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Review

Intraovarian actions of oestrogen

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Oestrogen regulates several hypothalamic and pituitary hormones, which in turn control ovarian functions. Oestrogen and its metabolites, such as catecholoestrogens, also have direct effects within the ovary. This review examines the roles of oestrogen in regulating ovarian folliculogenesis, ovulation and corpus luteum formation. Oestrogen promotes follicular development, which culminates in ovulation, by potentiating follicular development, granulosa cell expression of gonadotrophin receptors, steroidogenesis, and gap junction formation by granulosa cells, and by inhibiting granulosa cell apoptosis. In addition, oestrogen may be needed for corpus luteum formation and maintenance. Studies on mutant mice that either lack one or both of the known oestrogen receptors or are unable to synthesize oestrogen support some but not all of these prior inferences of the roles of oestrogen within the ovary. Although these transgenic mice have proved useful in determining some of the intraovarian actions of oestrogen, they present confounding problems, including hormonal imbalances, that hinder interpretation. Transgenic mice with conditional or tissue-directed mutations in their oestrogen receptors are needed to dissect the ovarian actions of oestrogen further. In addition, microarray technologies, combined with specific hormone treatment regimens are likely to provide an attractive, alternative approach to using mutant mice in clarifying the direct actions of oestrogen in the ovaries of other species.

Since the discovery of oestrogens in the early 1920s (Allen and Doisy, 1923), their effects have intrigued reproductive biologists. Classically, two approaches have been used to study the role of oestrogen within the ovary: surgical removal of the pituitary gland to eliminate confounding gonadotrophins, and pharmacological blockage of either the synthesis of oestrogen or its receptors both *in vivo* and *in vitro*. Although some studies have implicated a role for oestrogen within the ovary (Goldenberg *et al.*, 1972; Nakayama *et al.*, 1981; Nakano *et al.*, 1982; Gore-Langton and Daniel, 1990; Nayudu and Osborn, 1992; Hulshof *et al.*, 1995), others have not (Coney *et al.*, 1987; Spears *et al.*, 1998). Transgenic gene knockout technology has been used to investigate the intraovarian effects of oestrogen. Mice that lack either the P450aromatase gene (Fisher *et al.*, 1998; Honda *et al.*, 1998) or one or both of the known oestrogen receptors have been produced (Lubahn *et al.*, 1993; Krege *et al.*, 1998; Couse *et al.*, 1999; Dupont *et al.*, 2000; for review, see Rosenfeld *et al.*, 2001). The main ovarian events studied for oestrogen involvement have been folliculogenesis, steroidogenesis, ovulation and corpus luteum formation.

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Oestrogen synthesis, sites of action and metabolism

Androgens are produced by the thecal cells and, in general, they are taken up by the granulosa cells and converted by P450aromatase to various oestrogens with the main one being oestradiol (Fig. 1). In some species, such as pigs (Lautincik *et al.*, 1994; Shores and Hunter, 1999), chickens (Kato *et al.*, 1995) and tree shrews (Kimura *et al.*, 2000), the thecal cells can also synthesize oestrogen. This steriod hormone is then released into the vasculature and circulated to the uterus, hypothalamus, mammary gland and various other reproductive and non-reproductive organs. Oestrogens may also exert local effects within the ovary. Some oestrogens appear also to be metabolized into presumed inactive as well as active products, such as the catecholoestrogens (Zhu and Conney, 1998; Fig. 1).

Catecholoestrogens

The enzymes, 2-hydroxylase (cyp1a1) and 4-hydroxylase (cyp1b1) catalyse the formation of catecholoestrogens from oestradiol (Fig. 1). Both of these catecholoestrogens can bind to the two known oestrogen receptors, ERα and ERβ (Kuiper *et al.*, 1997). In addition, it has been proposed that they bind to a novel oestrogen receptor (Das *et al.*, 1997) and possibly

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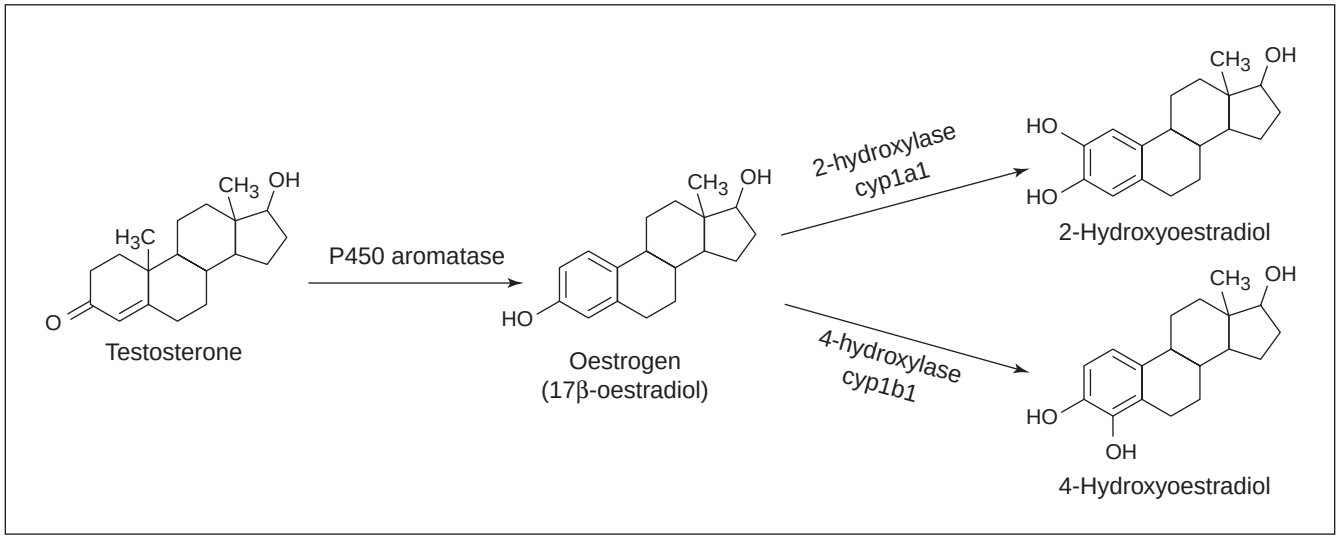


