An Antiestrogen-responsive Estrogen Receptor-α Mutant (D351Y) Shows Weak AF-2 Activity in the Presence of Tamoxifen*

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Estrogens act by binding two specific intracellular receptor proteins (ERs, hereafter referred to as ERα and ERβ) to ERα and ERβ; reviewed in Refs. 1–4). Both receptors are conditional transcription factors that work either by binding to specific estrogen response elements (EREs) within the promoters of estrogen-regulated genes or by enhancing the activity of heterologous transcription factors such as the AP-1 (Jun-Fos) complex (5, 6). Like other nuclear receptors, the ERs consist of three distinct domains, an N-terminal domain (NTD), a centrally located DNA binding domain (DBD), and a C-terminal ligand binding domain (LBD). Specific DNA recognition is mediated by the DBD, and transcriptional enhancement is mediated by the synergistic action of two separate activation functions, AF-1 and AF-2, that lie within the N-terminal domain and LBD, respectively. The overall process of transcriptional enhancement has two distinct hormone-dependent components. First, estrogens promote ERα dissociation from a heat shock protein-chaperonin complex that serves to restrict its activity. Second, AF-2 absolutely requires estrogens for its activity.

The ER activation functions work by recruiting coactivator proteins (7–10). AF-2 binds to members of the p160 coactivator family, including GRIP1 (TIF2/NCoA2), SRC-1 (NCoA1), and ACTR (pCIP/RAC3/AIB1/TRAM1), which bind to the histone acetyltransferases CBP/p300 and pCAF. The ER AF-2 functions also bind to other coactivators, including TRAP220 (11, 12), a component of the TRAP220-ARC-SMCC complex, PGC-1 (13), E6-AP (14), and others (reviewed in Ref. 10), although the significance of many of these interactions is not yet clear. In each case, AF-2 binds short amphipathic α-helices with the consensus LXXLL, termed nuclear receptor boxes (NR boxes), which are often reiterated several times within each coactivator. The AF-2 surface is composed a hydrophobic cleft made up of residues from helices 3–5 and 12 (15). A recent ERα co-crystal with a GRIP1 NR box peptide has revealed that there are two components of ERα/NR box recognition (16). First, lysine and glutamic acid residues, which lie within ERα helices 3 and 12, respectively, form a charge clamp that stabilizes the carbamyl backbone of the NR box peptide. Second, residues within the hydrophobic cleft interact with the NR box leucines. ERα AF-1, which is cell type-specific and constitutive (17), also binds to p160s but does not bind NR boxes (18–20). Instead, AF-1 recognizes a distinct surface that lies within the p160 C terminus (18).

Because estrogens stimulate the growth of about 50% of human breast tumors and also play a role in tumor incidence, drugs that antagonize estrogen action have found favor as breast cancer treatments and preventatives (21). Each of the available antiestrogens (including tamoxifen, raloxifene, and ICI 182,780) allows the ERs to bind to DNA but inhibits the ability of AF-2 to bind to coactivators (22). Tamoxifen and raloxifene do show some agonist activity at classical EREs, but this activity stems from AF-1 and not from residual AF-2 activity (17, 23–25). ER-tamoxifen and ER-raloxifene complex crystal structures have revealed the molecular basis of antiestrogen action (16, 26, 27). Unlike estrogens, which are completely buried within the LBD hydrophobic core, tamoxifen and raloxifene both possess a bulky side chain extension that protrudes through the LBD surface near the base of helix 12. This extension displaces helix 12, which rotates 110° and folds back

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Antiestrogens, including tamoxifen and raloxifene, block estrogen receptor (ER) action by blocking the interactions of an estrogen-dependent activation function (AF-2) with p160 coactivators. Although tamoxifen does show some agonist activity in the presence of ERs, this stems from a distinct constitutive activation function (AF-1) that lies within the ERα N terminus. Previous studies identified a naturally occurring mutation (D351Y) that allows ERα to perceive tamoxifen and raloxifene as estrogens. Here, we examine the contributions of ERα activation functions to the D351Y phenotype. We find that the AF-2 function of ERα D351Y lacks detectable tamoxifen-dependent activity when tested in isolation but does synergize with AF-1 to allow enhanced tamoxifen response. Weak tamoxifen-dependent interactions between the ERα D351Y AF-2 function and GRIP1, a representative p160, can be detected in glutathione S-transferase binding assays and mammalian two-hybrid assays. Furthermore, tamoxifen-dependent AF-2 activity can be detected in the presence of ERα D351Y and high levels of overexpressed GRIP1. We therefore propose that the D351Y mutation allows weak tamoxifen-dependent AF-2 activity but that this activity is only detectable when AF-1 is strong, and AF-1 and AF-2 synergize, or when p160s are overexpressed. We discuss the possible structural basis of this effect.

