

Chapter 3

Mechanisms of Hormone Action

Core concepts related to hormone mechanism of action are presented as the following topics:

- How Hormones Change Their Target Cells
- Hormones with Cell Surface Receptors
- Hormones with Intracellular Receptors

3.1. How Hormones Change Their Target Cells

Hormones are chemical messengers that invoke profound changes within target cells. There are two fundamental mechanisms by which such changes occur:

- **Activation of enzymes and other dynamic molecules:** Most enzymes shuttle between conformational states that are catalytically active versus inactive, *on* versus *off*. Many hormones affect their target cells by inducing such transitions, usually causing an activation of one of more enzymes. Because enzymes are catalytic and often serve to activate additional enzymes, a seemingly small change induced by hormone-receptor binding can lead to widespread consequences within the cell.
- **Modulation of gene expression:** Stimulating transcription of a group of genes clearly can alter a cell's phenotype by leading to a burst of synthesis of new proteins. Similarly, if transcription of a group of previously active genes is shut off, the corresponding proteins will soon disappear from the cell.

More specifically, when a receptor becomes bound to a hormone, it undergoes a conformational change which allows it to interact productively with other components of the cells, leading ultimately to an alteration in the physiologic state of the cell.

Considerable information about a how a hormone acts can be gained by knowing the type of receptor it uses. Despite the molecular diversity of hormones, all hormone receptors can be categorized into one of two types, based on their location within the cell. Thus, if introduced to a new steroid hormone, one can quickly deduce that it has an **intracellular receptor** and acts upon its target cells by affecting transcription.

3.2. Mechanism of Action: Hormones with Cell Surface Receptors

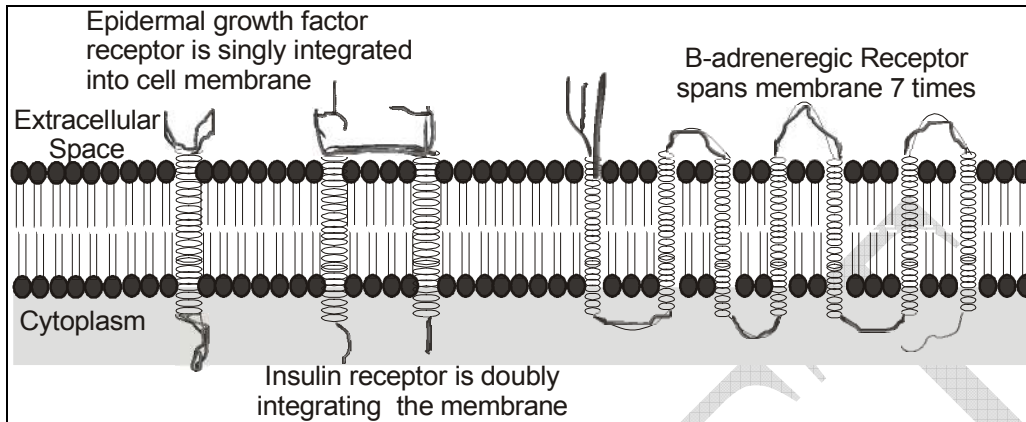
Protein and peptide hormones, catecholamines like epinephrine, and eicosanoids such as prostaglandins find their receptors at the surface of the plasma membrane of target cells. Binding of hormone to receptor initiates a series of events which leads to generation of so-called **second messengers** within the cell (the hormone is the first messenger). The second messengers then trigger a series of molecular interactions that alter the physiologic state of the cell through the process of **signal transduction**.

3.2.1. Structure of Cell Surface Receptors

Cell surface receptors are integral membrane proteins and, as such, have regions that contribute to three basic domains:

- **Extracellular domains:** Some of the residues exposed to the outside of the cell interact with and bind the hormone – in the ligand-binding domain.
- **Transmembrane domains:** Hydrophobic stretches of amino acids penetrates in the lipid bilayer as integral proteins and serve to anchor the receptor in the membrane.
- **Cytoplasmic or intracellular domains:** Tails or loops of the receptor that are within the cytoplasm react to hormone binding by interacting in some way with other molecules, leading to generation of second messengers. Cytoplasmic residues of the receptor are thus the **effector region** of the molecule.

Several distinctive variations in receptor structure have been identified. As depicted below, some receptors are simple, single-pass proteins; many growth factor receptors take this form. Others, such as the receptor for insulin, have more than one subunit. Another class, which includes the beta-adrenergic receptor, is threaded through the membrane seven times.



