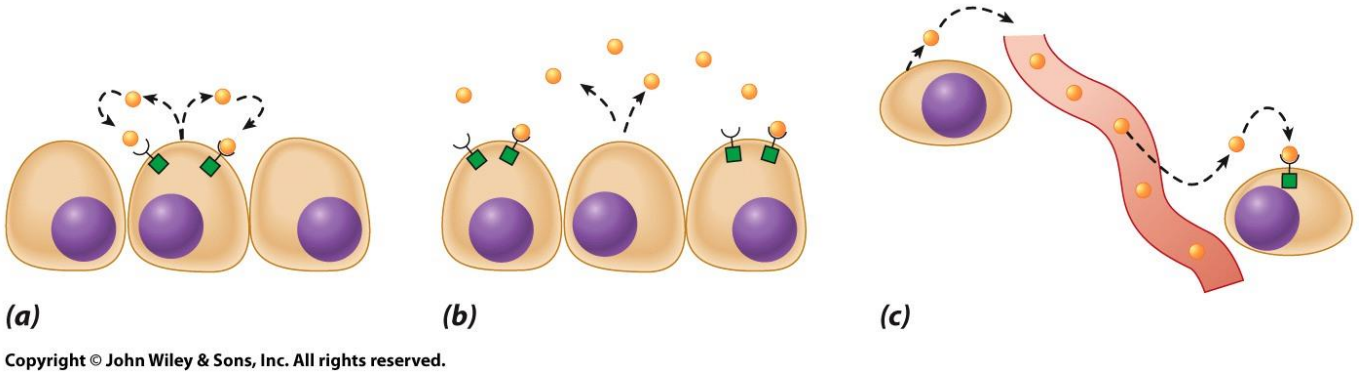
CHAPTER 15

Cell Signaling Pathways

15.1 | The Basic Elements of Cell Signaling Systems



Types of intercellular signaling: autocrine (left), paracrine (middle), and endocrine (right)

Extracellular messenger molecules transmit messages between cells.

In *autocrine* signaling, the cell has receptors on its surface that respond to the messenger.

During *paracrine* signaling, messenger molecules travel short distances through extracellular space.

During *endocrine* signaling, messenger molecules reach their target cells through the bloodstream.

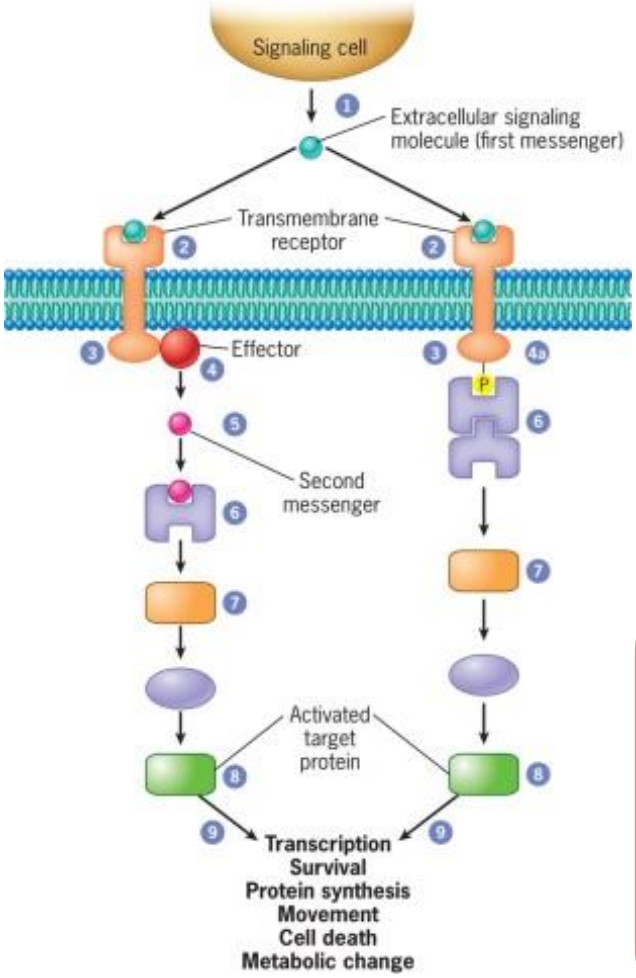
15.1 | The Basic Elements of Cell Signaling Systems

Receptors on or in target cells receive the message.

Some cell surface **receptors** generate an intracellular **second messenger** through an enzyme called an **effector**.

Second messengers are small substances that activate (or inactivate) specific proteins.

Other surface receptors recruit proteins to their intracellular domains at the plasma membrane.



An overview of the major signaling pathways by which extracellular messenger molecules can elicit intracellular responses

15.1 | The Basic Elements of Cell Signaling Systems

Signaling pathways consist of a series of proteins.

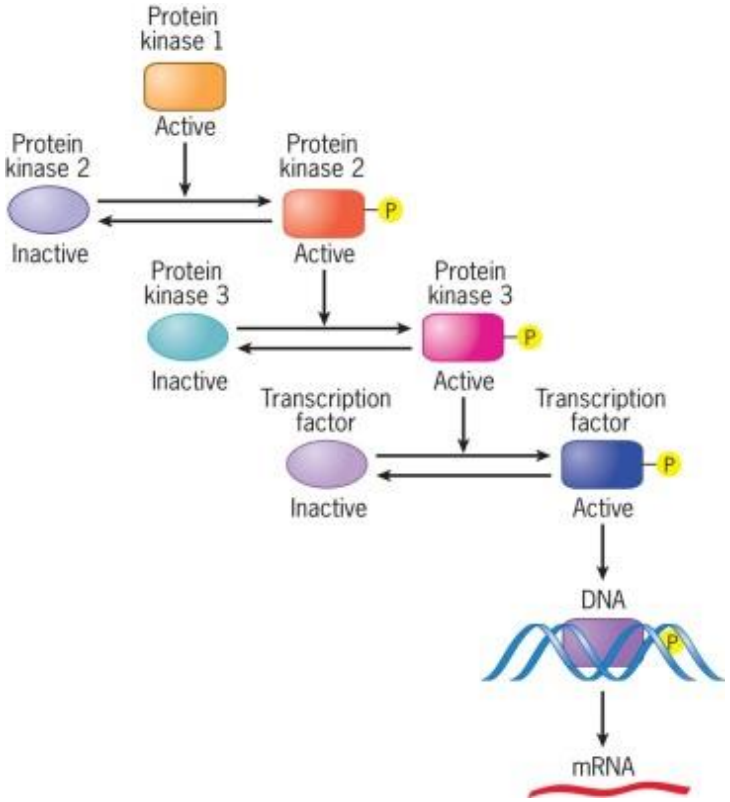
Each protein in a pathway alters the conformation of the next protein.

