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Review

Transcriptional activation of nuclear estrogen receptor and progesterone receptor and its regulation

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Abstract: Estrogen receptor (ER) and progesterone receptor (PR) are two important members of steroid receptors family, an evolutionarily conserved family of transcription factors. Upon binding to their ligands, ER and PR enter cell nucleus to interact with specific DNA element in the context of chromatin to initiate the transcription of diverse target genes, which largely depends on the timely recruitment of a wide range of cofactors. Moreover, the interactions between steroid hormones and their respective receptors also trigger post-translational modifications on these receptors to fine-tune their transcriptional activities. Besides the well-known phosphorylation modifications on tyrosine and serine/threonine residues, recent studies have identified several other covalent modifications, such as ubiquitylation and sumoylation. These post-translational modifications of steroid receptors affect its stability, subcellular localization, and/or cofactor recruitment; eventually influence the duration and extent of transcriptional activation. This review is to focus on the recent research progress on the transcriptional activation of nuclear ER and PR as well as their physiological functions in early pregnancy, which may help us to better understand related female reproductive diseases.

Key words: estrogen receptor; progesterone receptor; post-translational modifications; transcriptional activation; cofactors; early pregnancy

雌、孕激素受体的转录激活与调控

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摘 要：雌激素受体与孕激素受体都是类固醇激素受体这一进化上高度保守的转录因子家族的重要成员。当雌、孕激素受体分别与其配体在细胞浆中结合后进入核内，与靶基因上特异的DNA响应元件结合，并适时募集一些辅助转录因子，诱导特定基因转录表达，影响靶细胞的功能活动。雌、孕激素受体的转录活性还因其在蛋白翻译后所发生的不同修饰而改变。蛋白翻译后的修饰种类繁多。经典的修饰为丝/苏氨酸和酪氨酸残基的磷酸化修饰。近些年的研究发现，泛素化与类泛素化修饰对激素受体的稳定性、在亚细胞定位及其对辅助因子的募集等方面都发挥重要作用，并最终影响激素受体的转录活性。本文旨在对国内外近几年关于雌、孕激素受体的转录活性调控及其在早期妊娠中的生理意义进行综述，这将有助于理解雌、孕激素作用异常相关的女性生殖疾病。

关键词：雌激素受体；孕激素受体；翻译后修饰；转录激活；共辅助因子；早期妊娠
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1 Introduction

Dynamic coordination of gene networks involved in numerous physiological, developmental, and metabolic processes can be ascribed in a large part to a superfamily of ligand-activated transcription factors, the steroid receptors [1] including the androgen receptor (AR), estrogen receptor (ER), glucocorticoid receptor (GR), mineralocorticoid receptor (MR) and progesterone receptor (PR), which can induce or repress gene expression by binding to the respective response elements in chromatin [2]. ER, existing in two isoforms ERα and ERβ derived from distinct genes, mainly mediates the action of estrogens in ER-expressing tissues such as the mammary gland and the reproductive tract [3]. With respect to the PR, there are two main isoforms PR-A and PR-B that are derived from the same gene and mediate the actions of progestins in various pregnancy events as well as sexual behavior [4].

A generally accepted framework for nuclear receptor activation including the steroid receptor is that ligand-bound receptors via forming homodimers bind to hormone response elements located within the upstream promoter/enhancer sequences of target genes followed with recruitment of co-activating proteins, and eventually activate gene transcription [5]. In this respect, steroid receptors possess an evolutionarily conserved domain structure, which consists of DNA-binding domain responsible for the recognition of a specific DNA motif encoded in the genome, ligand-binding domain, and transactivation domains for transcription initiation [6]. In the absence of ligands, steroid receptors stay in the cytoplasm, forming complexes with chaperone proteins. Molecular chaperones and co-chaperones are typically known to assist the correct conformation of steroid receptors for ligand binding [7]. Following ligand binding, steroid receptors undergo conformational changes and translocate into the nucleus. The final outcome of steroid receptor activation is to modulate the transcription activity of target genes through recruiting general transcription factors and RNA polymerase II [8]. Although it is known that steroid receptors can interact directly with general transcription factors, there is overwhelming evidence that ligand-bound receptors need recruit co-regulators that modulate the transcriptional activities [1, 9]. Moreover, post-translational modification of ligand-bound steroid receptors is an important regulator loop for their functional activation. These covalent changes have shown to affect receptor stability, subcellular localization as well as the interactions with other proteins [10], pointing toward the complexity of ligand-receptor activation. In this review, we summarize recent research progress on transcriptional activation machinery of ER and PR, as well as their pathophysiological significance in various reproductive events.

2 Structures of ER and PR

2.1 ER

ERα and ERβ isoforms are encoded by two distinct genes in both mice and humans [11]. ERα is predominantly expressed in mammary glands, pituitary, hypothalamus, ovarian theca cells and reproductive tract. In contrast, ERβ is primarily expressed in ovarian granulosa cells, lung and prostate [3]. Transcription of the mouse ERα gene *in vivo* predominantly results in a single transcript of approximately 6.3 kb transcribed from 9 exons. This transcript encodes a protein of 599 amino acids with an approximate molecular mass of 66 kDa [12]. Human ERα consists of 595 amino acids and exhibits a similar molecular mass as mouse ERα [13, 14]. While human ERα gene has been mapped to chromosome 6 [15], mouse ERα gene is located on chromosome 10 [16]. The existence of multiple promoter and regulatory regions in the 59-untranslated sequences of the human and rat ERα has been described, but only a single open reading frame appears to exist [17, 18]. Previous studies indicated that the rodent ERβ was composed of 485 amino acids with an estimated molecular mass of 54 kDa and therefore was slightly smaller than the ERα [11, 19]. The majority of this difference in size between two ER isoforms was due to a significantly shorter N9 terminus in ERβ protein [20].

Similarly to most other nuclear receptors, ERs contain a domain with ligand-independent activation function (AF-1) at the N-terminus, a DNA-binding domain (DBD domain) followed by a hinge domain, and a ligand-binding/dimerization domain (LBD) at the C-terminus that contains a ligand-dependent transcription activation domain (AF-2) [21] (Fig. 1).

