A tale of two estrogen receptors (ERs): how differential ER-estrogen responsive element interactions contribute to subtype-specific transcriptional responses

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The interaction of ERα and ERβ with ERE constitutes the initial step in the canonical nuclear E2 signaling in which E2-ERβ is a weaker transactivator than E2-ERα. This perspective summarizes recent findings to discuss potential mechanisms that contribute to ER subtype-specific transcriptional responses.

Received December 15th, 2005; Accepted April 3rd, 2006; Published July 7th, 2006 | **Abbreviations:** **E2:** Estradiol 17β; **ER:** Estrogen Receptor; **ERE:** Estrogen Responsive Element; **SMRT:** Silencing Mediator of Retinoid and Thyroid Receptors; **NCoR:** Nuclear CoRepressor

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Estrogen receptors and estrogen signaling

Estradiol information is primarily conveyed by the members of a nuclear receptor superfamily, estrogen receptor (ER) α and β [Hall et al., 2001]. ERs are encoded by two distinct genes and expressed in the same and different tissues at varying levels. ERs consist of six functional domains. The structurally distinct amino terminal A/B domains (17% amino-acid identity) contain a ligand-independent transactivation function (AF1). The near identical central C region is the DNA binding domain (DBD). The flexible hinge, or D, domain contains a nuclear localization signal and links the C domain to the multi-functional carboxyl terminal (E/F) domain. E/F, which shows 56% amino-acid homology between ERs, is involved in ligand binding, dimerization, and ligand-dependent transactivation function (AF2).

ApoERs dimerize and translocate to the nucleus, likely as a part of large protein complexes [Zheng et al., 2005], independent of E2 [Bai and Giguere, 2003; Huang et al., 2005]. The nuclear apoERs are highly mobile molecules dynamically partitioned between intracellular target sites on chromatin and nuclear matrix [Stenoien et al., 2001]. ApoERs associate with ERE [Chen et al., 1999; Huang et al., 2005], permutations of a palindromic DNA sequence with three central non-specific nucleotides, 5'-GGTCAnnnTGACC-3' [Klinge, 2001].

ERα-mediated signaling

Elegant kinetic studies using the pS2 promoter as a model indicate that the engagement of apoERα with ERE occurs cyclically with short periods requiring both activating and repressing epigenetic processes [Metivier et al., 2004; Metivier et al., 2003; Reid et al., 2003]. ApoERα through the amino- and carboxyl-termini interacts [Webb et al., 1998; Yi et al., 2002b], albeit inefficiently, with highly mobile heterogeneous coregulator complexes [Stenoien et al., 2001] including protein and chromatin modifiers. Further protein alterations, including ubiquitination, of apoERα and associated coregulators, disassembles the transcription complex for proteasomal degradation [Lonard et al., 2000; Wang et al., 2001]. This is followed by promoter remodeling through the association of modifiers with basal transcription factors [Metivier et al., 2004; Metivier et al., 2003; Reid et al., 2003]. This oscillating promoter restructuring is suggested to provide a mechanism that enables a rapid adaptation of transcription to E2 [Metivier et al., 2004].

The binding of E2 to apoERα is accompanied by a conformational shift in the carboxyl-terminus that enhances the stability of the ERα dimer [Bai and Giguere, 2003], and generates a binding surface for stable interaction with cofactors [Hall et al., 2001]. E2 also increases the association of ERα with ERE [Chen et al., 1999; Huang et al., 2005]. Although mechanism is unclear, pre- and post-ERE binding events likely participate in the E2-mediated augmentation of ERα-ERE interactions. One possible pre-ERE binding event involves allosteric alteration of the folding or the stability of the DBD of ERα upon binding to E2 that lead to an increase in the population of the receptor capable of interacting with ERE. Alternatively, E2 mediates the dissociation of ERα from chaperones/nuclear matrix-associated proteins bound to the DBD, or to other regions that sterically block the DBD [Oesterreich, 2003; Pratt et al., 1996]. This could unmask DBD that allows ERα-ERE interactions. Additionally, E2 could influence the intermolecular association of ERα with protein complexes to enhance the stability of ERα-ERE interactions [Loven et al., 2003; Petz et al., 2002]. Pre-ERE binding events may also affect the partitioning of E2-ERα to chromatin from nuclear matrix, reflected as a decrease in the mobility of E2-ERα [Stenoien et al., 2001].

Kinetic studies further indicate that E2-ERα-ERE initiates a series of interdependent events that result in an extended periodicity of cyclic engagement [Metivier et al., 2004; Metivier et al., 2003; Reid et al., 2003]. This raises the possibility that post-ERE binding events also contribute to the E2-mediated increase in ERα-ERE interaction. In addition to the ability of the amino-terminus ERα to interact with coregulators independent of E2, the binding of E2 dramatically enhances the affinity of AF2 for coregulators [Yi et al., 2002b]. An effective recruitment of coregulators by both AF1 and AF2 could form a stable platform necessary for subsequent combinatorial recruitment of distinct mediators, integrators, and ubiquitin-proteasome pathway enzymes. Integrated actions of these complexes could extensively remodel chromatin leading to an increase in the duration of promoter occupancy of E2-ERα.

