Chapter 1
How Hormones, as Ancient Signalling Molecules, Regulate Diverse Biological Processes Through Evolution

Jamshed R. Tata

Abstract Hormones are chemical messengers that regulate and coordinate all major metabolic, growth and developmental activities of different populations of cells of an organism. Since most hormones of higher organisms can be detected in primitive organisms, it follows that hormones arose during evolution well before many of the functions they regulate in higher organisms. Two examples to illustrate this principle are the protein hormone prolactin and the iodothyronine thyroid hormone. The first regulates such diverse activities as lactation in mammals, crop sac development in birds and migration in fish; the second hormone controls metabolic rate in homeotherms and different functions in different tissues of the same developing individual as during amphibian metamorphosis, such as restructuring of the digestive system, gene switching for new blood proteins, new cell development during limb formation and programmed cell death in unwanted tissues in the tail and intestine. The acquisition of hormonal function during evolution is likely to have coincided with the appearance of hormonal receptors, which are the key to understanding the mechanism or specificity of action of a given hormone. The hormone-receptor complex for protein hormones can be considered to have co-evolved as a unit. Most animal hormone receptors can be divided into two classes according to their localization in the hormone's target cell: (a) those located in the cell membrane and (b) in those in the nucleus. Work based on the exploitation of recombinant DNA and cell transfection has established a high degree of homology between oncogenes and both membrane and nuclear receptors. An early consequence of the formation of hormone-membrane receptor complex is at the modulation of processes such as phosphorylation of proteins, as exemplified by the control of levels of cyclic AMP. Nuclear receptors control the chromatin structure of their target genes through interaction with or structural modification of chromatin. The key role of receptors in explaining hormone action has to be considered in the context of evolution of a system of molecular linguistics in intercellular communication.

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1.1 Introduction

Although it is only 110 years ago that the word **Hormone** was coined by Starling (1905) to mean *I stimulate*, the principles of Endocrinology had already emerged many decades earlier. In the 19th century, Claude Bernard's studies in Paris on the secretions of the dog pancreas and sugar metabolism had already established the presence of regulatory constituents in this secretory organ. Similarly, the work of Brown-Séquard, also in Paris and Adolphe Berthold in Germany, on testicular extracts established the presence of secretions that promoted and regulated sexual functions and cellular longevity in male accessory tissues (see Turner and Bagnara 1971; Baulieu and Kelly 1990). In the same century, several physicians described successful treatment of patients with certain disorders by administering extracts of animal endocrine tissues, such as the thyroid, adrenals and pancreas; they subsequently showed that these disorders were due to hormonal deficiencies. More recently, the study of hormones has led to enormous benefits to human health, social and economic progress, such as contraception, in vitro fertilization (IVF) and the availability of recombinant human hormones (Edwards et al. 1984; Pincus et al. 1958; Sharpe and Skakkebaeck 1993). At the same time, the subject of hormones and hormonally regulated development, has also found much interest among public health experts and the larger public, in view of the emerging evidence of various man-made chemicals that can act as hormone disruptors to cause a wide range of diseases and disorders in man and wildlife.

Starling's proposal of a new term sparked off enormous interest in determining the nature of endocrine secretions and soon led to the discovery of how these substances exerted their actions. In the first half of the 20th century, researchers thus concentrated their efforts on identifying the source of these internal messengers, with the result that many hormones have been named after the gland or organ from which they were secreted, e.g. thyroid, adrenal, etc. This system of nomenclature was not always perfect, since quite distinct hormones are now known to be secreted by the same gland, as, for example, the pituitary and pancreas. Scientists soon succeeded in deciphering the chemical nature of hormones. For example, Kendall purified and determined the structures of cortisone (a steroid) and thyroxine (an iodoamino acid), while Charles Harington first chemically synthesized a hormone: thyroxine. This breakthrough work was soon followed by the characterization of the nature and activity of the pancreatic hormone insulin—a protein—by Banting and Best, while in the 1920s and 1930s, Budenandt, Reichstein and Doisy discovered and characterized various steroid hormones, including oestrogen, testosterone and progesterone. The growing knowledge of the physiological actions also led to many hormones being named according to their actions, such as growth hormone and

prolactin. However, this nomenclature can still be unsatisfactory when a hormone exerts different actions in different target tissues or organisms at different developmental stages.

1.2 Evolution: Hormones Are Messengers but Do not Carry Any Information

It is obvious from studies on comparative endocrinology of the effects of endocrine organ extracts in heterologous tissues that many hormones have been conserved across species during evolution. This fact is central to the question of which came first in evolution: the hormone or its actions? One of the most striking illustrations of this generalization is provided by the hormone prolactin. As its name suggests, prolactin was named after its action in regulating lactation. But it also emerged that this protein hormone made in mammalian pituitary is also known to have potent action in regulating important physiological functions in other non-mammalian vertebrates. As depicted in Fig. 1.1, the major functions of this hormone in mammals are the regulation of lactation and luteotropic activity. Prolactin also regulates major functions in non-mammalian species (Gorbman and Bern 1962; Tata 1993, 1998). It stimulates crop-sac development in birds, induces "water drive" in terrestrial urodeles and regulates salt adaptation and melanogenesis in fish. Prolactins from lower vertebrates generally do not exert their function in higher forms, whereas mammalian prolactin is quite effective in fish and amphibia. Since the amino acid sequence of prolactins is known to vary in different species, this latter fact reflects a concomittant evolutionary variation in the structure of protein hormone receptors as well. It is at once obvious from Fig. 1.1 that the molecule of prolactin does not carry the information for such a wide variety of physiological activities that it regulates in different organisms, as, for example, salinity detection in fish, water drive in neotenic amphibia, metamorphosis in amphibians, crop sac development and function in birds, and lactation and female reproductive functions in mammals. The "information" content is the way in which the hormonal target tissue interprets the signal.