Receptor molecules are neither isolated by themselves nor fixed in one location of the plasma membrane. In some cases, other integral membrane proteins interact with the receptor to modulate its activity. Some types of receptors cluster together in the membrane after binding hormone. Finally, interaction of the hormone-bound receptor with other membrane or cytoplasmic proteins is the key to generation of second messengers and transduction of the hormonal signal.

3.2.2. Second Messenger Systems

Currently, four second messenger systems are recognized in cells, as summarized in the following table.

Second Messenger	Examples of Hormones Which Utilize This System
cyclic AMP	Epinephrine and norepinephrine, glucagon, luteinizing hormone, follicle stimulating hormone, thyroid-stimulating hormone, calcitonin, parathyroid hormone, antidiuretic hormone
Protein kinase activity	Testosterone, prolactin, oxytocin, erythropoietin, several growth factors
Inositol and/or phosphoinositides	Epinephrine and norepinephrine, angiotensin II, antidiuretic hormone, gonadotropin-releasing hormone, thyroid-releasing hormone.
cyclic GMP	Natriuretic hormone

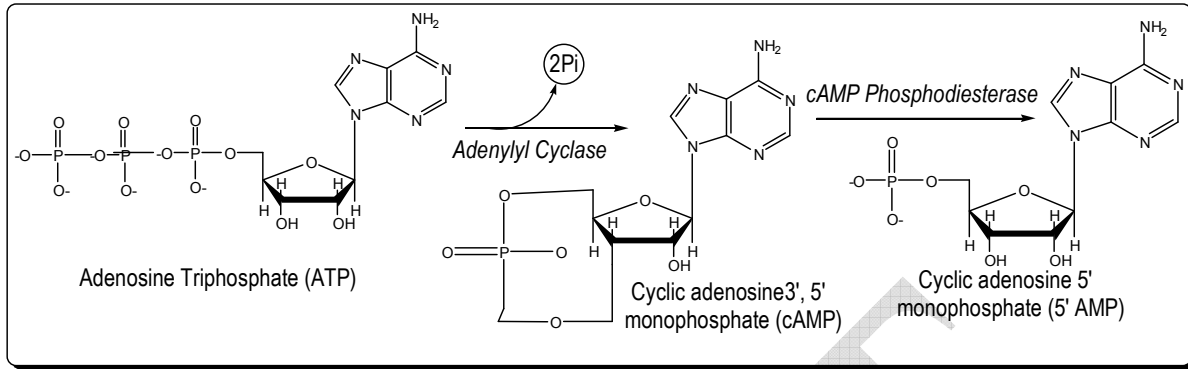
In all cases, the seemingly small signal generated by hormone binding its receptor is amplified within the cell into a cascade of actions that changes the cell's physiologic state. Presented below are two examples of second messenger systems commonly used by hormones. The examples used are of glucagon and insulin, both of which ultimately work through a molecular switch involving protein phosphorylation.

3.2.3. Cyclic AMP Second Messenger Systems

Cyclic adenosine monophosphate (cAMP) is a nucleotide generated from **ATP** through the action of the enzyme **adenylate cyclase**. The intracellular concentration of cAMP is increased or decreased by a variety of hormones and such fluctuations affect a variety of cellular processes. One prominent and important effect of elevated concentrations of cAMP is activation of a cAMP-dependent protein kinase called protein kinase A.

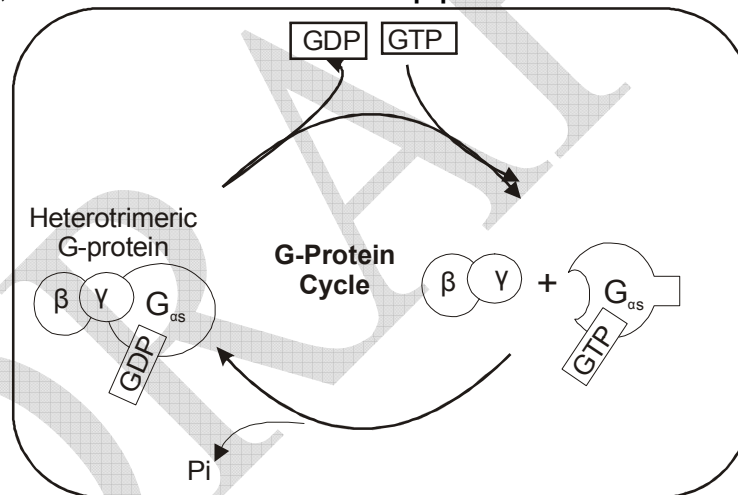
ATP consists of an adenosine molecule bound to a ribose sugar that has three terminal phosphate groups (hence triphosphate) attached to it at the 5' position. **Adenylate cyclase** hydrolyzes the terminal two phosphate groups and forms a **diester bond** connecting the phosphate group from the 5' to the 3' position. This is the active form

of this adenosine monophosphate. The phosphodiester bond is broken by **cAMP phosphodiesterase** forming 5'-AMP which does not activate any of cAMP's targets.



