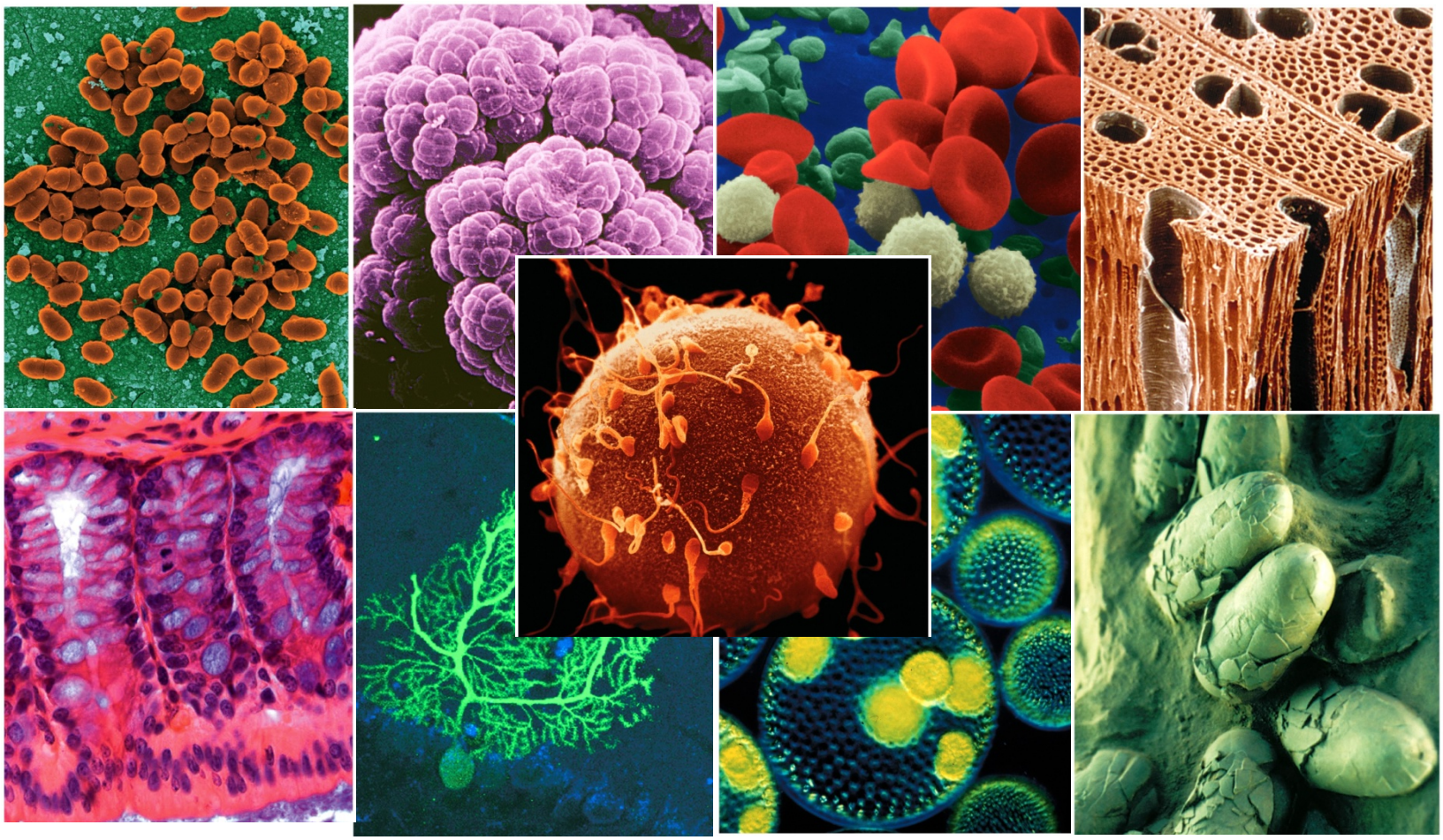
Lesson 14

Cell Signaling

The science of understanding how individual cells sense their environments and respond to stimuli...

... Or: how so many functionally different cells can come from one fertilized egg....?



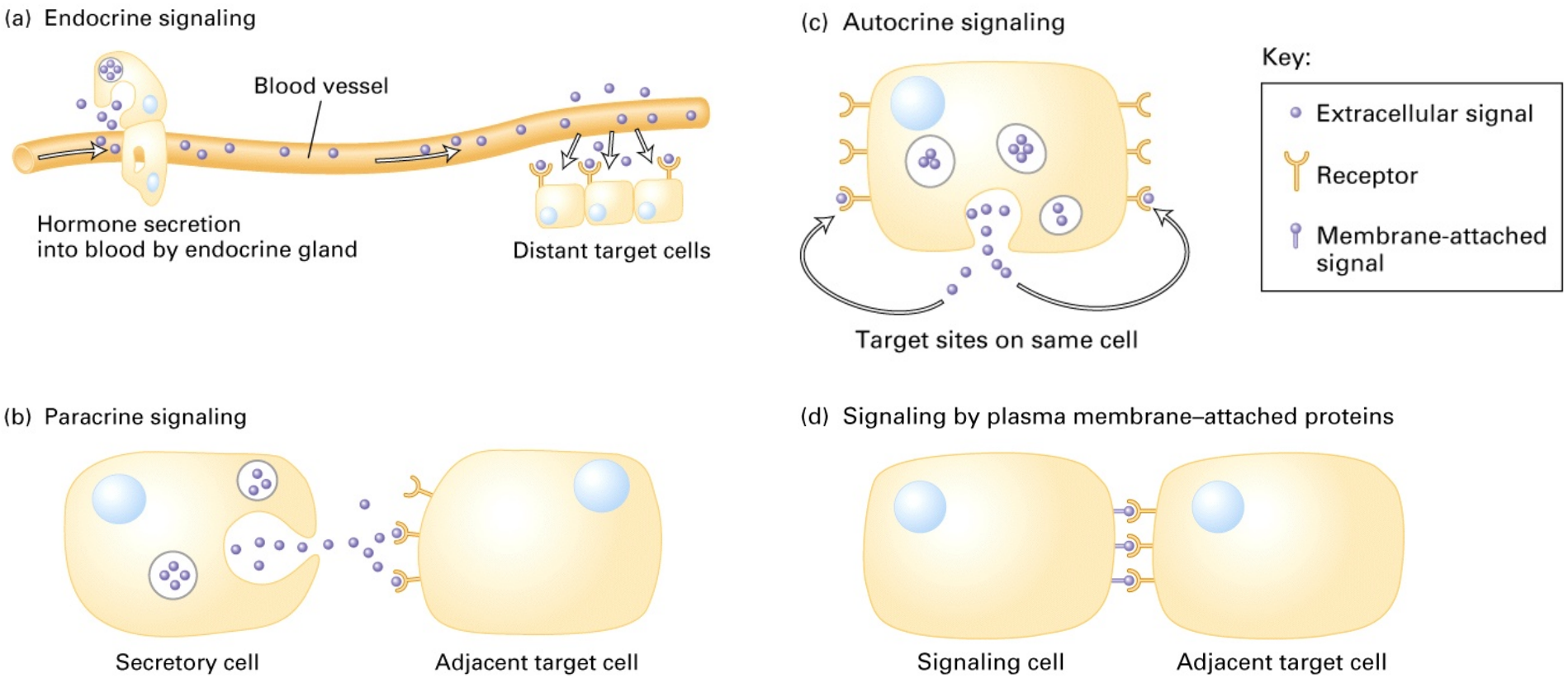
Communication by extracellular signaling usually involves eight steps

1. Synthesis of the signaling molecule by the signaling cell
2. Release of the signaling molecule by the signaling cell
3. Transport of the signal to the target cell
4. Binding of the signal by a specific receptor protein → conformational change
5. Initiation of intracellular signal-transduction pathways by the activated receptor
6. A change in cellular metabolism, function, structure, or development triggered immediately by the receptor-signal complex
7. Usually deactivation of the receptor
8. Removal of the signal, which usually terminates the cellular response

-Additionally:

Receptors also exist that bind and react to metabolites (oxygen, sugar, NO....) or physical stimuli (touch, light, heat....) In which case step 4-8 still apply

Signaling molecules operate over various distances in animals



Receptor proteins exhibit ligand-binding and effector specificity

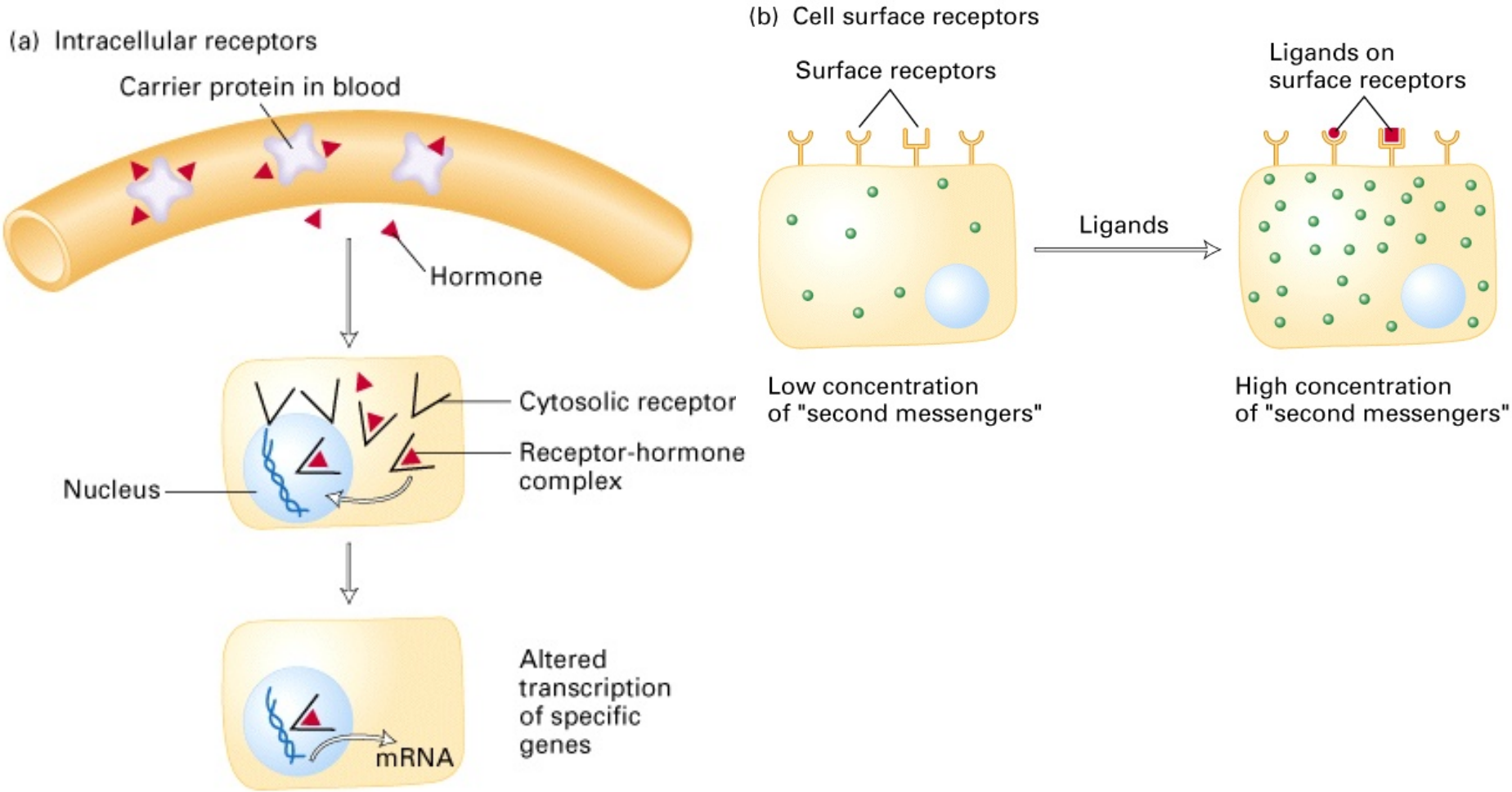
The synthesis, release, and degradation of hormones is regulated

TABLE 20-1 Characteristic Properties of Principal Types of Mammalian Hormones

Property	Steroids	Thyroxine	Peptides and Proteins	Catecholamines
Feedback regulation of synthesis	Yes	Yes	Yes	Yes
Storage of preformed hormone	Very little	Several weeks	One day	Several days, in adrenal medulla
Mechanism of secretion	Diffusion through plasma membrane	Proteolysis of thyroglobulin	Exocytosis of storage vesicles	Exocytosis of storage vesicles
Binding to plasma proteins	Yes	Yes	Rarely	No
Lifetime in blood plasma	Hours	Days	Minutes	Seconds
Time course of action	Hours to days	Days	Minutes to hours	Seconds or less
Receptors	Cytosolic or nuclear	Nuclear	Plasma membrane	Plasma membrane
Mechanism of action	Receptor-hormone complex controls transcription and stability of mRNAs		Hormone binding triggers synthesis of cytosolic second messengers or protein kinase activity	Hormone binding causes change in membrane potential or triggers synthesis of cytosolic second messengers

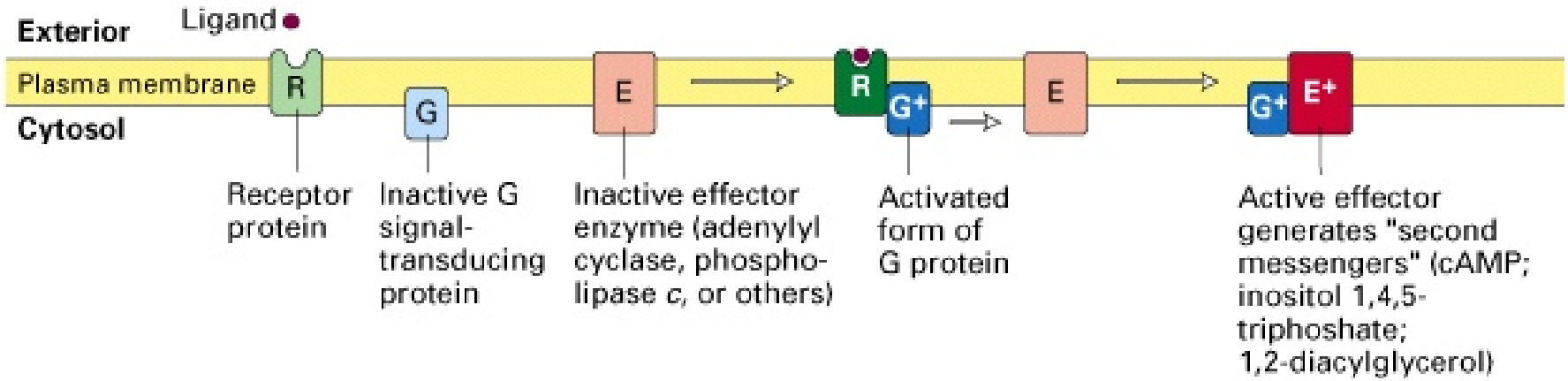
SOURCE: Adapted from E. L. Smith et al., 1983, *Principles of Biochemistry: Mammalian Biochemistry*, 6th ed., McGraw-Hill, p. 358. Reproduced by permission of McGraw-Hill.

