I. Introduction

Steroid hormones mediate a wide variety of effects in higher eucaryotes. They play a major role in the intercellular communication within complex organisms. Their mode of action includes interaction with their receptors in target cells, transport of the hormone-receptor complex to the nucleus and finally direct activation or inhibition of gene expression at the chromatin level. Each of these steps has been extensively studied in the last years. The cloning of the cDNA coding for some of these receptors has revealed that they belong to a still increasing multigene family which also includes thyroid hormone receptors, oncogene products like v-erbA and several developmental regulation factors in Drosophila, such as knirps and krüppel gene products.

Glucocorticoids are active in inducing gluconeogenesis in liver, they promote the development of various organs and are necessary for the growth of many cell types in vitro. The corresponding receptor is present in nearly every cell consistent with the action of these hormones. The glucocorticoid receptor (GR) has been
This review will discuss the current knowledge about the action of the GR, following step by step the molecular events during glucocorticoid induction, and relate some of the recent findings in this field.

II. The cytoplasmic receptor

II.A. Hormone binding

The first step in the cascade of events leading to induction of gene expression by glucocorticoids is the binding of the hormone to the receptor present in the cytoplasm of target cells. The domain of the GR involved in this process was shown to be the carboxy-terminal half of the molecule [2–4]. Attempts to localize this function more precisely have led to the identification of a proteolytic fragment of the rat GR ranging from Thr-537 to Arg-673 [5]. This 16 kDa fragment binds dexamethasone specifically with a 23-fold lower affinity as compared to the complete GR, suggesting that other parts of the GR are involved in high affinity hormone binding. Covalent labelling of the contact sites by using reactive hormone analogs has shown that amino acid residues Met-622, Cys-656 and Cys-754 of the rat GR interact with the steroid [6,7]. These residues are all positioned within hydrophobic segments of the hormone binding domain. The large region which is required for optimal hormone binding suggests that a three-dimensional protein structure is folded, which binds the hormone in a hydrophobic pocket.

Interestingly, the GR molecule is functional without the hormone binding domain. Deletion of this domain leads to a constitutive activator, which has nearly wild type trans-activation capacity also in the absence of hormone [2,3]. Chimeric proteins have been constructed which contain the DNA binding domain of the yeast transcription factor GAL4 joined to the C-terminal region of the human GR [8,9]. When this protein is expressed in eucaryotic cells, it confers glucocorticoid responsiveness to a reporter gene under the control of the GAL4 binding motif. Similar results were obtained using fusion proteins with the DNA binding domain of the bacterial LexA repressor [10]. The trans-activation of the E1a a protein fused to the rat GR hormone binding domain is also repressed in the absence of hormone [11]. Thus, it appears that the hormone binding domain acts as a repressor of trans-activation function in the absence of hormone and that this repression represents an intrinsic property of this domain, which can act on completely unrelated proteins as well.

II.B. Cytoplasmic multiprotein complex

In the absence of hormone, the GR is present in the cytosolic fraction of cell homogenates. After hormone administration, it is found in the nuclear fractions. In
Studies have shown that, after hormone binding, the receptor undergoes a modification which enables it to bind DNA [12]. This modification also occurs in living cells, proving that it represents a normal step in signal transduction [13]. This activation process has been the object of a large number of investigations, which were greatly facilitated by the finding that the unactivated form of the receptor is stabilized by sodium molybdate [14]. The unactivated receptor is present in the cytosol as a 9 S complex with a molecular mass of 310 kDa [15]. In contrast, the activated GR has a sedimentation coefficient of 3.2 S (4 S form) and a molecular mass of 90 kDa. Thus, GR activation involves dissociation of a large multiprotein complex. Using antibodies against the GR, it was concluded that the 9 S complex contains only one GR molecule [16] and constitutes therefore a heteromer composed of one hormone binding GR and two non-hormone-binding proteins of 90 kDa [17]. This receptor associated protein was subsequently shown to be the 90 kDa heat shock protein (hsp90) [18,19]. Since hsp90 is an abundant protein in cells, it was important to show that its association with the GR was not due to the use of molybdate for stabilization of the GR and did not represent an artifact of receptor purification. Direct evidence for a physiological association of hsp90 and GR came from two types of experiments: co-immunoabsorption of hsp90 with the GR using anti-GR antibodies even in the absence of molybdate [20], and the demonstration that newly synthesized radioactive hsp90 binds to the GR only after a 2 h in vivo incubation [20]. Moreover, the unactivated complex was demonstrated in living animals after short exposure to hormone [21] and by photochemical crosslinking performed with cytosolic fractions or with intact cells [22].

