*Proc. Natl. Acad. Sci. USA*
Vol. 92, pp. 4206–4210, May 1995
Biochemistry

# Specific mutations in the estrogen receptor change the properties of antiestrogens to full agonists

(tamoxifen/ICI 164,384/activation function/nuclear localization/hormone therapy)

ABDERRAHIM MAHFOUDI*†, EMMANUELLE ROULET*†, SOPHIE DAUVOIS†‡, MALCOLM G. PARKER‡, AND WALTER WAHLI*§¶

*Institut de Biologie Animale, Université de Lausanne, CH-1015 Lausanne, Switzerland; §Glaxo Institute for Molecular Biology, CH-1228 Plan-les-Ouates, Geneva, Switzerland; and ‡Molecular Endocrinology Laboratory, Imperial Cancer Research Fund, London, United Kingdom

*Communicated by Elwood V. Jensen, Institute for Hormone and Fertility Research, Hamburg, Germany, December 29, 1994 (received for review November 23, 1994)*

ABSTRACT **The estrogen receptor (ER) stimulates transcription of target genes by means of its two transcriptional activation domains, AF-1 in the N-terminal part of the receptor and AF-2 in its ligand-binding domain. AF-2 activity is dependent upon a putative amphipathic α-helix between residues 538 and 552 in the mouse ER. Point mutagenesis of conserved hydrophobic residues within this region reduces estrogen-dependent transcriptional activation without affecting hormone and DNA binding significantly. Here we show that these mutations dramatically alter the pharmacology of estrogen antagonists. Both tamoxifen and ICI 164,384 behave as strong agonists in HeLa cells expressing the ER mutants. In contrast to the wild-type ER, the mutant receptors maintain nuclear localization and DNA-binding activity after ICI 164,384 treatment. Structural alterations in AF-2 caused by gene mutations such as those described herein or by estrogen-independent signaling pathways may account for the insensitivity of some breast cancers to tamoxifen treatment.**

Estrogens regulate target cell proliferation, growth, and differentiation through a defined sequence of molecular events triggered by their binding to the intracellular estrogen receptor (ER) (1–7). The receptor functions directly as a ligand-dependent transcription factor as shown in different species (8–13). Transcriptional activation is mediated by AF-1 in the N-terminal domain and AF-2 in the C-terminal hormone-binding domain (1–7). Since the growth of approximately one third of breast tumors is stimulated by estrogen, a large number of antiestrogens have been developed as potential drugs for endocrine cancer therapy. In fact, the ER status helps to identify those tumors which may respond to hormone therapy in place of cytotoxic treatments or surgery (14, 15).

The antiestrogen tamoxifen is a leading therapeutic agent in the treatment of ER-positive primary breast cancers (14–17) and in large chemopreventive trials with healthy candidates (18, 19). Tamoxifen or its potent metabolite 4-hydroxytamoxifen (4-OHT) competes with estrogen for binding to ER and blocks the transcriptional activity of the receptor (15, 20–22). However, tamoxifen has a complex pharmacological behavior, as it can present either antagonistic or agonistic activities in a species-, tissue-, and cell type-dependent fashion (16, 20). Moreover, only half of ER-positive breast tumors respond to tamoxifen therapy, and a majority of them relapse into antiestrogen-unresponsive tumors even though ER is often still measurable by ligand-binding assays (14, 15). The unpredictability of the side effects of tamoxifen and the frequent recurrence of tumors in patients undergoing tamoxifen therapy have stimulated the search for more potent antiestrogens devoid of agonistic activity. Along this line of investigation, it was demonstrated that the estradiol derivatives ICI 164,384 and ICI 182,780 bind to the ER and are pure antagonists (21, 23, 24). They have been shown to reduce the cell content of ERs by increasing their turnover (25, 26), and this is accompanied by an inhibition of nuclear uptake of the receptor during nucleocytoplasmic shuttling (27).

In this paper, we demonstrate that certain point mutations in the ligand-binding domain of the ER alter dramatically the responsiveness of the mutated ERs not only to estrogen but more importantly to antiestrogens. Two mutants are described whose activation by estrogen is abolished but are instead strong transcriptional activators in response to stimulation by both 4-OHT and the pure antiestrogen ICI 164,384.