Hormones can be classified based on their solubility and receptor location

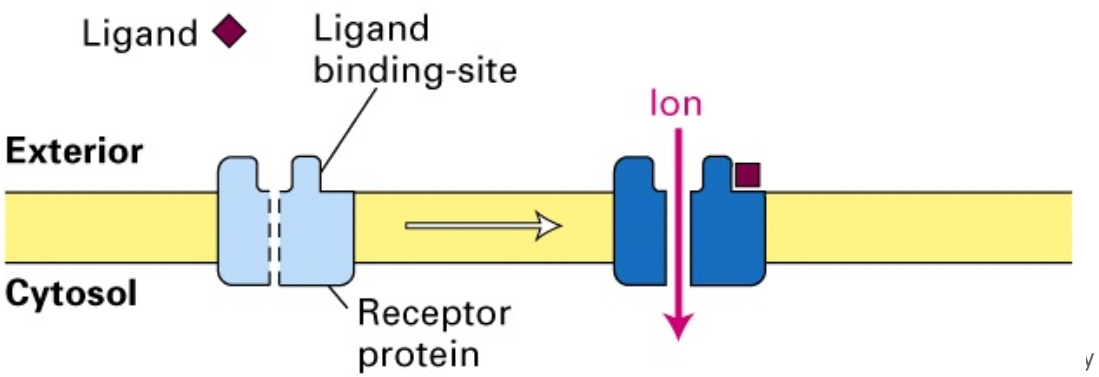


Cell-surface receptors belong to four major classes

(a) **G protein–coupled receptors** (epinephrine, glucagon, serotonin)



(b) **Ion-channel receptors** (acetylcholine)

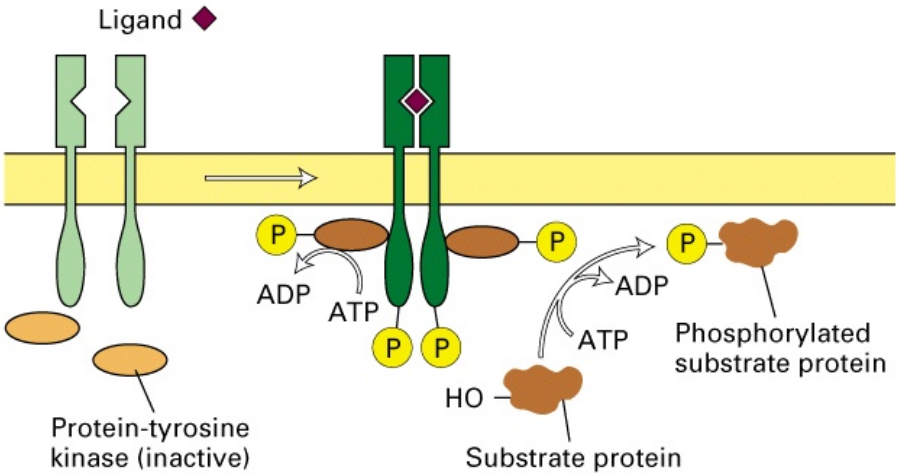


Several subclasses exist giving a total of approx a dozen different classes.

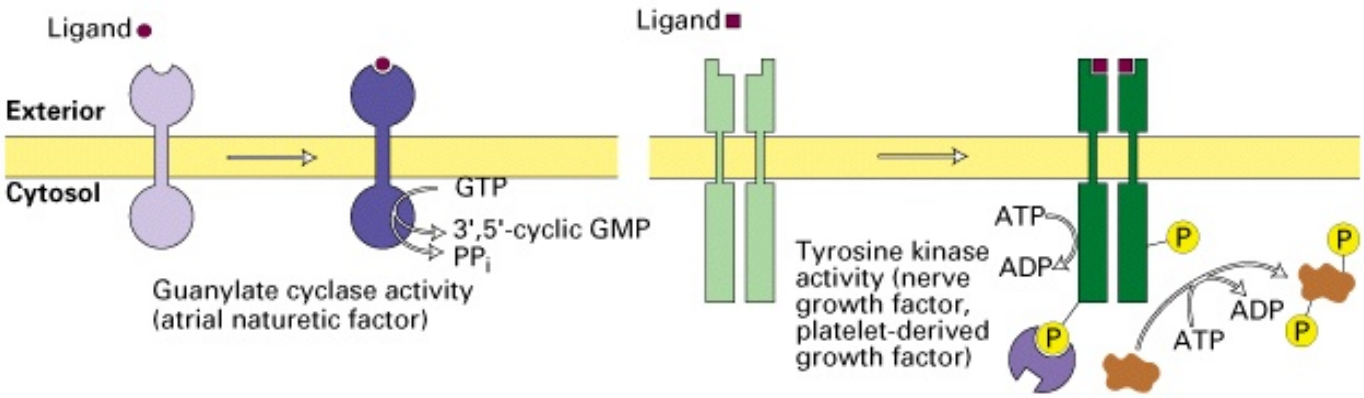
Several hundred variants of the downstream mediators exist, giving a wide and variable array of possible signaling cascades

Cell-surface receptors belong to four major classes

(c) **Tyrosine kinase–linked receptors** (erythropoietin, interferons)

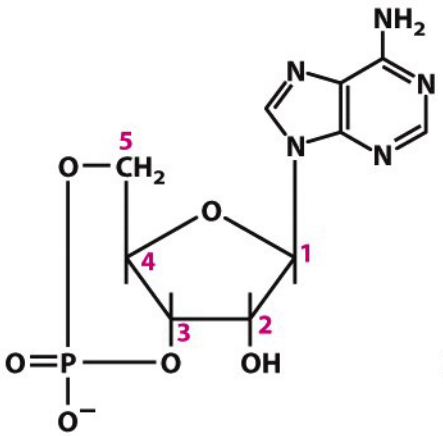


(d) **Receptors with intrinsic enzymatic activity**



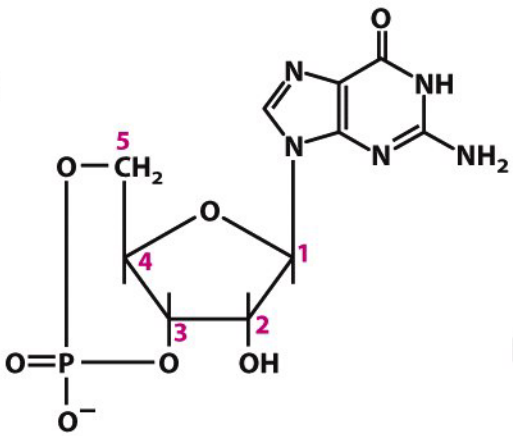
The effects of many hormones are mediated by

second messengers



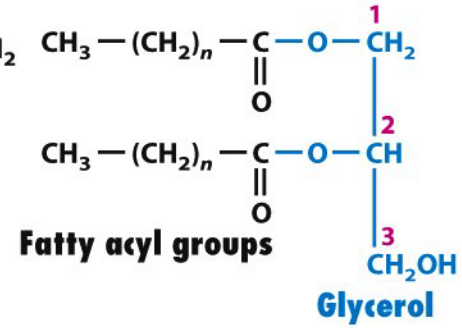
3′,5′-Cyclic AMP (cAMP)

Activates protein kinase A (PKA)



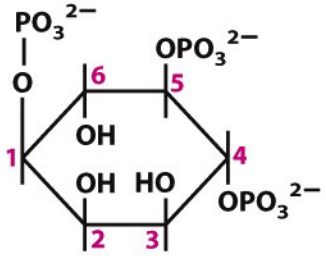
3′,5′-Cyclic GMP (cGMP)

Activates protein kinase G (PKG) and opens cation channels in rod cells



1,2-Diacylglycerol (DAG)

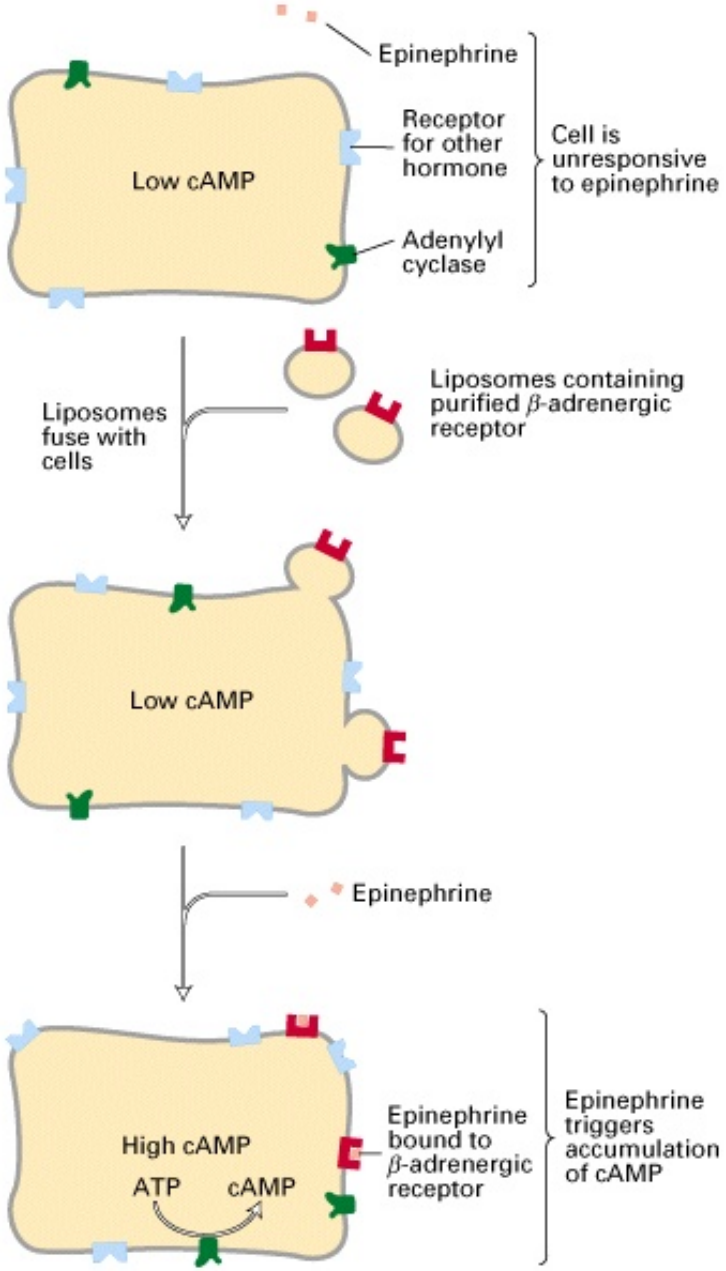
Activates protein kinase C (PKC)



Inositol 1,4,5-trisphosphate (IP_3)

Opens Ca^{2+} channels in the endoplasmic reticulum

Demonstration that receptors mediate the induction of cAMP synthesis



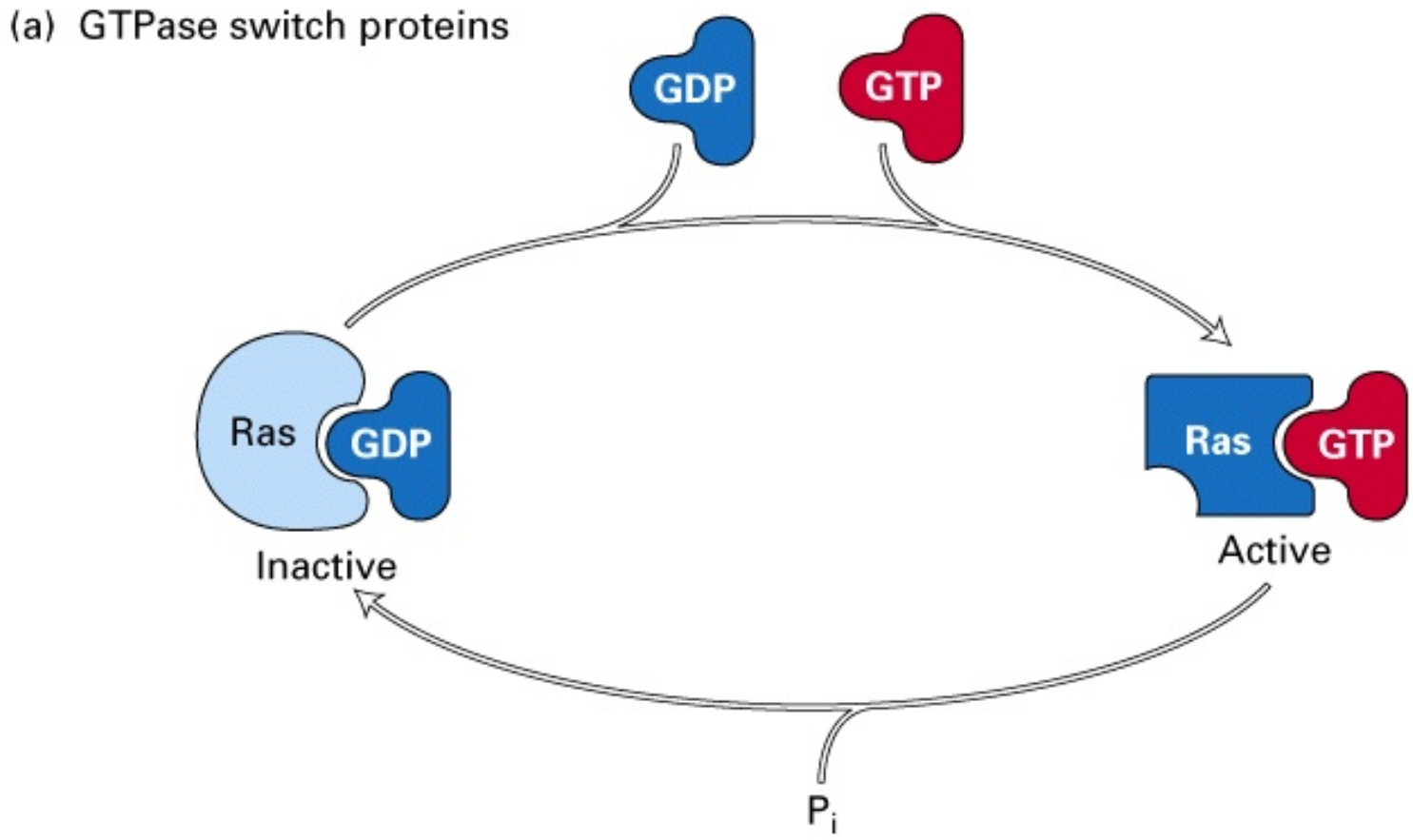
Cellular responses to cAMP vary among different cell types

TABLE 20-3 Metabolic Responses to Hormone-Induced Rise in cAMP in Various Tissues

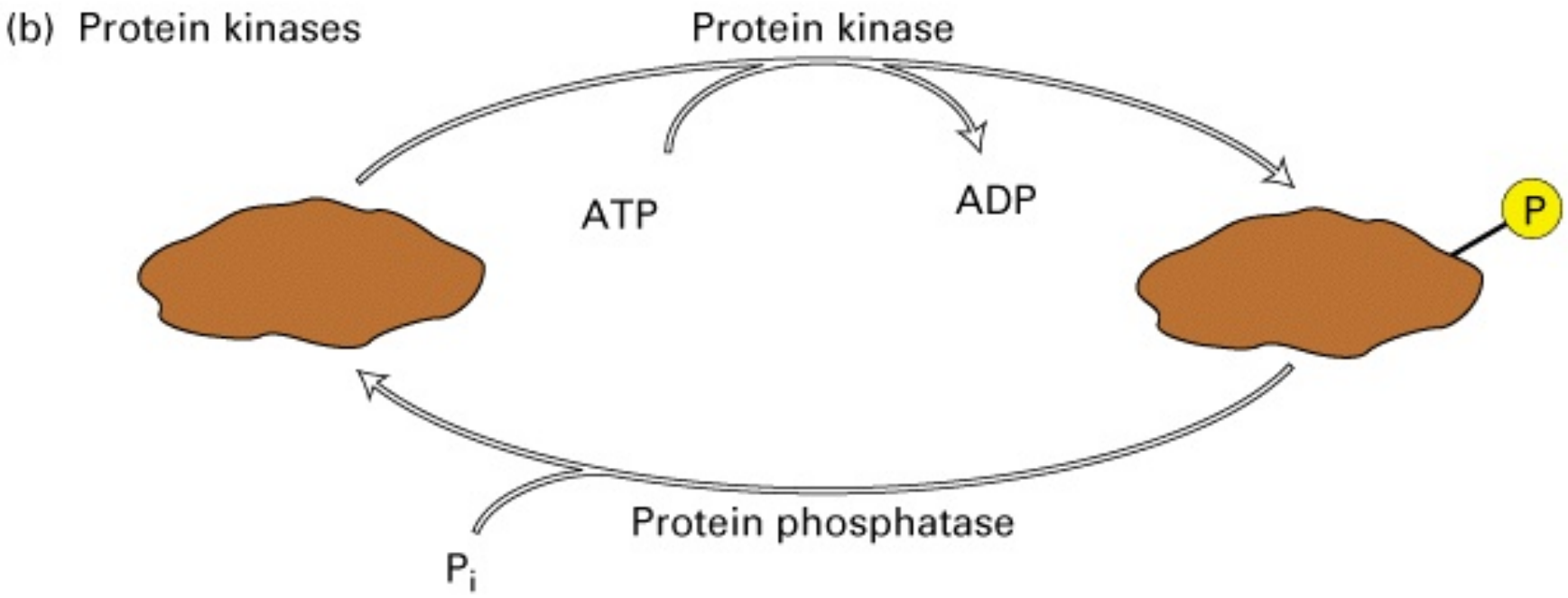
Tissue	Hormone Inducing Rise in cAMP	Metabolic Response
Adipose	Epinephrine; ACTH; glucagon	Increase in hydrolysis of triglyceride; decrease in amino acid uptake
Liver	Epinephrine; norepinephrine; glucagon	Increase in conversion of glycogen to glucose; inhibition of synthesis of glycogen; increase in amino acid uptake; increase in gluconeogenesis (synthesis of glucose from amino acids)
Ovarian follicle	FSH; LH	Increase in synthesis of estrogen, progesterone
Adrenal cortex	ACTH	Increase in synthesis of aldosterone, cortisol
Cardiac muscle cells	Epinephrine	Increase in contraction rate
Thyroid	TSH	Secretion of thyroxine
Bone cells	Parathyroid hormone	Increase in resorption of calcium from bone
Skeletal muscle	Epinephrine	Conversion of glycogen to glucose
Intestine	Epinephrine	Fluid secretion
Kidney	Vasopressin	Resorption of water
Blood platelets	Prostaglandin I	Inhibition of aggregation and secretion

SOURCE: E. W. Sutherland, 1972, *Science* **177**:401.