Protein conformation is usually altered by phosphorylation.

Kinases add phosphate groups while *phosphatases* remove them.

Target proteins ultimately receive a message to alter cell activity.

This overall process is called **signal transduction**.



Signal transduction pathway consist of kinases and phosphatases that change the conformations and activities of target proteins.

15.1 | The Basic Elements of Cell Signaling Systems

Protein phosphorylation can change protein behavior in different ways.

It can activate or inactivate an enzyme.

It can increase or decrease protein-protein interactions.

It can change the subcellular location of the protein .

It can trigger protein degradation.

Phosphorylation patterns differ between cell types (e.g. two different types of breast cancer cells).

15.2 | A Survey of Extracellular Messengers and Their Receptors

Extracellular messengers include:

Small molecules such as amino acids and their derivatives (e.g. acetylcholine, epinephrine, dopamine).

Gases such as NO and CO.

Steroids, derived from cholesterol, regulate sexual differentiation, pregnancy, carbohydrate metabolism.

Eicosanoids, derived from arachidonic acid, regulate processes including pain, inflammation, blood pressure, and blood clotting.

Various peptides and proteins, including transmembrane proteins on the surface of an interacting cell, extracellular matrix interacting proteins, and secreted proteins.

15.2 | A Survey of Extracellular Messengers and Their Receptors

Receptor types include:

G-protein coupled receptors (GPCRs), which contain seven transmembrane α helices and activate GTP-binding proteins.

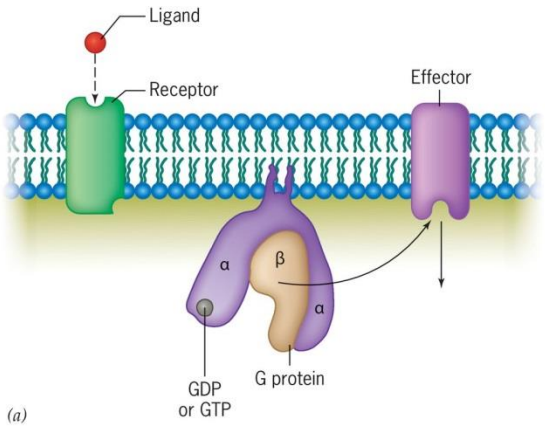
Receptor protein-tyrosine kinases (RTKs), which dimerize and activate their cytoplasmic protein-kinase domain to phosphorylate specific tyrosine residues of cytoplasmic substrate proteins.

Ligand gated channels, which conduct a flow of ions across the plasma membrane to change its potential.

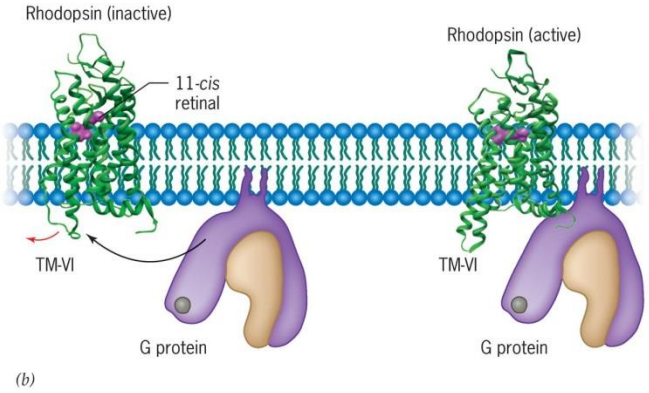
Steroid hormone receptors, which function as ligand-regulated transcription factors.

Specific receptors such as B-and T-cell receptors which are involved in the response to foreign antigens.

15.3 | Signal Transduction by G Protein-Coupled Receptors



Transducing signals through a heterotrimeric G protein.



A model depicting the activation of the GPCR rhodopsin

G protein-coupled receptors (**GPCRs**) constitute the single largest superfamily of proteins encoded by animal genomes.

GPCRs have seven α-helical transmembrane domains and interact with G proteins.

Natural ligands: hormones (both plant and animal), neurotransmitters, opium derivatives, chemoattractants (odorants, tastants, and photons).

15.3 | Signal Transduction by G Protein-Coupled Receptors

TABLE 15.1 Examples of Physiologic Processes Mediated by GPCRs and Heterotrimeric G Proteins

Stimulus	Receptor	Effector	Physiologic response
Epinephrine	β-Adrenergic receptor	Adenylyl cyclase	Glycogen breakdown
Serotonin	Serotonin receptor	Adenylyl cyclase	Behavioral sensitization and learning in *Aplysia*
Light	Rhodopsin	cGMP phosphodiesterase	Visual excitation
IgE–antigen complexes	Mast cell IgE receptor	Phospholipase C	Secretion
f-Met Peptide	Chemotactic receptor	Phospholipase C	Chemotaxis
Acetylcholine	Muscarinic receptor	Potassium channel	Slowing of pacemaker activity

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Ligands activate receptors that stimulate effectors to give rise to a physiological response

G protein-coupled receptors normally have their amino-terminus present on the outside of the cell, the seven α helices that traverse the plasma membrane connected by loops of varying length, and the carboxyl-terminus present on the inside of the cell.