## MATERIALS AND METHODS

**Receptor Expression Vectors and Reporter Gene Constructs.** The isolation of mouse ER (MOR) cDNA clones, the construction of mutants thereof, and their transfer into the pJ3Ω expression vector have been described (28, 29). Missense mutants of the full-length protein [MOR-(1–599)] were generated and named according to the position and identity of the substituted amino acid and the substituting residue—e.g., L543A indicates replacement of leucine at position 543 by alanine. Deletion mutants are described by the remaining amino acids—e.g., MOR-(121–599) is an N-terminal deletion mutant starting at methionine-121. The reporter construct pERE BLCAT contains the estrogen-responsive element (ERE) from the *Xenopus* vitellogenin A2 promoter (30, 31), which was cloned upstream of the herpes simplex virus thymidine kinase gene promoter driving expression of the chloramphenicol acetyltransferase (CAT) coding sequences (28). pRSV-luc encodes luciferase expressed under the control of the Rous sarcoma virus (RSV) promoter (32).

**Cell Cultures, Transient Cotransfections, and CAT Assays.** HeLa and Cos-1 cells were maintained routinely in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum (GIBCO). HeLa cells were transfected transiently by using the calcium phosphate coprecipitation method. Sixteen hours prior to transfection, cells were plated out at a density of $5 \times 10^5$ cells per 6-cm dish in phenol red-free DMEM containing 10% dextran/charcoal-stripped fetal calf serum. The transfected DNA included pERE BLCAT (4 μg), the internal control plasmid pRSV-luc (2 μg), the appropriate receptor expression vector pJ3Ω (0.25 μg), and pBluescript

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: ER, estrogen receptor; MOR, mouse ER; ERwt, wild-type ER; 4-OHT, 4-hydroxytamoxifen; ERE, estrogen-responsive element; WCE, whole cell extract; CAT, chloramphenicol acetyltransferase.

†A.M., E.R., and S.D. have contributed equally to this work.

¶To whom reprint requests should be addressed at: Institut de Biologie Animale, Université de Lausanne, Bâtiment de Biologie, CH-1015 Lausanne, Switzerland.

DNA (Stratagene) to a total of 12 μg of DNA per dish. Following transfection, the cells were maintained without added ligand, with 10 nM 17β-estradiol alone or together with 0.5 μM 4-OHT or 0.5 μM ICI 164,384, a gift from A.-E. Wakeling (ICI Pharmaceuticals, Macclesfield, U.K.). After a 36- to 40-hr incubation, cells were harvested and assayed for luciferase (32) and CAT (33) activities. For the production of receptor used in gel retardation assays, Cos-1 cells were electroporated with the receptor expression vectors and pRSV-luc as described (29). Whole cell extracts (WCEs) were prepared as described (29) and assayed for luciferase activity (32).

**DNA-Binding Analyses.** DNA-binding activity in WCE from Cos-1 cells that expressed wild-type or mutant MORs was measured by using a gel retardation assay. DNA-binding reaction mixtures containing 10–15 μg of WCE protein, 5 μg of poly(dI-dC), 10 mM Tris·HCl at pH 7.5, 100 mM KCl, 10% (vol/vol) glycerol, and 1 mM dithiothreitol in a final volume of 20 μl were incubated on ice for 20 min. $^{32}$P-radiolabeled double-stranded oligonucleotide probe (1 ng) containing a consensus ERE sequence (4) was then added and incubated for a further 30 min at room temperature. MOR–ERE complexes were resolved in 5% polyacrylamide gels that were then fixed, dried, and subjected to autoradiography.

**Western Blotting Analysis.** WCEs were prepared from transfected Cos-1 cells treated with 17β-estradiol, ICI 164,384, or vehicle alone and expressing the different forms of receptor. Proteins (20-μg samples) were separated on an SDS/10% (wt/vol) polyacrylamide gel and transferred to nitrocellulose. After blocking with phosphate-buffered saline containing 1% nonfat dried milk and 0.1% Tween-20, pH 7.5, receptors were detected with the monoclonal antibody H222 (34) (gift from Abbott Laboratories) and peroxidase-conjugated rabbit anti-rat IgG. The blots were washed in phosphate buffered saline containing 0.1% Tween-20, pH 7.5, and developed by enhanced chemiluminescence (ECL) reactions (Amersham).

**Indirect Immunofluorescence.** Transfected Cos-1 cells were plated on poly-(L-lysine)-precoated coverslips placed in tissue culture plates in phenol red-free DMEM, supplemented with 10% charcoal-stripped fetal calf serum. After 48 hr, cells were treated with 10 nM 17β-estradiol, or 0.1 μM ICI 164,384 for 1 hr. Cells were then fixed and permeabilized, and ER was detected by using the H222 antibody as described (27). Untransfected or transfected cells incubated without H222 antibody were included as controls.

