Endocr (2008) 33:1–8
DOI 10.1007/s12020-008-9054-1

REVIEW

The multifunctional estrogen receptor-alpha F domain

Debra F. Skafar · Changqing Zhao

Received: 27 November 2007 / Accepted: 4 March 2008 / Published online: 25 March 2008

Abstract The members of the nuclear receptor superfamily act as transcriptional regulatory factors and exhibit a multidomain structure characterized as domains A-E/F. This review focuses on a small, relatively understudied region at the extreme carboxy-terminus of the estrogen receptor (ER) alpha, the F domain. The F domain contributes to differences in the activity of ER alpha and beta subtypes; it is required for tamoxifen's agonist activity on an estrogen response element, and it modifies the receptor's interactions with coregulators including steroid receptor coactivator-1. The differences between the F domains of the ER alpha and beta subtypes and among the other members of the nuclear hormone receptor superfamily may offer opportunities for selective control of the activity of these proteins.

Keywords Estrogen receptor · Estradiol · Nuclear receptor superfamily · Transcription · MDA-MB-231 cell line · Tamoxifen

Estrogen receptors (ERs), of which there are two major subtypes, alpha and beta, are multidomain transcription regulators whose activity is regulated by the binding of ligands (Fig. 1) [1–3]. Studies using knockout mice and observations in human patients show that estrogen, acting through its receptors, plays a critical role in the reproductive system, the maintenance of bone density, and the central nervous system in both males and females. The receptors are also the target of the selective estrogen receptor modulator (SERM) tamoxifen, which is used for the prevention and treatment of breast cancer. Although newer drugs, such as the aromatase inhibitors, are more effective than tamoxifen for the treatment and prevention of breast cancer in post-menopausal women, the development of resistance maintains the need to develop additional preventive and therapeutic agents with fewer side effects. To do so, we need to understand the mechanisms through which estradiol, tamoxifen, and related ligands exert their tissue-specific effects, as well as the mechanisms through which resistance to tamoxifen, and similar ligands is mediated. This is especially critical for the prevention of primary tumors in women at high risk and for the prevention of recurrences, as drug therapy is maintained for a period of years.

This review focuses on a small, relatively understudied region at the extreme carboxy-terminus of the ERalpha, the F domain. Despite its relatively short length, the F domain strongly influences several activities of the ER: it contributes to differences in the activity of ER subtypes [4–6], it is required for tamoxifen's agonist activity on an estrogen response element (ERE) [7, 8], and it inhibits the dimerization of the ER and modifies its interactions with coregulators [4, 6, 9]. Most importantly, studies from our and other laboratories provide evidence that the F domain plays an important role in determining the response of the ERalpha to agonist and antagonist ligands. The members of the nuclear receptor superfamily act as transcriptional regulatory factors and exhibit a multidomain structure characterized as domains A-E/F (Fig. 1) [1, 2]. The F domain, which is present in certain members of this large superfamily, is located at the extreme carboxyl-terminus of the receptor distal to the larger ligand-binding domain (LBD, domain E) [10]. Among the nuclear receptors for which this region is present, substantial variability exists in

D. F. Skafar (✉) · C. Zhao
Department of Physiology, Wayne State University School of Medicine, 540 E. Canfield, Detroit, MI 48201, USA
e-mail: dskafar@med.wayne.edu

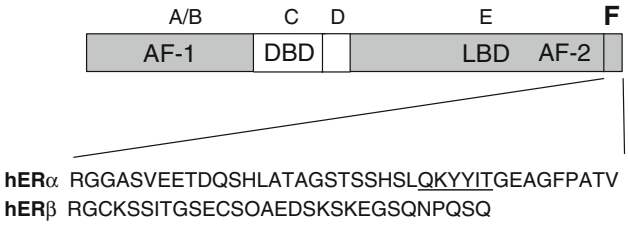

Fig. 1 Domain structure of the estrogen receptor and sequence of the F domain. Top, domains of the estrogen receptor. Middle, functions of the domains: AF-1, activation function-1; DBD, DNA-binding domain; LBD, ligand-binding domain; AF-2, activation function-2. Bottom, sequences of the ERalpha and ERbeta F domains. The conserved beta-strand-like region in the alpha subtype is underlined

both the length of the F domain—from 19 to more than 80 amino acids long—and its sequence, suggesting that this region may confer substantial specificity of activity. For example, the F domains in the alpha and beta subtypes of the human estrogen receptor (hER) are different in length, 42 residues vs. 30 residues, they exhibit <25% sequence identity, and they differ in predicted secondary structure [11, 12]. Although overall the F domain is poorly conserved, when the F domain of the ERalpha is compared among species, a predicted beta-strand-like region, QKYYIT, is conserved in species as diverse as human and chicken. The F domains of the ERalpha in the human, however, exhibit no significant homology with the F domains of fish ERalpha; indeed, the fish proteins have an F domain of approximately 80 residues, with a high content of proline residues. Although structural information is available for the DNA-binding domain and the ligand-binding domain of the hERalpha, no structural information has been published for the F domain of the hERalpha.