15.3 | Signal Transduction by G Protein-Coupled Receptors

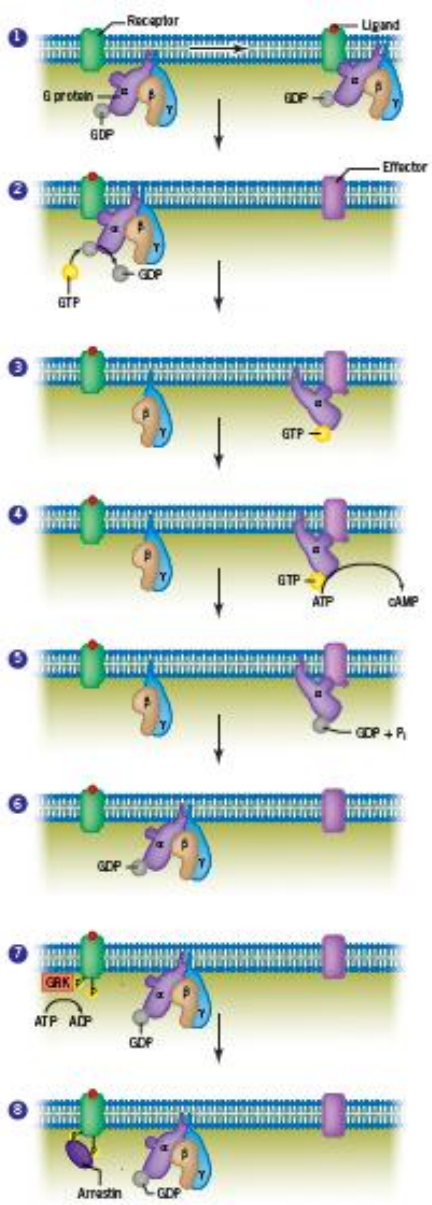
Receptors

Ligand binding to the receptor extracellular domain changes the conformation of its intracellular domain.

The receptor's affinity for G proteins increases, and the receptor binds the trimeric G protein.

A GDP is exchanged for GTP on the Gα subunit, thus activating it and promoting association with the effector.

One ligand-bound receptor can activate many G proteins.



Receptor-mediated activation (or inhibition) of effectors by means of heterotrimeric G proteins

15.3 | Signal Transduction by G Protein-Coupled Receptors

Receptors

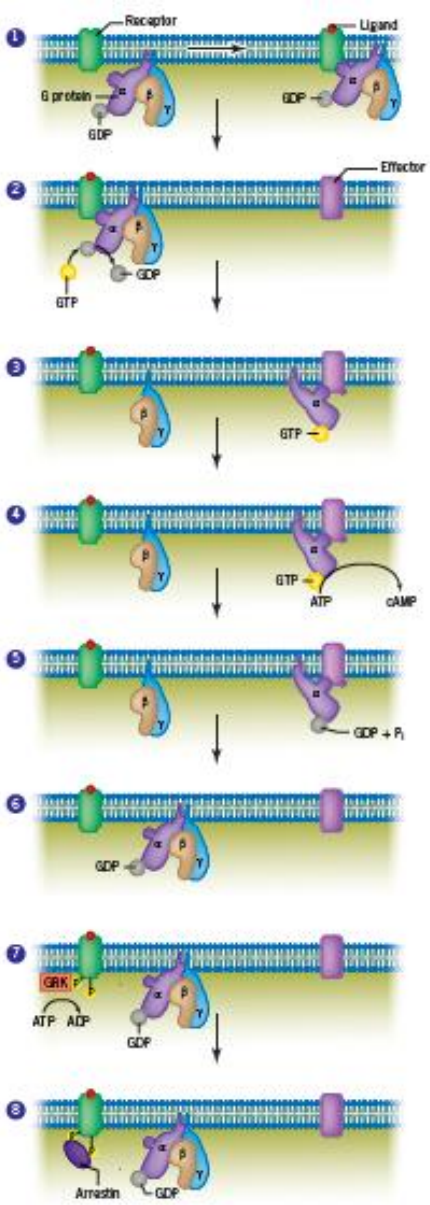
Termination of the response can occur through multiple processes.

Desensitization – by blocking active receptors from turning on additional G proteins.

G protein-coupled receptor kinase (GRK) activates a GPCR via phosphorylation.

Proteins called *arrestins* compete with G proteins to bind GPCRs.

Termination of the response is accelerated by *regulators of G protein signaling (RGSs)*.



Receptor-mediated activation (or inhibition) of effectors by means of heterotrimeric G proteins

15.3 | Signal Transduction by G Protein-Coupled Receptors

G proteins

Gα subunits can turn themselves off by hydrolysis of GTP to GDP and Pi, which causes a conformational change and a decreased affinity for the effector and an increased affinity for the Gβγ subunit.

Heterotrimeric G proteins function as molecular timers, and while active, Gα subunits can turn on downstream effectors.

Following its dissociation from the Gα subunit, the Gβγ complex also has a signaling function and it can couple to a number of different types of effectors, including PLCβ, K^+ and Ca^{2+} ion channels, and adenylyl cyclase.

15.3 | Signal Transduction by G Protein-Coupled Receptors

Bacterial Toxins

Bacterial Toxins, such as cholera toxin and pertussis virulence factors, target GPCRs and G proteins.

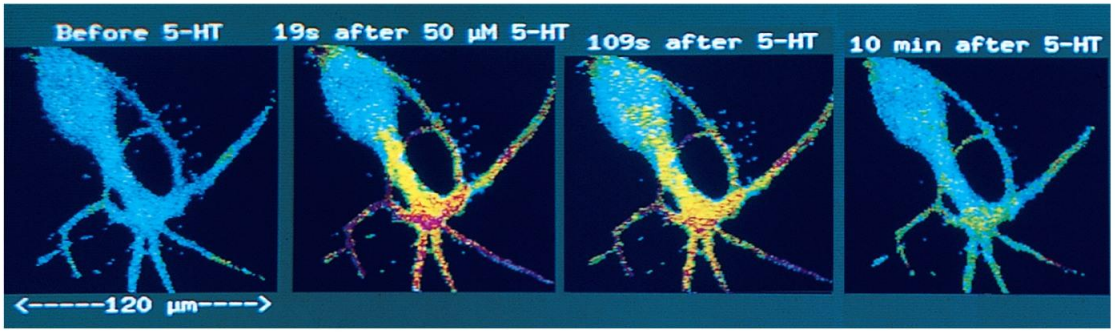
β Adrenergic Receptors stimulate Ga_s to activate adenylate cyclase. Ga_s is a target of *cholera toxin*, as the GTPase activity is inhibited.

Pathway is locked in stimulatory state, as adenylyl cyclase molecules remain in an activated mode, churning out cAMP, which causes the epithelial cells to secrete large volumes of fluid into the intestinal lumen.

α Adrenergic Receptors stimulate Ga_i to inhibit adenylate cyclase. Ga_i is a target of *pertussis toxin*.

The toxin also inactivates Gα subunits, thereby interfering with the signaling pathway that leads the host to mount a defensive response against the bacterial infection.

15.4 | Second Messengers



Localized formation of cAMP in response to the addition of an extracellular messenger molecule. Photographs of a sensory nerve cell (*Aplysia*)

Cyclic AMP is a second messenger that diffuses to other sites in the cell.

