Characterization of a Temperature-sensitive Mutation in the Hormone Binding Domain of the Human Estrogen Receptor

STUDIES IN CELL EXTRACTS AND INTACT CELLS AND THEIR IMPLICATIONS FOR HORMONE-DEPENDENT TRANSCRIPTIONAL ACTIVATION*

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A previous report from this laboratory (Reese, J. C., and Katzenellenbogen, B. S. (1991) J. Biol. Chem. 266, 10880–10887) identified an estrogen receptor (ER) mutant which had a similar binding affinity for estradiol as wild-type ER but displayed a dose-response shift for estradiol in transactivation studies. In this study, we have utilized hormone binding, DNA binding, and gene transfer experiments to further characterize this mutant, which contains an alanine substitution for a cysteine at amino acid 447 in the hormone binding domain of the receptor. Hormone binding studies indicate that the C447A receptor is a temperature-sensitive mutant, whose instability is only apparent at elevated temperatures, and that ligand can stabilize the mutant receptor. Western blot analysis reveals that the temperature-sensitive loss of hormone binding is not attributable to a degradation of receptor protein, but rather is an inactivation of the receptor’s hormone binding ability. In addition to the loss in the hormone binding capacity of the C447A mutant, this mutant shows a temperature-sensitive loss in the DNA binding ability of the receptor. Transactivation profiles of the mutant and wild-type receptors demonstrate that incubation of transfected cells with increasing concentrations of estradiol at more ambient temperatures shifts the mutant receptor’s dose-response curves to the left, converging on the wild-type curve. Hence, these transactivation studies reveal that the dose-response shift observed for this mutant in cells reflects the measured instability of the hormone binding and DNA interaction of the C447A mutant that can be demonstrated in vitro. In addition, this temperature-sensitive ER mutant is of interest in that its DNA binding is now ligand-dependent with the result that transcriptional activation now parallels receptor occupancy by ligand, which is similar to other steroid hormone receptors.

The estrogen receptor (ER) is a nuclear regulatory protein that mediates the actions of estrogens in target cells. The receptor-ligand complex binds as a dimer to a well defined 13-base pair palindromic sequence referred to as an estrogen response element (ERE). The binding of ligand is presumed to cause a conformational change in the receptor protein which results in a hormone-receptor complex that promotes transcription activation (or repression).

The cloning of the human estrogen receptor cDNA (1) has allowed the mapping of its functional domains through mutagenesis studies (2). The hormone binding domain (HBD) of the ER has been localized to the carboxy terminus of the receptor protein. Most earlier studies involved creating large deletions that provided critical, yet limited, information about this complex structure consisting of over 200 amino acids. More recently, a number of studies have focused on the roles of individual amino acids or regions in the HBD involved in ligand binding (3–5) and dimerization (6). More detailed analyses like these are necessary to identify amino acids important in the individual functions of the HBD, namely hormone binding, dimerization, nuclear localization, and transactivation.

A previous study by this laboratory had identified an ER mutant (C447A) in which the cysteine at position 447 had been replaced by a alanine. This C447A ER mutant had similar affinity for estradiol in vitro, while displaying a dose-response shift for estradiol in gene transfer/transactivation experiments (3). In this study we have examined the impairment of the C447A mutant in detail. The hormone binding, stability, DNA binding, and transactivating ability of this mutant were examined under a variety of conditions. The results of these studies indicate that the C447A mutant is a temperature-sensitive mutant, whose instability is apparent at elevated but not at more ambient temperatures. We report the characterization of a temperature-sensitive steroid receptor, whose demonstrated temperature sensitivity in cell-free extracts is also manifested in intact cells, and show how its analysis has revealed important information about ligand modulation of receptor activity.

**EXPERIMENTAL PROCEDURES**

**Chemicals and Materials—**Radioinert 17β-estradiol (E2) was obtained from Calbiochem. [2,4,5,6-3H]Estradiol (90 Ci/mmol), 13H1-2

*The abbreviations used are: ER, estrogen receptor; ERE, estrogen response element; E2, 17β-estradiol; CHO, Chinese hamster ovary; CAT, chloramphenicol acetyltransferase; WCE, whole cell extracts; HBD, hormone binding domain; WT, wild-type; DME, Dulbecco’s modified Eagle’s medium; SDS, sodium dodecyl sulfate; PBS, phosphate-buffered saline.

