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A STUDY OF THE EFFICIENCY OF DIFFERENT TYPES OF BACTERIAL EXPRESSION VECTORS, BASED ON THE "λP_L" AND "TAC" PROMOTERS.

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Several bacterial expression vectors have been constructed in our laboratory for the production of human pituitary hormones in genetically modified *E. coli*, each of them including different constitutive elements like promoter, ribosome binding site (SD), leader sequence, antibiotic resistance. Most of these vectors failed to produce human Prolactin (hPRL), a hormone that, according to literature data, is very difficult to be obtained in bacteria, especially in the periplasmic space. For this reason we started to study be performance of some of these vectors and of their constitutive elements in order to increase the expression yields of this hormone which is probably rapidly degraded in the bacterial environment.

A series of vectors based on "λP_L" promoter and the natural hPRL leader sequence, did not show any significant expression of this hormone, while the same vectors had produced high yields of human growth hormone (hGH).

A chimeric gene including hGH leader sequence together with the first 15 aminoacids (AA) of this protein, while lacking the first 30 AA of hPRL, did not show any improvement at all. This seems to indicate that the problem is not related with the promoter, SD or the type of leader sequence. Also other construction, either including or excluding the presence of the poly-adenylation site confirmed that this or other 3' terminal sequences is not influencing the stability of the mRNA being formed.

A second series of vectors, based on the "Tac" (trp/lac) promoter, a g10 translational enhancer element (tee) derived from bacteriophage T7, a "tag" sequence, including 6 His and a factor Xa cleavage site, which is useful for protein purification, and a Cellulase leader sequence, produced better results with a moderate expression of tag-hPRL in the periplasmic space. The same vector, without leader sequence, is expressing indeed high levels of tag-hPRL inside the cytoplasm, as insoluble inclusion bodies. Still the same vector, without "tag", is expressing very low amounts of authentic hPRL in the periplasm.

We are speculating the following:

a) hPRL is really extremely unstable when present in soluble form in *E. coli* cytoplasm or periplasmatic space;

b) the 12 AA tag sequence Ala-Ser-(His)6-Ile-Glu-Gly-Arg is having some stabilizing effect on the protein;

c) The combination "Tac" promoter/g10 tee is so efficient that, even rapidly degrading, significant amounts of tag-hPRL or authentic hPRL reach the periplasm;

d) The cellulase leader sequence may not be very efficient for our purpose.

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