Identification of Amino Acids in the Hormone Binding Domain of the Human Estrogen Receptor Important in Estrogen Binding*

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The initial step in the regulation of the transcriptional activity of the estrogen receptor (ER) is the binding of hormone. Previous studies have suggested that the region between amino acids 515 and 535 near the C terminus of the human ER is likely to be important in ligand binding. In order to explicitly define which amino acids in this region are critical for ligand recognition and binding, we have utilized alanine-scanning mutagenesis over the complete 515-535 region. The ability of these 21 mutants to activate transcription in response to the natural estrogen, 17β-estradiol (E2), was evaluated in cell co-transfection assays with estrogen-responsive reporter genes. In addition, their ability to bind E2 was also tested. Mutations at four sites in the 521-528 region had the greatest effects on E2-induced transcription, with L525A reducing responsiveness 250-fold, G521A and H524A 35-fold, and M528A 11-fold. Mutations at other sites had either no effect or a 4-fold or lesser reduction in sensitivity to E2 (M517A, Y526A, N532A, and P535A). Three of the mutants most affected in their transcriptional response, G521A, H524A, and M528A, showed a coordinate reduction in E2 binding affinity. E2 binding by the most affected mutant, L525A, could not be detected. Thus, the altered transcriptional response of these ER mutants appears to derive solely from an alteration in their affinity for the ligand E2. The four sites most affected by alanine substitution, 521, 524, 525, and 528, follow an α-helical periodicity, such that they would be positioned on one face of an α-helix. Furthermore, they correspond precisely to residues in an α-helix shown to be in contact with ligand in the recently described x-ray crystal structures of two other members of the nuclear hormone receptor superfamily, namely the retinoic acid receptor- and thyroid hormone receptor-ligand complexes. Our findings, which broaden observations to the steroid receptor family within the superfamily of nuclear receptors, suggest that this region of the estrogen receptor is in contact with its cognate ligand in a similar fashion.

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§The abbreviations used are: ER, estrogen receptor; hER, human ER; AF, activation function; CAT, chloramphenicol acetyltransferase; CMV, cytomegalovirus; ERE, estrogen response element; E2, 17β-estradiol; LBD, ligand binding domain; hRARα, human retinoid X receptor α; rTRα, rat thyroid hormone receptor-α1.
region of the receptor being important for ligand binding. For instance, mutating the glycine at position 525 of the mouse ER (Gly525 of the human ER) to arginine nearly completely abolished E₂ binding and activity, and deleting residues 521 and 522 (517 and 518 of the human ER) was shown to reduce E₂ binding by 200-fold (15).

Other experiments have demonstrated that regions more C-terminal and N-terminal in the LBD play important roles in hormone-dependent transactivation and receptor dimerization, respectively. A predicted amphipathic α-helix spanning residues 534–548 of the E domain appears to be critical in AF-2 function (16–18), and amino acids 507–518 of the mouse ER (503–514 of the human ER) have been implicated as being important in the dimerization of the receptor (17, 19).

In order to explicitly define which amino acid residues from positions 515–535 of the estrogen receptor are critical for ligand recognition and binding, we have utilized alanine-scanning mutagenesis (20). Alanine substitution is a more conservative mutation than has previously been utilized in the limited analyses in this region, and it has enabled us to assess the individual contributions of each of these 21 amino acids to the transcriptional activity induced by the natural ligand estradiol and the binding of this ligand. With this approach, we identified four amino acids that are of particular importance in ligand binding. Interestingly, their positioning suggests that these four residues lie along one face of an α-helix in the ER LBD and are likely to make contact with ligand. These results are interpreted in light of the recently published crystal structures of LBD-ligand complexes from two related receptors of the nuclear receptor superfamily (21–23).

