ENDOCRINOLOGY (HORMONES/RECEPTORS)

BIOCHEMISTRY

Cell-Cell Interactions

- For a coordinated function of cells in a tissue, tissues in an organ, organs in a system and systems in the body, cells need to be able to communicate with each other.
- Each cell should be capable of sending chemical signals to other cells and of receiving chemical signals from other cells, as well as signals (chemical or other) from its immediate environment.

- A cell can communicate signals to other cells in various ways.
- **Autocrine signaling**
- **Paracrine signaling**
- **Endocrine signaling**
- **Direct signaling**
- **Synaptic signaling**

Autocrine signaling

- is a way for a cell to alter its own extracellular environment, which in turn affects the way the cell functions. The cell secretes chemicals outside of its membrane and the presence of those chemicals on the outside modifies the behavior of that same cell. This process is important for growth.

Paracrine signaling

- is a way for a cell to affect the behavior of neighboring cells by secreting chemicals into the common intercellular space. This is an important process during embryonic development.

Endocrine signaling

- utilizes hormones. A cell secretes chemicals into the bloodstream. Those chemicals affect the behavior of distant target cells.

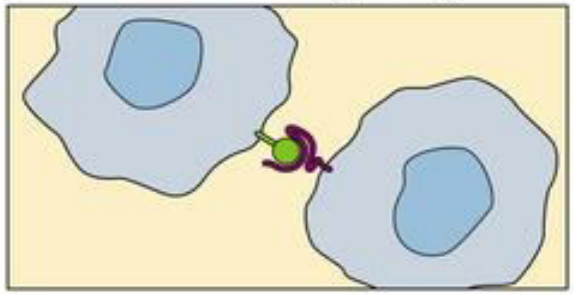
Direct signaling

- is a transfer of ions or small molecules from one cell to its neighbor through pores in the membrane. Those pores are built out of membrane proteins and are called **gap junctions**. This is the fastest mode of cell-cell communication and is found in places where extremely fast and well-coordinated activity of cells in needed. An example of this process can be found in the heart. The muscle cells in the heart communicate with each other via gap junctions which allows all heart cells to contract almost simultaneously.

Synaptic signaling

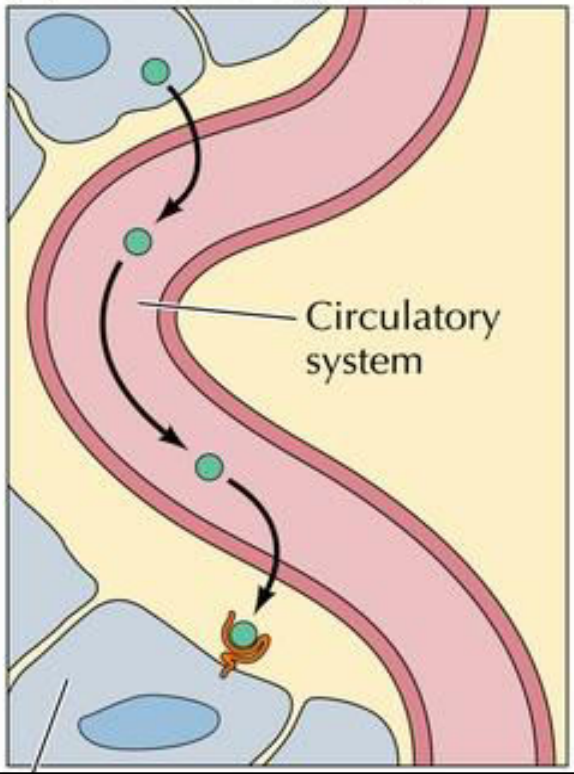
- is found in the nervous system. It is a highly specific and localized type of paracrine signaling between two nerve cells or between a nerve cell and a muscle cell.

Direct Cell-Cell Signaling

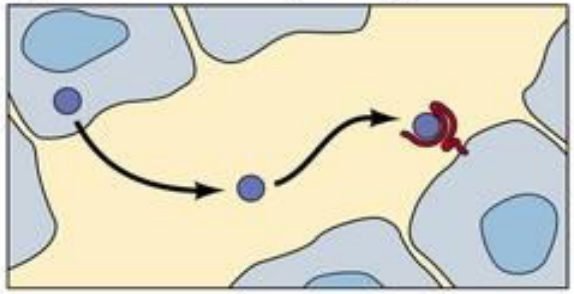


Signaling by Secreted Molecules

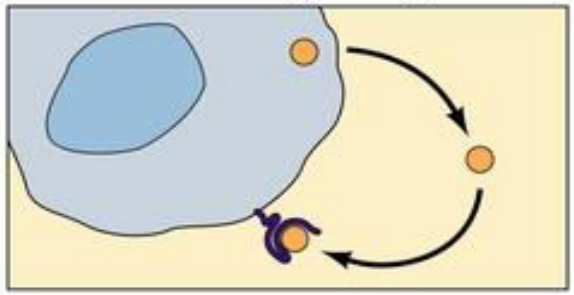
(A) Endocrine signaling



(B) Paracrine signaling



(C) Autocrine signaling



Target cells

Specific cells are affected by hormone and respond in unique fashion:

- Cells have specific receptors on membrane or in cell that respond to hormone
- Can have receptors for several different hormones
- Number of active receptors can change
  - Down-regulation - number of receptors decreases & target is less sensitive
  - Up-regulation - number increases & target is more sensitive

- Several factors determine the response of a target cell to a hormone. These can be thought of in two general ways:
- (1) as factors that affect **the concentration** of the hormone at the target cell and

- (2) as factors that affect the **actual response** of the target cell to the hormone

Determinants of the Concentration of a Hormone at the Target Cell

The rate of synthesis and secretion of the hormones.

The proximity of the target cell to the hormone source (dilution effect).

The dissociation constants of the hormone with specific plasma transport proteins (if any).

