Current Biology, Vol. 12, R657–R659, October 1, 2002, ©2002 Elsevier Science Ltd. All rights reserved. PII S0960-9822(02)01160-0

Wnt Signalling: Refocusing on Strabismus

Dispatch

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Vertebrate homologues of the Strabismus/van Gogh (stbm/vang) gene have been implicated in patterning and morphogenesis during gastrulation. Recent work shows that stbm/vang is mutated in zebrafish trilobite mutants and that stbm/vang is required for morphogenesis but not patterning during zebrafish gastrulation.

There is increasing evidence that vertebrate homologues of genes involved in the establishment of epithelial planar cell polarity (PCP) in *Drosophila* regulate gastrulation movements in vertebrates (reviewed in [1]). One of these genes, *stbm/vang*, encodes a unique transmembrane protein. *stbm/vang* is implicated in patterning and morphogenesis during zebrafish and *Xenopus* gastrulation by modulating canonical and non-canonical Wnt signalling pathways [2–4]. A recent study shows that the zebrafish *trilobite* (*tri*) locus encodes *stbm/vang,* providing new insight into the function of Stbm/Vang in regulating cellular re-arrangements within the gastrula and the developing hindbrain [5].

In vertebrate development, gastrulation is the first major morphogenetic process leading to the formation of the three germ layers, ectoderm, endoderm and mesoderm. One of the main cellular rearrangements underlying morphogenesis during gastrulation is convergent extension. The cellular basis of this process has been well studied in amphibians and teleosts (reviewed in [6]). Cells undergoing convergent extension move to the dorsal side of the gastrula (convergence) where they redistribute along the emerging anterior–posterior embryonic axis (extension). Convergent extension movements are driven by medio-lateral cell intercalations, accompanied by the elongation of cells along the medio-lateral axis [7,8].

The molecular mechanism that controls these gastrulation movements in vertebrates has only just begun to unravel. Various components of the Wnt signalling cascade that determine PCP in *Drosophila* epithelia also appear to regulate gastrulation movements in vertebrates [1]. Such genes include those encoding the Wnt receptor *frizzled* (*fz*), the intracellular signal transducer *dishevelled* (*dsh*), the small GTPases *rhoA* and *cdc42,* and the RhoA effector kinase *rho kinase 2* (*rok2*) (reviewed in [1]). Other genes that are part of the Wnt/PCP pathway in vertebrates but not yet implicated in PCP in *Drosophila* are *slb/wnt11* and *knypek/glypican4/6* [9,10].

Jessen *et al.* [5] used positional cloning to show that *tri* mutations disrupt the zebrafish *stbm/vang* gene. The

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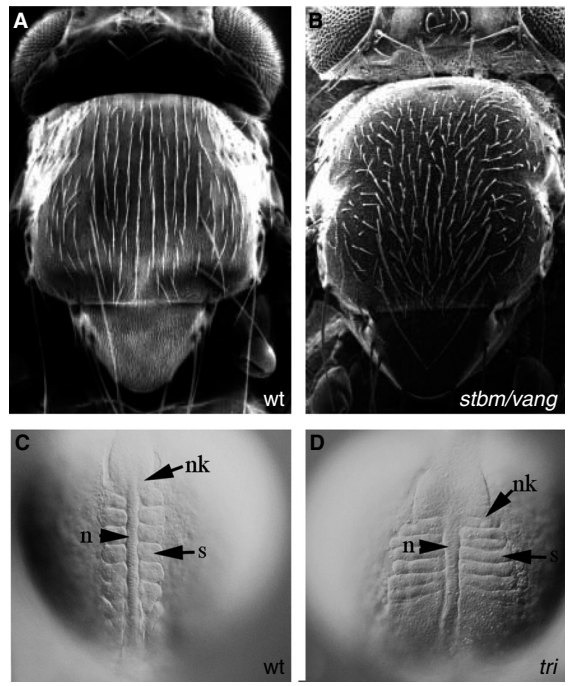

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Figure 1. Phenotypes of *Drosophila stbm/vang* and zebrafish *tri* (*stbm/vang*) mutants.