## RESULTS

Previous mutant analyses have shown that a segment of AF-2 comprising residues 538–552 in MOR is essential for hormone-dependent activation of transcription (29). This region, which is conserved in the ER from different species and also in several other nuclear receptors, contains two pairs of hydrophobic amino acids (L543/L544, M547/L548) and a negatively charged glutamic acid residue (E546) and is flanked by additional negatively charged aspartic acid residues, D542 and D549 (Fig. 1). Structural predictions indicate that this region folds into an amphipathic α-helix (29).

Substitution for the glutamic acid (E546A) or all three charged amino acids in this region (D542N/E546Q/D549N) did not significantly affect the transcriptional activity of the ER in HeLa cells (Fig. 2). Both 4-OHT and ICI 164,384 antagonized the estradiol induction, although ICI 164,384 was more potent (Fig. 2). In contrast, substitution of either one pair of hydrophobic residues, L543A/L544A or M547A/L548A, resulted in a markedly lower basal activity in the absence of hormone as compared to the wild-type ER (ERwt), and estradiol had only a weak effect on the transcriptional activity of these two mutants (Fig. 2), although hormone binding was not significantly affected (29). However, the transcriptional activity of these two ER mutants, unlike that of ERwt, was strongly stimulated by 4-OHT and ICI 164,384 to a level close to that of estradiol-activated ERwt (Fig. 2). This full agonist activity of both antiestrogens was also detected in chicken embryo fibroblasts and MCF-7 cells (data not shown).

The DNA-binding activity in WCE was determined for the different ER mutants that were transiently expressed in estradiol- or antiestrogen-treated Cos-1 cells (Fig. 3). As previously reported (26), the DNA-binding activity of the wild-type receptor was unaffected by treating cells with 17β-estradiol or 4-OHT, but fewer complexes were observed in the presence of ICI 164,384. In contrast, as shown in Fig. 3, ER(L543A/L544A) and ER(M547A/L548A) generated normal amounts of complexes after ICI 164,384 treatment, which correlates with their strong transcriptional activation properties (see Fig. 2). The mutant ER(E546A) and ER(D542N/E546Q/D549N) behaved like ERwt (data not shown).

Since ICI 164,384 has been shown to decrease the cellular levels of ER (25, 26), we determined the effect of the antiestrogens on the levels of mutant receptors by Western blotting to test if reduced binding activity is due to reduced receptor levels (Fig. 4*A*). The levels of ER(L543A/L544A) and

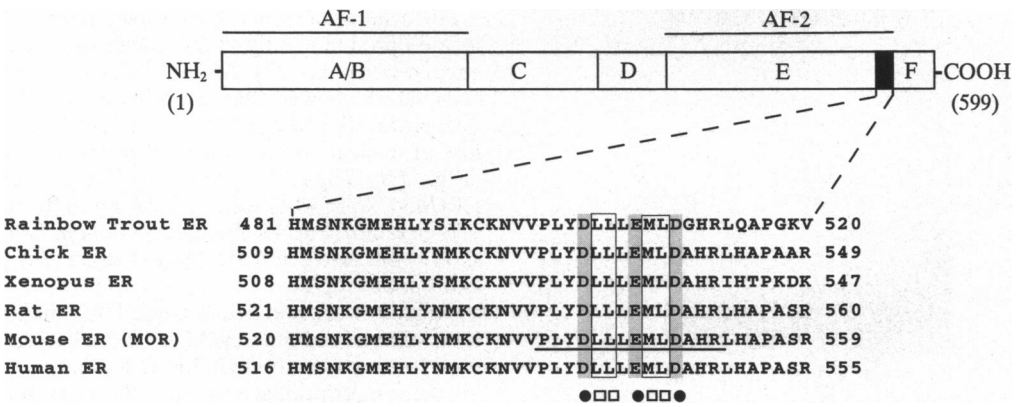


FIG. 1. Domain organization of the ER and amino acid sequence alignment of a highly conserved region of the ligand-binding domain from different species. The mouse ER (MOR), 599 amino acids long, is subdivided into six regions (A–F) (top scheme). The A/B domain carries a cell-type-dependent and promoter-specific transcriptional activation function termed AF-1, and the E domain contains the ligand-inducible transcriptional activation function AF-2. The AF-2 region, which is essential for transcriptional activation, is highly conserved as shown below by the alignment of a homologous region (amino acid numbers are indicated) of ER from rainbow trout (13), *Xenopus laevis* (11), chicken (10), rat (9), mouse (12), and human (8). The amino acid sequence underlined in the MOR represents a subregion that is essential for hormone-dependent activation of transcription (29). It contains the two pairs of hydrophobic amino acids (boxed and □) and their adjacent negatively charged amino acids (shaded and ●) whose substitution has been studied herein.