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protein A (low specific activity) and (dichloroacetyl-1,2-14C)chloroamphenicol (60–60 Ci/mmol) were from Du Pont-New England Nuclear. Fetal calf serum and media were from Sigma, calf serum was from HyClone Laboratories, and antibiotics were from Gibco. DNA restriction and modification enzymes were purchased from Bethesda Research Laboratories or U. S. Biochemical Corp.

Cell Culture Conditions and Transfections—Chinese hamster ovary cells (CHO) and COS-1 cells, estrogen receptor-deficient cell lines, were passaged in phenol-red free DME/F-12 tissue culture medium supplemented with 10% charcoal-dextran-treated fetal calf serum, penicillin (100 units/ml), and streptomycin (100 μg/ml).

CHO cells were plated at a density of 1.8 × 104 cells in 60-mm dishes in DME/F-12 + 5% charcoal-dextran-treated fetal calf serum, penicillin (100 units/ml), and streptomycin (100 μg/ml).

CHO cells were transfected at a density of 1.8 × 104 cells in 60-mm dishes in DME/F-12 + 5% charcoal-dextran-treateled fetal calf serum, penicillin (100 units/ml), and streptomycin (100 μg/ml).

CHOS cells were treated with 1 mol of cold ethyl acetate, and spotted on TLC plates (Polygram Si1 G, Sybron Corp.) and subjected to dimethyl sulfoxide shock and given fresh medium. Cells were harvested 40 h later, and extracts were prepared as described below.

DNA Constructs—All cloning was performed by standard procedures (9). The human ER cDNA clone hOR8 (provided by Dr. G. Greene of the University of Chicago) was modified as described in a previous publication (3). The human estrogen receptor expression vectors pCMV-ER (3) and pCMV-ER (8) are described elsewhere. The mutant C447A was created by oligonucleotide site-directed mutagenesis performed according to the method of Kunkel (10) with modifications (3).

The estrogen-responsive plasmid (ERE)–TATA-CAT was used in the transcriptional activation studies and was provided by Dr. D. Shapiro of the University of Illinois (11). The plasmid pCH110 (Pharmacia, LKB Biotechnology Inc.) was used as a β-galactosidase internal control for transcription efficiencies during all gene transfer experiments.

CAT Assays—Twenty-four hours after the addition of hormone or control vehicle, the plates were washed with cold PBS, collected in 1 ml of TNB (40 mM Tris-HCl, pH 7.5, 140 mM NaCl, 1.5 mM EDTA), and the pellet resuspended in 200–300 μl of cold 230 mM Tris-HCl, pH 7.5. Cells were lysed by three cycles of freeze-thaw. Aliquots of 30–50 μl were used in a β-galactosidase assay as described previously (3). CAT assays, performed when activity was normalized to protein or β-galactosidase activities, gave similar results (data not shown); however, we normalized CAT activities to β-galactosidase activities because we feel it more accurately corrects for transcription efficiencies. The expression of β-galactosidase was temperature-dependent, with the levels of β-galactosidase being greater at the more elevated temperatures (data not shown). Hence, extracts of volume containing different amounts of β-galactosidase activity were used in CAT assays in order to achieve comparable amounts of acetylated products among the temperatures examined. Extracts containing 50 units (from cells incubated at 37°C), 25 units (from cells incubated at 30°C), or 10 units (from cells incubated at 25°C) of β-galactosidase activity were used for the CAT assay (12).

Extracts were used with 0.1 μCi of [14C]chloramphenicol in a total volume of 95 μl. The reaction was started by the addition of 5 μl of 10 mM acetyl-CoA (U. S. Biochemical Corp.) and placed at 37°C for 90 min. The reaction was stopped by extracting with 1 ml of cold ethyl acetate. The supernatant (900 μl) was recovered and dried down. The residue was resuspended in 15 μl of ethyl acetate, and spotted on TLC plates (Polygram Sil G, Sybron–Brinkmann).

The reaction products were separated in a solvent of 95:5 chloroform:methanol (v/v). After autoradiography, radioactivity on the TLC plates was quantitated by a densitometer. β-galactosidase activity was expressed as percent conversion of chloramphenicol to its acetylated forms. Basal (control) level was determined as CAT activity in cells transfected with the reporter plasmid in the absence of ER plasmid.