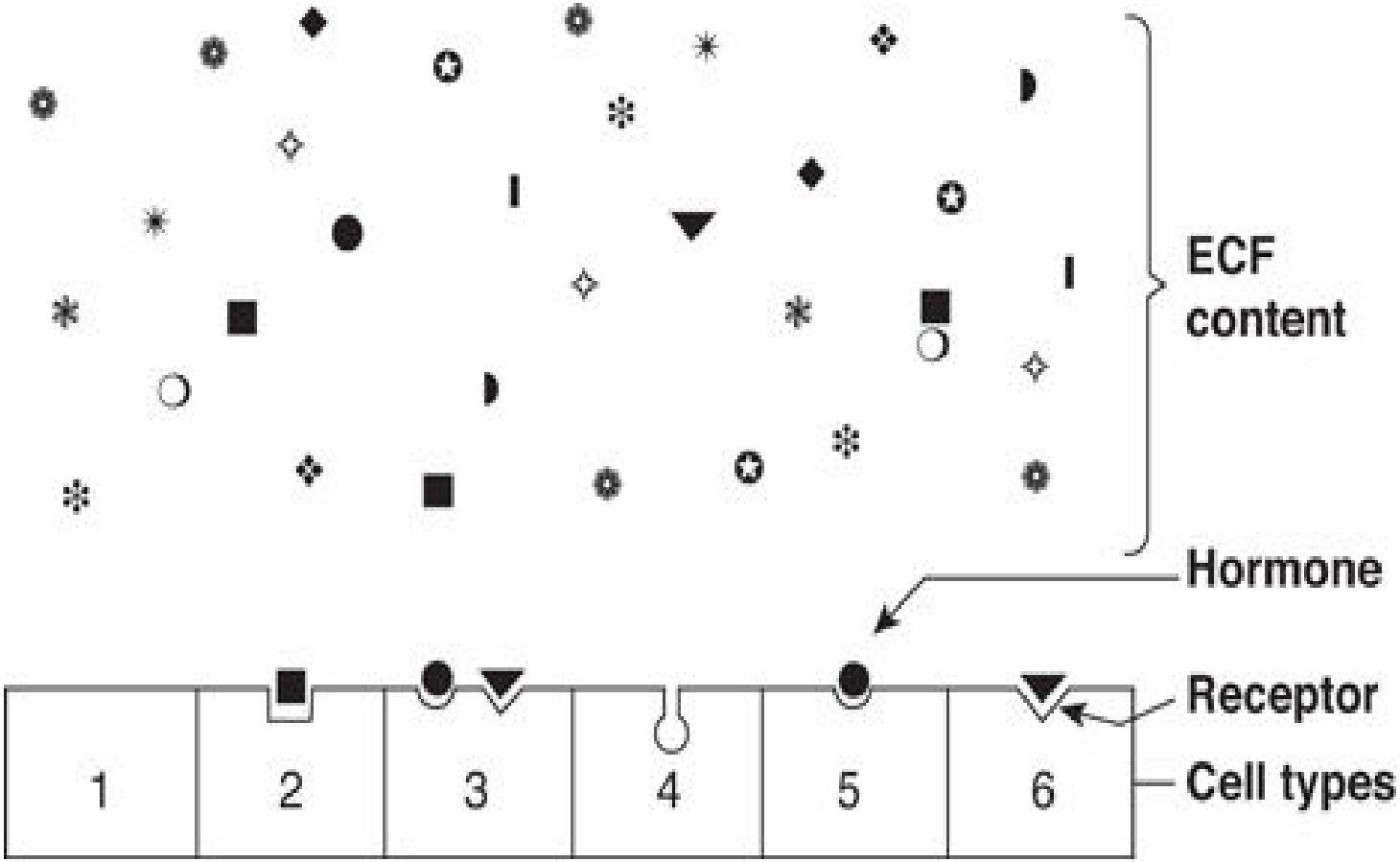
The conversion of inactive or suboptimally active forms of the hormone into the fully active form.

The rate of clearance from plasma by other tissues or by digestion, metabolism, or excretion

Determinants of the Target Cell Response

- The number, relative activity, and state of occupancy of the specific receptors on the plasma membrane or in the cytoplasm or nucleus.
- The metabolism (activation or inactivation) of the hormone in the target cell.
- The presence of other factors within the cell that are necessary for the hormone response.
- Up- or down-regulation of the receptor consequent to the interaction with the ligand.
- Post receptor desensitization of the cell, including down-regulation of the receptor.

- Hormones are present at very low concentrations in the extracellular fluid
- This concentration is much lower than that of the many structurally similar molecules (sterols, amino acids, peptides, proteins)
- high degree of discrimination is provided by cell-associated recognition molecules called receptors



Source: Murray RK, Bender DA, Botham KM, Kennelly PJ, Rodwell VW, Weil PA: *Harper's Illustrated Biochemistry, 28th Edition:* http://www.accessmedicine.com

- A target cell is defined by its ability to selectively bind a given hormone to its cognate receptor.

- Several biochemical **features of this interaction** are important in order for hormone-receptor interactions to be physiologically relevant:

- 1) binding should be specific i.e. displaceable by agonist or antagonist
- (2) binding should be saturable and
- (3) binding should occur within the concentration range of the expected biologic response.

How does a cell receive a signal?

- Some small molecules are capable of entering the cell through the plasma membrane.

- Some small hormones also enter the cell directly, by passing through the membrane. Examples are steroid hormones and thyroid hormones.

HORMONES (Biomedical Importance)

- Hormones are the chemical messengers of the body. They are defined as organic substances secreted into blood stream to control the metabolic and biological activities.

- These hormones are involved in transmission of information from one tissue to another and from cell to cell. These substances are produced in small amounts by various endocrine (ductless) glands in the body.

- They are delivered directly to the blood in minute quantities and are carried by the blood to various target organs where these exert physiological effect and control metabolic activities. Frequently their site of action is away from their origin.

- Hormones are required in trace amounts and are highly specific in their functions.
- The deficiency of any hormones leads to a particular disease, which can be cured by administration of that hormone.

- Hormones are released continuously or in short bursts, amount and frequency can vary.

- Circulating hormones are transported by blood in solution or attached to plasma proteins.

- Local hormones remain in interstitial fluid and act on nearby cells.
- Hormones produce slower response than nervous system but longer lasting effects.
- Hormones are inactivated by liver & excreted by kidneys.

- **Functions:**
  - Stimulate synthesis of enzymes or structural proteins.
  - Change rate of synthesis.
  - Inactivate or activate existing enzymes or protein channels.

General Properties Of Hormones

1. Hormone receptors
2. Half life (mins/ days)
3. Onset of action(immediate/ delayed)
4. Types of Action (specific/general)
5. Storage

6. Release of Hormones:

a.insoluble to soluble (T3-T4)

b. Storage granules (insulin, glucagon)

c. Passive diffusion down their concentration gradient. (steroid hormones)

7. Regulation of secretion:

a. Hypothalamus / anterior pituitary

b. Negative feedback mechanism

c. Concentration of ions (ca)

d. Changes in environment (melatonin- light, cold-thyroxine)

e. stimulation of sympathetic nervous system(adrenal medullary)

f. Afferent nerve impulses (oxytocin)

g. Cyclic rhythms of release- (ultradian (mins-hrs), circadian(24hrs), infradian(months – years)

8. Pre-Pro hormones/ Pro hormones:
9. Production of abnormal hormones
10. Ectopic production of Hormones
11. Hormonal Assays (radioimmunoassays, chemiluminescent methods)
12. Interactive effects

Target cells sensitive to several hormones may show interactive effects.

1. **Permissive effects** - first hormone enhances the effect of a later hormone action: example:

- Estrogen up-regulates progesterone receptors in uterus
- Thyroid hormone increases the effect of epinephrine on breakdown of triglycerides in adipocytes.

2. **Integrative effects** - hormones produce complementary effects on different tissues: Example:

- PTH and calcitriol increase ECF calcium.

3. **Synergistic effects** - two hormones acting together have a greater effect than the sum of the effects of each hormone acting independently. Example:

- Both FSH and estrogen necessary for normal oocyte development.
- FSH and testosterone together increase spermatogenesis than alone.

4. **Antagonistic effects** - one hormone opposes the action of the other hormone. Example:

- Insulin and glucagon.

