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Steroid hormone receptors: an update

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Steroid hormones (SHs) are lipophilic molecules derived from cholesterol and synthesized in the adrenal cortex (glucocorticoids, mineralocorticoids, and adrenal androgens), the testes (testicular androgens, oestrogen), and the ovary and placenta (oestrogens and progestagens or progestins). SHs reach their target cells via the blood, where they are bound to carrier proteins, and because of their lipophilic nature pass the cell membrane by simple diffusion. Within the target cells SHs bind to steroid hormone receptors (SHRs), the key mediators of SH action, which are complexed to chaperones, e.g. heat shock protein 90 (Hsp90), that help other proteins to fold and prevent aggregation. SHRs are intracellular transcription factors that can be activated, among other possibilities, by the specific and high affinity binding of ligand to exert positive or negative effects on the expression of target genes. Binding of agonistic or antagonistic ligands leads to different allosteric changes of SHRs making them competent to exert positive or negative effects on the expression of target genes by different mechanisms. (i) After dissociation of chaperones the liganded SHR–complexes can bind to chromatin organized DNA sequences in the vicinity of target genes, termed hormone response elements (HREs). The HRE-recruited hormone-receptor-complexes are then able to initiate chromatin remodelling and to relay activating or repressing signals to the target genes transcription machinery; (ii) through protein–protein interactions with other sequence-specific transcription factors, SHRs can also regulate the activity of many genes that are switched on, for instance, during stress or an inflammatory response; (iii) the SH response can also be integrated in the intracellular signalling network via cross-talk of SHRs with signal transduction pathways that transmit extracellular signals via membrane receptors and activation of protein kinase cascades to nuclear transcription factors that activate various target genes. By all these different mechanisms SHRs modulate numerous and specific responses in a large variety of cells, whereby their particular effect depends on the physiological, cellular and genetic context.

Key words: chromatin/cross-talk/steroid hormones/steroid hormone receptors/transcription factors

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Introduction

In mammals the gonads and adrenal gland produce five major groups of steroid hormones (SHs): oestrogens, progestins, androgens, glucocorticoids and mineralocorticoids. All these SHs regulate a large number of physiological processes in target cells equipped with the corresponding steroid hormone receptors (SHRs). The concept of target cells has been extended in the last years following the demonstration of functional SHRs in a large variety of cell types. For these, and many other crucial experiments, the availability of radioactively labelled SHs in the late 1950s was a key development. Using these compounds it was possible to follow the fate of the steroid hormones from their site of synthesis in the endocrine glands, through the blood circulation, up to their target tissues (Jensen and Jacobsen, 1962). Although SHs are extensively metabolized, particularly in the liver, it could be shown that in most cases the hormone itself, not a metabolite, produced the response via the modulation of gene expression mechanisms. The concept that steroid hormones are involved in transcriptional control was triggered by the observation that the insect steroid hormone ecdysone induces puffs in giant chromosomes (Clever and Karlson, 1960). A few years later, a two-step model was established that involved binding of the hormone to specific high-affinity SHRs within the target cells, followed by activation of the hormone–receptor complex in order to induce expression of hormone responsive genes (Noteboom and

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Trivial Name	Nomenclature Name	Accession Number
ERα	NR3A1	X03635
ERβ	NR3A2	U57439
ERRα/ ERR1	NR3B1	X51416
ERRβ/ ERR2	NR3B2	X51417
GR	NR3C1	X03225
MR	NR3C2	M16801
PR	NR3C3	M15716
AR	NR3C4	M20132

Figure 1. Trivial and nomenclature names of steroid hormone receptors (SHRs), according to the Nuclear Receptors Nomenclature Committee (1999). Current information can be found on the Nuclear Receptor Nomenclature Homepage (http://www.ens-lyon.fr/LBMC/LAUDET/nomenc.html). In manuscripts dealing with SHRs, it is recommended that the receptor(s) be identified by the official nomenclature at least once in the Summary and the Introduction. The three subgroups A, B and C are separated by stippled lines. Genebank accession numbers are given for the human mRNAs, except for NR3A2, which is from the rat.

Gorski, 1965; Jensen *et al.*, 1968). Almost 20 years later the receptors for glucocorticoids and oestrogens were cloned and thus became the first molecularly-defined transcription factors for RNA polymerase II (Hollenberg *et al.*, 1985; Walter *et al.*, 1985; Weinberger *et al.*, 1985a,b). At around the same time cloned SHR targets, e.g. human metallothionein gene and the mouse mammary tumour virus (MMTV) were used in DNA binding and gene transfer experiments to identify the first hormone response elements (HREs) (Chandler *et al.*, 1983; Payvar *et al.*, 1983; Scheidereit *et al.*, 1983; Karin *et al.*, 1984). HREs are short DNA sequence elements that convey direct transcriptional responsiveness to adjacent genes. During the last decade many details of the SHR signal transduction pathway, including new mechanisms, have been discovered. In this review we will summarize our present view of the various pathways by which SHRs modulate gene expression.

Domain structure of SHRs

Following the cloning of the receptors for glucocorticoids (GR) and oestrogens (ERα), receptors for androgens (AR), progestins (PR) and mineralocorticoids (MR) have been identified and extensively characterized. More recently, a second oestrogen receptor (ERβ) and two oestrogen-related receptors (ERRα/ERR1 and ERRβ/ERR2) have been characterized. All SHRs are characterized by a central DNA-binding domain (DBD), that targets the receptor to the HREs, and a ligand-binding domain (LBD), required for switching the receptors' functions (Beato, 1989). When the v-*erbA* oncogene was cloned it turned out to contain also a DBD and an LBD that were homologous to the cognate regions of the known SHRs. Later on the c-*erbA* locus was identified as the receptor for the thyroid hormone T3 which, like the SHs, is also a hormone found in the nucleus (Green and Chambon, 1986; Evans, 1988). The identification of another structurally related receptor for the vitamin A metabolite retinoic acid solidified the concept of a nuclear receptor superfamily. Today >60 different nuclear receptors are described, among them receptors for the known nuclear hormones and a vast number of so called orphan receptors with no, or unknown, and possibly novel ligands (Mangelsdorf *et al.*, 1995). With so many different receptors nomenclature has become a real problem. Therefore, the majority of leading researchers in the field have now agreed to use a unified nomenclature based on the system developed for the *P450* gene superfamily (Nuclear Receptors Nomenclature Committee, 1999). SHR and SHR-like receptors form the nuclear receptor (NR) subfamily 3 (NR3), consisting of three groups A, B, and C (Figure 1). NR3 includes two receptors for oestrogens (ERα and ERβ or NR3A1 and NR3A2 respectively), two oestrogen-related orphan receptors (ERRα/ERR1 and ERRβ/ERR2, or NR3B1 and NR3B2 respectively), and one receptor each for the other major steroid hormone classes: glucocorticoids (GR or NR3C1), mineralocorticoids (MR or NR3C2), progestins (PR or NR3C3), and androgens (AR or NR3C4).