**EXPERIMENTAL PROCEDURES**

**Plasmids and General Reagents**—The plasmids 2ERE-pS2-CAT (24), pCMV5HER (16), and pCMVβ (Clonetech, Palo Alto, CA) have been previously described. The vector pTZ19R was kindly provided by Dr. Byron Kemper (University of Illinois, Urbana, IL), and pBluescript II SK⁺ was from Stratagene (La Jolla, CA). Plasmids were purified for transfection using either CsCl gradient centrifugation or a plasmid preparation kit according to the manufacturer’s instructions (Qiagen, Chatsworth, CA). Restriction enzymes were purchased from Life Technologies, Inc. and Sigma. For Western analysis, nitrocellulose membrane was obtained from Millipore Corp. (Marlborough, MA), and the H226 antibody was kindly provided by Dr. Geoffrey Greene (University of Chicago), and rabbit anti-rat IgG was purchased from Zymed (San Francisco, CA). Radioisotopes for chloramphenicol acetyltransferase (CAT) assays, screening, hormone binding assays, and Western blotting were purchased from DuPont NEN and Amersham Corp. All other reagents were purchased from Sigma, Fisher (Pittsburgh, PA), and Amersham. Oligomer-directed Mutagenesis—The 1.8-kilobase ER-containing BamHI fragment from pCMV5HER was cloned into the BamHI site of pBluescript II SK⁺. Site-directed mutagenesis was then performed according to Kunkel et al. (26) using the oligonucleotides listed in Table I, omitting the listed ER alanine substituted oligonucleotides. Oligonucleotides were purchased from Life Technologies, Inc. Screening for the desired ER mutations was done by restriction enzyme analysis (Table I). Following mutagenesis, the ER cDNAs were excised from pBluescript II SK⁺ using BamHI and ligated into the BamHI site of the cytomegalovirus-driven expression vector, pCMV5, kindly provided by Dr. David Russell (University of Texas, Dallas, TX) (27). All ER mutations were then confirmed by dideoxy sequence analysis using a Sequenase 2.0 kit (U.S. Biochemical Corp./Amersham).

**Cell Culture and Transfections**—All transfections were done in ER-negative human breast cancer MDA-MB-231 cells. Cells were maintained and transfected as described previously (7). Cells were plated for transfection at a density of 3 × 10⁶ cells/100-mm dish and incubated for 40–48 h at 37°C with 5% CO₂. Transfections were performed using 2.0 μg of the reporter plasmid 2ERE-pS2-CAT, 0.8 μg of the β-galactosidase reporter plasmid pCMVβ, 0.1 μg of ER expression vector, and pTZ19R carrier plasmid to 15 μg of total DNA/100-mm diameter dish. Cells were incubated with calcium phosphate-precipitated DNA for 4 h and then subjected to a 2.5-min glycerol shock, using 20% glycerol in growth medium, followed by a 2.5-min rinse in Hank’s balanced salt solution and ligand treatment in growth medium. Cells were harvested 24 h after ligand treatment and lysed by three cycles of freezing on dry ice and thawing at 37°C. ER transactivation ability was determined by CAT activity of the whole cell lysates and assayed as described previously (28). CAT assays were normalized to β-galactosidase activity from the co-transfected pCMVβ plasmid. Western Analysis—231 cells were transfected in 100-mm dishes with 10 μg of ER expression vector, 0.8 μg of pCMVβ, and pTZ19R carrier plasmid to 15 μg of total DNA. Cells were then treated with hormone or ethanol vehicle and incubated for 24 h before harvesting in cold HBSS. The cells were centrifuged at 200 × g for 5 min and resuspended in 20 mM Tris (pH 7.4), 0.5 mM NaCl, 1.0 mM dithiothreitol, 10% glycerol (v/v), 50 μg/ml leupeptin, 50 μg/ml aprotinin, 2.5 μg/ml pepstatin, and 0.2% phenylmethylsulfonyl fluoride. Whole cell extracts were obtained by subjecting cells to three rounds of freezing on dry ice and thawing on wet ice followed by centrifugation at 15,000 × g to remove cell debris. Cell extracts for expression studies were normalized to β-galactosidase activity, from the co-transfected pCMVβ vector, before loading equivalent β-galactosidase units on a 10% SDS-polyacrylamide gel (29). Electrophoresis and Western blotting were done according to standard methods (30). Nitrocellulose blots were probed with the human ER-specific primary antibody H226 at 2.0 μg/ml and then incubated with rabbit anti-rat IgG (1 μg/ml), and detected with 125I-conjugated protein A.