General features of Hormone classes

GROUP –I

Types- steroids hormones

Solubility – Lipophilic

Transport Proteins- Yes

Plasma half life- long

Receptor- Intracellular

Mediator- Receptor- Hormone complex

GROUP –II

Types- Polypeptides, proteins, glycoproteins, catecholamine hormones

Solubility – Hydrophilic

Transport Proteins- No

Plasma half life- Short

Receptor- Plasma membrane

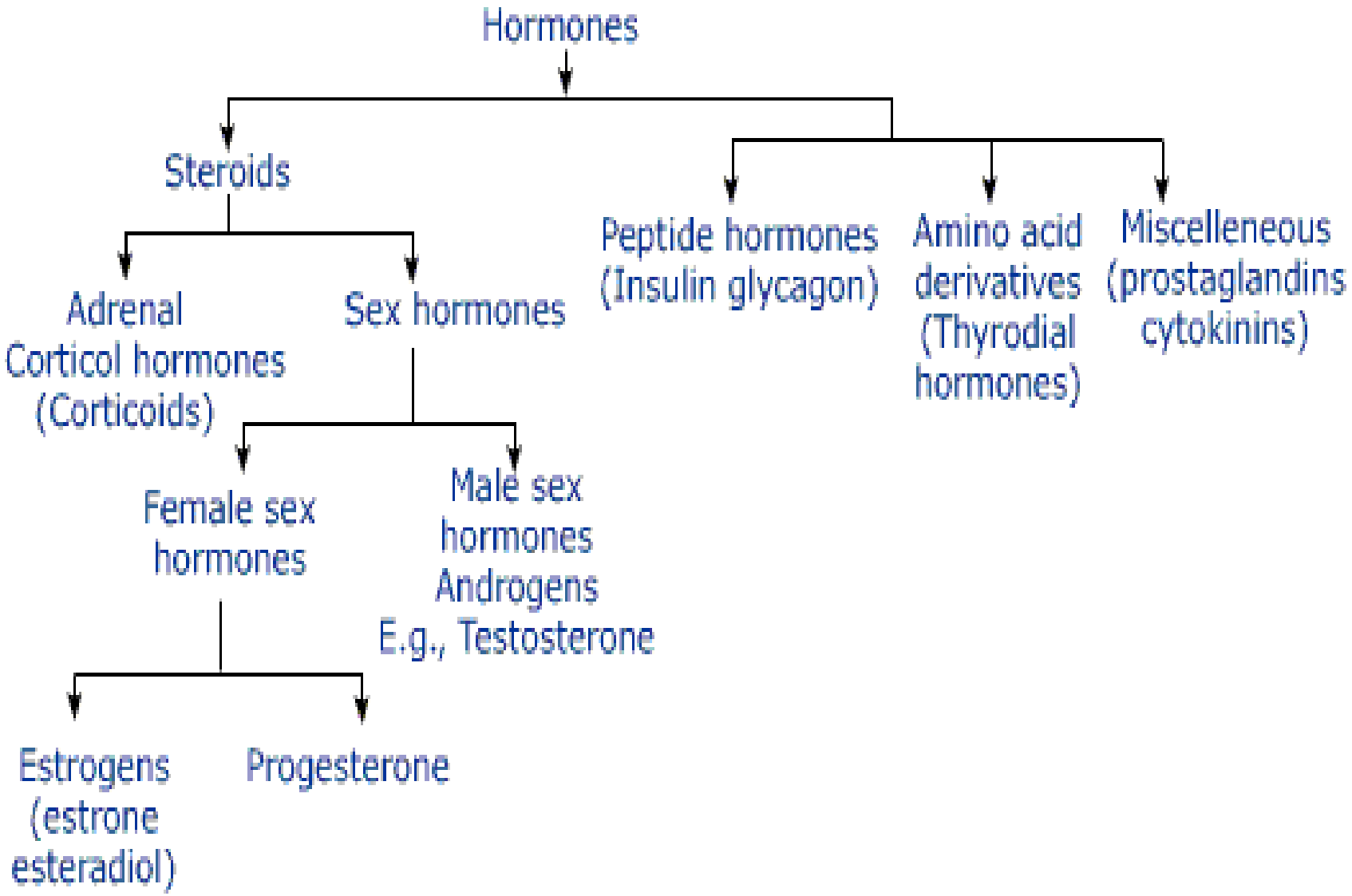
Mediator- cAMP, cGMP, Calcium and phosphatidylinostinol.

Classification of Hormones

- Hormones are classified on the basis of:

(i) Their structure.

(ii) Their site of activity in the cell.



Steroid hormones

1. **Sex hormones** - are divided into 2 groups

(i) Male sex hormones or Androgens (testosterone)

(ii) Female sex hormones- Estrogens/Progesterons

2. **Hormones of Adrenal Cortex –**
mineralocorticoids /glucocorticoids

Non steroid hormones

1. **Peptide hormones-**

- e.g. all hypothalamic, anterior pituitary, digestive hormones

2. **Amino acid derivatives**

- Amines - simplest form, derived from tyrosine or tryptophan
- e.g. Thyroid hormone, dopamine, epinephrine, melatonin

Hypothalamus functions as master co-ordinator of hormonal action. It produces at least 6 releasing factors or hormones.

- Thyrotropin releasing hormone (TRH)
- Corticotropin releasing hormone (CRH)
- Gonadotropin releasing hormone (G_nRH)
- Growth hormone releasing hormone (GRH)
- Growth hormone release inhibiting hormone (GRIH)
- Prolactin release inhibiting hormone (PRIH)

Domains present on the receptors

- All receptors have two functional domains:

1. Recognition domain
2. Coupling domain.

- **Recognition domain: it binds the hormone**
- **Coupling domain: it generates a signal that couples the hormone recognition to some intracellular function.**
- **Coupling means signal transduction.**
- **Receptors are proteins.**

Transduction:

The biochemical mechanism(s) that allow the transfer of information between an occupied hormone-receptor & the molecules within the cell that result in production of a cellular response.

Ligand
Receptor
Transducer
Effector

} **Amplifiers**

Change in metabolism
Change in transcription/gene-read out
Change in secondary hormone production

RECEPTORS

- These are proteins, to which hormones bind. **They are present in cell membranes, cytoplasm and nucleus, and serve two functions**.
- Firstly, they are required for **selectivity.** Secondly, they are connected to an **effecter mechanism** in the cell .

- Selectivity

- Effecter mechanism- receptor has got two domains.

1. Binding domain
2. Signal generation domain

Transduction System Concepts

Features of transduction that both **alter protein shape & function**

Allosteric changes
Phosphorylation

Membrane Receptors

usually for proteins & charged molecules
rapid response systems, sec-min

Intranuclear Receptors

lipids & hydrophobic hormones
longer term responses, min-days

Transduction Pathways Depend on Receptor Types

- **Ion Channels**
- **Intracellular/Intranuclear Receptor**
  - Steroids (sex, adrenal, vitamin D, sterols)
  - Thyronines (tri-iodothyronine)
- **G-Protein Receptors/Serpentine Receptors**
  - cGMP/NOS
  - cAMP/PKA/CREB
  - PLCβ/PKC/Calcium ion
- **Cytokine & GH Receptors**
  - JAK/STAT
  - TyrK
    - Ras/GAP/MEK/MAPK
    - RAC/Rho
    - PI3K
    - PLCγ/PKC

Cross-talk allows unique responses in specific tissues & /or at specific times.

- Translate information in hormone messages into language that can be interpreted & acted upon by target cells.

- For proteins, peptides, & hormones with a high ionic charge at neutral pH, receptors are usually integral membrane proteins in the cell surface. When hormones bind, **the receptors interact with membrane-bound or intracellular transducer proteins to begin the cascade of events leading to cellular response**

- Some membrane receptors, *e.g.*, the acetyl-choline receptor, act as ion channels that open or close in response to hormone binding & induce changes via changes of the intracellular ion/charge balance.

- For many lipophilic hormones, *e.g.*, steroids or thyronines, receptors are intracellular, usually intranuclear, proteins.
- When their specific *ligands* bind, the hormone-receptor complexes undergo conformational changes that allow them to interact with specific hormone recognition sites (HREs) in the DNA of the regulatory regions of certain genes

(Steroid, Retinoid and Thyroid have several functional domains):

- Binding of ligand
- Binding of DNA
- Binding of co regulator proteins(activation or inhibition)
- Binding of other proteins that specify intracellular trafficking of receptor.