(A,B) In wild-type (wt) flies (A), the bristles and hair of the thorax are uniformly oriented in space while in *stbm/vang* mutants (B), the polarity of these structures is severely disrupted. Pictures taken from [12]. (C,D) In zebrafish wild-type embryos (C), the notochord (n) and somites (s) are narrow and elongated while in *tri* (*stbm/vang*) mutant embryos (D), both notochord and somites are much broader and less elongated indicative of reduced convergent extension movements. nk, neural keel. Pictures taken from [20].

Drosophila stbm/vang gene encodes a novel protein with four transmembrane domains and a PDZ binding motif at the carboxyl terminus, which is required to establish polarity in the eye, legs, bristles and wing [11,12] (Figure 1A,B). Recent studies in zebrafish and *Xenopus* [2–4] have shown that *stbm/vang* is also required for convergent extension movements and cell fate specification during gastrulation. *stbm/vang* functions in these processes by modulating both the Wnt/PCP pathway and the canonical Wnt signalling pathway as shown by its ability to increase c-Jun phosphorylation and to repress β-catenin dependent transcription, respectively.

The *tri* locus was originally identified in two large-scale mutagenesis screens as a mutant having defective cell movements during gastrulation [13,14] (Figure 1C,D). Jessen *et al.* [5] showed that in *tri* mutant embryos, convergent extension movements are reduced while the specification of cell fates is unaffected. These observations contradict the conclusions of a previous study by Park and Moon [2],

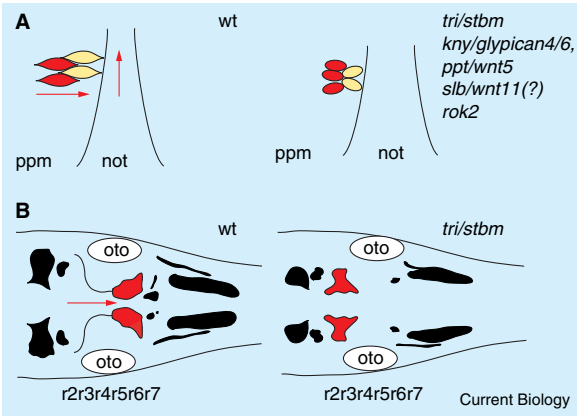

Figure 2. Schematic drawings of the cellular rearrangements underlying convergent extension movements during gastrulation and migration of branchiomotor neurons in the developing hindbrain.

(A) Wild-type (wt) cells in the posterior paraxial mesendoderm (ppm) undergo medio-lateral cell intercalations (red arrows) and exhibit a medio-lateral oriented and elongated cell morphology. In *tri* (*kny*, *ppt*) mutant embryos, the same cells exhibit reduced medio-lateral intercalations and are less oriented and elongated along the medio-lateral axis. (B) In the hindbrain of wt embryos, branchiomotor neurons migrate posteriorly from rhombomere 2 into rhombomere 5/6 (red arrow) while no such migration is observed in *tri* (*stbm/vang*) mutant embryos. r, rhombomere; oto, otic placode; not, notochord.

which claimed that zebrafish *stbm/vang* is also needed for anterior–posterior patterning of the gastrula by interfering with canonical Wnt signalling.

It is likely that these conflicting views are the results of different interpretations of the expression patterns of various marker genes within the developing neural plate. As both reduced convergent extension movements and alterations in the patterning of the neural axis can drastically change the expression domains of these marker genes, it is necessary to distinguish carefully between alterations in cell fate specification versus secondary effects due to morphogenetic changes. In the case of *stbm/vang*, the overwhelming experimental evidence [3–5] points to a role for *stbm/vang* in morphogenesis of the neural plate rather than in cell fate specification.

