The cell-specific activity of the estrogen receptor alpha may be fine-tuned by phosphorylation-induced structural gymnastics

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Abstract

The estrogen receptor alpha (ERalpha) regulates the transcription of target genes by recruiting coregulator proteins through several domains including the two activation functions AF1 and AF2. The contribution of the N-terminally located AF1 activity is particularly important in differentiated cells, and for ERalpha to integrate inputs from other signaling pathways. However, how the phosphorylation of key residues influences AF1 activity has long remained mysterious, in part because the naturally disordered AF1 domain has resisted a structural characterization. The recent discovery of two coregulators that are specific for a phosphorylated form of AF1 suggests that phosphorylation, possibly in conjunction with the subsequent binding of these coregulators, may enforce a stable structure. The binding of the "pioneer" coregulators might facilitate the subsequent recruitment of yet other coregulators. Different AF1 folds may be enabled by the combinatorial action of posttranslational modifications and coregulator binding thereby fine-tuning ERalpha activities in a cell- and promoter-specific fashion.

Reference

GBURCIK, Valentina, PICARD, Didier. The cell-specific activity of the estrogen receptor alpha may be fine-tuned by phosphorylation-induced structural gymnastics. *Nuclear Receptor Signaling*, 2006, vol. 4, p. e005

DOI: 10.1621/nrs.04005
PMID: 16604168
The cell-specific activity of the estrogen receptor α may be fine-tuned by phosphorylation-induced structural gymnastics

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The estrogen receptor α (ERα) regulates the transcription of target genes by recruiting coregulator proteins through several domains including the two activation functions AF1 and AF2. The contribution of the N-terminally located AF1 activity is particularly important in differentiated cells, and for ERα to integrate inputs from other signaling pathways. However, how the phosphorylation of key residues influences AF1 activity has long remained mysterious, in part because the naturally disordered AF1 domain has resisted a structural characterization. The recent discovery of two coregulators that are specific for a phosphorylated form of AF1 suggests that phosphorylation, possibly in conjunction with the subsequent binding of these coregulators, may enforce a stable structure. The binding of the “pioneer” coregulators might facilitate the subsequent recruitment of yet other coregulators. Different AF1 folds may be enabled by the combinatorial action of posttranslational modifications and coregulator binding thereby fine-tuning ERα activities in a cell- and promoter-specific fashion.

Cite this article: Nuclear Receptor Signaling (2006) 4, e005

Introduction

ERα is a member of the nuclear receptor superfamily, and mediates the responses to estrogens as well as a variety of other extracellular signals by signaling crosstalk. As a nuclear receptor, it harbors a receptor function, DNA-binding capacity and transcriptional activation functions all within the same molecule. Transcriptional regulation by ERα is mediated by the two activation functions AF1 and AF2. These activation functions represent docking surfaces on the receptor through which corepressors and coactivators are recruited. The particular combination of recruited coregulators determines the assembly of the general transcription machinery on the promoter and the resulting gene expression pattern.

AF2 lies within the ligand binding domain (LBD) of ERα and is induced upon binding of an agonist [Nagy and Schwabe, 2004; Steinmetz et al., 2001]. Depending on the exact chemical nature of the ligand and the precise allosteric rearrangements it induces in the LBD, coactivators or corepressors are recruited [Nettles and Greene, 2005]. The AF1 domain is located in the N-terminal region of ERα. The intrinsically constitutive activity of AF1 is unleashed by agonist binding to the LBD, but various signaling pathways also stimulate its activity, in part by direct phosphorylation of several serines [Ali et al., 1993; Bunone et al., 1996; Chen et al., 2000; Kato et al., 1995] (see also below). To the extent that AF1 can be dissected at all, different regions of AF1 have been shown to have distinct cell-type and promoter selectivity [McInerney and Katzenellenbogen, 1996; Metivier et al., 2000; Metzger et al., 1995; Tora et al., 1989].

Signaling crosstalk involves phosphorylation of AF1

During the last fifteen years, many investigators have reported that crosstalk between steroid- and growth factor-stimulated intracellular signaling pathways can affect the activity of nuclear receptors, and as a consequence the transcription of target genes [Cenni and Picard, 1999; Picard, 2003; Weigel and Zhang, 1998]. In the case of ERα, this involves the direct phosphorylation of the receptor, coactivators, and/or other regulatory proteins. A whole series of amino acid residues of ERα display basal and induced phosphorylation in response to ligands, growth factors and other regulatory molecules by MAPK, AKT, Rsk, protein kinases A and C, casein kinase II, CDK2, and CDK7 [Ali et al., 1993; Bunone et al., 1996; Campbell et al., 2001; Chen et al., 2000; Clark et al., 2001; Joel et al., 1998; Kato et al., 1995; Le Goff et al., 1994; Martin et al., 2000; Rogatsky et al., 1999; Tremblay et al., 1999]. Our understanding of the roles of all of these kinases and phosphorylation sites remains unclear. As far as AF1 is concerned, serine 118 (S118; numbering according to the sequence of the human ERα) is the main phosphorylation site that needs to be considered.

