CELL SIGNALING

How do cells receive and respond to signals from their surroundings.

Prokaryotes and unicellular eukaryotes are largely independent and autonomous.

In multicellular organisms there is a variety of signaling molecules that are secreted or expressed on the cell surface of one cell and bind to receptors expressed by other cells. These molecules integrate and coordinate the functions of the cells that make up the organism.

Modes of cell-cell signaling

1. **Direct** cell-cell or cell-matrix (integrins and cadherins)

2. **Indirect: Secreted molecules**.

A. Endocrine signaling. The signaling molecules are hormones secreted by endocrine cells and carried through the circulation system to act on target cells at distant body sites.

B. Paracrine signaling. The signaling molecules released by one cell act on neighboring target cells (neurotransmitters).

C. Autocrine signaling. Cells respond to signaling molecules that they themselves produce (response of the immune system to foreign antigens, and cancer cells).

In the following slides we describe several groups of signaling molecules

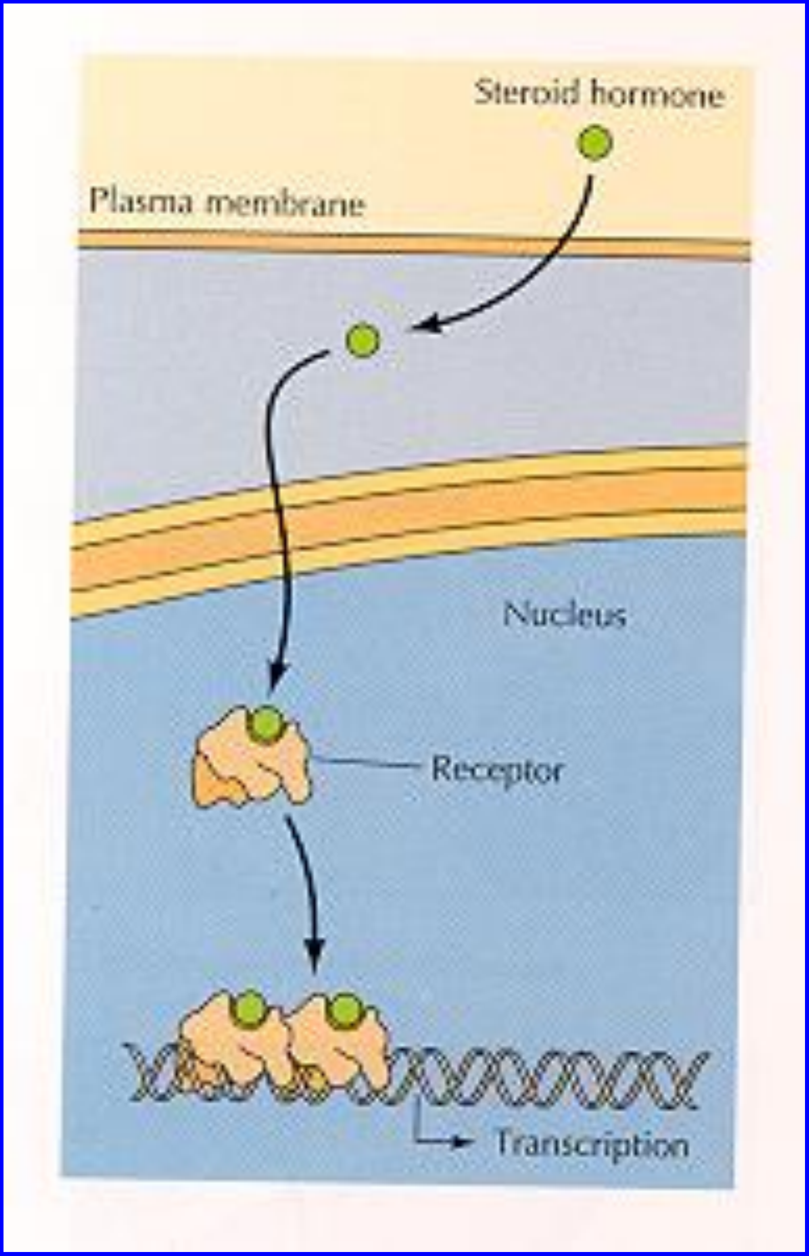
1. Steroid hormones

This class of molecules diffuse across the plasma membrane and bind to Receptors in the cytoplasm or nucleus. They are all synthesized from **cholesterol**.

They include sex steroids (estrogen, progesterone, testosterone)
corticosteroids (glucocorticoids and mineralcorticoids)

Thyroid hormone, vitamin D3, and retinoic acid have different structure and function but share the same mechanism of action with the other steroids.

Steroid Receptor Superfamily. They are transcription factors that function either as activators or repressors of transcription.



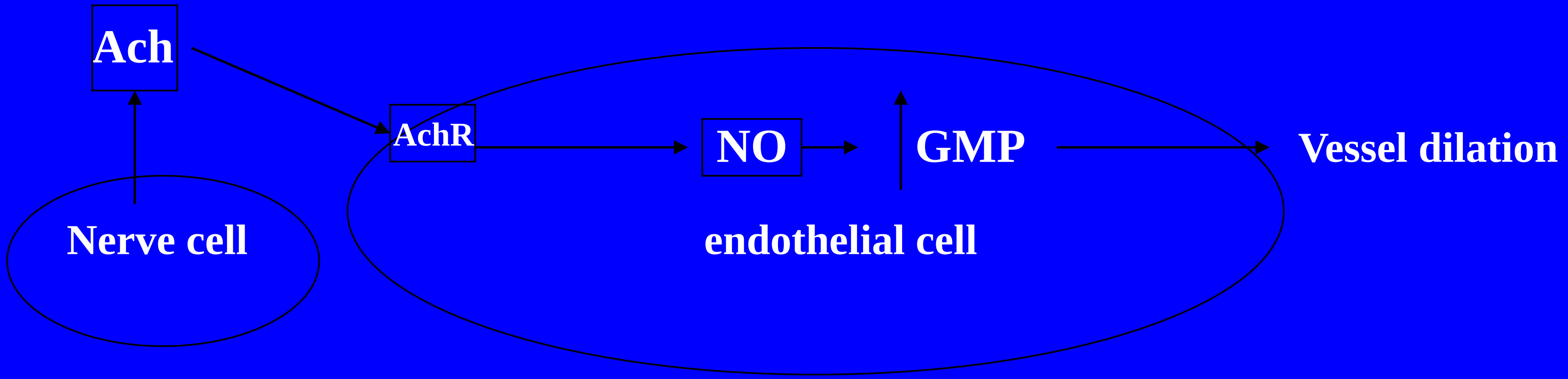
Steroid hormone
Plasma membrane
Nucleus
Receptor
Transcription

2. Nitric oxide (NO) and Carbon Monoxide (CO)

NO, a simple gas, is able to diffuse across the membrane, and alters the activity of intracellular target enzymes. It's extremely unstable, so its effects are local. Ex. It signals the dilation of blood vessels.

