Anti-Cancer Drugs, 1, pp. 127–132

Review paper

Nuclear hormone receptor variants: their role in malignancy and progression to hormone resistance in cancer

Mels Sluyser

Mels Sluyser is at the Division of Tumor Biology, The Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands. Tel: 31-20-51-29-111; fax: 31-20-17-26-25.

Mutations in functional domains of steroid and thyroid hormone receptors cause these molecules to become constitutive (hormone independent) transactivators of gene transcription. Mutations in, for example, the estrogen receptor (ER) may contribute to the loss of estrogen control of some breast carcinomas. The crucial factor in determining estrogen requirement for the growth of tumors may not be the presence or absence of estrogen-regulated ER, but whether the ERs present require estrogen to be able to cause transcription activation of growth regulatory genes. This also has implications for the therapy of breast cancer because it may be necessary to design effective anticancer agents to suppress the malignant effects of ER mutants.

Key words: Hormone receptor, estrogen receptor, breast cancer.

Introduction

The presence of mutant steroid and thyroid hormone receptors in various tumor tissues suggests that these mutants may be implicated in the process of malignancy.[1] Steroid and thyroid hormone receptors form part of a superfamily of DNA-binding proteins with a zinc-binding 'finger' motif.[2,3] This class also includes the oncogene v-*erb*A of the avian erythroblastosis virus (AEV) which is the malignant counterpart of the thyroid hormone (T_3) receptor c-*erb*A. Different domains are distinguished in these receptor molecules. These domains have been assigned A–F on the basis of degrees of homology between receptors, i.e. regions A, C, and E are highly conserved whereas this is less the case for regions B, D, and F.[2] This class of nuclear receptors also includes genes for the retinoic acid receptor (RAR), vitamin D_3 receptor (VDR), and dioxin receptor (Figure 1). Several new genes of this superfamily have recently been discovered including so-called 'orphan receptors' whose ligands are unknown.[3]

The genomic organization of these genes probably follows the eight-exon pattern found for the thyroid hormone receptor (TR) β gene,[4] estrogen receptor (ER) gene,[5] and progesterone receptor (PR) gene.[6].

Different functions have been delineated for different parts of the receptor molecules (Table 1). These functions and their possible role in malignancy are discussed below.

Transcription activation

As shown in Table 1, members of the steroid–thyroid hormone receptor superfamily function as hormone-dependent regulators of gene transcription. The receptors bind to enhancer elements which are located upstream of the basal promoters of the genes. In general, enhancer factors are believed to exert their activity by interacting with basic factors which are necessary for initiation of transcription by RNA polymerase.[7] Such interaction could either recruit these basic factors to the vicinity of a promoter (recruiting hypothesis) or activate them (activating hypothesis). Within these hypotheses it has been proposed that acidic activating domains (AADs) of gene regulatory proteins may interact with the basic TATA box factor.[8]

Several classes of domains have been described that are capable of mediating transcriptional activation. The activation domains of the yeast activators GAL4, GCN4, and herpes simplex virus VP16 protein are composed of acidic stretches of amino acids. However, not all activating domains

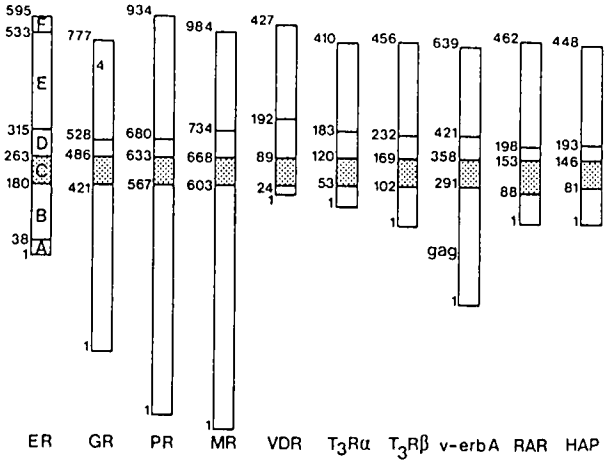

Figure 1 Schematic amino acid comparison of nuclear hormone receptors and related proteins. Primary amino acid sequences have been aligned on the basis of regions of maximum amino acid similarity. Amino acid numbers are those for the human receptors with the exception of v-*erb*A. Abbreviations: GR, glucocorticoid receptor; MR, mineralocorticoid receptor; VDR, vitamin D_3 receptor; T_3R, thyroid hormone receptor, RAR, retinoic acid receptor; HAP, hepatoma-associated protein (epithelial-type retinoic acid receptor, RAR_E). The DNA-binding (region C) regions are shaded.

are acidic; for example the activating regions of the human Sp1 and CTF/NF1 proteins contain glutamine- and proline-rich motifs, respectively.

