Ligand-induced estrogen receptor α degradation by the proteasome: new actors?

Mathilde Calligé and Hélène Richard-Foy

Corresponding Author: hrfoy@ibcg.biotoul.fr

Laboratoire de Biologie Moléculaire Eucaryote, Institut d'Exploration Fonctionnelle des Génomes, Toulouse, France

In this perspective we consider new aspects of ligand-induced estrogen receptor α (ERα) degradation. What are the possible roles of CSN5/Jab1 and the CSN complex in this process? We compare hormone (estrogen) or pure antagonist (fulvestrant) induced degradation of ERα and review the effects of kinase-inhibitors and CRM1-dependent nuclear export on ERα degradation and transcription activation. A model for ERα action integrating these new actors is proposed and the relation between hormone-induced ERα degradation and transcription-activation is discussed.

Received August 16th, 2005; Accepted Novemeber 15th, 2005; Published February 8th, 2006 | **Abbreviations:** **ALLN:** N-acetyl-leucyl-leucyl-norleucinal; **CKII:** Casein Kinase II; **CSN:** COP9 Signalosome; **CSN5/Jab1:** subunit 5 of CSN; **DRB:** 5,6-dichlorobenzimidazole riboside; **ERα:** estrogen receptor α; **PKD:** Protein Kinase D (also termed Protein Kinase C μ); **SRC3/AIB1:** Steroid receptor coactivator 3; **TBL1:** Transducin β-like protein 1; **TBLP1:** TBL1-related protein; **TFF1:** Trefoil Factor 1 (also termed pS2) |

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Introduction

Over the last few years, the involvement of the proteasome pathway in ligand-dependent nuclear receptor degradation has been established. This pathway entails first a polyubiquitination of the substrate, catalyzed by three factors (E1, E2 and E3), followed by its proteolysis by the proteasome. Recently, the role of the CSN complex that regulates the activity of a class of E3 ubiquitin ligases, the cullin RING ubiquitin ligase superfamily, was demonstrated. The cullin subunits of these ubiquitin ligases are modified by the conjugation of an ubiquitin-like protein, NEDD8. The neddylated subunit Cul1 is selectively degraded. Recent data demonstrate that CSN, via its subunit CSN5/Jab1, dennedylates cullin, thus increasing the activity of the E3 ligases [Wee et al., 2005].

Several lines of evidence suggest that CSN is involved in ligand-dependant nuclear receptor degradation. First, estrogen receptor α (ERα) degradation is dependant on the neddylation pathway [Fan et al., 2003]; second, CSN5/Jab1 interacts with both the progesterone receptor and the coactivator SRC1 and is itself a coactivator of the nuclear receptors [Fan et al., 2003]; third, Jab1/CSN5 increases hormone-induced ERα degradation [Fan et al., 2003].

The nature of the ligand affects ERα degradation differently: estradiol and the pure antagonist fulvestrant induce ERα degradation by the proteasome, whereas the mixed antagonist tamoxifen stabilizes Erα [Wijayaratne and McDonnell, 2001]. Thus, degradation may play an important role in ERα function and/or the action of its antagonists. ERα undergoes post translational modifications such as phosphorylation [Lannigan, 2003], acetylation [Wang et al., 2001] or sumoylation [Sentis et al., 2005]. However, the role of such modifications in targeting ERα for degradation remains unclear. A kinase activity, inhibited by curcumin, is associated to CSN. At least two curcumin-sensitive kinases, CKII and PKD, which co-purify with CSN, could contribute to this activity [Uhle et al., 2003]. CKII phosphorylates ERα on Ser167 in response to estradiol [Lannigan, 2003]. The inhibition of ERα degradation by curcumin and the co-immunoprecipitation of Jab1/CSN5 with ERα in the presence of curcumin, suggest that this kinase activity could participate in targeting ERα for degradation. In addition, the inhibition by curcumin of the interaction of ERα with its DNA target, points towards a role of this complex in transcription activation [Callige et al., 2005].

