# *Introduction to Clinical Endocrinology*

**Endocrinology is the study of endocrine glands.** Endocrine glands are a group of glands in the body which secrete **hormones**. The purpose of the secreted hormones is to evoke a specific response in other cells of the body which are located far away. As shown in the picture, the hormones are secreted into the blood stream giving them access to all other cells of the body.

### *Examples of Endocrine Glands and Their Hormones*

- **Pituitary Gland**. The pituitary is located at the base of the brain. Secretes thyroid stimulating hormone (TSH), follicle stimulating hormone (FSH), adrenocotropic hormone (ACTH), and others. parathyroid, parathyroid surgery, parathyroid hormone
  - Purpose: Control the activity of many other endocrine glands (thyroid, ovaries, adrenal, etc.).
- **Thyroid Gland.** Located in the front of the neck. Secretes thyroid hormone.
  - Purpose: Regulate the body's overall metabolism.
- **Parathyroid Glands.** There are 4 parathyroid glands located behind the thyroid. Secretes parathyroid hormone.
  - Purpose: Absolute control over calcium levels throughout the body.
- **Adrenal Glands.** There are 2 adrenal glands located on the top of each kidney. Inner part secretes adrenaline, outer part secretes aldosterone and cortisol.
  - Purpose: Maintain salt levels in the blood, maintain blood pressure, help control kidney function, control overall fluid concentrations in the body.
- **Endocrine Pancreas.** Located deep in the abdomen behind the stomach, the pancreas is primarily a digestive organ. It also contains extremely important endocrine cells which secrete: insulin, glucagon, somatostatin, and others.
  - Purpose: Control blood sugar and overall glucose metabolism, help control other endocrine cells of the digestive tract.

## Hormone Chemistry, Synthesis and Elimination

**Nature uses a diverse spectrum of molecules as hormones, and knowing the basic structure of a hormone imparts considerable knowledge about its receptor and mechanism of action**. Additionally, the simpler structures can often be exploited to generate similar molecules - agonists and antagonists - that are therapeutically valuable.

Like all molecules, hormones are synthesized, exist in a biologically active state for a time, and then degrade or are destroyed. Again, having an appreciation for the "halflife" and mode of elimination of a hormone aids in understanding its role in physiology and is critical when using hormones as drugs.

**Most commonly, hormones are categorized into four structural groups, with members of each group having many properties in common:**

- Peptides and proteins
- Steroids
- Amino acid derivatives
- Fatty acid derivatives - Eicosanoids

## Peptides and Proteins

**Peptide and protein hormones are, of course, products of translation.** They vary considerably in size and post-translational modifications, ranging from peptides as short as three amino acids to large, multisubunit glycoproteins.

Many protein hormones are synthesized as prohormones, then proteolytically clipped to generate their mature form. In other cases, the hormone is originally embedded within the sequence of a larger precursor, then released by multiple proteolytic cleavages.

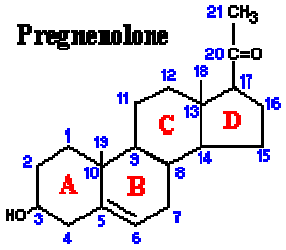
Peptide hormones are synthesized in endoplasmic reticulum, transferred to the Golgi and packaged into secretory vesicles for export. **They can be secreted by one of two pathways:**

- **Regulated secretion:** The cell stores hormone in secretory granules and releases them in "bursts" when stimulated. This is the most commonly used pathway and allows cells to secrete a large amount of hormone over a short period of time.
- **Constitutive secretion:** The cell does not store hormone, but secretes it from secretory vesicles as it is synthesized.

Most peptide hormones circulate unbound to other proteins, but exceptions exist; for example, insulin-like growth factor-1 binds to one of several binding proteins. In general, the halflife of circulating peptide hormones is only a few minutes.

## Steroids

**Steroids are lipids and, more specifically, derivatives of cholesterol.** Examples include the sex steroids such as testosterone and adrenal steroids such as cortisol.




The first and rate-limiting step in the synthesis of all steroid hormones is conversion of cholesterol to pregnenolone, which is illustrated here to demonstate the system of numbering rings and carbons for identification of different steroid hormones.

