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Prolactin stimulation of rat relaxin gene promoter activity in cultured rat luteinized granulosa cells.

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Bacground: Preprorelaxin (preproRLX) mRNA levels in the rat ovary increase markedly around midpregrancy in rats. This elevation is likely the result of placental lactogen (rPL) action, as the onset of preproRLX mRNA expression coincides with rPL secretion and is dependent on the placenta. Either rat placental lactogen or its pituitary equivalent, prolactin(PRL), stimulates production of preproRLX mRNA by luteinized rat ovarian granulosa cells in primary culture (Perers *et al*, Mol. Endocrinol., in press).

Objective: To understand the signal pathway and Transcription factors involved in PRL-stimulated preproRLX synthesis, we have cloned and begun characterizing the rat RLX gene.

Results: The gene has a single transcription start site, as determined by primer extension analysis, and a canonical TATA box, located 21 to 27 bases uostream from the start site. In preliminary studies using promoter/reporter constructs transiently transfected into rat luteinized granulosa cells in primary culture, we found that there are three regions of interest in the 5'-flanking sequence of the gene ; 1) the proximal promoter accounting for high levels of basal expression ; 2) a more distal region accounting for reduced expression ; and 3) an even more distal region involved in PRL stimulation (or derepression). Cotransfection with a stat5b expression plasmid enhanced relaxin promoter-deriven expression ; the effects of stat5b were increased by PRL treatment. None of the other stat types (1,3,5a) were effective in enhancing promoter activity. Finer mapping of the cis-acting sequences should allow us to identify the stat5b binding sites and to determine the identities of other trans-acting factors involved in regulation of RLX gene expression.

Conclusions: From these studies, we hope to develop a model explaining the interactions between signal pathways and transcription factors that lead to activation of RLX gene expression.