However, the formation of a stable and transcriptionally productive complex may not be sufficient to explain the E2-mediated increase in ERα-ERE interaction. Transcriptionally impaired ER variants with abrogated AF1 and/or AF2 display an E2-mediated increase in ERE binding and cyclical promoter occupancy that are indistinguishable from those observed with ERα [Huang et al., 2005; Metivier et al., 2004; Valley et al., 2005]. Since variant ERs undergo distinct proteasome-mediated degradations [Valley et al., 2005], a delay in the disassembly of the transcription complex could also extend the duration of E2-ERα promoter occupancy. Post-translational modifications, including phosphorylation, acetylation, sumoylation and/or ubiquitination, could influence the periodicity of the promoter occupancy of ERα by providing unique target surfaces for the recruitment of distinct coregulators that differentially modify the amplitude of transcription. Modifications could also affect the degradation of ERα independently from transcription. Although ApoERα and E2-ERα are degraded through the ubiquitin-proteasome pathway, E2 dramatically enhances the ubiquitination of ERα [Valley et al., 2005; Wijayaratne and McDonnell, 2001]. It is possible that a delay in the sequence of events leading to poly-ubiquitination could prolong the association of E2-ERα with the promoter. Lysine residues, for example, serve as common attachment sites for acetylation and sumoylation of the hinge domain of ERα, the latter of which is strictly dependent upon E2 binding [Sentis et al., 2005; Wang et al., 2001]. Since post-translational processing is a reversible and dynamic process, sumoylation or acetylation, prior to poly-ubiquitination, could modify ERα activity. These modifications could also disguise the recognition of the receptor as a proteolytic substrate for degradation, extending the promoter occupancy. Similarly, phosphorylation status of ERα could increase the duration of promoter engagement by uncoupling transactivation from degradation through the repression of poly-ubiquitination and subsequent ERα turnover [Valley et al., 2005].

We suggest that the E2-mediated increase in ERα-ERE interaction involves both pre- and post-ERE binding events that are manifested as increases in the population of ERα capable of interacting with ERE and in the periodicity of cyclic engagement of ERα with estrogen responsive promoters.

ERβ-mediated signaling

Although ERs show similar DNA and ligand binding properties *in vitro*, ERβ is less effective than ERα in inducing transcription from the ERE-dependent signaling pathway [McInerney et al., 1998; Yi et al., 2002a]. Despite a plethora of studies, comparatively little is known about mechanisms of ERβ action. It appears that AF1 is critical for defining the receptor-subtype specific activity. The amino-terminus of ERβ, in contrast to ERα, lacks significant transcriptional capacity and capability of functional interaction with the carboxyl-terminus [McInerney et al., 1998; Yi et al., 2002a]. Our recent study indicated that in contrast to ERα, the interaction of ERβ with ERE is independent of E2 and is impaired by its amino-terminus, contributing to transfection inefficiency of the receptor [Huang et al., 2005]. Although how the amino-terminus affects the binding of ERβ to ERE is not clear, the inter- and/or intramolecular interactions of the ERβ amino-terminus could sterically mask or allosterically affect the folding of the DBD. This could limit the population of ERβ capable of interacting with ERE. That truncation of the amino-terminus of ERβ dramatically enhanced the variant ERβ-ERE interaction compared to that observed with ERβ lends credence to this conclusion. However, the binding of the truncated ERβ to ERE remained independent of E2 unless AF2 was also obliterated. This implies that the binding of a factor(s) to the carboxyl-terminus induces a conformation in ERβ that resembles that mediated by the E2 binding. This contrasted to the amino-terminally truncated ERα with or without AF2 that showed an E2-mediated increase in ERE binding similar to ERα. Thus, although the amino-terminus of ERβ is a dominant region to impair ERβ-ERE interaction, the structural basis for the differential effect of E2 on ER-ERE interactions resides in the carboxyl-termini of ERs.

Studies indicate that ERα interacts, albeit weakly, with SMRT and NCoR, in the absence of E2 [Lavinsky et al., 1998; Webb et al., 2003]. The binding of E2 to ERα releases co-repressors from ERα. Apo-ERβ also interacts with SMRT/NCoR through a region that encompasses AF2 [Webb et al., 2003]. However, E2 does not promote the dissociation of corepressors from ERβ [Webb et al., 2003]. If indeed the conformation of the ERβ carboxyl-terminus bound to corepressors mimicked the conformation mediated by E2, the E2 effect on ERβ-ERE interaction would then be masked. This could explain why the interaction of ERβ with ERE appears to be independent of E2. Since, however, E2 is necessary for transactivation, E2 must also convert the inactive ERE-bound ERβ to a transcriptionally active state by concurrently recruiting coactivators through a distinct cofactor interacting surface(s). The presence of both coactivators and corepressors could be responsible for

the weak transactivity of E2-ERβ in the ERE-dependent signaling.

Although it is likely that ERβ engages with responsive promoters cyclically, the duration of promoter engagement may be similar for both apo- and E2-ERβ, as opposed to ERα, for which E2 is critical for determining the duration of promoter occupancy. The mode of promoter engagement of ERβ, however, could be modified by heterodimerization with ERα when co-expressed [Li et al., 2004] and by post-translational modifications [Tremblay et al., 1999] that likely alter pre- or post-ERE binding of ERβ.

Future studies directed to a better understanding of the mechanism of ER subtype-specific transcriptional responses will clarify these issues, and could provide a basis for the development of novel therapeutic approaches for estrogen target tissue malignancies.

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