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Mechanisms of Transcriptional Activation by Steroid Hormone Receptors

Aria Baniahmad and Ming-Jer Tsai

Department of Cell Biology, Baylor College of Medicine, Houston, Texas 77030

Abstract Steroid hormones are involved in cell growth, development, and differentiation. The hormonal signal is mediated by nuclear receptors which represent a specific class of transcription factors. During the last few years, the cloning of all the major steroid hormone receptors increased our insight into how the hormonal signal converts the receptor into a transcriptional activator. Good progress has been made towards understanding the mechanism of steroid hormone action. In this review we will discuss the role of heat shock proteins in the process of transcriptional activation, the mechanistic differences between the hormone (agonist) and the antihormone (antagonist), the resulting functional consequences, and a possible mode by which transcriptional activation is mediated. © 1993 Wiley-Liss, Inc.

Key words: steroid hormone receptors, antihormones, conformational change, gene transcription

CELL FREE SYSTEMS AND THE ROLE OF HORMONE

Members of the steroid/thyroid hormone receptor family are nuclear receptors which modulate gene activity in a hormone-dependent manner. This family includes receptors for steroid hormones, vitamin D, thyroid hormone, and retinoic acid. In addition, the so-called "orphan receptors" for which the putative ligand has not yet been identified belong to this family due to homologies in their amino acid sequences [for review see Evans, 1988; Beato, 1989; O'Malley, 1990]. To exert their diverse effects on reproduction, development, and differentiation, the hormone receptors link extracellular signals directly to transcriptional regulation [for review see Wahli and Martinez, 1991; Tsai et al., 1991]. The hormone response is mediated by binding of the ligand to its receptor, followed by the binding of the receptor-ligand complex to specific regulatory sequences, referred to as response elements, at the target genes. However, the mechanism by which the receptor regulates gene transcription remains largely unknown. One approach to analyzing the hormone-induced gene activation pathway is to reconstitute hormone- and receptor-dependent gene activation in vitro. Such a cell free system enables one to define precisely the conditions and the necessary minimal number of components for receptor action. It has allowed us to analyze the role of heat shock proteins (hsps) associated with the receptor, the detailed role of hormone and antihormone in the process of transactivation, and the mechanism of how transcriptional activation by steroid receptor occurs.

In vitro transcription from a target promoter regulated by a transcription factor belonging to the steroid receptor superfamily was first described for COUP-TF (chicken ovalbumin upstream promoter-transcription-factor) [Sagami et al., 1986]. This purified orphan receptor stimulated the synthesis of correctly initiated RNA from an ovalbumin promoter in HeLa cell nuclear extracts. Steroid receptor dependent cell free transcription systems were subsequently established using highly purified chicken progesterone receptor (PR) and HeLa nuclear extract. PR showed stimulation of a reporter, which contained the ovalbumin gene TATA box and multiple copies of a progesterone response element (PRE) [Klein-Hitpass et al., 1990; Bagchi et al., 1990]. Kalff et al. [1990] and Freedman et al. [1989] showed that the rat glucocorticoid DNA binding domain expressed in *E. coli* and PR isolated from rabbit uterus stimulated transcription of an MMTV-promoter in a cell free system. In these cases, transcriptional activation was dependent on the presence of a receptor binding site. Subsequently, a cell free system

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Address reprint requests to Ming-Jer Tsai, Department of Cell Biology, Baylor College of Medicine, Houston, TX 77030.

was developed for other steroid receptors, including the human glucocorticoid receptor (hGR) [Tsai et al., 1990] and the mouse estrogen receptor (ER) [Elliston et al., 1990] produced from insect cells with the baculovirus expression system. All these receptors strongly enhanced (greater than thirtyfold) gene activity in vitro.

A hormone dependent cell free transcription system, however, was first reported by Corthesy et al. [1988]. The sole addition of estradiol was sufficient to enhance RNA synthesis from a vitellogenin promoter in *Xenopus* liver nuclear extracts. In our laboratory, we were able to show progesterone-dependent activation of cell free transcription using extracts from the human breast cancer cell line T47D. Transcription could be induced efficiently after addition of 10–100 nM hormone [Bagchi et al., 1990a]. Similar results were obtained with human and chicken PR expressed in the baculovirus system [Elliston et al., 1992]. Thus, analogous to the in vivo situation, it is possible to activate gene transcription in a cell free system in both a hormone- and receptor-dependent manner. The necessary DNA elements for gene activation are only a PRE and a TATA box, suggesting that transcriptional activation by steroid receptors can be mediated without additional promoter elements.

