STRATEGIES FOR ACHIEVING HIGH-LEVEL EXPRESSION OF RECOMBINANT HUMAN PROLACTIN (hPRL) IN *E. COLI*

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Prolactin, originally identified as a hormone associated with pregnancy and lactation, has been shown to have effects on many different cell types, with biological activities as diverse as lactogenesis, immune regulation and osmoregulation. This hormone has been produced up to now with very low yields by laborious extraction procedures from pituitary glands where only approximately 0.1 mg are present. DNA recombinant techniques represent therefore an attractive alternative for hPRL production.

Our laboratory at IPEN-CNEN/S.P. has obtained, for the first time in bacterial periplasmic space, a bioactive form of prolactin with 12 extra N-terminal amino acids (Morganti et al. Biotechnol. Appl. Biochem. 1996; 23:67) and, at a much lower expression level, the authentic form of hPRL. No report exist, up to now showing high-level expression of authentic hPRL in *E. coli*.

In the present work we tried to investigate the influence that two different promoters may have on bacterially synthesized hPRL. The first is the "λP_L", activated by a temperature increase, while the second is the "tac" promoter (a hybrid of "trp" and "lac") activated by IPTG and that is joined to the T7 phage gene 10 leader (g10-L) which appears to act as a very efficient ribosome binding site. The "tag-hPRL" gene (cDNA), which already demonstrated an efficient cytoplasmic expression, was inserted in both vectors and tag-hPRL, accumulated as inclusion bodies, was determined by SDS-PAGE/densitometry. The "tac/g10-L" system offered a 5-10-fold higher expression level, possibly explaining why the "λP_L" vector series never produced significant amounts of periplasmic hPRL.

The influence of post-translational mechanisms (precursor processing and protein degradation) on hPRL periplasmic expression will also be discussed together with some preliminary data obtained on hPRL expression in mammalian cells.

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