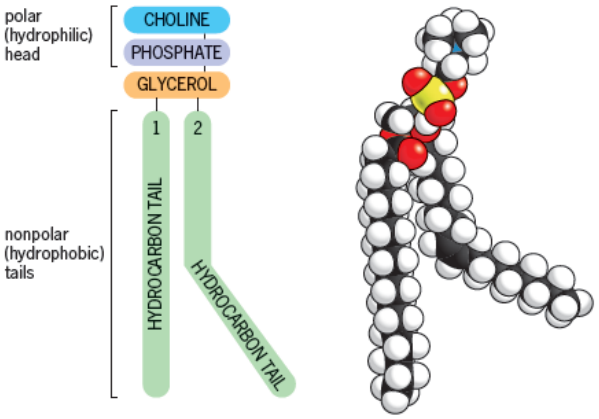
The synthesis of cyclic AMP follows the binding of a first messenger, a hormone or other ligand, to a receptor at the outer surface of the cell.

Second messengers enable cells to mount a large-scale, coordinated response following stimulation by a single extracellular ligand.

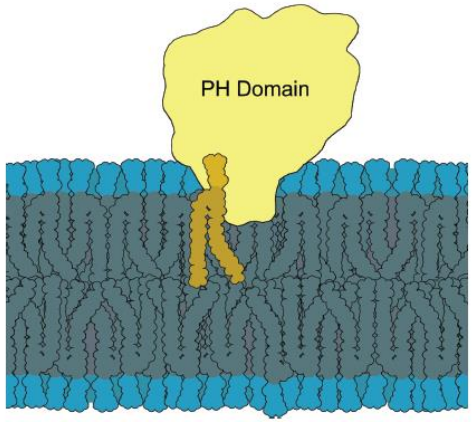
Other second messengers include Ca^{2+}, phosphoinositides, inositol trisphosphate, diacylglycerol, cGMP, and nitric oxide.

15.4 | Second Messengers

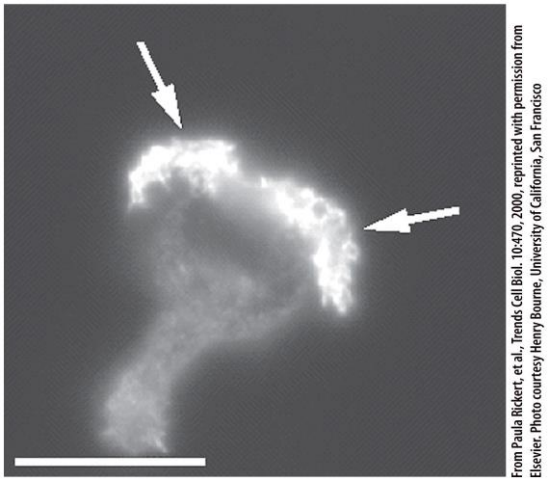
Phosphatidylinositol-Derived Second Messengers



Structure of a generalized phospholipid



Interaction: PLC enzyme and a phosphoinositide



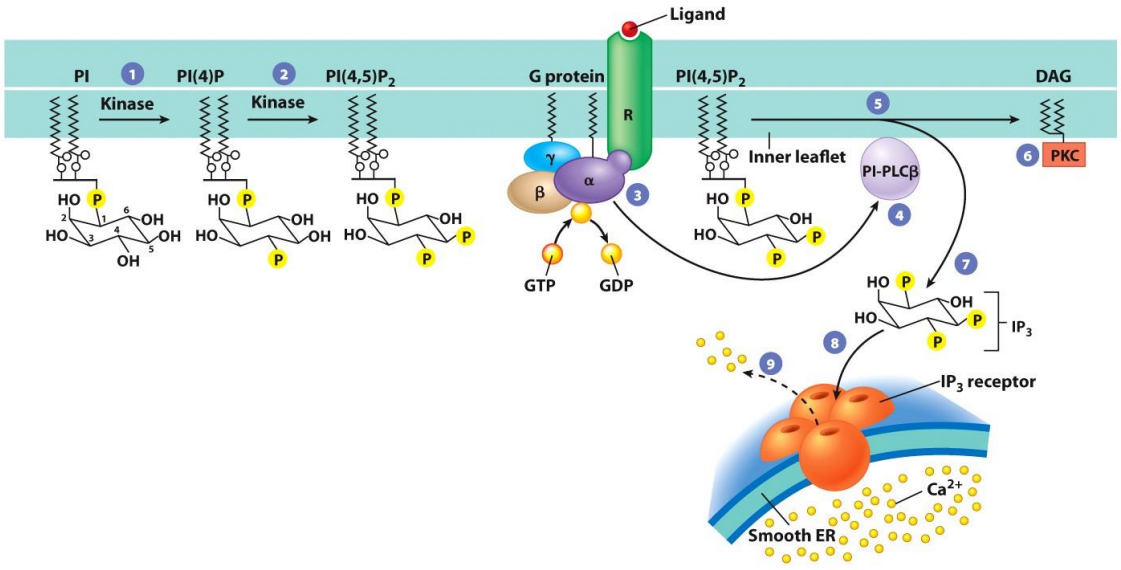
Chemoattractant generates PIP_3 at the cell leading edge

Some phospholipids of cell membranes are converted into second messengers by phospholipases (lipid-splitting), phospholipid kinases (lipid-phosphorylating), and phospholipid phosphatases (lipid-dephosphorylating).

Phosphorylated phosphoinositides, derived from phosphatidylinositol, form lipid-binding domains called **PH domains**.

15.4 | Second Messengers

Phosphatidylinositol-Derived Second Messengers



The generation of second messengers as a result of ligand-induced breakdown of phosphoinositides (PI) in the lipid bilayer.

Phosphatidylinositol-specific phospholipase C-β produces second messengers derived from phosphatidylinositol-inositol triphosphate (IP_3) and diacylglycerol (DAG).

DAG activates protein kinase C, which phosphorylates serine and threonine residues on target proteins.