The discovery that E2 and E3 factors and proteasome subunits associate with the transcriptional machinery, reinforces the hypothesis that the degradation of nuclear receptors could be linked to transcriptional activation and may be necessary for efficient transcriptional activity [Nawaz and O'Malley, 2004]. Here we discuss ERα degradation pathways in the presence of different ligands and the role of this degradation in ERα function.

Which E3 ligases are involved in ERα degradation?

The demonstration that the NEDD8 pathway is required for proteasome mediated degradation of ERα, suggests that the E3 ligases involved belong to the cullin RING ubiquitin ligase superfamily. Within this family, MDM2 and E6-AP, were identified as ERα coactivators [Nawaz et al., 1999; Saji et al., 2001]. MDM2 is also involved in the degradation of glucocorticoid and androgen receptors [Kinyamu and Archer, 2003; Lin et al., 2002] and thus is a good candidate for ERα polyubiquitination. ERα could also be a substrate for BRCA1/BARD1, another potential E3 ligase, which is recruited by Phospho-Pol II and is involved in the degradation of both chromatin proteins and active RNA polymerase II [Starita and Parvin, 2003]. One could speculate its involvement in ERα degradation since this degradation is concomitant with transcriptional activation. However BRCA1/BARD1 belongs to the HECT

E3 ligase family, and there is no evidence that the NEED8 pathway regulates its activity.

What role do coactivators and adaptors play in ERα degradation?

Transcription activation by ERα involves a number of co-regulators, in particular coactivators of the p160 family (SRC1/SRC2/SRC3). The activity of SRC3 (the major ERα coactivator in breast cancer cell lines) is regulated by phosphorylation [Wu et al., 2004]. Hormone-induced transcription requires the dissociation of a corepressor (N-CoR/SMRT) from ERα and its replacement by a coactivator from the p160 family. TBLR1, a protein that shares homology with TBL1 (Transducinβ-like1), selectively mediates corepressor/coactivator exchange upon ligand binding to nuclear receptors [Perissi et al., 2004].

Several observations converge towards a link between the recruitment of ERα cofactors and ligand-dependent degradation by the proteasome. Suppression of SRC3/AIB1 by siRNA leads to ERα stabilization in presence of estradiol [Shao et al., 2004] and SRC3/AIB1 itself is degraded by the proteasome in a hormone-dependent process [Perissi et al., 2004], suggesting a role for SRC3/AIB1 in ERα degradation. Nuclear receptor co-factors such as TBL1 and TBLR1 (originally identified as components of an N-CoR complex), besides their ERα-cofactor exchange activity, serve as specific adaptors for the recruitment of the conjugating/19S proteasome complex and thus participate in co-factor dynamics at the promoter during the transcription initiation process [Perissi et al., 2004].

In which cellular compartment is ERα degraded?

Inhibition of CRM1-dependent nuclear export by leptomycin B blocks estrogen-dependent, but not fulvestrant-induced degradation of ERα. This demonstrates that the two ligands induce ERα degradation through different pathways, taking place in different cellular compartments. In the presence of estradiol ERα is degraded by a cytoplasmic fraction of the proteasome, whereas in the presence of fulvestrant it is degraded in the nucleus [Callige et al., 2005]. These findings reinforce observations demonstrating rapid sequestration of the ERα-fulvestrant complex in a salt-insoluble, nuclear compartment [Giamarchi et al., 2002] and reduced mobility of the ERα-fulvestrant complex relative to the estradiol-ERα complex in the nucleus [Stenoien et al., 2001]. The fact that fulvestrant is unable to promote either chromatin remodeling over the TFF1 estrogen-regulated promoter [Giamarchi et al., 2002], or binding of ERα to its DNA target within the same promoter [Reid et al., 2003], indicates that the nuclear sub-compartment in which the fulvestrant-ERα complex is degraded, must be distinct from the sub-compartment in which transcription takes place. The nature of the nuclear compartment in which this degradation takes place remains unknown. To understand better the mechanism of action of the pure antiestrogen fulvestrant, several issues need to be elucidated: What is the nature of the compartment in which ERα is sequestered? Does fulvestrant play a direct role in such targeting, e.g., by directly recruiting a cofactor that changes the fate of ERα-fulvestrant complex? Do corepressors or coactivators play a role in this process (as suggested by the fact that suppression of SRC3/AIB1 by siRNA leads to ERα stabilization in presence of estradiol but not of fulvestrant) [Shao et al., 2004]?