Receptor Binding Studies—COS-1 cells transfected with the hER expression vector were harvested in PBS + 1.2 mM EDTA by scraping with a rubber policeman. Cells were placed on ice, collected by centrifugation at 100 x g, and washed in ice-cold PBS. Transfected COS cells were resuspended in a volume of 100 μl/100-mm plate in whole cell extract buffer (50 mM Tris-HCl, pH 7.4, 1.0 mM EDTA, 10 mM sodium molybdate, 0.5 mM β-mercaptoethanol, 500 mM NaCl, 10% glycerol, 10 μg/ml leupeptin, 5 μg/ml aprotinin, and 0.1 mM phenylmethylsulfonyl fluoride), homogenized by 25–30 strokes in a glass Dounce homogenizer (B pestle), and incubated on ice for 30 min. The homogenate was then centrifuged at 180,000 g at 4°C to yield whole cell extracts. Protein concentration was determined by the BCA reagent kit (Pierce Chemical Co.).

Hormone binding assays were performed as described (3, 8). Affinity of the receptor for estradiol was determined by the method of Scatchard (12).

Western Blotting—Whole cell extracts were fractionated on polycrylamide gels under reducing conditions. Slab gels containing 10% polyacrylamide and 0.1% SDS were used as described (14). Proteins were transferred from SDS gels to nitrocellulose by electrophoresis and visualized by Western blot analysis using the anti-ER monoclonal antibody H222 as described (15).

Gel Shift Assays—The glucocorticoid pCCAGGTCACAGTCGATCCCCTGAAAAATACCATCTTG-CATGCACTTCAG-CTGAGCTTTAAAACATACATTCCG-OH was annealed to its complementary sequence, gel-purified, and labeled with [32P]ATP (specific activity 6000 Ci/mmol) and diluted with radiolabeled oligonucleotide at 25,000 cpm/0.2 μg. This oligonucleotide contains a consensus ERE DNA response element.

Whole cell extracts were prepared as described above from transfected cells. Extract 1–2 μl; 0.5–1.0 μg of protein) was preincubated with or without ligand as described in the figure legends and then incubated on ice for 15 min in a reaction containing 20 mM Tris-HCl, pH 7.4, 1.0 mM dithiothreitol, 100 mM NaCl, 10% glycerol (v/v), 200 μg/ml bovine serum albumin, 0.50 μg of poly(dI-dC) in 15 μl. Radiolabeled ERE (0.5 ng) in 1 μl of TE was added and incubated for 20 min at room temperature. The samples were directly loaded onto a 4.5% acrylamide (30% acrylamide, 0.9% bisacrylamide) non-denaturing gel, using 0.5 X TBE as a buffer. The gel was run at 200 V at room temperature until the free probe approached the bottom of the gel. The gel was fixed in 20% methanol, 10% acetic acid (v/v) in water and dried onto filter paper before autoradiography.

RESULTS

Analysis of the Hormone Binding Properties of the C447A Receptor at Elevated Temperatures—Whole cell extracts (WCE) were prepared from COS-1 cells transfected with the expression vector coding for the wild-type (WT) or the C447A estrogen receptor (ER). The binding affinity of the expressed receptor for estradiol was measured in vitro at 37 and 25 °C. The affinity of both receptors for estradiol was comparable when examined under parallel conditions (Fig. 1 and Table 1), differing by less than 2-fold. This is in good agreement with our previous report (3). However, of note is the reduced number of binding sites in the C447A receptor preparation when ligand exposure was at 37 °C (Fig. 1, panel B).

The stability of the C447A mutant was, therefore, compared with WT at different temperatures. WCEs from transfected cells were preincubated in the presence or absence of hormone for 2 h on ice to "preload" the hormone-exposed receptor extracts, and all samples were placed at 25, 30, or 37 °C for 0, 15, and 30 min. At the indicated times, following exposure to the different temperatures, hormone was added to samples with or without hormone, and all samples were incubated for 2 h at 25 °C. ER binding was then measured by dextran-coated charcoal assay. Fig. 24 shows that while the WT receptor was stable throughout the 30-min period studied over the entire range of temperature conditions, the C447A mutant was unstable under some of these conditions. At 25 °C both the mutant and wild-type receptors were equally stable in both
FIG. 1. The affinity of the WT and C447A mutant receptors for E₂ at 25 and 37 °C. WCEs from cells transfected with the expression vectors coding for WT ER (filled circles) or the C447A ER mutant (open circles) were incubated with increasing concentrations of [³H]E₂ at 25 °C (panel A) or 37 °C (panel B) for 2 h or 30 min, respectively. Incubations were terminated by placing the samples on ice, and free steroid was removed by charcoal-dextran treatment. Affinities of the receptors for estradiol were estimated by the method of Scatchard (13).

