PKCδ is essential for Dishevelled function in a noncanonical Wnt pathway that regulates Xenopus convergent extension movements

Noriyuki Kinoshita,1 Hidekazu Iioka, Akira Miyakoshi, and Naoto Ueno

Department of Developmental Biology, National Institute for Basic Biology; Department of Molecular Biomechanics, The Graduate University for Advanced Studies, Myodaiji, Okazaki, Aichi 444-8585, Japan

Protein kinase C (PKC) has been implicated in the Wnt signaling pathway; however, its molecular role is poorly understood. We identified novel genes encoding 6-type PKC in the Xenopus EST databases. Loss of PKCδ function revealed that it was essential for convergent extension during gastrulation. We then examined the relationship between PKCδ and the Wnt pathway. PKCδ was translocated to the plasma membrane in response to Frizzled signaling. In addition, loss of PKCδ function inhibited the translocation of Dishevelled and the activation of c-Jun N-terminal kinase (JNK) by Frizzled. Furthermore, PKCδ formed a complex with Dishevelled, and the activation of PKCδ by phorbol ester was sufficient for Dishevelled translocation and JNK activation. Thus, PKCδ plays an essential role in the Wnt/JNK pathway by regulating the localization and activity of Dishevelled.

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During Xenopus gastrulation, mesodermal cells migrate to the inside of the embryo and move along the blastocoel roof. This movement is essential for embryonic morphogenetic processes such as the establishment of the three germ layers and body axes. The process involves highly integrated cell movements. One of the important mechanisms for this movement is convergent extension. As convergent extension begins, cells are polarized and aligned mediolaterally; this is followed by the intercalation of these polarized cells. This movement elongates the mesodermal tissue along the anteroposterior axis, producing a driving force for gastrulation movements [Wilson and Keller 1991; Shih and Keller 1992; Wallingford et al. 2002]. The regulation of the convergent extension movements is known to involve a noncanonical Wnt signaling pathway.

The Wnts are a family of secreted proteins that regulate many biological processes [Cadiyan and Nusse 1997]. Functional analyses in Xenopus suggest that the Wnt family can be divided into two functionally distinct groups. The first group of Wnts induces a secondary axis when ectopically expressed in embryos. They activate the canonical Wnt/β-catenin pathway and induce the transcription of target genes such as siamois and Xnr3 [Brannon and Kimelman 1996; Carnac et al. 1996; McKendry et al. 1997]. The second group of Wnts, which includes Xwnt5a and Xwnt11, activates the noncanonical Wnt signaling pathway that controls morphogenetic cell movements [Kuhl 2002; Tada et al. 2002]. It was shown in zebrafish that mutations in Wnt11/silberbrick and Wnt5a/pipetail inhibit normal gastrulation movements [Rauch et al. 1997; Heisenberg et al. 2000]. The noncanonical Wnt pathway branches into two cascades. One is the Wnt/JNK pathway, which involves c-Jun N-terminal kinase (JNK; Bourgois et al. 1998; Yamanaka et al. 2002). The other is the Wnt/Ca2+ pathway [Kuhl et al. 2000]. In Drosophila, the Wnt/JNK pathway is called the planar cell polarity (PCP) pathway, and it specifies cell polarities in epithelial cells and other types of cells [Adler 2002].

The Wnt signaling pathway is mediated by a seven-transmembrane Wnt receptor, Frizzled, and the signal is transmitted through a cytoplasmic protein, Dishevelled (Dsh), which plays pivotal roles in both the canonical and noncanonical Wnt pathways [Boutros and Mlodzik 1999; Wharton 2003]. In Drosophila, Dsh localizes to the membrane, and this localization is required for Dsh function (Axelrod 2001). Xenopus Dsh (Xdsh) is also translocated from the cytoplasm to the plasma membrane in response to a signal generated by some Frizzled receptors [Yang-Snyder et al. 1996; Axelrod et al. 1998;
Protein kinase C (PKC) is thought to be involved in the noncanonical Wnt signaling pathway, particularly in the Wnt/Ca\(^{2+}\) pathway, for several reasons. Xwnt5a and rat Frizzled2 activate the phosphatidylinositol pathway and increase the intracellular Ca\(^{2+}\) levels in zebrafish embryos [Slusarski et al. 1997a,b]. The phosphatidylinositol pathway and Ca\(^{2+}\) levels are closely related to PKC activation. In fact, overexpression of *Frizzled* causes the translocation of epitope-tagged PKCa from the cytoplasm to the plasma membrane in *Xenopus* embryos [Sheldahl et al. 1999; Medina et al. 2000]. Kuhl et al. (2001) showed that PKCa phosphorylated Dsh in vitro. In addition, the loss of *Xfz7* function leads to a defect in tissue separation during *Xenopus* gastrulation, which is rescued by the overexpression of PKCa (Winkbauer et al. 2001). PKC is also implicated in the Xwnt11 signaling pathway for *Xenopus* cardiogenesis [Pandur et al. 2002] and in the Dmtn4 pathway for *Drosophila* ovarian morphogenesis [Cohen et al. 2002].