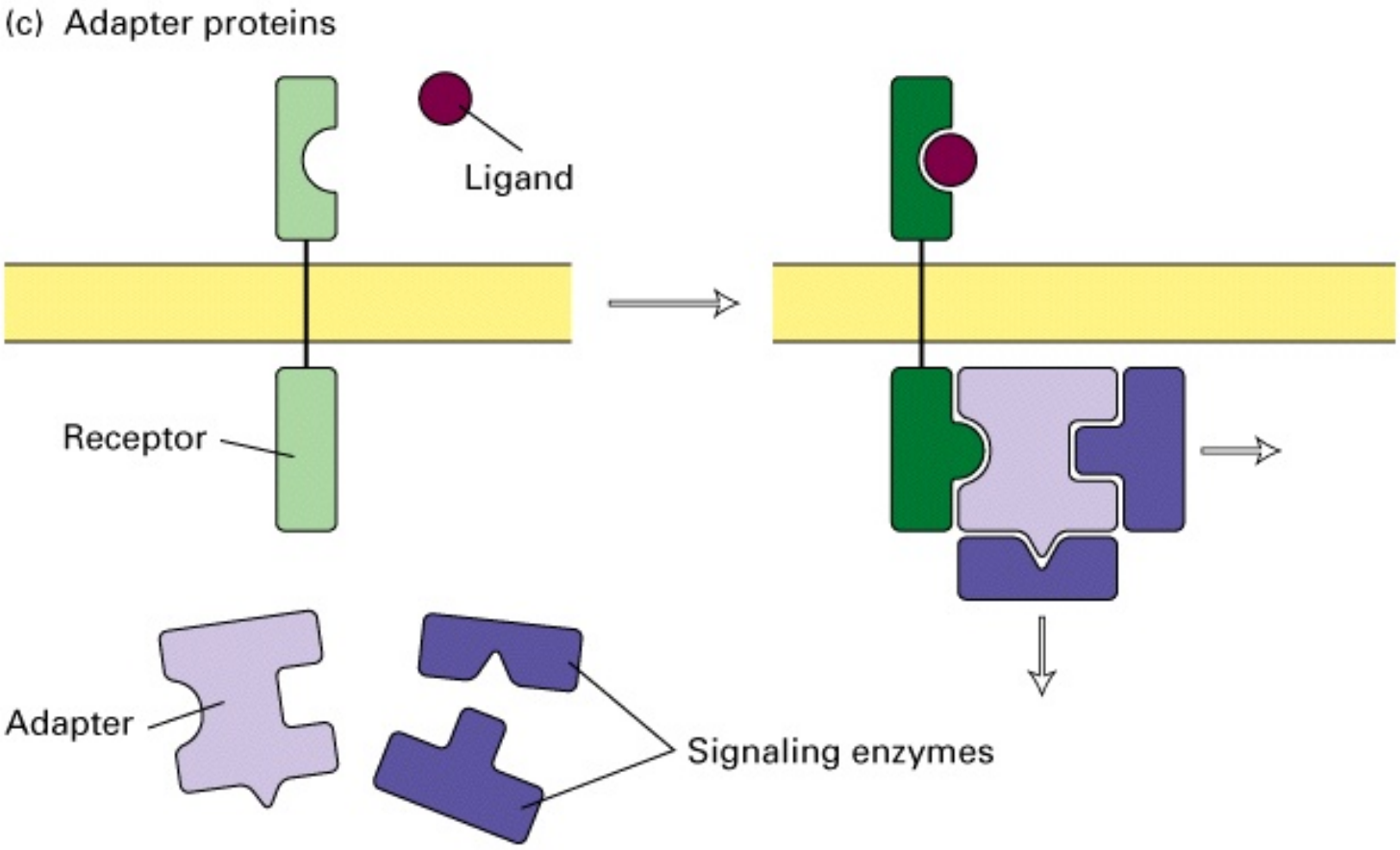
Other conserved proteins function in signal transduction: **GTPase switch proteins**



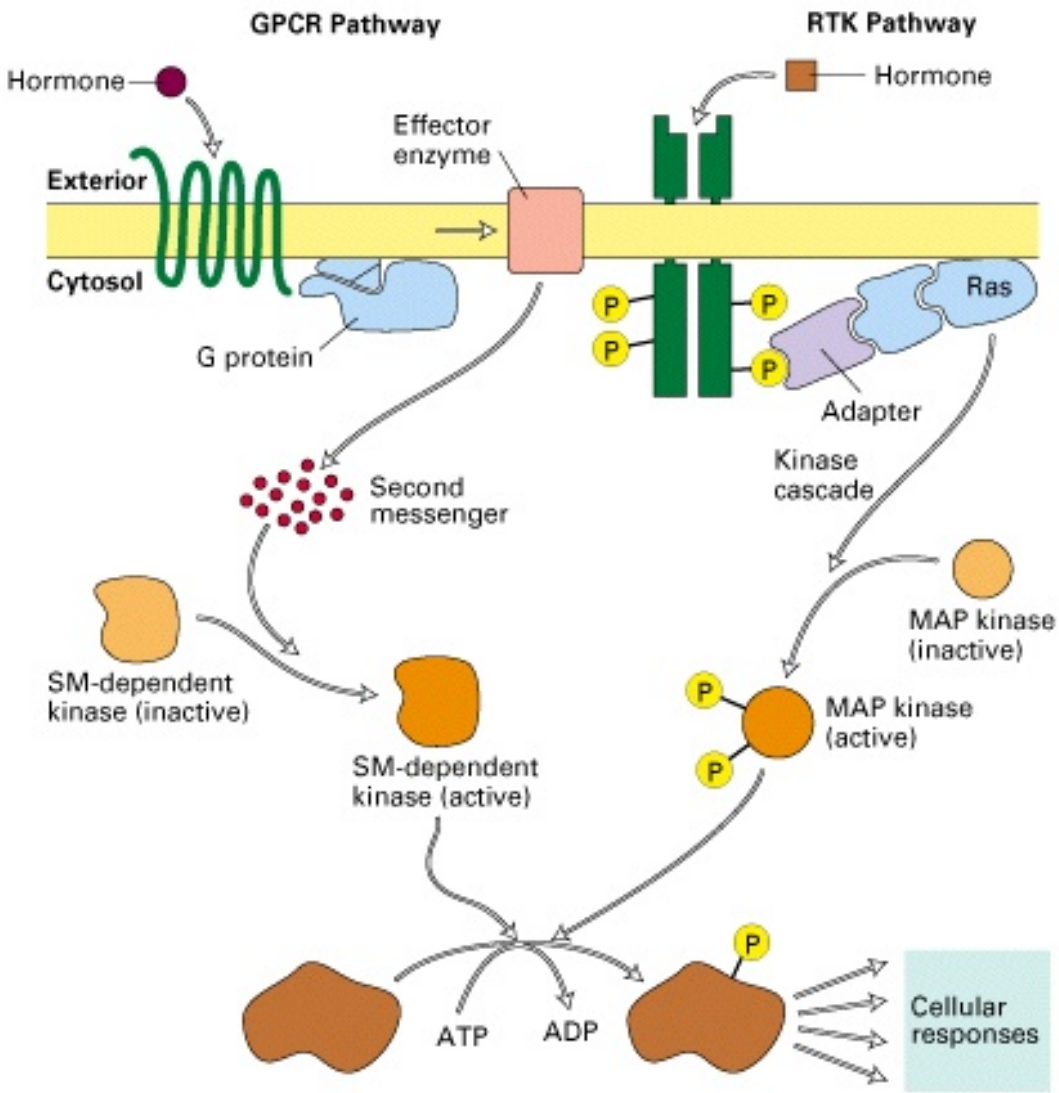
Other conserved proteins function in signal transduction: **protein kinases**



Other conserved proteins function in signal transduction: **adapter proteins**

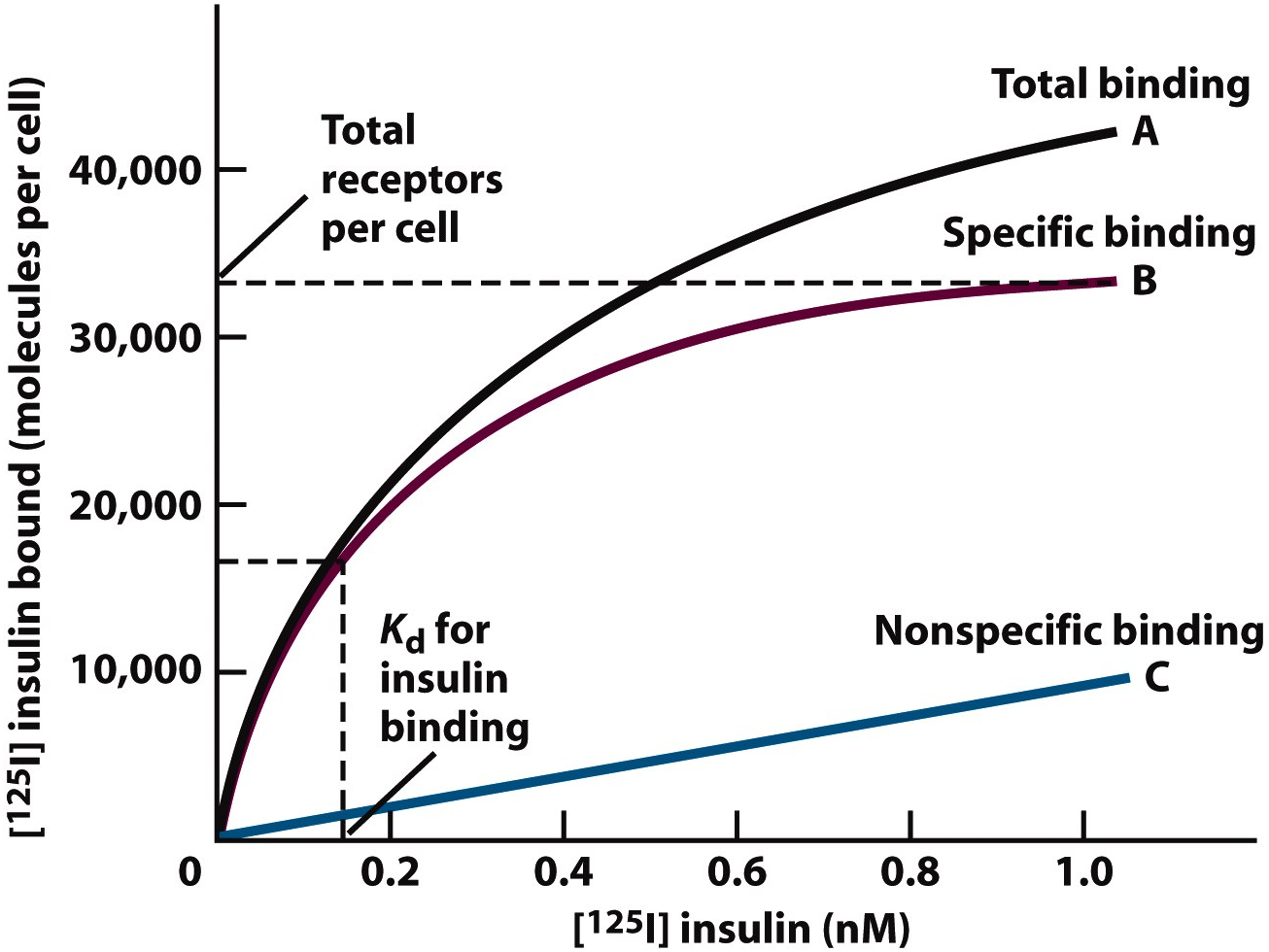


Common signaling pathways are targeted by different types of receptors

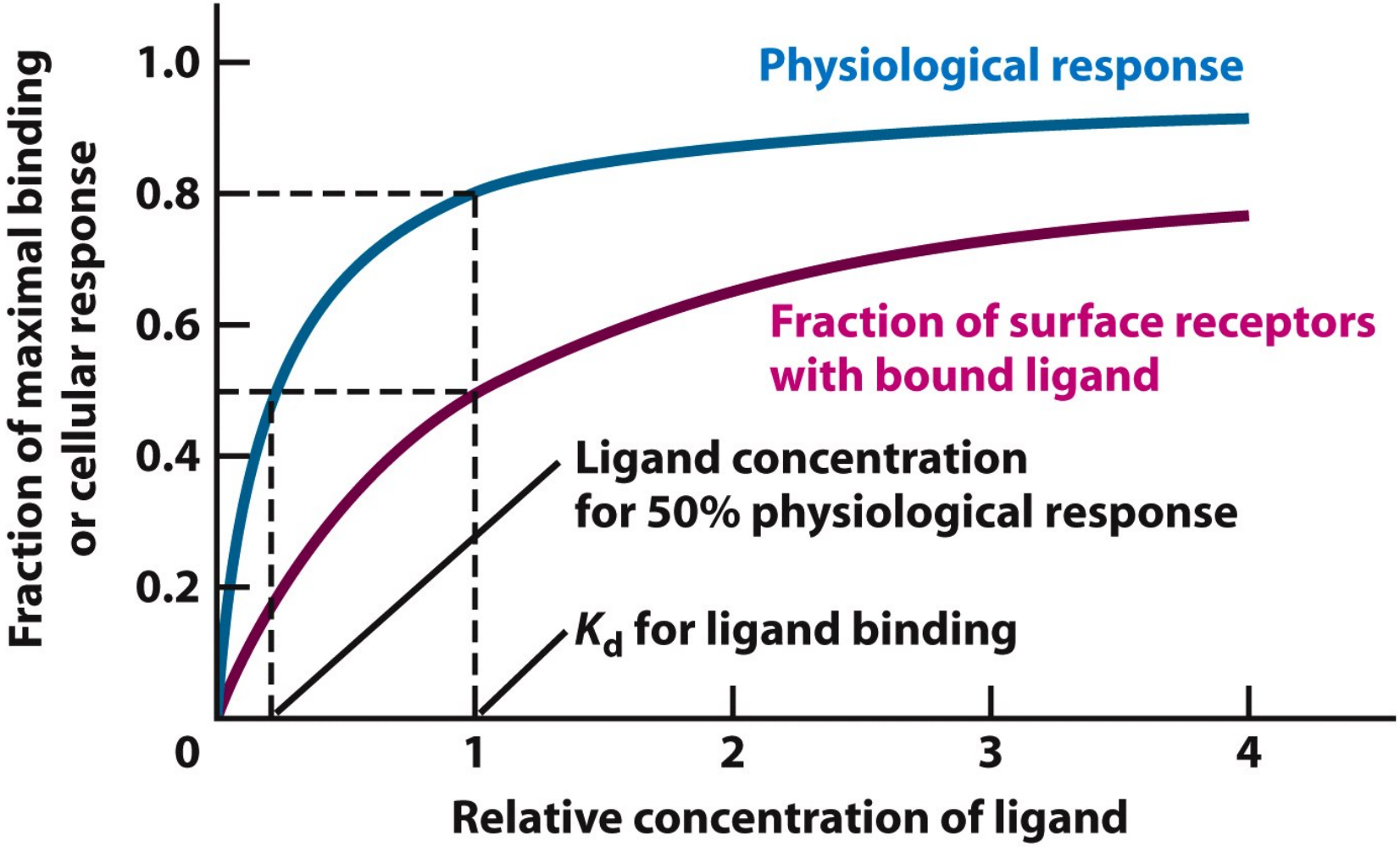


Identification and purification of cell-surface receptors

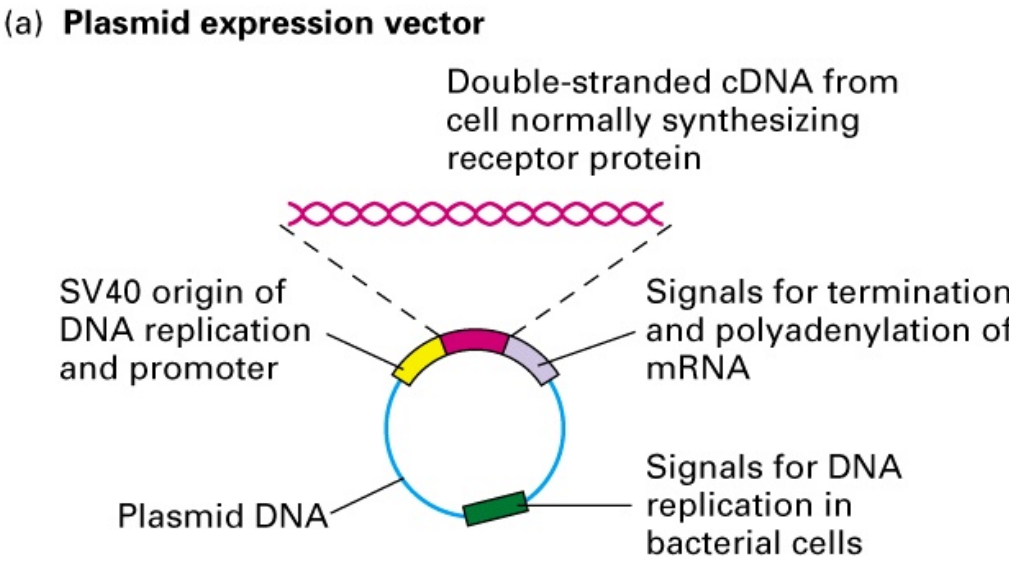
Hormone receptors are detected by binding assays