This cell free system enabled us to analyze the role of hsp's in the process of transactivation in more detail. The ligand free PR, like the other steroid hormone receptors, GR, ER, and the androgen receptor (AR), exist in an 8-10S complex in association with the hsp 90, hsp 70, and hsp 56 [Sanchez et al., 1985; Denis et al., 1987; Kost et al., 1989; Tai et al., 1986; Sanchez et al., 1990; Sanchez, 1990b] (see Fig. 1). Interestingly, hsp 56 was recently shown to have rotamase activity [Tai et al., 1992]. Hormone is believed to release the heat shock proteins from the receptor and to transform the receptor into a transcriptionally active form [Denis et al., 1988; Pratt et al., 1987]. To examine whether the release of the hsps from the receptor indeed results in a constitutively active receptor, we purified from T47D cells a hormone free receptor devoid of hsp90, 70, and 56 [Bagchi et al., 1990a]. In a cell free with HeLa nuclear extract reconstituted transcription system this purified receptor still required hormone for binding to its response element and for transcriptional activation. This clearly shows that there is an additional hormone-dependent step required after release of hsps from the receptor to achieve the biologically active form.

ACTION OF HORMONES AND ANTIHORMONES

The role of antihormone in the process of transcription is poorly understood. Addition of the antiprogestin and the antiglucocorticoid RU486 completely blocked transactivation by rabbit PR [Kalff et al., 1990]. In general, RU486 allows the receptor to bind with high affinity and specificity to its response element in vitro [El-Ashry et al., 1989; Bagchi et al., 1988, 1990b] and in vivo [Guiochon-Mantel et al., 1988]. Therefore, the block of activation has to occur at a different level after hsp dissociation and DNA binding. How then does the antihormone produce a transcriptionally inactive receptor? One possibility is that antihormone prevents phosphorylation of the receptor, which might be an important step in the normal transactivation process. Following hormone treatment in vivo or in vitro PR becomes hyperphosphorylated [Bagchi et al., 1992]. Using the antihormone RU486, however, similarly extensive hyperphosphorylation occurred. Although the phosphorylation pattern of the receptor might be different, this suggests that antagonism by the antihormone is not likely due to prevention of phosphorylation of the receptor.

Another possibility would be that antihormone and hormone change the structure of the receptor in different ways. There is an indication from band shift experiments showing that an antihormone-receptor-DNA complex migrated slightly faster than a hormone-receptor-DNA complex [El-Ashry et al., 1989]. In order to directly show a conformational distinction, we used a different approach. Labeled in vitro translated full-length human PR was produced and shown to be in an 8-10S complex. Addition of hormone converted it to the DNA binding 4S complex. The in vitro translated receptor bound hormone with similar affinity and specificity compared to the purified receptor. This receptor bound DNA hormone dependently and with high specificity, which was shown to be a characteristic of PR [Guiochon-Mantel et al., 1988; Meyer et al., 1990; Bagchi et al., 1990b].

We used limited proteolytic digestion of translated receptor to detect conformational changes by hormone or with the antihormone RU486. Interestingly, progesterone treatment induced a dramatic conformational change in the receptor