**Hormone Binding Assays**—Binding assays for estradiol and Scat-}

### RESULTS

Screen for Alanine-substituted Mutant Estrogen Receptors with Altered Transcriptional Activity—Alanine mutants in the ligand binding domain of the human estrogen receptor (hER; amino acids 515–535) were created by oligomer-directed mutagenesis of the ER cDNA (see Table I). The presence of the correct mutations was confirmed by restriction enzyme analysis and sequencing. The ability of each of the 21 mutant ERs to transactivate an ERE-dependent gene was first determined in a simple screening assay (Fig. 1 and Table II). Human breast cancer MDA-MB-231 cells, which lack endogenous ER, were cotransfected with one of the ER expression vectors together with an [ERE]₂-pS2-CAT reporter, and CAT activity in cells was monitored after treatment with a single concentration of estradiol (Fig. 1). For this screening we used 1 × 10⁻⁸ M E₂ and 100 ng of ER expression vector, since wild type ER reached near maximal activity at this concentration of E₂, and under these conditions the level of activation was independent of the amount of transfected ER DNA over the range of 50–400 ng (data not shown). CAT activity was very low in all receptors treated with control 0.1% ethanol vehicle and was induced 100–200-fold over control for wild type ER by the addition of E₂. Fig. 1 shows the transcriptional activity of each of the mutants. Alanine substitutions N-terminal to position 520 and C-terminal to position 529 showed little to no effect on the ability of the ER to activate transcription, although slight reductions in transactivation were observed for M517A, S518A, N532A, and P535A. As expected from previous work, changing the cysteine at position 530 to alanine had no effect (28). Larger effects on ER activity were observed when alanine was substituted at certain sites in the region spanning amino acids 521–
pressed as full-length stable proteins, Western blot analyses
In order to demonstrate that the mutant receptors were ex-
alterations in the protein that might affect its overall stability.
receptor activity that might be caused by global structural
21-amino acid region of the hER, we hoped to avoid changes in
conservative amino acid change, for each amino acid across this
528. Particularly affected were amino acids Gly521, His524, and
Leu525, with the latter mutant having almost no activity at 1 \times 10^{-9} M E_2. A lesser but still significant reduction in activity
was also observed for M528A.

Western Blot Analysis—By substituting alanine, a relatively
conservative amino acid change, for each amino acid across this
21-amino acid region of the hER, we hoped to avoid changes in
receptor activity that might be caused by global structural
alterations in the protein that might affect its overall stability.
In order to demonstrate that the mutant receptors were ex-
pressed as full-length stable proteins, Western blot analyses
were performed. Cells were transfected with ER expression
vector and treated with either ethanol control vehicle or hor-
mone. ER protein was detected using H226 antibody, which
recognizes an epitope in the B domain of the ER, well away
from the altered region. With the exceptions of K520A and
M528A, proved to be dose-shifted in their response to E2,

Surprisingly, despite its decreased transcriptional activity,
the G521A ER mutant was found to be present at higher levels
than wild type, both in the presence and absence of estradiol
(Fig. 2, compare lanes 1 and 2 with lanes 5 and 6). Conversely,
both K520A and L525A were present at low levels in the
absence of estradiol, although their levels increased somewhat
(for K520A) or to wild type levels (for L525A) in the presence of
1 \times 10^{-8} M E_2 (data not shown)). This result
indicates that the L525A protein is more stable in the presence
of estradiol, in contrast to wild type ER (Fig. 2).

Estriol Dose-Response Curves for the Mutant Receptors—
Since our goal was to identify residues that are important in
estrogen receptor ligand binding and recognition, we measured
the transcriptional activity of the mutant receptors over a
range of E_2 concentrations, from 1 \times 10^{-12} to 1 \times 10^{-7} M.
These dose-response curves enabled us to determine whether mu-
tants, which exhibited reduced activity at the single E_2 concen-
tration tested in Fig. 1, were dose-shifted in their response to
E_2 or were defective in their activation function. For wild type
ER, maximal activity was reached at an E_2 concentration be-
tween 1 \times 10^{-9} M and 1 \times 10^{-8} M, and half-maximal activity
(EC_{50}) was reached at about 1 \times 10^{-10} M E_2 (Fig. 3A). For
several of the mutant receptors that exhibited wild type or near
wild type activity at 1 \times 10^{-9} M E_2, dose-response curves
were not significantly different from wild type ER (K520A) or were
only slightly altered (Fig. 3, A and B, and data not shown).