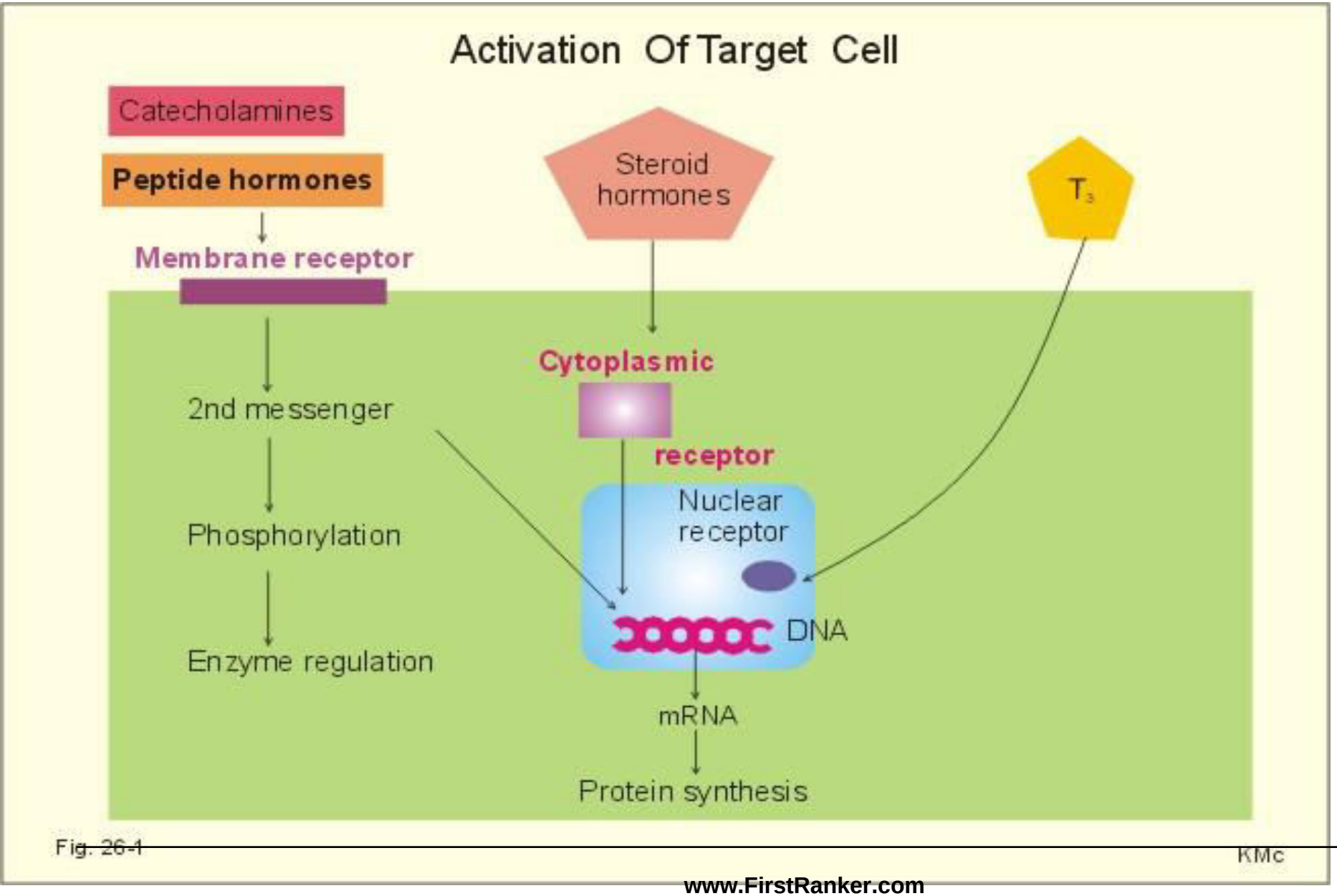


Fig. 26-1

KMc

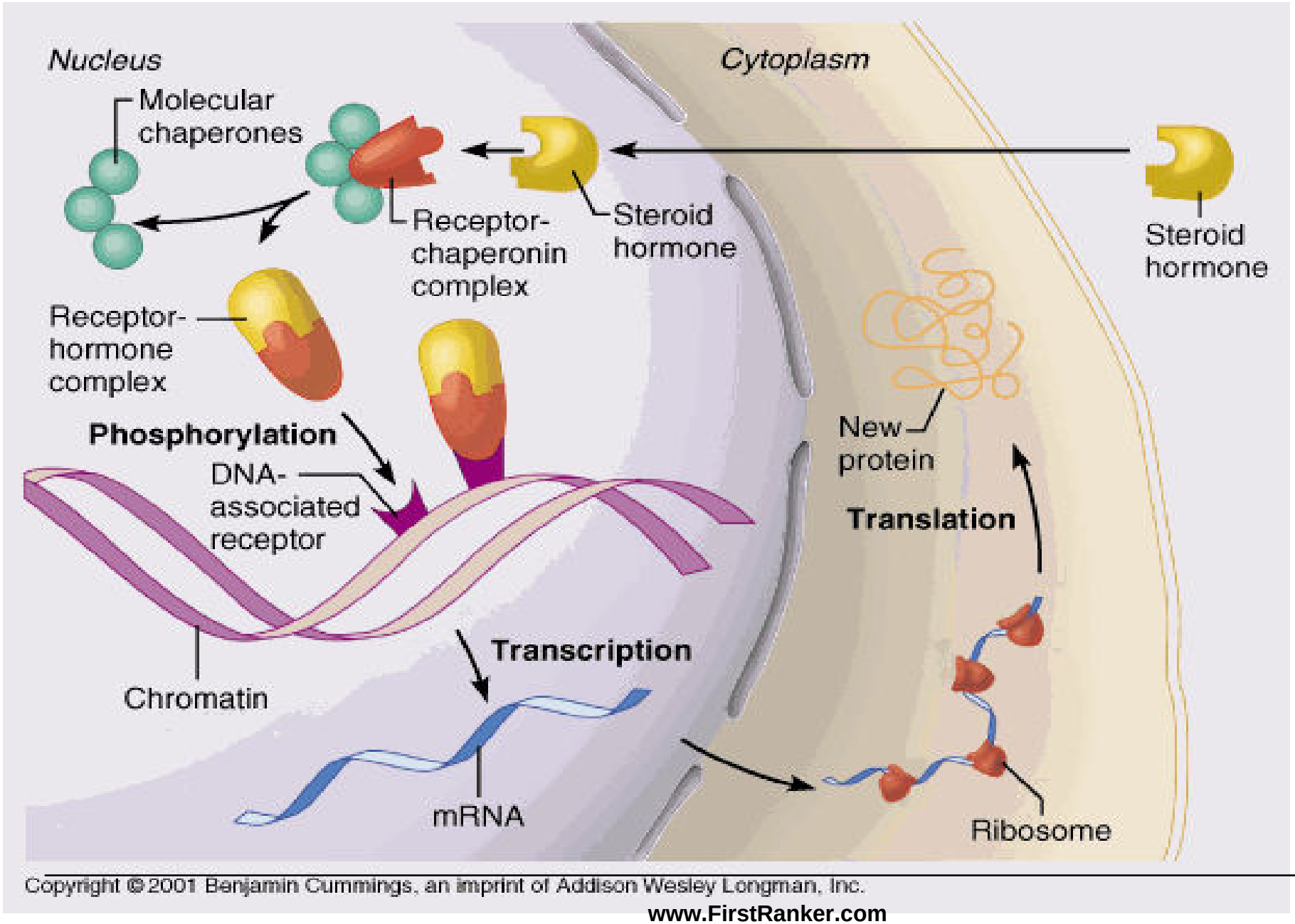
Steroid Hormones

- Steroid hormones are lipid soluble.
- Steroids can diffuse through the membrane
- They can cause:

Direct Gene Activation

- **Step-by-step**

1. Diffuse through the membrane
2. Binds & activates intracellular receptor.
3. Steroid-Receptor complex binds to DNA receptor protein
4. Activates a gene.
5. Gene transcribed into messenger RNA.
6. mRNA goes to the ribosomes
7. Translate mRNA into protein.