More precise analysis of the 9 S complex revealed the presence of an additional protein of 59 kDa which is more rapidly lost during cytosol preparation and exposure to high salt [23]. Using antibodies against p59 it was shown that this protein binds directly to hsp90 [24].

While the binding of hormone to the GR takes place in vitro at 0 °C, the activation requires a temperature of 37 °C, high salt or an acidic pH. The thermal activation requires hormone-binding to convert the GR to a DNA binding form [25]. Recent evidence [26,27] suggests the involvement of heat-stable, non-proteinaceous factors in the hormone-dependent GR activation. The loss of hsp90 by heat activation decreases about 10-fold the receptor’s affinity for the hormone, to a level which is also seen for bacterially expressed GR [28,29], indicat-

![Signal transduction pathway of glucocorticoid hormones. The different steps for gene induction by glucocorticoid hormones are depicted. The steroid diffuses through the cell membrane to the cytoplasm where it binds to the 9 S complex. This complex dissociates and gives rise to its components: two heat shock protein molecules hsp90, the p59 protein and the activated, hormone loaded receptor which assembles to form a homodimer. This complex is transported to the nucleus, binds to chromatin and finally interacts with the transcription machinery (shaded oval).](image-url)
ing that hsp90 association increases the affinity of the receptor for its hormone ligand. In this respect, the location of the hsp90 binding domain in the receptor was of interest. It was shown [30] that deletion of amino acids 532–697 comprising two-thirds of the hormone binding domain, or of only amino acids 568–616 [31] in the rat GR, completely abolished 9 S complex formation, i.e., hsp90 binding. This severely impeded hormone binding and yielded a constitutive receptor which is present in the nucleus in the absence of hormone.

Glutaraldehyde crosslinking of the activated receptor resulted in a shift of its sedimentation rate from 4 S to 6 S [32]. The same complex was obtained with or without crosslinking by gel-filtration. This suggests that the activated receptor, after dissociation from hsp90, is present as a homodimer.

The processes involved in signal transduction of glucocorticoid hormones from the outside of the cell to the target genes are outlined in Fig. 2.

II.C. Phosphorylation

Metabolic labelling experiments using radioactive orthophosphate revealed that the 92 kDa glucocorticoid binding protein is phosphorylated in vivo. The extent of phosphorylation and its significance remains unclear. Localization of phospho-amino acids in the rat GR has been attempted several times by specific proteolytic or chemical cleavage of purified rat GR [33–35]. It appears that the receptor contains mostly phosphoserines. Phosphorylation is seen mainly in the N-terminal half which contains a trans-activation domain, and to some extent in the DNA binding domain. Addition of hormone increases the phosphorylation of the trans-acting domain about 2- to 3-fold in vivo. A constitutive GR mutant lacking the hormone binding domain is also constitutively hyperphosphorylated. On the other hand, mutants lacking this region are very poor activators. Whether hormone-dependent phosphorylation has indeed a modulating effect on trans-activation remains to be shown. It should be noted, however, that the mouse GR is phosphorylated in a region immediately adjacent [36] to the trans-activating domain, which is homologous to the rat domain enh2 (see Fig. 1).

