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Minireview

Glucocorticoid Receptor: Retrospective and Perspective

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Glucocorticoids are secreted from the adrenal glomerular cortices under the control of the brain via limbic system, hypothalamus, and pituitary. They have a multitude of effects on a variety of cellular processes within the body. In liver, they stimulate enzymes and increase protein and glycogen contents. In many tissues, including muscle, their catabolic actions inhibit synthesis and stimulate degradation of protein and RNA[1]. The molecular mechanisms of action of glucocorticoids have been actively investigated. It is only in the 1960s, that the concept of receptor for glucocorticoids evolved. Upon entry into the target cells, glucocorticoids interact with intracellular receptors to form complexes which subsequently undergo activation and translocation to the nucleus where they interact with specific acceptor sites on chromatin and thus modulate gene expression[2]. The nuclear acceptor sites are regions of DNA sequences (termed as glucocorticoid response elements) in regulated genes. However, *in vivo*, non-DNA compounds may play an important role in the nuclear accumulation of steroid-receptor complexes. This nuclear binding capacity can be achieved *in vitro* at an elevated temperature[3,4] and under high ionic conditions[5,6], possibly by exposing positively charged amino acid residues on the surface of the receptor molecule[3,8], which in turn enhances the affinity for nuclei and polyanions such as DNA and DNA-cellulose[3,9]. This phenomenon occurs *in vivo* under physiological conditions and is believed to be rate limiting for nuclear binding[10-12].

Activation of Glucocorticoid Receptor

Although the exact mechanism of activation of receptors has not been ascertained, it is found to be controlled by phosphorylation/dephosphorylation[13,14], subunit dissociation[15,16], endogenous heat-stable cytoplasmic factor(s)[17] and presence of small RNA molecules[18]. This transformation event is accompanied by a change in the size of the receptor from an oligomer of 320 kDa to a monomer of 94-100 kDa[15,19,20]. A number of proposals have been made regarding the oligomeric nature of the untransformed glucocorticoid receptor. Based on hydrodynamic properties, some consider the receptor oligomer as a tetramer of identical steroid binding subunits[21]. However, untransformed glucocorticoid receptor from mouse L-cell cytosol has been reported as a heteromer that contains a 100 kDa steroid-binding protein and a 90 kDa steroid non-binding protein[22]. The steroid non-binding subunit of the L-cell receptor complex shows homology with the murine 90 kDa heat-shock protein[23]. The exact function of heat-shock protein is not known, although it is thought to be involved in maintaining the steroid receptor in an untransformed state. Recently, a dimer of 90 kDa steroid non-binding protein has been purified from the liver unactivated glucocorticoid receptor[24].

Activation of glucocorticoid receptor complexes is one of the initial events that occur after the binding of the hormone to the specific cytosolic receptor in the target cells. Several factors have been implicated in the process of activation/transformation (conversion of the receptor complex from the non-DNA binding form to a form that binds to the nucleus or DNA). The availability of the highly purified unactivated receptor complex has made possible a number of striking observations on the activation process. The purified liver unactivated receptor, when activated, binds to DNA/or nuclei to the extent of less than fifty percent of the crude receptor. However, when cytosol is replenished to the activated purified receptor complex, the extent of the receptor binding to DNA is greatly increased[25,26]. This indicates the presence of certain factor(s) in the crude cytosol which may be responsible for enhanced activation of receptors. A number of modulators that affect the receptor transformation have been searched. The ability of molybdate to affect glucocorticoid receptor function was first discovered in an experiment where this element was used as a phosphatase inhibitor[27]. Addition of molybdate stabi-

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lizes the steroid binding capacity of glucocorticoid receptor against temperature-dependent inactivation. It also inhibits the activation of the steroid-bound receptor to the DNA-binding state[28-30]. Several other transition metals, such as vanadate (VO_3) and tungstate (WO_4), also block the activation of receptor[31]. The molecular mechanism(s) by which molybdate prevents receptor activation is not yet clear. However, the accumulated evidence suggests the direct interaction of molybdate with the steroid receptor complexes to preserve the untransformed, steroid binding state. In doing so, it prevents the transformation-associated changes in physicochemical properties of steroid receptor complexes such as molecular size, isoelectric point and subunit dissociation. The unactivated glucocorticoid receptor has a characteristic sedimentation coefficient of 8-10S on sucrose gradient. This sedimentation coefficient falls to 4-5S upon activation of hormone-receptor complexes. Molybdate has been shown to stabilize 8-10S receptor forms[21,31]. An endogenous low molecular weight inhibitor, called modulator, has been implicated in the activation process. The modulator maintains the integrity of the unactivated heterologous receptor and stabilizes the steroid-receptor complex[17]. Recently, a modulator has been purified to near homogeneity from rat liver cytosol and its structure has been characterized[32]. It has a structure of an ether-linked glycerophosphoserine derivative. Its action is mimicked by molybdate when added exogenously to cell cytosol to stabilize and prevent the activation of the receptor complex[32].