What is the link between ERα degradation and estradiol induced transcription?

The pathways involved in ERα degradation differ, depending on the ligand (hormone or full antagonist, such as fulvestrant). A phosphorylation event catalysed by a curcumin-sensitive kinase, that could be CKII, is necessary for both estradiol- and fulvestrant-induced ERα degradation [Callige et al., 2005], making it unlikely that this phosphorylation plays a role in the targeting of ERα to different degradation-compartments. The exact role of SRC3/AIB1 in each step of the hormone-induced ERα degradation process remains to be determined. The fact that ERα-OH-tamoxifen complex (that cannot recruit coactivators) is not released from target promoters [Wijayaratne and McDonnell, 2001], along with the observations that SRC3 is co-degraded with ERα [Shao et al., 2004] and that suppression of SRC3 expression abrogates ERα degradation [Lonard et al., 2000], favours a central role for coactivators in ERα degradation. A possible role of the CSN in SRC3/AIB1 phosphorylation and degradation should be investigated.

Figure 1 presents a model summarizing the different steps that may be involved in estradiol-induced ERα degradation and the possible links with transcription activation. ERα-hormone complex interacts with a coactivator (SRC3/AIB1 in breast cancer cells) and with CSN5/Jab1. ERα and/or SRC3/AIB1 are phosphorylated by a curcumin-sensitive kinase, possibly associated with the CSN, leading to the binding of ERα to promoters of estrogen regulated genes. Curcumin prevents such binding either because ERα or SRC3/AIB1 phosphorylation is required for DNA binding, or because phosphorylation is required for a stable interaction between ERα and SRC3/AIB1. Upon transcription initiation, the promoter is cleared. Exchange factors such as TBL1 or TBLR1 should facilitate recruitment of coactivators by ERα and of ubiquitin ligase factors at the promoter. ERα and/or SRC3/AIB1 may be tagged for degradation as a result of a post-translational modification and/or a polyubiquitination. The complex containing ERα and SRC3/AIB1 is then exported to the cytoplasm. The CSN may promote export of ERα which lacks a nuclear export signal. Leptomycin B inhibits ERα degradation and increases hormone dependent transcription, suggesting that the modifications (putative tagging and/or polyubiquitination) can be reversed and the complex recycled. The CSN could be involved at that step through the recruitment of a deubiquitinating enzyme. In the

