Journal of Steroid Biochemistry & Molecular Biology 127 (2011) 223–230

Contents lists available at ScienceDirect

Journal of Steroid Biochemistry and Molecular Biology

journal homepage: www.elsevier.com/locate/jsbmb

Review

Signaling functions of ubiquitin in the 17β-estradiol (E2):estrogen receptor (ER) α network

Piergiorgio La Rosa, Filippo Acconcia*

Department of Biology, University Roma Tre, Viale Guglielmo Marconi, 446, I-00146, Rome, Italy

ARTICLE INFO

Article history:
Received 17 June 2011
Received in revised form 23 July 2011
Accepted 26 July 2011

Keywords:
17β-Estradiol
Estrogen receptor
Ubiquitin
Ubiquitination
Signal transduction
Cell proliferation

ABSTRACT

Protein posttranslational modifications (PTMs) are signaling alterations that allow coordinating the cellular responses with the changes in the extracellular environment. In this way, the posttranslationally-modified protein becomes a switch node in the transduction network activated by the specific extracellular stimuli. It is now clear that this is the case also for protein ubiquitination: this extremely versatile PTM controls cell physiology through the modulation of protein stability as well as through the modulation of the dynamics of the intracellular signaling cascades. Recent evidence clearly indicates that such a complex scheme appears to be valid also for the 17β-estradiol (E2):estrogen receptor (ER) α signal transduction pathways. Indeed, beside the long standing notion that ERα ubiquitination is required for the regulation of receptor stability, several laboratories, including our own, have clearly indicated that ERα ubiquitination also serves non-degradative functions. This review will reconsider the role of ubiquitination in E2:ERα signaling by particularly highlighting how the functions of the non-degradative ubiquitination impact on ERα activities and contribute to the modulation of E2-dependent physiological processes.

Contents

1. Introduction

Reversible posttranslational modification (PTM) is to date widely recognized as a mean to regulate protein activity. Although phosphorylation is the best studied PTM in mammalian cells, many other types of reversible PTMs such as acetylation, methylation, and ubiquitination exist and contribute to finely coordinate the physiological responses of cells with respect to the diverse environmental stimuli through the modulation of intensity, length and frequency of the intracellular signal transduction pathways. Such exquisite regulation is achieved because protein PTM is often an inducible process and because many proteins are multiply modified by different covalently attached groups, which generate multiple distinct protein states. Therefore the information content included in the initial environmental stimuli is increased and amplified and the posttranslationally-modified protein becomes an highly versatile node in the transduction of intracellular signaling [1]. PTMs-dependent regulation of protein function occurs because the

* Corresponding author. Tel.: +39 0657336320; fax: +39 0657336321.
E-mail address: acconcia@uniroma3.it (F. Acconcia).

0960-0760/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.jsbmb.2011.07.008

attached group can change protein activity (e.g., enzymatic activity) and/or alter the molecular landscape of the protein surface thus creating or even abrogating binding sites for both protein–protein interactions and protein:subcellular compartment associations.

Among other PTMs, ubiquitination of proteins is emerging as new mean to regulate different physiological processes not only by controlling protein stability and turnover through proteolytic degradation but also by modulating intracellular signaling in a non-degradative fashion [2]. Thus, understanding how non-proteolytic functions of ubiquitination impact on protein regulation represents to date a hot topic. Due to the novel discoveries that implicate non-degradative ubiquitination in the modulation of estrogen receptor α (ERα) signaling [3–7], this review will summarize the actual knowledge of the impact of ubiquitination in E2:ERα-based intracellular signaling, highlighting the new functions of the non-degradative ubiquitination in ERα activities.

2. The complexity of the ER-based signaling network

A key role in the control of development, sexual behavior, and reproductive functions is played by the sex hormone 17β-estradiol (E2). Interestingly, E2 is further able to exert a myriad of effects also in non-reproductive organs such as brain, liver, bone and heart to name a few. These pleiotropic hormone effects depend on the peculiar nature of the E2:ERs pathways [8].

ERα and ERβ are independent receptors encoded by different genes, display selective tissue expression, and can be often co-expressed at different levels in different tissues. In addition, it is now accepted that E2 can trigger contrasting effects when bound to the ERα or to the ERβ. For example, E2 is a mitogen when ERα is engaged in the mammary gland whereas it works as a pro-apoptotic factor when ERβ is activated in the colon [8–10].

The multifaceted action of E2 can be further ascribed to the structural plasticity of the ERs. ERα and ERβ are ligand-activated nuclear receptors with a structure composed by six modular domains. The *N*-terminal portion of the receptor (i.e., the A and B domains) does not display a folded structure and thus its intrinsic flexibility allows intermolecular and intramolecular interactions that are necessary for the activation of gene transcription. On the contrary, the DNA binding domain (i.e., DBD or C domain) consists of the repetition of two 'zinc finger' motifs, which are required for DNA binding to specific estrogen response element (ERE) whereas the ligand binding domain (i.e., LBD, or E domain) is mainly made of α-helices and contains the E2 binding sites. The DBD and the LBD are connected by a hinge region, which is the target of several different PTMs and appears to be a regulatory domain. Finally the very terminal *C*-end of the protein contains the so-called F domain whose functions are still largely unknown [8]. Two transcription activation functions (AFs) are endowed within the ER structure and direct both ligand-independent (i.e., the AF-1 region in the *N*-terminal portion) and ligand-dependent (i.e., AF-2 in the *N*-terminal portion) transcriptional activation by E2. The AF-1 and AF-2 regions allow the receptors to physically associate with diverse transcriptional co-factors (i.e., co-activators and co-repressors) that in turn are required to recruit the basal transcriptional apparatus. Receptors:co-factors association represents another important mechanism to diversify the E2-dependent effects. Indeed, several classes of co-activators and co-repressors with variable tissue expression exist and can act on either the ERα or the ERβ [8,11].

Remarkably, additional complexity is due to the mechanisms of the E2-ERs intracellular signaling, which are strictly dependent on ERs cellular localization. Nuclear and cytoplasmic localization defines the classic actions of E2. This model dictates that, in the absence of ligand, the ERs in the cytoplasm of E2-target cells associates with the heat shock proteins (e.g., Hsp90), which maintain the receptor in an inactive state. Binding of E2 to ERs induces the dissociation of the receptor from Hsps, dimerization, and translocation to the nucleus, where the ERs directly bind to the ERE sequence in the promoter of related genes and gene transcription occurs [12,13]. Remarkably, ERs can regulate gene transcription through the association to specific transcription factors such as Sp-1 and AP-1, without directly contacting DNA [11]. On the other hand, plasma membrane localization of the nuclear ERs allows E2 to elicit also extranuclear effects [14]. These E2 effects are independent on ER transcriptional activity, are activated quickly (i.e., in seconds to minutes) and are insensitive to transcription inhibitors. From a functional point of view, the E2-triggered extranuclear signaling (e.g., ERK/MAPK; PI3K/AKT) is necessary and sufficient for the E2-dependent control of several physiological processes (e.g., proliferation, apoptosis and differentiation) and occurs *in vivo* [8,14,15].