Mechanism.

Acetylcholine is released from the terminus of nerve cell in the blood vessel wall. The endothelial cells are stimulated to produce **NO** (from arginine), which causes an increased synthesis of **GMP**, a second messenger responsible for blood vessel dilation.

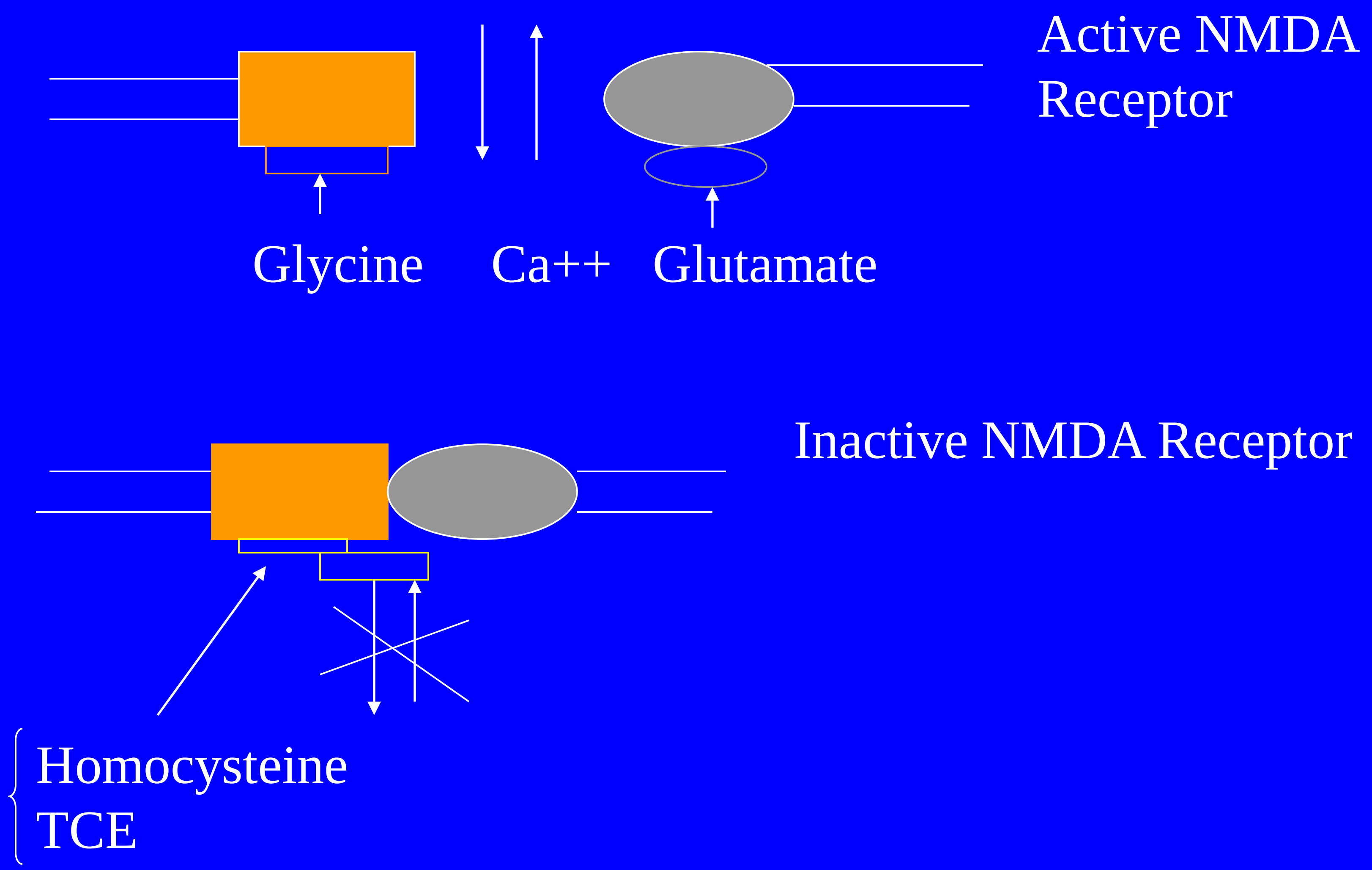


3. Neurotransmitters

They signal from neuron to neuron or from neuron to other target cell (ex. muscle cell).

Acetylcholine
Glycine
Glutamate
Dopamine
Epinephrine
Serotonin
Histamine
GABA.

Common features: **hydrophilic molecules that bind to cell surface receptors. The binding induces conformational changes that open ion channels → ion fluxes in the cell.**


Active NMDA
Receptor
Glycine
Ca++
Glutamate
Inactive NMDA Receptor
Homocysteine
TCE

4. EICOSANOIDS

This class of lipids act as signaling molecules that bind to cell surface molecules.

They include: **PROSTAGLANDINS**
PROSTACYCLIN
TROMBOXANES
LEUKOTRIENES.

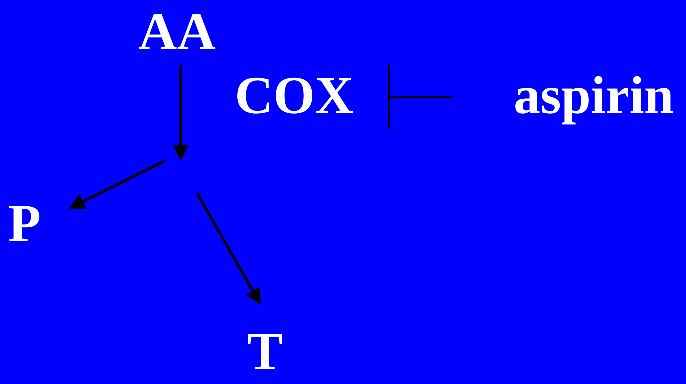
The eicosanoids are rapidly broken down and therefore act in **autocrine** or **paracrine** pathways. They stimulate a variety of responses in their target cells, including **blood platelet aggregation**, **inflammation, and smooth muscle contraction.**

Eicosanoids are synthesized from arachidonic acid. The first enzyme involved in their synthesis (cyclooxygenase, COX) is the target of **ASPIRIN**.