2.2 PR

Human PRs are encoded by a single gene located on chromosome 11 (11q22-q23). Expression of PR isoforms is controlled by two promoters to produce two major mRNA transcripts that encode two proteins: the full-length PR-B (116 kDa) that is controlled by the

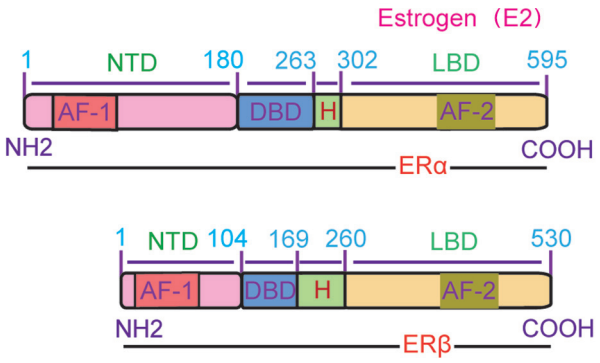

Fig. 1. Domain structures of estrogen receptor (ER). Diagram of translational start sites for human ERα and ERβ isoforms. ER is a modular protein consisting of a number of functional domains including the NTD (amino-terminal domain), DBD (DNA binding domain), H (hinge region), and LBD (ligand binding domain) as indicated. The presence of these domains and the activation function domains (AF-1, AF-2) allow for the unique function of the individual EGR isoforms. METc, methionine.

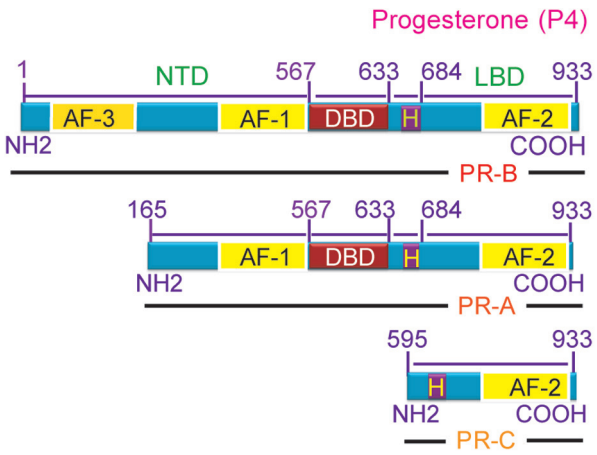

Fig. 2. Progesterone receptor isoforms. Diagram of translational start sites for human PR-A, PR-B, and PR-C isoforms. The numbers of the amino acids found at the boundaries in the individual receptors between the NTD (amino-terminal domain), DBD (DNA binding domain), H (hinge region), and LBD (ligand binding domain) are indicated. The presence of these domains and the activation domains (AF-1, AF-2, and AF-3) allow for the unique function of the individual PR isoforms. METc, methionine.

distal PR-B promoter region and initiated from the first AUG translational start codon, and PR-A (94 kDa) that is controlled by the proximal PR-A promoter region and initiated from the second AUG translational start codon that is 492 bases from the PR-A start codon [22]. Other PR isoforms are thought to be generated by the initiation of translation from further downstream AUG start sites (e.g. PR-C), exon splicing or exon insertions, respectively [22], but their physiologic relevance is uncertain.

Like the ER protein, both PR isoforms consist of multiple domains, such as the AF-1 in the N-terminus, the DBD and the ligand binding domain which contains AF-2. The PR-B isoform has an additional 164 amino acids in the N-terminus which contains an additional activation domain (AF-3) (Fig. 2) [23]. This region has been shown to endow a transactivation function that is specific to the PR-B protein, and plays an essential role in specifying target genes activated only by PR-B but not by PR-A [24, 25]. In fact, during embryo implantation and decidualization, evidence from genetic mouse models and *in vitro* manipulation of human uterine cells has demonstrated while PR-A is the main functional isoform in mice, PR-B is the functional one in humans, although both isoforms are simultaneously expressed in mouse uteri as well as in human endometrium [26, 27]. These findings suggest that PR-A and PR-B can differentially regulate the expression of targeting genes in response to progesterone, involving different transactivation capabilities in different targeting tissues [28, 29].

3 Transcriptional activation of ER and PR

3.1 ER activation

Estrogen may activate or repress the transcription of ER-targeting genes potentially by recruiting distinct classes of co-regulators that have chromatin remodeling properties. Structural and functional studies revealed that ER co-activators are recruited to estrogen-responsive genes through their interaction with activated receptors. In turn, the co-activator complex remodels the chromatin at this region through histone modification, facilitating RNA polymerase II-mediated transcription [30, 31]. With respect to the repressed genes, it has also been established that estrogen stimulates the selective association of ER with co-repressors [32, 33]. The interaction of these co-repressors prompts the binding of chromatin deacetylases and other repressive modification enzymes, therefore leading to transcriptional inhibition.

Like all other members of the nuclear receptor family, ERs can be activated upon ligand binding [34]. Importantly, ER-mediated transactivation can reach its maxi-

mal level only if ER is phosphorylated at various sites, even in the absence of estrogen binding (Fig. 3). The ER proteins are generally believed to shuttle between the cytoplasm and nucleus. *In vitro* experiments have demonstrated that ligand free ERα, like other steroid nuclear receptors, is maintained in a non-DNA binding form encompassed by a multi-chaperone complex organized around HSP90 [35]. However, little information is available with regard to ERβ. Upon ligand binding, ERα undergoes conformational changes that control its interaction with heat shock proteins and co-regulators. These interactions determine ER binding to the 13-bp estrogen response element sequence (ERE) within the promoter. ER-dimers dynamically and sequentially recruit various regulatory protein complexes contributing to chromatin remodeling, thereby strongly enhancing transcriptional activity [36]. Ligand-activation of ER may also stimulate the indirect binding of ER to DNA by protein-protein interactions with transcription factors such as AP-1 or Sp-1, which anchor the pre-initiation complex to ERE [37]. In addition, various ER variants may alter the estrogenic response. For example, ERα-36, an ERα variant lacking the N-terminal domain and a truncated ligand-binding C-terminal domain, has been implicated as a mediator of extranuclear (non-genomic) actions.

