Abstract

In this study, the large-scale whole-mount *in situ* hybridization was employed to screen an activin-treated ectoderm cDNA library of *Xenopus laevis*, leading to the isolation of 51 novel cDNA sequences of *Xenopus*. So this approach turned out to be practicable for identification of new genes from a cDNA library in a large scale. *XXBP-1*, *XMLP* and *XAT* were selected for further functional studies based on their suggestive expression pattern.

XXBP-1 is a novel basic leucine zipper transcription factor in *Xenopus*. It is a maternal factor and expressed on the dorsal blastopore lip and ventral ectoderm with exception of perspective neural plate in gastrula stages. RT-PCR indicated that *XXBP-1* is weakly expressed before gastrula stages, afterwards is up-regulated and kept in a persistent level during the embryonic development. Overexpression of *XXBP-1* leads to ventralization of the injected embryos as described for *BMP-4*. Moreover, *XXBP-1* and *BMP4* interact in a positive feedback loop. Consistent with mediating *BMP-4* signaling, the ectopic expression of *XXBP-1* partially recovers the expression of *epidermal keratin* in animal cap assay and converses the dorsolizaiton imposed by truncate BMP receptor I. Thus, we propose that the *XXBP-1* is downstream of BMP receptors, and plays roles in the inhibition of neural differentiation.

XXBP-1 functions as a transcriptional activator. When overexpressed ventrally, the effect of wild type *XXBP-1* can be phencopied by the XXBP1-VP16 containing the activator domain VP16 and the DNA binding domain of *XXBP-1*. Another fusion construct, XXBP1-Eve containing the DNA binding domain of *XXBP-1* and even-skipped repression domain induce neural markers *NCAM* and *nrp1* in animal cap assay and leads to the secondary axis when overexpressed ventrally in a low ratio (10%). This confirms that *XXBP-1* acts as an activator, playing negative roles in neural induction.

The XMLP may be a new number of the small MLP protein family. Using whole-mount *in situ* hybridization and RT-PCR, *XMLP* maternal transcripts were detected during the cleavage stages. After MBT the signals are restricted to the neural plate. Subsequently *XMLP* is expressed predominantly in the brain, somites, and

pronephros. Ectopic expression of *XMLP* results in eye and axis defects and as well as a change of the expression pattern of *Krox 20*. Apoptosis was induced by the injection of *XMLP*. Overexpression of mutant *XMLPs* indicated that this phenotype is correlated with its putative PSD domain and glycine at position 2. The loss-of-function of *XMLP* was studied by injection with a morpholino oligo complementary to *XMLP* mRNA, which revealed the malformations of anterior axis and eye defects. According to extirpation experiments the phenotypes might be correlated with disturbed morphogenetic movements rather than an inhibition of induction process. Overexpression of *XCYP26* resulted in a shift of the expression pattern of *XMLP*, showing the signal stripe of *XMLP* in injected half of the embryo getting diffuse or even disappeared. This observation suggests that retinoic acid plays an important role in the *XMLP* regulation. Taken together, XMLP may participate in pattern formation of the embryonic axis and the central nervous system.

XAT encodes *Xenopus* amidinotransferase which shares a highly conserved sequence with the homologues of human, chick and rat. Characterization of embryonic expression indicates that *XAT* is differentially expressed around the yolk plug including the dorsal blastopore area at early gastrula stages and is extensively expressed in the midline of the neural plate of early neurula stages. Sections reveal that its transcripts are located in the notochord. In the tailbud stage signals are found both in the notochord and the trunk area, whereas faint signals can be found in the cephalic part only.