CMLS, Cell. Mol. Life Sci. 59 (2002) 821–831
1420-682X/02/050821-11 $ 1.50 + 0.20/0
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CMLS Cellular and Molecular Life Sciences

Review

Human estrogen receptor-α: regulation by synthesis, modification and degradation

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Received 12 February 2002; accepted 14 March 2002

Abstract. This review aims to evaluate the impact that human estrogen receptor-α (ER-α) synthesis, modification and degradation has on estrogen-dependant physiological and pathological processes within the body. Estrogen signaling is transduced through estrogen receptors, which act as ligand-inducible transcription factors. The significance of different isoforms of ER-α that lack structural features of full-length ER-α are discussed. The influence of differential promoter usage on the amount and isoform of ER-α within individual cell types is also reviewed. Moreover, the potential role of phosphorylation, ubiquitination and acetylation in the function and dynamic turnover of ER-α is presented.

Key words. Estrogen receptor-α; regulation; splicing; isoform; tissue specificity; proteasome, post-translational modification; ubiquitination; phosphorylation; acetylation.

Introduction

The nuclear receptor superfamily includes more than 150 members that are key regulatory modulators of a diverse range of metabolic and physiological processes [reviewed in ref.1]. The estrogen receptor (ER), within the steroid receptor family, is widely distributed in evolutionary terms from chordates through to mammals [2, 3]. ERs mediate the developmental and physiological responses to the steroid hormone estrogen. Although estrogens are commonly recognized as pivotal in female reproductive physiology [4], they are also involved in male reproductive development and physiology [5], in bone [6], in lipid metabolism and in the maintenance of the cardiovascular [7] and neuronal systems [8]. ERs are known to participate in the pathology of several diseases. Estrogens have a proliferative effect in the endometrium and in the breast [9] and may also influence hyperplasia in the prostate [10]. Consequently, ERs have been found to be essential in the initiation and development of neoplasia in at least breast and endometrial cancer. Moreover, osteoporosis, atherosclerosis and Alzheimer's disease increase in incidence and severity with increasing age and are associated with the reduction of estrogen levels that occur in both males and females as they become older [11–13].

ERs are intracellular transcription factors that, on association with ligand, bind to estrogen-responsive target genes to modulate their expression. To date, two estrogen receptors, ER-α [14] (NR3A1 [15]) and ER-β (NR3A2 [16]), are known in humans. Separate genes located on different chromosomes encode each of these ERs. ER-α lies on chromosome 6 at the 6q25.1 locus, whereas ER-β is found on chromosome 14 at the boundary between 14q11.1 and 14q11.2. ER-β has been extensively reviewed elsewhere [17]. Unliganded ER-α exists in a monomeric form complexed with heat shock proteins (HSPs), principally HSP70 and HSP90, and is distributed between the nucleus and cytoplasm. On binding ligand,

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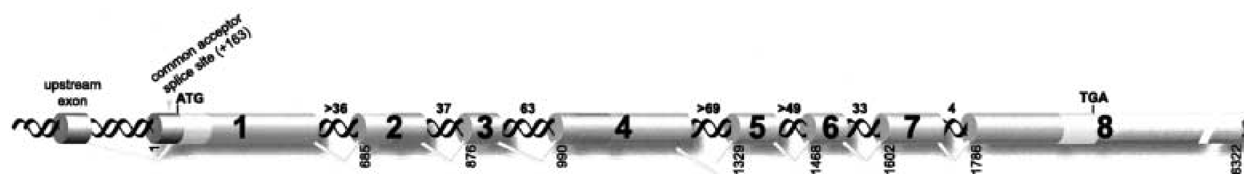
ER-α undergoes a major conformational change, dissociates from HSPs, dimerizes and locates to the nucleus [18]. ER-α is a modular protein consisting of an N-terminal domain that contains activating function 1, (AF-1), a zinc finger DNA-binding domain, a hinge region and a C-terminal ligand-binding domain (LBD) that also harbors the ligand-dependant activating function 2 (AF-2) region (fig. 1) [19]. On binding estradiol (E2), ER-α undergoes a major conformational change that effectively completes the folding pathway of this molecule [20]. Several studies have elucidated that estrogen agonists and antagonists induce distinct conformations in the structure of the LBD [21]. Ligand-activated ER-α interacts with co-activator complexes that in turn recruit the transcription machinery. Some co-activators that interact with liganded ER-α possess histone acetylase activity and act to modify local chromatin structure [22]. Consequently, the rate of target gene transcription is increased. AF-1 may also be autonomous in that it is able in certain circumstances to recruit co-activators to ER-α independently of association with ligand. However, in the context of the full-length receptor, AF-1 activity is also regulated by ligand-bound LBD and by the requirement for the receptor to be associated with an estrogen response element (ERE) in the target gene [23]. In addition to a direct ER-α/ERE interaction, ER-α can also transactivate gene transcription indirectly by protein/protein interactions. For example, ERs also modify transcription by influencing the activity of the AP-1 protein complex when associated with its cognate DNA response element [24]. Estrogen modulation of AP-1 activity requires both ER-α AF-1 and AF-2 transactivation surfaces, the same protein-protein contacts being used to recruit co-factors and the transcription machinery at EREs and at AP-1 sites (fig. 2).

Estrogens can also induce a rapid and transient activation of several signal transduction pathways. These effects, generally referred to as 'non-genomic,' appear not to involve direct ER-mediated gene transcription but involve cross-talk between signaling pathways [25, 26].

Genomic organization of human ER-α

The generation of human ER-α transcripts has recently been revealed to be complex, involving at least seven promoters and eight coding exons encompassing in total some 450 kb of chromosome 6 [27] (fig. 3). In general, alternative promoter usage results in mRNA variants that only differ in their 5′ untranslated region (UTR). This occurs as the first exon arising from each alternative pro-



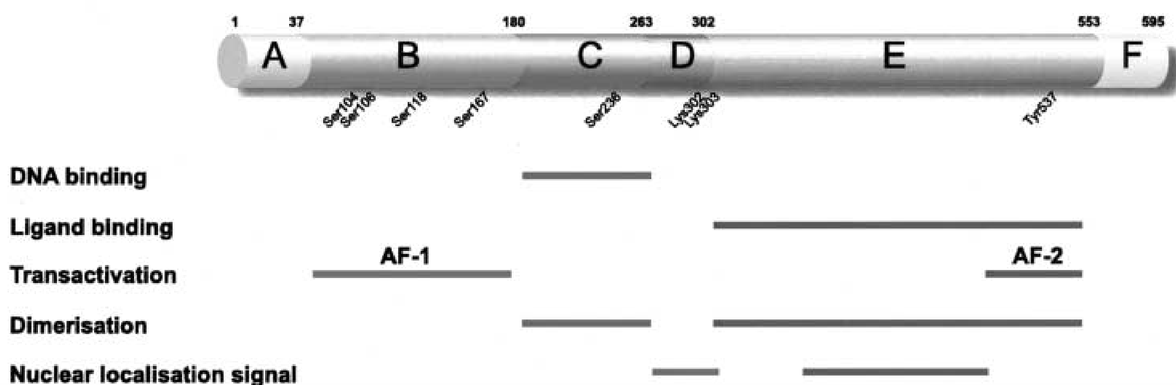


Figure 1. Genomic organization and domains of the human ER-α gene. The ER-α protein is derived from eight coding exons that encode six functional domains (A–F). Two major transactivation functions (AF), AF-1 (B domain) and AF-2 (E domain) are located in human ER-α. These generate surfaces that bind to co-factors, which in turn recruit the transcription machinery and act to modify the local chromatin structure of the target gene. The C domain contains two zinc finger motifs that bind to DNA, while the D domain is a hinge region between the N-terminal half of ER-α and the C-terminal ligand-binding domain (LBD) that is encoded by the E domain. Nuclear localization signals are found within the hinge region (D) and the LBD (E). Also shown are significant phosphorylation and acetylation sites within ER-α.