One of the initial steps of activation *in vitro* may be controlled by the release of the modulator causing a decrease in the affinity of the subunits of the heterologous receptor complex. This very step can be blocked by the addition of modulator or of molybdate[32]. The products of activation of glucocorticoid receptor have not been fully determined. During the process of activation the receptor oligomer (8-10S) is dissociated into an intermediate form (5S), probably bound to a low molecular weight RNA, which then is converted to a free steroid-bound subunit where its DNA binding domain is no longer occupied by other macromolecules[18,33]. Once the activated form of receptor is released, it translocates to the nucleus and binds to acceptor sites on the nuclear chromatin. The mechanism of receptor translocation is still unknown. Vitamin B − 6, pyridoxal phosphate, has been reported to be an inhibitor of nuclear translocation[34]. *In vivo* addition of pyridoxal phosphate prevents the nuclear uptake of the glucocorticoid receptor and blocks the induction of tyrosine aminotransferase in the rat liver[34]. Pyridoxal phosphate inhibits the nuclear uptake by interacting with an epsilon amino group of an essential lysine residue on the DNA binding domain of glucocorticoid receptor. The rat liver glucocorticoid receptor has a monomeric molecular weight of ~ 94 kDa, which exceeds the apparent exclusion limit of ~ 70 kDa for passive diffusion through nuclear pores[35]. Nuclear accumulation of proteins appears to be mediated by nuclear localization signals[36]. The protein segments are thought either to facilitate diffusion and intranuclear retention, or to interact with putative active transport machinery[37]. Although the equilibrium distribution of unoccupied receptor for steroid hormones is controversial[38-40], the glucocorticoid receptors are predominantly cytosolic under physiological conditions[41,42]. The nuclear translocation factors have been ascertained to lie within the polypeptide of rat glucocorticoid receptor[42]. Using a transient expression of cloned receptor cDNA, the subcellular distribution of various receptor constructs were monitored by immunofluorescence. Two distinct nuclear localization signals, NL1 and NL2, were defined. This nuclear translocation of glucocorticoid receptor is a hormone-dependent phenomenon[42].

Glucocorticoid Receptor at the Molecular Level

Domain structure—Due to advances in molecular techniques, it has been possible to characterize the structural and functional domains of the glucocorticoid receptor. The receptor polypeptide is made of 795 amino acid residues[43]. Biochemical, immunological, and genetic analyses of glucocorticoid receptor indicate three distinct functional domains, viz. the hormone binding, the DNA binding, and an immunologic domain[43]. At the protein level, these domains can be defined by limited proteolysis of purified rat liver glucocorticoid receptor[43]. The steroid-binding domain is isolated by proteolysis of glucocorticoid receptor with trypsin. It has a molecular weight of 25-27 kDa on SDA-polyacrylamide gel electrophoresis (SDS-PAGE). Digestion of glucocorticoid receptor (GR) with α-chymotrypsin, on the other hand, results in a proteolytic fragment of 39-40 kDa that contains both the steroid and the DNA binding sites. Further digestion of this fragment with trypsin results in a fragment that contains only the steroid binding domain. Recent analysis of cDNA clones for the human[44], rat[45] and mouse[46] GR have confirmed these three functional domains.

Steroid-binding domain—The steroid-binding domain corresponds to the carboxyl (-COOH) terminal part of the receptor polypeptide starting from ~ 518 to 795 amino acid residues. Using *in vitro* translation of receptor coding sequences, it has been demonstrated that the ligand affinity falls 30-fold upon deletion of 5 amino acids (791-795) and ~ 10^4-fold with a 29 amino acids deletion (767-795) from the C-terminus[47]. However, there are little effects on steroid binding of N-terminal truncation which imply that these sequences may indirectly contribute to conformational stability at the hormone binding site[47]. More surprisingly, the truncated polypeptides having just the hormone binding domains are sufficient for steroid binding[47]. The steroid binding domain also acts as a *cis* acting inhibitor of DNA binding domain that is reversed upon steroid binding[47]. Affinity labeling of glucocorticoid receptor with dexamethasone 21-mesylate shows that it reacts with cys[656] of the rat GR and cys[644] of the mouse GR[48]. It has also been demonstrated that glu[546] and Tyr[770] of the murine GR participate in forming the hormone binding site. One of the mutant receptors of the $^{r-}$type has glycine at position 546 and another has asparagine at 770 and show a defective hormone binding[47].

