ROLE OF ESTROGEN AND PROGESTERONE IN REPRODUCTION

Both the normal development and normal physiological states of reproductive tracts of adult females are heavily influenced by the effects of steroid hormones such as estrogens and progestins. Indeed coordinated and cascadic effects of cyclical changes in estrogens and progestins are critical for proper regulation of uterine physiology. Under the influence of ovarian hormones the oviduct, uterus, cervix and vagina serve to transport ovulated eggs, promote fertilization, provide an environment for implantation of fertilized eggs and development of fetus and facilitate parturition.

Estrogen action in uterus

Estrogen in human increases twice during the menstrual cycle: the first coincides with pre-ovulation period, the second occurs during the luteal phase of the cycle. Following menstruation, the ovary enters into the follicular phase of the ovarian cycle. In this phase another crop of ovarian follicles begin to mature and the levels of E2 rise; the oviduct and uterus enter into a proliferative phase in which the action of E2 dominate the oviductal and uterine physiology. Following ovulation, the ovary enters into luteal phase in which E2 levels decrease, whereas progesterone rises. In the oviducts, periods of high E2 promote an increase in epithelial mitotic activity, hypertrophy and ciliation of the mucosal epithelium. As oviduct prepares for the egg transport and fertilization, the uterus undergoes proliferative changes in both the myometrium and the endometrium. These include E2-induced synthesis of contractile proteins, creatinine phosphokinase, and ATPase in myometrial cells. it also promotes glycogen buildup and cellular hypertrophy.

Under the influence of E2, the endometrium undergoes extensive remodeling or proliferation, which prepares the luminal epithelium and stroma for implantation. During the initial phase (1-4 h) following E2 exposure in mice, changes in stromal tissue include hyperemia and water imbibition. In the later stages of E2 stimulation, there is an increase in DNA and protein synthesis with resultant hyperplasia and
hypertrophy. Morphologically, the epithelial cells become columnar, endometrial glands dilate, and the stromal cells exhibit some increased numbers of mitotic cells and edema. It has been suggested that the initial effects of E2 on epithelial hyperplasia are mediated through activation of stroma and subsequent effect of stroma on the epithelium. Another important effect of E2 during uterine proliferation is the induction of progesterone receptor synthesis, which allows the uterus to respond to elevated progesterone. Elevated E2 also regulate uterine expression of growth factors and growth factor receptors, such as epidermal growth factor (EGF) and epidermal growth factor receptor (EGF-R), in the uterus. These elevated levels of EGF appear to be partly responsible for the uterotropic effects induced by E2.

During proliferative phase, the dimensions of the endocervical canal change and the cervix undergoes increased vascularization, thickening of epidermal layers, and edema. Synthesis and secretion of cervical mucous also reaches its genith during the periovulatory period when E2 is at its nadir.

The vaginal epithelium also undergoes thickening and cornification associated with estradiol- induced increases in cell division of the basal epithelium as well as ER- mediated increase in keratin gene expression.

![Stages of estrous cycle](image)

**Fig. 14** Serum hormone (estradiol 17β and progesterone) levels during the estrous in the rat.

The Estrous cycle is the sequence of reproductive processes in mammalian species and, unlike the primate cycle, is characterized overtly by mating behaviour. It is a cascade of hormonal and behavioral events which are progesterone dominated, highly synchronized, and repetitive. Rats are seasonal polyestrus having continuous follicular availability. In the 4 day cycling rat, estradiol is at low basal level
throughout estrous and most of metestrus. In the evening of metestrus, however, the serum level starts to rise and continues to rise through diestrus, reaching a peak in the afternoon of proestrus (Fig. 14).

