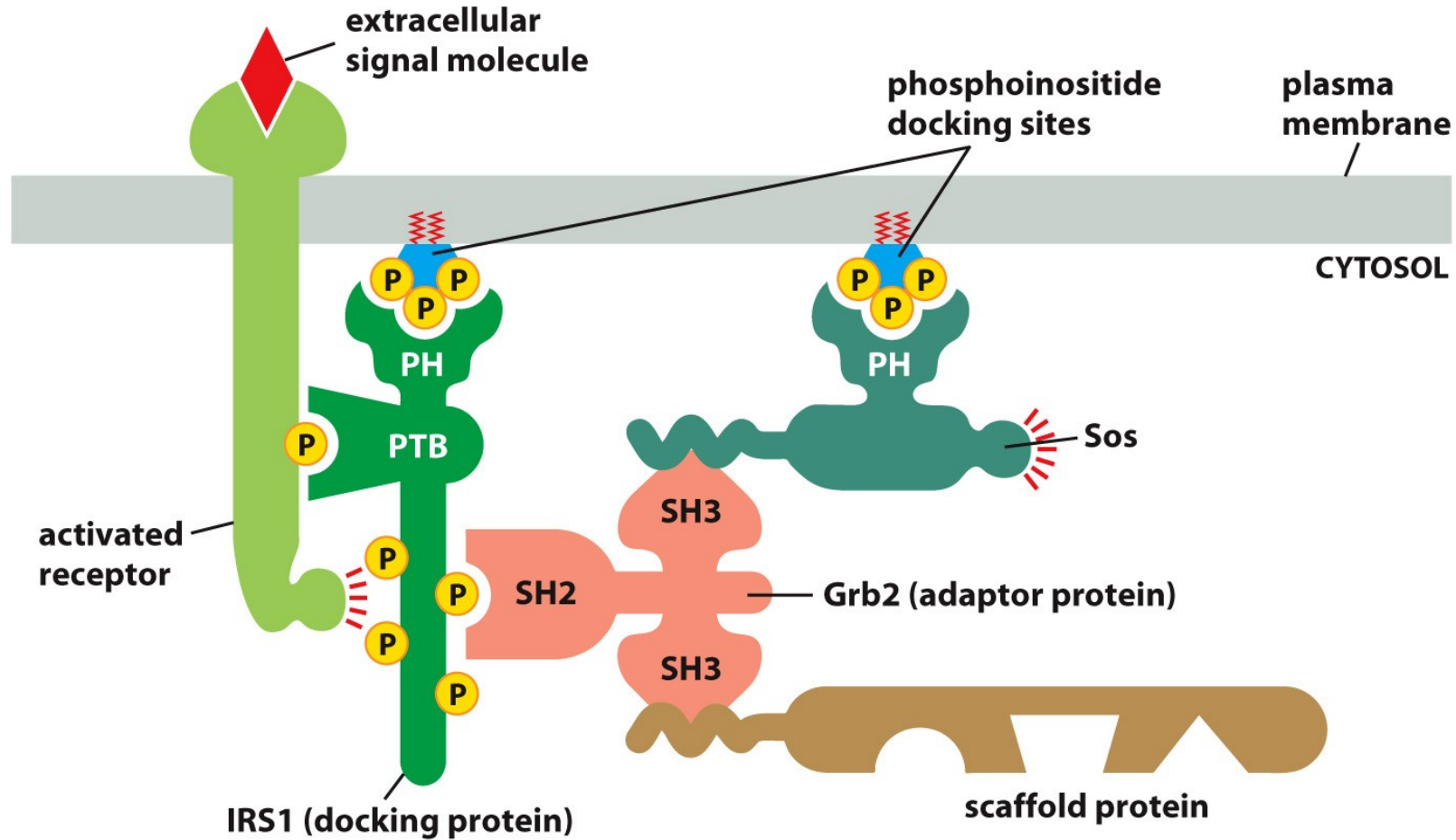


Figure 15-11 Molecular Biology of the Cell 6e (© Garland Science 2015)

Jednotlivé signální dráhy interagují a koordinují se

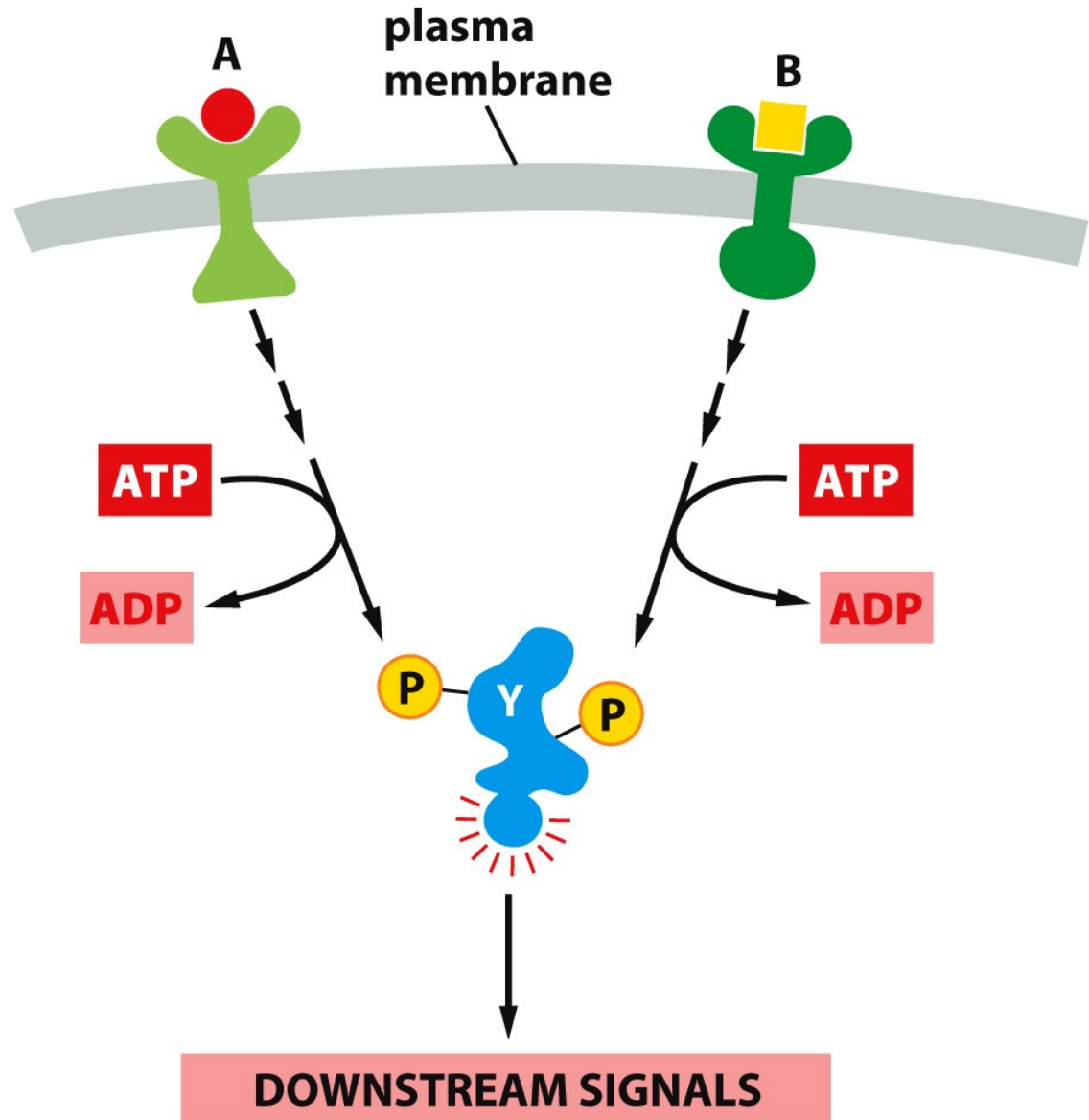


Figure 15-12 Molecular Biology of the Cell 6e (© Garland Science 2015)

Různé signální kaskády probíhají různou rychlostí

SIGNALIZACE

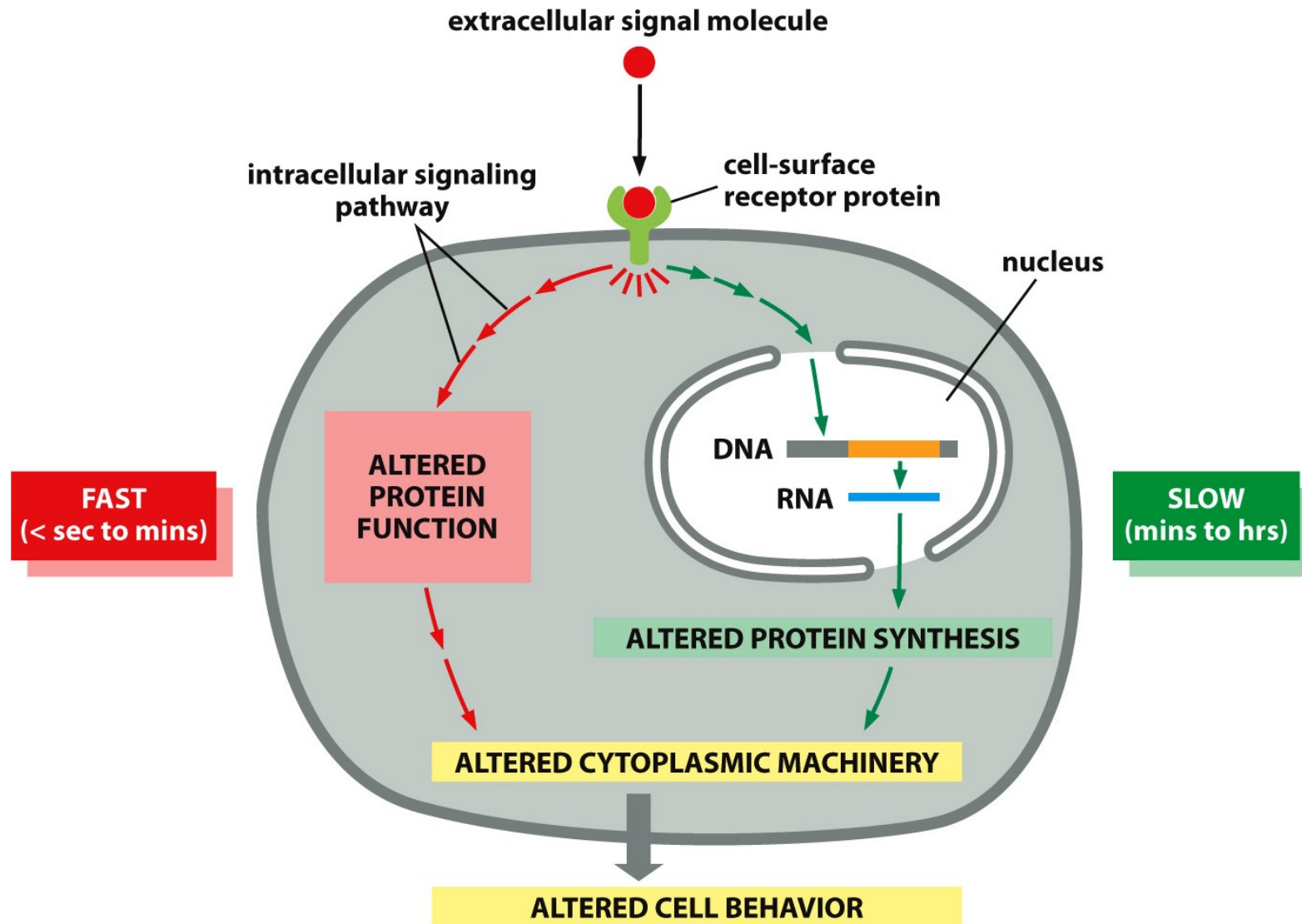


Figure 15-13 Molecular Biology of the Cell 6e (© Garland Science 2015)

Pozitivní a negativní zpětná vazba

- ▶ Jsou dva základní principy, které regulují intenzitu a délku trvání signálu

Pozitivní a negativní zpětná vazba

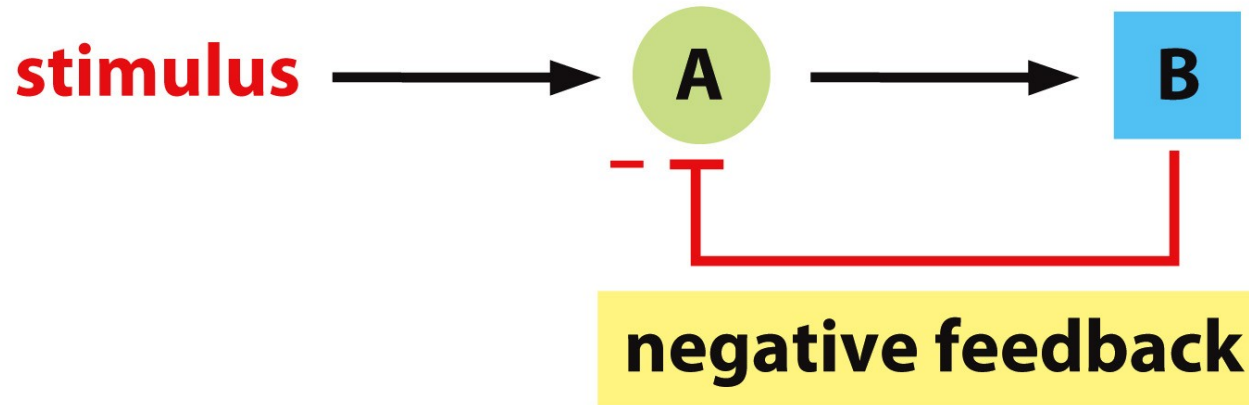
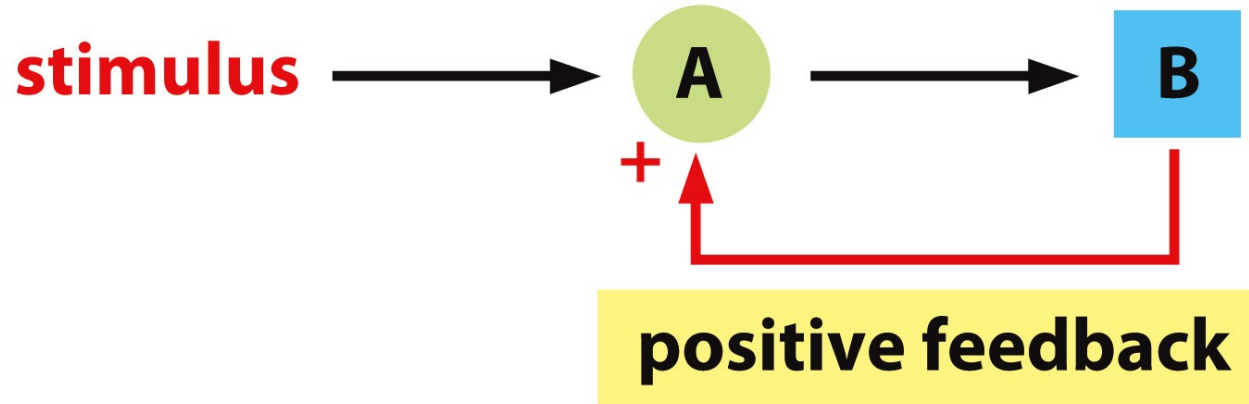


Figure 15-17 Molecular Biology of the Cell 6e (© Garland Science 2015)

POSITIVE FEEDBACK

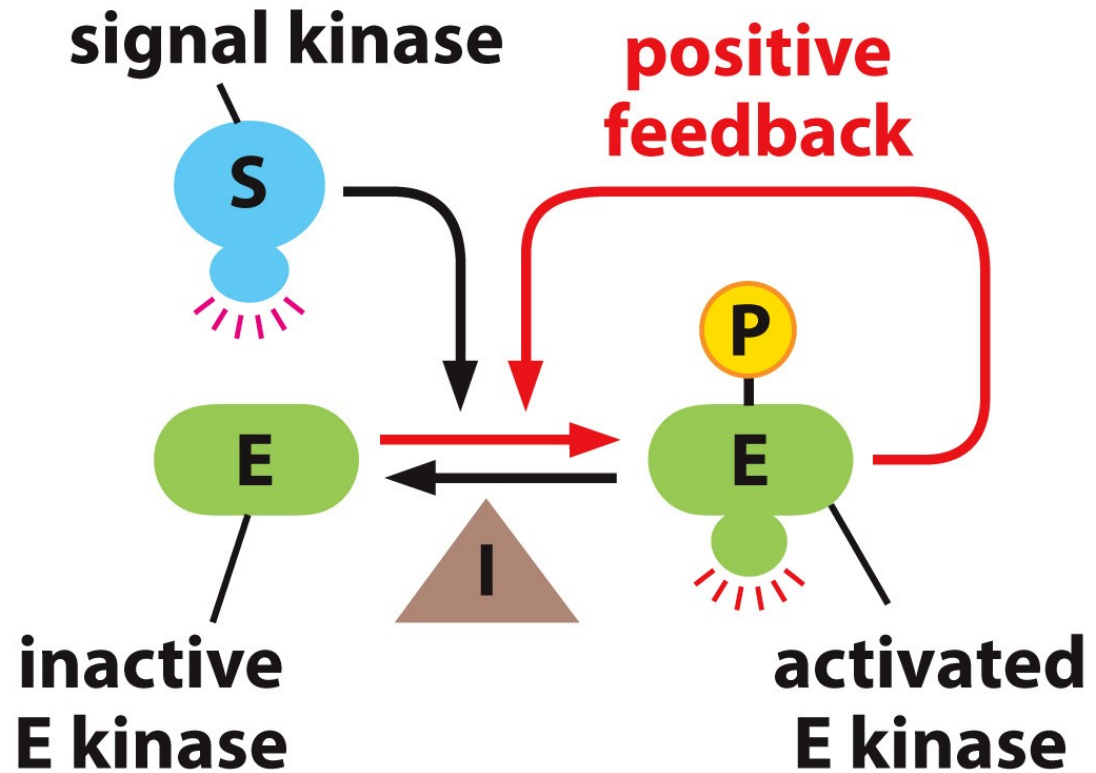


Figure 15-18a Molecular Biology of the Cell 6e (© Garland Science 2015)

Pozitivní a negativní zpětná vazba

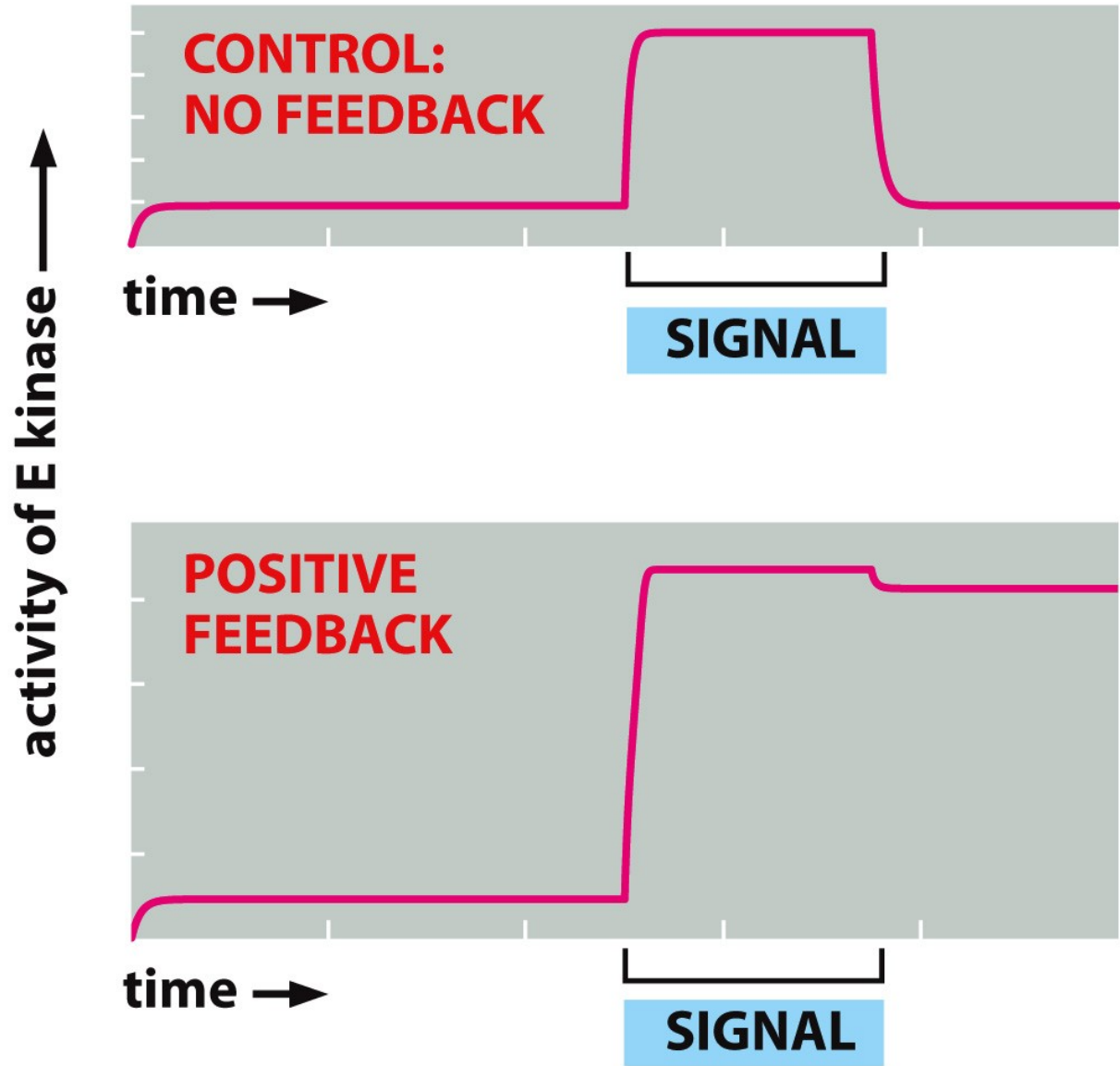


Figure 15-18b Molecular Biology of the Cell 6e (© Garland Science 2015)

