INTRODUCTION

In multicellular organisms, elaborate signaling mechanisms enable their cells to communicate with one another so as to coordinate their behavior for the benefit of the organism as a whole. Each cell in a multicellular animal is programmed during development to respond to a specific set of signals that act in various combinations to regulate the behavior of the cell and to determine whether the cell lives or dies and whether it proliferates or stays quiescent. These signals or signaling molecules include proteins, peptides, steroids, amino acids and their derivatives, fatty acids, retinoids etc.

The term hormone refers to a Greek verb herman, meaning "to stir up or excite". They are chemical messengers released from specialized group of cells to elicit response by interacting with cognate receptors in target cells. A class of hormones, called endocrine hormones, that arise in one tissue or gland and travel a considerable distance through circulation to reach a target cell having cognate receptors. Paracrine hormones on the other hand arise from a cell and travel a relatively small distance to interact with their cognate receptors on neighboring cell. Autocrine hormones, alternatively, are produced by the same cell that functions as the target cell for those hormones (Hardie 1990; Norman and Litwack 1997).

Regardless of the nature of hormone, the target cell responds by means of a specific protein called receptor (Levitski 1984). Receptor specifically binds the signaling molecule and then initiates a response in the target cell. The specific way by which a cell reacts to its environment varies according to the set of receptors that a cell possesses through which it is tuned to detect a particular subset of the available signal and also according to the intracellular machinery by which the cell integrates and interprets the information that it receives. Thus a single signaling molecule often has different effects on different target cells.

For any given hormone, an incredible array of biological responses can be modulated depending upon the phenotype of the target cell that possesses the cognate receptor. In any given target cell, only a small subset of genes will have their chromatin in an active or "open" configuration. Thus, while a hormone may modulate as many as 300 genes in a given organism, in a specific target cell perhaps only a few genes will be available for regulation (Bamberger et al 1996; Norman and Litwack 1997). Furthermore, each cell is bombarded with chemical signals that regulate diverse physiological responses. In general, these signals bind to cell-surface receptors that activate several intracellular signaling pathways, so that the intracellular signals generated from different receptors will interact with one another in many complex ways. An important consequence of this cross-talk is that it will not be easily disrupted by removing or changing a single signaling element in one of these pathways. In most cases the receptors are transmembrane proteins on the target cell surface; when they bind an
extracellular signaling molecule, they become activated so as to generate a cascade of intracellular signal that alter the behavior of the cell (Kahn 1976). Additionally, in some cases, receptors are inside the target cell and the signaling ligand has to enter the cell to activate them.

**Signaling through cell surface receptors**

All water-soluble signaling molecules (including neurotransmitters, protein/peptide hormones, and growth factors) bind to specific receptors on the surface of the target cells. Most cell-surface receptors belong to one of the two classes; enzyme-linked cell-surface receptors and G-protein-coupled receptors (Nishizuka 1992; La Marco and Vivanco 1996).

**Enzyme-linked cell-surface receptors**

Enzyme-linked cell-surface receptors (ELCSRs) are transmembrane proteins with their ligand-binding domain on the outer surface of the plasma membrane (La Marco and Vivanco 1996). Their cytosolic domain has either an intrinsic enzyme activity or associate directly with an enzyme. One of the best understood cell-surface receptor with intrinsic enzyme activity is the receptor tyrosine kinases (RTKs). The RTKs are a family of more than 50 different transmembrane polypeptides with in-built tyrosine kinase activity domain towards cytoplasmic side. Binding of cognate hormone results in diverse cellular responses (Geer et al 1994). All the members of the RTK family share common structural and functional domains Majority of growth factor receptors are RTKs. These include the receptors for epidermal growth factor (EGF), fibroblast growth factor (FGF), hepatocyte growth factor (HGF), insulin, insulin-like growth factor (IGF-1) and nerve growth factor (NGF). The insulin receptor belongs to the RTK family, with extramembrane α subunits those bind to insulin and the membrane spanning β subunits having RTK towards cytoplasmic side of the subunits (Hubbard et al 1994). Insulin is an important hormone not only in regulating glucose metabolism but also it plays diverse role, influencing cellular growth and development in animals.

**G-protein-coupled receptors**

G-protein-coupled receptor (GPCR) were discovered in the 1970s by Rodbell and Gilman and since then many hormones have been shown to transduce signals through these receptors. GPCRs are the largest family of cell-surface receptors, with more than 100 members have already been defined in mammals. Cloning studies have revealed several hundred GPCRs that are structurally and functionally related, with all displaying the seven-transmembrane helical (heptahelical, serpentine) motif. GPCR and their endogenous hormones are involved in
regulating a number of important physiological phenomena in almost every major tissues and organs.

**Signaling through Intracellular receptors**

Steroid hormones and the related lipophilic molecules, retinoids, vitamin D₃ and thyroid hormones, diffuse through the cell membrane and bind to specific cytosolic (corticosteroids) and nuclear (gonadal steroids, Vitamin D₃ and thyroid hormones) receptors. The hormone-receptor complexes then modulate the expression of target genes in the chromatin thus regulating cellular responses. Additionally, a number of receptors ('orphan receptors') have been identified for which the specific ligand has not been identified as yet.

**Orphan receptors**

Orphan receptors (ORs), discovered in 1988, represent a class of putative, ever growing class of intracellular nuclear receptors, whose defining characteristic is the lack of identifiable physiological regulatory ligands (LaMarco and Vivanco 1996). ORs are found in every metazoan species and were identified by applying low stringency hybridization screening and genetic and molecular cloning techniques. Some ORs do interact with some novel ligands, while others may represent constitutive activators/repressors or factors whose activities are modulated by post translational modifications (Mangelsdorf et al 1995; Mangelsdorf and Evans 1995; Enmark and Gustaffson 1996). Many ORs have structural domains similar to that of members of steroid/nuclear receptor superfamily. Several ORs have been characterized in recent years, some of which have been suggested to control vital physiological and developmental processes.

**Vitamin D receptors**

The vitamin D metabolite, 1,25-dihydroxy vitamin D₃ (calcitriol), regulates cell growth and differentiation, immune function and myriad cellular activities associated with mineral metabolism. These actions of vitamin D₃ are mediated by its cognate receptor in the nucleus called vitamin D receptor (VDR) (Pike and Sleator 1985). VDR belongs to the nuclear receptor superfamily. The ligand activated VDR can bind to DNA as homodimer or heterodimer (with RXR or RAR) in the direct repeat response element called vitamin D response element (VDRE), which may be a positive or negative regulator of cognate gene expression (Koszewski et al 2000).

**Retinoid receptors**

The retinoids [vitamin A (retinol) and its natural and synthetic derivatives] are essential for diverse biological processes, including, vision, reproduction, differentiation, metabolism, bone
development, hematopoiesis, and pattern formation during embryogenesis (Sporn et al 1984; Gudas et al 1994). The myriad roles of retinoids are mediated by retinoid receptors, which bind all-trans - and 9-cis-retinoic acid (RA). The retinoic acid receptor (RAR) (Petkovitch et al 1987; Giguere et al 1987) proteins belong to the steroid/nuclear receptor superfamily. Three different subtypes of RAR have been identified, RARα, RARβ and RARγ in mammals, birds and amphibians (Chambon 1994). Another nuclear receptor termed the retinoid X receptor (RXR), has been identified (Mangelsdorf et al 1990) which also exists in three different subtypes, RXRα, RXRβ and RXRγ. The RXR has a unique ability to form heterodimer with RARs, THRα, VDRs and an increasing number of ORs, indicating their potential physiological functions.

