

# Wnt11 stimulation induces polarized accumulation of Dishevelled at apical adherens junctions through Frizzled7

Hiroaki Yamanaka and Eisuke Nishida\*

Department of Cell and Developmental Biology, Graduate School of Biostudies, Kyoto University, Sakyo-ku, Kyoto 606-8502, Japan

**Dishevelled, an essential mediator of Wnt signaling, is shown to regulate vertebrate gastrulation movements by controlling cell polarity, but how cell polarization is regulated during gastrulation has remained unclear. Here, we show that Dishevelled accumulates in the apical region at cell–cell contacts in involuting mesodermal cells, and that Wnt11 stimulation induces the accumulation of Dishevelled at apical adherens junctions in *Xenopus* ectodermal explants. We also show that the accumulation of Dishevelled is suppressed by the depletion of the Wnt receptor Frizzled7 with a morpholino antisense oligonucleotide, and Frizzled7 itself also accumulates at apical adherens junctions in response to Wnt11. These results indicate that Wnt11 stimulation induces the accumulation of Dishevelled via the accumulation of Frizzled7. Our subsequent analysis shows that the DIX domain of Dishevelled is necessary for its translocation and accumulation in response to Wnt11. Our results suggest that Wnt11-induced polarized accumulation of Frizzled7 and Dishevelled at adherens junctions underlies the formation and maintenance of apicobasal cell polarity.**

## Introduction

The control of cell movements has a crucial role in morphogenesis during embryonic development. Previous studies have shown that non-canonical Wnt signaling pathway regulates the cell movements (Veeman *et al.* 2003; Saburi & McNeill 2005). Dishevelled, an essential component of the Wnt signaling pathways, is also shown to play a role in cell movements by controlling cell polarity during gastrulation (Sokol 1996; Heisenberg *et al.* 2000; Tada & Smith 2000; Wallingford *et al.* 2000). The mechanisms by which Dishevelled mediates non-canonical Wnt signaling and regulates cell polarity, however, have not been fully elucidated. Previous studies suggested the similarities between the non-canonical Wnt signaling pathway, which regulates vertebrate gastrulation, and the planar cell polarity pathway in *Drosophila melanogaster*, in which Dishevelled regulates cell polarity through establishing asymmetric localization of several cytoskeletal and adhesion molecules (Shimada *et al.* 2001; Mlodzik 2002; Bastock *et al.* 2003; Lawrence & Morel 2003). It has remained unclear, however, whether Dishevelled regulates cell polarity during vertebrate gastrulation by

a similar mechanism. In this study, we show that Dishevelled accumulates at the apical region of cell–cell contacts in involuting mesodermal cells, and that Wnt11 stimulation can induce the accumulation of Dishevelled via its DIX domain in *Xenopus* ectodermal explants. In preparation of this manuscript, a paper appeared, which reported that Wnt11 is able to induce accumulation of the Wnt receptor Frizzled7 at cell–cell contacts in zebrafish embryos (Witzel *et al.* 2006). We also show that Frizzled7 itself accumulates at apical adherens junctions in response to Wnt11 and demonstrate that Frizzled7 is necessary for the accumulation of Dishevelled. Thus, our results indicate that Wnt11 induces the polarized accumulation of Dishevelled through Frizzled7, which should contribute to the formation and maintenance of cell polarity during gastrulation movements.

## Results

### ***Xenopus* Dishevelled accumulates at apical adherens junctions in response to Wnt11**

Since it has been reported that Dishevelled translocates to the plasma membrane region from the cytoplasm during vertebrate gastrulation (Wallingford *et al.* 2000), we examined the subcellular localization of Dishevelled in more detail. To this end, we expressed GFP-tagged

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\*Correspondence: E-mail: L50174@sakura.kudpc.kyoto-u.ac.jp

