DOCTORAL DISSERTATION

Screening and characterization of novel genes involved in the embryogenesis of *Xenopus laevis*

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Abstract

Using a strategy of large-scale whole mount *in situ* hybridization, three genes were identified from a cDNA library constructed from endoderm-like tissue induced from activin treated animal caps. One gene, *XODC2*, encodes a paralogue to ubiquitous ODC (Genbank accession number: AF217544); another, *XCL-2*, encodes a tissue-specific m-type Calpain (Genbank accession number: AF212199); while the third one, *XETOR*, encodes a novel member of the ETO/MTG8 oncoprotein family (Genbank accession number: AF212198).

Spatial and temporal expressions of these genes were examined by ways of whole mount *in situ* hybridization and RT-PCR. Functional analyses were focused on *XCL-2* and *XETOR*.

Overexpression of wild-type *XCL-2* suggests that this gene is involved in gastrulation movement and convergent extension during gastrulation and neurulation. Overexpression of a dominant-negative mutant caused a phenotype morphologically similar to, but histologically different from, that caused by overexpression of wild-type *XCL-2*. The mutant phenotype can be rescued by injection of wild-type *XCL-2*. These data suggest that XCL-2 plays an important role in convergent extension movements during embryogenesis in *Xenopus laevis*.

XETOR is expressed during neurula stage in three bilaterally symmetrical stripes at each side of dorsal midline, a pattern similar to that of the genes involved in primary neurogenesis. Indeed, overexpression of *XETOR* or a series of truncated mutants led to the inhibition of primary neuron formation without

disruption of neural plates. Such an inhibitory effect is not mediated by lateral inhibition, but an independent action. Moreover, it was shown that *XETOR* and lateral inhibition antagonizes each other. Further evidence showed that expression of *XETOR* is activated or promoted by overexpressed proneural genes such as *Xngnr-1*, *Xash-3*, *Xath3*, and *XNeuroD*, and conversely, overexpressed *XETOR* inhibits the expression and function of *Xath3* and *XNeuroD*. The neuron inducing activity but not expression of *Xash-3* is inhibited in response to *XETOR* overexpression, while neither the expression nor the function of *Xngnr-1* is inhibited. Thus a negative feedback loop is established between XETOR and proneural genes. Blocking of XETOR function *in vivo* resulted in a neurogenic phenotype of an enlarged neurogenic domain without alteration in neuron density. Nevertheless, double depletion of XETOR and lateral inhibition led to primary neuron formation with an increased density in enlarged domains. Based on these data, it is concluded that during primary neurogenesis, lateral inhibition and XETOR comprise a dual inhibitory mechanism to refine the number and localization of primary neurons via repression of the expression and function of proneural genes.