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#The abbreviations used are: ER(s), estrogen receptor(s); AF, activation function; DBD, DNA binding domain; LBD, ligand binding domain; DES, diethylstilbestrol (DES); GST, glutathione S-transferase; PCR, polymerase chain reaction; PBS, phosphate-buffered saline; ERE, estrogen response elements; PAGE, polyacrylamide gel electrophoresis; NR, nuclear receptor.
into the remainder of the hydrophobic cleft thereby occluding the coactivator binding surface. Thus, the tamoxifen and raloxifen side chain extension plays a key role in the antiestrogenicity of both compounds. Interestingly, an aspartic acid residue, which lies at the base of ERα helix 3 (Asp-351), forms hydrogen bonds with a tertiary amine group in the tamoxifen and raloxifen extensions (16, 26). Moreover, Asp-351 was later found to be mutated to tyrosine in an MCF-7 breast tumor cell variant whose growth was stimulated, rather than inhibited, by tamoxifen, and the D351Y mutant allowed increased tamoxifen and raloxifen agonist activity at ERα-responsive genes (28–31). It was therefore proposed that Asp-351 plays an important role in securing the position of the tamoxifen and raloxifen side chain extensions and that the D351Y mutation allowed ERα to perceive tamoxifen and raloxifen as estrogens.

The molecular basis of the D351Y phenotype is not yet known. One possible explanation for the enhanced tamoxifen responses is that the D351Y mutant might allow AF-2 activity in the presence of both estrogens and antiestrogens (28, 29), although this has not been directly confirmed. Another possible explanation is that the D351Y mutant might indirectly enhance AF-1 activity. In this study, we examine the role of ERα activation functions in the D351Y mutant phenotype. We demonstrate that the enhanced tamoxifen responses that are characteristic of this mutant stem from synergy between AF-1 and very weak (<1% maximal) tamoxifen-dependent AF-2 activity. We discuss possible structural explanations for this effect.

**EXPERIMENTAL PROCEDURES**

**Materials**—Cells were grown in Dulbecco's modified Eagle's/Ham's F-12 1:1 mixture without phenol red (Sigma) supplemented with 10% iron-supplemented calf serum (Sigma) and penicillin/streptomycin. Extractions were performed on 1 ml isopro- pyl-1-thio-β-D-galactopyranoside was added at A590 = 0.7, and cultures were then induced for 4 h. Bacteria were then pelleted, resuspended in IPAB-80 (20 mM Hepes, 80 mM KCl, 6 mM MgCl2, 10% glycerol, 1 mM dithiothreitol, 1 mM ATP, 0.2 mM phenylmethylsulfonyl fluoride, and protease inhibitors, pH 7.9), and then sonicated mildly. The lysate was cleared by centrifugation at 12,000 rpm for 1 h in an SS34 rotor. The cleared supernatant was then incubated for 2 h at 4 °C with 500 μl of glutathione-Sepharose 4B beads, which had been prewashed with 5 volumes of PBS, 0.2% Triton X-100 and equilibrated with 5 volumes of IPAB-80 at 4 °C. The protein/bead mixture was then washed with 5 volumes of PBS, 0.05% Nonidet P-40 and resuspended in 1 ml of IPAB-80. Protein preparations were stored at −20 °C.

For binding assays a volume of bead suspension containing 3 μg of GST fusion protein was incubated at 4°C with 1–2 μl of [35S]-labeled protein in IPAB-80 supplemented with 20 μg/ml bovine serum albumin, either 5 μM tamoxifen, 100 nM estradiol, or vehicle, in a total final volume of 150 μl. After a 90-min incubation the beads were washed four times in IPAB-150 (identical to IPAB-80, but including 150 mM instead of 80 mM KCl) The beads were then resuspended in standard SDS-PAGE gel loading buffer. Bound proteins were then analyzed by SDS-PAGE and visualized by autoradiography, along with input protein controls.