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961

*Xenopus* Dishevelled (GFP-Xdsh) in *Xenopus* embryos. In sections of the embryo at stage 11, GFP-Xdsh was found to accumulate at the apical region of cell–cell contacts in involuting mesodermal cells (Fig. 1A). This polarized localization of Dishevelled has not been reported before. Since Wnt11, a non-canonical Wnt ligand, is expressed in involuting dorsal mesodermal cells and has been shown to be involved in the gastrulation cell movements (Heisenberg *et al.* 2000; Saka *et al.* 2000; Tada & Smith 2000; Ulrich *et al.* 2003), we ectopically expressed Wnt11 with GFP-Xdsh in *Xenopus* ectodermal explants to examine whether the accumulation of GFP-Xdsh was induced by Wnt11. Explants were stained with either anti- $\beta$ -catenin antibody or anti-ZO1 antibody, and sectioned. While GFP-Xdsh localized punctatedly in the cytoplasm in the absence of Wnt11, it was translocated and accumulated at the apical region of cell–cell contacts in the presence of Wnt11 (Fig. 1B,C). The accumulated GFP-Xdsh co-localized with  $\beta$ -catenin (Fig. 1B), but not with ZO1 (Fig. 1C), which is located more apically than  $\beta$ -catenin or GFP-Xdsh. These results indicate that the accumulated Dishevelled localizes at apical adherens junctions. To examine whether the translocation of Xdsh is induced by secreted Wnt11, we attached an explant expressing GFP-Xdsh to an explant expressing Wnt11 (Fig. 1D). GFP-Xdsh translocated to the cell–cell contacts when the attached with the explant expressing Wnt11 (Fig. 1E), indicating that GFP-Xdsh is able to translocate to cell–cell contacts in response to extracellular Wnt11.

### The Wnt receptor Frizzled7 transmits Wnt11 stimulation to Dishevelled

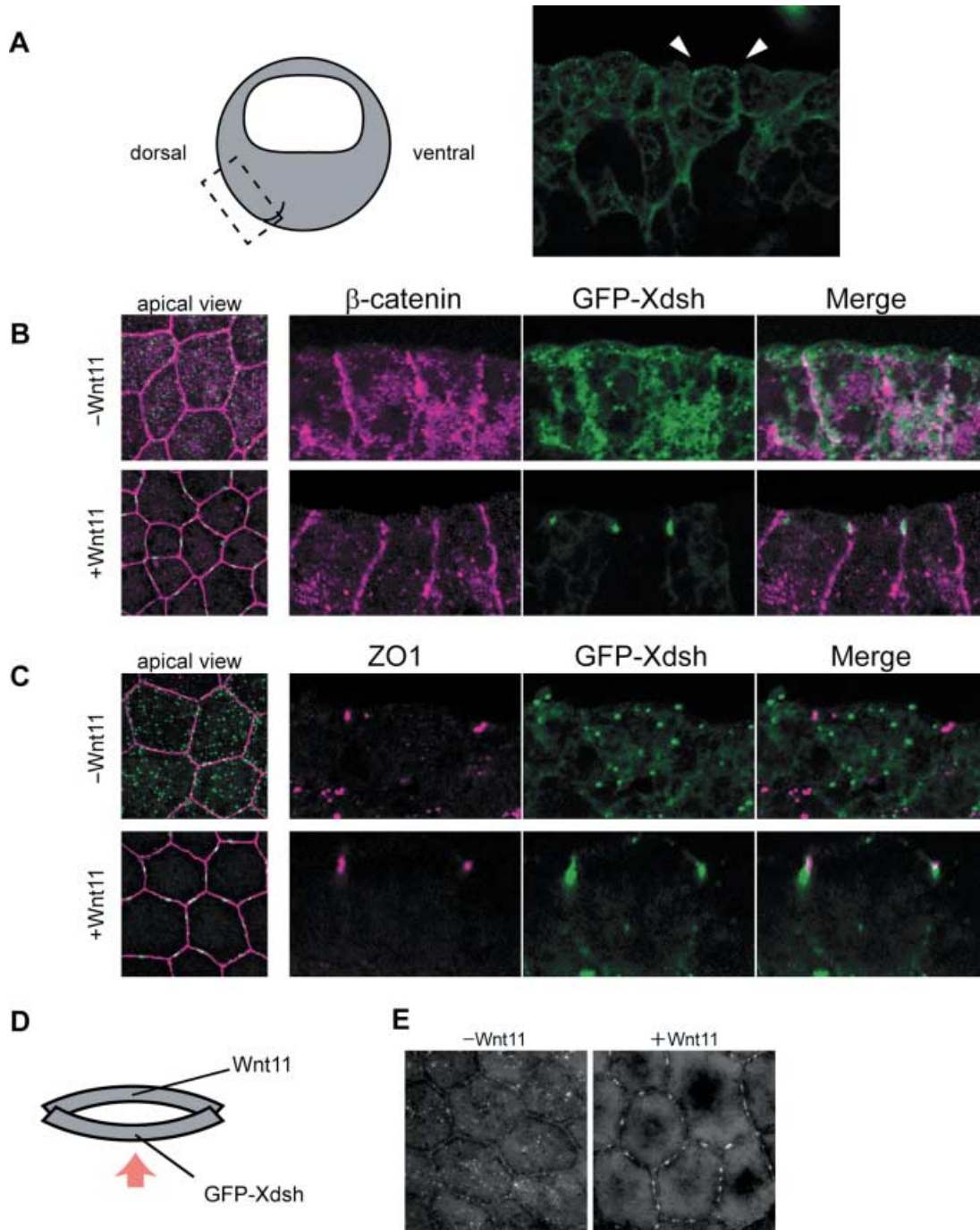
The Wnt receptors, Frizzled proteins, transmit signals to Dishevelled (Miller *et al.* 1999), and Frizzled7 has been shown to mediate Wnt11 signaling (Djiane *et al.* 2000; Penzo-Mendez *et al.* 2003; Witzel *et al.* 2006). To examine whether Frizzled7 is necessary for Wnt11 to localize Xdsh, we injected a morpholino antisense oligonucleotide (Fz7-MO) that has been shown to deplete endogenous Frizzled7 (Winklbauer *et al.* 2001). Depletion of Frizzled7 specifically suppressed the Wnt11-induced accumulation of GFP-Xdsh (Fig. 2A). A 5 mismatch MO (Fz7-5misMO) had little effect on the localization of GFP-Xdsh. These results indicate a requirement of Frizzled7 for Wnt11-induced translocation of Xdsh. We next examined the subcellular localization of Frizzled7 itself. We expressed GFP-tagged Frizzled7 (Fz7-GFP) in the explants. In the absence of Wnt11, Fz7-GFP localized uniformly at the plasma membrane (Fig. 2B, upper). When co-expressed with Wnt11, Fz7-GFP translocated and accumulated at discrete spots at