The results from one experiment comparing receptor activation 2B.

Transactivation Ability of Receptor at Different Temperatures—The transactivating activity of both mutant and wild-type receptor was compared at 37, 30, and 25 °C. Following transfection, cells were given hormones in their media, and the plates of cells were then placed at 37, 30, and 25 °C for 24 h. The cells were harvested, and the ß-galactosidase and CAT activities were measured in the samples. The morphology and density of the cells following a 24-h period were similar over the temperatures examined.

The results from one experiment comparing receptor activity as a function of culture temperature are shown in Fig. 4, and the results from several experiments are summarized in Table I. When cells are exposed to hormone at 37 °C, the C447A mutant displays a significant dose-response shift compared with wild-type receptor, requiring 30-fold higher estradiol concentrations to achieve half-maximal stimulation of

| Table I | Hormone binding and transactivation abilities of wild-type and C447A ER at various temperatures |
|------------------|---------------------------------------------------------------|
| Receptor       | Kₐ  E₂ binding (nM) | Half-maximal [E₂] transactivation (-fold) |
| Wild-type      | 0.63 ± 0.02         | 7.6 ± 0.9 × 10⁻¹² (26) |
| C447A          | 1.13 ± 0.04*       | 2.0 ± 0.5 × 10⁻¹⁰ (4.8) |
| C447A          | ND                 | 1.1 ± 0.4 × 10⁻¹¹ (4.8) |
| C447A          | 0.28 ± 0.06        | 7.4 ± 0.9 × 10⁻¹² (4.8) |
| C447A          | 0.41 ± 0.09*       | 1.8 ± 0.4 × 10⁻¹¹ (4.8) |

* p < 0.005 versus WT ER by Student's t test.
† p < 0.10 versus WT ER by Student's t test.
were preincubated on ice for 2 h in the absence of hormone binding with 100% equal to the specific binding measured in extracts of wild-type receptor decreases (Fig. 4). At maximal estradiol concentration between the mutant and wild-type receptors (closed symbols) to occupy the receptors. Data are expressed as the percent of specific binding to samples lacking ligand, and all tubes were placed at 25 °C for 2 h to occupy the receptors. Data are expressed as the percent of specific binding with 100% equal to the specific binding measured in extracts kept at 4 °C for 30 min, followed by the addition of hormone and incubation at 25 °C. B. WCEs were treated in the absence of hormone at 30 and 37 °C as described above, except that at the end of the 2-h incubation at 25 °C, SDS-PAGE loading solution was added and samples were heated and loaded onto a 10% SDS-PAGE gel. ER was radioinert estradiol ligand followed by an incubation at 30 °C for 30 min prior to the DNA binding assay. Protein-DNA complexes were separated on a 4.5% nondenaturing gel. The gel was run "long" (1.5x) to allow greater distinction between unliganded and liganded receptor-DNA complexes. Treatments are indicated above.

CAT activity (Fig. 4 and Table I). Interestingly, as the incubation temperature is decreased, the difference in the half-maximal estradiol concentration between the mutant and wild-type receptor decreases (Fig. 4). At 30 and 25 °C the difference in the estradiol dose-response curves for C447A ER versus wild-type ER is reduced to 4.8- and 2.4-fold, respectively (Fig. 4 and Table I). This is caused by an observed shift of the dose-response curve of the mutant receptor, as the E₅₀ concentration for half-maximal stimulation of transactivation activity of the WT receptor changed by less than 2-fold among the three different temperatures examined (Fig. 4 and Table I). At 25 °C, a temperature where the stability of the mutant receptor is equal to that of the wild-type, the minimal difference in the dose-response curves for estradiol-stimulated CAT activity reflects the very minimal difference in the measured in vitro hormone binding affinity of the receptors (Fig. 1 and Table I). These data provide evidence that the temperature instability of the mutant receptor demonstrated in vitro in terms of hormone and DNA binding is also manifested in transfected CHO cells in terms of impaired transactivation.

It is of interest that we found reproducibly that the magnitude of maximal hormonal induction of CAT activity was similar at 37 and 25 °C (Fig. 4), but when the cells were incubated with hormones at 30 °C, the maximal level of CAT activity was ~4-fold higher than that observed at 37 or 25 °C. This phenomenon has also been observed by others. Presently we do not know if this reflects a greater stability of the CAT gene products or of other factors inside the cells at 30 °C.

