Biochemical Pharmacology, Vol. 38, No. 19, pp. 3135–3143, 1989.
Printed in Great Britain.

0006-2952/89 $3.00 + 0.00

COMMENTARY

STEROID RECEPTORS

HOW TO BE BOTH A RECEPTOR AND A TRANSCRIPTION FACTOR

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Steroid hormones regulate a variety of developmental and physiological responses in organisms as diverse as fungi and humans. These effects are mediated by intracellular steroid receptor proteins which bind specifically and with high affinity to their cognate ligands. This interaction facilitates a temperature-dependent alteration in the receptor termed "transformation", resulting in the binding of the receptor to specific chromosomal sites and the regulation of gene expression (Fig. 1). Steroid receptors induce transcription when bound to DNA sequences termed hormone response elements (HREs) and inhibit transcription when bound to sequences termed negative HREs (nHREs). Hormone-responsive genes often encode enzymes and other regulatory proteins that determine developmental, metabolic or secretory activities of target cells. In this way, a complex series of changes can be initiated by altering the concentration of a single steroid hormone.

In this review, we will summarize some of the recent data concerning three key questions regarding steroid hormone action: (1) what are the functional domains of steroid receptors; (2) how does steroid binding regulate the activity of the receptor; and (3) how does the receptor regulate gene transcription. Because of space considerations it is impossible to review all of the data concerning these issues. Instead, we will focus primarily on recent studies on the structure and function of the glucocorticoid and the estrogen receptors. Additional information and some alternative viewpoints can be found in recent reviews [1–4].

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STRUCTURE OF STEROID RECEPTORS

Complementary DNA (cDNA) clones encoding a number of steroid receptors have been isolated, enabling detailed studies on the structure and function of these proteins. Analysis of these cDNAs showed that the sequence of a particular receptor is highly conserved between species, and that receptors for different hormones also share regions of homology. Based on their degree of homology, steroid receptors have been divided into six regions, A–F [5], as shown in Fig. 2. The observation that the most highly conserved region C contains homology with the viral oncogene *erbA* led to the discovery that the *erbA* proto-oncogene encodes a receptor for thyroid hormone [6, 7]. Using a variety of approaches, cDNAs encoding a number of related proteins have been cloned. These include the vitamin D_3 receptor, a second receptor for thyroid hormone, a receptor for the vertebrate morphogen retinoic acid, a protein related to the retinoic acid receptor, and two estrogen-receptor related proteins [2]. Thus, these proteins are members of a large family of ligand-activated

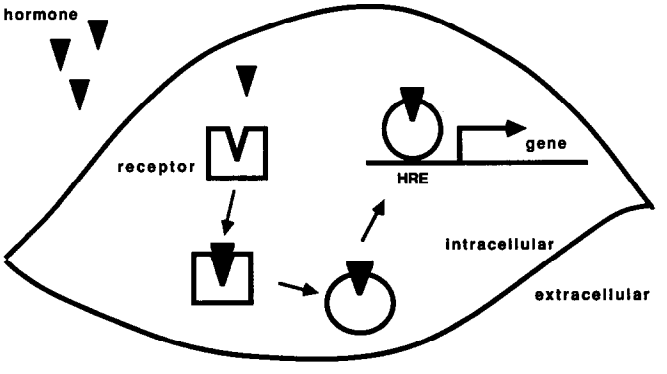

Fig. 1. Schematic representation of signal transduction by ligand-activated transcription factors.

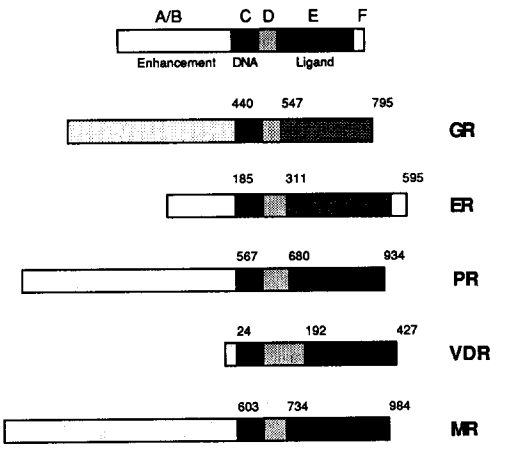

Fig. 2. Schematic representation of the ligand-activated transcription factor (LTF) family. LTFs have been divided into six regions (A–F, top) based upon the degree of their amino acid homology. Regions involved in DNA or hormone binding are indicated. For some LTFs, region A/B has been shown to mediate transcriptional enhancement. Comparisons of the primary sequence of the rat glucocorticoid receptor (GR), human estrogen receptor (ER), human progesterone receptor (PR), human vitamin D_3 receptor (VDR), and the human mineralocorticoid receptor (MR) are shown. The amino acid residues defining the boundaries of the A/B and C domains, the D and E regions, and the C-terminal residue are numbered. In each case, region C contains 66 amino acids.

transcription factors (LTFs) that may have evolved from a common ancestoral gene.

DNA binding domain

Rusconi and Yamamoto [8] showed that the DNA binding domain of the rat glucocorticoid receptor (GR) is located between amino acids 440 and 525. This includes the highly conserved region "C" of the nuclear receptor gene family as well as a portion of region D that contains an abundance of basic amino acids. When region C of the estrogen receptor (ER) is replaced with the corresponding region C of the GR, the chimeric receptor binds to glucocorticoid response elements (GREs) in the presence of estradiol [9]. Thus, region C is responsible for specific DNA binding. Mutational analyses indicate that region D is not required for specific binding, but it affects the affinity of the receptor for DNA [8]. In the LTF family, region C varies from 66 to 68 amino acids, and contains 20 invariant residues including 9 cysteines. Region C contains two motifs that are variations of "zinc fingers" identified in other nucleic acid binding proteins such as the *Xenopus* transcription factor IIIA (TFIIIA) [10, 11]. Indeed, Freedman *et al.* [12] have shown that region C of the GR does coordinate two Zn(II) ions in a tetrahedral fashion. In contrast to the TFIIIA finger structure, in which the zinc ion is coordinated by a pair of cysteines and a pair of histidines, LTF zinc fingers appear to coordinate the metal by two pairs of cysteines.

