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Physiological Action of Progesterone in Target Tissues*

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I. Introduction

THE STEROID hormone progesterone is a key component in the complex regulation of normal female reproductive function. Broadly speaking, the major physiological roles of progesterone in the mammal are 1) in the uterus and ovary: release of mature oocytes, facilitation of implantation, and maintenance of pregnancy, by promotion of uterine growth and suppression of myometrial contractility; 2) in the mammary gland: lobular-alveolar development in preparation for milk secretion and suppression of milk protein synthesis before parturition; and 3) in the brain: mediation of signals required for sexually responsive behavior. Recent evidence also supports a role for progesterone in modulation of bone mass (Table 1). The recent description of a mouse model carrying a null mutation of the progesterone receptor (PR) gene (1) has served to answer many of the complex questions of progesterone action *in vivo* and has confirmed the importance and diversity of roles of progesterone in normal female development and reproduction.

The pathways of progesterone action in target tissues are not well defined and, in many respects, distinctions remain to be made between the direct downstream targets of progesterone action and indirect consequences of progesterone regulation. Furthermore, as the effects of progesterone are mediated by its receptor and as PR is induced by estrogen in most target tissues, the delineation of specific progesterone effects, as distinct from those of estrogen, is similarly not clear. A number of reviews have described the molecular mechanisms of PR action (2–4), progesterone and progesterone antagonist effects on cellular proliferation (5, 6), and regulation of the concentration of progesterone-responsive proteins (7, 8). This review does not seek to replicate existing reviews of the molecular biology of PR action but will provide an overview of the physiological action of progesterone and its regulation of gene expression in target tissues.

II. Synthesis and Secretion of Progesterone

The ovary is the major site of synthesis and secretion of estrogen and progesterone in the mammal and gives rise to cyclical fluctuations in the levels of these hormones in the circulation (reviewed in Ref. 9). Primary follicles play a dual role in secreting both hormones as well as being responsible for the release of the ovum during the normal cycle. Before ovulation, granulosa cells in the follicle biosynthesize and secrete estrogen. After follicle rupture and release of the ovum, these granulosa cells mature to form the corpus luteum, which is responsible for secretion of progesterone and estrogen in the latter part of the cycle. In the human, if fertilization does not occur within 1 to 2 days, the corpus luteum will continue to enlarge for 10–12 days followed by regression of the gland and concomitant cessation of estro-

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TABLE 1. Physiological functions of progesterone

Tissue	Function
Uterus/ovaries	Release of oocytes
	Facilitation of implantation
	Maintenance of pregnancy: *via myometrial quietening*
	Stimulation of stromal regeneration: *luteal phase of cycle*
Mammary gland	Lobular alveolar development
	Suppression of milk protein synthesis during pregnancy
Brain	Mediation of sexual responsiveness
Bone	Regulation of bone mass: *prevention of bone loss*

gen and progesterone release. If fertilization occurs, the corpus luteum will continue to grow and function for the first 2 to 3 months of pregnancy. After this time it will slowly regress as the placenta assumes the role of hormonal biosynthesis for the maintenance of pregnancy.

The release of progesterone from the corpus luteum is influenced by a number of hormones. Primary among these is LH, the activity of which is mediated via its intracellular effects on cAMP (10, 11). FSH, PRL, prostaglandins, and β-adrenergic agents also play a role in the control of progesterone secretion (9). Intermediates such as activin, which is stimulated by FSH and inhibits progesterone secretion by granulosa cells, and follistatin, which is synthesized by granulosa cells and is able to bind activin, contribute to a complex pattern of regulation of progesterone secretion. At the time of implantation of the blastocyst in the rat uterus, increased progesterone synthesis is accompanied by induction of ovarian follistatin gene expression, which appears to help in maintaining progesterone secretion (12). However, it is not clear whether follistatin is induced by progesterone to prevent local inhibition of progesterone effects by activin in the uterus or whether follistatin prevents down-regulation of progesterone secretion from the corpus luteum.

Once released, progesterone is carried in the blood by transcortin (corticosteroid-binding globulin) in many species including humans. In the uterine fluid of the rabbit, between days 3 and 12 of pregnancy, an additional progesterone carrier, uteroglobin, is present. Uteroglobin has a postulated role in protection of the embryo during pregnancy (discussed in *Section IV*), by mechanisms that are still not clear (13, 14). A specific progesterone binding plasma protein has also been described in the pregnant guinea pig, which has significantly higher affinity for progesterone than corticosteroid binding globulin (15, 16) and is strongly induced from days 15 to 20 of pregnancy, remaining elevated until parturition at approximately day 65 (17). During this time it represents the major progesterone-binding protein in the guinea pig and is specifically synthesized by the placenta (17, 18). Synthesis of this protein appears to be under progesterone control since ovariectomy of pregnant animals, or parturition, causes a fall in progesterone binding protein levels concomitant with decreased progesterone levels (19).

III. The Progesterone Receptor

Progesterone effects are mediated by its nuclear receptor. Receptor proteins that specifically bind progesterone, and are induced by estrogen, were initially characterized in the mammalian uterus and chick oviduct in the early 1970s (20–23). O'Malley and co-workers showed that chick oviduct PR was a dimer composed of two receptor proteins, PR A and PR B, which each bound progesterone (24). In human breast cancer the PR A and B proteins, characterized *in vitro* (25) and *in vivo* (26), are detected with molecular masses of approximately 81 kDa and 115 kDa, respectively. The two PR proteins are encoded by a single gene in the human (Fig. 1), under the control of distinct promoters, each of which gives rise to a distinct subgroup of PR mRNA species (27). In contrast, only one PR protein has been described in the rabbit, which has high homology to PR B in the human (28, 29).

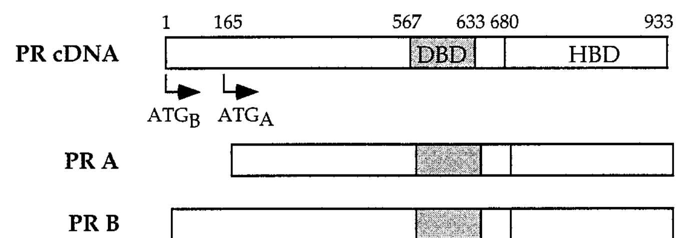

FIG. 1. The human PR cDNA and PR A and B proteins. The *arrows* indicate the translation start sites for PR B and PR A (27). DBD, DNA-binding domain; HBD, hormone-binding domain.

PR is a member of a large family of ligand-activated nuclear transcription regulators, which includes receptors for steroids, retinoids, thyroid hormones, and vitamin D. The genes are characterized by organization into specific functional domains that are conserved, to differing degrees, between species and family members. The most highly conserved region between receptor genes is a region in the center of the gene [Fig. 1, DBD (DNA-binding domain)] encoding two "zinc finger" DNA-binding motifs (30). Binding of progestins to the carboxyl-terminal ligand-binding domain of PR [Fig. 1, HBD (hormone-binding domain)] causes association of the progestin-complexed PR dimer with specific progestin response elements (PREs) in target genes, resulting in modulation of transcription of those genes (reviewed in Refs. 3 and 4).

Although both PR A and PR B bind progestins and interact with PREs, there is increasing evidence that they are functionally different. In transfection studies the two proteins have different abilities to activate progestin responsive promoters; these differences are promoter- and cell-specific (31–34), suggesting that cellular responsiveness to progestins may be modulated via alterations in the ratio of PR A and B expression. While PR B tends to be a stronger activator of target genes, PR A can act as a dominant repressor of PR B (33, 34), suggesting that high PR A expression may result in reduced progestin responsiveness and that PR A and PR B

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may thus be, respectively, an activator and repressor of progestin action. The repressor role of PR A extends beyond that on PR B, as PR A has been shown to diminish the response of other hormone receptors such as the androgen, glucocorticoid, mineralocorticoid, and estrogen receptors to their appropriate ligands (35–37).