3.2 PR activation

Prior to the presence of progesterone in the extracellular space, the PR protein resides within the cytoplasm. In the absence of ligand, PRs reside in the cytoplasm, forming a complex with chaperone proteins. These chaperones hold the receptor in an inactive state, primed to bind to ligands. These proteins consist of heat shock protein HSP90, a P23 chaperone protein, and one of four chaperones containing a tetratricopeptide repeat (TPR) domain [35]. The first step in this

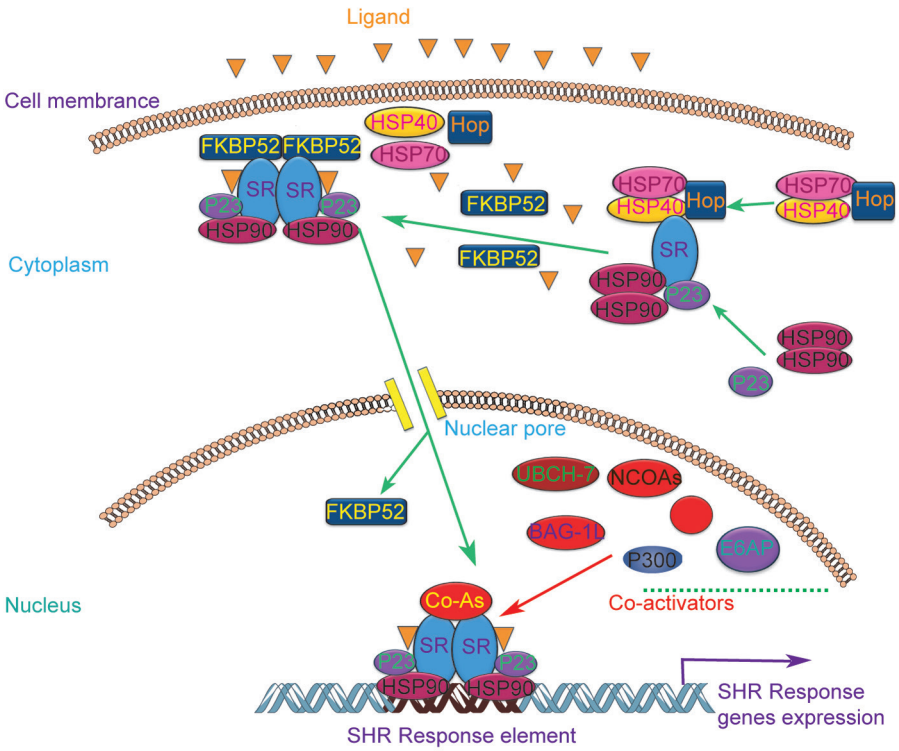

Fig. 3. A model depicting some of the key steps of the canonical pathway of steroid receptors. Binding of the HSP90, P23 and a preassembled complex of Hop, HSP70 and HSP40 assists a mature folding of the steroid receptor (SR). Addition of HSP90-dimers and P23 complete the assembled complex, termed the "foldosome". Release of Hop, HSP70 and HSP40 and addition of any one of the tetratricopeptide repeat (TPR)-containing cochaperone, for example FKBP52, which mediates translocation to the nucleus in a molecular complex was termed the "transportosome". Within the nucleus FKBP52 is released and the receptor binds to the response elements as an active dimer. Other co-activators, such as NCOAs, E6AP, UBCH-7, P300, enhance the activity of the SR most likely by stabilizing the active state of the receptors. The molecular chaperones HSP90 and HSP70 possibly also play a role in this process. SHR: steroid hormone receptor.

assembly is the formation of a molecular complex (HSP90, PR, HSP70, HSP40), termed the foldosome (Fig. 3).

Jensen two-step hypothesis on cytoplasmic-nuclear transportation of steroid receptors upon activation, together with the finding that molecular chaperones and co-chaperones bind to non-liganded receptors, collectively supported the concept that molecular chaperones confine steroid receptors in an inactive cytoplasmic state [38]. Upon ligand binding, PR undergoes a conformational change that triggers release from the chaperone complex and favors receptor dimerization. The receptor/chaperone complex is thought to move along the cytoskeleton to the nucleus in a format described as the transportosome [39]. The affinity of the FKBP52-receptor complex for dynein possibly determines transportation of the steroid receptors into the nucleus, and further stabilizes the PR in a high affinity form [40]. Dimerized hormone-receptor complexes translocate to the nucleus, where they bind to DNA and direct the recruitment of transcriptional co-activators, co-repressors, and the transcriptional machinery to modulate the expression of target genes [41].

Once in the nucleus, the steroid receptor/molecular chaperone complex dissociates and the steroid receptor is converted into a DNA binding form [41]. In the nucleus, molecular chaperones function as modulators of the DNA binding and transcriptional activities of steroid receptors [42]. The use of the HSP90-specific inhibitor GA blocked the transcriptional activity of this receptor on chromatin, demonstrating a crucial role of HSP90 in the nuclear function of the PR [42]. The DBD gives the receptor specificity for the target genes. This specificity is determined by which DNA sequences the DBD will recognize. These sequences or progesterone response elements (PREs) are located in enhancer/promoter regions of the target genes. This domain is responsible for linking the receptors to the cellular transcriptional machinery and regulates transcription of target genes.

Upon the binding of the receptor to the PRE, the activated receptor then interacts with co-activators, which will link the steroid hormone receptor to the basal transcriptional machinery of the cell. The co-activators not only link the receptor to the transcription machinery, but also facilitate transcription by covalently modifying chromatin. These co-activators have histone acetyltransferase (HAT) activity that functions to acetylate histone proteins, allowing the DNA to achieve a conformation that increases the accessibility of the target gene promoter to the activated receptor, and basal transcriptional machinery. This remodeling of the chromatin serves to facilitate the transcription of specific genes. These co-activators include members of the steroid receptor coactivator (SRC) family, CREB binding protein and related P300 protein (CBP/P300), high mobility group proteins (HMGs), and E3 ubiquitin protein ligases (E6AP and RPF-1). Thus, the entire process of steroid hormone receptor activation results in the enhanced transcription of specific target genes, as well as the degradation of the activated steroid hormone receptor [43].