Fig. 1. Synthesis of oestradiol and catecholoestrogens. Testosterone is converted by P450aromatase into oestrogen (oestradiol). Oestradiol can then be metabolized into inactive and active metabolites, such as 2-hydroxyoestradiol and 4-hydroxyoestradiol. The enzymes 2-hydroxylase and 4-hydroxylase catalyse the conversion of oestradiol to 2-hydroxyoestradiol and 4-hydroxyoestradiol, respectively. All of these enzymes are expressed by the granulosa cells.

even androgen receptor (Hudson and Hillier, 1985). Pig and human ovaries contain high concentrations of 2-hydroxylase and 4-hydroxylase (Hammond *et al.*, 1986; Muskhelishvili *et al.*, 2001), and certain ovarian responses appear to be induced directly by these steroid metabolites, as discussed further below. The pattern of expression of these enzymes has not been examined throughout the oestrous cycle.

Oestrogen receptors

Oestrogen was the first steroid hormone demonstrated to activate transcription by binding to a receptor, the oestrogen receptor (for review, see Jensen and DeSombre, 1973). The receptors with their bound hormone modulate transcription by binding to oestrogen response elements (ERE) present on oestrogen-inducible genes (Kumar *et al.*, 1987). The receptors can also act as co-regulators through binding to other transcription factors already attached to gene regulatory regions (Adler *et al.*, 1988; Feng *et al.*, 1998), and by ligand-independent mechanisms (Ignar-Trowbridge *et al.*, 1992). Although it was first believed that oestrogen receptors were predominantly cytoplasmic, it was later proposed that they are located exclusively within the nucleus (Welshons *et al.*, 1984; Greene *et al.*, 1986). It is now clear that these receptors can be found in both the cytoplasm and nucleus, with the ratio dependent upon the cell type and physiological conditions (Yamashita, 1998; Zieba *et al.*, 2000). Some studies also indicate that oestrogen receptors reside on the plasma membrane and modulate cellular activity without directly associating with DNA (Morley *et al.*, 1992; Levin, 1999; Pietras and Szego, 1999; Nadal *et al.*, 2000).

The oestrogen receptors are members of the superfamily of steroid nuclear receptors (Parker, 1995; Baker, 1997; White and Parker, 1998). All steroid receptors have a DNA-binding domain that is composed of two zinc fingers followed by a C-terminal ligand-binding domain (Parker, 1995; White and Parker, 1998). Currently, two oestrogen receptors, ERα (Green *et al.*, 1986; Greene *et al.*, 1986) and ERβ (Kuiper *et al.*, 1996; Mosselman *et al.*, 1996) have been cloned. These two receptors have considerable sequence identity in the DNA-binding domains, which permit both receptor types to interact with EREs of various genes. Sequence differences between the two receptors occur primarily in the N- and C-terminal regions (Kuiper *et al.*, 1996; Mosselman *et al.*, 1996).

There are many alternatively spliced forms of both ERα and ERβ (Chu and Fuller, 1997; Petersen *et al.*, 1997; Kos *et al.*, 2000; Poola *et al.*, 2000). The alternatively spliced forms of ERα have been identified predominantly in human breast cancer and other tumour cell lines (Kos *et al.*, 2000; Poola *et al.*, 2000) but not in normal ovarian cells. One alternative form of ERβ identified in rats lacks exon 4, which forms the second zinc finger of the DNA-binding domain, whereas another form has 18 additional codons in the ligand-binding domain, which results in a protein with only 10% of the oestradiol-binding affinity and 100- to 1000-fold lower transcriptional activation potential compared with the full length receptor (Petersen *et al.*, 1997). This form with 18 additional amino acids appears to be the predominant ERβ in various mouse organs, including the ovary (Lu *et al.*, 2000). A third form of ERβ described in rats carries a deletion of the second zinc finger, as well as the 18 additional codons in the ligand-binding domain (Petersen *et al.*, 1997) (Fig. 2). All three of these aforementioned forms have been identified in