There are interspecies differences in the relative expression of PR A and B in normal tissues. Approximately equimolar expression of PR A and B is observed in chick oviduct (38) and human uterus (39), and a similar ratio of expression is seen in cultured human breast cancer cells (25). In the rodent, PR A expression predominates over PR B in a ratio of 3:1 (40, 41). Alterations in the ratio of PR forms in the chick oviduct during late winter, or in aged nonlaying animals, results in a measurable decrease in PR functional activity (42, 43). In human breast tumors, the ratio of expression of PR A and B proteins differs markedly between patients (44). The biological importance of these different ratios of PR expression has not been extensively explored. Little is known of whether relative PR A and B expression is modulated *in vivo*, although in PR-positive breast cancer cells in culture PR B is preferentially stimulated by estradiol, resulting in a significant decrease in PR A to B ratio (45). Given the functional differences between the two PR proteins demonstrated *in vitro*, this suggests that the relative expression of PR A and B may influence cellular responsiveness to progesterone.

A. PR expression and regulation

PR expression has been described in tissues known to be progesterone responsive such as the uterus [mammalian endometrium (46–51) and myometrium (51, 52)]; the ovary [luteinizing granulosa cells and corpus luteum (53), preovulatory granulosa cells (54)]; and the chick oviduct (24) and bursa of Fabricius (55). Specific progestin binding has been described in other reproductive tissues such as testes (56) and vaginal tissue (57). PR has been described in normal and neoplastic breast (58–61), and in the brain, in the pituitary, ventromedial hypothalamus, and preoptic areas (62, 63). PR has also been described in other tissues where the action of progesterone is less well defined, including vascular endothelium (64) and rat thymus (65). Specific progestin binding has been detected in rabbit lung (66), rat pancreatic islets (67), and human osteoblast-like cells (68). A summary of the tissues and cell types in which PR has been detected is shown in Table 2.

The expression of PR, and therefore sensitivity to progestins, is under the control of estrogen, which increases, and progesterone, which decreases PR expression in most target tissues. PR protein is increased during proestrus or by exogenous estrogen administration in the mammalian uterus (52, 69–74). During the second half of the cycle, as serum progesterone levels increase, total PR levels in the uterus decrease, and this decrease can be brought on prematurely by treatment with progesterone (73). Furthermore, progesterone treatment can oppose the estrogen induction of PR (71). Although progesterone decreases total PR expression in the uterus, down-regulation is not observed in all cell types. During the follicular phase of the cycle, high levels of PR are present in the nuclei of epithelial and stromal cells of the human endometrium and in myometrial smooth muscle cells. In the mid- and late luteal phase, detection of PR in the luminal and glandular epithelium declines markedly, to undetectable levels; on the other hand, stromal and myometrial cells continue to express high levels of PR despite high circulating progesterone and absent estrogen receptor (ER) (48, 49, 51). By contrast with the uterus, PR levels do not decrease in the breast between the follicular and luteal phases (75–78).

TABLE 2. Tissues and cell types expressing PR

Tissue	Cell type	Reference
Uterus	Endometrium	(46–51)
	Myometrium	(51, 52)
Ovary	Luteinizing granulosa	(53)
	Preovulatory granulosa	(54)
	Corpus luteum	(53)
Reproductive tissues	Testes	(56)
	Vagina	(57)
Breast	Normal and neoplastic	(58–61)
Brain	Pituitary	(62, 63)
	Ventromedial Hypothalamus	(62, 63)
	Preoptic area	(62, 63)
Other	Vascular endothelium	(64)
	Thymus	(65)
	Pancreatic islets	(67)
	Osteoblast-like cells	(68)
	Lung	(66)
Chicken	Oviduct	(24)
	Bursa of Fabricius	(55)

IV. Progesterone Regulation of Gene Expression in the Uterus, Ovary, and Chick Oviduct

Progesterone has a central role in reproduction, being involved in ovulation, implantation, and pregnancy. Associated with this is the involvement of progesterone in regulation of uterine function during the menstrual cycle, by control of cyclical changes in proliferation and decidualization. Progesterone is essential for the development of decidual tissues, and if fertilization occurs, high circulating progesterone levels are important not only for facilitating implantation, but also for maintaining pregnancy by stimulating uterine growth and opposing the actions of factors involved in myometrial contraction. The mechanisms underlying these diverse and complex actions of progesterone are yet to be fully defined, but it is clear that progesterone can both stimulate and inhibit cell proliferation in the uterus, depending on the cell type and physiological context, and that it also plays a role in differentiated function. Some of the functions influenced by progesterone include the stimulation of glycogenesis (79, 80), cyclic nucleotide metabolism (8), protein synthesis and secretion (8), and cell cycle regulation (5), although the molecular mechanisms of these effects of progesterone are not known. Intracellular proteins known to be regulated by progesterone, and which therefore may be involved in mediation of its effects, include ER, estrogen metabolizing enzymes, α-fucosidase, and type II cAMP-dependent kinase. Secreted proteins include enzymes for protein, carbohydrate and prostaglandin metabolism, hydrolases, phosphatases, prostaglandins, plasminogen activator, and PRL (8).

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A. Progesterone effects on proliferation and decidualization in the uterus during the menstrual cycle

Changes in proliferative activities of the glandular epithelium and stromal elements of the human endometrium can be correlated directly with circulating levels of estrogen and progesterone (5), with estrogen stimulating proliferation and progesterone opposing the effects of estrogen and causing inhibition of proliferation. Potential mechanisms through which progesterone opposes estrogen action during the menstrual cycle and maintains the balance between the cyclical influences of estrogen and progesterone are discussed in *Section VIII*. The estrogen-stimulated follicular phase of the cycle is associated with high proliferative activity in both the epithelial and stromal cells (81). This is followed by a decline in proliferation in the first half of the progesterone-dominated luteal phase of the cycle. In the late luteal phase, while proliferative activity remains low in the epithelium, a second peak of proliferation, consistent with decidual changes, is seen in the stromal elements, associated with high serum levels of progesterone and presumably mediated by the continued expression of PR in those cells (48, 49, 51).

Specificity of the proliferative effects of progesterone in decidualization may be due in part to cell type-specific expression and regulation of growth factor receptors and their peptide ligands. Heparin binding epidermal growth factor (EGF)-like growth factor mRNA is specifically induced by progestins in uterine stromal cells (and is a mitogen in these cells) but, in contrast, is repressed by progesterone treatment in luminal and glandular epithelium (82).

PRL is likely to be involved in decidualization. PRL is secreted by both the endometrial stroma (83) and myometrium (84) during the normal cycle in the human and appears to be under the control of progesterone. Synthesis of PRL by the stroma is greatest during the mid- to late luteal phase (83) and can be induced, *in vitro*, in follicular phase endometrial tissue by treatment with progesterone (85). Furthermore, progesterone treatment of both follicular and luteal phase tissue explants results in PRL induction, which coincides with early morphological changes resembling the decidualization of cells during early pregnancy (86). The progesterone-mediated increase in PRL secretion from stromal cells is the result of increased PRL mRNA expression and is additive to the stimulatory effects of estrogen and relaxin (87). In contrast, progesterone inhibits PRL mRNA transcription by myometrial cells (84).