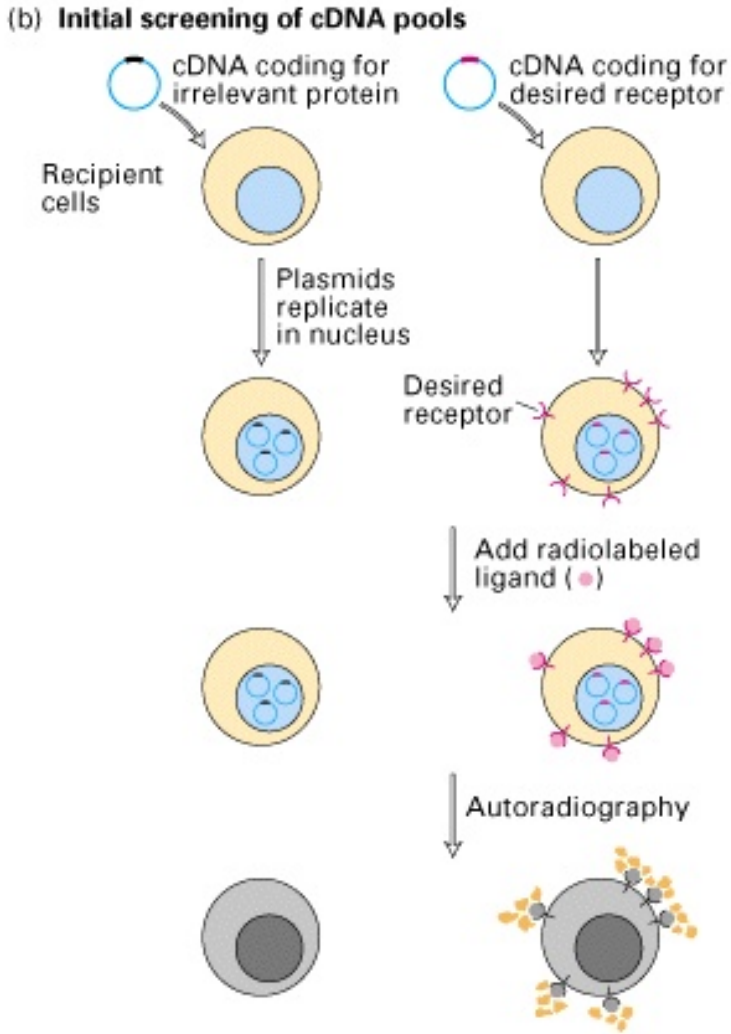


Maximal physiological response occurs when only a fraction of receptors are bound



Many receptors can be cloned without prior purification



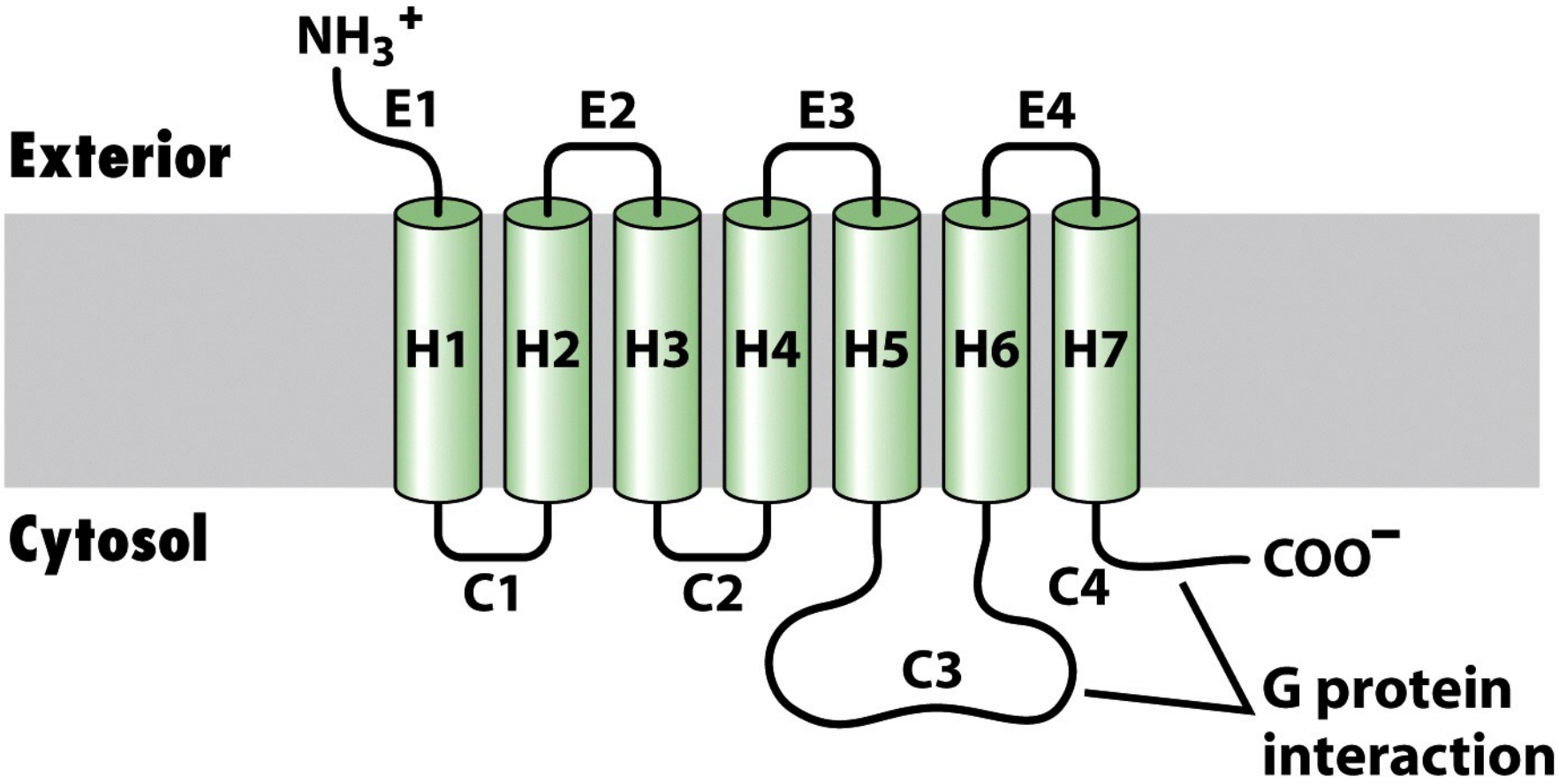


Receptors proteins also are purified by affinity techniques

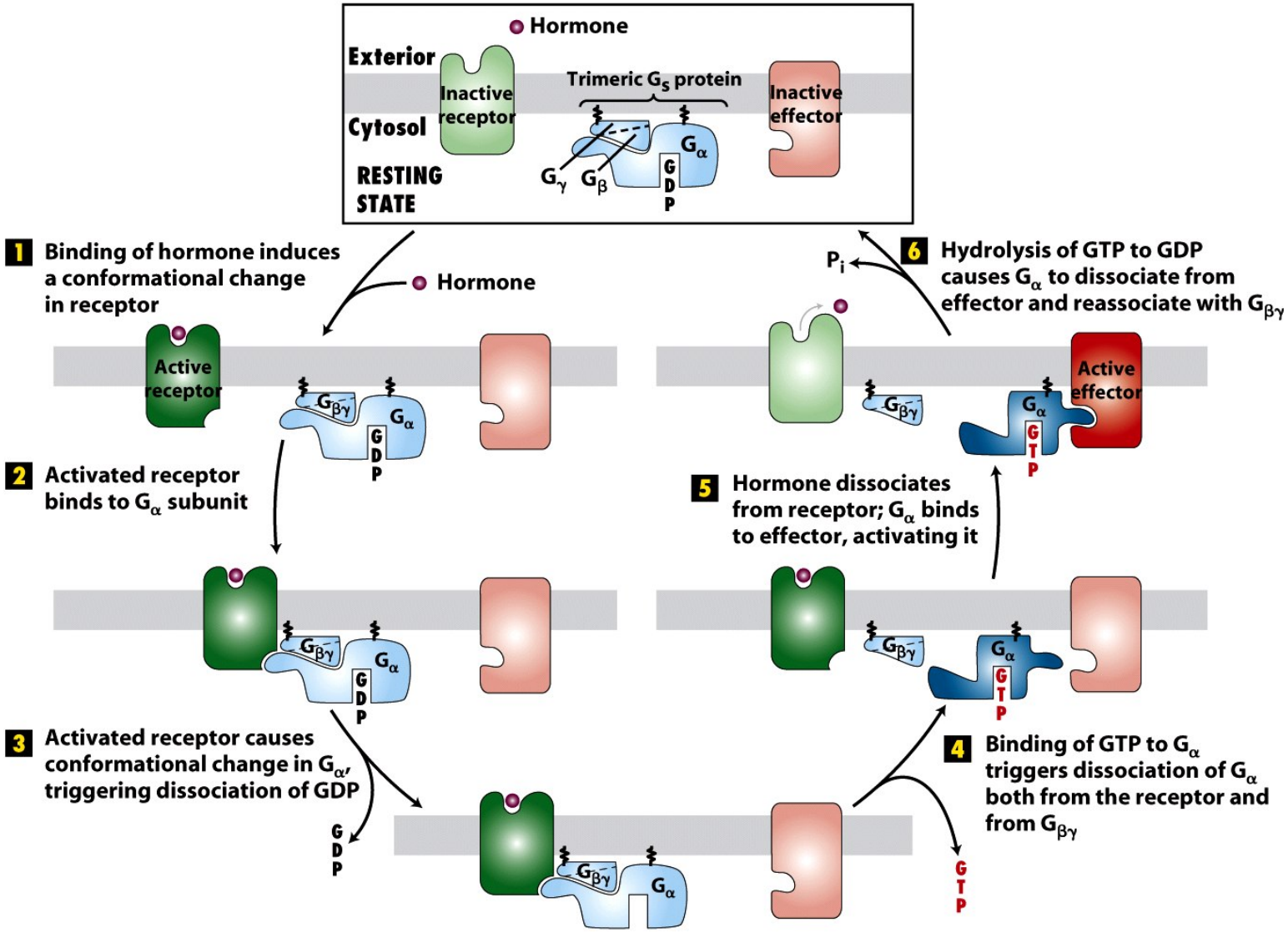
G protein-coupled receptors (GPCRs) and their effectors

- Many different mammalian cell-surface receptors are coupled to a trimeric signal-transducing G protein
- Ligand binding activates the receptor, which activates the G protein, which activates an effector enzyme to generate an intracellular second messenger
- All G protein-coupled receptors (GPCRs) contain **seven membrane-spanning regions** (= signature motif) with their N-terminus on the exoplasmic face and C-terminus on the cytosolic face
- GPCRs are involved in a range of signaling pathways, including light detection, odorant detection, and detection of certain hormones and neurotransmitters

Structure of GPCRs



Trimeric G proteins links GPCRs to an effector



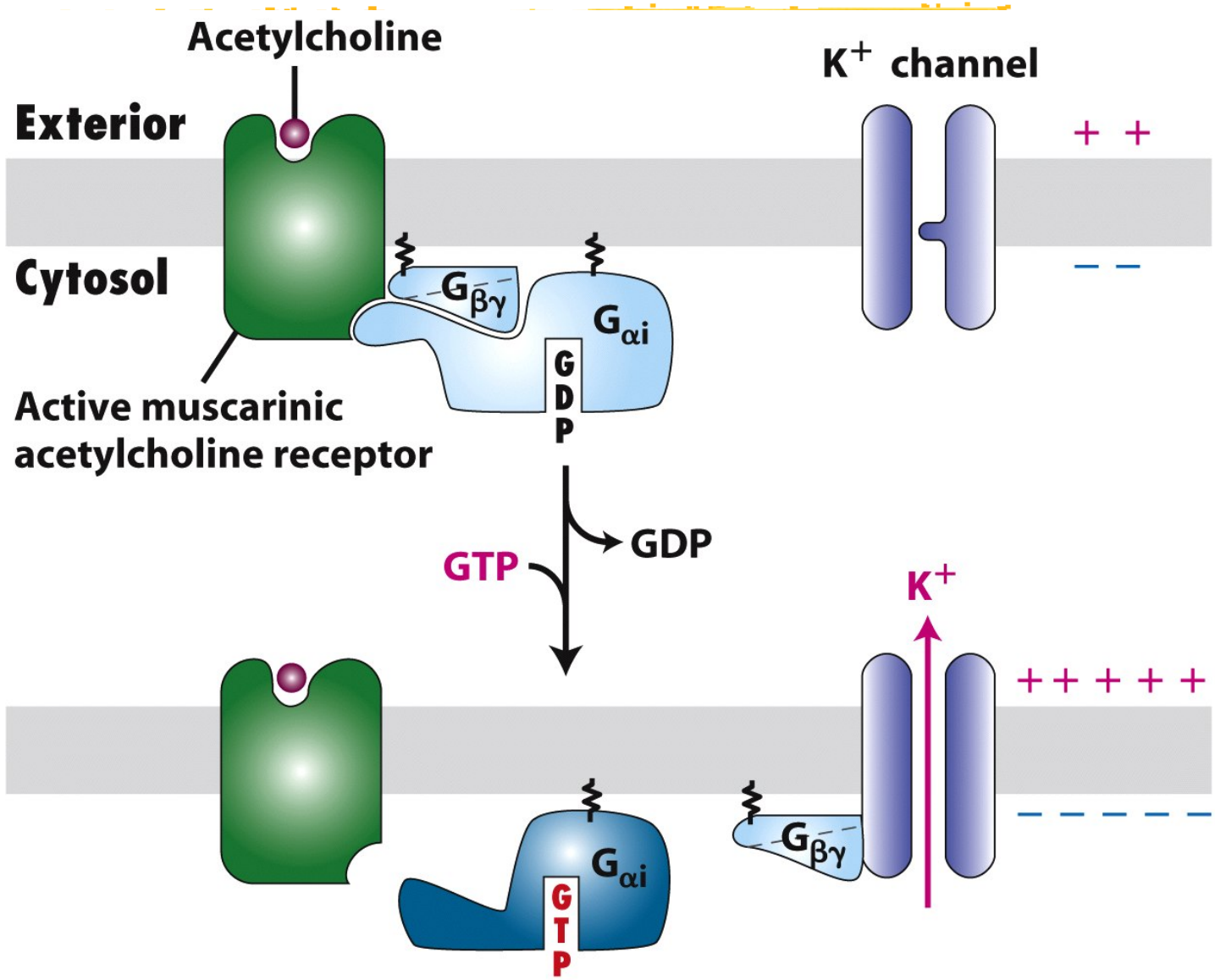
Different G proteins are regulated from different GPCRs