By analysing the *tri* mutant phenotype, Jessen *et al.* [5] further showed that *stbm/vang* is required for medio-lateral cell elongation and intercalations, which underlie effective convergent extension movements during gastrulation (Figure 2A). These findings support the assumption that the Frizzled signalling pathway, which establishes PCP in *Drosophila,* shares significant similarities with the Wnt signalling pathway, which regulates gastrulation movements in vertebrates. However, it is still unclear whether components of the Wnt/PCP signalling cascade can influence cell polarity in vertebrates.

In *Drosophila* wing discs, tissue polarity is manifested by a cortical polarization of epithelial cells along the proximal–distal axis, resulting in the polarized outgrowth of a single wing hair close to the distal edge of the cell (reviewed in [15]). Jessen *et al.* [5] showed that *stbm/vang* is needed for both the alignment of ectodermal cells along the medio-lateral axis and the directional movements of these cells towards the midline. Although this strongly implies that *stbm/vang* determines the polarity of ectodermal cells, it does not directly show that these cells have a polarity along the medio-lateral axis (medial versus lateral) nor that *stbm/vang* is involved in the establishment of such a polarity.

In *Drosophila,* the proximal–distal polarity of epithelial cells in the wing disc depends on the polarized distribution of components in the Fz/PCP pathway, such as Stbm/Vang, to the proximal and/or distal edges of these cells (reviewed in [15]). During vertebrate gastrulation, Stbm/Vang, together with other components of the Wnt/PCP pathway, localizes to the membrane of cells undergoing convergent extension movements [2]. However, no polarized distribution of Stbm/Vang, or of any of the other Wnt/PCP components, has yet been seen in these cells. Therefore, it might be possible that the Wnt/PCP pathway in vertebrates, unlike the Fz/PCP pathway in *Drosophila,* influences cellular morphologies without necessarily imposing cellular polarity. Alternatively, the Wnt/PCP pathway might function in a permissive way together with another instructive signal to establish cell polarity during gastrulation.

What are the signalling pathways through which *stbm/vang* acts in vertebrate development? In *Drosophila*, *stbm/vang* interacts with components of the Fz/PCP signalling cascade, but is not a simple linear component of this pathway [16]. Similar to the situation in *Drosophila*, Jessen *et al.* [5] showed that the *tri* gastrulation phenotype is modulated by components of the Wnt/PCP pathway, but that *stbm/vang* does not act directly upstream or downstream of any of these genes.

Interestingly, a later function of *stbm/vang* in regulating neuronal migration in the developing hindbrain [17] (Figure 2B) appears to be independent of the Wnt/PCP pathway suggesting that *stbm/vang* signals differently in the hindbrain as compared to the gastrula. Jessen *et al.* [5] attributed this difference to specific roles of *stbm/vang* during gastrulation and within the hindbrain. In the hindbrain, *stbm/vang* appears to influence migration of branchiomotor neurons without affecting cellular elongation, while *stbm/vang* is required during gastrulation for medio-lateral cell intercalation and elongation [5,17]. Evidence for a Wnt/Fz independent function of tissue polarity genes also comes from studies in *Drosophila* showing that the protocadherin *flamingo* is required for dendritic field formation independently of the Fz signalling cascade [18].

Important questions arise about the function of *stbm/vang* in vertebrate development. First, the mechanisms by which *stbm/vang* influences cellular morphologies and movements are still unclear. Can *stbm/vang* truly function independently of the Wnt/PCP pathway as suggested by the analysis of the hindbrain phenotype in *tri* mutant embryos? What are the downstream effectors and targets of *stbm/vang* function? In *Drosophila* for example, the tissue polarity gene *prickle* has been shown to act both as an agonist and antagonist of *stbm/vang* function during the establishment of PCP in the wing and eyes [11,19]. Finally, it will be

important to determine how seemingly unlocalized molecules, such as Stbm/Vang and other PCP components, can induce polarized changes in cell morphologies and migration during vertebrate development.

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