Whether the extranuclear and nuclear mechanisms of E2 integrate in a unique picture or represent parallel and synergic pathways still remains to be firmly clarified. Nonetheless, rapid E2-evoked activation of signaling pathways impinges on ERα phosphorylation and nuclear ERs co-regulatory proteins also play important roles in the modulation of ERs extranuclear effects. Therefore the interplay among the E2-ERs dependent nuclear and extranuclear mechanisms further demonstrate the intricacy of ER-signaling [11].

3. The complexity of the ubiquitin-based signaling network

Ubiquitination is a reversible PTM which occurs through the sequential activation of the enzymatic reactions that lead to the covalent attachment of the 8 kDa protein ubiquitin (Ub) *via* an isopeptide bond between the Ub *C*-terminal glycine (Gly) residue and the ε-amino group of a lysine (Lys) residue on the target protein. Three types of enzymes mediate this process: the Ub-activating enzyme (E_1) catalyzes the ATP-dependent conjugation of Ub on a cysteine (Cys) residue (i.e., thiol-ester conjugate) on E_1 and then transfers Ub to another Cys residue on a Ub-conjugating enzyme (E_2). In the final step of the ubiquitination cascade the E_2s work in concert with Ub-ligases (E_3) to specifically attach Ub to the target protein. Two types of Ub ligases exist. The RING-type E_3s bridge the E_2 and the substrate thus facilitating the direct transfer of Ub from the E_2. On the other hand, the HECT-type E_3s are first modified with Ub by E_2 and then transfer the Ub moiety on the target protein. Accordingly, the specificity of the ubiquitination cascade is ultimately due to the action of the E_3 alone or in association with its E_2 [16,17].

Additional complexity of the ubiquitination pathway is given by the possibility of the substrate to be modified with Ub in diverse ways: monoubiquitination (monoUbq) or multimonoubiquitination happen when Ub is appended to the target protein through one or several Lys residues, respectively [18]. Furthermore, E_3s can also form polyubiquitin (polyUb) chains on the substrate since Ub contains seven lysines that are available for chain formation. PolyUb chains can be different in nature since different Lys linkages give rise to different three-dimensional topologies of the resulting polyUb chain. Although all Lys residues are used for chain formation *in vivo* [19,20], structural data are available only for Lys48- and Lys63-based polyUb chains. Indeed, Lys48-linked chains adopt a closed conformation [21] whereas Lys63-linked Ub chains are linearly arranged head to tail [22]. From a functional point of view, each different Ub modification determines a different signaling outcome and thus a different possibility of modulation of the physiological process in which the modified protein is involved in [2]. Remarkably, all the Ub modifications are reversible

since the isopeptide bond can be targeted by the action of several de-ubiquitinating enzymes (i.e., isopeptidases, DUBs) that act on ubiquitinated substrates by removing Ub moieties [23]. Therefore, the ubiquitination cascade is a dynamic and modular process.

3.1. Ubiquitin as an intracellular second messenger

In general, ubiquitination influences the functions of the target proteins by either affecting their stability or endowing them with additionally signaling properties as well as by creating new surfaces for intermolecular interactions [24]. Thus Ub is acknowledged as an intracellular messenger, whose nature as a signal resides in several aspects of the Ub modification. For example, single or multiple Ub moieties or polyUb chains attached to the substrate create structural determinants that can be further used for molecular interactions.

For this reason, cells have evolved specific proteins that are able to decipher the message included in the new surfaces created by ubiquitination [2]. These proteins, which are called Ub receptors, bind to the ubiquitinated protein by contacting the Ub-modification through specific ubiquitin-binding domains (UBDs). Although many different UBDs exist and several new UBDs are being discovered, no specific conservation in terms of UBDs three-dimensional structure has still been recognized. As a matter of fact, UBDs appear to share a common 'shape': structural folds that have a regular secondary structure (e.g., α-helix) or a 'zinc-finger' motif, or both, are thought to be the only UBDs common features. Furthermore, UBDs are able to bind to both monoubiquitin and polyubiquitin with an affinity constant in the micro molar range (i.e., the UBD binding to Ub is very weak) but to date no single domain has been indicated to exclusively bind to one specific Ub modification [25], although preferential binding for Lys48- or Lys63-linked chains has been reported [26].

The UBDs located in the Ub-receptors work as signal transducers *via* protein–protein interaction in the ubiquitination pathway through several mechanisms. As examples, multiple UBDs in the ubiquitin receptors may recognize multiple monoUb moieties on the ubiquitinated substrate [27]. Alternatively, a ubiquitin receptor can simultaneously engage a single monoubiquitin through two different UBDs [28]. Thus, despite the UBDs weak binding affinity towards Ub, these high avidity modes of association among ubiquitinated substrates and ubiquitin receptors determine high affinity interactions relevant for cell physiology. UBDs also contribute to the amplification of the Ub-based signal since some ubiquitin receptors are able to bind to monoubiquitin and undergo monoUbq in a molecular process (i.e., coupled monoUbq) that requires an intact UBD [29].

3.2. Non-degradative ubiquitination contributes to intracellular signaling

Classically, the modification of intracellular proteins with a polyUb chain based on Lys48 linkage is the signal for the activation of the intracellular proteolytic pathway through the 26S proteasome. Thus ubiquitination is a mean to regulate protein stability and turnover. This concept is firmly established in the scientific community and a systematic description of such a mechanism (for a mechanistic review, see also [30]) is beyond the scope of the present review and thus will not be presented.

However, in recent years non-degradative functions of Ub as diverse as endocytosis, intracellular trafficking and DNA damage tolerance have been ascribed to modification of proteins *via* Lys63-linked polyUb chains as well as to the modification of the target substrate *via* monoubiquitin [2]. For example, one of the best characterized pathway in which the non-degradative function of Ub occurs is the ligand-induced intracellular trafficking of the epidermal growth factor (EGF) receptor (EGFR). Upon ligand activation, EGFR dimerizes and becomes phosphorylated. Besides creating binding sites for several signaling molecules (e.g., ERK/MAPK; PI3K/AKT), receptor phosphorylation also determines the recruitment of the RING E_3 Ub ligase c-Cbl to the EGFR [31]. As a consequence, EGFR becomes both multimonoubiquitinated and polyubiquitinated through Lys63-linked chains [32–34]. Ubiquitination of the EGFR drives receptor internalization and trafficking to the endosomal and the lysosomal compartments [24,35,36]. In parallel, ligand stimulation triggers the monoubiquitination of several ubiquitin receptors (e.g., eps15) which belong to the EGFR trafficking machinery and contribute to EGFR internalization and endosomal sorting [31]. As a net result, EGFR cellular levels are reduced and the ligand-dependent cellular processes (e.g., cell proliferation) occur.

4. The ERα and the Ub-based signaling network

From what mentioned above, the extracellular stimuli-mediated activation of the Ub-based network modulates different physiological processes through an intricate system of ubiquitinated proteins. This system creates a plethora of protein–protein interactions where the Ub-based modification, the activity of the Ub-modified protein and both the specific protein and Ub interactors all contribute to the signaling network dynamics.

Mounting evidence indicates that this molecular scheme can be operative also for the ERα-based signaling network. Indeed, ERα is a ubiquitinated protein, binds to UBDs containing proteins (i.e., RAP80; CUEDC2) [37,38] and may also have Ub-binding surfaces (our unpublished results), thus this hormone receptor also belongs to the Ub-based signaling network. Moreover, the ERα has long been recognized as a polyubiquitinated protein and more recently ERα monoubiquitination as well as multimonoubiquitination has been reported. As a consequence, degradative and non-degradative ubiquitination of the ERα occurs and impacts on receptor activities [3–7,39–41].