Upon binding ligand, dimerizing and entering into the nucleus, the nuclear receptor dimer binds to recognition sequences known as response elements. Nuclear receptor proteins have their own response elements, but at times, can cross-react with other response element [44]. Although response elements for a particular nuclear receptor, such as the PR, have a specific sequence motif, there is room for flexibility within the sequence. The PREs usually consist of a palindromic hormone response element of AGAACAnnnTGTTCT [45]. However, PR binding is not limited to the full PRE. Indeed, it was determined that PR can bind to promoters of known progesterone target genes such as *Lifr*, *Gata2*, *Cyp26a1*, and *Ihh* with just half the sequence of the normal PRE [46]. Additionally, it was identified that PR can also bind to promoters of known target genes *Egfr* and *Wnt7a* with no canonical PRE present [46] (Fig. 3).

Functional dissection of nuclear receptor co-regulators revealed that their transcriptional co-regulation was linked to histone acetylation. Histone modification and chromatin remodeling indicate that histone-modifying enzymes, including histone methylases and chromatin remodelers, are potential transcriptional co-regulators that interact directly and indirectly with nuclear receptors [47, 48].

Chromatin remodeling is a fundamental process of chromatin reorganization [49]. The chromatin state of a normal nucleosomal array is inhibitory for transcriptional events, but is convertible into an even more inactivated state (heterochromatin) by the action of packing nucleosomal arrays, which work through association with histone H1 and non-histone proteins. Conversely, the normal nucleosomal array can be loosened, exposing naked DNA in active chromatin states (euchromatin). Through a process termed histone-octamer sliding,

chromatin remodelers can induce the reversible organization of nucleosomal arrays without unwinding DNA [50]. Chromatin remodeling appears to be indispensable for dynamic gene activation and repression, and hence, chromatin remodelers are assumed to globally co-regulate DNA-binding transcription factors, at least indirectly. Three types of ATP-dependent chromatin remodelers have been reported to facilitate transcriptional events. Switch/Sucrose non-fermenting (SWI/SNF)-type and imitation switch (ISWI)-type complexes are known to participate in both transcription activation and repression [51].

PR-mediated transcriptional regulation is exerted through cyclical recruitment and dismissal of multiple co-regulator complexes with distinct enzymatic activities, including HATs, histone methylations (HMTs), histone deacetylases (HDACs), histone demethylases (HDMs), ATP-dependent chromatin remodelers, and histone chaperones. The other complexes with presently unknown enzymatic activities are also assumed to be involved. Furthermore, these co-regulatory complexes are under the control of diverse extracellular and intracellular signaling pathways, which can sense changes in both the external environment and nutritional status, and direct appropriate transcriptional responses [52].

4 Cofactors for ER and PR activation

Similar with the other transcription factors, both the ER and PR need to interact with the other proteins or cofactors in the nucleus to regulate the target gene expression. These cofactors could ensure the full activity of these hormone receptors through different mechanisms, such as mediating the epigenetic modification on target gene promoter which promotes the transcription, enhancing the binding of receptors to the responsive DNA element and regulating the level of active DNA bounded ER and PR.

4.1 SRCs

SRC/p160 family is a family of ligand-recruited co-activators of ER and PR. SRC/p160 family, the initially defined nuclear receptor co-activators, is structurally and functionally distinguishable from other molecules. A recurring structural feature of the co-activator proteins is a helical LXXLL motif, or nuclear receptor box [53] presenting from a single to several copies in many co-activators, which is implicated in their ligand-dependent recruitment by the AF2-embedded LBD domain of nuclear receptors. Moreover, several functional properties are common across different groups of co-activators. Acetyltransferase activity, for instance, with which co-activators are thought to target histones and other proteins to create a transcriptionally permissive environment at the promoter, is possessed by CBP [54], P300/CBP-associated factor (PCAF) [55], and members of the SRC family [56, 57].

SRC-1 is a widespread cofactor that can functionally interact with a wide variety of nuclear receptors and a plausible candidate for the biochemically-defined p160. However, the subsequent cloning of GRIP1/TIF2/SRC-2 [58] and p/CIP [59] (also designated ACTR/RAC3/AIB-1/TRAM-1/SRC-3 herein) suggested that the term p160 encompasses a novel family of structurally-related nuclear receptor co-activators, the SRC-1 family. SRC-1, SRC-2/GRIP-1/TIF2 and p/CIP/SRC-3 exhibit common properties in the transcriptional activation of a wide variety of nuclear receptors [60–62] (Table 1). This family has a number of structural features in common, and one of the most interesting is the presence of the PAS/bHLH domain in their N-termini. Members of the bHLH family are involved in regulation of cell differentiation and proliferation, and are characterized by the formation of homo or heterodimeric complexes with bHLH partners [63]. Like other PAS-bHLH proteins [64], SRC-1 and SRC-2 appear to be capable of forming multimeric complexes *in vivo* [65], but the role of the PAS domain in this interaction is unclear. The phosphorylation-dependent multi-mono-ubiquitination event of SRC-3 also influenced its co-activational function with ER [66].

4.2 CBP/P300

CBP/P300 plays a critical role in cell cycle regulation, cell differentiation and apoptosis and exhibits HAT activity [67, 68]. CBP/P300 also interacts with other HATs, such as PCAF [55], and acetylates components of the basal transcription machinery. CBP/P300 are ubiquitous, evolutionarily conserved transcriptional co-activators for a host of diverse transcription factors, including CREB (cAMP-response element-binding protein) [69], STAT-2 [70] and p53 [71, 72]. Moreover, CBP has been shown to exist in a stably preformed complex with RNA Pol II [73], suggesting that interaction of transcription factors with CBP, either directly or indirectly, might result in a direct link to basal transcription factors. It has been proposed that nuclear receptors might also require the mediation of CBP/P300 for efficient

