

What's new in estrogen receptor action in the female reproductive tract

Sylvia C Hewitt¹, Wipawee Winuthayanon² and Kenneth S Korach¹

¹Receptor Biology Group, Reproductive and Developmental Biology Laboratory, National Institute of Environmental Health Sciences, National Institutes of Health, Research Triangle Park, North Carolina 27709, USA

²School of Molecular Biosciences, College of Veterinary Medicine, Washington State University, Pullman, Washington 99164, USA

Correspondence should be addressed to K S Korach
Email
korach@niehs.nih.gov

Abstract

Estrogen receptor alpha (ER α) is a critical player in development and function of the female reproductive system. Perturbations in ER α response can affect wide-ranging aspects of health in humans as well as in livestock and wildlife. Because of its long-known and broad impact, ER α mechanisms of action continue to be the focus on cutting-edge research efforts. Consequently, novel insights have greatly advanced understanding of every aspect of estrogen signaling. In this review, we attempt to briefly outline the current understanding of ER α mediated mechanisms in the context of the female reproductive system.

Keywords

- ▶ estrogen receptors
- ▶ gene expression
- ▶ gene regulation
- ▶ hormone action
- ▶ uterus/endometrium

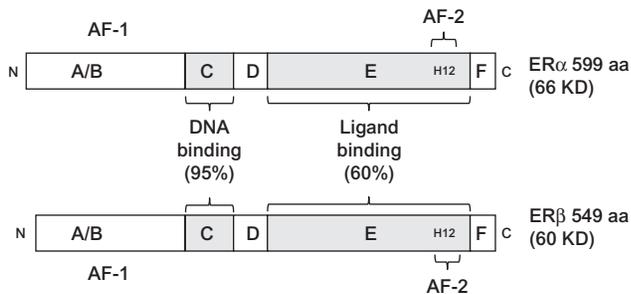
Journal of Molecular Endocrinology
(2016) **56**, R55–R71

Estrogen receptor

The vast majority of estrogen's activities are mediated by the estrogen receptor (ER), a member of the nuclear receptor family of hormone activated transcription factors. Our understanding of the physiological role of estrogen action has been greatly advanced by the generation of experimental mouse and rat models with knockout of receptors or coactivators either globally or in specific tissues and cells, or with knock-in expression of mutated forms of these molecules. These models, used in combination with microarray, RNA next generation sequencing (RNA-seq), and chromatin immunoprecipitation next generation sequencing (ChIP-seq) methods, allow comprehensive mapping of interaction of ERs with the chromatin landscape to impact genomic response. Together, these models and techniques have led to better understanding of the molecular details of ER roles in biological processes.

Estrogen receptor alpha (ER α) cDNA was the first described and cloned estrogen receptor (termed *ESR1* (ER α)) (Walter *et al.* 1985). A second ER gene, termed *ESR2* (ER β), was discovered in 1996 (Kuiper *et al.* 1996). ER α and ER β are not isoforms but rather distinct receptors encoded by two separate genes on different chromosomes. ER α is found on chromosome 6 in humans and chromosome 10 in mice. ER β is found on chromosome 14 in humans and chromosome 12 in mice. The ER α proteins are 595 and 599 amino acids in length in humans and mice respectively with an approximate molecular weight of 66 kDa (Fig. 1) (Heldring *et al.* 2007, Le Romancer *et al.* 2011, Gibson & Saunders 2012).

The *ESR2* encodes a receptor of 549 amino acids in rodents and 530 amino acids in humans, each with an approximate molecular weight of 60–63 kDa (Fig. 1) (Gibson & Saunders 2012). Therefore, ER β is slightly smaller than ER α , and most of these differences lie within the smaller N-terminus.

**Figure 1**

Structures of ER α and ER β protein with functional domains. Estrogen receptors ER α and ER β share a conserved domain structure. The A/B domain, at the amino terminus (N) of the protein contains AF-1. The C domain binds to DNA motifs called EREs. The D domain is called the hinge region, and contributes to DNA binding specificity and nuclear localization of the ERs. The E domain is called the ligand binding domain because it interacts with estrogen, through an arrangement of 11 α helices (H1, and H3 through H12). H12 in this region of the receptor is critical to mediating transcriptional activation via AF-2. At the carboxy terminus (C) is the F domain. The % homology shared between ER α and ER β in the C and E domains is shown.

Receptor structure

The estrogen receptors are composed of five functional domains (Fig. 1), an N-terminal domain (NTD) or A/B domain, the DNA-binding (DBD or C) domain, a hinge (D) region, LBD (LBD or E), and a C-terminal F domain (Laudet & Gronemeyer 2001, Aagaard *et al.* 2011, Hilser & Thompson 2011, Breliet *et al.* 2012, Helsen *et al.* 2012).

NTD or A/B domain

Crystallography of the ER NTD or A/B domain has been largely unsuccessful because this portion of the receptor is unstructured and fluctuates in aqueous solutions. However, evidence suggests that intramolecular interactions between the A/B and other receptor domains are likely to induce a more structured NTD (McEwan 2004, Aagaard *et al.* 2011, Hilser & Thompson 2011), as evidenced from recent cryogenic Electron Microscopy (cryo-EM) studies (Yi *et al.* 2015). Current models of ER signaling incorporate the flexibility of intrinsically disordered (ID) regions of the receptor, including the NTD, into a mechanism of allosteric interaction and coordination of ligand, DNA motif and ER domain functions (Aagaard *et al.* 2011, Hilser & Thompson 2011). The NTD contains the transcriptional activation function-1 (AF-1) domain and provides for cell- and promoter-specific activity of the receptor as well as a site for interaction with coreceptor proteins (Table 1). More recent

description of full-length ER α structure derived using cryo-EM indicates A/B domain is positioned near the LBD and facilitates recruitment of the steroid receptor transcriptional coactivator, SRC-3 (Yi *et al.* 2015). Post-translational modifications, such as phosphorylation, of the A/B domain can dramatically affect the overall behavior of the receptor and are thought to be an important mechanism for the modulation of AF-1 functions (Le Romancer *et al.* 2011).

DNA-binding or C domain

The C domain of the ER recognizes and binds to the cis-acting enhancer sequences, called estrogen responsive elements (EREs) (Helsen *et al.* 2012). The C domain contains two zinc fingers, each composed of four cysteine residues that chelate a single Zn² ion. Crystallography studies indicate a highly conserved structure consisting of dual α -helices positioned perpendicular to each other (Aagaard *et al.* 2011, Hilser & Thompson 2011, Helsen *et al.* 2012). Amino acids in the C-terminal 'knuckle' of the first zinc finger form the proximal box ('P-box') of the DNA binding domain and confer DNA sequence recognition specificity to the receptor for binding DNA sequences; hence, the proximal zinc finger is often referred as forming the 'recognition helix.' Amino acids at the N-terminal 'knuckle' of the second zinc finger form the distal box ('D-box') and are more specifically involved in differentiating the 'spacer' sequence within the ERE as well as providing a secondary interface for receptor dimerization.

The consensus motif (ERE) that ER binds is composed of a six-base pair (bp) palindromic sequence arranged as an inverted repeat and separated by a three-bp spacer, GGTCAnnnTGACC. The inverted-repeat arrangement of the ERE dictates that the ER homodimerizes in a 'head-to-head' position when bound to DNA. Structural analysis has revealed the importance of the 10–30 amino acid carboxy terminal extension (CTE) of the DBD in DNA interaction (Aagaard *et al.* 2011, Hilser & Thompson 2011, Helsen *et al.* 2012). Although this CTE region is variable between steroid receptors, it is crucial for DNA binding, particularly for sequence selectivity of DNA binding, by extending the interaction surfaces between the receptor and the DNA.

Hinge region or D domain

The above described CTE extends into the hinge region, which also contains a nuclear localization signal, and influences cellular compartmentalization of ER, as well as

Table 1 ER coregulator complexes. Adapted, with permission, from Binder AK, Winuthayanon W, Hewitt SC, Couse JF & Korach KS (2015) Steroid receptors in the uterus and ovary. In *Knobil and Neill's Physiology of Reproduction*, 4th Edn, pp 1099–1193. Eds TM Plant & AJ Zeleznik. Elsevier

Complex	Functions	Comments	References
Src1, Src2, Src3	Interact with Helix12 of agonist bound ER, interact with SWI/SNF, histone modifiers		Hsia <i>et al.</i> (2010) and Johnson & O'Malley (2012)
Mediator	'Bridges' ER and transcriptional 'machinery' (RNA Pol II) to control transcription	Made up of > 20 subunits, MED 1–31, arranged in three modules (head, middle, tail)	Malik & Roeder (2010) and Conaway & Conaway (2011)
SWI/SNF	Regulate access to enhancer sequences via chromatin remodeling, ATPase activity	Made up of 9+ subunits, examples include BRG1, BRM, BAF subunits	Roberts & Orkin (2004)
Histone modifiers	Modify histones to increase or decrease transcription	Acetyl transferase (HAT; e.g., p300/CBP), deacetylase (HDAC; e.g., NCoR), methyl transferase (e.g., PMRT/CARM), de-methylase	Barnes <i>et al.</i> (2005) and Wu & Zhang (2009)
26S proteasome	'Clears' transcriptional modulatory proteins to facilitate subsequent transcription, transcriptional termination	Structure made up of 20S catalytic core particles (CP), 19S regulatory particles (RP)	Keppler <i>et al.</i> (2011) and Kim <i>et al.</i> (2011a,b)

sites of post-translation modifications (Kim *et al.* 2006). Current mechanisms suggest this non-conserved and ID domain is important for intra-molecular allosteric interactions involving the N-terminal and LBD. This type of flexible structural interaction works to allow rapid response to diverse modulators governing changes in biological environments (Kumar & McEwan 2012).

LBD or E domain

The LBD or E domain of the ER is a highly structured multifunctional region that primarily serves to specifically bind estrogen and provide for hormone-dependent transcriptional activity through an activation function 2 (AF-2) domain located close to the C-terminus of the E domain. A strong receptor dimerization interface, sites for interaction with heat shock proteins, and nuclear localization signals are also within the E domain (Laudet & Gronemeyer 2001, Kumar & McEwan 2012). Structural studies indicate that the LBD is composed of 11 α -helices (H1, and H3 through H12) arranged in a three-layer α -helical sandwich to create a hydrophobic ligand-binding pocket near the C-terminus of the receptor (Huang *et al.* 2010). Receptor binding to an estrogen agonist leads to rearrangement of the LBD such that H11 is repositioned and H12 rotates back toward the core of the domain to form a 'lid' over the binding pocket. This agonist-induced repositioning of H12 leads to the formation of a hydrophobic cleft, or 'NR box,' by helices 3, 4, and 5 on the receptor surface, constituting the AF-2, which serves to recruit coactivators (Table 1) to the receptor complex.

In contrast, estrogen antagonists are unable to induce a similar repositioning of H12, leading to a receptor formation that is incompatible with coactivator recruitment and is therefore less likely to activate transcription. The LBDs of ER α and ER β exhibit ~60% homology (Fig. 1) but bind the endogenous estrogen, estradiol (E₂), with similar affinity (ER α , 0.1 nM; ER β , 0.4 nM) (Le Romancer *et al.* 2011, Gibson & Saunders 2012) indicating only a small portion of the LBD sequence governs the specificity of ligand binding. However, given the divergence in homology, it is not surprising that ER α and ER β exhibit measurable differences in their affinity for other endogenous steroids and xenoestrogens (Le Romancer *et al.* 2011, Gibson & Saunders 2012). Natural and synthetic steroidal and non-steroidal ER agonists and antagonists have been described, some of which show specificity or preference for one or the other ER subtype, illustrating differences between the LBDs of ER α and ER β and provide for conceptual pharmacological tools to discern the overall function of each ER. The most widely used ER sub-type selective ligands currently in use are propylpyrazole (PPT), an ER α selective agonist, and diarylpropionitrile (DPN), an agonist showing preference, but not exclusive selectivity, towards ER β (Stauffer *et al.* 2000, Meyers *et al.* 2001).