Although much evidence suggests that PKC is involved in the Wnt signaling pathway, the molecular roles of PKC in this pathway are not well understood. The PKC family is subdivided into three subfamilies: the classical, novel, and atypical PKCs (cPKC, nPKC, and aPKC, respectively). cPKC is activated by Ca\(^{2+}\) and diacylglycerol (DAG), nPKC is activated by DAG but not by Ca\(^{2+}\), and aPKC is not activated by these molecules [Kikkawa et al. 1989; Burns and Burns 1991; Nishizuka 1995; Newton 1997]. In *Xenopus*, cDNAs encoding PKCα and PKCβ, which belong to the cPKC subfamily, have been isolated [Chen et al. 1988], and shown to be involved in neural induction [Otto et al. 1988; Otto and Moon 1992]. However, their roles in the regulation of gastrulation movements are not clear. Thus, we searched for novel PKC genes that might have roles in the noncanonical Wnt signaling pathway. Here, we describe the identification and functional analyses of *Xenopus* PKCβ, which belongs to the nPKC subfamily. We demonstrate that PKCβ is essential for convergent extension, and that PKCβ regulates the function of Dishevelled in the Wnt/JNK pathway.

**Results**

**PKCβ is expressed during Xenopus embryogenesis**

Although PKC has been implicated in the noncanonical Wnt signaling pathway, its molecular role is poorly understood. It has been shown that Xwnt5a and rat Frizzled2 trigger the phosphatidylinositol pathway and induce an increase in intracellular Ca\(^{2+}\) [Slusarski et al. 1997a,b]. Among the PKC subfamilies, cPKC and nPKC are known to be activated by Ca\(^{2+}\) and/or diacylglycerol (DAG). For this reason, we searched our *Xenopus* EST database ([NIBB XDB, http://Xenopus.nibb.ac.jp]) to identify PKC family members that belong to the cPKC or nPKC subfamily. We found that in addition to PKCα and PKCβ, which have already been reported [Chen et al. 1988], the database included two novel cDNAs encoding nPKC family members. The predicted amino acid sequences of these two PKCs had 95% identity, suggesting that these are duplicated genes due to the tetraploidyism of *Xenopus laevis*. As described later, these two genes had indistinguishable activities in the tests we performed. These proteins are the most similar to mammalian 6-type PKC [Fig. 1A,B]. Thus, we designated these genes PKCβ1 and PKCβ2. It is known that the N-terminal regulatory domain of PKCs inhibits the kinase activity by masking the catalytic domain, and activators such as DAG release this autoinhibition by binding to the C1 domain [Kemp et al. 1994; Orr and Newton 1994; Nishizuka 1995; Newton 1997]. The regulatory domain of *Xenopus* PKCβ1/2, including the C1 domain, is highly homologous to that of human PKCβ, suggesting that these regulatory mechanisms are conserved. PKCβ is relatively similar to PKCα and PKCγ, which also belong to the nPKC family. This class of PKCs is found not only in vertebrates, but also in sea sponges [GenBank accession no. CAAB3557], *Aplysia* [GenBank accession no. 16975], *Hydra* [GenBank accession no. CAAB72926], *Drosophila* [GenBank accession no. NP_511171], and nematodes [GenBank accession no. NP_499868]. Thus, the nPKCs may have evolutionarily conserved regulatory mechanisms and functions distinct from those of other PKC subfamilies.