DNA binding domain—The DNA binding domain was similarly localized between 440-546 amino acid residues[46]. From genetic mutation studies, it has been shown that all the receptor derivatives that retained wild type segment (440-508) displayed full DNA binding activity and constitutive activation of glucocorticoid receptor regulated genes[46], whereas all mutations with alterations in this particular segment lacked DNA binding activity. DNA binding domain of the most steroid and thyroid hormone receptors is highly conserved[49,50]. The most striking feature is the conservation of cysteines, basic and hydrophobic amino acid residues. It has been demonstrated[51] that point mutations replacing two cysteines by histidines in the DNA binding domain of the human estrogen receptor prevent the gene activation by mutant receptor. A chimeric receptor construct, where the 66 amino acid region of the DNA binding domain of human estrogen receptor is replaced by the human glucocorticoid receptor DNA binding domain, results in the activation of glucocorticoid-inducible gene instead of an estrogen-inducible gene in the presence of oestradiol. A close homology has been observed between glucocorticoid and progesterone receptor (PR) DNA binding domain. This is well demonstrasted by the fact that both receptors can bind to the same nucleotide sequences in the long terminal repeat (LTR) region of the mouse mammary tumour virus[50]. Recently, it has been shown that the oestrogen and glucocorticoid response elements are also as closely related as the DNA binding domains of these hormone receptors[52]. Estrogen response element (ORE) to vitellogenin genes of frog and chicken can be converted to a glucocorticoid response element by substitution of one or two bases of ORE[52]. The homology between GR and mineralocorticoid receptor is even greater than that of GR and PR[53,54]. The DNA binding region of glucocorticoid receptor also shows analogy to the nucleic acid binding region of transcription factor (TF IIIA) from xenopus oocytes[54]. The DNA binding domain has a great significance in the mechanism of steroid action. In one of the n^t-(nuclear translocation deficient) mutants of mouse glucocorticoid receptor, Arg[484] in the DNA binding domain is replaced by histidine[46]. Most of the steroid receptors have in this position of the DNA binding domain arginine which may be directly involved in DNA interaction.

Immunologic domain—The immunologic domain (mostly amino terminal half) is not well characterized. It has been identified by its antigenicity. Generally polyclonal and monoclonal antibodies against the glucocorticoid receptor are directed to epitopes located in this domain. This domain has functional relationship with the hormone action. Variants of mouse lymphoma cells that are resistant to glucocorticoid exhibit a form of the receptor that can bind the steroid and interact with DNA but lacks the immunologic domain[55]. Interestingly, the immunologic domain and the DNA binding domain exhibit striking sequence homology with the erb-A gene of avian erythroblastoma virus[56,57]. This suggests that the hormone receptor and oncogene product are members of a new family of DNA binding proteins. The structural relatedness among selected regions of glucocorticoid receptor with other steroid receptors, thyroid hormone receptor and the erb-A oncogene suggest their evolutionary origin from a primordial receptor gene[49].

Glucocorticoid Receptor and Transcriptional Activation

Glucocorticoids modulate the gene expression of target cells through a cascade of regulatory events initiated by high affinity binding to their intracellular receptors. The hormone-receptor complex traverses the nuclear membrane and binds to its acceptor site usually located 100-300 bp upstream from the RNA polymerase start site, ulti-

mately causing the transcription of specific mRNAs that encode proteins involved in regulating proliferation, differentiation and physiological function of the target cells[2]. Glucocorticoid receptor is a class of cell-specific transacting transcription regulatory factor whose activity is controlled by specific binding of the hormone. The hormone-receptor complex appears to associate with promoter/enhancer elements of specific target genes and modulate their expression[2]. In the presence of hormone, the glucocorticoid receptor selectively regulates transcription by binding to specific DNA sequences, termed as glucocorticoid response elements (GREs), near hormone-responsive promoters[58]. GREs are transcriptional enhancer elements that are active only in the presence of the bound receptor. In some cases, the same DNA sequences have been shown to be involved in other hormone responses and to confer hormone inducibility to heterologous promoters linked to them. These regulatory DNA sequences are generally called as hormone regulatory elements (HREs)[59].