4.1. Degradative functions of the ubiquitin network in ERα signaling

The ERα is a polyubiquitinated protein. Indeed, ERα polyubiquitination happens as a result of E2 binding and receptor degradation occurs [39–41]. Furthermore, also the apoERα (i.e., unliganded receptor) undergoes polyubiquitination and proteolytic degradation [12,13]. Interestingly, these two different proteasomal-dependent pathways play different roles in the regulation of ERα degradation: polyubiquitination of the apoERα works as quality control system since it targets the misfolded neosynthesized ERα for proteasomal degradation whereas E2-induced ERα polyubiquitination and its resulting proteasomal-dependent degradation are necessary for receptor nuclear functions [42]. The role of 26S proteasome in the ability of ERα to regulate E2-dependent gene expression has been the object of debate. Indeed, the group of Gannon [12,13] defined that both the unliganded receptor and the E2-activated ERα pools contribute to the ability of ERα to act as a transcription factor since receptor polyubiquitination is the signal to allow ERα to cycle on and off its natural target promoters (i.e., ERE-containing promoters). As a consequence, inhibition of proteasome activity prevents E2-induced ERα transcriptional activity [12,13]. On the contrary, the group of Nephew reported that the inhibition of proteasomal proteolysis sustains E2-induced ERα transcriptional activity [43]. However, besides differences in the experimental settings [43], the group of Alarid showed that ERα transcriptional regulation and proteolysis represent a point of divergence that is controlled

by the phosphorylation of the Ser-118 ERα residue [41]. Because Ser-118 phosphorylated ERα is recruited to ERE containing promoters [44] and is necessary for enhanced association of ERα of transcriptional cofactors in the cell nucleus [45], it is possible that ERα polyubiquitination and proteasomal degradation may be dissociated while the ERα-mediated transcriptional process is actually occurring. Nevertheless, polyubiquitination-dependent 26S proteasome-mediated ERα breakdown not only determines the amount of ERα intracellular levels by controlling receptor turnover but also synchronizes E2-dependent ERα-mediated gene transcription with the E2-induced receptor degradation, which thus appears to be required for limiting the cellular response to E2 [39–41,43]. Hence, it should not be surprising that several enzymes of the ubiquitination cascades (i.e., E_2 and E_3) have been shown to be either associated with ERα or to be recruited together with ERα to its responsive promoters [12,46].

Three Ub conjugating enzymes (i.e., UBCH7, ubc4, ubc9) have a role in ERα signaling network. UBCH7 modulates steroid hormone receptor transcriptional activity and in particular upon E2 administration UBCH7 is recruited to ERE-containing gene promoters, thus contributing to the ERα-mediated activation of gene transcription [47]. A similar effect has been reported for ubc4, which is also recruited to ERα-responsive promoters but further promotes receptor degradation [48]. Remarkably, ubc9, which is the conjugating enzyme also for the small ubiquitin-like modifer-1 (SUMO-1), directly interacts with the ERα in an E2-dependent manner, enhances receptor transcriptional activity and contributes to the regulation of ERα proteasomal degradation [49,50].

Furthermore, numerous Ub E_3 ligases take part in the ERα-based signaling pathway. The Ub ligase complex containing the carboxyl terminus of Hsc70-interacting protein (CHIP) is responsible for polyubiquitination and proteasomal degradation of the apoERα [51]. Interestingly, E2 triggers the dissociation of CHIP from the ERα and addresses the receptor to the action of other ligases, which are required for the E2-dependent proteasomal degradation [42]. Besides CHIP, the human murine double minute 2 (hMdm2) influences ERα stability. Indeed, hMdm2 regulates breast cancer cell basal and E2-induced ERα degradation by mediating receptor polyubiquitination [52]. Furthermore, the physical interaction of hMdm2 with ERα is mediated by a bridge created by p53 and contributes to the enhancement of E2-induced ERα target gene transactivation [52]. The binding between ERα and the E_3 Ub ligase E6-associated protein (E6-AP), which is required for ERα proteolytic breakdown, has also been reported [53]. Moreover, E2 triggers the association of E6-AP with ERE-containing gene promotes and enhances gene transcription [13,53]. Similar functions have also been found for the estrogen-responsive finger protein (EFP) [54,55], an E_3 Ub ligase that can directly be induced by E2 [56]. Furthermore, ERα degradation can be triggered by receptor association with specific components of the cullin-based E_3 Ub ligase SCF complexes, which further play a role in the regulation of ERα transcriptional activity [57,58]. Remarkably, this picture is additionally complicated by the fact that some E_3 Ub ligases can even protect ERα from degradation. Indeed, siRNA experiments have revealed that the reduction in both Breast Cancer 1 (BRCA1) and in the WW domain containing the E_3 Ub protein ligase 1 (WWP1) results in a significant reduction in ERα intracellular levels [59,60], thus suggesting that ubiquitination has pleiotropic roles in ERα signaling.

This evidence clearly indicates that the 26S proteasome-based proteolytic pathway is intrinsically connected with ERα signaling and that receptor polyubiquitination is particularly required for receptor intra-nuclear dynamics as well as for the ERα nuclear functions and in turn for the E2:ERα-modulated physiological processes. As a consequence, targeting the Ub system in breast cancer is becoming an appealing pharmacological option especially in light of the fact that the expression of several E_3 Ub ligases often correlates with ERα expression [59,61–63]. Indeed, ERα-positive breast cancers are treated with drugs that destroy ERα thus reducing receptor tumor levels and tumor responsiveness to circulating E2 [64].

Finally it is interesting to note here that no information is available on the possible interplay between ERα polyubiquitination and E2:ERα-mediated extranuclear signaling.

4.1.1. The topology of the ERα polyubiquitin chain

Notwithstanding the huge amount of information that clearly indicates polyubiquitination of the ERα to be the signal for 26S proteasome-based receptor degradation, the direct evidence for the modification of the ERα with a chain of ubiquitins based on the Lys48 linkage has never been reported. Interestingly, the first evidence of an E2-induced ubiquitination of ER traces back more than 15 years ago when the Ub 26S proteasome system was starting to emerge as a mean to regulate protein turnover. At that time, immunoprecipitation of ER from cytoplasmic extracts of uterine cells revealed that E2 treatment induces a rapid (1–4 h) accumulation of high molecular species, which were detected by both ER and Ub antibody. Remarkably, modification of ER with Ub paralleled the decrease in total ER content, thus suggesting a Ub-dependent proteasomal degradation of ER induced by E2 stimulation [65].

