PREPARATION OF HUMAN PROLACTIN BY DNA-RECOMBINANT TECHNIQUES

L.E.M.F. Dias* and P. Bartolini
Divisão de Medicina - Departamento de Aplicação de Técnicas Nucleares em Ciências Biológicas - IPEN-CNEN/SP

Prolactin (hPrl) is a protein whose content, in human pituitary, is about 1/100 of the content of its main polypeptide hormone: human growth hormone (hGH), with which it shares similar structure and physico-chemical behaviour. For these reasons its extraction and purification is extremely difficult , providing very low yields of a labile and extremely expensive product.

DNA-recombinant techniques offer therefore a very interesting approach to hPrl expression and production in transformed bacteria. For this purpose a cDNA human pituitary library was prepared in phage Lambda gt-10 presenting, at the moment of the screening, a titre of ~10^8 plaque forming units.

For the screening of hPrl-cDNA two probes were used: rat PRL-cDNA (kindly donated by Dr. Joseph Martial, University of Liege, Belgium) and a synthetized 21-mer oligonucleotide, that should hybridize with a portion of the 5' non-coding sequence of hPRL-cDNA. Four different screening process were carried out using both probes each time , on nitrocellulose filters. After the last process,five positive clones were obtained, only one of which demonstrated to be full length, beginning about 16 nucleotides upstream from the ATG starting codon. This clone, after partial sequencing, via the method of Sanger, demonstrated to be hPRL-cDNA, and it is now being used for the construction of a bacterial expression vector.

Supported by CNPq