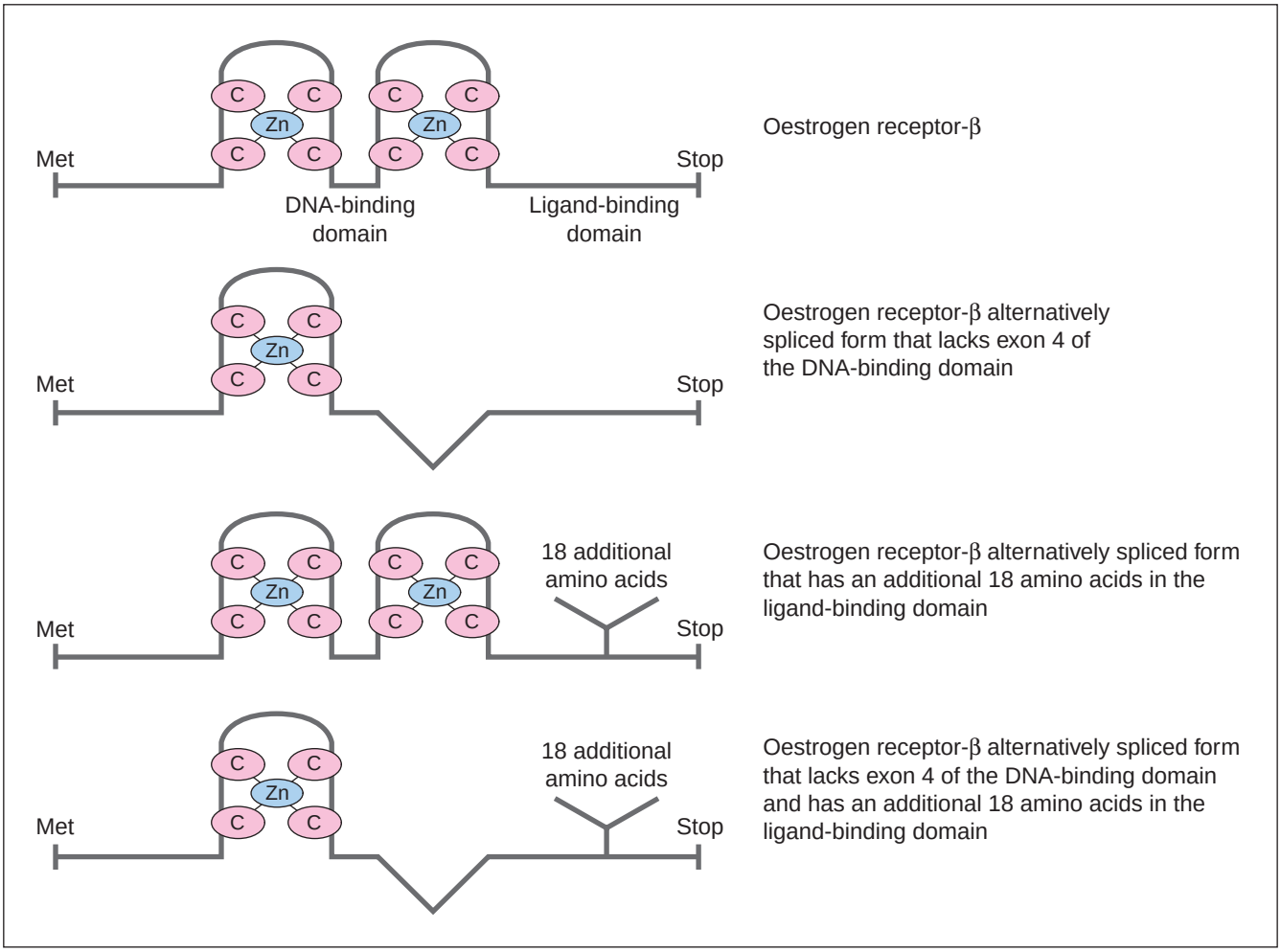


Fig. 2. Alternatively spliced forms of oestrogen receptor β (ERβ) identified in the rat ovary. Spliced forms that have a deletion of the second finger, which contributes to binding of the oestrogen responsive element on DNA, are presumably unable to induce transcription through the oestrogen response element. Other forms that have insertion of amino acids within the ligand-binding domain may have different binding affinities to the various endogenous oestrogens.

the rat ovary (Petersen *et al.*, 1997), and thus, each may regulate oestrogenic activity in the rodent ovary. In addition, other novel human ERβ forms, which differ in their C-terminal sequences, have been identified, and some of these are expressed in the human ovary (Moore *et al.*, 1998).

The discovery of ERβ raises the question as to whether mammals possess additional oestrogen receptors that have yet to be identified. No such genes have been identified to date in the human genome, although there is some evidence for at least one additional oestrogen receptor in the mouse ovary and uterus (Kudolo *et al.*, 1984a,b; Hillier *et al.*, 1989; Das *et al.*, 1997; Ghosh *et al.*, 1999; for review, see Rosenfeld *et al.*, 2001). Moreover, a third oestrogen receptor, ERγ, has been identified in fish (Hawkins *et al.*, 2000). ERγ is distinct from other fish ERαs and ERβs, but is quite closely related to ERβ. In mammals, alternatively spliced forms of ERα and ERβ that differ in their ligand-binding domains may represent novel oestrogen receptors because these mutations might alter their ability to interact with various endogenous oestrogens, their metabolites and antagonists.