**RESULTS**

**ERα D351Y-dependent Tamoxifen Responses Are Cell-specific**—To investigate the contribution of ER activation to the D351Y phenotype, we first examined the behavior of ERα D351Y in cell types that naturally allow distinct levels of AF-1 activity. In MDA-MB-453 breast tumor cells, which allow relatively strong AF-1 activity (18),2 ERα enhanced ER-dependent transcription even in the absence of exogenous ligand, and addition of estradiol or the synthetic estrogen DES gave further stimulation (Fig. 1A). Whereas the antiestrogens all suppressed the ERα-dependent constitutive activity, they did so to different degrees. Significant residual activity was retained in the presence of tamoxifen, modest activity in the presence of raloxifen, and none in the presence of the pure antiestrogen ICI 182,780 (hereafter, ICI). An ERα G400V mutant (34), which lacks constitutive activity, gave comparable levels of transcriptional activity to wild type ERα in the presence of each ligand.

In parallel, and in agreement with previous results (31), ERα D351Y showed no constitutive activity but did give estrogen activation that was comparable to wild type ERα. Here, however, tamoxifen gave significantly higher transcriptional activity. Raloxifene also gave higher transcriptional activity in the presence of ERα D351Y, although these effects were weaker than tamoxifen. No ICI activation was detected. Thus, our results confirm that ERα D351Y allows enhanced tamoxifen and raloxifen agonist activity in breast cells (28–31).

We then examined the behavior of the D351Y mutant in
FIG. 1. Analysis of ERα and ERα D351Y activity in different cells. A, transcriptional activity of ERα, ERα G400V and ERα D351Y in MDA-MB-453 breast cells. Expression vectors for each ER or empty pSG5 vector control (1 μg) were transiently transfected in MDA-MB-453 by electroporation along with ERE-II-LUC reporter gene (2 μg), shown in the schematic at the top of the diagram, and actin-β-galactosidase control (1 μg). The cells were treated with ethanolic vehicle, ICI 182,780 (0.1 μM), raloxifene (0.1 μM), tamoxifen (5 μM), estradiol (10 nM), or diethylstilbestrol (10 nM). Luciferase activity was determined in cell extracts using a single experiment. ERα is cell type-specific and correlates with AF-1 activity. The experiment was performed as described for Fig. 1A. B, interactions of ERα, ERα G400V, and ERα D351Y in HeLa cervical carcinoma cells. Expression vectors for each ER or empty pSG5 vector control (1 μg) were transiently transfected in HeLa cells by electroporation along with ERE-II-LUC reporter gene (2 μg) and analyzed by autoradiography.

Thus, in agreement with previous observations made in other cell types (17, 18, 23, 24, 39, 40), ERα-dependent tamoxifen activation stems from AF-1 in MDA-MB-453 cells. In parallel, ERα D351Y again showed comparable estrogen response and enhanced tamoxifen response. However, a DBD-LBD truncation containing the D351Y mutation showed only modest estrogen response and no tamoxifen response. Thus, AF-1 is absolutely required for the D351Y phenotype and the D351Y mutation does not allow strong tamoxifen-dependent AF-2 activity in vivo.

We next examined the interactions of ERα D351Y with GRIP1, a representative p160, in vitro (Fig. 2B). As expected, wild type ERα bound strongly to a bacterially expressed GST-GRIP1 fusion protein overlapping the NR box region in the presence of estradiol but only showed weak residual binding in the presence of tamoxifen. In parallel, ERα D351Y showed reduced binding to GRIP1 in the presence of estradiol. Moreover, although tamoxifen-liganded ERα D351Y did show a slight, but reproducible, increase in binding to GRIP1 (taken up below), this still represented a very weak interaction. We therefore conclude that ERα D351Y lacks strong tamoxifen-dependent AF-2 activity and that, in fact, ERα D351Y behaves as a partial AF-2 mutant even in the presence of estradiol.

The D351Y Mutant Phenotype Is Not Related to Reduced AF-2 Activity—Given that ERα D351Y showed both enhanced tamoxifen response and reduced AF-2 activity, we confirmed that the enhanced tamoxifen response was not a general con-
sequence of reduced AF-2 activity. Fig. 3 shows that ERα D351Y again showed enhanced tamoxifen response relative to either wild type ERα or ERα G400V in HeLa cells. ERs bearing mutations in the AF-2 charge clamp (ERα K362A and ERα E542K), or the AF-2 hydrophobic cleft (ERα V376R) showed the expected reduction in estrogen activation but no increase in tamoxifen response. Thus, the ERα D531Y phenotype is specific to this mutant and not related to reduced AF-2 activity.