Pregnenolone is formed on the inner membrane of mitochondria then shuttled back and forth between mitochondrion and the endoplasmic reticulum for further enzymatic transformations involved in synthesis of derivative steroid hormones.

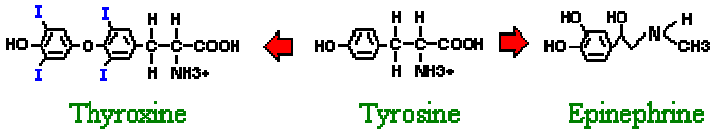
**Newly synthesized steroid hormones are rapidly secreted from the cell, with little if any storage**. Increases in secretion reflect accelerated rates of synthesis. Following secretion, all steroids bind to some extent to plasma proteins. This binding is often low affinity and non-specific (e.g. to albumin), but some steroids are transported by specific binding proteins, which clearly affects their halflife and rate of elimination.

Steroid hormones are typically eliminated by inactivating metabolic transformations and excretion in urine or bile.

## Amino Acid Derivatives

**There are two groups of hormones derived from the amino acid tyrosine:**

- **Thyroid hormones** are basically a "double" tyrosine with the critical incorporation of 3 or 4 iodine atoms.
- **Catecholamines** include epinephrine and norepinephrine, which are used as both hormones and neurotransmitters.



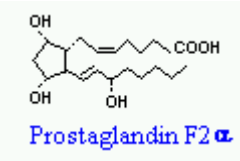

The pathways to synthesis of these hormones is provided in the sections on the thyroid gland and the adrenal medulla.

The circulating halflife of thyroid hormones is on the order of a few days. They are inactivated primarily by intracellular deiodinases. Catecholamines, on the other hand, are rapidly degraded, with circulating halflives of only a few minutes.

**Two other amino acids are used for synthesis of hormones:**

- **Tryptophan** is the precursor to serotonin and the pineal hormone melatonin
- **Glutamic acid** is converted to histamine

## Fatty Acid Derivatives - Eicosanoids




**Eicosanoids** are a large group of molecules derived from polyunsaturated fatty acids. **The principal groups of hormones of this class are prostaglandins, prostacyclins, leukotrienes and thromboxanes.**

**Arachadonic acid is the most abundant precursor for these hormones.** Stores of arachadonic acid are present in membrane lipids and released through the action of various lipases. The specific eicosanoids synthesized by a cell are dictated by the battery of processing enzymes expressed in that cell.

These hormones are rapidly inactivated by being metabolized, and are typically active for only a few seconds.

## Control of Endocrine Activity

**The physiologic effects of hormones depend largely on their concentration in blood and extracellular fluid.** Almost inevitably, disease results when hormone concentrations are either too high or too low, and precise control over circulating concentrations of hormones is therefore crucial.

**The concentration of hormone as seen by target cells is determined by three factors:**

- **Rate of production:** Synthesis and secretion of hormones are the most highly regulated aspect of endocrine control. Such control is mediated by positive and negative feedback circuits, as described below in more detail.
- **Rate of delivery:** An example of this effect is blood flow to a target organ or group of target cells - high blood flow delivers more hormone than low blood flow.
- **Rate of degradation and elimination:** Hormones, like all biomolecules, have characteristic rates of decay, and are metabolized and excreted from the body through several routes. Shutting off secretion of a hormone that has a very short halflife causes circulating hormone concentration to plummet, but if a hormone's biological halflife is long, effective concentrations persist for some time after secretion ceases.

## Feedback Control of Hormone Production

**Feedback circuits are at the root of most control mechanisms in physiology, and are particularly prominent in the endocrine system.** Instances of positive feedback certainly occur, but negative feedback is much more common.

Negative feedback is seen when the output of a pathway inhibits inputs to the pathway. The heating system in your home is a simple negative feedback circuit. When the furnace produces enough heat to elevate temperature above the set point of the thermostat, the thermostat is triggered and shuts off the furnace (heat is feeding back negatively on the source of heat). When temperature drops back below the set point, negative feedback is gone, and the furnace comes back on.