For oestrogens to act on specific cell types in the ovary, ovarian oestrogen receptors must be present, and oestrogen-induced gene activation generally occurs. The ovarian cell types in which ERα and ERβ have been identified are listed (Table 1). In all species studied to date, ERβ is the most abundant ovarian oestrogen receptor. This receptor is especially prominent in granulosa cells, but it is also expressed in luteal cells and, to a lesser extent, in thecal cells (see references in Table 1).

Regulation of ovarian oestrogen receptor expression

As in other oestrogen-responsive organs, such as the uterus, oestrogen downregulates granulosa cell expression of ERβ protein (Sharma *et al.*, 1999). In rodents, treatment

Table 1. Expression of oestrogen receptor α and β in ovarian cells of various species, as determined by *in situ* hybridization or immunohistochemistry

	Oestrogen receptor α			Oestrogen receptor β		
Ovarian cells	Expression	Species	Reference	Expression	Species	Reference
Granulosa cells of pre-antral follicles	Not detected	Marmoset	Saunders *et al.*, 2000	High	Human	Pelletier and El-Alfy, 2000; Saunders *et al.*, 2000; Taylor and Al-Azzawi, 2000
		Human	Pelletier and El-Alfy, 2000;			
		Rat	Saunders *et al.*, 2000			
			Sar and Welsch, 1999		Rat	Byers *et al.*, 1997; Sar and Welsch, 1999; Pelletier *et al.*, 2000
					Marmoset	Saunders *et al.*, 2000
Granulosa cells of antral follicles	Low	Human	Saunders *et al.*, 2000; Taylor and Al-Azzawi, 2000	High	Human	Pelletier and El-Alfy, 2000; Saunders *et al.*, 2000; Taylor and Al-Azzawi, 2000
		Marmoset	Saunders *et al.*, 2000		Marmoset	Saunders *et al.*, 2000
					Cow	Rosenfeld *et al.*, 1999
					Rat	Sar and Welsch, 1999; Pelletier *et al.*, 2000
	Not detected	Rat	Sar and Welsch, 1999			
		Human	Pelletier and El-Alfy, 2000			
Theca cells of antral follicles	Low	Rat	Pelletier *et al.*, 2000; Sar and Welsch, 1999	Low	Human	Saunders *et al.*, 2000; Taylor and Al-Azzawi, 2000;
		Human	Pelletier and El-Alfy, 2000		Marmoset	Saunders *et al.*, 2000
	Not detected	Marmoset	Saunders *et al.*, 2000; Taylor and Al-Azzawi, 2000	Not detected	Rat	Sar and Welsch, 1999
		Human	Saunders *et al.*, 2000; Taylor and Al-Azzawi, 2000			
Luteal cells	Low–moderate	Human	Hosokawa *et al.*, 2001	Low–moderate	Human	Saunders *et al.*, 2000; Taylor and Al-Azzawi, 2000; Hosokawa *et al.*, 2001
		Rat	Telleria *et al.*, 1998			
		Ewe	Zieba *et al.*, 2000			
					Marmoset	Saunders *et al.*, 2000
	Not detected		Human			Pelletier and El-Alfy, 2000;
			Taylor and Al-Azzawi, 2000	Not detected	Rat	Sar and Welsch, 1999
Germinal epithelium	Low	Rat	Sar and Welsch, 1999; Pelletier *et al.*, 2000	Low	Human	Pelletier and El-Alfy, 2000; Saunders *et al.*, 2000
		Human	Pelletier and El-Alfy, 2000; Saunders *et al.*, 2000		Marmoset	Saunders *et al.*, 2000
		Marmoset	Saunders *et al.*, 2000	Not detected	Rat	Sar and Welsch, 1999

with gonadotrophins, in particular LH, results in a marked decrease of ERβ in the ovary (Byers *et al.*, 1997; Fitzpatrick *et al.*, 1999). This effect is mediated through a cAMP-dependent pathway. Not surprisingly, addition of cAMP to cultured rat granulosa cells also decreases ERβ expression (O'Brien *et al.*, 1999). In contrast, when cattle are infused with ovulatory dosages of LH, ovarian ERβ concentrations are not altered (Manikkam *et al.*, 1999). Why this species difference occurs is not clear, but it may relate to the manner in which follicular maturation and selection occurs in the two species, that is, polyovulatory rodents versus generally monovulatory cattle. ERβ may regulate early follicular growth in rodents, whereas it may exert control over both early and late follicular development in cattle.

Mice that lack LH–hCG receptors have a marked decrease in ovarian ERα mRNA, but an increase in ovarian ERβ mRNA expression, as determined by northern blot analysis (Lei *et al.*, 2001). Oestrogen and progesterone treatment of these mice restores ERα mRNA to the amounts found in wild-type mice, but ERβ mRNA remains increased. These findings support the contention that LH signalling is needed in mice to maintain proper ovarian ERβ mRNA concentrations. Concomitantly, the absence of ERα results in increased serum concentrations of LH (Rissman *et al.*, 1997; Couse and Korach, 1999), and thus, ERα is needed to maintain normal LH concentrations.