Aspirin actions:
-reduces inflammation and pain (inhibition of prostaglandins)
- reduces platelet aggregation and blood clotting (tromboxanes)

Applications:

- prevention of strokes
- reduce the frequency of colon cancer



NEWSBREAK

Function of Cell Surface Receptors

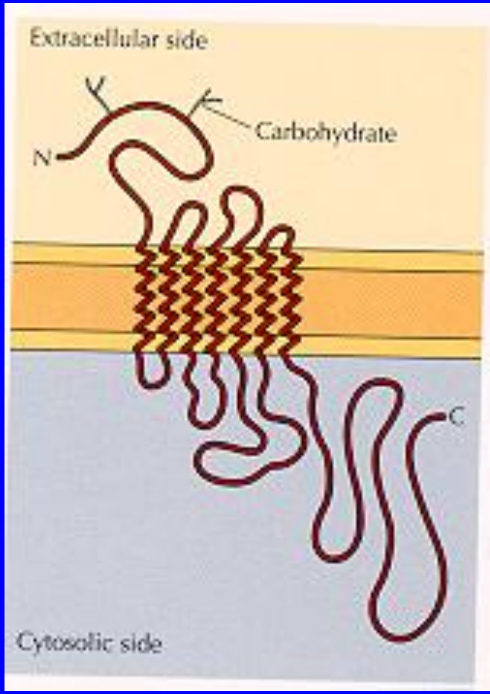
Neurotransmitters
Peptide Hormones
Growth Factors

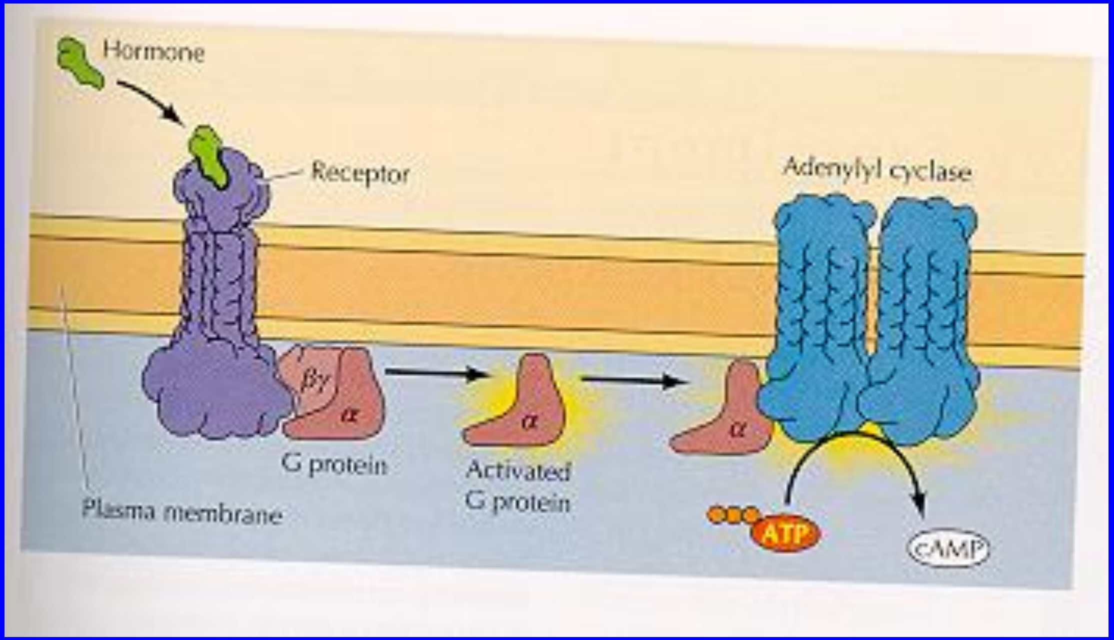
-Ligand-gated ion channels that directly control ion flux across the plasma membrane (NEUROTRANSMITTERS)

-Other receptors initiate a cascade of events ultimately affecting gene expression (PEPTIDE HORMONES AND GROWTH FACTORS)