15.6 | Regulation of Blood Glucose Levels

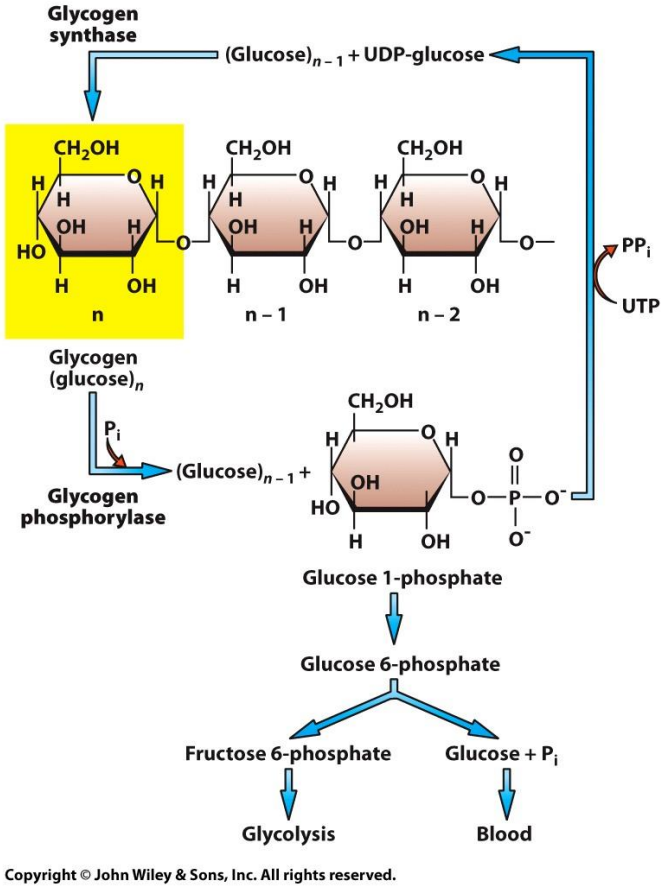
Different stimuli acting on the same target cell may induce the same response.

Glucagon and epinephrine bind to different receptors on the same cell.

Both hormones stimulate glycogen breakdown and inhibit its synthesis.

cAMP is activated by the G protein of both hormone receptors.

Responses are amplified by signal cascades.



The reactions that lead to glucose storage or mobilization.

15.6 | Regulation of Blood Glucose Levels

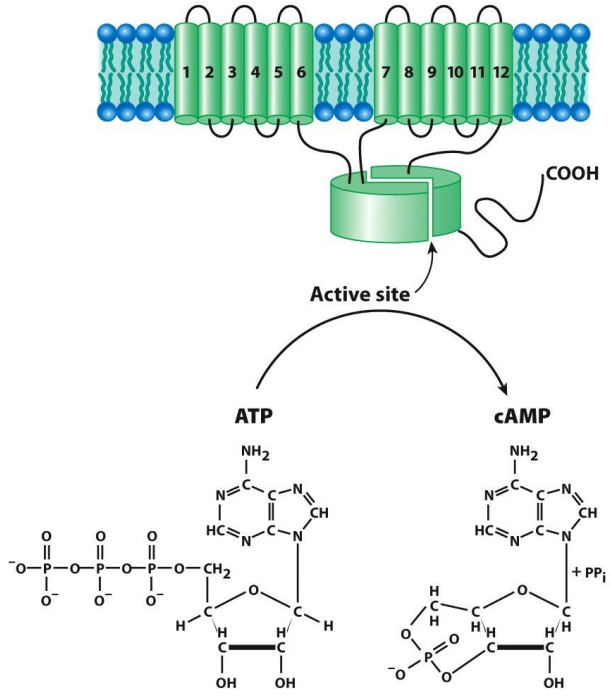
Glucose Mobilization: An Example of a Response Induced by cAMP

cAMP is synthesized by the effector enzyme *adenylyl cyclase*.

Adenylyl cyclase is an integral membrane protein whose catalytic domain resides at the inner surface of the plasma membrane.

cAMP evokes a *reaction cascade* that leads to glucose mobilization.

cAMP breakdown is accomplished by a phosphodiesterase.



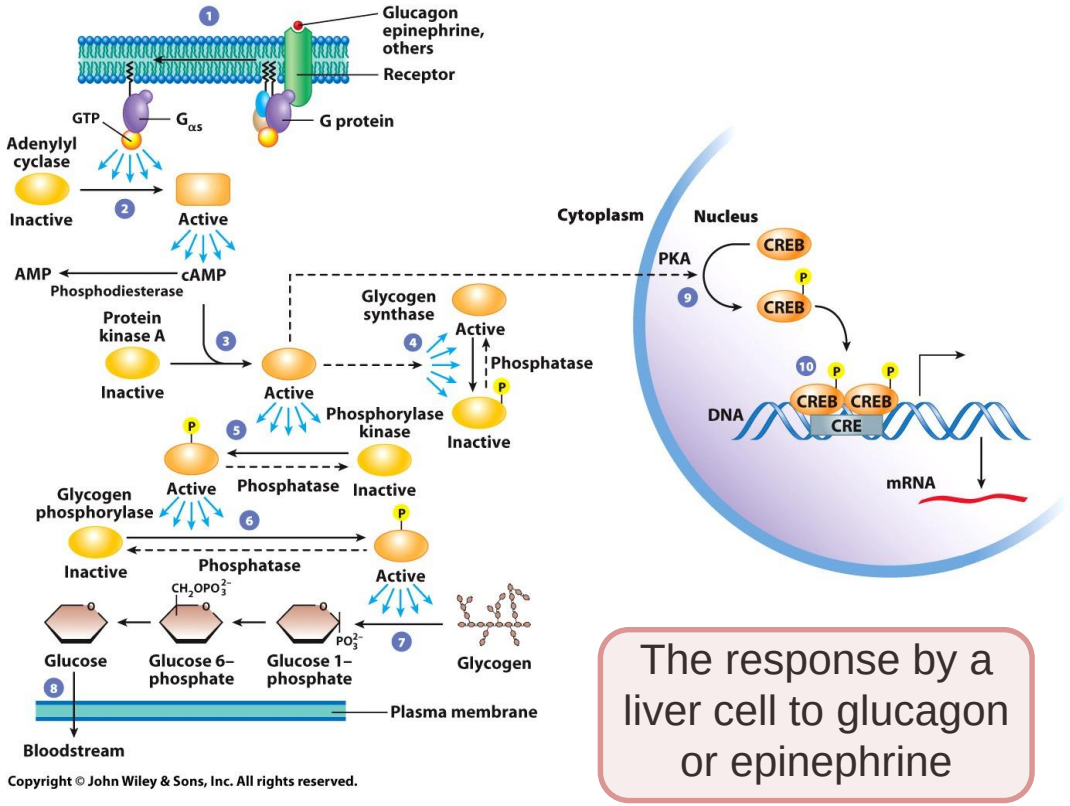
Formation of cyclic AMP from ATP is catalyzed by adenylyl cyclase, an integral membrane protein that consists of two parts

15.6 | Regulation of Blood Glucose Levels

Glucose Mobilization: An Example of a Response Induced by cAMP

The first step in the *reaction cascade* occurs as the hormone binds to its receptor, activating a Gαs subunit, which activates adenylyl cyclase.