cell–cell contacts (Fig. 2B, lower). This accumulation pattern is similar to the recently reported pattern of Frizzled7 accumulation in zebrafish embryos (Witzel *et al.* 2006). In sections, while Fz7-GFP mainly localized at the basolateral plasma membrane in the absence of Wnt11, it accumulated at the apical region in the presence of Wnt11 (Fig. 2C). The accumulated Fz7-GFP co-localized with  $\beta$ -catenin (Fig. 2C). We also made GFP-tagged Frizzled3 (Fz3-GFP) and expressed it with Wnt11. Fz3-GFP did not translocate or accumulate at all (data not shown). The accumulated pattern of Fz7-GFP was very similar to the Wnt11-induced accumulated pattern of GFP-Xdsh. In fact, when CFP-tagged Xdsh (CFP-Xdsh) and YFP-tagged Frizzled7 (Fz7-YFP) were co-expressed, CFP-Xdsh and Fz7-YFP completely co-localized at the cell–cell contacts (Fig. 2D). Our results here are consistent with a recent report showing Wnt11-induced accumulation of Frizzled7 in zebrafish embryos (Witzel *et al.* 2006), and further reveal that both Dishevelled and Frizzled7 translocate to the apical region and accumulate at apical adherens junctions in response to Wnt11 stimulation.