Intraovarian effects of oestrogen

Folliculogenesis

As the ovarian follicles grow and differentiate, increasing amounts of oestrogen are produced (Fortune, 1994), which, in turn, upregulate the synthesis and release of the pituitary gonadotrophins, FSH and LH, thereby promoting ovarian follicular growth. On the basis of some reports, oestrogen directly increases the number and size of ovarian follicles *in vivo* and the size of rat, mouse and bovine follicles in culture (Goldenberg *et al.*, 1972; Nakayama *et al.*, 1981; Nakano *et al.*, 1982; Gore-Langton and Daniel, 1990; Nayudu and Osborn, 1992; Hulshof *et al.*, 1995). However, other studies on rat and ovarian follicles have not supported this finding (Coney *et al.*, 1987; Spears *et al.*, 1998).

If oestrogen is indeed directly promoting ovarian follicular growth, it is uncertain which oestrogen receptors are involved. Both the ERα knockout (ERαKO) (Rosenfeld *et al.*, 2000) and ERβKO (Krege *et al.*, 1998) mice exhibit follicular development to the Graafian stage, albeit to a lesser extent than in wild-type sibling mice. However, ERαβ double KO mice are infertile because of follicular arrest (Couse *et al.*, 1999; Dupont *et al.*, 2000). Nevertheless, early follicular growth and development occurs in these mice, even though mature Graafian follicles do not form. Collectively, these results indicate that, in mice, ERα or ERβ can compensate, at least partially, for the absence of the other to provide mature ovulatory follicles, but may not be necessary for the earlier stages of follicular growth.

Aromatase knockout (ArKO) mice can complete folliculogenesis without exogenous oestrogens, but the process is impaired, with fewer follicles reaching maturity than in wild-type controls (Fisher *et al.*, 1998; Britt *et al.*, 2000). ArKO mice that are treated with oestradiol every 4 days from 4 weeks of age for 1 month have increased numbers of follicles compared with untreated ArKO mice. However, these treated mice still do not develop corpora lutea (Toda *et al.*, in press). On the basis of these data, folliculogenesis can occur, albeit sub-normally, in the absence of either oestrogen or one of its two known receptors.