Table 1. Phenotypes of ER, PR and cofactor mutant mice

Gene	Genotype	Phenotype	References
Nr3c3 (Pgr)	PRKO	Impaired implantation/decidualization/infertility	[168] [27]
	PR-AKO	Impaired implantation/decidualization/infertility	[27]
	PR-BKO	Normal implantation/decidualization	[27]
	$PR^{f/f}$		
	Wnt7a-Cre	Impaired implantation/decidualization/infertility and the inability to cease estrogen-induced epithelial cell proliferation	[180]
*Nr3a1 (Esr*1*)*	ERαKO	Infertile/hypoplastic/no implantation/no decidual response persists with progesterone priming	[181]
Nr3a2 (Esr2)	ERβKO	Normal fertility uterine phenotype/exaggerated estrogen responsiveness	[182]
Nr3a1 (Esr1)	$ERα^{f/f}$		
	Wnt7a-Cre	Infertile/Enhanced uterine apoptosis/Impaired decidualization due to control stromal proliferation and differentiation	[163, 183]
Ube3a (E6AP)	E6APKO	Reduced male reproductive function/female subfertility/mammary gland development defect	[94]
Ncoa1	$SRC\text{-}1^{f/f}$ PR-Cre	Partial hormone resistance/impaired implantation/decidualization/ infertility	[184]
Ncoa2	$SRC\text{-}2^{f/f}$ PR-Cre	Endometrial decidualization defect/impaired implantation/decidualization/ infertility	[185, 186]
Ncoa3	$SRC\text{-}3^{f/f}$ PR-Cre	Female subfertility/mammary gland development defect	[187]
Fkbp4	Fkbp52KO	Implantation/decidualization defect/uterine progesterone resistance	[188–190]
Ncor-6	$SRC\text{-}6^{f/f}$ PR-Cre	Implantation/decidualization defect/increased estrogen sensitivity causes infertility	[191]

transactivation [74, 75]. It was shown that CBP, interacting weakly with nuclear receptors in a ligand-dependent manner, could enhance nuclear receptor-mediated transactivation, and was capable of binding to SRC-1 directly. CBP/P300 are proposed to be common integrators for distinct but convergent signaling pathways, functioning to integrate multiple different signals into an appropriate response at a common promoter [75]. The role of CBP in steroid receptor signaling indicates that CBP and SRC-1 synergistically activate transcription from ER and PR regulated promoters [76]. However biochemical analysis suggests that CBP and SRC-1 exist in largely distinct preformed complexes [65], and it may be possible that they interact only transiently when recruited by ligand-bound receptors at the promoter.

4.3 Forkhead box protein A1 (FOXA1)

FOXA1 (also known as HNF3α), a member of the forkhead family of transcription factors, is expressed in many organs and plays a key role in development. FOXA proteins are the most studied pioneer transcription factors that bind to chromatin and enable the potential gene expressional activity. FOXA1 recruitment to chromatin is mediated by the epigenetic signature consisting of mono and di-methylated histone H3 on lysine 4 (H3K4me1/me2), which is a transcriptional active mark [77]. The pioneering properties of FOXA1 reside on its protein structure, which contains a winged helix domain that can structurally mimic linker histone, and thus permits its stable interaction with histone H3 and H4 with high affinity [78, 79]. The high chromatin affinity of FOXA1 is a unique feature that allows it binding to the specific DNA sequences on the nucleosome core and displaces the linker histones, leading to de-compaction of chromatin and facilitating the binding by other transcription factors. In breast cancer cell lines that are hormone-sensitive and resistant, almost all ER-chromatin interactions and estrogen induced gene expression changes are dependent on the expression of FOXA1 [80]. Therefore, FOXA1 is a major determinant of ER activity in breast cancer.

4.4 GATA

The GATA family is composed of six highly conserved transcription factors (GATA-1 to GATA-6) identified in vertebrates, which bind to the DNA sequence (A/T) GATA (A/G) via two zinc-finger domains [81]. In the breast, GATA-3 is expressed in luminal tumors [82]. However, the mechanism of GATA-3 action or its potential role as a pioneer factor of ER and PR has not been described yet. Meanwhile, GATA-4 has been shown to have pioneering properties during early

development [83] and for ER binding in U2OS osteosarcoma cell line [84, 85], which stably expresses exogenous ER and very low levels of FOXA1 [80]. Interestingly, recent work has identified RunX1 as a mediator for ER-DNA interaction in MDA-MB-231 breast cancer cell line [86] which stably expresses exogenous ER and is negative for the expression of FOXA1. These results support the idea that distinct pioneer proteins influence ER binding in FOXA1-negative tissues.

4.5 E6-associated protein (E6AP)

E6AP is a 100 kDa cellular protein that mediates the interaction of the human papilloma virus with p53. The association of p53 with E6AP promotes the specific ubiquitination and subsequent proteolytic degradation of p53 *in vitro* [87, 88]. E6AP also functions as a ligand-activated co-activator for the steroid hormone receptors ER, AR, PR and growth hormone receptor (GHR) [89–91]. It is co-recruited by ER/PR to promoters that contain an ERE/PRE [92, 93]. A link between E6AP and ER/PR levels and/or activity has been genetically established: compared with wild-type littermates, E6AP-null animals show increased ER/PR protein levels in the mammary tissue but defective estrogen action (Table 1), aberrant ovulation, defective uterine growth and reduced fertility [94]. By contrast, transgenic E6AP overexpression reduces ER levels in mouse mammary tissue [90]. Src kinase accelerates estrogen dependent ER proteolysis [95]. Estrogen/progesterone stimulates rapid Src kinase activation, and Src kinase phosphorylates ER/PR to facilitate their binding to E6AP. This complex is then recruited to a subset of ER/PR target gene promoters, leading to their transcriptional activation [92]. The interaction between ER with E6AP also catalyzes rapid ER ubiquitylation in biochemical assays and in cells. Furthermore, the expression of a mutant (Y537F) ER results in increased ER stability but reduced binding to E6AP and reduced target gene activation [92]. This study was the first to indicate that the crosstalk between ER and a specific kinase could mediate ER phosphorylation to promote the recruitment of a dual-role co-activator that also drives ER degradation [92]. Although other studies have reported that ER Y537F is functional in ERE luciferase assays, such studies did not take into account the increased steady-state levels of ER Y537F when considering its transcriptional efficiency [96, 97]. These data support a model in which ER transcriptional activation can be coupled to receptor degradation as a mechanism to fine-tune ER action. The possibility also exists that Y537 phosphorylation could also modulate the interaction of ER with other ubiquitin ligase. These works suggests that receptor action and receptor levels are not synonymous. After ligand binding, ER transcriptional activity is maintained despite ongoing proteolysis and decreasing ER levels, introducing the possibility that hormonally sensitive tissues may not always have readily detectable levels of ER protein [96, 97].