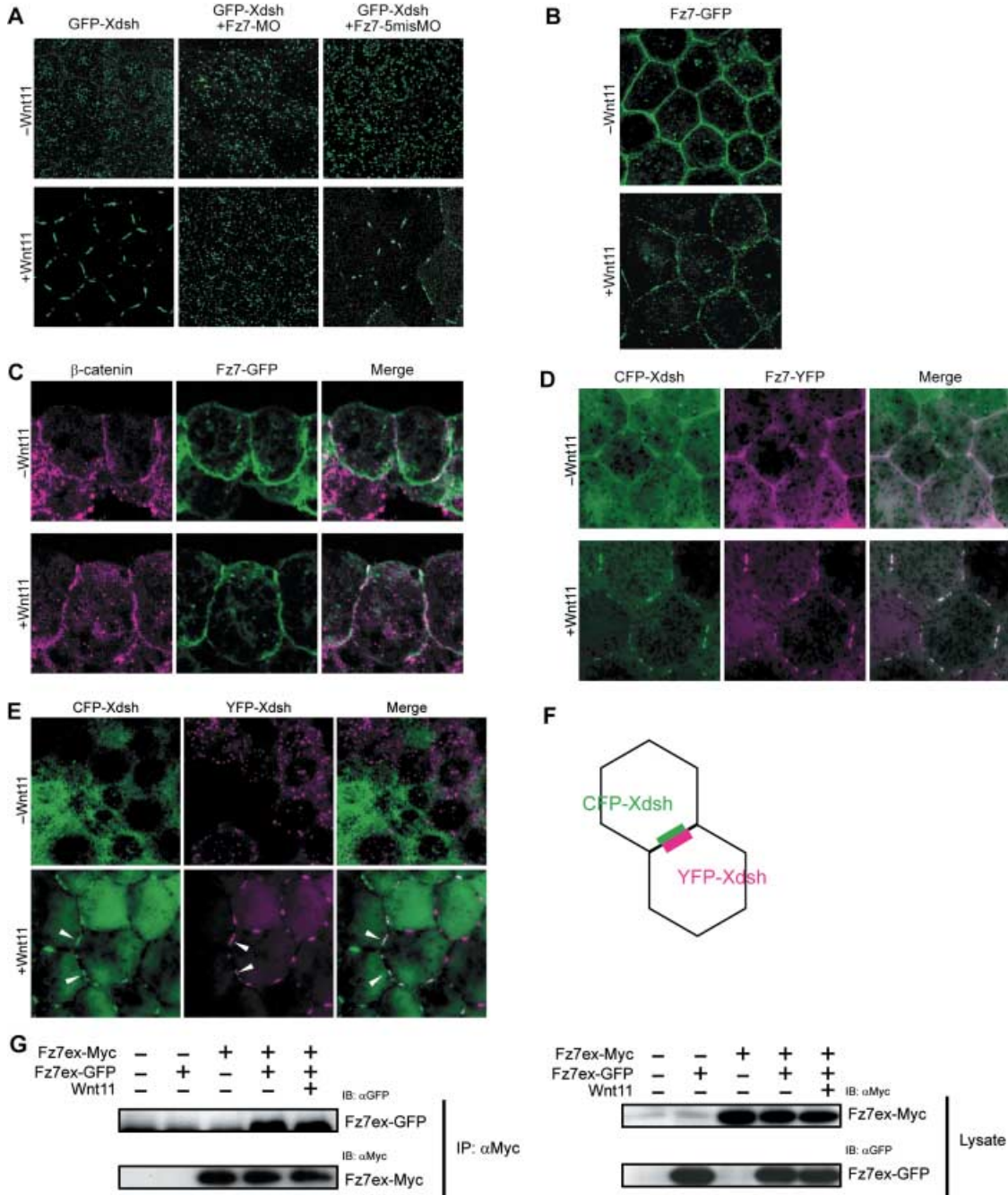
To investigate how Dishevelled localizes at cell–cell contacts, we expressed CFP-Xdsh and YFP-Xdsh in different cells by injecting each mRNA into different blastomeres at 4-cell stage. CFP-Xdsh and YFP-Xdsh in neighbor cells accumulated at cell–cell contact regions when co-expressed with Wnt11 (Fig. 2E, arrowheads), indicating that Dishevelled accumulates in each side of the same cell–cell contacts in response to Wnt11 (Fig. 2F). Because Dishevelled accumulation at cell–cell contacts depends on Frizzled7, we assumed that Frizzled7 in each side of cell–cell contacts should associate with each other to facilitate the accumulation of Frizzled7 at the both sides, leading to Dishevelled accumulation at the same contact sites. To test this idea, we assayed the binding ability of an extracellular domain of Frizzled7 (Fz7ex) by a co-immunoprecipitation assay. When GFP-tagged Fz7ex (Fz7ex-GFP) and Myc-tagged Fz7ex (Fz7ex-Myc) co-expressed in ectodermal explants were subjected to the precipitation assay, they co-immunoprecipitated with each other (Fig. 2G). This result suggests that Frizzled7 is able to undergo dimerization or oligomerization via its extracellular domain.