Specific recruitment of coregulators by the phosphorylated AF1

The key question is how the phosphorylation of ERα AF1 modulates its transcriptional activity. The mechanistic answer might depend on how this phosphorylation comes about, and on whether or not AF2 is also activated by cognate hormone, but it seemed reasonable from the beginning to speculate that the phosphorylation of S118
Recruitment by phosphorylation-induced structural gymnastics

In contrast to other nuclear receptor domains, there are no high-resolution structures available to date for the AF1 domain of any member of the nuclear receptor superfamily [Lavery and McEwan, 2005]. Its structure may be naturally disordered. AF1 domains appear to be structurally flexible with little stable secondary structure. This structural flexibility may provide the possibility for multiple different interactions [Dunker et al., 2002; Dyson and Wright, 2005]. Since different partner proteins may induce different conformations, they may in turn depend on cellular and promoter context. Moreover, it is possible that the AF1 domain requires specific post-translational modifications in order to be fully active [Kumar and Thompson, 2003]. Phosphorylation of AF1 may increase its helical content, which has been shown to correlate with increased activation potency in case of the peroxisome-proliferator activated receptors (PPARs) [Gelman et al., 2005].

Are SF3a120 or SPBP novel phosphoserine binding proteins? Competition and truncation experiments (data not shown) suggest that SPBP recognizes a specific AF1 fold induced by phosphorylation rather than the immediate context of the phosphorylated serine itself (see Figure 1). If we hypothesize that the phosphorylation of AF1 induces a conformational change or stabilization resulting in the generation of a docking site for a cofactor, several predictions are worth considering. Cofactors that interact with the phosphorylated AF1 might facilitate each other’s recruitment (Figure 1A). Anchoring one cofactor might further stabilize or structure the domain [Gelman et al., 2005; Lavery and McEwan, 2005], and allow the subsequent binding of a second factor (Figure 1B and C).

In a different context, it had already been suggested that the recruitment of coactivators could facilitate the subsequent recruitment of other coactivators or even corepressors [Perissi and Rosenfeld, 2005]. Indeed, some of our preliminary results with combinations of SPBP and coactivators support this speculation (data not shown). We suggest that upon AF1 phosphorylation, coactivators such as SF3a120 are recruited first. They then facilitate the recruitment of SPBP, which acts as a corepressor, most likely by recruiting other corepressors such as NCOR [Gburcik et al., 2005]. The end result is that the strength and the duration of ERα activity are dampened. In this and other situations, alternative scenarios with an inverse order of binding or with cyclical exchanges are also conceivable.

This phosphorylation-induced gymnastics may itself be influenced by and complement additional “outside” inputs into AF1 structure. For example, binding of JDP-2 to the DNA binding domain of the progesterone receptor increases the helical contents of the N-terminus and AF-1 activity [Wardell et al., 2005], and sequence-specific allosteric effects of the DNA response element on receptor conformation have been recognized as a general principle for several nuclear receptors [Lefstin and Yamamoto, 1998] including ERα [Wood et al., 1998].

Physiological implications

There may be many physiological consequences of this structural gymnastics induced by signaling crosstalk. To illustrate this point it should be sufficient to mention a few. In differentiated cells, AF1 may be the major transactivation function of ERα [Merot et al., 2004; Pendaries et al., 2002]. Therefore, SPBP might be an important determinant of the cell-specific activity pattern of ERα in differentiated cells. It might also play an important role in the organ-specific activity pattern of ERα during the estrous cycle, which has recently been monitored in a transgenic mouse model with a luciferase reporter gene under the control of activated ERs [Ciana et al., 2003]. Interestingly, the reporter activity in reproductive organs was synchronized with estrogen levels, while the peak of ER-dependent activity in non-reproductive organs did not correlate with estrogen levels. It was speculated that the latter activity might be due to ligand-independent activation of ERs by growth factors such as IGF-I. Whereas SPBP is not expressed in reproductive organs [Rekdal et al., 2000], the expression of SPBP might be cyclically induced in non-reproductive organs during the estrous cycle repressing ERα activity when estrogen peaks.

Signaling crosstalk of ERα with growth factors is also thought to contribute to resistance to endocrine therapy in breast cancer by stimulating AF1 phosphorylation and activity [Osborne et al., 2003; Osborne et al., 2005; Shou et al., 2004]. Hence, antiestrogen-resistant ERα-positive
breast tumors would be expected to have lower SPBP levels. In contrast, ERα-negative breast tumors would not be adversely affected by the presence of SPBP. Rather they would benefit from the activating effects of SPBP on other growth-promoting transcription factors such as c-Jun [Rekdal et al., 2000]. Indeed, as we have previously pointed out [Gburcik et al., 2005], this inverse correlation between SPBP and ERα expression can be seen in a microarray analysis of breast tumor samples [van’t Veer et al., 2002].

Outlook
The models discussed in this essay have several practical as well as biological implications. For example, the identification of certain AF1 coregulators may only be possible in the presence of a first-line coregulator. Moreover, solving the structure of AF1 may require solving the structure of a complex between a phosphorylated AF1 and a coregulator. At a more mechanistic and physiological level, it will be interesting to fill in the details of how signaling crosstalk induces structural changes in AF1, and how this contributes to specifying and fine-tuning the physiological functions of ERα.

Acknowledgements
This work was supported by the Swiss National Science Foundation, Krebsforschung Schweiz, the Fondation Médic, and the Canton de Genève.

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