GENERATION OF TRANSGENIC MICE CARRYING THE E. coli *LacZ* GENE DRIVEN BY A Drosophila melanogaster *PATCHED* GENE PROMOTER

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Little is known about the mechanisms that control pattern formation in *Drosòphila* imaginal discs. However, several classes of developmental genes are known to play a role in this process (1). The segment polarity genes constitute one of such classes. Position differences within each polarity segment are regulated by intercellular interactions (2). Molecular basis of these signalling mechanisms are poorly understood, but the activity of several other genes notably patched and hedgehog has been documented to be involved in the process (3). Patched plays an important role in the regulation of wingless, the *Drosophila* homologue of murine wint-1 gene, a developmental control gene required for proper pattern formation within individual segments, and its expression is localized in the developing spinal cord and brain (4). In this study we examine the pattern of expression of the lacZ reporter gene under the control of a *Drosophila* patched gene promoter, during early stages of development in mice.

Transgenic mice were generated by standard DNA microinjection. The genetic construct comprises the cDNA coding for *E. coli* β-galactosidase, flanked by *Drosophila* P-elements, under the control of a *Drosophila* patched gene promoter. Transgenic mice were identified among founder animals by dot blot hybridization, for further studies on copy number and transgene integrity, we used Southern blot. The only transgenic founder female was mated to non-transgenic male, and F1 progeny was produced. A homozygous line was established, for that, transgenic heterozygous F1 siblings were mated and the F2 progeny was analyzed by Southern blot. After hybridization with a fragment of the patched gene promoter, membranes were washed and re-hybridized with a probe containing glyceraldehyde phosphatedehydrogenase gene (GPDH). Quantitation of the intensity of the hybridization signals was performed using Anablot test (ICID, Cuba). After normalization of the readings and quantitation, homozygous animals were identified and mated to establish lines.

The pattern of expression of lacZ gene was assayed from day 9 to 16 of embryonic development in foetuses obtained from the mating of transgenic F1 females. At fixed times foetuses were poured out from the uterus, washed several times in PBS, fixed with 4 % paraformaldehyde, 0.4 % glutaraldehyde for 2 hours at 4 °C, rinsed again in PBS, and stained for β-gal activity, using chromogenic substrate (X-gal). Starting from day 12, foetuses were cut longitudinally in two halves before fixation. After staining, DNA was extracted by phenol/chloroform from proteinase K-treated foetuses and PCR was performed using specific primers for *E.coli* lacZ gene.

Out of a total of 99 founder animals, only one was identified as transgenic in the dot blots. Southern analysis showed that the transgene was integrated in a single chromosomic location, without obvious rearrangements, the copy number was estimated to be about 30 copies. The transgene was transmitted to the F1 progeny in a Mendelian fashion, and a homozygous line was established.

Expression of the lacZ gene was studied using F1 heterozygous foetuses during the period from day 9 to 16 of embryonic development. This period is crucial for the formation of the neural system of the foetus, and it includes the time during which wint-1 gene is expressed in mice. Since we assayed F1 foetuses, among them were both negative and transgenic foetuses. Transgenic status of the foetuses was corroborated by PCR.

A mild blue staining in the forebrain and cerebellum of transgenic foetuses, was detected. Other areas of mild staining included the area of the nostrils, sternum and the umbilical cord. No staining was detected in control foetuses, however, a disperse and milder signal was seen in some of the control animals, if stained for larger periods of time (overnight, for instances). We can not rule out the possibility of some false PCR-negative foetuses accounting for this background β-gal activity. To overcome this problem, we are at present studying the pattern of expression of the lacZ gene, using homozygous foetuses.

1. Ingham PW *et al.* Nature 1991;353:184-187.
2. Sampedro J and Guerrero I. Nature 1991;353:187-190.
3. Hama Ch *et al.* Genes and Development 1990; 4:1079-1093.
4. Capdevila J *et al.* EMBO J 1994;13:71-82.
5. Ingham PW. Nature 1988;335:25-34.