Progesterone may influence uterine proliferation and differentiation during the menstrual cycle by regulation of proteases or matrix proteins. Progestins suppress expression of stromelysins in endometrial stromal cells and induce transforming growth factor-β (TGFβ) in these cells, resulting in down-regulation of matrilysin expression in endometrial epithelium in stromal-epithelial cocultures (88, 89). Thrombospondin-1, an extracellular matrix glycoprotein that is expressed in vascular endothelium and inhibits angiogenesis, is induced by progestins in endometrial stromal cells (90). Furthermore, expression of thrombospondin-1 in endometrial stroma *in vivo* is correlated with stage of the menstrual cycle, with strongest expression seen in the luteal phase, suggesting that progesterone-mediated induction of thrombospondin-1 influences cyclical regulation of vascular formation and differentiation in this tissue.

B. Progesterone regulation of insulin-like growth factor (IGF) pathways in the endometrium

The inhibitory effects of progesterone on estrogen-mediated cell proliferation in the endometrium during the menstrual cycle may be mediated by opposition of estrogen action, as discussed in *Section VII*, but modulation of growth factor pathways may also play a role. The proliferative effects of IGFs are specifically controlled by progesterone, principally through regulation of IGF-binding protein I (IGFBP-I). In humans, IGFBP-I is expressed in a cyclical fashion in endometrial stromal cells, with the highest expression seen in the mid- to late luteal phase (91). IGFBP-I may act in a paracrine fashion to prevent epithelial cell proliferation during the late luteal phase, since progestins increase IGFBP-I secretion from endometrial stromal cells both *in vitro* (92) and *in vivo* (93). It is postulated that by binding to IGF-I, IGFBP-I prevents binding of the growth factor to its receptor, resulting in decreased cellular responsiveness to IGF-I (91, 94). Progesterone and IGFBP-I may also form an autocrine loop controlling stromal cell proliferation at the end of the luteal phase, since IGFBP-I treatment blocks the proliferative effects of both IGF-I and progestins on stromal cells in culture (95). Alternatively, it has been postulated that IGFBP-I may play a role in tissue remodeling toward the end of the cycle, by binding $\alpha5\beta1$-integrin, a specific cellular receptor for the extracellular matrix protein fibronectin, and thus altering cell motility (96, 97).

C. Control of ovulation

The presence of PR in most follicular cell types and in the corpus luteum of the human ovary (98) suggests that the process of ovulation is regulated by progesterone, an interpretation confirmed by studies on PR null mice, which fail to ovulate despite the presence of mature preovulatory follicles (1). Relaxin is increased in the endometrium of nonpregnant women during mid to late secretory phase and is postulated to be progesterone-dependent (99). Studies with rat granulosa cells in culture suggest that the increase in relaxin may facilitate follicle rupture by increasing the secretion of plasminogen activator, collagenase, proteoglycanase, and β-glucuronidase (100). This suggestion is supported by reports in mice that treatment with epostane, which inhibits 3β-hydroxysteroid dehydrogenase, resulting in decreased serum progesterone levels, inhibits the activities of serine proteases, kallikrein, and plasminogen activator and suppresses ovulation (101). Treatment with progesterone relieves the suppression, just as treatment with the antiprogestin RU 38486 suppresses ovulation (102), an inhibition associated with decreased protease activity (103), implying that progesterone is responsible for their induction.

The formation of the corpus luteum represents a distinct intraovarian process and appears to be progesterone-dependent. Expression of PR is induced by LH in granulosa cells of mature preovulatory follicles (104), and PR is detectable in the primate corpus luteum despite high local progesterone

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concentrations (53, 105). Granulosa cells from mature preovulatory follicles of PR null mice show an inability to luteinize correctly despite prolonged exposure to gonadotropins (1).

D. Implantation, uterine proliferation, and early pregnancy

Progesterone has a major role in the endometrium in preparation for implantation of a fertilized ovum, and in many species a decrease in circulating progesterone at the time of fertilization is sufficient to delay implantation (106). Progesterone is important in promoting and maintaining implantation through effects on both the maternal uterus and on the developing blastocyst. Progesterone facilitates implantation by stimulating the synthesis of enzymes responsible for lysis of the zona pellucida. However, while progesterone is known to be essential for implantation to occur, lysis of the zona is not the crucial step in this process, suggesting that other essential progesterone-mediated events are yet to be described in the initiation of implantation (106). PRL plays a role in implantation, and this is supported by recent observations that female mice that are PRL receptor null have complete failure of embryonic implantation, leading to sterility (107).

The induction of uterine cell proliferation in early pregnancy may be mediated by locally produced growth factors, many of which are under progesterone control. Furthermore, cell type-specific expression of growth factor receptors controls cellular sensitivity to the autocrine/paracrine effects of growth factors. Progesterone induction of growth factor secretion from the luminal and glandular epithelium in the mouse endometrium promotes proliferation of the EGF receptor-positive blastocyst trophectoderm to facilitate implantation (108). In early pregnancy, EGF receptor mRNA is also induced in the stroma of the maternal uterus by progesterone, but not in the luminal or glandular epithelium (108). It has been suggested that the hemopoietic growth factor, colony stimulating factor-I, exerts a paracrine influence on the growth and differentiation of the placental trophoblast, and its secretion from the luminal and glandular epithelium in the mouse is regulated by estrogen and progesterone (109). In the first 2 days of pregnancy in the mouse, IGF-I is secreted from the luminal and glandular epithelium of the uterus under estrogen stimulation and may contribute to effects on the blastocyst. After this time, secretion from the epithelium declines and significantly greater synthesis and secretion of IGF-I by the stroma are induced by progesterone, resulting in increased proliferation and enlargement of the uterus (110). It is postulated that increased growth factor receptor expression in the stroma mediates the effects of EGF, TGFα, and heparin-binding EGF-like growth factor from the epithelium and IGF-I from the stroma, resulting in tissue-specific stimulation of proliferation.

The molecular mechanisms of progesterone action during pregnancy have been studied intensively in the rabbit uterus. In particular, uteroglobin, which is transcriptionally regulated by progesterone, has been well characterized at both the cellular and molecular levels. Uteroglobin is expressed between days 3 and 12 of pregnancy in the rabbit uterus. It is a dimer of two identical 8-kDa subunits and incorporates two FeII ions into its normal structure. Although it shows acid phosphatase activity, its primary role has been suggested to be the binding and transport of progesterone and its metabolite 5-α-pregnane-3,20-dione, which protects the blastocyst from the high levels of circulating progesterone required for maintenance of pregnancy (13). Alternatively, it has been hypothesized that uteroglobin may protect the embryo from maternal immune and inflammatory response during implantation by contributing to the inhibition of phospholipase A2 activity—a key point in the regulation of these response pathways (14).

Expression of uteroglobin is almost exclusively confined to the rabbit uterus, the exception being the lung where its expression is constitutive and is not regulated by progesterone (13). The uteroglobin gene is encoded by three exons (111), and progesterone regulation of the gene is mediated via binding of PR to specific regulatory elements in the 5′-flanking region of the gene 2 to 3 kb upstream of the start of transcription (112). Other progesterone-stimulated proteins are also postulated to bind the uteroglobin gene in positions more proximal to the promoter and to function as *trans*-acting factors in progesterone regulation of the gene (113). The binding of these proteins may be a mechanism by which the strict tissue specificity of uteroglobin expression is maintained (114). Protein binding to the regulatory region of the uteroglobin gene is also modulated by other pregnancy-associated proteins. PRL, acting through its receptor, augments progesterone effects by increasing protein binding to the uteroglobin promoter (115). Uteroglobin is expressed only in the rabbit, although when transgenically expressed in the mouse, the uteroglobin promoter is specifically regulated by progesterone in the uterus (116). A homolog of uteroglobin, the Clara cell 10-kDa protein (CC10), has been described in the human. However, it is expressed primarily in the lung, and the 5′-flanking regions of the gene, which correspond to the PREs described in rabbit uteroglobin, are only partially conserved, resulting in a lack of progesterone responsiveness (117).