TABLE 15-1 Major Classes of Mammalian Trimeric G Proteins and Their Effectors*

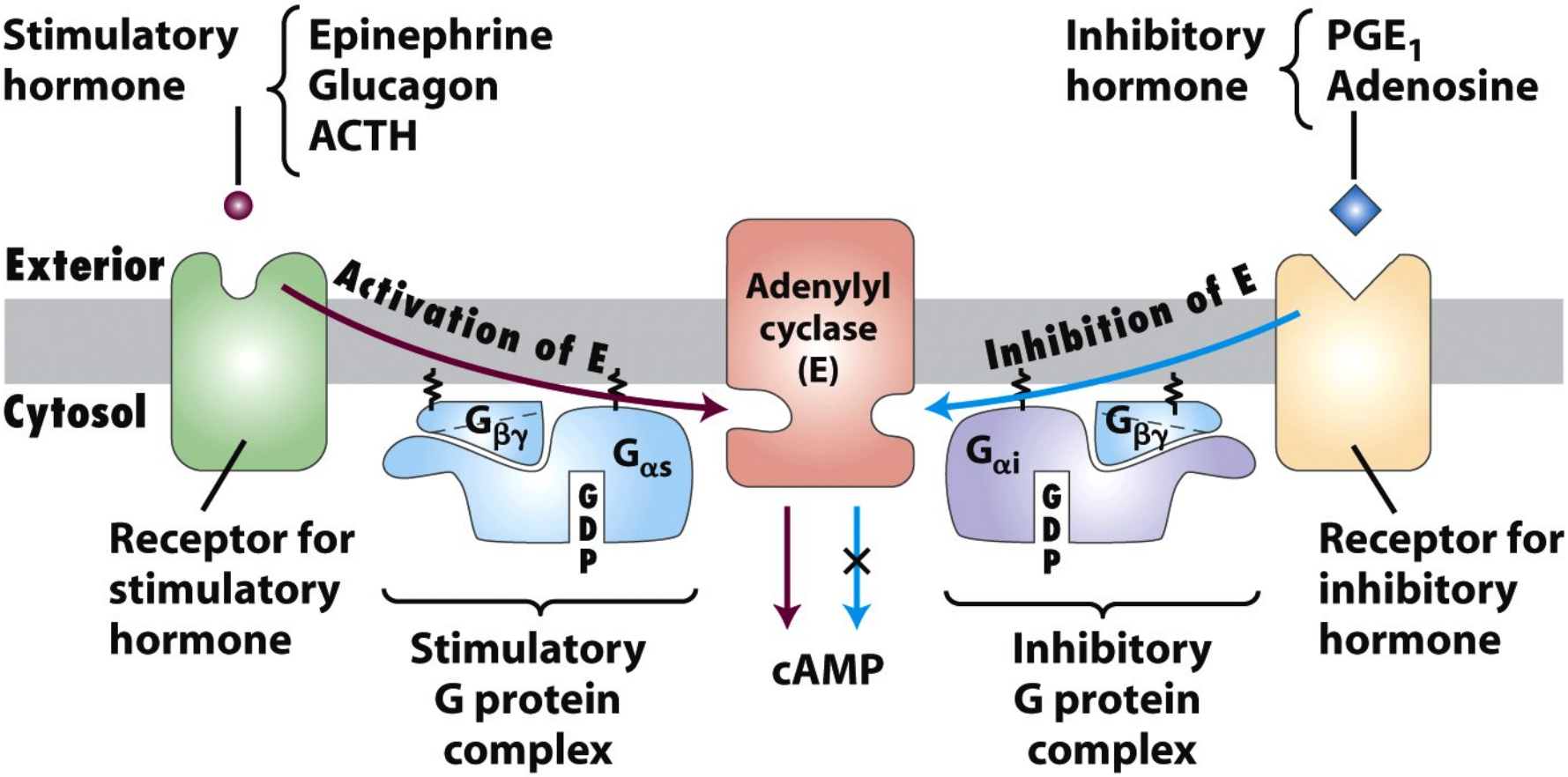
G_α CLASS	ASSOCIATED EFFECTOR	2ND MESSENGER	RECEPTOR EXAMPLES
$G_{\alpha s}$	Adenylyl cyclase	cAMP (increased)	β-Adrenergic (epinephrine) receptor; receptors for glucagon, serotonin, vasopressin
$G_{\alpha i}$	Adenylyl cyclase K^+ channel ($G_{\beta\gamma}$ activates effector)	cAMP (decreased) Change in membrane potential	α_2-Adrenergic receptor Muscarinic acetylcholine receptor
$G_{\alpha olf}$	Adenylyl cyclase	cAMP (increased)	Odorant receptors in nose
$G_{\alpha q}$	Phospholipase C	IP_3, DAG (increased)	α_1-Adrenergic receptor
$G_{\alpha o}$	Phospholipase C	IP_3, DAG (increased)	Acetylcholine receptor in endothelial cells
$G_{\alpha t}$	cGMP phosphodiesterase	cGMP (decreased)	Rhodopsin (light receptor) in rod cells

*A given G_α subclass may be associated with more than one effector protein. To date, only one major $G_{\alpha s}$ has been identified, but multiple $G_{\alpha q}$ and $G_{\alpha i}$ proteins have been described. Effector proteins commonly are regulated by G_α but in some cases by $G_{\beta\gamma}$ or the combined action of G_α and $G_{\beta\gamma}$.
IP_3 = inositol 1,4,5-trisphosphate; DAG = 1,2-diacylglycerol.
SOURCES: See L. Birnbaumer, 1992, *Cell* **71**:1069; Z. Farfel et al., 1999, *New Eng. J. Med.* **340**:1012; and K. Pierce et al., 2002, *Nature Rev. Mol. Cell Biol.* **3**:639.

The effectors of GPCRs are either ion channels…

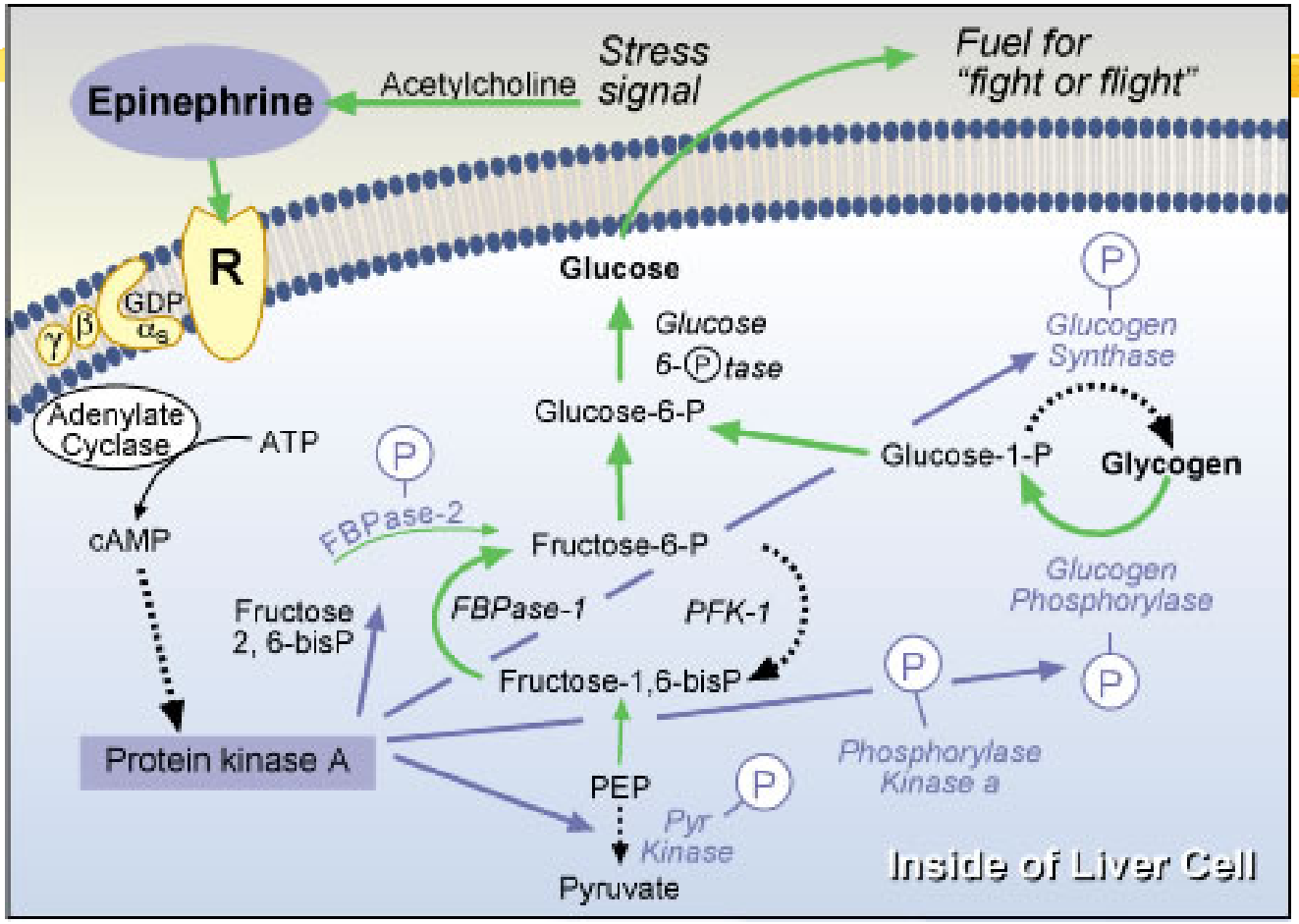


…or enzymes generating second messengers



Adenylyl cyclase is stimulated and inhibited by different receptor-ligand complexes

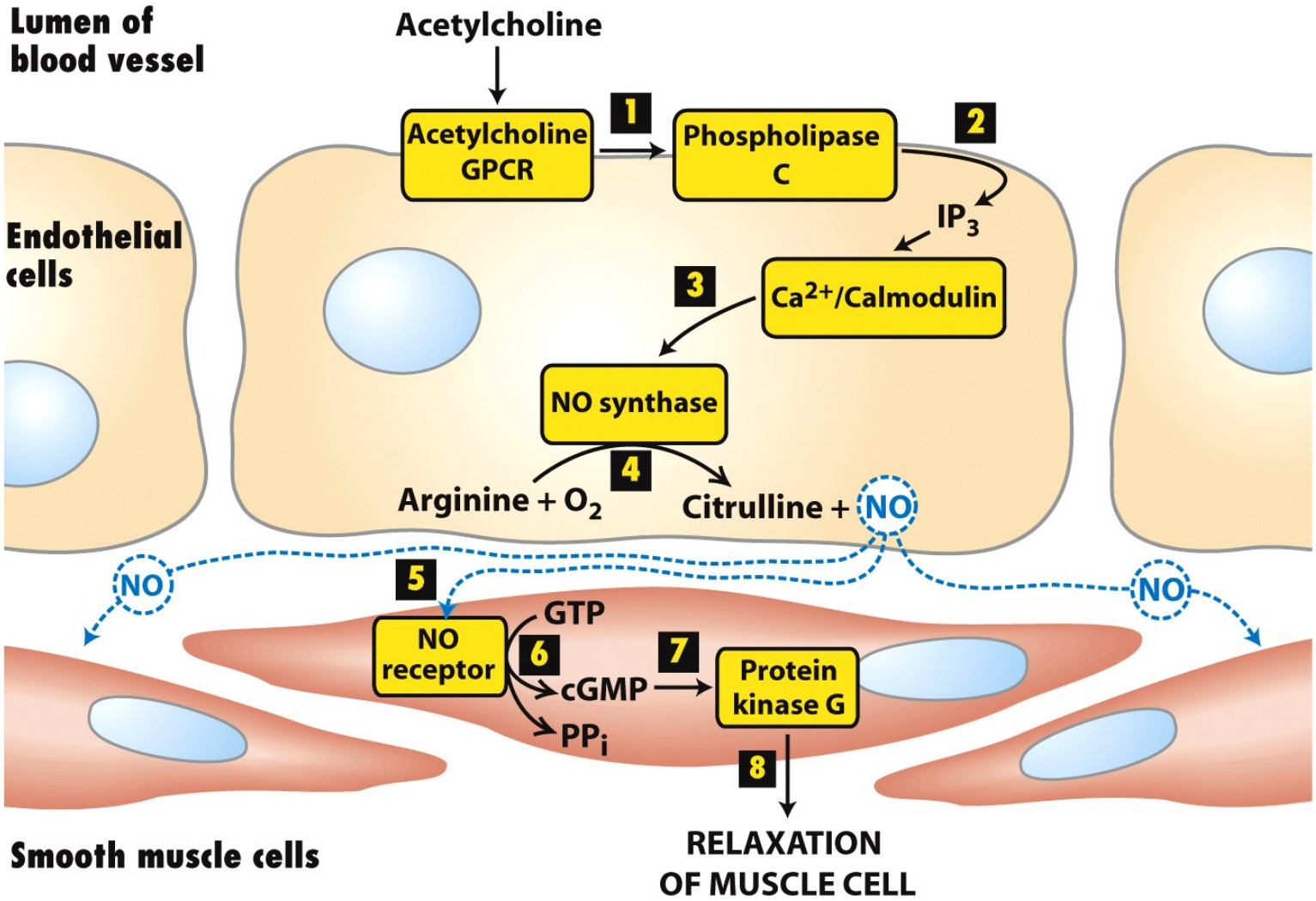
Example1: β-adrenergic receptors



Epinephrine is inducing the „fight-or-flight“ response

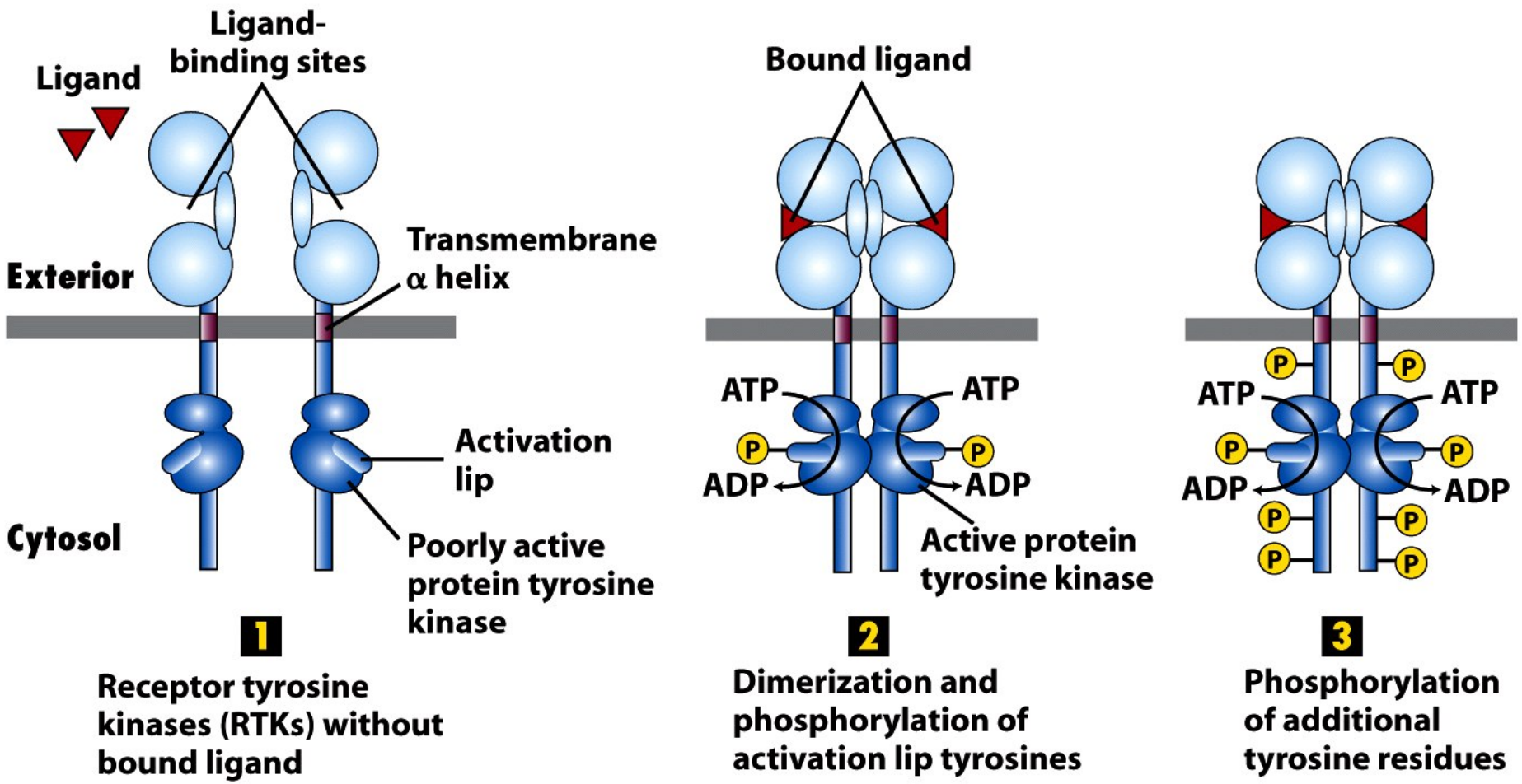
Effector: Adenylyl cyclase; 2nd messenger: cAMP;
Activates: Protein Kinase A