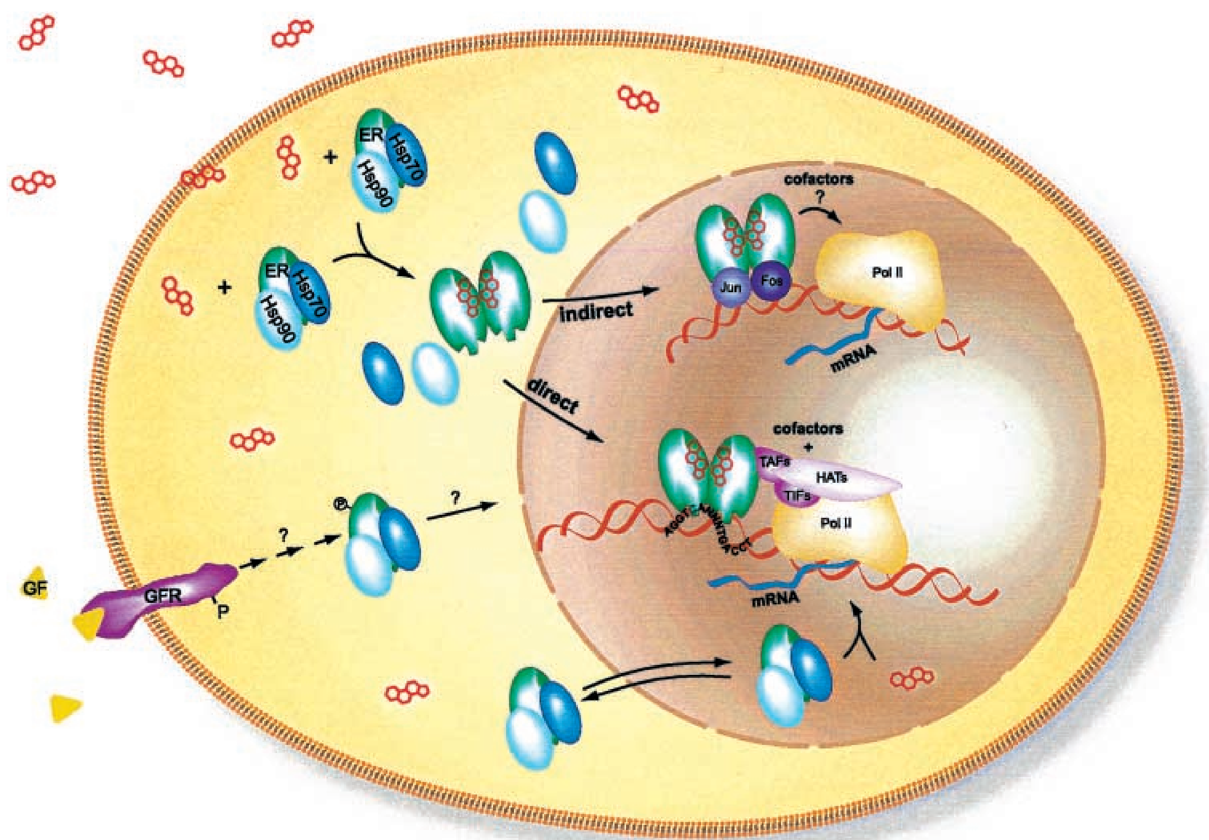


Figure 2. ER-α can transactivate estrogen-responsive genes either directly or indirectly. Unliganded ER-α exists in association with heat shock proteins (HSPs) in a monomeric form, distributed between the nucleus and the cytoplasm. On binding ligand, ER-α undergoes a major conformational change, dissociates from HSPs, dimerizes and translocates to the nucleus. Liganded ER-α can activate transcription of responsive genes either by directly binding to an estrogen-responsive consensus sequence or indirectly by associating with AP-1 (illustrated here as a jun/fos heterodimer) bound to its cognate DNA sequence. In both cases, liganded ER-α recruits co-factors that in turn activate transcription and act to modify local chromatin structure. Cross-talk occurs between other signaling pathways and estrogen-mediated responses, as ER-α is a substrate for kinases involved in extracellular signal transduction. Phosphorylation on serine, threonine and tyrosine modulates ER-α, generally to enhance ligand-dependent transactivation. GF, growth factor; GFR, growth factor receptor; TAF, transcription-activating factor; TIF, transcription intermediary factor; HAT, histone acetyl transferase; PolII, DNA-dependent RNA polymerase II.

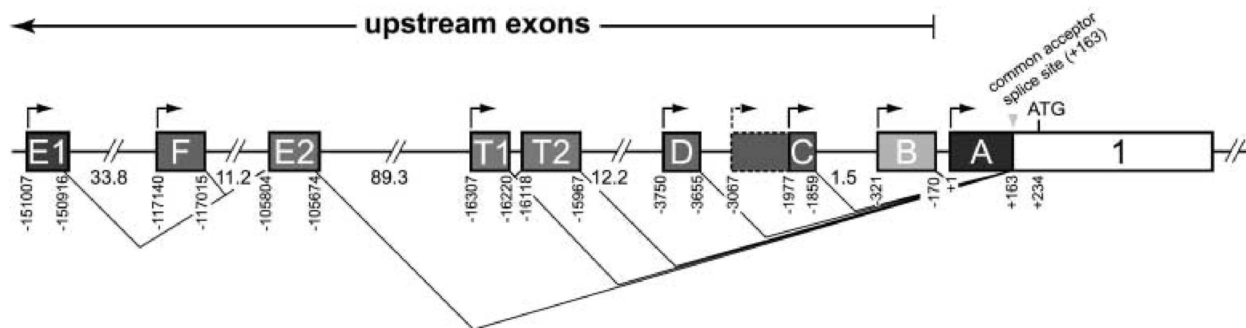


Figure 3. Genomic organization of the human ER-α promoter region. The location of multiple promoters and corresponding upstream exons of the human ER-α gene is shown. Upstream exons are represented by shaded boxes and their promoters shown as arrows. The intronic distance between exons is given in kb. The numbers below the exon boxes indicate experimentally defined 5′ start sites, or splice donor or acceptor sites involved in generating mature ER-α mRNA with the distance from the originally described transcription start site at +1 in base pairs. Also indicated is the common splice acceptor site at +163 which all 5′ untranslated regions can splice to.

moter is non-coding and is spliced to a common splice acceptor site upstream of the initiation codon of ER-α. Consequently, in all cases, this results in the expression of the full-length 66-kDa ER-α receptor protein. Quantitative analysis, as opposed to RT-PCR, in various cell lines and tissues reveals that there is a distinct expression profile of ER-α in individual cell lines and tissues. The relative level of ER-α transcripts varies up to 200-fold between MCF-7 and primary human osteoblasts [28]. Furthermore, promoter utilization also varies between different cell types. For example, whereas the A promoter is the major promoter utilized in MCF-7 cells, the E promoter is the predominant promoter used in the generation of ER-α mRNA. The relative expression levels and promoter usage for several human cell lines and tissues are shown in figure 4.