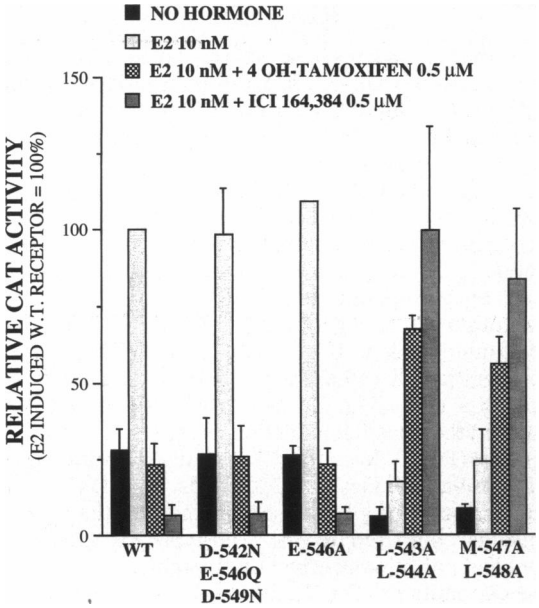


FIG. 2. Effect of antiestrogens on transcriptional stimulation of an ERE-controlled reporter gene by wild-type and mutant MOR-(1–599). HeLa cells cotransfected with the reporter gene and expression vectors for wild-type or mutated receptors were maintained without added ligand (no hormone) or in the presence of 10 nM 17β-estradiol (E2) alone, or together with 0.5 μM 4-OHT or 0.5 μM ICI 164,384. 4-OHT and ICI 164,384 alone had the same effects as when combined with 17β-estradiol (not shown). CAT activities, measured in WCE after normalization based on luciferase activity, represent the mean of four independent experiments with SEM error bars. The activity of the reporter gene in the presence of 17β-estradiol-induced wild-type MOR-(1–599) was taken as 100% activity.

ER(M547A/L548A) were similar irrespective of the ligand bound, whereas the levels of ERwt were markedly reduced after ICI 164,384 treatment. Thus it appears that, in contrast to ERwt, the turnover of the mutant receptors is unaffected by the pure antiestrogen.

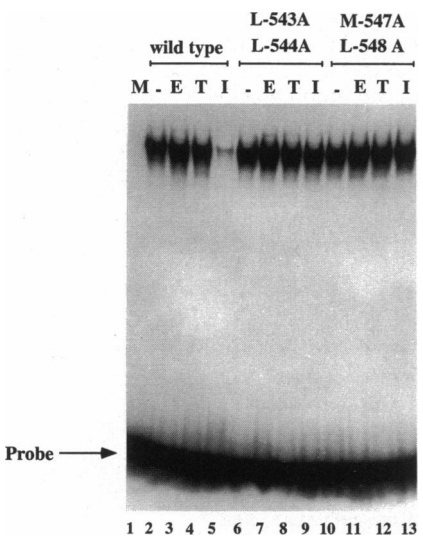


FIG. 3. Effect of antiestrogens on the DNA-binding activity of wild-type and mutant ERs. MOR-(1–599) (lanes 2–5), L543A/L544A (lanes 6–9), and M547A/L548A (lanes 10–13) were produced in Cos-1 cells cultured in hormone-free medium (carrier alone; -), 10 nM 17β-estradiol (E), 0.5 μM 4-OHT (T), or 0.5 μM ICI 164,384 (I). M is for an extract prepared from mock-transfected cells. Equivalent amounts of WCEs were assayed for their DNA-binding activity in a gel-shift assay using a consensus ERE probe. The position of the free probe is indicated.