NEGATIVE FEEDBACK

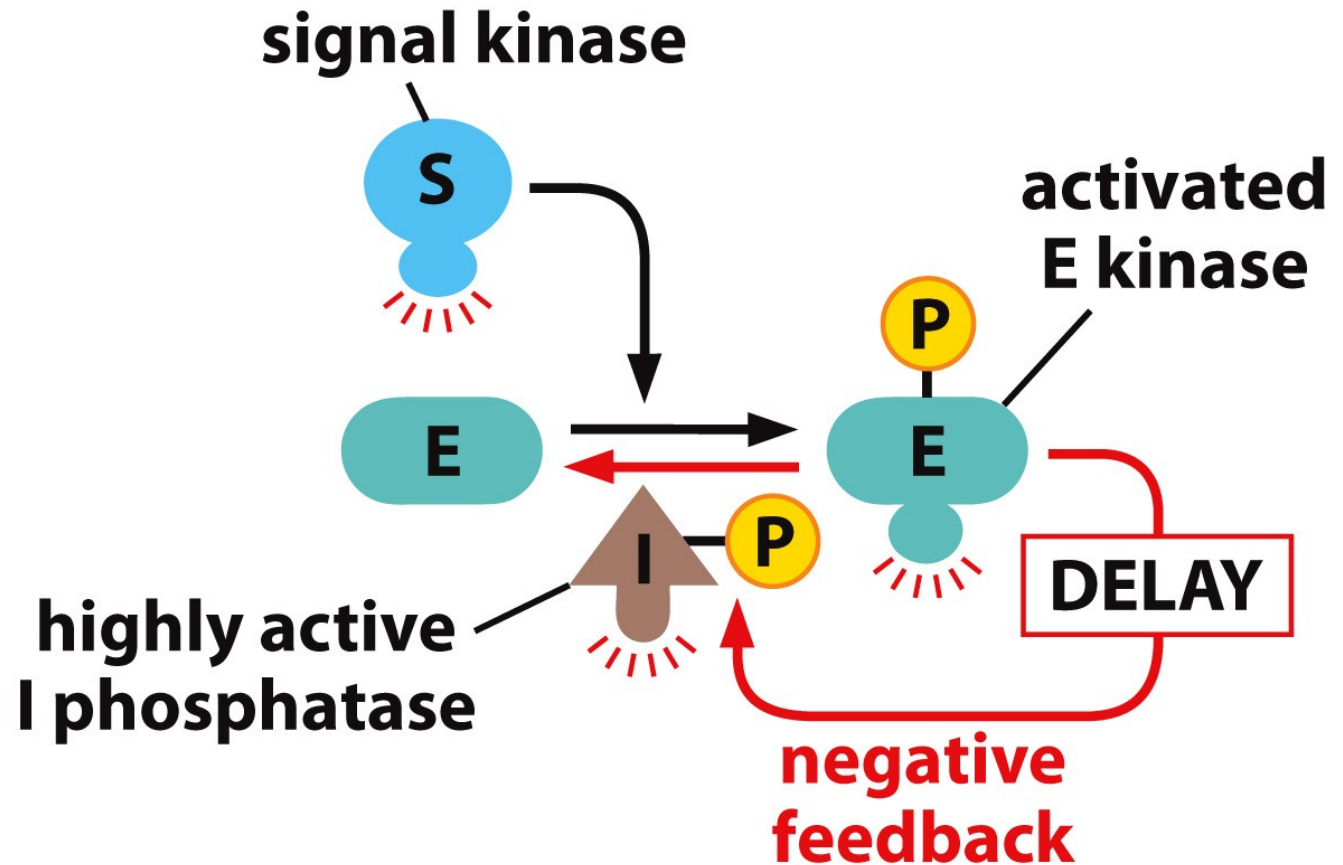


Figure 15-18c Molecular Biology of the Cell 6e (© Garland Science 2015)

Pozitivní a negativní zpětná vazba

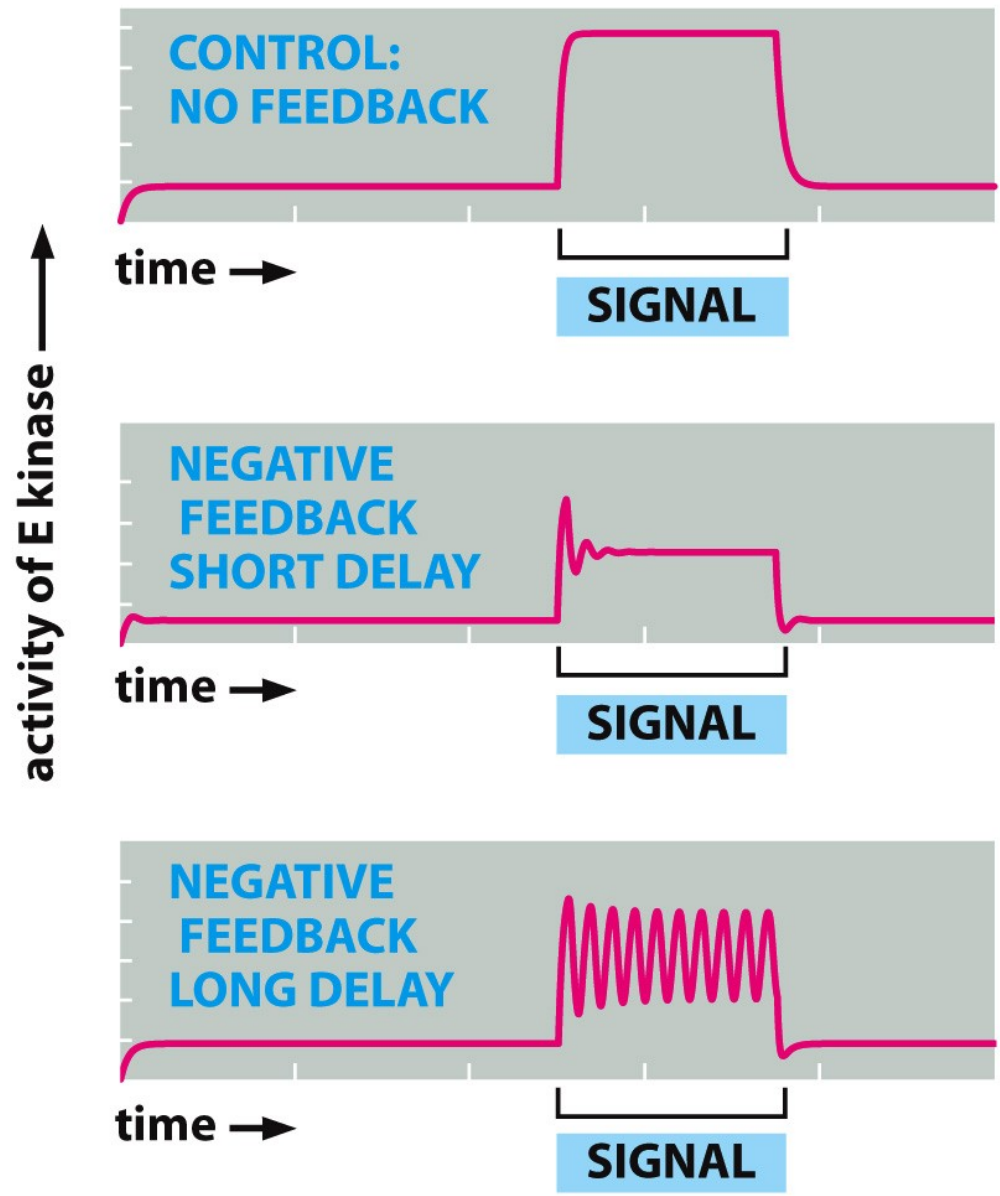
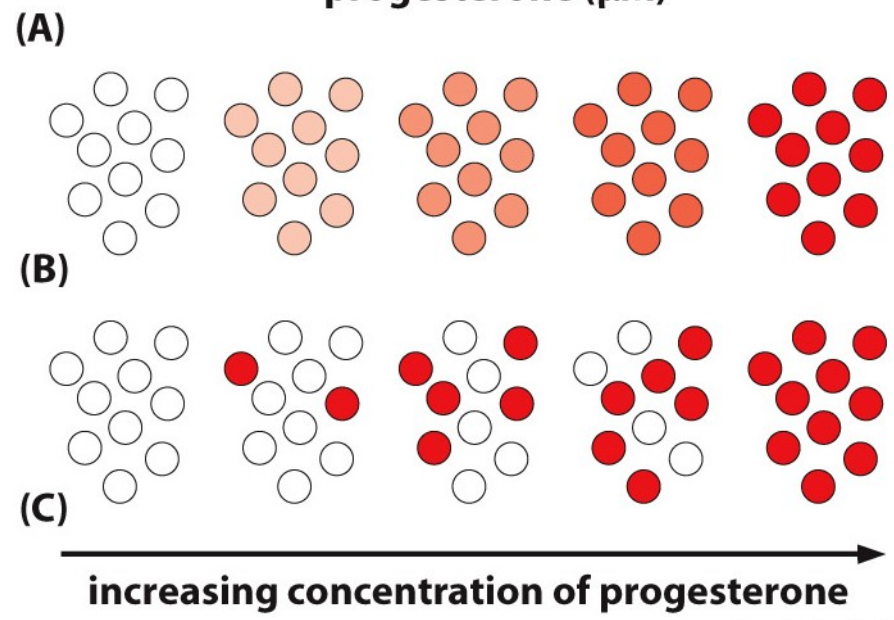
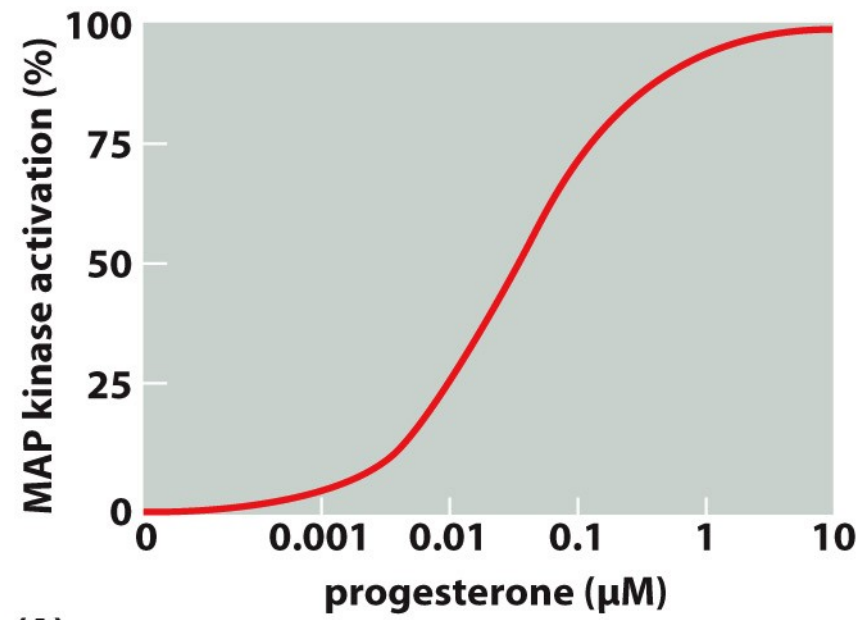


Figure 15-18d Molecular Biology of the Cell 6e (© Garland Science 2015)

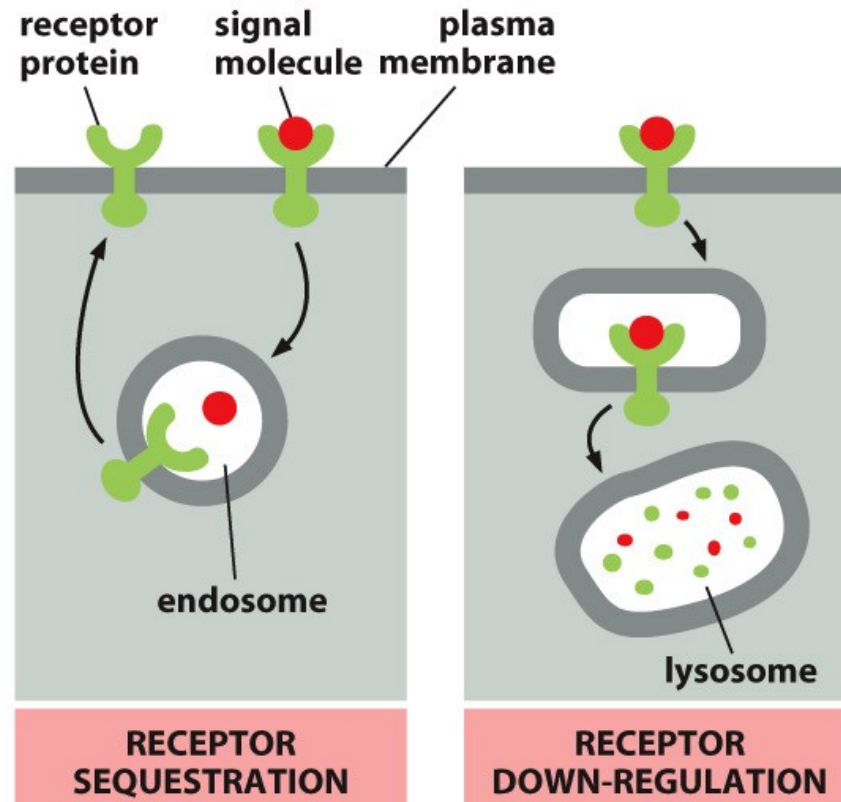
Pozitivní a negativní zpětná vazba



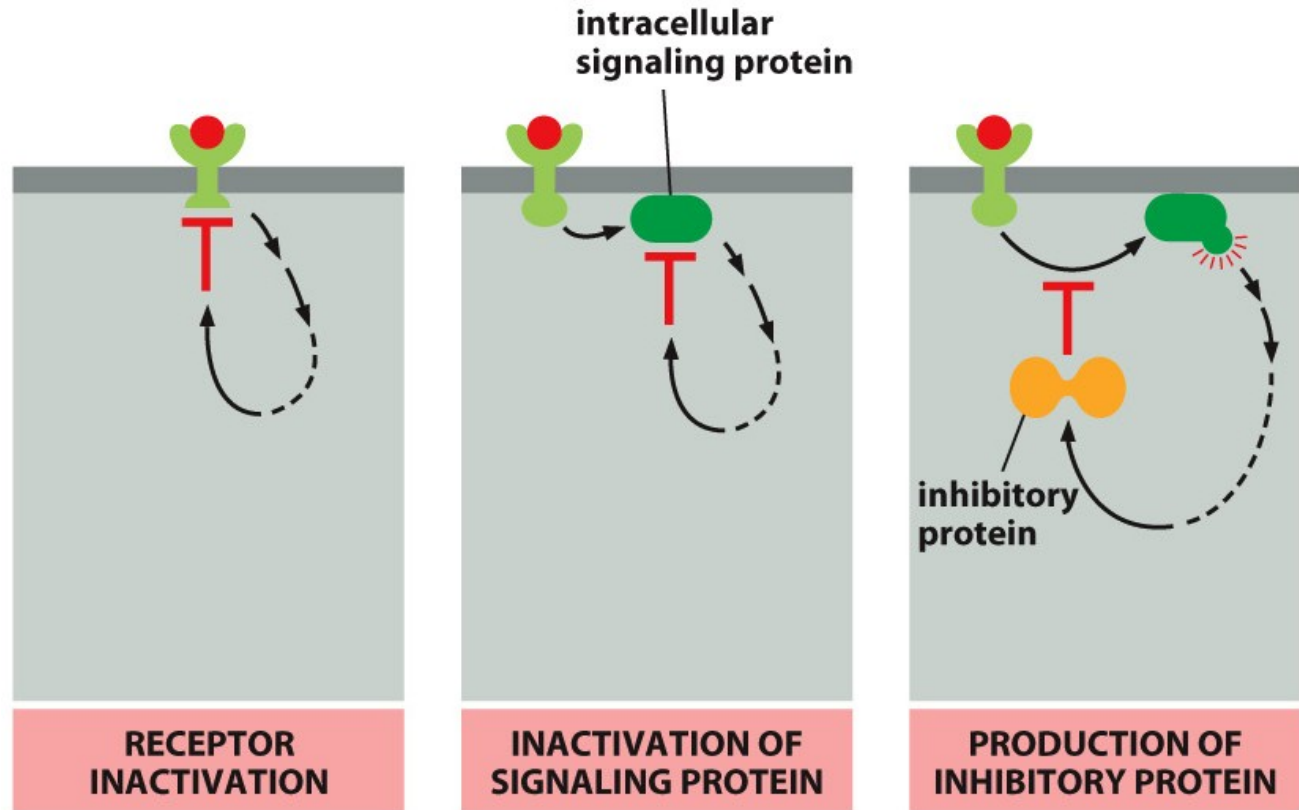
Mechanismy negativní zpětné vazby

- Internalizace receptoru
- Degradace receptoru
- Inaktivace receptoru
- Inaktivace signálního proteinu
- Produkce inhibitoru
- Aj.

Mechanismy negativní zpětné vazby



Mechanismy negativní zpětné vazby



Druhý posel

molecule	measured context	diffusion coefficient ($\mu\text{m}^2/\text{s}$)	BNID
H ₂ O	water	2000	104087, 106703
H ₂ O	nucleus of chicken erythrocyte	200	104645
H ⁺ (from H ₃ O ⁺ to H ₂ O)	water	7000	106702
O ₂	water	2000	104440
CO ₂	water	2000	102625
tRNA (≈ 20 kDa)	water	100	107933, 107935
protein (≈ 30 kDa GFP)	water	100	100301
protein (≈ 30 kDa GFP)	eukaryotic cell (CHO) cytoplasm	30	101997
protein (≈ 30 kDa GFP)	rat liver mitochondria	30	100300
protein (NLS-EGFP)	cytoplasm of <i>D. melanogaster</i> embryo	20	109209
protein (≈ 30 kDa)	<i>E. coli</i> cytoplasm	7–8	100193, 107985
protein (≈ 40 kDa)	<i>E. coli</i> cytoplasm	2–4	107985
protein (≈ 70 –250 kDa)	<i>E. coli</i> cytoplasm	0.4–2	107985
protein (≈ 140 kDa Tar-YFP)	<i>E. coli</i> membrane	0.2	107985
protein (≈ 70 kDa LacY-YFP)	<i>E. coli</i> membrane	0.03	107985
fluorescent dye (carboxy-fluorescein)	<i>A. thaliana</i> cell wall	30	105033
fluorescent dye (carboxy-fluorescein)	<i>A. thaliana</i> mature root epidermis	3	105034
transcription factor (LacI)	movement along DNA (1D, <i>in vitro</i>)	0.04 ($4 \times 10^5 \text{ bp}^2\text{s}^{-1}$)	102036
morphogen (bicoid-GFP)	cytoplasm of <i>D. melanogaster</i> embryo	7	109199
morphogen (wingless)	wing imaginal disk of <i>D. melanogaster</i>	0.05	101072
mRNA	HeLa nucleus	0.03–0.10	107613
mRNA	various localizations and sizes	0.005–1	110667
ribosome	<i>E. coli</i>	0.04	108596

Table 4-1 A compilation of empirical diffusion constants that shows the dependence on size and cellular context.

Druhý posel

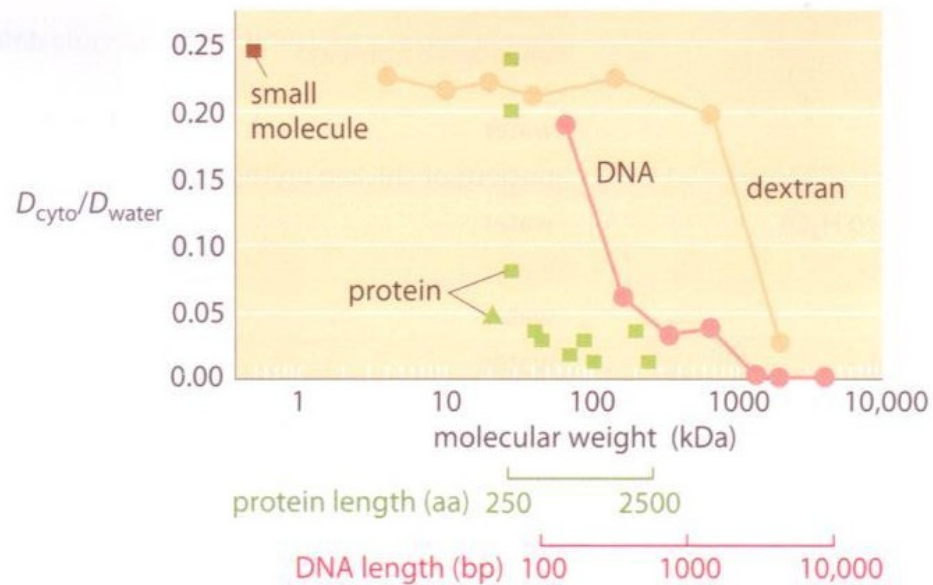


Figure 4-2 The decrease in the diffusion constant in the cytoplasm with respect to water as molecular weight increases. (Adapted from Verkman AS [2002] *Trends Biochem Sci* 27:27–33.)