Not only is the nature of response determined by the targets in different species but no two tissues within the same individual organism may respond to the hormone in the same manner. Amphibian metamorphosis is a good example to illustrate this point. The discovery by Gudernatsch in 1912 that extracts of horse thyroid tissue can induce the complete metamorphosis of frog tadpoles. The availability of synthetic thyroid hormone further established the extraordinary multiplicity of responses to this hormone, as can be discerned from Table 1.1. Thyroid hormone, which is present in primitive organisms, such as amphioxus, fish, birds, amphibians and mammals, regulates very different physiological processes, such as moulting in birds, metamorphosis in amphibia and basal metabolic rate in mammals.

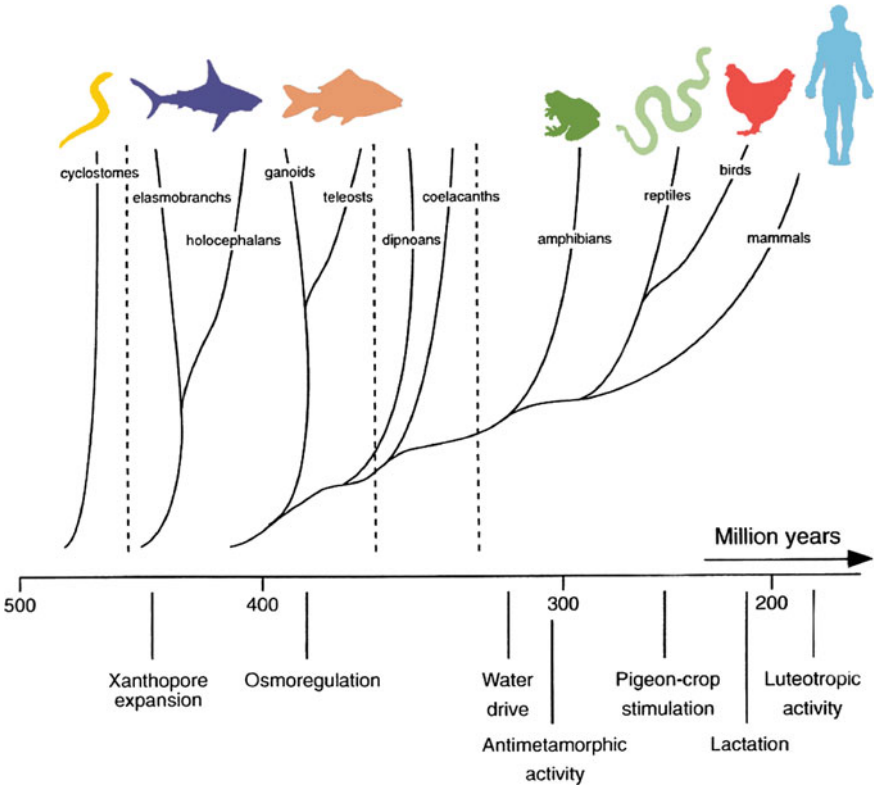


Fig. 1.1 Acquisition by a hormone of different regulatory physiological functions in different species during evolution, as exemplified here for the hormone prolactin. At the *bottom* of the figure is the evolutionary time scale, below which are given the important regulatory functions of prolactin. See Tata (1998) for further details

Table 1.1 Multiplicity of physiological and biochemical actions of thyroid hormone

Growth and developmental actions	Metabolic actions
Rate of postnatal growth of many mammalian species	Regulation of basal metabolic rate and avian tissues
Functional and biochemical maturation of foetal brain and bone	Movement of water and Na^+ ions across cell membranes
	Calcium and phosphorous metabolism
Morphogenesis, gene switching and cell death in amphibian metamorphosis	Regulation of metabolism of cholesterol and other lipids
Control of molting in birds	Nitrogen (urea, creatine) metabolism
Regulation of synthesis of mitochondrial respiratory enzymes and membranes	Control of oxidative phosphorylation and energy metabolism

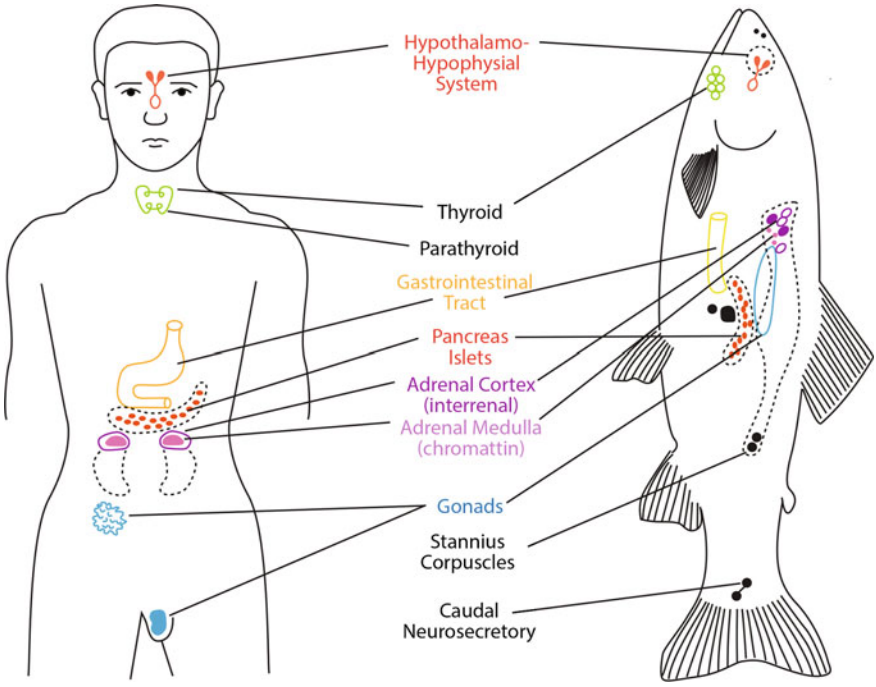
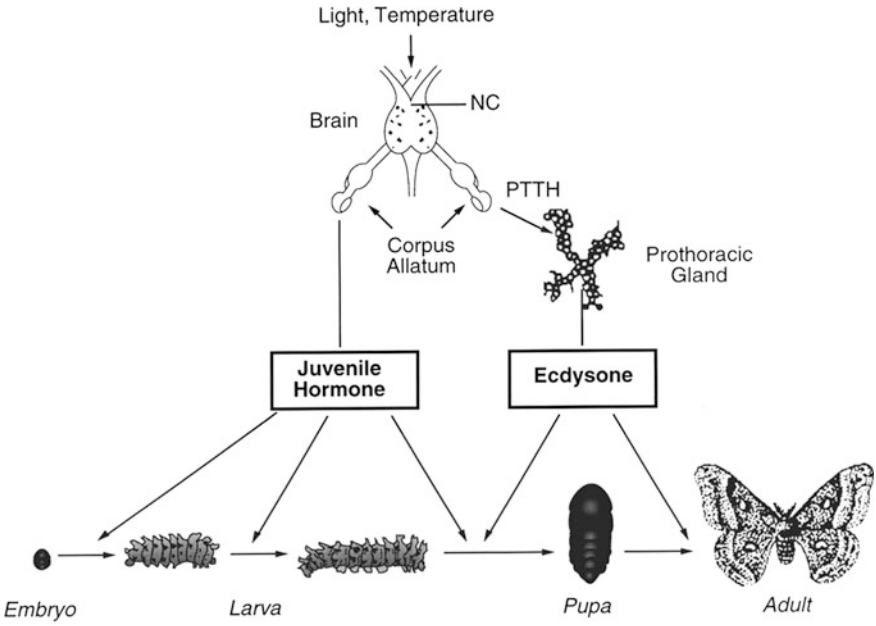