### The DIX domain is necessary and sufficient to accumulate in response to Wnt11

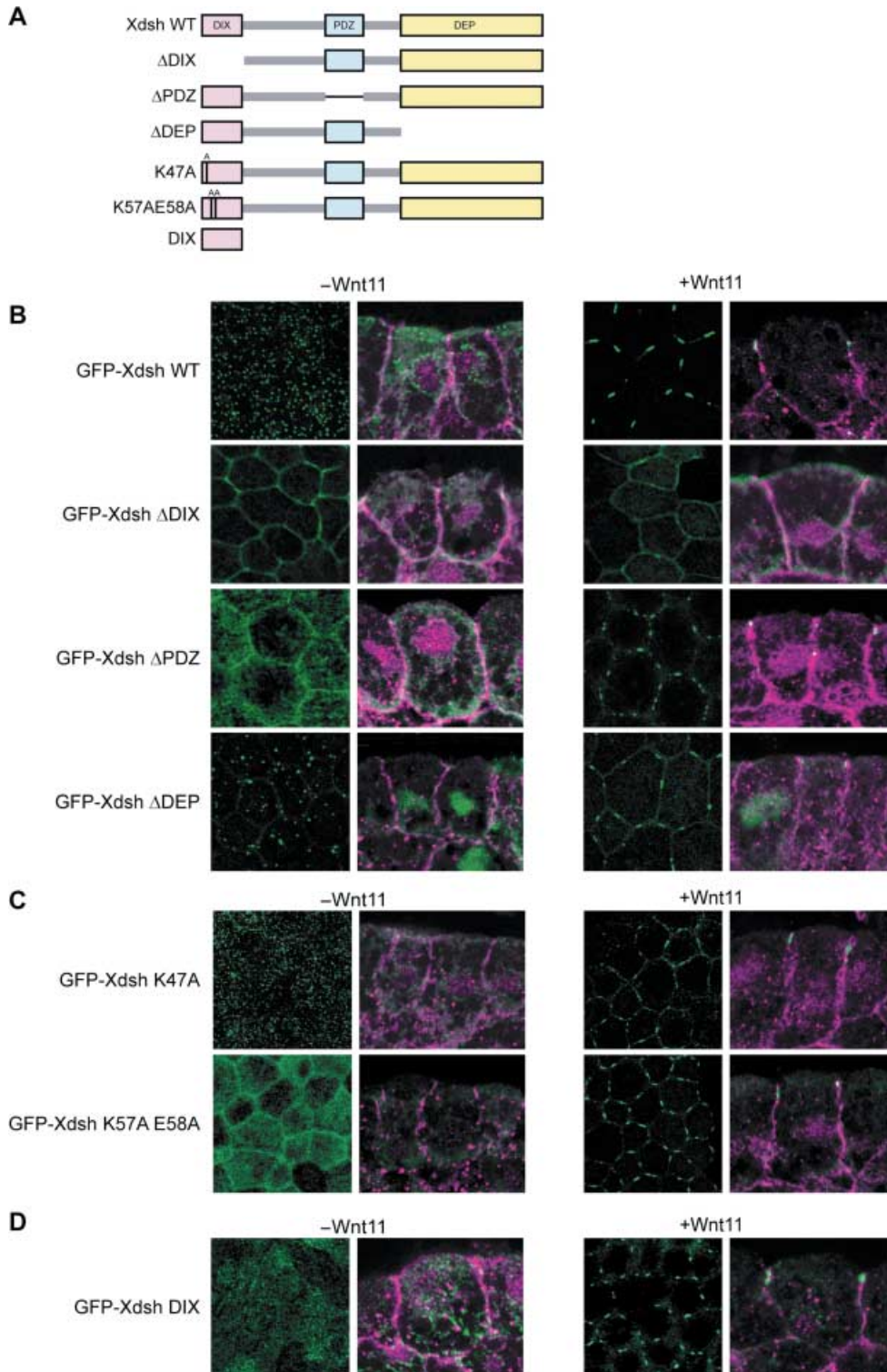
Dishevelled consists of three domains, the DIX, PDZ and DEP domains. To examine which domain is important for Wnt11-induced polarized accumulation, we expressed GFP-tagged deletion mutants of Xdsh with and without Wnt11 in *Xenopus* ectodermal explants (Fig. 3A).



**Figure 1** Wnt11 induces the accumulation of GFP-Xdsh at apical adherens junctions. (A) GFP-Xdsh was expressed in the dorsal marginal zone of *Xenopus* embryos. GFP-Xdsh RNA (100 pg) was injected into dorsal marginal zone. Embryos were sectioned as shown in the experimental scheme (left). GFP-Xdsh in involuting mesodermal cells (right). (B, C) GFP-Xdsh in ectodermal explants in the absence and presence of Wnt11 (green). RNAs (GFP-Xdsh 200 pg, Wnt11-HA 400 pg) were injected into the animal pole. Explants were stained with anti- $\beta$ -catenin antibody (B, magenta), or anti-ZO1 antibody (C, magenta). Apical views of ectodermal explants (left panels). Sectioned ectodermal explants (right panels). (D, E) Secreted Wnt11 induces the accumulation of GFP-Xdsh. (D) Experimental scheme (a red arrow indicates the direction of observation). (E) GFP-Xdsh in ectodermal explants, which were attached with ectodermal explants expressing (+Wnt11) or not expressing Wnt11 (-Wnt11).