The F domain and ER activity

Interest in the F domain has increased since the discovery of the beta subtype of the ER [11, 12]. As mentioned above, the F domains of the two subtypes are different in length and have only limited sequence homology (Fig. 1). Moreover, they have quite different predicted secondary structures [8]. The F domain of the hERalpha is predicted to contain an alpha-helical region and an extended beta-strand-like region, separated by regions of random coil, as well as a very short extended region near the extreme C-terminus of the protein; the F domain of the ERbeta is predicted to be almost entirely random coil, with only a very short extended region near the extreme C-terminus of the protein [8]. Several recent studies support the idea that the F domain is in part responsible for the differences in the biological activity of the ERalpha and ERbeta. Notably, deleting and swapping the F domains of the ERalpha and ERbeta provided evidence that the F domain is in part responsible for the differences in the biological activity of the two subtypes on AP-1 sites in response to estradiol (E2), tamoxifen and raloxifene [5]. On an AP-1 site, the F domain-deleted ERbeta is activated by tamoxifen and raloxifene, whereas the wt ERbeta is activated only by raloxifene; the ERalpha F domain-deleted receptor is activated by raloxifene, whereas the wt ERalpha is not activated by raloxifene, but by E2 and tamoxifen. In addition, putting the ERalpha F domain on the ERbeta did not change its ligand preferences for activation, while putting the ERbeta F domain on ERalpha eliminated E2's stimulatory activity. This shows that the F domain does not exert its activity independently, but does so in concert with the other domains of the ER.

The effects of deleting or mutating the F domain on transcription activation by the hERalpha are complex. Studies in which the entire F domain has been deleted indicate that it is not required for either ligand binding or transcriptional activation on an ERE-driven promoter [7, 8, 10]. Indeed, in some cases, deletion of the F domain enhances receptor activity [5, 9, 10]. By contrast, Safe's laboratory has shown that deleting residues 579–595 of the F domain eliminates the ability of the hERalpha to activate transcription via interaction with Sp1 [13]. Our laboratory and others have shown that deleting or mutating the F domain alters the responses of the ER to E2 and to 4-hydroxytamoxifen (4-OHT, the active metabolite of tamoxifen), as well as the relative agonist and antagonist activity of combined E2 and 4-OHT administration [8]. Compared with the wt hERalpha, the F domain-deleted ER requires less 4-OHT in MDA-MB-231 and CHO cells, and more 4-OHT in 3T3 and HeLa cells, to repress E2's stimulatory activity on an ERE_2-pS2 (trefoil factor-1) promoter construct [7]. This is consistent with other observations showing that the F domain-deleted ER requires less E2 than the wt ER to overcome tamoxifen's inhibitory activity in HeLa cells [8]. Certain mutations of the F domain can impair responses to E2 in a cell-specific manner [8, 14, 15]. The G566W/G586W mutant exhibits similar responses to E2 and 4OHT as does the F domain deletion mutant in four different cell lines, MDA-MB-231, CHO, 3T3, and HeLa, on ERE_2-pS2 and ERE_2-PR_{distal} promoters. In CHO and 231 cells, deleting the F domain reduced the agonist activity of 4OHT and increased the antagonist activity of antiestrogens, while in 3T3 and HeLa cells, antiestrogens became less potent antagonists of E2 [7]. In addition, Wrenn and Katzenellenbogen [15] have shown that although the S554fs ER, a frameshift mutant whose F domain contains 35 codons not present in the F domain of the wt ERalpha, is a potent mediator of E2-stimulated transactivation in yeast, its activity in CHO cells is markedly impaired despite the demonstration of an almost normal E2-binding affinity in both cell types. Ince

et al. [14] showed that when the same mutant is coexpressed with the wild-type (wt) receptor, transactivation is suppressed, indicating that the frameshift mutant exhibits dominant-negative activity.

In striking contrast with the observation that the F domain of the hERalpha is not essential for the response to E2 on an ERE-driven reporter, deleting this domain or specific point mutations within this domain eliminates the ability of tamoxifen to act as an agonist on an ERE [7, 8]. Furthermore, using a yeast fusion-protein assay, Nichols et al. have shown that repositioning of the F domain is a critical component of the antagonist activity of tamoxifen [16]. This is consistent with work from Montano et al. [7], showing that deleting the F domain altered the agonist versus antagonist profile of 4-OHT in a cell-specific manner, and work from Schwartz et al. [8], showing that a specific mutation within the F domain, Q565P, increased the antagonist activity of 4-OHT. Thus, the F domain is a key modulator of the ability of the hERalpha to respond to tamoxifen in a cell-specific manner.

Note that, although deleting the F domain alters the transcriptional activity of the hERalpha, the F-domain-deleted ERalpha and the wt ERalpha have similar abilities to induce distortions in DNA and directed bend angles [17]. Deletion of the F domain does not alter the half-life of the ERalpha [18], nor does it alter down-regulation of the ER by E2 and the effect of the proteasome inhibitor MG132 [19]. Of note, Long and Nephew [20] have recently shown that fulvestrant (ICI 182,780)-mediated degradation of the ERalpha is not affected by F domain deletion. These results provide strong evidence that the effects of deleting the F domain are not due to alterations in the stability, turnover, and/or degradation of the hERalpha.

The region of the F domain involved in E2 and 4-OHT's agonist activity was further delineated using serial truncation of the receptor (Fig. 2) [21]. Serial truncation of the F domain by introducing stop codons within the ERalpha coding sequence, Q580stop, G572stop, and S554stop, did not eliminate the ability of E2 to stimulate transcription in a transient cotransfection assay using an ERE-driven reporter in HeLa cells [21]. There were no differences among the truncated receptors in their activity in the presence of E2. However, the activity of the truncated ERs in the presence of E2 was strikingly reduced, compared with the wt protein. Western immunoblotting showed that the mutated receptors were expressed at levels comparable to or greater than that of the wt ER. Thus, these results show that a region within the C-terminal 16 residues of the ER increases the activity of the ER in the presence of E2 on an ERE-driven promoter.