Druhý posel

- ▶ Malé molekuly, které jsou produkovány enzymatickými reakcemi nebo uvolňovány iontovými kanály
- ▶ Slouží k amplifikaci signálu
- ▶ Jejich produkce i destrukce jsou lokalizovány, což umožňuje jejich kontrolu v čase a prostoru

Příklady nejznámějších signálních kaskád: Mechanismy přenosu signálu a regulace

	cAMP System	Phosphoinositol system	cGMP System	Tyrosine kinase system
First Messenger (signální molekula)	ACTH, ANP, CRH, CT, FSH, Glucagon, hCG, LH, MSH, PTH, TSH	AGT, GnRH, GHRH, Oxytocin, TRH	ANP, Nitric oxide	INS, IGF, PDGF
Signal Transducer	GPCR	GPCR	-	RTK
Primary effector	Adenylyl cyclase	Phospholipase C	guanylate cyclase	RasGEF (Grb2-Sos)
Second messenger (druhý posel)	cAMP (cyclic adenosine monophosphate)	IP3; DAG; Ca²⁺	cGMP	Ras.GTP (Small G Protein)
Secondary effector	protein kinase A	protein kinase C; CaM kinase	protein kinase G	MAP3K (c-Raf)

Receptory spřažené s G proteiny (GPCR)

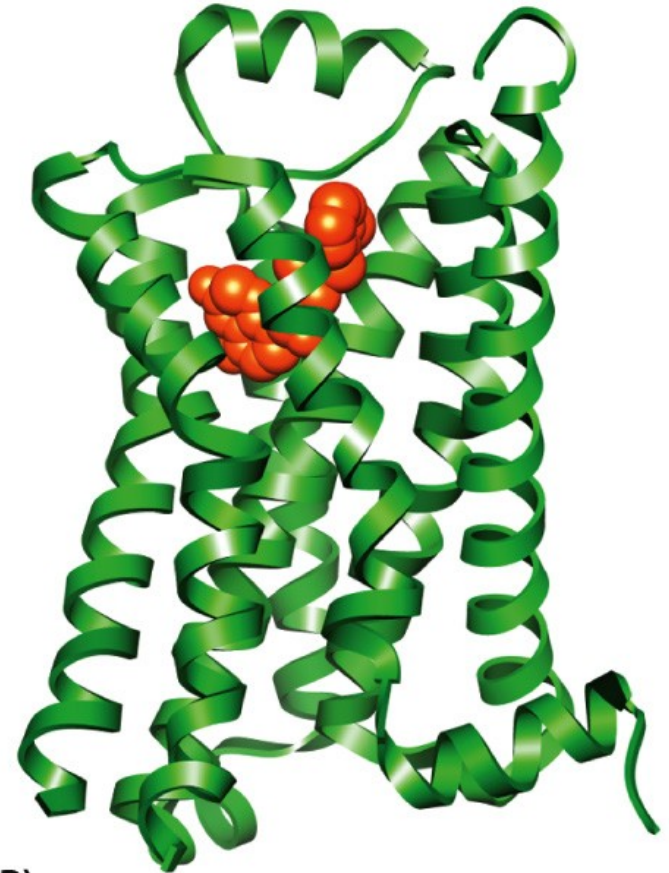
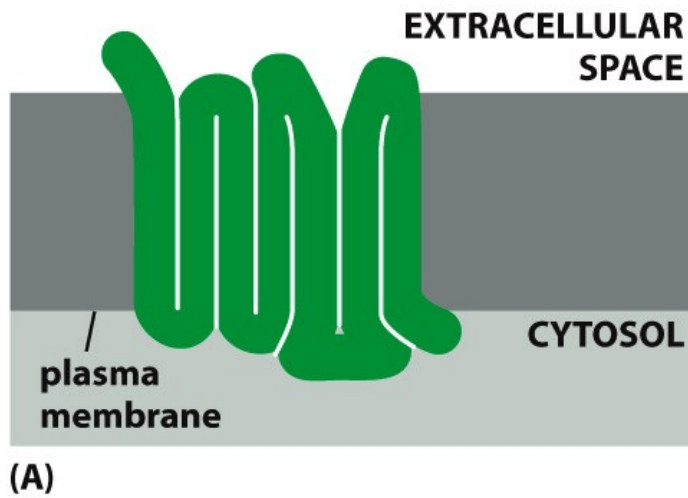
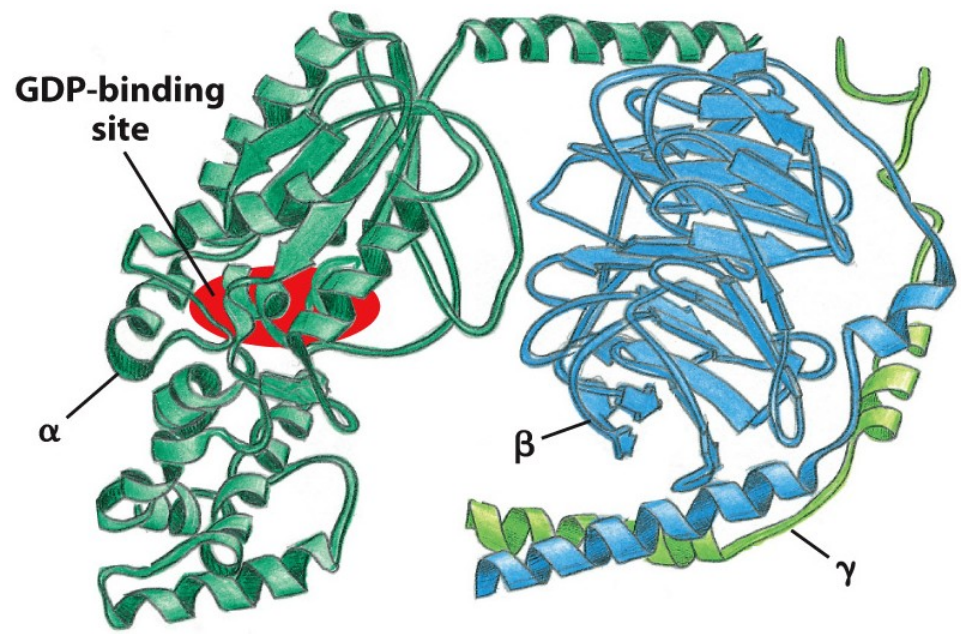
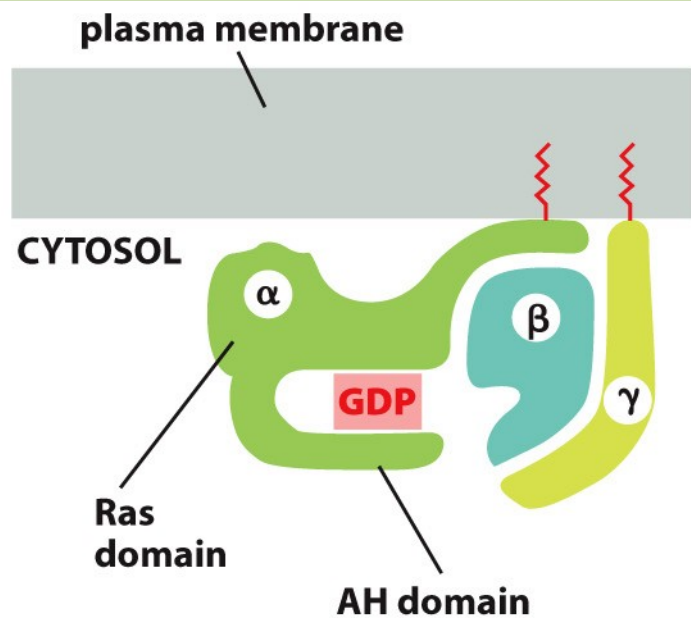


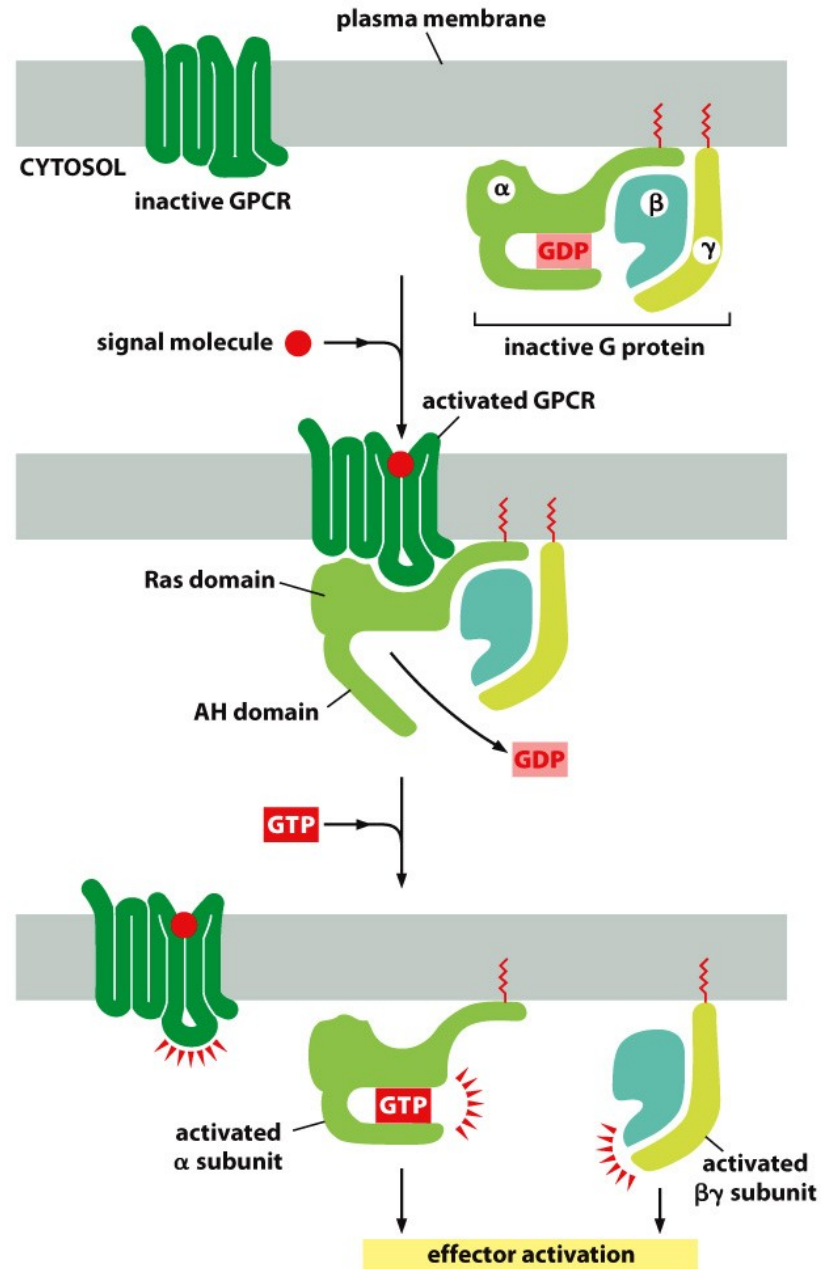
Figure 15-21 Molecular Biology of the Cell 6e (© Garland Science 2015)

Receptory spřažené s G proteiny (GPCR)



Receptory spřažené s G proteiny – trimerické G proteiny

SIGNALIZACE

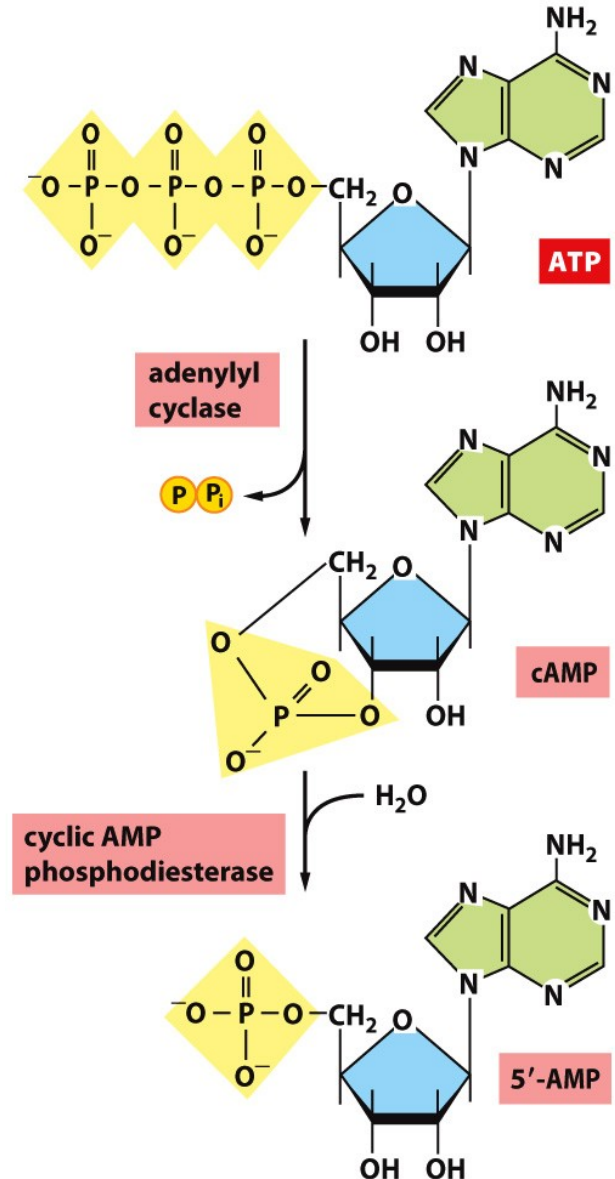


Zajímavost:

If you had to make a wild guess about the target of a certain drug, your best odds are with “G-protein coupled receptor.” Drugs targeting members of this integral membrane protein superfamily, which transmit chemical signals into a wide array of different cell types, represent the core of modern medicine. They account for the majority of best-selling drugs and about 40% of all prescription pharmaceuticals on the market.

Cyklický AMP – adenyl cykláza vs. fosfodiesteráza

cAMP je typický druhý posel
asociovaný s GPCR



Cyklický AMP – aktivuje protein kinázu A (PKA)

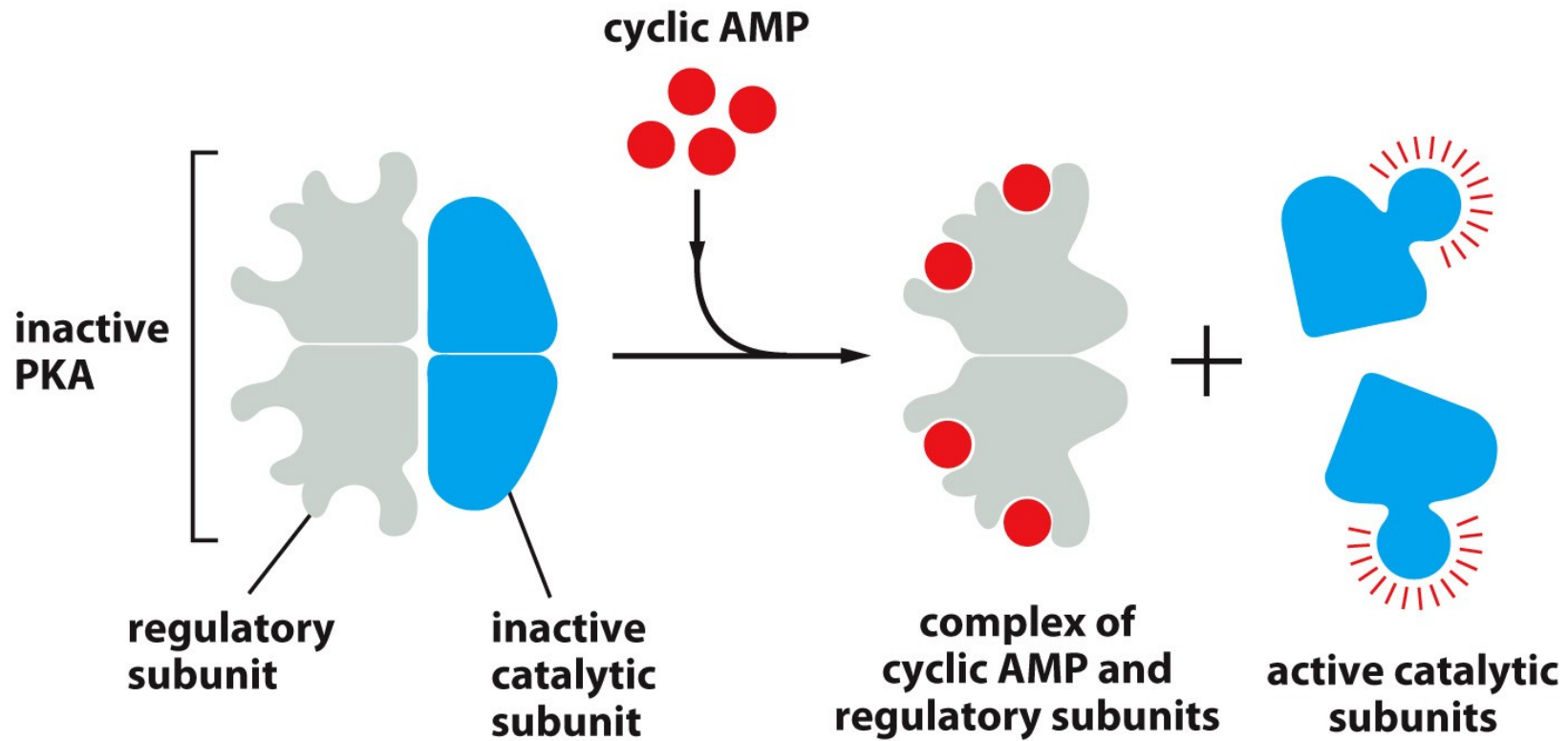


Figure 15-26 Molecular Biology of the Cell 6e (© Garland Science 2015)

SIGNALIZACE

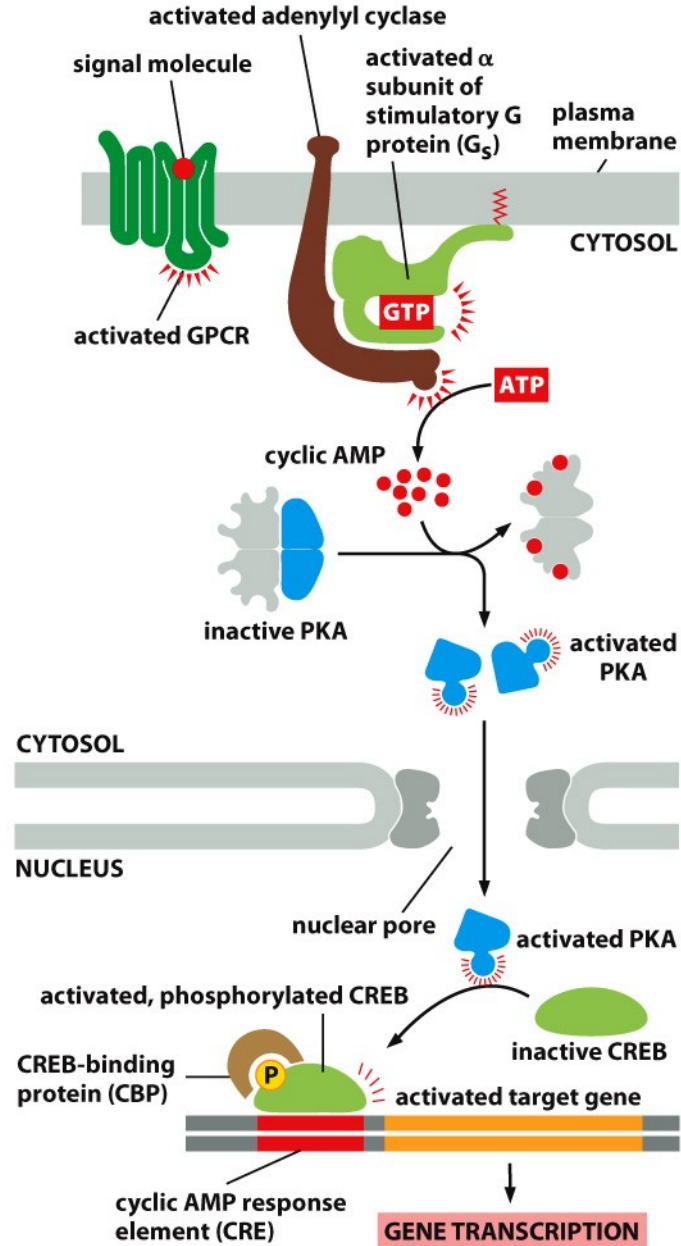


TABLE 15-1 Some Hormone-induced Cell Responses Mediated by Cyclic AMP

Target tissue	Hormone	Major response
Thyroid gland	Thyroid-stimulating hormone (TSH)	Thyroid hormone synthesis and secretion
Adrenal cortex	Adrenocorticotrophic hormone (ACTH)	Cortisol secretion
Ovary	Luteinizing hormone (LH)	Progesterone secretion
Muscle	Adrenaline	Glycogen breakdown
Bone	Parathormone	Bone resorption
Heart	Adrenaline	Increase in heart rate and force of contraction
Liver	Glucagon	Glycogen breakdown
Kidney	Vasopressin	Water resorption
Fat	Adrenaline, ACTH, glucagon, TSH	Triglyceride breakdown

Table 15-1 Molecular Biology of the Cell 6e (© Garland Science 2015)

GPCR aktivují i lipidické druhé posly přes fosfolipázu C

SIGNALIZACE

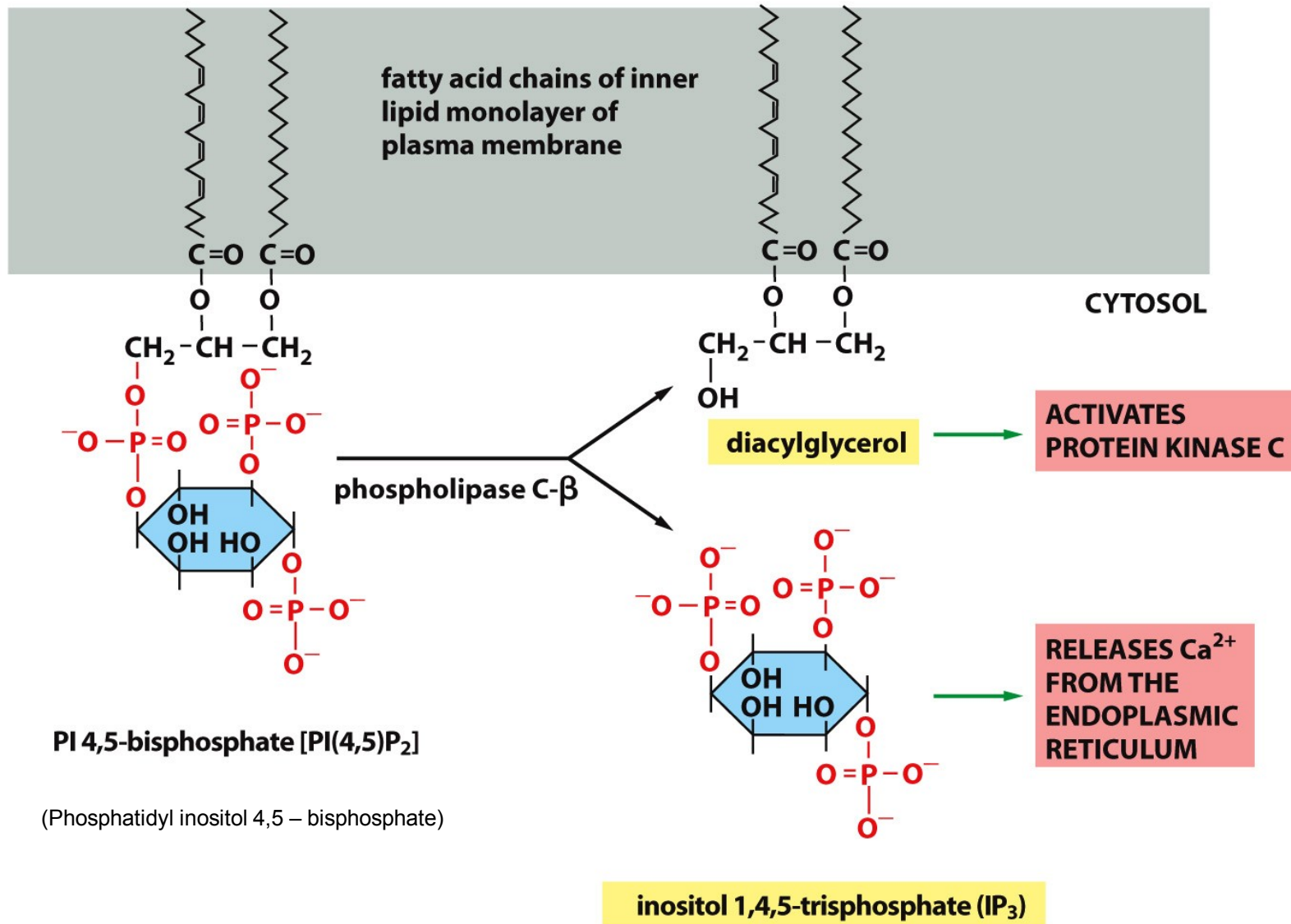


Figure 15-28 Molecular Biology of the Cell 6e (© Garland Science 2015)