All steroid hormone receptors are modular proteins composed of distinct regions as shown in Figure 2. When the chicken oestrogen receptor cDNA became available, the comparison with the human oestrogen and glucocorticoid receptor sequences led to the region A–F nomenclature used for all members of the whole nuclear receptor superfamily until today (Krust *et al.*, 1986; Green and Chambon, 1987; Kumar *et al.*, 1987). From numerous functional and structural analyses it became clear that these distinct regions correspond to functional and structural units called domains. Region C, the DNA binding domain, and region E, the ligand binding domain, display a high degree of sequence conservation, whereas no significant conservation was detected between the paralogous SHRs for the regions A/B, D and F. Region D is considered as a flexible hinge region between the DNA and ligand binding domains. Its very amino terminus is an integral part of the DNA-binding domain and involved in DBD dimerization. The F region is essential for hormone binding in the PR, GR and AR but not in the ERα. Recent work indicates that this domain is also important for the discrimination between agonistic and antagonistic hormone ligands (Nichols *et al.*, 1998). The A region is highly conserved only between chicken and human oestrogen receptors, but this distinction is much less clear in the other steroid receptors. Therefore, regions A and B are combined into an A/B region in most cases.

Regions C and E are not only responsible for DNA- and ligand binding respectively, but do encode other functions as well

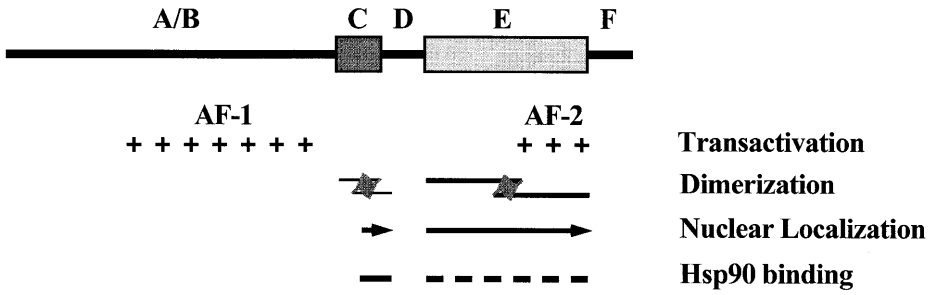

Figure 2. Steroid hormone receptor (SHR) domain structure and structure–function relationships. Domains are numbered A to F, originally based on the comparison of human oestrogen and glucocorticoid receptor sequences. Domain C (dark grey box) is the DNA-binding domain (DBD) and domain E (light grey box) is the ligand-binding domain (LBD). AF-1 and -2 are the transcription activation functions 1 and 2. Indicated regions are required for receptor dimerization (symbolized by hooked lines), nuclear localization (arrows) and heat shock protein (Hsp)90 binding (dashed lines).

(Figure 2). The intracellular distribution of steroid receptors is the result of nuclear–cytoplasmic diffusion and ATP-dependent cytoplasmic–nuclear shuttling (Guiochon-Mantel *et al.*, 1991). At equilibrium the majority of ER, AR and PR is in the nucleus due to the presence of so-called nuclear localization signals (NLSs) that are believed to be required for nuclear pore recognition. The number and location of NLSs varies among SHRs but often a constitutive NLS is located at the border of region C–D, whereas a second NLS in the LBD is ligand dependent. Intracellular localization is less clear for the GR and MR because hormone-induced nuclear translocation has been reported in these cases.

All unliganded SHRs are associated with a large multiprotein complex of chaperones, including heat shock protein 90 (Hsp90), which maintains the receptor in an inactive state but keeps it well prepared for hormone binding (Pratt and Toft, 1997). Again the region at the border of region C to D together with the LBD are required for an SHR–Hsp90 interaction to take place. Most likely these chaperones play an active role in keeping the SHRs functional (Godowski and Picard, 1989).

Ligand binding confers transcriptional competence onto SHRs that is exerted in most receptors by two independent transactivation functions, a constitutively active one in the A/B-region located close to the DBD, referred to as activation function 1 (AF-1, also called τ-1 or enh-1) and a ligand-inducible activation function in the LBD, called AF-2. The two AFs act synergistically and connect the receptor to the transcription apparatus via direct interactions with basal transcription factors, sequence-specific transcription factors and/or transcriptional co-activators (see below).

Receptor isoforms and variants

PR was the first SHR shown to exist in two common isoforms generated by differential promoter usage (Kastner *et al.*, 1990). One promoter initiates transcription at positions +1 and +15 of the gene which gives rise to the longer isoform B, PR-B (Figure 3). The second promoter initiates human PR transcripts between +737 and +842 encoding the 164 amino acid residues shorter hPR form A, PR-A. Both PR isoforms show high affinity for the natural ligand progesterone and the synthetic agonist R5020. Only PR-B harbours a third activation function at its specific amino terminus, termed AF-3, that functions in a promoter and cell-specific manner (Sartorius *et al.*, 1994). Both isoforms, therefore, display