Based on these results, as well as on mutational analysis of the receptor DNA-binding domain [13], a metal coordination scheme for LTFs has been proposed (Fig. 3). The proximal finger is rich in hydrophobic residues, whereas the distal finger is smaller and contains numerous basic amino acids. Where information is available regarding the genomic structure of the particular receptor gene, it has been found that each finger is encoded by a different exon (Refs. 14–16; and M. Jacobson and K. R. Yamamoto, personal communication*). A third exon encodes the arginine and lysine rich region following the second finger. It seems likely that the receptor DNA binding domain may have evolved from smaller, functionally distinct units. In fact, Green *et al.* have shown that the amino acids important for specific DNA binding are largely encoded by exon CI; they propose that the distal finger contributes to DNA binding by non-specific interaction with DNA and by facilitating dimerization of the receptor [17].

N-terminal domain

The A/B region of the LTF family is the least conserved and ranges from 24 to 603 amino acids (vitamin D_3 receptor and mineralocorticoid receptor respectively). This region of the rat GR is encoded by a single exon and is separated from the exons of

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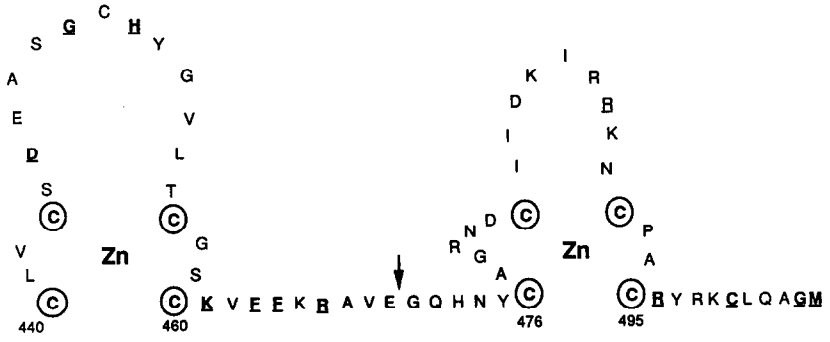

Fig. 3. Predicted structure of the DNA binding domain of the LTF family. The amino acid sequence in single letter code of residues 440–505 of the rat GR is shown. For each finger, two pairs of cysteine residues are proposed to coordinate a single zinc atom (Zn). These 8 cysteines are conserved in all members of the LTF family identified so far. Other residues that are conserved are underlined. The DNA binding domain is split into two exons, CI and CII; the boundary of these exons is indicated by the arrow.

the DNA binding domain by an intron of >100 kb (M. Jacobson and K. R. Yamamoto, personal communication*). Given this extensive divergence, the A/B region of different LTFs will likely exhibit functional differences. The A/B region may also confer variability within a particular receptor type in that the mRNAs for the progesterone receptor (PR), the GR and possibly the ER appear to utilize a number of sites to initiate translation, resulting in receptor subfamilies differing at their amino termini [18–20]. The mRNA encoding the chicken PR is translated into a 786 amino acid full length species (form B) and an internally initiated species (form A) which lacks the N-terminal 127 amino acids. While both forms efficiently induce transcription by binding to the mammary tumor virus (MTV) LTR, only the smaller form A induces expression from the ovalbumin gene [21]. Thus, the N-terminal 127 amino acids contained in form B appear to influence target gene activation. It is unclear if the PR form B is defective for binding to the ovalbumin PRE or if it fails to enhance transcription when bound. Analysis of the ER provides further evidence that the A/B region affects target gene activation. Deletion of the ER A/B region has little effect on the estrogen-regulated expression of the vitellogenin gene, but it blocks induction of the S2 gene [22].

The mechanism by which the A/B region influences target gene activation remains obscure. For the GR, genetic experiments discussed below show that a "transcriptional activation domain" is contained within this region. However, biochemical approaches suggest that this domain can also influence target gene activation indirectly by affecting the ability of the GR to bind DNA. GR derivatives lacking the A/B region exhibit an increased affinity for non-specific DNA and a reduced affinity for specific binding sites resulting in at least a 200-fold net reduction in binding selectivity [23, 24]. We note that similar explanations may account for the differences in target gene activation by PR and ER derivatives described above. If the A/B region contributes to selective ERE binding by the ER, then derivatives lacking this region may not bind low affinity EREs *in vivo*. In fact, the ER does have a lower affinity for the S2 ERE than for the vitellogenin ERE [25].

Structure of the ligand-binding domain

LTFs bind molecules as different as steroid hormones and retinoic acid. Nevertheless, the ligand binding domain is the second-most conserved domain amongst all known members of the LTF superfamily. The hormone binding domains occupy approximately 25 kD at the receptor C-termini and are generally rich in hydrophobic amino acids [2–4].

The GR, the PR, and the thyroid receptor (TR) ligand binding domains are encoded by several exons ([14–16, 26, 27]; M. Jacobson and K. R. Yamamoto, personal communication*), raising the possibility that they evolved as a composite of distinct subdomains. However, the integrity of the entire domain is important for hormone binding. Deletion of just a few amino acids from the C-terminus of the GR drastically reduces the binding affinity, and most point or insertion mutations throughout the domain are deficient if not fully defective for ligand binding [8, 28, 29]. Indeed, even sequences N-terminal to the conserved region of the GR steroid binding domain, while not necessary for binding *per se*, influence the binding affinity [8]. In contrast to the GR, the ER contains a short segment of about 40 amino acids (domain F) at the extreme C-terminus of the receptor that appears to be dispensable for hormone binding and receptor activity in general [5, 22]. In the future, the identification and characterization of such "tails" in other members of the LTF family may help to shed some light on the role of this extra domain.