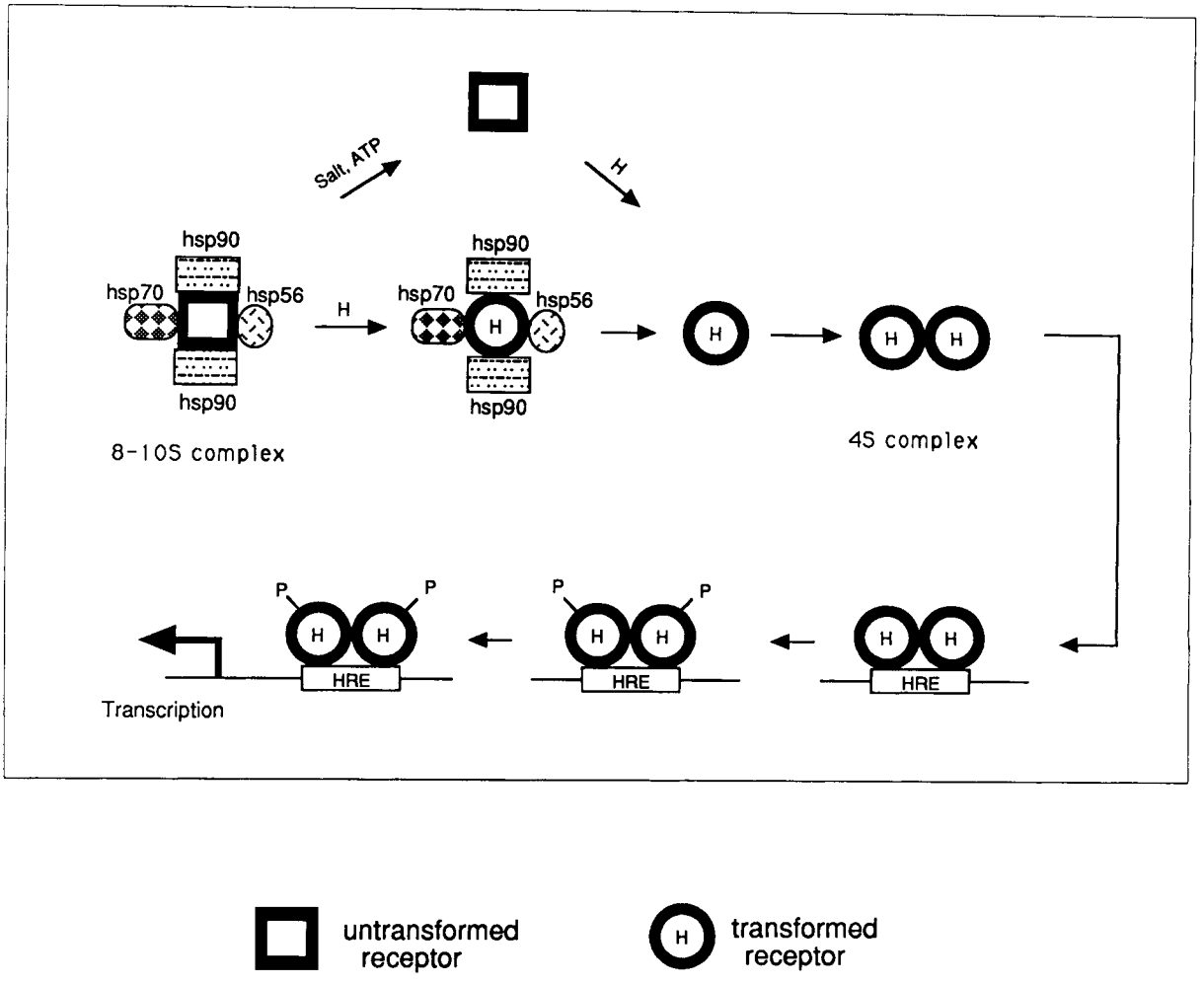

Fig. 1. Role of hormone in steroid receptor activation. Progesterone receptor exists in a transcriptionally inactive 8-10S complex in association with heat shock proteins hsp90, hsp70, and hsp56 in the absence of hormone. Addition of hormone (H) changes the conformation of the receptor followed by dissociation of hsps. However, a receptor free of hsps, which can be achieved after salt and ATP treatment, still requires hormone for transcriptional activation. The hormone loaded receptor binds to its response element (HRE), becomes phosphorylated (P), and activates gene transcription (represented by an arrow).

structure. A prominent protease-resistant band of about 30 kD was observed after addition of hormone [Allan et al., in press]. This resistant fragment was not dependent on the presence of DNA. Antihormone induced an equally dramatic but distinct change in the receptor conformation. A protease resistant band of about 27 kD was induced by the antihormone RU486. Similar results were observed with other agonists (R5020 or RU27987) or antagonists (Org 31376 and Org 31806) [Allan et al., in press]. To generalize these conclusions, the estrogen receptor was analyzed similarly after addition of the corresponding agonists and antagonists. The structure of this receptor was also changed dramatically, the protease resistant fragment induced by antihormones being 2 kD smaller than that induced by agonists. Thus, the natural ligand of a steroid receptor induces a dramatic conformational change in the receptor compared to the ligand free form. The conformational change induced by antihormones, however, is different. These data are consistent with our hypothesis that antihormones such as RU486 block transcriptional activation by inducing a different receptor structure. This emphasizes that a specific conformational change of the receptor has to precede transcriptional activation.