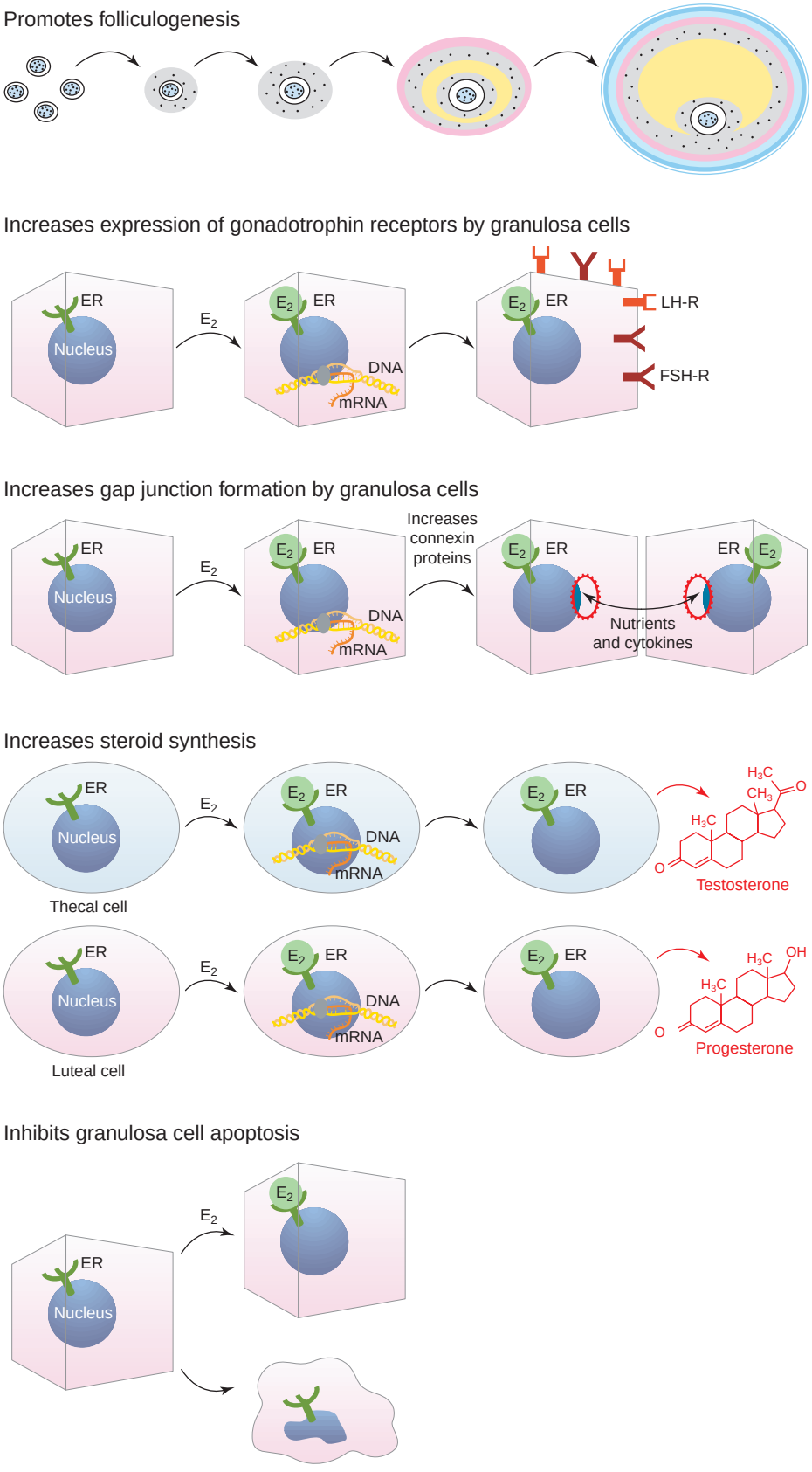
Gonadotrophin receptor expression

Growing follicles express gonadotrophin receptors. Before selection, follicles express FSH receptors, but LH receptors emerge later in the dominant follicles. In cattle, when the dominant follicles are first selected, FSH reaches its nadir (Fortune, 1994) while serum concentrations of LH begin to increase. It is presumed that only those follicles that express LH receptors in granulosa cells can be rescued and, in time, ovulated.

Oestrogen increases follicular expression of both FSH and LH receptors in rat granulosa cells (Richards *et al.*, 1976, 1979). Those follicles that first begin to produce significant amounts of oestrogen are more likely to possess more gonadotrophin receptors. Therefore, whether or not a particular follicle expresses oestrogen receptors may control its fate. However, ERαKO mice have increased expression of granulosa and thecal LH receptor (Schomberg *et al.*, 1999), again indicating a lack of ERα involvement in control of follicular growth. These mice also have increased serum oestrogen concentrations, which may increase granulosa cell expression of LH receptor by acting through ERβ or one of the putative additional receptor types. There is currently no information on the ovarian expression of FSH and LH receptors in ERβKO and ERαβ double KO mice. If oestrogen acts through ERβ to upregulate gonadotrophin receptor expression, ERβ mutant mice presumably would have decreased expression of FSH and LH receptors.

Steroid production

Ovarian steroid hormones are produced throughout the oestrous cycle. During folliculogenesis, the thecal cells produce androgens, which are converted into oestrogens by P450aromatase in the granulosa cells. Both oestradiol (Fortune and Hansel, 1979; Leung and Armstrong, 1980; Welsh *et al.*, 1983; Roberts and Skinner, 1990) and catecholoestrogens (Spicer and Hammond, 1987; Tekpetey and Armstrong, 1994) can regulate the production of androgen and progesterone within bovine, rat and pig ovaries. Oestrogen is known to increase CYP17 expression by rat theca cells (Johnson and Crane, 1995). The oestrogen-driven increase in progesterone presumably promotes corpus luteum formation and maintenance through luteal progesterone receptors (Duffy and Stouffer, 1995; Smith *et al.*, 1995).



Gap junctions

As the follicles continue to grow and proliferate, the avascular granulosa cell lining becomes removed from the nutrient-providing interstitial blood vessels. Gap junctions permit transfer of nutrients and cytokines to and from the granulosa cells and developing oocytes (Albertini and Anderson, 1974; Anderson and Albertini, 1976). Oestrogen controls granulosa cell gap junction formation (Merk *et al.*, 1972; Burghardt and Anderson, 1981). Gap junctions are composed of various connexin proteins: connexin 43 is a major granulosa and luteal gap junction protein in bovine (Nuttinck *et al.*, 2000), ovine (Grazul-Bilska *et al.*, 1998), pig (Lenhart *et al.*, 1998) and rat (Mayerhofer and Garfield, 1995) ovaries, and is oestrogen-regulated (Yu *et al.*, 1994). One method whereby oestrogen might potentiate gap junction formation between granulosa cells is through induction of this protein. However, connexin 43 protein expression has not been reported in any of the oestrogen receptor or P450arom mutant female mice.

Apoptosis

Most follicles do not reach the ovulatory stage but instead become atretic owing to apoptosis of the granulosa cell lining (Kaipia and Hsueh, 1997; Amsterdam *et al.*, 1999). This process is normally tightly regulated with anti- and pro-apoptotic factors balancing each other, and with crosstalk among multiple intracellular pathways determining the final outcome of the cell (Kaipia and Hsueh, 1997; Amsterdam *et al.*, 1999). Oestrogen inhibits granulosa cell apoptosis (Billig *et al.*, 1993). In contrast, androgens promote apoptosis (Billig *et al.*, 1993).

As with other ovarian processes, it is uncertain which oestrogen receptors are mediating this oestrogenic anti-apoptotic effect. In addition, it is not clear whether there are physiological repercussions if the anti-apoptotic mechanism is perturbed. In 10–12-week-old ArKO mice, many apoptotic granulosa cells are present in large antral follicles (Britt *et al.*, 2000), and by 21–23 weeks of age, these mice have fewer antral follicles than do wild-type controls. Predictably, ArKO mice have increased ovarian expression of pro-apoptotic genes, such as *p53* and *Bax*, compared with wild-type mice (Toda *et al.*, in press). At 1 year of age, the ArKO mice have a reduced number of primary follicles, all of which contain numerous apoptotic cells (Britt *et al.*, 2000). By contrast, ERαKO mice have similar numbers of apoptotic granulosa cells when compared with wild-type control mice (Schomberg *et al.*, 1999). Although oestrogen may be essential for preventing the demise of ovarian follicles, this protection is clearly not conferred through ERα.