In the following slides we'll describe different types of cell Surface receptors

1. G-Protein – Coupled Receptors
(Largest family of cell surface receptors)

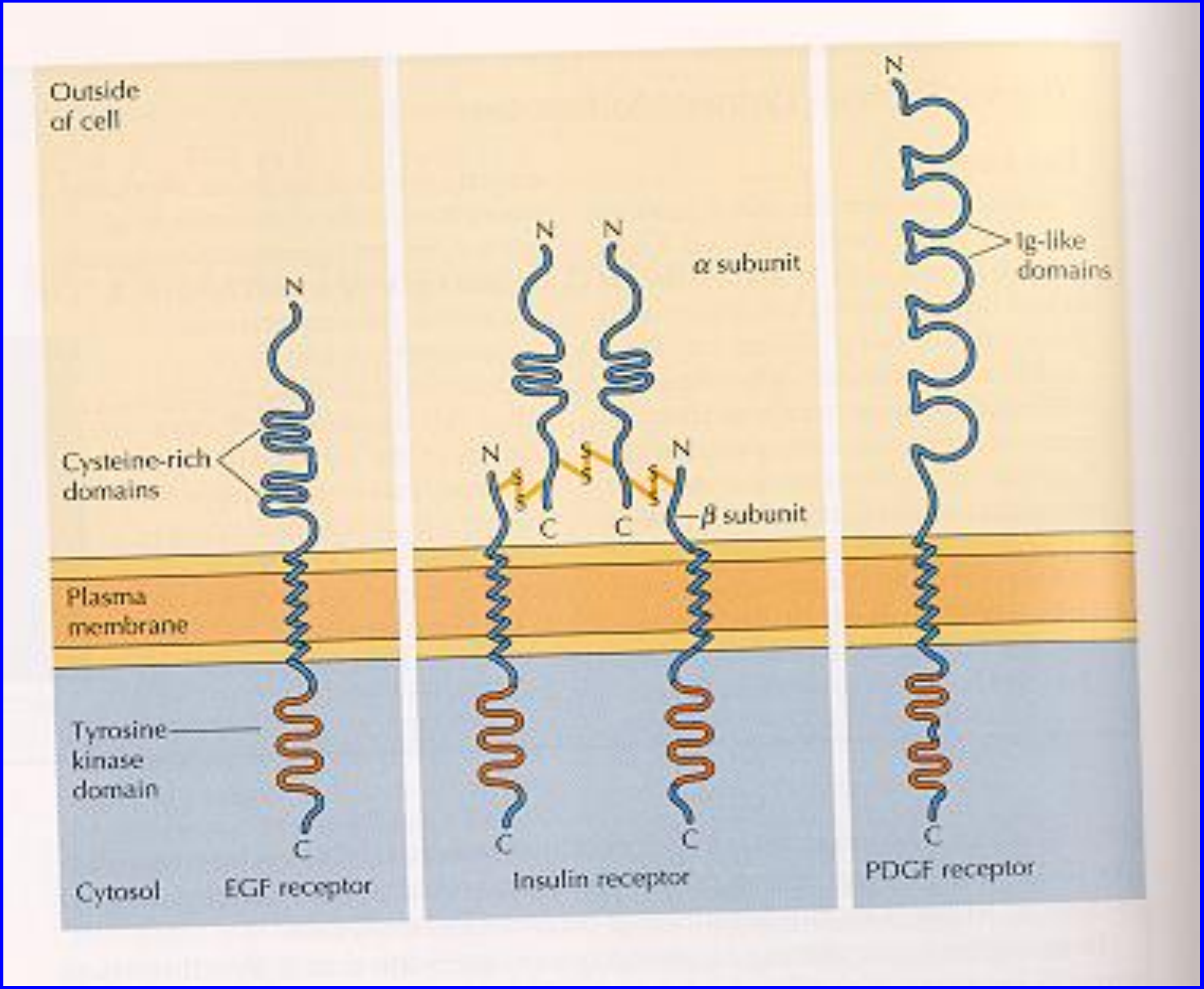




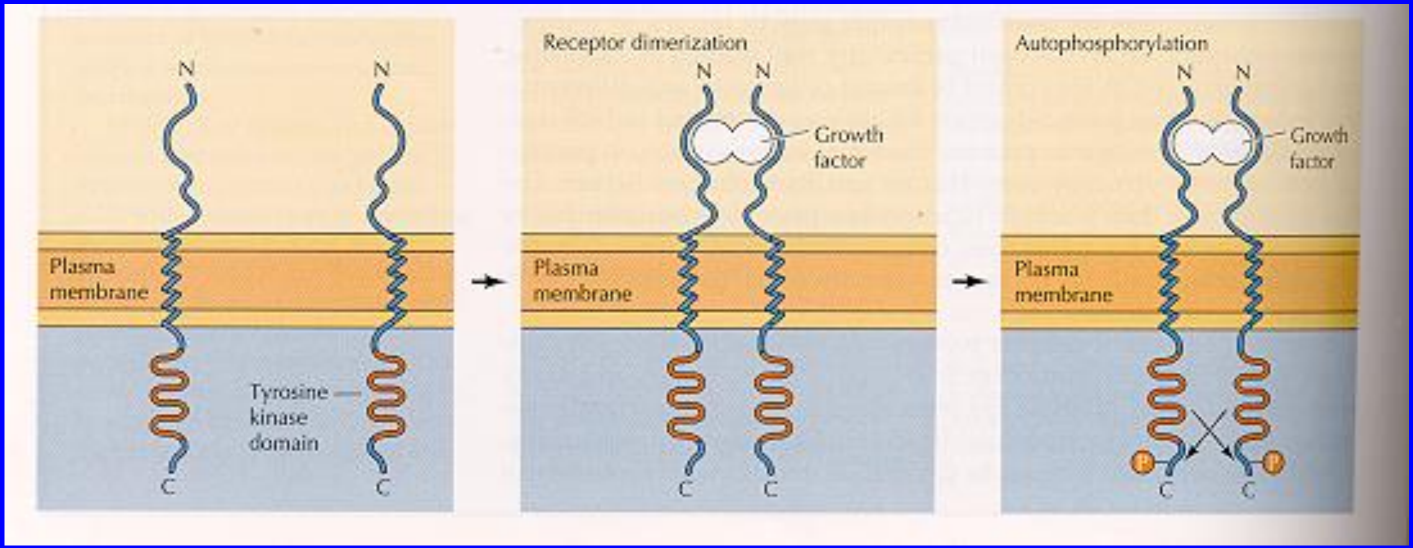
cAMP is a second messenger that mediates cellular responses to a variety of hormones.

2. Receptor Protein-Tyrosine Kinase
(They are directly linked to intracellular enzymes)
They phosphorylate their substrate proteins on tyrosine residues (peptide growth factors).

Common structure:
N-terminal extracellular ligand-binding domain
A single trans-membrane α helix
A cytosolic C-terminal domain with kinase activity



Outside of cell
N
Cysteine-rich domains
Plasma membrane
Tyrosine kinase domain
C
Cytosol
EGF receptor
N
N
α subunit
N
N
S S
S S
S S
C
C
β subunit
C
C
Insulin receptor
N
Ig-like domains
C
PDGF receptor



