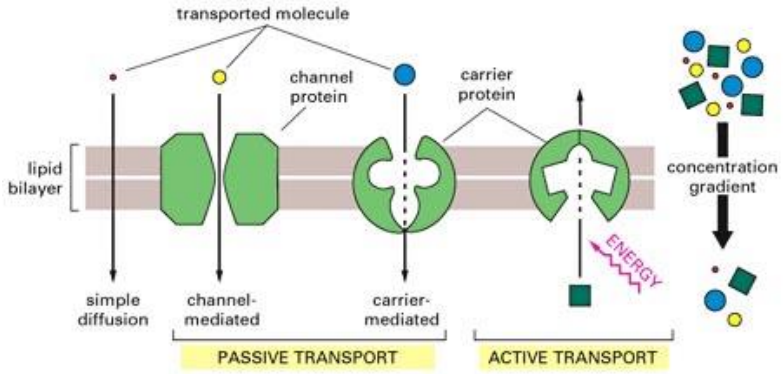
Membrane transport

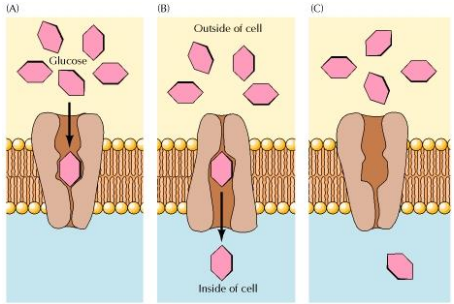
Two main categories of molecule transport exist in cells, active transport and passive transport.



Molecular biologyof the cell by Alberts et al.

Passive transport of small molecules
Transport proteins, each specialized for a certain molecule, can transport polar molecules across the membrane.
Uniports move solutes from one side to another.
Cotransport systems work by simultaneously sending two solutes across the lipid bilayer.
There are two types of cotransport systems - symport, in which the solutes are sent in the same direction, or antiport, in which they are sent in opposite directions.
These transport proteins work passively, meaning that the cell doesn't have to expend energy sending the solute in or out.

Model for the facilitated diffusion of glucose
The glucose transporter alternates between two conformations in which a glucose-binding site is alternately exposed on the outside and the inside of the cell.
Glucose binds to a site exposed on the outside of the plasma membrane and the transporter undergoes a conformational change such that the glucose-binding site faces the inside of the cell and glucose is released into the cytosol.
Then the transporter then returns to its original conformation.



The cell: A molecular approach by Cooper.

Primary Active Transport
The membrane proteins that bind to and transport the molecules or ions against their gradients posses the enzymatic activity to hydrolyze or break down ATP to harness its energy.
Known as **ATPase**, these transporters **are ATP-powered pumps that directly hydrolyze ATP to ADP and inorganic phosphate.**
Four classes of transport proteins function as ATP-powered pumps to transport ions and molecules against their concentration gradient. All have ATP-binding sites on the cytoplasmic side of the membrane.

Class	Substrate(s) transported
P F V ABC*	Ions (H^+, Na^+, K^+, Ca^{+2}) H^+ only H^+ only Ions, drugs, xenobiotics The ABC transporters are associated with multidrug resistance (they pumping nonpolar toxic molecules out of the cell). MDR is major limitation in cancer therapy.

Active Transport
The sodium-potassium pump in conjunction with the potassium leak channel, allows the cell the control it's membrane potential.
The sodium-potassium-ATPase pumps sodium out and potassium in, which creates a high concentration of potassium inside the cell, and a low concentration outside.
The potassium leak channel allows the potassium to leak out (so to even out the concentrations), which gives the cell a negative charge on the inside.

Secondary Active Transport
The process by which **ion gradients** generated by ATP-powered pumps **are used to power transport** of other molecules and ions against their own concentration gradients.
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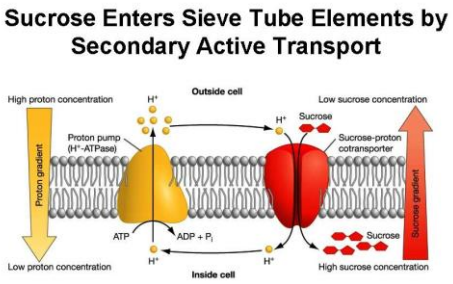


Active secondary transport driven by the Na^+ gradient is responsible for the uptake of glucose from the intestinal lumen. The transporter coordinately binds and transports 1 glucose and 2 Na^+ into the cell. The transport of Na^+ in the energetically favorable direction drives the uptake of glucose against its concentration gradient.