**Progesterone action in uterus**

Progesterone has a central role in complex regulation of female reproductive function being involved in ovulation, implantation and pregnancy. Associated with this is the involvement of progesterone in the regulation of uterine function during the reproductive cycle (estrous or menstrual), by control of cyclical changes in proliferation and decidualization. And if fertilization occurs, high circulating levels of progesterone are important not only for facilitating implantation, but also maintaining pregnancy by stimulating uterine growth and opposing the action of factors involved in myometrial contraction. It can both stimulate as well as inhibit cell proliferation in uterine tissue depending on the cell types and physiological context. It also plays a major role in cell differentiation. Various functions regulated by progesterone are glycogenesis (Demers et al., 1977; Shapiro et al., 1980), cyclic nucleotide metabolism, protein synthesis and secretion (Savouret et al., 1990) and cell cycle regulation (Clarke and Sutherland, 1990). Intracellular proteins known to be regulated by progesterone include estrogen receptor, estrogen metabolizing enzyme, α-fucosidase and type II cAMP dependent kinase. Secreted proteins include enzymes for protein, carbohydrate and prostaglandin metabolism, hydrolases, phosphatases, prostaglandin, plasminogen activator and PRL (Savouret et al., 1990).

Proliferation activities of glandular and stromal elements of human endometrium are regulated by circulating levels of estrogen and progesterone. In the late luteal phase progesterone stimulate proliferative activity in stromal elements while it is low in epithelium (Lessey et al., 1988). Specificity of the proliferative effects of progesterone in decidualization may be due in part to cell type specific action and regulation of growth factor receptors and their peptide ligands.

Heparin binding epidermal growth factor (EGF) like growth factor mRNA is induced by progestin in stromal cells but repressed in luminal and glandular epithelium (Zhang et al., 1994). Progesterone may influence uterine proliferation and differentiation during the menstrual cycle by regulation of proteases and matrix proteins. Progesterone suppress expression of stromolysin in endometrial cells and
induce transforming growth factor β (TGFβ) in these cells, resulting in down regulation of matrilysin expression in endometrial epithelium and stromal-epithelial co-cultures (Osteen et al., 1994; Burner et al., 1995). Progestin induces thrombospondin-1, an extracellular matrix glycoprotein that is expressed in vascular endothelium and inhibits angiogenesis, in endometrial stroma during late luteal phase, hence influencing cyclical regulation of vascular formation and differentiation in this tissue (Iruela-Arispe et al., 1996). Besides opposing estrogen action, progesterone carries out inhibition in the endometrial cell proliferation, by modulating growth factor pathways. It controls the proliferative effects of insulin like growth factors (IGFs) by regulating IGF- binding protein I (IGFBP-I), whose expression is in cyclical fashion in endometrial stromal cells, with the highest expression seen in mid-to-late luteal phase (Giudice et al., 1991). By binding to IGF-1, IGFBP-1 prevents its binding to its receptor and decreases cellular responsiveness to IGF-1. It acts in paracrine fashion to prevent epithelial cell proliferation during late luteal phase, and as autocrine fashion to prevent stromal cell proliferation at the end of luteal phase.

Progesterone has a major role in the endometrium in preparation for implantation of a fertilized ovum, and in many species a decrease in circulatory progesterone at the time of fertilization is sufficient to delay implantation (Rothchild, 1983). The induction of uterine cell proliferation in early pregnancy may be mediated by locally produced growth factors, many of which are under progesterone control. For example, EGF, and its receptor (in stroma), hemopoietic growth factor, colony stimulating factor-I. These exert a paracrine influence on growth and differentiation of the placental trophoblast, and its secretions from luminal and glandular epithelium by estrogen and progesterone (Das et al., 1994; Pollard et al., 1987).

Progesterone suppresses myometrial contractility during pregnancy, and a number of mechanisms exist whereby this may be mediated, including progesterone effects on intracellular calcium concentration, and levels of prostaglandins, relaxin and oxytocin. It increases the expression of calcitonin, a Ca$$^{++}$$ binding peptide hormone and lowers the calcium levels in the uterus and suppresses the gene expression of Ca transporter calbindin-D9K. It also suppresses the synthesis of PGF2α and its action.
Role of Estrogen Receptors in Uterus as Evidenced by Knockout Models

The estrogen receptor isoform ERα was first suggested as a mediator of estrogen action by Jensen and Jacobson in 1962 (Jensen and Jacobson, 1962). A second subtype of ER was discovered in 1996 by Kuiper et al and named ERβ (Details are given in Page 4, Section I). ERα is the predominant ER in the rat uterus, oviduct and vagina / cervix. ERβ is abundantly expressed in ovary, and weakly expressed in uterus and vagina / cervix and sparsely expressed in oviduct during the estrous cycle (Wang et al., 2000).