Glucocorticoid regulatory elements (GREs) were discovered in the mouse mammary tumour virus (MMTV) whose RNA synthesis was glucocorticoid dependent[58,60,61]. Using gene transfer experiments with the cloned proviral DNA, it is observed that the long terminal repeat (LTR) region has receptor binding sites. These regions also convey glucocorticoid-dependent induction upon a variety of linked heterologous promoters[58,60,61]. The receptor binding sequences are located between 50 and 200 bp upstream from the transcriptional start site[60,62]. GREs are also found within and downstream of the MMTV gene[2]. DNase I footprinting experiments have shown several binding sites for the purified glucocorticoid receptor within the hormone-inducible segment of DNA[58,63]. The nucleotide sequences (5′-TGTTCT-3′) are essentially conserved in all the binding sites[63]. Guanine residue within the hexanucleotide is shown to be the direct contact point for the receptor[64]. The role of GREs in glucocorticoid-mediated regulation of many genes has been studied. The 5′-flanking region of the human metallothionein-IIA gene confers glucocorticoid inducibility to a heterologous promoter[65,66]. Using 5′-deletion analysis the most responsive sequences have been identified between positions -236 and -268 upstream from the transcription start point[67]. The direct contact point for glucocorticoid receptor and DNA was the guanine within the hexanucleotide (5′-TGTTCT-3′) as observed in the case of MMTV-LTR. Using gene transfer and purified glucocorticoid receptor, it has been shown that two glucocorticoid response elements (GREs) are located at 2.5 kb upstream from the transcription initiation site of tyrosine aminotransferase (TAT) gene[68]. Although the proximal GRE has no inherent capacity by itself to stimulate transcription, when present in conjunction with the distal GRE, this element synergistically enhances glucocorticoid induction of gene expression. This cooperativity of the two GREs is maintained when they are transposed upstream of a heterologous promoter. An oligonucleotide of 22 bp representing the distal GRE is sufficient to confer glucocorticoid inducibility. Mapping of DNase I hypersensitive sites indicate local alterations in the structure of chromatin at the GREs as a consequence of hormone treatment. These TAT-GREs are located far upstream ($\sim$2.5 kb) from the promoter for TAT gene. This makes the TAT-GREs unique as most of the GREs/HREs for other genes are as close as $\sim$300 bp. When the TAT-GREs are moved closer to their cognate promoter or to a heterologous promoter inducibility strongly increases[68]. A possible explanation for such an observation may be that bringing GREs closer to promoter easily facilitates the changes at the transcription start site by acting on initiation factors.

In general, there is a consensus that most of the hormone receptors act as transacting enhancer proteins and interact with enhancer sequences upstream from the transcription site. It has been shown that a human gene encoding an interferon-induced 15 kDa protein has enhancer element between -115 and -96, just upstream from the CA-AT box. On transferring, this region confers interferon inducibility on a heterologous promoter of thymidine kinase when cloned in either orientation upstream or downstream from the gene[69]. The chick oviduct progesterone receptor binds preferentially to the sequences immediately flanking the ovalbumin promoter[70]. These 5′-flanking sequences are needed for induction by both progesterone and estrogen[71]. A role for the 5′-flanking sequences in glucocorticoid regulation has been demonstrated for growth hormone gene[72]. One of the glucocorticoid regulatory elements in the growth hormone gene is located downstream from the regulated promoter[72]. Generally, the glucocorticoid regulatory elements of different genes are located at variable distances from the transcriptional start site. Some are very far while most are located very close to the transcriptional start point. The exact mechanism(s) of the transcriptional regulation by these transacting proteins are