Drug Transport
Many drug transporters function as primary and secondary active transporters. The structures, function and tissue distribution vary widely.

Drug transporters	Transport
Solute carriers (SLC's): PEPT Peptide transporters OATP Organic anion transporting polypeptides Organic ions transporters H+/organic cation antiporters ABC transporters	Secondary active transort Proton (H+) and di- and tri-peptide cotransport B-lactam antibiotics (penicilin), ACE (angitenisn converting enzyme) inhibitors Amphipathic organic coumpounds such as: bile salts, steroids, thyroid hormones Organic ions Metformin – drug to treat type 2 diabetes Organic cations are excreted and H+ are taken up *Primary active transport* Export of ions, drugs and xenobiotics anticancer-, antiviral-, immunosuppressive agents, calcium channels blockers

Channel proteins
Form open pores in the membrane, allowing small molecules of the appropriate size and charge to pass freely through the lipid bilayer.
Ion channels are highly selective because narrow pores in the channel restrict passage to ions of the appropriate size and charge. Thus, specific channel proteins allow the passage of Na+, K+, Ca2+, and Cl- across the membrane.
Transport through channels is extremely rapid.

Most ion channels are not permanently open.
Ion channels are regulated by "gates" that transiently open in response to specific stimuli.
Channels called **ligand-gated channels** open in response to the binding of neurotransmitters or other signaling molecules.
Voltage-gated channels open in response to changes in electric potential across the plasma membrane.

Macromolecules Transport
To transport the macromolecules (proteins, polynucleotides, and polysaccharides), cells rely on **active transport**.
There are two basic means of active transport – by **exocytosis** and by **endocytosis**.
Exocytosis involves sending macromolecules out of the cell, while the opposite applies to endocytosis.
There are two types of endocytosis:
pinocytosis involves ingesting small molecules and/or fluids surrounding the cell in a process known as fluid-phase endocytosis;
phagocytosis involves the ingestion of large molecules, such as microorganisms or cell debris using large vesicles, or vacuoles.

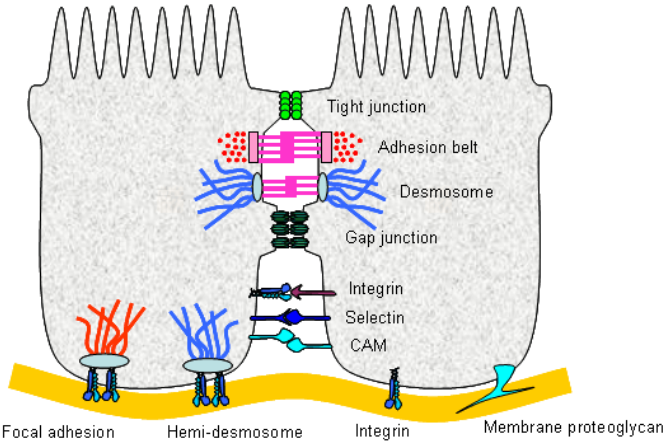
Receptor-mediated endocytosis
The macromolecules to be internalized first bind to specific cell surface receptors, allowing the cell to select what molecules to take and what to reject. These receptors are concentrated in specialized regions of the plasma membrane, called clathrin-coated pits.
The pits bud from the membrane to form small clathrin-coated vesicles containing the receptors and their bound macromolecules (ligands).
The clathrin-coated vesicles then fuse with early endosomes, in which their contents are sorted for transport to lysosomes or recycling to the plasma membrane.
Many receptors (like the LDL receptor) being returned to the plasma membrane following dissociation of their bound ligands in early endosomes.
The recycling of these receptors results in the continuous internalization of their ligands.

Cell-to-cell signaling

In all multicellular organisms, survival depends on an elaborate intercellular communication network that coordinates the growth, differentiation, and metabolism of the multitude of cells in diverse tissues and organs.
Cells within small groups often communicate by **direct cell-cell contact**.
Specialized junctions in the plasma membranes of adjacent cells permit them to exchange small molecules and to coordinate metabolic responses; other junctions between adjacent cells determine the shape and rigidity of many tissues.