**Feedback loops are used extensively to regulate secretion of hormones** in the hypothalamic-pituitary axis. An important example of a negative feedback loop is seen in control of thyroid hormone secretion. The thyroid hormones thyroxine and triiodothyronine ("T4 and T3") are synthesized and secreted by thyroid glands and affect metabolism throughout the body. The basic mechanisms for control in this system (illustrated to the right) are:

- Neurons in the hypothalamus secrete thyroid releasing hormone (TRH), which stimulates cells in the anterior pituitary to secrete thyroid-stimulating hormone (TSH).
- TSH binds to receptors on epithelial cells in the thyroid gland, stimulating synthesis and secretion of thyroid hormones, which affect probably all cells in the body.
- When blood concentrations of thyroid hormones increase above a certain threshold, TRH-secreting neurons in the hypothalamus are inhibited and stop secreting TRH. ***This is an example of "negative feedback".***

Inhibition of TRH secretion leads to shut-off of TSH secretion, which leads to shut-off of thyroid hormone secretion. As thyroid hormone levels decay below the threshold, negative feedback is relieved, TRH secretion starts again, leading to TSH secretion ...

**Another type of feedback is seen in endocrine systems that regulate concentrations of**

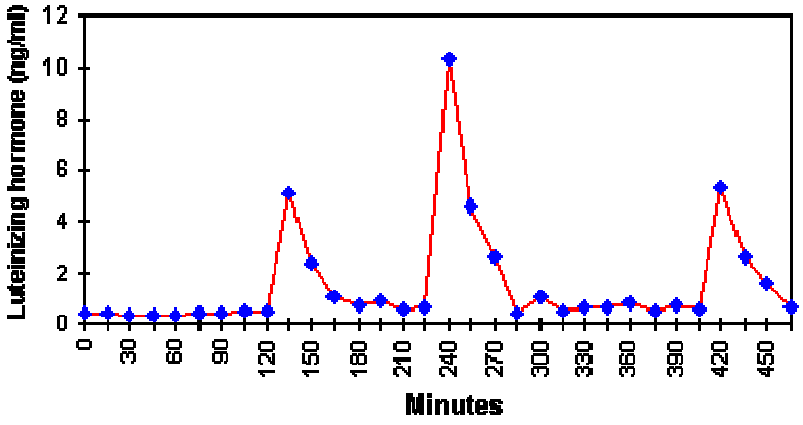
**blood components such as glucose.** Drink a glass of milk or eat a piece of bread and the following (simplified) series of events will occur:

- Glucose from the ingested lactose or sucrose is absorbed in the intestine and the level of glucose in blood rises.
- Elevation of blood glucose concentration stimulates endocrine cells in the pancreas to release insulin.
- Insulin has the major effect of facilitating entry of glucose into many cells of the body - as a result, blood glucose levels fall.
- When the level of blood glucose falls sufficiently, the stimulus for insulin release disappears and insulin is no longer secreted.

**Numerous other examples of specific endocrine feedback circuits are presented in the sections on specific hormones or endocrine organs.**

## Hormone Profiles: Concentrations Over Time

**One important consequence of the feedback controls that govern hormone concentrations and the fact that hormones have a limited lifespan or halflife is that most hormones are secreted in "pulses".** The following graph depicts concentrations of luteinizing hormone in the blood of a female dog over a period of 8 hours, with samples collected every 15 minutes:

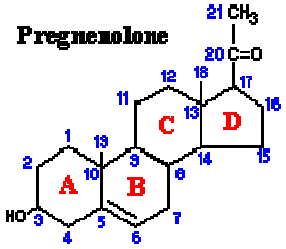



The pulsatile nature of luteinizing hormone secretion in this animal is evident. Luteinizing hormone is secreted from the anterior pituitary and critically involved in reproductive function; the frequency and amplitude of pulses are quite different at different stages of the reproductive cycle.