Next, the effect of the same serial truncations on the weak agonist activity of 4-OHT on an ERE-driven reporter was tested in HeLa cells (Fig. 2) [21]. All truncated hERs

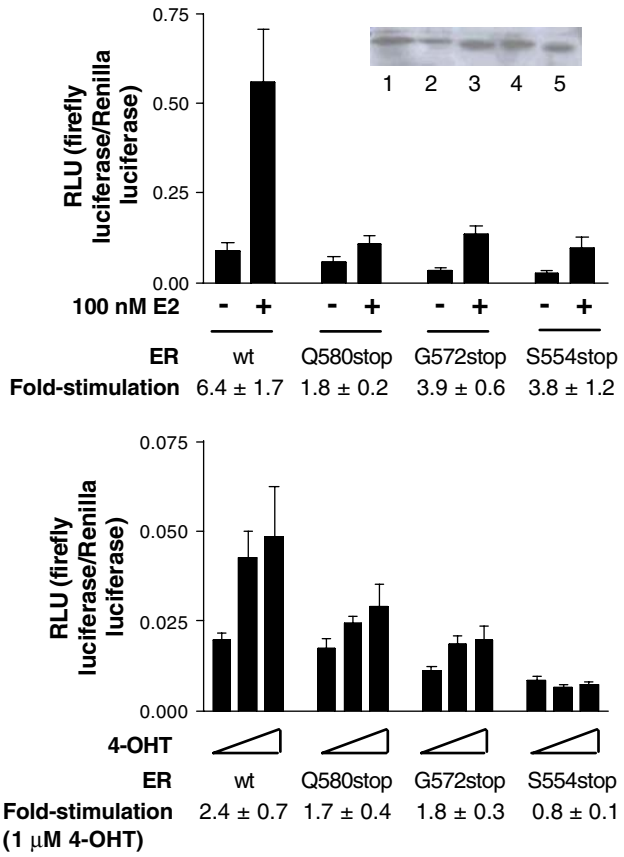

Fig. 2 Response of hERalpha truncation mutants to E2 and 4-OHT. The activity of the wt hERalpha and F domain truncation mutants on an ERE-driven promoter in the absence of ligand and in response to E2 (100 nM) (top) or 4-OHT (10 nM, 1 μM) (bottom) was measured after transient transfection into HeLa cells. Lane 1, MCF-α cells; 2, wt hERalpha; 3, G572stop; 4, Q580stop; 5, S554stop. From Koide and Zhao et al. [21], used with permission

exhibited less activity than the wt ER in the presence of 4OHT. Furthermore, 4-OHT increased the activity of the wt hER (~2.4-fold), the Q580stop (~1.7-fold), and the G572stop (~1.8-fold) mutants, but not the S554stop mutant (~0.8-fold). There was no difference in the 4-OHT-stimulated activity of the Q580stop and G572stop mutants, but the activity of each of these mutants was less than the activity of the wt hER but greater than the activity of the S554stop mutant. Thus, two regions within the F domain are involved in the agonist activity of 4-OHT on an ERE-driven promoter: one region within the C-terminal 16 residues of the hER increases the response of the hER to 4-OHT, and a second region between S554 and G572 is essential for the agonist activity of 4-OHT. These results are consistent with previous reports that mutations within the 554–572 region of the F domain impair (S559A/E562A) or eliminate (Q565P, G556W/G586W) 4-OHT agonism [7, 8]. Note that the G556W/G586W mutant, which has lost 4-OHT agonist activity, alters both these regions [7].

Taken together, these results show that at least two regions within the F domain of the hERα modulate the response of the hERα to ligands. One region, from Q580 to V595, increases the activity of the ER in the presence of either E2 or 4OHT on an ERE-driven reporter. Most interestingly, a different region, between S554 and G572, is essential for the agonist activity of 4OHT on an ERE, yet is dispensable for the agonist activity of E2 on this same promoter.

The F domain and crystallographic studies

The overall poor conservation in sequence and length, combined with the relative lack of structural and functional information, makes the F domain one of the least well-understood segments in the multidomain structure of nuclear receptors. The F domain of the ERbeta is present in the constructs used for crystallographic studies, but it was not visualized [22].

Although crystallographic structures of the hERalpha do not include the F domain, the fact that the F domain immediately follows the LBD allows us to examine the structures of the ERalpha LBD in the light of some suggested roles for the F domain. The C-terminus in the crystallographic structure of the diethylstilbestrol (DES)-bound hERalpha LBD is located at the dimerization interface of the ER monomers [23, 24]. This suggests that the F domain of the agonist-bound ERalpha plays a role in receptor dimerization [9]. In addition, the ERalpha is an allosteric protein that binds E2 with a high degree of positive cooperativity, which indicates that information is transferred efficiently between subunits of the homodimer [25]. The position of the F domain relative to the dimer interface suggests it could be involved in site-site interactions between subunits. These ideas are supported by studies from Peters and Khan, who showed that deleting the F domain increased dimerization in a yeast two-hybrid assay, and from Schwartz et al., who showed that the Q565P mutant lost positive cooperativity of [3H]estradiol binding ([8, 9]; see below). Further, since the C-terminal helix of the ERalpha LBD is reoriented in the crystallographic structures of the 4-OHT- and raloxifene-bound ER LBDs compared with its position in the E2- and DES-bound structures [23, 24], the F domain may also become reoriented and thus play a role in the response of the receptor to these ligands, as shown by Montano et al. [7] and Schwartz et al. [8].