unclear. Several possible explanations have been advanced. Based on the role of protein-protein interaction in prokaryotic gene regulation[73], it has been postulated that the hormone receptor bound to an enhancer sequence would be recognized by some protein factor that is itself part of the transcriptional machinery[58]. This explanation has certain advantages in correlating the tissue specific response of the same hormone, i.e. the different cells may have a different set of these transcriptional factors[59]. It is hard to explain the role of protien-protein interactions when the receptor binds several kilobases away from the start site in either direction (upstream/downstream) of the regulated gene[58]. However, using electron microscopy to observe progesterone receptor binding to regulatory regions of uteroglobin and mouse mammary tumour virus genes, it has been demonstrated that DNA loops are formed when the hormone regulatory elements (HREs) are at a distance from one another[74]. This, in common with certain prokaryotic systems where several regulatory proteins bind to specific DNA sites and interact with one another to regulate the binding of RNA polymerase to DNA, protein-protein interactions may be important in steroid hormone regulation of gene transcription[74]. An alternative explanation may be a conformational change in the promoter region upon binding of hormone-receptor complexes to specific DNA sequences[59]. It has been observed that the enzyme topoisomerase I accumulated in the active regions of chromatin[75], thus indicating the control of the topological state of the DNA in active chromatin. The binding of the transcriptional factor TF III A to the 5S RNA gene of Xenopus appears to change the topological state of the DNA in chromatin[76]. Glucocorticoid receptor-DNA interaction may alter the configuration of DNA or chromatin in the vicinity of the binding sites, which may create an active transcriptional enhancer[2].

Glucocorticoid Receptor Mutants

Receptor mutants are generally derived from cell culture systems. In general, mouse lymphoma cells of T-cell origin and human lymphoblastic leukemia cells are very useful in screening the receptor mutants[77,78]. These cells show growth inhibition and cell lysis in response to glucocorticoids. During this process, some cells acquire resistance to the hormone. Many of these resistant cells were found to carry receptor defets of different types. The major abnormalities which have been observed are: r^- (receptor deficient), nt^- (nuclear transfer deficient), nt^i (increased nuclear transfer), and act^l (activation labile). The r^- phenotype has been characterized by a very low or undetectable hormone binding. Certain r^- variants have an identical polypeptide as wild type glucocorticoid receptor with the molecular weight of 94 kDa and show cross-reactivity with anti receptor antibodies. However, they do not bind to hormone[54,79]. This indicates the defect in the hormone binding site of the glucocorticoid receptor[54,79]. The nt^- type receptors show a defective nuclear binding and have low affinity for DNA. In contrast, the nt^i receptors have increased binding to nuclei and very high affinity for DNA[80,81]. Receptors of the act^l type are very labile to the conditions (heat, salt, *p*H, dilutions) which normally activate receptor-hormone complexes to a form which has more affinity for polyanions such as DNA and DNA-cellulose[82]. These studies of receptor mutants have provided a great deal of molecular and cellular aspects of physiological functions in different normal and abnormal cell types.

Phosphorylation of Glucocorticoid Receptors

Glucocorticoid receptor has been found to be a phosphoprotein[83–86]. Previously, many laboratories have suggested that phosphorylation/dephosphorylation play crucial roles in regulating glucocorticoid receptor functions including steroid binding and activation/transformation of GR complexes. The initial observation regarding the energy dependency of the steroid binding to GR was made by Munck and his group[87,88]. Incubation of thymus cells at 37°C under anaerobic conditions caused a rapid decrease in the levels of cortisol binding, and these levels could be restored by O_2 supply. These reversible changes in the binding of a glucocorticoid were correlated with the changes in the concentration of ATP. Similar reversible changes in glucocorticoid binding were also observed in thymus cells treated with 2,4-dinitrophenol instead of exposure to anaerobic conditions[89]. These observations led to the proposal of continuous recycling of glucocorticoid receptor between active and inactive states[90]. The active state may be generated by phosphorylation reaction which utilizes ATP. The recycling or reactivation of glucocorticoid receptor is further supported when human IM-9 lymphoblast cells incubated in glucose-free media into a nitrogen atmosphere lost both their ability to bind a synthetic glucocorticoid and their ATP levels. The loss of receptor function and ATP levels were restored upon addition of glucose and oxygen to the medium. This inactivation of receptors in intact cells was inhibited by phosphatase inhibitors such

as molybdate, fluoride, vanadate and ATP[91]. These studies pointed out that inactivation of receptors may result from a dephosphorylation. This was supported by the observation that pretreatment of rat liver cytosol with purified exogenous calf thymus alkaline phosphatase elicits inactivation of glucocorticoid binding capacity[27]. However, the potential site for dephosphorylation of receptor and/or non-receptor component was not detailed as the studies were performed with crude receptor preparation. In another set of experiments, addition of ATP and dithiothreitol potentiate/reactivate the glucocorticoid binding capacity of inactivated mouse L-cell receptors. These studies led to the speculation that the binding of glucocorticoid in these cells requires phosphorylation as well as reduction of the receptor and/or some non-receptor component required for glucocorticoid bindings[92].