E. Myometrial contractility

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. Increases in free intracellular calcium, if unopposed, lead to myometrial contraction. Induction and secretion of calcitonin, a peptide hormone involved in calcium homeostasis, are postulated to lower free calcium levels in the uterus, thereby preventing contraction (118). In the rat uterus, expression of calcitonin is induced in glandular epithelial cells during early pregnancy. This effect can also be achieved by progesterone treatment after estrogen priming, suggesting that progesterone is primarily responsible (118). It is also postulated that suppression of gene expression of the calcium transporter calbindin-D9k prevents increases in intracellular calcium and therefore contributes to prevention of myometrial contraction. Calbindin-D9k mRNA expression in the pregnant rat uterus decreases significantly with increasing endogenous progesterone levels, and this de-

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crease can be blocked by the antagonist RU 38486, suggesting that the effect is PR-mediated (119). Furthermore, calbindin-D9k levels in rat uterus are lowest during the progesterone-dominated diestrus phase of the cycle, and estrogen induction of calbindin-D9k mRNA can be blocked by the progesterone agonist R5020 (120).

Progesterone inhibits prostaglandin synthesis and activity in the pregnant sheep and therefore decreases myometrial contractility. This inhibition is mediated by a number of pathways that include blocking prostaglandin action, decreasing prostaglandin synthesis, and increasing its rate of inactivation. Progesterone is thought to stimulate prostaglandin 15-dehydrogenase, which catalyzes prostaglandin oxidation and results in inactivation (121). Progesterone opposes the effects of prostaglandins in the human uterus, during pregnancy, and in the luteal phase of the cycle by decreasing the levels of prostaglandins F2α and E in the endometrium. Furthermore, estrogen stimulation of prostaglandin F2α expression in the luteal phase of the cycle in the human endometrium is inhibited by progesterone (122). A fall in progesterone levels at the end of pregnancy is associated with increased prostaglandin synthase activity and prostaglandin F2α production, leading to parturition (121). The antiprogestin RU 38486 antagonizes all the actions of progesterone on prostaglandin synthesis and catabolism and stimulates prostaglandin production, resulting in its abortifacient effect (123).

Prostaglandin effects are mediated by prostaglandin receptors and indirectly via oxytocin receptors, proteins that are also regulated by steroid hormones. Oxytocin receptors are decreased by progesterone in uteri of ovariectomized ewes (124). Oxytocin receptor levels are also inhibited in the human uterus by blocking PGF2α production; conversely, PGF2α induction of luteolysis results in decreased plasma progesterone and a parallel increase of oxytocin receptors (125). Angiotensin II receptors are increased in the rabbit uterus by estrogen priming, resulting in increased contractile sensitivity. This effect is blocked by progesterone treatment, and progesterone alone markedly decreases angiotensin II receptor expression (126). Similarly, atrial natriuretic factor receptors are decreased by progesterone in the rat myometrium during pregnancy, resulting in refractoriness to the tocolytic effects of atrial natriuretic factor on the uterus. It has been postulated that this is mediated by abrogation of estrogen induction of these receptors (127).

During pregnancy the adrenergic system is involved in myometrial quietening. Progesterone increases transcription of β-adrenergic receptors in myometrium from late pregnant rats, resulting in increased sensitivity to adrenergic agents (128). Relaxin is also important in inhibiting spontaneous or prostaglandin-induced myometrial contraction, contributing to the maintenance of implantation and early pregnancy by increasing the collagen framework and distensibility of the uterus (106). The corpus luteum, placenta, and decidua are major relaxin-containing tissues during pregnancy, and progesterone has been shown to be responsible for maintaining relaxin levels (99).

In summary, progesterone has diverse roles in the uterus and ovary at every stage of reproductive function (Table 1). Modulation of cyclical proliferation during the menstrual cycle, regulation of ovulation, stromal growth and decidual formation, promotion and maintenance of implantation, uterine growth, and prevention of myometrial contractility are all dependent upon specific gene regulation by progesterone. It is apparent that transcriptional regulation by progesterone is central to cell-specific growth regulation and involves the coordination of growth factors and their receptors in a complicated array of autocrine and paracrine effects. Similarly, the local signals controlling prostaglandin effects on myometrial contraction involve gene regulation by progesterone at many distinct levels, from regulation of oxytocin signaling to control of prostaglandin synthesis and promotion of calcium homeostasis. Current understanding of the involvement of progesterone in these processes is fragmentary, and the interrelationships between the many regulatory steps largely remain to be described.

F. Chick oviduct

In the chick oviduct, progesterone induces the synthesis of egg-white proteins, including ovalbumin, conalbumin, lysozyme, and ovomucoid, and it is postulated that induction is mediated by binding of PR to the genes encoding these proteins (129). Hormonal induction of ovalbumin, in particular, has been well described in this system, and its cDNA was one of the first to be fully sequenced (130, 131). The ovalbumin gene 5′-flanking region contains several PR-binding regions, as well as regions that are postulated to bind other proteins and thus influence progesterone and estrogen regulation of the gene (129, 132, 133). Two regions within the ovalbumin 5′-flanking sequences mediate induction of a reporter gene by progesterone in transfection studies (31, 134). Estrogen is also able to activate transcription through these regions (31, 135) and in some cells acts additively with progesterone-bound PR A, but not PR B, to induce ovalbumin gene transcription (31). It is not clear whether PR and ER act through overlapping or distinct motifs in these regions. Estrogen action on the proximal steroid-responsive region of the ovalbumin gene involves interaction of the fos-jun complex with this region (136). It has been reported that progestins down-regulate c-jun transcription in estrogen-withdrawn chick oviduct (137), although the implications of this regulation for estrogen control of ovalbumin expression have not been explored.

V. Progesterone Action in the Breast

The major developmental role of progesterone in the normal breast has been postulated to be the formation of lobular-alveolar structures during pregnancy (138). This is supported by the observation that mammary glands in PR null mice develop ductal structures that are relatively similar to the wild type but fail to form an interductal lobular-alveolar structure upon exposure to estrogen and progesterone (1). The influence of progesterone is likely to be proliferative in this process, mediated by progesterone regulation of cell cycle genes, growth factors, and growth factor receptors. Progesterone also exerts a differentiating effect on the breast through its role in lactation. The role of progesterone in

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differentiated function at other times has not been extensively explored.

In comparison with the uterus, there is less known of the mechanisms through which progesterone exerts its effect in the breast, primarily because of the difficulty of obtaining normal breast tissue and the relative paucity of models of progesterone action in the normal breast. Breast cancer cells have been used extensively as models to examine the role of growth factors and growth factor receptors in mediating progesterone effects. However, the limitation of studying progesterone regulation of gene expression in malignant cells, often derived from metastatic lesions, is the difficulty in extrapolating results to the normal breast. An illustration of this is the difference in progesterone effects on the PRL receptor in breast cancer cells and normal mammary gland. In T-47D and MCF-7 cells, progestins increase PRL receptor levels (139), whereas in the normal mammary gland of pseudopregnant rabbits, progestins antagonize PRL induction of PRL receptors (140). Another example is the demonstrated decrease in PR associated with exposure to progestins in breast cancer cells (141–143), which contrasts with the persistence of PR in the breast in the luteal phase of the cycle (75–78).