Crystallographic information is available for the F domain of the human progesterone receptor (hPR) and part of the F domain of the retinoic acid receptor (RAR). In the progesterone-bound hPR, the F domain is present as an extended beta strand at the dimer interface and prevents the PR dimer from adopting the interface used by the ER dimer [26]. In a structure of the RAR, the F domain extends toward and contacts the other monomer in the dimer [27, 28]. These observations are generally consistent with the idea that the F domain can interact with the LBD, thereby modulating its activity.

Covalent modifications within the F domain

Another key mechanism for modulating the activity of the hERalpha is through covalent modification. Jiang and Hart have demonstrated that the mouse ERalpha is subject to O-GlcNAcation on Thr575 and that Ser576 may also be glycosylated [29]. Interestingly, the non-glycosylated form of the ERalpha, but not the glycosylated form, bound to an estrogen response element in vitro [29]. In addition, Rowan's laboratory has shown that Ser559 of the human ERa is phosphorylated, and that elimination of phosphorylation by mutation to alanine increases the basal activity, while eliminating E2-stimulated activity, of the receptor [30].

The F domain in HNF4alpha

Functional studies of the hepatocyte nuclear factor 4alpha (HNF4alpha) provide additional support for the idea that the F domain is a functionally and biologically important modulatory region within the nuclear receptors [31–33]. HNF4alpha contains one of the longest F domains among the nuclear receptors, >60 residues. Alternative splicing gives rise to two forms of the HNF4alpha, 1 and 2, that differ in their F domains; the HNF4alpha2 contains a 10-amino acid insertion in the middle of F that is absent in HNF4alpha1. In transient transfection assays, the HNF4alpha2 variant activates transcription fourfold better than does the HNF4alpha1 variant, and is more responsive to stimulation by the coactivators GRIP1 (glucocorticoid receptor-interacting protein 1) and CBP (cyclic AMP response element-binding protein-binding protein) [31]. Most interestingly, a mutation in the F domain of HNF4alpha2, V393I, is associated with the development of maturity-onset diabetes of the young (MODY) [34]. In transient transfection assays on three different promoters, the V393I mutant of the HNF4alpha2 exhibits a 50% reduction in transactivation activity, as compared with the wt protein [34].

A sequence similar to a repressor sequence found in PR, glucocorticoid receptor, mineralocorticoid receptor, and androgen receptor—but not ER—was identified near the C-terminal end of the F domain in HNF4alpha, though the effects of mutating this sequence were not studied [31].

Other studies from Sladek's laboratory have shown that the F domain of HNF4alpha1 functionally interacts with coactivators as well as with corepressors [33]. In a model of the HNF4alpha1, the F domain wraps around the LBD, and so is positioned to affect both dimerization and coregulator interactions [32]. Of note, although there is no sequence homology with the hERalpha, the V393I mutation in the F domain of the HNF4alpha2 associated with MODY occurs in a predicted beta strand region [31], which suggests it is a region of biological and clinical importance.

The F domain and ligand binding

Although several laboratories have looked for independent actions of the F domain of either the ERalpha or the HNF4alpha1, no evidence for such has yet been found [10]. Rather, the data support a model in which the F domain interacts with other region(s) of the receptor, particularly the LBD. Several laboratories have investigated LBD-F domain interactions by studying the effects of deleting or mutating the F domain on the affinity of a nuclear receptor for ligands. Deletion of the F domain of the RARalpha does not alter its affinity for the agonist all-trans retinoic acid (ATRA) [27, 28]. However, truncation of the F domain of the HNF4alpha is reported to reduce its affinity for long-chain fatty acyl-CoAs, and increase its affinity for unesterified long-chain fatty acids, reversing the specificity shown by the full-length protein [35]. In that same report, Petrescu et al. [35] show that truncation of the HNF4alpha F domain eliminates cooperative interactions between receptor monomers. By contrast, competitive binding assays as well as direct binding assays using transiently transfected ER revealed no difference in affinity of the F domain-deleted ER for either E2 or 4-OHT [7, 18]. In addition, the observed cell-specific effects of F domain truncation and mutation discussed above do not support the concept that truncating or mutating the ERalpha F domain alters ER function simply by altering ligand affinity. To further test the effects of truncating or mutating the F domain on the ligand-binding properties of the hERalpha, we analyzed the functional effects of F domain deletion and mutation on the interaction of the hERalpha with ligands in vitro [8] by expressing selected F domain mutant ERs as histidine-tagged fusion proteins via a baculovirus expression vector in *Sf9* insect cells, and measuring their affinity for E2 and the cooperativity of hormone-binding in vitro [8]. Similar biochemical studies of the interactions of receptors with ligands have been a sensitive method of detecting alterations in receptor conformation, and have provided important information on receptor dimerization and interactions between receptor subunits [25, 36–40].