4.6 Murine double minute clone 2 (MDM2)

MDM2 was initially cloned from a transformed 3T3 cell line, which is a single-subunit RING finger E3 protein, identified as a p53-interacting protein [98]. This multifunctional protein also promotes ER-mediated transcription and receptor proteolysis. Overexpression of MDM2 often occurs in breast cancer tissue and cell lines, but has not been shown to inversely correlate with ER levels. MDM2 functions as an ER co-activator [99] and can directly interact with ER in a ternary complex with p53 to regulate ER turnover [100]. Estrogen activates the cyclic co-recruitment of MDM2 and ER to the ERE motif of the target TFF1 promoter [93]. MDM2 was recently shown to bind to ER and increase ER-Sp1-mediated transcriptional activation in MCF-7 and ZR-75 breast cancer cells [98]. To date, the spectrum of ER target genes that are governed by the MDM2-ER interaction remains unknown. Furthermore, the relevance of this interaction to hormone-regulated cancers and its potential as a target for therapeutic intervention has not been explored.

4.7 Breast cancer 1 protein (BRCA1)

Germline mutations in BRCA1 predispose individuals to familial breast and ovarian cancers [101], and BRCA1 is involved in DNA repair [102]. BRCA1 binds to ER, and this complex has been postulated to have a role in DNA damage repair [103, 104]. BRCA1 can function as a transcriptional regulator [105], but it also binds to BARD1 to form a dimeric RING finger E3 ubiquitin ligase. Several lines of evidence suggest that BRCA1 functions as an E3 ligase for ER [106, 107]. ER is an *in vitro* substrate for the BRCA1-BARD1 ubiquitin ligase, and cancer-predisposing BRCA1 mutations that affect the RING motif abrogate its *in vitro* E3 ligase function towards ER [107, 108]. Although BRCA1-BARD1 can function as an E3 ligase *in vitro*, the effects of BRCA1 on ER transcriptional activity are controversial. It can both repress [109] and activate ER-mediated transcription in different cellular contexts [110]. BRCA1 can function as a co-repressor of ER-mediated transcription, but the

ectopic overexpression of either p300 or CBP reverses the inhibition of ER activity by BRCA1 [110]. Additional research has shown that estrogen-bound ER recruits BRCA1 into a transcriptional activation complex that contains the co-activator CBP [111], but the subset of ER target genes that are co-regulated by BRCA1 has not been fully defined. BRCA1 appears to function as either a co-activator or a co-repressor of other steroid receptors in different cellular contexts [112]. Most BRCA1-mutant breast cancers are ER negative [113]. This has been postulated to result from transcriptional repression of *Esr1* by mutant BRCA1 [114], whereas wild-type BRCA1 activates *Esr1*. Interestingly, estrogen action appears to contribute to breast cancer development in *Brca1*-mutant carriers, since the risk of BRCA1-mutant breast cancer is decreased by prophylactic oophorectomy and by tamoxifen treatment [113, 115, 116]. BRCA1 may serve a dual role as a co-activator and E3 ligase for ER to mediate constitutive estrogenic action, coupled to ER loss. This warrants further investigation since it would have substantial therapeutic implications.

5 Post-translational modifications of ER and PR upon activation

As the critical regulator for the reproductive process, both the ER and PR proteins were undergoing diverse modification responsive to many signaling transduction pathways in ligand-dependent and/or -independent manners. These covalent modification including phosphorylation, ubiquitination, methylation and other newly identified can influence the stability, subcellular localization, and/or affinity for the interact partners and many other activities, thus affecting these receptors' functions.

5.1 Ubiquitination

Ubiquitin is a small 76 amino-acid protein that can be reversibly attached to other proteins and lies at the core of an elaborate post-translational modification pathway. Ubiquitination of proteins is a sequential enzymatic cascade involving a ubiquitin activating enzyme (E1), a ubiquitin conjugating enzyme (E2) and a ubiquitin ligase (E3) [117, 118].

Nuclear receptors are common targets of ubiquitination. The E2 enzymes UBCH5 and UBCH7 (also known as UBE2L3) have proven to be critical for ER/PR receptor-dependent transcriptional activities [119]. PR activities were stimulated by the yeast E3 ubiquitin ligase RSP5, as well as its human homologs hRPF1 [120] and E6AP [89]. Co-expression of UBCH7 and E6AP enhance PR transcription synergistically, and SRC-1 functions as co-activator for PR also requires UBCH7 [121].

But the most common effect of PR poly-ubiquitination is ligand-dependent downregulation of nuclear receptor [122]. In breast cancer cells, the half-life of unliganded PR is about 21 h, but falls to about 6 h after ligand binding [123] due to their accelerated degradation by proteasomes [124]. Thus, like other transcription factors, PR degradation is closely linked to high activity. Besides targeting the receptors, proteasomal degradation also influences multiple other factors critical to transcriptional activity including RNA polymerase II, which was recruited to receptor-bound promoters [125].

Similarly, ER is rapidly ubiquitylated and degraded after estrogen binding [126]. Ligand binding rapidly signals ER ubiquitylation and ubiquitylated ER cycles on and off ERE promoter sites to activate target gene transcription [127]. Ubiquitin ligases MDM2 [100], SCF-SKP2 [128], and E6AP [89] promote estrogen-induced transcriptional activity. A number of other CUL-RING ligases have also been shown to govern ER stability, such as CUL4B [129], CUL5 [130], CUL7 [131]. In addition, some other ligases also function as steroid hormone receptor co-activators, such as ubiquitin-conjugating enzyme UBCH7 [121], thyroid hormone receptor-interacting protein 1 (TRIP1; also known as SUG1) [132] and so on. After ligand binding, ER transcriptional activity is maintained despite ongoing proteolysis and decreasing ER levels, introducing the possibility that hormonally sensitive tissues may not always have readily detectable levels of ER protein [133].