However, it is interesting to note that these data still hold up as the unique clear evidence for a direct time-dependent polyubiquitin modification of ER by E2 *in vitro* and *in vivo*. Indeed, 'endogenous' ERα ubiquitination has been next studied by exploiting experimental settings in which either the ubiquitination machinery (i.e., Ub and ligases) and/or the substrate (i.e., ERα) were overrepresented with respect to the physiological conditions or the 26S proteasome was chemically inhibited (e.g., MG-132 cell treatment) [13,39,40,48,49,66–68]. However, given the complexity of the Ub-based system (see above), exogenous overexpression of those components in cells as well as the inhibition of the mechanism by which cells regulate the turnover of the intracellular proteins (i.e., 26S proteasome inhibition) could not accurately recapitulate endogenous protein ubiquitination [69]. Remarkably, this technical issue has been recently bypassed by the development of polyubiquitin linkage-specific antibodies, which are able to selectively recognize proteins polyubiquitinated through either Lys48- or Lys63-based polyUb chains [69].

Therefore, we recently used these two powerful reagents to analyze the topology of the polyUb-modification of the endogenous ERα in ductal carcinoma cells (MCF-7) [6]. In our experimental model (i.e., ductal carcinoma cells, MCF-7), immunofluorescence staining did not demonstrate any direct modification of the ERα with either a Lys48- or a Lys63-based polyubiquitin chain both under basal as well as under E2-treated conditions while the intracellular localization for the Lys48-based chains (i.e., nuclear) and for the Lys63-based chains (i.e., cytoplasmic) ([6]; unpublished results) corresponded to what originally reported [69]. However, co-localization of the ERα with Ub was observed when cells were stained with an anti-Ub antibody that recognizes all the Ub-based modifications [6]. On this basis, we concluded that polyubiquitination is not the major form of the Ub-based modification of the endogenous ERα (see below). These observations do not differ from the ones made by many other investigators who used 26S proteasome inhibitors to emphasize the basal and E2-induced ERα polyubiquitination [13,39,40,48,49,66–68] but rather indicate that only a small pool of the receptor could be polyubiquitinated with Lys48-based polyUb chains (or possibly Lys63-based polyUb chains) and suggest that the ERα proteolytic clearance is a very fast process. Accordingly, E2 reduces the ERα half-life from 24 h to 2 h [13], thus rapidly down-regulating receptor intracellular levels.

4.2. Non-degradative functions of the ubiquitin network in ERα signaling

The first evidence for a non-degradative Ub-based modification of the ERα came by the pioneering work from Klevit's group [3]. In an attempt to identify the ubiquitination substrates of the BRCA1/BARD1 complex, the authors noticed that BRCA1 interacts with and regulates ERα and progesterone receptor (PR) transcriptional activation and that among all the known BRCA1-interactors, only ERα and PR have expression profiles similar to BRCA1 mutation-associated breast tumors. Subsequent *in vitro* ubiquitination assays coupled with mass spectrometry analysis revealed that the Lys302 (and most likely also Lys303) of the ERα LBD is monoubiquitinated by the BRCA1/BARD1 complex and that BRCA1 breast cancer-predisposing mutations prevent ERα monoUbq [3]. Consistently with the *in vitro* data, ERα monoUbq was later confirmed to occur also in cell lines [4–7]. These discoveries not only suggested that the regulation of ERα activity by BRCA1, possibly through receptor monoUbq, could have significant implications in controlling E2-dependent breast cancer cell proliferation but also opened the possibility that ERα monoUbq may impact on ERα signaling.

As a consequence, subsequent work was aimed at understanding the impact of ERα monoUbq in ERα activities. However, the analysis of the ability of monoUbq in modulating ERα nuclear functions and receptor turnover produced contrasting results [4,5,68,70]. Indeed, the mutation in the ERα *in vitro* monoUbq sites (i.e., Lys302 and Lys303 residues) determines a mutant receptor that can have either an increased or a decreased transcriptional activity depending on the cell type used [4,68,71,72]. Furthermore, since in addition to monoUbq the BRCA1/BARD complex is able to direct the synthesis of Lys63- or Lys48-linked polyUb chains depending on the E_2 that interacts with BRCA1 [73], a role for ERα monoUbq in receptor turnover was also evaluated. Although the mutation of *in vitro* ERα monoUbq sites protects ERα from ligand-induced proteolytic degradation as much as the reduction in BRCA1 cellular levels does [5,68], siRNA-dependent reduction in BRCA1 expression has been also found to cause a concomitant reduction in ERα expression levels [70].

Remarkably, these contrasting results can be ascribed to the fact that most of the experiments were done under conditions in which either ERα or BRCA1 were exogenously overexpressed and/or the 26S proteasome was inhibited [4,5,68]. As a consequence the activity of the Ub-based signaling network could have been unbalanced because the experimental systems become saturated with the components required for the Ub-based reactions [6,7]. Accordingly, under conditions in which both ERα and BRCA1 are overexpressed, the Lys302,303Ala mutation strongly reduces ERα monoUbq [4] whereas under condition in which both ERα and Ub are overexpressed, the Lys302,303Ala mutation greatly enhances ERα ubiquitination [68].

For these reasons, we decided to minimize the manipulation of the Ub system by using an alternative approach. The use of the double ubiquitin binding domain (RUZ:MIU) of the Rabex5 [28] as a powerful Ub-binding reagent suited to this purpose and allowed us to demonstrate that ERα monoUbq is the main Ub-based modification of the endogenous ERα [6,7]. More importantly, monoUbq does not function as a proteolytic mark in ERα signaling since E2 rapidly reduces the amount of the pool of the monoubiquitinated ERα with a kinetic (30 min) faster than the one required for the E2-triggered ERα degradation (2 h) [6]. Considering that ERα monoUbq could play a role in ERα nuclear functions [4,68], and that the rapid modulation of this ERα PTM suggested a role for monoUbq in ERα rapid extranuclear signaling, the analysis of the impact of monoUbq in E2:ERα signaling was conducted in stable clones expressing either the wild type ERα or the receptor mutated in the monoUbq sites [6,7]. Remarkably, we observed that monoUbq is required for the E2-induced association of ERα to the insulin like growth factor receptor (IGF-1-R) and for the activation of the E2-induced ERα extranuclear signaling (i.e., AKT activation) that controls cyclin D1 transcription, G1-to-S phase transition, cell cycle progression and cell proliferation. Moreover, lack of ERα monoUbq also prevents the E2-dependent ERα Ser-118 phosphorylation and consequently ERα transcriptional activity [6,7]. In turn, endogenous ERα monoUbq is critical for the E2-dependent nuclear and extranuclear ERα activities. In particular, monoUbq appears to be a limiting signal for the activation of the ERα-mediated E2 effects. Indeed, on E2 binding, ERα monoUbq is removed and as a consequence, the extent of the E2-activated ERα extranuclear signaling is limited. In parallel, since the monoubiquitinated ERα displays a particulate nuclear localization, which may correspond to the sites where the receptor is transcriptionally active, the E2-dependent reduction in ERα monoUbq would address the receptor to the sites where transcription needs to efficiently take place and at the same time limit the ERα transcriptional activity, thus synchronizing the nuclear ERα effects [6,7].

This evidence, together with the discovery that BRCA1 breast cancer-predisposing mutations determines a non-monoubiquitinated ERα [3], strongly indicates that monoubiquitination must be regarded as a new signaling modifier for the E2:ERα-based signal transduction pathway required to elicit the regulation of cell proliferation. Therefore targeting ERα monoUbq could represent a new potential pharmacological option for the treatment of E2-related cancers.

