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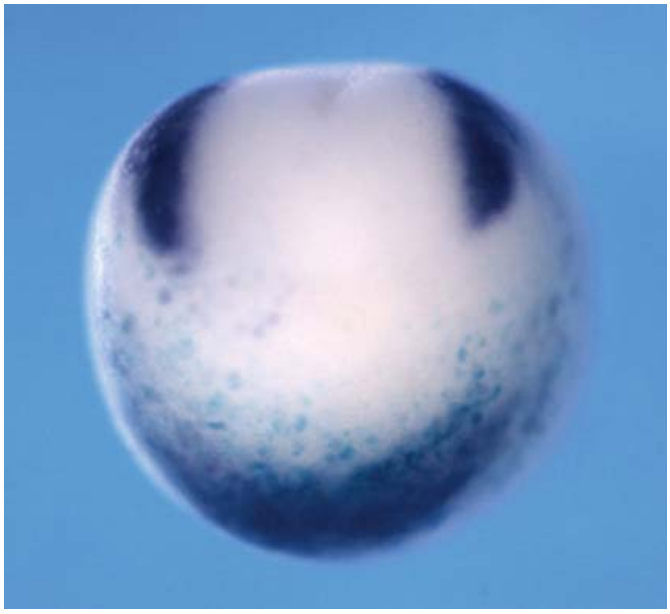
Crest commencement: *Pax3* and *Zic1* co-activate neural crest differentiation

April 19, 2005 – Early in vertebrate development, the foundations of the nervous system are laid down in specific regions of the embryonic body. A sheet of epithelial tissue rolls into a cylinder, forming the neural tube, the structure that will give rise to the central nervous system. A migratory population of cells called the neural crest develops slightly later, before spreading throughout the body to create the peripheral and autonomic nervous systems, as well as a range of other tissues including facial cartilage and bone, the pigmented cells called melanocytes, and the adrenal medulla. Despite the importance of the neural crest, however, the molecular signals that function upstream in the multistep process of the specification and demarcation of its developmental field have remained a mystery.



Ectopic ventral expression (bottom) of neural crest marker *Foxd3* in stage 15 *Xenopus* embryo co-injected with *Pax3* and *Zic1* (anterior view)

A host of regulatory molecules, including members of the BMP and Wnt signaling families, have been implicated in this determination process, and a pair of transcriptional factors, *Foxd3* and *Slug*, has been identified as definitive markers of the presumptive neural crest, but the factors that define its exact boundaries have stayed out of reach. Now, in a study published in the online edition of the journal *Development*, Yoshiki Sasai (Group Director, Laboratory for Organogenesis and Neurogenesis) and colleagues in the RIKEN CDB (Kobe, Japan) report the identification of a pair of overlapping regulatory signals that seem to initiate the neural crest developmental program in the African clawed frog, *Xenopus laevis*.

Earlier studies in the same laboratory had suggested a role for *Zic*-family factors, and they focused on *Zic1*, which is expressed in the dorsal ectodermal region of the gastrulating embryo, the site of prospective neural development. A second molecule, *Pax3*, shows a similar but distinct pattern of expression in about the same region and embryonic stages, leading the Sasai group to narrow their search to these candidates. Preliminary tests showed that an increase in BMP signaling, a potent neural inhibitor, suppressed the expression of both, while the suppression of BMP caused an

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expansion of their range toward the ventral side of the embryo. Conversely, the soluble factor Wnt caused *Pax3* and the presumptive neural crest marker, *Foxd3*, to be expanded beyond their normal anterior limits.

They next looked at the effects of gain of *Pax3* and *Zic1* function in the developing frog, and found that both were able to trigger neural crest differentiation, as evidenced by the expression of *Foxd3* and *Slug* prior to the late gastrula stage, when those markers normally first appear, as well as in the typically non-neural ventral region. When misexpressed singly, both *Pax3* and *Zic1* showed the ability to trigger an ectopic expansion of *Foxd3* and *Slug* in the dorsal region, but that effect did not extend to the ventral side. On direct injection of both *Pax3* and *Zic1* into the ventral side of animal blastomeres from very early embryos they found that the factors in combination could indeed induce neural crest markers even in the ventral side, indicating the potency and directness of their effect.

Sasai et al followed up by studying how a loss of these molecules' function might affect the neural crest in otherwise normal embryos by injecting morpholino (MO) antisense oligonucleotides (a method of inhibiting the function of specific genes by interfering with the translation of the proteins they encode). The injection of either *Pax3* or *Zic1* MOs was sufficient to suppress the expression of the marker *Foxd3*, while the loss of function of either of the two factors had no discernible effects on the expression of the other, suggesting that both must be active to achieve normal determination of the neural crest.

Animal cap assays, which provide an in vitro model of many aspects of early *Xenopus* development, helped to clarify the details of the molecular interactions at work. Finding that *Pax3* alone failed to induce *Foxd3*, as it had in vivo, they began to search for the missing signals needed to achieve that effect. When they co-injected *Wnt3a* (a known factor in neural crest differentiation), they found not only that *Pax3* now strongly induced *Foxd3*, but also that *Zic1* began to be expressed. Injection of *Zic1* alone into untreated animal caps was able to induce *Foxd3*, but only weakly, an effect that was strongly complemented by co-injection with *Wnt3a*. Interestingly, the inductive action of these factors acting alone could be blocked by increasing the activity of the neural inhibitor, BMP4, but the combination of *Pax3*, *Zic1* and *Wnt3a* proved able to induce *Foxd3* robustly even in the face of an antagonistic BMP signal.

By interfering with gene function in dissociated cells, the group tested whether this co-activity between *Pax3* and *Zic1* in Wnt treated cells relied on external signals. Morpholino blockade of Zic1 in *Pax3*-injected and Wnt-treated single cells resulted in the loss of *Foxd3* induction, while cells exposed to all three signals continued to express Foxd3, indicating that the *Pax3*, *Zic1* and *Wnt3a* effect is cell-autonomous. The critical role of the endogenous Wnt cascade was shown by the loss of *Foxd3* induction when Wnt signaling was disrupted by morpholino knockdown of β–catenin, a Wnt downstream factor.

This comprehensive and compelling set of evidence points strongly to a modus of neural crest differentiation involving the close cooperation between *Pax3* and *Zic1* in the presence of Wnt signaling in the pre-neural embryo. That this trio of signals operates even in the presence of inhibitory BMP signal suggests that the combination is a powerful determinant of the prospective neural crest, and the question of exactly how the Pax3-Zic1 partnership overrides BMP on the molecular level represents an intriguing question for further study.