II.D. Nuclear translocation

Nuclear localization of GR is under hormonal control. Two domains of the rat GR have been identified that are involved in this process, referred to as NL1 and NL2 [37]. NL1 maps to a 28 amino acids segment next to the DNA binding domain. Fusion of NL1 to an unrelated protein mediates nuclear translocation also in the absence of hormone. However, in the intact GR the function of NL1 seems to be repressed by the hormone-binding domain. NL2, in contrast, is closely associated with the hormone-binding domain and its function is hormone-dependent. Thus, the nuclear translocation contributes to the steroid control of GR action. The importance of this translocation step was further analyzed using recombinant proteins. A rearranged rat GR with NL1 and the hormone-binding domain being repositioned to the N-terminus of the receptor or a chimeric receptor which contains an additional nuclear localization signal from the SV 40 T antigen are both constitutively present in the nucleus, nevertheless, transcriptional activation was still hormone-dependent [11]. Thus, although in the natural GR nuclear localization is hormone-dependent, it is not sufficient to yield an active receptor. Chromatin-binding and/or transactivation seem to be hormone-controlled as well.

III. The nuclear receptor

Regulation of gene transcription by steroid hormone receptors is mediated by specific DNA sequences located in the vicinity of the regulated gene [38]. Such transcriptional control regions have been identified in a variety of genes and the comparison of these sequences has allowed the identification of a consensus for GR binding sites. The binding of steroid receptors to these consensus sequences has been confirmed by DNase I and DMS methylation protection experiments using purified hormone-loaded receptor [39–49]. Finally, synthetic oligonucleotides cloned in front of the herpes simplex thymidine kinase (tk) promoter have been found to confer responsiveness to glucocorticoids and progestins [59] to this otherwise unresponsive transcription unit. The so-defined glucocorticoid responsive element (GRE) consists of a palindromic sequence of 15 basepairs (bp), which has been shown to bind a dimer of the receptor [51,52]. The consensus glucocorticoid receptor binding site is the sequence GGTTACANNNTGTTC, which can also mediate the action of progesterone, androsterone and mineralocorticoid receptors [50,53–55].

The influence of hormone on the DNA binding ability of the GR is a matter of dispute. On the one hand, purified steroid-free GR binds upon heat activation specifically to a GRE in vitro [56]. The DNase I footprints were indistinguishable from those obtained using hormone-loaded receptor or GR loaded with the antagonist RU486. On the other hand, in vivo methylation protection experiments carried out on the rat tyrosine aminotransferase gene revealed that protein interaction with the corresponding GREs was only visible after hormone administration [46]. Thus, although the unliganded receptor has the potential to bind DNA, it seems that in vivo it is hindered in doing so, possibly by confinement to the cytoplasm or the association with hsp90. More recently, the issue was analyzed in vitro for more detail [57]. Binding studies using specific or...
specific DNA revealed that addition of the hormone influences the kinetics of the DNA–protein interaction, both the on-rate and the off-rate are accelerated. Thus, formation and separation of the complex is faster in the presence of hormone with a slight increase in overall affinity. This effect is not so pronounced when an antagonist is used. It is conceivable that the hormone-loaded receptor is able to scan the genome more rapidly in order to detect its site of action.

III.A. DNA binding domain

The cloning of the cDNA coding for GR allowed to elucidate the region required for its DNA binding. It lies in a central basic domain which is rich in cysteines and is found highly conserved within the entire steroid receptor family and between different species. Study of deletion mutants and chimeric proteins [58–61] showed that this region represents indeed the DNA binding domain (see Fig. 1) and is responsible for targeting the receptor to the regulated gene. It contains two motifs which show some similarity to the ‘zinc-finger’ motif of the Xenopus transcription factor TFIIIA involved in DNA binding. The coordination of GR with zinc was also demonstrated [62]. However, in contrast to TFIIIA, which involves histidine residues, the structure in the GR is obtained by coordination of four cysteines to one zinc atom. By site-directed mutagenesis it was shown that seven out of the eight cysteines are absolutely required for receptor function [63,64]. Only substitution with histidines maintained partial activity. These results strongly support the model of a zinc coordinated finger as the DNA binding structure of the GR. A detailed analysis of the contact points between GR and the MMTV promoter revealed that the receptor contacts the DNA mainly in the major groove of the double helix [49] (Fig. 3).

