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UTILIZATION OF DI AND TRICISTRONIC VECTORS FOR THE EXPRESSION OF RECOMBINANT HUMAN THYROTROPIN (rec-hTSH) IN CHO CELLS.

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The availability of large quantities of rec-hTSH, a heterodimeric glycoprotein composed of non-covalently linked α- and β-subunits, is extremely important in the diagnosis and therapy of thyroid carcinoma, via stimulation of thyroidal radioiodine uptake.

This glycoprotein was expressed in Chinese hamster ovary (CHO) cells using for the first time systems based on di and tricistronic expression vectors, containing internal ribosome entry sites (IRES) isolated from the encefalomiocardites (EMC) virus and amplifiable markers genes such as dihydrofolate reductase (DHFR) and adenosine deaminase (ADA).

The first strategy utilized was the cotransfection of DHFR$^-$ CHO cells with two dicistronic vectors (pEDdc-α-EMC-DHFR and pEAdc-βhTSH-EMC-ADA). After the cells had been submitted to gene amplification in culture medium containing stepwise increments of methotrexate (MTX), it was possible to isolate clones that presented a secretion level up to 5.7 ± 1.0μg hTSH/10^6 cells/day, the highest ever reported for the expression of this glycoprotein hormone. The production in a hollow-fiber bioreactor was set up in order to obtain a useful protein secretion and permit a preliminary physico-chemical, immunological and biological characterization.

We also constructed tricistronic vectors (pEDdc-α-EMC-βhTSH-EMC-DHFR and pEDdc-βhTSH-EMC-α-EMC-DHFR) and transfected the same DHFR$^-$ CHO cells having already isolated some hTSH-secreting clones. Now we intend to perform the gene amplification procedure with MTX and compare the expression levels based on the mechanism by which the same transcription unit containing dicistronic or tricistonic mRNA can be translated in mammalian cells.

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