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EXPRESSION OF RECOMBINANT HUMAN THYROTROPIN (REC-hTSH) IN CHO CELLS USING DICISTRONIC mRNA EXPRESSION VECTORS WITH AMPLIFIABLE MARKERS.

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High level recombinant protein expression in mammalian cells can be obtained by selection of stable clones followed by gene amplification of transfected DNA. Recombinant human thyrotropin (rec-hTSH), a heterodimeric glycoprotein composed of an α and β subunit, was expressed in CHO cells using a system based on dicistronic mRNA expression vectors containing an internal ribosome entry site (IRES) of the encefalomiocarditis (ECM) virus and amplifiable markers genes like dihydrofolate reductase (DHFR) or adenosine deaminase (ADA). These plasmids denominated respectively pEDdc and pEAdc were kindly donated by the Genetics Institute (Cambridge, MA, USA). The vectors pEDdc- α subunit cDNA and pEAdc- β subunit cDNA were cotransfected into dhfr^r CHO DXB11 cells. After methotrexate (MTX) amplification using 0.03; 0.06; 0.1; 0.2; 0.5 and 1.0 μ M MTX, we obtained some clones which were able to secrete up to 6 μ g hTSH/10⁶ cells/day in dishes, determined by immunoradiometric assay, a value comparable to the highest ever reported for this glycoprotein (4.5 μ g hTSH/10⁶ cells/day).

Cell culture and hTSH production in a hollow fiber bioreactor (Cellmax Artificial Capillary Module, Laguna Hills, CA, USA) is now being set up in order to obtain an useful rec-hTSH expression, being able to fully characterise this protein, which is extremely important in the diagnosis and therapy of thyroid cancer.

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