The most well characterized estrogen target tissues are those of the mammalian female reproductive tract. As shown by knockout studies, αERKO and βERKO female mice exhibit a properly differentiated female reproductive tract possessing the constituent structures (Lubahn et al., 1993; Krege et al., 1998). However, estrogen insensitivity has severely disrupted sexual maturation of the whole reproductive tract in the αERKO females and ovarian function in the βERKO females.

The uteri of both αERKO and βERKO females possess all three definitive uterine compartments, the myometrium, endometrial stroma and epithelium. However, in the αERKO, each is hypoplastic and results in whole uterine weights that are approximately half that recorded for wild type littermates. In contrast, the uteri of βERKO females appear normal and are able to undergo the cyclic changes associated with the ovarian steroid hormone. Therefore, perinatal development of the female reproductive tract in the mouse appears to be independent of ERα and ERβ actions. However, estrogen responsiveness and subsequent sexual maturity in the uterus is due to ERα gene, and has been ablated by disruption of the ERα gene. The αERKO endometrial stroma is characterized by a less organized structure and hypotrophy, with a sparse distribution of uterine gland compared with that of the wild type (Korach et al., 1996). Luminal and glandular epithelial cells in the αERKO uterus most often appear healthy, but are consistently cuboidal and lack the normal “estrogenised” morphology of tall columnar shape and basally located nucleus. And ERβ is unable to provide a compensatory role in the αERKO uterus.
The response of the ovariectomized rodent uterus to estradiol has been well documented and is often described as biphasic, with the initial phase consisting of effects that become apparent with in the first 6 h of a single estrogen treatment (Clark and Markaverich, 1988). These include metabolic responses in the form of increased water imbibition, vascular permeability and hyperemia, prostaglandin release, glucose metabolism, and eosinophil infiltration. A series of biosynthetic responses are also characteristic of the first phase and include RNA polymerase and chromatin activity, lipid and protein synthesis and increased glucose-6-phosphate dehydrogenase activity. As several of the above processes continue, they are accompanied in parallel, by dramatic increases in RNA and DNA synthesis, mitosis and cellular hyperplasia and hypertrophy that peak 24-72 h after exposure. However knockout studies provide strong evidence for the requirement of ERα in the full response (Couse et al., 1995).

The mitotic and stimulatory action of estradiol in the uterus is a complex process involving increase RNA polymerase and ribosomal activity, resulting in regulation of a plethora of genes. Two such examples are the genes encoding the progesterone receptor. (Kastner et al., 1990; Kraus et al., 1993) and the secretory protein, lectoferrin (Liu and Teng, 1992). But in the uteri of αERKO mice these genes are not up regulated by estradiol (Couse et al., 1995).

However, it must be noted that constitutive levels of PR and lectoferrin mRNA are present in αERKO uteri, suggesting that these genes are also under the influence of pathways independent of ERα.

ER isoforms in rat uterus during estrous cycle

In the uterus ERα mRNA levels are highest in proestrus when plasma E2 level is at highest, and lowest in the metestrus stage when the plasma progesterone concentration starts to increase (Sahlin et al., 1994). Also E2 causes increased concentrations of both ERα and progesterone receptor (Mergman et al., 1992) and it is likely that the rise in circulating E2 levels during the cycle is responsible for triggering the major up regulation of ERα in all cell types.

The pattern of ERβ expression is similar to that of ERα in endometrium and myometrial smooth muscle cells (Wang et al., 2000), but ERβ expression is much less predominant and shows no cyclic changes.
ER mRNA and protein levels in various target tissues can be influenced by several physiological factors, including estrogen. The effect of estrogen on ER concentration varies among different tissues and cell types. In liver cells, for example, E2 up regulates ER (Barton and Shapiro, 1988). In estrogen-responsive human breast cancer cells and rat fibroblasts (Borras et al., 1994; Saceda et al., 1989) ER is down regulated by E2. The rat uterus has served as an excellent model for studying the effect of E2 on ER mRNA and receptor protein expression. Both up regulation and down regulation of ER by E2 have been reported in rat uterus, depending upon the physiological state of the animal and experimental system employed.

