AMPLIFICATION OF hTSH α- AND β-SUBUNIT GENES BY PCR, USING PITUITARY cDNA, FOR RECOMBINANT hTSH PREPARATION AND PRODUCTION OF THE α-SUBUNIT IN CHO CELLS.

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Human thyrotropin (hTSH) is a member of a family of pituitary and placental heterodimeric glycoproteins hormones which have a common α-subunit but differ in their hormone-specific β-subunit. Being present in extremely low amounts in the human pituitary together other hormones (luteotropin and follitropin), whose similar physico-chemical structures make its purification difficult, the preparation of the recombinant hTSH (rec hTSH) by DNA recombinant techniques is particularly interesting. This recombinant hormone can fully replace the extracted hormone, as tracer and standard in diagnostic "in vitro" immunoassay systems and stimulate ^{131}I uptake in thyroid carcinoma diagnosis and therapy.

In our laboratory, after the unsuccessful screening of classical libraries for the hTSH β-subunit in particular, we isolated and amplified α- and β-subunits by Polymerase Chain Reaction (PCR), using human pituitary cDNA. Specific primers pairs (0.5 μM) for α- and β-subunits were designed and amplification was carried out by PCR in 50μl (30-70 ng) under specific denaturation, annealing and extension conditions. The PCR amplified genes were subsequently introduced into appropriate expression vectors kindly donated from the Genetics Institute (Boston, MA, USA), in order to transfect Chinese hamster ovary (CHO) cells. Choosing a strategy of sequential transfection, selection and amplification, we isolated some high producing clones for the α- subunit, whose gene was inserted into one of these vectors (pEDdc) for the obtainment of a stable α chain-secreting transformed CHO cell line.

Supported by IAEA (Vienna, Austria), FAPESP (São Paulo, Brazil) and CNPq (Brasília, Brazil)