**Figure 2** Wnt11 induces the translocation and accumulation of GFP-Xdsh through Fz7. (A) GFP-Xdsh in ectodermal explants injected with Fz7-MO or Fz7-5misMO, in the absence (upper) or presence of Wnt11 (lower). RNAs (GFP-Xdsh 200 pg, Wnt11-HA 400 pg) and Fz7MO (40 ng) were injected into the animal pole. (B) Fz7-GFP in ectodermal explants in the absence (upper) or presence of Wnt11 (lower). RNAs (Fz7-GFP 500 pg, Wnt11-HA 400 pg) were injected into the animal pole. (C) Fz7-GFP in sectioned ectodermal explants (green). Explants were stained with anti- $\beta$ -catenin antibody (magenta). (D) CFP-Xdsh (green) and Fz7-YFP (magenta) in ectodermal explants in the absence (upper) or presence of Wnt11 (lower). RNAs (CFP-Xdsh 200 pg, Fz7-YFP 500 pg, Wnt11-HA 400 pg) were injected into the animal pole. (E) CFP-Xdsh (green) and YFP-Xdsh (magenta) in ectodermal explants in the absence (upper) or presence of Wnt11 (lower). RNA of either CFP-Xdsh (100 pg) or YFP-Xdsh (100 pg) was injected into two of four blastomeres of 4-cell stage embryos with Wnt11-HA (200 pg). (F) A model for the localization of Xdsh at cell-cell contacts. (G) Co-immunoprecipitation assay using Fz7ex-GFP and Fz7ex-Myc. Expression levels of the injected RNAs are shown in the lysate.



**Figure 3** The DIX domain is necessary and sufficient for the Xdsh translocation in response to Wnt11. (A) Schematic illustration of constructs of Xdsh mutants. (B–D) GFP-Xdsh mutants in ectodermal explants in the absence or presence of Wnt11 (green). Explants were stained with anti- $\beta$ -catenin antibody (magenta) and sectioned. Each Xdsh mutant RNA (200 pg) and Wnt11-HA (400 pg) were injected into the animal pole.

The explants were observed apically or stained with anti- $\beta$ -catenin antibody and sectioned. In the absence of Wnt11, GFP-Xdsh  $\Delta$ DIX localized to the plasma membrane, GFP-Xdsh  $\Delta$ PDZ was broadly distributed in the cytoplasm and partially localized to the plasma membrane, and GFP-Xdsh  $\Delta$ DEP localized punctatedly within the cell (Fig. 3B, -Wnt11). When co-expressed with Wnt11, both GFP-Xdsh  $\Delta$ PDZ and GFP-Xdsh  $\Delta$ DEP translocated and accumulated at the apical region of the cell-cell contacts, like GFP-Xdsh WT. In contrast, GFP-Xdsh  $\Delta$ DIX did not respond to Wnt11 stimulation and remained unchanged (Fig. 3B, +Wnt11). These results indicate that the DIX domain is required for the polarized accumulation of Dishevelled in response to Wnt11. Since there are an actin binding motif and a lipid binding motif in the DIX domain (Capelluto *et al.* 2002), we then examined whether these motifs are involved in the Wnt11-induced change in Dishevelled localization. Xdsh K47A and Xdsh K57AE58A, which are defective in actin binding and lipid binding, respectively (Capelluto *et al.* 2002), were expressed with and without Wnt11. Without Wnt11, GFP-Xdsh K47A localized in the cytoplasm, like GFP-Xdsh WT, and GFP-Xdsh K57AE58A localized in the cytoplasm diffusely. Both mutants translocated and accumulated at the apical region of cell-cell contacts, like GFP-Xdsh WT (Fig. 3C, +Wnt11). Thus, these motifs are dispensable for the Wnt11-dependent change in the subcellular localization of Dishevelled. Finally, we expressed a GFP-tagged version of the DIX domain alone (GFP-Xdsh DIX) in explants. Surprisingly, GFP-Xdsh DIX translocated from the cytoplasm to the plasma membrane region and accumulated at apical adherens junctions in response to Wnt11 stimulation (Fig. 3D). This behavior of GFP-Xdsh is indistinguishable from that of GFP-Xdsh WT. Thus, the DIX domain is not only necessary but also sufficient for Wnt11-induced polarized accumulation of Dishevelled.

## Discussion

Dishevelled, a direct downstream component of the Wnt receptor Frizzled, has been shown to be essential for the establishment of cell polarity during vertebrate gastrulation (Dollar *et al.* 2005); however, how Dishevelled mediates Wnt signaling in cell polarization has been unknown (Wallingford & Habas 2005). Our finding of polarized accumulation of Dishevelled via Frizzled7 at apical adherens junctions in response to Wnt11 is the first demonstration of polarized localization of the components of Wnt signaling, and suggests a molecular basis underlying cell polarization during gastrulation. Our result

that Frizzled7 could form a dimer or an oligomer via its extracellular domain suggests the possibility that Frizzled7 oligomerization could facilitate its accumulation at cell-cell contacts. A recent study has shown that Wnt11-induced accumulation of Frizzled7 increases cell contact persistence (Witzel *et al.* 2006). We can assume that the increase of cell contact persistence is also caused by the interaction between the extracellular domain of accumulated Frizzled7. Our results here also demonstrate that the DIX domain of Dishevelled is able to translocate and accumulate at apical adherens junctions in response to Wnt11. This unexpected finding suggests that the DIX domain functions as a sensor in Wnt11-Frizzled7 signaling. How polarized accumulation of Dishevelled regulates cell polarity should be elucidated in future studies.