Domain swapping experiments between different receptors and point mutation analysis allowed to functionally dissect the DNA-binding domain of the GR. It appears that the first (amino-terminal) finger is involved in the distinction between ERE (estrogen responsive element) and GRE. Three residues at the C-terminus of the first finger and in the inter-finger region are sufficient for specific GRE recognition. The second finger seems to stabilize the interaction [65,66]. Later studies [67] revealed that the second finger is involved in distinction between GRE or ERE and TRE (thyroid hormone responsive element). Recently, these data were combined with structural nuclear magnetic resonance data obtained using the GR DNA binding domain expressed in Escherichia coli in order to obtain a three-

![Fig. 3. A model of the dimeric DNA binding domain bound to DNA. A dimer of the glucocorticoid receptor DNA-binding domain consisting each of two ‘zinc-finger’ regions (thick line) and two α-helical regions (shaded cylinders) is shown on top of the DNA with the receptor-binding sequence indicated. The double headed arrow points out the region of symmetry for both, the DNA sequence and the receptor dimer. The solution structure of the glucocorticoid receptor DNA-binding domain has been determined by nuclear magnetic resonance spectroscopy and distance geometry [68]. The results predict that one of the two α-helices within a receptor monomer serves as a recognition helix located in the major groove of the DNA. Furthermore, one region of a ‘zinc-finger’ is in close contact with the corresponding region of the second receptor monomer (central part of figure). In this model one of the ‘zinc-fingers’ is pointing away from the DNA and possibly interacting with other proteins.](image-url)
dimensional picture of this structure [68]. The occurrence of zinc coordinated fingers was confirmed, but the region contacting the DNA was shown to be an α-helical structure located between the two fingers (Fig. 3). Furthermore the model predicts that in a homodimeric complex bound to a palindromic GRE, the amino acids located at the N-terminal base of the second finger are in close contact.

III.B. Trans-activation

The domains responsible for the trans-activation function of the GR have been identified by analysis of deletion mutants and of chimeric proteins (Fig. 1.). The fact that the N-terminal trans-acting domain τ₁ in the human GR corresponds exactly to the enh2 domain of the rat GR makes it probable that the corresponding region in the mouse GR is also involved in this function. The N-terminus of both human [9] and rat [10] GR has been shown to confer constitutive activation when fused to unrelated DNA-binding domains, again supporting the notion of a linear domain structure of the GR. C-terminal deletions of human, rat and mouse receptors lead to constitutive activators as long as the hormone-binding domain is absent [4,9,10]. As already mentioned, the hormone-binding domain contains an inactivating function possibly mediated by the hsp90 protein, which also binds to this region. The τ₂ domain defined for the human GR represents an intact trans-activation domain on its own, since it remains functional when moved to a different location within the receptor [9]. Its activity is increased when multimerized or when used in combination with τ₁. It is unclear whether the corresponding rat region contains a similar activity. The fact that amino acids 524 to 795 fused to the lexA DNA-binding domain mediates some hormone dependent activation [10] supports this possibility. The rat GR domain DNA/enh 1 retains much of the wild type activity [3], the DNA-binding activity and the activation function could not be further delineated. Interestingly most of these domains, except enh1, have a slightly acidic character, in accordance with the observation that many trans-acting domains carry a nett negative charge [69]. This negative charge might be increased by phosphorylation of amino acids residues within these regions contributing to the transcription activating potential of the receptor.

III.C. Synergism

A GRE conferred glucocorticoid inducibility to a reporter gene when cloned close to the TATA box

Fig. 4. Model of a hormone regulatory unit. Different arrangements and compositions of binding sites for transcription factors (TF) and glucocorticoid receptor (R) within a hormone regulatory unit lead to different activities in gene expression. Strength of transcriptional activation is indicated by the strength of the curved arrows, synergism by the combined arrows. (A) Synergetic activity of two receptor dimers is depicted, (B) indicates a synergistic activity of a receptor dimer with another transcription factor and (C) a non-synergistic arrangement. (D) In case of three binding sites only one pair at a time may synergize.
(within 100 bp), but not when cloned at a position further upstream [70]. Similarly, induction by glucocorticoids was independent of the topology of the reporter plasmid when the GRE was close to the transcription start site, but revealed a topological dependence when it was located further upstream [71]. This suggests that additional components of the transcription machinery are involved in regulation by those HREs which are naturally located at large distances from the promoter which they control.