Studies with steroid hormone receptors have demonstrated transactivation regions in the A–B region, in the DNA-binding C region and in the hormone-binding E region. In the glucocorticoid receptor, transactivating functions (called τ1 and τ2, respectively) have been reported which are acidic in nature.[9] By contrast, the transcription-activating functions TAF-1 and TAF-2, discovered in the ER, do not contain highly acidic regions. TAF-1 and TAF-2 of human ER are not only structurally but also functionally distinct from AADs such as are present in the glucocorticoid receptor (GR), GAL4 or GAL-VP16. Analysis of the ability of TAF-1 and TAF-2 of human ER to synergize with themselves and with the AADs of GAL4 and GAL-VP16 indicates that the properties of TAF-1 and TAF-2 are different from those of AADs.[10]

Table 1 Functional domains of steroid hormone receptors

Domain	Function
A–B	• Transcription activation by TAF-1 and/or τ1[9,10,12,25]
C	• DNA-binding[34]
	• Transcription activation[13–15]
D	• Nuclear localization[20,21]
	• Weak hormone-independent dimerization[23]
E–F	• Hormone-binding[34]
	• Nuclear localization[20,21]
	• Dimerization[22,23,49]
	• Hormone-dependent transcription activation by TAF-2 and/or τ2[9,10,12,25]
	• Promotion of high affinity DNA-binding[22,49]
	• Repression of other activators[49]
	• Binding of hsp90[30]

Experiments suggest that transcriptional activators function by interacting with intermediary factors present in limiting amounts in the cell. For example, expression of high levels of the acidic activators GAL4 or GAL-VP16 inhibit ('squelch') transcription from other genes that do not contain a cognate GAL4 binding site. TAF-1 and TAF-2 of human ER also show squelching: they compete with transcriptional activation by PR and GR when using PR and GR reporter genes that do not bind ER.[11]

It is possible to distinguish distinct transactivating functions on the basis of the fact that they 'squelch' in different manners. Such analyses indicate that the transactivation function in the A–B region of human GR is not a single function but is in fact composed of two separate weak activation functions which act together to give the activation seen in this domain. This also applies to the transactivation function located in the hormone-binding region of GR. Therefore, the hGR is composed of several activation functions: in addition to the weak AADs present in both the A–B and the E regions (τ1 and τ2) there are additional transcriptional activation functions located in these regions with properties similar to those of the ER TAF-1 and TAF-2, respectively.[12,13] See Table 1.

Transactivation is also associated with the DNA-binding domains (region C) of GR[13,14] and ER. For example, region C of ER confers both DNA binding and transcriptional activation of the estrogen-responsive rat prolactin gene.[15]

The GR can not only have a positive effect on gene induction, but can also repress gene expression. In some cases this is thought to involve direct interaction of the GR with a site on the DNA that has been named 'negative GRE'.[29] However, in other cases other mechanisms seem to be responsible. For example, the anti-inflammatory effect of glucocorticoids occurs by down-modulation of the transcription factor AP-1 (*jun*/*fos*) via direct interaction between the GR and AP-1. This is a mutual effect, i.e. while the GR is a potent inhibitor of AP-1 activity, both the proto-

oncogenes c-*jun* and c-*fos* are potent repressors of GR activity.[27–29]

The presence of several activating and repressing domains within the same receptor molecule probably greatly increases the potential of these receptors to respond in a cell-specific manner to a variety of regulatory stimuli, and to activate or repress differentially the promoters of the various hormone-responsive genes.

Recent studies have revealed how some anti-estrogenic drugs act on ER. One of these drugs, the 4-hydroxy derivative of tamoxifen, has proved to be predominantly anti-estrogenic by binding to the hormone-binding domain of ER, thereby blocking estrogen binding and TAF-2 activation. However, (4-hydroxy)tamoxifen is not a pure antagonist but can behave as an agonist in certain test systems and animal models via the cell-type- and promoter-context-dependent activity of TAF-1.[46]

Nuclear localization and DNA binding

The region on steroid and thyroid hormone receptor which directly and specifically binds to DNA (region C) is highly conserved and consists of about 70 amino acid residues.[2] Region C comprises two zinc finger motifs, each of which coordinates a zinc atom with four conserved cysteine residues.[16,17] Nuclear magnetic resonance studies have revealed that the DNA-binding region consists of a globular body from which the finger regions extend.