5. The ERα and the ubiquitin-like modifiers (Ubls) network

In addition to ubiquitin, other ubiquitin-related proteins intersect the ERα-based signaling network. These proteins, which are known as ubiquitin-like modifiers (Ubls) (e.g., SUMO, Nedd8, ISG15), possess a biochemistry that is very similar to Ub although some differences exist [74,75]. Indeed, Ubls all have essentially the same Ub three-dimensional structure, are attached to substrates *via* enzymatic pathways similar to the ubiquitination cascade that catalyze the formation of an isopeptide bond between the Ubl terminal glycine and the target Lys of the substrate. Interestingly, there are also many ubiquitin-related proteins in which the ubiquitin-like domain (ULD) is part of a larger polypeptide but, usually, is neither processed nor covalently attached to other proteins. Such ULDs confer properties on a protein that are similar to those from a transferable UBL, including the ability to bind to specific target proteins. Like Ub, Ubls function as modifiers of different signal transduction pathways by targeting many different substrate proteins and thus they play critical roles in the regulation of many cellular processes, including transcription, DNA repair, and cell-cycle control (for more detailed reviews, see also: [74,75]).

The ERα has been found to be modified with SUMO-1 on the Lys266, Lys268, Lys299, Lys302, and Lys303 residues by the SUMO-1 E_3 ligases PIAS1 and PIAS3 (protein inhibitor of activated signal transducer and activator of transcription). Interestingly, this receptor modification is E2-dependent, occurs on overexpressed and endogenous ERα and is important for the modulation of the ERα-based nuclear activity [76,77]. Remarkably, the role of SUMOylation in mediating the proteolysis of the SUMO-modified protein is not clear [78,79]. However, BRCA1 can be SUMOylated by ubc9 and BRCA1 SUMOylation may play a role in E2-induced ERα degradation [80]. Nonetheless, if modification of the ERα with SUMO targets the receptor for 26S proteasome-mediated degradation has not been clarified. As in the case of other proteins [79], ERα SUMOylated Lys residues act as acceptors not only for SUMO-1 but also for Ub and for methyl and acetyl groups [81]. It is therefore possible that a

cross-talk among the different PTMs, acting sequentially and/or in concert, as a mechanism to regulate ERα functions could occur but this still remains to be elucidated [81]. Alternatively, different ERα pools of differentially posttranslationally modified receptors may exist and differentially modulate ERα activities.

Other Ubls [74] i.e., the neural precursor cell expressed developmentally down-regulated (NEDD8) [82,83], the interferon-stimulated gene product of 15 kDa (ISG15) [84] and the spliceosome component splicing factor 3a p120 (SF3aP120) [85] are not covalently attached to ERα but have been found to meet the ERα-based signaling network. In this respect, the NEDD8 pathway appears to work in concert with the ubiquitin proteasome system to modulate ERα turnover and cellular responses to E2. Indeed, the ubiquitin-activating enzyme (Uba)3, the catalytic subunit of the NEDD8-activating enzyme, binds to ERα and inhibits its transcriptional activity through a process involving receptor polyubiquitination and degradation by the 26S proteasome [82,83]. Furthermore, the E_3 Ub ligase EFP, which is inducible upon E2 administration and regulates some ERα activities [54,55], can work also as an ISG15 E_3 ligase [84] and the SF3aP120 is a ERα co-activator that physically associates with Ser-118 phosphorylated receptor and enhances ERα -mediated RNA splicing [85].

Finally, it is important to note here that the possible influence of Ubls in the regulation of the ERα extranuclear functions has not been evaluated. Moreover, whether or not ERβ, which is not ubiquitinated [6,7], may be modified with other Ubls is completely unknown.

6. Conclusions and perspectives

The estrogen receptors (ERα and ERβ) are devoid of any enzymatic activity but are able to mediate the pleiotropic effects of the sex steroid hormone E2 by activating a complex network of intracellular mechanisms. Interestingly, it is becoming increasingly clear that the PTM pattern of the estrogen receptors as well as its ligand-dependent modulation is critical for the regulation of ER activities.

Indeed, many PTMs occur on the ERα: this hormone receptor is either phosphorylated, basically on all serine, threonine (Thr) and tyrosine (Tyr) residues, palmitoylated, acetylated, methylated, myristoylated, nitrosylated, or glycosylated [8,86]. Moreover, the amount of the posttranslationally-modified receptors can be hormonally modified: as examples, E2 changes both the phosphorylation and the palmitoylation status of the ERα [8,87,88], thus controlling ERα activation. ERα ubiquitination also follows this scheme with E2 inducing on one hand receptor polyubiquitination and 26S proteasome-dependent degradation and on the other hand reducing ERα monoubiquitination and the extent of the ERα-based signaling. Finally, although less is known for the ERβ, this other ER isoform also undergoes different PTMs (e.g., phosphorylation, palmitoylation) [89,90]. However, ERβ has never been found to be ubiquitinated [7,10], thus suggesting that the difference in ERα/ERβ ubiquitination pattern may further recapitulate the differences in ERα/ERβ signaling and functions.

In conclusion, a 'code' of PTMs on ER exists [81]. The challenge is now to understand how the receptor PTMs cross-talk to finely tune nuclear and extranuclear ER activities which are required for the E2 pleiotropic functions.

Acknowledgments

This study was supported by grants from Ateneo Roma Tre to FA. The authors wish to thank Prof Maria Marino who heavily contributed to the ideas presented through critically discussions of the data reviewed here and Dr Viviana Trezza for helpful discussion and manuscript editing.