Thyroid hormone receptors
Thyroid hormones (T₃ and T₄) are known to be important modulators of developmental processes in humans and several other organisms. Thyroid hormones also play a crucial role in the development and maturation of the nervous system, however, very little is known about the role of this hormone in adult brain (Calza et al 1997). The physiological actions of thyroid hormones are carried out by specific nuclear localized receptors called thyroid hormone receptors (THRαs). T₃ binds to THRα with higher affinity (10 times higher) than T₄ and hence, is more biologically potent. The THRαs have been isolated from brain, pituitary, lung, liver, pancreas and testes. THR belongs to the nuclear receptor superfamily and several isoforms of this receptor have been identified, THRα1, THRα2, and THRβ1, and THRβ2 (Glauser and Barakat 1997).

Steroid receptors
The steroids, glucocorticoid, mineralocorticoid, androgen, estrogen and progesterone act at the cellular level through the mediation of their respective cognate intracellular receptors. The glucocorticoid receptor (GR), androgen receptor (AR), estrogen receptor (ER) and progesterone receptor (PR) all belong to the nuclear receptor superfamily.

Androgens play critical role in several stages of development and maintenance of male phenotype (Brinkmann et al 1999). The biological action of androgens are mediated by the AR, a 110 kDa phosphoprotein, which upon ligand binding, leads to transcriptional activation of androgen responsive genes (McPhaul 1999).

Estrogens are the principle feminizing hormones involved in regulation of physiological functions, such as, secondary sexual development, maintenance of female phenotype and pregnancy. The wide range of action is mediated by the estrogen receptors (ERs). Two isoforms of ER are known, ERα and ERβ (Cowley and Parker 1999), with similar transcriptional activities. ER has been described to be present in a number of tissues, such as, ovary, prostates, cerebral cortex and hippocampus.
Progesterone through the mediation of PR has principal targets on the uterus and ovary, where it has a central role in reproduction, being involved in ovulation, implantation and pregnancy. Associated with this is the involvement of progesterone in regulation of uterine function during the menstrual cycle. Other tissues showing responsiveness towards progesterone are breasts and brain.

Glucocorticoids exert most of their effects on target cell through the mediation of glucocorticoid receptor (GR). GR is the most thoroughly studied steroid receptor from the nuclear receptor superfamily. The detailed knowledge of GR structure and function including recent developments are given below:

**Glucocorticoid receptors**

The study of glucocorticoid receptor (GR) assumes great significance on account of the myriad physiological and biochemical role of the glucocorticoid (GC) hormones, influencing almost each and every tissues and organs. GR is a housekeeping protein and functionally acts as a transcriptional regulatory protein, and hence, most of the known actions of GCs ultimately culminate in regulating target gene expression in responsive tissues. Thus, changes in GR functions may automatically modulate the expression of GR responsive genes (Tronche et al 1998; Kellendonk et al 1999).

Tremendous pace of work on the GR-mediated gene expression during the last 10 years, reveal the ever-growing complexity of this process. It is now substantially clear that, the GR requires numerous other basal transcriptional factors to co-regulate gene expression (Bamberger et al 1996; McNally et al 2000). Also, some of the effect of GCs through GR on target genes may be through the process of cross-talk (Cella et al 1998). Recent advances have also suggested that for some responsive genes, the GRs need not interact physically with the DNA and that the GR binding to DNA is not essential for survival (Tronche et al 1998).

**Glucocorticoids and physiology**

GCs are synthesized and released into the circulation by the zona fasciculata of the adrenal cortical cells. Upon binding to its cognate receptor, they act on a variety of different target cells and regulate normal metabolic and other functions leading to homeostasis, differentiation and growth of animal tissues through modulation of gene expression. Increased circulating level of GCs lead to several pathological consequences, such as Cushing’s syndrome in humans. Therefore, GC level is precisely controlled by an endocrine cascade, the hypothalamo-pituitary-adrenal axis (HPA) (Fink 1997). GCs control their production via feedback, exerted at the levels of hypothalamus and pituitary. GCs are an absolute necessity for the maintenance of homeostasis and their coordinate actions allow the body to respond to internal and external alterations.
In liver, GCs are primarily gluconeogenic, where they stimulate transcription of genes for enzymes like phosphoenolpyruvate carboxykinase (PEPCK), glucose 6-phosphatase and tyrosine aminotransferase (TAT). In kidney, they enhance reabsorption of Na⁺ and increase K⁺ and H⁺ elimination. In adipocytes, they stimulate lipolysis by facilitating the action of epinephrine and norepinephrine and in peripheral tissues they inhibit glucose uptake. In cardiovascular system, they influence vascular permeability, maintain blood pressure and increase hemoglobin and number of RBCs. In the brain, GCs have been suggested to influence emotions and cognitive processes like learning and memory (Sapolsky 1996; Kellendonk et al 1999; Welberg and Seckl 2001). They also regulate sleep patterns and neuronal firing in different groups of animals. However, elevated GC levels appear to impair brain functions. On the immune system, they are involved in the suppression of inflammation and immune responses, by inhibiting the action of lymphokines and acting as lymphotoxic (Barnes and Adcock 1993).

GCs are not only involved in controlling adult physiology, but also have been shown to influence critical developmental processes. Important roles are suggested, for example, lung maturation, chromaffin cell differentiation and erythroblast proliferation (Haagsman 1991; Wessely et al 1997). The physiological effects of glucocorticoids are mediated by a receptor of approximately 94 kDa molecular mass, mainly localized in the cytoplasm of the target cells, called the glucocorticoid receptor (GR). GR is present in all tissues that are targets of glucocorticoid action. Tissues that lack functional receptors, or are receptor deficient, failed to respond to the normal circulating levels of hormones. The identification of intracellular receptors was made possible by the use of radiolabelled hormones by Jensen (1960) that could specifically bind to its cognate receptor. The intracellular localization of GR was uncertain before its discovery in the rat thymic cytosol (Munck 1961). It is now well established that they are predominantly present in the cytoplasmic compartment, however, there is a constant nucleo-cytoplasmic shuttling of the receptor even in hormone unbound form (Scherrer et al 1990). The GR phylogenetically belongs to the steroid/nuclear receptor superfamily. This superfamily is the single largest class of eukaryotic transcription factors, and has been divided into three types. Type I represent the GR, MR, ER, AR, PR and THR localized in the cytoplasm or nucleus. Type II includes the VDR, RAR, and RXR, whereas the type III represents the so-called “orphan receptors”, for which most of the ligands are not yet fully characterized. The GR is the most thoroughly studied receptor of this superfamily, and its structure, function and mode of action is best understood.

The 9S GR
The presence of unliganded GR as 9S receptor complexes was first reported in the rat hepatoma cell cytosol (Baxter and Tomkins 1971). This observation was rapidly confirmed for
GR in the cytosol of rat liver (Beato and Feigelson 1972), brain (Chytil and Toft 1972'), and mammary gland (Gardner and Witliff 1973). Although, it was known that cytosolic GR existed in the 9S complex and that the temperature-transformed GR extracted from nuclei with salt was 4S, investigators did not focus on the dissociation of the 9S complex as the transforming event. Rather, they focussed on the other half of the coin, i.e., the acquisition of DNA/nuclear binding activity. The unliganded 9S GR is a part of a multiprotein complex that consists of a 94 kDa steroid binding protein, 2 molecules of Hsp90, one Hsp70 and one Hsp56 (Pratt 1993; Hutchison et al 1994; Prima et al 2000). In addition, other less-well characterized proteins have been found to be involved in this complex. In the absence of hormone, this oligomeric complex undergoes constant cycles of dissociation and ATP and Hsp70-dependent reassociation (Hu et al 1994).

The 9S GR is much less stable in cytosol preparations compared to the 9S forms of ER and PR. The instability of the 9S GR complex, however, turned out to be an advantage. The GR-Hsp90 complex is required to maintain the high affinity competence of the steroid-binding pocket. Dissociation of Hsp90 from the unliganded receptor results in the loss of cytosolic steroid binding activity (Bresnick et al 1989). It was the study of this instability of cytosolic glucocorticoid-binding activity that led to the discovery of agents that prevent the loss of steroid binding activity, most notably, molybdate and some other metal oxyanions (Nielsen et al 1977a,b). Molybdate has been widely used for the purification of untransformed GR and for identification of Hsp90 component of the 9S complex.