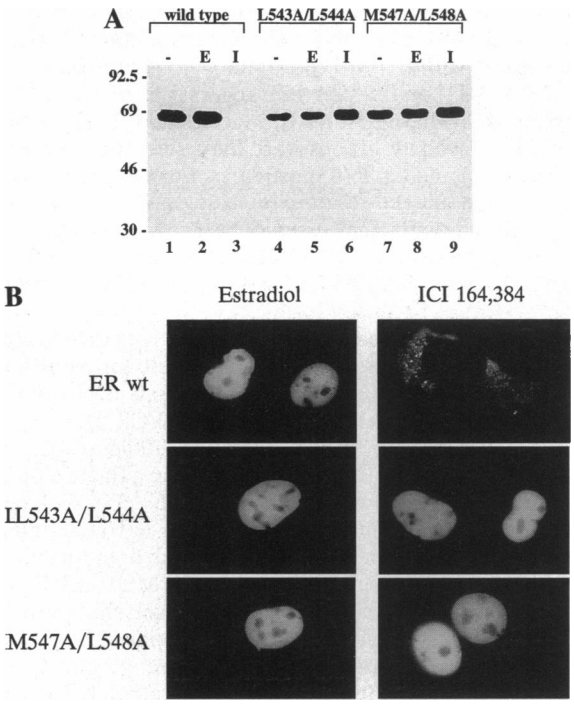


FIG. 4. Effect of ICI 164,384 on wild-type and mutant receptor protein levels and subcellular localization determined by Western blotting (*A*) and immunocytochemistry (*B*). (*A*) Cos-1 cells, transiently expressing wild-type MOR-(1–599) (lanes 1–3), L543A/L544A (lanes 4–6), and M547A/L548A (lanes 7–9) were cultured and treated with 10 nM 17β-estradiol (E), 0.5 μM ICI 164,384 (I), or carrier alone (-). The amounts of wild-type and mutant receptors in equivalent amounts of WCE were determined by Western blot analysis using the H222 antibody (Abbott Laboratories). The position of molecular mass markers is shown (in kDa). (*B*) MOR-(1–599) (ERwt) (*Top*), ER(L543A/L544A) (*Middle*), and ER(M547A/L548A (*Bottom*) were transiently expressed in Cos-1 cells as in Fig. 3. After treatment with 10 nM 17β-estradiol or 100 nM ICI 164,384 for 1 hr, cells were fixed and the proteins were detected by indirect immunofluorescence using the H222 antibody. Wild-type and mutant ERs exhibited a nuclear staining in the absence (not shown) or in the presence of estradiol (*Left*). ICI 164,384 altered the subcellular distribution of the wild-type protein, which was detected at a low level only in the cytoplasm (*Top Right*). However, nuclear localization and levels of ER(L543A/L544A) and ER(M547A/L548A) were not affected by ICI 164,484 treatment (*Middle* and *Bottom Right*).

Histochemical studies have shown that the ERwt protein is located predominantly in the nucleus in the absence or presence of hormone (27). A similar study with transfected Cos-1 cells, which showed that, while 4-OHT had no effect on the nuclear localization of ERwt, ICI 164,384 resulted in a rapid loss of nuclear in favor of cytoplasmic localization of ERwt (Fig. 4*B, Top*). In contrast, ER(L543A/L544A) and ER(M547A/L548A) were nuclear not only in the presence of 17β-estradiol (Fig. 4*B, Middle* and *Bottom Left*) or 4-OHT (not shown) but also after ICI 164,384 treatment (Fig. 4*B, Middle* and *Bottom Right*).

Previous work had demonstrated that the agonist activity of tamoxifen is mediated by AF-1 (20). Therefore, we tested the importance of this domain in the mutant receptors by analyzing the transcriptional activity of the same mutations as above but in MOR-(121–599), which lacks AF-1. The DNA-binding activity of these mutant receptors was similar to that of the mutated full-length receptors irrespective of the ligand bound (data not shown). However, mutations in the charged residues of the truncated receptor reduced the basal activity of the reporter gene compared to that obtained with the wild-type receptor but showed a similar 3- to 4-fold response to estradiol (Fig. 5). Both antiestrogens abolished the estrogen-dependent