References

[1] T. Hunter, The age of crosstalk: phosphorylation, ubiquitination, and beyond, Mol. Cell 28 (2007) 730–738.
[2] T. Woelk, S. Sigismund, L. Penengo, S. Polo, The ubiquitination code: a signalling problem, Cell Div. 2 (2007).
[3] C.M. Eakin, M.J. MacCoss, G.L. Finney, R.E. Klevit, Estrogen receptor alpha is a putative substrate for the BRCA1 ubiquitin ligase, Proc. Natl. Acad. Sci. U.S.A. 104 (2007) 5794–5799.
[4] Y.X. Ma, S.J. Fan, C.Y. Hu, Q.H. Meng, S.A. Fuqua, R.G. Pestell, Y.A. Tomita, E.M. Rosen, BRCA1 regulates acetylation and ubiquitination of estrogen receptor-alpha, Mol. Endocrinol. 24 (2010) 76–90.
[5] E. Dizin, I. Irminger-Finger, Negative feedback loop of BRCA1-BARD1 ubiquitin ligase on estrogen receptor alpha stability and activity antagonized by cancer-associated isoform of BARD1, Int. J. Biochem. Cell Biol. 42 (2010) 693–700.
[6] P. La Rosa, M. Marino, F. Acconcia, 17beta-estradiol regulates estrogen receptor alpha monoubiquitination, IUBMB Life 63 (2011) 49–53.
[7] P. La Rosa, V. Pesiri, M. Marino, F. Acconcia, 17beta-estradiol-induced cell proliferation requires estrogen receptor (ER) alpha monoubiquitination, Cell. Signal. 23 (2011) 1128–1135.
[8] P. Ascenzi, A. Bocedi, M. Marino, Structure–function relationship of estrogen receptor alpha and beta: impact on human health, Mol. Aspects Med. 27 (2006) 299–402.
[9] J.A. Gustafsson, Estrogen receptor beta - a new dimension in estrogen mechanism of action, J. Endocrinol. 163 (1999) 379–383.
[10] P. Galluzzo, F. Caiazza, S. Moreno, M. Marino, Role of ERbeta palmitoylation in the inhibition of human colon cancer cell proliferation, Endocr. Relat. Cancer 14 (2007) 153–167.
[11] F. Acconcia, R. Kumar, Signaling regulation of genomic and nongenomic functions of estrogen receptors, Cancer Lett. 238 (2006) 1–14.
[12] R. Metivier, G. Penot, M.R. Hubner, G. Reid, H. Brand, M. Kos, F. Gannon, Estrogen receptor-alpha directs ordered, cyclical, and combinatorial recruitment of cofactors on a natural target promoter, Cell 115 (2003) 751–763.
[13] G. Reid, M.R. Hubner, R. Metivier, H. Brand, S. Denger, D. Manu, J. Beaudouin, J. Ellenberg, F. Gannon, Cyclic, proteasome-mediated turnover of unliganded and liganded ERalpha on responsive promoters is an integral feature of estrogen signaling, Mol. Cell 11 (2003) 695–707.
[14] S.R. Hammes, E.R. Levin, Extranuclear steroid receptors: nature and actions, Endocr. Rev. 28 (2007) 726–741.
[15] K.L. Chambliss, Q. Wu, S. Oltmann, E.S. Konaniah, M. Umetani, K.S. Korach, G.D. Thomas, C. Mineo, I.S. Yuhanna, S.H. Kim, Z. Madak-Erdogan, A. Maggi, S.P. Dineen, C.L. Roland, D.Y. Hui, R.A. Brekken, J.A. Katzenellenbogen, B.S. Katzenellenbogen, P.W. Shaul, Non-nuclear estrogen receptor alpha signaling promotes cardiovascular protection but not uterine or breast cancer growth in mice, J. Clin. Invest. 120 (2010) 2319–2330.
[16] A. Hershko, H. Heller, S. Elias, A. Ciechanover, Components of ubiquitin-protein ligase system. Resolution, affinity purification, and role in protein breakdown, J. Biol. Chem. 258 (1983) 8206–8214.
[17] S. Sigismund, S. Polo, P.P. Di Fiore, Signaling through monoubiquitination, Curr. Top. Microbiol. Immunol. (2004) 149–185.
[18] L. Hicke, Protein regulation by monoubiquitin, Nat. Rev. Mol. Cell. Biol. 2 (2001) 195–201.
[19] J. Peng, D. Schwartz, J.E. Elias, C.C. Thoreen, D. Cheng, G. Marsischky, J. Roelofs, D. Finley, S.P. Gygi, A proteomics approach to understanding protein ubiquitination, Nat. Biotechnol. 21 (2003) 921–926.
[20] P. Xu, J. Peng, Dissecting the ubiquitin pathway by mass spectrometry, Biochim. Biophys. Acta 1764 (2006) 1940–1947.
[21] M.J. Eddins, R. Varadan, D. Fushman, C.M. Pickart, C. Wolberger, Crystal structure and solution NMR studies of Lys48-linked tetraubiquitin at neutral pH, J. Mol. Biol. 367 (2007) 204–211.
[22] T. Tenno, K. Fujiwara, H. Tochio, K. Iwai, E.H. Morita, H. Hayashi, S. Murata, H. Hiroaki, M. Sato, K. Tanaka, M. Shirakawa, Structural basis for distinct roles of Lys63- and Lys48-linked polyubiquitin chains, Genes Cells 9 (2004) 865–875.
[23] K.D. Wilkinson, Ubiquitination and deubiquitination: targeting of proteins for degradation by the proteasome, Semin. Cell. Dev. Biol. 11 (2000) 141–148.
[24] F. Acconcia, S. Sigismund, S. Polo, Ubiquitin in trafficking: the network at work, Exp. Cell Res. 315 (2009) 1610–1618.
[25] L. Hicke, H.L. Schubert, C.P. Hill, Ubiquitin-binding domains, Nat. Rev. Mol. Cell. Biol. 6 (2005) 610–621.
[26] S. Raasi, I. Orlov, K.G. Fleming, C.M. Pickart, Binding of polyubiquitin chains to ubiquitin-associated (UBA) domains of HHR23A, J. Mol. Biol. 341 (2004) 1367–1379.
[27] J.H. Hurley, S. Lee, G. Prag, Ubiquitin-binding domains, Biochem. J. 399 (2006) 361–372.
[28] L. Penengo, M. Mapelli, A.G. Murachelli, S. Confalonieri, L. Magri, A. Musacchio, P.P. Di Fiore, S. Polo, T.R. Schneider, Crystal structure of the ubiquitin binding domains of rabex-5 reveals two modes of interaction with ubiquitin, Cell 124 (2006) 1183–1195.
[29] T. Woelk, B. Oldrini, E. Maspero, S. Confalonieri, E. Cavallaro, P.P. Di Fiore, S. Polo, Molecular mechanisms of coupled monoubiquitination, Nat. Cell. Biol. 8 (2006) 1246–1254.