Consistent with the apparent complexity of the ligand binding domain, three widely separated residues in the rat GR have been cross-linked to bound glucocorticoid agonists: dexamethasone mesylate through its reactive mesylate group at the 21-position to Cys-656 [30–32], and triamcinolone acetonide by UV cross-linking to Met-622 and Cys-754 [32]. A comparison of the steroid binding domains of PR and GR reveals a 47% sequence identity as well as a remarkably similar hydropathy profile with the notable exception of the region around Cys-656. Indeed, there is considerable cross-reactivity with respect to hormone binding between the GR and the PR but not the ER whose hydropathy profile and respective steroid ligand are quite different. Given that glucocorticoids differ from progestins by the presence of a hydroxyl group at the 21-position, it has been proposed that the hydrophobic pockets around Met-622 and Cys-754 interact with the A ring common to both progestins and glucocorticoids whereas Cys-656 interacts with the glucocorticoid-specific side group at the 21-position [32].

Steroidogenic enzymes and steroid binding proteins might be expected to share with steroid receptors the portion of the steroid binding pocket that is involved in recognizing the common determinants of steroid hormones. In fact, sequences matching the consensus polypeptide sequence

$$L\begin{matrix}P\\L\end{matrix}LL \pm 000\begin{matrix}R\,D\\K\,E\end{matrix}0L\begin{matrix}R\\K\end{matrix}L \pm PV$$

(0, any uncharged amino acid; ±, any charged amino acid) can be found in a number of steroidogenic enzymes, in sex steroid binding proteins, and in steroid receptors [33]. In the rat GR, for example, it maps to a segment around amino acid 700. A notable exception is the corticosteroid binding protein (CBG), which lacks a segment with significant homology to the consensus sequence. As the functional dissection of CBG proceeds, it will be interesting to see whether other structural similarities with the GR can be identified that are not readily detectable at the level of the primary sequence.

Ligands have yet to be found for several putative LTF cDNA clones that were isolated by low-stringency hybridization with probes specific for region C (DNA binding domain). The assumption that such ligands exist is based on the finding that they also share significant homology with other LTFs within the C-terminal region. For example, several cDNAs have been cloned which encode variants of the TR

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but are unable to bind thyroid hormone ([26, 27]; see also Refs 2 and 3). As the result of differential mRNA splicing, the C-terminal portion of the hormone binding domain has been replaced with other sequences. While it is possible that an as yet unidentified ligand binds to this type of chimeric hormone binding domain, it is also worth considering that there could be members of the LTF family which do not bind any ligand. A special case of such a protein is v-*erbA*. Unable to bind hormone due to a number of point mutations in the thyroid hormone binding domain [34], it appears to be a constitutively active derivative of its normal cellular counterpart c-*erbA* (=TR).

LIGAND REGULATION OF RECEPTOR ACTIVITY

Hormonally induced transformation of receptors

Biochemical analyses have established that steroid receptors undergo a poorly understood temperature-dependent alteration following hormone binding, a process termed "transformation". Operationally, transformation converts receptor from a 9S to a 4S complex in sucrose gradients and confers upon receptor the ability to bind to DNA *in vitro* and to bind to nuclei in a relatively salt-resistant manner [1]. In addition to the untransformed receptor, the 9S complex as opposed to the 4S complex contains the heat shock protein hsp90 [35–39], a very abundant cytosolic protein. Receptor phosphorylation and oligomerization have also been proposed to play important roles in receptor transformation [40, 41]. Partly because of the difficulty of purifying unliganded receptors, the mechanism by which the hormone effects receptor transformation has remained unknown. Recent developments promise to shed some light on it and are discussed in the following paragraphs.

Direct regulation of several receptor activities by the hormone binding domain

Picard and Yamamoto [42] have shown that the GR is cytoplasmic in the absence of hormone and that it rapidly localizes to the nucleus upon hormone binding and transformation. However, hormonal control of the DNA binding/transcriptional regulatory activities was maintained even under conditions in which GR was constitutively nuclear. This result shows that nuclear localization of the GR is necessary but not sufficient for signal transduction by the receptor and that the DNA binding and/or the transcriptional regulatory functions are regulated directly, consistent with the early finding that *in vitro* DNA binding by the GR is hormone-dependent ([43]; see also Ref. 44). Interestingly, the ER and the PR are nuclear proteins even in the absence of hormone [discussed in Ref. 42]. The significance of the cytoplasmic localization of the unliganded GR remains unclear. It is conceivable that the GR fulfills a cytoplasmic function as well which would be active in the absence of hormone. Alternatively, the cytoplasmic localization of unliganded GR may facilitate its encounter with hormone and/or determine the kinetics of the hormone response.

Two signals, NL1 and NL2, which mediate nuclear localization have been mapped in the GR [42]. NL1 is a short segment which mediates constitutive nuclear localization of an unrelated protein such as β-galactosidase; NL2 overlaps with the steroid binding domain and is strictly dependent on hormone for activity. Remarkably, NL1 mediates nuclear localization of receptor derivatives lacking the steroid binding domain, but appears to be repressed in the context of the full-length receptor in the absence of hormone. This explains why the unliganded receptor is cytoplasmic.

Mechanism of hormone regulation

The finding that deletion mutants of the GR lacking >190 C-terminal amino acids [45] or a large internal portion [46] of the steroid binding domain are both constitutively nuclear and transcriptionally active came as a big surprise. This suggested that the nuclear localization and the DNA binding and/or the transcriptional regulatory functions may be repressed in the absence of hormone rather than induced by an allosteric effect upon hormone binding.

Indeed, the functional domains of both the GR and the ER can be grossly rearranged without loss of activity. The transcriptional regulatory activity of derivatives in which the steroid binding domain has been moved from its C-terminal position to the N-terminus are still subject to hormonal control [47, 48]. Furthermore, hormone regulation can even be conferred upon a heterologous protein. The DNA binding activity of the *LexA* DNA binding domain ([49]; and D. Picard, unpublished results) and the transcriptional regulatory activity of the adenoviral E1A protein [47] become hormone-dependent by fusion to the steroid binding domain of the GR. Thus, in addition to the hormone binding and nuclear localization activities, a "protein inactivation" function also resides within the steroid binding domain of the GR. As a "regulatory cassette", it can repress several activities resident on the same polypeptide in a hormone-reversible fashion.

