EXPRESSION OF A NOVEL otx-RELATED GENE IN MAMMALIAN BRAIN DEVELOPMENT

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Introduction

The morphogenesis of the CNS and the differentiation of the neural structures are highly complex processes and until very recently little was known about the development of most anterior regions of the body, even in files. Gene candidates for the identification of forebrain regions have been isolated, they are homologs of Drosophila genes controlling head development, one of them is orthodenticle (otd), in mouse there are two otd-related genes, otx 1 and otx 2 which homeodomains are strikingly similar to the otd homeobox. Here we report a novel gene otd-related, that also shares sequences from gooseberry homeobox and some data about its expression is discussed.

Materials and Methods

Mice

C57/B16 mice were mated between 2 100 and 1 000 h. Day 0,5 post-coitum was assumed to begin at the middle of the day of vaginal plugging.

Pregnant female mice were killed by cervical dislocation and embryos were collected in ice-cold PBS under a dissection microscope and fixed overnight in 4 % paraformaldehyde.

PCR cloning of the homeobox

A novel sequence had been cloned using a degenerated primers strategy by RT-PCR. Preparation of RNA from mouse embryos was performed using the LiCl procedure (1). New sequence was gained by means of 5'RACE System (Rapid Amplification of cDNA Ends, GIBCO BRL) with the most divergent sequence, it was subcloned in pGEM-T vector (PROMEGA), a convenient system to the cloning of PCR products, and sequenced using Pharmacia sequencing kit.

Whole mount in situ hybridization

T7 or SP6 derived *in vitro* transcribed sense or antisense 130 nt probes were generated from the pGEM-T vector labeled with digoxigenin together with an alkaline phosphatase-coupled antidigoxigenin antibody (2). 10 µg of mouse genomic DNA were digested with BamHI and EcoRI and a Southern-blot was performed using the same probe. The band obtained around 2,8 kb or 3 kb in the other digestion, was cloned in a plasmid library using pUC18 EcoRI-BAP or pUCBamHI-BAP as vectors and transformed in electrocompetent cells TOP10F (*in vitro* gen).

Results and Discussion

The identification of a novel mouse gene is reported, it contains a homeodomain homologous to that of otd (3) and gsb-p (4) showing a high homology with the otx family members previously reported (5), although in the sequence found between Helix 1 and Helix 2 there is a residue glutamin that resemble homeobox gooseberry like. It has been reported a novel homeobox gene with this feature, otp, which shares sequence from two different homeodomains (6).

Expression study was done with a probe containing just a fragment of homeobox sequence and a very restrictive pattern was obtained in E12,5 mouse embryos in the prospective cerebral cortex, there is a co-expression pattern with otx 1 that could suggest these genes act in a combinatorial fashion, the presence and/or identity of a particular head segment may depend on the comb-ination of genes it express. A more detailed expression study has to be done with the genomic fragment cloned.

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