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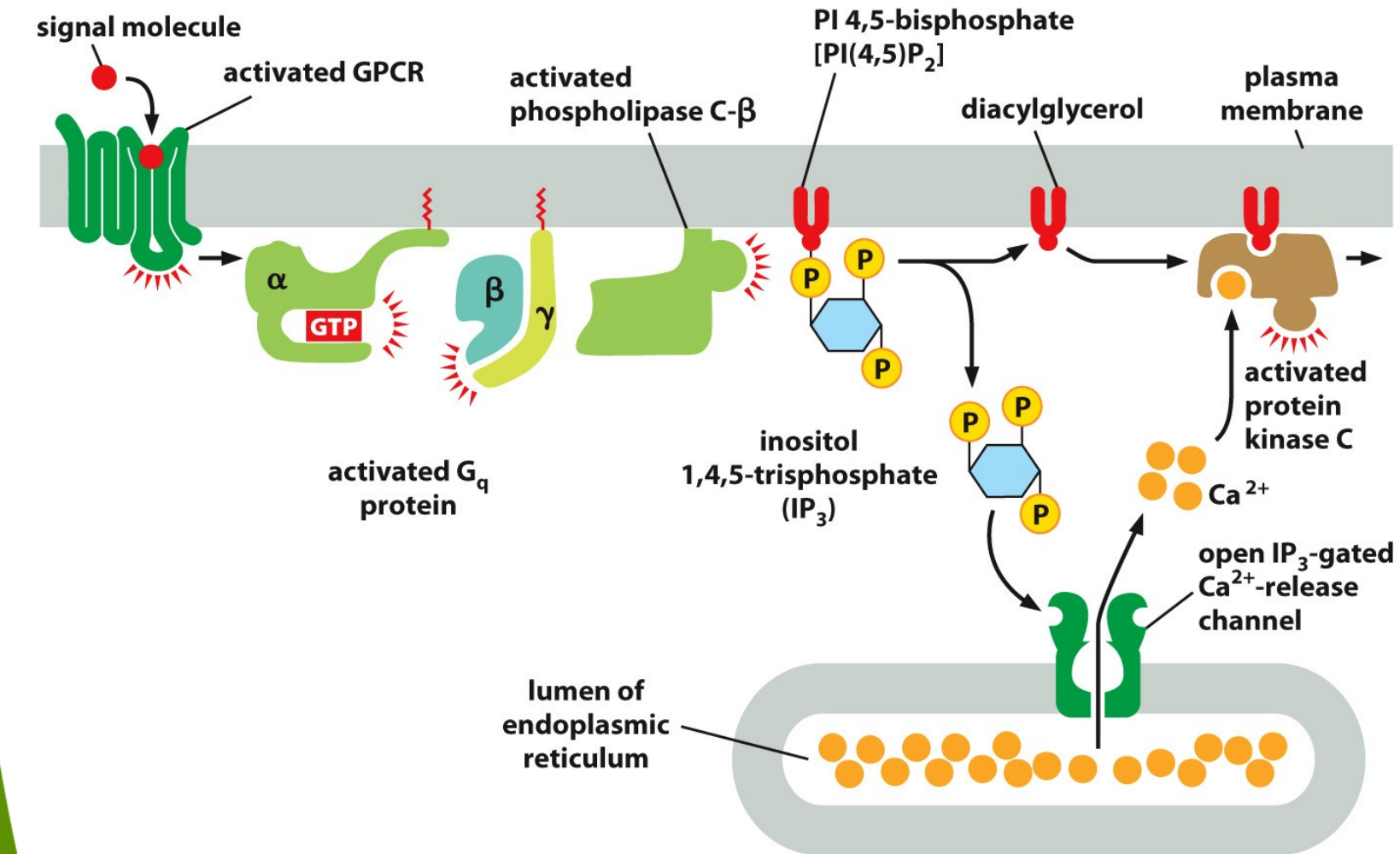


Figure 15-29 Molecular Biology of the Cell 6e (© Garland Science 2015)

Ionty Ca^{2+} jsou hlavním anorganickým druhým poslem

- Vápníkové vlny po oplození vajíčka

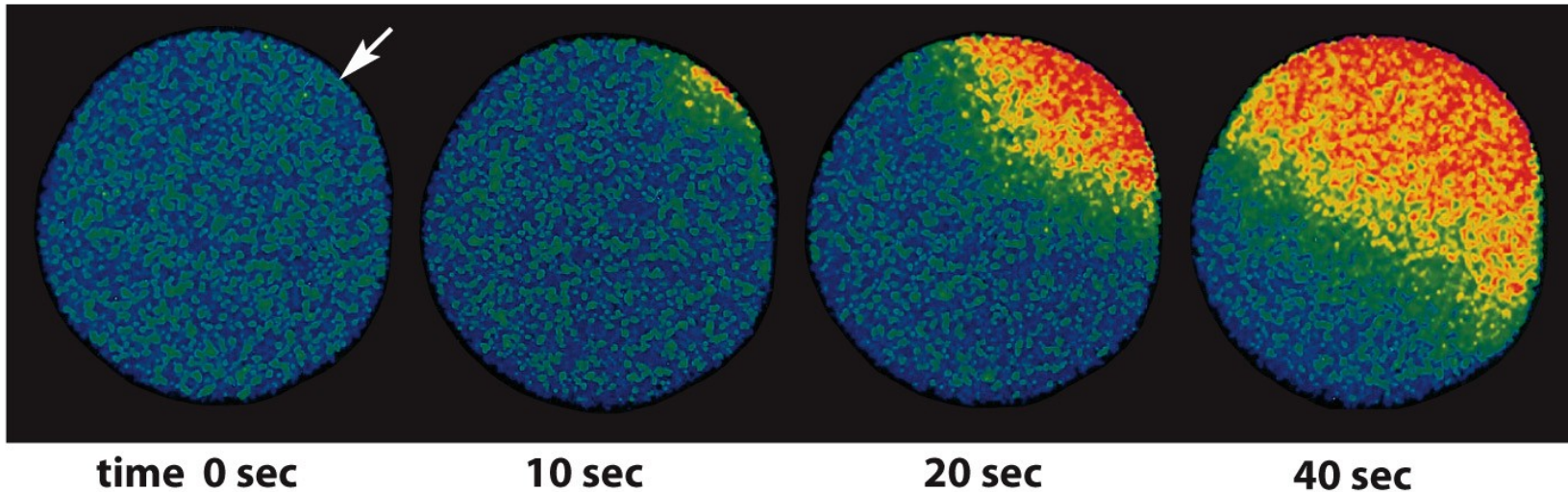


Figure 15-30 Molecular Biology of the Cell 6e (© Garland Science 2015)

SIGNALIZACE

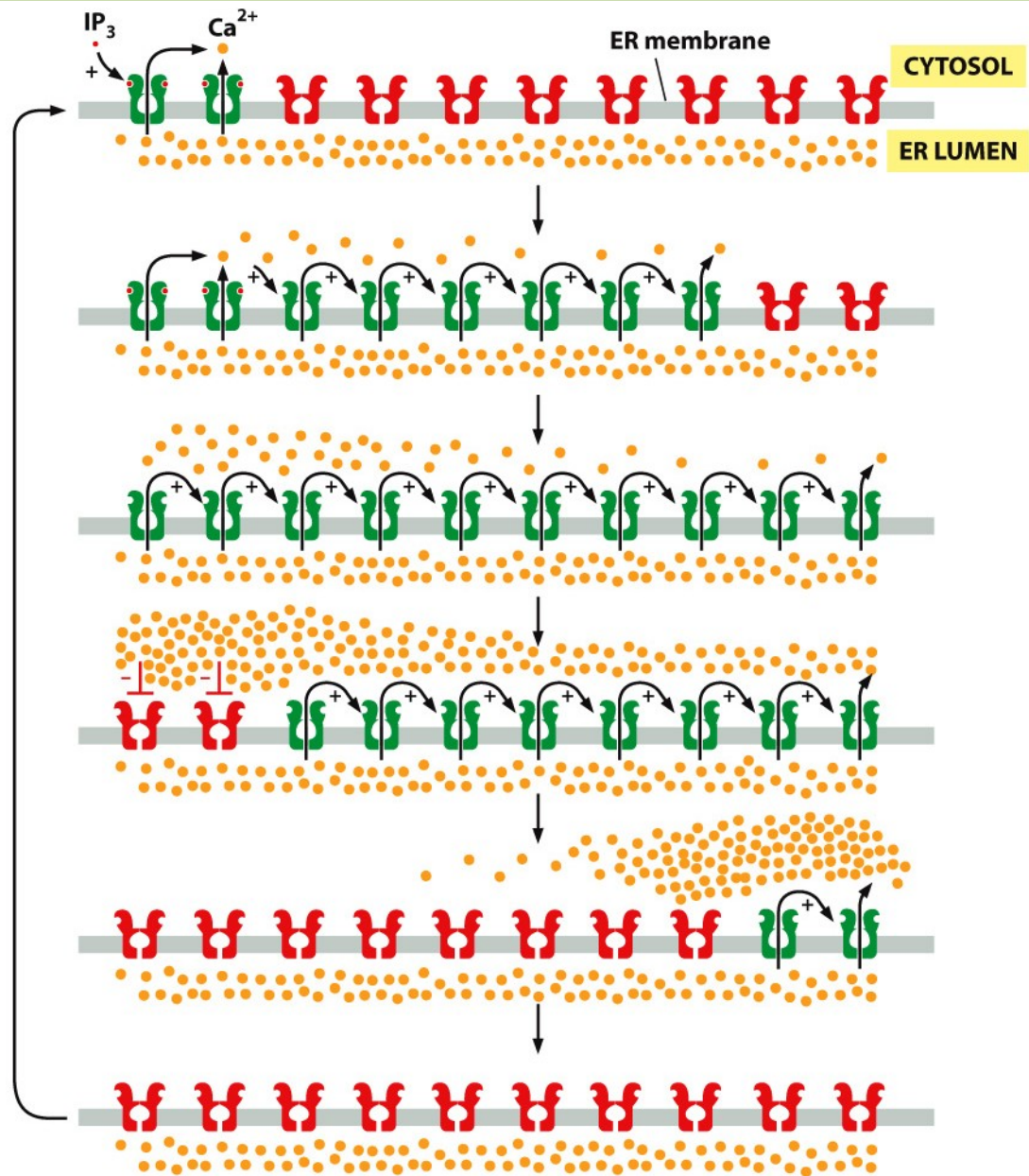


Figure 15-31 Molecular Biology of the Cell 6e (© Garland Science 2015)

SIGNALIZACE

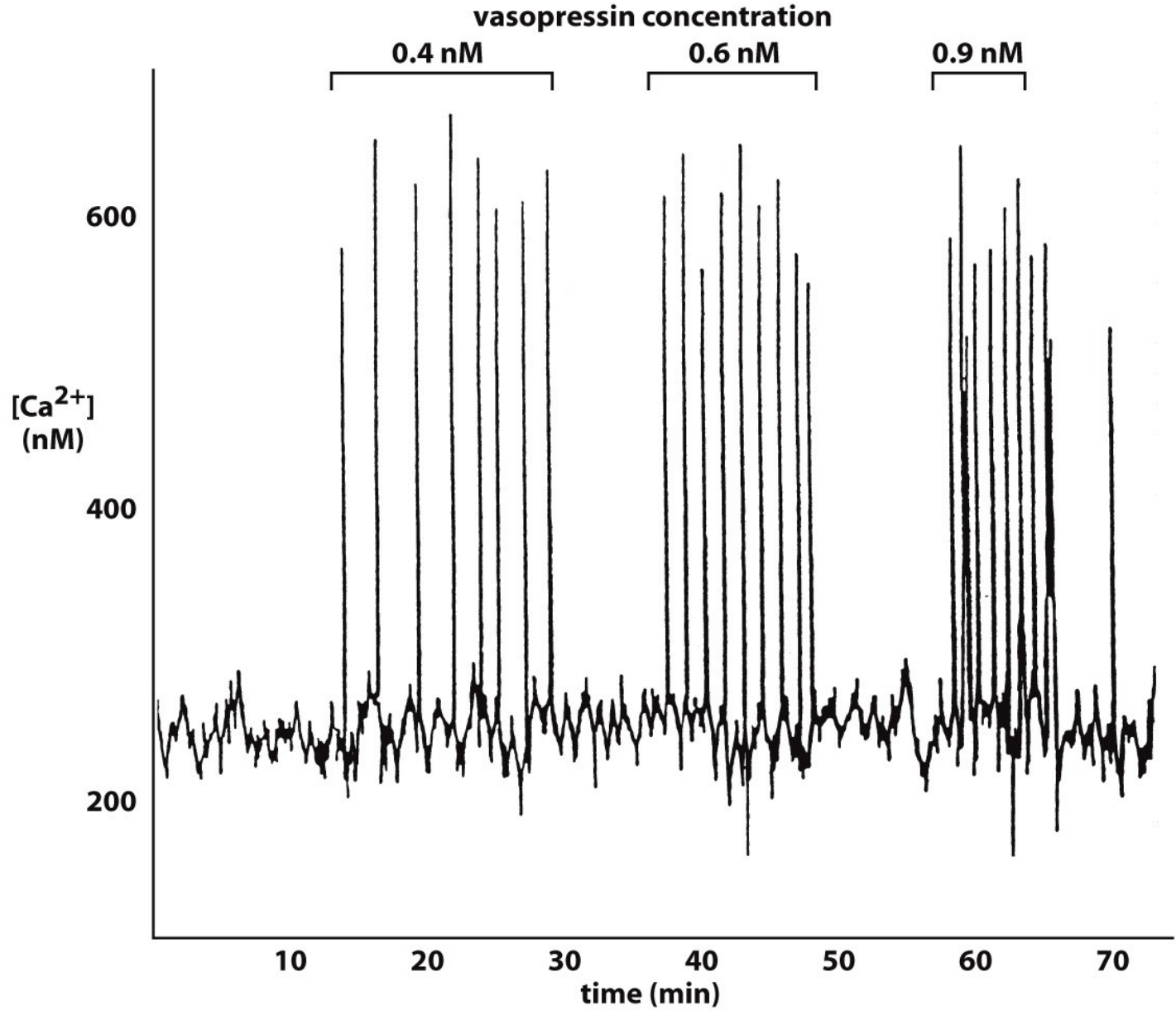


Figure 15-32 Molecular Biology of the Cell 6e (© Garland Science 2015)

Vápníkové senzory - calmodulin

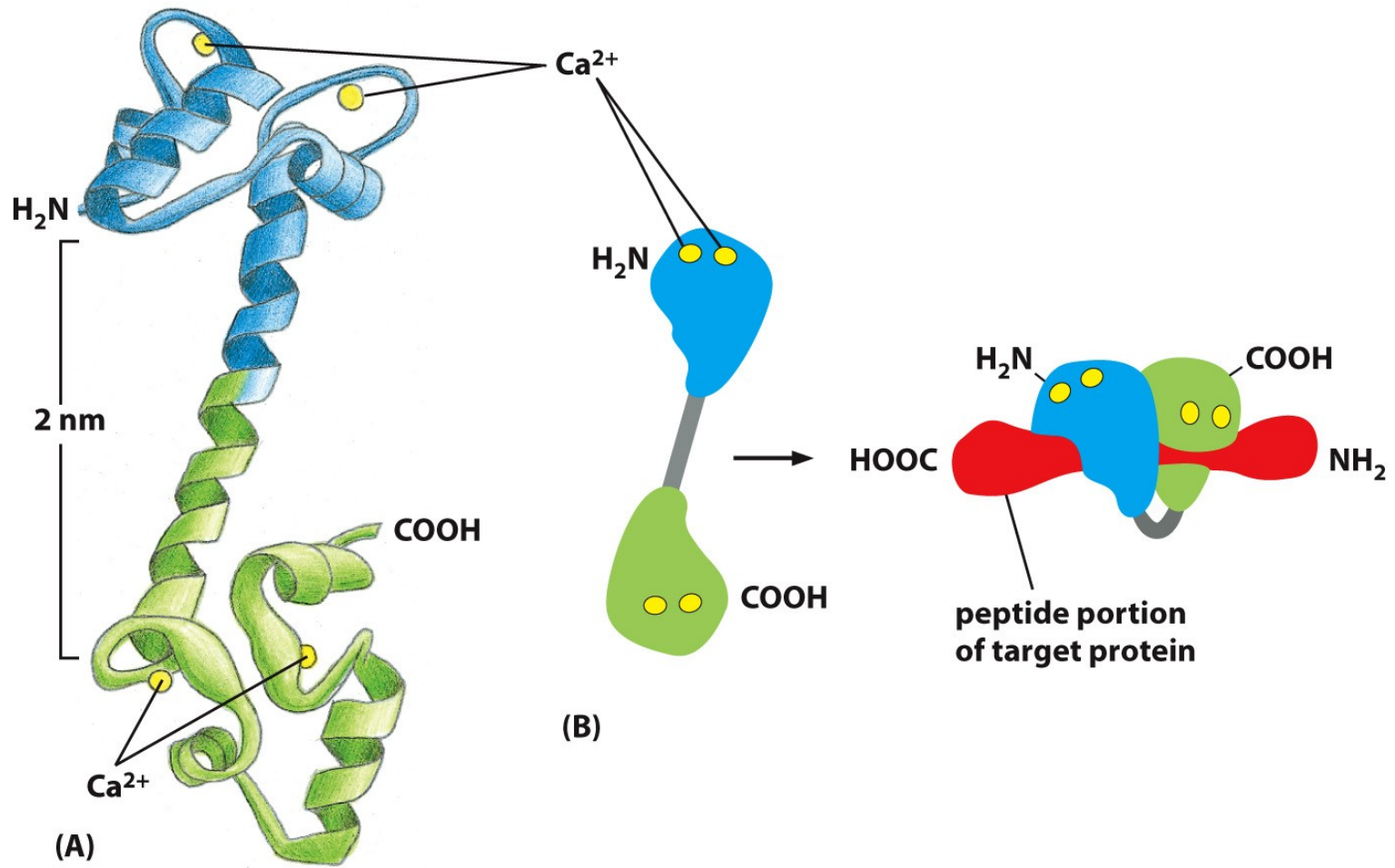


Figure 15-33 Molecular Biology of the Cell 6e (© Garland Science 2015)

Vápníkové senzory – CaMK (Ca/Calmodulin-dependent kinases)