**DISCUSSION**

The HBD is the largest and the most complex of the proposed domain structures which characterize the family of nuclear receptors, and it is one whose structure and function are not well understood. Only recently have there been studies that have begun to probe this region in some detail (3-6). While both hormones and antihormones bind to this region, and the binding of one is mutually exclusive with the other, only the former is able to produce a productive ligand-receptor complex (3, 4, 16, 17). Some residues in the HBD have been identified that appear to comprise the ligand binding core and interact with the general ring structure associated with steroid hormones and with the side chains of the ligand, which encode the ligand's specificity and biocharacter (3-6, 18, 19). Relatively little attention has been directed, however, to the study of residues in the HBD involved in maintaining the higher order structure/stability of steroid receptors. Separation of the local interaction versus more global structural roles of individual amino acids in a receptor presents a challenge that can be addressed by site specific mutational changes. Mutational changes in residues critical to global structure have often led to temperature-sensitive mutants, and the study of these mutants has provided considerable information about the biological system in which they operate (20, 21).

Although there are naturally occurring nuclear receptor mutants that have characteristics which may suggest they have temperature-sensitive hormone binding mutations (22-24), very few temperature-sensitive receptors have been well characterized (25). This study has analyzed, in detail, a temperature-sensitive estrogen receptor (ER) mutant. The C447A ER's hormone binding function is impaired at elevated but not at more ambient temperatures, and the receptor's instability in vitro in terms of ligand binding and specific DNA
The C447A Mutant Is Unstable at Elevated Temperatures in Vitro—The loss of hormone binding by this mutant at 37 and 30 °C in the unoccupied state can be prevented by ligand (Fig. 2A). This result is similar to the mutation present in the original ER cDNA clone, 008A (25). However, this G400V mutant (termed Val996) receptor is much more labile than the C447A mutant. The G400V mutant loses its hormone binding capacity at 25 °C in the absence of ligand while the C447A mutant is as stable as the wild-type receptor at 25 °C. Although the authors did not directly show that the G400V mutant is unstable at 37 °C as well as at 25 °C, it is assumed that this is the case based on receptor transactivation studies in HeLa cells (25). Western blot analysis of extracts containing C447A mutant and wild-type receptor revealed that the loss of hormone binding at elevated temperatures is not accounted for by a loss of intact receptor protein, therefore indicating that the loss in binding is due to a destabilization of the HBD and not from the degradation of receptor protein.

The observation that the ligand is capable of preventing the loss of the receptor's hormone binding capacity provides evidence that the ligand is capable of influencing the three-dimensional structure of the HBD and/or of stabilizing the receptor.

While it is difficult to say what role Cys447 plays in the maintenance of receptor stability, it is unlikely that it is involved in the formation of disulfide bridges within the HBD, because mutation of any one of the other cysteines in the HBD (Cys391, Cys517, or Cys539) fails to have a similar effect (3). Of relevance, a naturally occurring glucocorticoid receptor mutant was isolated from a mouse lymphoma cell line which has a Gly substitution for Cys447 in the HBD of the mouse glucocorticoid receptor, and this mutant was found to be nonfunctional in transactivation studies (26). Examination of the expressed mutant protein in transfected COS cells revealed that the C742G mutant was completely unstable, existing as fragments of the intact receptor (26). In contrast to the glucocorticoid receptor mutant, the C447A ER mutant is functional and exists in its intact form when expressed in COS cells, indicating that the C447A mutant is not altered identically to the C742G GR mutant. However, the C447A ER and the C742G glucocorticoid receptor mutants do provide evidence that cysteines are important in the preservation of receptor stability and function.