However, mRNA transcripts that encode the 66-kDa form of ER-α are not the only possibility. Three variant forms of ER-α with molecular weights of 66, 46 and 39 kDa are expressed in osteoblasts [28]. The main promoter utilized for the expression of ER-α in osteoblasts is the F promoter, which lies approximately 117 kb upstream of the originally described transcription start site. Splicing of the 5′ UTR resulting from the F promoter to the splice acceptor site in exon 2 results in skipping of exon 1 and the formation of a shorter ER-α isoform. This alternative splicing event generates an mRNA that has an AUG in a favorable Kozak sequence for translational initiation in frame with the remainder of the open reading frame of ER-α. The translation of this variant mRNA results in the expression of the 46-kDa ER-α isoform [28, 29].

In addition to human osteoblasts, the 46-kDa isoform is also present in the human breast carcinoma cell line MCF-7 [29]. Whereas the hER-α46 corresponds to approximately one-third of the transcripts expressed in osteoblasts, it represents around one-tenth of the total ER-α mRNA transcripts in MCF-7 cells. The generation of alternative ER-α transcripts is largely unexplored at present and it remains possible that further promoters and perhaps even protein-coding exons exist that may be used in a selected range of cell types or tissues in the generation of alternative protein isoforms of ER-α.

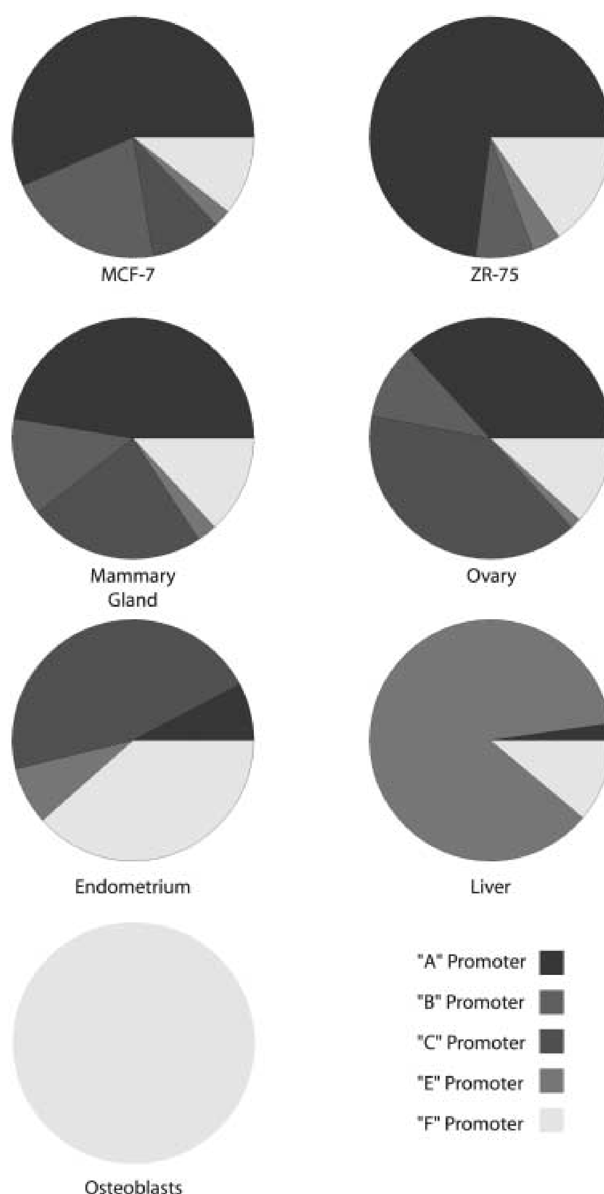


Figure 4. Differential promoter usage in human cell lines and tissues. The contribution that known promoters make to ER-α mRNA synthesis in different cell types is shown. Whereas the promoters clustered within 2 kb of the common splice acceptor site (A, B and C) are predominantly utilized in cell lines and tissues expressing relatively high levels of ER-α, more distal promoters (E and F) contribute most to the pool of ER-α mRNA in liver and in primary osteoblasts, where ER-α mRNA is less abundant.

Known functional consequences of ER-α isoforms

hER-α46 homodimers have been demonstrated to bind to an ERE and hER-α46 can form heterodimers with hER-α66 [28, 29]. Interestingly, hER-α46 homodimers show a higher affinity for an ERE than hERα66 homodimers. Furthermore, the hER-α46/66 heterodimer forms preferentially as compared to the hER-α66 homodimer. These effects have been demonstrated both in vitro and in vivo [28, 29]. To evaluate the potential physiological consequences of this interaction, both hER-α66 and hER-α46 receptor isoforms were evaluated for their ability to transactivate an ERE reporter construct in transient transfection assays in different cell lines.

Individual cell lines differ in their expression of co-factors that interact with ER-α. The endometrial carcinoma line HeLa, for example, has a co-factor profile such that transactivation on an ERE by ER-α predominantly occurs through co-factors associating with the AF-2 surface,

while in the liver cell line HepG2, transactivation is primarily dependant on AF-1. Other cells, such as the osteosarcoma cell line SaOS, have a mixed context, containing a co-factor profile that can respond to both AF-1 and AF-2 [28]. In an AF-1-dependant context, hER-α46 acts to competitively inhibit transactiviation mediated by liganded hER-α66. However, as anticipated, this effect does not occur in an AF-2-dependant environment [29]. The response of an individual cell to E2 is therefore clearly dependant on the level and profile of ER-α isoforms and also on the spectrum of co-factors within the cell.

Regulation of ER-α mRNA transcription

There is at present only a limited amount of information known about how ER-α transcription is regulated. As the A promoter was the first promoter to be identified, and is the major promoter used in cell lines expressing relatively high levels of ER-α mRNA transcripts, some of the *cis*- and *trans*-acting elements that are involved in regulation of ER expression have been characterized. As differential expression of ER-α transcripts occurs between different cell types, regulation at the level of individual promoters must be a key event in ER-α mRNA formation. This is likely to be controlled by both cellular transcription factors and by epigenetic phenomena, given that the ER-α promoter region encompasses such a sizeable genetic unit. For example, the E promoter, almost exclusively used in liver and which lies some 150 kb from the first coding exon of ER-α, may be epigenetically repressed in other tissues.

How are these processes regulated in a cell?

The mechanisms controlling the regulation of ER-α expression remain to some extent poorly understood. Although the expression of ER-α is differentially regulated within individual cell types, the role that individual promoters, *cis*-acting elements, transcription factors, ER-α itself and cellular context have on the complex regulation of ER-α expression remains incomplete. The recognition that estrogen signaling is transduced within different cells through the differential expression of ER-α and the individual co-factor profile within the cell could be potentially useful in targeting individual pathological situations.