To determine the expression patterns during *Xenopus* development, we performed reverse transcriptase PCR (RT–PCR) using primers whose sequences were common to PKCβ1 and PKCβ2. As shown in Figure 1C, PKCβ was expressed from the two-cell stage through the tadpole stage. In situ hybridization using probes for PKCβ1 and PKCβ2 revealed that they were ubiquitously expressed [Fig. 1D]. PKCβ1 and PKCβ2 were strongly expressed in the mesoderm and ectoderm during gastrulation, indicating their possible involvement in the regulation of gastrulation movements.

**Overexpression of PKCβ lacking the catalytic domain inhibits gastrulation movements**

To test whether PKCβ is involved in the regulation of gastrulation movements, we made an expression construct for PKCβ1 lacking the catalytic domain [PKCβΔC]. The N-terminal regulatory domain of PKCs includes pseudosubstrate and C1 domains. The pseudosubstrate domain interacts with the kinase domain and inhibits the catalytic activity [Kemp et al. 1994; Orr and Newton 1994]. The C1 domain interacts with DAG and other activators. A mutant lacking the catalytic domain was
expected to function as a dominant-negative form by binding to the catalytic domain of a native protein through its pseudosubstrate domain and/or by competitive binding to the activators. RNA encoding PKC\(\beta_1\)/H9254/H9004 was synthesized in vitro and injected into the two dorsal blastomeres of four-cell embryos. As shown in Figure 2A, PKC\(\beta_1\)/H9254/H9004 severely inhibited gastrulation movements. Involution of the mesoderm was impaired, and the blastopore remained open or showed delayed closing. The same phenotype was observed in embryos injected with PKC\(\beta_2\)/H9254 lacking the catalytic domain (data not shown). The phenotype was rescued by full-length PKC\(\beta_1\)/H9254 (Fig. 2A) or full-length PKC\(\beta_2\)/H9254 (data not shown), suggesting that PKC\(\beta\Delta C\) functioned as a dominant-negative mutant of PKC\(\beta\). It has been reported that a similar gastrulation-defect phenotype is caused by loss-of-function of the noncanonical Wnt signaling components, such as Xwnt11 [Tada and Smith 2000] and Xfz7 [Djiane et al. 2000].

To test whether PKC\(\alpha\) or PKC\(\beta\) has an activity similar to that of PKC\(\beta\), we constructed mutant genes encoding PKC\(\alpha\) and PKC\(\beta\) lacking the catalytic domain [PKC\(\alpha\Delta C\) and PKC\(\beta\Delta C\), respectively], expecting that they would function as dominant-negative mutants of their respective native forms. We injected the same amount of mRNA encoding PKC\(\alpha\Delta C\)/H9251/H9004, PKC\(\beta\Delta C\)/H9252/H9004, or PKC\(\beta\Delta C\)/H9254/H9004 into Xenopus embryos. Although comparable levels of the mutant proteins were detected by Western blotting, PKC\(\alpha\Delta C\)/H9251/H9004 and PKC\(\beta\Delta C\)/H9252/H9004 did not have any effects on gastrulation, unlike PKC\(\beta\Delta C\)/H9254/H9004 (data not shown). This result

Figure 1. PKC\(\beta\) is expressed during Xenopus embryogenesis. [A] Sequence alignment of Xenopus PKC\(\beta_1\) and human PKC\(\beta\). The DAG-binding C1 domain is underlined. The pseudosubstrate region is indicated by dots. [B] Sequence comparison between Xenopus PKC\(\beta\) and some human PKC family members. Xenopus PKC\(\beta_1\) is the most similar to human PKC\(\beta\) in both the regulatory and the catalytic domains. [C] RT–PCR analysis of PKC\(\beta\) expression during Xenopus development. Primers whose sequences were common between PKC\(\beta_1\) and PKC\(\beta_2\) were used. Stages are according to Nieuwkool and Faber [1994]. [D] In situ hybridization probing with PKC\(\beta_1\) and PKC\(\beta_2\) showing their ubiquitous expression.
suggests that the role in gastrulation movements may be PKCδ-specific.