All of the mutations that resulted in significantly reduced
activity of receptor at 1 \times 10^{-9} M E_2, G521A, H524A, L525A,
and M528A, proved to be dose-shifted in their response to E_2,
requiring elevated levels of E_2 relative to wild type ER in order
to reach half-maximal and maximal activity (Fig. 3, C and D). Of these four mutants, the M528A dose-response was shifted the least, while the L525A ER was the most defective in E2 response. L525A required $1 \times 10^{-7}$ M or more estradiol to reach wild type activity, and its dose-response curve was shifted to the right more than 250-fold (Fig. 3D). Both G521A and H524A were dose-shifted nearly 35-fold (Fig. 3C). As might be expected for mutations that cause a defect only in affinity for ligand, each mutant receptor achieved wild type or near wild type activity in the presence of sufficiently high concentrations of estradiol. Over the concentration ranges we studied, none of the 21 mutant receptors exhibited higher activity than wild type ER at any E2 concentration (Fig. 3 and data not shown).

Binding of Estradiol by the Mutant Receptors—The affinities of the mutant estrogen receptors for estradiol were determined by saturation binding and Scatchard analysis. Cells were transfected with ER expression vector, and cell extracts were incubated with 1 nM to 10 nM $[^{3}H]$estradiol in the presence or absence of excess racinoid estradiol. The $K_d$ values are given in Table II and represent the average of three independent experiments. Our observed $K_d$ of 0.12 nM for wild type ER is in close agreement with previously published values (32) and with the level of E2 necessary to achieve half-maximal stimulation in our transient transfection assays (Fig. 3A). The dissociation constants calculated for the mutant receptors ($K_d$ values) correlated well with their predicted binding based on the estradiol dose-response curves in the co-transfection assay ($EC_{50}$ values), as shown in Fig. 4. M528A, with a $K_d$ of 0.45 nM, was the least impaired in hormone binding and in sensitivity to E2-induced transactivation, followed by G521A and H524A, which had $K_d$ values of 0.78 nM and 1.40 nM, respectively. These values represent 4–12-fold decreases in hormone binding and 10–35-fold decreases in sensitivity to E2-induced transactivation, compared with wild type ER.

We were unable to obtain a $K_d$ value for the L525A mutant for two reasons. First, the protein was unstable in the absence of hormone, limiting the quantity of receptor present in cell extracts used in the hormone binding assays (Fig. 2). Second, L525A bound estradiol extremely poorly. Considering the fact that transactivation response of L525A is dose-shifted by a factor of nearly 250, it is not surprising that we experienced difficulty with this mutant in our hormone binding experiments. Dose-response data suggest that the $K_d$ for estradiol binding by this mutant is at least 100-fold greater than for wild type ER (i.e. >10 nM).

**DISCUSSION**

The initial step in the regulation of the transcriptional activity by the estrogen receptor is the binding of ligand. Therefore, determining which residues of the ER are involved in ligand binding will be essential in elucidating the mechanism of receptor activity. Our findings, using alanine-scanning mutagenesis, suggest that amino acids in the region 521–528 of the human ER are intimately involved in the recognition and binding of hormone and also provide evidence that this region of the ER is $\alpha$-helical in its ligand-occupied form.

Specifically, we have identified four amino acid residues, Gly522, His524, Leu525, and Met528, that when mutated to alanine, resulted in a significantly reduced sensitivity of the ER to E2-stimulated transcription activation. However, despite requiring higher levels of E2 to elicit a response, each of the mutant receptors reached wild type activity at sufficiently high E2 concentrations. This suggests that the mutant ERs are impaired in terms of hormone binding rather than AF-2 transactivation function or DNA binding. Substitution of alanine for other amino acids in the region 521–528 of the human ER are intimately involved in the recognition and binding of hormone and also provide evidence that this region of the ER is $\alpha$-helical in its ligand-occupied form.