Cell-cell adhesion is a selective process – cells adhere only to other cells of specific types.
Selective cell-cell adhesion is mediated by transmembrane proteins called **cell adhesion molecules**, which can be divided into four major groups: the selectins, the integrins, the immunoglobulin (Ig) superfamily and the cadherins.
Cells adhere to each other *via*:

- Cell adhesion molecules
- Junctional adhesion mechanisms



www.cellbiology.med.unsw.edu.au

Tight junctions are the closely associated areas of two cells whose membranes join together forming a virtually impermeable barrier to fluid.
Function:

- They hold cells together;
- They help to maintain the polarity of cells;
- They prevent the passage of molecules and ions through the space between cells.

Tight junctions play this role in maintaining the blood-brain barrier.

Adherens junctions provide strong mechanical attachments between adjacent cells.
Lie just below tight junctions.
Present mainly in epithelial cells (they hold epithelial cells together).
They hold cardiac muscle cells tightly together as the heart expands and contracts.
They seem to be responsible for contact inhibition.

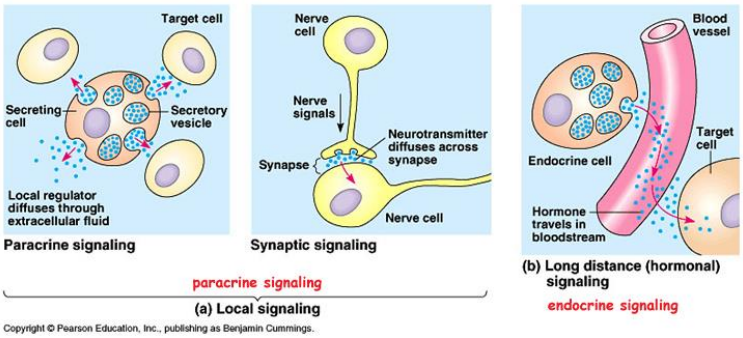
A **gap junction** or **nexus** is a specialized intercellular connection between a multitude of animal cell-types.
It directly connects the cytoplasm of two cells, which allows various molecules and ions to pass freely between cells.

Desmosomes & **hemidesmosomes**
Strong adhesions found typically in epithelial cells and other cell types that are subjected to stress or shear (e.g., cardiac muscle, ephitelium of skin).
In epithelia, keratins intermediate filaments form junctions that:
hold cells together – **desmosomes**,
or attach cells to matrix – **hemidesmosomes**.

Extracellular signaling molecules are the substances synthesized and released by **signaling cells** and produce a specific response only in **target cells** that have receptors for the signaling molecules. An enormous variety of chemicals, including small molecules (e.g., amino acid derivatives, acetylcholine), peptides, and proteins, are used in this type of cell-to-cell communication.

In animals, signaling by extracellular, secreted molecules can be classified into three types, based on the distance over which the signal acts:

- **endocrine,**
- **paracrine,**
- **autocrine**.



Communication by extracellular signals usually involves six steps:
(1) synthesis
(2) release of the signaling molecule by the signaling cell;
(3) transport of the signal to the target cell;
(4) detection of the signal by a specific receptor protein;
(5) a change in cellular metabolism, function, development triggered by the receptor-signal complex;
(6) removal of the signal, which often terminates the cellular response.

Signaling molecules

1. Because of their hydrophobic character, **the steroid hormones**, thyroid hormone, vitamin D3, and retinoic acid are able to enter cells by diffusing across the plasma membrane. Once inside the cell, they bind to intracellular receptors that are expressed by the hormonally responsive target cells.

2. The gas **nitric oxide** (NO) is a major paracrine signaling molecule in the nervous, immune, and circulatory systems.
NO diffuses across the plasma membrane of its target cells.
NO reacts with iron bound to the active site of the enzyme **guanylyl cyclase**. This increases enzymatic activity, resulting in synthesis of the second messenger cyclic GMP, which induces muscle cell relaxation and blood vessel dilation.
Another simple gas, **carbon monoxide** (CO), also functions as a signaling molecule in the nervous system. CO is closely related to NO and appears to act similarly as a neurotransmitter and mediator of blood vessel dilation.