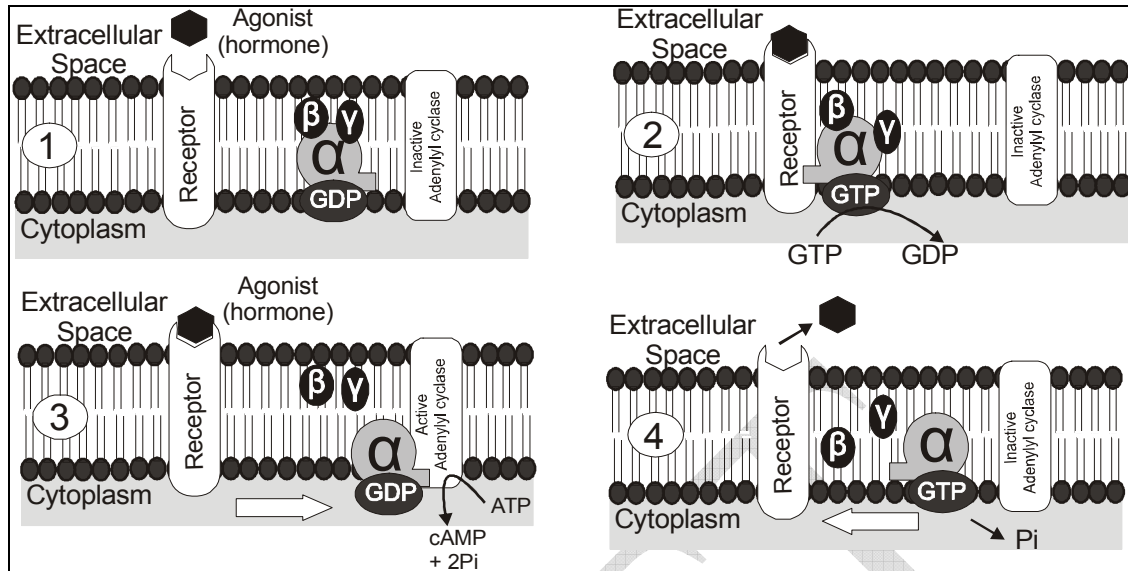
3.2.4. G Proteins & the G Protein Cycle

G proteins are localized in the cell membrane where they mediate the function of G protein linked receptors (GPCRs). G proteins are **heterotrimeric** because they consist of three subunits that are different. The subunits are called α , β , γ . The α subunit is the enzymatic component. It binds GTP and hydrolyzes it to GDP. The β and γ subunits typically remain bound to each other and associate with the α subunit when it is bound to GDP. When GTP displaces the GDP, the α subunit dissociates from the β/γ subunits.



3.2.5. cAMP as a Second Messenger

Below is a graphic showing how cAMP is generated as a second (intracellular) messenger in eucaryotes. A ligand binds to a receptor causing a conformational change that allows the G protein to associate with it. This interaction leads to a change in the conformation of the G α subunit causing GTP to replace GDP which leads to the dissociation of the GTP-G α s from the G β/γ subunits. As shown in this figure, the G α and G γ subunits are linked to the membrane by lipid groups. The "s" on the G α subunit indicates it stimulates cAMP synthesis by adenylyl cyclase. In keeping with this, the GTP-G α s subunit binds to adenylyl cyclase (AC). This activates AC leading to the synthesis of cAMP from ATP.