**Table II**

| Receptor | Transactivation activity | Hormone binding |
|----------|-------------------------|-----------------|
|          | Transactivation ability | $EC_{50}$ | Fold difference | $K_d$ | Fold difference |
| Wild type | 100% | 0.082 ± 0.03 | 1.0 | 0.12 ± 0.03 | 0.12 | 1.0 |
| R515A    | 96 ± 7 | 0.28 ± 0.04 | 3.4 | 0.78 ± 0.02 | 6.5 |
| H516A    | 94 ± 7 | 0.75 ± 0.004 | 0.9 | 1.4 ± 0.04 | 12 |
| S518A    | 85 ± 2 | 0.34 ± 0.01 | 4.0 | — | — |
| L525A    | 4 ± 1 | 0.45 ± 0.03 | 250 | — | — |
| G521A    | 3 ± 17 | 0.91 ± 0.02 | 11 | 2.1 |
| E523A    | 2 ± 11 | 0.02 ± 0.01 | 0.2 | 0.23 ± 0.01 | 2.8 |

* Transactivation of the mutant receptors relative to wild type at 1 $\times$ 10$^{-6}$ M E2, which is set at 100%.

**a** Effective concentration of E2 required for the receptor to reach half-maximal activity in the transactivation assay. Values are obtained from E2 dose-response data and are only given where full dose response assays were done.

**b** Where no value is given, $K_d$ is not determined.

**c** $K_d$, hormone binding assays performed, but specific binding by the L525A receptor protein was too low to obtain an accurate $K_d$ value.
the LBD, affect transactivation but not hormone binding by the receptor (16, 18).

Other laboratories have previously examined some mutations in this 515–535 region of the ER. For instance, Danielian et al. (15) showed that changing the methionine at position 532 of the mouse ER (Met528 in hER) to an arginine impaired hormone binding to the same degree as our M528A mutation, increasing its ¥ value for estradiol 3.7-fold. Interestingly, these two very different amino acid substitutions, small hydrophobic versus large negatively charged, at the position corresponding to hER Met528, affected the ER to a similarly modest extent, suggesting that this methionine plays an important, although not critical, role in estradiol binding.

The Gly521 residue has also been previously identified as potentially important in ligand binding. Changing the corresponding residue in the mouse ER (Gly225) to arginine has been shown to render mER incapable of binding E2 or of activating transcription even in the presence of ¥ M E2 (15, 17). For this reason, it has been assumed that this glycine plays a critical role either in the structure of the ligand binding domain or in hormone binding directly. Our more conservative alanine substitution had a much less severe effect on ER activity than the mER G525R mutation; the G521A mutant exhibited only a 50–60% reduction in activity at ¥ ¥ M E2. Furthermore, the affinity of our G521A mutant receptor for E2 was only 5.5-fold lower than that of wild type ER. Surprisingly, when comparing the levels of ER protein in transfected cells, G521A was found to be considerably more abundant than wild type ER, both in the presence and absence of E2. This G521A substitution must therefore alter the structure of the ER in some way that makes the protein more resistant to degradation, possibly by stabilizing an a-helix.

Our findings can be interpreted in the context of three recently reported x-ray crystallographic structures of the ligand binding domains of other members of the nuclear receptor superfamily, namely the LBD of the human retinoid X receptor a (hRXRa) without ligand and the LBD-ligand complexes of the human retinoic acid receptor g (hRARg) and rat thyroid hormone receptor-a (rTRAa) (21–23). Although these three receptors all have nonsteroidal ligands and have relatively modest amino acid sequence identity and similarity to the hER, it is anticipated that the LBDs of all nuclear hormone receptors will share a similar tertiary structure (33). A sequence alignment encompassing the hER region 515–535 is given in Fig. 5, together with an annotation of secondary structure elements described in the three reports and an identification of the residues in hRARg and rTRAa that are reported to be in contact with the ligand.