5.2 Sumoylation

Sumo proteins are about 10 kDa in size and resemble the three-dimensional structure of ubiquitin [134], functioning as a reversible post-translational protein modifier [135]. Unlike ubiquitin, sumoylation does not target proteins for degradation. Rather, sumoylation plays multiple roles in protein stabilization, subcellular localization, nuclear translocation, nuclear body formation and modulation (usually inhibition) of transcriptional activity [136].

PR sumoylation has a suppressive effect on transcription [137]. Sumoylated wild-type PRs have relatively low transcriptional activity compared to non-sumoylated mutants. Sumoylation of PR is especially important in

regulating activity of promoters with multiple PREs rather than promoters with a single PRE. PIAS1 functions as a sumo E3 ligase for PRs to inhibit their transcriptional activity and silencing of endogenous PIAS1 with siRNAs enhances the activity of wild-type PRs but has little effect on the activity of sumoylation-deficient PR mutants [138]. UBCH-9, acting as the E2 enzyme in the sumoylation cascade, also acts as a co-regulator through recruitment of co-activators [139].

5.3 Phosphorylation

Regulation of PR phosphorylation is complex in view of the multiple constitutive and ligand-stimulated sites [140]. PR-B, the longest of the human PR isoforms, are 933 amino acids in length and contain at least 14 phosphorylation sites; mostly at serine (Ser, S) residues located in the N-terminus [141]. Substitution of Ser294 in the amino-terminal domain by Ala decreases PR transcriptional activity by 50%–90% in a target gene specific manner [142], increases protein stability [143], and enhances PR sumoylation at K388 [144]. Interestingly, although this amino acid is common to both PR-B and PR-A, only the longer PR-B isoform is efficiently phosphorylated at this site [145]. Phosphorylation of Ser345 promotes association of PR with Sp1 in target genes that lack canonical PREs [146]; Ser81, 162, 190 and 400 are considered to be basal sites phosphorylated in the absence of hormones. Ser102, 294 and 345 are ligand-dependent sites phosphorylated 1–2 h after binding of hormones to the LBD [147, 148]. Specific kinases responsible for phosphorylation of select sites have been identified but others remain unknown. Ligand-dependent kinases include CDK2, MAPK, PKA and PKC [149, 150]. Functional roles for phospho-Ser345 and Ser400 have also been described. Ser345 for example, is phosphorylated by progestin-dependent rapid membrane signaling cascades that activate EGFR, c-Src and MAPK pathways and allow PR to target growth promoting genes that lack canonical PREs [146]. Since the phosphorylation state of individual sites may control the transcription of only a subset of endogenous genes, under restricted physiological conditions and in tissue specific ways, discovering the true *in vivo* function of every post-translational modification on a site-by-site basis is a prodigious task.

The AF-2 domain on the C-terminal of ER is phosphorylated and thus activated by ligand binding of estrogen; meanwhile, the N-terminus AF-1 is activated by phosphorylation at several residues [151]. Most post-translational modifications occur in the N-terminus upon ligand binding, and ligand-independent growth factor signaling pathways [152]. Substitution of Ser residues 104, 106 and 118 by Ala reduces transcriptional activity as measured by an ER responsive reporter [153] and reduces co-activation of AF-1 by co-activators like p160 and CBP [154]. Phosphorylation of the hinge site, Ser294, enhances ER activity measured by using a reporter [155], and p38 MAPK-mediated phosphorylation of Ser294 stimulates ubiquitination and turnover of ER [131]. Moreover, AKT-stimulated S167 phosphorylation can also mediate binding of ER with co-activator SRC-3 in the presence of estrogen; thus increasing ER transcription activity [156, 157]. Concerning ERβ in some contexts, Ser105Ala mutant shows reduced reporter activity relative to wild type form, and a Ser105Glu mutant exhibits enhanced reporter activity as well as the ability to reduce migration of breast cancer cells [158]. Two serine residues of ERβ phosphorylated by the MAPK pathway and leading to enhanced interaction with the co-activator SRC-1 in the absence of estrogen have been identified [159]. Still, functional study of human ERβ phosphorylation remains largely uncovered.

6 ER and PR in early pregnancy

6.1 ER in early pregnancy

The role of ERs was largely promoted by studies from the genetic mouse models. The ERα knockout (KO) mouse has a hypo plastic uterus and is infertile due to multiple defects including implantation failure [160]. In contrast, the ERβ KO mouse maintains normal implantation, further suggesting estrogen signals primarily via the ERα isoform in uterine function [161] (Table 1). In the mouse, early uterine responses to estrogen include transcription of early cell cycle genes, hyperemia, infiltration of immune cells and water imbibition into the uterine tissue. Later responses include the further infiltration of immune cells, increased uterine weight and the induction of late cell cycle genes resulting in robust DNA synthesis and mitosis of epithelial cells [162]. Consequently, uterine epithelium specific ERα KO displayed a significant increase in apoptosis. This evidence suggests the role of epithelial ER is to prevent epithelial apoptosis and ensuring a full epithelial response, while stromal ER is responsible for estrogen-driven epithelial proliferation [163]. These observa-

tions are consistent with the tissue reconstitution studies and collectively illustrate the differential roles for ER during implantation. Prior to the implantation occurs, an estrogen surge on day 4 in mice was indispensable for the successful implantation, which was similarly observed in the human beings [164]. Many studies have confirmed that the nidatory estrogen surge, mediated by ER, could induce glandular secretion of leukemia inhibitory factor (LIF), which is required to initiate the window of receptivity [165]. LIF expression is also high in humans around the time of implantation [164]. Clinical data has shown that endometria of women with unexplained infertility and multiple implantation failures often display significantly lower levels of LIF during the mid-secretory phase of their menstrual cycle when compared to healthy fertile controls [166].