[30] M.H. Glickman, A. Ciechanover, The ubiquitin-proteasome proteolytic pathway: destruction for the sake of construction, Physiol. Rev. 82 (2002) 373–428.
[31] C. Le Roy, J.L. Wrana, Clathrin- and non-clathrin-mediated endocytic regulation of cell signalling, Nat. Rev. Mol. Cell. Biol. 6 (2005) 112–126.
[32] K. Haglund, S. Sigismund, S. Polo, I. Szymkiewicz, P.P. Di Fiore, I. Dikic, Multiple monoubiquitination of RTKs is sufficient for their endocytosis and degradation, Nat. Cell Biol. 5 (2003) 461–466.
[33] F. Huang, D. Kirkpatrick, X. Jiang, S. Gygi, A. Sorkin, Differential regulation of EGF receptor internalization and degradation by multiubiquitination within the kinase domain, Mol. Cell 21 (2006) 737–748.
[34] E. Argenzio, T. Bange, B. Oldrini, F. Bianchi, R. Peesari, S. Mari, P.P. Di Fiore, M. Mann, S. Polo, Proteomic snapshot of the EGF-induced ubiquitin network, Mol. Syst. Biol. 7 (2011) 462.
[35] S. Sigismund, T. Woelk, C. Puri, E. Maspero, C. Tacchetti, P. Transidico, P.P. Di Fiore, S. Polo, Clathrin-independent endocytosis of ubiquitinated cargos, Proc. Natl. Acad. Sci. U.S.A. 102 (2005) 2760–2765.
[36] S. Sigismund, E. Argenzio, D. Tosoni, E. Cavallaro, S. Polo, P.P. Di Fiore, Clathrin-mediated internalization is essential for sustained EGFR signaling but dispensable for degradation, Dev. Cell 15 (2008) 209–219.
[37] J. Yan, Y.S. Kim, X.P. Yang, M. Albers, M. Koegl, A.M. Jetten, Ubiquitin-interaction motifs of RAP80 are critical in its regulation of estrogen receptor alpha, Nucleic Acids Res. 35 (2007) 1673–1686.
[38] X. Pan, T. Zhou, Y.H. Tai, C. Wang, J. Zhao, Y. Cao, Y. Chen, P.J. Zhang, M. Yu, C. Zhen, R. Mu, Z.F. Bai, H.Y. Li, A.L. Li, B. Liang, Z. Jian, W.N. Zhang, J.H. Man, Y.F. Gao, W.L. Gong, L.X. Wei, X.M. Zhang, Elevated expression of CUEDC2 protein confers endocrine resistance in breast cancer, Nat. Med. 17 (2011) 708–714.
[39] E.T. Alarid, N. Bakopoulos, N. Solodin, Proteasome-mediated proteolysis of estrogen receptor: a novel component in autologous down-regulation, Mol. Endocrinol. 13 (1999) 1522–1534.
[40] M.T. Preisler-Mashek, N. Solodin, B.L. Stark, M.K. Tyriver, E.T. Alarid, Ligand-specific regulation of proteasome-mediated proteolysis of estrogen receptor-alpha, Am. J. Physiol. Endocrinol. Metab. 282 (2002) E891–E898.
[41] C.C. Valley, R. Metivier, N.M. Solodin, A.M. Fowler, M.T. Mashek, L. Hill, E.T. Alarid, Differential regulation of estrogen-inducible proteolysis and transcription by the estrogen receptor alpha N terminus, Mol. Cell. Biol. 25 (2005) 5417–5428.
[42] Y. Tateishi, Y. Kawabe, T. Chiba, S. Murata, K. Ichikawa, A. Murayama, K. Tanaka, T. Baba, S. Kato, J. Yanagisawa, Ligand-dependent switching of ubiquitin-proteasome pathways for estrogen receptor, EMBO J. 23 (2004) 4813–4823.
[43] M. Fan, H. Nakshatri, K.P. Nephew, Inhibiting proteasomal proteolysis sustains estrogen receptor-alpha activation, Mol. Endocrinol. 18 (2004) 2603–2615.
[44] G.E. Weitsman, L. Li, G.P. Skliris, J.R. Davie, K. Ung, Y. Niu, L. Curtis-Snell, L. Tomes, P.H. Watson, L.C. Murphy, Estrogen receptor-alpha phosphorylated at Ser118 is present at the promoters of estrogen-regulated genes and is not altered due to HER-2 overexpression, Cancer Res. 66 (2006) 10162–10170.
[45] M. Dutertre, C.L. Smith, Ligand-independent interactions of p160/steroid receptor coactivators and CREB-binding protein (CBP) with estrogen receptor-alpha: regulation by phosphorylation sites in the A/B region depends on other receptor domains, Mol. Endocrinol. 17 (2003) 1296–1314.
[46] T. Ohta, M. Fukuda, Ubiquitin and breast cancer, Oncogene 23 (2004) 2079–2088.
[47] S. Verma, A. Ismail, X. Gao, G. Fu, X. Li, B.W. O'Malley, Z. Nawaz, The ubiquitin-conjugating enzyme UBCH7 acts as a coactivator for steroid hormone receptors, Mol. Cell. Biol. 24 (2004) 8716–8726.
[48] M. Luo, M. Koh, J. Feng, Q. Wu, P. Melamed, Cross talk in hormonally regulated gene transcription through induction of estrogen receptor ubiquitylation, Mol. Cell. Biol. 25 (2005) 7386–7398.
[49] Z. Nawaz, D.M. Lonard, A.P. Dennis, C.L. Smith, B.W. O'Malley, Proteasome-dependent degradation of the human estrogen receptor, Proc. Natl. Acad. Sci. U.S.A. 96 (1999) 1858–1862.
[50] S. Kobayashi, H. Shibata, K. Yokota, N. Suda, A. Murai, I. Kurihara, I. Saito, T. Saruta, FHL2, UBC9, and PIAS1 are novel estrogen receptor alpha-interacting proteins, Endocr. Res. 30 (2004) 617–621.
[51] M. Fan, A. Park, K.P. Nephew, CHIP (carboxyl terminus of Hsc70-interacting protein) promotes basal and geldanamycin-induced degradation of estrogen receptor-alpha, Mol. Endocrinol. 19 (2005) 2901–2914.
[52] V. Duong, N. Boulle, S. Daujat, J. Chauvet, S. Bonnet, H. Neel, V. Cavailles, Differential regulation of estrogen receptor alpha turnover and transactivation by Mdm2 and stress-inducing agents, Cancer Res. 67 (2007) 5513–5521.
[53] L. Li, Z. Li, P.M. Howley, D.B. Sacks, E6AP and calmodulin reciprocally regulate estrogen receptor stability, J. Biol. Chem. 281 (2006) 1978–1985.
[54] A. Nakajima, S. Maruyama, M. Bohgaki, N. Miyajima, T. Tsukiyama, N. Sakuragi, S. Hatakeyama, Ligand-dependent transcription of estrogen receptor alpha is mediated by the ubiquitin ligase EFP, Biochem. Biophys. Res. Commun. 357 (2007) 245–251.
[55] K.W. Zhao, D. Sikriwal, X. Dong, P. Guo, X. Sun, J.T. Dong, Estrogen causes degradation of KLF5 by inducing the E3 ubiquitin ligase EFP in ER-positive breast cancer cells, Biochem. J. (2011).
[56] S. Inoue, A. Orimo, T. Hosoi, S. Kondo, H. Toyoshima, T. Kondo, A. Ikegami, Y. Ouchi, H. Orimo, M. Muramatsu, Genomic binding-site cloning reveals an estrogen-responsive gene that encodes a RING finger protein, Proc. Natl. Acad. Sci. U.S.A. 90 (1993) 11117–11121.
[57] B. Byun, Y. Jung, Repression of transcriptional activity of estrogen receptor alpha by a Cullin3/SPOP ubiquitin E3 ligase complex, Mol. Cells 25 (2008) 289–293.
[58] K. Yoshida, Characterization of estrogen-induced F-box protein FBXO45, Oncol. Rep. 14 (2005) 531–535.
[59] C. Chen, Z. Zhou, C.E. Sheehan, E. Slodkowska, C.B. Sheehan, A. Boguniewicz, J.S. Ross, Overexpression of WWP1 is associated with the estrogen receptor and insulin-like growth factor receptor 1 in breast carcinoma, Int. J. Cancer 124 (2009) 2829–2836.
[60] J.J. Gorski, R.D. Kennedy, A.M. Hosey, D.P. Harkin, The complex relationship between BRCA1 and ERalpha in hereditary breast cancer, Clin. Cancer Res. 15 (2009) 1514–1518.
[61] K. Sato, E. Rajendra, T. Ohta, The UPS: a promising target for breast cancer treatment, BMC Biochem. 9 (Suppl. 1) (2008) S2.
[62] D. Hoeller, I. Dikic, Targeting the ubiquitin system in cancer therapy, Nature 458 (2009) 438–444.
[63] A.M. Burger, F. Kona, Y. Amemiya, Y. Gao, S. Bacopulos, A.K. Seth, Role of the BCA2 ubiquitin E3 ligase in hormone responsive breast cancer, Open Cancer J. 3 (2010) 116–123.
[64] R. Kumar, Another tie that binds the MTA family to breast cancer, Cell 113 (2003) 142–143.
[65] P.B. Nirmala, R.V. Thampan, Ubiquitination of the rat uterine estrogen receptor: dependence on estradiol, Biochem. Biophys. Res. Commun. 213 (1995) 24–31.
[66] D.M. Lonard, Z. Nawaz, C.L. Smith, B.W. O'Malley, The 26S proteasome is required for estrogen receptor-alpha and coactivator turnover and for efficient estrogen receptor-alpha transactivation, Mol. Cell 5 (2000) 939–948.
[67] A.L. Wijayaratne, D.P. McDonnell, The human estrogen receptor-alpha is a ubiquitinated protein whose stability is affected differentially by agonists, antagonists, and selective estrogen receptor modulators, J. Biol. Chem. 276 (2001) 35684–35692.
[68] N.B Berry, M. Fan, K.P. Nephew, Estrogen receptor-alpha hinge-region lysines 302 and 303 regulate receptor degradation by the proteasome, Mol. Endocrinol. 22 (2008) 1535–1551.
[69] K. Newton, M.L. Matsumoto, I.E. Wertz, D.S. Kirkpatrick, J.R. Lill, J. Tan, D. Dugger, N. Gordon, S.S. Sidhu, F.A. Fellouse, L. Komuves, D.M. French, R.E. Ferrando, C. Lam, D. Compaan, C. Yu, I. Bosanac, S.G. Hymowitz, R.F. Kelley, V.M. Dixit, Ubiquitin chain editing revealed by polyubiquitin linkage-specific antibodies, Cell 134 (2008) 668–678.
[70] A.M. Hosey, J.J. Gorski, M.M. Murray, J.E. Quinn, W.Y. Chung, G.E. Stewart, C.R. James, S.M. Farragher, J.M. Mulligan, A.N. Scott, P.A. Dervan, P.G. Johnston, F.J. Couch, P.A. Daly, E. Kay, A. McCann, P.B. Mullan, D.P. Harkin, Molecular basis for estrogen receptor alpha deficiency in BRCA1-linked breast cancer, J. Natl. Cancer Inst. 99 (2007) 1683–1694.
[71] M.H. Herynk, T. Hopp, Y.K. Cui, A.R. Niu, A. Corona-Rodriguez, S.A.W. Fuqua, A hypersensitive estrogen receptor alpha mutation that alters dynamic protein interactions, Breast Cancer Res. Treat. 122 (2009) 381–393.
[72] Y. Cui, M. Zhang, R. Pestell, E.M. Curran, W.V. Welshons, S.A. Fuqua, Phosphorylation of estrogen receptor alpha blocks its acetylation and regulates estrogen sensitivity, Cancer Res. 64 (2004) 9199–9208.
[73] D.E. Christensen, P.S. Brzovic, R.E. Klevit, E2-BRCA1 RING interactions dictate synthesis of mono- or specific polyubiquitin chain linkages, Nat. Struct. Mol. Biol. 14 (2007) 941–948.
[74] O. Kerscher, R. Felberbaum, M. Hochstrasser, Modification of proteins by ubiquitin and ubiquitin-like proteins, Annu. Rev. Cell. Dev. Biol. 22 (2006) 159–180.
[75] M. Hochstrasser, Origin and function of ubiquitin-like proteins, Nature 458 (2009) 422–429.
[76] S. Sentis, M. Le Romancer, C. Bianchin, M.C. Rostan, L. Corbo, Sumoylation of the estrogen receptor alpha hinge region regulates its transcriptional activity, Mol. Endocrinol. 19 (2005) 2671–2684.
[77] H. Zhu, J.N. Zhou, SUMO1 enhances 17-beta estradiol's effect on CRH promoter activation through estrogen receptors, Neuro Endocrinol. Lett. 29 (2008) 230–234.
[78] L. Shimshon, A. Michaeli, R. Hadar, S.L. Nutt, Y. David, A. Navon, A. Waisman, B. Tirosh, SUMOylation of Blimp-1 promotes its proteasomal degradation, FEBS Lett. (2011).
[79] R.T. Hay, SUMO: a history of modification, Mol. Cell 18 (2005) 1–12.
[80] J. Xu, T. Watkins, A. Reddy, E.S. Reddy, V.N. Rao, A novel mechanism whereby BRCA1/1a/1b fine tunes the dynamic complex interplay between SUMO-dependent/independent activities of Ubc9 on E2-induced ERalpha activation/repression and degradation in breast cancer cells, Int. J. Oncol. 34 (2009) 939–949.
[81] M. Le Romancer, C. Poulard, P. Cohen, S. Sentis, J.M. Renoir, L. Corbo, Cracking the estrogen receptor's posttranslational code in breast tumors, Endocr. Rev. (2011).
[82] M. Fan, R.M. Bigsby, K.P. Nephew, The NEDD8 pathway is required for proteasome-mediated degradation of human estrogen receptor (ER)-alpha and essential for the antiproliferative activity of ICI 182, 780 in ERalpha-positive breast cancer cells, Mol. Endocrinol. 17 (2003) 356–365.
[83] M. Fan, X. Long, J.A. Bailey, C.A. Reed, E. Osborne, E.A. Gize, E.A. Kirk, R.M. Bigsby, K.P. Nephew, The activating enzyme of NEDD8 inhibits steroid receptor function, Mol. Endocrinol. 16 (2002) 315–330.
[84] W. Zou, D.E. Zhang, The interferon-inducible ubiquitin-protein isopeptide ligase (E3) EFP also functions as an ISG15 E3 ligase, J. Biol. Chem. 281 (2006) 3989–3994.
[85] Y. Masuhiro, Y. Mezaki, M. Sakari, K. Takeyama, T. Yoshida, K. Inoue, J. Yanagisawa, S. Hanazawa, W.B. O'Malley, S. Kato, Splicing potentiation by growth factor signals via estrogen receptor phosphorylation, Proc. Natl. Acad. Sci. U.S.A. 102 (2005) 8126–8131.