The GR isoforms
GR structure polymorphism has been extensively studied in human. The two isoforms of GR discovered were termed hGR alpha (hGRα) and hGR beta (hGRβ), that are generated by alternative splicing of human GR pre-mRNA (Hollenberg et al 1985; Encio and Detera-Wadleigh 1991). The hGRα is the predominant form while the hGRβ is a minor variant (Rivers et al 1999). These two protein isoforms have the first 727 amino acids in common and thus, contain both the transactivation and the DBD. The hGRβ is the non-hormone binding splice variant that differs from the wild-type hGRα only at the carboxyl terminus. The carboxyl terminus of hGRβ contain a unique 15 amino acid sequences with replacement of the last 50 amino acids of hGRα (Hollenberg et al 1985). This alteration renders hGRβ unable to bind glucocorticoid hormone. The hGRβ mRNA and protein were shown to be physiologically relevant (Bamberger et al 1995; Castro et al 1996). Indeed, in human multiple cell types, hGRβ was found to inhibit the hGRα-mediated activation of the MMTV promoter (Oakley et al 1999). In the absence of glucocorticoids, hGRβ binds to the GRE containing DNA with a greater affinity than hGRα. Also glucocorticoid treatment was found to increase hGRα but not hGRβ binding to DNA. It was also demonstrated that hGRα and hGRβ can physically associate with
each other in a heterodimer. It is now clear that the dominant negative activity of hGRβ resides within its unique 15 amino acid carboxyl-terminal sequence.

Another novel variant of human GR has been recently reported through analysis of cDNA from different tissues. This variant, termed hGR gamma (hGRγ), which is generated as a result of alternative splicing where three bases are retained from the intron separating exons 3 & 4 (Rivers et al 1999). These three bases code for an additional amino acid (arginine) in the DBD of the receptor. This novel isoform of hGR have been found to be expressed at a relatively high level (4-9% of total GR) in different tissues. Otto et al (1997) reported the absence of hGRβ in mice using reverse transcriptase PCR. However, the role of hGRβ and hGRγ in the mediation of glucocorticoid actions remains uncertain.

**Modular structure of GR**
Molecular cloning and structure/function analyses have revealed that the members of the steroid/nuclear receptor superfamily have a characteristic common functional domain structure. GR was the first member of the superfamily whose characteristic three-dimensional model was described (Bamberger et al 1996). This includes a variable N-terminal domain (modulatory domain), often important for transactivation of transcription; a central well conserved DNA binding domain (DBD), crucial for recognition of specific DNA sequences and protein-protein interactions; and at the C-terminal end, a ligand/hormone binding domain (LBD/HBD), important for hormone binding, protein-protein interactions and additional transactivation activity.

![A schematic domain structure of glucocorticoid receptor](image)

**The ligand-binding domain**
Glucocorticoids, being lipophilic hormones, cross the plasma membrane of target cells and interact with the high affinity intracellular cytoplasmic GR. The domain which bind the hormone is referred to as ligand binding domain (LBD) or hormone binding domain (HBD), which extends for approximately 250 amino acids located at the C-terminal end of the receptor. Extensive studies using partial proteolysis have revealed that hormone binding induces a
unique conformational change at the C-terminus of the receptor (Allan et al 1992; Vegeto et al 1992). HBD is very complex and participates in diverse functions (Simons 1989). It possesses the crucial property of hormone recognition and binding, and ensures both specificity and selectivity of physiologic response. The HBD is thought of as a "molecular switch" that, upon hormone binding, shifts the receptor to a transcriptionally active state (Mangelsdorf et al 1995). The conformational competence of HBD is maintained by its association with multiple chaperone protein system, most notably two Hsp90, one each of Hsp70, and Hsp56 (immunophilin) and p23 (an acidic protein) (Biola and Pallardy 2000). It has been reported that at least three other proteins are required for formation of active Hsp90-steroid receptor complex (Prima et al 2000). In addition, Hsp90 maintains the receptor in an inactive form in the absence of ligand (Kang et al 1999). The HBD is rich in cysteine and methionine and plays important role in hormone binding to the pocket. Several of these amino acids have been characterized in human (Cys638 and 665) and rat (Cys640, 656,661,674 and met622) (Yu et al 1995).

The DNA-binding domain
GR is in many ways indistinguishable from other eukaryotic transcription factors that regulate gene expression. In most cases, GR selectively binds to DNA, primarily as dimers. Moreover, they posses identifiable activation functions (AFs) that confer transactivation potential to the DNA binding domain (DBD). The structure of DBD was first revealed by NMR studies and later by X-ray crystallography (Hard et al 1990; Luisi et al 1991; Schwabe et al 1993). The ~70 amino acid DBD can be expressed in bacteria as a functional recombinant protein fragment. The isolated domain contains two Zn\(^{2+}\) atoms tetrahedrally coordinated by conserved cysteine residues (zinc fingers), exhibits sequence specific binding to GREs and contains amino acids necessary for dimerization (La Marco and Vivanco 1996). The structure of the DBD obtained by NMR spectroscopy indicates that this domain exists as a monomer in solution (Hard et al 1990). The DBD is a globular structure and can be subdivided into two modules: the first module contains both the zinc finger motifs for DNA binding, the second module is involved in phosphate contacts and receptor dimerization. Both these modules contain loop structure referred as P-loop and D-loop, respectively. The ability to dimerize depends upon the D-loop, a stretch of five amino acids. Several contacts made by the D-loop residues at the dimerization interface stabilize receptor dimers and thereby allowing cooperative DNA binding (Dahlman-Wright et al 1991; Luisi et al 1991). Mutations within the D-loop have a lethal effect on cooperative binding to GREs, but do not completely abolish DNA binding (Dahlman-Wright et al 1991).

The N-terminal domain
The N-terminal end of GR is highly variable in sequence and length (~439 amino acids) and contains the transcription activation function (AF1 or tau1) motif. This motif along with other motif, AF2 in the C-terminal end, presumably modulate target genes by interacting with coactivators or other transactivators (Hollenberg and Evans 1988). AF1/tau1 has been shown to be critical for target gene specificity (Dahlman-Wright et al 1995). AF2, present at the distal C-terminal end of the HBD has been shown to be important for hormone binding and hormone-dependent transactivation (Lanz and Rusconi 1994). The role of AF2 in transcriptional activation by GR has been extensively studied and reveals that it undergoes a conformational change upon hormone binding. This enables the receptor to bind to a series of coactivator proteins, such as steroid receptor coactivator-1 (SRC-1) and CREB-binding protein (CBP) (Glass et al 1997).

**Glucocorticoid action mechanism**

At the cellular level, most known effects of glucocorticoids are mediated by a ~94 kDa intracellular phosphoprotein, the GR. In the hormone-bound state, the GRs specifically bind to and modulate the activity of target gene promoters and are, therefore known as ligand-regulated transcriptional regulatory protein. Glucocorticoids act on the target cells with a mechanism that may be summarized in the following steps:

i) Free glucocorticoids enter the target cell by passive diffusion through the plasma membrane and bind non-covalently to the high affinity cytoplasmic GR to form hormone-receptor complex.

ii) The hormone-receptor complex undergoes activation/transformation, a process that involves conformational changes leading to dissociation of chaperone proteins and subsequent nuclear translocation of the activated hormone-receptor complex.

iii) Inside the nucleus, the activated hormone-receptor complex interacts with the specific DNA sequences in the chromatin called glucocorticoid response elements (GREs) of the target gene.

iv) The modulation of gene expression by protein-protein interactions and ultimately, generating cellular response(s).