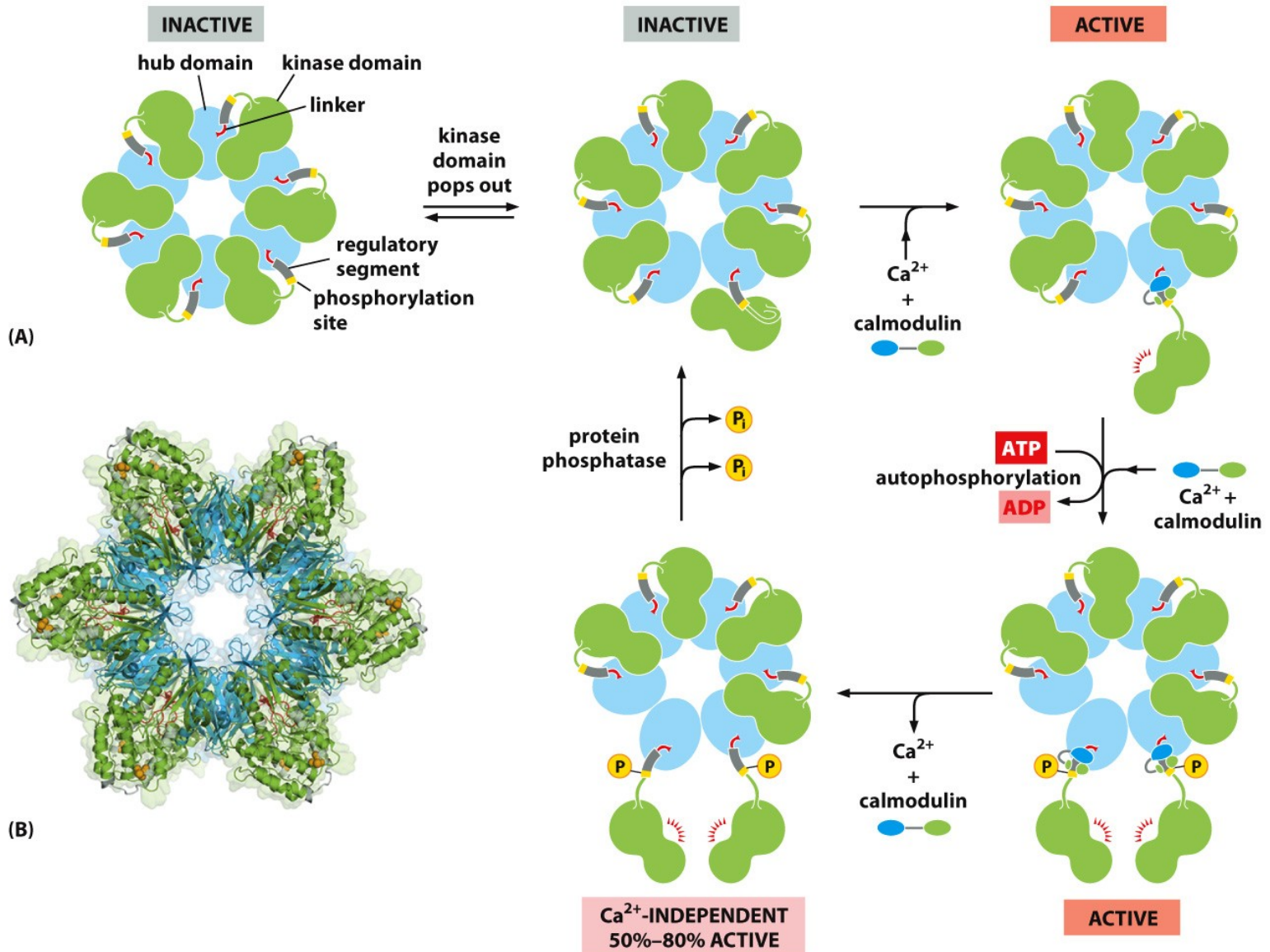
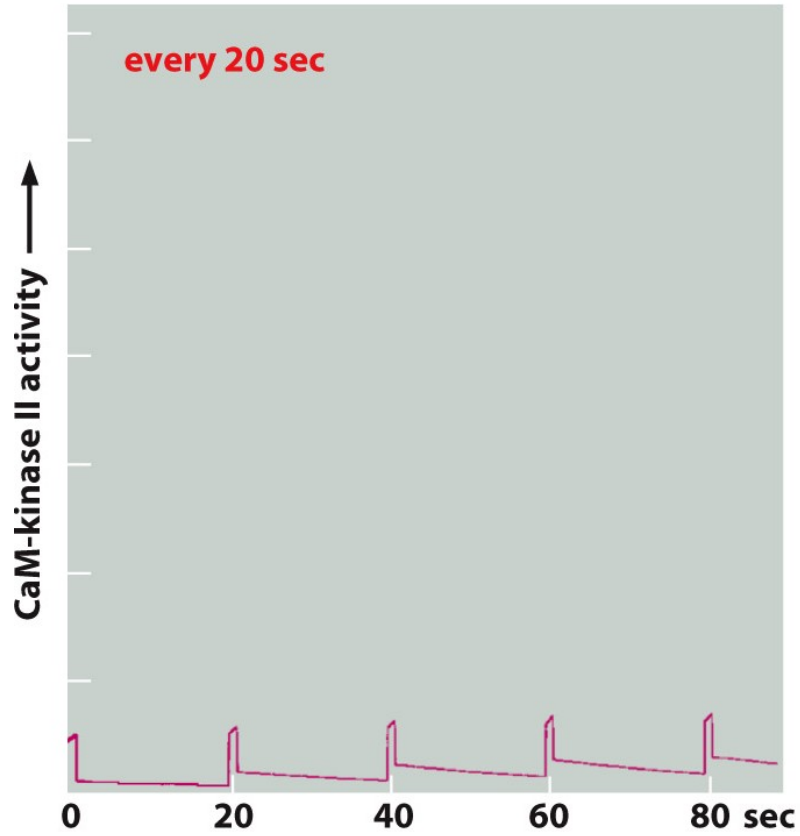
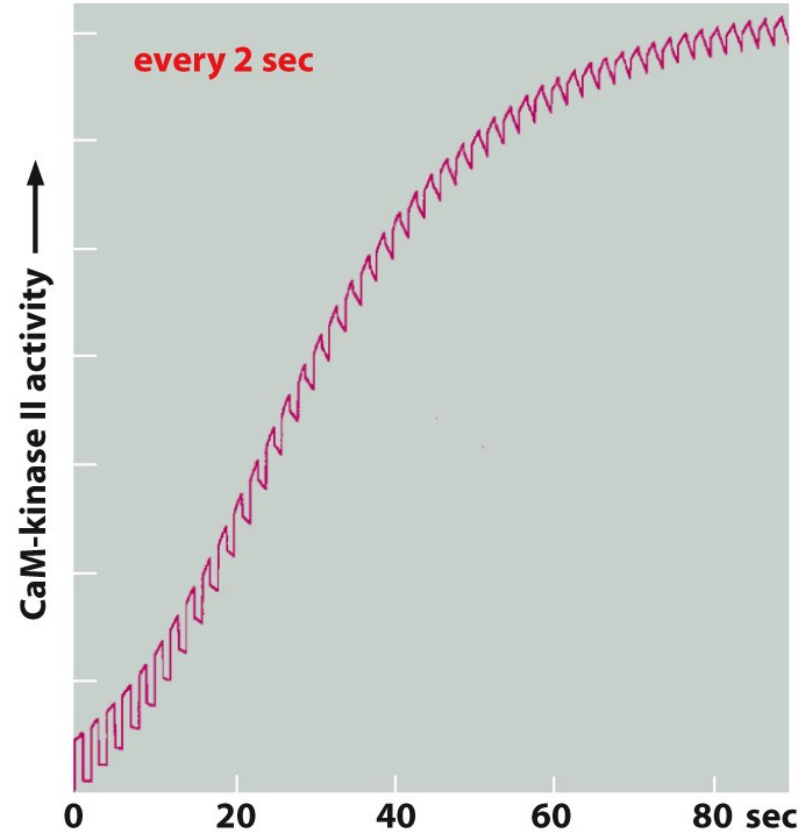


Figure 15-34 Molecular Biology of the Cell 6e (© Garland Science 2015)

SIGNALIZACE



(A) low-frequency Ca^{2+} oscillations



(B) high-frequency Ca^{2+} oscillations

Figure 15-35 Molecular Biology of the Cell 6e (© Garland Science 2015)

TABLE 15–2 Some Cell Responses in Which GPCRs Activate PLC β

Target tissue	Signal molecule	Major response
Liver	Vasopressin	Glycogen breakdown
Pancreas	Acetylcholine	Amylase secretion
Smooth muscle	Acetylcholine	Muscle contraction
Blood platelets	Thrombin	Platelet aggregation

Table 15-2 Molecular Biology of the Cell 6e (© Garland Science 2015)

Cyklický GMP je druhý posel zprostředkovávající vidění

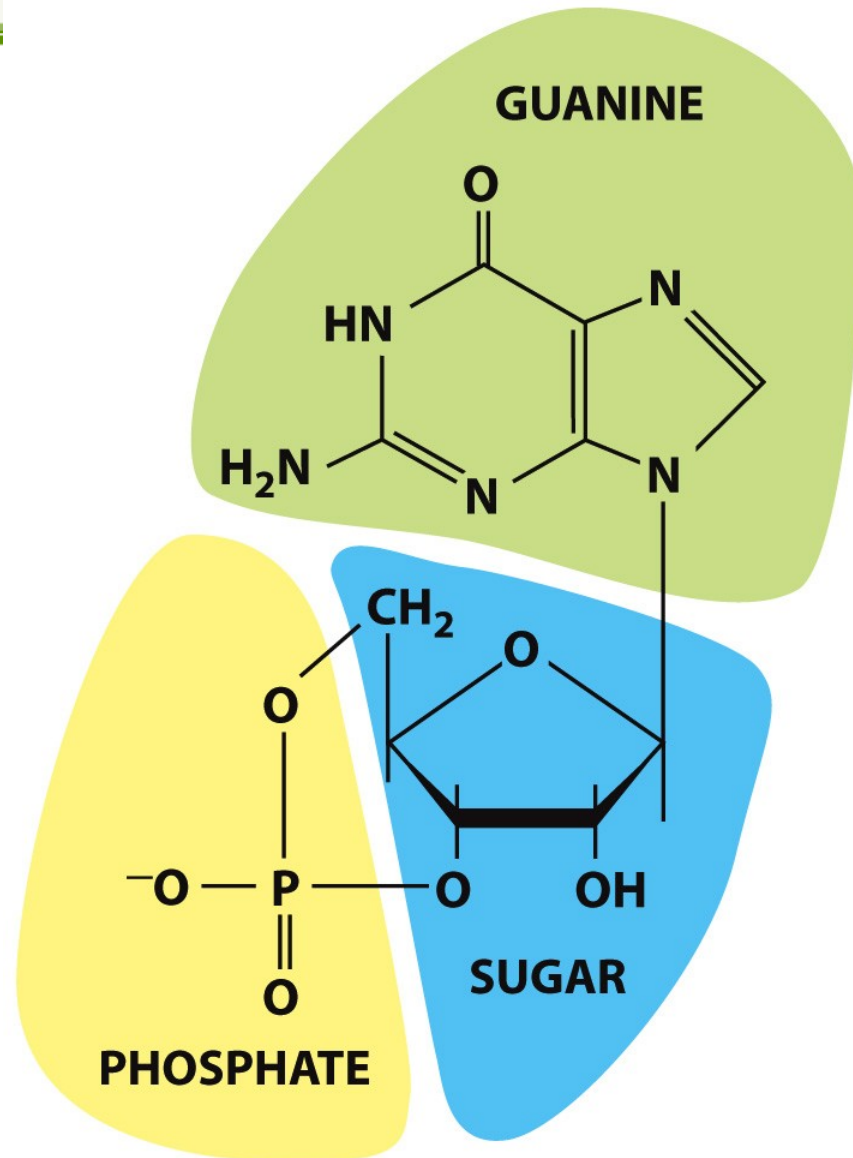
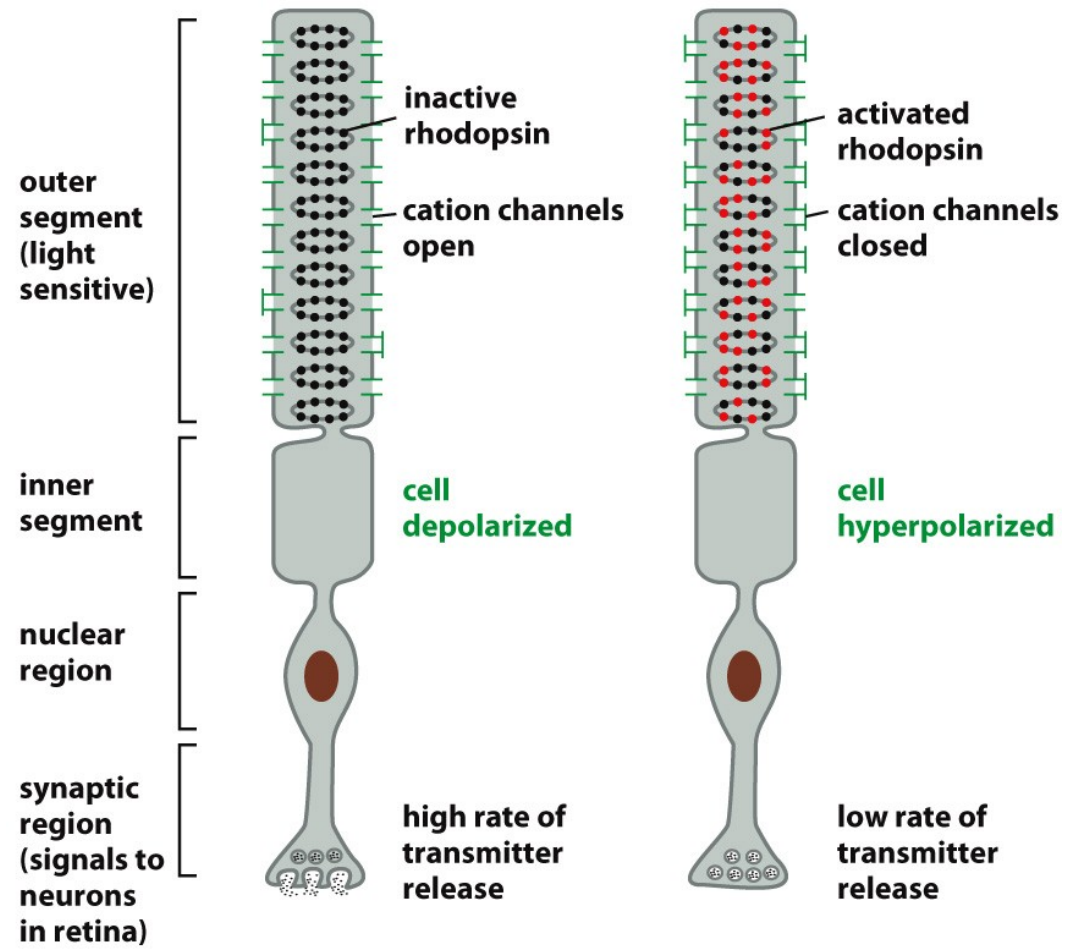
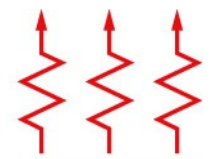


Figure 15-37 Molecular Biology of the Cell 6e (© Garland Science 2015)

Morfologie tyčinek



DARK

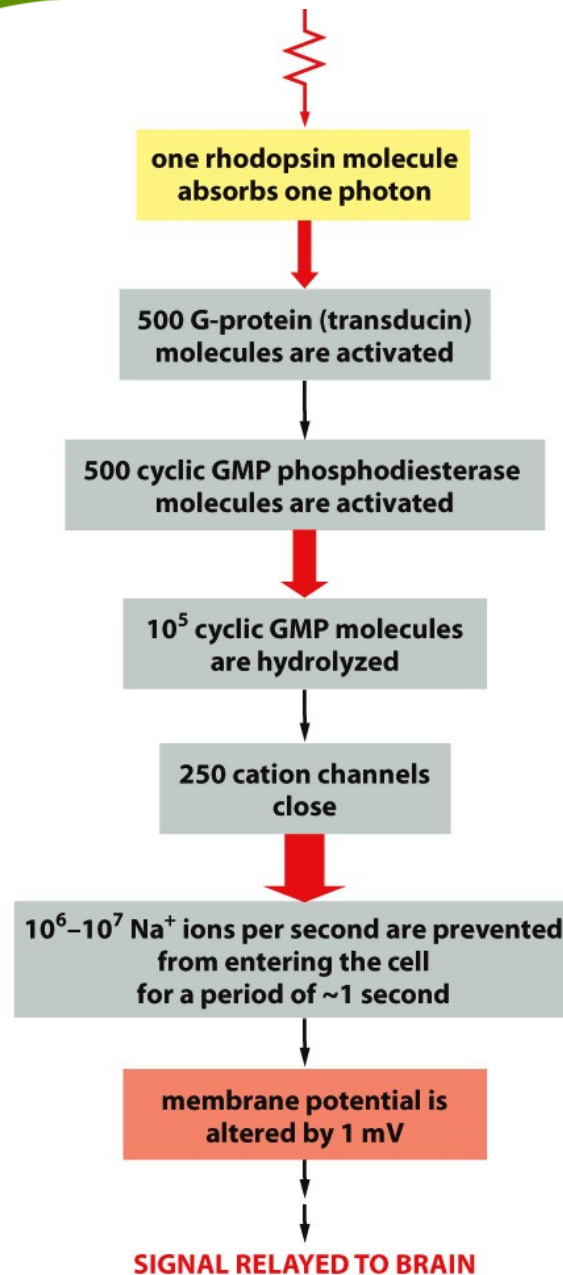


LIGHT

Aktivace tyčinky světlem (upraveno z Wiki)

- ◆ Po dopadu světla na sítnici dochází k jeho **absorpci**. Tyčinky jsou neuvěřitelně citlivé, reagují na dopad jediného fotonu.
- ◆ Za tmy jsou Na kanály otevřeny (jsou závislé na cGMP)
- ◆ Absorpce vede k **excitaci** membrány, která se projeví **izomerací 11-cis-retinalu** na **ALL-TRANS-RETINAL**. Dochází tak ke změně jeho geometrie (Schiffova báze s opsinem se posune o 0,5 nm). Energie fotonu se tedy transformovala na pohyb atomů. Světelná aktivace je velice rychlá a přitom složitá. Během milisekund proběhne řada fotochemických reakcí, jejichž meziprodukty (bathorodopsin, lumirodopsin, metapodopsin I, metarodopsin II) vykazují různá maxima od 500 do 380 nm.
- ◆ Následujícím důsledkem dopadu fotonu je **odpoutání barviva od bílkoviny**. Trans-izomer už nezapadá do vazebného místa. Rhodopsin se tak rozpadá na opsin a all-trans-retinal.
- ◆ Takto aktivovaný rhodopsin dále **aktivuje G-protein TRANSDUCIN**
- ◆ Kaskáda pokračuje aktivací **FOSFODIESTERÁZY (PDE)**, která **hydrolyzuje cGMP** na **NECYKLICKÝ 5'-GMP**.
- ◆ Původně otevřený **kanál pro Na⁺ ionty se uzavírá**, tok iontů se zastaví.
- ◆ Následkem je **HYPERPOLARIZACE** membrány,

SIGNALIZACE



- jedna molekula rodopsinu absorbuje jeden foton
- je aktivováno 500 G-proteinových molekul
- dále je aktivováno 500 molekul fosfodiesterázy cyklického GMP
- 10^5 molekul cyklického GMP je hydrolyzováno
- uzavírá se 250 kationtových kanálů
- 10^6 - 10^7 kationtům Na^+ za sekundu je zbráněno vstoupit do buňky po dobu 1 sekundy
- membránový potenciál buňky je změněn o 1mV
- signál je přenášen do mozku

Shrnutí základních funkcí trimerických G proteinů

TABLE 15-3 Four Major Families of Trimeric G Proteins*

Family	Some family members	Subunits that mediate action	Some functions
I	G _s	α	Activates adenylyl cyclase; activates Ca ²⁺ channels
	G _{olf}	α	Activates adenylyl cyclase in olfactory sensory neurons
II	G _i	α	Inhibits adenylyl cyclase
		βγ	Activates K ⁺ channels
	G _o	βγ	Activates K ⁺ channels; inactivates Ca ²⁺ channels
		α and βγ	Activates phospholipase C-β
G _t (transducin)	α	Activates cyclic GMP phosphodiesterase in vertebrate rod photoreceptors	
III	G _q	α	Activates phospholipase C-β
IV	G _{12/13}	α	Activates Rho family monomeric GTPases (via Rho-GEF) to regulate the actin cytoskeleton

*Families are determined by amino acid sequence relatedness of the α subunits. Only selected examples are included. About 20 α subunits and at least 6 β subunits and 11 γ subunits have been described in humans.

BUNĚČNÁ SIGNALIZACE: **Konec prvního dílu**

Receptorové tyrosin kinázy

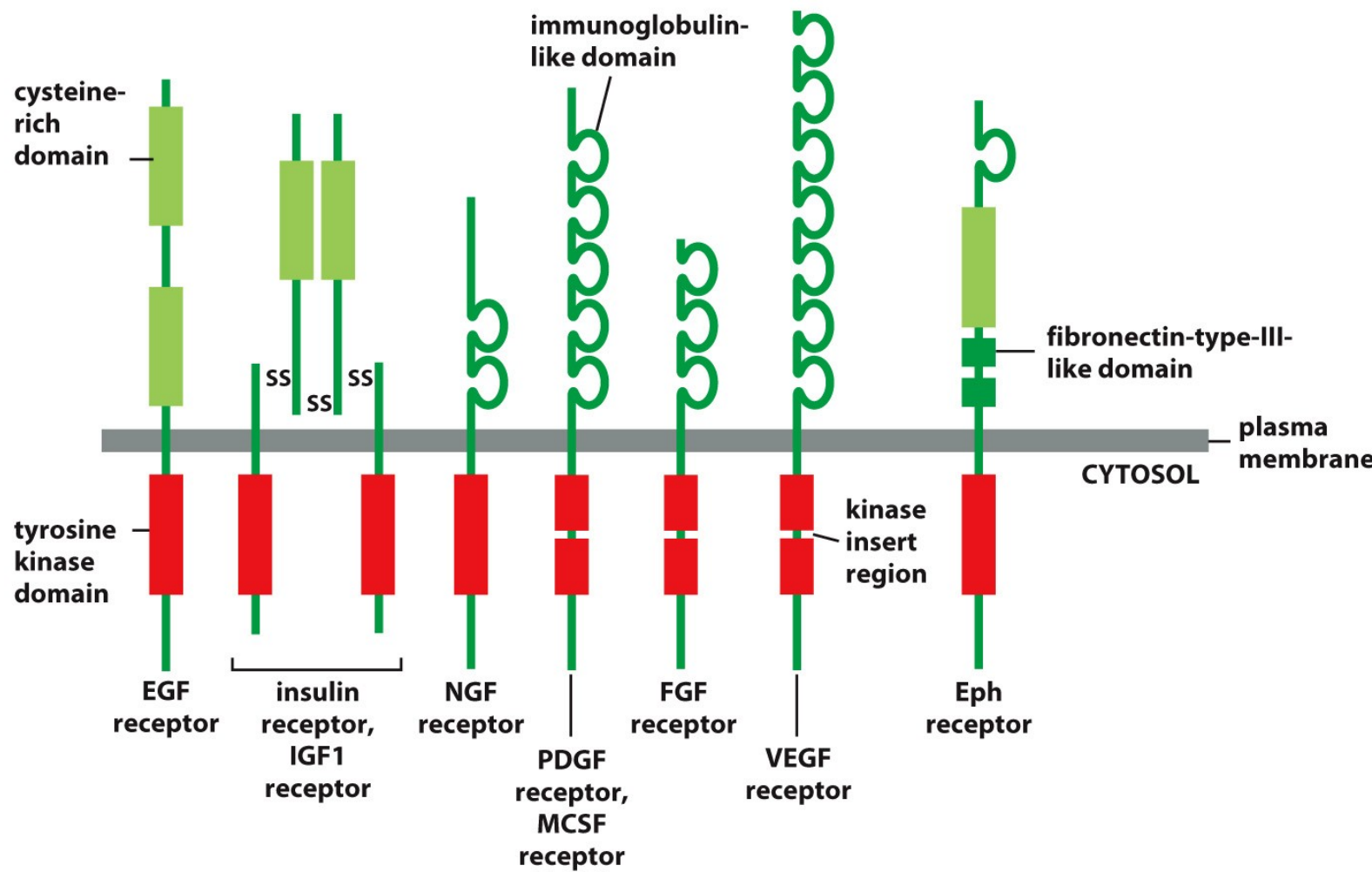


Figure 15-43 Molecular Biology of the Cell 6e (© Garland Science 2015)