A. Effect of progesterone on proliferation of the normal breast

In addition to the developmental role of progesterone in formation of lobular-alveolar structures, there is an increasing body of *in vivo* evidence that supports a role for progesterone in the induction of cyclical proliferation in the breast. A number of studies have examined the effects of cyclical hormonal changes during the menstrual cycle on DNA synthesis in normal breast epithelium, and there is general agreement between studies that an increase in DNA synthesis is seen in the late luteal phase of the natural cycle (144–148). The increase in DNA synthesis is consistent with the observation of a cyclical increase in the number of epithelial mitoses, which peaks toward the end of the luteal phase and is followed by an increase in apoptotic activity (149, 150). These *in vivo* data are further supported by the observation that high circulating progesterone levels during pregnancy are responsible for inducing marked lobular-alveolar development of the breast in preparation for lactation (151).

In contrast to this, a recent study examined proliferation in breast tissue from patients who had received percutaneous estrogen and progesterone administration to the breast before surgery (152). Epithelial mitoses and expression of proliferating cell nuclear antigen were lowest in progesterone-treated samples, compared with both untreated controls and those receiving estrogen or estrogen plus progesterone (152). However, these data should be interpreted with caution: while this is clearly an *in vivo* study, the duration of hormone administration, the percutaneous route of administration, and the levels of hormones applied were likely to result in tissue levels of hormones different from those found during the menstrual cycle, with attendant difficulty in extension of these effects on proliferative parameters to those observed in the breast during natural cycles.

In vitro studies of the involvement of progesterone in breast epithelial proliferation have produced inconsistent results (Table 3 and Ref. 5). Although estrogen consistently increases proliferation of normal breast epithelium *in vitro*, the progesterone effects either alone or combined with estrogen have been variable. Progesterone has been found to increase DNA synthesis in normal mammary epithelium in organ culture (153). However, progesterone either decreases, or has no effect on, the proliferation of normal breast epithelium explanted into nude mice (154, 155). Gompel *et al* (156), who examined the effects of estrogen and the progestin R5020 on the growth of cultured normal breast epithelial cells, found that estrogen and progesterone had opposing effects, estrogen increasing and progesterone decreasing cell proliferation.

It is not known why the *in vivo* evidence in support of a role for progesterone in cell proliferation in the breast is difficult to reproduce *in vitro*, but a contributing factor may be the limitations inherent in attempts to reproduce the physiological environment of breast tissue, including paracrine effects from surrounding stroma on epithelial cell proliferation. Further studies on determinants of cell proliferation in the breast and the involvement of ovarian hormones in this process are required to reconcile the discrepancies in the published data on the role of progesterone in cell proliferation. The effects of estrogen and progesterone on proliferation in the breast, in the *in vitro* and *in vivo* models used, are summarized in Table 3.

TABLE 3. Effects of estrogen and progesterone on cell proliferation in the breast

Experimental model	Effect of hormone on proliferation	
	Progesterone	Estrogen
In vivo		
Follicular phase (estrogen-dominated)	NA	No increase
Luteal phase (progesterone-dominated)	Increase	NA
Pregnancy (high progesterone)	Increase	NA
Percutaneous hormone administration	Decrease	Increase
In vitro		
DNA synthesis in organ culture	Increase	Increase
Explants in nude mice	Decrease or no change	Increase
Normal mammary cells in tissue culture	Decrease	Increase

Summary of hormone effects on proliferation in the breast *in vivo* and *in vitro* from Refs. 5, 144–156. NA, Not applicable.

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B. Progesterone regulation of genes associated with cell cycle progression

Insights into the mechanisms underlying proliferative effects of progesterone in the breast have been obtained from studying the effects of progestins on the cell cycle, primarily in breast cancer cells in culture. A transient increase in cell cycle progression is seen in PR-positive T-47D breast cancer cells after administration of progesterone, which is correlated with a short-lived induction of genes associated with cell cycle progression (157–159). This can be demonstrated by progestin treatment of cells that have been growth arrested in G1 phase by serum deprivation and then released by treatment with insulin, which is a strong mitogen in these cells. Under these conditions, the cells are stimulated into a single round of synchronized progression through the cell cycle, an effect accompanied by transient increases in expression of cell cycle-regulatory genes, such as cyclins and cyclin-dependent kinases, and of protooncogenes associated with proliferative activity, such as c-*myc* and c-*fos* (158, 159). The progestin induction of cell cycle progression reflects an increased rate of progression of cells already in transit through the cycle, rather than increased numbers of cells entering S phase. Furthermore, while progestins potentiate the insulin-mediated increase in cyclin D1 mRNA levels, the timing of the cyclin D1 induction remains the same as seen with insulin alone (159). Therefore, although the increases in cyclin D1 and c-*myc* expression resulting from progestin treatment can be blocked by RU 38468, demonstrating that the progestin effects are PR-mediated, it is unlikely that cell cycle genes are direct targets of progesterone action. The temporal relationship between progestin regulation of cell cycle progression and expression of cell cycle genes is summarized schematically in Fig. 2.

In addition to stimulating expression of genes associated with cell cycle progression, progestins may act by inhibiting the expression of genes responsible for suppression of cell growth. Expression of the tumor suppressor protein p53 is decreased by progestins in T-47D breast cancer cells (160), suggesting that progestins may stimulate proliferation of these cells by removing the inhibitory effects of this protein. Furthermore, loss of progestin responsiveness, resulting in constitutive down-regulation of p53, may be a mechanism by which increased, unregulated proliferation may occur.

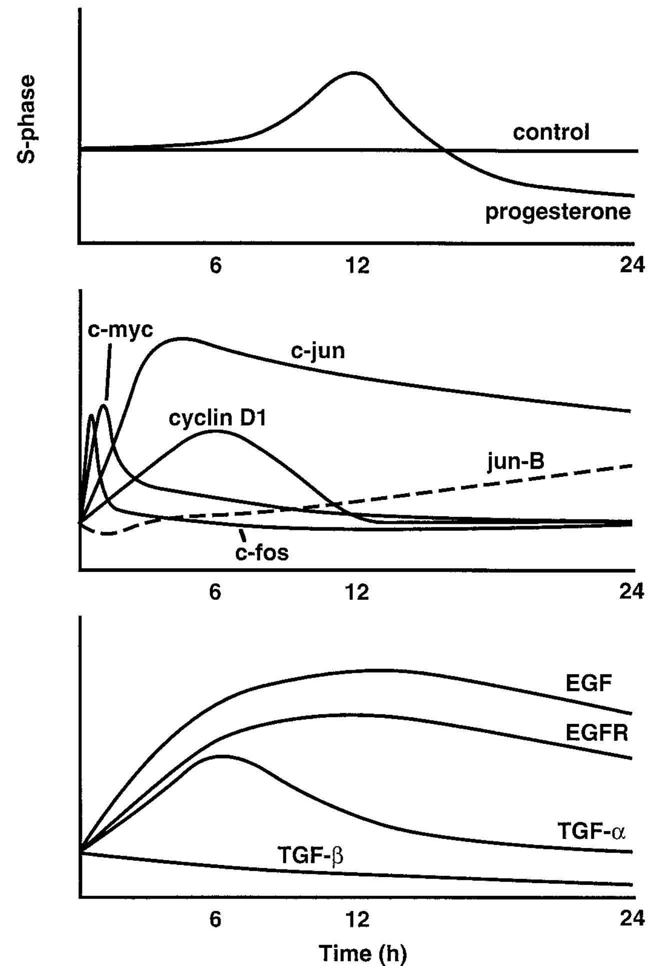

FIG. 2. Relationship between progestin regulation of cell cycle progression, cell cycle gene expression, and growth factor gene expression in breast cancer cell lines. Schematic representation of progestin regulation of cell cycle- related genes (*middle panel*) and growth factor and growth factor receptor genes (*lower panel*), and comparison to timing of progestin-induced cell cycle progression into S phase (*upper panel*). EGF, Epidermal growth factor; EGFR, EGF receptor; TGFα, transforming growth factor-α; TGFβ, transforming growth factor-β. [Redrawn from information contained in Refs. 157–159, 161, 164, and 231].