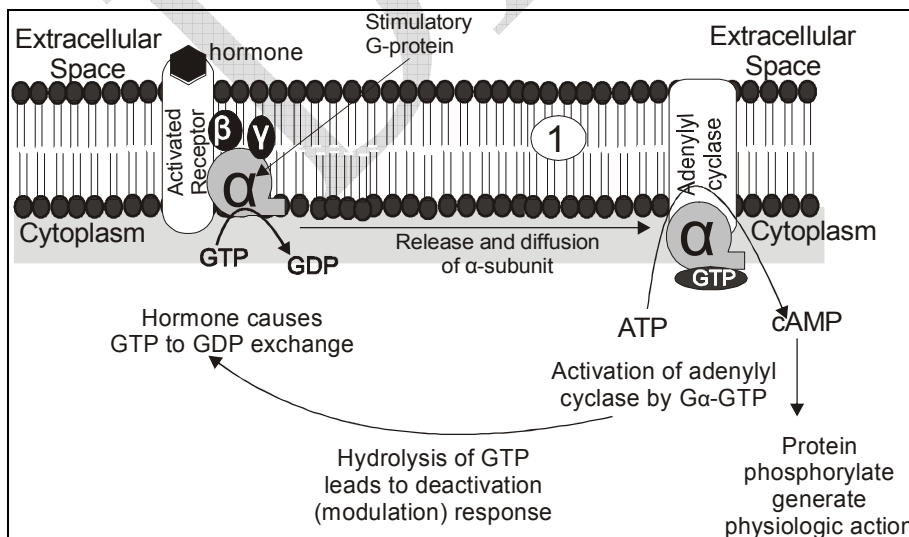


3.2.6. Generalized Mechanism of Action of a Hormone

Now, let's put this information together to understand the mechanism of action of a hormone like glucagon:

- Glucagon binds its receptor in the plasma membrane of target cells (e.g. hepatocytes).
- Bound receptor interacts with and, through a set of G proteins, turns on adenylate cyclase, which is also an integral membrane protein.
- Activated adenylate cyclase begins to convert ATP to cyclic AMP, resulting in an elevated intracellular concentration of cAMP.
- High levels of cAMP in the cytosol make it probable that protein kinase A will be bound by cAMP and therefore catalytically active.
- Active protein kinase A circulate in the cell adding phosphates to other enzymes, thereby changing their conformation and modulating their catalytic activity generating a physiologic activity.
- Levels of cAMP decrease due to destruction by cAMP-phosphodiesterase and the inactivation of adenylate cyclase.

In the above example, the hormone's action was to modify the activity of pre-existing components in the cell. Elevations in cAMP also have important effects on transcription of certain genes.



3.2.7. Example of Glucagon & cAMP Signaling in the Liver

The level of glucose in the blood is regulated by glucagon and insulin. Insulin stimulates the uptake of glucose and its conversion to glycogen, the storage carbohydrate, by the enzyme glycogen synthase (GS). Glucagon stimulates liver cells to break down the glycogen to glucose when blood glucose levels drop. To do this, glucagon binds to its receptor activating the G protein cycle, leading to the activation of adenylyl cyclase and the formation of cAMP.

The cAMP binds to protein kinase A (PKA), a cAMP-dependent protein kinase, activating it. PKA in turn phosphorylates other downstream target proteins including phosphorylase kinase (PhosK) and glycogen synthase (GS). The phosphorylation of PhosK leads to its activation. Conversely, phosphorylation of GS causes its inhibition stopping the formation of glycogen. The activated PhosK then phosphorylates the next kinase in the chain, glycogen phosphorylase kinase (GPhos). Phosphorylation of GPhos activates the enzyme leading to the release of glucose subunits from glycogen. Thus a chain of phosphorylations leads to the activation of some downstream signaling components while inhibiting others.

3.3. Tyrosine Kinase Second Messenger Systems

The receptors for several protein hormones are themselves protein kinases which are switched on by binding of hormone. The kinase activity associated with such receptors results in phosphorylation of tyrosine residues on other proteins. **Insulin** is an example of a hormone whose receptor is a tyrosine kinase. The hormone binds to domains exposed on the cell's surface, resulting in a conformational change that activates kinase domains located in the cytoplasmic regions of the receptor. In many cases, the receptor phosphorylates itself as part of the kinase activation process. The activated receptor phosphorylates a variety of intracellular targets, many of which are enzymes that become activated or are inactivated upon phosphorylation.

Following binding of hormone, the receptor undergoes a conformational change, phosphorylates itself, then phosphorylates a variety of intracellular targets. As with cAMP second messenger systems, activation of receptor tyrosine kinases leads to rapid modulation in a number of target proteins within the cell. Some of the targets of receptor kinases are protein phosphatases which, upon activation by receptor tyrosine kinase, become competent to remove phosphates from other proteins and alter their activity. Again, a seemingly small change due to hormone binding is amplified into a multitude of effects within the cell.