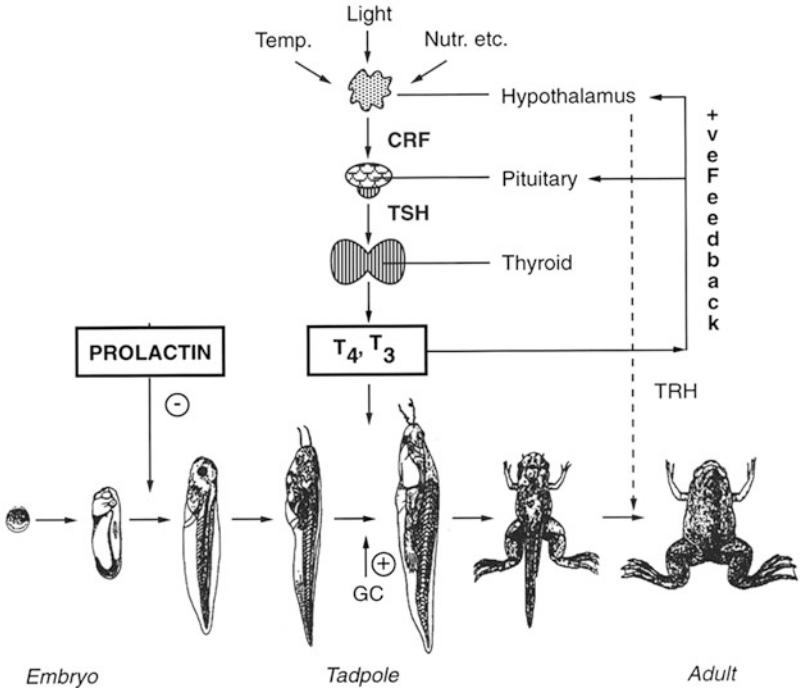
Fig. 1.2 Conservation of endocrine glands in two evolutionarily distant vertebrates, producing similar hormones but whose actions may vary according to the species and target tissue (Gorbman and Bern 1962)

Furthermore, even in the same individual organism, a given hormone may exert different, if not opposing actions, as illustrated by thyroid hormone in the initiation of cell death in the frog tadpole tail and gills, while simultaneously promoting differentiation and growth of limbs in the immature limb buds. Another example to illustrate this point of conservation of both the hormonal signal and its receptor through evolution is the anti-metamorphic action of human prolactin in thyroid hormone-induced amphibian metamorphosis in the tadpole of the amphibian *Xenopus*, both in vivo and in organ cultures (Tata 1993, 1996). It is also an important feature of endocrinology that both the internal secretions and the organs producing them were conserved across species (see Fig. 1.2).

1.3 Environmental Cues and Endocrine Cascades

A major principle of endocrinology involves the interplay and feedback between the central nervous and endocrine systems. Most of the external physical signals, such as photoperiodicity, temperature, etc., are transmitted to the CNS, where neurotransmitters act on specific target neurons, which in turn produce neurohormones, and set


Light, Temperature
Brain
NC
PTTH
Corpus
Allatum
Prothoracic
Gland
Juvenile
Hormone
Ecdysone
Embryo
Larva
Pupa
Adult


Light
Temp.
Nutr. etc.
Hypothalamus
CRF
Pituitary
TSH
Thyroid
+ve Feedback
PROLACTIN
T4, T3
TRH
GC
Embryo
Tadpole
Adult

◂**Fig. 1.3** Schemes depicting the similar patterns of hormonal regulation of metamorphosis in **a** insect and **b** amphibian. In response to environmental cues the brain of the moth larva stimulates specialized cells to secrete Juvenile Hormone and the hormone ecdysone. The balance between these two hormones determines the initiation of formation of the adult insect (here depicted for the moth). Similarly, in the frog tadpole, environmental factors trigger the sequential release hormones in the brain which stimulate the thyroid gland to secrete thyroid hormone (T3, T4). Although not fully understood, the hormone Prolactin prevents or slows down the action thyroid hormone. Other hormones also exert a modulatory effect. At the tissue and cellular levels the same hormone regulates new tissue formation and programmed cell death. Other abbreviations and details to be found in Tata (1998)

up a cascade of endocrine secretions Hormones can thus be considered as intermediaries in the transfer of information from the environment to the organism. Their overall purpose is to coordinate and integrate the activities of metabolic and developmental processes in diverse target cells in response to environmental signals. By the 1950s, important associations between the neural and hormonal signalling pathways were established in vertebrates for a variety of hypothalamic "releasing" hormones or factors. It is an interesting fact that some neurotransmitters, such as serotonin, are also signalling molecules in plants, while many animal hormones are found in primitive organisms and obviously have been put to different uses through evolution (Gorbman and Bern 1962; Barrington 1964).