Corpus luteum formation and maintenance

Early studies with hypophysectomized or X-irradiated rabbits and rats with hypothalamic lesions indicated that oestrogen directly regulates corpus luteum formation and controls luteal maintenance (Robson, 1937; Bogdanove, 1966; Keyes and Nalbandov, 1967). Unexpectedly, ERβKO mice demonstrate normal corpus luteum development, but have an overall reduced fertility (Krege *et al.,* 1998). Pre-pubertal ERαKO mice treated with superovulatory dosages of gonadotrophins also develop steroidogenically functional corpora lutea, but they remain infertile (Rosenfeld *et al.*, 2000). Thus, the phenotypes of these mutant mice do not corroborate earlier studies. In contrast, ArKO and ERαβ double KO mice do not undergo luteinization and corpus luteum formation (Fisher *et al.*, 1998; Couse *et al.*, 1999; Britt *et al.*, 2000; Dupont *et al.*, 2000). Taken together, these findings indicate that oestrogen is indeed needed for corpus luteum formation and maintenance, and that it likely acts non-selectively through ERα or ERβ with one oestrogen receptor compensating for the absence of the other. If both receptors are lacking, oestrogen cannot stimulate luteinization.

Non-genomic actions of oestrogen within the ovary

The complex physiological responses to oestrogen are generally presumed to involve transcriptional regulation of many genes. However, oestrogen-binding to putative membrane oestrogen receptors (Levin, 1999) may also induce non-genomic–non-transcriptionally mediated responses. In cultured granulosa cells, oestrogen has been implicated in causing a rapid increase in intracellular Ca^{2+} concentrations (Morley *et al.*, 1992). As this response occurs within seconds of treating the cells with oestrogen, a reasonable conclusion is that it is occurring without transcriptional activation, that is, it is elicited through some existing membrane-associated response. The demonstration that ERα associates with the regulatory subunit of phosphatidylinositol-3-OH kinase (PI(3)K) in the presence of ligand may provide at least a partial explanation for the non-genomic effects of oestrogen (Simoncini *et al.*, 2000), and thus provide another dimension to oestrogenic action within the ovary. Non-genomic responses have not yet been studied in transgenic mice.

Confounding problems in interpreting ovarian phenotypes in oestrogen- and oestrogen receptor-deficient mice

Although these knockout mice have proved invaluable in elucidating some of the roles of oestrogen within the ovary,

Fig. 3. Illustration of the proposed intraovarian actions of oestrogen. On the basis of past pharmacological, surgical, and culture approaches, oestrogen modulates many ovarian responses, including promoting folliculogenesis, increasing granulosa cell gonadotrophin receptor expression, increasing gap junction formation between granulosa cells, increasing steroid production by both thecal and luteal cells, and inhibiting granulosa cell apoptosis. E_2: oestrogen; LH-R: LH receptor, FSH-R: FSH receptor.

confounding factors hinder interpretation of the resulting ovarian phenotypes. In rodents, only one P450aromatase gene has been identified and knocked out (Youngblood *et al.*, 1989; Fisher *et al.*, 1998; Honda *et al.*, 1998). However, such mice are likely to have been exposed *in utero* to maternal oestrogen and postnatally to other sources of oestrogen, including compounds in the environment and diet, which would hinder interpreting the reproductive phenotypes of the adult animals. Furthermore, it is now apparent that there are non-aromatized steroid molecules, such as hermaphrodiol, that have both androgenic and oestrogenic activity (Lima *et al.*, 2000; Rosenberg-Zand *et al.*, 2000). Therefore, proper interpretation of ovarian phenotypes in ArKO mice requires exclusion of all sources of both aromatic and non-aromatic oestrogenic molecules. In addition, ArKO mice have secondary hormonal imbalances, such as increased serum concentrations of LH, FSH and androgen, which may account for some of the ovarian pathology (Fisher *et al.*, 1998).

The ERKO mice also have secondary hormonal imbalances, including an increase in serum LH, which is present in both ERαKO (Rissman *et al.*, 1997; Couse and Korach, 1999) and ERαβ double KO (Couse *et al.*, 1999a) mice. In ERKO mice, all receptors that bind oestrogen have to be considered and deleted before it is possible to make accurate inferences about which oestrogen receptors mediate the intraovarian effects of oestrogen. Novel oestrogen receptors may be discovered once the mouse and human genomic sequences are fully annotated and the properties of forms arising by alternative splicing elucidated. It is interesting to note that the orphan oestrogen-related receptors (ERRα, β, and γ), the endogenous ligands of which are unknown, have been demonstrated, albeit with low affinity, to bind diethylstilboestrol (DES) but not oestradiol (Tremblay *et al.*, 2001). Thus, the ERRs may play a role in oestrogenic actions within the ovary, although as yet these receptors have not been examined fully in the ovary.