A detailed analysis of the long terminal repeat (LTR) of the mouse mammary tumor virus (MMTV) revealed the presence of several glucocorticoid receptor binding sites [39,40,72–77], the progressive deletion of which resulted in a gradual loss of inducibility [73,78]. Similar observations were made during the analysis of other glucocorticoid regulated genes including the chicken lysozyme gene [42,44], the rabbit urothelial gene [41], the rat tyrosine aminotransferase (TAT) gene [48] and the rat tryptophan oxygenase (TO) gene [47]. In the case of the TAT gene, induction mediated by two GREs could only be explained by cooperative effects. The presence of different HREs in the chicken vitellogenin II gene allowed the demonstration of a synergism in induction in the presence of both steroids [79]. All these observations indicate that several hormone receptor binding sites can cooperate to yield the strong induction observed in natural genes.

In order to study the synergism in a more defined environment, synthetic regulatory units consisting of several receptor binding sites were constructed. In each case, the combination of two palindromic GREs showed a synergistic effect [70,80] (Fig. 4A). Further addition of one or two GREs, however, did not lead to a further increase in induction [70,81].

During the study of sequences responsible for glucocorticoid regulation of the rat tryptophan oxygenase (TO) gene, a puzzling observation was made [47]: A 5'-deletion mutant containing the intact receptor binding site as determined by footprinting experiments failed to show inducibility by glucocorticoids. A construct containing additional 5' sequences was, however, regulated by the steroid. These observations suggested that a sequence adjacent to the GRE contributes to hormone induction of the TO gene. Footprinting experiments in this region using nuclear extracts revealed binding of a factor recognizing a CACCC-sequence [82] identical to that found in the human β-globin gene [83].

Another example of such a functional cooperation between receptor and other transcription factors was seen in the MMTV promoter. Deletion of an NFI-binding site in the vicinity of a GRE almost completely abolished the inducibility by glucocorticoids [84,85]. Mutations at this site, or in some other non-receptor binding sequences impeded the function of the regulatory element [86]. Different mutations had varying effects on the inducibility depending on the type of receptor involved. This indicates that requirements for neighbouring sequences are receptor specific [49].

In vivo analysis of the TAT gene revealed steroid-dependent changes in the pattern of specific sites protected from DMS methylation. In addition to the glucocorticoid receptor binding sites, other sequence elements were protected upon glucocorticoid induction [46]. One of these regions is a CAAT-box immediately adjacent to a GRE, which was shown in transfection experiments to be essential for inducibility in its natural environment [70].

Since the detailed analysis of MMTV and the TO and TAT genes showed the importance of non-receptor binding sites for steroid induction, a computer search for known transcription factor binding sites in the neighbourhood of glucocorticoid responsive elements was carried out. For several other genes potential binding sequences were detected in the vicinity of receptor binding sites. These observations supported the idea that the strength of a hormone responsive unit (HRU) [87] is determined by both receptor and non-receptor binding sites. Combinations of a receptor binding site with binding sites for other transcription factors (CACCC-box factor, NF1, CAAT-box factor, Sp1, Oct1) showed strong synergistic effects on steroid induction [80] (Fig. 4B). The best cooperation is seen between two GREs, the other sequences cooperate to varying extents.

Interestingly, the effects of the different transcription factors were shown to be strongly dependent on the cell line, probably reflecting the relative abundance of the various factors in these cell lines [70]. These observations offer a possible explanation for the variable inducibility of natural genes in different cell types, which does not always correlate with corresponding amounts of receptor in the cell [88,89].

Thus, it appears that in many cases a hormone responsive unit is composed of one or several receptor binding sites intermingled with binding sites for other transcription factors which act synergistically to yield the observed expression and inducibility pattern.