It was important to determine where in the receptor the conformational change occurred and, most of all, what part of the receptor was

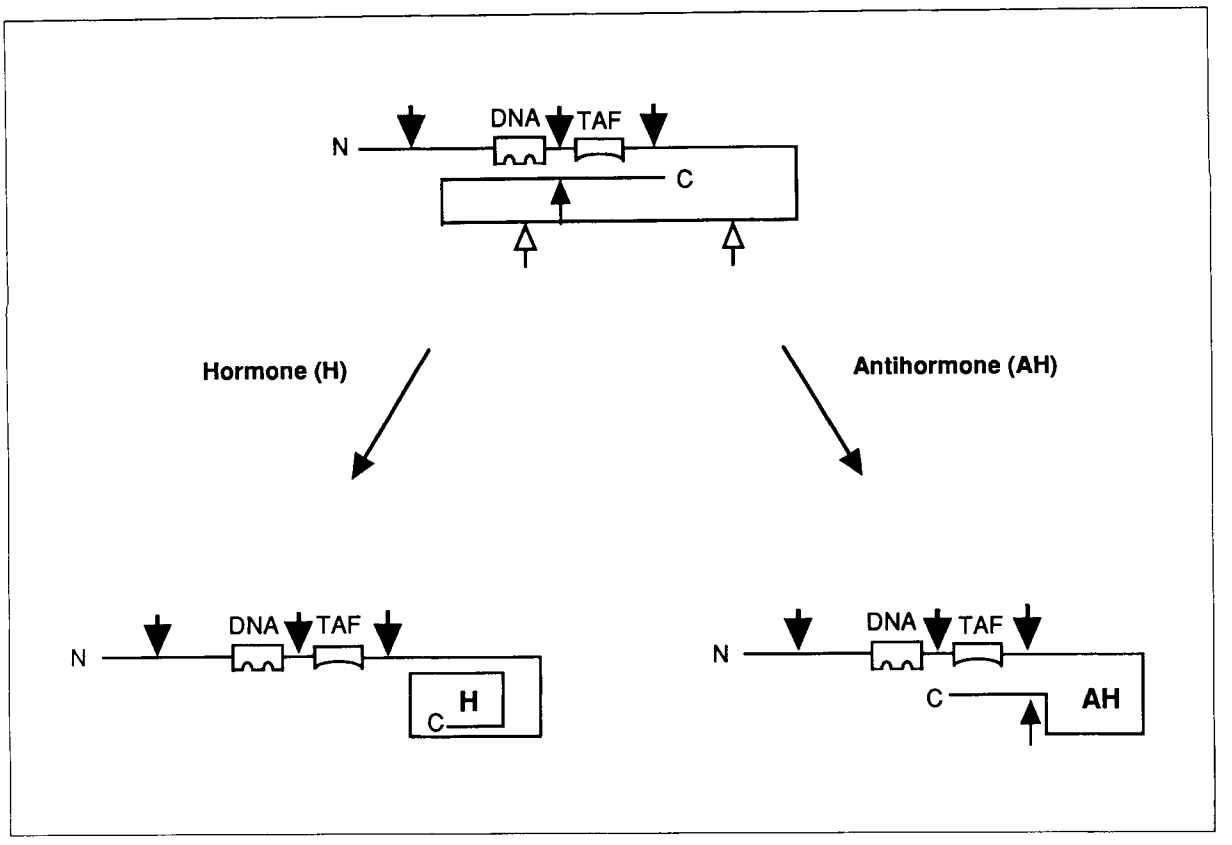

Fig. 2. A model for distinct interactions of the progesterone receptor with hormone and antihormone. In the absence of hormone, the C-terminal tail of the receptor inhibits intrinsic transactivation (TAF) and DNA binding activites. Hormone (H) interacts with the ligand binding domain and thereby relieves the repression. Antihormone (AH), however, induces a different conformational change, allowing DNA binding but not transactivation. Arrows represent putative protease cleavage sites.

involved in the distinction between hormone and antihormone. For this purpose we took advantage of various antibodies obtained from Dean Edwards, which recognize different parts of the receptor for immunoprecipitation assays after limited proteolytic digestion. One antibody, C-262, recognized the 30 kD but not the 27 kD proteolytic fragment, which appeared only after antagonist treatment. This antibody was raised against the last 14 amino acids (aa) of the carboxy-terminal (C-terminal) domain of PR. In addition, this antibody bound to the full-length receptor only in the presence of antihormone, whereas progesterone prevented the recognition of the receptor by the C-262-antibody [Vegeto et al., 1992]. This suggests that the C-terminus is accessible after antihormone treatment. Thus, we have been able to show that the ligand-induced structural change occurs in the ligand binding domain. The hormone-specific resistant fragment consisted of the entire ligand binding domain and possibly the hinge region. Furthermore, the distinction in the conformational structure of hormone and antihormone bound PR is located at the very C-terminal end of the receptor. These results led to the hypothesis that antihormones such as RU486 functionally antagonize because of a different conformation at the C-terminus of steroid hormone receptors (Fig. 2). The C-terminus is accessible to both proteases and antibody and therefore may be not necessary for the binding to antihormones. If that hypothesis is correct, C-terminal deletion of the receptor will still allow binding of the antagonist but not of the agonist.