3. **The neurotransmitters** carry signals between neurons or from neurons to other types of target cells (such as muscle cells).
They are a diverse group of small hydrophilic molecules that bind to cell surface receptors.
The release of neurotransmitters is signaled by the arrival of an action potential at the terminus of a neuron. The neurotransmitters then diffuse across the synaptic cleft and bind to receptors on the target cell surface.

4. **Peptide** hormones, neuropeptides, and growth factors are signaling molecules, which are unable to cross the plasma membrane of their target cells, so they act by binding to cell surface receptors.

5. Several types of **lipids** serve as signaling molecules that, in contrast to the steroid hormones, act by binding to cell surface receptors.
The most important of these molecules are members of a class of lipids called **the eicosanoids**, which includes: prostaglandins, prostacyclin, thromboxanes, and leukotrienes.
The eicosanoids are rapidly broken down and therefore act locally in autocrine or paracrine signaling pathways.
They stimulate a variety of responses in their target cells, including blood platelet aggregation, inflammation, and smooth-muscle contraction.

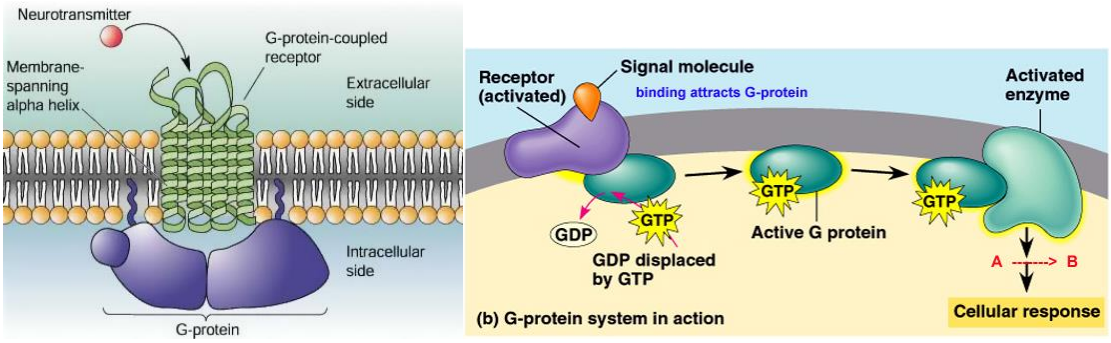
Membrane receptors

Most ligands responsible for cell-cell signaling bind to receptors on the surface of their target cells.
The cell surface receptor, which binds the signaling molecule sends a signal that alters the behavior of the target cell.

G Protein-Coupled Receptors

The largest family of cell surface receptors transmit signals to intracellular targets via the intermediary action of guanine nucleotide-binding proteins called G proteins.
The G protein-coupled receptors are structurally and functionally related proteins characterized by seven membrane-spanning α helices.

The binding of ligands to the extracellular domain of these receptors induces a conformational that allows the cytosolic domain of the receptor to bind to a G protein associated with the inner face of the plasma membrane.
Heterotrimeric G proteins: in the resting state the α subunit is bound to GDP in a complex with β and γ subunits. Guanine nucleotides regulate G protein activity. The activated GTP-bound α subunit dissociates from β and γ and interact with their targets to elicit an intracellular response.



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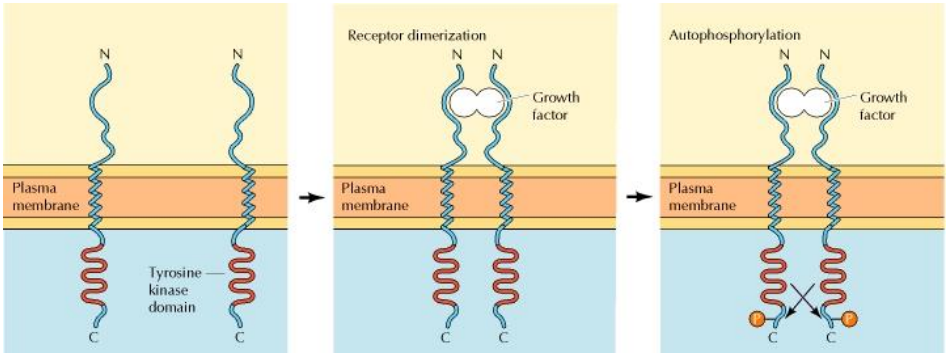
Receptor Protein-Tyrosine Kinases