III.D. Arrangement of cooperating binding sites

For a better understanding of synergism in transcription activation, it is important to define the requirements for this phenomenon. First of all, the influence of the relative arrangement of the binding sites was investigated by comparing constructs containing the CACCC-box either downstream from a GRE, upstream from a GRE or in two copies flanking a GRE. All these constructs showed a similar induction by dexamethasone [82]. This result indicates that the relative orientation and arrangement of GRE and CACCC-box is of no importance for the functional cooperativity. An additional CACCC-box does not further increase induc-
bility. This might be related to the fact discussed earlier that two GREs show a clear synergism, while more binding sites do not further increase inducibility. Thus, within several transcription factor binding sites only two at a time may be involved in synergism, and this synergism occurs independently of their relative arrangement to one another (Fig. 4D).

It is feasible that functional cooperativity may involve direct interaction between transcription factors. This possibility was examined by testing the glucocorticoid or progesterone induction of constructs containing a CACCC-box at various distances from a GRE/PRE. In both cases a clear distance dependence of synergism is seen which shows a cyclic pattern with a period of about 10 bp corresponding to one complete turn of the double helix. The induction maxima are different for the two receptors analyzed [80,82]. These results suggest a requirement for a stereospecific alignment and a direct or indirect protein–protein interaction of the two factors (Fig. 4C). The different optima observed for the two receptors probably reflect differences in their sizes and/or binding geometry. An observation which was made during analysis of the MMTV HRU [49] leads to a similar interpretation. These authors observed that individual mutations in the GRE sequences or in their vicinity as well as insertion of 5 bp between two groups of GREs differentially affect glucocorticoid and progesterone inducibility. The relative responsiveness to progesterones or glucocorticoids of each of several GRE’s in such a HRU may then be determined by its spatial relationship to companion transcription factor binding sites.

A different result is seen by changing the spacing between a GRE and the TATA box of the tk promoter. A strong distance dependence, but no apparent periodicity comparable to that seen within an upstream HRE was observed [55]. It seems that the interaction of receptors with the TATA box factor does not require a stereospecific alignment, an observation which has also been made with other transcription factors upstream of the TATA box [90–92].

**III.E. Functional synergism and DNA-binding affinity**

The simplest explanation for the synergism between two transcription factors would be cooperative binding to two adjacent binding sites. This possibility was investigated using the gel retardation method. Cooperative binding to a double GRE by purified glucocorticoid receptor was demonstrated [93]. In addition, these authors showed a dependence of the increased binding affinity on the space between the binding sites; the affinities changed in a cyclical pattern with a periodicity at 10 bp intervals. Moreover, the stability of the formed DNA-protein complex was increased for two properly-spaced GREs. Thus, it appears that the functional synergism of two HREs is due, at least in part, to the increase of binding affinity of the receptor to the regulatory element.

To analyze whether the synergism of non-receptor factors with a receptor is similarly mediated by increased DNA binding affinities, gel retardation experiments addressing this question were carried out. For combinations of a GRE with several different transcription factor binding sites no indication for cooperative DNA-binding of the two factors was found (Baniamad, C., Muller, M. and Renkawitz, R., unpublished results). Similar observations were reported concerning the binding of GR and NF1 to the MMTV enhancer. No binding cooperativity was detected, in fact, rather a binding inhibition of one protein for the other was apparent [94]. Thus, it appears that the functional synergism of a GRE with another transcription factor binding site, in contrast to that of a dimerized GRE, cannot be explained by an increase in DNA binding affinity of either factor.

Recently, we were able to show that intrinsic transactivation, measured on a reporter construct containing one isolated GRE, and synergism, as determined using a reporter gene carrying a CACCC-box adjacent to the GRE, are differently affected by several receptor deletions. These results suggest that synergism and transactivation constitute two different functions of the same molecule.

**III.F. Binding to chromatin**

It was shown that hormone administration altered the in vivo DNase I sensitivity of MMTV DNA, suggesting that specific GR–DNA interaction may alter the configuration of DNA or chromatin in the vicinity of the binding sites, thereby creating an active transcriptional enhancer in vivo [95]. The study of the MMTV promoter placed on an episomal vector revealed that nucleosomes are specifically positioned over this sequence in uninduced cells [97]. Additional evidence indicated that the nucleosome is displaced (or modified) upon hormone induction. Experiments using reconstituted nucleosomes support the idea that the GR is able to bind to chromatin in vivo [97]. As already mentioned the MMTV promoter contains a NF1 binding site which is necessary for hormone induction [85].