Deleting the F domain (S554stop mutant) increased the affinity of the receptor for E2 and did not eliminate the cooperativity of the interaction: both the wt and S554stop mutant exhibit curved Scatchard plots and Hill coefficients greater than one [8], which are characteristic of a positive-cooperative binding mechanism. Several other laboratories and a commercial supplier of purified hERalpha report a positive cooperative-binding mechanism for E2, having Hill coefficients between 1.5 and 1.8 as well as curved Scatchard plots [25, 41–44]. By contrast, a mutation of the ERalpha, Q565P, eliminated positive cooperativity of E2 binding, though it had no effect on the affinity for E2; the Hill coefficient was reduced to a value near one and the Scatchard plot was changed from a curve to a straight line [8]. The loss of site-site interactions could stem from a reduced ability or inability to form a dimer, disruption of allosteric communication between subunits, or a combination of the two [44]. The positive cooperative-binding mechanism of the S554stop mutant shows that removing the F domain in its entirety does not interfere with site-site interactions of the hERalpha. Indeed, removal of the F domain is reported to enhance dimerization of the hERalpha [9]. This is different than the HNF4alpha, wherein deletion of the F domain eliminated positive cooperativity [35].

Effects of the F domain on intermolecular interactions with coregulators and other proteins

One key step in the mechanism of action of the ERs is the recruitment of coactivator proteins. Though the effects of the F domain on transcription are complex, relatively few studies have examined the influence of the F domain on ER-coregulator interactions. Two studies have shown that the ERalpha and ERbeta exhibit different affinities for the mammalian Mediator subunit TRAP220, which in turn is dependent on the F domain [4, 6]. As mentioned above, Safe's laboratory has shown that deleting residues 579–595 of the F domain eliminates the ability of the hERα to activate transcription via interaction with Sp1 [13]. In addition, not only is the F domain required for interaction of the ERalpha with Sp1, but a fusion protein containing sequences corresponding to the C-terminal 17 residues of the F domain of the hERalpha (aa 579–595) can block the activity of the ER on an Sp1-linked reporter [13]. The F domain has also been reported to promote the interaction between the ERalpha and the coactivator TAF-Iβ [45] and inhibit the interaction between the hERalpha and the coregulator RIP140 [9]. In other members of the nuclear receptor superfamily, a frame-shift mutation of the F domain of RARbeta results in enhanced corepressor binding to the receptor [28]. Deletion of the F domain of

RARalpha results in increased coactivator binding, reduced corepressor binding, and increased constitutive and ligand-stimulated transactivation [27]. Furthermore, the work of Sladek's laboratory shows that the F domain of the HNF4alpha1 interacts with the corepressor SMRT [33].

To further investigate the role of the F domain in interactions with coregulators, our laboratory tested the sensitivity of receptors having truncations in the F domain to overexpressed steroid receptor coactivator (SRC)-1 using a transient cotransfection assay in HeLa cells [21]. The activity of the Q580stop mutant in the presence of overexpressed SRC-1 and E2 was reduced compared with the activity of the wt protein. Thus, removing the C-terminal 16 residues of the ERalpha reduced its sensitivity to overexpressed SRC-1. These results are consistent with the observation that removal of the same C-terminal 16 residues reduced the activity of the ER in the presence of E2, as well as the ability of E2 to stimulate transcription. In the presence of E2 and SRC1, the activity of the G572stop and S554stop mutants was greater than the activity of the Q580stop mutant, but was less than that of the wt ERalpha. This is reminiscent of the observation that the ability of the G572stop and S554stop mutants to respond to E2, expressed as fold-stimulation, was greater than that of the Q580stop mutant and was similar to that of the wt protein. These results show that regions 580–595 are stimulatory for E2 and 4OHT-stimulated transcription, as well as interaction with SRC-1. Our results [8, 21] have also shown that residues 554–572 are required for 4-OHT agonist activity.

The above studies show that the regions from 580 to 595 are important in the function of the ERalpha and its interaction with SRC-1. Using a yeast two-hybrid assay, Dr. Shohei Koide (University of Chicago) has shown that truncation of the F domain within the predicted beta-strand-like region does not eliminate the ability of the E2-bound ERalpha to react with probes (the SRC-1 receptor-interacting domain and monobodies, which are ERalpha LBD-binding proteins engineered on a fibronectin backbone) that recognize the agonist-bound conformation of the ERalpha, but rather, increased the reactivity with such probes in the absence of ligand [21, 46]. Taken together, these results show that the extreme C-terminal region of the ERalpha modulates the reactivity of the ERalpha and its LBD with full-length SRC-1, the receptor-interaction region of SRC-1, and engineered protein probes that recognize the active conformation of the LBD.

Role of the F domain in the activity of the hERalpha on an endogenous promoter

The previous studies analyzed the interaction between the ER and coregulators using exogenous reporters and transiently cotransfected mammalian cells, or yeast. To determine the role of the extreme C-terminal region of the F domain in transcription of an endogenous gene in a more native chromatin context, we developed cell lines stably expressing the wt hERalpha or the truncated Q580stop hERalpha, which displays an attenuated response to E2 and reduced interaction with SRC-1 in transient transfection studies [21]. We then examined transcription and recruitment of the hERα to the endogenous promoter of the pS2 gene in the absence of ligand, and in the presence of E2, in MDA-MB-231 cell lines stably expressing the wt or truncated hERalpha.

Cells were first screened using the ability to stimulate transcription of an ERE-driven promoter in response to E2 (Fig. 3). This first screening identified clones that expressed functional ER. Lines were then examined by Western immunoblotting for the level of ERalpha, and clones expressing comparable amounts of wt and truncated ER were isolated (Fig. 3). The level of endogenous pS2 mRNA was then determined by quantitative real-time PCR, and the results in the absence and presence of E2 were quantified by the delta Ct method (Fig. 4).