Receptorové tyrosin kinázy

TABLE 15-4 Some Signal Proteins That Act Via RTKs		
Signal protein family	Receptor family	Some representative responses
Epidermal growth factor (EGF)	EGF receptors	Stimulates cell survival, growth, proliferation, or differentiation of various cell types; acts as inductive signal in development
Insulin	Insulin receptor	Stimulates carbohydrate utilization and protein synthesis
Insulin-like growth factor (IGF1)	IGF receptor-1	Stimulates cell growth and survival in many cell types
Nerve growth factor (NGF)	Trk receptors	Stimulates survival and growth of some neurons
Platelet-derived growth factor (PDGF)	PDGF receptors	Stimulates survival, growth, proliferation, and migration of various cell types
Macrophage-colony-stimulating factor (MCSF)	MCSF receptor	Stimulates monocyte/macrophage proliferation and differentiation
Fibroblast growth factor (FGF)	FGF receptors	Stimulates proliferation of various cell types; inhibits differentiation of some precursor cells; acts as inductive signal in development
Vascular endothelial growth factor (VEGF)	VEGF receptors	Stimulates angiogenesis
Ephrin	Eph receptors	Stimulates angiogenesis; guides cell and axon migration

Table 15-4 Molecular Biology of the Cell 6e (© Garland Science 2015)

SIGNALIZACE

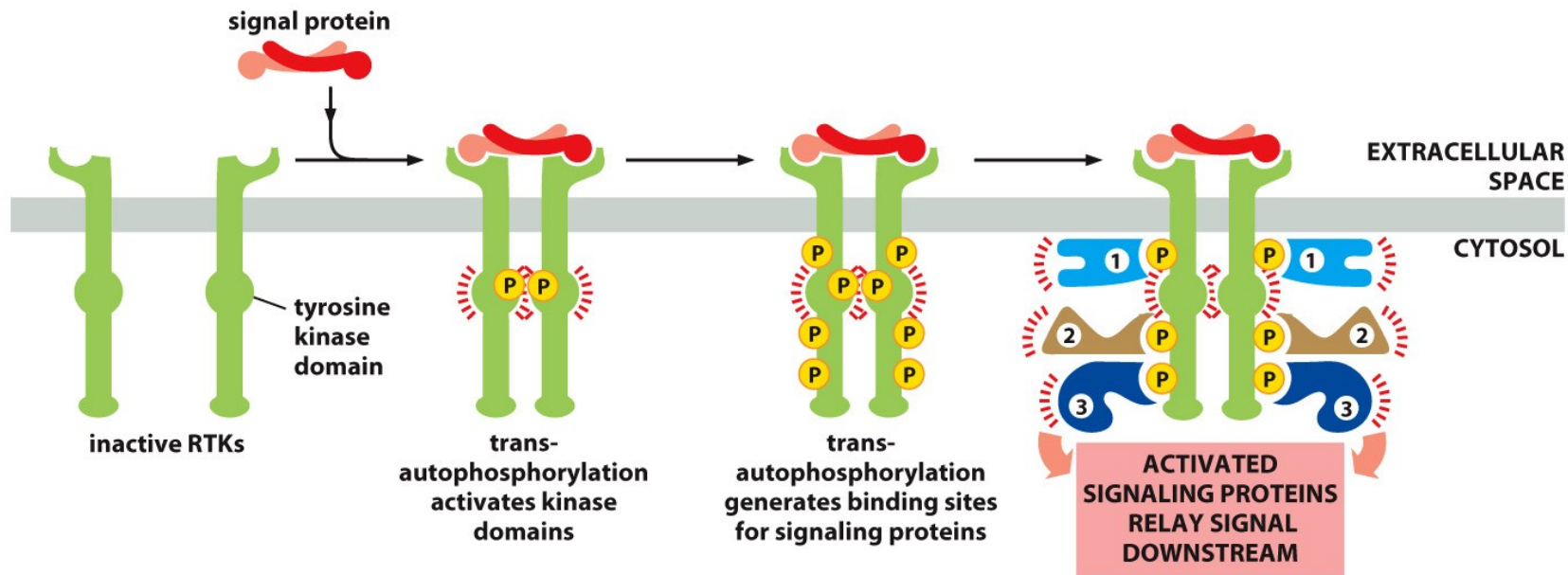


Figure 15-44 Molecular Biology of the Cell 6e (© Garland Science 2015)

SIGNALIZACE

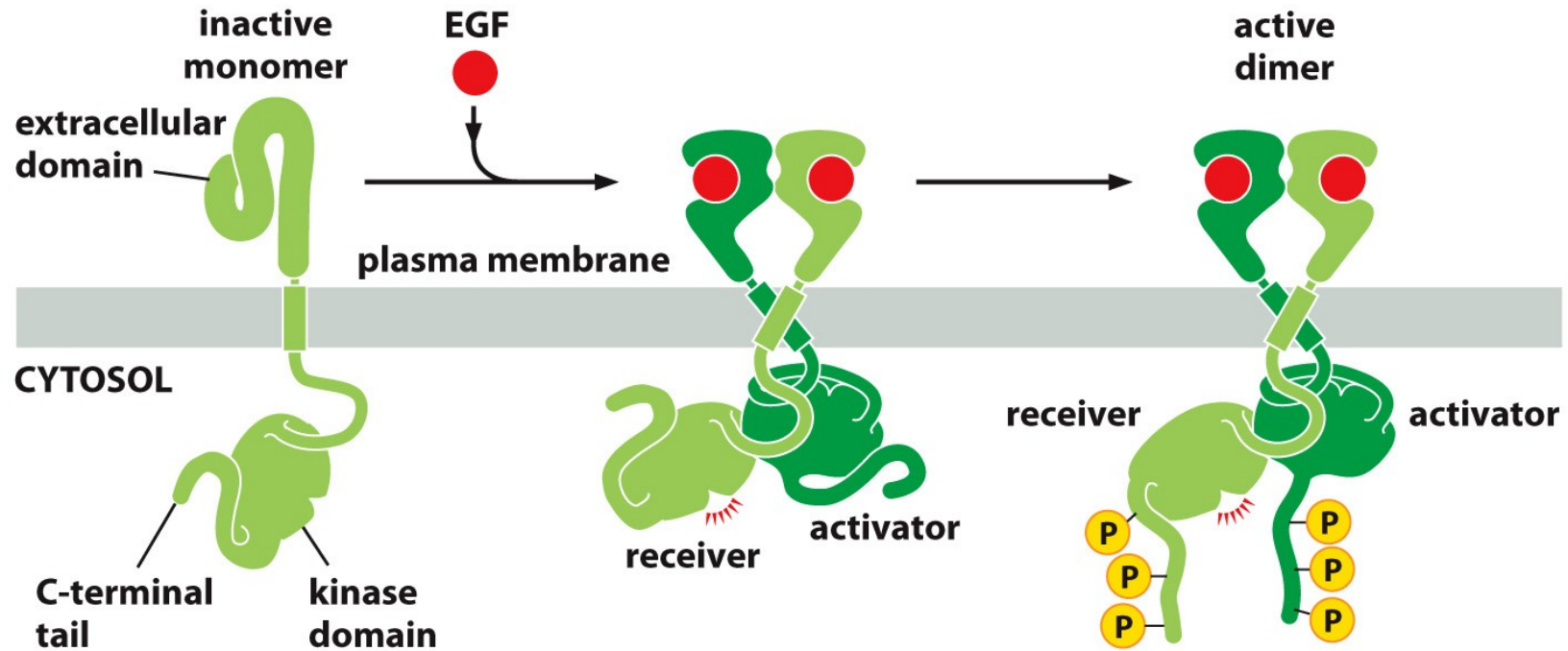


Figure 15-45 Molecular Biology of the Cell 6e (© Garland Science 2015)

SIGNALIZACE

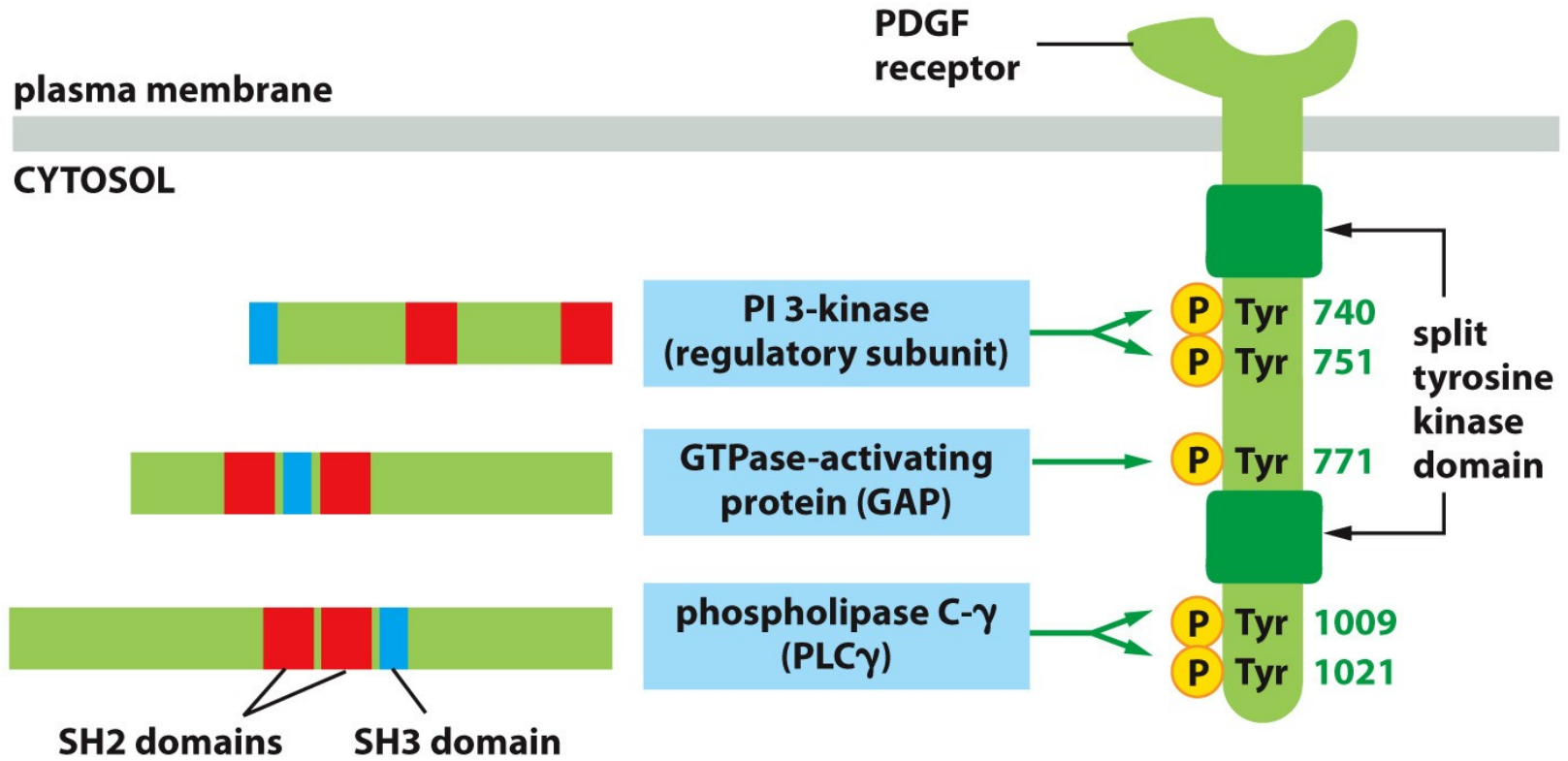


Figure 15-46a Molecular Biology of the Cell 6e (© Garland Science 2015)

Doména SH2 rozpoznává fosfo-tyrosin

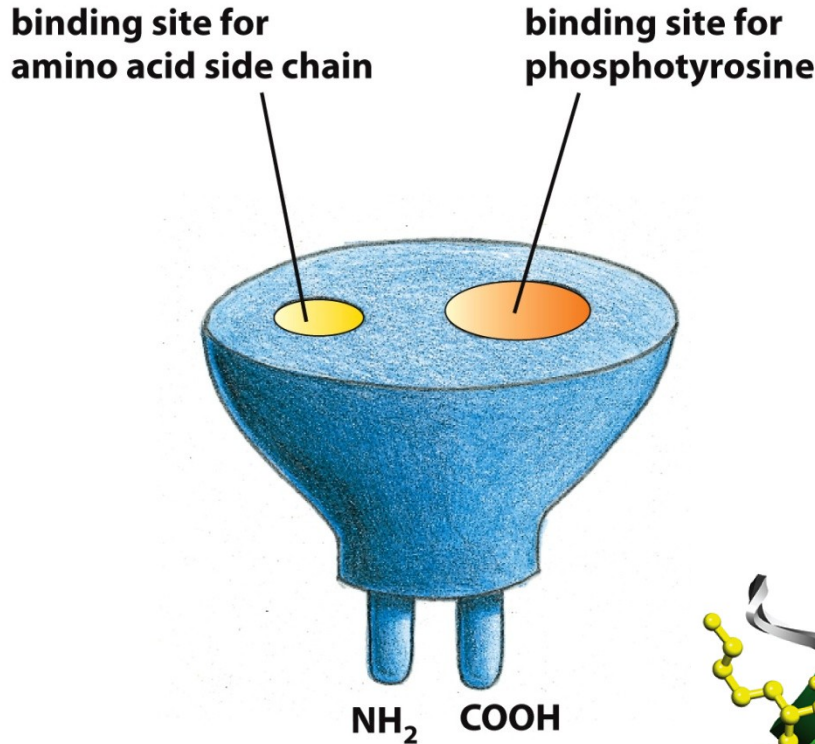


Figure 15-46c Molecular Biology of the Cell 6e (© Garland Science 2015)

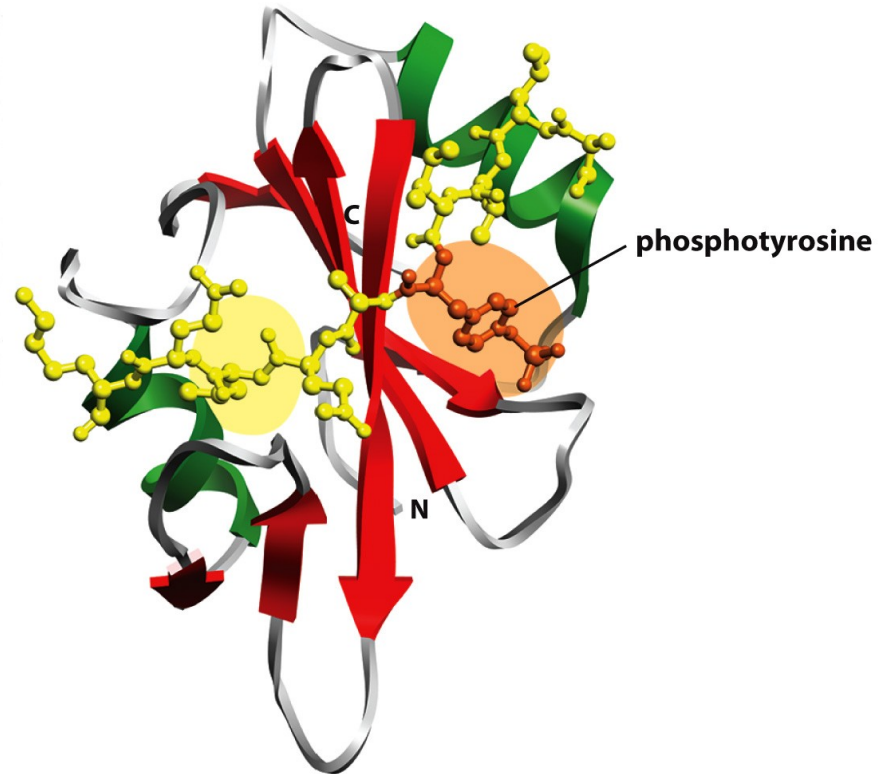


Figure 15-46b Molecular Biology of the Cell 6e (© Garland Science 2015)

Aktivace malé GTPázy z rodiny Ras

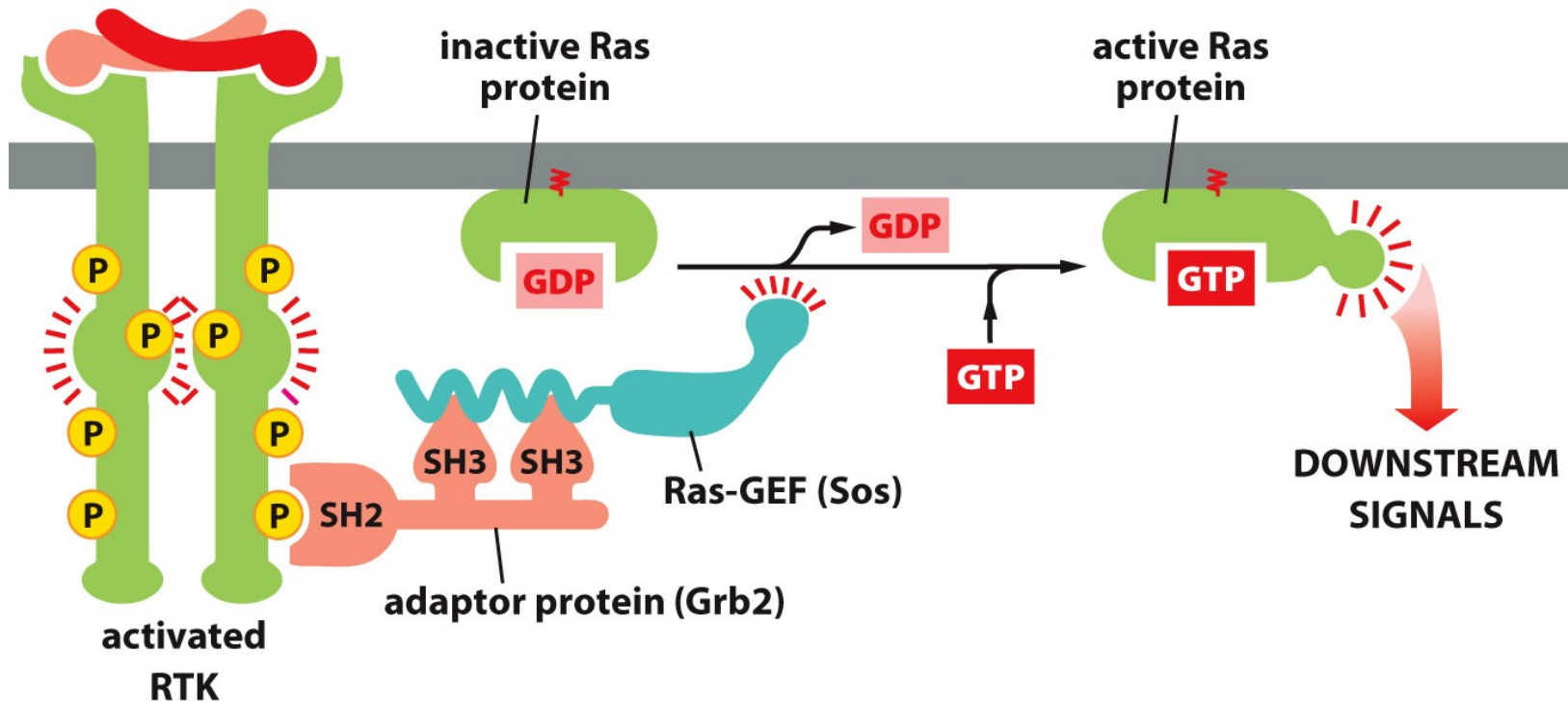


Figure 15-47 Molecular Biology of the Cell 6e (© Garland Science 2015)