Several groups have reported that supraphysiological doses of E2 suppress ERα mRNA and protein in homogenates of whole rodent uterus (Medlock et al., 1991; Rosser et al., 1993; Shupnik et al., 1989; Zhou et al., 1993). After hormone treatment, receptor mRNA and protein suppression occurred primarily in the uterine epithelia of both adult and immature rats (Nephew et al., 2000). In the adult rat uterus, the decreased expression in ERα mRNA at 6 h occurred throughout the three uterine compartment making the change apparent even on the total uterine RNA level. The decrease in ERα protein after E2 treatment of adult and immature rats occurred primarily in the luminal and glandular epithelium. The periluminal stromal cells in the adult uterus and the majority of stromal cells of the juvenile uterus continued to express ERα protein, which would be expected based on the fact that the proliferative response of the uterine epithelium is mediated by stromal ERα (Cooke et al., 1998; Bigsby and Cunha, 1986). The functional implication of the decrease in ERα mRNA and protein in uterine epithelial cells may be linked to DNA synthesis and cell proliferation. In immature rats, a second injection of E2 18 h after an initial hormone treatment caused a delay in epithelial DNA synthesis; the authors concluded that the level of ER must decrease before the onset of DNA synthesis (Nephew et al., 2000). Furthermore, the initial cell-type specific decrease in ERα is very similar to what has been observed for c-Jun, an immediate early gene, at both the level of mRNA (Nephew et al., 1994) and protein (Yamashita et al., 1996). Perhaps a transient decrease in the expression of the transcription factors, ERα and c-Jun, plays a role in allowing uterine epithelia to undergo cell proliferation. It must be noted however, that epithelial cell proliferation results from a stromally derived signal following estrogen
stimulation. There was no change, or even a slight increase in the stromal cell expression of ERα.

There are contradictions in the time course of E2 regulation of ERα in rat uterus. A decrease in ERα mRNA in rat uterus was reported at 6 h (Nephew et al., 2000). According to some, decrease in ERα mRNA has been observed as early as after a supraphysiological dose of E2 (Copland et al., 1987; Rosser et al., 1993) and Shupnik et al observed a decrease in uterine ERα mRNA at 24 h, but not at 4 h after a high dose of E2 (Shupnik et al., 1989). According to Wang et al (1999) the uterine mRNA level was significantly upregulated by E2 in ovx rats after 24 h treatment. This is consistent with the results from previous studies (Shughrue et al., 1998). The mechanism of E2-mediated up-regulation of ERα is still unclear, but the most well supported mechanism of estrogen up-regulation of target gene expression involves enhanced rates of transcription. E2 may also up-regulate endometrial ERα gene expression post-transcriptionally (Ing et al., 1996).

In contrast to some ERα mRNA trends, the stronger immunoreactivity of ERα showed the highest concentration in the stroma and glandular epithelium in the ovx rats. E2 decreases ERα protein in glandular epithelium whereas the ERα mRNA level seemed to increase. The negative effect on the intensity of ERα immunostaining in glandular epithelium agrees with a previous study in the mouse (Tibbetts et al., 1998). The contrasting results between mRNA and protein levels of ERα in glandular epithelium could be explained by a temporally different regulation by E2. It has been shown that an increase in endometrial ERα mRNA levels precedes enhanced ERα protein concentration during the preovulatory estrogen surge in ewes (Ott et al., 1993). E2 suppressed steady state of ERα mRNA caused a decrease in ERα protein levels in the rat uterus. The short half lives of both mRNA and protein products of ERα gene make levels susceptible to transcriptional and / or post transcriptional regulation (Zhou et al., 1993; Borras et al., 1994).

ERβ expression in the rat uterus was much weaker than that of ERα. Oopherectomy slightly upregulated ERα mRNA (Shupnik et al., 1989) and decreased ERβ mRNA expression in the rat uterus (Shughrue et al., 1998). Estrogen and / or progesterone had no effect on the ERβ mRNA expression in the various uterine compartments, although the total mRNA level in the rat uterus was higher in the 24 h
E\textsubscript{2} treatment group as compared to 48 h progesterone and estrogen + progesterone treatment groups. According to Wang \textit{et al} (1999) ER\textbeta protein expression in the uterine compartment is similar to that of ER\textalpha. ER\textbeta was observed in the luminal epithelium and glandular epithelium and was distributed throughout the stroma with weaker positive immunostaining than for ER\textalpha (Saunders \textit{et al}., 1997). But according to Hiroi \textit{et al}., (1999) ER\textbeta expression only in the nuclei of the glandular epithelium, the ER\textbeta protein level being too low to detect in the luminal epithelium of the intact rat uterus.

