Int. J. Dev. Biol. 45: 225-227 (2001)

Regulation of convergent extension in *Xenopus* by Wnt5a and Frizzled-8 is independent of the canonical Wnt pathway

JOHN B. WALLINGFORD, KEVIN M. VOGELI and RICHARD M. HARLAND*

Department of Molecular and Cell Biology. University of California, Berkeley, U.S.A.

ABSTRACT The Wnt signaling pathway is increasingly recognized as a highly branched signaling network. Experimental uncoupling of the different branches of this pathway has proven difficult, as many single components are shared downstream by multiple, distinct pathways. In this report, we demonstrate that the upstream Wnt antagonists Xwnt5a and Nxfz-8, which inhibit normal morphogenetic movements during *Xenopus* gastrulation, act independently of the canonical Wnt signaling pathway. This finding is important, as it highlights the promiscuity of upstream Wnt signaling components and further establishes an important role for non-canonical Wnt signaling in *Xenopus* morphogenesis.

KEY WORDS: *Gastrulation, Convergent Extension, Wnt, Frizzled, Xenopus.*

Molecules of the Wnt signal transduction cascade are critical to establishing proper cell fate and cell polarity during embryogenesis, and the complexity of Wnt signaling mechanisms continues to increase. One pathway -the canonical Wnt pathway- leads from an extracellular Wnt, through a Frizzled receptor and Dishevelled (Dsh), to stabilization of β-catenin, regulation of transcription, and changes in cell fate (Fig. 1). Another pathway diverges at Dsh and signals via small GTPases and the jun N-terminal kinase to regulate cell polarity and cell shape (Peifer and Polakis, 2000). Yet another pathway has been identified in vertebrates in which certain Wnts and Frizzleds activate G-proteins and PKC and elicit calcium release (Slusarski *et al.*, 1997). Evidence for still other non-canonical Wnt signals has also been found in *C. elegans* (Schlesinger *et al.*, 1999) and in the chick limb bud (Kengaku *et al.*, 1998).

At least two different Wnt pathways are at work in the early *Xenopus* embryo. *Xenopus* dishevelled (Xdsh) signals via the canonical pathway to specify dorsal structures during early development (Miller *et al.*, 1999), while non-canonical Wnt signaling is required later to establish normal polarity in cells undergoing convergent extension during gastrulation (Wallingford *et al.*, 2000; Tada and Smith, 2000). Nonetheless, just how upstream mediators of Wnt signaling communicate with Xdsh and other downstream players during *Xenopus* gastrulation remains unresolved. For example, *Xenopus* Frizzled-7 appears to play a role in convergent extension, but can signal via both canonical and non-canonical Wnt pathways (Medina *et al.*, 2000). It is therefore important to understand the mode of action by which upstream Wnt signaling components affect convergent extension.

Recently, molecules such as *Xenopus* Wnt5a (Xwnt-5a), as well as experimentally engineered constructs such as Nxfz-8 (a mutant of *Xenopus* Frizzled-8) have been shown to inhibit convergent extension when expressed in *Xenopus* dorsal mesoderm. Interestingly, these molecules have also been identified as antagonists of Wnt signaling based on their ability to inhibit the formation of secondary axes in response to ectopic Wnt expression in *Xenopus* embryos (Deardorff *et al.*, 1998; Du *et al.*, 1995; Torres *et al.*, 1996). These data could be explained in two different ways. First, it is possible that canonical Wnt signaling is involved in both dorsoventral axis induction and control of convergent extension. On the other hand, it is also possible that these upstream Wnt antagonists are capable of repressing both canonical and non-canonical signaling.

To distinguish between these two possibilities, we examined convergent extension in open-face "Keller" explants (Fig. 2 A,B; Keller *et al.*, 1992) from embryos expressing a variety of Wnt signal transduction components. Expression of either Nxfz-8 or Xwnt5a in Keller explants severely inhibited convergent extension (Fig. 2 C,D), consistent with previous results (Deardorff *et al.*, 1998; Du *et al.*, 1995; Moon *et al.*, 1993). The effect of Nxfz-8 on convergent extension was rescued by co-expression of Xdsh (Fig. 2E), a downstream component shared by both canonical and non-canonical Wnt signaling pathways (Fig. 1). If Nxfz-8 and Xwnt5a inhibit convergent extension by disrupting canonical Wnt signaling, then downstream activation specifically of the canonical pathway should

Abbreviations used in this paper: Dsh, dishevelled; Fz, frizzled; Nxfz, a mutant of *Xenopus* Frizzled-8; PKC, protein kinase C.