**Binding of hormone to GR**

The adrenal cortex synthesizes and releases glucocorticoids into the blood circulation under the precise control of HPA-axis (Miller and Tyrrel 1995). In plasma, these hormones are bound to corticosteroid binding globulin (CBG), also known as transcortin. The bound form functions as a circulating reservoir of hormones that keeps a supply of free hormones available to tissues. Glucocorticoids, being liposoluble, cross the plasma membrane of target cells by
passive diffusion and bind to its high affinity cognate receptor in the cytoplasm. The unbound GR exists as an inactive hetero-oligomeric complex in the cytoplasm due to its association with receptor associated proteins (RAPs) or chaperones (two Hsp90 & one Hsp70), co-chaperones (Hop & Hsp40) and several other polypeptides such as immunophilin (Cyp40 & FKBP59) and p23 to achieve a high affinity hormone-binding state (Prima et al 2000; Biola and Pallardy 2000). This complex dissociates in response to hormone binding into a holo-GR that translocates to the nucleus, and regulates the activity of glucocorticoid responsive genes (Sarlis et al 1999).

**Activation of hormone-bound GR complex**

It is obligatory for the hormone action that GR must be able to assume at least two states- one that is active and one that is inactive- with the binding of the hormone promoting the activation/transformation from the inactive to the active form. Transformation of steroid receptors has been described to occur under physiological conditions (Munck and Foley 1979). As mentioned earlier, it is the inactive form of GR that binds to the hormone, which subsequently causes the associated proteins to dissociate from the steroid receptor complex under physiological intracellular condition, a process termed as activation or transformation (Tsai and O'Malley 1994; Pratt and Toft 1997). The existence of GR as inactive complex in the cytoplasm in association with Hsp's is to facilitate the folding of the HBD into a high-affinity steroid binding conformation. The activation of GR is a crucial step in GC action, as it is a rate-limiting step for nuclear or chromatin binding. There is an absolute requirement of hormone binding to GR to allow the activation to occur and the same was subsequently demonstrated by genomic footprinting that hormone was required for receptor binding to specific response elements in intact cells (Bamberger et al 1996; Pratt and Toft 1997).

In vitro, activation can be made to occur by several artifactual transforming conditions. Thermal activation at 25°C of cytosol containing hormone-bound GR causes the dissociation of bound chaperones and subsequent activation (Milgrom et al 1979). Salt such as ammonium sulfate, dialysis, gel filtration and elevated pH promoted GR transformation in vitro. The transformed 4S receptor exhibits increased binding affinity for isolated nuclei, chromatin, DNA-cellulose, phosphocellulose and ATP-Sepharose. Salt at 0°C also causes dissociation of Hsp 90 from the receptor and is accompanied by concomitant and proportional generation of the DNA-binding state (Meshinchi et al 1990). Transformation of hepatic GR from rat by ammonium sulfate at 0°C also increased the nuclear binding ability (Dahmer et al 1981; Sanchez et al 1987), by dissociating Hsp90 from the GR and subsequent precipitation (at ~30% saturation). It was later demonstrated by Western blotting that, hormone-free GR may also be transformed to a state that binds DNA-cellulose (Sanchez et al 1987). Dilution, gel filtration and dialysis all transform cytosolic GR by reducing the concentration of, or elimination of, a small heat stable cytocolic factor that, like molybdate, stabilizes the 9S GR complex (Bailly et al 1977). Cake et
al (1976) showed that passage of cytosol through gel filtration column transformed the GR. Both dilution and gel filtration of cytosol facilitated GR activation by elimination of low molecular weight cytosolic inhibitor (Sato et al 1980). Later, Bodine and Litwack (1988), purified an active factor from rat liver as a novel ether aminophosphoglyceride which inhibited activation of (Litwack 1988).

**Models of GR activation/transformation**

Numerous diverse mechanisms for GR transformation have been proposed, including a conformational change in the receptor (Atger and Milgram 1976; Bailly et al 1980), dissociation of receptor oligomer (Holbrook et al 1983; Vedeckis 1983), dissociation of macromolecular or low molecular weight inhibitor (Sato et al 1980; Sekula et al 1981), and receptor dephosphorylation. Milgram et al (1973) suggested that GR transformation consisted of a simple change in the conformation of 4S receptor molecule induced by hormone, in his 'equilibrium model of transformation'. However, now we know that during the transformation process, the HBD undergoes a conformational change, and the process is reversed only by a complex protein-folding reaction involving Hsp90, Hsp70, and other proteins. Milgram's equilibrium model of transformation turned out to be under cloud. Another observation by Atger and Milgram (1976), where they have examined the energy changes that accompany the binding of hormone to the receptor and the subsequent heat transformation (at 25°C), and found that binding of hormone to the receptor requires a moderate thermodynamic activation energy. Moreover, the complex corresponds to a striking lower level of free energy. Therefore, a high energy of activation is required for receptor transformation, however, the transformed receptor is at a level of free energy similar to that of untransformed receptor. They concluded that mainly the binding of hormone drives this overall reaction to the receptor, which is accompanied by a large change in free energy. It is now clear that the Hsp90-bound HBD is in the high affinity steroid-binding conformation, and that an important energy barrier that must be overcome in transformation of the receptor is provided by the non-covalent bonds responsible for the protein-protein interaction between GR and Hsp90.

The actual process of GR transformation in vivo is still unclear and complex. There seems to be little doubt that the models of receptor transformation based on purely cytosolic observations will be simplistic. However, it is only through examining hormone-mediated dissociation of more purified receptor heterorocomplexes and through studying the reversal of this transformation with purified Hsp chaperone system, that eventually a correct molecular model describing how the steroid hormone causes receptor transformation could be developed.

*In vitro modulation of GR activation*
Nishigori and Toft (1980) first reported inhibition of PR transformation by molybdate. Subsequently, both heat and salt transformation of cytosolic GR were inhibited by molybdate (John and Moudgil 1979; Chong and Lipman 1981-82). Tungstate and vanadate were also active in causing inhibition of transformation (Murakami 1982). The effect of molybdate was reversible, and it was effective only when added before transformation; that is, addition of molybdate after transformation did not influence DNA binding of activated receptor. Studies by Raaka et al. (1985) on the effect of molybdate on receptor transformation in intact cells revealed reduced nuclear accumulation of GR after steroid treatment. Various activators of in vitro GR transformation have also been identified which include the nucleoside triphosphates such as ATP, GTP, CTP etc.

**Nuclear translocation**

Activated GR is ultimately destined to migrate to the nucleus, where they modulate target gene expression upon binding to chromatin. Nuclear translocation is now understood to be a complex process, which probably utilizes the nuclear localization signals (NLS) sequences in the receptor itself (Picard and Yamamoto 1987), and bidirectional shuttling of the receptor into and out of the nucleus occurs constantly (DeFranco et al. 1995). The NLS, NL1 overlaps with the C-terminal end of the receptor DBD (Tsai and O’Malley 1994) and additional NLS, called NL2 (a ligand-dependent NLS) has been identified in the HBD, whose sequence has not been delimited. Nuclear localization also appears to be dependent in large part on the nuclear matrix (van Steensel 1995), and other nuclear components (van Steensel 1995). In the ‘heterocomplex model of receptor translocation’, Pratt et al. (1992) proposed that the receptor migrates to the nucleus in association with Hsp90 and the immunophilin (Cyp40) acting as a protein transport unit or ‘transportosome’ (Pratt 1993). This model of receptor migration was further supported by the work of Kang et al. (1994), in which Hsp90 was targeted to the nucleus by fusion to the nucleoplasmin NLS. Recent work by Kang et al. (1999), revealed that GR, after in vivo activation, was still able to reassociate with Hsp90, suggesting that this interaction plays a role in intact cells, probably in translocation and receptor recycling. Also, it has been proposed that an intact cytoskeleton is required for nuclear translocation. Use of a fused chimera of green fluorescent protein (GFP) and GR, under physiological conditions to test the notion that Hsp90 is required for the activated GR translocation along the intact cytoskeletal tract has yielded positive results (Galigniana et al. 1998), indicating that the GFP-GR complex utilizes the Hsp90 activity. Geldanamycin, a Hsp90-binding benzoquinone ansamycin, inhibits the activated receptor complex migration to the nucleus from the cytoplasmic compartment, indicating that, a possible interaction of Hsp90 with the activated receptor is required for nuclear translocation (Czar et al. 1997). However, in cells without intact cytoskeletal system, the GFP-GR complex was reported to migrate through the cytoplasm by diffusion.
**Modulation of nuclear translocation**