Figure 1.3 shows the extraordinary similarity of how information originating as changes in the environment (such as temperature, photoperiodicity, nutritional elements, etc.) is transmitted via the central nervous system, or other sensory apparatus, and then to the target organs via the synthesis and secretion of specific hormones. In insects, certain neurosecretory cells in the larval or pupal brain act on the *corpus allatum* to produce a hormone called prothoracicotropic hormone (PTTH) which acts on specialized cells of the prothoracic gland. The latter when stimulated by PTTH will, in turn, secrete a group of steroid hormones termed ecdysteroids (the principal member being ecdysone) which induce metamorphosis. At well-defined periods of the developmental progression leading to metamorphosis, different cells in the invertebrate larval and pupal brain stimulate the *corpus allatum* to produce and secrete a group of terpenoid compounds collectively termed juvenile hormone or JH. It is significant that JH will counteract many biochemical actions of ecdysteroids at the cellular level.

In amphibians (and other metamorphosing vertebrates) it has been known since the discovery by Gudernatsch in 1912 of the precocious induction of metamorphosis in frog tadpoles that the process is under hormonal control (Gorbman and Bern 1962; Tata 1993). With the recognition of the central role played by the hypothalamus-pituitary-thyroid axis in vertebrates, the link between environmental signals and the initiation of metamorphosis could also be traced to the central nervous system through the intermediary of hypothalamic hormones TRH (thyrotropin releasing hormone), CRF (corticotropin releasing factor) and TSH (thyrotropic hormone) made in the pituitary.

1.4 Receptors: Key to the Understanding of the Mechanism of Hormone Action

Early studies on the mechanism of hormone action sought to uncover a unique mode of action for all hormones and vitamins, which often involved adding a given hormone or active principle to isolated tissues, cell homogenates or sub-cellular preparations. The availability of purified enzymes in the 1930s and 1940s allowed studying direct hormone-enzyme interactions. For a few years, it was thought that hormones induced allosteric or conformational changes in proteins, as, for example, the effects of insulin on hexokinase. These studies were eventually vitiated by the very high concentrations of hormones needed to elicit a direct effect on a given enzyme, nor was this approach compatible with the high degree of tissue specificity exhibited by hormones. By the end of the 1950s there was little enthusiasm for the idea of direct hormone-enzyme interactions as the basis for a common mode of action (Tata 1986, 1998).

1.5 Membrane Receptors

Hormone receptors have been commonly classified into two major groups: cell membrane and nuclear receptors. Receptors for protein hormones and growth factors, such as insulin, epidermal growth factor, growth hormone and prolactin, as well as many neurotransmitters, are all located in the target cell membrane; most of them are products of the oncogenes *v-erb*B, *v-ros* and *v-mpl*. Many membrane receptors are closely linked to adenylyl cyclase and G-proteins, and, through these, to cytoplasmic protein phophokinases, which transfer extracellular signals to the intracellular regulatory machinery (Parker 1996).

In the early 1940s, Levine had proposed that insulin controlled sugar metabolism by regulating its transport into the target cell, a proposal which led several years later to the concept that proteins and smaller peptide signals interact with the cell membrane. The discovery of cyclic AMP by Sutherland in 1956 as a "second messenger" of adrenaline and glucagon, followed by the discovery that adenylyl cyclase, the enzyme synthesizing cyclic AMP, was located in the plasma membrane, further consolidated the view that the cell membrane was a major site of action for many hormones (Sutherland 1972; Beavo and Brunton 2002). With the discovery of other secondary signalling molecules, such as inositol trisphosphate, G-proteins, oncogenes and the advent of gene cloning and sequencing technologies, it soon became possible to identify and characterize several membrane hormone receptors. Binding of the ligand to these receptors initiates a cascade of protein phosphorylation and de-phosphorylations in the cytoplasm, eventually leading to the physiological action of the hormone (Parker 1996; Hunter 1997, Fig. 1.4).

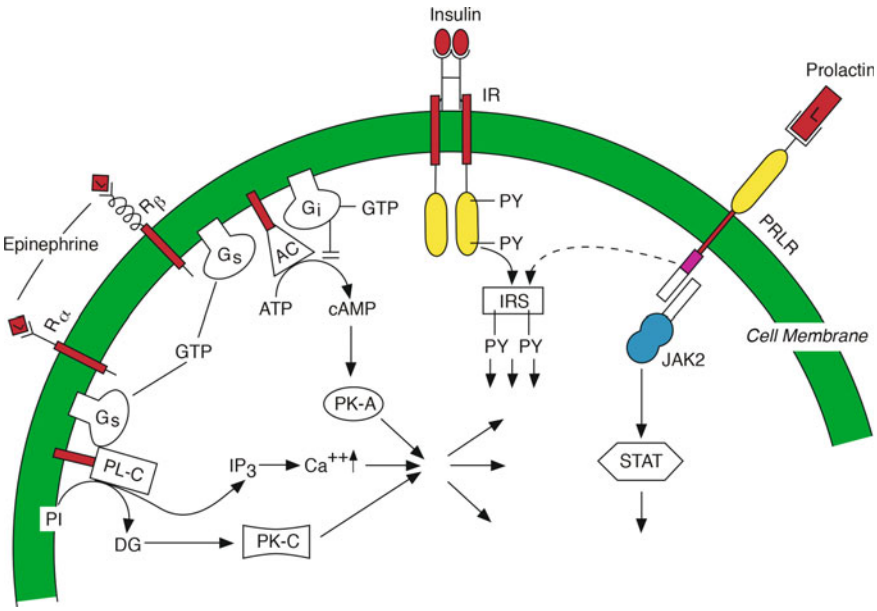


Fig. 1.4 A simplified diagram to illustrate "second messenger" and protein phosphorylation pathways associated with target cell membrane receptors for three hormones: epinephrine (adrenaline), insulin and prolactin. *R£VRß, IR* and *PRLR* denote receptors for epinephrine, insulin and prolactin, respectively; *Gs* and *Gi* are stimulatory and inhibitory G proteins; *PL-C*, phospholipase C; *AC*, adenylyl cyclase; *PI*, phophatidyl inositide; *IP3*, inositide trisphosphate; *DG*, diacylglycerol; *PK-A* and *PK-C*, phosphokinases A and C; *cAMP*, cyclic AMP; *PY*, phosphotyrosine; *IRS*, insulin receptor substrate protein; *JAK*, Janus kinase; *STAT*, signal transduction and transcription factor. See Tata (2005) for details

