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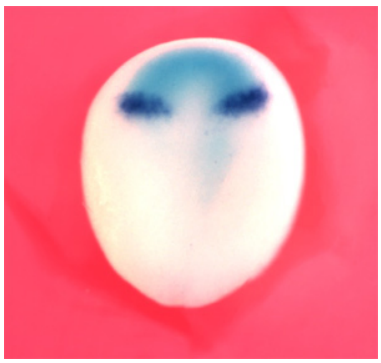
XsalF comes to the fore in brain regionalization

July 13, 2004 – In vertebrates, the nervous system is divided into distinct regions patterned in a head-to-tail direction. Embryologists have long been interested in working out the means by which this regionalization is achieved, and for years the dominant theory has involved a two-step mechanism in which neural tissues are first induced lengthwise down the entire body axis, followed by a transformation step where a second signal specifies the posterior identities of the neural tissue, which can be induced by a number of factors including members of the Wnt and FGF gene families, and retinoic acid.

While there is much evidence to support this model, a number of recent studies have indicated that the specification of the forebrain (an anterior structure, presumed to be induced by the initial signal in the activation-transformation model) requires additional regulatory inputs as well. New work by scientists in the Laboratory for Organogenesis and Neurogenesis (Group Director, Yoshiki Sasai) at the RIKEN Center for Developmental Biology (CDB; Kobe, Japan) showing anterior neural specifying activity in the African clawed frog, *Xenopus laevis*, lends weight to the revisionist argument.



Xenopus embryo double-stained for *XsalF* and mid- and hindbrain marker gene, *Pax2*, showing overlap in the posterior region of XsalF expression.

In an article published in the July 13 issue of *Developmental Cell*, Takayuki Onai et al. report that *XsalF*, the *Xenopus* homolog of *spalt*, a homeotic gene known to function in anterior-posterior segment identity in *Drosophila*, regulates the expression of forebrain and midbrain-specific genes. A series of experiments in which *XsalF* was misexpressed, deleted and its function blocked, showed direct linkage between *XsalF* expression and forebrain/midbrain identity.

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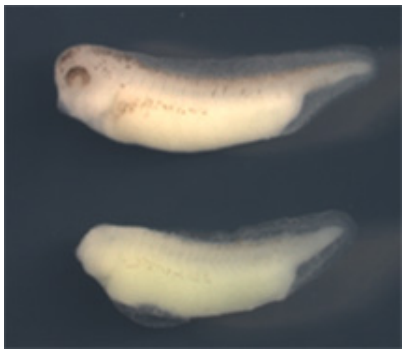
XsalF was originally identified in a screen of the frog anterior neural plate, a structure that appears early in neural development. Sequencing of the gene, and analysis of the timing and spatial pattern of its expression showed that it codes a transcription factor related to Spalt and is expressed in the incipient forebrain and midbrain at precisely those developmental stages when brain regions are specified. When members of the Sasai lab overexpressed the gene by injecting its mRNA into early embryos, they found it caused the expanded expression of genes specific to anterior brain regions while suppressing the expression of more posterior markers.



Interference with *XsalF* gene function results in incomplete head development in the *Xenopus* embryo (bottom); normal embryo shown at top

Onai et al. next went on to test the effects of the loss of *XsalF* function. Disabling the gene by the deletion of functional domains resulted in embryos with significant reductions in anterior neural structures. Conditional loss-of-function experiments showed that the gene's expression was required in the early and mid neurula stages, when the developing brain undergoes regionalization. They then confirmed the specificity of this requirement for *XsalF* by injecting morpholinos (short nucleotide chains that block the function of a targeted gene), which gave similar results to the earlier loss-of-function studies.

These preliminary findings prompted the lab to look into the molecular mechanisms behind *XsalF*'s anterior specifying activity, which they suspected was linked to the inhibition of the Wnt cascade (a signaling pathway that posteriorizes neural tissues). They focused on two factors, GSK3β and Tcf3, known to antagonize Wnt signaling in certain contexts, and found that the expression of both factors was dependent on *XsalF*. Gain- and loss-of-function studies reconfirmed the connection between XsalF and these Wnt antagonists, showing that XsalF alters the receptivity of anterior neural cells to Wnt signaling by regulating the expression of *GSK3β* and *Tcf3*, making these cells resistant to the posteriorizing effects of Wnt.

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The signaling networks at work in this competitive regional determination appear to be intricate and involved in two linked, but distinct, aspects of regions-specific transcriptional regulation – the switching on of fore- and midbrain-specific genes, and the suppression of posterior genes. The Sasai lab now plans to conduct further studies to develop a detailed model for understanding both the direct and mediated effects of *XsalF* in neural patterning.

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