*With reference to clinical endocrinology, examination of the graph should also demonstrate the caution necessary in interpreting endocrine data based on isolated samples.*

**A pulsatile pattern of secretion is seen for virtually all hormones, with variations in pulse characteristics that reflect specific physiologic states.** In addition to the short-term pulses discussed here, longer-term temporal oscillations or endocrine rhythms are also commonly observed and undoubtedly important in both normal and pathologic states.

## Steroidogenesis



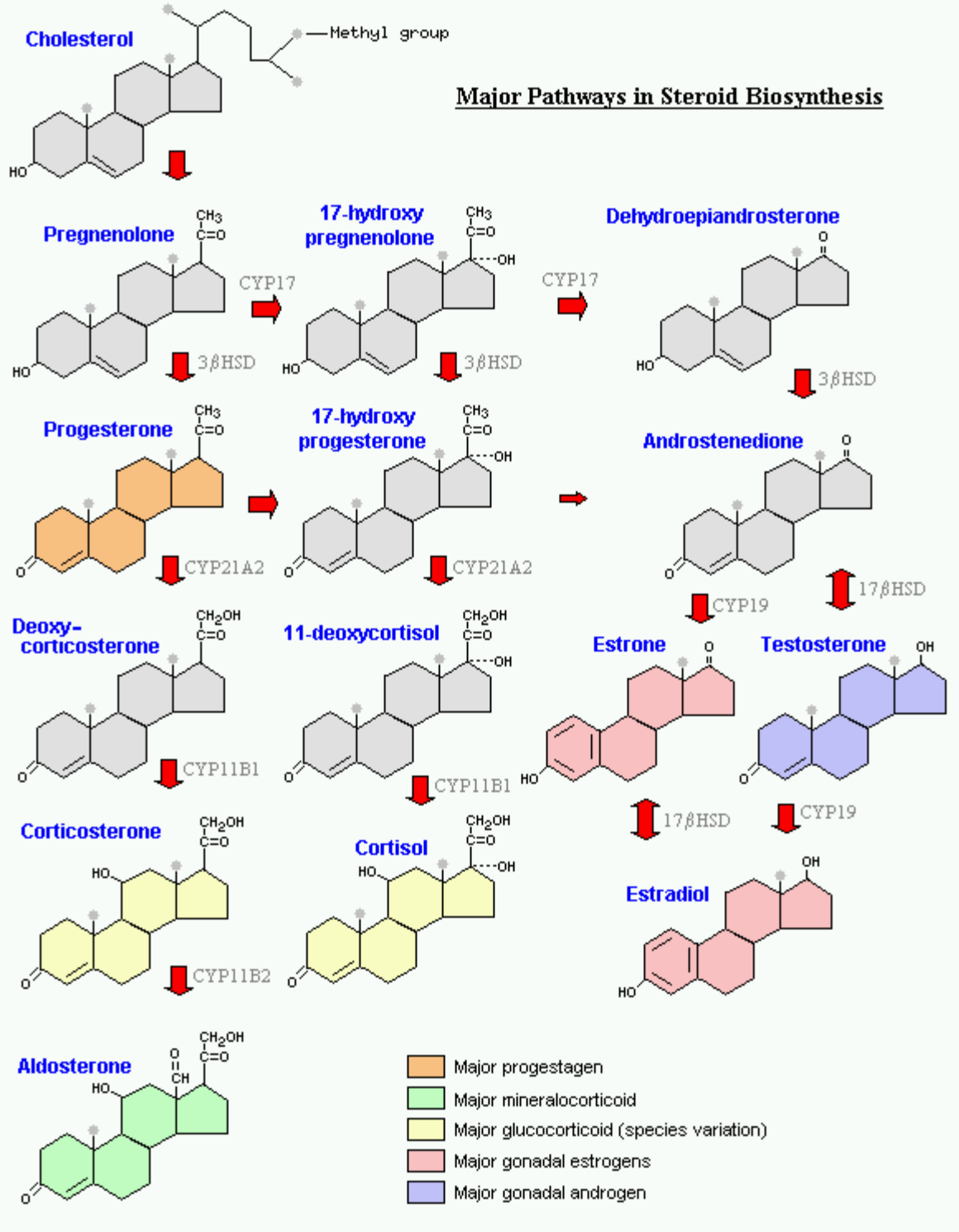

**Steroid hormones are derivatives of cholesterol** that are synthesized by a variety of tissues, most prominently the adrenal gland and gonads. The cholesterol precursor comes from cholesterol synthesized within the cell from acetate, from cholesterol ester stores in intracellular lipid droplets or from uptake of cholesterol-containing low density lipoproteins. Lipoproteins taken up from plasma are most important when steroidogenic cells are chronically stimulated. The basic cyclopentanoperhydrophenanthrene ring structure and carbon numbering system of all steroid hormones is depicted to the right, using pregnenolone as an example. Pregnenolone is an example of what is called a "C-21 steroid" because it has 21 carbons. Similarly, a steroid such as testosterone (see below) is referred to as a "C-19 steroid".

Biosynthesis of steroid hormones requires a battery of oxidative enzymes located in both mitochondria and endoplasmic reticulum. **The rate-limiting step in this process is the transport of free cholesterol from the cytoplasm into mitochondria.** Within mitochondria, cholesterol is converted to pregnenolone by an enzyme in the inner membrane called CYP11A1. **Pregnenolone itself is not a hormone, but is the immediate precursor for the synthesis of all of the steroid hormones.**

Typically, endocrinologists classify steroid hormones into five groups of molecules, based primarily on the receptor to which they bind:

- **Glucocorticoids**; cortisol is the major representative in most mammals
- **Mineralocorticoids**; aldosterone being most prominent
- **Androgens** such as testosterone
- **Estrogens**, including estrodiol and estrone
