RIKEN Center for Developmental Biology (CDB)
2-2-3 Minatojima-minamimachi, Chuo-ku, Kobe 650-0047, Japan

Wnt Amp: XTsh3 enhances Wnt signaling in *Xenopus* axis determination

April 25, 2007 – The formation of the dorsal-ventral axis is one of the most important developmental milestones in early embryogenesis. The establishment of this axis distinguishing the back from the belly side is known to rely on the unequal distribution and subsequently the asymmetric action of the canonical Wnt pathway, which is known to be triggered as early as sperm entry. But the initial differences in Wnt intensity along the D-V axis, at only about two-fold, appear to be too slight to account for the dramatic polarization of the embryo that they induce, suggesting that other factors may act to boost the Wnt signal.

An important piece has been fitted into this puzzle in a new study by Takayuki Onai and Mami Matsuo-Takasaki and colleagues in the Laboratory for Organogenesis and Neurogenesis (Yoshiki Sasai; Group Director) that shows how the factor XTsh3 functions as a molecular adjuvant, amplifying the effects of canonical Wnt signaling in DV axis determination in the embryo of the African clawed frog, *Xenopus laevis*, by increasing the nuclear accumulation of the transcriptional activator, β-catenin.

Injection of antisense morpholinos against XTsh3 interfere with normal axis formation (right)

The XTsh3 protein belongs to a family of zinc-finger gene products whose founding member, Teashirt (Tsh), was first identified in the fruit fly *Drosophila* as a homeotic selector specifying thoracic segment identity, and was subsequently shown to bind to Armadillo, the fly homolog of the Wnt downstream factor β-catenin. While the frog genome includes at least three members of the Tsh family, their patterns of expression vary, and it was the distinct strong expression of one of these, *XTsh3*, in the dorsal aspect of the early embryo that caught the Sasai group's attention and prompted their search for a possible role in D-V axis determination. In situ hybridization showed XTsh3 transcripts throughout the animal region of cleavage-stage embryos, and that by early gastrulation, its expression was concentrated strongly in the dorsal ectoderm and marginal zone even as it gradually faded in the ventral half of the embryo.

Studying the effect of its loss of function, Onai and Matsuo-Takasaki next interfered with the dorsally-expressed XTsh3 using antisense morpholinos against its mRNA sequence, and found that this resulted in the ventralization of normally dorsal tissues. Looking at the genetic level as well, they found that signature dorsal genes such as *Chordin* and *Goosecoid* were downregulated in the morphant, while the expression of ventral markers encroached into dorsal territory, suggesting that XTsh3 plays an important part in setting up the DV axis by supporting dorsal specification.

The mechanism by which it does this, however, remained unclear. Past work in *Drosophila* had showed possible involvement of Tsh in canonical Wnt signaling through its binding with β-catenin, prompting the group to explore the possibility of a similar scenario in the very different context of amphibian development. They discovered that the activity of Wnt-mediated genes was upregulated on the forced expression of XTsh3, and that this effect was reliant on the β-catenin step of the Wnt pathway, indicating that the two might work together as co-factors.

Contact: Douglas Sipp sipp@cdb.riken.jp TEL: +81-78-306-3043
RIKEN CDB, Office for Science Communications and International Affairs

On entering the nucleus, β-catenin binds with factors such as Tcf and activates the transcription of target genes. Interestingly, however, the β-catenin protein does not exhibit any of the typical localization sequences that serve as entry passes into the nucleus, which led Onai and Matsuo-Takasaki to ask whether this might be XTsh3's role. Using an animal-cap assay as an in vitro model of the early embryo, they determined that XTsh3 accumulates predominantly in the nucleus, and that this preferential localization was unaffected by either overexpressing or knocking down Wnt pathway proteins. Significantly, the converse was not true—injection of XTsh3 dramatically increased the accumulation of β-catenin in the nucleus, while loss of XTsh3 function had the opposite effect.

This set of findings, which were reported in *The EMBO Journal*, tells the story of how dorsally expressed XTsh3 enhances canonical Wnt signaling by promoting the entry of β-catenin into the nucleus, thus playing a decisive role in the determination of the D-V axis. The work by Onai and Matsuo-Takasaki et al is also the first report of a Tsh-family protein's function in a vertebrate model, adding a new dimension to the body of knowledge that has been built up using *Drosophila* genetics. "We still don't know how XTsh3 is causing β-catenin to collect in the nucleus," admits Sasai, "It could be protecting it from degradation, or promoting its nuclear transport. Its expression pattern also suggest it may be working in the caudal specification of the central nervous system, so we'll be very interested to find out if its enhancement of Wnt signaling is involved in other developmental processes as well."

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