***Address correspondence to:** Department of Molecular and Cell Biology. 401 Barker Hall, University of California, Berkeley, CA. 94720. FAX: +1-510-643-6334. e-mail: harland@socrates.berkeley.edu

0214-6282/2001/$25.00

Printed in Spain
www.ijdb.ehu.es

also rescue the effect. Dominant-negative (DN)-GSK3 is a potent activator of canonical Wnt signaling (Dominguez *et al.*, 1995; He *et al.*, 1995; Pierce and Kimelman, 1995), however co-expression of DN-GSK3 with Xwnt5a or with Nxfz-8 failed to rescue convergent extension (Fig. 2 F,G). This result suggests that Xwnt5a and Nxfz-8 do not inhibit morphogenesis by down-regulating a canonical Wnt signal.

In *Drosophila*, overexpression of either wild-type Dsh or wild-type Frizzled inhibits the non-canonical Wnt signaling that establishes planar polarity, but does not perturb the canonically transduced wingless pathway (Axelrod *et al.*, 1998; Krasnow and Adler, 1994). Similar effects were observed with Xdsh in the dorsal mesoderm of *Xenopus* (Wallingford *et al.*, 2000). Consistent with a role for Xfz-8 in non-canonical Wnt signaling, overexpression of wild-type Xfz-8 in the dorsal mesoderm strongly inhibited convergent extension (Fig. 2H). Furthermore, dorsal expression of wild-type Xfz-8 elicited a whole-embryo morphology very similar to that elicited by Nxfz-8 (Fig. 3 A-C). These results are similar to those obtained with wild-type Xfz-7 (Medina *et al.*, 2000), suggesting that, *in vivo*, both Xfz-8 and Xfz-7 control convergent extension via non-canonical Wnt pathways.

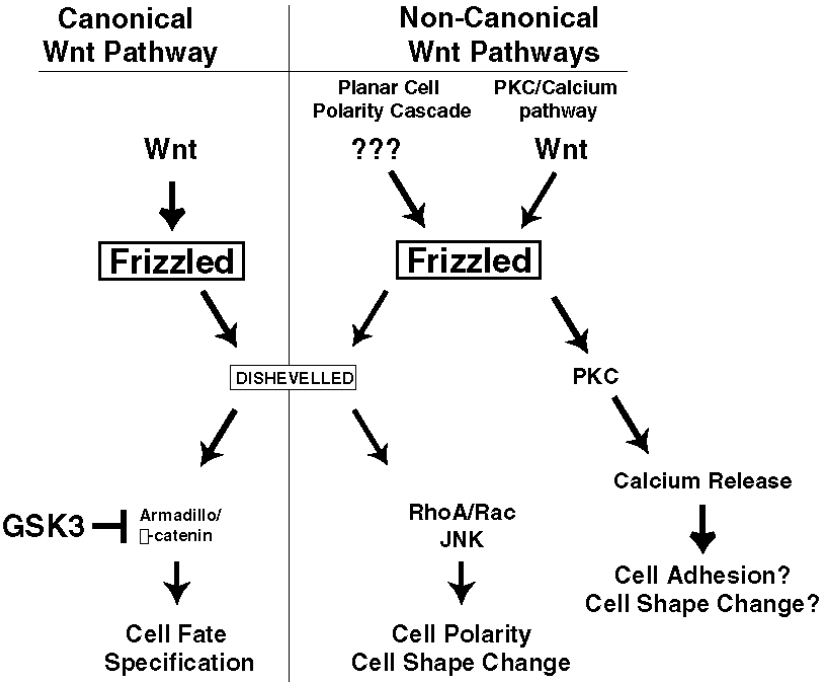

Fig. 1. Canonical and non-canonical Wnt pathways. *For review, see Peifer and Polakis, 2000.*

Gastrula-stage canonical Wnt signals can ventralize cell fates (Christian *et al.*, 1991; Smith and Harland, 1991), and ventral mesoderm converges and extends only very weakly (Keller and Danilchik, 1988). To control for the possibility that the Frizzled overexpression phenotypes in *Xenopus* were due to gastrula-stage hyper-activation of the canonical Wnt pathway, we activated the canonical wnt pathway by expression of DN-GSK3 alone and assessed the effect on convergent extension. Expression of DN-GSK3 did not inhibit convergent extension of Keller explants (Fig. 2I), indicating that overstimulation of the canonical Wnt pathway does not inhibit convergent extension.