**Role of ER in decidual cell reaction**

The influence of ovarian steroids, mainly progesterone, with additional stimulation from the blastocyst, transforms stromal cells of the endometrium into decidual cells. Decidual cells are essential for successful growth of the early embryo from the time of implantation through establishment of the placenta. The mechanism of decidual cell function however remained to be explored.

Although the role of estrogen in the mechanism of decidualization has not been defined, it is likely that estrogen induces gene required for decidual transformation. However, the gene targets of estrogen in initiating and maintaining the deciduoma have not been identified. There is evidence that estrogen can induce several physiological responses that might be involved in implantation and decidual transformation. These responses include histamine and prostaglandin release (Weitlauf, 1988), increases vascular permeability (Milligran and Mirembe, 1985), and induction of various growth factor receptor ligands (Wang \textit{et al}., 1994; Das \textit{et al}., 1995; Lim \textit{et al}., 1997).

The decidual response in the ERKO uterus was unexpected, since estrogen priming was thought to be essential for a decidual transformation. The increase in wet weight following the decidualization response was more robust in the ERKO group than in the wild type group (Curtis \textit{et al}., 1999). When no estrogen was used either at priming or with progesterone, all the ERKO uteri responded, while none of the wild type uteri responded. Thus, estrogen action through ER\textalpha is not necessary for decidualization in ERKO. The ERKO uterus is fully progesterone-responsive in
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terms of gene regulation and morphological changes of the tissues despite the absence of ERα.

The function of progesterone during implantation and reproduction is strongly associated with estrogen and ERα signaling. Decidualization reaction is estrogen-dependent in the wild type, but not in the ERKO uterus (Curtis et al., 1999). Some studies have demonstrated the necessity for estrogen priming (at a time mimicking estrous) as well as low dose of estrogen (at the time mimicking the peri-implantation period) to induce decidualization with oil (Finn, 1966; Milligran and Mirembe, 1985). However, more traumatic stimuli, such as crushing the uterine horns, can produce decidualization without estrogen priming in the mouse (Finn, 1965). This indicates that the mechanism of decidualization does not absolutely require estrogen. The threshold level of stimulus required for initiation of decidualization might be lowered by estrogen treatment in a wild type. Similarly, the estrogen-independent decidualization in the ERKO may indicate that the ovx ERKO uterine tissue is more sensitive to a decidualization stimulus so that the threshold level of trauma required to initiate decidualization is as low as that of an estrogen primed wild type.

Induction of PR, especially in the priming phase of the experiment, has been proposed to be a key component of the mechanism. Although PR is increased in both the stroma and epithelium, ERα-mediated induction of PR seems to occur mainly in the stroma, since antiestrogen treatment blocks stromal but not epithelial induction of PR mRNA (Das et al., 1998). There is also evidence that ERα-independent estrogen induction of PR might occur in the stroma, as increase in PR protein has been reported in the stroma following estrogen treatment of both wild type and ERKO uteri (Tibbetts et al., 1998; Kurita et al., 1998). The discrepancy between these results may indicate a post-transcriptional effect of estradiol on stromal ER. Clearly the amount of PR exposed in ERKO uterus is sufficient to mediate decidualization with or without estradiol induction. Interestingly, the stromal PR has been shown, through tissue recombination, to be the mediator of inhibition of estrogen-induced mitosis.

Regulation of ER during decidualization

The influence of ovarian steroids, mainly progesterone, with additional stimulation from the blastocyst, transforms stromal cells of the endometrium into
decidual cells. Decidual cells are essential for successful growth of the early embryo from the time of implantation through establishment of the placenta.

Previous studies have shown the presence of estrogen binding sites in the decidua of rats (Talley et al., 1977; Armstrong and Villee, 1978; Martel et al., 1984). This binding activity is probably due to both ER subtypes, which are largely confined to the antimesometrial cells where blastocyst apposition, a process that depends upon estradiol and progesterone, takes place.