The ability of the Q580stop mutant to stimulate transcription of the pS2 gene was substantially reduced, compared with the wt hERalpha (Fig. 4). This parallels the effect observed on the transcription of an ERE-driven promoter.

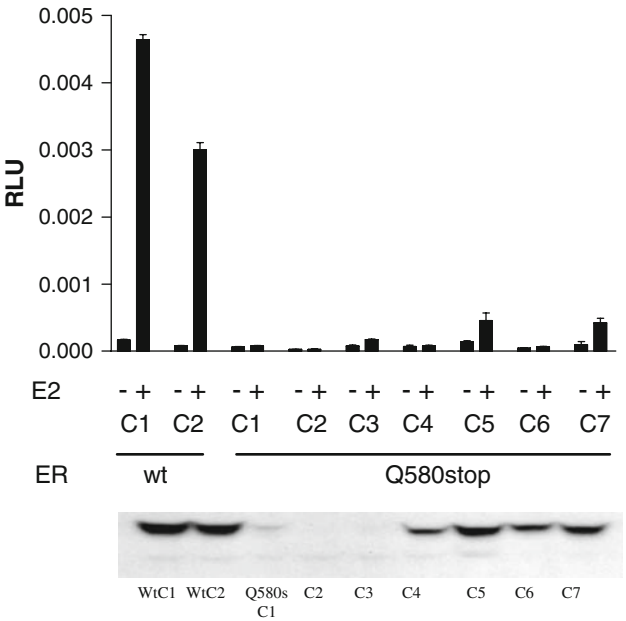

Fig. 3 Generation of MDA-MB-231 cell lines stably expressing the wt or the Q580stop hERalpha. (Top) Activity of an ERE-driven promoter in response to E2. (Bottom) Western blot against the hERalpha. Clones 5 and 7 express levels of functional mutated ER similar to those expressed by clones 1 and 2 of the wt ER, and were selected for further study

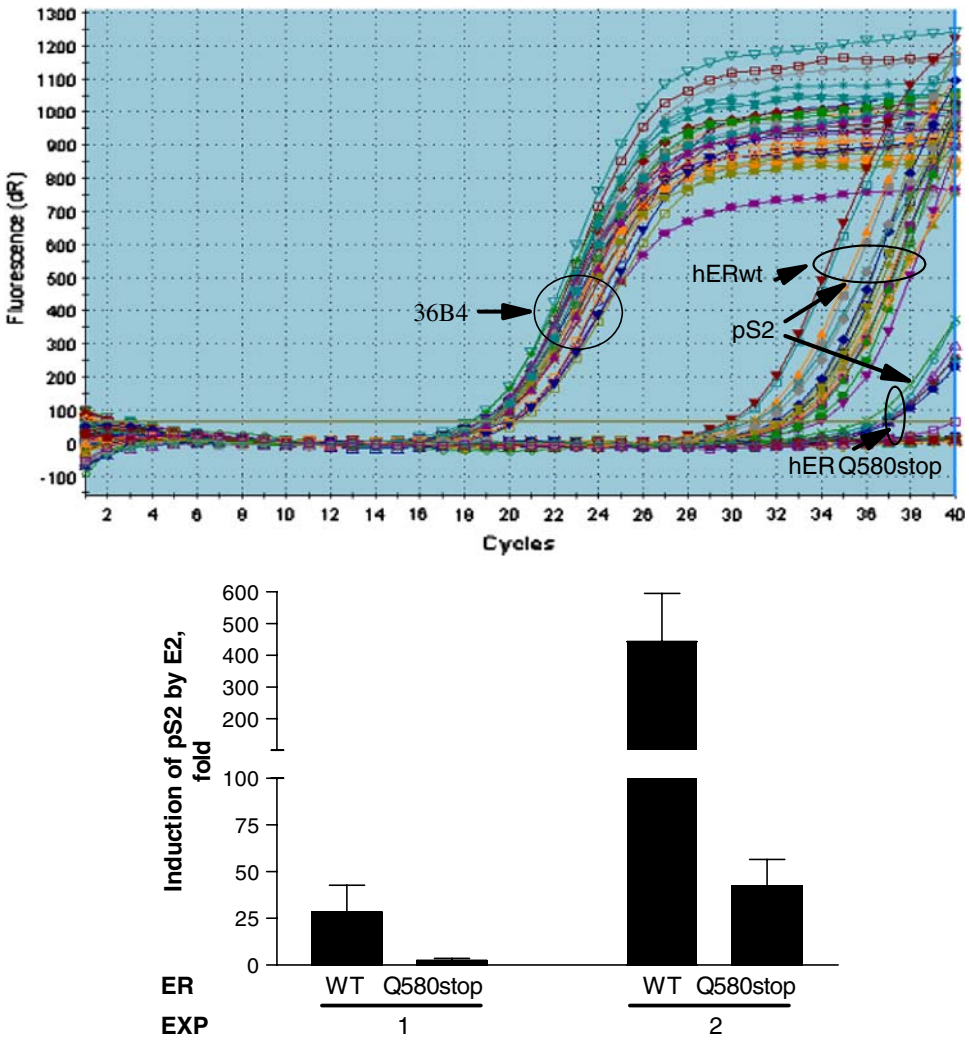

Fig. 4 Truncating the hERalpha F domain induced lower levels of pS2 mRNA in response to E2. (Top) Amplification plots of pS2 mRNA and 36B4 mRNA (ribosomal protein control) in MDA-MB-231 cell lines stably expressing the wt or truncated (Q580stop) hERalpha. The mRNA levels were measured by quantitative RT-PCR in the absence and presence of 1 μM E2. (Bottom) Stimulation of pS2 mRNA accumulation by E2, normalized to the level of mRNA for the ribosomal protein, 36B4, was reduced in the cells expressing the Q580stop mutant compared with the wt hERalpha. Adapted from Koide and Zhao et al. [21]