To determine whether this gastrulation-defective phenotype was caused by a defect in mesodermal differentiation, the expression of mesodermal markers, we used in situ hybridization to examine a pan-mesodermal marker, Xbra, and dorsal mesodermal markers chordin (chd) and goosecoid (gsc). At the gastrula stage, PKCδΔC-injected embryos expressed these markers at the same level as control embryos (Fig. 2B). In tadpoles, the noto-
chord and somites were differentiated in the PKC\(\Delta C\)-injected embryos, but the extension of these tissues was severely inhibited (Fig. 2C). These results indicated that the phenotype was caused not by a defect in mesoderm differentiation, but by a defect in morphogenetic movements.

We then tested whether PKC\(\Delta C\) inhibited the elongation of animal caps by activin. In this system, when the dorsal mesoderm is induced in animal cap explants by activin, the explants elongate by convergent extension movements. PKC\(\Delta C\) expression inhibited the elongation of explants by activin without affecting the induction of mesodermal markers (Fig. 2D,E). A similar effect has been observed as a result of inhibition of the noncanonical Wnt pathway. Our results suggest that PKC\(\delta\) is also required for the convergent extension.

PKC\(\delta\) antisense morpholino also blocked gastrulation movements and convergent extension

To confirm that the effects of PKC\(\Delta C\) were due to the depletion of PKC\(\delta\) activity, we made antisense morpholino oligonucleotides [MOs] for PKC\(\delta\) and tested their effects on development. Because the highly homologous PKC\(\delta 1\) and PKC\(\delta 2\) are both expressed, MOs for both PKC\(\delta 1\) and PKC\(\delta 2\) were prepared. First, we confirmed that these MOs inhibited the translation of mRNA that has the corresponding sequences. As shown in Figure 3A, each MO blocked the production of its respective GFP-tagged PKC\(\delta\), but unrelated GFP was not affected.

To inhibit PKC\(\delta\) synthesis in embryos, the MOs for PKC\(\delta 1\) and PKC\(\delta 2\) were mixed at an equimolar ratio and injected into four-cell embryos (the mixed MOs will be

![Figure 3](https://genesdev.cshlp.org/10.1101/gad.162585.14667)

**Figure 3.** PKC\(\delta\) antisense morpholino also blocked gastrulation movements. (A) Morpholino oligonucleotides [MOs] for PKC\(\delta 1\) and PKC\(\delta 2\) inhibited the translation of mRNA that had the corresponding sequences. RNA encoding GFP-tagged PKC\(\delta\) and unrelated GFP were coinjected with or without each MO. PKC\(\delta 1\) (left panel) and PKC\(\delta 2\) MO (right panel) blocked the production of each GFP-tagged PKC\(\delta\), but unrelated GFP was not affected. (B) Control MO or PKC\(\delta\) MO (20 ng each) was injected into four-cell embryos (panels a, b, respectively). The PKC\(\delta\) MO caused a gastrulation-defective phenotype that was indistinguishable from that of PKC\(\Delta C\)-injected embryos. This phenotype was rescued by 1 ng of full-length PKC\(\delta\) RNA (panel c). (C) In situ hybridization of early gastrula embryos probed with chordin (chd), goosecoid (gsc), and Xbra. The left and middle panels show 20 ng of control or PKC\(\delta\) MO was injected into all four blastomeres of four-cell embryos. (Right) To trace the cell lineage, mRNA encoding β-gal with a nuclear localization signal was coinjected with PKC\(\delta\) MO into two dorsal blastomeres of four-cell embryos. Twenty nanograms of MO were injected into the two dorsal blastomeres of four-cell embryos.
PKC\(\delta\) is translocated to the membrane in response to Xfz7 signaling and interacts with Xdsh