To account for these findings, and to incorporate the biochemical data, it has been proposed [47] that the inactivation by the hormone binding domain is mediated by hsp90 (Fig. 4). Consistent with this view, hsp90 appears to associate selectively with the unliganded hormone binding domain [35–39], and the DNA binding and transcriptional regulatory activities of the GR correlate inversely with its association with hsp90 [38, 46, 50]. Thus, hsp90, alone or in conjunction with additional as yet unidentified factors, may interfere with receptor function by steric hindrance; alternatively, hsp90 could cause the unliganded receptor to assume or to maintain an unfolded conformation. The "unfoldase" model ascribes to hsp90 an activity similar to that proposed for certain other heat shock and related proteins [discussed in Ref. 47]. Furthermore, it implies that the unfolded conformation is the active one for the steroid binding domain itself. Hormone binding presumably triggers a conformational change in the steroid binding domain resulting in the release of hsp90. According to the "unfoldase" model, this would allow appropriate folding and thus derepression of the remainder of the polypeptide chain. A key feature of these models is that the mechanism

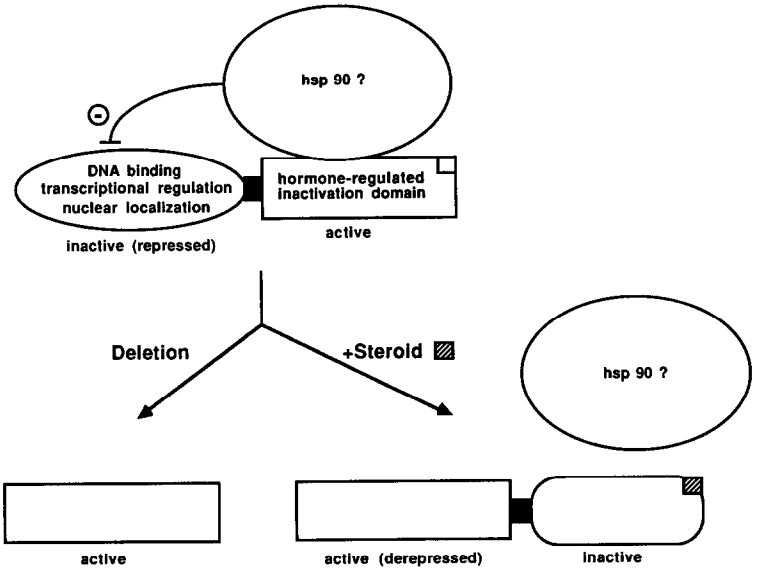

Fig. 4. Model for inactivation of receptor functions by the unliganded hormone binding domain. The heat-shock protein hsp90 is proposed to mediate this novel inactivation function. This figure is modified from Fig. 5 in Ref. 47.

of signal transduction is based on a rather unspecific inactivation function. In contrast to a mechanism involving allosteric regulation, this would clearly allow different regulatory domains to evolve more freely. Both models are consistent with the notion that the interaction with hsp90 may also be required to maintain the steroid binding domain in a favorable conformation for hormone binding.

A test of these ideas will require characterization of the receptor–hsp90 interaction. Given that mammalian ER [51] and GR ([52], and M. J. Garabedian and K. R. Yamamoto, personal communication*) work in yeast in a hormone-dependent fashion, these questions can now be addressed with powerful genetic means.

TRANSCRIPTIONAL REGULATION BY STEROID RECEPTORS

In this section, we shall try to summarize the current information regarding the nature of the DNA binding sites for steroid receptors and the functions that steroid receptors mediate when bound to DNA.

The HRE, an inducible enhancer element

It was shown initially that purified rat liver GR bound with high affinity to specific regions of the mammary tumor virus (MTV) DNA [53–56]. These binding sequences conferred hormone-regulated transcriptional enhancement when linked to heterologous promoters and were therefore termed glucocorticoid response elements or GREs [57]. Subsequently, response elements for other LTFs were identified; together these are referred to as hormone response elements (HREs) [2]. The consensus binding sites for some GREs and EREs (GGTACANNNTGTTCT and GGTCANNNTG$^{A}_{T}$CC respectively) are near or perfect palindromes separated by three base pairs [58–60]. Martinez *et al.* [61] have shown that mutation of just two base pairs is sufficient to convert an ERE to a GRE. The ER and the GR appear to bind as a dimer to their respective HRE, consistent with the palindromic nature of these binding sites ([24, 62]; and J. LaBaer and K. R. Yamamoto, personal communication*).

More recently, Sakai *et al.* [63] showed that the GR also binds specifically to sites within the promoter for prolactin, a gene whose transcription is repressed by glucocorticoid hormones. These sites confer hormone-mediated repression when linked to heterologous promoters, and have been termed negative GREs or nGREs. nGREs show little sequence homology with GREs, although the range of apparent affinities of purified GR for nGREs is similar to that observed for GREs identified in MTV DNA. Thus, the GR appears to bind GREs and nGREs with similar affinities but elicits dramatically different effects.

Transcriptional activation domains of steroid receptors

Three types of experiments, deletion mapping, "domain swapping", and targeted mutagenesis have been utilized to determine the regions of steroid receptors involved in transcriptional activation. The results suggest that some receptors contain multiple activation domains and that the role of a particular domain within a receptor may vary depending on the target gene.

Deletion analysis showed that expression of a small region of the GR termed *enh1*, which overlaps the

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DNA binding domain, is sufficient to mediate transcriptional enhancement at GREs, albeit at levels of only a few percent of the intact protein [64]. Deletions or mutations within the A/B region of the GR result in reduced levels of transcriptional activation, suggesting that this domain modulates transcriptional enhancement [28, 64]. However, the analysis of A/B deletion or insertion mutations does not permit direct evaluation of the function of this region. For example, the A/B region may act indirectly by affecting the avidity of DNA binding by the LTF or directly, perhaps by interacting with other components of the transcriptional machinery.