In a different approach, Cordingley et al. [98] showed that in vivo protection against exonuclease III digestion through the NF1 binding site could only be seen in chromatin isolated from hormone-induced cells, although the factor is also present in uninduced cells. This suggests that the NF1 binding site is inaccessible in uninduced cells. Recently, the structure of nucleo-protein complexes formed with the MMTV promoter was analyzed in in vitro reconstituted chromatin [99]. While the GR binds naked DNA and reconstituted
nucleosomes with equal affinity, NF1 in contrast only binds efficiently to free DNA, as demonstrated by gel retardation and DNase I footprinting experiments. Precise analysis of the positioning of the nucleosome revealed that the NF1 binding site in the absence of GR is hidden by the histone complex and that GR binding results in an increased accessibility of the promoter proximal end of the DNA. It is possible that in this case the major function of the GR is the displacement of the nucleosome from the promoter in order to allow a strong activator protein to bind to its otherwise hidden binding site. Whether the receptor then leaves the complex or stays to contribute to the overall trans-activation remains to be shown.

III. G. Negative regulation

Some physiologically relevant genes are negatively-regulated by glucocorticoids. Since the main interest has been focussed on positive regulation, only recently transcriptional repression has been studied more intensely. Functional data obtained by transfection experiments and DNA binding analysis are available for four systems.

The most extensively characterized system is the repression of the human glycoprotein α-subunit gene by glucocorticoids. It was shown in this case that glucocorticoid inhibition was dependent on the presence of a functional cAMP responsive element (CRE) closely adjacent to or overlapping the GRE [100]. This system was also used to analyze the transcriptional inhibition of several human GR mutants. Deletion of the DNA binding domain abolishes repression and deletion of the hormone binding domain weakens the inhibition. However, the replacement of the C-terminus by an unrelated protein stretch fully restores the negative regulation [101]. The N-terminal trans-activation domain was not required. Thus, negative regulation could be due to the GR causing steric hindrance to the binding of a positive factor to the CRE. Similar results were obtained for other systems. In the case of the bovine prolactin gene [102], the rat α1-fetoprotein [103] and the rat pro-opiomelanocortin gene [104], it was shown that the GREs are always located close to or overlap with binding sites for other transcription factors (Fig. 5B). Although binding competition remains to be experimentally proven, it is reasonable to assume that negative regulation mediated by the GR is due to the displac-

![Diagram of transcriptional effects](image)

Fig. 5. Positive and negative transcriptional effects of the receptor. The different types of interaction between receptor (R) and other transcription factors (TF) are depicted. (A) Binding of the receptor dimer immediately adjacent to a transcription factor leads to synergistic activation, the strength of transcriptional activation is indicated by the strength of the curved arrows, synergism by the combined arrows. (B) Binding of the receptor to the GRE displaces a positive transcription factor from a synergizing complex of two transcription factors (TFa and TFB) and thereby represses gene activity; the remaining receptor dimer and TFB are bound in a non-synergistic arrangement. (C) Binding of the receptor to a positive transcription factor via protein–protein interaction blocks the activation signal and thereby represses transcription.
ment of a positive transcription factor. Why in this case the receptor does not mediate its own positive effect remains an unsolved question. Possibly the sequence of the GRE itself plays a crucial role as indicated by a preliminary consensus for a negative GRE which is distinct from that of a positive GRE [38,102]. Alternatively, the location of the GRE or the lack of neighbouring synergizing factors might be important (Fig. 5B).

A different type of negative regulation has been described which does not involve DNA binding of the receptor. Treatment of mouse fibroblasts with phorbol esters, ultraviolet light, TPA or serum growth factors induces dramatic changes in their gene expression pattern. One example is the induction of the collagenase gene mediated by binding of API protein to the promoter, which can be counteracted by addition of glucocorticoids in the absence of a GR binding site [105]. GR lacking the DNA binding domain is also functional in repression. Direct interaction between API and GR in the presence of hormone could be demonstrated by coimmunoprecipitation. Thus, it appears that API and GR form a complex which is unable to trans-activate (Fig. 5C). Similarly, glucocorticoid induction can be repressed by overexpression of API. These observations offer a first hint to a possible explanation for the anti-tumor-promoting and anti-inflammatory actions of glucocorticoids.