6.2 PR in early pregnancy

PR regulation of gene expression occurs by direct binding of the receptor in the regulatory promoter regions of targets genes [167], and PR binding ChIP-Seq datasets were overlapped with microarray gene expression comparing genes significantly induced by acute progesterone treatment. This analysis confirmed PR binding on both up-regulated (*Gata2*, *Egfr*, *Ihh*, *Fkbp5*, *Areg*, *Hand2*) and down-regulated (*Pgr*, *Wnt7a*, *Lifr*) progesterone target genes. Expression of both PR isoforms is observed in the murine uterus, and ablation of both isoforms (PR KO) results in multiple reproductive abnormalities, including a hyperplastic response to estrogen and an implantation defect [168] (Table 1). However, specific ablation of PR-A (PR-A KO) and PR-B (PR-B KO) individually has shed light on the role of these isoforms in the mouse uterus. Ablation of PR-B resulted in no discernible uterine phenotype and displayed normal fertility. However, PR-B KO mice display reduced pregnancy-associated mammary gland morphogenesis, indicating PR-B is a major regulator of mammary gland maturation during pregnancy [169]. Ablation of PR-A phenocopies the PR KO mouse uterine phenotype and indicates that the PR-A isoform is the predominant functional isoform in the mouse uterus [27]. Collectively, these observations identify functional differences between these two isoforms in response to progesterone in the regulation of epithelial proliferation during early pregnancy. Vascular permeability is frequently associated with inflammation and triggered by a cohort of secreted permeability factors. Endothelial expressed PR mediates local vascular permeability in response to progesterone, and it is demonstrated that PR activation of NR4A1 (Nur77/TR3) triggers barrier instability in the endothelium [170]. In addition, aberrant activation of canonical Notch1 signaling in the mouse uterus decreases PR expression by promotor hypermethylation and leads to infertility [171] and MicroRNA-200a serves a key role in the decline of PR function leading to term and preterm labor [172].

The anti-proliferative action of progesterone in the endometrium has also been the focus of extensive research for its potential therapeutic role in regulating progression of estrogen-dependent pathologies; such as endometrial cancer and endometriosis [173]. Contrasting mechanisms have been proposed for the progesterone-mediated inhibition of estrogen inducing epithelial cell proliferation. One line of evidence supports a paracrine mechanism while the alternative proposes a direct role of PR in the uterine epithelial cells. The paracrine mechanism for the inhibition of epithelial cell proliferation was strongly supported when the basic helix-loop-helix transcription factor Hand2 was shown to play an essential role in the regulation of growth factor signaling in a progesterone-dependent manner, conditional ablation of Hand2 using the $PR^{cre/+}Hand2^{f/f}$ ($Hand2^{d/d}$) mouse model results in infertility due to an implantation defect and abnormal epithelium proliferation [174]. Collectively these results identified a complicated mechanism of stromal-epithelial crosstalk regulating proliferation during implantation.

Therefore, PR downstream signaling is not only required for proper embryo attachment, but also is important in the support and development of the implanted embryo in decidualization. Besides the critical function of PR during the implantation, PR was also the key regulator for the stromal cell differentiation during the decidualization. Knockout mice have been pivotal in demonstrating that members of the bone morphogenetic protein (BMP) and wingless related MMTV integration site (Wnt) family are critical for these processes within early pregnancy under the control of PR signaling [175]. Importantly, progesterone induces stromal cell decidualization in the late luteal phase and is essential for maintenance of the decidual phenotype in human [176]. Moreover, loss of PR expression in decidual cells at term is thought to cause functional progesterone withdrawal that triggers inflammation at the maternal-fetal interface leading to

parturition [177].

As discussed above, progesterone affects normal uterine function via a finely tuned and tissue/cell type specific balance between PR-A and PR-B mediated transcriptional activities. Most pathophysiological conditions of myometrium and endometrium are responsive to progesterone, albeit in an abnormal manner. PR-mediated progesterone actions vary according to the cell type.

7 Conclusions

This review illustrates the mechanisms of how ER and PR affect the expression of downstream genes. ER and PR both have the conservative structure of steroid receptors, including ligand-independent AF-1, DBD domain, LBD domain that contains a ligand-dependent AF-2. Possessing these special structure domains, ER and PR can play specific physiological functions in certain periods: mammogenesis, menstrual cycle, early pregnancy and so on. Activated ER and PR interact with various cofactors, not only widespread cofactors as SRCs, CBP/P300 and FOXA1, but also many ubiquitin ligases as E6AP, MDM2 and BRCA1 discovered in recent years. Additionally, post-translational modifications as mentioned ubiquitination, sumoylation and phosphorylation of ER and PR are essential for their activation. Recent advances have identified mechanisms that link the functions of ER and PR in early pregnancy.

As described above, *in vitro* analyses of the molecular biology of ER and PR have defined the mechanisms by which ER and PR regulate the transcription of specific target genes. Important to the understanding of the role of ER and PR in regulating uterine physiology is the identification of the specific target genes responding to hormones. Over the last decades, the mouse has emerged as a model system to investigate these hormone receptors in uterine biology *in vivo*. The mouse models will be used to better understand how the expression of the steroid hormone receptors is regulated in the uterus during pregnancy and the function of these receptors in regulating uterine biology.

Development of genetically engineered mouse models lacking ER, PR and their target genes has provided a wealth of information regarding the role of estrogen and progesterone regulated pathways in related diseases. ER and PR are widely distributed in various tissues and organs, especially in breast and uteri. Progesterone also could induce adult mammary stem cell expansion (MaSCs) during the reproductive cycle, where MaSCs are putative targets for cell transformation events leading to breast cancer [178, 179]. There are many subtypes of breast cancer, existing as ER-positive and ER-negative ones. Researches on the structure and functional mechanisms of ER can be correlated with clinical outcomes. ER signaling and its crosstalk with various signaling pathways have been clinically associated with poor clinical outcomes and resistance to anti-estrogen therapies. Therefore, affecting either kinases or phosphatases regulating ER might help in treating patients with resistance to these therapies.

Future prospective clinical sequencing studies with large cohorts of tumors refractory to different hormonal therapies will clarify the association of the mutations with mechanisms of endocrine resistance. Specific inhibition of site modification on ER will offer new ideas for the treatment of ER positive breast cancers. As such, next-generation anti-estrogens are currently being tested in preclinical and clinical settings with promising results. In addition to anti-estrogens, further structure modeling studies will contribute to a better understanding of the conformation of ER and determine whether peptide derivatives can be tested as alternative targeted therapies.

Finally, given the crucial role of co-activators in the ligand-independent activation of the ER and PR, compounds that targeting co-activators may prove be effective strategy in reversing ER and PR mutant-driven endocrine resistance in many cancers and reproductive defects.

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