The activated enzyme catalyzes the formation of cAMP molecules, which diffuse into the cytoplasm where they bind a cAMP-dependent protein kinase (*protein kinase A*, *PKA*).



The response by a liver cell to glucagon or epinephrine

15.6 | Regulation of Blood Glucose Levels

Signal Amplification

Binding of a single hormone molecule can activate a number of G proteins, each of which can activate an adenylyl cyclase effector, each of which can produce a large number of cAMP messengers quickly.

The production of a second messenger provides a mechanism to greatly amplify the signal generated from the original message.

cAMP molecules activate PKA, which phosphorylates a large number of phosphorylase kinase molecules, which in turn phosphorylate an even larger number of glycogen phosphorylase molecules, which in turn can catalyze the formation of a much larger number of glucose phosphates.

What begins as a barely perceptible stimulus at the cell surface is rapidly transformed into a major mobilization of glucose within the cell.

15.8 | Protein-Tyrosine Phosphorylation as a Mechanism for Signal Transduction

Protein-tyrosine kinases, which phosphorylate tyrosine residues on target proteins, can be divided in two groups: receptor protein-tyrosine kinases (RTKs), integral membrane proteins with a single transmembrane helix and an extracellular ligand binding domain, and non-receptor (or *cytoplasmic*) protein-tyrosine kinases.

The human genome encodes nearly 60 RTKs and 32 non-receptor TKs.

RTKs are activated directly by extracellular growth and differentiation factors such as epidermal growth factor (EGF) and platelet-derived growth factor (PDGF) or by metabolic regulators such as insulin.

Non-receptor protein-tyrosine kinases are regulated indirectly by extracellular signals, and they control processes as diverse as the immune response, cell adhesion, and neuronal cell migration.

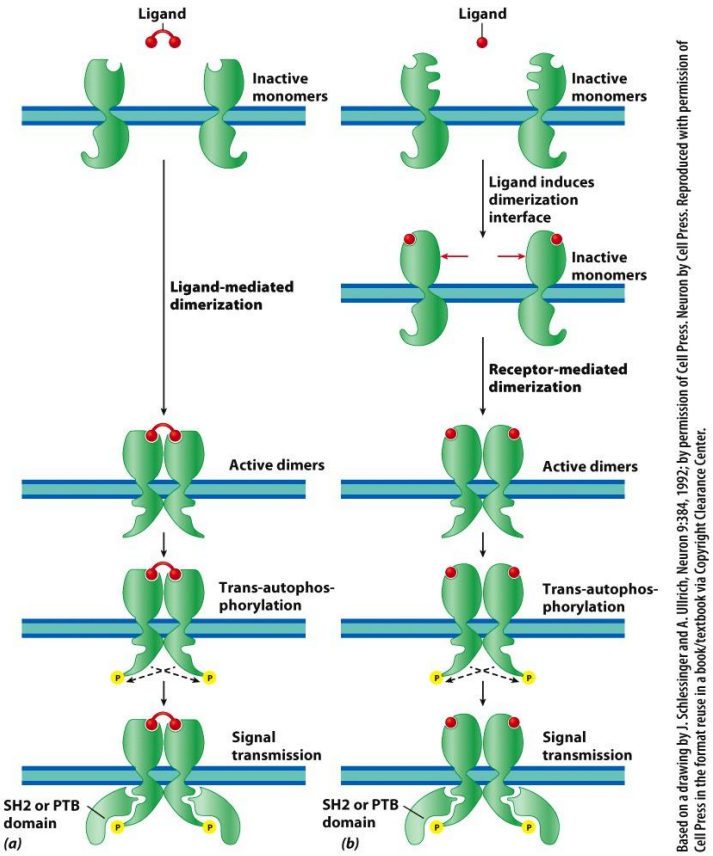
15.8 | Protein-Tyrosine Phosphorylation as a Mechanism for Signal Transduction

Receptor Dimerization

Ligand binding results in dimerization of the extracellular ligand-binding domains of a pair of receptors.

Two mechanisms for receptor dimerization: ligand-mediated dimerization (e.g. PDGF) and receptor-mediated dimerization (e.g., EGF).

For most RTKs, dimerization brings two kinase domains in close contact for trans-autophosphorylation; the protein kinase activity of one receptor phosphorylates tyrosine residues in the cytoplasmic domain of the other receptor.



Steps in the activation of a receptor protein-tyrosine kinase (RTK)

15.8 | Protein-Tyrosine Phosphorylation as a Mechanism for Signal Transduction

Protein Kinase Activation

Autophosphorylation sites can regulate the receptor's kinase activity or serve as binding sites for cytoplasmic signaling molecules.

Kinase activity is usually controlled by autophosphorylation on tyrosine residues that are present in the activation loop of the kinase domain.

Following its phosphorylation, the activation loop is stabilized in a position away from the substrate-binding site, resulting in activation of the kinase domain.

The receptor subunits then phosphorylate each other on tyrosine residues that are present in regions adjacent to the kinase domain; these sites act as binding sites for cellular signaling proteins.