1.6 Nuclear Receptors

At almost the same time as cyclic AMP was discovered, Knox demonstrated that glucocorticoids regulate hepatic metabolism by selectively enhancing the synthesis of the enzyme tyrosine aminotransferase (Knox et al. 1956). New methods to study cell-free protein synthesis, and the availability of specific transcription inhibitors, allowed a more precise analysis of how growth and developmental hormones influenced protein synthesis in their respective target cells. The resulting observations that all steroid and thyroid hormones, administered in vivo affect the protein synthesizing machinery in vitro soon shifted the attention to transcriptional control (Tata 1986). That hormonal signals regulate transcription rather than translation, first became evident in the early 1960s from work demonstrating that puffing of polytenic chromosomes in larval salivary glands of insects is regulated by the steroid moulting hormone ecdysone (Ashburner 1974). Kinetics of labelling of nuclear RNA further revealed that all steroid and thyroid hormones strongly influence the formation and turnover of messenger RNA. In the mid-1960s several

investigators were able to reproduce the transcriptional effects of steroid and thyroid hormones in cell-free transcription systems using isolated nuclei and nuclear extracts from target tissues (Tata 1986, 1998). In the 1970s and 1980s, the laboratories of Chambon and O'Malley described in detail how oestrogen activates tissue-specifically and selectively the genes for egg white protein (ovalbumin, conalbumin, ovomucoid) and yolk proteins (O'Malley 1978; LeMeur et al. 1981; Tata 1998; Chambon 2004).

Although we know much about the details of the transcriptional machinery, it would be impossible to understand how hormones or other signals regulate gene expression without a reasonable knowledge of the structure and function of their receptors. First evidence for the existence of nuclear receptors came in 1961 from the work of Jensen and colleagues in which they tracked radioactively-labelled oestradiol-17ß in female sexual tissues, and found that it forms a complex in the nucleus with a protein, which fulfilled the criteria for a receptor (Jensen 2004). The cloning of receptors for oestrogen, glucocorticoids and thyroid hormone in the 1980s in the laboratories of Chambon, Evans and Vennström, showed that all nuclear receptors are cellular homologues of the oncogene *v-erb*A and function as ligand-activated zinc-finger transcription factors. Today, more than 30 nuclear receptors encoded by this gene superfamily have been cloned, sequenced and many obtained as pure recombinant proteins (Mangelsdorf et al. 1995; Benoit et al. 2004; Chambon 2004), including several 'orphan' receptors whose ligands have not yet been identified. What is most remarkable for these nuclear hormone receptors is their high degree of target gene specificity, which is achieved by a precise spacing of nucleotide repeats in the target gene promoter's hormone response element (HRE) that interacts with the DNA-binding domain (DBD) of the receptor.

As depicted in Fig. 1.5, the central DNA-binding domain a nuclear hormone receptor is the most conserved region, while the 3′-terminal ligand—binding domain is the most variable (not shown in Fig. 1.5). Nuclear hormone receptors can be sub-divided into two groups, according to whether they form cytoplasmic complexes with hsp90 (heat-shock proteins of around 90 kDa molecular weight), and can be active as monomer, homodimer or heterodimers. All vertebrate steroid hormone receptors, belong to the first category, while the liganded receptors for retinoic acid, thyroid hormone (TR), vitamin D_3 (VDR) and peroxisome prolifetor (PPAR) function as heterodimers with RXR, the 9-cis-retinoic acid receptor (RXR does not have to be liganded to function as heterodimer). At first, only the second group of receptors were found to exist as multiple isoforms, the multiplicity residing in the N-terminus of the receptor, but more recent work has shown that the same holds true for other steroid receptors.

An interesting question arises as to how the high degree of target gene specificity for a given hormone and its receptor is achieved. The answer lies in the highly precise spacing of nucleotide repeats in the hormone response element (HRE) of the promoter of the target gene and the DNA-binding domain (DBD) of the receptor which recognizes it. A consensus hexanucleotide sequence, usually present as a pair, is the most common feature of all the known HREs but the sequence of each hexad, and the relative position of the two hexads, exhibit considerable variability