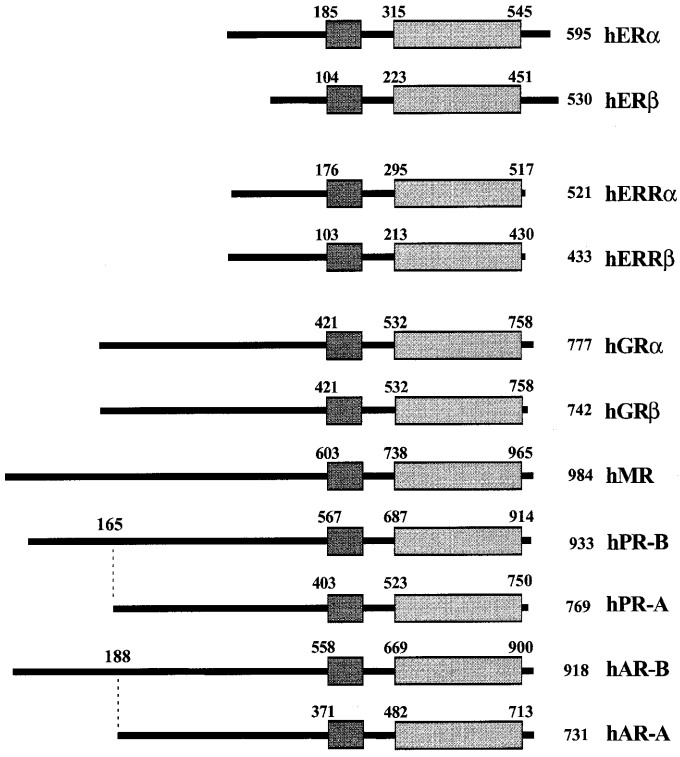

Figure 3. The human steroid hormone receptor family including isoforms and variants. The numbers refer to amino acid positions. Highlighted are the DNA binding domain (dark grey box) and the ligand binding domain (light grey box). The A/B- and F-domains are drawn to scale. The name of each receptor is indicated: h = human; ER = oestrogen receptor; ERR = oestrogen-related receptor; GR = glucocorticoid receptor; MR = mineralocorticoid receptor; PR-A/PR-B = progesterone receptor form A and B; AR-A/AR-B = androgen receptor form A and B.

differential target gene specificity. First regarded as an exception in the family, differential promoter usage and alternative splicing are now the rule for all members.

The human ERα (hERα, Figure 3) was cloned from a cDNA expression library produced from the breast cancer cell line MCF-7 (Walter *et al.*, 1985). Recently, a second oestrogen receptor, ERβ, was characterized which is very similar to ERα in terms of structure and function but also shows subtle and important

functional differences (Kuiper *et al*., 1996; Mosselman *et al*., 1996; Ogawa *et al*., 1998). Both receptors bind the ligands, oestradiol, diethylstilboestrol, oestriol, and oestrone with high affinity (Paech *et al*., 1997) and can form heterodimers on HREs (Pettersson *et al*., 1997). cDNA clones for the oestrogen-related receptors α and β were isolated from a human testis cDNA library, using the human (h) ERα DNA-binding domain as a probe. Moreover, breast cancer cells as well as other transformed oestrogen target cells contain truncated variants of hERα, whose relation to the progression of the malignancy is still unclear (Pfeffer *et al*., 1995; Vladusic *et al*., 1998).

For the glucocorticoid receptor, two different classes of cDNAs have been described in humans, hGRα and β, which are the result of alternative splicing from a single gene transcript (Hollenberg *et al*., 1985; Bamberger *et al*., 1995; Oakley *et al*., 1996). Both isoforms are identical up to amino acid 727 and then diverge with hGRα being slightly larger (777 amino acids) than hGRβ (742 amino acids). In contrast to hGRα, that is commonly referred to as the *bona fide* hGR, hGRβ was long dismissed as a cloning artefact but is now shown to be expressed at modest but varying levels in a range of tissues. As hGRβ does not bind hormone and is transcriptionally inactive, it acts as a ligand-independent negative regulator of glucocorticoid action in transfection experiments. However, the β-isoform is not conserved across species and the relative expression of both isoforms is not known. hGRα shows high affinity for the artificial glucocorticoid dexamethasone, moderate affinity for the physiological steroids cortisol and corticosterone, and low affinity for mineralocorticoids and progesterone. In contrast to all other SHRs, GRα dimerizes only weakly, which is generally believed to be due to the lack of a strong dimerization interface within the LBD. The monomeric form of GRα is involved in gene repression via interaction with other sequence-specific transcription factors, such as AP1 (see below).

The human mineralocorticoid and androgen receptors were cloned 2–3 years after GR (Arriza *et al*., 1987; Chang *et al*., 1988; Lubahn *et al*., 1988; Patel *et al*., 1989; Faber *et al*., 1991). hAR exists in two isoforms hAR-A and hAR-B which are structurally analogous to the two hPR isoforms (Wilson and McPhaul, 1996). However, in contrast to hPR-A/B, hAR-A is expressed at substantially lower levels than the B-form, and its contribution to androgen action is not known. The hAR binds the two naturally occurring ligands dihydrotestosterone and testosterone with high affinity whereas the hMR shows high and equivalent affinity for aldosterone, physiological corticosteroids, and progesterone.

DNA and chromatin binding

Initially all members of the SHR subfamily were thought to bind as homodimers to the palindromic HREs within the promoters of target genes (Figure 4), in contrast to the other members of the nuclear receptor family which bind as heterodimers to their cognate sequences (Beato, 1989). But with the identification of the oestrogen-related receptors ERRα and ERRβ the family now contains two orphan members that have been reported to bind to DNA as monomers (Johnston *et al.*, 1997) as well as homodimers (Vanacker *et al.*, 1999). Moreover, for the two closely related oestrogen receptors ERα and ERβ it could be shown that they

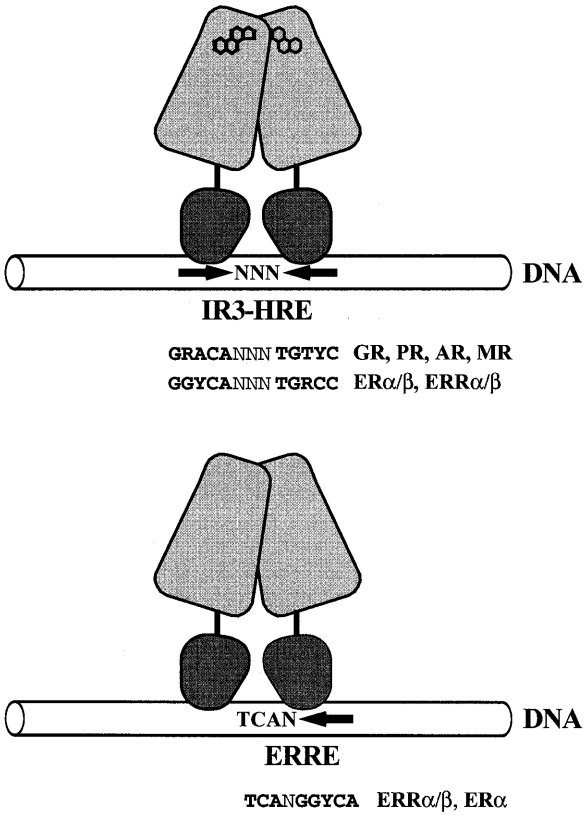

Figure 4. Illustration of a steroid hormone receptors (SHR) homodimer bound to its cognate hormone response element (HRE). The HRE of all SHRs (top panel) is of an inverted repeat (IR) type. The half-sites (arrows) are separated by three unspecified (N) nucleotides (IR3-HRE). The hormone ligand is indicated by a sterane formula. The HRE sequences recognized by the different SHRs are given underneath the DNA bar. Both ERRs can also bind to an ERE, but, together with ERα, can also bind to one extended oestrogen response element half-site, called an oestrogen-related response element, ERRE (bottom panel).

form heterodimers (Pettersson *et al*., 1997). It is also well known that the predominant form of the GRα is monomeric in solution and that dimerization occurs only after binding to an HRE, accounting for the co-operativity observed during DNA-binding. Responsible for dimerization are a weak dimerization region encoded within the DBD and a dimerization interface provided by the LBD, the strength of which varies among receptors and seems to be weaker for GR.