Numerous endogenous factors have been identified, which indicate that they modulate the nuclear migration of activated GR. Pyridoxal 5-phosphate (PLP) an active form of vitamin B6, was one of the early identified agent, which has been implicated to be an inhibitor of GR nuclear translocation (Milgram and Atger 1975; Goldl et al 1977). Rats deficient in PLP, were found to have higher rate of migration of receptor from the cytoplasmic compartment to the nuclear, and under opposing condition of elevated PLP concentration, a decreased nuclear migration was observed (Maksymowych et al 1990). Possible mode of such inhibition may be the influence of PLP with the NLS domain of the receptor (Allgood et al 1990). Recently, an endogenous protein factor termed macromolecular-translocation inhibitor (MTI), has been identified in rat hepatocytes, with some role in modulating the nuclear translocation of GR. Three species of MTI (MTI-I, MTI-II & MTI-III) have been separated, out of which MTI-II from rat liver is a 11.5 kDa Zn²⁺-binding acidic protein (ZnBP, or parathymosin) (Okamoto and Isohashi 2000), and an inhibitor of activated GR translocation to the nucleus. Interleukin-1 alpha was also demonstrated to inhibit dexamethasone-induced GR migration in cell lines (Pariante et al 1999). Retinoic acid was shown to increase the nuclear translocation of activated rat hepatic GR (Chambon 1994; Audouin-Chevallier et al 1995). Nuclear translocation has been found to increase in cells treated with Hsp56 binding drug FK506 (Hutchison et al 1993; Ning and Sanchez 1993) and a similar observation was found with an antibiotic, cyclosporin A (Renoir et al 1995). Geldanamycin, has been found to impede the glucocorticoid-bound receptors to the nucleus in L-cells as measured by indirect immunofluorescence with anti-receptor antibody and by a shift of specifically bound [³H]triamcinolone acetonide from the cytosolic to the nuclear fraction. However, the exact mechanism of its action is not completely understood. In cell-free experiments, it has been shown that geldanamycin prevents the association of the p23 component of the heterocomplex assembly system with Hsp90 (Johnson and Toft 1995).

**DNA binding**

Once the activated GR complex reaches the nucleus, the final effect culminates in its binding to specific DNA sequences (GRE) in the chromatin, ultimately leading to modulation of responsive gene transcription. Under physiological conditions, the occupancy of the HBD is thought to promote the binding of receptor to DNA, as deletions within the HBD in the human estrogen receptor failed to induce transcription, indicating that the HBD is indispensable for DNA binding (Pratt and Toft 1997). It has been observed that hormone-free GR binds specifically to MMTV-LTR promoter and therefore it was suggested that the function of hormone in vivo could be to modulate nuclear partitioning of the receptor (Willmann and Beato 1986). The interaction of GR with the GRE of a target gene was retarded in the absence of hormone, indicating that the hormone requirement is absolute (Becker et al 1986).
Mutational analysis of GREs revealed them to be a 15 base pair (bp) palindromic sequence [5' GGTACANNNTGTTCT 3'] composed of 6 bp half sites, separated by a 3 bp spacer (Beato et al 1995). This structure of GRE suggests that GR binds to it as a dimer. However, dimerization occurs only after binding to palindromic GREs (Dahlman-Wright et al 1991).

Modulation of DNA binding

The interaction of GR with chromatin is modulated by a number of parameters, such as state of phosphorylation of the receptor and other factors, which vary in response to activation of other signal transduction pathways. Cytosolic GR, being a phosphoprotein, is said to be hyperphosphorylated upon hormone binding (Orti et al 1993). However, the mechanism by which hyperphosphorylated GR promotes its ability to bind to DNA is not clear. The cAMP/protein kinase A (PKA) pathway stimulation has been reported to influence GR binding to its target GRE (Rangarajan et al 1992; Reisfeld and Vardimon 1994). Footprinting studies in hepatoma cells revealed that GR binding to GRE of the tyrosine aminotransferase (TAT) gene promoter requires the activation of PKA (Espinas et al 1995), and the effect of PKA is not on the receptor itself, but, perhaps on the phosphorylation of factors interacting with the receptor (Moyer et al 1993).

Various endogenous factors have also been identified, which suggest their role as modulators of DNA binding of activated GR. PLP has been identified as an inhibitor of DNA binding in vivo, since, it leads to ~50% decrease in glucocorticoid-induced transactivation (Allgood et al 1993; Tully et al 1994). ATP-stimulated translocation promoter (ASTP), a 93 kDa histone-binding protein, isolated from rat hepatocytes, has been reported to raise the nuclear binding of activated GR in vitro (Martin et al 1993), which could, perhaps, stimulate the same in intact cells. Also, recent finding that MTI-II (a Zn$^{2+}$ binding acidic protein or parathymosin), isolated from rat liver could modulate DNA-binding in vivo, as, in vitro studies have shown that it inhibits activated GR binding to DNA containing GRE and nuclei (Okamoto and Isohashi 2000). Another report recently revealed that melatonin, inhibits the binding of activated ER to ERE in the DNA (Rato et al 1999), with the possibility of similar action on GR binding to DNA sequences (Asainz et al 1999).

Numerous chemical agents have been identified, which modulate the binding of activated GR to DNA as assessed by using DNA-cellulose, isolated nuclei, ATP-Sepharose etc. in vitro. Methylxanthines like theophylline, aminophylline and caffeine have been shown to greatly enhance the binding of activated GR complex from rat liver cytosol to DNA-cellulose and isolated nuclei (Cake and Litwack 1978), with similar effect of GR from kidney and thymus. Covalent binding of biotin to purified GR decreases the nuclear binding capacity by as much as 50% (Hapgood and Holt 1987). Various other agents also block the activated GR binding to DNA-cellulose and nuclei, like aurintricarboxylic acid, methyl methanethiosulfonate and O-phenanthroline (Moudgil et al 1984).
Glucocorticoid response elements

Cloning and analysis of the MMTV genome and the human metallothionein II_A gene, led to identification of first hormone response elements (HREs), in this case called the glucocorticoid response elements (GREs), present in the 5’-regulatory region of the gene, which serve as GR binding sites and inducible enhancer elements (Yamamoto 1985; Beato et al 1995). Transactivation by the GR requires binding of receptor dimers to specific palindromic sequences in the cis-regulatory region of target genes called GREs. The ability to dimerize (post-binding dimerization) depends on the D-loop, a stretch of five amino acid residues located in the DBD. It has been found that mutations within this region have a lethal effect on cooperative binding to palindromic GREs (Dahlman-Wright et al 1991). In the classical model, GR transactivates transcription by binding to the classical or positive GRE, as in TAT and PEPCK genes (Beato 1995), however, it was found that some genes are negatively regulated by the GR and do not contain a classical GRE (Reichardt et al 1998). Subsequently, it was proposed that the transrepression action of GR are mediated by utilizing different classes of response elements, namely, negative, composite, and tethering GREs (Diamond et al 1990; Miner et al 1991). The negative GREs involve direct DNA binding of the GR as exhibited in the POMC gene (Drouin et al 1993). The composite elements, where the GR contacts the DNA together with another transcription factor, as in case of proliferin gene (Diamond et al 1990), whereas in the tethering elements, repression is facilitated by protein-protein interaction without direct GR binding to the DNA, for example, in genes regulated by AP-1 (Schreiber et al 1995) and NFkB (Auphan et al 1995).