Deletion analysis of small stretches of amino acids at the very C-terminus of the hPR led to a receptor with altered hormone responsiveness. Deletion of 42 aa abolished the binding of progesterone but had no effect on binding of the antagonist RU486 [Vegeto et al., 1992]. Functional characterization showed that this receptor mutant induced transcription upon addition of RU486 but not with its natural hormone

progesterone [Vegeto et al., 1992]. Our finding that the receptor undergoes different structural changes is strongly supported by this functional analysis. Thus, we can conclude that the conformation of the C-terminus determines the transcriptional activity of steroid hormone receptors. This leads to the hypothesis presented in Figure 2, that the C-terminus of hPR contains an inhibitory domain that directly blocks the transactivation domains of the receptor or perhaps interacts with an additional, as yet unidentified factor, which keeps the receptor in an inactive form. Hormone removes the inhibitory effect by binding to the C-terminal end [Vegeto et al., 1992].

STEROID HORMONE RECEPTOR INTERACTION WITH BASAL TRANSCRIPTION FACTORS

The mechanism of transcriptional activation by steroid hormone receptors is largely unknown. The basal transcription factor TFIID has been implicated as a target for transactivation [for review see Greenblatt, 1991]. Recombinant TFIID (TBP) has been shown to directly interact with the acidic activation domain of the herpes virus VP16 [Stringer et al., 1990] and the adenovirus E1a proteins [Lee et al., 1991; Horikoshi et al., 1991]. Recent work shows that the binding of TFIIB seems to be the rate limiting step in the process of transcriptional activation [Lin and Green, 1991a]. Furthermore, recombinant human TFIIB also interacts directly with the VP16 activation domain [Lin et al., 1991b], suggesting that both TFIIB and TFIID may be targets of gene activation. It was shown that PR, GR, and ER stabilize the formation of preinitiation complexes on DNA [Klein-Hitpass et al., 1990; Tsai et al., 1990; Elliston et al., 1990]. An insight into how that stabilization may occur derived from studies with the orphan receptor COUP-TF. Purified COUP-TF needed an additional factor (the S300-II factor) for full activation of the ovalbumin promoter in a cell free transcription system. S300-II itself did not bind to DNA in a sequence specific manner, but it stabilized the binding of COUP-TF to its response element. Cloning and sequencing of the S300-II factor showed, surprisingly, that it is the general transcription factor TFIIB [Ing et al., in press]. Protein-protein interaction studies revealed a specific and a direct interaction between COUP-TFI and S300II/TFIIB [Ing et al., in press]. In addition, we showed that other members of the steroid receptor family such as the progesterone and estrogen receptor also interact specifically with recombinant TFIIB. Using an estrogen receptor amino-terminal deletion mutant expressed in *E. coli* and crude HeLa nuclear extract as a natural source of TFIIB, we found that TFIIB was selectively retained by the estrogen receptor bound to a column with the glutathione S-transferase system [Smith and Johnson, 1988]. TFIIB was eluted at a high ionic strength of 270 mM [Ing et al., in press], indicating that high stringency was needed to disrupt the protein-protein interaction. This suggests that members of the steroid receptor superfamily interact specifically with the general transcription factor TFIIB. This type of interaction may facilitate the process of transcriptional activation of responsive genes.

FUTURE PROSPECTS

Although significant advances have been made, many questions regarding the regulation of gene transcription by steroid hormone receptors remain unsolved. Some major questions are: How does interaction with basal transcription factor activate gene transcription? What is the role of putative adaptors or coactivators? What is the role of phosphorylation or dephosphorylation? How does the newly identified inhibitory domain of PR function? Once a gene has been induced, what is the turn-off mechanism? The dissection of proteins into functional domains, combined with the use of cell free transcription with purified factors or recombinant proteins, will allow us to answer these questions in the near future.

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