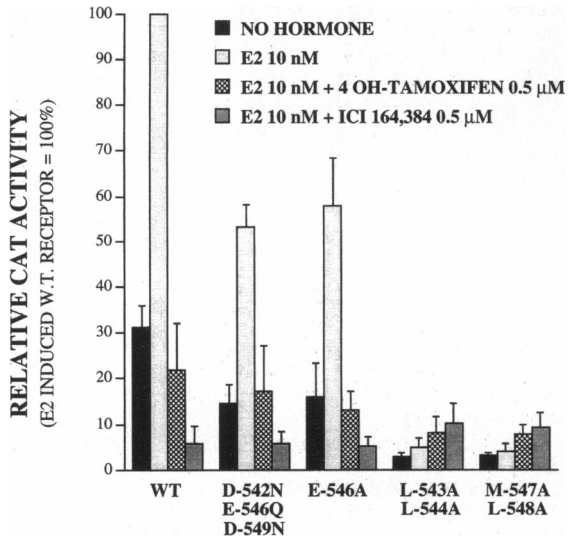


FIG. 5. An intact AF-1 domain is a prerequisite for transcriptional stimulation by L543A/L544A and M547A/L548A ER mutants activated by antiestrogens. The mutations L543A/L544A and M547A/L548A were introduced into a truncated receptor lacking the N-terminal AF-1 region [MOR-(121–599)] and their activity was compared to that of the wild-type truncated version (WT). HeLa cells cotransfected with the reporter gene and expression vectors for wild-type or mutated receptors were maintained without added ligand (no hormone) or in the presence of 10 nM 17β-estradiol (E2) alone, or together with 0.5 μM 4-OHT or 0.5 μM ICI 164,384 as indicated. 4-OHT and ICI 164,384 alone had the same effects as when combined with 17β-estradiol (not shown). The mean of normalized CAT activities from four independent experiments with SEM error bars is presented. The activity of the reporter gene in presence of 17β-estradiol-induced wild-type MOR-(121–599) was taken as 100% activity.

activation, with ICI 164,384 being more effective. As expected ER(L543A/L544A) and ER(M547A/L548A) failed to stimulate transcription in the presence of estradiol, but, in contrast to the corresponding full-length mutated receptors, they had only a weak effect in the presence of antiestrogens. Thus, we conclude that AF-1 is required to mediate the action of both tamoxifen and ICI 164,384 in ER(L543A/L544A) and ER(M547A/L548A) (Table 1).

## DISCUSSION

Point mutations within the conserved region required for hormone-dependent activation of the ER dramatically change the pharmacology of antiestrogens. Mutation of either one of the two pairs of hydrophobic amino acids present in this region (L543/L544 or M547/L548) is sufficient to convert the ER into a transcriptionally potent molecule after activation not only by tamoxifen with its known mixed agonist/antagonist activity but also by the pure antiestrogen ICI 164,384. Such mutations protect the ER from the enhanced turnover and nuclear exclusion induced by ICI 164,384. These characteristics of the mutants are independent of AF-1, since they are also a feature of the mutants lacking AF-1. However, the AF-1 region is required, in combination with AF-2, for a transcriptionally active configuration of the mutants, which is dependent on tamoxifen or ICI 164,384 activation. This suggests that AF-1, whose activity is minimal in the wild-type receptor in a number of cell types (this work; refs. 29 and 35), together with AF-2 is involved in the formation of a strong transcriptional interface induced by the antiestrogens in the mutant receptors. Other mutations in this region have been described that alter the binding of estradiol or tamoxifen (36–38), but we know of no other report of mutations which change the sensitivity of the ER to antiestrogens from antagonist to agonist.

The regulated transcription of protein coding genes by transcriptional activators involves the assembly of a preinitiation complex (39, 40). Numerous basal transcription factors are required for transcriptional initiation by RNA polymerase II (41), and it is thought that these are likely targets for the transcriptional activators, including nuclear receptors (42–47). Alternative targets, distinct from the basal transcription factors, are also suggested by so-called transcriptional interference or squelching experiments (48, 49). Potential candidates include proteins that have recently been shown to interact directly with the hormone-binding domain in the presence of estrogen (50, 51). It will be interesting to determine if the interaction of these proteins with mutant receptors is enhanced by antiestrogens. However, since AF-1 is required to allow the mutant receptors to stimulate transcription, it is conceivable that the AF-1 and mutant AF-2 domains generate a distinct activation function in the presence of antiestrogens, and the activation region contacts a distinct target protein.

The most important question raised by our results is whether similar mutations occur in breast tumors and, if so, whether they account for tamoxifen resistance. Recently, the analysis of ER coding sequences in tamoxifen-resistant tumors by using the technique of single-stranded chain polymorphism (SSCP) revealed that mutations were infrequent and were unlikely to account for most tamoxifen resistance encountered (52, 53). Nevertheless, since SSCP is estimated to detect only about 85% of the gene mutations, it is important that more tumors are analyzed and by alternative techniques. Since the hydrophobic mutations might manifest themselves as tumor flare, the most likely type of tumors in which these mutations may play a role are those which show a reduced growth or even regression upon tamoxifen withdrawal.

