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BACTERIAL EXPRESSION AND PURIFICATION OF A CYTOPLASMIC FORM OF HUMAN PROLACTIN : A STUDY ON THE UTILIZATION OF DIFFERENT VECTOR ELEMENTS.

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Large-scale production of hPrl has recently become possible with the use of recombinant DNA techenology. Our laboratory has been the first in absolute to obtain recombinant human prolactin in bacterial periplasmic space, the expression level, however, always being quite low when compared to cytoplasmic expression.

In order to study and improve expression efficiency in general, different vectors, directing the synthesis of an active form of human prolactin (taghPrl) *in E. coli* cytoplasm, were constructed and analyzed especially for what concerns their constitutive elements. We developed a series of three expression vectors, all including an identical λP_L promotor and taghPrl gene. Each construction was prepared to evaluate the action of specific elements, like the ribosome-binding site (Shine and Dalgarno), the T7 gene 10 translation enhancer, the transcription terminator (g32) and the antibiotic resistence gene. Comparing the obtained expression efficiencies with those presented by other two vectors obtained in this same laboratory and based on the tac promotor, indications were found that the combination g32-kanamycin resistence gene can be responsible the highest expression levels ever reported : up to 130 $\mu g/ml.A_{600}$.

A solubilization-renaturation methodology was set up obtaining a total yield of about 42% of the oxidised, properly folded form of the hormone. A comparison betweem two techinques, one based on inclusion body solubilization with N-lauryl sarcosine and the other utilizing 8 M urea, indicated that the latter is more suitable for obtaining good quality, monomeric human prolactin.

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