- **Progestogens** (also known a progestins) such as progesterone

The biosynthetic pathways for major representatives of these classes of steroid hormones is depicted in the following diagram. Be aware that a variety of related molecules exist, some of which may have significant effects, particularly in certain pathologic conditions.




# Mechanisms of Hormone Action

Immediately after discovery of a new hormone, a majority of effort is devoted to delineating its

sites of synthesis and target cells, and in characterizing the myriad of physiologic responses it invokes. **An equally important area of study is to determine precisely how the hormone acts to change the physiologic state of its target cells - its *mechanism of action*.**

Understanding mechanism of action is itself a broad task, encompassing structure and function of the receptor, how the bound receptor transduces a signal inside the cell and the end effectors of that signal. This information is not only of great interest to basic science, but critical to understanding and treating diseases of the endocrine system, and in using hormones as drugs.

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

- **How Do Hormones Change Their Target Cells?**
- **Hormones with Cell Surface Receptors**

**Hormones with Intracellular Receptors**

## How Do Hormones Change Their Target Cells?

**Hormones are chemical messengers that invoke profound changes within target cells. How is this accomplished?** 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:

| Location of Receptor | Classes of Hormones | Principle Mechanism of Action |
|---|---|---|
| **Cell surface receptors** (plasma membrane) | Proteins and peptides, catecholamines and eicosanoids | Generation of *second messengers* which alter the activity of other molecules - usually enzymes - within the cell |
| **Intracellular receptors** (cytoplasm and/or nucleus) | Steroids and thyroid hormones | Alter transcriptional activity of responsive genes |

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.

**Additional detail on mechanism of action for these two groups of hormones and receptors are found in the next sections and in discussions about specific endocrine systems.**