In some cases, binding of hormone to a surface receptor induces a tyrosine kinase cascade even though the receptor is not itself a tyrosine kinase. The **growth hormone** receptor is one example of such a system - the interaction of growth hormone with its receptor leads to activation of cytoplasmic tyrosine kinases, with results conceptually similar to that seen with receptor kinases.

3.3.1. cAMP Regulation of PKA

Protein kinase A is a primary target of cAMP in a diverse number of tissues and cell types. In its inactive state two regulatory subunits keep two substrate binding (catalytic) subunits in an inactive state. A pseudosubstrate domain in the regulatory subunits binds to the catalytic domain of the catalytic subunits. Binding of cAMP to the regulatory subunits changes the conformation of the pseudosubstrate domains resulting in the disassociation of the now active PKA catalytic subunits. The active PKA can now phosphorylate target proteins. In addition to the two the two targets that function in glucose mobilization, phosphorylase kinase and glycogen synthase, PKA has many other substrate proteins that it can phosphorylate. These include, protein phosphatase-1 (regulation of glucose metabolism), heart muscle troponin (contraction), myosin light chain kinase (muscle contraction), phosphofructokinase (anaerobic metabolism), and CREB (transcription factor).

3.3.2. Fate of the Hormone-Receptor Complex

Normal cell function depends upon second messenger cascades being transient events. Indeed, a number of cancers are associated with receptors that continually stimulate second messenger systems. One important part of negative regulation on hormone action is that cell surface receptors are internalized. In many cases, internalization is stimulated by hormone binding.

Internalization occurs by **endocytosis** through structures called **coated pits**. The resulting **endosomes** (sometimes called "receptosomes") may fuse with lysosomes, leading to destruction of the receptor and hormone. In other cases, the hormone dissociates and the receptor is recycled by fusion of the endosome back into the plasma membrane.

3.4. Mechanism of Action: Hormones with Intracellular Receptors

Receptors for **steroid** and **thyroid** hormones are located **inside target cells, in the cytoplasm or nucleus**, and function as **ligand-dependent transcription factors**. ie, the hormone-receptor complex binds to promoter regions of responsive genes and stimulate or sometimes inhibit transcription from those genes. **Thus, the mechanism of action of these hormones is to modulate gene expression in target cells.** By selectively affecting transcription from a battery of genes, the concentration of those respective proteins are altered, which clearly can change the phenotype of the cell.

3.4.1. Structure of Intracellular Receptors

Steroid and thyroid hormone receptors are members of a large group of transcription factors. In some cases, multiple forms of a given receptor are expressed in cells, adding to the complexity of the response. All of these receptors are composed of a single polypeptide chain that has, in the simplest analysis, three distinct domains:

- **The amino-terminus:** In most cases, this region is involved in activating or stimulating transcription by interacting with other components of the transcriptional machinery. The sequence is highly variable among different receptors.
- **DNA binding domain:** Amino acids in this region are responsible for binding of the receptor to specific sequences of DNA.
- **The carboxy-terminus or ligand-binding domain:** This is the region that binds hormone.



In addition to these three core domains, two other important regions of the receptor protein are a **nuclear localization sequence**, which targets the protein to nucleus, and a **dimerization domain**, which is responsible for bringing two receptors together in a form capable of binding DNA.

3.4.2. Hormone-Receptor Binding and Interactions with DNA

Being lipids, steroid hormones enter the cell by simple diffusion across the plasma membrane. Thyroid hormones enter the cell by facilitated diffusion. The receptors exist either in the cytoplasm or nucleus, which is where they meet the hormone. When hormone binds to receptor, a characteristic series of events occurs:

- **Receptor activation** is the term used to describe conformational changes in the receptor induced by binding hormone. The major consequence of activation is that the receptor becomes competent to bind DNA.
- **Activated receptors bind to hormone response elements**, which are short specific sequences of DNA which are located in promoters of hormone-responsive genes. In most cases, hormone-receptor complexes bind DNA in pairs.
- **Transcription from those genes to which the receptor is bound is affected.** Most commonly, receptor binding stimulates transcription. The hormone-receptor complex thus functions as a transcription factor.

There are a number of variations on the themes described above, depending on the specific receptor in question, e.g., in the absence of hormone, some intracellular receptors do bind their hormone response elements loosely and silence transcription, but, when complexed to hormone, become activated and strongly stimulate transcription. Some receptors bind DNA not with another of their kind, but with different intracellular receptor.