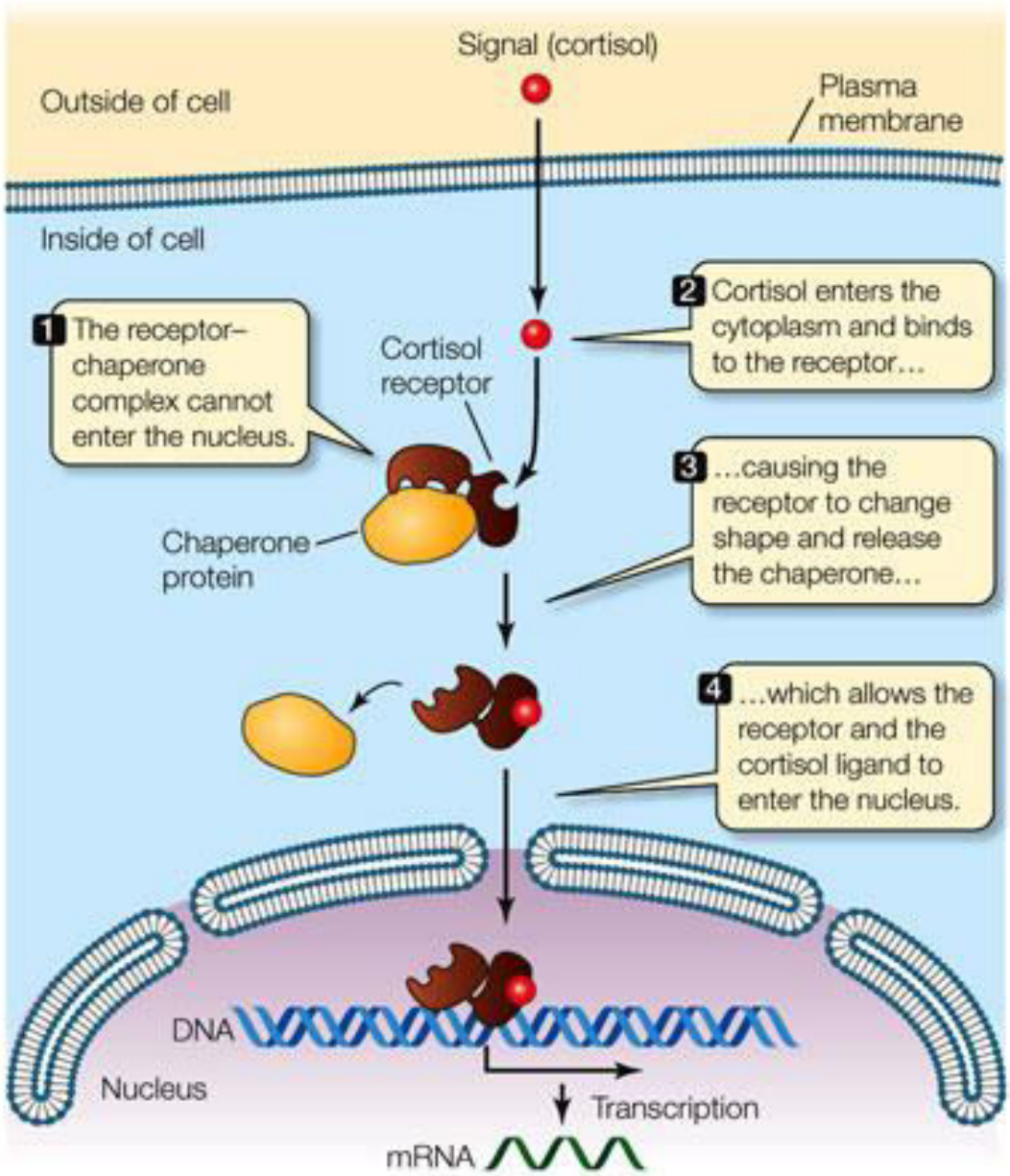
Cytoplasmic Receptors.

- Once inside the cell, they (Steroid hormones) bind **cytoplasmic receptors**.
- This causes receptor activation.
- Binding dislodges a protein that inhibits the expression of the gene at that segment (heat shock 90 protein).

- The hormone-receptors complex then enters the nucleus and binds to a particular sequence on the DNA.

- This sequence is called hormone response element (HRE).

- This receptor which has hormone bound to it and DNA sequence now serves as a binding site for other co activator proteins.
- Thus the gene begins to be transcribed and translated, and a new protein appears in the cell and assumes its normal function within it (or gets secreted).



LIFE 8e, Figure 15.8

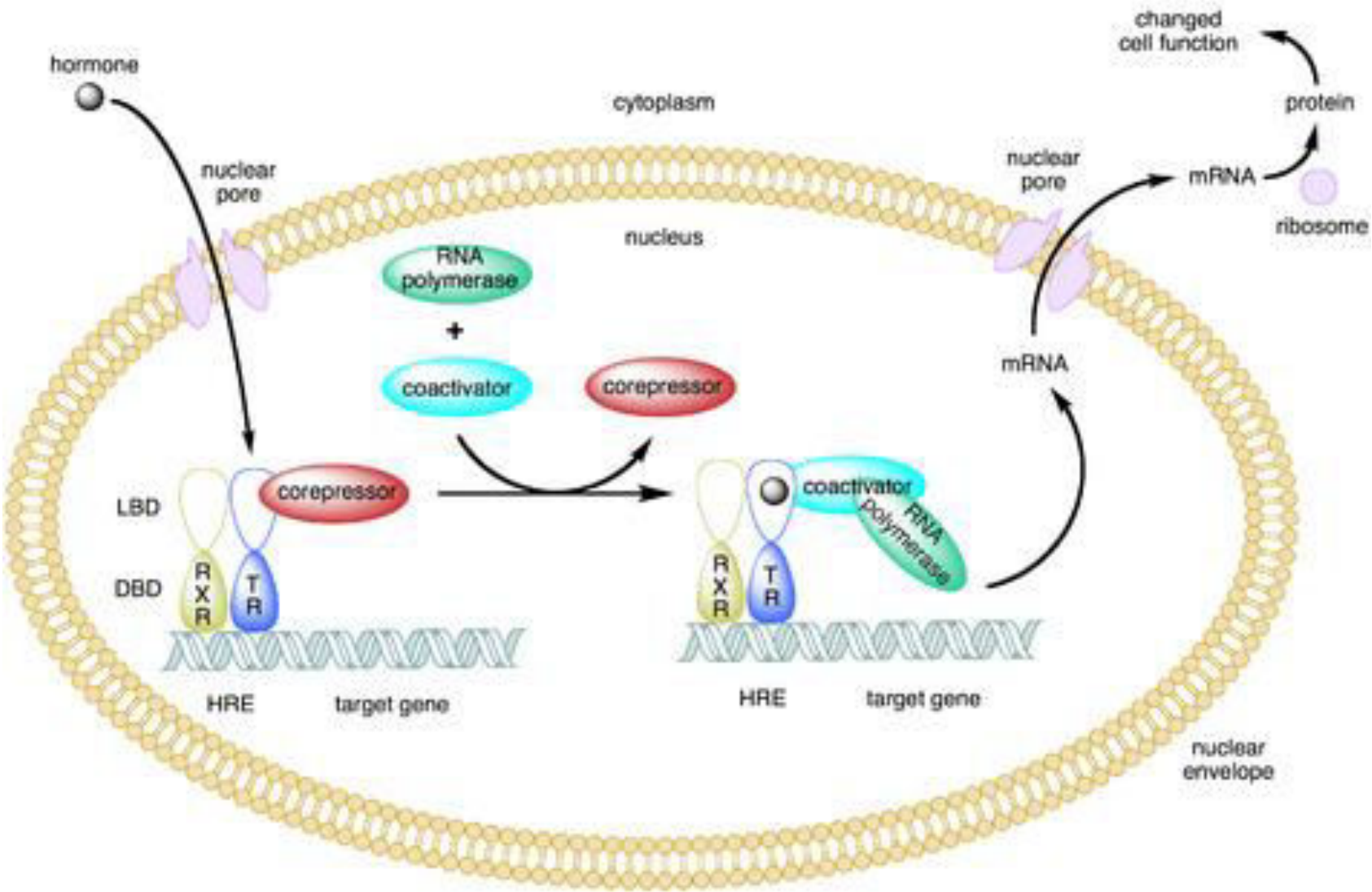
- The action of nuclear receptors is slow, as it takes some hours for the whole process to occur. The effect is long-lasting (or even permanent) and changes the properties of the cell. This type of process is important in **development, differentiation and maturation** of cells, e.g. gametes (eggs and sperm cells).