Residues Gly521, His524, Leu525, and Met528 of the hER all correspond to positions identified as ligand contact residues in the hRARg structure, Gly395, Arg396, Ala397, and Leu400. Positions 521 and 528 of the hER also correspond to ligand contact sites in the rTRAa structure, residues His389 and Met398. This correlation highlights a basic similarity with which at least a portion of these nuclear receptors interact with their ligands, whether steroidal or not, and suggests that Gly521, His524, Leu525, and maybe Met528 are in fact ligand contact sites in the hER.

It is interesting to note the location of these presumed ligand binding sites in the hER.
contact residues in the hER within the context of the LBD secondary and tertiary structure shown in the crystal structures. By analogy to the hRARγ and rTRα1 ligand-receptor complexes, the hER residue positions 521, 524, 525, and 528 lie in an α-helical region. In the helical face map of the residues in the 515–535 region of the hER (shown in Fig. 6), it is clear that the three residues most affected by mutation, Gly521, His524, and Leu528, are arranged as a compact unit on one face of two successive turns of a putative α-helix. Met525, which is of somewhat lesser importance in hormone binding and transactivation, is also located in this same facial region, but on the next helical turn. Alanine substitution of certain residues further removed from this facial region, Met517, Ser518, Tyr520, Asn522, and Pro525, have a modest but detectable effect on E2-induced transcriptional activity of the receptor (Fig. 1) and may likely be affecting ligand binding, although to a lesser degree. Interestingly, all of these residues, except one (Tyr526), are on the same face of the α-helix as Gly521, His524, and Leu528.

In any case, given the helical periodicity of the sites where mutation to alanine most affects ligand binding, and the homology of this region of the hER with α-helical regions in hRARγ and rTRα1, it seems most likely that the 521–528 region of the hER will adopt an α-helical secondary structure in the hER-estradiol complex. However, not all of these residues may be in a helix in the absence of ligand. Residues 521–525 of the hER correspond to a loop region between helices 10 and 11 of the unliganded hRXRa crystal structure but that this region is α-helical in the ligand-bound forms of hRARγ and rTRα1.

FIG. 4. Correlation between E2-induced transcriptional activation and E2 binding for wild type and mutant ERs. The Kd values for E2 binding and the concentration of E2 required to induce half-maximal transcriptional activity (EC50) are displayed in a log-log plot. The linearity of this plot indicates that E2 binding affinity to the various ERs and ER sensitivity to E2-induced transcriptional activation are correlated.

FIG. 5. Amino acid residues required for E2 binding by the hER correspond to residues of RAR and TRR that have been shown to contact ligand. A sequence alignment of hER with hRARγ, rTRα1, and hRXRα (taken from Ref. 33) is shown along with representations of secondary α-helical structure as revealed by x-ray crystallography. Shaded residues in the hER sequence identify amino acids that we have shown are important in E2 binding by the ER. These residues correspond exactly with ligand contact sites identified in the hRARγ and rTRα1 crystal structures (boxed residues). Note that amino acids 521–525 of the hER correspond to a loop region between helices 10 and 11 of the unliganded hRXRα crystal structure but that this region is α-helical in the ligand-bound forms of hRARγ and rTRα1.

FIG. 6. Residues in the region from 515 to 535 of the hER that are most important in E2 binding reside in a compact unit on one face of a proposed α-helix. A helical face map of the 515–535 region of the hER is shown (α-helix split longitudinally and opened up). Darkly shaded circles represent positions where alanine substitution inhibits E2 activity of the receptor 40–95%. Lightly shaded circles represent positions where alanine substitution inhibits E2 activity of the receptor 20–40%.
with other regions of estradiol that are within or near the A-ring.

It will be informative to discover whether the changes that affect E2 binding by the ER also affect the binding of other estrogens or antiestrogens, or whether different amino acids are involved in interactions with ligands of different structures.