F domain

Among the sex steroid receptors, only ERs possess a well-defined F domain (Fig. 1). This region is relatively unstructured with little known function, although some data indicate a role in coactivator recruitment,

dimerization and receptor stability (Katzenellenbogen *et al.* 2000, Koide *et al.* 2007, Yang *et al.* 2008, Kumar *et al.* 2011, Arao *et al.* 2013).

Coregulatory complexes

All steroid receptors interact with coregulatory molecules, coactivators, and corepressors (Hsia *et al.* 2010, George *et al.* 2011). The primary coactivator interaction for steroid receptors is with a family of p160/SRC (steroid receptor coactivator) 1, 2, and 3 coactivators (Lonard & O'Malley 2005, Bulynko & O'Malley 2011, Johnson & O'Malley 2012). SRC1 (NCOA1), SRC2 (GRIP1 and TIF2), and SRC3 (pCIP, RAC3, ACTR, TRAM, and A1B1) interact with helix 12 of ERs via 'LXXLL' motifs in their nuclear receptor interacting domains, which are leucine rich regions with 'X' designating any amino acid (Johnson & O'Malley 2012). SRCs also contain activation domains that recruit secondary molecules such as p300, and a bHLH-PAS motif within the N-terminal region, which can interact with other transcription factors (Johnson & O'Malley 2012). ERs and SRCs function as a nexus interacting with massive multimeric complexes, including the SWI/SNF chromatin remodeler, mediator complex, or proteasomes (Table 1) (Bulynko & O'Malley 2011). These interactions coordinate the specific functions necessary to allow appropriate gene and cell selective access to chromatin, via modifications of histones or members of coregulatory complexes (O'Malley *et al.* 2012). In this way, coactivators dynamically mediate and coordinate processes necessary to accomplish transcription, including initiation, elongation, termination, and clearing or turnover of the transcriptional modulators.

Mechanisms of estrogen response

Our understanding of the mechanisms by which estrogens influence cell function and behavior has expanded profoundly since initial models of ligand-dependent activation, which is now referred to as the 'classical' or ligand dependent direct DNA binding model of receptor function (Fig. 2). In the years since, numerous discoveries primarily in cell-based systems have been made that illuminate the complexity of ER signaling in cells and tissues. The entrée into the 'omics' era has facilitated massive expansion for the study of transcriptional regulation and chromatin remodeling. In addition, several alternative receptor signaling mechanisms that diverge from the classic model have become apparent, including 'tethering' of the ER to heterologous DNA-bound

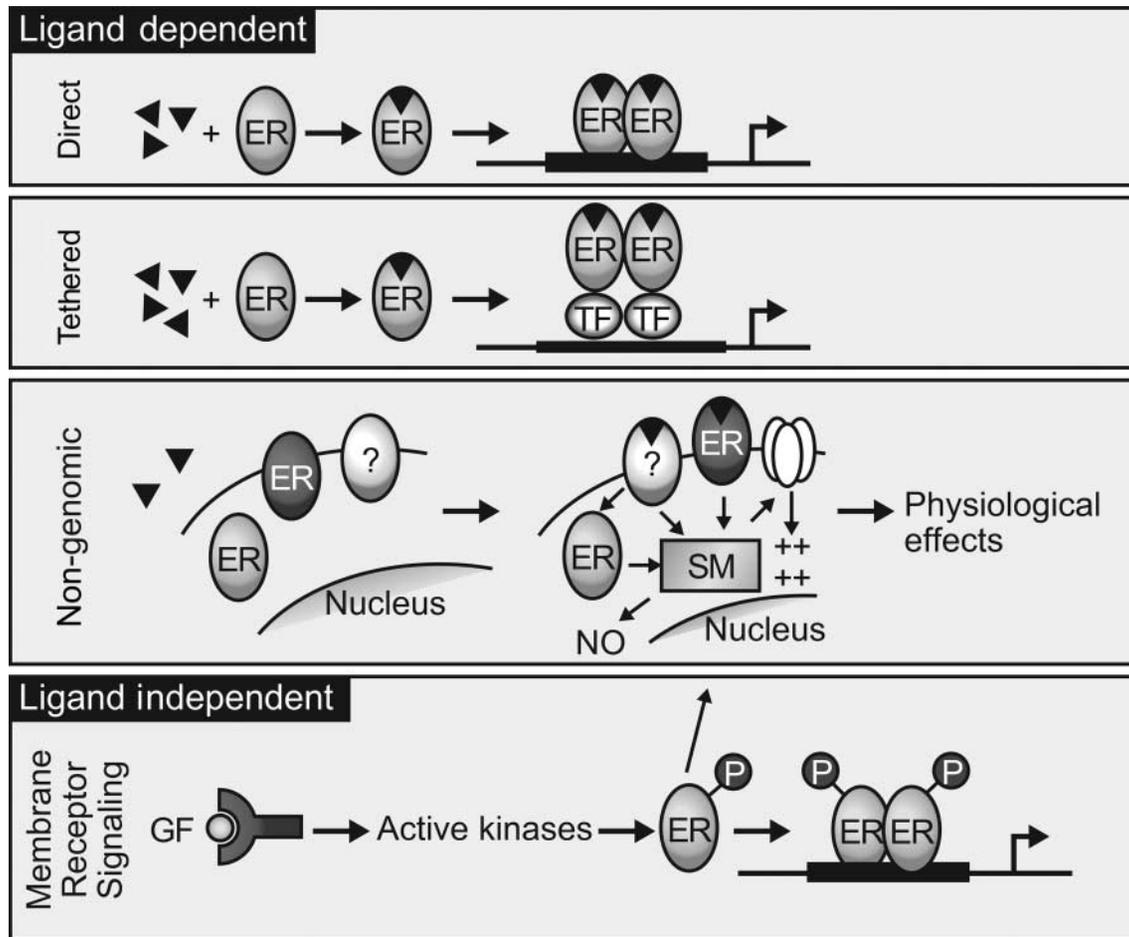
transcription factors to provide for regulation of genes that lack ERE sequences (Fig. 2); plasma membrane estrogen signaling, often referred to as 'nongenomic' steroid actions and ligand-independent 'cross-talk' with intracellular and second messenger systems that provide for ER activation in the absence of the cognate steroid ligand (Fig. 2). These modes of ER responses as currently understood are discussed below.

Ligand-dependent actions: direct or classical

In the classic model of estrogen response (Figs 2 and 3) estrogen ligands diffuse across the plasma and nuclear membranes to bind ER, primarily localized to the nucleus, resulting in a conformational change in the receptor, transforming it to an 'activated' state that interacts with chromatin via ERE motifs and transcriptional mediators. ERs seem to be preferentially recruited to open regions of chromatin (Biddie *et al.* 2010). Studies using MCF7 breast cancer cells indicate that FoxA1 acts as a pioneering factor, providing accessible regions in the chromatin that recruit ER α (Fig. 3) (Carroll *et al.* 2005, Carroll & Brown 2006, Fu *et al.* 2011, Zaret & Carroll 2011). The ligand-ERE-bound receptor complex then engages coactivator molecules as described above (Johnson & O'Malley 2012) leading to modulation of transcription rates of responding genes. This classic steroid receptor mechanism is dependent on the functions of both AF-1 and AF-2 domains of the receptor, which synergize via the recruitment of coactivator proteins, most notably the p160 family members (Johnson & O'Malley 2012). Depending on the cell and target gene promoter context, the DNA-bound receptor complex may positively or negatively affect expression of the downstream target gene. Initially, study of ER mediated gene regulation was carried out on a gene-by-gene basis using a handful of known hormone regulated transcripts. Now, after numerous comprehensive analyses of hormonally regulated transcriptional profiles, using microarray and more recently RNA-seq, thousands of ER targets have been found in various cell lines and tissues.

Indirect/tethered actions (ERE independent)

In *in vitro* reporter gene systems, ligand-activated ER can modulate the expression of genes that lack a conspicuous ERE within their promoter (Kushner *et al.* 2000, Safe & Kim 2004, 2008). This mechanism of ERE-independent steroid receptor activation is postulated to involve a 'tethering' of the ligand-activated receptor to transcription factors that are directly bound to DNA via their respective response

**Figure 2**

Ligand-dependent and ligand-independent nuclear receptor mechanisms. The direct 'classic' model of ER action involves direct interaction between ER bound to estrogen (triangles) and ERE; the tethered pathway utilizes indirect 'tethering' of ER to genes via interactions with other transcription factors (TF). 'Nongenomic' signaling is initiated by membrane-localized receptors modulating extranuclear second messenger (SM) signaling pathways. Ligand-independent responses occur as a result of transduction

elements (Fig. 2). However, the ER $\alpha^{EAAE/EAAE}$ mouse, which is mutated in the ER α DBD and lacks ERE binding, does not exhibit estrogen response *in vivo*, indicating the tethering mechanism, at least on its own, is unable to mediate hormonal responses (Ahlbory-Dieker *et al.* 2009, Hewitt *et al.* 2014) and is likely complimentary to the direct DNA stimulated responses.

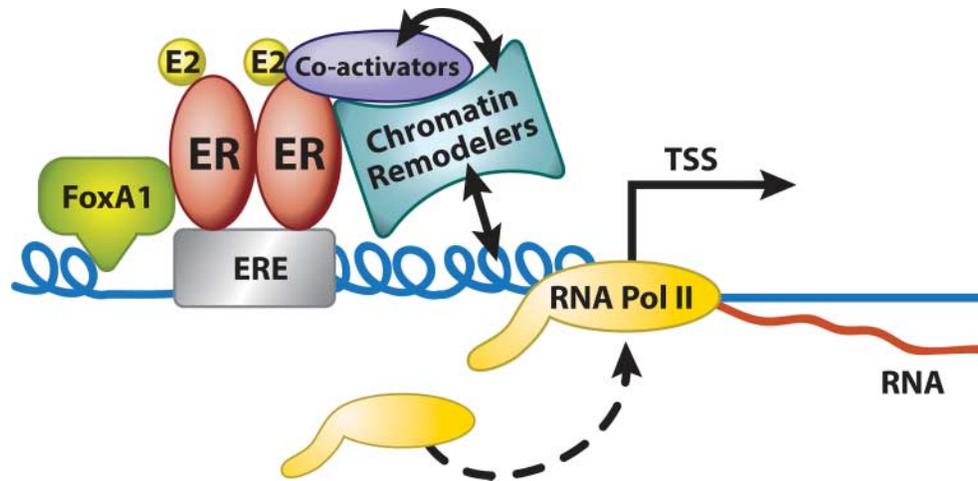
Non-genomic actions

Rapid effects of E₂ have been described, including a rapid activation of endothelial nitric oxide synthase in endothelial cells (Levin 2011) and potentiation of nerve conductance (Takeo & Sakuma 1995, Kim *et al.* 2011a,b).

of membrane receptor signaling, such as growth factors (GF), to nuclear ER. Adapted, with permission, from Heldring N, Pike A, Andersson S, Matthews J, Cheng G, Hartman J, Tujague M, Ström A, Treuter E, Warner M & Gustafsson JA (2007) Estrogen receptors: how do they signal and what are their targets. *Physiological Reviews* 87 905–931. Copyright 2007 The American Physiological Society (APS). All rights reserved.

Because these estrogen effects occur within minutes, they have been thought not to involve direct ER activation of gene transcription, they are often collectively referred to as representing 'non-genomic' pathways of estrogen action. Questions remain concerning whether the membrane-associated receptors mediating these events are identical or variant forms of the ER or instead distinct receptors altogether.

One potential mediator of rapid membrane localized hormone response is the G protein coupled ER (GPER, originally referred to as GPR30), which is activated by E₂ (Prossnitz & Barton 2011). *Gper* null mice lack reproductive phenotypes (Langer *et al.* 2010), although effects on the degrees of uterine responses elicited by E₂ have been

**Figure 3**

Model of chromatin dynamics in ER mediated transcription. FoxA1 interacts with chromatin, providing access for ER to nearby EREs. ER then interacts with transcriptional coactivators and chromatin modifying enzymes to open up transcription start sites (TSS) for RNA polymerase II (PolII), allowing

initiation of transcription. Adapted, with permission, from Wall EH, Hewitt SC, Case LK, Lin CY, Korach KS & Teuscher C (2014) The role of genetics in estrogen responses: a critical piece of an intricate puzzle. *FASEB Journal* 28 5042–5054.

observed with G15, a GPER selective antagonist, suggesting a potential role for GPER in modulating ER α mediated responsiveness (Gao *et al.* 2011).