Table 1. Activation of full-length [MOR-(1–599)] and AF-1-truncated [MOR-(121–599)] wild-type and mutant ERs by 17β-estradiol (E2) or antiestrogens (4-OHT and ICI 164,384)

| Receptor | Fold induction, activated receptor vs. no hormone | | | | | |
|---|---|---|---|---|---|---|
| | E2 (10 nM) | | E2 (10 nM) + 4-OHT (0.5 μM) | | E2 (10 nM) + ICI 164,384 (0.5 μM) | |
| MOR-(1–599) | 3.7 ± 1.0 | 1.1 | 0.8 ± 0.2 | 1.1 | 0.2 ± 0.1 | 1.0 |
| MOR-(121–599) | 3.3 ± 0.5 | | 0.7 ± 0.4 | | 0.2 ± 0.2 | |
| MOR-(1–599)(L543A/L544A) | 3.0 ± 0.9 | 1.9 | 12.1 ± 4.2 | 4.7 | 17.8 ± 7.9 | 5.1 |
| MOR-(121–599)(L543A/L544A) | 1.6 ± 0.4 | | 2.6 ± 0.7 | | 3.4 ± 0.8 | |
| MOR-(1–599)(M547A/L548A) | 2.6 ± 0.9 | 2.0 | 6.2 ± 0.3 | 2.4 | 9.3 ± 2.5 | 4.2 |
| MOR-(121–599)(M547A/L548A) | 1.3 ± 0.5 | | 2.6 ± 0.4 | | 3.0 ± 0.8 | |

The stimulation is expressed as fold induction over the noninduced level. Values represent the mean ± SEM of four independent experiments. Numbers to the right of braces are the relative contribution of AF-1 in the ligand-dependent effects. Note that MOR-(121–599) is induced by estradiol as efficiently as MOR-(1–599). Deletion of AF-1 abolished the induction of L543A/L544A and M547A/L548A mutants by estradiol and resulted in a marked loss of activation by 4-OHT and ICI 164,384.

We thank B. Desvergne for critical reading of the manuscript. This work was supported by the Swiss National Science Foundation and the Etat de Vaud.