The C regions of nuclear hormone receptors bind to specific hormone responsive elements (HREs) on DNA which occur frequently in the 5′ flanking regions of hormone responsive genes. These sequences are generally variations of a perfect palindrome with a short interspace. For example, the glucocorticoid response element (GRE) is represented by AG(A/G)ACAnnnTGT(T/C)CT and the estrogen response element (ERE) is AGGTCAnnnTGACCT. Discrimination among specific HREs is determined by three amino acids at the base of the first finger. When these three amino acids in the ER are replaced with the corresponding amino acids of the GR, the resulting ER no longer recognizes EREs, but recognizes GREs instead.

Five amino acids in the second zinc finger probably serve in discriminating between HREs with different half-site spacing.

A number of nuclear localization signals have been described, including one encoded in region D that may be conserved in all steroid receptors.[20,21] Nuclear localization signals allow entry of the receptor molecules into the nucleus, their site of action.

Hormone binding and dimerization

The ligand-binding domain or E domain encompasses about 210 amino acid residues and determines the ligand-binding specificity of each receptor. The hormone-binding and dimerization functions are contained within overlapping regions, but are not entirely coincident. Hormone binding does not appear to be required for dimerization and specific DNA binding controlled by the E region.[25] This is indicated by the finding that certain mouse ER mutants fail to bind estradiol but both dimerize and bind to DNA with high affinity. Perhaps these mutants can copy the estradiol-induced three-dimensional conformation of the wild-type receptor and so be able to bind to DNA and dimerize in a constitutive manner.

Although ligand binding does not appear to be absolutely required for dimerization, it may stabilize the receptor dimers.[49]

The anti-estrogenic drug ICI 164,384 has been reported to act by preventing ER dimerization.[51]

The dimerization domain in region E has certain features in common with both the leucine zipper and the helix–loop–helix motif which have been proposed as dimerization structures in other DNA-binding proteins.[19,22,24]

Normal gene vs oncogene

In order to elucidate which mutations are responsible for turning hormone receptor genes into oncogenes, it is useful to compare the normal gene with its oncogenic counterpart. This can be done, for instance, with the thyroid hormone (T_3) receptor and its oncogenic counterpart, the v-*erb*A oncoprotein. The *erb*A oncogene of the AEV arrests erythroid differentiation by interfering with the expression of erythrocyte-specific genes.[31] Whereas the thyroid hormone receptor modulates erythroid differentiation and erythrocyte-specific gene expression in a T_3-dependent manner,[32] the v-*erb*A oncoprotein has lost the ability to regulate

erythrocyte gene transcription in response to T_3, and represses transcription in the absence of T_3.

Compared with the thyroid hormone receptor, v-*erb*A lacks both amino- and carboxy-terminal sequences, and possesses additional amino-terminal *gag*-derived sequences and internal amino acid codon differences.[35] The v-*erb*A has lost a cluster of amino acid residues that can form an amphipathic α-helix, i.e. four negatively charged amino acids projecting to one side of the helix within an otherwise largely hydrophobic environment. The region is adjacent to a stretch of leucine and methionine residues (spaced by a multiple of seven amino acids) that may serve as a potential 'leucine' zipper-like dimerization interface.[32] Intriguingly, although the v-*erb*A protein has lost the ability to bind thyroid hormone owing to alterations in the carboxy-terminal domain, the remnants of the hormone-binding domain in v-*erb* have retained their ability to regulate the properties of this protein. Genetic lesions in the carboxy-terminal domain of v-*erb*A inhibit both nuclear localization and DNA-binding and lead to an intermediate transformed phenotype in AEV-infected cells.[36] Studies on carboxy-terminally-truncated v-*erb*A mutants also show that presence of the carboxy-terminal region is a prerequisite for v-*erb*A to act as an oncogene.[33] This region may be essential by interfering with the binding of thyroid hormone receptors to DNA, and so contributing to malignant transformation of the cell.[33] On the contrary, the mutated DNA-binding domain of v-*erb*A also appears to be partly responsible, in particular the Gly73 → Ser mutation in this region.[52]