Some cell surface receptors are directly linked to intracellular enzymes.
The largest family of such enzyme-linked receptors are the receptor protein-tyrosine kinases, which phosphorylate their substrate proteins on tyrosine residues.
This family includes the receptors for most polypeptide growth factors, so protein-tyrosine phosphorylation has been particularly well studied as a signaling mechanism involved in the control of animal cell growth and differentiation.
Growth factor binding induces receptor dimerization, which results in receptor autophosphorylation as the two polypeptide chains phosphorylate one another.



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The association of downstream signaling molecules with receptor protein-tyrosine kinases is mediated by protein domains that bind to specific phosphotyrosine-containing peptides.
The best-characterized of these domains are called SH2 domains (for *S*rc *h*omology *2*) because they were first recognized in protein-tyrosine kinases related to Src, the oncogenic protein of Rous sarcoma virus.
SH2 domains bind to specific phosphotyrosine-containing peptides of the activated receptors.
The resulting association can have several effects: it localizes the SH2-containing proteins to the plasma membrane, leads to their association with other proteins, promotes their phosphorylation, and stimulates their enzymatic activities.

Cytokine receptors and nonreceptor protein-tyrosine kinases
Many receptors act by stimulating intracellular protein-tyrosine kinases with which they are noncovalently associated.
This family of receptors (called the cytokine receptor superfamily) includes the receptors for most cytokines and for some polypeptide hormones (e.g., GH).
The cytokine receptors contain N-terminal extracellular ligand-binding domains, single transmembrane α helices, and C-terminal cytosolic domains.
The cytosolic domains of the cytokine receptors are devoid of any known catalytic activity. Instead, the cytokine receptors function in association with nonreceptor protein-tyrosine kinases, which are activated as a result of ligand binding.
The nonreceptor protein-tyrosine kinases fall into two major families: **the Src family**, which consists of Src and eight closely related proteins and **the Janus kinase**, or JAK, family.

Receptors linked to other enzymatic activities
Receptors associated with other enzymatic activities include protein-tyrosine phosphatases, protein-serine/threonine kinases, and guanylyl cyclases.

Protein-tyrosine phosphatases remove phosphate groups from phosphotyrosine residues, thus acting to counterbalance the effects of protein-tyrosine kinases.
In many cases, they play negative regulatory roles in cell signaling pathways by terminating the signals initiated by protein-tyrosine phosphorylation.

The receptors for transforming growth factor β (TGF-β) are protein kinases that phosphorylate serine or threonine, rather than tyrosine, residues on their substrate proteins. TGF-β controls proliferation and differentiation of a variety of cell types, generally inhibiting proliferation of their target cells.

Some peptide ligands bind to receptors whose cytosolic domains are **guanylyl cyclases**, which catalyze formation of cyclic GMP.

Other receptors bind to cytoplasmic proteins with additional biochemical activities. For example, the cytokine tumor necrosis factor (TNF) induces cell death, as a way of eliminating damaged or unwanted cells from tissues. The receptors for TNF and related death-signaling molecules are associated with specific proteases, which triggers the activation of additional downstream proteases, ultimately leading to degradation of a variety of intracellular proteins and death of the cell.

Pathways of intracellular signal transduction

Most cell surface receptors stimulate intracellular target enzymes.
These intracellular enzymes serve as downstream signaling elements that propagate and amplify the signal initiated by ligand binding.
In most cases, a chain of reactions transmits signals from the cell surface to a variety of intracellular targets—a process called intracellular signal transduction.
The targets of such signaling pathways frequently include transcription factors that function to regulate gene expression.