The control of ER-α expression is best understood in breast cancer, where ER-α plays a pivotal role in the initiation and progression of this neoplasia. An increase in ER-α expression is seen with increasing proliferative capacity in breast hyperplasia and in tumors [30], although many breast cancers progress to estrogen independence with concomitant modification of ER-α expression [31]. Two promoter/transcription complexes have been identified as playing a role in the differential expression of ER-α in ER-α-positive and -negative breast carcinoma cell lines. Tang et al. [32] demonstrated that a 35-bp element at position –3.7 kb acts as a major enhancer element required for the high level of ER-α expression in ER-α^+ breast cancer cell lines. These authors further demonstrated that this enhancer binds AP-1 and that adjacent sequences bind to other unknown factors. It is tantalizing to note that overexpresion of c-jun in MCF-7 cells results in the loss of ER-α expression and a gain of estrogen independence [33]. Further, deConinck et al. [34] described a *cis*-acting element located in the region of the ER-α gene corresponding to the 5′UTR around +190 [34]. A transcription factor, identified as AP-2γ [35], that is expressed in ER-positive breast and endometrial cell lines is at least partly responsible for enhancement of ER-α transcription in AP2-γ-positive cell lines. However, not all ER-α-positive cell lines express AP2-γ [36], indicating that multiple mechanisms contribute to the high level expression of ER-α in tumor cells. An example of a transcription factor that selectively contributes to promoter-specific ER-α expression in breast carcinoma cell lines is ERBF-1, whose enhancer element is located at –1.9 kbp [37]. The authors showed that this transcription factor enhances a distal promoter element (promoter C in accordance with the recently described genomic organization of ER-α [27]). ERBF-1 selectively increases only C promoter activity which is very low in breast carcinoma cell lines, such as ZR-75, that do not express ERBF-1 and apparently, in consequence, do not generate significant levels of C hER-α mRNA.

Further mechanisms that potentially regulate gene expression, at a transcriptional level, are DNA methylation and chromatin condensation of promoter regions. Epigenetic silencing, either through hypermethylation of CpG islands associated with ER-α promoters [38, 39] or by chromosome condensation through nucleosome acetylation can act to preclude the expression of ER-α. It is clear that, between the distal promoter region (some 151 kb [27]) and the region containing the coding exons (140 kb [40]), the ER-α gene encompasses a sizable genetic unit. It is possible that some ER-α promoters need to be separated from each other by a significant distance so that epigenetic events involved in either the activation or suppression of individual promoters during differentiation apply to a single promoter in a certain cell type. Chromosome condensation associated with epigenetic silencing usually extends over a minimum of several kb [41]. Interestingly, four different promoters (A, B, C and D) are localized within a 3.7-kb region, perhaps suggesting that promoter clustering occurs to allow single epigenetic effects to apply to genes that are expressed by multiple promoters.

Autoregulation of ER-α expression

It appears that the 5′ upstream exons of the ER-α gene play an important role in the tissue-specific expression profile through a number of *cis*-acting elements. The 5′UTRs that are encoded by these upstream exons of ER-α negatively affect the translation of these mRNA variants. For example, the T1 and T2 5′UTRs, which are specifically expressed in testis, reduce the translation of the main open reading frame in that tissue [M. Kos, unpublished data]. Activated ER is known to regulate the expression of target genes such as the progesterone receptor, cyclin D1 and transforming growth factor-β but little is known about the auto-regulation of its own expression. This effect has been described for the C promoter of hER-α66, where exogenous hER-α66 increased the transactivation of a luciferase reporter construct [42]. Additionally, hER-α66 increased F promoter activity 50-fold in a ligand-dependant manner, suggesting that estradiol can specifically up-regulate ER-α expression in cells, such as osteoblasts, where the F promoter is predominantly used [43]. In contrast to estradiol-mediated up-regulation of ER-α promoters, full-length chicken ER-α down-regulates expression of the chicken A promoter [44].

mRNA stability

The 3′UTR of ER-α is unusually long, having twice the length (4.3 kb) of the coding region (2 kb). Some regions of the 3′UTR show extensive homology between species. These observations prompted an evaluation for a potential functional role of the 3′UTR in the post-transcriptional control of ER-α expression. Sequence analysis shows that the 3′UTR harbors more AU-rich sequences than are present in the coding region, which is usually a reliable indicator of mRNA destabilization. AU-rich motifs or AUUUA sequences were initially identified as having a functional role in relatively instable transcripts that encode proto-oncogenes (c-fos and c-myc) and cytokines, where rapid turnover is required to achieve a fast response in mRNA levels in reaction to extra-cellular signals [45, 46].

A destabilizing role of ER-α 3′UTR sequence was demonstrated recently [47]. The decay kinetics of mRNAs containing the 3′UTR of ER-α were studied in order to delineate destabilizing regions. The presence of a complete hER-α 3′UTR reduced the half-life of a reporter mRNA from greater than 24 h to 3 h. Destabilizing structures are restricted to a 1-kb region within the 4.3-kb ER-α mRNA 3′UTR. A repeat of four AUUUA motifs, anticipated to be essential for destablilization, was found not to be responsible for this effect. However, a co-operative effect of different regions of this 1-kb sub-fragment was mandatory for the observed destabilizing effect on ER-α mRNA.

Mechanisms of protein degradation

The major mechanism for targeted degradation of rapidly turned over proteins is the ubiquitin-proteasome pathway [20,48]. Proteins are marked for degradation by the proteasome through the action of ubiquitin ligases, which covalently attach the highly conserved 8.6-kDa ubiquitin protein to the ε-amino group of lysine on the targeted protein [49, 50]. A single ubiquitin-activating enzyme (UBA) activates ubiquitin in mammalian cells before transferring ubiquitin to one of several ubiquitin-conjugating enzymes. Finally, ubiquitination of the target protein is achieved through the action of ubiquitin protein ligases, specific substrate adaptor proteins that complete ubiquitin conjugation. Further ubiquitin moieties are then added to form ubiquitin chains [51]. Subsequently, polyubiquitinated proteins are recognized and degraded by the multi-subunit 26S proteasome complex [52, 53].

Estrogens modulate the stability of the ER. In the absence of ligand, the half-life of ER-α is approximately 5 days compared to around 3 h on binding ligand [54, 55]. Degradation of ER can be inhibited by the proteasome inhibitors MG132 and lactocystin indicating that the major route of clearance of liganded ER-α is through the proteasome pathway [56–58]. Ubiquitination also apparently affects the transcriptional competence and mobility of ER-α, suggesting either that targeted degradation directly plays a critical role in transactivation or that a block in degradation prevents release of ubiquitinated ER-α from responsive promoters [59].

There are three observations that associate proteasome activity with ER-α transactivation. First, several proteins that interact with steroid receptors are also components of the ubiquitin/proteasome degradation pathway. These are SUG1/TRIP1 [60, 61], RSP5/RPF1 [62], E6-AP [63] and UBC9 [64, 65]. RSP5/RPF1 and E6-AP are both E3-type ubiquitin ligases that stimulate nuclear hormone-dependant gene transcription. In addition to proteasome-mediated degradation of ER-α, other cofactors (SRC-1, TIFII, RAC3 and CBP) that associate with ER-α are also degraded through proteasome action [66]. Second, the proteasome inhibitors MG132 and lactacystin abrogate transactivation by ER-α [66], implying that proteasome degradation is necessary for ER-α-mediated transcription. Further, use of a cell line expressing a temperature-sensitive mutant form of UBA indicated that conjugation of ubiquitin might be a pre-requisite for transcriptional competence [66]. Finally, fluorescence recovery after photo-bleaching (FRAP) clearly demonstrated that, in contrast to unliganded ER-α which is highly mobile, the mobility of ER-α is severely impaired by treatment with either ICI 182,780, a pure estrogen antagonist, or by MG132. Conversely, E2 and the partial estrogen antagonist 4-hydroxytamoxifen only slightly impair the mobility of ER-α [59]. These authors also demonstrate that

treatment with E2, MG132 or actinomycin D results in ER-α association, often in a poly-ubiquitinated form, with nuclear components resistant to extraction with high salt after detergent and DNAse treatment, indicating that liganded ER-α becomes tightly associated with components of the nuclear matrix.