In this report we demonstrate that the inhibition of convergent extension by the Wnt antagonists Xwnt5a and Nxfz-8 in the dorsal mesoderm of *Xenopus* is independent of canonical Wnt signaling. Because these molecules are also potent inhibitors of canonical Wnt signaling (Deardorff *et al.*, 1998; Torres *et al.*, 1996), these data demonstrate that such antagonists can effectively disrupt signaling via both canonical and non-canonical downstream Wnt pathways. This point is important in that it highlights the promiscuity of these signaling components in commonly used assays. As such, when using these reagents to investigate the mechanisms of normal development in the *Xenopus* embryo, it will be important to acknowledge that the downstream events being affected may involve

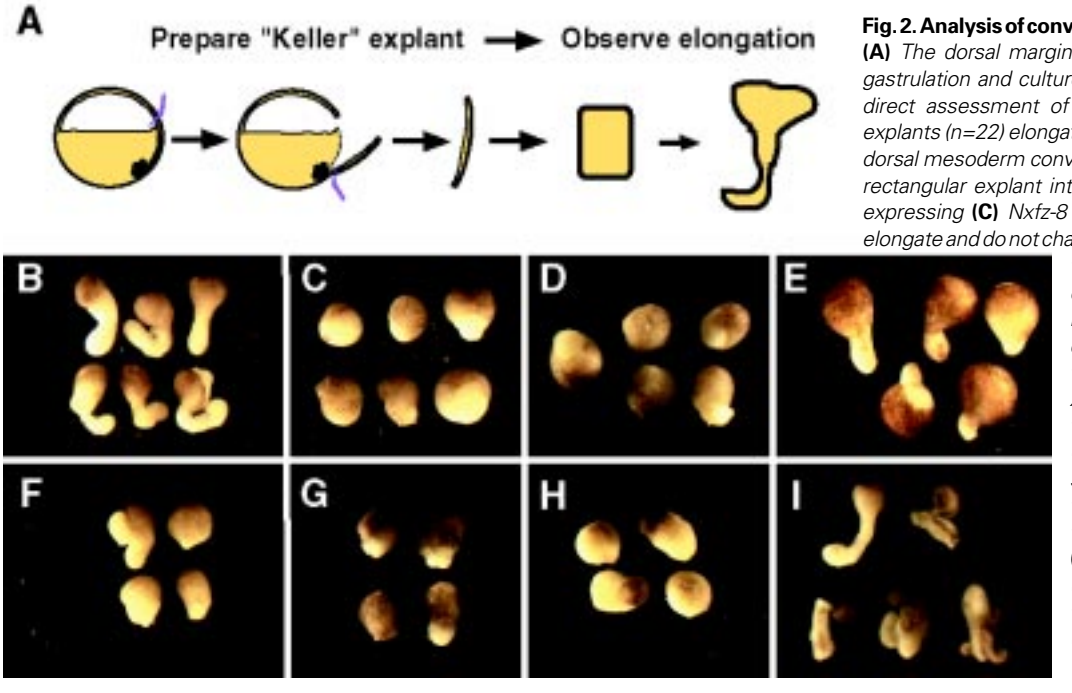

Fig. 2. Analysis of convergent extension in Keller explants. **(A)** *The dorsal marginal zone is removed at the onset of gastrulation and cultured until late neurula stages allowing direct assessment of convergent extension.* **(B)** *Control explants (n=22) elongate significantly and change shape; the dorsal mesoderm converges and extends, transforming the rectangular explant into a head and a tail region. Explants expressing* **(C)** *Nxfz-8 (n=17) or* **(D)** *Xwnt5a (n=26) fail to elongate and do not change shape.* **(E)** *Co-expression of Xdsh with Nxfz-8 (n=7) rescues convergent extension, and all explants extend a mesodermal tail and elongate to some degree. Co-expression of DN-GSK with* **(F)** *Nxfz-8 (n=11) or with* **(G)** *Xwnt5a (n=6) does not rescue convergent extension. The dose of DN-GSK used was sufficient to induce secondary axes when injected ventrally (not shown), indicating activation of the canonical Wnt pathway.* **(H)** *Overexpression of wild-type Xfz-8 (n=16) inhibits convergent extension, while* **(I)** *overexpression of DN-GSK3 (n=29) alone does not.*

non-canonical as well as more traditional Wnt signal transduction pathways.

For example, Xwnt5a expression increases intracellular calcium concentrations (Slusarski *et al.*, 1997), so our results raise the intriguing possibility that calcium signaling may play a role in coordinating convergent extension, perhaps by modulating another non-canonical Wnt signaling cascade. Indeed, both Wnt11 and Dsh regulate convergent extension via a non-canonical Wnt cascade similar to that which controls planar polarity in *Drosophila* (Heisenberg *et al.*, 2000; Tada and Smith, 2000; Wallingford *et al.*, 2000), and Xdsh has been demonstrated to control cell polarity in the dorsal mesoderm (Wallingford *et al.*, 2000). The present study implicates both Xwnt5a and Xfz-8 as additional, important mediators of these events.