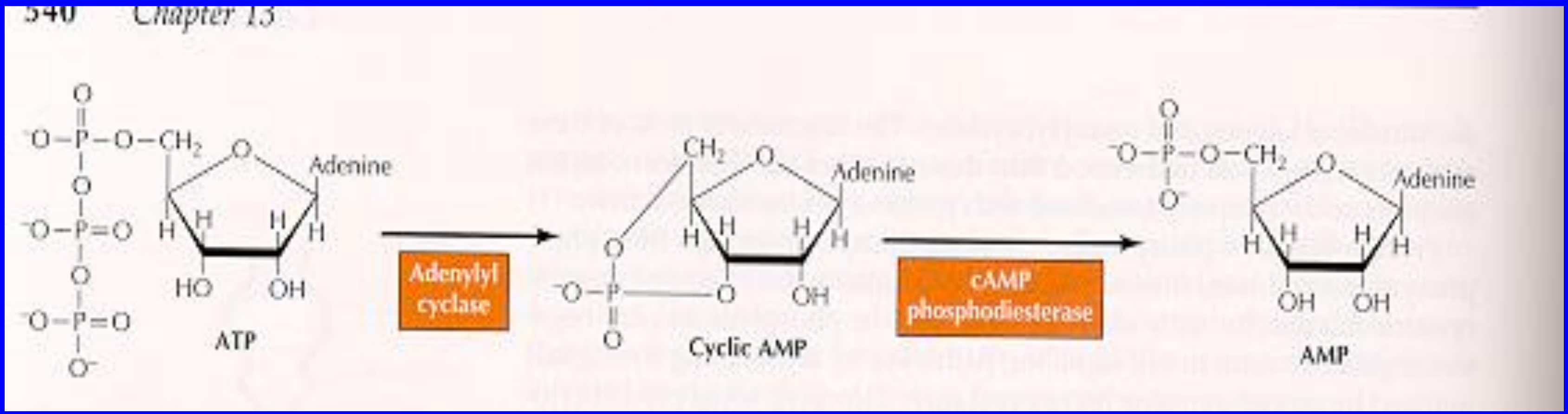
Growth factor binding induces receptor dimerization, which results in receptor autophosphorylation as the two polypeptide chains phosphorylate each other.

Pathways of intracellular Signal Transduction

From the receptor to intracellular enzymes that propagate and amplify the signal initiated by ligand binding. The chain of signaling molecules often ends at the nucleus where transcription factors bind to DNA and regulate gene expression.

Many pathways are well conserved among species from lower invertebrates to humans.

The cAMP pathway : Second messengers and Protein phosphorylation.



Cyclic AMP is synthesized from ATP by **adenylyl cyclase** and degraded to AMP by cAMP phosphodiesterase.

This is an example of a cAMP mediated pathway: Protein kinase A is activated by 4 molecules of cAMP that bind to the regulatory subunits releasing the catalytic subunits of the protein kinase A.

The active protein kinase phosphorylates two key enzymes that control degradation of glycogen.

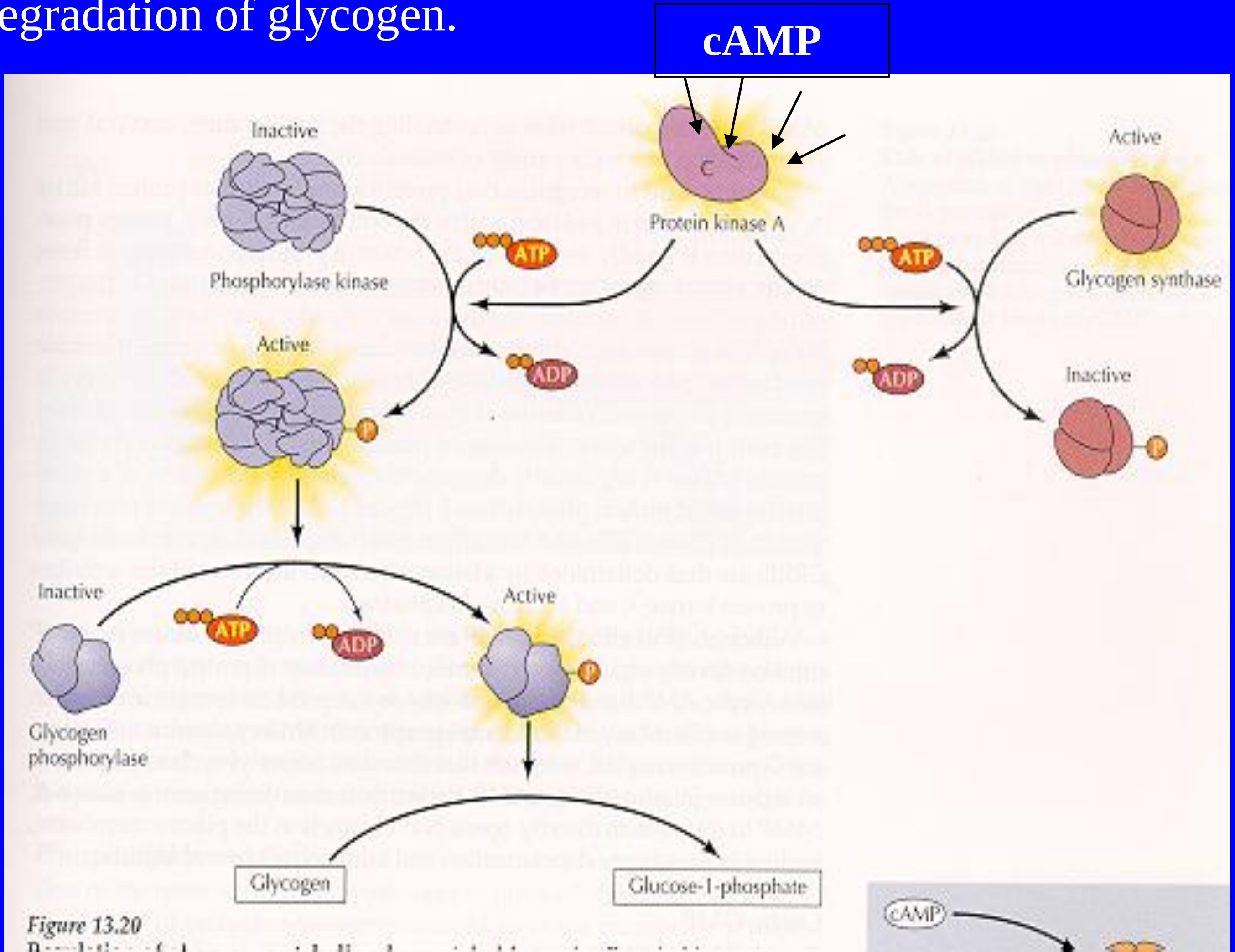
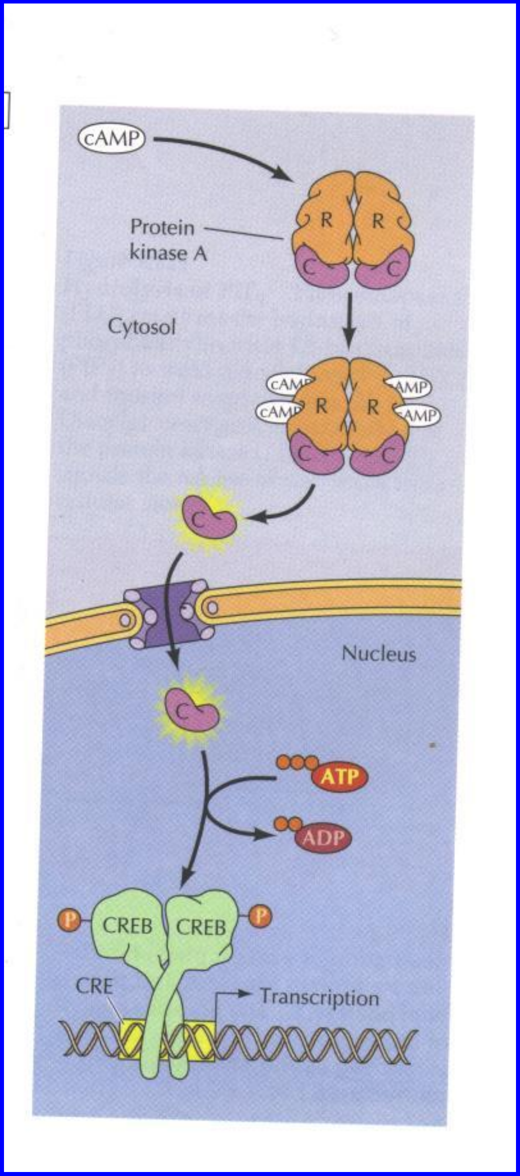
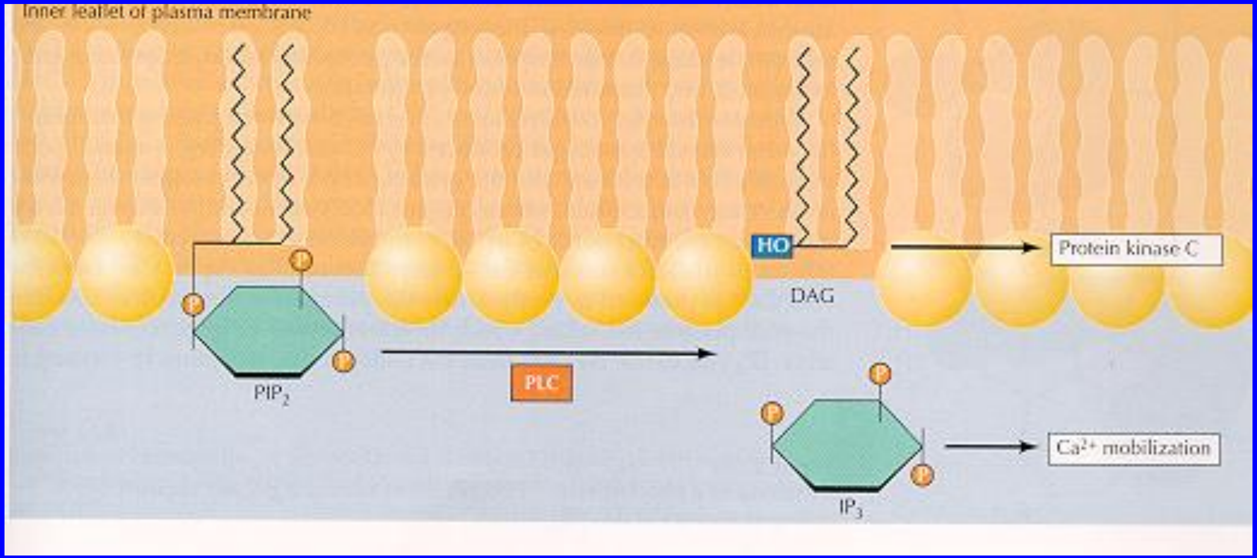