Hormone receptor variants

Hormone receptor variants have been found in a number of tumor tissues. ER and PR mutants have been reported in human breast cancer biopsies and cell lines.[37 43] T47D human breast cancer cell lines contain several mutant ERs which include two different frame-shift mutations. The first would encode an ER truncated just beyond the second zinc finger of the DNA-binding domain near the end of exon 3. The second is truncated in the hormone-binding domain near the end of exon 5. A third mutant has an in-frame deletion from the nuclear localization signal of exon 4 to the end of exon 5. None of these forms could be detected by ligand-binding or clinical immunoassays of ER.[43]

An ER variant lacking exon 5 has been detected as the predominant ERmRNA in biopsies from three ER−/PR+ breast tumors. The variant ER activated transcription of a normally estrogen-dependent gene construct in yeast cells in an estrogen-independent manner.[37]

Discussion

Hormone receptor mutants may act as oncogenes in breast cancer and also may be responsible for the loss of hormone responsiveness of some tumors during their progression.[45] The data reviewed here suggest that receptor variants which have a mutation in the hormone-binding domain may act as constitutive transcription activators via the transactivating domains that are still intact in the receptor molecule. In this respect it is of interest that an ER mutant lacking the hormone-binding domain can activate the c-*fos* gene promoter in the absence of estrogen.[47] This raises the possibility that one of the ways in which mutant hormone receptors participate in tumor progression is by causing constitutive expression of oncogenes such as *fos*.

Besides containing a constitutive activator function, the ER has recently been shown to contain a constitutive DNA binding function that enables the ER to bind and thereby to repress other activators of the ERE in the absence of estradiol.[26] Since mutations in the ligand-binding domain strongly affect the DNA binding and repressor capacity of ER, a lack of repressor function may disturb important steps in transcriptional regulation and eventually lead to uncontrolled proliferation.[26]

Current methods for determining the hormonal responsiveness of tumors are based on assays of hormone receptors. For example, among the breast cancers of postmenopausal women, a majority (75%) contain ERs. Among these, about 50–60% possess PRs. Although the presence of PR in addition to ER improves the predictability of hormone dependency of a tumor, this relationship is far from absolute as about 30% of ER+/PR+ tumors fail to respond to hormone therapy.[48] It can be inferred from these results that the crucial factor in determining estrogen requirement for the growth of tumors may not be the presence or absence of normally estrogen-regulated ER molecules, but whether the ERs present in a tumor require estrogen to be able to cause transcription activation of growth regulatory genes.

The ability to 'run amok'[44] may not only apply to steroid and thyroid hormone receptors, but also

to other members of the DNA-binding 'finger' protein family.[50] Deviant members of this class of proteins may be regarded as the products of dominant negative oncogenes.[51]

The presence of products of these 'dononcs'[1] obviously poses serious difficulties in designing effective therapies of cancer. In case of breast cancer, the anti-estrogens in current use presumably destroy only the tumor cells that have ERs with intact hormone-binding domain, leaving cells with mutated ERs undisturbed.

It will be a challenge for oncologists to discover or design anti-cancer agents that can neutralize the malignant effects of these mutant receptors, by for instance suppressing their TAF-1/τ1 functions or restoring the hormonal regulation of TAF-2/τ2.