Many conditions such as heat, salt, dilutions and *p*H which accelerate inactivation of unbound receptors also accelerate activation and/or transformation of bound glucocorticoid receptor complexes to a DNA binding form. It was speculated that if phosphorylation of the receptor is required for steroid binding, activation of glucocorticoid-receptor complexes may involve a dephosphorylation[14,92]. The importance of dephosphorylation in activation of glucocorticoid receptor was also studied with the use of phosphatase inhibitors such as molybdate and tungstate which block the heat-induced receptor activation[14,92]. The exact mechanism(s) by which molybdate and tungstate block the activation of bound hormone-receptor complexes is not well understood. The potential role of dephosphorylation in glucocorticoid receptor activation has been questioned[93,94]. The number of phosphate groups is almost the same in both the non-DNA binding and the DNA binding forms of the receptor[94,95]. This does not exclude the possibility of a dephosphorylation of some other non-receptor component such as an endogenous inhibitor which may influence the activation of the hormone-receptor complexes[95]. Furthermore, the possibility of site specific phosphorylation/dephosphorylation with an equal number of phosphate groups cannot be ruled out.

Physiological Implications

Glucocorticoids influence a wide variety of cell types and metabolic pathways. They are involved in catabolic reactions, mainly the mobilization of amino acids from peripheral tissues, caused by increased degradation and decreased synthesis of proteins in most of the tissues excluding liver. Increased glucocorticoids cause a loss of protein from the muscle, supportive tissues and skin and lead to weakness, oesteoporosis and skin dystrophy. Glucocorticoids have been implicated in various normal and pathologic conditions, normal and abnormal growth, development and aging processes. Since the actions of glucocorticoids are mediated by the intracellular receptor, much of the adaptive responsiveness have been attributed to the receptor and post-receptor events. Decreased glucocorticoid binding and receptor activation have been reported in the brain of genetically diabetic MDB/MDB mice[96]. Increased cytosolic binding sites for glucocorticoids have been observed in several muscular atrophies. The occurrence of quantitative as well as qualitative changes in the glucocorticoid receptor has been described during developent and aging[97-100].

Glucocorticoid hormones elicit a series of cellular responses in various immunological functions. They induce growth inhibition and cell lysis in certain lymphomas, leukemias and immature thymocytes[77,78]. This process requires functional intracellular receptor proteins and involves activation and nuclear translocation steps[78]. Glucocorticoid-mediated cell death is not unique to the cell culture system but is also observed in the whole organism. In a series of experiments, the loss of hippocampal glucocorticoid receptor has been correlated with the loss of specific hippocampal neuronal cell population as a function of age. These neuronal deaths in aged animals have been associated with the increased stress-induced glucocorticoids with age[101]. The exact mechanism(s) of cell death by glucocorticoids are not very clear, but may partly be mediated through glucocorticoid receptors. The importance of glucocorticoid receptors in cell lysis is attributed by growing certain rodents and human cell lines at relatively high concentration of glucocorticoids. It has been observed that certain cell lines are resistant to glucocorticoids due to the dysfunction of receptors either at the initial binding, activation and/or nuclear translocation levels. In a recent experiment, the loss of steroid responsiveness has been found to be independent of the loss of steroid receptor function. Using a steroid regulated chimeric gene transfection into both responsive and unresponsive cells, it has been shown that the steroid-insensitive cells contain functional glucocorticoid receptor and that the defect exists at the level of transcriptional control[102].

Summary

The advent of molecular techniques such as purification of proteins to homogeneity, production of monoclonal antibodies, molecular characterization by Western blotting, Southwestern blotting, cDNA cloning and site-specific mutagenesis has made possible to dissect the various domains of the glucocorticoid receptor. A significant progress has been made regarding receptor-mediated enhancement of gene expression. This has opened a new insight into an enhancer element (called glucocorticoid response elements) where the glucocorticoid-receptor complex interacts with the specific DNA sequences. These sequences are usually located a few basepairs upstream from the transcription start site. In spite of great advances in the glucocorticoid receptor field, there are numerous questions, viz. (i) the subunit structure of the unactivated receptor, (ii) association and role of heat-shock protein with the receptor, (iii) role of phosphorylation and dephosphorylation, (iv) presence of endogenous modulators of receptor activation and nuclear translocation, and (v) the gene regulation in a cell and tissue specific manner, remain to be answered before getting a complete picture of its mechanisms of action.

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