SIGNALIZACE

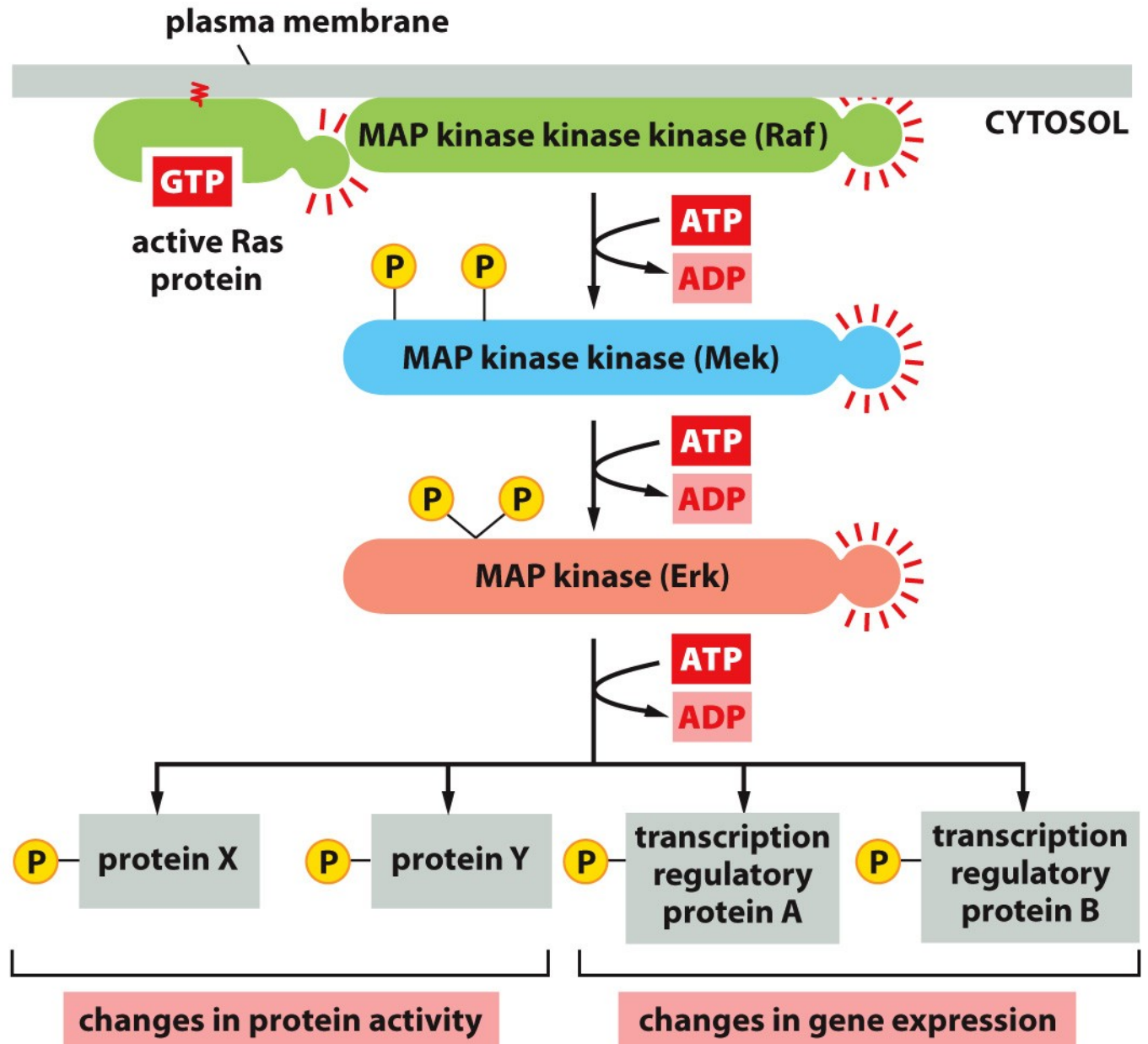
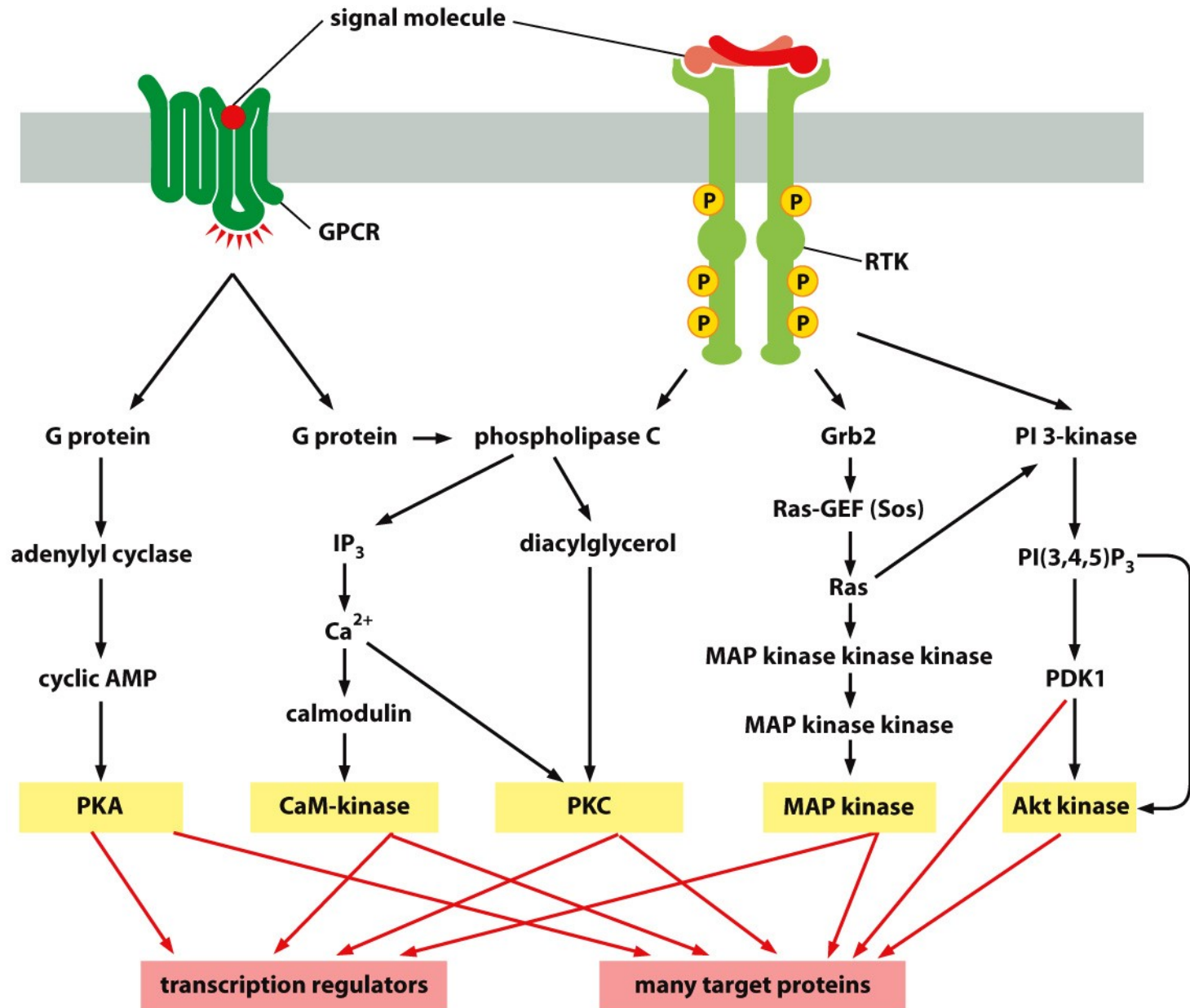


TABLE 15-5 The Ras Superfamily of Monomeric GTPases

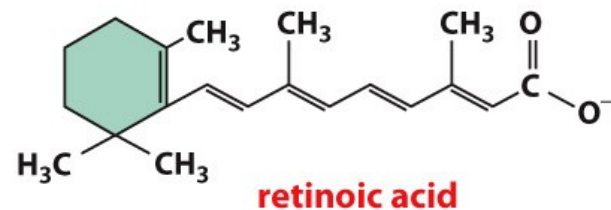
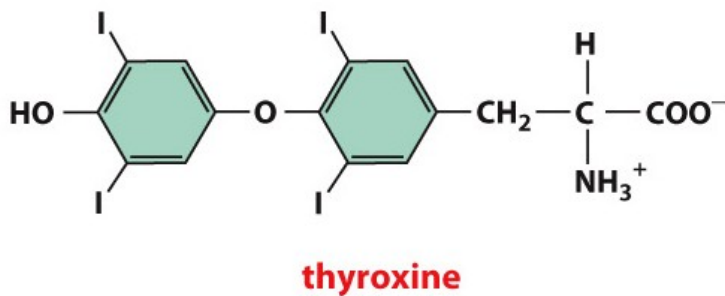
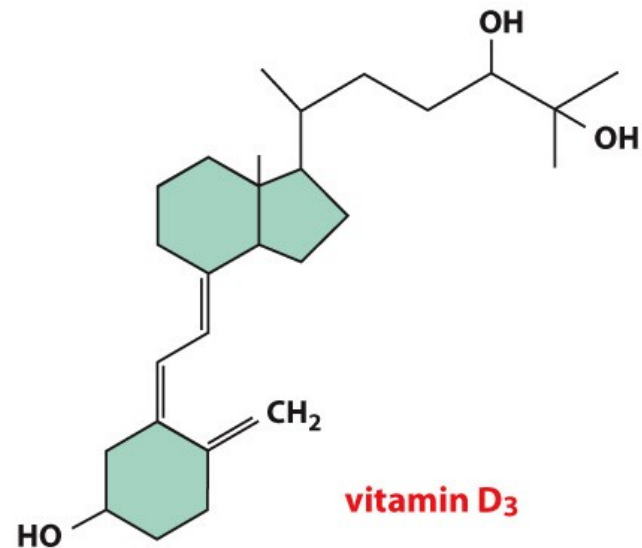
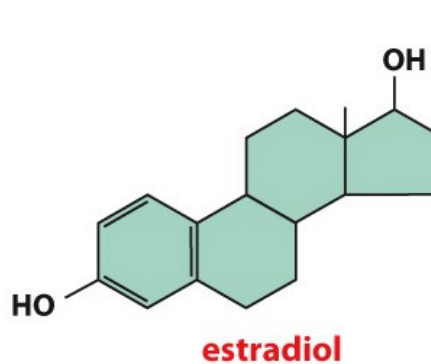
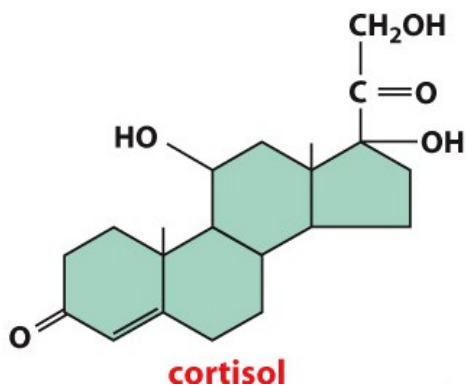
Family	Some family members	Some functions
Ras	H-Ras, K-Ras, N-Ras	Relay signals from RTKs
	Rheb	Activates mTOR to stimulate cell growth
	Rap1	Activated by a cyclic-AMP-dependent GEF; influences cell adhesion by activating integrins
Rho*	Rho, Rac, Cdc42	Relay signals from surface receptors to the cytoskeleton and elsewhere
ARF*	ARF1-ARF6	Regulate assembly of protein coats on intracellular vesicles
Rab*	Rab1-60	Regulate intracellular vesicle traffic
Ran*	Ran	Regulates mitotic spindle assembly and nuclear transport of RNAs and proteins

***The Rho family is discussed in Chapter 16, the ARF and Rab proteins in Chapter 13, and Ran in Chapters 12 and 17. The three-dimensional structure of Ras is shown in Figure 3-67.**

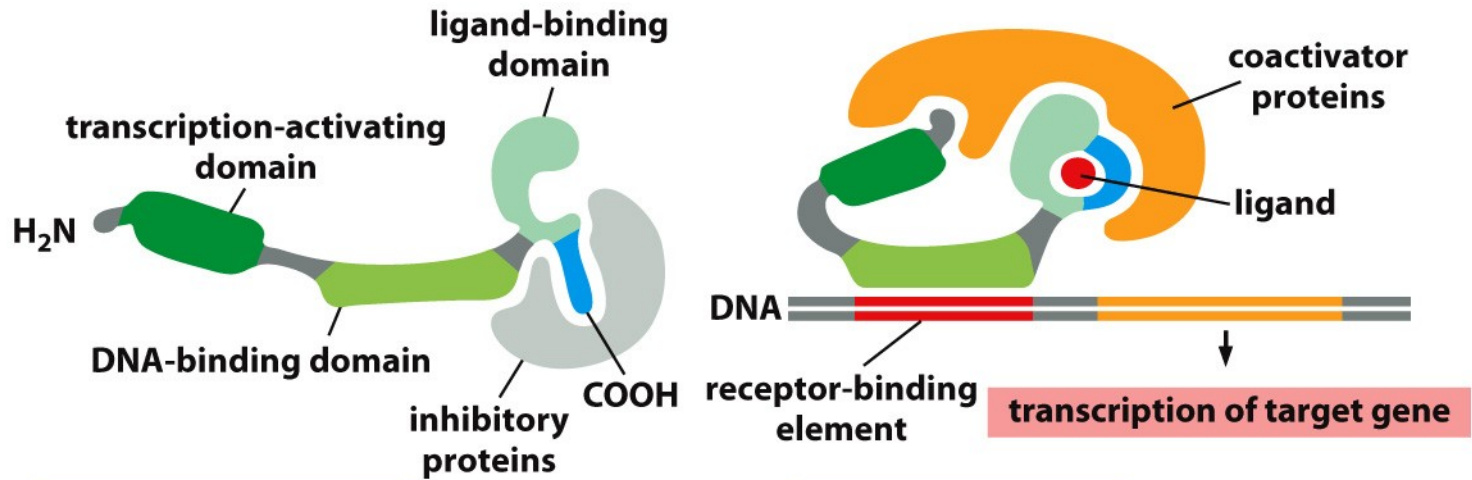
SIGNALIZACE



Signalizace přes jaderné receptory

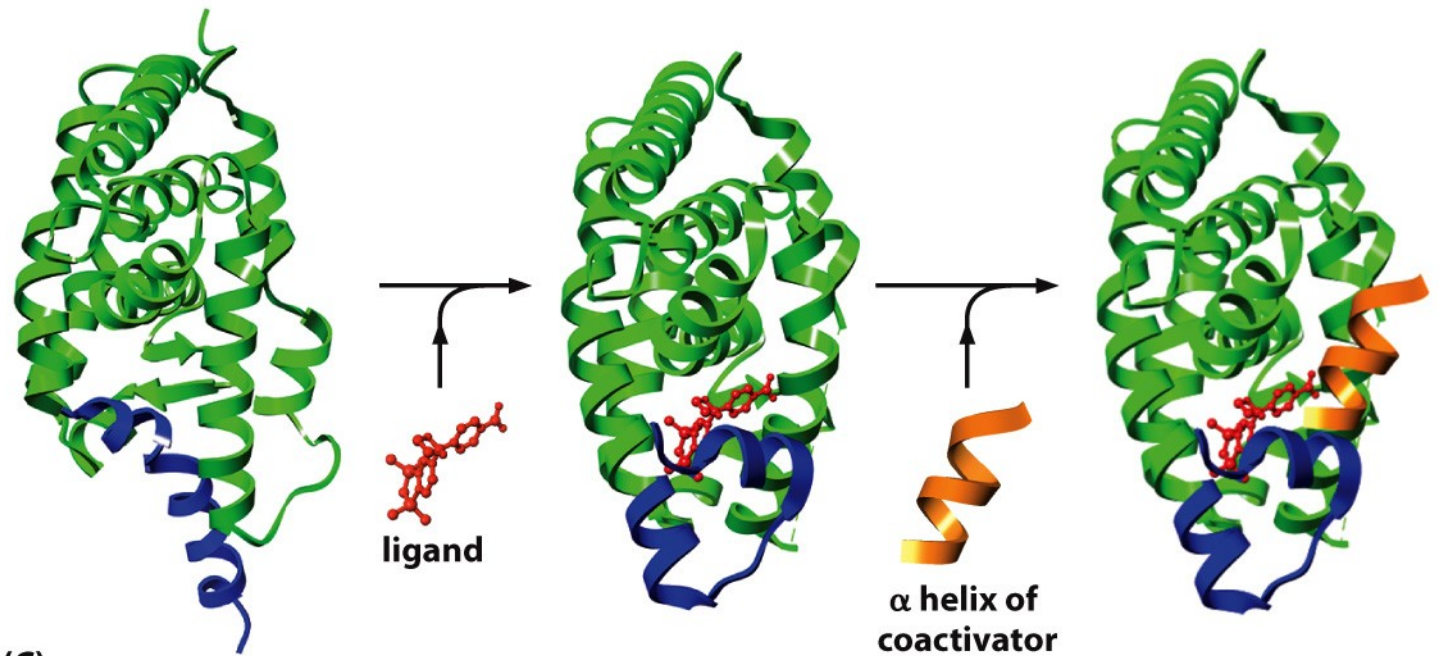


SIGNALIZACE



(A) **INACTIVE RECEPTOR**

(B) **ACTIVE RECEPTOR**



(C)

BUNĚČNÁ SIGNALIZACE:

Díl II

**Krátká exkurze do signálních drah
významných v imunologii a vývojové
biologii**

Signalizace přes cytokinové receptory – JAK/STAT signální kaskáda

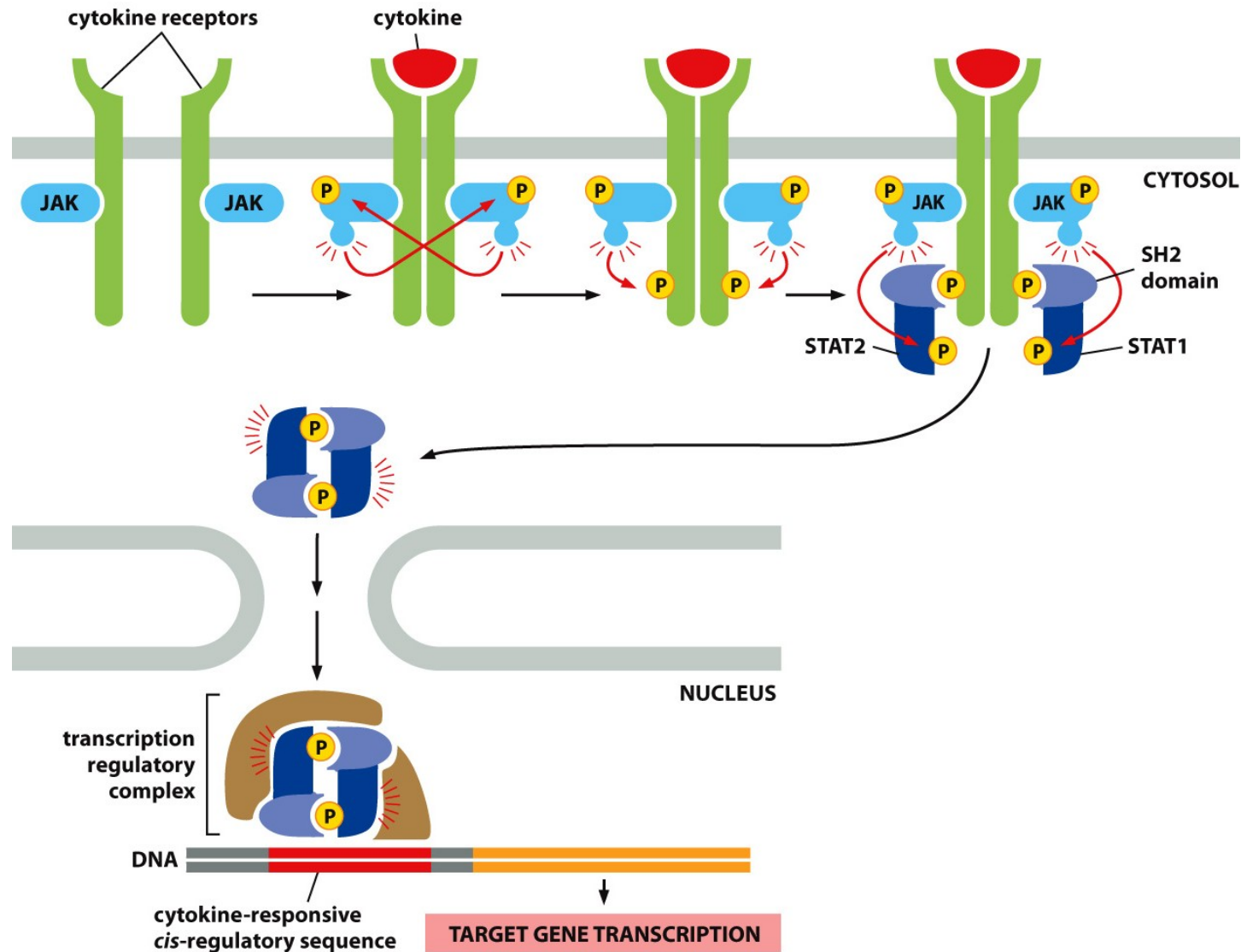
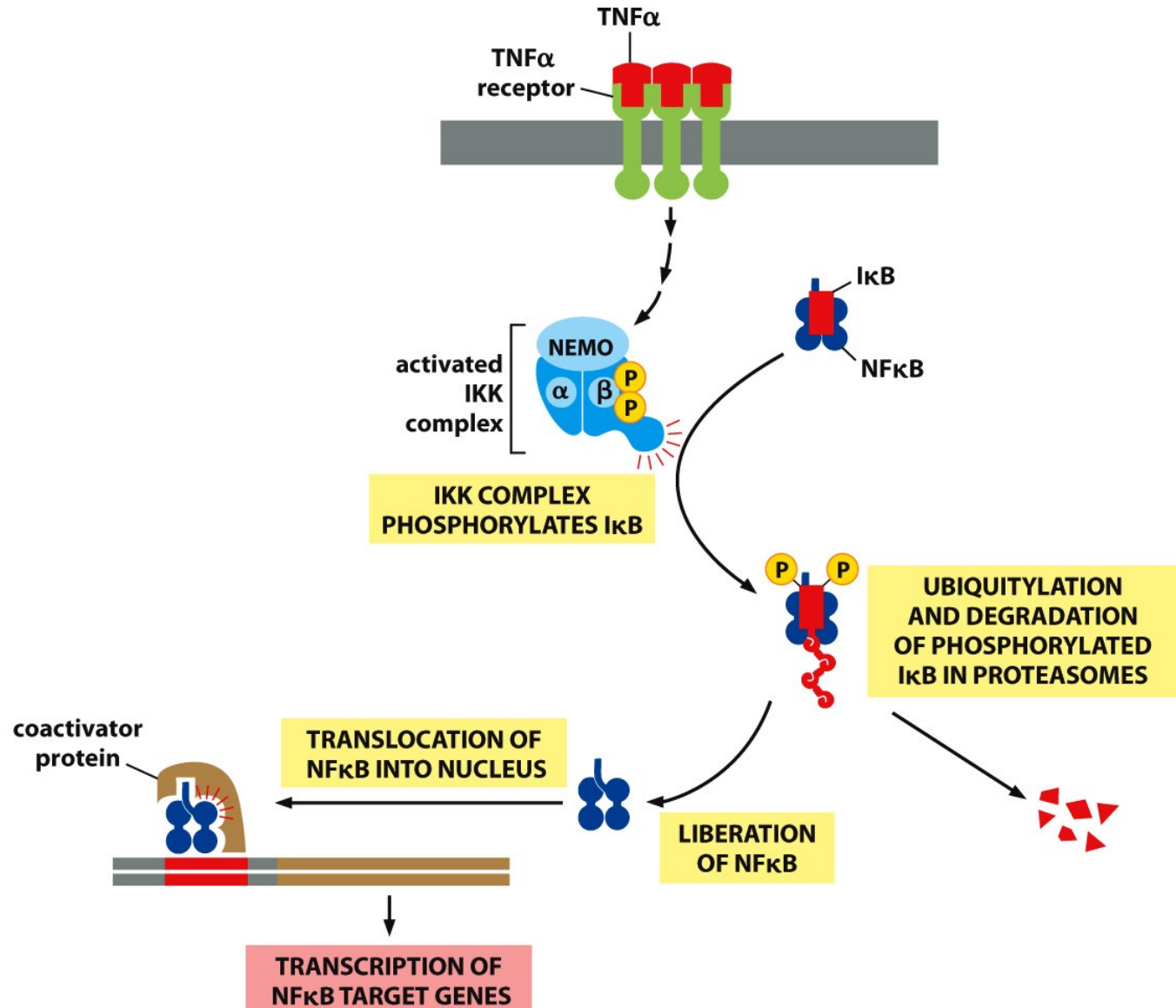


TABLE 15-6 Some Extracellular Signal Proteins That Act Through Cytokine Receptors and the JAK-STAT Signaling Pathway