We wish to discuss these observations and present a hypothetical model for the potential role of proteolysis in ER-mediated gene transactiviation (fig. 5). Liganded ER-α, initially highly mobile, associates with an ERE. Cofactors involved in recruiting the transcriptional machinery then associate with the ER-α/DNA complex. Subsequent acetylase activity (e.g., by p300) results in the direct acetylation of ER-α [67]. Co-factor binding consequently results in the ubiquitination of ER-α. We predict that this event results in ER-α becoming transcriptionally incompetent, perhaps because co-factor binding to ER-α is disrupted. If subsequent degradation of ubiquitinated ER-α does not occur, transcription ceases, as the responsive promoter is loaded with transcriptionally incompetent ER-α. In support of this hypothesis, regions of ER-α responsible for proteasome-mediated degradation have been shown to be co-incident with regions involved in coactivator binding. Furthermore, transcriptional repression by actinomycin D, which prevents RNA polymerase II from initiating transcription, also prevents proteasome degradation of ER-α, suggesting that initiation of transcription is an integral event in marking ER-α for degra-

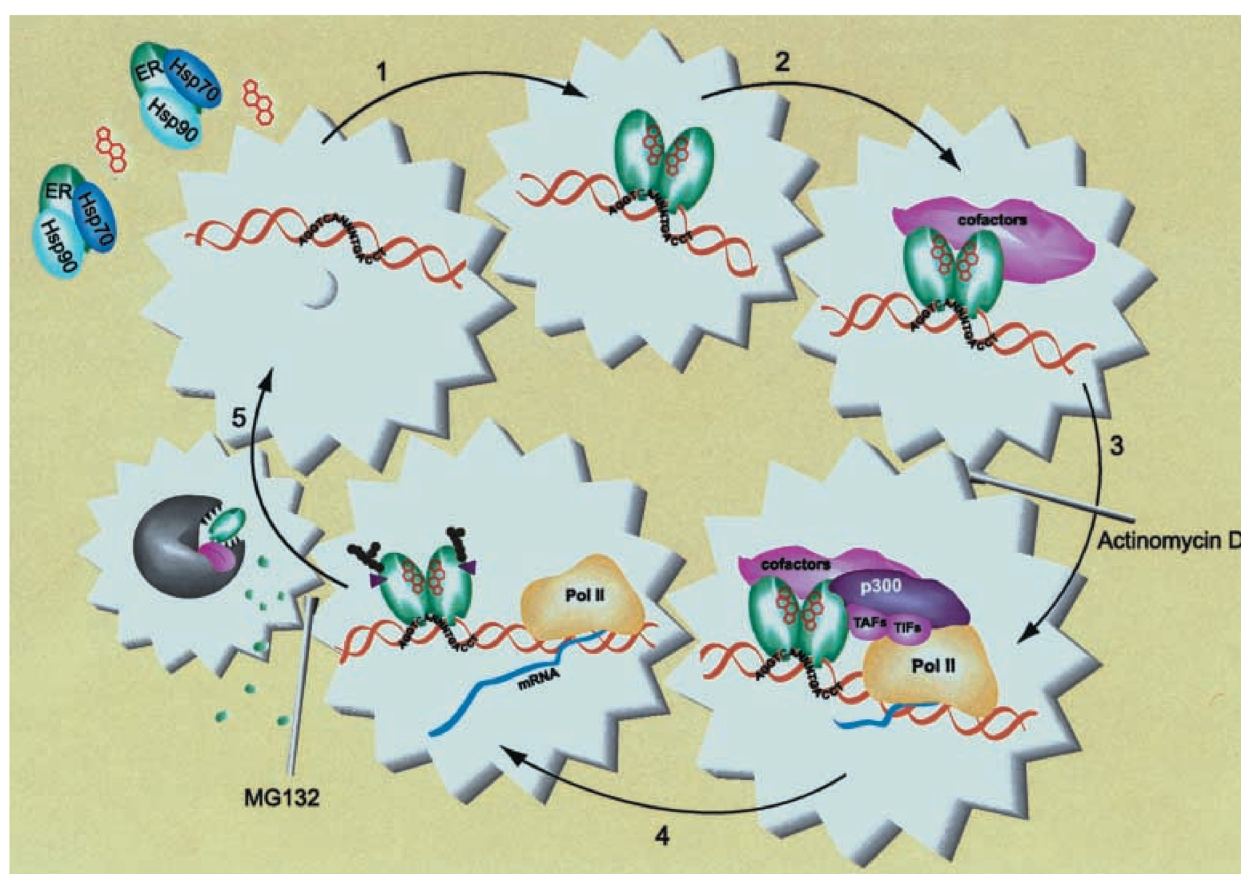


Figure 5. Co-factor recruitment, transcription, proteolysis and a transcription cycle. ER-α, on association with ligand, dimerizes and binds to a responsive promoter through association with an ERE (**1**). This DNA/ER-α/ligand complex then recruits co-factors (**2**) that in turn associate with the basal transcription machinery (**3**). We suggest that the acts of co-factor recruitment and/or transcription mark ER-α (indicated by purple triangles) such that ubiquitination of ER-α then occurs (**4**; ubiquitin moieties are indicated by brown circles). A post-translational modification of ER-α, such as acetylation, may be the physical event involved in this switch in the transcriptional competence of ER-α. We further propose that ubiquinated ER-α is transcriptionally incompetent and that until it is cleared from the promoter by proteasome action (**5**), transcription does not occur. This scheme may explain some paradoxical findings. All drugs indicated preclude both ER-α-mediated gene transcription and the degradation of ER-α, indicating that clearance of transcriptionally marked ER-α from estrogen responsive promoters is essential for a subsequent transcription to occur. 4-(OH)-tamoxifen may act to block recruitment of factors that mark ER-α for proteasome degradation, explaining why 4-(OH)-tamoxifen treatment of cells results in partial antagonism (co-factors still associate with AF-1) and in the accumulation of ER-α within the cell. Actinomycin D blocks PolII from leaving the assembled transcription complex, thereby stalling ER-α in the transcription cycle before it is targeted for degradation. MG132 is a potent inhibitor of proteasome-mediated degradation.

dation through the ubiquitin/proteasome pathway. It is implicit that degradation of ER-α that has been involved in a transcriptional event has to occur for the promoter to become available for further ER-α-mediated transcription. This model also explains the immobilization of ER-α on treatment with MG132 and actinomycin D. When ubiquitination of ER-α occurs in relation to transcriptional initiation remains unclear. Also not known is the extent to which ER-α ubiquitination is required to prevent transcriptional competence or to trigger proteasome-mediated degradation. As chains of ubiquitin form on ER-α, these events may take several transcription cycles to achieve a switch to degradation. The finding that genetic ablation of the steroid receptor co-activator ubiquitin ligase E6-AP results in tissue-selective steroid hormone resistance [68] also fits with this proposed model. A process where transcription is limited by the inactivation of trans-acting factors, such as ER-α, provides exquisite control of key transcriptional processes.