GRE Consensus sequence

<table>
<thead>
<tr>
<th>Gene</th>
<th>Consensus Sequence</th>
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<tbody>
<tr>
<td>MMTV</td>
<td>TGGTTT GGTATC AAA TGTCTCT GATCTG</td>
</tr>
<tr>
<td>hGH</td>
<td>CCTTTG GGCACA ATG TGTCTCT GAGGGG</td>
</tr>
<tr>
<td>TO</td>
<td>CTCATA TGCAAG CCG AGTTCT AGTGAG</td>
</tr>
<tr>
<td>TAT</td>
<td>CTCTGC TGTAAG GGA TGTCTCT AGCTAC</td>
</tr>
</tbody>
</table>

MMTV: mouse mammary tumor virus
hGH: human growth hormone
TO: tryptophan oxygenase
TAT: tyrosine aminotransferase

There have been reports on the existence of the so-called glucocorticoid modulatory elements (GMEs) upstream of the GREs (Simons et al 1992; Szapary et al 1992). The GME of the rat TAT gene is located at 3.6 kbp to 1 kbp upstream of GREs. The GME has the unique transcriptional properties of modulating GR action, and it has been found that the expression of
GME activity involves the binding of two unique proteins, GME-1 and GME-2 (Zeng et al 2000). It was previously reported that GME and GRE mediate transcriptional activation synergistically, however, recent findings have revealed that the mechanisms of expression of GME and GRE activation probably utilize parallel, rather than common pathways.

**Regulation of gene expression**

In the classical model of gene regulation, GR homodimers transactivate transcription by binding to GREs in the promoter region of glucocorticoid responsive genes (Beato 1989). The role of activated GR is to recruit and maintain a preinitiation complex at the promoter of target genes. Transactivation is probably mediated through interaction of DNA-bound GR homodimers with basal transcriptional machinery coactivators and other transcription factors (Beato et al 1995) directly as in the case of transcription factor IIb (TF IIb). Various independent lines of evidence have suggested that liganded nuclear receptors are capable of directly contacting basal transcription factors, many of which appear to be cell-specific in their expression patterns (Jacq et al 1994; May et al 1996). The characterization of a 170 kDa GR-associated protein (GRIP-170), which stimulated GR transactivation in vitro, suggested that these endogenous cofactors were functionally limiting (Eggert et al 1995). The interaction of GRE-bound GR homodimers may be indirect, as in the case of steroid receptor coactivator (SRC-I) and CREB binding protein (CBP) (McKenna et al 1999). This interaction is presumably sufficient to stabilize the preinitiation complex on the promoter and, thus, to stimulate transcription by RNA polymerase II (Tsai and O'Malley 1994).

In addition, binding of activated GR homodimer to the GRE can induce a rearrangement of the chromatin structure in the respective promoter region, then allowing access to other transcription factors to interact to the previously inaccessible DNA (Li and Rosen 1994; Truss et al 1995). Furthermore, eukaryotic genes are required to exist in configurations that maximize access to the promoter regions with which DNA binding transcription factors specifically interact. These genes are organized into structurally repressed nucleosomes, which allow strict access of transcriptional proteins to key regions of genes, thereby allowing precise regulation of such genes. Covalent modifications (like acetylation) of nucleosomal histones have been suggested to reduce the affinity for DNA and to be an important preface to transcriptional activation in vivo. The link between chromatin disruption and transcriptional activity is now well established. CBP, p300 etc contain histone acetyltransferase activity, which indicates that GRs might function in part by recruiting these proteins and directing nucleosomal modification at their target promoters (Bannister and Kouzarides 1996; Yang et al 1996).

Promoters of many glucocorticoid responsive genes contain negative GREs (nGREs) (Tsai and O'Malley 1994). nGREs are also specific DNA sequences that bind activated GRs and lead to inhibition of transcription of responsive genes. A classic case is the nGRE in the pro-opiomelanocortin (POMC) gene promoter (Drouin 1993). It has been reported that instead of
binding as GR homodimer to nGRE, three molecules of GR interact with nGRE leading to inhibition of POMC gene expression.

Interestingly, in the last decade, several groups found that GR also regulates transcription by protein-protein interaction without directly binding to DNA (Jonat et al 1990). This is true for the antiinflammatory/immunosuppressive effects of glucocorticoids, where activating protein-1 (AP-1) regulated genes like collagenase-3, whose transcriptional regulation by proinflammatory cytokines can be repressed by GR. However, the interaction between GR and AP-1 is direct or needs an intermediary factor is unknown and perhaps might vary from gene to gene (Kamei et al 1996). Activated GR has also been reported to be involved with the interference of functions of other transcription factors such as NF-κB, CREB etc (Caldenhoven et al 1995; Stocklin et al 1996). Also, the protein-protein interaction between GR and other transcription factors is not always a negative regulation, but can also synergize with other factors as in the case of jun homodimers or STAT-5 (Pearce and Yamamoto 1993; Stocklin et al 1996).

The emerging scenario indicates several possible interactions of GR with GRE and transcription factors, and also with a large number of intermediary factors. These molecular events are only partly understood. It now appears that transcriptional control by GRs is a multistep process, a fact reflected in the diversity of coregulators with which GR interacts in myriad manner.

Is the DNA binding essential for survival?
The presence of intact GR is essential for survival, as experiments involving generation of GR-deficient mice (GR\(^{-}\)) have demonstrated (Cole et al 1995). In humans, several GR mutations have been described, suggesting that GR may be indispensable for life (Reichardt et al 1998). The generation of two independent mutant mouse lines, one involving the insertion of neomycin resistance cassette into exon II (GR\(^{\text{hypo}}\)), and the other involving deletion of exon III, which encodes the first zinc finger of the DBD (GR\(^{\text{null}}\)). The GR\(^{\text{null}}\) leads to complete inactivation of GR. Interestingly, all the GR\(^{\text{null}}\) mice die shortly after birth, whereas 20% of GR\(^{\text{hypo/hypo}}\) survived until adulthood, suggesting the indispensability of DNA binding. As mentioned earlier, genes like TAT, PEPCK etc are positively regulated by the classical GREs, where the GR binding to DNA is an absolute necessity. To study the in vivo relevance of DNA binding, a point mutation was introduced into the mouse GR gene. This mutation was generated by substitution of alanine458 by a threonine (A458T) in the D-loop (Reichardt et al 1998). This substitution resulted in a dimerization-defective GR (GR\(^{\text{dm}}\)), that could no longer bind cooperatively to palindromic GREs (Heck et al 1994). It was subsequently shown by Reichardt et al (1998) that the GR\(^{\text{dm/dm}}\) mice could not respond to GC with regard to induction of TAT mRNA. Also, it was shown that, there was a large decrease in the inducibility of the MMTV-based promoter, however, the residual induction observed could be due to the fact that GR\(^{\text{dm}}\) can bind non-cooperatively to the multiple GREs in the MMTV promoter.
The GR\textsuperscript{dim/dim} mice, however, appeared relatively normal and healthy under standard laboratory conditions and strikingly, survived. The most notable conclusion was that the GRE-mediated gene activation is not essential for development and survival. Unlike the GR\textsuperscript{dim/dim} mice, the GR\textsuperscript{V} mice die shortly after birth due to respiratory failure because of immature lungs. However, the GR\textsuperscript{dim/dim} mice showed normal differentiation of lungs (Reichardt et al 1998), indicating that the GRE-mediated gene induction is not the basis for GC-induced lung maturation. This indicates that the DNA-binding of GR is not essential for survival. Moreover, the GR\textsuperscript{dim/dim} mice did not express gluconeogenic enzymes, indicating that these enzymes are not necessary for survival under standard laboratory conditions. However, induction of gluconeogenic enzymes may be required for viability under stressful conditions. Furthermore, the AP-1-GR complex-mediated transrepression was intact in the GR\textsuperscript{dim/dim} mice, indicating that the AP-1 inducible genes are regulated by protein-protein interactions.