Each of the ER AF-2 mutants did show reduced constitutive activity. Coupled with previous observations of reduced constitutive activity in another AF-2 mutant (18), in ERα G400V (34), in ERs bearing other mutations at position Asp-351 (31), and in ERs bearing mutations in the LBD dimerization interface, our results suggest that this effect is not specific to the D351Y mutant and is probably a general consequence of disturbances of the ERα-LBD surface.

Tamoxifen-dependent Interaction of ERα D351Y with LXXLL Motifs—Although the D351Y mutant did not allow strong tamoxifen-dependent AF-2 activity, it was noteworthy that tamoxifen activation in the presence of the ERα D351Y mutant exceeded the level of constitutive activation in the presence of isolated AF-1 and that tamoxifen-ligated ERα D351Y showed a slight increase in weak residual interactions with the GRIP1 NR box region (see Fig. 2). Together, these results pointed to an active role for the ERα-LBD in the D351Y mutant phenotype and suggested that the D351Y mutant might allow very weak tamoxifen-dependent AF-2 activity. We therefore examined ERα D351Y binding to GRIP1 in mammalian two-hybrid assays, which are sensitive enough to detect relatively weak interactions.

Fig. 4 shows a Gal fusion protein containing the GRIP1 NR box region efficiently recruited a VP16-LBD fusion protein to the promoter in the presence of estradiol. Moreover, the GRIP1 NR box region completely failed to recruit the VP16-LBD fusion in the presence of tamoxifen (Fig. 4, inset), even in the presence of high levels of the VP16-LBD fusion protein. In parallel, the GRIP1 NR box region also recruited a similar VP16-LBD D351Y mutant fusion protein in the presence of estradiol. The overall level of estrogen-dependent recruitment ranged from 10 to 20% of wild type ERα-LBD, consistent with the notion that D351Y behaves as a partial AF-2 mutant. More importantly, the GRIP1 NR box region now also recruited the mutated VP16-LBD fusion protein weakly in the presence of tamoxifen (up to 2–3 times over background, see Fig. 4, inset). This suggests that the D351Y mutant permits low levels of tamoxifen-dependent AF-2 activity. We estimate that this level of AF-2 activity is less than 1% of wild type ERα in the presence of estradiol.

We then asked whether the D351Y mutant might also affect ERα interactions with other types of coactivators. The LXXLL motifs of different ERα coactivators fall into one of three homology groups, which differ according to their receptor interaction preferences (11, 36, 41). Class I includes p160s, and class II includes TRAP220, and class III includes PGC-1. In accordance with previous results (11), the VP16-LBD fusion protein interacted strongly with idealized class I (D2) and class III peptides (Phe-6) and more weakly with a class II peptide (D47/F6) in the presence of estradiol but not tamoxifen (Fig. 5). In each case, the D351Y mutant reduced estrogen-dependent ERα interactions with each peptide and allowed, in the best, weak tamoxifen-dependent interactions (Fig. 5, inset). Thus, the D351Y mutant does not alter the overall spectrum of ERα/coactivator recognition, but rather acts as a generalized partial AF-2 mutant that allows very weak interactions with LXXLL motifs in the presence of tamoxifen.

GRIP1 Enhances AF-2 Activity in the Presence of Estrogens and Antiestrogens in the Context of the ERα D351Y Mutant—To confirm that the weak tamoxifen-dependent ERα-LBD/NR box interactions played a role in the D351Y phenotype, we examined the effects of GRIP1 overexpression upon isolated ERα AF-2. Fig. 6 shows that wild type GRIP1 enhanced the overall level of estrogen response with the isolated ERα DBD-LBD region but gave no tamoxifen or raloxifene response. In parallel, a GRIP1 NR box mutant showed markedly reduced potentiation of estrogen activation. This is consistent with the notion that GRIP1 potentiation of AF-2 activity requires intact NR boxes (35).