The receptors for progestins, glucocorticoids, mineralocorticoids, and androgens bind to the same HREs, which originally were described as glucocorticoid responsive element (GRE) (Scheidereit *et al*., 1983; Karin *et al*., 1984). GREs are composed of hexanucleotide halves (TGTYCT) arranged as inverted (palindromic) repeats and separated by three non-conserved base pairs, abbreviated as inverted repeat-3 (IR-3) (Beato, 1989). As the sixth base pair of each half-palindrome is not well conserved and its identity is not essential for specific binding (Truss *et al*., 1991), a more proper description would be to view each half palindrome as a pentanucleotide TGTYC (where Y stands for a pyrimidine base, C or T) with the half-sites separated by three non-conserved base pairs (IR-3). The oestrogen

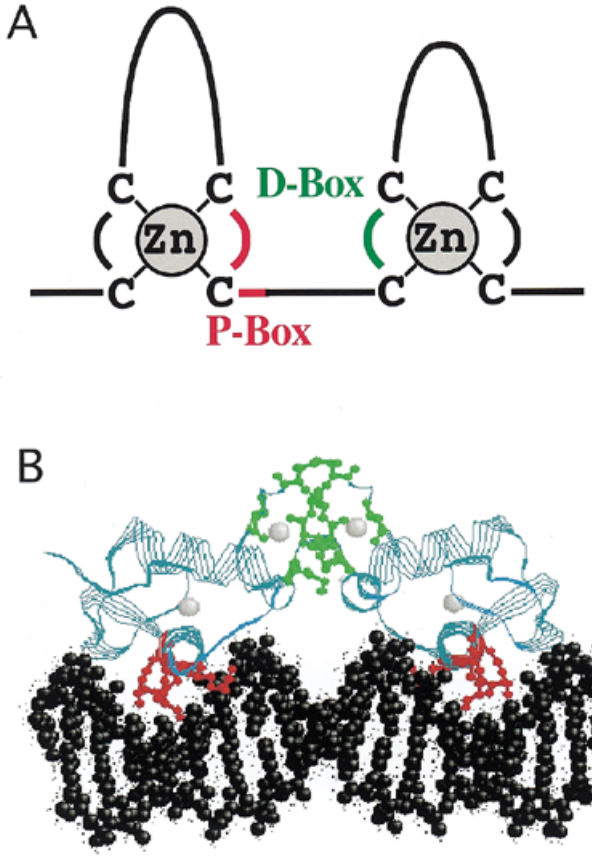

Figure 5. (**A**) The DNA-binding domain of human oestrogen receptor (ER)α is characterized by two steroid hormone receptor-specific zinc fingers. Four cysteines each tetrahedrally co-ordinate two zinc ions (grey). The proximal box (P-box) responsible for specific DNA-recognition is shown in red, the distal box (D-box) mediating DBD-dimerization is shown in green. (**B**) The secondary structure explodes into a view of the crystal structure of the hERα's DBD (blue) bound to DNA (black). The four amino acid side chains of the P-box interacting with DNA bases are again shown in red. These residues are part of an α-helix responsible for specific DNA-sequence recognition which is positioned within the DNAs major groove (perpendicular to the paper plane). A second amphipathic α-helix can be seen to cross the recognition helix with the red P-box residues at a right angle (within the paper plane). Located between the two helices are the green D-box residues promoting DBD dimerization.

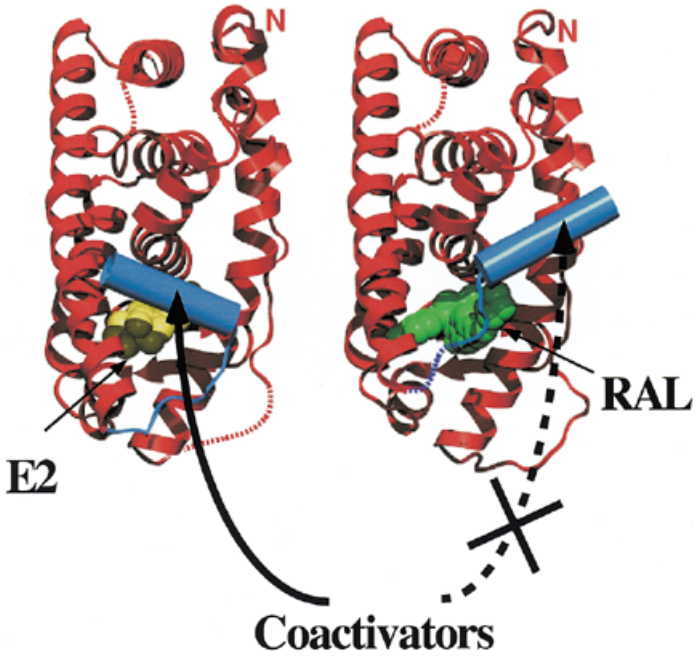

Figure 6. Crystal structure of the ligand binding domain of human oestrogen receptor (ER)α complexed with the natural ligand 17β-oestradiol (E2, yellow, left) and the anti-hormone raloxifene (RAL, green, right). Decisive for a productive interaction with co-activators is the proper positioning of helix 12 (blue) over the ligand binding pocket. (Figure courtesy of R.Hubbard and A.Pike, University of York, UK). N = *N*-terminus.

responsive element (ERE) recognized by both oestrogen receptors contains the half-site TGRCC (where R stands for a purine base, A or G), which deviates only in the third position from the GRE half-site (Figure 4). Each HRE half-site is recognized by one receptor monomer (Luisi *et al*., 1991). GR and PR discriminate their half-site from that of an ERE mainly by a hydrophobic interaction with the methyl group of the thymine in position 3 of each pentanucleotide half (TG**T**YC) that is not present in EREs (Truss *et al*., 1990, 1992). The half-site spacing of five base pairs is such that an SHR-homodimer in head-to-head orientation binds both half-sites on the same face of the DNA double helix contacting a narrow sector of the helix circumference (Scheidereit *et al*., 1983).