Phosphorylation and acetylation

Phosphorylation of ER-α can modify the activity of this ligand-activated transcription factor. In general terms, phosphorylation integrates estrogen signaling into a complex cross-talk network with other signaling pathways. ER-α is a substrate for both serine/threonine and tyrosine kinases and is phosphorylated at multiple sites. This can affect either AF-1, thereby modulating ligand-independent activation of ER-α, or AF-2, where DNA binding and ligand binding is located. The physiological state of the cell apparently determines the specific sites that are modified.

Phosphorylation of ER-α is enhanced in response to growth factors or to cytokine treatment [69–72]. Cytokine-induced phosphorylation of ER-α was first reported to involve epidermal growth factor (EGF) in mouse uterus [69]. EGF reproduces many of the effects of estrogen in uterus, indicating that cross-signaling between growth factor and steroid pathways is important in normal physiology. Ser118 and Ser167 have been most widely characterized among the sites known to be phosphorylated in ER-α. Arnold et al. [73] demonstrated that Ser118 does not require ER-α to be associated with ligand for phosphorylation to occur, whereas phosphorylation of Ser167 is dependant on ER-α being associated with ligand. Kato et al. [70] reported that Ser118 can be phosphorylated both in vitro and in vivo through the Ras/MAPK cascade of the signal transduction pathways. These studies indicate that phosphorylation by the MAPK pathways influences receptor action by mechanisms that are not ligand dependant. Interestingly, there is some indication that phosphorylation of Ser118, following stimulation of the MAP kinase pathway, is sufficient to result in ligand-independent transactivation to around half of the activation observed with E2 in HeLa cells [71]. Joel et al. [72] reported that phosphorylation of Ser118 by MAPK enhances ligand-induced transactivation approximately 2.5-fold. In contrast, phosphorylation of Ser167 by casein kinase II increases the binding affinity of liganded ER-α to an ERE. However, the role of phosphorylation at other sites of ER-α, such as Ser104, 106, and 158 is less clearly defined as the effects of phosphorylation on these sites are dependant on the cells that were used in these studies, again indicating that cell-specific phosphorylation can occur.

In addition to studies that show the impact of Ser/Thr phosphorylation on transcriptional activation, others suggest that dimerization and DNA binding are also affected by Tyr537 phosphorylation [74, 75]. These authors suggest that phosphorylation at Tyr537 is regulated by potentially oncogenic tyrosine kinases such as $p60^{c\text{-}src}$ and $p56^{lck}$. In addition, phosphatases can also modulate the function of ER in proliferative processes.

It has been recently shown that ER-α may also be acetylated [67]. Ligand-activated ER-α recruits co-factors that have intrinsic histone acetylase activity. Histone acetylation likely influences local chromatin structure resulting in enhanced transcriptional activity. However, the cofactor p300 but not P/CAF, both of which have intrinsic acetylase activity, were found to directly acetylate lysine residues at position 302/303 at the boundary between the hinge region and the LBD. The ER-α acetylation motif is conserved in a subset of nuclear receptors suggesting that acetylation can contribute to transcriptional regulation within this group. Surprisingly, mutagenesis of these residues to either neutral (K to A, Q or T) or to arginine (R), in the presence of exogenous p300, resulted in an increased transactivation capacity of the mutant ER-α [67]. A possible explanation for enhancement of transcriptional activity is that acetylation contributes to the ligand-mediated downregulation of ER-α by proteasome activity. If ER-α is unable to be acetylated, transcription may be potentiated by an increase in the time that transcriptionally competent ER-α resides on a responsive promoter.

Future prospects

More than three decades of intensive research have resulted in a considerable understanding of the role of estrogens in physiological and pathological processes. Frequently, however, disparate and apparently contradictory results have been difficult to comprehend because of the lack of a comprehensive hypothesis that integrated all aspects of ER function. In this review, we have attempted to provide such a framework for the assessment of the data of others and as a guide to future studies. Nonetheless, these

efforts to date have yielded considerable benefits in terms of medical treatment and in drug development. However, given the important role that estrogens play in both health and disease, considerable scope remains to increase our understanding of estrogen signaling and to develop and exploit further therapeutic strategies in the future.

We are beginning to recognize that the generation of ER-α mRNA variants and protein isoforms is complex and likely to reflect the requirements of estrogen signaling in individual cells and tissues. Further investigation is required to evaluate the role of such processes during development, normal physiology, disease and aging. There is also increasing awareness of the adaptive role different cell contexts have in E2 signaling. Such differences must reflect the co-factor profile within individual cell types. Results from transcriptome- and proteome-wide analysis of different cell types and tissues should clarify why substantial differences occur between target tissues and may result in the identification of novel targets for prophylactic and therapeutic interventions. Although much has been learned using model systems, it is becoming increasingly evident that research will have to focus on specific tissues and primary cell types. Answering these challenges should contribute greatly to understanding the tissue-specific effects of estrogens in health and in disease.