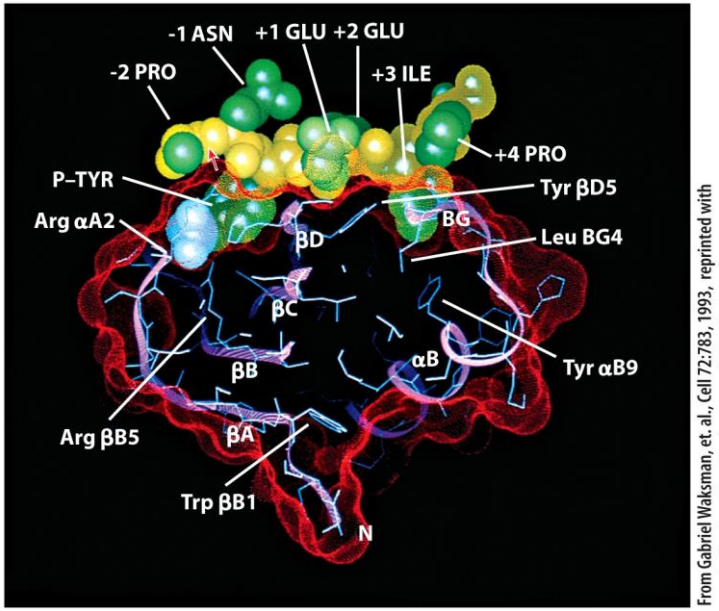
15.8 | Protein-Tyrosine Phosphorylation as a Mechanism for Signal Transduction

Phosphotyrosine-Dependent Protein-Protein Interactions

Phosphorylated tyrosines bind effector proteins that have either a *Src-homology 2* (*SH2*) *domain* or a *phosphotyrosine-binding* (*PTB*) *domain.*

SH2 domains are composed of roughly 100 amino acids and contain a conserved binding-pocket that accommodates a phosphorylated tyrosine residue.

PTB domains can bind to phosphorylated tyrosine residues and are usually present as part of an Asn-Pro-X-Tyr motif.



From Gabriel Waksman, et. al., Cell 72:783, 1993, reprinted with permission from Elsevier. Courtesy of John Kuriyan.

The interaction between SH2 domain and a peptide contain a phosphotyrosine

15.8 | Protein-Tyrosine Phosphorylation as a Mechanism for Signal Transduction

Activation of Downstream Signaling Pathways

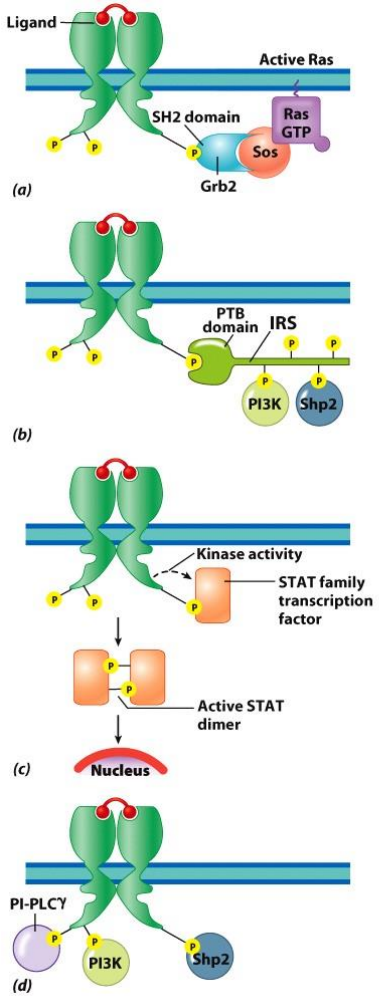
SH2 and PTB domain proteins include:

1) Adaptor proteins that bind other proteins.

2) Docking proteins that supply receptors with other tyrosine phosphorylation sites.

3) Signaling enzymes (kinases) that lead to changes in cell.

4) Transcription factors



A diversity of signaling proteins. Cells contain numerous proteins with SH2 or PTB domains that bind to phosphorylated tyrosine residues

15.8 | Protein-Tyrosine Phosphorylation as a Mechanism for Signal Transduction

Ending the Response

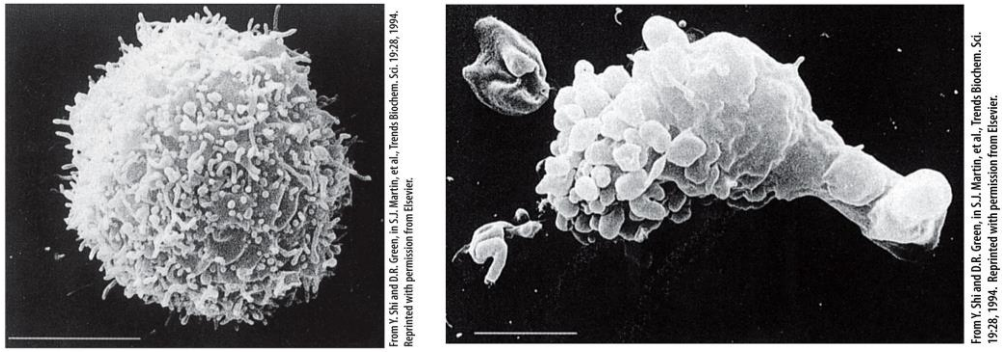
Signal transduction by RTKs is usually terminated by internalization of the receptor, primarily through clathrin-mediated endocytosis.

Some RTKs may bind to the clathrin adaptor protein AP-2, or may be targeted by ubiquitination by ubiquitin ligases through SH2 domains or adaptor proteins.

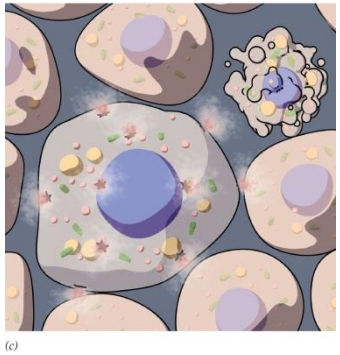
Internalized RTKs can have several alternate fates; they can be degraded in lysosomes, returned to the plasma membrane, or become part of endosomal signaling complexes and engage in continued intracellular signaling.

Two important downstream signaling pathways: 1)the Ras-MAP kinase pathway is probably the best characterized signaling cascade, and 2) the insulin receptor-mediate cascade.