- In contrast hormones such as: Thyroid and Retinoids go directly into the nucleus.

- Their receptor is already bound to HRE, but along with a co –repressor protein which fails to activate transcription.

- The association of the ligand with the receptor results in the dissociation of the co repressor.

- Now this receptor- ligand complex can bind other co activator proteins and transcription begins.



Cell Surface Receptors

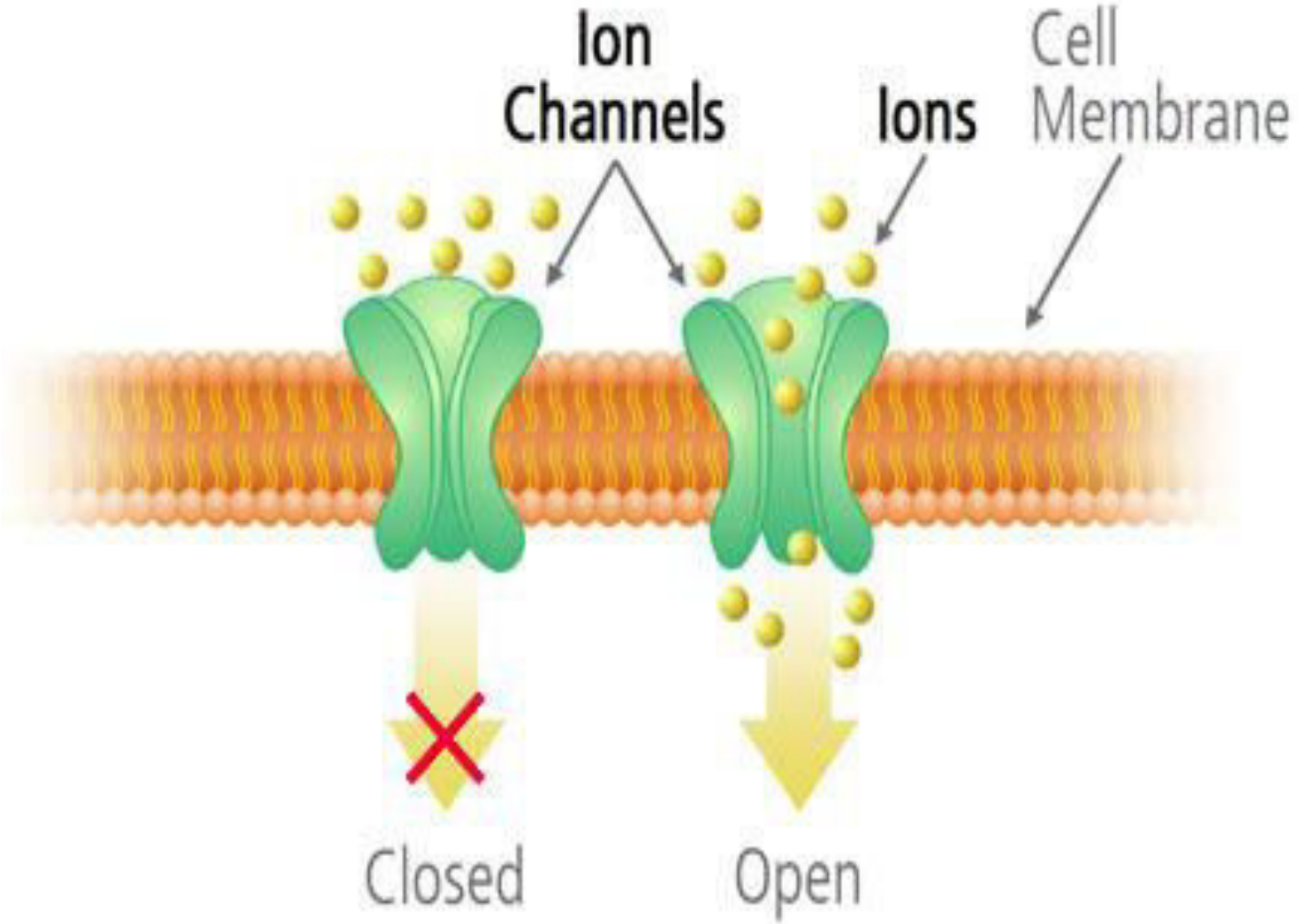
- There are three types of cell surface receptors:

1. Ion channel receptors,
2. Transmembrane receptors,
3. Receptors that are kinases or bind kinases.

Ion channel receptors:

- When a signaling molecule binds to an **ion channel** on the outside of the cell, this triggers the change of the 3D conformation of the protein and the channel opens, allowing the ions to move in or out of the cell following their electrical gradients and thus altering the polarization of the cell membrane.

- Some ion channels respond to non-chemical stimuli in the same way, including changes in electrical charge or mechanical disturbance of the membrane.


Ion
Channels
Ions
Cell
Membrane
Closed
Open

Receptors that are kinases or bind kinases:

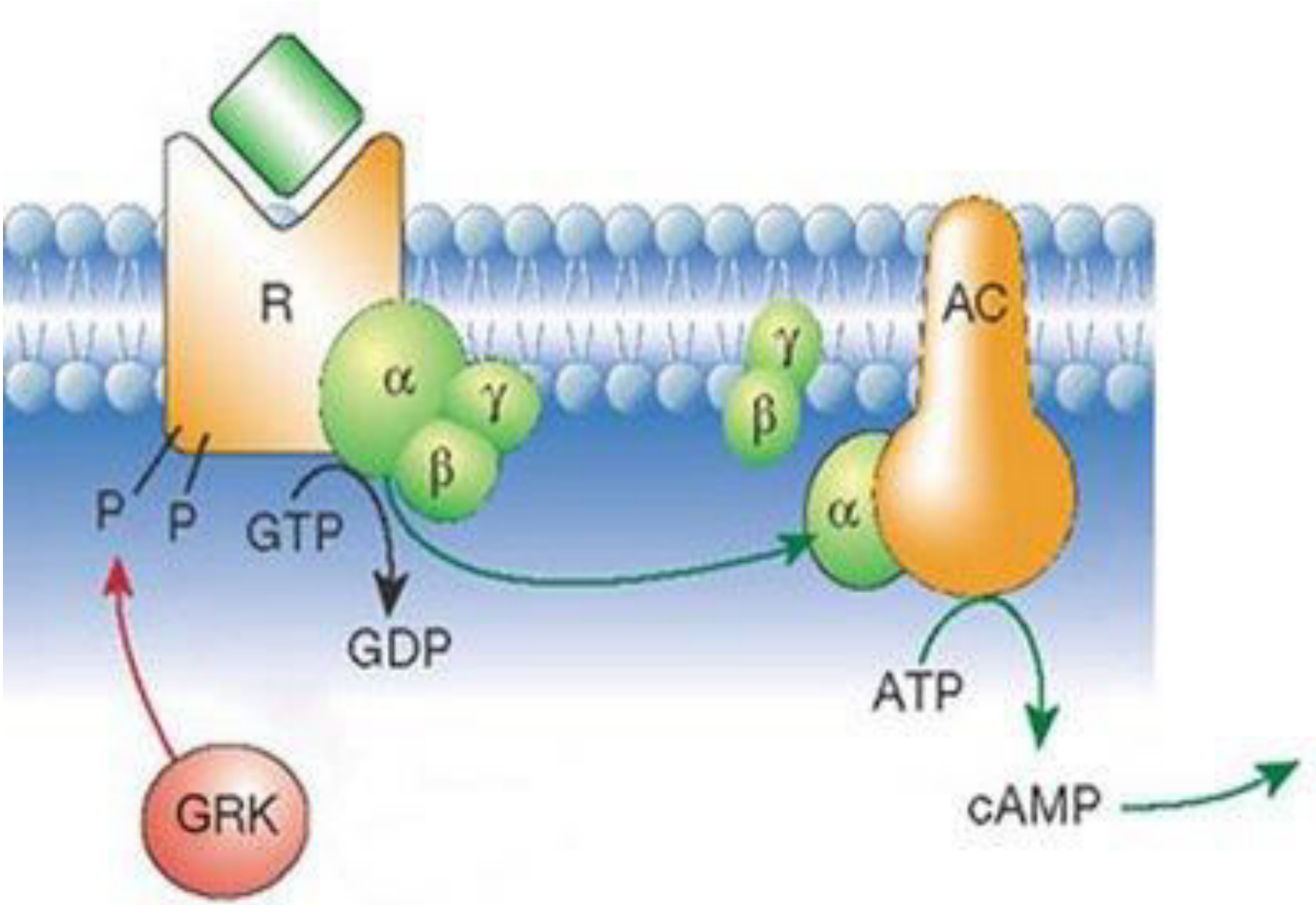
- When a signaling chemical binds to the **membrane receptor** protein on the outside of the cell, this triggers a change in the 3D conformation of that protein, which in turn, triggers a chemical reaction on the inside of the cell.