## Experimental procedures

### Plasmid construction

The Xdsh cDNA was kindly given by Dr S.Y. Sokol. GFP-Xdsh, YFP-Xdsh and CFP-Xdsh were constructed by inserting the coding sequence of Xdsh into pCS2-GFP, pCS2-YFP and pCS2-CFP. GFP-tagged deletion mutants of Xdsh were constructed as follows. GFP- $\Delta$ DIX (bp 1–246 deleted); A PCR fragment (EcoRI-247–2211–EcoRI) was inserted into the EcoRI site of pCS2-GFP. GFP-Xdsh  $\Delta$ PDZ (bp 787–1026 deleted); PCR fragments (EcoRI-1–786–BamHI and BglII-1027–2211–AGGCC-TGCGCCCT-StuI) were combined and inserted into the EcoRI-StuI site of pCS2-GFP. GFP-Xdsh  $\Delta$ DEP (bp 1288–2211 deleted); A PCR fragment (EcoRI-1–1287–TGAAGGCCTGCGCCCT-StuI) was inserted into the EcoRI-StuI site of pCS2-GFP. GFP-Xdsh DIX (bp 247–2211 deleted); A PCR fragment (EcoRI-1–246–TGA–BglII) was inserted into the EcoRI-BamHI site of pCS2-GFP. GFP-Xdsh K47A was constructed by replacing Lys47 with alanine. GFP-Xdsh K57AE58A was constructed by replacing Lys57 and Glu58 with alanines. These mutations were made by site-directed mutagenesis (Quick Change<sup>TM</sup>, Stratagene) and confirmed by DNA sequencing. The Wnt11 cDNA was isolated by PCR amplification, and inserted into pCS2-HA. The Fz7 cDNA was isolated by PCR amplification, and inserted into pCS2-GFP and pCS2-YFP. Fz7ex-Myc and Fz7ex-GFP were constructed by inserting a PCR fragment of Fz7ex (BamHI-1–66–EcoRI) into the BamHI-EcoRI site of pCS2-Myc and pCS2-GFP.

### Embryonic manipulation and microinjection

Embryos were *in vitro* fertilized, dejellied and cultured in 0.1 $\times$  MBS. *In vitro* synthesis of capped mRNA was performed using the Ambion mMESSAGE mMACHINE kit. The RNAs were injected into 4-cell stage embryos. Ectodermal explants were excised from stage 8 embryos and cultured in 0.5 $\times$  Sater's modified blastocoel buffer until stage 14. Morpholino oligonucleotides

were mixed with RNAs and injected into embryos. Fz7-MO and Fz7-5misMO had following sequences: (Fz7-MO; 5'-CCAACAA-GTGATCTCTGGACAGCAG-3') and (Fz7-5misMO; 5'-CCAAGAACTGATCTGTGGAGAGGAG-3') (Winklbauer *et al.* 2001).

### Immunofluorescence

The ectodermal explants were fixed in MEMFA and incubated in methanol at  $-20^{\circ}\text{C}$  for 6 h. Explants were washed and blocked in TBS containing 2% skim milk and 2.5% DMSO, and were incubated with rabbit anti- $\beta$ -catenin antibody (H-102, Santa Cruz Biotechnology, Inc.) or mouse anti-ZO1 antibody (kindly given by Dr S. Tsukita) overnight at  $4^{\circ}\text{C}$  in the same buffer. The explants were then washed in TBS, were incubated with Alexa Fluor 594 goat anti-rabbit IgG or anti-mouse IgG, and were cryosectioned.

### Co-immunoprecipitation assay

RNAs of Fz7ex-Myc (250 pg) and Fz7ex-GFP (250 pg) were injected into the animal pole of 4-cell stage embryos. Fifteen ectodermal explants were harvested at stage 14 and lysed in buffer (20 mM HEPES pH 7.5, 100 mM NaCl, 1.5 mM  $\text{MgCl}_2$ , 1 mM EGTA, 10 mM sodium pyrophosphate, 10% glycerol, 10 mM NaF, 1 mM vanadate, 1 mM PMSF, 0.5% aprotinin, 5  $\mu\text{g}/\text{mL}$  leupeptin and 1% NP-40). Mouse anti-myc antibody (9E10, Santa Cruz Biotechnology, Inc.) was used for immunoprecipitation. The immunoprecipitates were washed 3 times with the same buffer containing no detergent, and were subjected to immunoblotting with mouse anti-GFP antibody (JL-8, Clontech Laboratories, Inc.).