We adopted an alternative approach to determine directly if the A/B region of the GR contained a transcriptional activation function. Chimeric genes were constructed that encoded proteins containing portions of the GR fused to the DNA-binding domain of the bacterial *LexA* protein [49]. *LexA* itself does not stimulate transcription when bound to its cognate binding site. However, proteins containing the receptor A/B region fused to *LexA* induce transcription from promoters containing *LexA* binding sites. The ability of the A/B region to confer a transcriptional activation function upon a heterologous DNA binding domain is direct proof that this region contains an activation function. This activity was further localized to a 213 amino acid portion of the A/B region termed "*enh2*". *Enh2* does not exhibit strong amino acid homology with other members of the nuclear receptor family, nor with activation domains identified in the yeast regulatory proteins *GAL4* or *GCN4*. However, like the *GAL4* and the *GCN4* activation domains, *enh2* contains an abundance of negatively charged amino acids (see Ref. 65 for review). It is noteworthy that the mineralocorticoid receptor and the PR contain regions with a similar charge distribution; whether these function as "activation domains" remains to be determined.

Using a similar strategy, Webster *et al.* [48] have shown that the hormone binding domains of the ER and the GR also contain activation domains, which we refer to as *enh3*. *Enh3* functions when the hormone binding domain is occupied by agonists but not by antagonists, suggesting that only an agonist can induce the proper conformation of *enh3*.

What is the purpose of this functional redundancy in activation domains? It is possible that these domains function by a similar mechanism to increase the overall efficiency of transcriptional enhancement by the GR. Alternatively, *enh1*, *enh2*, and *enh3* may interact with different components of the transcriptional machinery, perhaps leading to promoter- or cell-specific activation.

Non-receptor factors involved in transcriptional enhancement

Eukaryotic promoters contain binding sites for multiple factors; some factors are common to nearly all genes transcribed by RNA polymerase II (general transcription factors), whereas others are found in only a subset of these genes. In principle, steroid receptors may enhance transcription by facilitating the binding of a transcription factor to its target site and/or by modifying the activity of a factor that is already bound to DNA. One approach to identify such putative factors is to determine if the binding sites for non-receptor factors are required for receptor-mediated enhancement from a particular promoter. Interestingly, the requirement for specialized transcription factors may differ depending on the relative strength and position of a GRE. For example, in some cases a single GRE located within 50 base pairs of the TATA box (the binding site for factor TFIID) is sufficient to render a heterologous promoter inducible by glucocorticoids (Ref. 66; S. D. Jones and K. R. Yamamoto, personal communication*). However, if positioned further than 100 base pairs from a TATA box, this same GRE may have only marginal effects [66]. In many cases, this "distance effect" can be overcome by either increasing the number of GREs or by including binding sites for other specialized transcription factors [66, 67]. Thus, specialized transcription factors appear to augment the effects of HREs in some but not all cases. Therefore, these factors are not an absolute requirement for LTF-mediated transcriptional enhancement.

Mechanism of transcriptional induction by LTFs

There are a number of models to explain how an activator increases the rate of initiation of transcription by RNA polymerase. Two points to consider for this discussion are: (1) Different activation domains of the receptor could function in different contexts and by different mechanisms. (2) Because the GR and the ER function both in mammalian cells and in yeast at least one of these mechanisms is strongly conserved [51, 52].

The first model is that LTFs act as "docking sites" for other transcription factors and, in this manner, increase the local concentration of a rate-limiting transcription factor near a promoter. Obvious candidates for these factors include RNA polymerase itself or proteins that form the stable transcription complex. There is no direct evidence that steroid receptors interact with such factors. The identification, expression and purification of LTF transcriptional activation domains should aid in biochemical experiments to address this model directly.

Alternatively, activators may alter the structure and activity of general transcription factors when bound to DNA. It has been suggested that regulatory factors containing acidic "activation domains" can alter the conformation of TFIID bound to the TATA element *in vitro* [68, 69]. Because of the chemical similarity, it is possible that *enh2* could similarly affect TFIID. It has not been proven that this alteration in the conformation of TFIID affects transcription *in vivo*.

A third model invokes that binding of the LTF to its HRE removes a repressor of transcription. Hager and colleagues have proposed that a nucleosome is displaced when the GR binds to the MTV GRE, thus facilitating the binding of the CTF transcription factor [70]. Finally, the LTF may induce a structural change in the DNA which increases the binding of other transcription factors.

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These models are not mutually exclusive. For instance, the binding of the LTF to its HRE could alter chromatin structure and increase the availability of the promoter to other transcription factors. LTFs may further enhance transcription through direct interaction with specialized transcription factors and/or TFIID. This obviously speculative model accounts for the observation that the GR contains multiple activation domains that share no obvious sequence or chemical similarity.

Transcriptional repression by the glucocorticoid receptor

As discussed above, the GR represses transcription when bound to nGREs. In direct contrast to GREs, fusion of an nGRE to a heterologous promoter results in an increased rate of transcription from that promoter in the *absence* of ligand. Upon addition of ligand, transcription from these promoters is reduced. To account for these observations, Sakai *et al.* [63] have proposed that nGREs contain overlapping binding sites for the GR and other transcription factors. In the absence of hormone, the non-receptor factors occupy nGREs and enhance transcription from linked promoters; upon hormone treatment, the GR blocks the activity of these factors either by competing for overlapping binding sites or by forming an inactive complex when bound. Mellon and colleagues have proposed a similar model to account for the GR-mediated repression of the human gene for the glycoprotein hormone alpha-subunit [71]. In this case, the GR binding site overlaps with a binding site for a cAMP responsive factor.

If this model is correct, why does the receptor itself fail to induce transcription when bound to nGREs? One possibility is that the GR is locked into an inactive complex with other factors when bound to nGREs. Alternatively, the GR bound to an nGRE may adopt a different conformation than the GR bound to a GRE. In this model, the nGRE induces a conformation in the GR which prevents the functioning of the transcriptional activation domain. Interestingly, we have isolated a mutant of the GR that fails to induce transcription but can efficiently repress transcription from the prolactin nGREs [72]. This mutant binds to GREs with wildtype affinity *in vitro* and appears to bind to GREs *in vivo*. It is interesting to speculate that the conformation of this mutant GR bound to a GRE mimics that of the wildtype GR bound to an nGRE in that it blocks the activity of the transcriptional activation domains.