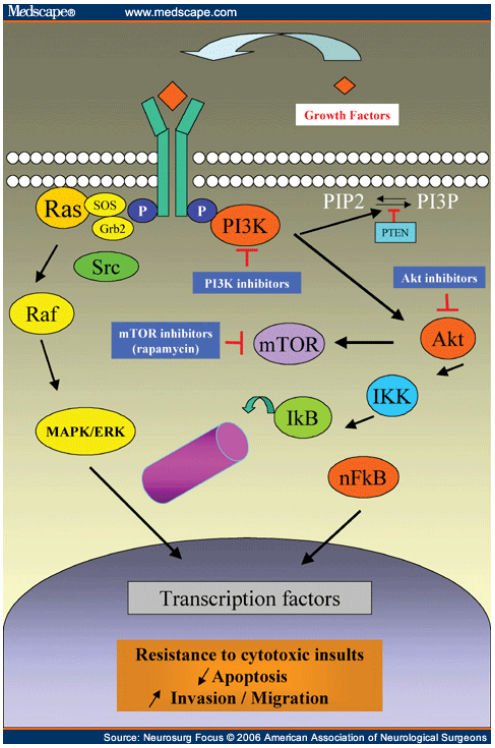
The cAMP pathway: second messengers and protein phosphorylation
Intracellular signaling was first elucidated by studies of the action of hormones such as epinephrine, which signals the breakdown of glycogen to glucose in anticipation of muscular activity.
Action of epinephrine is mediated by an increase in the intracellular concentration of cyclic AMP (cAMP), what suggested that cAMP is a second messenger in hormonal signaling (the first messenger being the hormone itself).
Cyclic AMP is formed from ATP by the action of adenylyl cyclase and degraded to AMP by cAMP phosphodiesterase
Increases in cAMP activate the transcription of specific target genes that contain a regulatory sequence called **the cAMP response element**, or **CRE**.
In this case, the signal is carried from the cytoplasm to the nucleus by the catalytic subunit of protein kinase A, which is able to enter the nucleus following its release from the regulatory subunit.
Within the nucleus, protein kinase A phosphorylates a transcription factor called CREB (for CRE-*b*inding protein), leading to the activation of cAMP-inducible genes.
Such regulation of gene expression by cAMP plays important roles in controlling the proliferation, survival, and differentiation of a wide variety of animal cells.

Cyclic GMP
Cyclic GMP is formed from GTP by guanylyl cyclases and degraded to GMP by a phosphodiesterase.
Stimulation of these guanylyl cyclases leads to elevated levels of cGMP, which then mediate biological responses, such as blood vessel dilation.
The action of cGMP is frequently mediated by activation of a cGMP-dependent protein kinase, although cGMP can also act to regulate other targets, including ion channels.

Phospholipids and Ca2+
One of the most widespread pathways of intracellular signaling is based on the use of second messengers derived from the membrane phospholipid phosphatidylinositol 4,5-bisphosphate (PIP2).
A variety of hormones and growth factors stimulate the hydrolysis of PIP2 by phospholipase C—a reaction that produces two distinct second messengers, **diacylglycerol** (DAG) and **inositol 1,4,5-trisphosphate** (IP3).
DAG and IP3 stimulate distinct downstream signaling pathways (protein kinase C and Ca2+ mobilization, respectively).
The diacylglycerol activates protein-serine/threonine kinases belonging to the **protein kinase C** (PKC) family, many of which play important roles in the control of cell growth and differentiation.
PKC then activates other intracellular targets, including a cascade of protein kinases known as the MAP kinase pathway, leading to transcription factor phosphorylation, changes in gene expression, and stimulation of cell proliferation.
Inositol 1,4,5-trisphosphate, is a small polar molecule that is released into the cytosol, where it acts to signal the release of Ca2+ from intracellular stores. As a result of binding to endoplasmic reticulum receptors cytosolic Ca2+ levels increase from 0.1 *μM* to about 1 *μM*, which affects the activities of a variety of target proteins, including protein kinases and phosphatases.

Ras, Raf, and the MAP kinase pathway
The MAP kinase pathway refers to a cascade of protein kinases that are highly conserved in evolution and play central roles in signal transduction in all eukaryotic cells, ranging from yeasts to humans.
The central elements in the pathway are a family of **protein-serine/threonine kinases** called the **MAP** kinases (for **mitogen-activated protein kinases**) that are activated in response to a variety of growth factors and other signaling molecules.

The best-characterized forms of MAP kinase in mammalian cells belong to the **ERK** (**extracellular signal-regulated kinase**) family. ERK activation plays a central role in signaling cell proliferation induced by growth factors that act through either protein-tyrosine kinase or G protein-coupled receptors.
Activation of ERK is mediated by two upstream protein kinases, which are coupled to growth factor receptors by a GTP-binding protein called **Ras**.
Activation of Ras leads to activation of the **Raf** protein-serine/threonine kinase, which phosphorylates and activates a second protein kinase called MEK (for *M*AP kinase/*E*RK *k*inase).
Once activated, ERK phosphorylates a variety of targets, including other protein kinases and transcription factors.