Recent discovery of the second type of receptor, ERβ prompted numerous investigations of how these two types of ER are related to different physiological functions. Gibori’s group analysed the expression of decidual ERα and ERβ, studied their regulation by prolactin and steroid hormone and examined the ability of decidual ERβ to transduce the estradiol signal to the progesterone receptor. They showed that the ER isoforms are co-expressed in decidua during pseudopregnancy (Tessier et al., 2000). The expression of ERβ in the rat decidua contrasts with reports from the mouse decidua, where very low, if any, expression of ERβ could be detected (Tan et al., 1999).

As estradiol plays a key role in the induction of the PR, it is not clear whether in mice; ERβ is responsible for such an effect. It is clear, however, that in rat decidual cells, estradiol causes a marked stimulation of PR mRNA expression through ERβ, as seen in decidua derived cell line (GG-AD) (Tessier et al., 2000). Decidual ERβ was shown to transduce a strong estradiol inhibition of IL-6 and gp130 expression (Deb et al., 1999). This estradiol effect prevents the decidual production and action of IL-6, a cytokine known to be determined to the normal progress of pregnancy. ERβ may also play an important role in sensitizing the uterus to progesterone action by inducing the PR, a process critical for the initiation and maintenance of the decidual reaction (Tessier et al., 2000).

**Effect of Progesterone on ER**

Progesterone inhibits ER expression in the uterus (Hsueh et al., 1975; Evans and Leavitt, 1980) and widely used to prevent estradiol-induced endometrial cell proliferation, has opposite effects on the two ER subtypes in the decidua. It decreased ERα expression in a dose-related manner; it concomitantly upregulates that of ERβ.
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(Tessier et al., 2000). This finding may be of physiological importance and may explain at least in part why some progesterone target cells can remain responsive to estradiol despite progesterone treatment (Kumar et al., 1998; Hargreoves et al., 1998). The differential effect of progesterone on the two ER subtypes may also be the reason why the ovaries express high levels of ERβ and little ERα, as this gland is subjected to very high levels of locally produced progesterone.

Antagonism of estrogen action by progesterone

Progesterone opposes the effect of estrogen and causing inhibition of estrogen action, thereby proliferation of uterine cells and maintains the balance between the cyclical influences of estrogen and progesterone. It abrogates estrogen induction of many of known hormone-responsive genes, by decreasing the uterine hormone levels as observed in rats (Hsues et al., 1975; Mester et al., 1974; Hsues et al., 1976). This effect is mediated by down regulation of cytoplasmic and nuclear ER protein concentration, decreasing the active estrogen concentration (Clarke and Sutherland, 1990) and antagonizing the action of ER at the molecular level. Progesterone shortens the ER protein half-life in hamster decidual cell, suggesting direct destabilization of ER by progestin (Takeda and Leavitt, 1986) as well as it decreases ER protein by decreasing cellular ER mRNA levels in breast cancer cells, likely to reflect decreased transcription of the ER genes (Read et al., 1989).

Progesterone opposes ER mediated gene regulatory events, probably by sequestering transcription factors in rabbit that are essential for estrogen action (Savouret et al., 1994). On the other hand progesterone and estrogen effects on human PR appear to be distinct, since estrogen primarily regulates the PR-B promoter, (Graham et al., 1995), whereas progestin regulate both PR isoforms in breast cancer cells. However, there is considerable variability between reports showing repressive effects of progesterone on ER. According to some reports PR-A but not PR-B had estrogenic effects on endogenous ER activation of a minimal estrogen responsive reporter gene (McDonnell and Goldman, 1994; Wen et al., 1994). While in some reports PR-B and not PR-A was found to repress activation of the reporter by estrogen (Chalbos and Galtier, 1994).
Effect of SERMs on ER

The transcriptional activity of ERs in the estrogen signaling pathways depends on dimerization, where the ERα / ERα and ERβ / ERβ homodimers, or ERα / ERβ heterodimers, bind directly to the classical estrogen responsive element (ERE) in the target genes (Pettersson et al., 1997). Varying ratio of ERα and ERβ in different cells result in different populations of homo- and hetero-dimers, which could constitute a mechanism for the tissue and cell specific actions of not only estrogens, but also of anti-estrogens (Pettersson et al., 1997). In vitro studies with tamoxifen have demonstrated that its partial agonist activity is manifested by ERα, and is completely abolished upon the co-expression of ERβ with ERα (Hall and McDonnell, 1999). Also it reduces the expression of ERβ hence reduced repression from ERβ results in an unopposed proliferation from ERα activity, an effect that could be responsible for the increased risk of endometrial carcinoma after tamoxifen treatment.