The two oestrogen-related receptors ERRα and ERRβ were initially reported to bind to an oestrogen-related response element (ERRE) as monomers (Johnston *et al*., 1997). An ERRE can be viewed as a 5′-extended ERE half-site (Figure 4) that is also recognized by ERα but not by ERβ (Vanacker *et al*., 1999). Conversely, the two ERRs recognize also an ERE, with both half-sites being required for binding of homodimers. Whereas ERα and ERβ can bind to an ERE as heterodimers, ERα is only able to bind to an ERRE as a homodimer, as it was also shown for ERRα. Therefore, ERs and ERRs have common DNA-binding but not ligand-binding properties.

The DNA-binding domain of SHRs comprises ~80 amino acids encoded by region C plus some 14 *N*-terminal amino acids of region D (Figure 5). Domain C contains two patterns that are reminiscent of, but clearly distinguishable from, the zinc finger motifs first observed in the *Xenopus laevis* transcription factor IIIA (Beato, 1989). The SHR zinc fingers are also able to tetrahedrally co-ordinate a zinc atom but are of the type (Cys_2-Cys_2) (Luisi *et al*., 1991; Schwabe *et al*., 1993). Only very few amino acids, termed the proximal (P)-box, within the first SHR zinc finger are responsible for specific recognition of the cognate HRE (Figure 5). Another set of amino acids, called the distal (D)-box within the second SHR zinc finger, forms the weak dimerization interface of the DNA-binding domain (Figure 5).

The nature of DNA binding, i.e. the interaction with a narrow sector of one side of the DNA double helix, should enable SHRs to bind to HREs organized in chromatin, provided the region of the major groove contacted by the receptor is exposed on the surface of nucleosomes (Beato and Eisfeld, 1997). This prediction has been confirmed experimentally (Piña *et al*., 1990a,b). A precise rotational orientation of some HREs in chromatin has been described *in vitro* and *in vivo* and has been shown to be the main determinant of SHR binding to chromatin (Piña *et al*., 1990a;

Truss *et al.*, 1995). This property is not shared by other transcription factors, such as nuclear factor 1 (NF1), which exhibit higher affinity for DNA and a larger number of contacts with DNA. As these proteins embrace the DNA double helix they cannot access their target sites in chromatin, independently of their rotational orientation (Beato and Eisfeld, 1997; Eisfeld *et al.*, 1997). Therefore, the DNA nucleotide sequence and its specific packaging in chromatin determine the interaction of certain regulatory sites with their cognate factors.

Ligand binding

Although not proven formally, it is believed that the lipophilic steroid hormones as well as synthetic compounds with agonistic or antagonistic effects enter the target cell by simple diffusion and bind, within the cytoplasm, to a multiprotein complex of chaperones and SHR. Hormone binding induces a transformation of the SHR complexes that is associated with an increase in affinity for DNA and a decrease in complex size. For instance, the unliganded hERα migrates as an 8S complex on low-salt sucrose gradients but can be activated with high salt, temperature or hormone to yield a more compact and proteinase resistant 4S form. Hormone-induced transformation of the 8S receptor complex appears to reflect loss of the associated chaperone Hsp90 followed by a tight association with the nuclear compartment. The 3D-structure of the LBDs of hERα and hPR complexed with their natural ligands have been determined (Brzozowski *et al.*, 1997; Tanenbaum *et al.*, 1998). Like for other known nuclear receptors the LBDs of SHRs are folded into a three-layered antiparallel α-helical sandwich that creates a wedge-shaped molecular scaffold with the ligand binding cavity at the narrower end of the domain (Figure 6). This cavity is completely partitioned from the external environment and is closed by helix 12 of the LBD, operating as a 'lid' after hormone has entered the binding pocket. The relocation of this amphipathic helix 12 over the hormone binding site generates (a) new surface(s) that allows co-activators (see below) to bind to the LBD, thereby mediating the activity of activation function 2 which forms the core of helix 12. Binding of anti-oestrogens, such as Raloxifen, to ERα generates a different conformational change leading to an orientation of helix 12 incompatible with co-activator binding (Figure 6). The LBD dimerization interfaces are formed by α-helices that line up and/or intertwine and differ slightly between ERα and PR. Knowledge of the atomic structure of the LBD of SHRs and of the structural changes required for receptor activation is being heavily exploited by the pharmaceutical industry to design specific and efficient agonistic and antagonist ligands.

Activation of transcription

To regulate transcription, agonist-liganded SHRs must talk to the general transcription machinery which has to gain access to the chromatin-organized target promoters to form the transcription preinitiation complex at the transcription start point. This can be achieved either by a direct contact between SHRs and the general transcription factors (GTFs), or by means of co-activators, also called transcription intermediary factors (TIFs), mediators or bridging factors. Though direct interactions of SHRs with

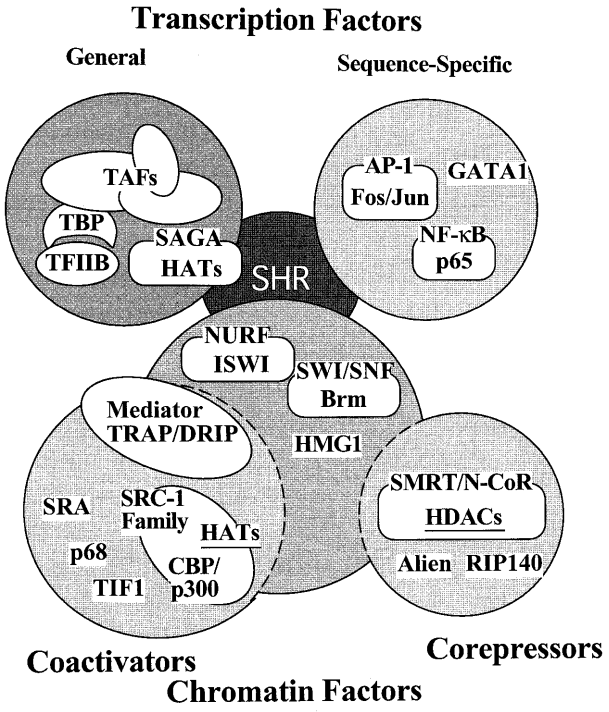

Figure 7. Overview over nuclear partners of steroid hormone receptors required for activation or repression of transcription. For each class (general transcription factors, sequence-specific transcription factors, co-activators, co-repressors and chromatin factors) typical examples are shown. See text for abbreviations.

components of the GTFs have been described (Beato and Sánchez-Pacheco, 1996), their physiological significance remains obscure and recent efforts have been mainly devoted to identify and characterize SHR co-activators. Interactions with other sequence-specific transcription factors or chromatin factors can also function as interpreters between SHRs and the general transcription machinery.