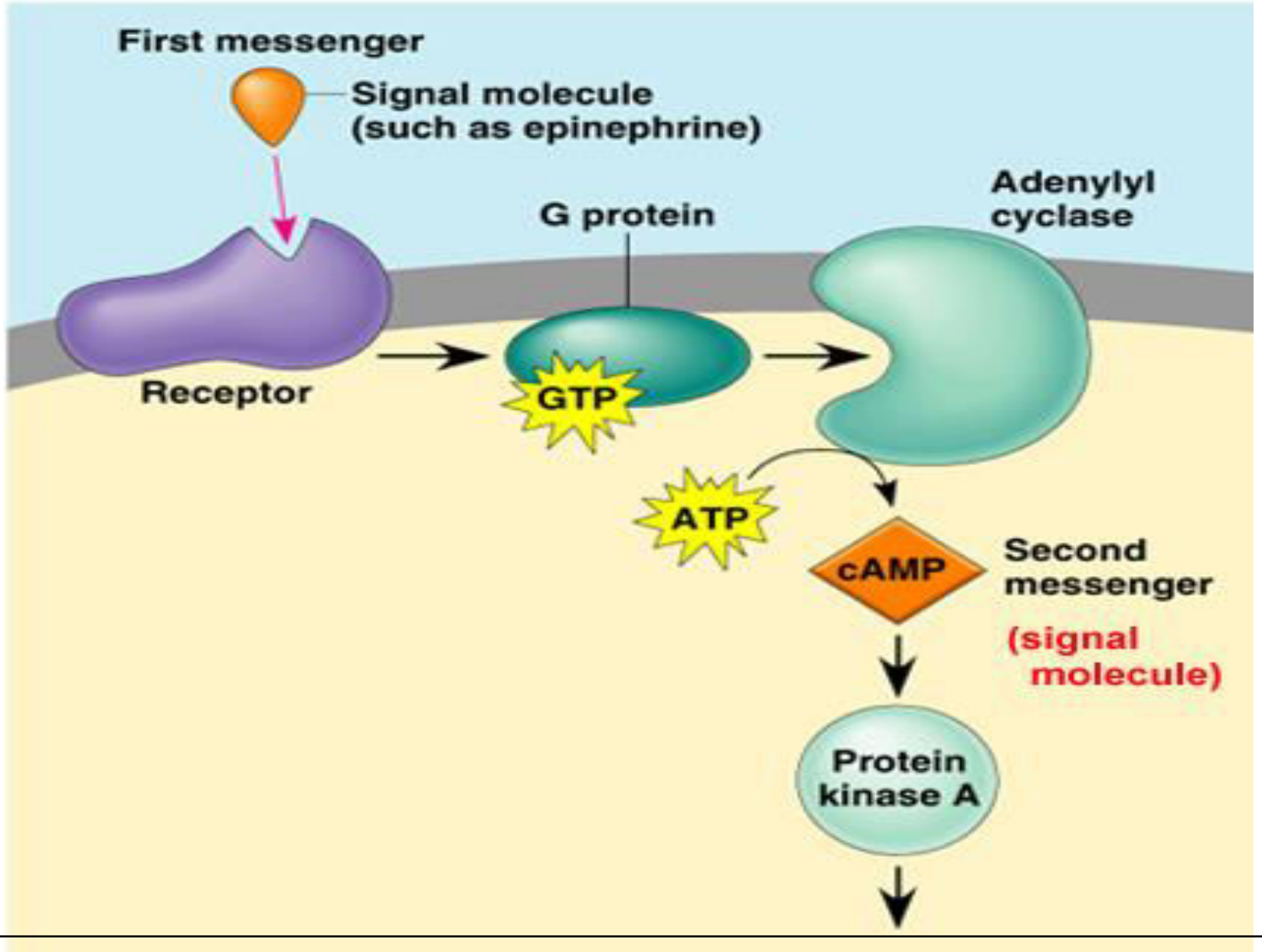
- Their main features is that the **intracellular domain of the receptor is a kinase**, that is activated when the messenger binds to the extracellular domain.
  Receptor kinase phosphorylates an amino acid residue that is present on the receptor or an associated protein.
- Message is transmitted through signal transducer proteins.

- **Transmembrane proteins** include **G protein-linked receptors** and they are seven-pass trans membrane proteins. This means that the polypeptide chain traverses the membrane seven times. When a chemical - a hormone or a pharmaceutical agent - binds to the receptor on the outside of the cell, this triggers a series of chemical reactions:

- including the movement and binding of the G-protein.
- transformation of GTP into GDP and
- activation of second messengers.

- Second messengers (e.g., cyclic AMP) start a cascade of enzymatic reactions leading to the cellular response. This signaling method is quite fast and, it amplifies the signal.