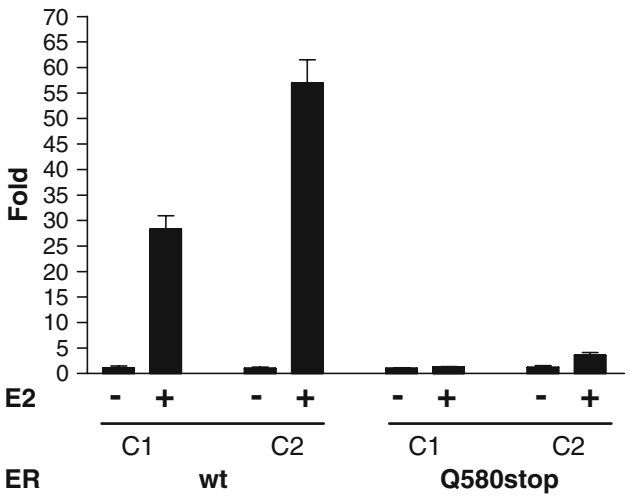

Fig. 5 Truncating the hERalpha F domain reduced its recruitment to the pS2 promoter. Binding of the hERalpha to the pS2 promoter was measured by chromatin immunoprecipitation

The ability of the hERalpha to bind the pS2 promoter was then examined by chromatin immunoprecipitation (Fig. 5). The Q580stop mutant exhibited a reduced ability to occupy the pS2 promoter in the presence of E2 than did the wt protein. Thus, the reduced transcription of the pS2 gene is likely to result from reduced recruitment of the hER to the promoter due to truncation of the F domain.

Conclusions

Drugs that target the ligand-binding domain of the estrogen receptor-alpha are successfully used in the prevention and treatment of breast cancer, yet the development of resistance to these compounds maintains the need to develop additional therapeutic agents. The F domain of the estrogen receptor-alpha, in concert with other receptor domains, modulates several activities of the protein, including ligand binding, interaction with coregulatory proteins including SRC-1, transcription activation, and accumulation at an

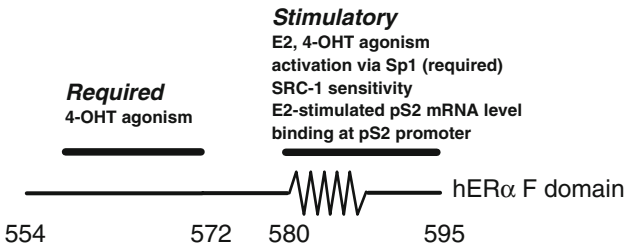

Fig. 6 Regions within the F domain of the hERalpha involved in the activity of the receptor. The predicted extended beta-strand-like region, amino acids 580–585, is denoted by jagged lines

endogenous promoter (Fig. 6). Notably, regions within the F domain are crucial in determining the response of the receptor to E2 and 4-OHT (Fig. 6). The differences between the F domains of the ER alpha and beta subtypes and among the other members of the nuclear hormone receptor superfamily may offer opportunities for selective control of the activity of these proteins.

Acknowledgments Many thanks to the Department of Defense Breast Cancer Research Program and the National Institutes of Health for funding, and the members of the Skafar laboratory for their excellent work.