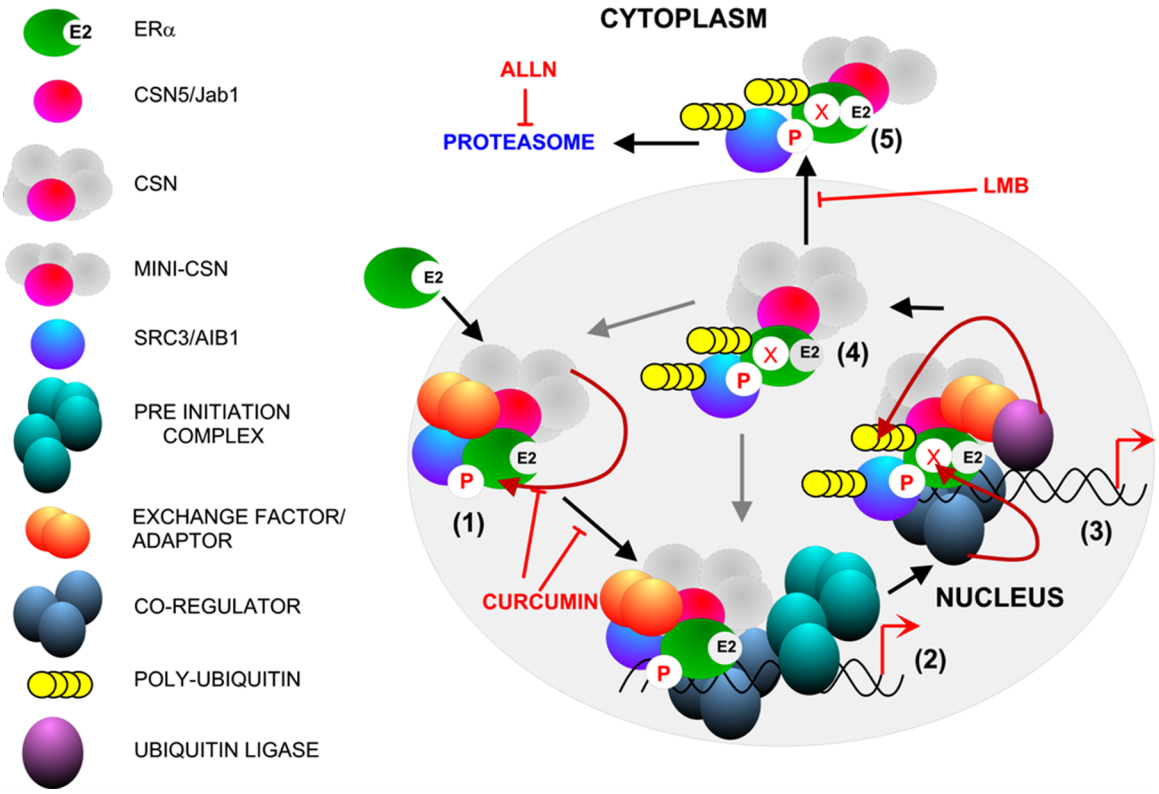

Figure 1. Estradiol-induced ERα degradation and transcriptional activation: a model.

cytoplasm, the ERα-hormone complex is degraded by the proteasome.

Conclusion

Controversial results regarding the involvement of ERα degradation in transcriptional activation have been published. A first set of results suggests that ERα degradation is required to sustain transcription [Lonard et al., 2000; Reid et al., 2003], yet another set concludes that ERα degradation has no general direct effect on transcription [Alarid et al., 2003; Callige et al., 2005; Fan et al., 2003]. It should be noted that the experiments suggesting that ERα degradation is required to sustain transcription, used treatment times with proteasome inhibitors or DRB, for longer than 12 hours [Lonard et al., 2000; Reid et al., 2003]. Such a long treatment time may have pleiotropic effects on the cell and affect multiple components of the transcription machinery. In contrast, the experiments suggesting that ERα degradation has no influence on transcription, used much shorter treatment times (less than 3 hours), minimizing general effects of the inhibitors used. ERα degradation seems rather to be the consequence of transcription initiation, as shown by the inhibitory effect of curcumin, rather than transcription per se, since DRB that blocks transcription does not prevent hormone-dependent ERα degradation [Alarid et al., 2003; Callige et al., 2005]. In addition, the nature of the transcription factors recruited, along with ERα, as well as the architecture of each promoter seem to be important, suggesting that the effect of proteasome inhibitors on transcription results from the degradation of co-regulators, rather than from that of ERα [Callige et al., 2005; Fan et al., 2003].

Whatever the role ERα degradation plays at the molecular level of transcription activation, ligand induced ERα degradation may be of importance at the physiological level. Estradiol forms a stable complex with ERα. This hormone-receptor complex should sustain transcription for extended periods of time. Degradation of the ERα-hormone complex at each round of transcription could be a way for the cell to fine tune transcription activation in response to rapid changes in hormone concentration.

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