ER Cross-Talk with Growth Factors

Estradiol and growth factors (EGF, IGF) are known mitogens in the rodent reproductive tract (Green and Chambon, 1991; Murphy and Ghahary, 1990). These polypeptide growth factors may act as autocrine or paracrine mediator of biological action of estrogens. The estrogen induces mRNA and protein for peptide growth factors Epidermal growth factor (EGF), Transforming growth factor α (TGFα) and Insulin like growth factor-I (IGF-I) (DiAugustine et al., 1988; Nelson et al., 1992; Murphy and Ghahary, 1990) and their receptor (Mukku and Stancel, 1985; Michels et al., 1993) in rodent uterus and hence regulate steroid hormone in uterine biology. Further, EGF mimics the effects of estrogen in the mouse reproductive tract in terms of increased DNA synthesis and cornification of vaginal epithelium (Nelson et al., 1991) lectoferrin gene expression, and phosphatidylinositol lipid metabolism in ovariectomized mice (Ignar-Trowbridge et al., 1992) as well as increased phosphorylation and nuclear retention of the estrogen receptor (ER).
Progesterone Receptor Isoforms in Uterus

Physiological effects of progesterone are mediated by interaction of the hormone with specific intracellular progesterone receptors (PRs). It is expressed as two distinct isoforms, PR-A and PR-B, that arise from a single gene with alternative start site with two distinct promoters. These receptors are nuclear receptor superfamily of transcription factors (Conneely et al., 1987; Kastner et al., 1990).

The gene is characterized by organization into specific functional domains that are conserved, to different degrees, between species and family members. PR-A and PR-B isoforms differ in that the PR-B protein contains additional sequences of amino acids at its amino terminus (Fig. 15). This PR-B specific domain encodes a third transactivation function (AF-3) that is absent in PR-A (Sartorius et al., 1994). Recent evidence has demonstrated that AF-3 allows binding of a subset of coactivators to PR-B that is not efficiently received by progestin bound PR-A (Giangrande et al., 2000) and PR-A can act as a repressor of PR B (Vegeto et al., 1993).

Some studies reported a third smaller progesterone receptor isoform having molecular weight 45kD-50kD (Wei et al., 1990). Several models of how PR-C could act as a selective repressor of progestin action have been proposed. A simple model provides that PR-C homodimers are basically transactivationally inactive and act as progestin binding sink to curtail P₄- binding to active receptor forms via mass action competition (Wei et al., 1996). Or PR-C may associate with a repressor protein(s), which in turn alters transcriptional efficiency of PR complexes for certain ER and PR.
A third model is that PR-C could form homodimer and/or heterodimers with PR-B and PR-A receptors with altered affinity for cis transactivational elements.

**Physiological role of progesterone receptor isoforms**

Although both PR-A and PR-B bind progestin and interact with PREs, but these two proteins have different ability to activate progestin responsive promoter in a promoter-specific and cell-specific manner. When expressed in equimolar ratio in cells, the A and B proteins can dimerize and bind DNA as three species: A: A or B: B homodimer or A: B heterodimer. Thus cellular responsiveness to progestins may be modulated via alteration in the ratio of PR-A and PR-B. A growing body of evidence has accumulated in recent years demonstrating that the PR-A and PR-B proteins are functionally different when in vitro. PR-B has been shown to function as a strong activator of transcription of several PR-dependent promoters and in a variety of cell types in which PR-A is inactive. Thus A and B may regulate different physiological target tissues. Second, when A and B proteins are coexpressed in cultured cells, in certain promoter contexts in which agonist bound PR-A is inactive, the protein can act as a dominant repressor of PR-B activity (Vegeto et al., 1993). While antagonist bound PR-A is inactive, antagonist bound PR-B can be converted to a strongly active transcription factor by modulating intracellular phosphorylation pathway in PR-B containing cells. Thus they respond differently to progestin antagonists (Giangrande and McDonnell, 1999).