Wild type GRIP1 also enhanced the overall level of estrogen-dependent AF-2 activity in the presence of ERα DBD-LBD D351Y mutant. Here, however, significant tamoxifen and raloxifene activation were also detected. This suggests that antiestrogen-dependent AF-2 activity is obtained in the presence of overexpressed GRIP1. Moreover, the GRIP1 NR box mutant failed to potentiate the ERα D351Y-dependent antiestrogen responses. This suggests that the antiestrogen-depend-
**ERα interactions with LXXLL motifs in mammalian two-hybrid assays.** HeLa cells were transfected with 1 μg of expression vector for various Gal-LXXLL fusion proteins (bait), 5 μg of expression vector for the VP16 activation domain or VP16-ERα-LBD fusion protein and VP16-ERα-LBD D351Y fusions (prey), 2 μg of GalRE-LUC, and 1 μg of actin β-galactosidase. The transfection components are shown in the schematic at the top of the diagram. Luciferase activities were determined as before. In this case the split is used to reveal lower luciferase activities. The inset shows tamoxifen responses on an expanded scale.

**Fig. 5.** ERα interactions with LXXLL motifs in mammalian two-hybrid assays. HeLa cells were transfected with 1 μg of expression vector for various Gal-LXXLL fusion proteins (bait), 5 μg of expression vector for the VP16 activation domain or VP16-ERα-LBD fusion protein and VP16-ERα-LBD D351Y fusions (prey), 2 μg of GalRE-LUC, and 1 μg of actin β-galactosidase. The transfection components are shown in the schematic at the top of the diagram. Luciferase activities were determined as before. In this case the split is used to reveal lower luciferase activities. The inset shows tamoxifen responses on an expanded scale.

**Fig. 6.** Effects of GRIP1 overexpression on ERα D351Y AF-2 activity. Transcriptional activity of ERE-II-LUC reporter gene (2 μg) in HeLa cells determined, as before, in the presence of 1 μg of empty pSG5 vector (none) or expression vectors for the ERα DBD-LBD region or the equivalent DBD-LBD D351Y mutant. Also included in the transfection were either 5 μg of empty pSG5 vector or expression vectors for full-length GRIP1 or GRIP1 NR box mutant containing alanine substitutions in the proximal leucines of NR boxes 2 and 3 (LXXLL → LXXAA).

**Fig. 7.** Effects of GRIP1 overexpression on ERα and ERα D351Y transcriptional activity. Transcriptional activity of the ERE-II-LUC reporter was determined in the presence of 1 μg of empty pSG5 expression vector or expression vectors for ERα or ERα D351Y. GRIP1 expression vectors were also included, as in Fig. 6.

**DISCUSSION**

Antiestrogens work by interacting with the ER ligand-binding pocket, thereby competing with endogenous estrogens and inactivating AF-2 (10, 16, 22, 26, 27, 42). A key determinant of antiestrogenicity is the presence of a bulky side chain extension on the antiestrogen molecule. Recent crystal structures of the ERα-LBD complexed with either tamoxifen or raloxifene (16, 26), and the ERβ-LBD complexed with raloxifene (27), have revealed that the extension protrudes through the ER surface and displaces helix 12, which occludes the coactivator binding surface. The ERα crystal structures have also revealed that Asp-351 formed hydrogen bonds with the tamoxifen and raloxifene extension, leading to the suggestion that Asp-351 might...

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play a key role in the behavior of antiestrogens. The discovery that a tamoxifen-stimulated MCF-7 variant cell line contained a mutated ERα with a tyrosine substitution at residue 351 strengthened this view and suggested that it was important to understand the role of this residue in antiestrogen action (28-31).

In this study, we have examined the contribution of ERα activation functions to the D351Y mutant phenotype. It is well established that tamoxifen activation at classical ER-responsive reporters stems from AF-1 activity (17). We found that the strength of ERα D351Y-dependent tamoxifen activation correlates with the strength of AF-1 in different cells and that tamoxifen activation could not be observed with ERα D351Y truncations that lack AF-1. These results indicate that AF-1 also plays an important role ERα D351Y-dependent tamoxifen responses. Moreover, ERα D351Y does not allow strong tamoxifen-dependent AF-2 activity in vitro, and we and others (31) also found that ERα D351Y does not allow strong tamoxifen-dependent interactions between ERα and GRIP1 in vitro. Thus, the D351Y mutation does not allow ERα to perceive tamoxifen exactly as wild type ERα perceives estrogen. Nonetheless, several lines of evidence did point to an unusual role for AF-2 in the D351Y phenotype. First, ERα D351Y gives higher levels of tamoxifen activation than would be expected from AF-1 alone. This result also suggests that AF-1 and AF-2 synergize, or when p160s are overexpressed. It is therefore not surprising that the D351Y mutant phenotype should be especially prominent in breast cells, which show relatively strong AF-1 activity and also contain elevated levels of AIB1 protein, one of the p160 coactivators (43).