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REFERENCES

1. Evans, R. M. (1988) Science 240, 889–895
2. Tsai, M.-J., and O’Malley, B. W. (1994) Annu. Rev. Biochem. 63, 451–486
3. Gronemeyer, H. (1991) Annu. Rev. Genet. 25, 89–123
4. Truss, M., and Beato, M. (1993) Endocr. Rev. 14, 459–479
5. Tsukerman, M. T., Esty, A., Santiso-Mere, D., Danielian, P., Parker, M. G., Stein, R. B., Pike, J. W., and McDonnell, D. P. (1994) Mol. Endocrinol. 8, 21–30
6. Berry, M., Metzger, D., and Chambon, P. (1990) EMBO J. 9, 2811–2818
7. Montano, M. M., Müller-Voll, V., Trobaugh, A., and Katzenellenbogen, B. S. (1995) Mol. Endocrinol. 9, 814–825
8. Fujimoto, N., and Katzenellenbogen, B. S. (1994) Mol. Endocrinol. 8, 296–304
9. Katzenellenbogen, B. S. (1996) Biol. Reprod. 54, 287–293
10. Sarrel, P. M., Lufkin, E. G., Oursler, M. J., and Keefe, D. (1994) Sci. Am. Sci. Med. 1, 44–53
11. Reed, L. D., and Katzenellenbogen, B. S. (1991) in Genes, Oncogenes, and Hormones: Advances in Cellular and Molecular Biology of Breast Cancer (Dickson, R. B., and Lippman, M. E., eds) pp. 277–299, Kluwer Academic Publishers, Boston
12. Harlow, K. W., Smith, D. N., Katzenellenbogen, J. A., Greene, G. L., and Katzenellenbogen, B. S. (1989) J. Biol. Chem. 264, 17476–17485
13. Reese, J. C., Wooge, C. H., and Katzenellenbogen, B. S. (1992) Mol. Endocrinol. 6, 2160–2166
14. Pakdel, F., Reese, J. C., and Katzenellenbogen, B. S. (1993) Mol. Endocrinol. 7, 1408–1417
15. Danielian, P. S., White, R., Hoare, S. A., Fawell, S. E., and Parker, M. G. (1993) Mol. Endocrinol. 7, 232–240
16. Wrenn, C. K., and Katzenellenbogen, B. S. (1993) J. Biol. Chem. 268, 24089–24098
17. Fawell, S. E., Lee, J. A., White, R., and Parker, M. G. (1990) Cell 60, 953–962
18. Danielian, P. S., White, R., Lee, J. A., and Parker, M. G. (1992) EMBO J. 11, 1025–1033
19. Lee, J. A., Fawell, S. E., White, R., and Parker, M. G. (1990) Mol. Cell. Biol. 10, 5529–5531
20. Wels, J. A. (1991) Methods Enzymol. 202, 390–410
21. Bourguet, W., Ruff, M., Chambon, P., Gronemeyer, H., and Moras, D. (1995) Nature 375, 377–382
22. Renaud, J.-P., Rochef, N., Ruff, M., Vivat, V., Chambon, P., Gronemeyer, H., and Moras, D. (1995) Nature 378, 681–689
23. Wagner, R. L., Aprieti, J. W., McGrath, M. E., West, B. L., Baxter, J. D., and Fletterick, R. J. (1995) Nature 378, 690–697
24. Montano, M. M., Ekema, K., Krueger, K. D., Keller, A. L., and Katzenellenbogen, B. S. (1996) Mol. Endocrinol. 10, 230–242
25. Mead, D. A., Szczesna-Skorpa, E., and Kemper, B. (1986) Protein Eng. 1, 67–74
26. Kunkel, T. A., Roberts, J. D., and Zakour, R. A. (1987) Methods Enzymol. 154, 367–382
27. Andersson, S., Davis, D. L., Dahlback, H., Jornvall, H., and Russell, D. W. (1989) J. Biol. Chem. 264, 8222–8229
28. Reese, J. C., and Katzenellenbogen, B. S. (1991) J. Biol. Chem. 266, 10880–10887
29. Laemmli, U. K. (1970) Nature 227, 680–685
30. Wrenn, C., and Katzenellenbogen, B. S. (1990) Mol. Endocrinol. 4, 1647–1654
31. Scatchard, G. (1949) Ann. N. Y. Acad. Sci. 51, 660–672
32. Pakdel, F., and Katzenellenbogen, B. S. (1992) J. Biol. Chem. 267, 3429–3437
33. Wurtz, J.-M., Bourguet, W., Renaud, J.-P., Vivat, V., Chambon, P., Moras, D., and Gronemeyer, H. (1996) Nat. Struct. Biol. 3, 87–94