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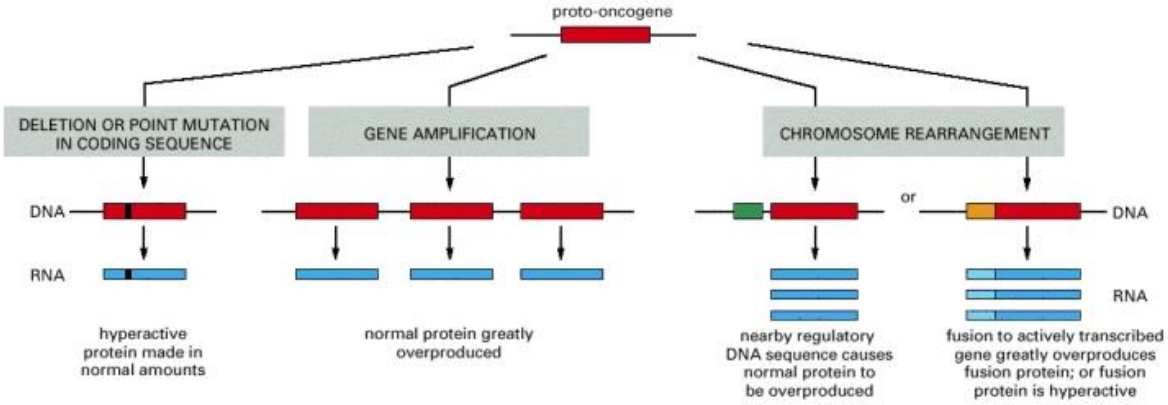
Mutations in *ras* genes are implicated in the development of human cancers

Ras proteins were first identified as the oncogenic proteins of tumor viruses that cause sarcomas in rats (Ras – *ra*t *s*arcoma virus).
Mutation of the *ras* genes remain one of the most common genetic abnormalities in human tumors.
Mutated *ras* oncogenes are found in about 20–30% of all human cancers, including approximately 25% of lung cancers, 50% of colon cancers, and more than 90% of pancreatic cancers.
Microinjection of active Ras protein directly induces proliferation of normal mammalian cells.
Conversely, interference with Ras function blocks growth factor-induced cell proliferation.
Thus, Ras is not only capable of inducing the abnormal growth characteristic of cancer cells, but also appears to be required for the response of normal cells to growth factor stimulation

Drugs specifically targeted against the oncogene proteins
Interruption of Ras signaling has become a focus for the development of anticancer agents.
One potentially effective approach involves the prevention of the localization of Ras through inhibition of protein farnesyltransferase (FTase), the enzyme which catalyzes post-translational modification of Ras to enable localization of Ras proteins to the inner plasma membrane.
Lonafarnib is a novel, orally active, FTase inhibitor. The agent has shown marked *in vitro* and *in vivo* antitumor activity and was chosen for further development and is currently undergoing phase II/III development for the treatment of cancer.
Lonafarnib Sorbera, L.A., Castaner, J.

Three ways in which a proto-oncogene can be made overactive to convert it into an oncogene



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At least 100 cancer-critical genes that can be converted into oncogenes by an activating mutation

Formation of the Bcr-Abl Gene by Translocation

In chronic myologenous leukemia, parts of chromosomes 9 and 22 are reciprocally exchanged, causing the *bcr* and *abl* genes to fuse.
The protein kinase encoded by the *bcr-abl* gene is expressed at higher levels in cells having this translocation than is the c-*abl* gene in normal cells.
Recent clinical trials of a specific inhibitor of the Bcr-Abl kinase have shown dramatic results; more than 90% of patients responded well to the treatment.

The ability of cells to perceive and correctly respond to their microenvironment is the basis of development, tissue repair, and immunity as well as normal tissue homeostasis.
Errors in cellular information processing are responsible for diseases such as cancer, autoimmunity, and diabetes. By understanding cell signaling, diseases may be treated effectively and, theoretically, artificial tissues may be yielded.