1 Mangelsdorf D. J., Thummel C., Beato M., Herrlich P., Schutz G., Umesono K. et al. (1995) The nuclear receptor superfamily: the second decade. Cell **83:** 835–839
2 Laudet V. (1997) Evolution of the nuclear receptor superfamily: early diversification from an ancestral orphan receptor. J. Mol. Endocrinol. **19:** 207–226
3 Escriva H., Delaunay F. and Laudet V. (2000) Ligand binding and nuclear receptor evolution. Bioessays **22:** 717–727
4 Nilsson S., Makela S., Treuter E., Tujague M., Thomsen J., Andersson G. et al. (2001) Mechanisms of estrogen action. Physiol. Rev. **81:** 1535–1565
5 Lombardi G., Zarrilli S., Colao A., Paesano L., Di Somma C., Rossi F. et al. (2001) Estrogens and health in males. Mol. Cell Endocrinol. **178:** 51–55
6 Juul A. (2001) The effects of oestrogens on linear bone growth. Hum. Reprod. Update **7:** 303–313
7 Rosano G. M. and Fini M. (2001) Comparative cardiovascular effects of different progestins in menopause. Int. J. Fertil. Womens Med. **46:** 248–256
8 Green P. S. and Simpkins J. W. (2000) Estrogens and estrogen-like non-feminizing compounds: their role in the prevention and treatment of Alzheimer's disease. Ann. N. Y. Acad. Sci. **924:** 93–98
9 Kuter I. (2001) Breast cancer. Oncologist. **6:** 338–346
10 Sciarra F. and Toscano V. (2000) Role of estrogens in human benign prostatic hyperplasia. Arch. Androl **44:** 213–220
11 Honjo H., Kikuchi N., Hosoda T., Kariya K., Kinoshita Y., Iwasa K. et al. (2001) Alzheimer's disease and estrogen. J. Steroid Biochem. Mol. Biol. **76:** 227–230
12 Bian Z., Nilsson S. and Gustafsson J. A. (2001) Selective estrogen receptor modulators and coronary heart disease. Trends Cardiovasc. Med. **11:** 196–202
13 Jordan V. C., Gapstur S. and Morrow M. (2001) Selective estrogen receptor modulation and reduction in risk of breast cancer, osteoporosis, and coronary heart disease. J. Natl. Cancer Inst. **93:** 1449–1457
14 Green S., Walter P., Kumar V., Krust A., Bornert J M., Argos P. et al. (1986) Human oestrogen receptor cDNA: sequence, expression and homology to v-erb-A. Nature **320:** 134–139
15 Nuclear Receptor Committee (1999) A unified nomenclature system for the nuclear receptor superfamily. Cell **97:** 161–163
16 Mosselman S., Polman J. and Dijkema R. (1996) ER beta: identification and characterization of a novel human estrogen receptor. FEBS Lett. **392:** 49–53
17 Nilsson S. and Gustafsson J. A. (2000) Estrogen receptor transcription and transactivation: basic aspects of estrogen action. Breast Cancer Res. **2:** 360–366
18 Htun H., Holth L. T., Walker D., Davie J. R. and Hager G. L. (1999) Direct visualization of the human estrogen receptor alpha reveals a role for ligand in the nuclear distribution of the receptor. Mol. Biol. Cell **10:** 471–486
19 Giguere V., Yang N., Segui P. and Evans R. M. (1988) Identification of a new class of steroid hormone receptors. Nature **331:** 91–94
20 Brzozowski A. M., Pike A. C., Dauter Z., Hubbard R. E., Bonn T., Engstrom O. et al. (1997) Molecular basis of agonism and antagonism in the oestrogen receptor. Nature **389:** 753–758
21 Gangloff M., Ruff M., Eiler S., Duclaud S., Wurtz J. M. and Moras D. (2001) Crystal structure of a mutant hERalpha ligand-binding domain reveals key structural features for the mechanism of partial agonism. J. Biol. Chem. **276:** 15059–15065
22 Chen H., Tini M. and Evans R. M. (2001) HATs on and beyond chromatin. Curr. Opin. Cell Biol. **13:** 218–224
23 Beato M. (1991) Transcriptional control by nuclear receptors. FASEB J. **5:** 2044–2051
24 Webb P., Nguyen P., Valentine C., Lopez G. N., Kwok G. R., McInerney E. et al. (1999) The estrogen receptor enhances AP-1 activity by two distinct mechanisms with different requirements for receptor transactivation functions. Mol. Endocrinol. **13:** 1672–1685
25 Coleman K. M. and Smith C. L. (2001) Intracellular signaling pathways: nongenomic actions of estrogens and ligand-independent activation of estrogen receptors. Front. Biosci. **6:** D1379–D1391
26 Moggs J. G. and Orphanides G. (2001) Estrogen receptors: orchestrators of pleiotropic cellular responses. EMBO Rep. **2:** 775–781
27 Kos M., Reid G., Denger S. and Gannon F. (2001) Minireview: genomic organization of the human ERalpha gene promoter region. Mol. Endocrinol. **15:** 2057–2063
28 Denger S., Reid G., Kos M., Flouriot G., Parsch D., Brand H. et al. (2001) ERalpha gene expression in human primary osteoblasts: evidence for the expression of two receptor proteins. Mol. Endocrinol. **15:** 2064–2077
29 Flouriot G., Brand H., Denger S., Metivier R., Kos M., Reid G. et al. (2000) Identification of a new isoform of the human estrogen receptor-alpha (hER-alpha) that is encoded by distinct transcripts and that is able to repress hER-alpha activation function 1. EMBO J. **19:** 4688–4700
30 Chappell S. A., Johnson S. M., Shaw J. A. and Walker R. A. (2000) Expression of oestrogen receptor alpha variants in non-malignant breast and early invasive breast carcinomas. J. Pathol. **192:** 159–165
31 Piccart M. J., Trivedi S., Maaroufi Y., Debbaudt A., Veenstra S. and Leclercq G. (1998) Evolution towards hormone independence of the MXT mouse mammary tumor is associated with a gradual change in its estrogen receptor molecular polymorphism. Cancer Biochem. Biophys. **16:** 169–182
32 Tang Z., Treilleux I. and Brown M. (1997) A transcriptional enhancer required for the differential expression of the human estrogen receptor in breast cancers. Mol. Cell Biol. **17:** 1274–1280

