ISOLATION OF A NEURAL-SPECIFIC GENE OF *XENOPUS LAEVIS* BY REPRESENTATIONAL DIFFERENCE ANALYSIS

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Neural induction of the competent ectoderm can be considered as the third rnajor phase of vertebrate embryonic development. In *Xenopus laevis*, the south african clawed frog, the first phase is the establishment of the primary asymmetries like dorsal-ventral, anterior-posterior and inside-outside. This process is followed by the induction of the mesoderm between the ectoderm in the animal hemisphere and the endoderm in the vegetal half. The dorsal mesoderm then induces the neighbouring competent ectoderm to form neural structures. Although there are many substances described, which have a neural inductive effect on isolated ectoderm, there is still little known about the complex interactions that leads to the determination of the central nervous system *in vivo*. Our main interest is focussed on the isolation of genes, which may play an important role in induction and in the response to those inductive signals within the target cells.

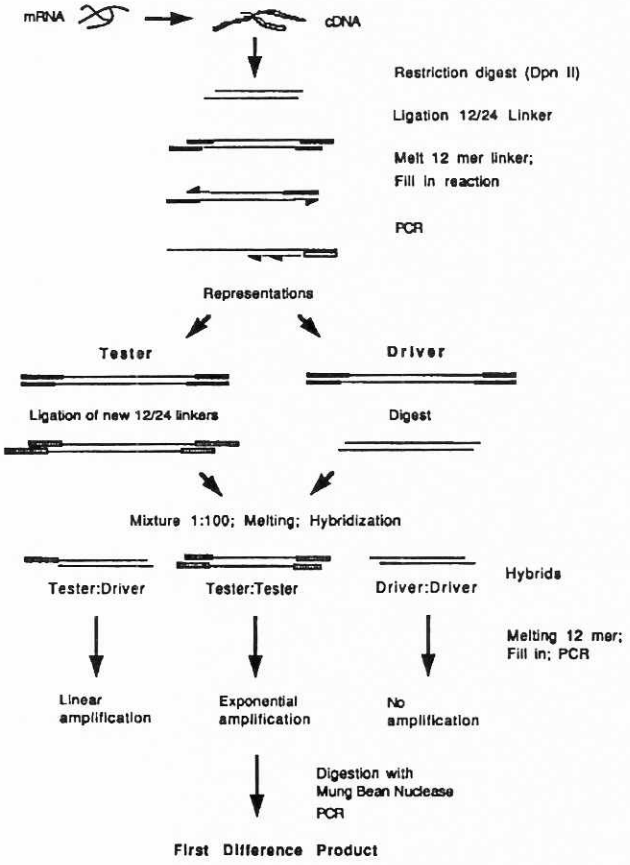

Figure 1: Schematic diagramm of the RDA-PCR method. Solid boxes show the oligonucleotides used to generate the tester and driver representations. Hatched boxes represent the oligonucleotides used to generate the difference products. Single half-arrows symbolize fill in reactions. Double half-arrows indicate DNA amplification. To generate second and third difference products, previous obtained products are reintroduced into the process at the tester stage.

To approach these questions we used the representational difference analysis (RDA; Hubank and Schatz, 1994) to detect tissue specific cDNAs in the inductive dorsal mesoderm and the reacting ectoderm. The RDA-PCR method is a process of subtraction coupled to PCR-amplification capable to isolate the differences between two cDNA populations with the advantage that only small amounts of RNA are required. We have dissected the neuroectoderm, chordamesoderm and ventral ectoderm from *X. laevis* stage 12 gastrulae (Nieuwkoop and Faber,1967). Total RNA was prepared to generate ds cDNA. The RDA-PCR method was performed according to Hubank and Schatz (1994) as shown in Figure 1. The neuroectoderm specific approach was done with the cDNA derivated from the neuroectoderm as the tester and chordamesoderm/ventral ectoderm as the driver. Three repeats of subtractive hybridization and PCR-amplification were carried out with increasing ratios of the driver. The third difference product was separated on an agarose gel, the resulting fragments were eluted and cloned into the pBluescript KS$^+$II vector. The transcriptional labelling was done by using the T3/T7 RNA polymerases and DIG- UTP to test the expression pattern of the isolated clones in the whole mount *in situ* hybridization assay. The nervous system specific clone N5/4 (242 bp) was sequenced and primers were generated to screen a *X. laevis* stage 29/31 ZAP Express™ cDNA library (by courtesy of Th. Hollemann and T. Pieler) by using a PCR-coupled method.

We were able to isolate a 1.691 kb cDNA, which contained an open reading frame coding for 309 amino acids encompassing a HMG box characteristic of the sox family. The deduced amino acid sequence shows a high homology to other sox genes, espacially to the chicken *sox3* gene within the HMG box (96 per cent). The nucleotide sequence of *xlsox3* has been deposited in the EMBL Data Library with accession number Y07542 (publ. in prep.). In order to clarify the expression pattern of *xlsox3* we are performing whole mount *in situ* hybridization assays and RT-PCR. Preliminary results (data not shown) suggest a role in the neural development of *X. laevis*.

References

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Nieuwkoop, P. D. and Faber, P. (1967) Normal Table of *Xenopus laevis* (Daudin) 2nd edition. North-Holland Publ. Co., Amsterdam