## Mechanism of Action: Hormones with Cell Surface Receptors

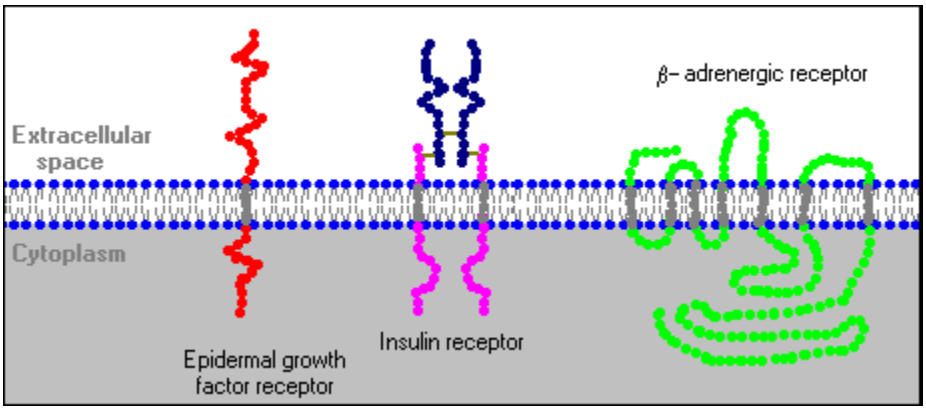
**Protein and peptide hormones, catecholamines like epinephrine, and eicosanoids such as prostaglandins find their receptors decorating the plasma membrane of target cells.** Binding of hormone to receptor initiates a series of events which leads to generation of so-called ***second messengers*** within 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. Another term used to describe this entire process is ***signal transduction***.

### 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 - another term for these regions is the ***ligand-binding domain***.
- **Transmembrane domains:** Hydrophobic stretches of amino acids are "comfortable" in the lipid bilayer 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, as elaborated below, 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.

## Second Messenger Systems

**Consider what would happen if, late at night, you noticed a building on fire.** Hopefully, you would dial 911 or a similar emergency number. You would inform the dispatcher of the fire, and the dispatcher would, in turn, contact and "activate" a number of firemen. The firefighters would then rapidly go to work pouring water on the fire, setting up roadblocks and the like. They would also probably activate other "players", such as police and fire investigators that would come in later to try and determine the cause of the fire. Importantly, once the fire is out (or the building totally destroyed), the firemen go back to the station and to sleep.

**The community response to a fire is, at least in some ways, analogous to a second messenger system** involved in a hormone's action. In the scenario described, you are the "first messenger", the dispatcher is "receptor", the firefighters are "second messengers".

**Currently, four second messenger systems are recognized in cells**, as summarized in the table below. *Note that not only do multiple hormones utilize the same second messenger system, but a single hormone can utilize more than one system.* Understanding how cells integrate signals from several hormones into a coherent biological response remains a challenge.

| 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** | Insulin, growth hormone, prolactin, oxytocin, erythropoietin, several growth factors |
| **Calcium and/or phosphoinositides** | Epinephrine and norepinephrine, angiotensin II, antidiuretic hormone, gonadotropin-releasing hormone, thyroid-releasing hormone. |
| **Cyclic GMP** | Atrial naturetic hormone, nitric oxide |

**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. Be aware that in both cases, a very complex system is being simplified considerably.

## 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.**

Protein kinase A is nominally in an catalytically-inactive state, but becomes active when it binds cAMP. Upon activation, protein kinase A phosphorylates a number of other proteins, many of which are themselves enzymes that are either activated or suppressed by being phosphorylated. Such changes in enzymatic activity within the cell clearly alter its state.

**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 "runs around the cell" adding phosphates to other enzymes, thereby changing their conformation and modulating their catalytic activity - - - *abracadabra, the cell has been changed!*
- 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.

## 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.

As was seen with cAMP second messenger systems, activation of receptor tyrosine kinases leads to rapid modulation in a number of target proteins within the cell. Interestingly, 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 through 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.

### 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 stimuated 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, it appears that the hormone dissociates and the receptor is recycled by fusion of the endosome back into the plasma membrane.

## 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*.** That is to say, 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.

### Structure of Intracellular Receptors

**Steroid and thyroid hormone receptors are members of a large group ("superfamily") 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 simplist 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 the protein to nucleus, and a dimerization domain, which is responsible for latching two receptors together in a form capable of binding DNA.

## 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, as shown in the figure below.
- **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.

**As might be expected, there are a number of variations on the themes described above, depending on the specific receptor in question.** For example, 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.

As a specific example, consider glucocorticoids, a type of steroid hormone that probably affects the physiology of all cells in the body. The image to the right depicts a pair of glucocorticoid receptors (blue and green on the top) bound to their DNA hormone response element (bottom). The two steroid hormones are not visible in this depiction.

The consensus sequence of the hormone response element in this case (called a *glucocorticoid response element*) is GGTACANNNTGTTCT, where N is any nucleotide.