There are interspecies differences in the relative expression of PR-A and B in normal tissue. Almost equimolar expression of PR-A and B is observed in chick oviduct and human uterus (Schrader and O’Malley, 1972; Feil et al., 1988). In Rodent PR-A expression predominates over PR-B in a ratio of 3:1 (Ilchenchuk and Walters, 1987). While only one PR protein has been described in the rabbit, which has high homology to PR-B in the human (Loosfelt et al., 1984).

Progesterone receptor is mainly involved in uterine implantation and decidualization of uterine stromal cells in response to progesterone. As studied in PR-AKO mice, PR-A expression in the uterus is found to be required to mediate the decidualization response to progesterone. Progesterone increases the expression of three genes calcitonin (CT), histidine decarboxylase (HDC) and amphiregulin (AR) in
uterus epithelium in association with uterine receptivity (Das et al., 1995; Paria et al., 1998; Zhu et al., 1998) and inhibited glycoprotein, lectoferrin (LF) expression. Ablation of PR-A results in loss of CT and AR expression associated with uterine epithelial receptivity, while regulation of HDC and LF was fully retained. Thus PR-B transcription factor activity is different to PR-A transcription factor activity. And the uterine defects observed in PR-AKO mice are due to differences in PR-B transcription factor activity rather than to differences in expression of the protein relative to PR-A.

From the PR-AKO studies now it has been clear that expression of the PR-B protein alone in the uterus results in a gain of proliferative activity (Mulac-lericevic et al., 2000). And uterine expression of the PR-A isoform is required not only to oppose estrogen- induced proliferation but also that induced by progesterone acting through the PR-B protein. Thus PR-A has the capacity to diminish overall P4 as well as E2 responsiveness in uterus in a tissue specific manner.

**Regulation of PR isoforms by estrogen**

Progesterone receptor is one of most well- documented estrogen regulated gene. In many target tissues, both normal and neoplastic, PR is induced by estrogen and is widely recognized as a marker for estrogen action (Graham and Clarke, 1997). Regulation of the PR gene by estrogen has been extensively studied. Within the PR gene promoter clusters of estrogen response element (ERE) half sites are present, which are essential for transactivation of the PR gene by liganded estrogen receptor (Kraus et al., 1993). Because the PR protein level is thought to be a critical determinant of sensitivity to P4, almost all hormone treatment protocols designed to elicit effects of P4 usually involved previous estrogen priming to induce PR.

Both progesterone and estrogen are essential for establishment and maintenance of pregnancy, where as P4 is required throughout pregnancy. E2 is essential only during early pregnancy, especially around the time of implantation. In rodents, E2 is essential in addition to P4 to ready the uterus for implantation of the blastocyst. Decidualization can be induced artificially by a combination of P4 treatment and a mechanical traumatization of the uterine horn in the absence of estrogen (Shelesnyak, 1963). On the other hand, E2 is required for natural decidualization during normal pregnancy or artificial decidualization induced by mild stimuli such as intrauterine oil injection (Finn, 1966). The continued presence of E2
reduces the dosage of $P_4$ required to maintain pregnancy after implantation and $E_2$ deficiency can be overcome by high doses of $P_4$ (Martin and Finn, 1997). Thus $E_2$ increases the sensitivity of uterine cells to the effects of $P_4$, perhaps by regulating PR level.

Using knockout studies it has been shown that the $\alpha$ERKO uterine response to estrogen, including induction of the progesterone receptor gene (PR) was impaired. Although PR is present in the ERKO uterus at about 60% of that present in ER$\alpha$ containing wild-type controls (Curtis et al., 1999). But these uteri fail to implant experimentally transferred donor embryo into hormone primed $\alpha$ERKO uterus, indicating that implantation requires ER$\alpha$.

Regulation of PR isoforms by antiprogestin

The precise mechanism by which antiprogestin block progesterone action at receptor level is not much known. It has been demonstrated that in mice, treatment with the PR antagonist RU 486 in vivo resulted in down-regulation of both isoforms i.e. PR-A as well as PR-B (Ruijin et al., 2003). Both PR-A and PR-B respond differently to antiprogestin (Conneely et al., 2001).