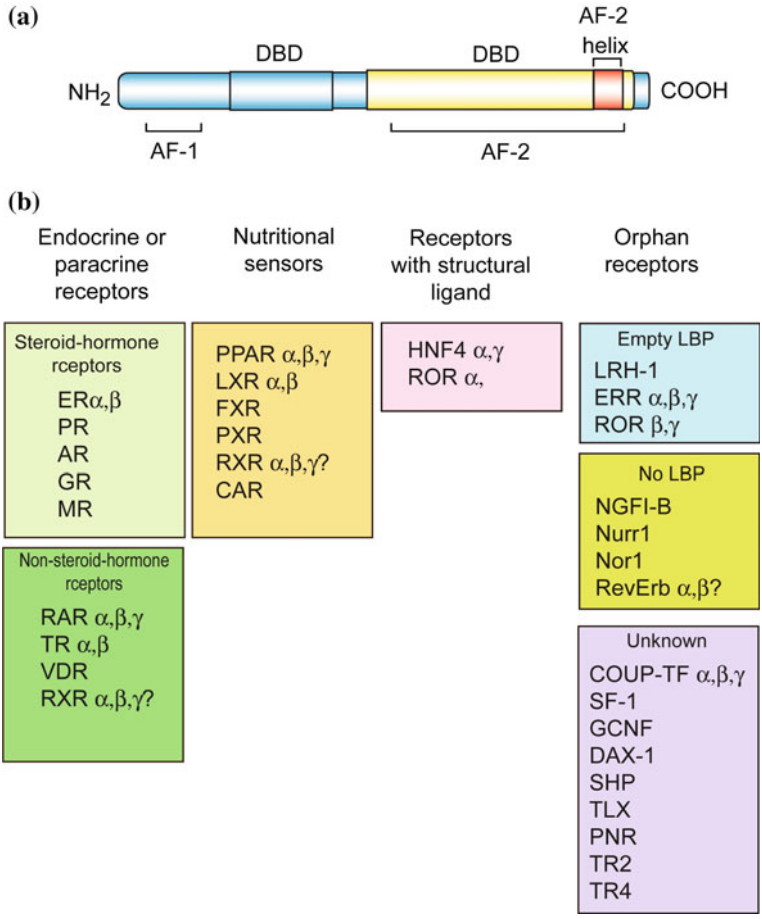


Fig. 1.5 **a** Representation of the common structural features of all members of the supergene family as ligand-activated transcription factors. *DBD* DNA-binding domain; *AF* transcription activating factors. The ligand-binding domain (LBD) is located at the COOH terminus. **b** Different categories of nuclear receptors divided by Benoit et al. (2004) into four sub-groups according to the nature of the signalling molecules. Only the two groups on the *left in green boxes* refer to hormonal signals and only these will be considered in this article. ER, PR, AR, GR and MR in the *light green box* are receptors for oestrogen, progesterone, androgen, glucocorticoids and mineralocorticoids, respectively. RAR, TR, VDR and RXR in the *dark green box* are receptors for retinoic acid, thyroid hormone, vitamin D_3 and 9-cis retinoic acid, respectively. For all other receptors and abbreviations refer to Benoit et al. (2004)

to generate the high degree of specificity of interaction between the receptor and its target gene. The HREs recognized by the receptors of steroid hormones oestrogen, progesterone, glucocorticoid, androgen and mineralocorticoid (ER, PR, GR, AR, MR) in the top green box in Fig. 1.5 are characterized by a 15-nucleotide motif consisting of two hexads in a palindromic configuration separated by 3 nucleotides.

The hexad sequences show a variability within this group of receptors. On the other hand, and more interestingly, the HREs of the non-steroid receptors shown in the bottom green box in Fig. 1.5 (TR, RAR, RXR, VDR, PPAR) all share the same AGGTCA hexad of ERE but are organized as direct repeats (DRs) separated by one to five nucleotides. This arrangement of HREs explains the fine discrimination of target genes by the heterodimers formed by each of these receptors with RXR and which confers an extraordinary hormonal specificity. It is a most remarkable example of selective transcriptional regulation (Mangelsdorf et al. 1995; Tata 1998). Dramatic confirmation of the above biochemical findings regarding the interaction between DBD of nuclear receptors and their cognate HREs has been provided in the last decade by NMR spectroscopy and x-ray crystal structure analysis (Chakravarti et al. 1996; Evans 2004).

What is most remarkable for these nuclear hormone receptors is their high degree of target gene specificity, which is achieved by a precise spacing of nucleotide repeats in the target gene promoter's hormone response element (HRE) that interacts with the DNA-binding domain (DBD) of the receptor. As shown in Fig. 1.6, large proteins termed CBP (CREB binding protein) and p300 are thought to form bridges between nuclear hormone receptors and other transcription factors. Other important elements of this complex are the p160 nuclear receptor coactivator and the 270 kDa nuclear receptor co-repressor (N-CoR) (McKenna and O'Malley 2002). The CBP/p300 complex serves to integrate multiple signalling pathways in the cell nucleus and many more such modulators will most likely be discovered in the very near future, forming even more complex structures with nuclear receptors.

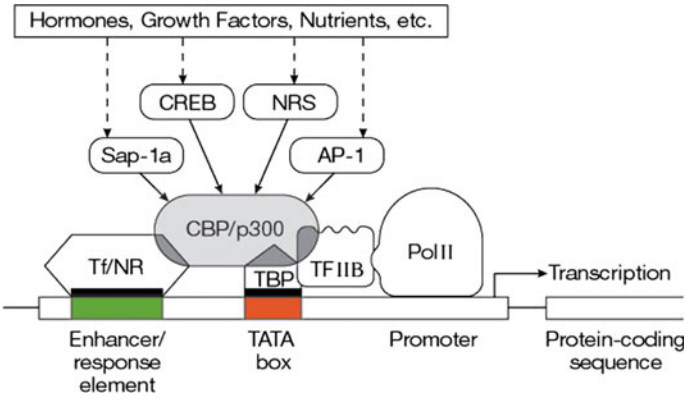


Fig. 1.6 How nuclear hormone receptors are thought to form complexes with other factors to regulate transcription. A bridging protein such as CBP/p300 would be in close contact with nuclear receptors and transcription factors (*Tf*) that recognize specific DNA sequences, TATA box binding protein (*TBP*) and transcription factor IIB (*TFIIB*), which would form a complex with RNA polymerase II. CBP/p300 is thought to form complexes with other transcription factors without involving DNA, such as CREB, AP-1 and Sap-1a. The activities of many of these components are modified by phosphorylation. See Tata (1998) and Evans (2004) for further details