OUTLOOK

Given the modular structure of LTFs, it is not unexpected that corresponding domains of different LTFs acquired additional functions relative to the "primordial" receptor through divergent evolution. For instance, nuclear localization activity may have come to overlap the hormone binding domain of some LTFs but not others. Similarly, some characteristics of signal transduction and transcriptional regulation may also differ.

However, members of the LTF superfamily, and steroid receptors in particularly, appear sufficiently similar structurally and biochemically to warrant the assumption that the overall mechanism by which they transduce the signal and regulate gene expression may be the same for all of them.

Therefore, research in the future will undoubtedly benefit from the possibility of comparing the results obtained with various LTFs. A major focus will be to define the function of factors that associate with LTFs before (e.g. hsp90) and after hormone binding. Moreover, the structural differences that might occur upon binding of LTFs to HREs or nHREs, and the promoter- and the tissue-specific interactions of LTFs will receive much attention. The potential to express and purify large quantities of individual LTFs should greatly facilitate novel approaches to these issues.

Acknowledgements—We are deeply indebted to Keith R. Yamamoto who has been an invaluable teacher; without his encouragement and help this work would not have been possible. We also thank all the members of Keith's laboratory for communicating unpublished results. Postdoctoral support was from the NIH (P. J. G.) and the Swiss National Research Foundation (D. P.).

REFERENCES

1. Yamamoto KR, Steroid receptor regulated transcription of specific genes and gene networks. *Annu Rev Genet* **19**: 209–252, 1985.
2. Evans RM, The steroid and thyroid hormone receptor superfamily. *Science* **240**: 889–895, 1988.
3. Green S and Chambon P, Nuclear receptors enhance our understanding of transcription regulation. *Trends Genet* **4**: 309–314, 1988.
4. Miesfeld R, The structure and function of steroid receptor proteins. *CRC Crit Rev Biochem and Molec Biol* **24**: 101–117, 1989.
5. Krust A, Green S, Argos P, Kumar V, Walter P, Bornet J-M and Chambon P, The chicken oestrogen receptor sequence: homology with v-*erb*A and the human oestrogen and glucocorticoid receptors. *EMBO J* **5**: 891–897, 1986.
6. Sap J, Muñoz A, Damm K, Goldberg Y, Ghysdael J, Leutz A, Beug H and Vennström B, The c-*erb*A protein is a high-affinity receptor for thyroid hormone. *Nature* **324**: 635–640, 1986.
7. Weinberger C, Thompson CC, Ong ES, Lebo R, Gruol DJ and Evans RM, The c-*erb*A gene encodes a thyroid hormone receptor. *Nature* **324**: 641–646, 1986.
8. Rusconi S and Yamamoto KR, Functional dissection of the hormone and DNA binding activities of the glucocorticoid receptor. *EMBO J* **6**: 1309–1315, 1987.
9. Green S and Chambon P, Oestradiol induction of a glucocorticoid-responsive gene by a chimaeric receptor. *Nature* **325**: 75–78, 1987.
10. Brown RS, Sander C and Argos P, The primary structure of transcription factor TFIIIA has 12 consecutive repeats. *FEBS* Lett **186**: 271–274, 1985.
11. Miller J, McLachlan AD and Klug A, Repetitive zinc-binding domains in the protein transcription factor IIIA from *Xenopus* oocytes. *EMBO J* **4**: 1609–1614, 1985.
12. Freedman LP, Luisi BF, Korszun ZR, Basavappa R, Sigler PB and Yamamoto KR, The function and structure of the metal coordination sites within the glucocorticoid receptor DNA binding domain. *Nature* **334**: 543–546, 1988.
13. Severne Y, Wieland S, Schaffner W and Rusconi S, Metal binding finger structures in the glucocorticoid receptor defined by site-directed mutagenesis. *EMBO J* **8**: 2503–2508, 1988.