References

1. Sluyser M. Steroid/thyroid receptor-like proteins with oncogenic potential: a review. *Cancer Res* 1990; **50**: 451–8.
2. Green S, Gronemeyer H, Chambon P. Structure and function of steroid hormone receptors. In: M Sluyser, ed *Growth Factors and Oncogenes in Breast Cancer*, Chichester: Ellis Horwood Series in Biomedicine, 1987: 7–28.
3. Denner L, Ji H. Steroid receptors, transcription factors, and gene expression. Meeting report. *Cancer Res* 1990; **50**: 6430–5.
4. Sakurai A, Nakai A, DeGroot LJ. Structural analysis of human thyroid hormone receptor β gene. *Mol Cell Endocrinol* 1990; **71**: 83–91.
5. Ponglikitmongkol M, Green S, Chambon P. Genomic organization of the human estrogen receptor gene. *EMBO J* 1988; **7**: 73385–8.
6. Huckaby CS, Conneely OMN, Beattie WG, *et al.* Structure of the chromosomal chicken progesterone receptor gene. *Proc Natl Acad Sci USA* 1987; **84**: 8380–4.
7. Lillie JW, Green MR. Activator's target in sight. *Nature (London)* 1989; **341**: 279–80.
8. Schaffner W. How do different transcription factors binding the same DNA sequence sort out their jobs? *Trends Genet* 1989; **5**: 37–9.
9. Hollenberg SM, Evans RM. Multiple and cooperative trans-activation domains of the human glucocorticoid receptor. *Cell* 1988; **55**: 899–906.
10. Tora L, White J, Brou C, *et al.* The human estrogen receptor has two independent nonacidic transcriptional activation functions. *Cell* 1989; **59**: 477–87.
11. Meyer ME, Gronemeyer H, Turcotte B, *et al.* Steroid hormone receptors compete for factors that mediate their enhancer function. *Cell* 1989; **57**: 433–42.
12. Tasset D, Tora L, Fromental C, Scheer E, Chambon P. Distinct classes of transcriptional activating domains function by different mechanisms. *Cell* 1990; **62**: 1177–87.
13. Hollenberg SM, Weinberger C, Ong ES, *et al.* Primary structure and expression of a functional human glucocorticoid receptor cDNA. *Nature (London)* 1985; **318**: 635–41.
14. Miesfeld R, Godowski PJ, Maler BA, Yamamoto KR. Glucocorticoid receptor mutants that define a small region sufficient for enhancer function. *Science* 1987; **236**: 423–7.
15. Waterman ML, Adler S, Nelson C, *et al.* A single domain of the estrogen receptor confers deoxyribonucleic acid binding and transcriptional activation of the rat prolactin gene. *Mol. Endocrinol.* 1988; **2**: 14–21.
16. Freedman LP, Luisi BF, Koszun ZR, *et al.* The function and structure of the metal coordination sites with the glucocorticoid receptor DNA binding domain. *Nature (London)* 1988; **334**: 543–6.
17. Dahlman K, Strömstedt PE, Rae C, *et al.* High level expression in *Escherichia coli* of the DNA-binding domain of the glucocorticoid receptor in a functional form from utilizing domain-specific cleavage of a fusion protein. *J Biol Chem* 1989; **264**: 804–9.
18. Härd T, Kellenbach E, Boelens R, *et al.* Solution structure of the glucocorticoid receptor DNA-binding domain. *Science* 1990; **249**: 157–60.
19. Landschutz WH, Johnson PF, McKnight SL. The leucine zipper: a hypothetical structure common to a new class of DNA binding proteins. *Science* 1988; **240**: 1759–64.
20. Picard D, Yamamoto KR. Two signals mediate hormone-dependent nuclear localization of the glucocorticoid receptor. *EMBO J* 1987; **6**: 3333–40.
21. Guiochon-Mantel A, Loosfelt H, Lescop P. *et al.* Mechanisms of nuclear localization of the progesterone receptor: evidence for interaction between monomers. *Cell* 1989; **57**: 1147–54.
22. Fawell SE, Lees, JA, White R, Parker M. Characterization and colocalization of steroid binding and dimerization activities in the mouse estrogen receptor. *Cell* 1990; **60**: 953–62.
23. Kumar V, Chambon P. The estrogen receptor binds tightly to its response element as a ligand-inducible homodimer. *Cell* 1988; **55**: 145–56.
24. Murre C, McCaw PS, Baltimore D. A new DNA binding and dimerization motif in immunoglobulin enhancer binding, *daughterless*, *MyoD* and *myc* proteins. *Cell* 1989; **56**: 777–83.
25. Lees JA, Fawell SE, Parker MG. Identification of two transactivation domains in the mouse oestrogen receptor. *Nucl Acids Res* 1989; **17**: 5477–88.
26. Tzukerman M, Zhang X, Hermann T, Wils KN, Graupner G, Pfahl M. The human estrogen receptor has two transcriptional activator and repressor functions in the absence of ligand. *New Biol* 1990; **2**: 613–20.
27. Jonat C, Rahmsdorf HJ, Park KK, *et al.* Antitumor promotion and antiinflammation: down-modulation of AP-1 (Fos/Jun) activity by glucocorticoid hormone. *Cell* 1990; **62**: 1189–204.
28. Schüle R, Rangarajan P, Kliewer S, *et al.* Functional antagonism between oncoprotein c-Jun and the glucocorticoid receptor. *Cell* 1990; **62**: 1217–26.
29. Yang-Yen HF, Chambard JC, Sun YL, *et al.* Transcriptional interference between c-Jun and the glucocorticoid receptor: mutual inhibition of DNA binding due to direct protein–protein interaction. *Cell* 1990; **62**: 1205–15.
30. Housley PR, Sanchez ER, Danielsen M, *et al.* Evidence that the conserved region in the steroid binding domain of the glucocorticoid receptor is required for both optimal binding of hsp90 and protection from proteolytic cleavage. *J Biol Chem* 1990; **265**: 12778–81.
31. Zenke M, Kahn P, Disela C, *et al.* V-*erb*A specifically suppresses transcription of the avian erythrocyte anion transporter (band 3) gene. *Cell* 1988; **52**: 107–19.
32. Zenke M, Munoz A, Sap J, Vennström B, Beug H. V-*erb*A oncogene activation entails the loss of hormone-