Figure 13.20

Another example of a signaling pathway mediated by cAMP



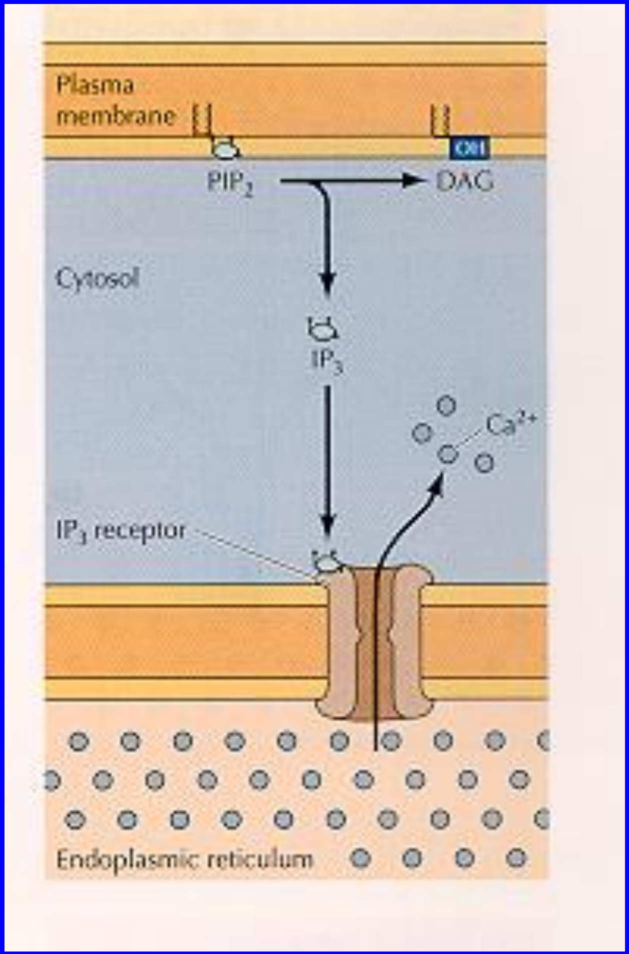
2.Phospholipids and Ca++ (another group of second messengers)



PIP2: PHOSPHATIDYLINOSITOL 4,5-BIPHOSPHATE (a component of the plasma membrane)

Hydrolysis of PIP2 is activated by PLC (Phospholipase C), and yields diacylglycerol and inositol phosphate (IP3).