References

1. R.M. Evans, Science **240**, 889–895 (1988)
2. S. Nilsson, J.-A. Gustafsson, Breast Cancer Res **2**, 360–366 (2000)
3. B.S. Katzenellenbogen, J.A. Katzenellenbogen, Breast Cancer Res. **2**, 335–344 (2000)
4. E. Treuter, L. Johansson, J.S. Thomsen, A. Warnmark, J. Leers, M. Pelto-Huikko, M. Sjoberg, A.P. Wright, G. Spyrou, J.A. Gustafsson, J. Biol. Chem. **274**, 6667–6677 (1999)
5. R.V. Weatherman, T.S. Scanlan, J. Biol. Chem. **276**, 3827–3832 (2001)
6. A. Warnmark, T. Almlof, J. Leers, J.-A. Gustafsson, E. Treuter, J. Biol. Chem. **276**, 23397–23404 (2001)
7. M.M. Montano, V. Muller, A. Trobaugh, B.S. Katzenellenbogen, Mol. Endocrinol. **9**, 814–825 (1995)
8. J.A. Schwartz, L. Zhong, S. Deighton-Collins, C. Zhao, D.F. Skafar, J. Biol. Chem. **277**, 13202–13209 (2002)
9. G.A. Peters, S.A. Khan, Mol. Endrocrinol. **13**, 286–296 (1999)
10. V. Kumar, S. Green, G. Stack, M. Berry, J.R. Jin, P. Chambon, Cell **51**, 941–951 (1987)
11. G.G. Kuiper, E. Enmark, M. Pelto-Huikko, S. Nilsson, J.-A. Gustafsson, Proc. Natl. Acad. Sci. USA **93**, 5925–5930 (1996)
12. S. Mosselman, J. Polman, R. Dijkema, FEBS Lett. **392**, 49–53 (1996)
13. K. Kim, N. Thu, B. Saville, S. Safe, Mol. Endocrinol. **17**, 804–817 (2003)
14. B.A. Ince, D.J. Schodin, D.J. Shapiro, B.S. Katzenellenbogen, Endocrinology **136**, 3194–3199 (1995)
15. C.K. Wrenn, B.S. Katzenellenbogen, J. Biol. Chem. **268**, 24089–24098 (1993)
16. M. Nichols, J.M. Rientjes, A.F. Stewart, EMBO J. **17**, 765–773 (1998)
17. S.J. Potthoff, L.E. Romine, A.M. Nardulli, Mol. Endocrinol. **10**, 1095–1106 (1996)
18. F. Pakdel, P. le Goff, B.S. Katzenellenbogen, J. Steroid Biochem. Mol. Biol. **46**, 663–672 (1993)
19. D.M. Lonard, Z. Nawaz, C.L. Smith, B.W. O'Malley, Mol. Cell. **5**, 939–948 (2000)
20. X. Long, K.P. Nephew, J. Biol. Chem. **281**, 9607–9615 (2006)
21. A. Koide, C. Zhao, M. Naganuma, J. Abrams, S. Deighton-Collins, D.F. Skafar, S. Koide, Mol. Endocrinol. **21**, 829–842 (2007)
22. A.C. Pike, A.M. Brzozowski, R.E. Hubbard, T. Bonn, A.G. Thorsell, O. Engstrom, J. Ljunggren, J. Gustafsson, M. Carlquist, EMBO J. **18**, 4608–4618 (1999)
23. A.M. Brzozowski, A.C.W. Pike, Z. Dauter, R.E. Hubbard, T. Bonn, O. Engstrom, L. Ohman, G.L. Greene, J.-A. Gustafsson, M. Carlquist, Nature (London) **390**, 753–758 (1997)
24. A.K. Shiau, D. Barstad, P.M. Loria, L. Cheng, P.J. Kushner, D.A. Agard, G.L. Greene, Cell **95**, 927–937 (1998)
25. A.C. Notides, N. Lerner, D.E. Hamilton, Proc. Natl. Acad. Sci. USA **78**, 4926–4930 (1981)
26. S.P. Williams, P.B. Sigler, Nature **393**, 392–396 (1998)
27. B. Ferboud, M.L. Privalsky, Mol. Endocrinol. **18**, 2839–2853 (2004)
28. B. Farboud, H. Hauksdottir, Y. Wu, M.L. Privalsky, Mol. Cell. Biol. **23**, 2844–2858 (2003)
29. M.S. Jiang, G.W. Hart, J. Biol. Chem. **272**, 2421–2428 (1997)
30. C.C. Williams, C.L. Smith, B.G. Rowan, Abstracts of the 89th Annual Meeting of the Endocrine Society, #258 (2007)
31. F.M. Sladek, M.D. Ruse, L. Nepomuceno, S.-H. Huang, M.R. Stallcup, Mol. Cell. Biol. **19**, 6509–6522 (1999)
32. A.A. Bogan, Q. Dallas-Yang, M.D. Ruse, Y. Maeda, G. Jiang, L. Nepomuceno, T.S. Scanlan, F.E. Cohen, F.M. Sladek, J. Mol. Biol. **302**, 831–851 (2000)
33. M.D. Ruse, M.L. Privalsky, F.M. Sladek, Mol. Cell. Biol. **22**, 1626–1638 (2002)
34. E.H. Hani, L. Suaud, P. Boutin, J.-C. Chevre, E. Durand, A. Philippi, F. Demenais, N. Vionnet, H. Furuta, G. Velho, G.I. Gell, B. Laine, P. Froguel, J. Clin. Invest. **101**, 521–526 (1998)
35. A.D. Petrescu, R. Hertz, J. Bar-Tana, F. Schroeder, A.B. Kier, J. Biol. Chem. **280**, 16714–16727 (2005)
36. J.A. Schwartz, D.F. Skafar, Biochemistry **32**, 10109–10115 (1993)
37. J.A. Schwartz, D.F. Skafar, Biochemistry **33**, 13267–13273 (1994)
38. B.M. Weichman, A.C. Notides, J. Biol. Chem. **252**, 8856–8862 (1977)
39. B.M. Weichman, A.C. Notides, Biochemistry **18**, 220–225 (1979)
40. J.D. Obourn, N.J. Koszewski, A.C. Notides, Biochemistry **32**, 6229–6236 (1993)
41. M.E. Brandt, L.E. Vickery, J. Biol. Chem. **272**, 4843–4849 (1997)
42. C.C. Zhang, K.A. Glenn, M.A. Kuntz, D.J. Shapiro, J. Steroid Biochem. Mol. Biol. **74**, 169–178 (2000)
43. R. Bolger, T.E. Wiese, K. Ervin, S. Nestich, W. Checovich, Environ. Health Perspect. **106**, 551–557 (1998)
44. A.V. Hill, J. Physiol. **40**, iv–vii (1910)
45. M.A. Loven, N. Muster, J.R. Yates, A. Nardulli, Mol. Endocrinol. **17**, 67–78 (2003)
46. A. Koide, S. Abbatiello, L. Rothgery, S. Koide, Proc. Natl. Acad. Sci. USA **99**, 1253–1258 (2002)