Example2: Nitric oxide/cGMP pathway in arterial smooth muscle cells

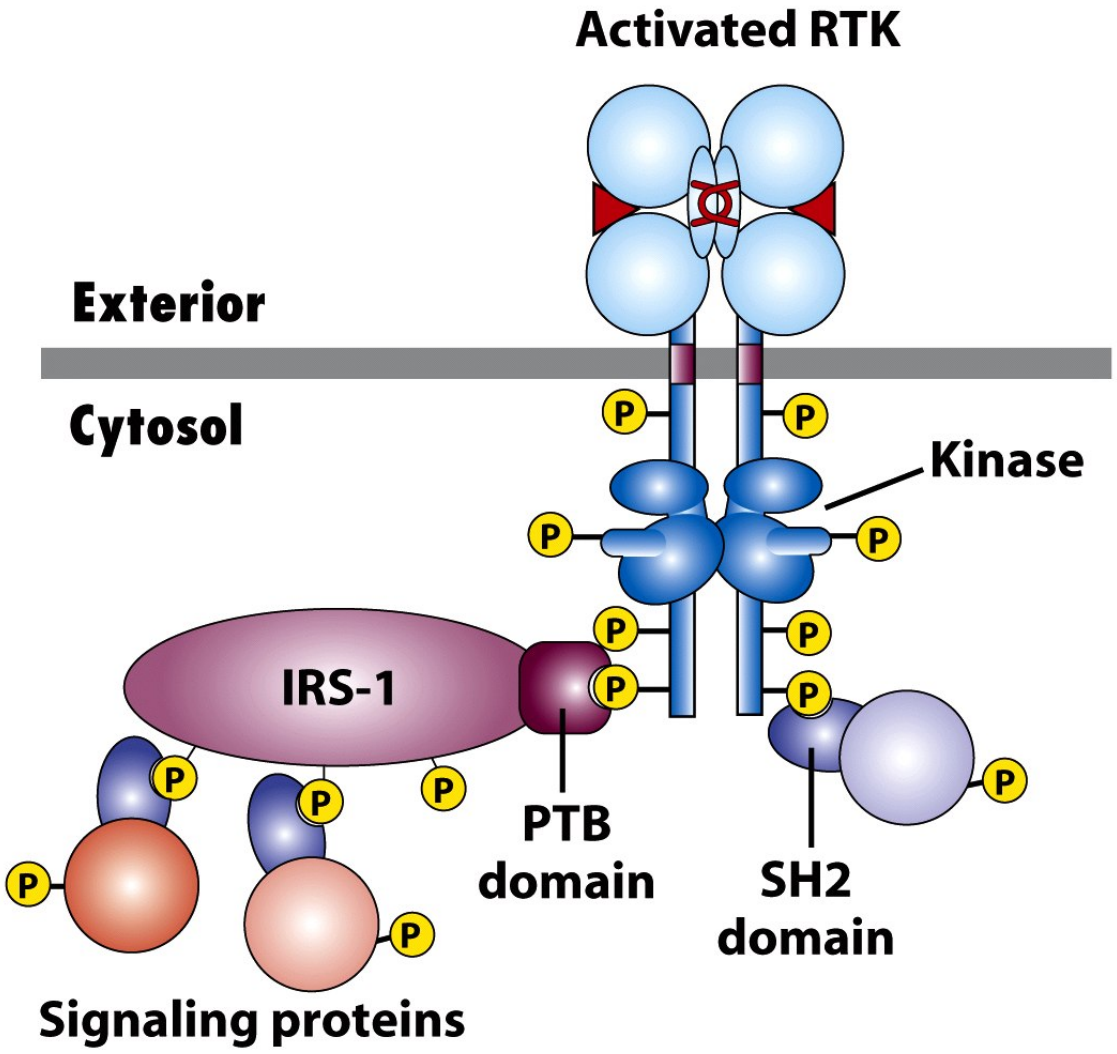


Effector: Phospholipase C; 2nd messenger: Ca^{2+};
Activates: Calmodulin/NO Synthase

Ligand binding leads to autophosphorylation of RTKs



Intracellular signal-transduction proteins are activated by activated RTKs



Examples of signaling pathways and their response to proper physiological stimuli

- Insulin and the regulatin of blood glucose homeostasis
- Regulation of glycogenolysis
- mTOR pathway
- MAP kinase signaling pathway
- CREB pathway
- NF-κB signaling pathway

Interaction and regulation of signaling pathways

- The effects of activation of GPCRs and RTKs is more branched* than a simple step-by-step cascade
- Stimulation of either GPCRs or RTKs often leads to production of multiple second messengers, and both types of receptors promote or inhibit production of many of the same second messengers
- The same cellular response may be induced by multiple signaling pathways
- Interaction of different signaling pathways permits fine-tuning of cellular activities

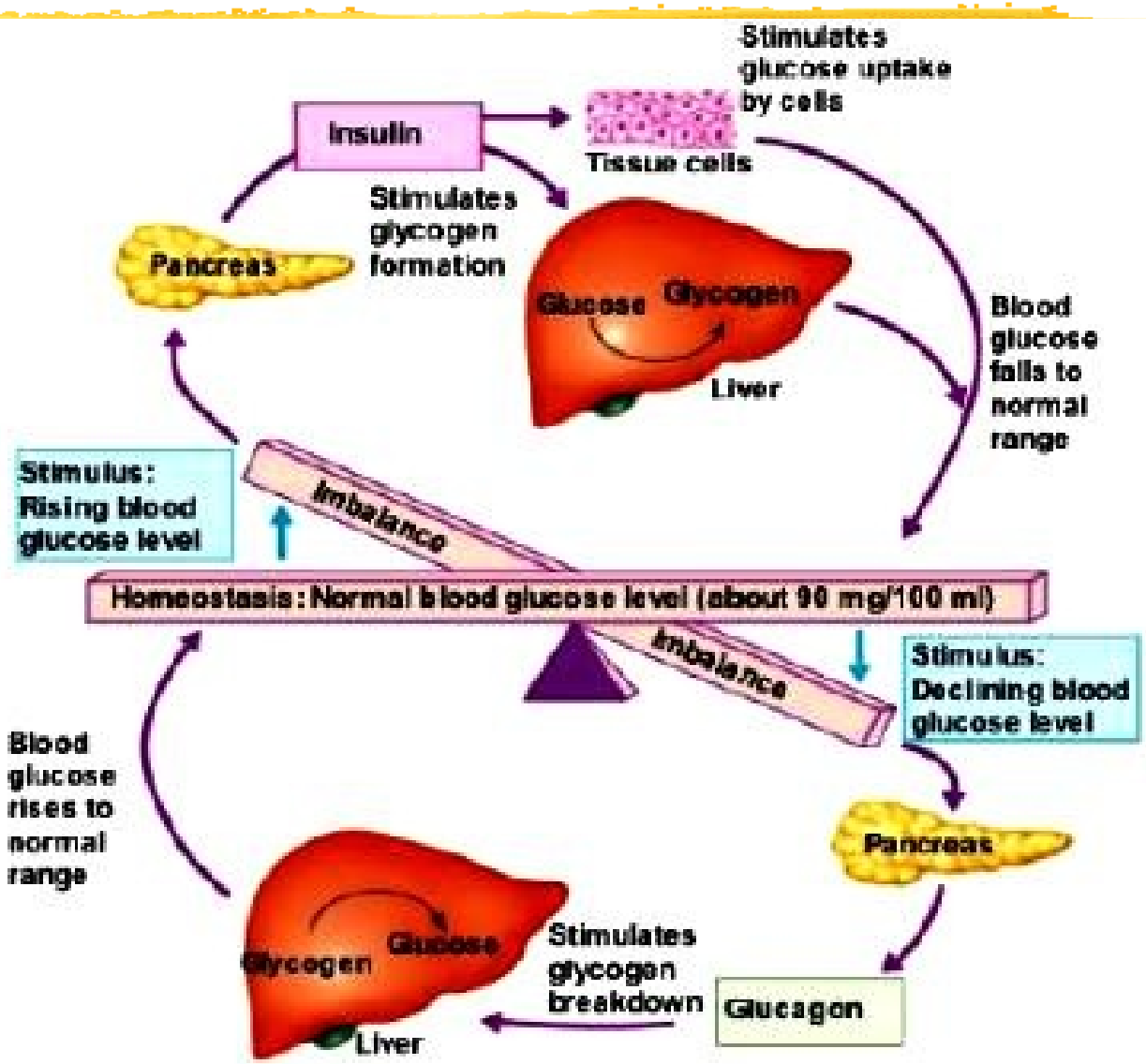
* Cell signaling has been compared to the subway map of Tokyo....

From plasma membrane to nucleus

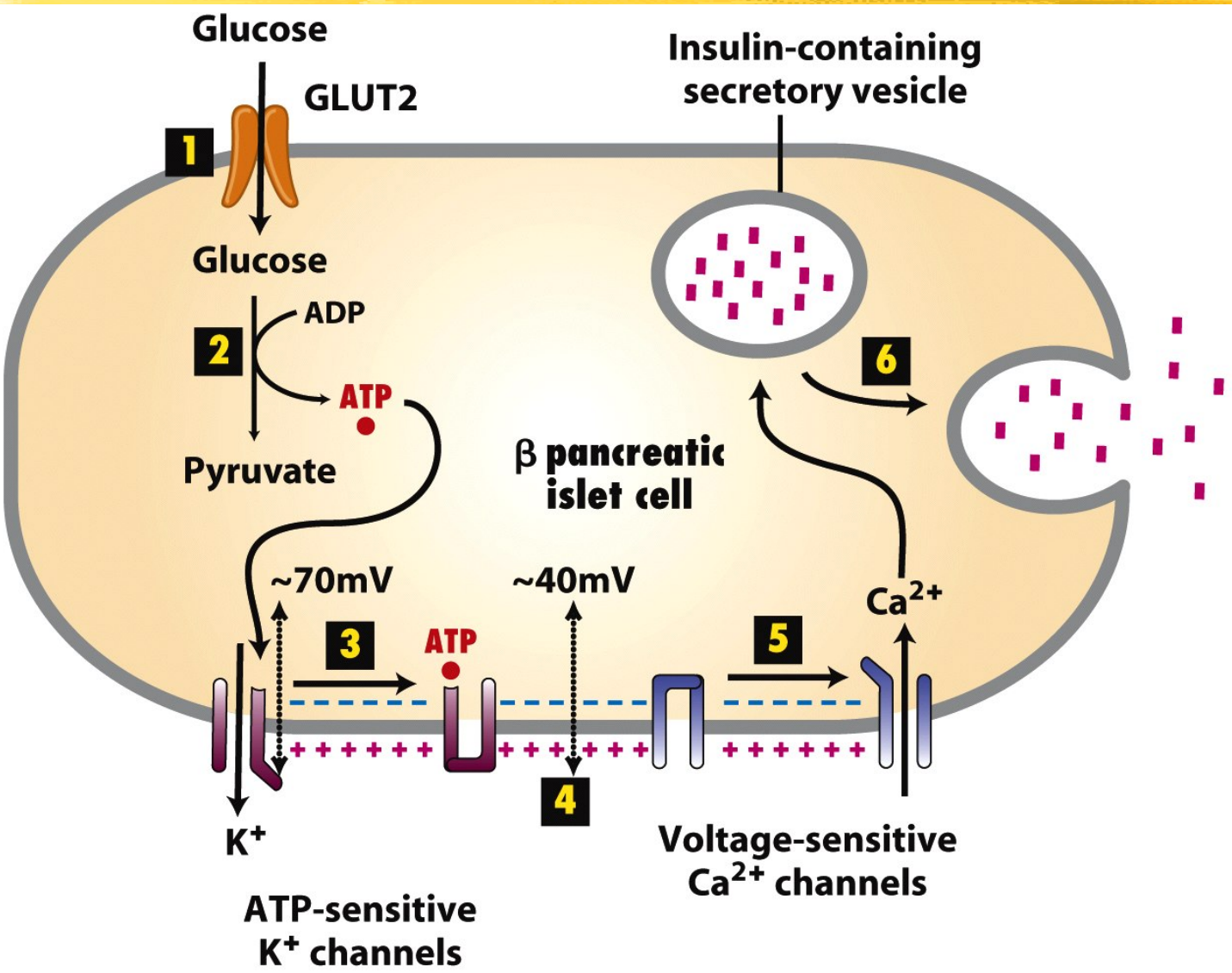
- Many cellular responses induced by water-soluble hormones, growth factors, and neurotransmitters result from their effects on gene expression

- Such pathways usually involve activation of protein kinases that directly or indirectly phosphorylate specific transcription factors

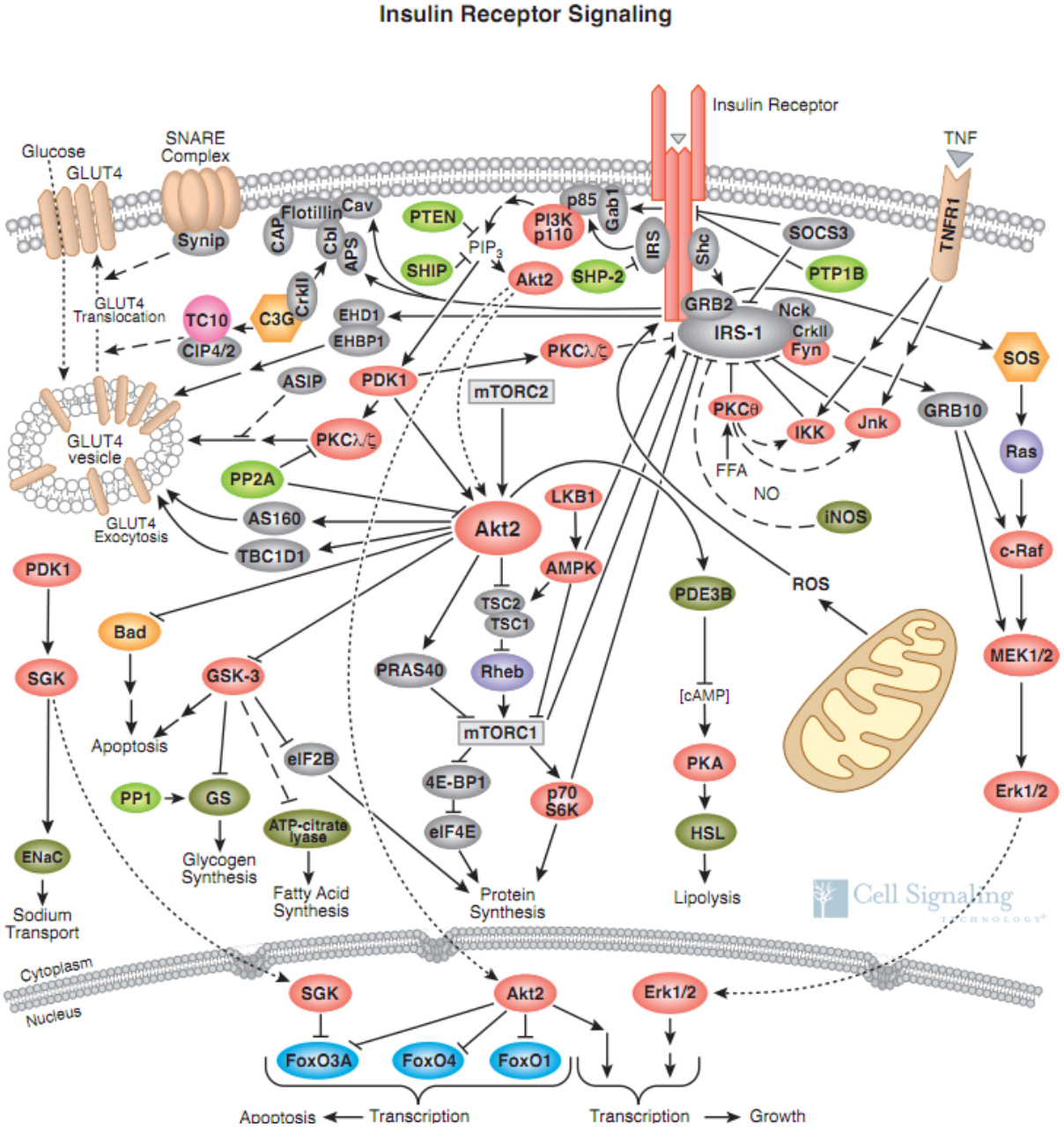
Action of insulin to manage glucose homeostasis

