Identification of Progesterone-Regulated Genes in the Uterus

Cindee R. Funk, Bert W. O’Malley, and Francesco J. DeMayo

Progesterone plays a critical role in transforming the nonpregnant uterus into an enriched environment specifically suited for the developing embryo. Its role in inducing and maintaining the pregnant state is paramount in mammalian systems and to a large extent, determines the success of any given pregnancy in the steps following fertilization. For years, reproductive biologists have tried to elucidate the actual mechanisms and the signaling pathways involved in mediating progesterone’s activity. Yet little is currently known about the cascade of events which initiate and communicate the inductive effects of this uterine steroid. It has only been in the past few years that molecular targets of progesterone regulation have really been identified and characterized with respect to mediating pregnancy (1). Although this area of research is clearly burgeoning forward with the advances of gene knock out techniques and the identification of essential “pregnancy” genes, the small handful of genes known to be essential for pregnancy lends only suggestive information as to what signaling pathways are essential mediators of a successful pregnancy.

This chapter first presents a brief review of the importance of progesterone during the early stages of pregnancy, traditionally exemplified by the use of RU486 as a progesterone antagonist, but more convincingly demonstrated by the pleiotropic reproductive phenotype of the progesterone receptor knock-out mouse. Then, it presents an overview of some of the currently available methodology utilizing steroid receptor mutant mice to isolate, identify, and characterize new targets of progesterone action with the ultimate goal of understanding the signaling pathways involved in the establishment of pregnancy.

Overview and Importance of Progesterone

Immediately following implantation and prior to development of the fetoplacenta unit, the growing embryo is completely dependent on the maternal uterine environment for both protection and nourishment. Later, the pla-
Placenta makes a direct link between the fetal unit and the maternal blood supply, which is maintained until parturition. In most species, the placenta takes on an endocrine role, by which it assumes the role of steroid biosynthesis and assures the maintenance of pregnancy throughout birth. The uterine environment is therefore a crucial player in determining the success rate of a given pregnancy, and this in turn is critically dependent on the intricate interplay between the two female steroid hormones—estrogen and progesterone. After estrogen initiates the uterine transformation, progesterone is absolutely required to further sensitize the endometrial component through a series of steps that in the rodent requires about two days. Progesterone has been traditionally regarded as the “pregnancy” hormone because of its roles during both implantation and placental maintenance which is continued throughout birth. Following fertilization, progesterone induced responses ultimately determine the success of a given pregnancy (2).

The importance of progesterone is further exemplified in clinical situations in which progesterone antagonists have been used to both prevent implantation of a fertilized ovum, and to prematurely terminate an already established pregnancy (3). The aging uterus also underscores the importance of progesterone with respect to embryo implantation, whereby the natural decline of circulating hormone in aging women negatively influences the success rates associated with in vitro fertilizations. These poor rates have been drastically improved by administering a two-fold increase in the dose of progesterone in these older recipients whereby they slightly exceeded the success rates observed in younger women (4).

Regulation of Progesterone Activity

In the uterus, progesterone mediates its effects through the progesterone receptor which, when activated, migrates to the nucleus and initiates transcription of progesterone target genes. The gene encoding progesterone receptor is thought to be in single copy, producing at least nine independent transcripts through differential splicing (5). Although as many as four different protein isoforms of the receptor have been reported in various species (6,7), the two largest forms, PR-A (81–83 kDa), and PR-B (116–120 kDa) are the most abundant forms found in human, chick, and rodent tissue. They are thought to regulate gene transcription either by transcriptional activation through PR-B or by repression through the dominant repression of PR-B by the PR-A isoform. Consequently, the relative proportion of the PR isoforms in any given target cell will ultimately determine if a specific gene will be expressed in the cell upon hormonal stimulation (6). In general, the level of available progesterone receptor is induced in most reproductive tissue by estrogens, growth factors and cAMP, but the level of available receptor decreases in response to progesterone, and may therefore explain the continued