15.15 | Apoptosis (Programmed Cell Death)



EM: normal (**left**) and apoptotic (**right**) T-cell hybridoma



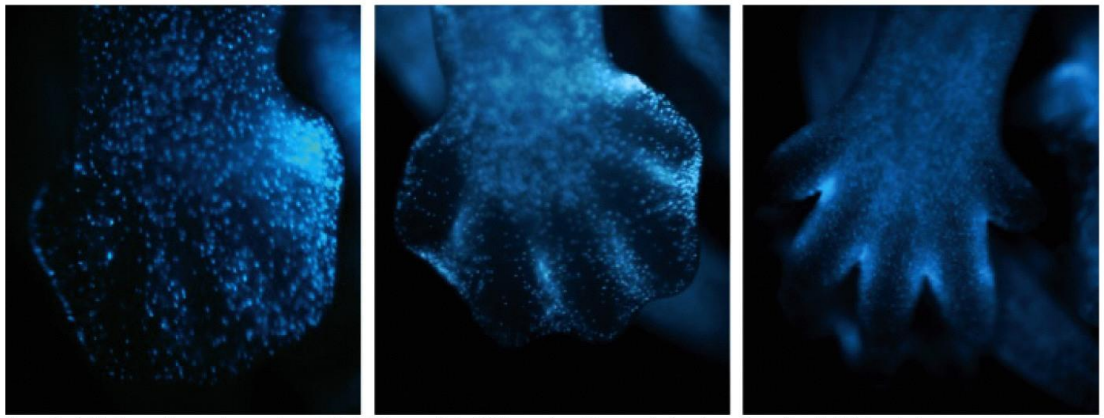
Morphological differences apoptosis (upper right) and necroptosis (center)

Apoptosis is an ordered process involving cell shrinkage, loss of adhesion to other cells, dissection of chromatin, and engulfment by phagocytosis.

Apoptotic changes are activated by proteolytic enzymes, **caspases**, which target:

- *Protein kinases*, some of which cause detachment of cells.
- *Lamins*, which line the nuclear envelope.
- *Proteins of the cytoskeleton*
- *Caspase activated DNase (CAD)*

15.15 | Apoptosis (Programmed Cell Death)



From David A. Hume, Nature Innumology 9:13, 2008; copyright 2008, reprinted by permission from Macmillan Publishers Ltd.

Apoptosis carves out the structure of the mammalian digits: tracking cyan fluorescent macrophages

Apoptosis is needed during embryonic development to form structure, organs and tissues (e.g. spaces between digits, pruning unneeded neurons).

Apoptosis is also active in the adult, where about 10^{10}-10^{11} cells die per day.

Reduced or elevated apoptosis is linked to several human diseases: *Cancer; Parkinson's, Alzheimer's, and Huntington's diseases; Diabetes type* I

15.15 | Apoptosis (Programmed Cell Death)

The Extrinsic Pathway of Apoptosis

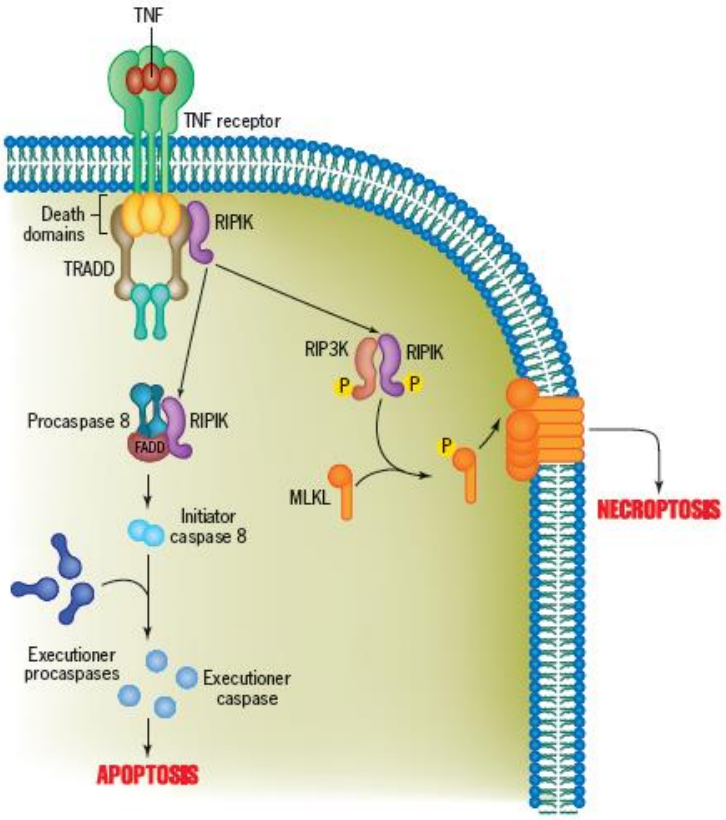
The *extrinsic pathway* of apoptosis is initiated by external stimuli.

As an example, the death ligand tumor necrosis factor (TNF) is detected by a TNF cell surface receptor.

Bound TNF receptors recruit "procaspases" to the intracellular domain of the receptor.

Procaspases convert other procaspases to caspases.

Caspases activate executioner caspases, leading to apoptosis.



The extrinsic (receptor-mediated) pathway of apoptosis

15.15 | Apoptosis (Programmed Cell Death)

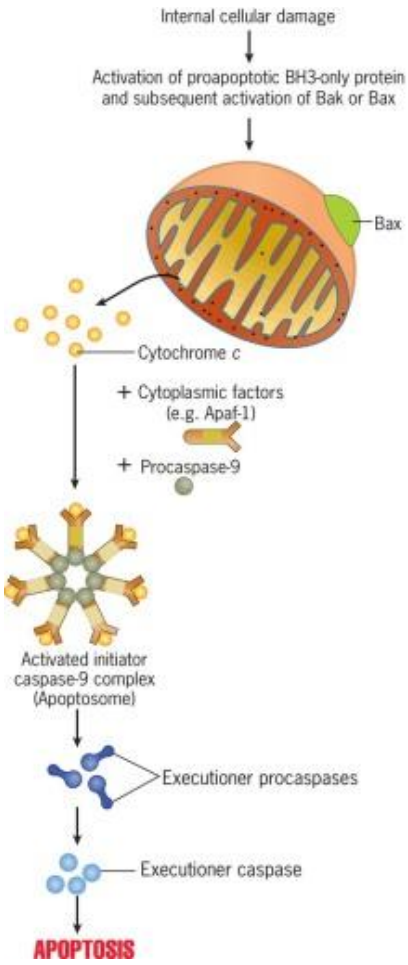
The Intrinsic Pathway of Apoptosis

The *intrinsic pathway* of apoptosis is initiated by intracellular stimuli, e.g. DNA damage.

Proapoptotic proteins stimulate mitochondria to leak proteins, mostly cytochrome *c*.

Once in the cytosol, cytochrome *c* forms part of a multiprotein complex called the *apoptosome*, that also includes several molecules of procaspase-9.

Release of apoptotic mitochondrial proteins irreversibly commits the cell to apoptosis.



The intrinsic (mitochondria-mediated) pathway of apoptosis