1.7 Chromatin Structure and Hormonal Regulation

The higher order of organization of genes within the nucleus and the growing number of cross-interactions among regulatory factors have focused attention on the important role of chromatin structure in hormonal regulation of gene expression. Studies from Chambon's laboratory in the mid-1980s on the DNase I-hypersensitive regions of the chicken ovalbumin gene promoter led to the first understanding of how oestrogen reversibly modifies the chromatin structure of this target gene in a tissue-specific manner (see Chambon 2004). Later studies provided evidence that the function of glucocorticoid receptor in regulating the target gene promoter is determined by the manner in which it itself is organized within the chromatin structure (Beato 1996). Recent work on how steroid and thyroid hormones modify the chromatin structure of their target genes has provided evidence that the HREs of the glucocorticoid receptor are highly organized in phased nucleosomes. The binding of the hormone to its nuclear receptor causes an alteration in the chromatin structure, such that it induces the incorporation of other transcription factors into chromatin, thus facilitating the transcription of the hormone-regulated gene promoter (Beato 1996; Wolffe 2000; Evans 2004). So far, most conclusions drawn from chromatin experiments are based on indirect observations in vitro. The recent development of chromatin immunoprecipitation (ChIP; Sachs and Shi 2000) is therefore a new promising technique. Workers in Gannon's laboratory have used it to show that the oestrogen receptor activates its target gene in a cyclical manner, in that the receptor-ligand complex is continuously removed by a protease to be replaced by a new receptor (Métivier et al. 2003; Reid et al. 2003).

According to a simple model proposed by Wolffe (2000), the HREs are highly organised in phased nucleosomes and the binding of the hormone to its nuclear receptor causes an alteration in the chromatin structure such that it will induce the binding of non-receptor transcription factors, such as NGF-1 and OTF-1, and thus allow the transcription of the hormone-regulated gene promoter. Similarly, as depicted in Fig. 1.7, Wolffe's group has suggested that both the silencing and activation of the Xenopus thyroid hormone TRß gene is determined by processes controlling nucleosome assembly (see Wolffe 2000). As pointed out earlier (see Fig. 1.3), thyroid hormone is obligatory hormonal signal for all tissues of the amphibian larva to develop to the adult organism. The conclusions of such chromatin studies have largely been inferred indirectly from techniques such as cross-linking to determine points of contact between DNA and protein. More direct information of the spatial organization and mobility of receptors is needed before we can draw precise conclusions as to the role played by chromatin rearrangements within the nucleus in vivo.

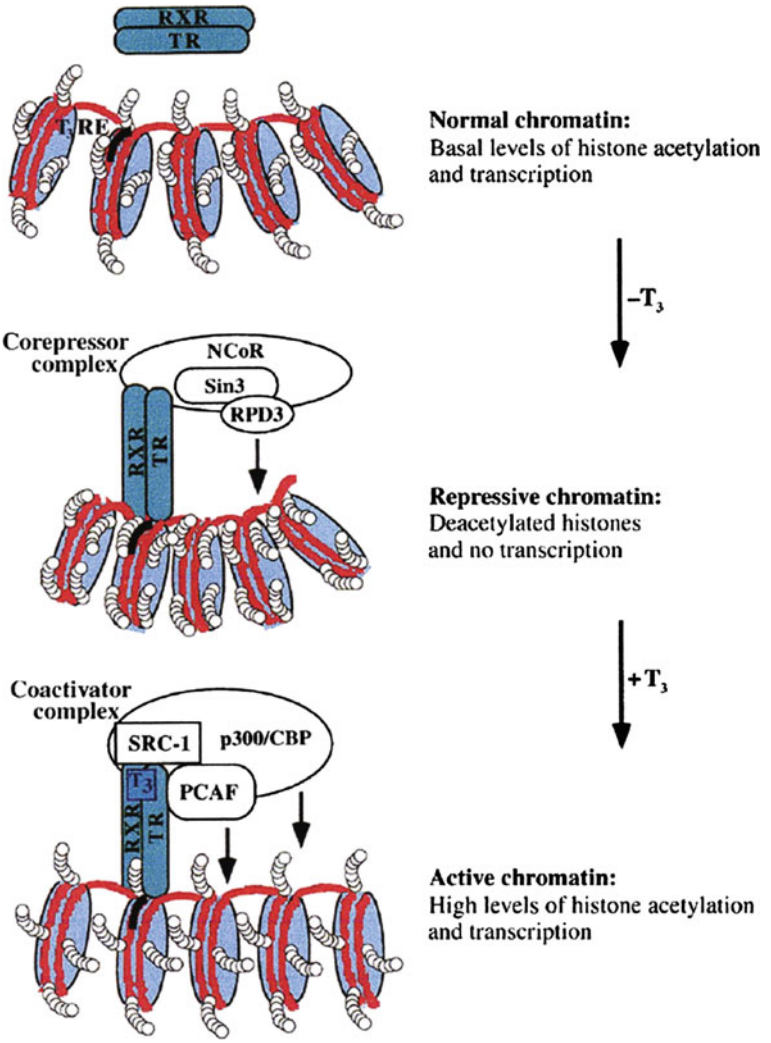


Fig. 1.7 How a ligand-activated nuclear receptor could modify the higher-order structure of chromatin. The packaging of DNA into chromatin is visualized in three transcriptionally active states: normal, repressive and active. In this example, the region of chromatin chosen contains the thyroid hormone receptor (TR)/RXR heterodimer, with or without its ligand triiodothyronine (T_3), bound to the thyroid responsive element (TRE) in the target gene. In normal chromatin, histone acetylation is at its basal level and so is the transcriptional activity. In the absence of T_3 (as during early stages of development), chromatin exists in its condensed and transcriptionally repressive form whereby the histones are in a largely deacetylated state with no transcription of the TR's target gene. In the presence of T_3 the chromatin is now active with elevated levels of histone acetylation and transcription. The other components are proteins that form 'corepressor' and 'coactivator' complexes with complexes with the TR/RXR receptor heterodimer. For more details see Wolffe (2000)

1.8 Integration of the Membrane- and Nuclear Receptor-Linked Hormonal Pathways

The functions of many transcription factors and co-regulators of nuclear receptors are regulated by protein phosphorylation (Wu et al. 2004). Since the processes of protein modifications are often linked to membrane components, this phenomenon is a reflection of the existence of a wider network that links membrane and nuclear receptor into complex signal transduction pathways. In this context, and as illustrated in Fig. 1.8, work, from Darnell's laboratory has highlighted the importance