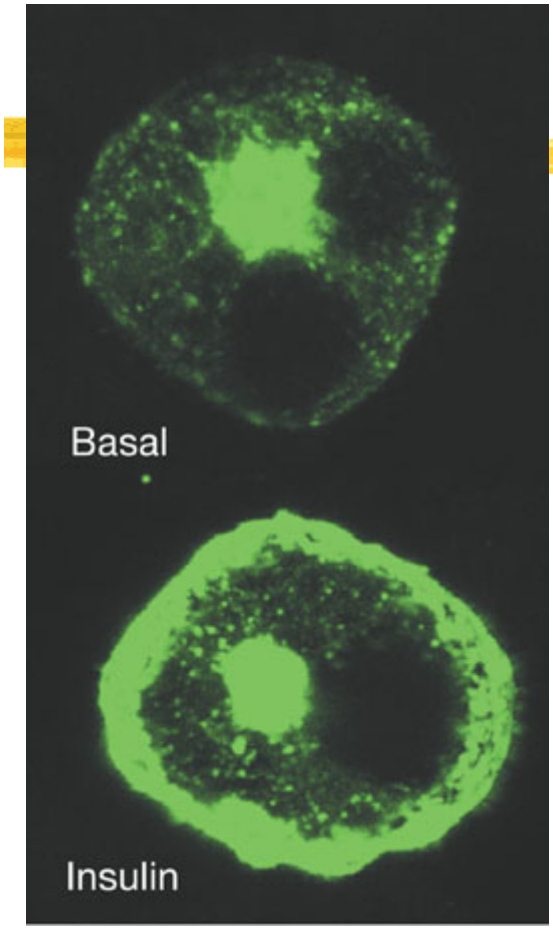


Secretion of Insulin in Response to a Rise in Blood Glucose



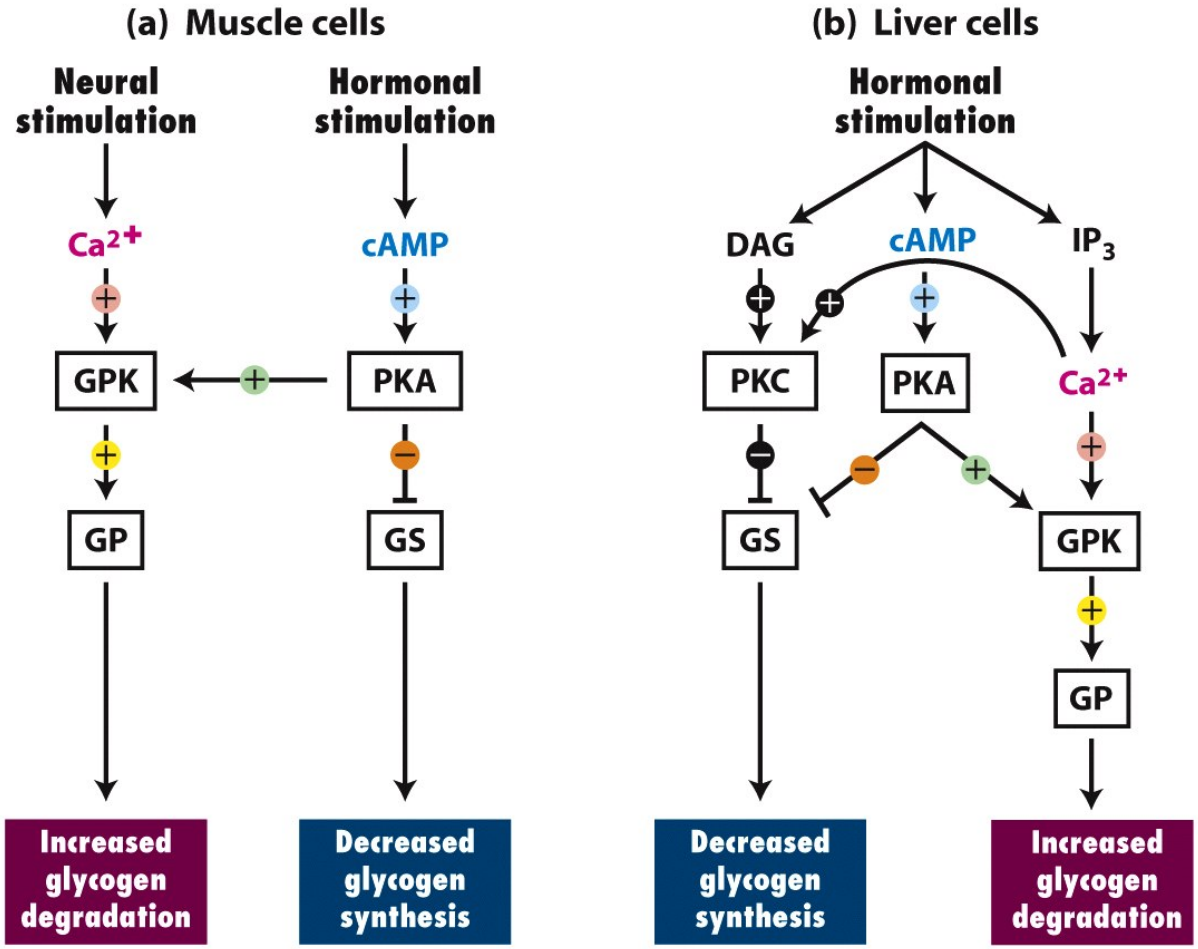
Example containing all levels of regulatin of protein activity





GFP-tagged GLUT4

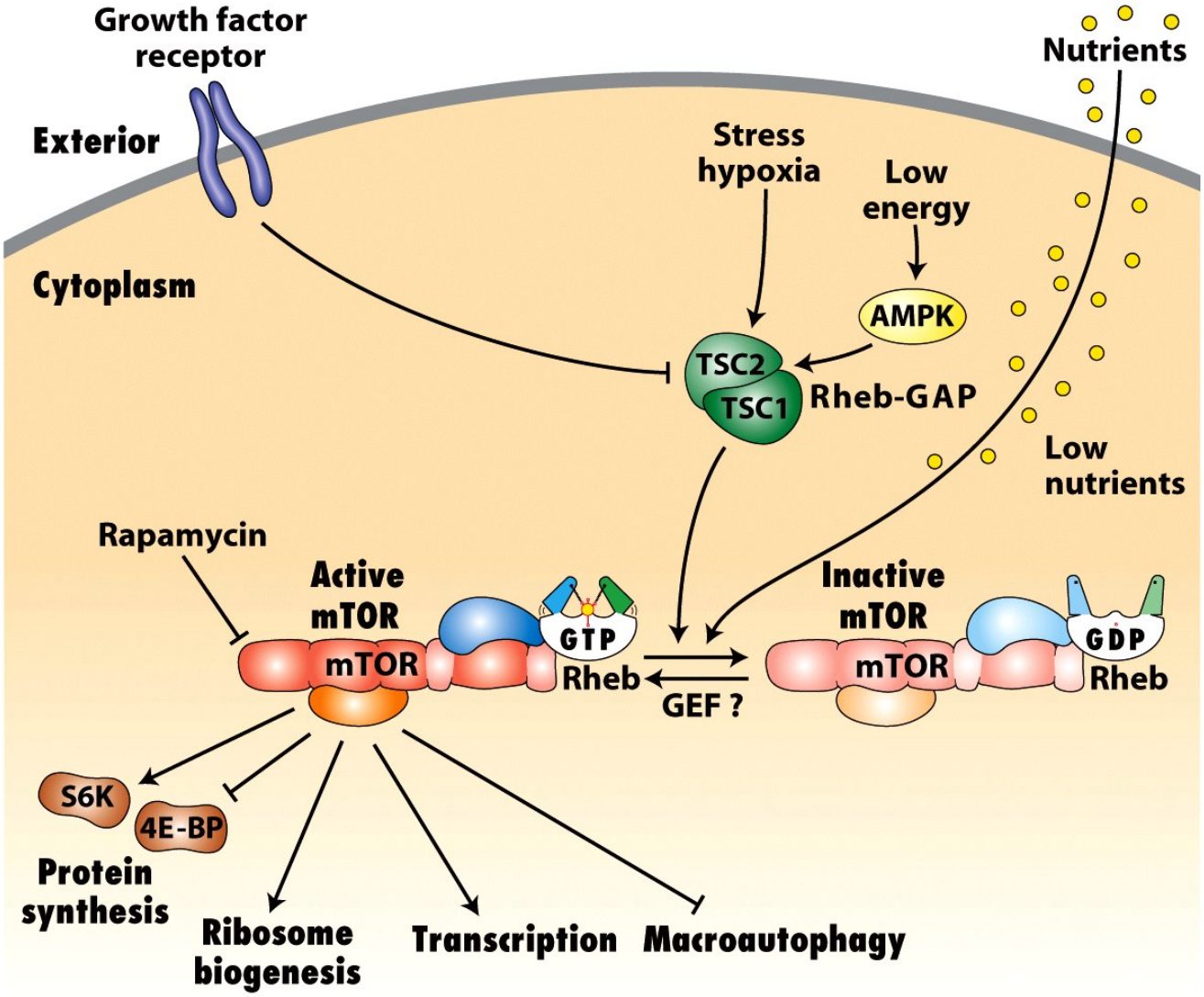
Integration of cellular responses: Glycogenolysis is promoted by multiple second messengers



Abbreviations:

PKA	Protein kinase A	GP	Glycogen phosphorylase
GPK	Glycogen phosphorylase kinase	GS	Glycogen synthase

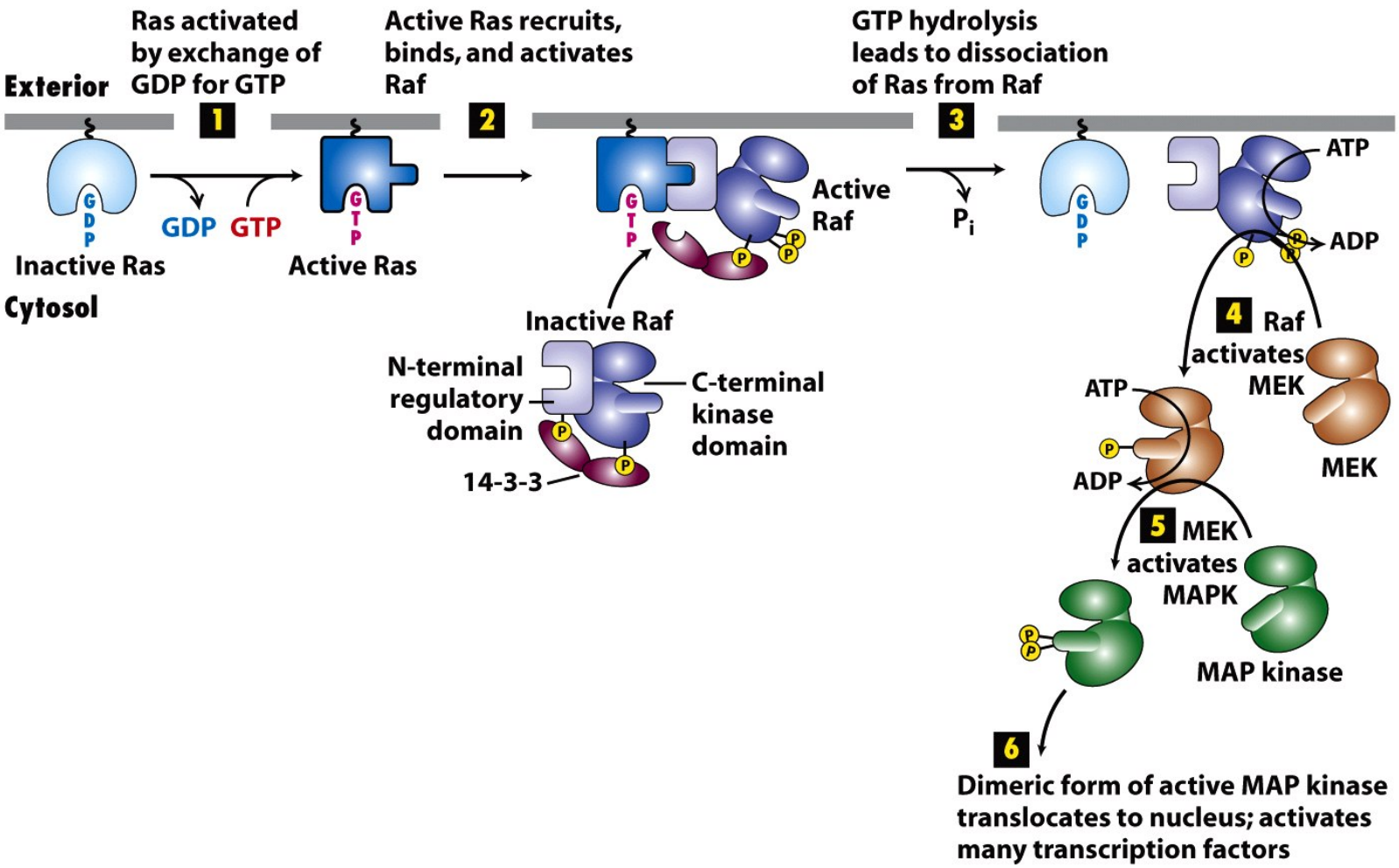
mTOR pathway regulates protein biosynthesis and cell proliferation



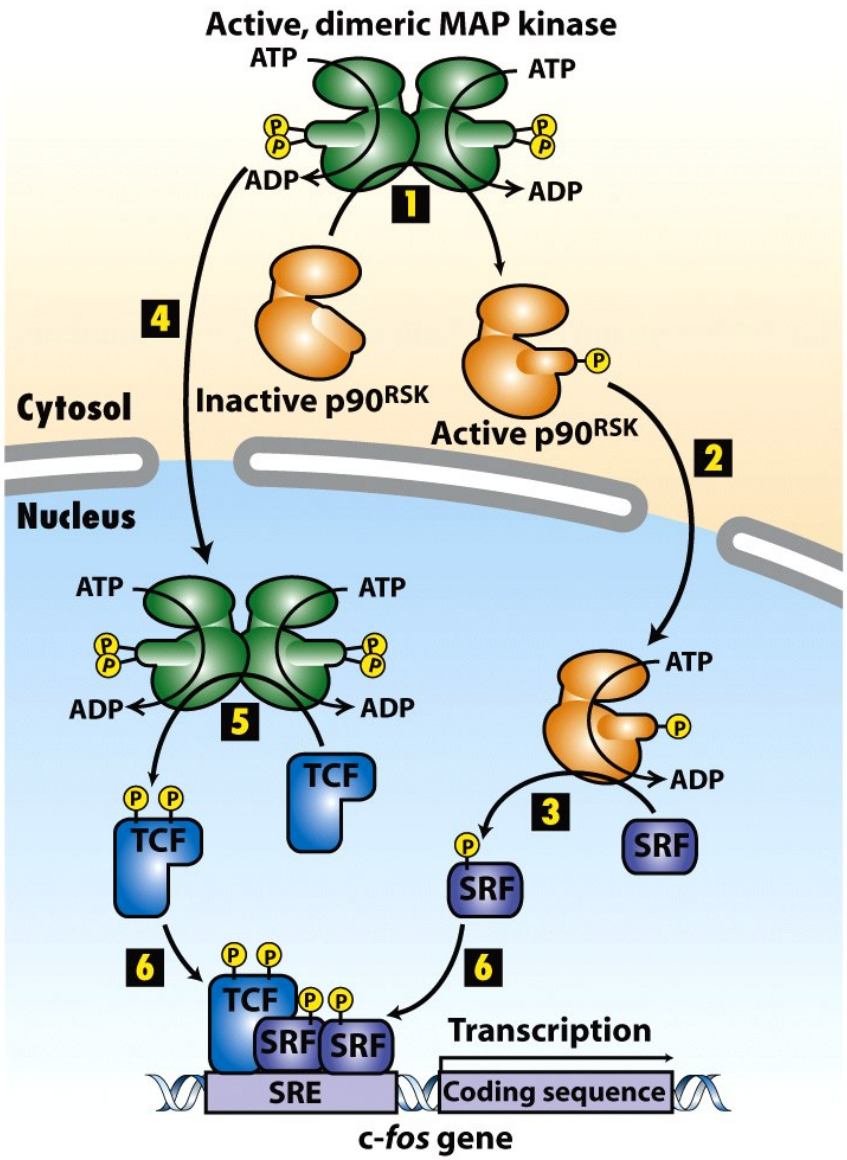
Mitogen activated protein (MAP) kinase pathways