The estrogen receptor was also shown to repress the prolactin gene in the absence of DNA binding [106]. In this case the target of repression may be the pituitary specific transcription factor (Pit-1). Using a chimeric receptor containing a fragment from amino acid 486 to 531 of the human GR in the homologous region of the estrogen receptor this repression was also observed.

Steroid receptor-mediated repression of induction by other steroid receptors requires overexpression of the interfering receptor and may act at another level, i.e., on an intermediary protein transmitting the steroid induction from the DNA-bound receptor to the transcription initiation complex [107]. Overexpression of the progesterone receptor (PR), of GR or ER interferes with the induction mediated by each of the other receptors. Since it was impossible to demonstrate heterodimer formation or any other kind of interaction between activating and interfering receptor the model of inactivating an intermediary factor was favored.

The influence of the hormone on receptor function is difficult to define. GR complex formation with hsp90 facilitates hormone-binding, which in turn induces dissociation. The wild type GR-steroid complex then migrates to the nucleus in a form which is competent for DNA binding and trans-activation (Fig. 2). In contrast, chimeric receptors carrying the hormone-binding domain which are brought to the nucleus in the absence of hormone, are inactive. Receptors lacking the hormone-binding domain are constitutively nuclear and functional. Thus, it appears that the hormone-binding domain is able to inactivate the receptor in the absence of hormone even if the receptor is present in the nucleus. A possible mechanism would be association with hsp90, which was proposed to have a protein unfolding activity, thereby inactivating bound factors [11]. Other heat shock proteins also seem to be involved in such folding/unfolding events [108]. The association of these chimeric GR proteins with hsp90 in the nucleus remains to be shown. In the case of the dioxin receptor, such an association with hsp90 in the nucleus was detected [109].

Experiments using antibodies against the DNA binding domain indicated that the GR was only recognized after its dissociation from hsp90, suggesting that this region of the GR is hidden in the unactivated complex [110]. Alternatively the unfolding activity of hsp90 in the unactivated complex might account for the lack of antibody recognition.

The results showing a modulation of DNA binding affinity and kinetics argue for an additional role of hormone-binding, which would not only be limited to inducing the dissociation from hsp90. To answer these questions, the availability of in vitro transcription systems [111] displaying hormone responsiveness upon addition of purified activated receptor is of great importance. In addition, the study of precise mechanisms of gene activation and repression as well as synergism with neighboring factors, in naked DNA or possibly in nucleosomes, will now be possible.

The GR seems to be embedded in a complicated network of transcriptional regulatory factors. Interactions with other transcription factors, which might be cell-specific, and which bind near the receptor to DNA leading to synergistic activation, or the displacement of factors from overlapping binding sites, illustrate the interplay of different proteins. A further complexity is added by the observed interactions between transcription factors which do not require DNA binding. Activation is not an isolated phenomenon due to one protein, but is carefully tuned by the effects of DNA-binding or non-binding factors present in the cell. A striking example is given by the antagonistic effects of GR and API possibly reflecting the antagonism between cell differentiation and proliferation.

The fate of the hormone-receptor complex after its
binding to DNA and the regulation of the receptor gene itself represent two questions which have been barely addressed till now. Receptor mRNA levels have been shown to decrease upon dexamethasone treatment [112, 113]. In addition a decrease of the half life of the GR protein was shown.

Very clearly, our understanding of glucocorticoid hormone action has increased during the last 10 years, but a lot of questions still remain unanswered. The precise roles of hsp90/receptor complex formation, of the bound hormone and of receptor phosphorylation have to be determined. Detailed mechanisms for gene activation and repression are still unknown. Interactions with other cellular components, also other transcription factors, have to be characterized in detail. Each step of the signal transduction pathway from receptor gene transcription to the action of DNA-bound hormone-receptor complex may be subject to its own regulation.

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