Glucocorticoid sensitivity determinants

GRs are essential for glucocorticoid-induced changes to occur, but hormonal sensitivity is not guaranteed simply by the presence of the receptors. There is, in general, a good correlation between the level of GRs in a cell and cellular sensitivity to glucocorticoids. However, other factors may modulate glucocorticoid sensitivity, including the presence of nonfunctional or modified receptors and other cellular factors that modify receptor function. Glucocorticoid sensitivity basically refers to the extent to which an already glucocorticoid responsive system responds to glucocorticoids. In general, sensitivity of tissues to steroid hormones may be important in both physiologic and pathologic conditions. In the case of glucocorticoids, changes in tissue's sensitivity may participate in the maintenance of resting and stress-related homeostasis (Chrousos 1995). The level of intracellular hormone available is an important parameter, since, they, in appropriate concentration must bind to the specific receptor to be able to transduce signal in the target cells. Kidney cells are primarily mineralocorticoid responsive, but glucocorticoids are also able to enter kidney cells to exert their effects. However, the intracellular glucocorticoids in renal cells are metabolized to an inactive derivative, rendering the kidney specifically mineralocorticoid responsive even though they contain functional GRs. This inactivation of glucocorticoids is achieved by 11\beta-hydroxysteroid dehydrogenases (11\beta-HSD) (Funder et al 1988; Albiston et al 1994), which exists in at least two isoforms, 11\beta-HSD 1 & 11\beta-HSD 2. 11\beta-HSD 1 is also reported to be expressed in tissues other than the kidney, where they may modulate glucocorticoid sensitivity.

The magnitude of GR-mediated cellular response is also dependent on the intracellular level of GR (Vanderbilt et al 1987). The expression level of GR varies in a tissue- and age-specific manner. GRs were to be expressed in a number of tissues including brain, liver, kidney, skeletal muscle etc., with the thymus, expressing in highest number. Tissue-specific level of GR may be dependent on the presence of various factor(s) which modulate the expression of
GR mRNA and/or the stability of the receptor protein itself. Glucocorticoids themselves cause down-regulation of the receptor, as shown in cells from intact animals (Burnstein et al 1991; Burnstein and Cidlowski 1992). At least, there are three mechanisms that take part in glucocorticoid-mediated down-regulation of GR. At the transcriptional level, glucocorticoid seems to repress the expression of GR gene by interference with AP-1- and/or AP-2-mediated pathway of GR gene expression (Vig et al 1994; Nobukuni et al 1995). It was also reported that the binding of activated GR to sequences within the structural gene, rather than within the consensus GREs, which are absent in the GR gene, may modulate its expression (Encio and Detera-Wadleigh 1991; Nobukuni et al 1995). Also, the presence of glucocorticoids may reduce the half-life of GR (Mcintyre and Samuels 1985). Other than glucocorticoids, estrogen was shown to repress the expression of GR in the anterior pituitary (Peiffer and Bardin 1987). Age-dependent alteration in GR level is also an important regulatory mechanism for glucocorticoid sensitivity. In most animals, especially in rodents, GR level was found to increase with increasing age, reaching a maximum in adults and then a gradual decline is observed. This variation in GR level may be due to the change in the endogenous modulators of GR itself (Kalimi et al 1988).

Untransformed GR in the cytoplasm is a phosphoprotein, which has been reported to become hyperphosphorylated upon hormone binding and dissociation of chaperone proteins (Bamberger et al 1996). However, the role of GR phosphorylation in determining its biological function is not clearly established, and there seems to be a consensus that GR phosphorylation status codetermines its subcellular location rather than its gene regulatory activity.

How GRs search for a target site in the genome?
GRs functions as transcription factors by binding to specific DNA sequences (GREs), generally upstream of transcription initiation sites. In order to bind these DNA elements, the receptor must first locate these sites in the genome. In vertebrates, this search entails locating a small fraction of functional binding sites from billions of base pairs of DNA, requiring this receptor to sample an immensely vast number of possible binding sites in a very short period of time. Nevertheless, GRs are able to bind their specific binding sites very rapidly. However, there is no unanimous agreement for the exact mechanism employed to locate specific binding sites in the chromatin. Given below a brief discussion on some of the models proposed to explain this search mechanism.

One model, the cycling model (Berg and Hippe! 1985), proposes that GRs bind target elements by repeated cycles of association and dissociation, until, a high affinity site is found. This mechanism is commonly assumed to be the search tactics employed by site-specific DNA-binding proteins. This model predicts that the search will be controlled solely by the intrinsic rate of receptor dissociation from nonspecific DNA sites. However, the explanation made by this model was not very satisfactory. A second model, the sliding model, involves the receptor
sliding along the DNA chain and conducting a one-dimensional search until it encounters a specific binding site. This model has an advantage that, it is highly efficient for locating a binding site over a limited distance along the DNA molecule. However, the sliding model indicates that this mechanism may not be an efficient strategy for a long distance search. The third model, the intersegment transfer mechanism proposes that GRs search for target site in the genome by intersegment transfer (Lieberman and Nordeen 1997). In this model, receptor dimers bind nonspecific DNA sequences and search for a target site by binding to a second strand of DNA before dissociating from the first. This has an advantage that high concentration of DNA favors, rather than hinders, the search, by increasing the apparent dissociation rate of the receptor, unlike the cycling and sliding model, in which rate of receptor dissociation is intrinsic and not affected by exogenous DNA. Using the purified DBD fragment of rat GR, it was found that receptor dissociation from DNA was highly dependent on the concentration of DNA in solution, supporting the intersegment transfer model (Lieberman and Nordeen 1997). However, this model is yet to be studied with full-length GR, nonetheless, it is an attractive idea for the search of GR to its specific binding site.

**Nucleocytoplasmic trafficking of steroid-free GR**

The binding of hormone to GR is a transient process, and the loss of hormone from the receptor leads to recycling of the receptor into the Hsp-containing oligomeric complex (Scherrer et al 1990). When liganded GR are shuttling proteins that traffic continuously between nucleus and cytoplasm (Madan and DeFranco 1993). Steroid receptors are differentially localized in the cell in absence of cognate ligand. Unliganded ER and PR are nuclear, whereas GR, MR, and AR are cytoplasmic. The molecular basis of these differences in localization is not well understood. The predominant localization of naive GR in cytoplasm is probably due to the masking of one of its NLS, NL1 by Hsp90, which somehow prevents its translocation to the nucleus (Pratt 1993; Czar et al 1995). This explanation is further supported by a study demonstrating that Hsp association of GR prevents the binding of an NL1-specific antibody (Hache et al 1999). However, other studies do not favor this simple model, for example, over expression of GR in many cell lines results in the nuclear translocation of the Hsp-associated receptor without apparent change in other properties (Sanchez et al 1990). Studies involving nucleocytoplasmic trafficking of hormone-free GR prior to hormone treatment and following hormone withdrawal have yielded encouraging results (Hache et al 1999), indicating that Hsp-associated GR complexes are not sufficient to prevent the trafficking across the nuclear membrane. Hache et al (1999) observed that following the withdrawal of its endogenous ligand, cortisol or the hormone antagonist RU486, GRs were able to rapidly recycle into the Hsp-associated, hormone-responsive complexes. However, the redistribution of GR to the cytoplasm, upon cortisol withdrawal was very slow, with absolutely nil in the case of RU486. The reason attributed was not due to the defect in export machinery, since in both the
instances, the complexed nuclear GRs migrated normally between heterologous nuclei (heterokaryon) in cell fusion experiments. Furthermore, the fusion of a heterologous protein (nuclear retention signal) to the N-terminus of GR stimulated the transfer of latent receptor to the nucleus in the absence of ligand. These studies strongly suggest that the localization of GR to the cytoplasmic compartment is attributed by precise regulation of the rates of transfer of GR across the nuclear membrane and/or by active retention that occurs independently from the association of GR with Hsp's.