Co-activators

Co-activators are supposed to bridge between DNA-bound sequence-specific transcription factors and GTFs. The suspicion that SHRs may require such co-activators for activation was derived from the 'squelching' phenomenon (Gill and Ptashne, 1988). Squelching refers to the observation that excess receptor can inhibit its own transactivation as well as transcription by other transactivators (Wright *et al.*, 1991). One possible explanation for this behaviour is that additional factors, which are present in limited amounts and required for transactivation, are trapped by excess SHRs in unproductive complexes.

Today many of these transcription intermediary factors are known. One of the best characterized examples is the steroid receptor co-activator-1 (SRC-1) (Onate *et al.*, 1995). SRC1 interacts with the AF-2 of PR, GR and ERα in a ligand-dependent fashion and enhances their hormone-dependent transcriptional activities without altering the basal activity of a target promoter. Moreover, by co-expressing SRC-1 it is possible to reactivate a target promoter that is squelched by excess SHR. Another important and general co-activator shown to interact with SHRs

and SRC-1 is the CREB (for cAMP responsive element binding protein) binding protein (CBP), and the related protein p300 (Kamei *et al*., 1996), which both interact with another potential co-activator, i.e. p300/CBP-associated factor (PCAF) (Yang *et al*., 1996; Blanco *et al*., 1998). Interestingly, SRC-1, as well as CBP and p300 exhibit intrinsic histone acetyltransferase (HAT) activity (Bannister and Kouzarides, 1996; Ogryzko *et al*., 1996; Spencer *et al*., 1997).

For the binding of at least a subset of these co-activators to agonist-liganded SHRs the presence of one or more nuclear receptor (NR) boxes with the amino acid signature motif, LXXLL, are necessary and sufficient (Heery *et al*., 1997). After binding of an agonistic ligand to the SHR, helix 12, containing the AF-2 core of the receptor, is relocated (see above), thus creating a new interface that is poised for interaction with the signature motif of a co-activator. Binding of an antagonistic ligand leads to a different orientation of helix 12 incompatible with efficient binding of co-activators (Shiau *et al*., 1998).

Recently the co-activator field has entered a new dimension with the discovery of a number of multiprotein complexes involved in general transcriptional activation. Two main classes of global co-activators can be distinguished: (i) those interacting with the sequence-specific transcription factors; and (ii) those interacting with the general transcriptional machinery on core promoter elements. To the latter class belong the TATA-box binding protein associated factors (TAFs), which are found in at least two different multiprotein complexes, the general transcription factor D for RNA polymerase II (TFIID) complex and the Spt, Ada, Gcn5 activation (SAGA) complex (Struhl and Moqtaderi, 1998). In the TFIID complex, TAFs are associated with the TATA-box binding protein (TBP), whereas in the SAGA complex they interact with several other polypeptides involved in chromatin remodelling, including HATs, e.g. PCAF and general control non-repressed (GCN5). A similar redundancy or promiscuity is found within the first class of co-activators, which interact directly with SHRs. The large multicomponent thyroid hormone receptor associated protein (TRAP) complex, was isolated a few years ago via its stable intracellular association with ligand-activated thyroid hormone receptor (TR) (Fondell *et al*., 1996; Ito *et al*., 1999). TRAP also interacts with SHRs and is very similar or identical to several other co-activator complexes: (i) suppressor of RNA polymerase b (Srb)/mediator co-activator complex (SMCC), identified by independent affinity purification methods and functional assays with viral protein 16 from the Herpes simplex virus (VP16) and p53 (Gu *et al*., 1999); (ii) vitamin D receptor interacting protein (DRIP) isolated via its interaction with the vitamin D3 receptor (Rachez *et al*., 1999; Rachez *et al*., 1998); (iii) activator-recruited co-factor (ARC) and co-factor required for specificity protein 1 (Sp1) activation (CRSP), described as co-activators of other transcription factors, e.g. Sp1 and sterol regulating element binding protein (SREBP) (Naar *et al*., 1999) (Ryu *et al*., 1999); (iv) the histone deacetylase containing negative regulator of activated transcription (NAT) complex (Sun *et al*., 1998); (v) the recently identified human 30 polypeptide mediator complex containing suppressor of ras (Sur2)/DRIP130 (Boyer *et al*., 1999); (vi) and the general positive co-factor (PC2) (Kretzschmar *et al*., 1994). All these complexes share a small subset of components of the mediator (Kim *et al*., 1994) and Srb complexes (Thompson *et al*., 1993), both originally identified in yeast, that are partly associated with RNA polymerase II holoenzyme complexes. Thus, some of the confusion generated by the multiplicity of co-activator complexes is reduced by the observation that all these complexes share a common and stable core, but differ with respect to various subsets of other components. Most likely these variable components provide the necessary specificity of co-activator function.

The components within the co-activator complexes interacting with some of the sequence-specific transcription factors have been defined. A region of TRAP220/DRIP205 has been identified that contains two conventional NR boxes, NR1 and NR2, which share the LXXLL motifs but differ in the flanking amino acid sequence (Yuan *et al*., 1998). This region interacts with the AF-2 region of thyroid receptor (TR)α, VDR and SHRs in a ligand-dependent manner. On the other hand, the *N*-terminal AF-1 transactivation function of GR interacts with DRIP150, thus offering a possible explanation for the transcriptional synergism between the two transactivation domains of GR in response to hormone (Hittelman *et al*., 1999). Neither purified DRIP complex nor the TRAP complex (Fondell *et al*., 1999; Rachez *et al*., 1999) exhibit HAT activity. Along with observations that the TRAP and SMCC complexes mediate activator functions on DNA templates, these results support an earlier model (Yuan *et al*., 1998; Fondell *et al*., 1999) suggesting that these complexes act in steps of the transactivation process subsequent to chromatin remodelling (Freedman, 1999).