C. Progesterone regulation of growth factors and growth factor receptors in the breast

Growth factors and growth factor receptors have been proposed as candidate mediators of progesterone effects on cell proliferation. EGF mRNA (161) and EGF receptor protein (162) and mRNA (163) are elevated by progestins in T-47D breast cancer cells. Progestins also increase expression of TGFα mRNA and modestly decrease that of TGFβ in T-47D breast cancer cells (164). The effect is time- and dose-dependent and can be inhibited by RU 38486. The implications of these effects were at first unclear, since it is believed that EGF and TGFα stimulate and TGFβ inhibits the growth of breast epithelial cells, and yet progesterone inhibits T-47D cell growth. However, more recent data suggest that progestins have both stimulatory and inhibitory effects on breast cancer cell growth (158), and that increased growth factor expression may be associated with the transient growth stimulation of these cells by progestins, before growth inhibition. It has also been suggested that overexpression of EGF and TGFα may represent one mechanism by which breast cancer cells acquire progestin resistance, as the development of resistance by a subline of T-47D-5 cells coincided with elevated levels of these factors (165). The above data must be interpreted with caution as in many cases regulation of mRNA expression only has been demonstrated, and increased mRNA levels may not necessarily lead to increased concentrations of biologically active signal or receptor. It is also noteworthy that the timing of the modulation in growth factor and growth factor receptor levels is not consistent with these factors having a primary role in mediating progesterone actions on cell proliferation: the increased expression of

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growth factors and their receptors occurs after the changes in cell cycle gene expression and is not associated closely with the increase in S phase distribution. The timing of growth factor gene regulation by progestins is summarized schematically in Fig. 2.

Progesterone also increases insulin receptor expression in both T-47D cells (166) and the subline T-47Dco (167). Although progesterone alone inhibits growth of these cells, cotreatment with progestin and insulin resulted in a synergistic induction of T-47D cell growth, suggesting that the progesterone-mediated increase in insulin receptor expression may result in greater sensitivity to the mitogenic effects of insulin (166, 168). This is consistent with the ability of progestins to potentiate insulin effects on synchronously growing breast cancer cell cultures (159). These effects have been postulated to have negative implications for the therapeutic use of progestins, since growth-stimulatory effects may be seen in breast tumors that express elevated levels of insulin receptors and IGF receptors.

In contrast to the effects of progestins on EGF and insulin receptor pathways, which are postulated to lead to increased cell proliferation, progestins are generally believed to inhibit the mitogenic effects of IGFs in breast cancer cells. This is in keeping with the observations in the uterus (see *Section IV*) and may be a mechanism through which progesterone-mediated inhibition of cell proliferation in breast cancer cells takes place. However, while in the uterus IGFBP1 is likely to be involved in progesterone modulation of IGF action, in breast cancer cells IGFBP1 is not widely expressed (169, 170). Nevertheless, if IGFBP1 is expressed, it has been postulated to inhibit the mitogenic effects of IGF-I in breast tumors (171, 172), similar to its action in the endometrium. Progestin inhibition of the mitogenic effects of IGFs in breast cancer cells is likely to occur by modulation of IGF receptor concentrations. While breast cancer cells express type I IGF receptors, few express IGF-I; *in vivo* this is thought to be contributed by surrounding stromal cells, producing a paracrine mitogenic effect on tumor growth (173). Progestins decrease expression of IGF receptors in T-47D cells and increase IGF-II, which produces a further down-regulation of IGF receptor (168, 174).

In summary, although there is evidence in support of a role for progesterone in cell proliferation in the breast, the underlying mechanisms are not clear, and the lack of appropriate models has slowed progress in this area. Much of the data on mechanisms of progestin action in the breast are derived from studies on breast cancer cells in culture: although these provide important information, the validity of extending this information to the normal breast has yet to be fully tested. Modulation of the cell cycle is associated with progesterone effects on cell proliferation: it remains to be determined whether direct PR involvement or, more likely, some other progesterone-regulated factor or factors underlies these effects. Furthermore, while progestins increase the expression of EGF and its receptor, the timing of these effects argues against their having a causative role in mediating progestin effects on cell proliferation. However, although the role of EGF pathways in mediating progesterone effects are unclear, progestins have been shown consistently to inhibit the mitogenic activity of the IGF pathways in both breast and uterus.

D. Markers of progestin action in the breast

Genes that are independently regulated by progestins in the mammary gland and, therefore, may act as markers of progestin responsiveness have been sought for their potential therapeutic or prognostic value in breast cancer. Two such candidate genes are those encoding the enzymes fatty acid synthetase and alkaline phosphatase.

Tissue-unspecific isoforms of alkaline phosphatase are detectable in the normal breast and breast milk and are induced by progestins in rat endometrial cells (175). In contrast, placental-type alkaline phosphatase activity is induced by estrogen but not progestins in the Ishikawa endometrial carcinoma cell line (176). Di Lorenzo and co-workers (177, 178) reported that tissue-unspecific alkaline phosphatase activity was induced by progestins in T47D breast cancer cells, and that this induction was accompanied by the acquisition of a differentiated, secretory phenotype. The increase in activity was due to increased expression of alkaline phosphatase mRNA, resulting in new alkaline phosphatase protein synthesis (178) rather than increased activity of existing enzyme. The progestin induction of alkaline phosphatase activity has not been characterized *in vivo*, and the physiological significance of its activity, the type of isoforms expressed in breast tumors, and the clinical significance of alkaline phosphatase activity in breast cancer remain to be determined.

Fatty acid synthetase was cloned from the MCF-7 breast cancer cell line by a subtractive hybridization strategy designed to detect progestin-regulated genes (179). It was subsequently shown (180) that the fatty acid synthetase protein had also been identified previously by [^{35}S]methionine labeling as a progestin-responsive protein in both MCF-7 and T-47D breast cancer cells (181). Progestins rapidly induce fatty acid synthetase both transcriptionally and posttranscriptionally, with an increase in gene transcription detectable as early as 15 min after hormone treatment, and a concomitant stabilization of mRNA (182). Furthermore, the progestin antagonist RU 38486 decreased basal transcription, stabilized existing mRNA, and blocked progestin induction of fatty acid synthetase, demonstrating that the effect was PR-mediated. Fatty acid synthetase catalyzes the conversion of acetyl-CoA and malonyl-CoA into fatty acid, and its induction in breast cancer cells is accompanied by increased lipid synthesis and the accumulation of lipid droplets (183). The enzyme is postulated to be a marker of differentiation and progestin responsiveness in breast cancer (184). As in the case of alkaline phosphatase, the clinical significance of fatty acid synthetase expression in breast cancer remains to be demonstrated. Studies have revealed that fatty acid synthetase mRNA levels in breast cancer tissues, measured by *in situ* hybridization, are not correlated to ER or PR levels or to node involvement. However, the enzyme may act as a marker of proliferation in benign mastopathies, since its expression is higher in cysts and lobules than in ducts (184).

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E. Progesterone effects on lactation

In the normal breast, progesterone acts synergistically with estrogen and PRL during pregnancy to prepare for lactation by promoting lobuloalveolar development (185). Progesterone also acts as an anti-PRL by preventing the synthesis of milk proteins in mid- to late pregnancy (7) and by inhibiting PRL secretion in women expressing abnormally high circulating PRL (186). In pseudopregnant rabbits, progesterone antagonizes PRL induction of PRL receptors (140). A sudden fall in circulating progesterone accompanies parturition and is associated with a concurrent increase in PRL secretion and the onset of lactation.