1. Evans, R. M. (1988) *Science* **240,** 889–895.
2. Kumar, V. & Chambon, P. (1988) *Cell* **55,** 145–156.
3. Ham, J. & Parker, M. G. (1989) *Curr. Opin. Cell Biol.* **1,** 503–511.
4. Martinez, E. & Wahli, W. (1989) *EMBO J.* **8,** 3781–3791.
5. Beato, M. (1989) *Cell* **56,** 335–344.
6. Martinez, E. & Wahli, W. (1991) in *Nuclear Hormone Receptors*, ed. Parker, M. G. (Academic, London), pp. 125–153.
7. Wahli, W. & Martinez, E. (1991) *FASEB J.* **5,** 2243–2249.
8. Green, S., Walter, P., Kumar, V., Krust, A., Bornet, J.-M., Argous, P. & Chambon, P. (1986) *Nature (London)* **320,** 134–139.
9. Koike, S., Sakai, M. & Muramatsu, M. (1987) *Nucleic Acids Res.* **15,** 2499–2513.
10. Krust, A., Green, S., Argos, P., Kumar, V., Walter, P., Bornet, J. M. & Chambon, P. (1986) *EMBO J.* **5,** 891–897.
11. Weiler, I. J., Lew, D. & Shapiro, D. J. (1987) *Mol. Endocrinol.* **1,** 355–362.
12. White, R., Lees, J. A., Neadham, M., Ham, J. & Parker, M. G. (1987) *Mol. Endocrinol.* **1,** 735–744.
13. Pakdel, F., Le Gac, F., Le Goff, P. & Valotaire, Y. (1990) *Mol. Cell. Endocrinol.* **71,** 195–204.
14. Morrow, M. & Jordan, V. C. (1993) *Arch. Surg.* **128,** 1187–1191.
15. Fuqua, S. A. W., Chumness, E. C. & McGuire, W. L. (1993) *J. Cell. Biochem.* **51,** 135–139.
16. Jordan, V. C. & Murphy, C. S. (1990) *Endocr. Rev.* **11,** 577–610.
17. Baum, M., Brinkley, D. M., Dossett, J. A., McPherson, K., Patterson, J. S., Rubens, R., Smiddy, F. G., Stoll, B. A., Wilson, A., Lea, J. C., Richards, D. & Ellis, S. H. (1983) *Lancet* **i,** 257–261.
18. Powles, T. J. (1992) *Lancet* **340,** 1145–1147.
19. Spern, M. B. (1993) *Lancet* **342,** 1211–1212.
20. Berry, M., Metzger, D. & Chambon, P. (1990) *EMBO J.* **9,** 2811–2818.
21. Wakeling, A. E. & Bowler, J. (1988) *J. Steroid Biochem.* **30,** 141–148.
22. Borgna, J.-L. & Rochefort, H. (1980) *Mol. Cell. Endocrinol.* **20,** 71–85.
23. Bowler, J., Lilley, T. J., Pittam, J. D. & Wakeling, A. E. (1989) *Steroids* **54,** 71–99.
24. Wakeling, A. E., Dukes, M. & Bowler, J. (1991) *J. Cancer Res.* **51,** 3767–3873.
25. Gibson, M. K., Nemmers, L. A., Beckman, W. C., Jr., Davis, V. L., Curtis, S. W. & Korach, K. S. (1991) *Endocrinology* **129,** 2000–2010.
26. Dauvois, S., Danielian, P. S., White, R. & Parker, M. G. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 4037–4041.
27. Dauvois, S., White, R. & Parker, M. G. (1993) *J. Cell Sci.* **106,** 1377–1388.
28. Lees, J. A., Fawell, S. E. & Parker, M. G. (1989) *Nucleic Acids Res.* **17,** 5477–5488.
29. Danielian, P. S., White, R., Lees, J. A. & Parker, M. G. (1992) *EMBO J.* **11,** 1025–1033.
30. Walker, P., Germond, J. E., Brown-Luedi, M., Givel, F. & Wahli, W. (1984) *Nucleic Acids Res.* **12,** 8611–8626.
31. Klein-Hitpass, L., Schorpp, M., Wagner, U. & Ryffel, G. U. (1986) *Cell* **46,** 1053–1061.
32. De Wet, J. R., Wood, K. K., Pemua, M., Helsinki, R. R. & Subromani, S. (1987) *Mol. Cell. Biol.* **7,** 725–737.
33. Gorman, C. M., Moffat, L. F. & Howard, B. H. (1982) *Mol. Cell. Biol.* **2,** 1044–1051.
34. Greene, G. L., Sobel, N. B., King, W. J. & Jensen, E. V. (1984) *J. Steroid Biochem.* **20,** 51–56.
35. Tora, L., White, J., Brov, C., Tasset, D., Webster, D., Sheen, E. & Chambon, P. (1989) *Cell* **59,** 477–487.
36. Wrenn, D. K. & Katzenellenbogen, B. S. (1993) *J. Biol. Chem.* **268,** 24089–24098.
37. Pakdel, F. & Katzenellenbogen, B. S. (1992) *J. Biol. Chem.* **267,** 3429–3437.
38. Danielian, P. S., White, R., Hoare, S. A., Fawell, S. E. & Parker, M. G. (1993) *Mol. Endocrinol.* **7,** 232–240.
39. Ptashne, M. (1988) *Nature (London)* **335,** 683–689.
40. Mitchell, P. J. & Tjian, R. (1989) *Science* **245,** 371–378.
41. Zawel, L. & Reinberg, D. (1992) *Curr. Opin. Cell Biol.* **4,** 488–495.
42. Ing, N. H., Beekman, J. M., Tsai, S. Y., Tsai, M.-J. & O'Malley, B. W. (1992) *J. Biol. Chem.* **267,** 17617–17623.
43. Sadovsky, Y., Webb, P., Lopez, G., Baxter, J. D., Cavaillès, V., Parker, M. G. & Kushner, P. J. (1995) *Mol. Cell. Biol.* **15,** 1554–1563.
44. Pugh, B. F. & Tjian, R. (1990) *Cell* **61,** 1187–1197.
45. Goodrich, J. A., Hoey, T., Thut, C. J., Admon, A. & Tjian, R. (1993) *Cell* **75,** 519–530.
46. Hoey, T., Weinzeri, R. O. J., Gill, G., Chen, J.-L., Dynlacht, B. D. & Tjian, B. (1993) *Cell* **71,** 247–260.
47. Jacq, X., Brou, C., Lutz, Y., Davidson, I., Chambon, P. & Tora, L. (1994) *Cell* **79,** 101–117.
48. Tasset, D., Tora, L., Fromental, C., Scheer, E. & Chambon, P. (1990) *Cell* **62,** 1177–1187.
49. Martin, K. J., Lillie, J. W. & Green, M. R. (1990) *Nature (London)* **346,** 147–152.
50. Halachmi, S., Marden, E., Martin, G., MacKay, C., Abbondanza, C. & Brown, M. (1994) *Science* **264,** 1455–1458.
51. Cavaillès, V., Dauvois, S., Danielian, P. S. & Parker, M. G. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 10009–10013.
52. Karnik, P. S., Kulkarni, S., Liu, X. P., Budd, G. T. & Bukowski, R. M. (1994) *Cancer Res.* **54,** 349–353.
53. Wolf, D. M. & Jordan, V. C. (1994) *Br. Cancer Res. Treat.* **31,** 129–138.