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### References

Bastock, R., Strutt, H. & Strutt, D. (2003) Strabismus is asymmetrically localised and binds to Prickle and Dishevelled during *Drosophila* planar polarity patterning. *Development* **130**, 3007–3014.

Capelluto, D.G.S., Kutateladze, T.G., Habas, R., Finkielstein, C.V., He, X. & Overduin, M. (2002) The DIX domain targets dishevelled to actin stress fibres and vesicular membranes. *Nature* **419**, 726–729.

Djiane, A., Riou, J., Umbhauer, M., Boucaut, J. & Shi, D. (2000) Role of frizzled 7 in the regulation of convergent extension movements during gastrulation in *Xenopus laevis*. *Development* **127**, 3091–3100.

Dollar, G.L., Weber, U., Mlodzik, M. & Sokol, S.Y. (2005) Regulation of Lethal giant larvae by Dishevelled. *Nature* **437**, 1376–1380.

Heisenberg, C.P., Tada, M., Rauch, G.J., Saude, L., Concha, M.L., Geisler, R., Stemple, D.L., Smith, J.C. & Wilson, S.W. (2000) Silberblick/Wnt11 mediates convergent extension movements during zebrafish gastrulation. *Nature* **405**, 76–81.

Lawrence, N. & Morel, V. (2003) Dorsal closure and convergent extension: two polarised morphogenetic movements controlled by similar mechanisms? *Mech. Dev.* **120**, 1385–1393.

Miller, J.R., Hocking, A.M., Brown, J.D. & Moon, R.T. (1999) Mechanism and function of signal transduction by the Wnt/ $\beta$ -catenin and Wnt/ $\text{Ca}^{2+}$  pathways. *Oncogene* **18**, 7860–7872.

Mlodzik, M. (2002) Planar cell polarization: do the same mechanisms regulate *Drosophila* tissue polarity and vertebrate gastrulation? *Trends Genet.* **18**, 564–571.

Penzo-Mendez, A., Umbhauer, M., Djiane, A., Boucaut, J.C. & Riou, J.F. (2003) Activation of  $\text{G}\beta\gamma$  signaling downstream of Wnt-11/Xfz7 regulates Cdc42 activity during *Xenopus* gastrulation. *Dev. Biol.* **257**, 302–314.

Saburi, S. & McNeill, H. (2005) Organising cells into tissues: new roles for cell adhesion molecules in planar cell polarity. *Curr. Opin. Cell Biol.* **17**, 482–488.

Saka, Y., Tada, M. & Smith, J.C. (2000) A screen for targets of the *Xenopus* T-box gene Xbra. *Mech. Dev.* **93**, 27–39.

Shimada, Y., Usui, T., Yanagawa, S., Takeichi, M. & Uemura, T. (2001) Asymmetric colocalization of Flamingo, a seven-pass transmembrane cadherin, and Dishevelled in planar cell polarization. *Curr. Biol.* **11**, 859–863.

Sokol, S.Y. (1996) Analysis of Dishevelled signalling pathways during *Xenopus* development. *Curr. Biol.* **6**, 1456–1467.

Tada, M. & 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.

Ulrich, F., Concha, M.L., Heid, P.J., Voss, E., Witzel, S., Roehl, H., Tada, M., Wilson, S.W., Adams, R.J., Soll, D.R. & Heisenberg, C.P. (2003) Slb/Wnt11 controls hypoblast cell migration and morphogenesis at the onset of zebrafish gastrulation. *Development* **130**, 5375–5384.

Veeman, M.T., Axelrod, J.D. & Moon, R.T. (2003) A second canon. Functions and mechanisms of  $\beta$ -catenin-independent Wnt signaling. *Dev. Cell* **5**, 367–377.

Wallingford, J.B. & Habas, R. (2005) The developmental biology of Dishevelled: an enigmatic protein governing cell fate and cell polarity. *Development* **132**, 4421–4436.

Wallingford, J.B., Rowning, B.A., Vogeli, K.M., Rothbacher, U., Fraser, S.E. & Harland, R.M. (2000) Dishevelled controls cell polarity during *Xenopus* gastrulation. *Nature* **405**, 81–85.

Winklbauer, R., Medina, A., Swain, R. K. & Steinbeisser, H. (2001) Frizzled-7 signalling controls tissue separation during *Xenopus* gastrulation. *Nature* **413**, 856–860.

Witzel, S., Zimyanin, V., Carreira-Barbosa, F., Tada, M. & Heisenberg, C.P. (2006) Wnt11 controls cell contact persistence by local accumulation of Frizzled 7 at the plasma membrane. *J. Cell Biol.* **175**, 791–802.

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