α-Lactalbumin, part of the lactose synthetase complex, is involved in lactogenesis after parturition and is induced by PRL, insulin, and glucocorticoids. Glucocorticoid induction of α-lactalbumin synthesis is specifically blocked by progestins in midpregnant rat mammary gland explants (187). The progesterone effect is seen at low concentrations, suggesting that it is PR-mediated. Several putative glucocorticoid/progesterone response elements have been identified in the α-lactalbumin gene (188), and PR is postulated to compete with GR for binding of these elements, resulting in antagonism of glucocorticoid effects (187, 189). cAMP levels are increased by progesterone during pregnancy and are also able to block hormonal stimulation of α-lactalbumin (138). In the final days of pregnancy and during lactation, α-lactalbumin synthesis increases significantly and is insensitive to induction by glucocorticoids or suppression by progestins (190). In the mouse, the lack of effect of progesterone on α-lactalbumin during lactation is attributed to a specific suppression of PR expression, which is refractory to stimulation by exogenous estrogen (191). However, this does not explain the observed loss of α-lactalbumin suppression during late pregnancy, when PR remains high (190). Another major milk protein, β-casein, is also under progesterone control. β-Casein mRNA expression is blocked by progesterone during pregnancy (192), presumably through the binding of pregnancy-specific factors to the casein gene promoter (193). This repressor effect is lost upon parturition, and β-casein mRNA expression is greatly increased during lactation.

In summary, studies of the physiological actions of progesterone in the breast have been focused primarily on the roles of this hormone in cell proliferation and milk protein regulation (Table 1). While the involvement of progesterone in the regulation of some milk protein genes has been defined and there are data *in vitro* describing genes likely to be implicated in control of cell proliferation, on the whole the molecular action of progesterone *in vivo* in the breast is poorly understood, there are few experimental models of normal breast physiology available, and genes that are direct targets of progesterone action have not been described in the normal breast *in vivo*.

VI. Progesterone Effects in the Brain

Estrogen and progesterone control specific brain functions involved in reproductive behavior. In guinea-pigs and rats, sequential secretion of estrogen and progesterone during the estrous cycle induces the sexually receptive lordosis response that coincides with ovulation. Induction of PR expression in the ventromedial hypothalamus and preoptic area, by estrogen or by sequential exposure to estrogen and progesterone, follows a time course that is correlated with the observed increase in lordosis response (194–196). Furthermore, while sequential treatment with estrogen and progesterone initially results in an even greater induction of PR than with estrogen alone (195, 196), receptor levels are later down-regulated, and this is accompanied by a parallel decrease in lordosis behavior (197, 198). A second progesterone treatment is ineffective on both PR and behavior (198). These data suggest the direct involvement of PR and progesterone in the regulation of sexual behavior. This is supported by the finding that sexual behavior can be abrogated by the direct delivery of antisense PR oligonucleotides to the cerebral ventricle of the rat brain (199). Sequential administration of estrogen and progesterone to these animals elicited a reduced or absent lordosis response, and the suppression was dependent on the oligonucleotide dose. Furthermore, PR was significantly lower in the hypothalamus of these animals. Similarly, PR null mice show a complete lack of lordosis response after hormone administration (1), demonstrating an absolute requirement for PR expression.

The mechanisms by which progesterone acts in the brain are not fully defined; however, progesterone is known to affect the expression of a number of proteins. Progesterone stimulates γ-aminobutyric acid (GABA) signaling pathways in specific areas of the brain. Progesterone-mediated increases in $GABA_A$ receptor binding sites in a number of regions of the brain, including some areas where PR expression is low or absent, are postulated to contribute to stimulation of lordosis behavior in rats and hamsters, suppression of aggressive behavior, and induction of the release of GnRH (200–202). Part of this effect may be mediated by direct interaction between 5α-reduced progesterone metabolites and $GABA_A$ receptor complexes in PR-negative regions of the brain (Refs. 202 and 203 and references therein) as well as by PR in areas such as the hypothalamus. Sequential estrogen and progesterone treatment, but not estrogen alone, potentiates oxytocin induction of norepinephrine release from the ventromedial hypothalamus (204), which in turn mediates hormone-dependent sexual behavior via noradrenergic projections. Progesterone and estrogen may also regulate behavior by affecting synthesis of POMC, the precursor of β-endorphin, in the ventromedial hypothalamus; estrogen down-regulates the synthesis of this peptide, and preliminary data suggest that progesterone prevents this down-regulation (205). β-Endorphin decreases pituitary secretion of LH and FSH. Adenylate cyclase activity and cAMP levels are rapidly increased (206) and serotonin turnover is down-regulated (207) by progesterone in the ventromedial hypothalamus-preoptic area in rats, and both are implicated in increased sexual receptivity.

Progesterone also affects gene expression in areas of the brain not involved in sexual behavior. PR is detectable in the cortex, hypothalamus, and pituitary within the first few days of postnatal life in rats and in the cortex may play a role in early learning patterns (63). Furthermore, progesterone treatment increases β-adrenergic receptors in the rat cortex, postulated to be involved in modulation of emotional activity

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(208). A recent report suggested that the rat PR isoforms have different functions in different areas of the brain. The study found that PR A is more highly expressed in the hypothalamus-preoptic area, whereas PR B predominates in the cortex (209). Rat PR A and B have been demonstrated *in vitro* to be differently inducible by estrogen (210, 211), suggesting that hormone regulation of PR expression may differ between the hypothalamus and cortex.

In summary, progesterone regulates signals in the brain involving sexually responsive behavior. The most well defined aspect of progesterone effects on this process are PR-mediated effects in the hypothalamus and preoptic area. Progesterone effects in the brain may also be mediated by nonclassic mechanisms of action such as direct interaction of progesterone metabolites with other receptors, such as $GABA_A$ receptors. Furthermore, the relative expression of PR A and B may be important in determining progesterone effects in specific sites in the brain.

VII. Progesterone Effects on Bone

Expression of both ER and PR in normal human osteoblast-like cells has been reported (68, 212), and several lines of evidence support a role for steroid hormones in regulating the expression and function of matrix proteins and metalloproteinases involved in bone remodeling and resorption. Estrogen down-regulation of mRNA for bone matrix-associated proteins has been reported to decrease bone resorption and formation, resulting in a net slowing of the rate of loss of bone mass (213, 214) and thereby supporting the use of estrogen agonists in the prevention of osteoporosis in postmenopausal women.

Progesterone may have a role in bone matrix regulation, via its effect on metalloproteinases. As mentioned earlier, progestins regulate proteinase activity in the uterus, suppressing expression of stromelysins in endometrial stromal cells and inducing TGFβ, resulting in down-regulation of matrilysin expression in the endometrial epithelium component of stromal-epithelial cocultures (88, 89). The demonstration that a sequence contained in the 5′-flanking region of the mouse gene encoding the bone matrix protein, osteonectin, can act as a PRE *in vitro* suggests that progestins may also regulate this protein *in vivo* (215).

It has been suggested that progesterone regulation of bone remodeling may also be indirectly facilitated by the ability of progesterone to act as a ligand for the glucocorticoid receptor. Glucocorticoids have been implicated in the process of bone loss through their ability to block 1,25-$(OH)_2$-vitamin D-induced osteocalcin synthesis (216) and to prevent attachment of osteoblasts to matrix proteins, including osteonectin, possibly through down-regulation of β1-integrin and other cell surface attachment factors (217). Glucocorticoids also increase bone sialoprotein mRNA levels in rat osteosarcoma cells, an effect that can be blocked by 1,25-$(OH)_2$-vitamin D and has been postulated to contribute to acceleration in maturation of preosteoblasts and ultimately to contribute to bone loss (218). Progesterone has been postulated to antagonize glucocorticoid-mediated effects in bone, resulting in abrogation of glucocorticoid-induced bone loss (219).