33 Smith L. M., Wise S. C., Hendricks D. T., Sabichi A. L., Bos T., Reddy P. et al. (1999) cJun overexpression in MCF-7 breast cancer cells produces a tumorigenic, invasive and hormone resistant phenotype. Oncogene **18:** 6063–6070
34 deConinck E. C., McPherson L. A. and Weigel R. J. (1995) Transcriptional regulation of estrogen receptor in breast carcinomas. Mol. Cell Biol. **15:** 2191–2196
35 McPherson L. A., Baichwal V. R. and Weigel R. J. (1997) Identification of ERF-1 as a member of the AP2 transcription factor family. Proc. Natl. Acad. Sci. USA **94:** 4342–4347
36 Turner B. C., Zhang J., Gumbs A. A., Maher M. G., Kaplan L., Carter D. et al. (1998) Expression of AP-2 transcription factors in human breast cancer correlates with the regulation of multiple growth factor signalling pathways. Cancer Res. **58:** 5466–5472
37 Tanimoto K., Eguchi H., Yoshida T., Hajiro-Nakanishi K. and Hayashi S. (1999) Regulation of estrogen receptor alpha gene mediated by promoter B responsible for its enhanced expression in human breast cancer. Nucleic Acids Res. **27:** 903–909
38 Ottaviano Y. L., Issa J. P., Parl F. F., Smith H. S., Baylin S. B. and Davidson N. E. (1994) Methylation of the estrogen receptor gene CpG island marks loss of estrogen receptor expression in human breast cancer cells. Cancer Res. **54:** 2552–2555
39 Ferguson A. T., Lapidus R. G., Baylin S. B. and Davidson N. E. (1995) Demethylation of the estrogen receptor gene in estrogen receptor-negative breast cancer cells can reactivate estrogen receptor gene expression. Cancer Res. **55:** 2279–2283
40 Ponglikitmongkol M., Green S. and Chambon P. (1988) Genomic organization of the human oestrogen receptor gene. EMBO J. **7:** 3385–3388
41 Rice J. C. and Allis C. D. (2001) Histone methylation versus histone acetylation: new insights into epigenetic regulation. Curr. Opin. Cell Biol. **13:** 263–273
42 Castles C. G., Oesterreich S., Hansen R. and Fuqua S. A. (1997) Auto-regulation of the estrogen receptor promoter. J. Steroid Biochem. Mol. Biol. **62:** 155–163
43 Denger S., Reid G., Brand H., Kos M. and Gannon F. (2001) Tissue-specific expression of human ERalpha and ERbeta in the male. Mol. Cell Endocrinol. **178:** 155–160
44 Griffin C., Flouriot G., Sonntag-Buck V. and Gannon F. (1999) Two functionally different protein isoforms are produced from the chicken estrogen receptor-alpha gene. Mol. Endocrinol. **13:** 1571–1587
45 Wilson T. and Treisman R. (1988) Removal of poly(A) and consequent degradation of c-fos mRNA facilitated by 3′ AU-rich sequences. Nature **336:** 396–399
46 Jones T. R. and Cole M. D. (1987) Rapid cytoplasmic turnover of c-myc mRNA: requirement of the 3′ untranslated sequences. Mol. Cell Biol. **7:** 4513–4521
47 Kenealy M. R., Flouriot G., Sonntag-Buck V., Dandekar T., Brand H. and Gannon F. (2000) The 3′-untranslated region of the human estrogen receptor alpha gene mediates rapid messenger ribonucleic acid turnover. Endocrinology **141:** 2805–2813
48 Haas A. L. and Siepmann T. J. (1997) Pathways of ubiquitin conjugation. FASEB J. **11:** 1257–1268
49 Huibregtse J. M., Scheffner M., Beaudenon S. and Howley P. M. (1995) A family of proteins structurally and functionally related to the E6-AP ubiquitin-protein ligase. Proc. Natl. Acad. Sci. USA **92:** 2563–2567
50 Scheffner M., Nuber U. and Huibregtse J. M. (1995) Protein ubiquitination involving an E1-E2-E3 enzyme ubiquitin thioester cascade. Nature **373:** 81–83
51 Ciechanover A., Orian A. and Schwartz A. L. (2000) The ubiquitin-proteasome pathway in mammals: mechanisms of action and involvement in pathogenesis of human diseases. In: Proteasomes: The World of Regulatory Proteolysis, 216–235, Hilt W. and Wolf D. H. (eds), Eurekah. com
52 Chen Z. J., Parent L. and Maniatis T. (1996) Site-specific phosphorylation of IkappaBalpha by a novel ubiquitination-dependent protein kinase activity. Cell **84:** 853–862
53 Kim T. K. and Maniatis T. (1996) Regulation of interferon-gamma-activated STAT1 by the ubiquitin- proteasome pathway. Science **273:** 1717–1719
54 Nirmala P. B. and Thampan R. V. (1995) Ubiquitination of the rat uterine estrogen receptor: dependence on estradiol. Biochem. Biophys. Res. Commun. **213:** 24–31
55 Pakdel F., Le Goff P. and Katzenellenbogen B. S. (1993) An assessment of the role of domain F and PEST sequences in estrogen receptor half-life and bioactivity. J. Steroid Biochem. Mol. Biol. **46:** 663–672
56 Alarid E. T., Bakopoulos N. and Solodin N. (1999) Proteasome-mediated proteolysis of estrogen receptor: a novel component in autologous down-regulation. Mol. Endocrinol. **13:** 1522–1534
57 El Khissiin A. and Leclercq G. (1999) Implication of proteasome in estrogen receptor degradation. FEBS Lett. **448:** 160–166
58 Nawaz Z., Lonard D. M., Dennis A. P., Smith C. L. and O'Malley B. W. (1999) Proteasome-dependent degradation of the human estrogen receptor. Proc. Natl. Acad. Sci. USA **96:** 1858–1862
59 Stenoien D. L., Patel K., Mancini M. G., Dutertre M., Smith C. L., O'Malley B. W. et al. (2001) FRAP reveals that mobility of oestrogen receptor-alpha is ligand- and proteasome-dependent. Nat. Cell Biol. **3:** 15–23
60 Lee J. W., Ryan F., Swaffield J. C., Johnston S. A. and Moore D. D. (1995) Interaction of thyroid-hormone receptor with a conserved transcriptional mediator. Nature **374:** 91–94
61 vom Baur E., Zechel C., Heery D., Heine M. J., Garnier J. M., Vivat V. et al. (1996) Differential ligand-dependent interactions between the AF-2 activating domain of nuclear receptors and the putative transcriptional intermediary factors mSUG1 and TIF1. EMBO J. **15:** 110–124
62 Imhof M. O. and McDonnell D. P. (1996) Yeast RSP5 and its human homolog hRPF1 potentiate hormone-dependent activation of transcription by human progesterone and glucocorticoid receptors. Mol. Cell Biol. **16:** 2594–2605
63 Nawaz Z., Lonard D. M., Smith C. L., Lev-Lehman E., Tsai S. Y., Tsai M. J. et al. (1999) The Angelman syndrome-associated protein, E6-AP, is a coactivator for the nuclear hormone receptor superfamily. Mol. Cell Biol. **19:** 1182–1189
64 Gottlicher M., Heck S., Doucas V., Wade E., Kullmann M., Cato A. C. et al. (1996) Interaction of the Ubc9 human homologue with c-Jun and with the glucocorticoid receptor. Steroids **61:** 257–262
65 Poukka H., Aarnisalo P., Karvonen U., Palvimo J. J. and Janne O. A. (1999) Ubc9 interacts with the androgen receptor and activates receptor-dependent transcription. J. Biol. Chem. **274:** 19441–19446
66 Lonard D. M., Nawaz Z., Smith C. L. and O'Malley B. W. (2000) The 26S proteasome is required for estrogen receptor-alpha and coactivator turnover and for efficient estrogen receptor-alpha transactivation. Mol. Cell **5:** 939–948
67 Wang C., Fu M., Angeletti R. H., Siconolfi-Baez L., Reutens A. T., Albanese C. et al. (2001) Direct acetylation of the estrogen receptor alpha hinge region by p300 regulates transactivation and hormone sensitivity. J. Biol. Chem. **276:** 18375–18383
68 Smith C. L., DeVera D. G., Lamb D. J., Nawaz Z., Jiang Y. H., Beaudet A. L. et al. (2002) Genetic ablation of the steroid receptor coactivator-ubiquitin ligase, E6-AP, results in tissue-selective steroid hormone resistance and defects in reproduction. Mol. Cell Biol. **22:** 525–535
69 Ignar-Trowbridge D. M., Nelson K. G., Bidwell M. C., Curtis S. W., Washburn T. F., McLachlan J. A. et al. (1992) Coupling of dual signaling pathways: epidermal growth factor action in-

volves the estrogen receptor. Proc. Natl. Acad. Sci. USA **89:** 4658–4662

70 Kato S., Endoh H., Masuhiro Y., Kitamoto T., Uchiyama S., Sasaki H. et al. (1995) Activation of the estrogen receptor through phosphorylation by mitogen-activated protein kinase. Science **270:** 1491–1494

71 Bunone G., Briand P. A., Miksicek R. J. and Picard D. (1996) Activation of the unliganded estrogen receptor by EGF involves the MAP kinase pathway and direct phosphorylation. EMBO J. **15:** 2174–2183

72 Joel P. B., Traish A. M. and Lannigan D. A. (1998) Estradiol-induced phosphorylation of serine 118 in the estrogen receptor is independent of p42/p44 mitogen-activated protein kinase. J. Biol. Chem. **273:** 13317–13323

73 Arnold S. F., Obourn J. D., Jaffe H. and Notides A. C. (1995) Phosphorylation of the human estrogen receptor by mitogen-activated protein kinase and casein kinase II: consequence on DNA binding. J. Steroid Biochem. Mol. Biol. **55:** 163–172

74 Arnold S. F., Obourn J. D., Jaffe H. and Notides A. C. (1995) Phosphorylation of the human estrogen receptor on tyrosine 537 in vivo and by src family tyrosine kinases in vitro. Mol. Endocrinol. **9:** 24–33

75 Yudt M. R., Vorojeikina D., Zhong L., Skafar D. F., Sasson S., Gasiewicz T. A. et al. (1999) Function of estrogen receptor tyrosine 537 in hormone binding, DNA binding, and transactivation. Biochemistry **38:** 14146–14156

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