Receptor Function in Cell Extracts and in Intact Cells—If ER were rate-limiting in transactivation assays, one might assume that receptor instability would cause a reduction in the number of functional receptor molecules per cell and, therefore, would cause a reduction in the magnitude of the induction of the reporter gene (27). However, this is not true for the C447A mutant. The C447A mutant is able to induce a transgene to levels comparable with the wild-type receptor (Ref. 3 and Fig. 4) and is only impaired in its dose response for estradiol. An alteration in the dose-response curve usually reflects an alteration in the affinity of the receptor for its ligand. However, an examination of the affinity of the C447A receptor for estradiol at 25 and 37 °C indicates that the mutant has roughly the same affinity for estradiol as the wild-type receptor. So, to examine if the instability of the receptor in cell extracts also manifests itself in intact cells, we examined the ability of the mutant and wild-type receptors to activate a transgene when estradiol exposure of cells was conducted at 25, 30, and 37 °C. Since the C447A receptor is as stable as the wild-type receptor at 25 °C and partially stable at 30 °C, we expected that as estradiol exposure was conducted at lower temperatures, the dose-response curves of the two receptors would converge. We did in fact find that incubation of the cells at more ambient temperatures during estradiol exposure caused the curves of the wild-type and the C447A mutant to converge (Fig. 4 and Table I), which was primarily due to a shift in the dose-response curve of the mutant. The convergence of the dose-response curves at more ambient temperatures provides strong evidence that the observed instability of the mutant receptor in vitro and its reduced binding to ERE-DNA are the cause of the alteration in transactivation properties of the receptor.

Mechanism of Action of the C447A Mutant: Implications for the Estrogen Receptor and Other Steroid Receptors—The studies with receptor preparations in vitro as well as the studies in transfected cells indicate that the instability of the C447A receptor causes the dose-response shift of this mutant for estradiol in transactivation studies. The dose-response shift

![Graph showing transactivation profiles of the wild-type and C447A ER at various temperatures](image-url)
Temperature-sensitive Estrogen Receptor

reflects the requirement for the C447A receptor to be occupied by ligand to function. The DNA binding assays indicate that the C447A receptor displays hormone dependence of DNA binding at elevated temperatures (Fig. 3). Hence, the transactivation profile of this mutant reflects the occupancy of the C447A mutant receptor by hormone, leading to a half-maximal stimulatory estradiol concentration ($K_d = 4 \times 10^{-10}$ m) that is similar to the equilibrium dissociation constant for $E_2$ binding ($K_d = 2.6 \times 10^{-10}$ m). This addresses the issue of the disparity between the half-maximal $E_2$ concentrations in transactivation studies for WT ER (6–8 × 10^{-12} m) and the $K_d$ (2.6 × 10^{-10} m) value for estradiol binding, which in theory should be similar.

Two ways in which the action of the estrogen receptor is unique from the other steroid hormone receptors are the following: 1) the receptor is capable of binding to DNA in the absence of ligand (6, 8, 28–30), and 2) the estradiol concentration required for a half-maximal response in transactivation studies and the equilibrium dissociation constant ($K_d$) for hormone binding are roughly 2 orders of magnitude apart (3, 25). In contrast to the ER, both the progesterone receptor and the glucocorticoid receptor require hormone to bind to DNA (31–33) and their half-maximal ligand concentrations reflect their $K_d$ values between the ER and the other steroid receptors most likely do not stem from different protein sequences or dissimilar transactivation domains but may reflect differences in their transformation to the DNA binding state; the implication is that a rate-limiting step in the activation pathway for steroid hormone receptors may exist and require the conversion of the receptor to a DNA binding state. In the case of the glucocorticoid receptor and progesterone receptor, it may involve a hormone-dependent dissociation of non-receptor proteins from the receptor (36–39) or the recruitment of factors that assist in their DNA binding activities (33). Recent studies have provided evidence of intermediate glucocorticoid and progesterone receptor complexes which assemble prior to the activation of the receptor complex (38–40). However, in the case of the ER, the receptor may already be "prebound" to DNA in the absence of ligand. By overcoming the rate-limiting step of transformation to the DNA binding state, maximal transactivation activity at lower ligand concentrations may be possible. The binding of ER to DNA in the absence of ligand may be one explanation for the hormone-independent activity of the receptor (25, 41).

In summary, Cys47 plays a vital role in maintaining the higher order structure of the estrogen receptor's HBD. Mutation of this residue leads to a receptor that is unstable at elevated temperatures and displays ligand-dependent DNA binding. Presumably, the "conversion" of this receptor to a form that requires hormone to bind to DNA causes a shift in the dose-response curve for estradiol that mirrors the occupancy of the receptor by ligand. Study of this temperature-sensitive receptor has provided important insights regarding the key issue of ligand modulation of receptor activity in whole cells and shows that the mutant receptor is more like other steroid hormone receptors in that DNA binding is now ligand-modulated, with the result that transcriptional activity reflects the fractional saturation of receptor by hormone.

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