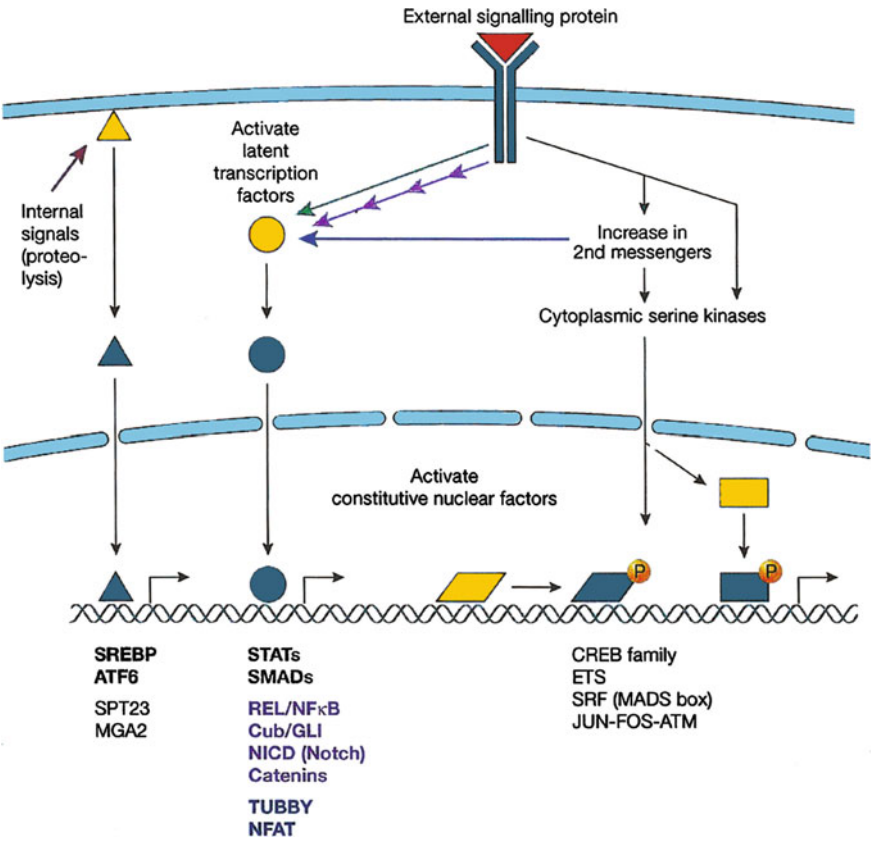


Fig. 1.8 According to this model, proposed by Brivanlou and Darnell (2002), latent intracellular regulatory factors, which include nuclear receptors, transcription factors and other modulators of nuclear functions, are activated or inhibited by post-translational modifications such as phosphorylation, proteolysis or through 'second messenger'-dependent functions. The latter would be regulated by signals impinging on the cell membrane. Thus, for example, the activities of nuclear proteins, receptors and transcription factors can be modified by phosphorylation by a convergence of functions operating at the levels of cell membrane and nucleus. For definitions of the abbreviations and other details see Brivanlou and Darnell (2002)

of protein phosphorylation in the control of gene expression, for example, as seen with the JAK/STAT pathway and that of nuclear factors, such as CREB (Brivanlou and Darnell 2002). The importance of the JAK/STAT pathway and the control of gene expression in general by transcription factors is a good example of how such complex interactions bring together nuclear and extra-nuclear regulatory processes and thus allow the setting up of a network linking membrane and nuclear receptor signal transduction pathways.

It is clear from what has been said above that hormone receptors now occupy a central role in our current concepts of signalling mechanisms. One should add that equally important is the complex of the target cell's signalling component that is linked to the receptor. Over the past 25 year, the application of gene cloning, cell transfection, transgenic and gene knock-out techniques, x-ray crystallography and NMR analysis of DNA-protein and protein-protein interactions have significantly advanced our understanding of the structure and function of receptors beyond all expectations. It therefore follows that our further understanding of the mechanism of hormone action will be a function of our changing perception of the different branches of the science of genomics, cell structure and cell signalling processes.

1.9 Hormones and Their Actions Through Evolution

While considering the progress resulting from the above advances in the future, the question of whether or not the hormone molecule itself carries the information for how the target cell has to respond needs to be kept in mind. The answer to this question is most convincingly answered by the example, depicted in Fig. 1.1, of the wide variety of actions that have been ascribed to the hormone prolactin through evolution—from a marine worm to man. Several similar examples of species-wide multiplicity of action can be found for many other hormones (see Gorbman and Bern 1962; Barrington 1964; Tata 1986). Not only is this multiplicity of action seen in different species of organisms, but is easily discerned in different tissues of the same organism, or even in the same tissue at different developmental stages of same tissue. The importance of this latter point concerning hormone action is most obvious from the example given in Table 1.1 on the induction and regulation of metamorphosis by thyroid hormone in amphibians. Thus the genes whose expression is regulated by this hormone in the larval and adult frog liver and intestine are nowhere similar. At the whole tissue level, the same hormone activates cell death in the larval muscle, skin and nerve cells of the tail and gills, while at the same time promoting the growth and development of the same cells in limb buds and lungs. The chemical structure of both prolactin and thyroid hormone is the same in the most primitive organism in which they are made as in man. These examples serve quite categorically that the hormone molecule in itself does not carry any information for its target cells. It serves as a trigger or signal for the responding cell to initiate the first step in the complex between the hormone receptor and its key partner that initiates the chain of downstream responses and

events that lead to the physiological action of the hormone. Thus, the focus has to shift to the complex of the hormone receptor and the immediate cellular regulatory element in order to explain how the actions of an ancient hormone molecule have varied through evolution. This conclusion lies at the heart of the current research on various facets of the mechanism of hormone action. At the same time, this research will continue to reflect our knowledge of cellular regulatory mechanisms at any one given time. Conversely, many important advances in biochemistry, cell and molecular biology are also the result of work on hormones.

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