We stress that the actual levels of ERα D351Y tamoxifen-dependent AF-2 activity are very small (<1% wild type). Although it may seem paradoxical that such low levels of AF-2 activity are sufficient for tamoxifen-dependent transcriptional activation, similar behaviors have been noted before. AF-1 often completely masks the phenotype of partial AF-2 mutants in the context of full-length ERα (44) and even partially masks the phenotype of some strong AF-2 mutants (11). Indeed, the fact that the D351Y mutant shows reduced binding to GRIP1 in vitro, but allows normal levels of estrogen response in vivo, illustrates this principle. It is also well established that GRIP1 overexpression suppresses the phenotypes of many partial ERα AF-2 mutants and can even partially mask the phenotype of strong AF-2 mutants (15, 45).

It will be interesting to ask exactly how the D351Y mutant allows AF-2 activity in the presence of tamoxifen. Presently, we favor the idea that the Asp-351 residue helps secure the position of the tamoxifen side chain extension and that this, in turn, helps secure helix 12 in the inactive position. We speculate that the D351Y mutant allows increased mobility of the tamoxifen extension and that this, in turn, allows increased mobility of helix 12. In principle, the D351Y mutation might allow helix 12 of the antiestrogen-liganded ERα D351Y mutant to adopt a position that resembles the estrogen-liganded ERα, at least for some of the time. Alternatively, the D351Y mutation might lead to complete displacement of helix 12 in the presence of antiestrogens, and thereby promote inefficient interactions between the helix 3,4,5 region of the hydrophobic cleft and the p160 NR box. It is even conceivable that the substituent tyrosine residue itself could make novel stabilizing contacts with p160s in either of these configurations.

We, and others (31), have also shown that ERα D351Y shows markedly reduced constitutive activity. This is a common phenotype that is also observed in ERα G400V (34), in ERα AF-2 mutants (Fig. 3 and Ref. 18), in other ERα Asp-351 mutants (31) and in ERs bearing mutations in the LBD dimerization interface. It is known that the ERα G400V phenotype stems from increased association with inhibitory heat shock proteins (46), and because heat shock proteins bind solvent exposed hydrophobic regions, it is likely that lack of constitutive activity is indicative of exposure of hydrophobic residues upon the ERα-LBD surface. Nonetheless, ERα D351Y is well expressed, with normal affinity for estradiol and antiestrogens, suggesting that its overall conformation is relatively normal (28, 29, 31). We therefore speculate that an altered position of helix 12 could expose the AF-2 hydrophobic cleft and target the D351Y mutant to the heat shock complex in the absence of hormone.

Finally, our studies also allow us to draw some conclusions about the behavior of wild type ERα. First, we have been unable to detect any association between the tamoxifen-ligated wild type ER with LXXLL motifs whatsoever. We have also confirmed that the overall level of tamoxifen-ligated AF-1 activity correlates with AF-1 activity. Thus, our results agree with the idea that tamoxifen agonist activity stems AF-1 and not from weak residual AF-2 activity. Second, we have confirmed that the ERα D351Y mutant allows increased agonist activity in the presence of tamoxifen.
ence of tamoxifen and raloxifene but not ICI (28, 29, 31). This suggests that tamoxifen and ICI must work by different mechanisms. Third, ERα D351Y also behaved as a partial AF-2 mutant. Our unpublished studies\(^a\) also reveal that the equivalent ERβ mutant (D303Y) also behaves as a partial AF-2 mutant and, given that a vitamin D receptor bearing a mutation in the equivalent residue also behaves as a partial AF-2 mutant (47), we suggest that the same residue could help stabilize helix 12 positioning in many nuclear receptors. Finally, our results also address the mechanism of estrogen-dependent regulation of cell division. It is known that ERα D351Y allows tamoxifen to mimic the stimulatory effects of estrogens upon MCF-7 cells and also the inhibitory effects of estrogens upon MDA-MB-231 cells (28, 29). Since ERα upon MDA-MB-231 cells (28, 29), it will be interesting to ask how the same ERα/coactivator interactions lead to opposite growth responses in closely related cell types.

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