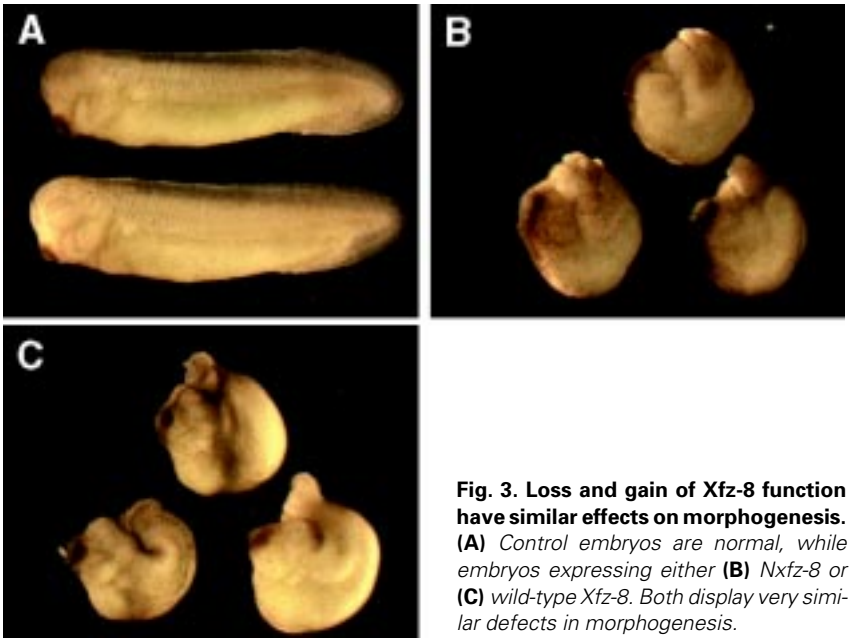

Fig. 3. Loss and gain of Xfz-8 function have similar effects on morphogenesis. **(A)** *Control embryos are normal, while embryos expressing either* **(B)** *Nxfz-8 or* **(C)** *wild-type Xfz-8. Both display very similar defects in morphogenesis.*

Materials and Methods

Xenopus embryos were injected into the two dorsal blastomeres of the 4-cell embryo with 1 ng *in vitro* synthesized mRNA. Embryos were co-injected with 100 pg of GFP mRNA as a tracer. Embryos were reared to stage 10.5 and Keller explants removed as described (Wallingford *et al.*, 2000) and cultured to stage 20 in 1X Steinberg's. Only GFP-positive explants were scored. Data shown are representative samples from two or more independent experiments.

Acknowledgments

We thank Peter Klein for Nxfz-8 and Xfz-8 plasmids and Elena Casey and Karen Liu for critical reading of the manuscript. This work was supported by the NIH (GM42341); JBW was supported by the American Cancer Society (PF-99-350-01-DDC). KMV was supported by the Nathan and Violet David Scholars program at UC Berkeley.

References

AXELROD, J.D., MILLER, J.R., SHULMAN, J.M., MOON, R.T., and PERRIMON, N. (1998). Differential recruitment of Dishevelled provides signaling specificity in the planar cell polarity and Wingless signaling pathways. *Genes Dev.* 12: 2610-22.

CHRISTIAN, J.L., MCMAHON, J.A., MCMAHON, A.P., and MOON, R.T. (1991). Xwnt-8, a *Xenopus* Wnt-1/int-1-related gene responsive to mesoderm-inducing growth factors, may play a role in ventral mesodermal patterning during embryogenesis. *Development* 111: 1045-55.

DEARDORFF, M.A., TAN, C., CONRAD, L.J. and KLEIN, P.S. (1998). Frizzled-8 is expressed in the Spemann organizer and plays a role in early morphogenesis. *Development* 125: 2687-700.

DOMINGUEZ, I., ITOH, K. and SOKOL, S.Y. (1995). Role of glycogen synthase kinase 3 beta as a negative regulator of dorsoventral axis formation in *Xenopus* embryos. *Proc. Natl. Acad. Sci USA* 92: 8498-502.

DU, S.J., PURCELL, S.M., CHRISTIAN, J.L., MCGREW, L.L. and MOON, R.T. (1995). Identification of distinct classes and functional domains of Wnts through expression of wild-type and chimeric proteins in *Xenopus* embryos. *Mol. Cell Biol.* 15: 2625-34.