Ligand independent actions: membrane receptor cross-talk

Peptide growth factors are able to activate ER α -mediated gene expression via mitogen-activated protein kinase activation of ER α in the absence of E₂ (Fig. 2). Likewise, growth factors are able to mimic the effects of E₂ in the rodent uterus via E₂ independent activation of ER α (Curtis & Korach 1999, Fox *et al.* 2009). In some cases, the MAP kinase protein ERK is corecruited to chromatin with ER α (Madak-Erdogan *et al.* 2011). Ligand-independent activation of estrogen receptors is believed to rely largely on cellular kinase pathways that alter the phosphorylation state of the receptor and/or its associated proteins (e.g., coactivators, heat shock proteins) (Fig. 2).

Uterine response to E₂

Utilizing animal models to follow and manipulate estrogen responsiveness is one way to understand and describe mechanisms of estrogen responses. The reproductive function of the mouse has been especially well studied and characterized in this manner.

Treatment of ovariectomized mice with estrogens (e.g., E₂ or diethylstilbestrol – DES) has long served as an

experimental model to mimic the uterine events that occur during the estrous phase of the rodent cycle or immediately after the preovulatory E₂ surge. Morphological and biochemical changes occur in the rodent uterus after estrogen stimulation following an established biphasic temporal pattern (Hewitt *et al.* 2003). Estrogen-stimulated changes in the rodent uterus that occur early, within the first 6 h after treatment, include increases in nuclear ER occupancy, water imbibition, vascular permeability and hyperemia, prostaglandin release, glucose metabolism, eosinophil infiltration, gene expression (e.g., *c-fos*), lipid and protein synthesis. ER α ChIP-Seq profiles from *in vivo* studies of uterine tissues show that in the unstimulated state the receptor pre-occupies chromatin sites in the absence of hormone and that E₂ treatment increases ER α recruitment (Hewitt *et al.* 2012). The above processes are followed by responses that peak after 24–72 h and include dramatic increases in RNA and DNA synthesis, epithelial proliferation, and differentiation of epithelial cells toward a more columnar secretory phenotype, dramatic increases in uterine weight, and continued gene expression.

Changes in uterine gene expression

The dramatic physiological changes that occur in the uterus in response to steroid hormones are presumably the ultimate effects of equally dramatic changes in gene expression among the uterine cells. It is unlikely that the

E₂-ER complex is directly involved in mediating the whole genomic response in the uterus but more plausibly serves to stimulate a cascade of downstream signaling pathways that act to amplify the estrogen action. However, early investigations of the genomic response to estrogens in the rodent uterus discovered a handful of genes that are directly regulated via the classic ER mode of action, including progesterone receptor (*Pgr*) and lactoferrin or lactotransferrin (*Ltf*). Microarray analysis has significantly advanced understanding of genomic response of the rodent uterus to E₂. Numerous studies have used microarray techniques to map the global gene expression patterns after estrogen exposure in the uterus and largely demonstrate that the biphasic uterine response to estrogens, so well characterized by physiological indicators above, is mirrored by the global changes in gene expression (Andrade *et al.* 2002, Fertuck *et al.* 2003, Hewitt *et al.* 2003, Watanabe *et al.* 2003, Ho Hong *et al.* 2004, Moggs *et al.* 2004, Hewitt *et al.* 2005, Hong *et al.* 2006). The clearly defined patterns of early and late response genes found in mouse uterine tissues are completely lacking in ER α -null (α ERKO, Ex3 α ERKO) uteri (Hewitt *et al.* 2003, 2010a,b). The identified genes fall into functional groups, including signal transduction, gene transcription, metabolism, protein synthesis and processing, immune function, and cell cycle. The expression levels of a striking number of genes are actively repressed by estrogen in the mouse uterus, and these effects were absent in ER α -null uteri or are relieved by cotreatment with ER antagonists in the presence of ER α , indicating that ER α is also actively involved in transcriptional repression as part of mediating the physiological responses (Hewitt *et al.* 2003, 2010a,b).

Whole transcriptome analyses are now routinely incorporated into studies of disruptions in signaling pathways underlying uterine phenotypes of mouse models such as those described in Table 2. Thus, microarray comparisons have now become just one of many tools employed for investigation of uterine functions.

Chip-seq

Evaluation of sites of transcription factor interaction with chromatin, by enriching a DNA binding protein, such as ER α , that has been crosslinked *in situ* to chromatin, with immunoprecipitation (ChIP), followed by hybridizing the associated DNA to a chip tiled with promoter region sequences (ChIP-Chip) or by 'next generation' massively parallel sequencing (ChIP-seq), have been developed and widely utilized to study sites of ER interaction (Farnham 2009, Park 2009, Biddie *et al.* 2010, Green & Han 2011,

Martens *et al.* 2011, Meyer *et al.* 2012). Initial studies focused on ER α binding in MCF7 breast cancer cells, and several similar studies followed, which are summarized and compared in several review articles (Deblois & Giguere 2008, Cheung & Kraus 2010, Gao & Dahlman-Wright 2011, Tang *et al.* 2011, Gilfillan *et al.* 2012). These articles reported that most sites were distal from transcriptional start sites (TSS), or were in intronic regions, rather than adjacent to TSS, as models of ER regulation of target transcripts had hypothesized. These comprehensive maps of cis-acting transcriptional regulators have been dubbed 'cistromes.' The initial ER α cistrome-associated sequences were evaluated for enrichment of transcription factor motifs and confirmed binding to the experimentally defined 'ERE' sequence. In the case of the MCF7 tumor cells, enrichment of motifs for forkhead binding factors (Fox) was apparent as mentioned in the earlier section. Owing to the abundant expression of the FoxA1 member of the Fox family, a potential role for FoxA1 in estrogen response was pursued with an arsenal of bioinformatic, Next Gen sequencing and biological studies that demonstrated FoxA1's role as 'pioneer,' creating accessible regions of the chromatin that were subsequently targeted by ER α (Lupien *et al.* 2009, Zaret & Carroll 2011).

ChIP-seq analysis is examining the ER α binding sites in mouse uterine tissue indicated that, much like the MCF7 breast cancer study, most ER α sites were not proximal to TSS (Hewitt *et al.* 2012). ERs bind to thousands of sites within the cellular chromatin, and not all potential EREs in every cell bind ER. Rather, it is apparent that chromatin exhibits 'pre-opened' regions destined to recruit ER (Grontved & Hager 2012). For ER in MCF7 and FoxA1 can establish ER accessible regions. The accessible chromatin regions are colocalized within nuclear 'hubs,' which seem to optimize frequency of interaction with ER (Grontved & Hager 2012). ChIP-seq is also used to locate other molecules involved in chromatin remodeling and transcriptional regulation, and to examine activating or repressive histone modifications or 'marks.' These maps of relative locations and dynamics of ER and chromatin components greatly enhance our understanding of hormone response mechanisms (Deblois & Giguere 2008, Green & Han 2011, Martens *et al.* 2011, Gilfillan *et al.* 2012, Meyer *et al.* 2012).

Uterine phenotypes in mouse models of disrupted estrogen signaling

Mouse models of disrupted ER signaling have proven invaluable to experimental investigation of estrogen

Table 2 Uterine phenotypes in mice null or mutated for estrogen receptors or estrogen signaling. Adapted, with permission, from Binder AK, Winuthayanon W, Hewitt SC, Couse JF & Korach KS (2015) Steroid receptors in the uterus and ovary. In *Knobil and Neill's Physiology of Reproduction*, 4th Edn, pp 1099–1193. Eds TM Plant & AJ Zeleznik. Elsevier

Mutated or null for sex steroid receptors and signaling	Uterine phenotypes	References
<i>Esr1</i> ^{-/-} (homozygous null alleles for ER α : α ERKO and Ex3 α ERKO)	Normal uterine development but exhibits hypoplastic uteri Insensitive to the proliferative and differentiating effects of endogenous, growth factors and exogenous E ₂ Implantation defect ^a Lack decidualization Infertile	Lubahn <i>et al.</i> (1993), Curtis <i>et al.</i> (1999), Dupont <i>et al.</i> (2000), Curtis Hewitt <i>et al.</i> (2002), Hewitt <i>et al.</i> (2010a,b), Antonson <i>et al.</i> (2012)
<i>NERKI</i> ^{+/-} (one mutated allele of two-point mutation in ER α DBD and one WT allele)	Normal uterine development but exhibits hyperplastic uteri Hypersensitive to estrogen Infertile	Jakacka <i>et al.</i> (2002)
<i>KIKO</i> (<i>ER</i> ^{AA/-}) (one mutated allele of two-point mutation in DNA binding domain of ER α and one ER α KO allele)	Normal uterine development Insensitive to the proliferative effects of exogenous E ₂ treatment ER ^{AA} binds HRE and induces genes that are normally progesterone responsive Infertile	O'Brien <i>et al.</i> (2006) and Hewitt <i>et al.</i> (2010a,b)
<i>ER</i> α ^{EAAE/EAAE} (homozygous animal of four-point mutation of DBD ER α)	Normal uterine development but exhibits hypoplastic uteri Loss of E ₂ -induced uterine transcripts Infertile	Ahlbory-Dieker <i>et al.</i> (2009)
<i>ER</i> α AF-1 ^o (deletion of amino acids 2–128 on ER α)	Normal uterine development and architecture Blunted E ₂ response Infertile	Billon-Gales <i>et al.</i> (2009) and Abot <i>et al.</i> (2013)
<i>ER</i> α AF-2 ^o (deletion of amino acids 543–549 on ER α)	Normal uterine development but exhibits hypoplastic uteri Insensitive to E ₂ treatment Infertile	Billon-Gales <i>et al.</i> (2011)
<i>ENERKI</i> (<i>ER</i> α ^{G525L}) (homozygous animal of one point mutation in LBD of ER α)	Normal uterine development but exhibits hypoplastic uteri Insensitive to E ₂ treatment IGF1 induced slight uterine epithelial proliferation compared to control littermates (non-homogenous pattern) Infertile	Sinkevicius <i>et al.</i> (2008)
<i>AF2ER</i> ^{KIKI} (homozygous knock-in of two-point mutation in LBD of ER α)	Normal uterine development but exhibits hypoplastic uteri Insensitive to E ₂ treatment ER antagonists and partial agonist (ICI 182,780 and TAM) induced uterine epithelial proliferation Growth factor did not induce the uterine epithelial cell proliferation Infertile	Arao <i>et al.</i> (2011)
<i>ER</i> α <i>Epi-cKO</i> (epithelial cell specific deletion of ER α using <i>Wnt7a</i> ^{Cre+} ; <i>Esr1</i> ^{fl/fl} mouse model)	Normal uterine development Sensitive to E ₂ - and growth factor-induced epithelial cell proliferation Lack full uterine growth response to E ₂ Selective loss of E ₂ -target gene response Implantation and decidualization defects Infertile	Winuthayanon <i>et al.</i> (2010 2014) and Pawar <i>et al.</i> (2015)
<i>Esr1</i> ^{ald} (uterine deletion of ER α using <i>Pgr</i> ^{Cre+} ; <i>Esr1</i> ^{fl/fl} mouse model)	Normal uterine development Hypoplastic uteri Defective decidual response	Pawar <i>et al.</i> (2015)
<i>Esr2</i> ^{-/-} (homozygous null alleles for ER β : β ERKO, Ex3 β ERKO, and ^b ER β _{ST} ^{L-/L-})	Exhibit grossly normal uterine development and function Sensitive to E ₂ treatment Some <i>Esr2</i> ^{-/-} lines reported elevated uterine epithelial proliferation after E treatment compared with WT Some are complete sterile (due to ovarian phenotype)	Krege <i>et al.</i> (1998), Dupont <i>et al.</i> (2000), Wada-Hiraike <i>et al.</i> (2006) and Antal <i>et al.</i> (2008)
$\alpha\beta$ ERKO (homozygous null for both ER α and ER β)	Normal uterine development but exhibit hypoplastic uteri, similar to those of <i>Esr1</i> ^{-/-} . Insensitive to E ₂ , infertile	Couse <i>et al.</i> (1999) and Dupont <i>et al.</i> (2000)
<i>Cyp19a1</i> ^{-/-} (homozygous null aromatase: ArKO)	Normal uterine development but exhibits hypoplastic uteri Sensitive to E ₂ -induced epithelial cell proliferation Infertile	Fisher <i>et al.</i> (1998) and Toda <i>et al.</i> (2001)
<i>Esr1</i> C541A palmitoylation deficient mutants	C451A-ER α normal uterine development, E ₂ growth response Nuclear-only ER α [NOER] hypoplastic ER α -null like uterus	Adlanmerini <i>et al.</i> (2014) and Pedram <i>et al.</i> (2014)

^a α ERKO females have a similar uterine phenotype to the newer Ex3 α ERKO except for maintaining decidualization response, which may due to the splice variants in the original α ERKO that retains ER activities.