Ultimate resolution of these difficulties may depend on the creation of more sophisticated approaches to producing mutant mice that have ovarian cell-specific and conditional ablation of oestrogen receptors. Production of such mice would permit a thorough analysis of the effects of oestrogen-binding to oestrogen receptors throughout the oestrous cycle and pregnancy and would have minimal confounding hormonal imbalances. Transgenic mice with tamoxifen-dependent Cre recombinase fused to a mutated ligand-binding domain of human oestrogen receptor have been produced (Metzger and Chambon, 2001) and, when these mice are treated with tamoxifen, the chromosomally integrated gene, which is flanked by loxP sites, is excised.

Studying the intraovarian actions of oestrogen in other species

It is worth considering whether the mouse is the best model with which to study the intraovarian effects of oestrogen. Is it possible, for example, to extrapolate from responses seen in rodents to other species? Although women, cows and mares are generally monovulatory, rodents are polyovulatory, and thus, the regulation of folliculogenesis may be different. How then can the *in vivo* effects of oestrogen in the ovaries of other species be studied, where the targeted deletion of genes is not currently possible? Microarray technology could be invaluable in this respect and provide comparison of oestrogen-regulated genes in various ovarian cells during puberty, the oestrous cycle, pregnancy and in response to oestrogenic treatment. This technique could be used in combination with pharmacological blockage of one or more oestrogen receptors to provide multiple snapshots of the intraovarian effects of oestrogen. An ovarian gene database (http://ovary.stanford.edu; Melner and Korach, 2000) has been established that may assist in the identification of all the oestrogen-regulated ovarian genes.

Regulation of oestrogen action within the ovary

Although there is ample evidence that oestrogen acts within the ovary, it also originates there, and is present in highest concentrations in this organ. Such quantities would in theory continually saturate and activate any cognate receptors, and potentially result in either continual transcriptional activation or other consequences such as desensitization. Some regulation of this system must occur to prevent such scenarios. One regulatory step could be at the receptor level. Oestrogen receptors may be required for only a limited period of follicular development but persist thereafter in a sub-functional state.

Co-activators may provide another level of regulation. These factors act in concert with oestrogen receptors to induce transcription of oestrogen-responsive genes. Various transcription factors, such as SRC-1, GRIP1, RAC3, p300, SPA AIB1, RIP140, SMRT, Fas-associated protein-tyrosine phophatase-1 (FAP-1), and co-activator independent of AF-2 function (CIA) that regulate oestrogen-responsive genes have been identified (Rey *et al.*, 2000; Hlaing *et al.*, 2001; Sauve *et al.*, 2001). Possibly, it is their specific expression pattern and not that of the cognate receptors that governs the oestrogen responsiveness of granulosa and other ovarian cells. Differential expression of some of the oestrogen receptor co-activators supports this hypothesis (Hlaing *et al.*, 2001). Although granulosa, thecal and stromal cells in ovine ovaries express SRC-1, RIP140 and SPA mRNA, granulosa cells have the highest mRNA expression for these co-activators (Hlaing *et al.*, 2001). Similarly, the ovine corpus luteum expresses smaller amounts of SRC-1 and RIP1240 mRNA than do follicular cells (Hlaing *et al.*, 2001).

A third form of regulation may be through metabolism of oestrogen by the *cyp* enzymes into various active and inactive metabolites. Compounds, such as the catecholoestrogens, may bind oestrogen receptors (Kuiper *et al.*, 1997), androgen receptor (Hudson and Hillier, 1985) and other novel steroid receptors (Das *et al.*, 1997, 2000), resulting in responses both quantitatively and qualitatively distinct from the parental steroid. How efficiently the various ovarian cells convert

oestradiol into these metabolites might provide another level of control.

In summary, selective expression of oestrogen receptors, co-activational factors, and metabolic conversion of oestradiol may regulate the intraovarian effects of oestrogen. Similar processes could be invoked in other tissues in which a hormone acts within its originating tissue. For example, progesterone receptors are found in the bovine and primate corpus luteum (Smith *et al.*, 1995; Duffy and Stouffer, 1995).

Conclusions

The intraovarian effects of oestrogen have been examined. Early work indicated that oestrogen directly regulates ovarian folliculogenesis, corpus luteum formation, granulosa cell expression of the gonadotrophin receptors, gap junction formation between granulosa cells, granulosa cell apoptosis and steroid production by granulosa, thecal and luteal cells (Fig. 3). These actions are presumably regulated by specific oestrogen receptors. ERβ is the predominant form of oestrogen receptor found in the ovary (Byers *et al.*, 1997; Couse *et al.*, 1997).

Aromatase-deficient and oestrogen receptor-deficient mice have been created to study the effects of oestrogen. Although results from these mice support a role for oestrogen in the ovary, some of the data are equivocal, such that the phenotypes cannot always be directly attributed to the absence of the gene, since secondary hormonal imbalances and other confounding factors invariably occur in these mice. More sophisticated, tissue-targeted knockout mice are needed to elucidate the roles of oestrogen within the ovary. The involvement of oestrogen in ovarian physiology needs to be examined in several species before any firm and general conclusions can be drawn about its intraovarian effects.

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