14. Ponglikitmongkol M, Green S and Chambon P, Genomic organization of the human estrogen receptor gene. *EMBO J* **7**: 3385–3388, 1988.
15. Huckaby CS, Conneely OM, Beattie WG, Dobson ADW, Tsai MJ and O'Malley BW, Structure of the chromosomal chicken progesterone receptor gene. *Proc Natl Acad Sci USA* **84**: 8380–8384, 1987.
16. Jeltsch JM, Krozowski Z, Quiran-Stricker C, Gronemeyer H, Simpson RJ, Garnier JM, Krust A, Jacob F and Chambon P, Cloning of the chicken progesterone receptor. *Proc Natl Acad Sci USA* **83**: 5424–5428, 1986.
17. Green S, Kumar V, Theulaz I, Wahli W and Chambon P, The N-terminal DNA-binding "zinc finger" of the oestrogen and glucocorticoid receptors determines target gene specificity. *EMBO J* **7**: 3037–3044, 1988.
18. Gronemeyer H, Turcotte B, Quirin-Stricker C, Bocquel RT, Meyer ME, Krozowski Z, Jeltsch L, Garnier JM and Chambon P, The chicken progesterone receptor: sequence, expression and functional analysis. *EMBO J* **6**: 3985–3994, 1987.
19. Miesfeld R, Rusconi S, Godowski PJ, Maler BA, Okret S, Wikström A-C, Gustafsson J-Å and Yamamoto KR, Genetic complementation of a glucocorticoid receptor deficiency by expression of cloned receptor cDNA. *Cell* **46**: 389–399, 1986.
20. Conneely OM, Maxwell BL, Toft DO, Schrader WT and O'Malley BW, The A and B forms of the chicken progesterone receptor arise by alternate initiation of translation of a unique mRNA. *Biochem Biophys Res Commun* **149**: 493–501, 1987.
21. Tora L, Gronemeyer H, Turcotte B, Gaub M-P and Chambon P, The N-terminal region of the chicken progesterone receptor specifies target gene activation. *Nature* **333**: 185–188, 1988.
22. Kumar V, Green S, Stack G, Berry M, Jin J R and Chambon P, Functional domains of the human estrogen receptor. *Cell* **51**: 941–951, 1987.
23. Payvar F P and Wrange Ö, Relative selectivities and efficiencies of DNA binding by purified intact and protease-cleaved glucocorticoid receptor. In: *Steroid Hormone Receptors: Structure and Function* (Eds. Eriksson H and Gustafsson J-Å), pp. 267–285. Elsevier/North Holland, Amsterdam, 1983.
24. Danielsen M, Northrop JP, Jonklaas J and Ringold GM, Domains of the glucocorticoid receptor involved in specific and non-specific DNA binding, hormone activation and transcriptional enhancement. *Mol Endocrinol* **1**: 816–822, 1987.
25. Kumar V and Chambon P, The oestrogen receptor binds tightly to its responsive element as a ligand-induced homodimer. *Cell* **55**: 145–156, 1988.
26. Izumo S and Mahdavi V, Thyroid hormone receptor α isoforms generated by alternative splicing differentially activate myosin HC gene transcription. *Nature* **334**: 539–542, 1988.
27. Mitsuhashi T, Tennyson GE and Nikodem VM, Alternative splicing generates messages encoding rat c-erbA proteins that do not bind thyroid hormone. *Proc Natl Acad Sci USA* **85**: 5804–5808, 1988.
28. Giguere V, Hollenberg SM, Rosenfeld MG and Evans RM, Functional domains of the human glucocorticoid receptor. *Cell* **46**: 645–652, 1986.
29. Danielsen M, Northrop JP and Ringold GM, The mouse glucocorticoid receptor: mapping of functional domains by cloning, sequencing and expression of wild-type and mutant receptor proteins. *EMBO J* **5**: 2513–2522, 1986.
30. Simons SS Jr, Selective covalent labeling of cysteines in bovine serum albumin and in hepatoma tissue culture cell glucocorticoid receptors by dexamethasone 21-mesylate. *J Biol Chem* **262**: 9669–9675, 1987.
31. Simons SS Jr, Pumphrey JG, Rudikoff S and Eisen HJ, Identification of cysteine 656 as the amino acid of hepatoma tissue culture cell glucocorticoid receptors that is covalently labeled by dexamethasone 21-mesylate. *J Biol Chem* **262**: 9676–9680, 1987.
32. Carlstedt-Duke J, Stromstedt PE, Persson B, Cederlund E, Gustafsson J-Å and Jornvall H, Identification of hormone-interacting amino acid residues within the steroid-binding domain of the glucocorticoid receptor in relation to other steroid hormone receptors. *J Biol Chem* **263**: 6842–6846, 1988.
33. Picado-Leonard J and Miller WL, Homologous sequences in steroidogenic enzymes, steroid receptors and a steroid binding protein suggest a consensus steroid-binding sequence. *Mol Endocrinol* **2**: 1145–1150, 1988.
34. Muñoz A, Zenke M, Gehring U, Sap J, Beug H and Vennström B, Characterization of the hormone-binding domain of the chicken c-*erb*A/thyroid hormone receptor protein. *EMBO J* **7**: 155–159, 1988.
35. Catelli MG, Binart N, Jung-Testas I, Renoir JM, Baulieu EE, Feramisco JR and Welch WJ, The common 90-kD protein component of non-transformed "8S" steroid receptors is a heat-shock protein. *EMBO J* **4**: 3131–3135, 1985.
36. Schuh S, Yonemoto W, Brugge J, Bauer VJ, Riehl RM, Sullivan WP and Toft DO, A 90,000-dalton binding protein common to both steroid receptors and the Rous sarcoma virus transforming protein $pp60^{v\text{-}src}$. *J Biol Chem* **260**: 14292–14296, 1985.
37. Sanchez ER, Toft DO, Schlesinger MJ and Pratt WB, The 90 kD non-steroid-binding phosphoprotein that binds to the untransformed glucocorticoid receptor in molybdate-stabilized L-cell cytosol is the murine 90 kD heat shock protein. *J Biol Chem* **260**: 12398–12401, 1985.
38. Sanchez ER, Meshinchi S, Tienrungroj W, Schlesinger MJ, Toft DO and Pratt WB, Relationship of the 90 kD murine heat shock protein to the untransformed and transformed states of the L-cell glucocorticoid receptor. *J Biol Chem* **262**: 6986–6991, 1987.
39. Howard KJ and Distelhorst CW, Evidence for intracellular association of the glucocorticoid receptor with the 90 kD heat shock protein. *J Biol Chem* **263**: 3474–3481, 1988.
40. Munck A, Wira CH, Young DA, Mosher KM, Hallahan C and Bell PA, Glucocorticoid receptor complexes and the earliest steps in the action of glucocorticoids on thymus cells. *J Steroid Biochem* **3**: 567–578, 1972.
41. Vedeckis WV, Subunit dissociation as a possible mechanism of glucocorticoid receptor activation. *Biochemistry* **22**: 1983–1989, 1983.
42. Picard D and Yamamoto KR, Two signals mediate hormone-dependent nuclear localization of the glucocorticoid receptor. *EMBO J* **6**: 3333–3340, 1987.
43. Yamamoto KR, Stampfer MR and Tomkins GM, Receptors from glucocorticoid-sensitive lymphoma cells and two classes of insensitive clones: physical and DNA-binding properties. *Proc Natl Acad Sci USA* **71**: 3901–3905, 1974.
44. Denis M, Poellinger L, Wikström AC and Gustafsson J-Å, Requirement of hormone for thermal conversion of the glucocorticoid receptor to a DNA-binding state. *Nature* **333**: 686–688, 1988.
45. Godowski PJ, Rusconi S, Miesfeld R and Yamamoto KR, Glucocorticoid receptor mutants that are constitutive activators of transcriptional enhancement. *Nature* **325**: 365–368, 1987.
46. Pratt WB, Jolly DJ, Pratt DV, Hollenberg SM, Giguere V, Cadepond FM, Schweizer-Groyer G, Catelli MG, Evans RM and Baulieu EE, A region in the steroid binding domain determines formation of the non-DNA-binding, 9S glucocorticoid receptor complex. *J Biol Chem* **263**: 267–273, 1988.