- Almost all RTKs can activate Ras/MAP kinase pathway
- Ras is a monomeric GTPase switched on by RTKs
- Activated Ras induces a kinase signal cascade that culminates in activation of MAP kinases (MAPKs)
- MAP kinases are a serine/threonine kinases that can translocate into the nucleus and phosphorylate many different proteins, including transcription factors that regulate gene expression
- The MAPK family is very large and contains many different members with both overlapping and specific functions

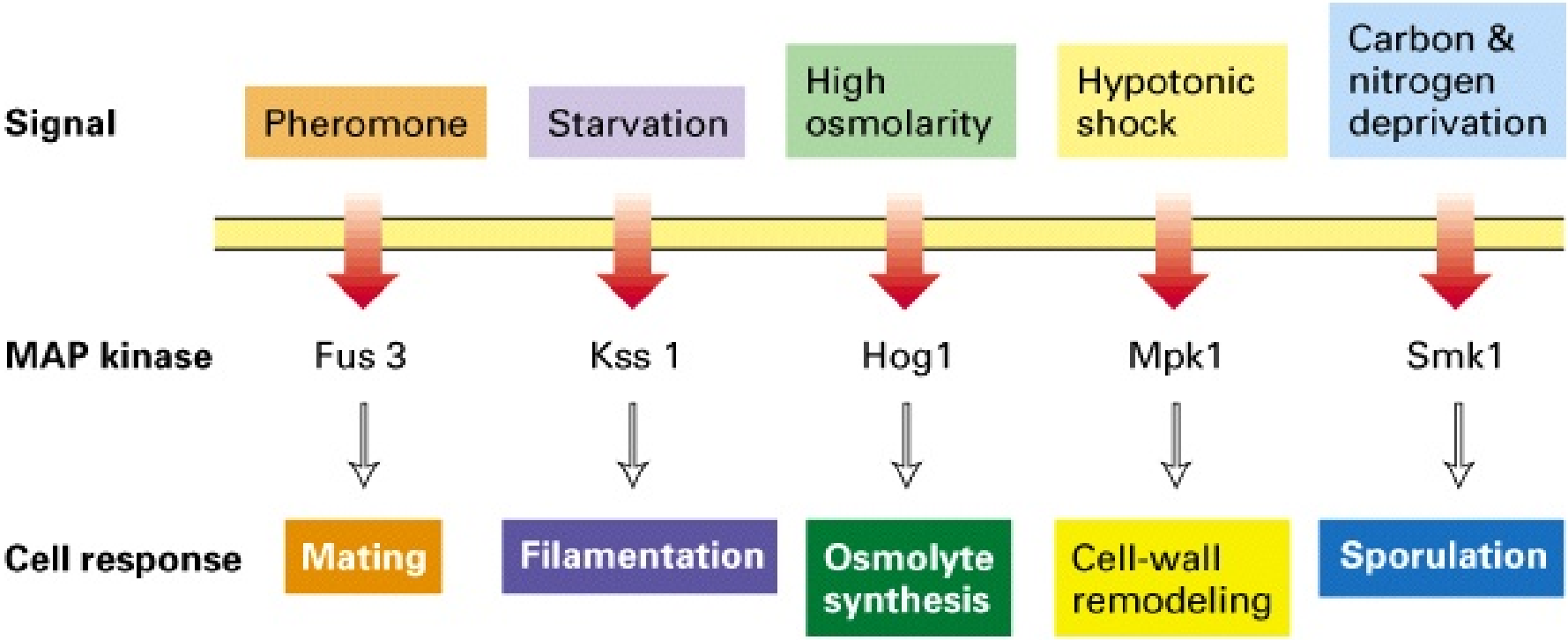
Signals pass from activated Ras to a cascade of protein kinases



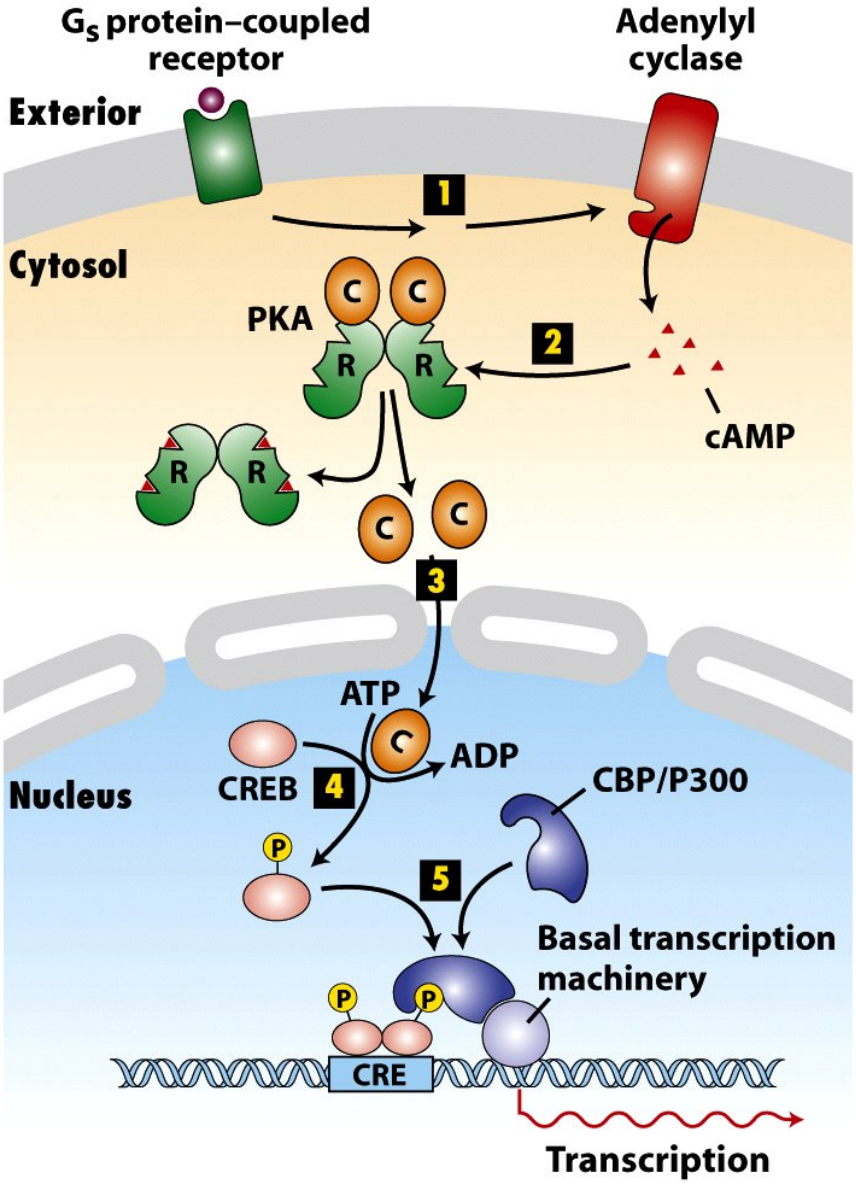
MAP kinase regulates the activity of many transcription factors



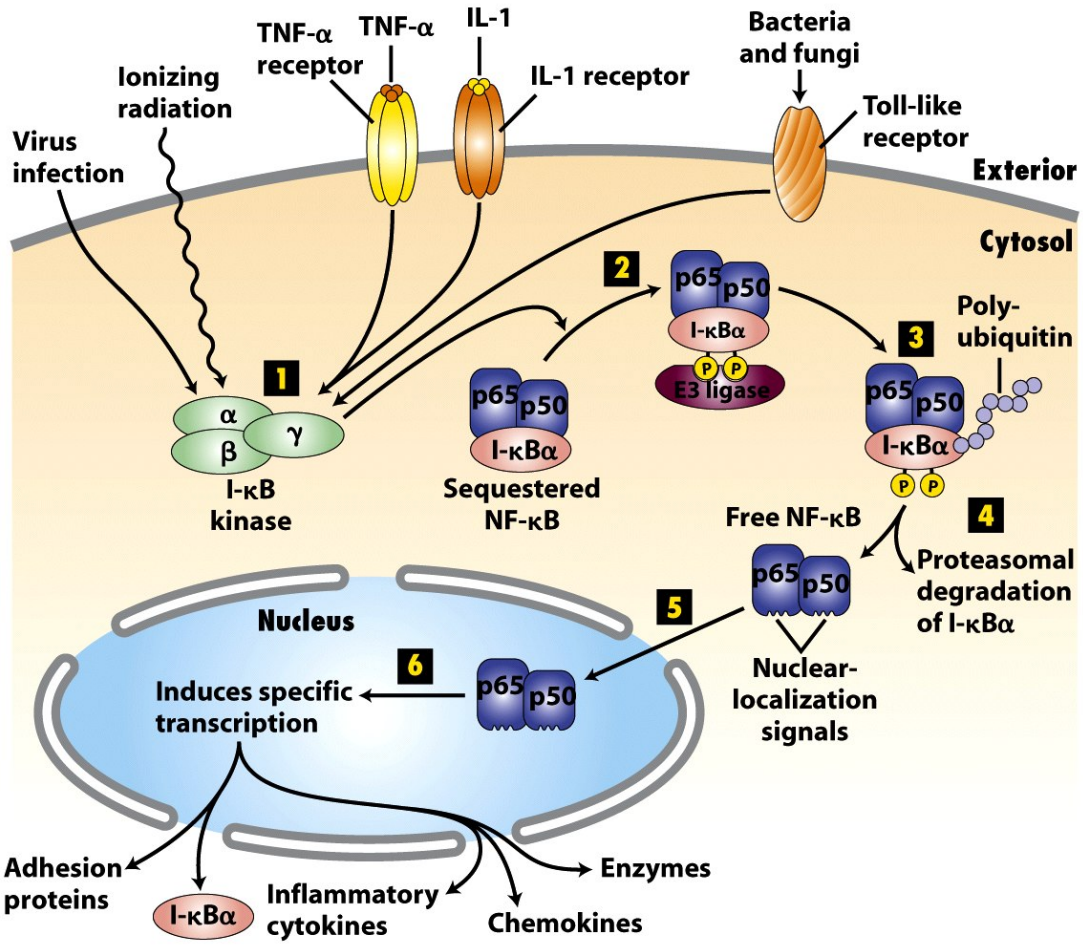
Multiple MAP kinase pathways are found in eukaryotic cells



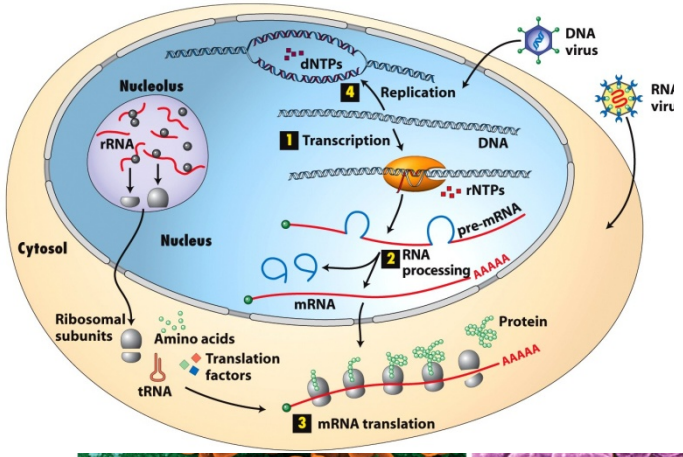
CREB links cAMP signals to transcription



The NF-κB signaling pathway is activated through phosphorylation-dependent protein degradation



1. Activation of the trimeric I-κB kinase
2. I- κB kinase phosphorylates the inhibitory regulator: I-κBα which can then bind ab E3 ubiquitin ligase
3. I-κBα is poly-ubiquitylated
4. Polyubiquitylation directs proteosomal degradation
5. Release of I-κBα unmasks the nuclear localization signal of NF-κB
6. In the nucleus NF- κB binds its cognate response elements in the promoters of several genes, activating their transcription. – including the gene for I- κBα which acts to terminate the signaling (negative feed-back)



+ Receptor-mediated responses

to external stimuli =

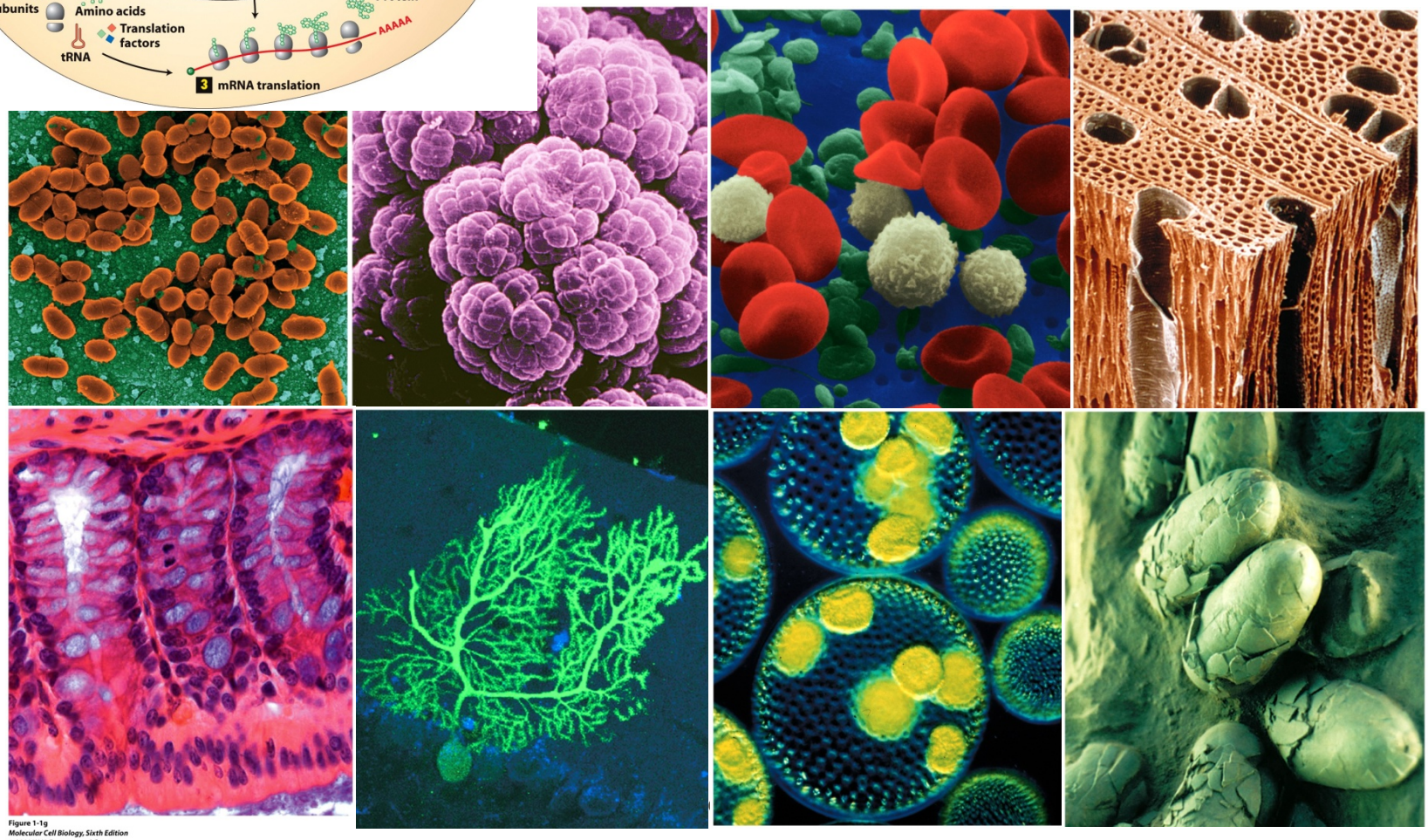


Figure 1-1g
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