[86] C. Atsriku, D.J. Britton, J.M. Held, B. Schilling, G.K. Scott, B.W. Gibson, C.C. Benz, M.A. Baldwin, Systematic mapping of posttranslational modifications in human estrogen receptor-alpha with emphasis on novel phosphorylation sites, Mol. Cell. Proteomics 8 (2009) 467–480.
[87] F. Acconcia, P. Ascenzi, A. Bocedi, E. Spisni, V. Tomasi, A. Trentalance, P. Visca, M. Marino, Palmitoylation-dependent estrogen receptor alpha membrane localization: regulation by 17 beta-estradiol, Mol. Biol. Cell 16 (2005) 231–237.
[88] S. Ali, D. Metzger, J.M. Bornert, P. Chambon, Modulation of transcriptional activation by ligand-dependent phosphorylation of the human oestrogen receptor A/B region, EMBO J. 12 (1993) 1153–1160.
[89] F. Caiazza, P. Galluzzo, S. Lorenzetti, M. Marino, 17beta-estradiol induces ERbeta up-regulation via p38/MAPK activation in colon cancer cells, Biochem. Biophys. Res. Commun. 359 (2007) 102–107.
[90] M. Sanchez, N. Picard, K. Sauve, A. Tremblay, Challenging estrogen receptor beta with phosphorylation, Trends Endocrinol. Metab. 21 (2011) 104–110.