DAG: Diacylglycerol activates the protein kinase C family, that play a crucial role in cell growth and differentiation.



Plasma membrane
OH
PIP$_2$
DAG
Cytosol
IP$_3$
Ca^{2+}
IP$_3$ receptor
Endoplasmic reticulum

Pathways of intracellular signal transduction

cAMP protein kinase A glycogen metabolism
gene expression (CREB)

Phospholipids and calcium PLC PIP2 IP3 calcium
DAG protein kinase C

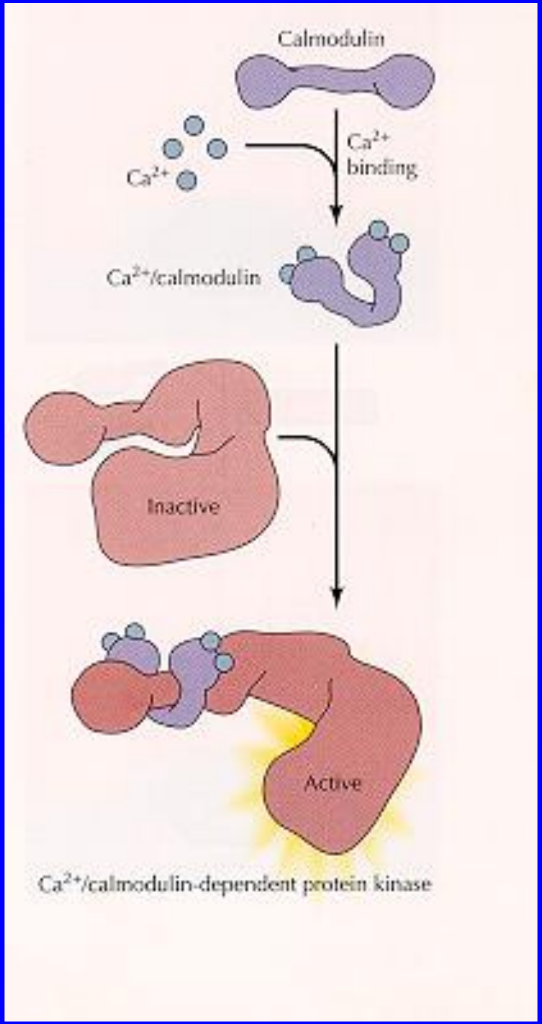
Ca++

Calcium cellular concentration is maintained low by pumps that transport calcium across the plasma membrane and from the cytosol inside the endoplasmic reticulum (ER).

High concentrations of calcium activate the functions of proteins including protein **kinase** and **phosphatases.**

Many of the effects of calcium are mediated by the Ca++-binding protein **calmodulin,** which is activated by calcium binding when the concentration of cytosolic calcium increases from 0.1 to 0.5 micromolar.

Calmodulin, in turn, binds to a variety of target proteins including protein kinases (CaM).



One of the proteins activated by Ca/calmodulin is a kinase called CaM

Ca2+ binding proteins and Ca2+ dependent pathways - conserved systems responsive to the level of Ca2+ ion in the cell

CaM is a protein kinase involved in most of the important signaling pathways in the cell.

CaM targets include:

- cyclic nucleotide metabolism (cAMP)
- signal transduction pathways involving phosphorylation and dephosphorylation
- calcium transport (carried out by the plasma membrane Ca2+ pump)
- nitric oxide pathway
- regulation of cytoskeletal proteins

Most of these systems are <u>evolutionarily conserved</u>, with counterparts in vertebrates as well as non-vertebrate species. The conservation of the principle Ca2+ reactive systems through evolution bespeaks their importance in cell function.

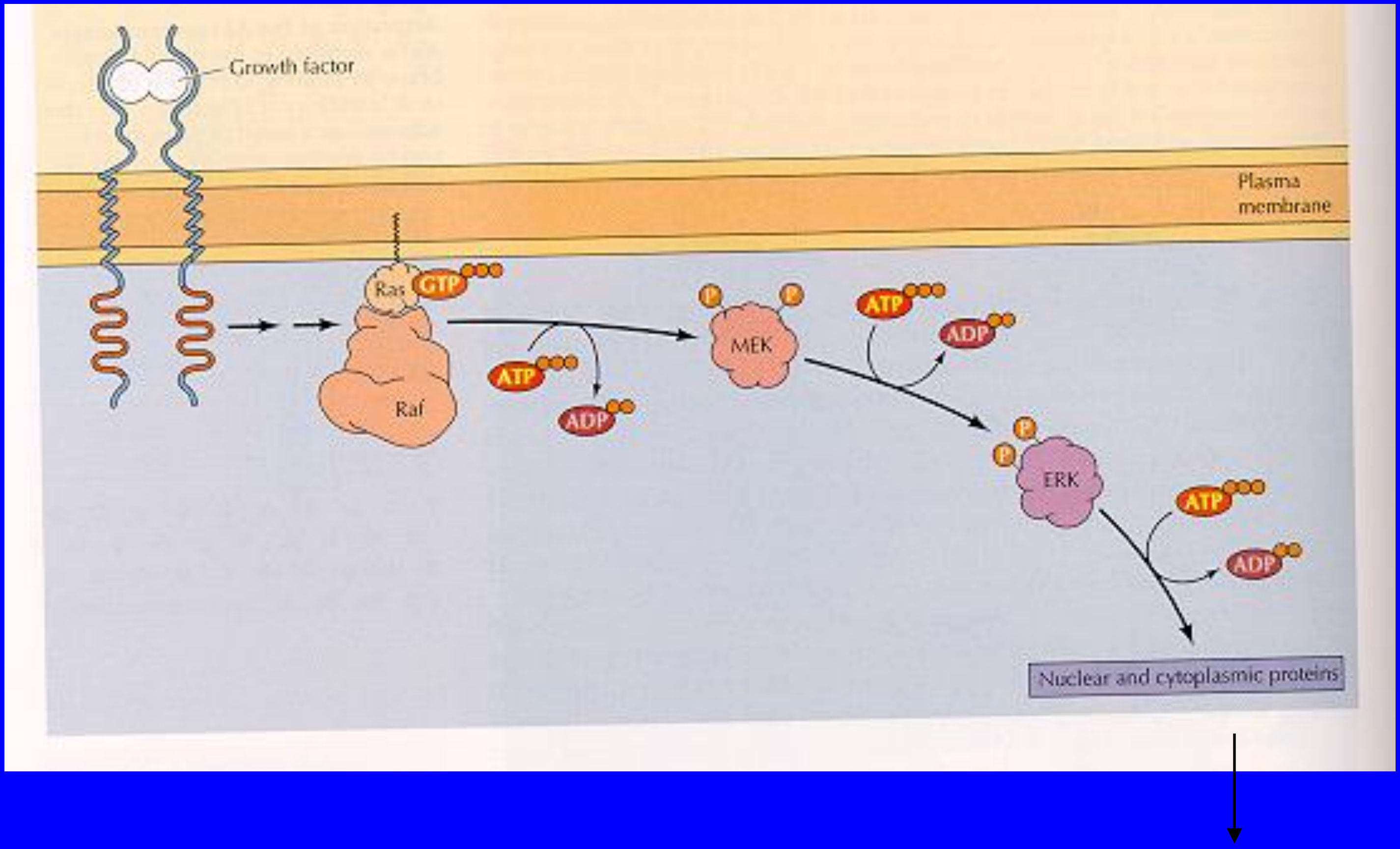
Third and last example of signaling pathways mediated by phosphorylation events.