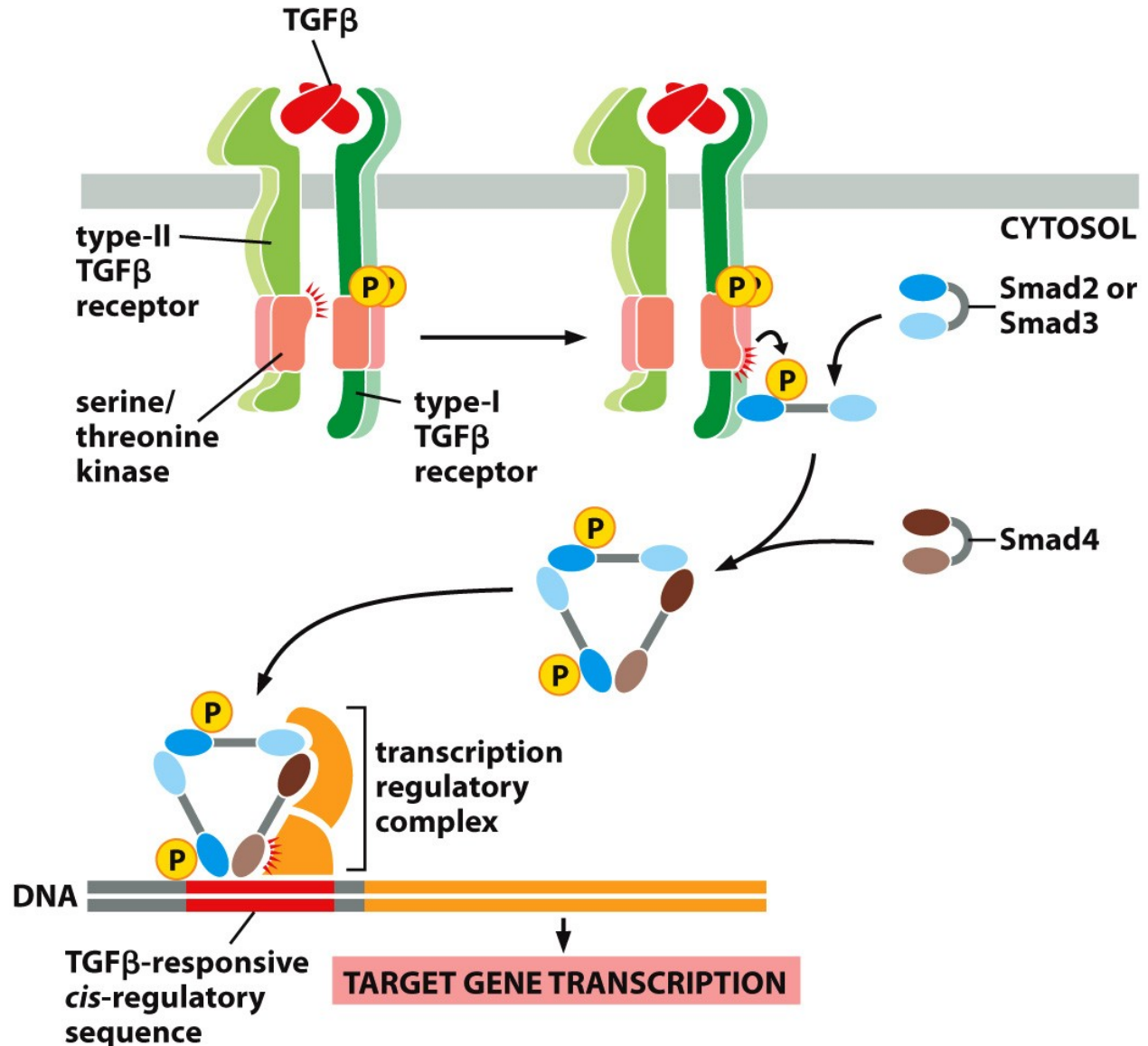
Signal protein	Receptor-associated JAKs	STATs activated	Some responses
Interferon- γ (IFN γ)	JAK1 and JAK2	STAT1	Activates macrophages
Interferon- α (IFN α)	Tyk2 and JAK2	STAT1 and STAT2	Increases cell resistance to viral infection
Erythropoietin	JAK2	STAT5	Stimulates production of erythrocytes
Prolactin	JAK1 and JAK2	STAT5	Stimulates milk production
Growth hormone	JAK2	STAT1 and STAT5	Stimulates growth by inducing IGF1 production
Granulocyte-Macrophage-Colony-Stimulating Factor (GMCSF)	JAK2	STAT5	Stimulates production of granulocytes and macrophages

Table 15-6 Molecular Biology of the Cell 6e (© Garland Science 2015)

Signalizace přes NFκB (indukovaná tumor necrosis factor)



TGF (transformující růstový faktor)/BMP (bone-morphogenetic protein) signalizace



Signalizace Notch

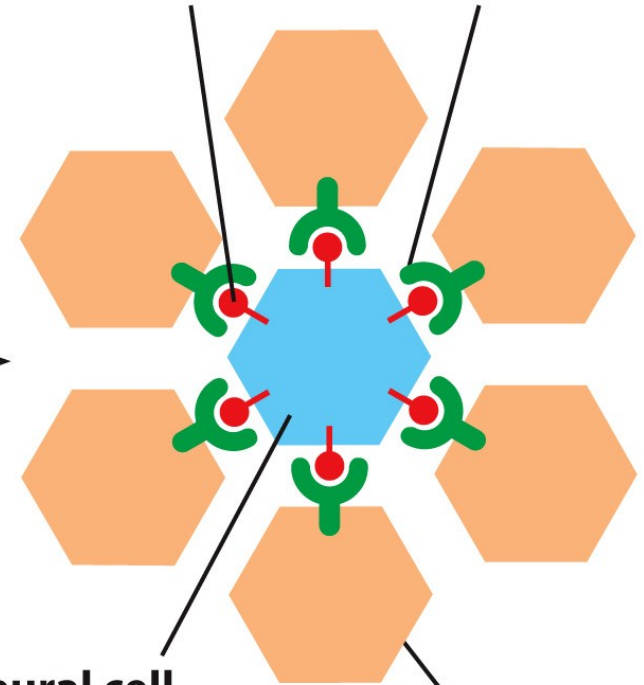


**unspecified
epithelial cells**



**transmembrane
inhibitory signal
protein (Delta)**

**receptor
protein
(Notch)**



**neural cell
developing from
epithelial cell**

**inhibited
epithelial cell**

Figure 15-58 Molecular Biology of the Cell 6e (© Garland Science 2015)

Signalizace Notch

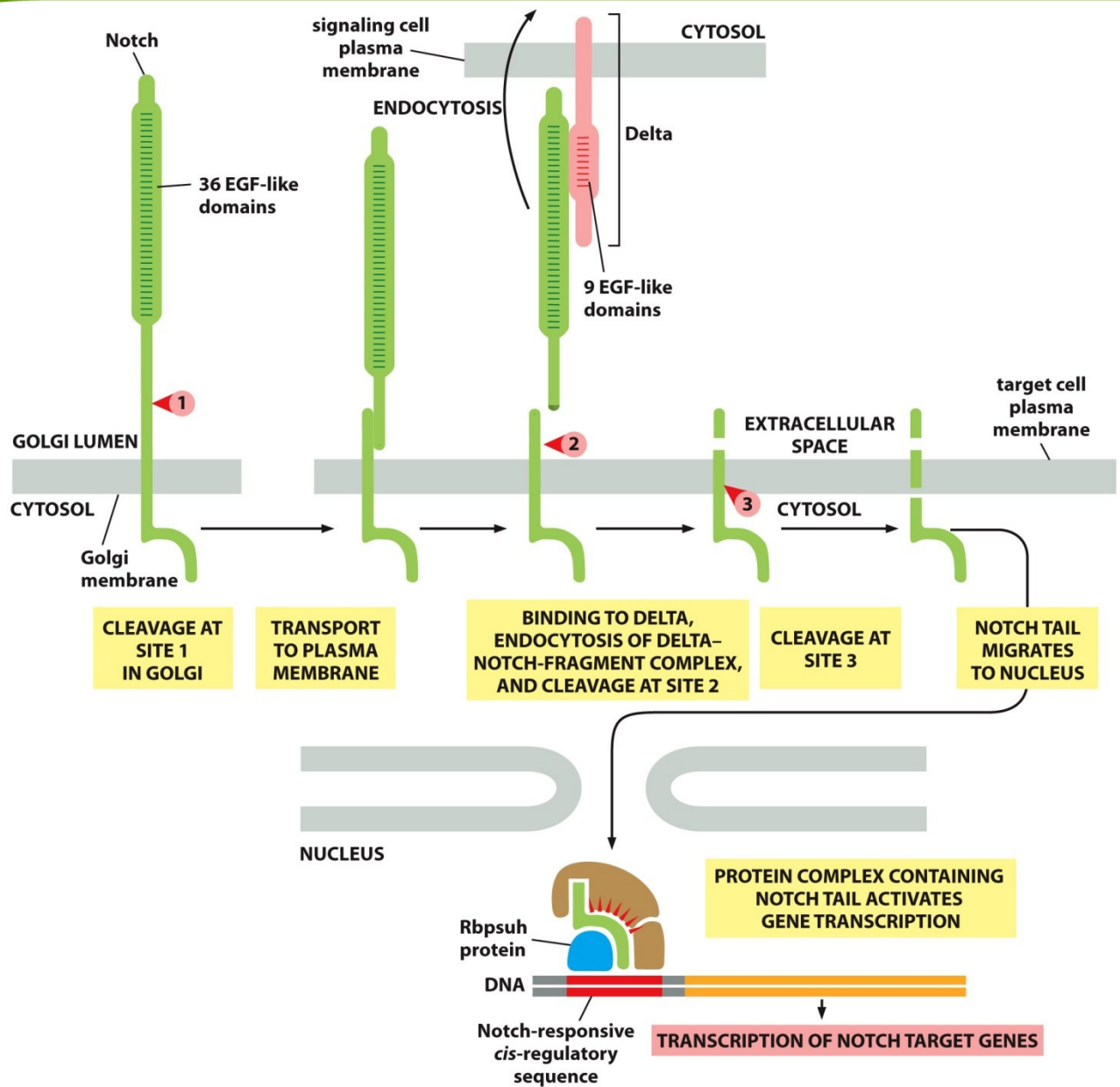
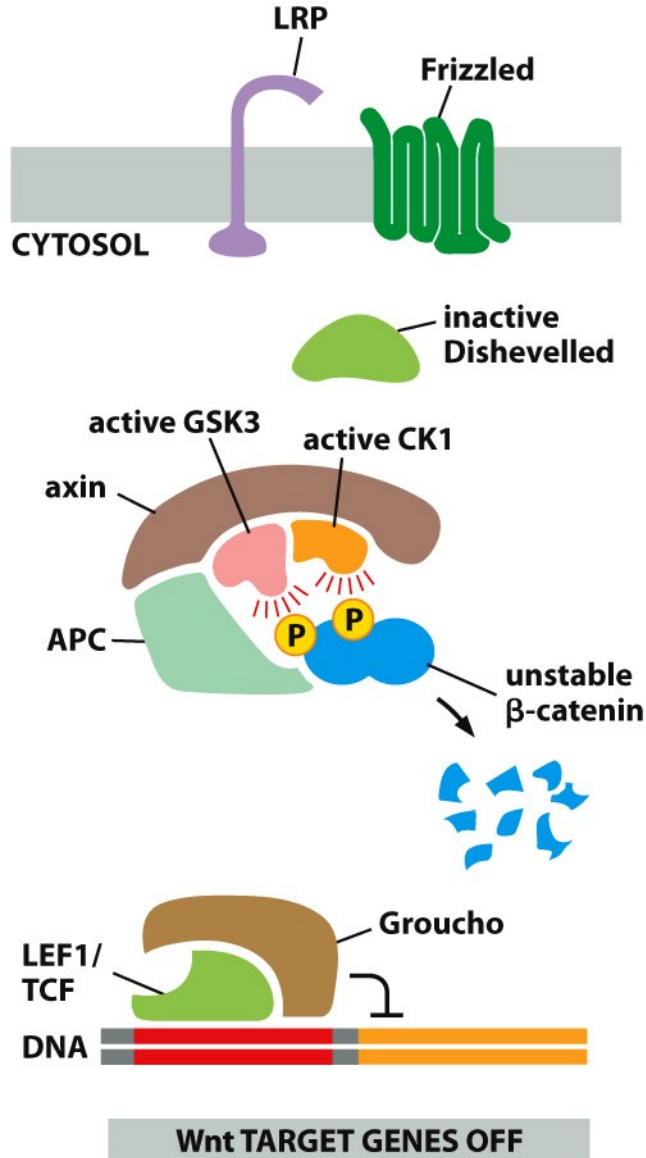


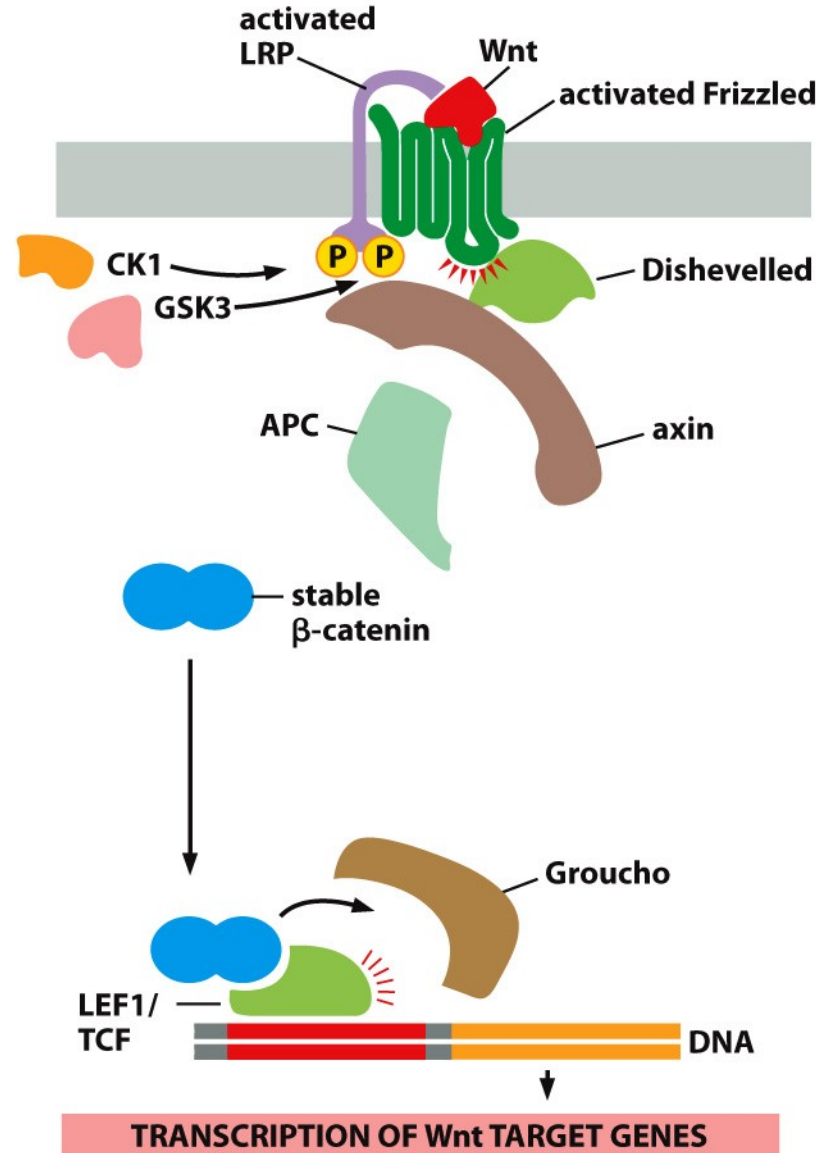
Figure 15-59 Molecular Biology of the Cell 6e (© Garland Science 2015)

Signalizace Wnt

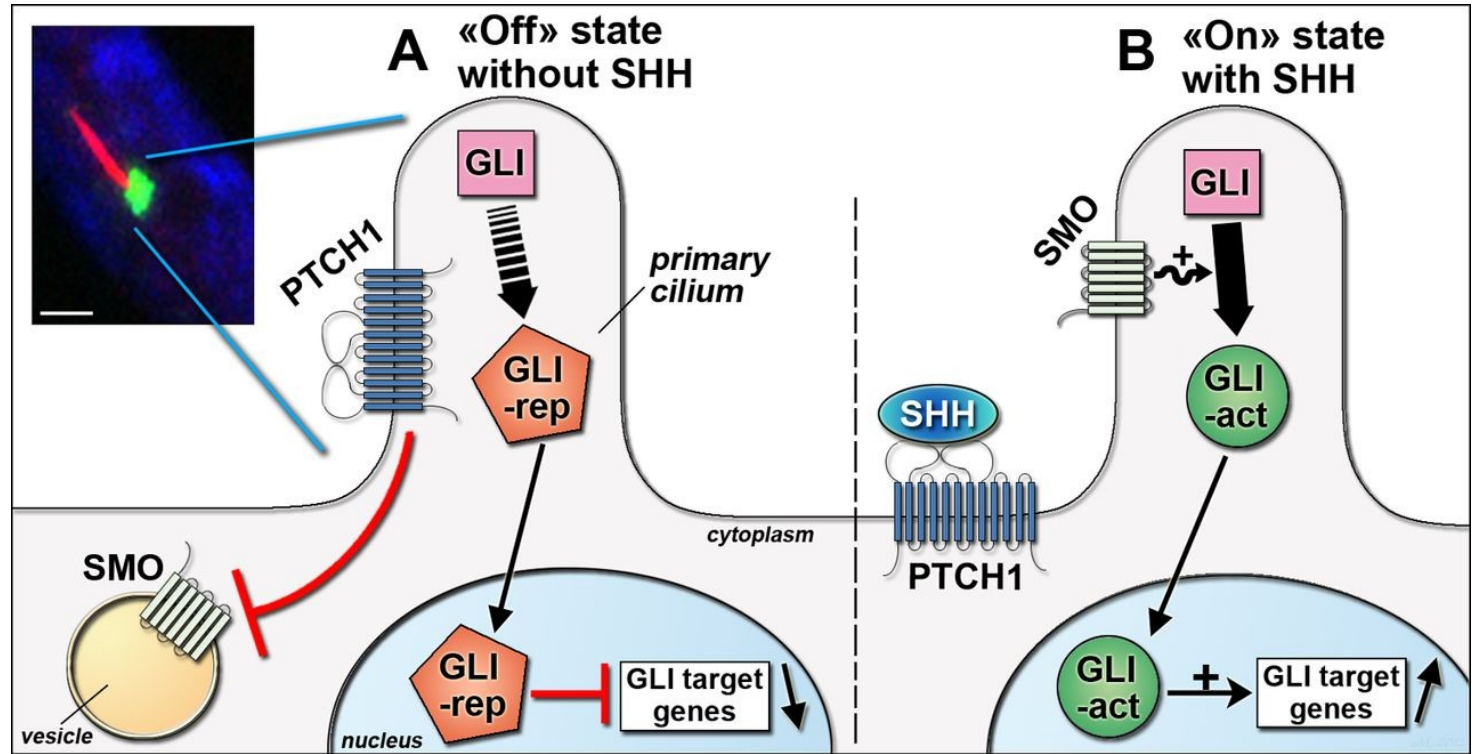
(A) WITHOUT Wnt SIGNAL



(B) WITH Wnt SIGNAL



Signalizace Hedgehog (vyžaduje primární cilium)



SIGNALIZACE

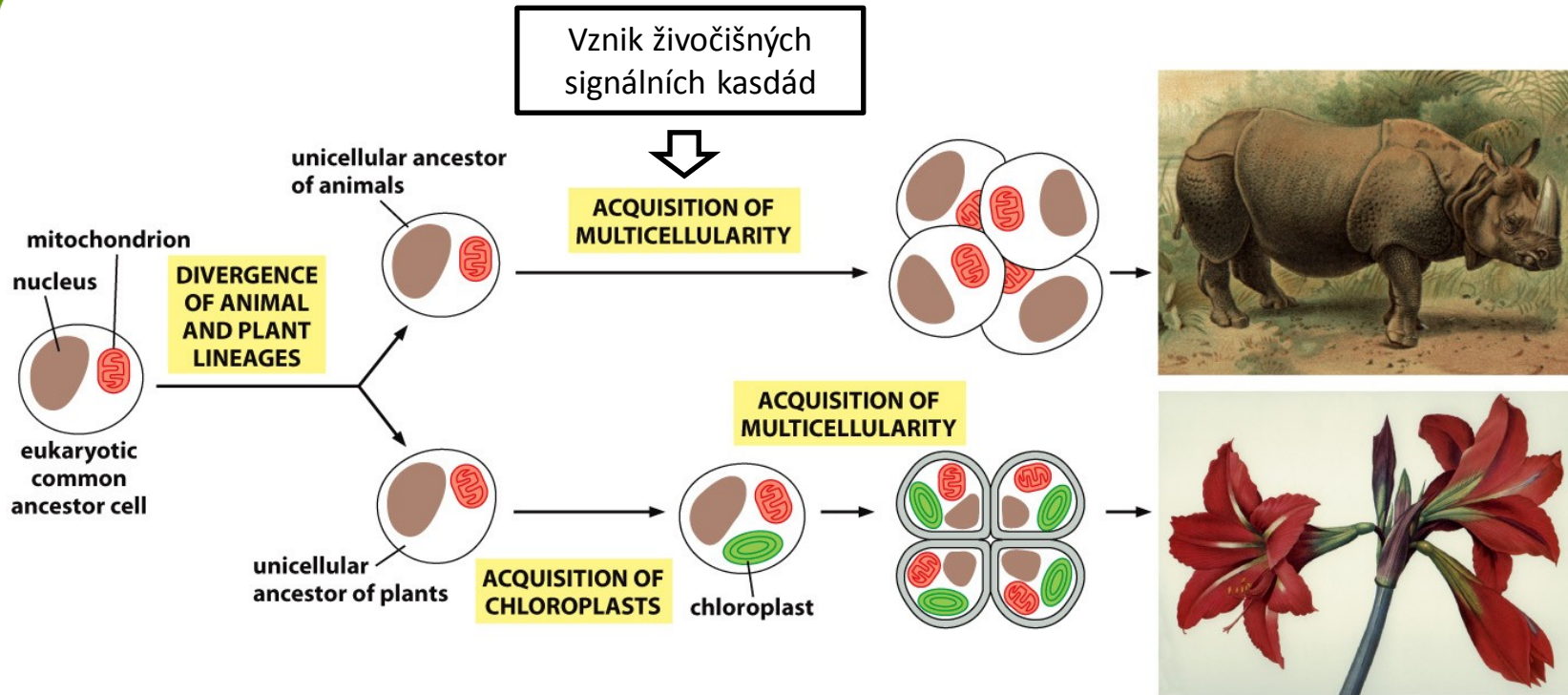


Figure 15-68 Molecular Biology of the Cell 6e (© Garland Science 2015)