Cross-talk with other signaling pathways
Biological regulation is generally exerted through combinatorial events. Interaction of regulatory pathways with individual transcriptional regulatory protein culminates in cell specific gene expression. The regulation of GR function is impinged upon by numerous other signaling pathways making it a complex, multifaceted event. By modulating GR signal pathway, cross-talk mediators may participate in defining the sensitivity of a cell to glucocorticoids either in a tissue-specific or generalized fashion. There is no consensus in how hormone binding influences the various steps in GR action and on whether hormone binding is the only way of activating the receptor. It seems that nuclear receptors can bind to target DNA sequences even in the absence of ligand or when complexed with antagonistic ligand. Recently it was demonstrated that GR is activated in the absence of hormone through signals originating from cell-surface receptors (Tsai and O'Malley 1994). These membrane signaling can modulate the activity of the GR complex by mechanism possibly involving phosphorylation/dephosphorylation. The biochemical modulation of GR is suggested to be achieved by phosphorylation (activation) and dephosphorylation (inactivation) at seven different phosphorylation sites (Ort et al 1992; Hu et al 1994; Webster et al 1997). Glucocorticoids being anti-inflammatory agents is frequently administered on human subjects suffering from asthma. In asthma, treatment regimen containing glucocorticoids and β2-agonists results in better symptom control. These clinical observations suggest an interaction of both classes of drugs at a molecular level. GR being a cytoplasmic receptor, the β2-adrenergic receptor (β2-AR) is a cell-surface G-protein-coupled receptor (GPCR), and transducing signal through adenylate cyclase that elevates the concentration of cAMP. Recently, ligand-independent activation of GR by the β2-AR agonists, such as salbutamol and salmeterol in primary lung fibroblasts and vascular smooth muscle cells has been reported (Eickelberg et al 1999). Treatment of cells with the above drugs resulted in increased translocation of hormone-free GR into the nucleus, and binding to GRE, as revealed by histochemical and Western blotting experiments. The effects of salbutamol and salmeterol were mediated upon binding to the β2-AR, because blocking β2-AR with propranol abrogated GR activation. However, the molecular mechanism of this activation is not demonstrated, but it is assumed that β2-AR activation leads to increase in
the level of cAMP, protein kinase A (PKA) and calmodulin (CaM) (Della-Rocca et al 1997; Eickelberg et al 1999). The pathway involving CaM, a cytosolic calcium binding protein, has gained considerable importance, as CaM directly activated GR with a mechanism suggested to be the phosphorylation of specific tyrosine residues in GR (Ning and Sanchez 1995). These demonstration opens out the possibilities of cross-talk between signal transduction pathways involving PKAs, CaM and steroid hormone action.

The idea of cross-talk between the intracellular steroid action cascade and the cell-surface protein/peptide hormone action cascade arose and visualized the inter-relation among the protein/peptide and steroid hormone actions (Sharma 1993, 1999). The protein/peptide hormone modifiers can modulate steroid hormone action. It has been reported earlier that the protein kinase C activators and inhibitors modulate the glucocorticoid-dependent regulation of TAT and TO in cultured rat hepatocytes (Sharma et al 1990; Sharma 1991). Several others have also observed that the protein kinases are central to these cross-talks, as most of the steroid receptors are phosphoproteins and their phosphorylation might control the activation and affinity of these receptors to DNA response elements. Selected steroid receptors can be activated in a ligand-independent manner by a membrane agonist. Dopamine has been reported to mimic the action of progesterone in activating the progesterone receptors while 8-bromo-cAMP has been demonstrated to mediate progesterone receptor-dependent transcription in the absence of progesterone (Denner et al 1990; Power et al 1991).

More recently, in a significant deviation, the receptors for steroid hormones were also found to be located on the membrane surfaces of certain cell types such as spermatozoa, oocytes, endometrial cells and granulosa cells (Revelli et al 1998; Sharma 1999). The non-genomic effects of 17β-estradiol, progesterone, testosterone and androstenedione on these reproductive cell types are well-documented (Revelli et al 1998; Sharma 1999). Grazzini et al (1998) have shown that progesterone inhibits oxytocin signaling by binding to the membrane-bound oxytocin receptor and changing the conformation such that oxytocin does not interact efficiently to its own receptor.

A number of cell-surface receptors are also known to activate transcription factors, but act through an enzymatic mechanism. A classic case is the mode of action of cytokines, which upon binding to the transmembrane cytokine receptors activate its inbuilt tyrosine kinase activity, which in turn phosphorylate a latent cytosolic transcription factor known as Stat (signal transducers and activators of transcription) (Stocklin et al 1999). Phosphorylated Stat monomers dimerize and translocate to the nucleus and assume the ability to bind to specific DNA sequences in target gene promoters. Stat5, a unique Stat molecule is activated by several essential cytokines, and has been demonstrated to functionally interact with the GR, giving rise to cooperation between GRs and Stat mediated pathways (Cella et al 1998). Stat5 acts both as a coactivator and corepressor of GR-mediated pathway of target gene expression modulation. The β-casein gene promoter expression requires GR, which acts as transcriptional activator for
Stat5 and enhances Stat5 dependent transcription of this gene promoter, but independent of a GRE (Cella et al 1998). Conversely, Stat5 molecule act as a corepressor of GR, since its binding to GR diverts the protein-protein complex from binding to GRE and therefore interferes with gene transcription, as in the case of MMTV-LTR transcription (Stocklin et al 1996; Stocklin et al 1997). This interaction between glucocorticoid- and cytokine-mediated transcriptional pathways may suggest tissue- and cell-specific activity of these extracellular signals.

Glucocorticoids, being immunosuppressive agent, are known to trigger apoptosis in T cells through GRs (Helmberg et al 1995). However, recently it was demonstrated that this apoptotic activity of glucocorticoids is blocked by the activation of T cell antigen receptor (TCR) (Jamieson and Yamamoto 2000), that suggests cross-talk between these two distinct signal transduction pathways. It was shown that the TCR activation of mitogen-activated protein kinase kinase cascade (MAPKK) via Ras protein is involved in inhibition of GR-mediated apoptosis in T cell lines. Also, the activation of various components (TCR, Ras and MAPKK1) changes the GR-mediated transcription. These findings reveal the importance of the convergence of the signal transduction pathways.

The above information's give an insight into the role of GR in vivo and help decipher the molecular mechanisms underlying its action. There is a clear role for agents that modulate GR function. The emerging picture shows different modulators as important agents that regulate GR-mediated signaling pathways. This thesis displays a biochemical attempt to study the modulation of GRs in mice using different endogenous/exogenous agents and also by diabetic and senescent state.

The entire work was performed with the following objectives:

i) To study the effects of dl-dithiothreitol (DTT), 2-mercaptoethanol (ME) and reduced glutathione (GSH) on hormone binding to glucocorticoid receptor (GR) and on the stabilization of hormone-receptor complexes as a function of time.

ii) Study the activation process of GR by heat and salt, and its modulation by cadmium, selenite, arsenite, leupeptin, polyunsaturated fatty acids (PUFAs) and pyrophosphate (PPi).

iii) Modulate the activated GR complexes binding to acceptor sites by pyridoxal 5-phosphate (PLP), aurintricarboxylic acid (ATA) and methyl methanethiosulfonate (MMTS).

iv) Induce diabetes by streptozotocin (STZ) and study the modulatory role of diabetes, on GR level, affinity and activation (by heat and salt) in diabetic and control animals.
v) To study the modulatory effect of aging on GR level, affinity, activation (by heat), activation modulation by PUFAs, and DNase I digestion in young and old animals.