Ras, Raf, and the MAP Kinase Pathway

It refers to a cascade of protein kinases that are highly conserved in evolution and play central roles in signal transduction in all eucaryotic cells.

The **central elements in the pathway are a family of protein-serine/threonine kinases called the MAP kinases.**

Best example is the activation of the ERK MAP kinases



Phosphorylation of transcription factors in the nucleus will either induce or inhibit gene expression.

Signal transduction and the cytoskeleton

Components of the cytoskeleton act as both receptors and targets in cell signaling pathways, integrating cell shape and movement with other cellular responses.

Regulation of the actin cytoskeleton.

Remodeling of the actin cyoskeleton is involved in processes like wound healing, embryonic development, metastatic invasion.

Regulation of Programmed Cell Death or APOPTOSIS

This process is responsible for balancing cell proliferation and maintaining constant cell numbers in tissues undergoing cell turnover.

It is also a defense mechanism against virus-infected cells and damaged cells.

During development, cells no longer necessary (larval tissue) or unwanted (tissue between the digits) are eliminated by apoptosis.

Regulation of apoptosis is mediated by the integrated activity of a variety of signaling pathways, some acting to induce cell death and others to promote cell survival.

How to recognize apoptosis in a cell.

During apoptosis, chromosomal DNA is fragmented as a result of cleavage between nucleosomes. The chromatin condenses and the nucleus breaks up in small pieces. The cell shrinks and breaks up in small membrane-enclosed fragments called apoptotic bodies. These fragments are engulfed and digested by macrophages.

Proteins involved in apoptosis.

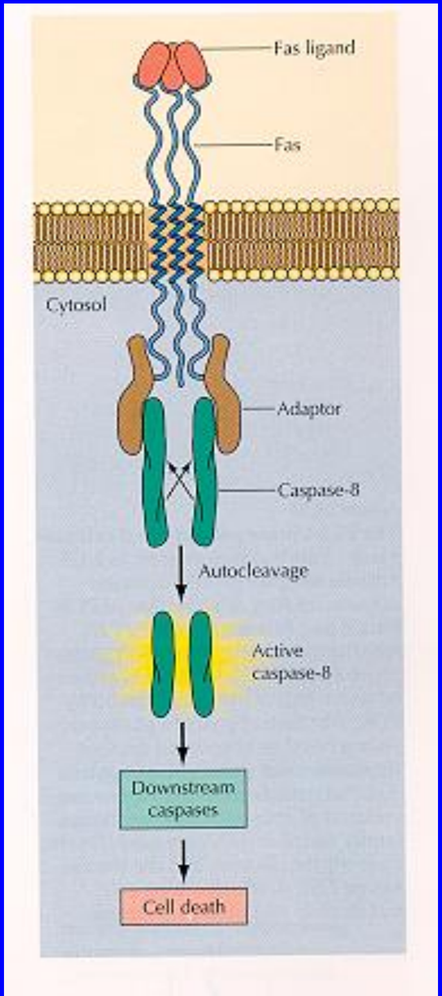
CASPASES. They all have **C**ysteine residues at their active site, and cleave after **Asp**artic Acid residues in the substrate protein. They are the ultimate effectors of apoptosis, cleaving more than 40 different target proteins.

Caspase activity is regulated by the **Bcl-2 family.** Some members of this family promote apoptosis, while others inhibit it.

Promotion of apoptosis involves mitochondrial damage, cytochrome c release, and caspase activation.

Cell death Receptors

Some secreted polypeptides signal apoptosis by activating receptors that directly induce cell death. These molecules belong to the **Tumor necrosis factor (TNF)** family. Best characterized member: **Fas.**



Summary

Signaling molecules and their receptors;

-Modes of cell-cell signaling (endocrine, paracrine, and autocrine)
-Steroid hormones and steroid receptor superfamily
-Nitric oxide and carbon oxide (paracrine signaling molecules important in the nervous system.)
-Neurotransmitters (hydrophilic, carry signals between neurons or neuron and other cell type, often bind to ion channels)
-Peptide hormones and growth factors (widest variety of signaling molecules)
-Eicosanoids (paracrine and autocrine; aspirin inhibits their function)

Function of cell surface receptors

G-protein-coupled receptors: transmit signals to intracellular targets via the intermediary actions of G proteins.

Receptor protein tyrosine-kinase: the receptor for most growth factors

PATHWAYS OF INTRACELLULAR SIGNAL TRANSDUCTION

The c-AMP pathway: important second messenger, mediates response to a variety of hormones and odorants. Most of its actions are mediated by protein kinase A, which phosphorylates both metabolic enzymes and the transcription factor CREB.

Phospholipids and Calcium

Ras, Raf, and MAP kinase. Involved in phosphorylation of cytosolic and nuclear proteins, including transcription factors.

SIGNAL TRANSDUCTION AND THE CYTOSKELETON

Integrins and signal transduction: they bind to the extracellular matrix and stimulate protein kinases and other downstream signaling

Regulation of actin : growth factors induce alterations in cell movement and shape by remodeling the actin cytoskeleton

REGULATION OF PROGRAMMED CELL DEATH

Caspases and apoptosis