^bER β _{ST}^{L-/L-} females are the only line of ER β knockout animals that reported to be completely sterile.

actions and the contribution of each ER form to these functions (Table 2). In addition to the ER-null models are lines of mice that lack the capacity to synthesize E₂ due to disruption of the *Cyp19* gene (Fisher *et al.* 1998, Toda *et al.* 2001). Below we will describe how these different mouse models have helped to delineate the biological role of ER mechanisms in estrogen hormone action.

ER α null patients and mice

Only one male patient and one female patient with ER α mutation have been described (Smith *et al.* 1994, Quaynor *et al.* 2013). The male patient's mutation is a true null since no ER α protein is expressed due to the mutation generating a premature stop codon in the A/B domain. The female patient has a single point mutation in her ER α LBD that results in decreased activity by reducing the receptors affinity for coactivator proteins more than 200-fold.

There are currently numerous reported lines of ER α -null mice and additional lines of mice with mutations in functional domains of ER α . Three separate lines of ER α -null mice were generated: the α ERKO, first described by Lubahn *et al.* (1993), the ER α KO (or Ex3 α ERKO), described by Dupont *et al.* (2000) and by Hewitt *et al.* (2010a,b), and ER α ^{-/-} described by Antonson *et al.* (2012). Homologous recombination was employed to disrupt ER α (α ERKO), or cre-mediated recombination was used to completely excise exon 3, which encodes the ER DNA binding domain (Dupont *et al.* 2000, Hewitt *et al.* 2010a,b, Antonson *et al.* 2012) of the murine *Esr1* (ER α) gene (ER α KO, Ex3 α ERKO, and ER α ^{-/-}). The uterine estrogenic response in α ERKO females differs from the latter two lines, but the overall spectrum of phenotypes are the same, as α ERKO animals have minimal level of truncated ER α protein produced from a splice variant, which preserves some residual biological functions (Couse *et al.* 1995), but all ER α null female mice are infertile. Recently, an ER α null rat has been derived using zinc finger nuclease (ZFN) genome editing. All phenotypes in the ER α null rats examined thus far were previously seen in the ER α null mice, including infertility due to hypoplastic uteri, polycystic ovaries, and ovulation defects (Rumi *et al.* 2014). The female patient with homozygous ER α mutation also has cystic ovaries and a small uterus despite elevated circulating serum E₂ (Quaynor *et al.* 2013).

The essential role of ER α in uterine response to estrogen is indicated by the loss of early phase effects of water imbibition and hyperemia as well as the late-phase effects of increased DNA synthesis and epithelial proliferation in ER α -null uteri (Couse *et al.* 1995, Korach *et al.*

1996, Hewitt *et al.* 2010a,b). The α ERKO model was the first test of a prevailing hypothesis that early uterine effects were non-receptor mediated (Lubahn *et al.* 1993). Lack of these early responses of water imbibition, hyperemia, and eosinophil infiltration in α ERKO indicated that ER α was involved in some manner and these responses clearly require the estrogen receptor. Additionally, ovariectomized mice normally exhibit a three- to four-fold increase in uterine weight after three daily treatments with E₂ or DES, whereas no such response is observed in the uteri of ER α -null females (Lubahn *et al.* 1993, Korach 1994, Hewitt *et al.* 2010a,b). Uteri of mice that lack ER α just in uterine epithelial cells (*Wnt7a*^{Cre+}; *Esr1*^{f/f}, called ER α Epi-cKO) have an initial proliferative response to estrogen, but full uterine response is impaired, as the growth after 3 days of estrogen treatment is significantly less than expected (Winuthayanon *et al.* 2010). The total lack of response to estrogens in ER α -null uteri as well as a lack of late biological response in epithelial ER α knockout uteri provide strong evidence that ER α is required to mediate the full biochemical and biological uterine response to estrogens (Hewitt *et al.* 2010a,b, Winuthayanon *et al.* 2010, 2014).

Numerous studies have demonstrated some of the molecular mechanisms of E₂-induced uterine epithelial cell proliferative responses in animal models. The transcription factor CCAAT enhancer binding protein beta (C/EBP β) is involved in hormone-induced uterine proliferation (Mantena *et al.* 2006). Maximum uterine expression of C/EBP β is induced 1 h after E₂ treatment in both epithelial and stromal cells (Mantena *et al.* 2006, Ramathal *et al.* 2010). ICI 182,786 (ER antagonist) strongly inhibited E₂-induced *Cebpb* transcript in the uterus suggesting an ER-dependent expression of C/EBP β (Bagchi *et al.* 2006). In addition, loss of epithelial ER α in the uterus did not alter E₂-induced *Cebpb* expression, indicating that *Cebpb* expression is independent of epithelial ER (Winuthayanon *et al.* 2010), and suggesting the stimulation was through a paracrine mechanism via stromal ER α . This points to the action of estrogen through ER α as the major mediator of C/EBP β expression in the uterus. Indeed, the deletion of C/EBP β (C/EBP β ^{-/-}) leads to a lack of the E-induced uterine proliferative response (Mantena *et al.* 2006) as reflected by the absence of mitotic activity, S-phase activity and an increase in apoptotic activity in the uterine epithelial cells (Ramathal *et al.* 2010). In addition to a blunted uterine growth response to hormones, the C/EBP β ^{-/-} females also exhibit complete infertility (Bagchi *et al.* 2006), due to implantation and decidualization defects (Mantena *et al.* 2006).

Pan *et al.* (2006) demonstrated that the uterine expression of minichromosome maintenance proteins (MCMs), a complex required for DNA synthesis initiation, is induced after E₂ treatment, specifically MCM2 and MCM3. MCM2 activity is crucial and required for DNA synthesis in uterine epithelial cells (Ray & Pollard 2012). Further study demonstrated E₂-mediated induction of the transcription factor KLF4, which then targets the *Mcm2* promoter (Ray & Pollard 2012).

Mice lacking ERβ

ERβ-null mice have provided insight into the importance of ERβ to female fertility and studies to date indicate ERβ plays a particularly important role in ovarian function. Four different lines of ERβ-null mice have been described. The βERKO mouse, made using homologous recombination was first described by Krege *et al.* (1998), and the ERβKO or Ex3βERKO, was described by Dupont *et al.* (2000), and by Binder *et al.* (2013). Cre-mediated recombination was employed in both lines to disrupt exon 3 (Dupont *et al.* 2000, Binder *et al.* 2013) of the murine *Esr2* (ERβ) gene. As described to date, the reproductive, endocrine, and ovarian phenotypes of both lines are indistinguishable, with both exhibiting female subfertility. Shughrue *et al.* (2002) reported the third line of ERβKO animals, however, no uterine or ovarian phenotypes were reported (Shughrue *et al.* 2002). Recently, ERβKO_{ST}^{L-/L-} animals, which contain *LoxP* sites flanking exon 3 of *Esr2*, were generated using the *Cre/loxP* recombination system (Antal *et al.* 2008). Interestingly, female mice from this recently described ERβKO_{ST}^{L-/L-} colony were reported to be sterile due to an ovarian defect while Ex3βERKO (Binder *et al.* 2013) are subfertile, due to ovulatory defects.

Mice lacking ER α and β

The two reported lines of compound ER-null mice are the αβERKO, described by Couse *et al.* (1999), and the ERαβKO, described by Dupont *et al.* (2000). Both were generated by cross breeding animals heterozygous for the respective individual ER-null mice and as described to date, exhibit comparable reproductive, endocrine, and ovarian phenotypes. The most striking phenotype is the unique trans-differentiation of the ovarian granulosa cells to sertoli-like cells in follicles of αβERKO females which is age dependent. To date, no manipulation of the individual αERKO or βERKO mouse lines can reproduce this novel phenotype. This model clearly

uncovered that both ER signaling systems are required to maintain the proper differentiation state of the adult granulosa cells.

Mice lacking Cyp19

Estrogens are produced by aromatase cytochrome P450, the product of *Cyp19* gene. Female mice with disruption of circulating estrogen production exhibit altered reproduction (Fisher *et al.* 1998, Honda *et al.* 1998, Toda *et al.* 2001). There are three animal models of Cyp19-null mice (called ArKO). Fisher *et al.* (1998) reported the first mouse line in 1998, which disrupted exon 9 of *Cyp19* gene, as the region is highly conserved. Later, Honda *et al.* (1998) reported a mouse line with targeted disruption of exons 1 and 2 of the *Cyp19* gene. Subsequently, Toda *et al.* (2001) generated the most recent mouse line of Cyp19-null in 2001 with a targeted disruption of exon 9 of the *Cyp19* gene. These ArKO female phenotypes are indistinguishable (Fisher *et al.* 1998, Honda *et al.* 1998, Toda *et al.* 2001), with similarity to the αβERKO mice with a clear metabolic syndrome (Couse *et al.* 1999) and infertility due to ovarian dysfunction marked by cystic follicles and a failure to respond to exogenous gonadotropins. Interestingly, the phenotype of the original ArKO mice (Fisher *et al.* 1998) were also shown to exhibit the same age related ovarian phenotype (Britt *et al.* 2002) as the αβERKO mice, indicating that hormone mediated ER action is required.

Female reproductive phenotypes in mice with disrupted estrogen signaling

Females within each respective model exhibit a similar phenotypic syndrome. Female mice lacking ERα or aromatase are infertile due to dysfunction of numerous physiological systems, including the ovary and uterus, whereas ERβ-null females exhibit reduction or loss of fecundity that is largely attributable to ovarian dysfunction. A level of caution is warranted when making phenotypic comparisons between the ER-null and Cyp19-null models because sensitivity to maternally derived estrogens may provide a more normal developmental environment during gestation in Cyp19-null mice and sensitivity to dietary estrogens during adulthood is able to abate several phenotypes in Cyp19-null mice (Britt *et al.* 2002).

The reported uterine phenotypes of these models are summarized in Table 2. All lines of ER-null females exhibit uteri that possess the expected tissue compartments, myometrium, endometrial stroma, and epithelium

(Couse & Korach 1999, Hewitt *et al.* 2010a,b). However, in females lacking functional ER α or Cyp19, uteri are overtly hypoplastic and exhibit severely reduced weights relative to wild-type littermates (Fisher *et al.* 1998, Couse & Korach 1999, Britt *et al.* 2001, Toda *et al.* 2001), whereas ER β -null uteri are grossly normal and normally responsive to ovarian-derived steroids (Couse & Korach 1999). The uterus of ER α -null females is severely hypotrophic, poorly organized, and possesses a paucity of glandular structures (Korach *et al.* 1996, Hewitt *et al.* 2010a,b). The luminal and glandular epithelial cells in ER α -null uteri are severely immature with fewer glands present in the adults (Nanjappa *et al.* 2015) and consistently exhibit a cuboidal morphology, vs the tall columnar morphology and basal location of the nucleus of an 'estrogenized' epithelium in WT uteri. Therefore, fetal, neonatal, and perinatal development of the female reproductive tract in mice is largely independent of ER α - and ER β -mediated actions, but estrogen responsiveness and sexual maturation of the adult uterus are ablated after the loss of functional ER α . The totality of the ER α -null phenotype and lack of any overt uterine abnormalities in ER β -null females suggest that ER β has little meaningful function in mediating estrogen actions in the uterus. Moreover, ER $\alpha\beta$ -null also demonstrated a similar uterine phenotype as ER α -null (Walker & Korach 2004). Weihua *et al.* (2000) reported that ER β -null females exhibited a slightly aberrant uterine growth response after estrogen replacement; however, the uterine bioassay was conducted in immature intact, not ovariectomized adult, animals. In addition, Wada-Hiraike *et al.* (2006) showed that in immature females, loss of ER β leads to increased uterine epithelial proliferation induced by E₂ compared with WT uteri. Although ER β -null females are subfertile, when pregnancies are established they are sustained to term (Krege *et al.* 1998), indicating uterine competence. More recent findings suggest that loss of ER β leads to complete sterility due to a defect in ovarian function (Dupont *et al.* 2000, Antal *et al.* 2008).