The recently discovered steroid receptor RNA co-activator (SRA) (Lanz *et al*., 1999), interacts with the AF-1 of SHRs and also with SRC-1, suggesting a connection between SHRs and further steps in RNA processing. Another potential co-activator for the AF-1of SHRs is p68, an RNA helicase that appears to be specific for ERα (Endoh *et al*., 1999). Its in-vitro interaction with the *N*-terminal domain was potentiated when the domain was phosphorylated by mitogen-activated protein kinase (MAPK) implying that it may play a role in cross-coupling between oestrogen and epidermal growth factor (EGF)/insulin-like growth factor I (IGF-I) signalling pathways which have been shown to phosphorylate and enhance the activity of AF-1 (Kato *et al*., 1995).

Co-repressors

For some members of the nuclear receptor family, e.g. TR and the retinoid acid receptors (RARs), forming heterodimers with the retinoic X receptor (RXR), it is well known that they repress basal transcription in the unliganded state. Recently, it could be shown that this silencing effect is mediated by co-repressors that were called nuclear receptor co-repressor (N-CoR) and silencing mediator for retinoid and thyroid hormone receptors (SMRT) (Chen and Evans, 1995; Hörlein *et al*., 1995). N-CoR and SMRT associate with DNA-bound TR/RXR or RAR/RXR heterodimers in the absence of hormone, but not in its presence. SMRT and N-CoR are multiprotein complexes that exhibit histone deacetylase activity. The same proteins are also implicated in SH action as it was demonstrated that overexpression of N-CoR and SMRT represses the partial agonistic activity of both ERα bound to the anti-hormone tamoxifen and of PR bound to the antagonist RU486 (Jackson *et al*., 1997; Lavinsky *et al*., 1998). Because up to now all investigations in this field have used artificial assays, e.g. yeast

two-hybrid screens and transfection experiments, these results have to be considered with caution in terms of their in-vivo relevance.

A novel co-repressor, Alien, first identified in *Drosophila*, is distinct from N-CoR/SMRT and has a different expression profile (Dressel *et al*., 1999). The protein potentiates silencing by TR in the absence of hormone, but not by RAR, and harbours an autonomous silencing domain. It may also regulate the activity of a number of nuclear receptors in *Drosophila* since it also interacts with the ecdysone receptor and seven-up. Evidence is accumulating to suggest that the receptor interacting 140 kDa protein (RIP140), which interacts with activated SHRs, also functions as a repressor (Cavailles *et al*., 1995). RIP140 antagonizes the ability of the GR to stimulate transcription from both GRE- and AP1-based reporter genes (Subramaniam *et al*., 1999).

Transactivation by SHRs not only appears to be a simple net activation but the sum of relief from repression by co-repressors and activation by co-activators. The switch from the repressed to the activated state is promoted by the hormone ligand through an allosteric change in the SHR structure. SHRs can exist in a multitude of conformations depending on the nature of bound ligand. The various interaction surfaces are mainly but not exclusively determined by helix 12, and most likely the *C*-terminus beyond helix 12, the F region.

Interactions with sequence-specific transcription factors

In addition to their HRE-mediated effects, SHRs control the activity of natural promoters also through positive and negative interactions with other sequence-specific transcription factors (Figure 7) (Beato *et al*., 1995). Particularly interesting is an interaction that represses the activity of both partners as exemplified in the case of hGR and the heterodimeric transcription factor activator protein-1 (AP-1). Similar interactions have been described between GR and the p65 subunit of the transcription factor NF-κB and the transcription factor GATA-1. From experiments with numerous SHR mutants it appears that repression is most likely mediated by a protein-protein interaction of the target transcription factor with an SHR monomer. Because most immunomodulatory genes as well as genes involved in inflammation are positively regulated by the transcription factors AP-1 and NF-κB, it is well conceivable that the immunosuppressive and anti-inflammatory activities of glucocorticoids are mediated through inhibition of AP-1 and NF-κB mediated transactivation by GR.

Many of the functions of glucocorticoids prevent an over-reaction of defence mechanisms to stress and moderate the stress response. Until recently it was believed that these glucocorticoid effects were of a genomic nature, mediated by its receptor binding to glucocortiocid response elements (GREs) in target genes. Given the multiple important functions of glucocorticoids it was therefore not surprising to learn that GR-deficient mice, generated using gene knock-out techniques, are not viable and die shortly after birth (Cole *et al*., 1995). But the cause of death (respiratory failure due to lack of lung maturation) does not seem to depend on genomic effects mediated by GREs. Contrary to the GR-deficient mice, homozygous mice that express a mutated GR that is not able to dimerize appear almost normal and healthy under standard laboratory conditions (Reichardt *et al*., 1998). This dimerization-defective GR (GR^{dim}) can no longer bind efficiently to palindromic GREs, and these mutant mice do not respond to the administration of glucocorticoids by the induction of well characterized hormone responsive genes. Clearly, GRE-mediated gene activation is not required for development and survival. It will be interesting to investigate how homozygous GR^{dim} mice react to acute stress.

Interaction with chromatin factors

The interaction of SHRs with DNA, the general transcription machinery, co-repressors and co-activators as well as sequence-specific transcription factors takes place in the nucleus with its DNA compacted into chromatin. Genetic analyses have demonstrated a widespread involvement of chromatin structure in gene regulation in general. For the GR it was shown that components of the so-called mating-type switching/sucrose non-fermenting (SWI/SNF) complex, a set of pleiotropic transactivators that counteract repressive functions of chromatin (Peterson and Tamkun, 1995), are required for transactivation in yeast (Yoshinaga *et al*., 1992). In human cells lacking brahma (hBrm), the homologue of the yeast SWI/SNF complex subunit SWI2 and the *Drosophila* homeotic protein brahma, transactivation by GR is weak and can be selectively enhanced by ectopic expression of hBrm (Muchardt and Yaniv, 1993). Like SWI2 in yeast, hBrm is part of a large multiprotein complex that mediates ATP-dependent disruption of a nucleosome. In addition to the SWI/SNF complex, other ATP-dependent chromatin remodelling machines have been described (Pazin and Kadonaga, 1997), which could mediate gene regulation by steroid hormones. As mentioned above, in an in-vitro system derived from *Drosophila* embryos an interaction between progesterone receptor and the nucleosome remodelling factor (NURF) has been shown to be involved in activation of MMTV chromatin transcription (Di Croce *et al*., 1999). These results document the link between SHRs and the cellular machinery involved in chromatin dynamics.