dependent regulator activity of c-*erb*A. *Cell* 1990; **61**: 1035–49.
33. Forrest D, Munoz A, Raynoschek C, Vennström B, Beug H. Requirement for the C-terminal domain of the v-*erb*A oncogene protein for biological function and transcriptional repression. *Oncogene* 1990; **5**: 309–16.
34. Kumar V, Green S, Stack G, *et al.* Functional domains of the human estrogen receptor. *Cell* 1987; **51**: 941–51.
35. Sap J, Munoz A, Damm K, *et al.* The c-*erb*A protein is a high affinity receptor for thyroid hormone. *Nature (London)* 1986; **324**: 635–40.
36. Boucher P, Privalsky ML. Mapping of functional domains within the v-*erb*A oncogene protein: the remnants of the hormone binding domain play multiple, vital roles in protein action. *Oncogene* 1990; **5**: 1301–11.
37. Fuqua SAW, Fitzgerald SD, Chamness GC, *et al.* A variant human breast tumor estrogen receptor with constitutive transcriptional activity. *Cancer Res.* In press.
38. Hill SM, Fuqua SAW, Chamness GC, *et al.* Estrogen receptor expression in human breast cancer associated with an estrogen receptor gene restriction fragment length polymorphism. *Cancer Res* 1989; **49**: 145–8.
39. Raam S, Robert N, Pappas CA, Tamura H. Defective estrogen receptors in human mammary cancers: their significance in defining hormone dependence. *J Natl Cancer Inst* 1988; **80**: 756–61.
40. Garcia T, Sanchez M, Cox JL, *et al.* Identification of a variant form of the human estrogen receptor with an amino acid replacement. *Nucl Acids Res* 1989; **17**: 8364.
41. Murphy LC. Mechanisms associated with resistance to the antiproliferative effects of antiestrogens and progestins in human breast cancer. *J Cancer Clin Oncol* 1990; **116** (suppl II): 962.
42. Fuqua SAW, Falette NF, McGuire WL. Sensitive detection of estrogen receptor RNA by polymerase chain reaction assay. *J Natl Cancer Inst* 1990; **82**: 858–61.
43. Graham ML, Krett NL, Miller LA, *et al.* T47D^{CO} cells, genetically unstable and containing estrogen receptor mutations, are a model for the progression of breast cancers to hormone resistance. *Cancer Res* 1990; **50**: 6208–17.
44. Bishop JM. Oncogenes as hormone receptors. *Nature (London)* 1986; **321**: 112–3.
45. Sluyser M, Mester J. Oncogenes homologous to steroid receptors? *Nature (London)* 1985; **315**: 546.
46. Berry M, Metzger D, Chambon P. Role of the two activating domains of the oestrogen receptor in the cell-type and promoter-context dependent agonistic activity of the anti-oestrogen 4-hydroxytamoxifen. *EMBO J* 1990; **9**: 2811–8.
47. Weisz A, Rosales R. Identification of an estrogen response element upstream of the human c-*fos* gene that binds the estrogen receptor and the AP-1 transcription factor. *Nucl Acids Res* 1990; **18**: 5097–106.
48. King RJB, Stewart JF, Millis RR, *et al.* Quantitative comparison of estradiol and progesterone receptor content of primary and metastatic human breast tumors in relation to response to endocrine treatment. *Breast Cancer Res Treat* 1982; **2**: 339–46.
49. Forman BM, Samuels HH. Dimerization among nuclear hormone receptors. *New Biol* 1990; **2**: 587–94.
50. Issemann I, Green S. Activation of a member of the steroid hormone receptor superfamily by peroxisome proliferators. *Nature (London)* 1990; **347**: 645–650.
51. Fawell SE, White R, Hoare S, Sydenham M, Page M, Parker M. Inhibition of estrogen receptor-DNA binding by the 'pure' antiestrogen ICI 164,384 appears to be mediated by impaired receptor dimerization. *Proc Natl Acad Sci USA* 1990; **87**: 6883–7.

(Received 15 October 1990; accepted 1 November 1990)