Mice with uterine specific deletion of ER α

Selectively deleting ER α in the uterus postpubertally, using the Cre/LoxP recombination system, by crossing Pgr^{Cre+} with *Esr1*^{f/f} animals (*Esr1*^{d/d}), leads to a hypoplastic uterus that lacks a decidual response (Pawar *et al.* 2015). Our laboratory has described uterine epithelial cell selective deletion of ER α , using the Cre/LoxP recombination system, by crossing *Wnt7a*^{Cre+} (Huang *et al.* 2012) with *Esr1*^{f/f} animals (Hewitt *et al.* 2010a,b) (ER α Epi-cKO). The expression of ER α in the uterine luminal and

glandular epithelium of these animals was ablated, while the ER α expression in the stromal cells and other uterine cells remains intact (Winuthayanon *et al.* 2010). The epithelial ER α was ablated not only in the uterus in this mouse line (Winuthayanon *et al.* 2010), but also in the oviduct (Winuthayanon *et al.* 2015). As expected, based on findings in the global ER α knockouts, loss of uterine epithelial ER α has no effect on female reproductive tract development. Uterine histological analysis showed a similar uterine morphology as WT control (Winuthayanon *et al.* 2010). The ER α Epi-cKO uteri are sensitive to 24 h treatment of E₂, as the uterine epithelial proliferation is preserved. However, ER α Epi-cKO uteri lack a complete uterine response to E₂, following a 3-day uterine bioassay, which demonstrated a blunted growth response and increased apoptotic activity in ER α Epi-cKO compared with the control uteri. Additionally, a lack of ER α expression in the uterine epithelial cells contributes to complete infertility, due to oviduct, and uterine implantation and decidualization defects (Winuthayanon *et al.* 2010, Pawar *et al.* 2015, Winuthayanon *et al.* 2015). This suggests that uterine epithelial ER α is dispensable for early uterine proliferative responses but crucial for a complete adult biological response induced by E₂, as well as for establishing pregnancy.

Mice with mutated DNA binding domains of ER α

To date, there are two mouse lines with mutations that are designed to disrupt the DNA binding function of the ER α that have been 'knocked-in' (KI) at the ER α gene locus. The first line was generated by replacing critical P-box amino acids E207 and G208 with alanines (ER α ^{AA}). This line was named 'non-genomic ER knock-in' (NERKI), as these mutations were intended to restrict ER α signaling to the non-genomic and tethered mechanisms. Female NERKI^{+/-} animals that have one mutated allele and one WT allele (Jakacka *et al.* 2002) were infertile, exhibiting a highly novel hyperplastic uterine phenotype, so NERKI^{+/-} males were crossed with ER α null heterozygous (WT/KO) females to produce mice with one NERKI mutated allele and one deleted *Esr1* allele, called ER α KIKO or ER α ^{AA/-} as described by O'Brien *et al.* (2006). The second line of DNA-binding domain knock-in animals were created through mutation of four amino acids in the first zinc finger of the *Esr1* gene, substituting Y at position 201 with E, and in the critical P box, K at position 210 with A, K at position 214 with A, and R at position 215 with E as described by Ahlbory-Dieker *et al.* (2009; called ER α ^{EAAE/EAAE}).

The $\text{NERKI}^{+/-}$ females have normal uterine development but exhibit hyperplastic uteri, and are hypersensitive to estrogen (Jakacka *et al.* 2002). These $\text{NERKI}^{+/-}$ are infertile and exhibit a uterine abnormality of enlarged hyperplastic endometrial glands despite possessing normal levels of circulating sex steroids.

$\text{ER}\alpha^{\text{AA}/-}$ females have normal uterine development. Initially, O'Brien *et al.* (2006) reported that $\text{ER}\alpha^{\text{AA}/-}$ females, with mutation of the DNA binding domain, maintained proliferative responses induced by E_2 . However, in subsequent studies, no uterine proliferation was observed (Hewitt *et al.* 2009, 2010). Ahlbory-Dieker *et al.* (2009) showed that, unlike the $\text{NERKI}^{+/-}$, females heterozygous for the $\text{ER}\alpha^{\text{EAAE}}$ mutation are fertile. The homozygous $\text{ER}\alpha^{\text{EAAE/EAAE}}$ females have normal reproductive tract development but uteri are severely hypoplastic, similar to global $\text{ER}\alpha$ -null uteri. Additionally, $\text{ER}\alpha^{\text{EAAE/EAAE}}$ uteri do not respond to E treatment, as normally estrogen-responsive uterine and liver genes are not regulated in $\text{ER}\alpha^{\text{EAAE/EAAE}}$ (Ahlbory-Dieker *et al.* 2009, Hewitt *et al.* 2014). The females from these two mouse lines with point mutations in the DNA binding domain of $\text{ER}\alpha$ are infertile. Thus the physiological function of the DNA binding domain of $\text{ER}\alpha$ is crucial for female reproduction. $\text{ER}\alpha$ ChIP-seq analysis of the $\text{ER}\alpha^{\text{AA}/-}$ uterus revealed that the DBD mutation, rather than completely disrupting DNA binding instead altered the motif specificity, so that $\text{ER}\alpha^{\text{AA}}$ could bind HRE motifs normally occupied by progesterone receptor (Pgr or PR). Additionally, this HRE binding lead to E_2 regulation of uterine transcripts that are normally progesterone responsive (Hewitt *et al.* 2014). This novel $\text{ER}\alpha^{\text{AA}}$ binding activity may also explain the hyperplastic phenotype of the heterozygous $\text{ER}\alpha^{\text{AA}/+}$ females where the normally activated uterine HRE sites are occupied by the mutant $\text{ER}\alpha^{\text{AA}}$ and thus blocking the dampening activity of uterine PR at those sites. Adding to this abnormal regulation is the expression of $\text{ER}\alpha^{\text{AA}}$ in all uterine cells at all times, whereas, the PR is restricted at times, to epithelial cells and is dynamically induced in the stromal cells during the estrous cycle. Additionally, the phenotype also indicates the specificity of the action at the HRE requires the proper activity of the PR to elicit the dampening action.

Mice with mutated AF-1 or AF-2 domains of $\text{ER}\alpha$

As discussed in the Receptor structure section, AF-1 and AF-2 are important for ER transcriptional activity (Fig. 1). Amino acids 2–128 were deleted from exon 1 of *Esr1*,

which removes the AF-1 domain, and knocked into a mouse line (called $\text{ER}\alpha\text{AF-1}^\circ$) (Billon-Gales *et al.* 2009). There are three reported mouse lines with mutation in the AF-2 domain of $\text{ER}\alpha$. One with a single point mutation in $\text{ER}\alpha$ of G at position 525 to L in the ligand binding domain (LBD), called 'estrogen-nonresponsive $\text{ER}\alpha$ knock-in or ENERKI' ($\text{ER}\alpha^{\text{G525L}}$) (Sinkevicius *et al.* 2008). Amino acids 543–549 were deleted from the LBD of $\text{ER}\alpha$, removing helix 12 and thus AF-2 functionality, to create a second mouse line (called $\text{ER}\alpha\text{AF-2}^\circ$) (Billon-Gales *et al.* 2011). Two point mutations in the AF-2 of the LBD of $\text{ER}\alpha$ were knocked into a mouse (L543A and L544A, called $\text{AF2ER}^{\text{KI/KI}}$ animals) (Arao *et al.* 2011). $\text{ER}\alpha\text{AF-1}^\circ$, $\text{ER}\alpha^{\text{G525L}}$, $\text{ER}\alpha\text{AF-2}^\circ$, and $\text{AF2ER}^{\text{KI/KI}}$ females are all sterile (Sinkevicius *et al.* 2008, Billon-Gales *et al.* 2009, Arao *et al.* 2011, Billon-Gales *et al.* 2011).

$\text{ER}\alpha\text{AF-1}^\circ$ females exhibited minimal uterine wet weight gain compared with $\text{ER}^{+/+}$ uteri after treatment with E_2 pellets for two consecutive weeks, while $\text{ER}\alpha\text{AF-2}^\circ$ females did not respond (Billon-Gales *et al.* 2009, 2011, Abot *et al.* 2013). This indicates that the $\text{ER}\alpha$ AF-2 functional domain contributes to minimal uterine weight increase induced by E_2 in the absence of AF-1. Both lines of AF-2 mutated animals ($\text{ER}\alpha^{\text{G525L}}$ and $\text{AF2ER}^{\text{KI/KI}}$) display severely hypoplastic uteri, and lack uterine growth response to E_2 treatment (Sinkevicius *et al.* 2008, Arao *et al.* 2011, Billon-Gales *et al.* 2011). Interestingly, uterine wet weight can be increased by using the synthetic $\text{ER}\alpha$ agonist PPT in $\text{ER}\alpha^{\text{G525L}}$ or by using the ER antagonists ICI 182,780 or tamoxifen in $\text{AF2ER}^{\text{KI/KI}}$ females (Sinkevicius *et al.* 2008, Arao *et al.* 2011). The ability of the antagonists to mediate responses seems to be due to a unique conformation of the LBD of the AF2ER that leads to AF-1-dependent transcriptional activity (Arao *et al.* 2011, 2013). Arao *et al.* (2011) also demonstrated that the uterine response to ICI or tamoxifen includes increased DNA synthesis in the uterine epithelial cells of $\text{AF2ER}^{\text{KI/KI}}$. The growth factor IGF-1 induced minimal uterine epithelial proliferation in $\text{ER}\alpha^{\text{G525L}}$, and was not seen in $\text{AF2ER}^{\text{KI/KI}}$ uteri (Sinkevicius *et al.* 2008, Arao *et al.* 2011). Together, these findings indicated that both AF-1 and AF-2 activation domains of $\text{ER}\alpha$ contribute to a normal regulation of the complete biological response of uterine growth and reproductive functions. As the AF domains mediate ER-coregulator interaction (Table 1), this emphasizes the importance of effective $\text{ER}\alpha$ coactivator protein recruitment for successful uterine E_2 response. Similarly, mice lacking sufficient SRC-1 coactivator ($\text{SRC1}^{-/-}$), exhibit measurably diminished uterine response to E_2 (Xu *et al.* 1998).

Mice with altered localization of ER α

A mutated mouse ER α that remains sequestered outside the nucleus (ER α H2NES), is unable to mediate transcriptional responses in a cell based assay, but maintains estrogen induced MAPK phosphorylation (Burns *et al.* 2011). Targeting steroid receptors to the membrane involves palmitoylation, which is facilitated by HSP27 (Levin 2011). The palmitoylation promotes interaction with caveolin-1, which then results in localization of the receptor in membrane caveolin rafts. Two laboratories have mutated the palmitoylation site of the mouse ER α , and created knock in mouse models to study the effect of disabling this mechanism *in vivo* (Adlanmerini *et al.* 2014, Pedram *et al.* 2014). Both mouse lines have ovarian defects, but differ in several aspects (Table 2). Both involved knocking in an ER α with the same mutation of cysteine 451 to alanine. The first, C451A-ER α , exhibits normal uterine development and E₂ induced growth response (Adlanmerini *et al.* 2014), whereas the nuclear-only ER α (NOER) has a hypoplastic ER α -null like uterus that fails to respond to E₂ (Pedram *et al.* 2014). Both models have elevation in LH, but only the NOER has elevated E₂. These mixed results remain to be reconciled to definitively illustrate the role of membrane associated ER α in these physiological systems.