Apart from the SWI/SNF complex and related ATP-dependent chromatin remodelling machines, increasing evidence has been accumulated in recent years implicating the modification of histones in the regulation of gene transcription. Histones are composed of a histone fold domain, involved in wrapping the DNA in nucleosomes, and an amino-terminal tail rich in lysine that is protruding out of the nucleosome. Acetylation of the tail lysines greatly reduces the affinity of the histone tails for DNA and is believed to render DNA more accessible to transcription factors while still maintaining a nucleosomal architecture. Moreover, some of the identified co-activators for the SHRs like CBP/p300, PCAF and SRC-1 as well as the largest subunit of the pivotal general transcription factor TFIID, $TAF_{II}230/250$, turned out to be histone acetyl transferases (HATs). Oestrogen- and anti-oestrogen-regulated, AF-2-dependent transcriptional activation by purified full-length human ERα has been reproduced with chromatin templates *in vitro* (Kraus and Kadonaga, 1998). With this system, purified human p300 was observed to act synergistically with ligand-activated ERα to enhance transcription. When transcription was limited to a single round, p300 and ERα were found to enhance the efficiency of transcription initiation in a co-operative manner. On the other hand, when transcription reinitiation was allowed to occur, ERα,

but not p300, was able to increase the number of rounds of transcription (Kraus and Kadonaga, 1998).

On the MMTV promoter a functional synergism between GR or PR-B and nuclear factor 1 (NF1) is essential for hormonal induction *in vivo* and depends on the organization of the promoter into positioned nucleosomes (Chávez *et al*., 1995; Chávez and Beato, 1997). Using minichromosomes assembled in extracts from *Drosophila* embryos the synergism can be reproduced *in vitro* and is dependent on preincubation of the minichromosomes with purified receptor in the presence of the extract and ATP (Di Croce *et al*., 1999). Intriguingly, the synergism can also be detected with truncated NF1 containing just the DNA binding domain and lacking any of the known transactivation domains. DNase I footprinting experiments show that the receptors bind synergistically with NF1 or its DNA binding domain to the minichromosomes, while they compete for the naked MMTV promoter (Di Croce *et al*., 1999). From the known ATP-dependent chromatin remodelling machines, neither SWI/SNF nor chromatin-accessibility complex (CHRAC) appear to play a role in this context, while the receptor recruits NURF to the MMTV promoter in chromatin. NURF induces an ATP-dependent remodelling of chromatin resulting in an unstable or transient opening of the promoter nucleosome. It seems that NF1 plays only a structural role acting as a wedge to stabilize the open conformation of chromatin, thus facilitating full occupancy of the HREs. It is the full loading with hormone receptors, which leads to optimal induction without a direct participation of transactivation functions of NF1. In subsequent steps other co-activators, such as TRAP/DRIP or HAT-containing complexes may be required for efficient transcription, but there does not seem to be an involvement of histone acetylation in the early steps of chromatin opening and loading with sequence-specific transcription factors (Bartsch *et al*., 1996).

All these observations lead to the current two-step model for transcriptional activation by SHRs that is built upon the hormone-mediated recruitment of co-activators and other transcription factors with chromatin remodelling or HAT-activity resulting in the local destabilization of repressive histone–DNA interactions, followed by direct or most likely co-activator-mediated interactions with the basal transcription machinery.

Cross-talk with other signal transduction pathways

As the experiments with GR knock out mice and mutant GR^{dim} mice have shown, in addition to GRE-mediated gene regulation at least the glucocorticoid receptor must exert important effects via protein–protein interactions (see above). These interactions could also be modulated by phosphorylation because all steroid hormone receptors are known to be phosphoproteins (Weigel, 1996) and may affect the activity of protein kinases. Most of the identified phosphorylation sites are serine and threonine residues, but some of the family members are also phosphorylated on tyrosine. In case of the chicken PR for instance four phosphorylated serines have been identified common to both isoforms, PR-A and PR-B. The two amino terminal sites are only moderately phosphorylated in the absence of hormone, whereas after hormone treatment an increase in phosphorylation of these sites and the appearance of two new phosphorylation sites are observed. Mutation of these serine residues results in a cell- and promoter-specific variation of receptor activity when tested in transfection experiments.

Modulation of kinase activity can also cause activation of some SHRs even in the absence of hormone. IGF treatment of ovariectomized mice for example results in nuclear translocation and an altered phosphorylation state of the ERα (Aronica and Katzenellenbogen, 1993). In transient transfection studies it could be shown that EGF can activate the ERα (Bunone *et al*., 1996). Conversely, it was found that in ERα-containing MCF-7 mammary tumour cells, oestradiol stimulates the c-Src kinase and mitogen-activated protein (MAP) kinase signal transduction pathways within minutes (Migliaccio *et al*., 1996). Here, the ERα was shown to directly interact with c-Src. Likewise, in the PR-positive T47D mammary tumour cell line progestins rapidly and reversibly stimulate the c-Src/p21ras/Erk-2 pathway (Migliaccio *et al*., 1998). This activation not only requires the PR-B but also ligand-free ERα. In contrast to ERα, PR-B does not interact with c-Src, but with the ERα via its amino terminal part not present in PR-A. The transactivation function AF-3 of PR-B is not required to activate this signal transduction pathway (Migliaccio *et al*., 1998). Therefore, extensive cross-talk takes place between peptide growth factors and SHRs which is essential for the mitogenic effects of steroid hormones in breast cancer cells (Castoria *et al*., 1999) and for the steroid-like effects of growth factors.

Conclusions

Two main developments have been emphasized in this review, which have originated from recent experimental results. On the one hand the unexpected complexity of the transcriptional regulatory processes modulated by steroid hormones, and on the other the pivotal role of SHRs in the regulation of multiple cellular functions due to their integration in the signalling networks of many different cells. The first aspect has lead to the identification of several kinds of cellular machines required for negotiating transcription in the context of chromatin. Surprisingly, SHRs do not use just one of these machines for exerting their effects on transcription but rather seem to have the potential to interact with multiple machines. Whether this really means that they do act at several steps in the regulation of a particular gene, remains to be established. The second aspect has brought to the centre of our attention events in steroid hormone action taking place in the cytoplasmic compartment and involving protein-protein interactions with other signalling pathways, which may be essential to understand some of the important effects of hormones, for instance, on cancer cell proliferation.

The enormous progress in our molecular understanding of regulatory pathways involving SHRs has not yet resulted in a complete elucidation of the physiological functions of steroid hormones. However, the methodologies available today should facilitate this task in the future. In particular, tissue-specific and conditional mutation of specific SHRs will clarify the participation of various receptor domains in the physiological regulation within the intact animal.

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