HE, X., SAINT-JEANNET, J.P., WOODGETT, J.R., VARMUS, H.E. and DAWID, I.B. (1995). Glycogen synthase kinase-3 and dorsoventral patterning in *Xenopus* embryos. *Nature* 374: 617-22.

HEISENBERG, C.-P., TADA, M., RAUCH, G.-J., SAUDE, L., CONCHA, M.L., GEISLER, R., STEMPLE, D.L., SMITH, J.C. and WILSON, S.W. (2000). Silberblick/Wnt11 activity mediates convergent extension movements during zebrafish gastrulation. *Nature* 405: 76-81.

KELLER, R. and DANILCHIK, M. (1988). Regional expression, pattern and timing of convergence and extension during gastrulation of *Xenopus* laevis. *Development* 103: 193-209.

KELLER, R., SHIH, J. and DOMINGO, C. (1992). The patterning and functioning of protrusive activity during convergence and extension of the *Xenopus* organiser. *Dev. Suppl.* 81-91.

KENGAKU, M., CAPDEVILA, J., RODRIQUEZ-ESTEBAN, C., DE LA PEÑA, J., JOHNSON, R., IZPISUA BELMONTE, J.C. and TABIN, C.J. (1998). Distinct WNT pathways regulating AER formation and dorsoventral polarity in the chick limb bud. *Science* 280: 1274-1277.

KRASNOW, R.E. and ADLER, P.N. (1994). A single frizzled protein has a dual function in tissue polarity. *Development* 120: 1883-1893.

MEDINA, A., REINTSCH, W. and STEINBEISSER, H. (2000). *Xenopus* frizzled 7 can act in canonical and non-canonical Wnt signaling pathways: implications on early patterning and morphogenesis. *Mech. Dev.* 92: 227-237.

MILLER, J.R., ROWNING, B.A., LARABELL, C.A., YANG-SNYDER, J.A., BATES, R.L. and MOON, R.T. (1999). Establishment of the dorsal-ventral axis in *Xenopus* embryos coincides with the dorsal enrichment of dishevelled that is dependent on cortical rotation. *J. Cell Biol.* 146: 427-37.

MOON, R.T., CAMPBELL, R.M., CHRISTIAN, J.L., MCGREW, L.L., SHIH, J. and FRASER, S. (1993). Xwnt-5A: a maternal Wnt that affects morphogenetic movements after overexpression in embryos of *Xenopus* laevis. *Development* 119: 97-111.

PEIFER, M. and POLAKIS, P. (2000). Wnt signaling in oncogenesis and embryogenesis—a look outside the nucleus. *Science* 287: 1606-9.

PIERCE, S.B. and KIMELMAN, D. (1995). Regulation of Spemann organizer formation by the intracellular kinase Xgsk-3. *Development* 121: 755-65.

SCHLESINGER, A., SHELTON, C.A., MALOOF, J.N., MENEGHINI, M. and BOWERMAN, B. (1999). Wnt pathway components orient a mitotic spindle in the early Caenorhabditis elegans embryo without requiring gene transcription in the responding cell. *Genes Dev.* 13: 2028-38.

SLUSARSKI, D.C., CORCES, V.G. and MOON, R.T. (1997). Interaction of Wnt and a Frizzled homologue triggers G-protein-linked phosphatidylinositol signalling. *Nature* 390: 410-3.

SMITH, W.C. and HARLAND, R.M. (1991). Injected Xwnt-8 RNA acts early in *Xenopus* embryos to promote formation of a vegetal dorsalizing center. *Cell* 67: 753-65.

TADA, M. and SMITH, J.C. (2000). Xwnt11 is a target of *Xenopus* Brachyury: regulation of gastrulation movements via dishevelled, but not through the canonical Wnt pathway. *Development* 127: 2227-2238.

TORRES, M.A., YANG-SNYDER, J.A., PURCELL, S.M., DEMARAIS, A.A., MCGREW, L.L. and MOON, R.T. (1996). Activities of the Wnt-1 class of secreted signaling factors are antagonized by the Wnt-5A class and by a dominant negative cadherin in early *Xenopus* development. *J. Cell Biol.* 133: 1123-37.

WALLINGFORD, J.B., ROWNING, B.A., VOGELI, K.M., ROTHBÄCHER, U., FRASER, S.E. and HARLAND, R.M. (2000). Dishevelled controls cell polarity during *Xenopus* gastrulation. *Nature* 405: 81-85