Conclusion

Female reproduction is a complex staged series of physiological responses occurring in multiple organ systems activated by estrogen and estrogen receptors. Cell based studies have uncovered that cellular signaling mechanisms for ER are multifaceted regarding gene regulation. Because of the complexity with what is known about female reproduction and fertility, the mechanisms and activities cannot be clearly studied or tested in cell based systems. The development of gene targeting has allowed the evaluation of the physiological roles of estrogen action and ER functionality under natural biological conditions. It is now apparent from the experimental and clinical reports outlined in this review that the primary mediator of female reproduction is ER α . What functional aspects of the ER α action are required will be forthcoming with the continued use of new technologies and experimental approaches, which will lead to a better understanding for the potential origins of infertility, reproductive tract disease and development of reproductive therapeutics.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

Funding

Research support for studies reported in this review was provided by the Division of Intramural Research of the NIEHS/NIH 1ZIAES070065 to K S K.

References

- Aagaard MM, Siersbaek R & Mandrup S 2011 Molecular basis for gene-specific transactivation by nuclear receptors. *Biochimica et Biophysica Acta* **1812** 824–835. (doi:10.1016/j.bbdis.2010.12.018)
- Abot A, Fontaine C, Raymond-Letron I, Flouriot G, Adlanmerini M, Buscato M, Otto C, Berges H, Laurell H, Gourdy P *et al.* 2013 The AF-1 activation function of estrogen receptor α is necessary and sufficient for uterine epithelial cell proliferation *in vivo*. *Endocrinology* **154** 2222–2233. (doi:10.1210/en.2012-2059)
- Adlanmerini M, Solinhac R, Abot A, Fabre A, Raymond-Letron I, Guihot AL, Boudou F, Sautier L, Vessieres E, Kim SH *et al.* 2014 Mutation of the palmitoylation site of estrogen receptor α *in vivo* reveals tissue-specific roles for membrane versus nuclear actions. *PNAS* **111** E283–E290. (doi:10.1073/pnas.1322057111)
- Ahlbory-Dieker DL, Stride BD, Leder G, Schkoldow J, Trolenberg S, Seidel H, Otto C, Sommer A, Parker MG, Schutz G *et al.* 2009 DNA binding by estrogen receptor- α is essential for the transcriptional response to estrogen in the liver and the uterus. *Molecular Endocrinology* **23** 1544–1555. (doi:10.1210/me.2009-0045)
- Andrade PM, Silva I, Borra RC, de Lima GR & Baracat EC 2002 Estrogen regulation of uterine genes *in vivo* detected by complementary DNA array. *Hormone and Metabolic Research* **34** 238–244. (doi:10.1055/s-2002-32136)
- Antal MC, Krust A, Chambon P & Mark M 2008 Sterility and absence of histopathological defects in nonreproductive organs of a mouse ER β -null mutant. *PNAS* **105** 2433–2438. (doi:10.1073/pnas.0712029105)
- Antonson P, Omoto Y, Humire P & Gustafsson JA 2012 Generation of ER α -floxed and knockout mice using the Cre/LoxP system. *Biochemical and Biophysical Research Communications* **424** 710–716. (doi:10.1016/j.bbrc.2012.07.016)
- Arao Y, Hamilton KJ, Ray MK, Scott G, Mishina Y & Korach KS 2011 Estrogen receptor α AF-2 mutation results in antagonist reversal and reveals tissue selective function of estrogen receptor modulators. *PNAS* **108** 14986–14991. (doi:10.1073/pnas.1109180108)
- Arao Y, Hamilton KJ, Coons LA & Korach KS 2013 Estrogen receptor α L543A, L544A mutation changes antagonists to agonists which correlates with the ligand binding domain dimerization associated with DNA binding activity. *Journal of Biological Chemistry* **288** 21105–21116. (doi:10.1074/jbc.M113.463455)
- Bagchi MK, Mantena SR, Kannan A & Bagchi IC 2006 Role of C/EBP β in steroid-induced cell proliferation and differentiation in the uterus: Functional implications for establishment of early pregnancy. *Placenta* **27** A13–A13.
- Barnes PJ, Adcock IM & Ito K 2005 Histone acetylation and deacetylation: importance in inflammatory lung diseases. *European Respiratory Journal* **25** 552–563. (doi:10.1183/09031936.05.00117504)
- Biddie SC, John S & Hager GL 2010 Genome-wide mechanisms of nuclear receptor action. *Trends in endocrinology and metabolism* **21** 3–9. (doi:10.1016/j.tem.2009.08.006)
- Billon-Gales A, Fontaine C, Filipe C, Douin-Echinard V, Fouque MJ, Flouriot G, Gourdy P, Lenfant F, Laurell H, Krust A *et al.* 2009 The transactivating function 1 of estrogen receptor α is dispensable for

- the vasculoprotective actions of 17 β -estradiol. *PNAS* **106** 2053–2058. (doi:10.1073/pnas.0808742106)
- Billon-Gales A, Krust A, Fontaine C, Abot A, Flouriot G, Toutain C, Berges H, Gadeau AP, Lenfant F, Gourdy P *et al.* 2011 Activation function 2 (AF2) of estrogen receptor- α is required for the atheroprotective action of estradiol but not to accelerate endothelial healing. *PNAS* **108** 13311–13316. (doi:10.1073/pnas.1105632108)
- Binder AK, Rodriguez KF, Hamilton KJ, Stockton PS, Reed CE & Korach KS 2013 The absence of ER- β results in altered gene expression in ovarian granulosa cells isolated from *in vivo* preovulatory follicles. *Endocrinology* **154** 2174–2187. (doi:10.1210/en.2012-2256)
- Binder AK, Winuthayanon W, Hewitt SC, Couse JF & Korach KS. 2015 Steroid receptors in the uterus and ovary. In *Knobil and Neill's Physiology of Reproduction*, pp 1099–1193. Eds TM Plant, AJ Zeleznik: Elsevier.
- Brelivet Y, Rochel N & Moras D 2012 Structural analysis of nuclear receptors: From isolated domains to integral proteins. *Molecular and Cellular Endocrinology* **348** 466–473. (doi:10.1016/j.mce.2011.08.015)
- Britt KL, Drummond AE, Dyson M, Wreford NG, Jones ME, Simpson ER & Findlay JK 2001 The ovarian phenotype of the aromatase knockout (ArKO) mouse. *Journal of Steroid Biochemistry and Molecular Biology* **79** 181–185. (doi:10.1016/S0960-0760(01)00158-3)
- Britt KL, Kerr J, O'Donnell L, Jones ME, Drummond AE, Davis SR, Simpson ER & Findlay JK 2002 Estrogen regulates development of the somatic cell phenotype in the eutherian ovary. *FASEB Journal* **16** 1389–1397. (doi:10.1096/fj.01-0992com)
- Bulyanko YA & O'Malley BW 2011 Nuclear receptor coactivators: structural and functional biochemistry. *Biochemistry* **50** 313–328. (doi:10.1021/bi101762x)
- Burns KA, Li Y, Arai Y, Petrovich RM & Korach KS 2011 Selective mutations in estrogen receptor α D-domain alters nuclear translocation and non-estrogen response element gene regulatory mechanisms. *Journal of Biological Chemistry* **286** 12640–12649. (doi:10.1074/jbc.M110.187773)
- Carroll JS & Brown M 2006 Estrogen receptor target gene: an evolving concept. *Molecular Endocrinology* **20** 1707–1714. (doi:10.1210/me.2005-0334)
- Carroll JS, Liu XS, Brodsky AS, Li W, Meyer CA, Szary AJ, Eeckhoutte J, Shao W, Hestermann EV, Geistlinger TR *et al.* 2005 Chromosome-wide mapping of estrogen receptor binding reveals long-range regulation requiring the forkhead protein FoxA1. *Cell* **122** 33–43. (doi:10.1016/j.cell.2005.05.008)
- Cheung E & Kraus WL 2010 Genomic analyses of hormone signaling and gene regulation. *Annual Review of Physiology* **72** 191–218. (doi:10.1146/annurev-physiol-021909-135840)
- Conaway RC & Conaway JW 2011 Function and regulation of the Mediator complex. *Current Opinion in Genetics & Development* **21** 225–230. (doi:10.1016/j.gde.2011.01.013)
- Couse JF & Korach KS 1999 Estrogen receptor null mice: what have we learned and where will they lead us? *Endocrine Reviews* **20** 358–417. (doi:10.1210/edrv.20.3.0370)
- Couse JF, Curtis SW, Washburn TF, Lindzey J, Golding TS, Lubahn DB, Smithies O & Korach KS 1995 Analysis of transcription and estrogen insensitivity in the female mouse after targeted disruption of the estrogen receptor gene. *Molecular Endocrinology* **9** 1441–1454. (doi:10.1210/mend.9.11.8584021)
- Couse JF, Hewitt SC, Bunch DO, Sar M, Walker VR, Davis BJ & Korach KS 1999 Postnatal sex reversal of the ovaries in mice lacking estrogen receptors α and β . *Science* **286** 2328–2331. (doi:10.1126/science.286.5448.2328)
- Curtis SH & Korach KS. 1999 Steroid receptor knockout models: Phenotypes and responses illustrate interactions between receptor signaling pathways *in vivo*. In *Hormones and Signaling*, pp 357–380. Ed BW O'Malley. San Diego, CA, USA: Academic Press.
- Curtis SW, Clark J, Myers P & Korach KS 1999 Disruption of estrogen signaling does not prevent progesterone action in the estrogen receptor α knockout mouse uterus. *PNAS* **96** 3646–3651. (doi:10.1073/pnas.96.7.3646)
- Curtis Hewitt S, Goulding EH, Eddy EM & Korach KS 2002 Studies using the estrogen receptor α knockout uterus demonstrate that implantation but not decidualization-associated signaling is estrogen dependent. *Biology of Reproduction* **67** 1268–1277. (doi:10.1095/biolreprod67.4.1268)
- Deblois G & Giguere V 2008 Nuclear receptor location analyses in mammalian genomes: from gene regulation to regulatory networks. *Molecular Endocrinology* **22** 1999–2011. (doi:10.1210/me.2007-0546)
- Dupont S, Krust A, Gansmuller A, Dierich A, Chambon P & Mark M 2000 Effect of single and compound knockouts of estrogen receptors α (ER α) and β (ER β) on mouse reproductive phenotypes. *Development* **127** 4277–4291.
- Farnham PJ 2009 Insights from genomic profiling of transcription factors. *Nature Reviews. Genetics* **10** 605–616. (doi:10.1038/nrg2636)
- Fertuck KC, Eckel JE, Gennings C & Zacharewski TR 2003 Identification of temporal patterns of gene expression in the uteri of immature, ovariectomized mice following exposure to ethynylestradiol. *Physiological Genomics* **15** 127–141. (doi:10.1152/physiolgenomics.00058.2003)
- Fisher CR, Graves KH, Parlow AF & Simpson ER 1998 Characterization of mice deficient in aromatase (ArKO) because of targeted disruption of the cyp19 gene. *PNAS* **95** 6965–6970. (doi:10.1073/pnas.95.12.6965)
- Fox EM, Andrade J & Shupnik MA 2009 Novel actions of estrogen to promote proliferation: integration of cytoplasmic and nuclear pathways. *Steroids* **74** 622–627. (doi:10.1016/j.steroids.2008.10.014)
- Fu X, Huang C & Schiff R 2011 More on FOX News: FOXA1 on the horizon of estrogen receptor function and endocrine response. *Breast Cancer Research* **13** 307. (doi:10.1186/bcr2849)
- Gao H & Dahlman-Wright K 2011 The gene regulatory networks controlled by estrogens. *Molecular and Cellular Endocrinology* **334** 83–90. (doi:10.1016/j.mce.2010.09.002)
- Gao F, Ma X, Ostmann AB & Das SK 2011 GPR30 activation opposes estrogen-dependent uterine growth via inhibition of stromal ERK1/2 and estrogen receptor α (ER α) phosphorylation signals. *Endocrinology* **152** 1434–1447. (doi:10.1210/en.2010-1368)
- George CL, Lightman SL & Biddie SC 2011 Transcription factor interactions in genomic nuclear receptor function. *Epigenomics* **3** 471–485. (doi:10.2217/epi.11.66)
- Gibson DA & Saunders PT 2012 Estrogen dependent signaling in reproductive tissues - a role for estrogen receptors and estrogen related receptors. *Molecular and Cellular Endocrinology* **348** 361–372. (doi:10.1016/j.mce.2011.09.026)
- Gilfillan S, Fiorito E & Hurtado A 2012 Functional genomic methods to study estrogen receptor activity. *Journal of Mammary Gland Biology and Neoplasia* **17** 147–153. (doi:10.1007/s10911-012-9254-4)
- Green CD & Han JD 2011 Epigenetic regulation by nuclear receptors. *Epigenomics* **3** 59–72. (doi:10.2217/epi.10.75)
- Gronqvist L & Hager GL 2012 Impact of chromatin structure on PR signaling: transition from local to global analysis. *Molecular and Cellular Endocrinology* **357** 30–36. (doi:10.1016/j.mce.2011.09.006)
- Heldring N, Pike A, Andersson S, Matthews J, Cheng G, Hartman J, Tujague M, Strom A, Treuter E, Warner M *et al.* 2007 Estrogen receptors: how do they signal and what are their targets. *Physiological Reviews* **87** 905–931. (doi:10.1152/physrev.00026.2006)
- Helsen C, Kerkhofs S, Clinckemalie L, Spans L, Laurent M, Boonen S, Vanderschueren D & Claessens F 2012 Structural basis for nuclear hormone receptor DNA binding. *Molecular and Cellular Endocrinology* **348** 411–417. (doi:10.1016/j.mce.2011.07.025)
- Hewitt SC, Deroo BJ, Hansen K, Collins J, Grissom S, Afshari CA & Korach KS 2003 Estrogen receptor-dependent genomic responses in the uterus mirror the biphasic physiological response to estrogen. *Molecular Endocrinology* **17** 2070–2083. (doi:10.1210/me.2003-0146)
- Hewitt SC, Collins J, Grissom S, Deroo B & Korach KS 2005 Global uterine genomics *in vivo*: microarray evaluation of the estrogen receptor α -growth factor cross-talk mechanism. *Molecular Endocrinology* **19** 657–668. (doi:10.1210/me.2004-0142)