47. Picard D, Salser SJ and Yamamoto KR, A movable and regulable inactivation function within the steroid binding domain of the glucocorticoid receptor. *Cell* **54**: 1073–1080, 1988.
48. Webster NJG, Green S, Jin JR and Chambon P, The hormone-binding domains of the estrogen and glucocorticoid receptors contain an inducible transcription activation function. *Cell* **54**: 199–207, 1988.
49. Godowski PJ, Picard D and Yamamoto KR, Signal transduction and transcriptional regulation by glucocorticoid receptor-LexA fusion proteins. *Science* **241**: 812–816, 1988.
50. Tienrungroj W, Sanchez ER, Housley PR, Harrison RW and Pratt WB, Glucocorticoid receptor phosphorylation, transformation, and DNA binding. *J Biol Chem* **262**: 17342–17349, 1987.
51. Metzger D, White JH and Chambon P, The human oestrogen receptor functions in yeast. *Nature* **334**: 31–36, 1988.
52. Schena M and Yamamoto KR, Mammalian glucocorticoid receptor derivatives enhance transcription in yeast. *Science* **241**: 965–967, 1988.
53. Geisse S, Scheidereit C, Westphal HM, Hynes NE, Groner B and Beato M, Glucocorticoid receptors recognize DNA sequences in and around murine mammary tumor virus DNA. *EMBO J* **1**: 1613–1619, 1982.
54. Govindan MV, Spiess E and Majors J, Purified glucocorticoid receptor–hormone complex from rat liver cytosol binds specifically to cloned mouse mammary tumor virus long terminal repeats *in vitro*. *Proc Natl Acad Sci USA* **79**: 5157–5161, 1982.
55. Payvar FP, Wrange Ö, Carlstedt-Duke J, Okert S, Gustafsson J-Å and Yamamoto KR, Purified glucocorticoid receptors bind selectively *in vitro* to a cloned DNA fragment whose transcription is regulated by glucocorticoids *in vitro*. *Proc Natl Acad Sci USA* **78**: 6628–6632, 1981.
56. Pfahl M, Specific binding of the glucocorticoid–receptor complex to the mouse mammary tumor proviral promoter region. *Cell* **31**: 475–482, 1982.
57. Chandler VL, Maler BA and Yamamoto KR, DNA sequences bound specifically by glucocorticoid receptor *in vitro* render a heterologous promoter hormone responsive *in vivo*. *Cell* **33**: 489–499, 1983.
58. Karin M, Haslinger A, Holtgreve A, Richards RI, Krauter P, Westphal HM and Beato M, Characterization of DNA sequences through which cadmium and glucocorticoid hormones induce human metallothionein-IIa gene. *Nature* **308**: 513–519, 1984.
59. Jantzen HM, Strähle U, Gloss B, Stewart F, Schmid W, Boshart M, Miksicek R and Schütz G, Cooperativity of glucocorticoid response elements located far upstream of the tyrosine aminotransferase gene. *Cell* **49**: 29–38, 1987.
60. Walker P, Germond J-E, Brown-Luedi M, Givel F and Wahli W, Sequence homologies in the region preceding the transcription initiation site of the liver estrogen-responsive vitellogenin and apo-VLDLII genes. *Nucleic Acids Res* **12**: 8611–8626, 1984.
61. Martinez E, Françoise G and Wahli W, The estrogen responsive elements as an inducible enhancer: DNA sequence requirements and its conversion to a glucocorticoid responsive element. *EMBO J* **6**: 3719–3727, 1987.
62. Tsai WY, Carlstedt-Duke J, Weigel NL, Dahlman K, Gustafsson J-Å, Tsai M-J and O'Malley B, Molecular interactions of steroid hormone receptor with its enhancer element: evidence for receptor dimer formation. *Cell* **55**: 361–369, 1988.
63. Sakai DD, Helms S, Carlstedt-Duke J, Gustafsson J-Å, Rottman FM and Yamamoto KR, Hormone-mediated repression: a negative glucocorticoid response element from the bovine prolacting gene. *Genes Dev* **2**: 1144–1154, 1988.
64. Miesfeld R, Godowski PJ, Maler BA and Yamamoto KR, Glucocorticoid receptor mutants that define a small region sufficient for enhancer activation. *Science* **236**: 423–426, 1987.
65. Ptashne M, How eukaryotic transcriptional activators work. *Nature* **335**: 683–689, 1988.
66. Strähle U, Schmid W and Schütz G, Synergistic action of the glucocorticoid receptor with transcription factors. *EMBO J* **6**: 3389–3395, 1988.
67. Schüle R, Müller M, Kaltschmidt C and Renkawitz R, Many transcription factors interact synergistically with steroid receptors. *Science* **242**: 1418–1420, 1988.
68. Horikoshi M, Carey M, Kakidani H and Roeder RG, Mechanism of action of a yeast activator: direct effect of Ga4 derivatives on mammalian TFIID–promoter interactions. *Cell* **54**: 665–669, 1988.
69. Horikoshi M, Hai T, Lin Y-S, Green MR and Roeder RG, Transcription factor ATF interacts with the TATA factor to facilitate establishment of a preinitiation complex. *Cell* **54**: 1033–1042, 1988.
70. Cordingley MG, Riegel AT and Hager GL, Steroid-dependent interaction of transcription factors with the inducible promoter of mouse mammary tumor virus *in vivo*. *Cell* **48**: 261–217, 1987.
71. Akerblom IE, Slater EP, Beato M, Baxter JD and Mellon PL, Negative regulation by glucocorticoids through interference with a cAMP responsive enhancer. *Science* **241**: 350–353, 1988.
72. Godowski PJ, Sakai D and Yamamoto KR, Signal transduction and transcriptional regulation by the glucocorticoid receptor. In: *DNA–Protein Interactions in Transcription. UCLA Symposium on Molecular and Cellular Biology*, New series (Ed. Gralla J), Vol. 95, pp. 197–210. Alan R. Liss, New York, 1988.