In summary, although the data are preliminary at this stage, progesterone appears to modulate bone remodeling, resulting in protection against bone loss. This effect appears to be mediated by PR expression in osteoblasts, as well as through binding to glucocorticoid receptor and perhaps reducing the influence of glucocorticoids.

VIII. Antiestrogen Action of Progesterone

Many of the effects of progesterone are thought to be due to its ability to oppose the action of estrogen, particularly in the uterus. Progesterone abrogates estrogen induction of many of the known hormone-responsive genes, and this effect is mediated by down-regulation of cytoplasmic and nuclear ER protein concentrations, decreasing the active estrogen concentration (reviewed in Ref. 5) and antagonizing the action of ER at the molecular level. The importance of this balance between estrogen- and progesterone-mediated effects is demonstrated by the extensive proliferation of the luminal and glandular epithelium, consistent with unopposed estrogen effects, in the uterus of ovariectomized PR null mice (1).

A. Inhibition of ER expression

The mechanism of progesterone action on ER was initially elucidated in the mammalian uterus. Uterine ER levels were decreased by administration of progesterone to estrogen-treated rats (220). Progesterone also antagonized estrogen induction of ER in the rat myometrium and in whole rat uterus (221, 222). Furthermore, administration of a synthetic progestin, medroxyprogesterone acetate, to women undergoing curettage during the follicular phase of the menstrual cycle resulted in decreased endometrial ER levels (223). A decrease in ER in hamster decidual cells, due to progesterone-mediated shortening of the ER protein half-life, suggested direct destabilization of ER by progestins (224). The progesterone-mediated decrease in ER protein has been shown more recently in breast cancer cells to result from decreased cellular ER mRNA levels (225), likely to reflect decreased transcription of the ER gene, since the effect was seen rapidly without shortening of the ER mRNA half-life (226).

B. Progesterone inhibition of the molecular action of ER

As well as directly reducing ER concentration, progesterone opposes ER-mediated gene-regulatory events, although the molecular mechanisms of this antagonism are not clear. In terms of this effect, the best defined model is the regulation of PR itself. Progestins inhibit and estrogens stimulate rabbit PR gene expression through the same region in the rabbit PR promoter (29), although this effect is mediated without binding of PR to this region, suggesting that PR may sequester transcription factors that are essential for estrogen action (227). By contrast, progesterone and estrogen effects on human PR appear to be distinct, since estrogen primarily regulates the PR B promoter (45), whereas progestins regulate both PR isoforms in breast cancer cells.

Other recent demonstrations that PR can inhibit transcrip-

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tional activation by ER of estrogen-responsive promoters without binding to DNA support the view that PR may act by sequestering transcription factors required for ER activity (36, 37, 228, 229). However, the repressive effects of progesterone appear to be promoter- and cell-specific, and there is considerable variability between reports. McDonnell and Goldman (36) reported that PR A but not PR B, in the presence of either progesterone or antiprogestins, lessened the ability of estrogen to induce an estrogen-responsive reporter when the two constructs were transfected into CV-1 or HS578T cells, but not HepG2 cells. PR A had similar antiestrogenic effects on endogenous ER activation of a minimal estrogen-responsive reporter in MCF-7 breast cancer cells in the presence of RU 38486 (37). However, when the estrogen-responsive region of the pS2 gene was used as a reporter in MCF-7 cells, PR B and not PR A repressed activation of the reporter by estrogen (229). The reason for this variability is not yet known, but it is possible that specific accessory proteins involved in transcriptional activation by ER and PR differ between cell types or are expressed at different concentrations, resulting in variable effects. PR A has been demonstrated to have similar repressive effects on other members of the nuclear receptor family, including those for androgens, mineralocorticoids, and glucocorticoids (35–37), although the physiological significance of this observation remains to be determined.

IX. Summary and Conclusion

Examination of the regulation of gene expression by progesterone has revealed the complexity of the diverse roles of this hormone in the control of female reproductive function. The actions of progesterone have been characterized in the most detail in the uterus, where progesterone participates in the cyclical control of proliferation and differentiation during the menstrual cycle and plays a key role in pregnancy, being involved in ovulation, implantation, uterine growth, and maintenance of pregnancy. In the uterus, progesterone both stimulates and inhibits cell proliferation. It promotes differentiated function and, although the mechanisms underlying progesterone opposition of estrogen action have been investigated, most of the mechanisms underlying the diverse roles of progesterone in the uterus still require elucidation. In the breast, progesterone is primarily responsible for development of the lobular-alveolar structures during puberty and pregnancy, but also plays a role in cyclical control of proliferation during the menstrual cycle by mechanisms that are poorly understood. In the brain, the effects of progesterone on sexually receptive behavior have been established in animal studies, but information on the role of progesterone in the human brain is limited. Similarly, although progesterone is likely to play a role in bone remodeling, there is a paucity of information on this at present.

The molecular mechanisms of progesterone action have been described only for a small number of well defined target genes, examples being the uteroglobin and ovalbumin genes, which have been studied in detail. However, progesterone is clearly involved in the regulation of a considerable number of genes about which little is known. Furthermore, the distinction between direct and indirect targets of progesterone action in cellular processes is largely yet to be made. By separating gene-regulatory events, which are central to the physiological effects of progesterone, from the secondary consequences of progesterone action, it may be possible to define the determinants of response to progesterone in normal and malignant cells.

X. Future Directions

Although significant progress has been made in understanding the physiological actions of progesterone in the mammalian reproductive system and the molecular structure and function of PR, there are still marked gaps in knowledge. There is a great deal to be learned about the biology and hormone responsiveness of the normal breast, and the relative paucity of models has been a limitation in this regard. Importantly, there is a need for more information on the human breast, both normal and malignant, to provide baseline information that will be beneficial in model development, as well as in a better understanding of the physiology of hormone action in the breast.

More information is needed on the significance of PR A and PR B expression. While PR involvement in regulating a host of physiological events has been described, there is little known of the individual roles of the PR proteins PR A and PR B in mediating these effects. While *in vitro* work suggests that these proteins have different functions and that PR A may be a repressor of PR B in particular, and more generally of other members of the nuclear receptor family, there are no data on the relative activities of PR A and B *in vivo*. Furthermore, it is not known whether PR A and B are expressed in all target tissues, and within target tissues whether cells express one or both of these proteins. The relative expression of the two PR proteins has not been examined to date in most tissues of the reproductive system. Addressing these questions, which rely on single cell analysis, poses significant technical challenges that will necessitate continued development and refinement of immunohistochemical, *in situ*, and other methodologies.

The mechanism of PR action may also depend on an array of other proteins, such as the recently described nuclear receptor coactivators and corepressors. Nuclear receptors interact with coregulatory proteins, which may function as intermediates in transcription (230). If they play a role in the transcriptional activity of PR, it is likely that they will be expressed in progesterone target tissues, although this has yet to be described. The role of coregulatory proteins in progesterone action needs further investigation to clarify whether progesterone regulates coregulatory protein expression and whether tissue levels of coregulatory proteins play a role in modulation of progesterone action.

Progesterone has both proliferative and differentiating actions, and identification of genes whose transcription is directly modulated by progesterone is an essential first step in understanding progesterone effects on these complex processes. The number of described genes that are directly modulated by progesterone is small, and many of the effects of progesterone described in this review are likely to be indirect

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consequences of the action of as yet undescribed gene products. New information on genes directly regulated by progesterone is urgently required, and techniques such as differential display PCR and similar approaches by which to identify progesterone-regulated transcripts, despite their limitations, are likely to yield important new knowledge in the near future.

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