- Hewitt SC, O'Brien JE, Jameson JL, Kissling GE & Korach KS 2009 Selective disruption of ER α DNA-binding activity alters uterine responsiveness to estradiol. *Molecular Endocrinology* **23** 2111–2116. (doi:10.1210/me.2009-0356)
- Hewitt SC, Kissling GE, Fieselman KE, Jayes FL, Gerrish KE & Korach KS 2010a Biological and biochemical consequences of global deletion of exon 3 from the ER α gene. *FASEB Journal* **24** 4660–4667. (doi:10.1096/fj.10-163428)
- Hewitt SC, Li Y, Li L & Korach KS 2010b Estrogen-mediated regulation of Igf1 transcription and uterine growth involves direct binding of estrogen receptor α to estrogen-responsive elements. *Journal of Biological Chemistry* **285** 2676–2685. (doi:10.1074/jbc.M109.043471)
- Hewitt SC, Li L, Grimm SA, Chen Y, Liu L, Li Y, Bushel PR, Fargo D & Korach KS 2012 Research resource: whole-genome estrogen receptor α binding in mouse uterine tissue revealed by ChIP-Seq. *Molecular Endocrinology* **26** 887–898. (doi:10.1210/me.2011-1311)
- Hewitt SC, Li L, Grimm SA, Winuthayanon W, Hamilton KJ, Pockette B, Rubel CA, Pedersen LC, Fargo D, Lanz RB *et al.* 2014 Novel DNA motif binding activity observed *in vivo* with an estrogen receptor α mutant mouse. *Molecular Endocrinology* **28** 899–911. (doi:10.1210/me.2014-1051)
- Hilser VJ & Thompson EB 2011 Structural dynamics, intrinsic disorder, and allostery in nuclear receptors as transcription factors. *Journal of Biological Chemistry* **286** 39675–39682. (doi:10.1074/jbc.R111.278929)
- Hong SH, Nah HY, Lee JY, Gye MC, Kim CH & Kim MK 2004 Analysis of estrogen-regulated genes in mouse uterus using cDNA microarray and laser capture microdissection. *Journal of Endocrinology* **181** 157–167. (doi:10.1677/joe.0.1810157)
- Honda S, Harada N, Ito S, Takagi Y & Maeda S 1998 Disruption of sexual behavior in male aromatase-deficient mice lacking exons 1 and 2 of the cyp19 gene. *Biochemical and Biophysical Research Communications* **252** 445–449. (doi:10.1006/bbrc.1998.9672)
- Hong EJ, Park SH, Choi KC, Leung PC & Jeung EB 2006 Identification of estrogen-regulated genes by microarray analysis of the uterus of immature rats exposed to endocrine disrupting chemicals. *Reproductive Biology and Endocrinology* **4** 49. (doi:10.1186/1477-7827-4-49)
- Hsia EY, Goodson ML, Zou JX, Privalsky ML & Chen HW 2010 Nuclear receptor coregulators as a new paradigm for therapeutic targeting. *Advanced Drug Delivery Reviews* **62** 1227–1237. (doi:10.1016/j.addr.2010.09.016)
- Huang PX, Chandra V & Rastinejad F 2010 Structural overview of the nuclear receptor superfamily: insights into physiology and therapeutics. *Annual Review of Physiology* **72** 247–272. (doi:10.1146/annurev-physiol-021909-135917)
- Huang CC, Orvis GD, Wang Y & Behringer RR 2012 Stromal-to-epithelial transition during postpartum endometrial regeneration. *PLoS ONE* **7** e44285. (doi:10.1371/journal.pone.0044285)
- Jakacka M, Ito M, Martinson F, Ishikawa T, Lee EJ & Jameson JL 2002 An estrogen receptor (ER) α deoxyribonucleic acid-binding domain knock-in mutation provides evidence for nonclassical ER pathway signaling *in vivo*. *Molecular Endocrinology* **16** 2188–2201. (doi:10.1210/me.2001-0174)
- Johnson AB & O'Malley BW 2012 Steroid receptor coactivators 1, 2, and 3: Critical regulators of nuclear receptor activity and steroid receptor modulator (SRM)-based cancer therapy. *Molecular and Cellular Endocrinology* **348** 430–439. (doi:10.1016/j.mce.2011.04.021)
- Katzenellenbogen BS, Choi IH, Delage-Mourroux R, Ediger TR, Martini PG, Montano M, Sun J, Weis K & Katzenellenbogen JA 2000 Molecular mechanisms of estrogen action: selective ligands and receptor pharmacology. *Journal of Steroid Biochemistry and Molecular Biology* **74** 279–285. (doi:10.1016/S0960-0760(00)00104-7)
- Keppeler BR, Archer TK & Kinyamu HK 2011 Emerging roles of the 26S proteasome in nuclear hormone receptor-regulated transcription. *Biochimica et Biophysica Acta* **1809** 109–118. (doi:10.1016/j.bbaggm.2010.08.005)
- Kim MY, Woo EM, Chong YT, Homenko DR & Kraus WL 2006 Acetylation of estrogen receptor α by p300 at lysines 266 and 268 enhances the deoxyribonucleic acid binding and transactivation activities of the receptor. *Molecular Endocrinology* **20** 1479–1493. (doi:10.1210/me.2005-0531)
- Kim HM, Yu Y & Cheng Y 2011a Structure characterization of the 26S proteasome. *Biochimica et Biophysica Acta* **1809** 67–79. (doi:10.1016/j.bbaggm.2010.08.008)
- Kim H, Ku SY, Sung JJ, Kim SH, Choi YM, Kim JG & Moon SY 2011b Association between hormone therapy and nerve conduction study parameters in postmenopausal women. *Climacteric* **14** 488–491. (doi:10.3109/13697137.2011.553972)
- Koide A, Zhao C, Naganuma M, Abrams J, Deighton-Collins S, Skafar DF & Koide S 2007 Identification of regions within the F domain of the human estrogen receptor α that are important for modulating transactivation and protein-protein interactions. *Molecular Endocrinology* **21** 829–842. (doi:10.1210/me.2006-0203)
- Korach KS 1994 Insights from the study of animals lacking functional estrogen receptor. *Science* **266** 1524–1527. (doi:10.1126/science.7985022)
- Korach KS, Couse JF, Curtis SW, Washburn TF, Lindzey J, Kimbro KS, Eddy EM, Migliaccio S, Snedeker SM, Lubahn DB *et al.* 1996 Estrogen receptor gene disruption: molecular characterization and experimental and clinical phenotypes. *Recent Progress in Hormone Research* **51** 159–186; discussion 186–158.
- Krege JH, Hodgin JB, Couse JF, Enmark E, Warner M, Mahler JF, Sar M, Korach KS, Gustafsson JA & Smithies O 1998 Generation and reproductive phenotypes of mice lacking estrogen receptor β . *PNAS* **95** 15677–15682. (doi:10.1073/pnas.95.26.15677)
- Kuiper GG, Enmark E, Peltö-Huikko M, Nilsson S & Gustafsson JA 1996 Cloning of a novel receptor expressed in rat prostate and ovary. *PNAS* **93** 5925–5930. (doi:10.1073/pnas.93.12.5925)
- Kumar R & McEwan IJ 2012 Allosteric Modulators of steroid hormone receptors: structural dynamics and gene regulation. *Endocrine Reviews* **33** 271–299. (doi:10.1210/er.2011-1033)
- Kumar R, Zakharov MN, Khan SH, Miki R, Jang H, Toraldo G, Singh R, Bhasin S & Jasuja R 2011 The dynamic structure of the estrogen receptor. *Journal of Amino Acids* **2011** 812540. (doi:10.4061/2011/812540)
- Kushner PJ, Agard DA, Greene GL, Scanlan TS, Shiau AK, Uht RM & Webb P 2000 Estrogen receptor pathways to AP-1. *Journal of Steroid Biochemistry and Molecular Biology* **74** 311–317. (doi:10.1016/S0960-0760(00)00108-4)
- Langer G, Bader B, Meoli L, Isensee J, Delbeck M, Noppinger PR & Otto C 2010 A critical review of fundamental controversies in the field of GPR30 research. *Steroids* **75** 603–610. (doi:10.1016/j.steroids.2009.12.006)
- Laudet V & Gronemeyer H 2001 *The Nuclear Receptor FactsBook*. Cambridge, MA, USA: Academic Press.
- Le Romancer M, Poulard C, Cohen P, Sentis S, Renoir JM & Corbo L 2011 Cracking the estrogen receptor's posttranslational code in breast tumors. *Endocrine Reviews* **32** 597–622. (doi:10.1210/er.2010-0016)
- Levin ER 2011 Minireview: extranuclear steroid receptors: roles in modulation of cell functions. *Molecular Endocrinology* **25** 377–384. (doi:10.1210/me.2010-0284)
- Lonard DM & O'Malley BW 2005 Expanding functional diversity of the coactivators. *Trends in Biochemical Sciences* **30** 126–132. (doi:10.1016/j.tibs.2005.01.001)
- Lubahn DB, Moyer JS, Golding TS, Couse JF, Korach KS & Smithies O 1993 Alteration of reproductive function but not prenatal sexual development after insertional disruption of the mouse estrogen receptor gene. *PNAS* **90** 11162–11166. (doi:10.1073/pnas.90.23.11162)
- Lupien M, Eeckhoutte J, Meyer CA, Krum SA, Rhodes DR, Liu XS & Brown M 2009 Coactivator function defines the active estrogen receptor α cistrome. *Molecular and Cellular Biology* **29** 3413–3423. (doi:10.1128/MCB.00020-09)