First messenger
Signal molecule
(such as epinephrine)
G protein
Adenylyl
cyclase
Receptor
GTP
ATP
cAMP
Second
messenger
(signal
molecule)
Protein
kinase A

G- protein receptors

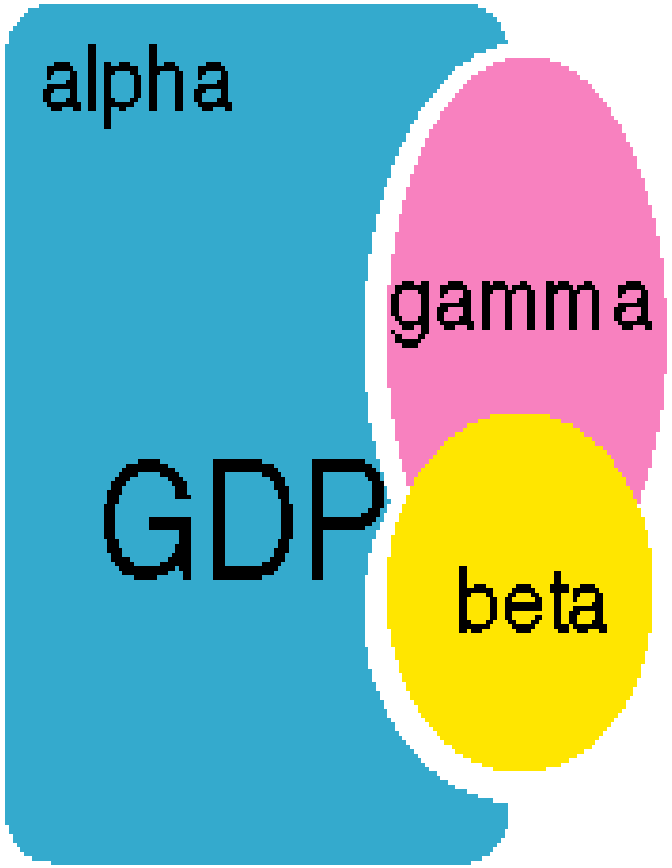
A. **Basic G-protein Receptor**

1. whole family of receptors
2. All use same basic pattern

a. ligand binds to receptor (outer surface of cell).

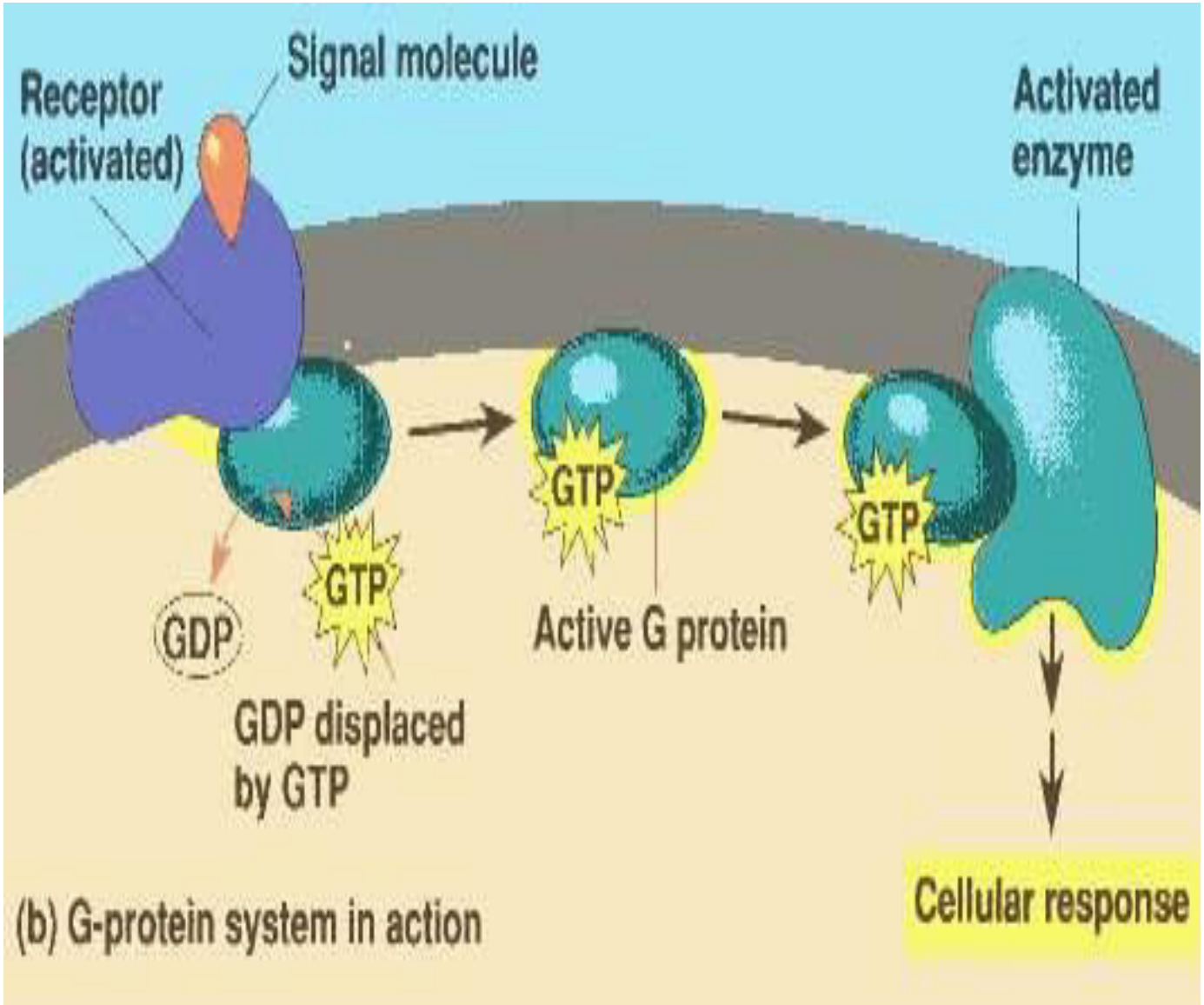
b. receptor **changes shape** (inner surface of cell).

- shape change allows receptor **to bind inactive G-protein**
- **inactive G-protein = G-alpha + GDP + G-beta + G-gamma**



Inactive Heterotrimeric G-protein

- **inactive G-protein binds** to receptor
- **receptor activates G-protein**
- G-alpha drops GDP, picks up GTP
- when G-alpha binds GTP --> G-beta and G-gamma are released.


Receptor
(activated)
Signal molecule
Activated
enzyme
GTP
GTP
GTP
GDP
Active G protein
GDP displaced
by GTP
Cellular response
(b) G-protein system in action

- G-alpha + GTP is released from receptor into cytoplasm
- G-alpha + GTP = active G-protein.
- activated G-protein binds to target protein . target protein's activity is altered - might be stimulated or might be inhibited .

Adenylyl Cyclase

- Different peptide hormones can either stimulate or inhibit the production of cAMP from adenylyl cyclase.

- There are two parallel systems that converge upon a single catalytic molecule – (C).

- These parallel systems are inhibitory or stimulatory.

- Each consists of a receptor and (**R** -Rs or Ri) and a regulatory complex (**G**- Gs or Gi).

- **G-complex** is again composed of three subunits- α,β and γ.

- It is basically the α-subunit that is either stimulatory or inhibitory.

- α-subunit binds the GDP or GTP.

- When the hormone binds to the receptor conformational change occurs in the G complex and it binds GTP instead of GDP.

- This binding occurs to the α-subunit and it dissociates from β and γ subunit.

- The **αs** protein has intrinsic GTPase activity and it catalyses the conversion of GTP- GDP

- The three subunits again recombine, and is again ready for another cycle of activation.

- Cholera and pertussis toxins catalyze ADP ribosylation of **αs** and **αi-2**.

- Due to which in **αs** intrinic GTPase activity is disrupted and it cannot associate with its other subunits.

- In the **αi-2** dissociation is prevented, and αs activity is unopposed.

- GPCRs are implicated in a number of diseases and are major targets for the pharmaceutical companies.

Clinical applications of hormones

- Distribution of estrogens and progesterone in *contraceptives* (P pills) is world-wide. Estrogens are widely used to relieve postmenopausal discomfort.
- Females with osteoporosis are treated with calcitonin, because calcitonin inhibits osteoclastic bone resorption.
- Insulin is a lifesaver for diabetics, and it is produced and distributed as pure human insulin.

- In the affluent areas of the world many women deliver their babies following an oxytocin infusion.
- estrogens and gonadotropins are used in treatment of sterility and menstrual disturbances.
- Huggins received the Nobel Prize in 1966 for the introduction of a new form of cancer therapy in which sex hormones are used to retard their growth. He used androgens for breast cancer and estrogens for prostate cancer.