

## **Role of Activating Protein-1 and Antisense Progesterone Receptor (PR) mRNA in the Regulation of PR Isoforms in Mouse Mammary Epithelial Cells**

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The progesterone receptor (PR) is an important transcription factor involved in key stages of mammary gland development and breast cancer. We recently discovered that the mouse PR locus contains an antisense mRNA transcript (PR<sup>antisense</sup> mRNA) which overlaps with the beginning of the PR gene. This unusual arrangement may be important because antisense RNA potentially modulates gene expression through a variety of novel mechanisms including transcriptional interference, mRNA stability, and translational efficiency. Adding to this complexity, there are two PR isoforms (PRA and PRB) whose expression in the mouse mammary gland is temporally and spatially regulated during development. PR isoform expression is also under hormonal influence, with PRA expression dominating in pubertal and virgin adult mice and PRB expression being restricted primarily to the period of alveologenesis during mid-pregnancy. Since PR<sup>antisense</sup> mRNA overlaps with the promoters (gene regulatory regions) that are believed to control PRA and PRB expression through separate PRA and PRB mRNA transcripts, antisense transcription may influence the balance between these two PR isoforms. Superimposed on these hormonal influences are important growth factor pathways, many of which ultimately converge on the Activating Protein-1 (AP-1) family of transcription factors, consisting of homologous or heterologous dimers between several jun and fos family members. Expression of the mouse PR gene is regulated not just by steroid hormones (estrogen and progesterone), but also by growth regulatory pathways that signal through AP-1 and other transcription factors. Therefore, we decided to examine the effect of steroid hormones and co-expression of jun and fos subunits on the activity of both the sense and antisense promoters, and on their respective mRNA transcripts. Our underlying hypothesis is that a change in the composition of AP-1 subunits is, at least in part, responsible for the shift from PRA to PRB expression during alveologenesis, perhaps due to their ability to regulate the activity of the PR<sup>antisense</sup> promoter. To test this hypothesis, we characterized the behavior of the sense and antisense promoters in response to co-expression of different AP-1 isoforms, and to determine if the presence of PR<sup>antisense</sup> mRNA has any effect on the activity of the sense-strand PR promoters, on the level of PR sense-strand mRNA, or on the expression of PRA or PRB protein itself. Our results suggest that PR<sup>antisense</sup> mRNA can indeed interfere with the efficiency of PR protein expression and that it likely does so by a co-transcriptional or post-transcriptional mechanism.

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