- Madak-Erdogan Z, Lupien M, Stossi F, Brown M & Katzenellenbogen BS 2011 Genomic collaboration of estrogen receptor α and extracellular signal-regulated kinase 2 in regulating gene and proliferation programs. *Molecular and Cellular Biology* **31** 226–236. (doi:10.1128/MCB.00821-10)
- Malik S & Roeder RG 2010 The metazoan mediator co-activator complex as an integrative hub for transcriptional regulation. *Nature Reviews. Genetics* **11** 761–772. (doi:10.1038/nrg2901)
- Mantena SR, Kannan A, Cheon YP, Li Q, Johnson PF, Bagchi IC & Bagchi MK 2006 C/EBP β is a critical mediator of steroid hormone-regulated cell proliferation and differentiation in the uterine epithelium and stroma. *PNAS* **103** 1870–1875. (doi:10.1073/pnas.0507261103)
- Martens JH, Rao NA & Stunnenberg HG 2011 Genome-wide interplay of nuclear receptors with the epigenome. *Biochimica et Biophysica Acta* **1812** 818–823. (doi:10.1016/j.bbadis.2010.10.005)
- McEwan IJ 2004 Molecular mechanisms of androgen receptor-mediated gene regulation: structure-function analysis of the AF-1 domain. *Endocrine-Related Cancer* **11** 281–293. (doi:10.1677/erc.0.0110281)
- Meyer CA, Tang Q & Liu XS 2012 Minireview: applications of next-generation sequencing on studies of nuclear receptor regulation and function. *Molecular Endocrinology* **26** 1651–1659. (doi:10.1210/me.2012-1150)
- Meyers MJ, Sun J, Carlson KE, Marriner GA, Katzenellenbogen BS & Katzenellenbogen JA 2001 Estrogen receptor- β potency-selective ligands: Structure- activity relationship studies of diarylpropionitriles and their acetylene and polar analogues. *Journal of Medicinal Chemistry* **44** 4230–4251. (doi:10.1021/jm010254a)
- Moggs JG, Tinwell H, Spurway T, Chang HS, Pate I, Lim FL, Moore DJ, Soames A, Stuckey R, Currie R *et al.* 2004 Phenotypic anchoring of gene expression changes during estrogen-induced uterine growth. *Environmental Health Perspectives* **112** 1589–1606. (doi:10.1289/ehp.7345)
- Nanjappa MK, Medrano TI, March AG & Cooke PS 2015 Neonatal uterine and vaginal cell proliferation and adenogenesis are independent of estrogen receptor 1 (ESR1) in the mouse. *Biology of Reproduction* **92** 78. (doi:10.1095/biolreprod.114.125724)
- O'Brien JE, Peterson TJ, Tong MH, Lee EJ, Pfaff LE, Hewitt SC, Korach KS, Weiss J & Jameson JL 2006 Estrogen-induced proliferation of uterine epithelial cells is independent of estrogen receptor α binding to classical estrogen response elements. *Journal of Biological Chemistry* **281** 26683–26692. (doi:10.1074/jbc.M601522200)
- O'Malley BW, Malovannaya A & Qin J 2012 Minireview: nuclear receptor and coregulator proteomics–2012 and beyond. *Molecular Endocrinology* **26** 1646–1650. (doi:10.1210/me.2012-1114)
- Pan H, Deng Y & Pollard JW 2006 Progesterone blocks estrogen-induced DNA synthesis through the inhibition of replication licensing. *PNAS* **103** 14021–14026. (doi:10.1073/pnas.0601271103)
- Park PJ 2009 ChIP-seq: advantages and challenges of a maturing technology. *Nature Reviews. Genetics* **10** 669–680. (doi:10.1038/nrg2641)
- Pawar S, Laws MJ, Bagchi IC & Bagchi MK 2015 Uterine epithelial estrogen receptor- α controls decidualization via a paracrine mechanism. *Molecular Endocrinology* **29** 1362–1374. (doi:10.1210/me.2015-1142)
- Pedram A, Razandi M, Lewis M, Hammes S & Levin ER 2014 Membrane-localized estrogen receptor α is required for normal organ development and function. *Developmental Cell* **29** 482–490. (doi:10.1016/j.devcel.2014.04.016)
- Prossnitz ER & Barton M 2011 The G-protein-coupled estrogen receptor GPER in health and disease. *Nature Reviews. Endocrinology* **7** 715–726. (doi:10.1038/nrendo.2011.122)
- Quaynor SD, Stradtman EW Jr, Kim HG, Shen Y, Chorich LP, Schreihoffer DA & Layman LC 2013 Delayed puberty and estrogen resistance in a woman with estrogen receptor α variant. *New England Journal of Medicine* **369** 164–171. (doi:10.1056/NEJMoa1303611)
- Ramathal C, Bagchi IC & Bagchi MK 2010 Lack of CCAAT enhancer binding protein β (C/EBP β) in uterine epithelial cells impairs estrogen-induced DNA replication, induces DNA damage response pathways, and promotes apoptosis. *Molecular and Cellular Biology* **30** 1607–1619. (doi:10.1128/MCB.00872-09)
- Ray S & Pollard JW 2012 KLF15 negatively regulates estrogen-induced epithelial cell proliferation by inhibition of DNA replication licensing. *PNAS* **109** E1334–E1343. (doi:10.1073/pnas.1118515109)
- Roberts CW & Orkin SH 2004 The SWI/SNF complex – chromatin and cancer. *Nature Reviews. Cancer* **4** 133–142. (doi:10.1038/nrc1273)
- Rumi MA, Dhakal P, Kubota K, Chakraborty D, Lei T, Larson MA, Wolfe MW, Roby KF, Vivian JL & Soares MJ 2014 Generation of ESR1-knockout rats using zinc finger nuclease-mediated genome editing. *Biology of Reproduction* **155** 1991–1999. (doi:10.1210/en.2013-2150)
- Safe S & Kim K 2004 Nuclear receptor-mediated transactivation through interaction with Sp proteins. *Progress in Nucleic Acid Research and Molecular Biology* **77** 1–36. (doi:10.1016/S0079-6603(04)77001-4)
- Safe S & Kim K 2008 Non-classical genomic estrogen receptor (ER)/specificity protein and ER/activating protein-1 signaling pathways. *Journal of Molecular Endocrinology* **41** 263–275. (doi:10.1677/JME-08-0103)
- Shughrue PJ, Askew GR, Dellovade TL & Merchenthaler I 2002 Estrogen-binding sites and their functional capacity in estrogen receptor double knockout mouse brain. *Endocrinology* **143** 1643–1650. (doi:10.1210/endo.143.5.8772)
- Sinkevicius KW, Burdette JE, Woloszyn K, Hewitt SC, Hamilton K, Sugg SL, Temple KA, Wondisford FE, Korach KS, Woodruff TK *et al.* 2008 An estrogen receptor- α knock-in mutation provides evidence of ligand-independent signaling and allows modulation of ligand-induced pathways *in vivo*. *Endocrinology* **149** 2970–2979. (doi:10.1210/en.2007-1526)
- Smith EP, Boyd J, Frank GR, Takahashi H, Cohen RM, Specker B, Williams TC, Lubahn DB & Korach KS 1994 Estrogen resistance caused by a mutation in the estrogen-receptor gene in a man. *New England Journal of Medicine* **331** 1056–1061. (doi:10.1056/NEJM199410203311604)
- Stauffer SR, Coletta CJ, Tedesco R, Nishiguchi G, Carlson K, Sun J, Katzenellenbogen BS & Katzenellenbogen JA 2000 Pyrazole ligands: structure-affinity/activity relationships and estrogen receptor- α -selective agonists. *Journal of Medicinal Chemistry* **43** 4934–4947. (doi:10.1021/jm000170m)
- Takeo T & Sakuma Y 1995 Diametrically opposite effects of estrogen on the excitability of female rat medial and lateral preoptic neurons with axons to the midbrain locomotor region. *Neuroscience Research* **22** 73–80. (doi:10.1016/0168-0102(95)00885-W)
- Tang Q, Chen Y, Meyer C, Geistlinger T, Lupien M, Wang Q, Liu T, Zhang Y, Brown M & Liu XS 2011 A comprehensive view of nuclear receptor cancer cistromes. *Cancer Research* **71** 6940–6947. (doi:10.1158/0008-5472.CAN-11-2091)
- Toda K, Takeda K, Okada T, Akira S, Saibara T, Kaname T, Yamamura K, Onishi S & Shizuta Y 2001 Targeted disruption of the aromatase P450 gene (Cyp19) in mice and their ovarian and uterine responses to 17 β -oestradiol. *Journal of Endocrinology* **170** 99–111. (doi:10.1677/joe.0.1700099)
- Wada-Hiraike O, Hiraike H, Okinaga H, Imamov O, Barros RP, Morani A, Omoto Y, Warner M & Gustafsson JA 2006 Role of estrogen receptor β in uterine stroma and epithelium: insights from estrogen receptor $\beta^{-/-}$ mice. *PNAS* **103** 18350–18355. (doi:10.1073/pnas.0608861103)
- Walker VR & Korach KS 2004 Estrogen receptor knockout mice as a model for endocrine research. *ILAR Journal* **45** 455–461. (doi:10.1093/ilar.45.4.455)
- Wall EH, Hewitt SC, Case LK, Lin CY, Korach KS & Teuscher C 2014 The role of genetics in estrogen responses: a critical piece of an intricate puzzle. *FASEB Journal* **28** 5042–5054. (doi:10.1096/fj.14-260307)
- Walter P, Green S, Greene G, Krust A, Bornert JM, Jeltsch JM, Staub A, Jensen E, Scraze G, Waterfield M *et al.* 1985 Cloning of the human estrogen receptor cDNA. *PNAS* **82** 7889–7893. (doi:10.1073/pnas.82.23.7889)
- Watanabe H, Suzuki A, Kobayashi M, Takahashi E, Itamoto M, Lubahn DB, Handa H & Guchi T 2003 Analysis of temporal changes in the expression of estrogen-regulated genes in the uterus. *Journal of Molecular Endocrinology* **30** 347–358. (doi:10.1677/jme.0.0300347)
- Weihua Z, Saji S, Makinen S, Cheng G, Jensen EV, Warner M & Gustafsson JA 2000 Estrogen receptor (ER) β , a modulator of ER α in the uterus. *PNAS* **97** 5936–5941. (doi:10.1073/pnas.97.11.5936)

- Winuthayanon W, Hewitt SC, Orvis GD, Behringer RR & Korach KS 2010 Uterine epithelial estrogen receptor α is dispensable for proliferation but essential for complete biological and biochemical responses. *PNAS* **107** 19272–19277. (doi:10.1073/pnas.1013226107)
- Winuthayanon W, Hewitt SC & Korach KS 2014 Uterine epithelial cell estrogen receptor α -dependent and -independent genomic profiles that underlie estrogen responses in mice. *Biology of Reproduction* **91** 110. (doi:10.1095/biolreprod.114.120170)
- Winuthayanon W, Bernhardt ML, Padilla-Banks E, Myers PH, Edin ML, Hewitt SC, Korach KS & Williams CJ 2015 Oviductal estrogen receptor α signaling prevents protease-mediated embryo death. *eLife* **4** e10453. (doi:10.7554/eLife.10453)
- Wu SC & Zhang Y 2009 Minireview: role of protein methylation and demethylation in nuclear hormone signaling. *Molecular Endocrinology* **23** 1323–1334. (doi:10.1210/me.2009-0131)
- Xu J, Qiu Y, DeMayo FJ, Tsai SY, Tsai MJ & O'Malley BW 1998 Partial hormone resistance in mice with disruption of the steroid receptor coactivator-1 (SRC-1) gene. *Science* **279** 1922–1925. (doi:10.1126/science.279.5358.1922)
- Yang J, Singleton DW, Shaughnessy EA & Khan SA 2008 The F-domain of estrogen receptor- α inhibits ligand induced receptor dimerization. *Molecular and Cellular Endocrinology* **295** 94–100. (doi:10.1016/j.mce.2008.08.001)
- Yi P, Wang Z, Feng Q, Pintilie GD, Foulds CE, Lanz RB, Ludtke SJ, Schmid MF, Chiu W & O'Malley BW 2015 Structure of a biologically active estrogen receptor-coactivator complex on DNA. *Molecular Cell* **57** 1047–1058. (doi:10.1016/j.molcel.2015.01.025)
- Zaret KS & Carroll JS 2011 Pioneer transcription factors: establishing competence for gene expression. *Genes and Development* **25** 2227–2241. (doi:10.1101/gad.176826.111)

Received in final form 10 November 2015

Accepted 19 November 2015

Accepted Preprint published online 19 November 2015