The inhibition of convergent extension by the loss of PKC\(\delta\) function strongly implies that PKC\(\delta\) plays a role closely related to the noncanonical signaling pathway. It has been shown that Frizzled translocates Xdsh and PKC\(\delta\) from the cytoplasm to the plasma membrane in \textit{Xenopus} embryos (Axelrod et al. 1998; Sheldahl et al. 1999; Medina and Steinbeisser 2000; Medina et al. 2000). To examine whether Frizzled also regulates the subcellular localization of PKC\(\delta\), Flag-tagged PKC\(\delta\) and myc-tagged Xdsh were coexpressed with or without Xfz7 in animal cap explants of \textit{Xenopus} embryos. The localization of the tagged proteins was then observed with a laser-scanning confocal microscope [Fig. 4A]. In the absence of Xfz7 mRNA, PKC\(\delta\) and Xdsh were mainly in the cytoplasm. Interestingly, however, when Xfz7 was coexpressed, they were mostly localized to the plasma membrane. In general, this class of PKC binds to and is activated by DAG that is produced on the membrane upon extracellular signaling. Thus, DAG may be produced by Xfz7 and then localize PKC\(\delta\) to the membrane, which further implies that PKC\(\delta\) is involved in the Wnt/Xfz7 pathway.

The above finding that the cotranslocation of PKC\(\delta\) and Xdsh from the cytoplasm to the membrane depended on Xfz7 function prompted us to examine whether these proteins might interact with each other. To test this possibility, Flag-tagged PKC\(\delta\) and myc-tagged Xdsh were expressed in HEK293T cells and immunoprecipitation was performed. As shown in Figure 4B, PKC\(\delta\) and Xdsh were coimmunoprecipitated. When the Flag and myc tags on PKC\(\delta\) and Xdsh were exchanged, we obtained essentially the same result [data not shown]. These findings indicated that PKC\(\delta\) and Xdsh form a complex. To test whether the activation of PKC\(\delta\) by the phorbol ester PMA (phorbol 12-myristate 13-acetate) altered this binding property, we treated transfected HEK293T cells with PMA and performed an immunoprecipitation. PMA is known to be a potent activator for PKC\(\delta\) and other members of the novel and classical PKC subfamilies (Kikkawa et al. 1989; Bell and Burns 1991; Zhang et al. 1995). As shown in Figure 4C, PMA treatment did not change the amount of coimmunoprecipitated Xdsh and PKC\(\delta\). In addition, a kinase-negative mutant of PKC\(\delta\) was also coinmunoprecipitated with Xdsh in HEK293T lysates [data not shown], indicating that this physical interaction may not depend on the activity of PKC\(\delta\). Taken together, these results suggest that PKC\(\delta\) and
Xdsh form a complex, and this complex is translocated to the membrane upon activation of the Xfz7 signal.

**PKCδ is required for Xdsh activation by Xfz7 signaling**

The precise molecular mechanisms of the membrane localization of Xdsh by Wnt/Xfz7 signaling are not known. To investigate this issue, we next tested whether PKCδ is required for the Xfz7-dependent membrane localization of Xdsh. PKCδ MO was coinjected with myc-tagged Xdsh and Xfz7 mRNAs, and localization of Xdsh to the animal cap cells was observed. As shown in Figure 5A, the coinjection of PKCδ MO blocked the membrane localization of Xdsh in response to Xfz7.

Xdsh is a phosphoprotein whose phosphorylated state is elevated [hyperphosphorylated] upon the activation of the noncanonical Wnt signaling pathway [Yanagawa et al. 1995; Willert et al. 1997; Rothbacher et al. 2000; Tada and Smith 2000]. This increase in phosphorylation can be monitored by a mobility shift of the Xdsh protein in SDS polyacrylamide gel electrophoresis (SDS-PAGE). To test whether PKCδ affects the phosphorylation state of Xdsh, myc-tagged Xdsh mRNA was coinjected with PKCδ MO or PKCδΔC mRNA into four-cell embryos. Animal cap explants were isolated at around stage 10, and their extracts were subjected to SDS-PAGE [Fig. 5B]. The myc-tagged Xdsh protein was detected by Western blotting using an anti-myc antibody. Two bands were detected in the Xdsh-injected samples. In the absence of Xfz7, the lower band was more intense than the upper band. When Xfz7 was coinjected, the upper band became much more intense. This indicated that Xdsh was hyperphosphorylated by Xfz7 signaling. The coinjection of PKCδΔC or PKCδ MO blocked this hyperphosphorylation.
PKCδ is required for the activation of Xdsh. The results indicated that PKCδ is required for both the membrane localization and the phosphorylation of Xdsh, suggesting that PKCδ is essential for the signaling from Xfz7 to Xdsh.

If the Xdsh function requires PKCδ activity, the activation of JNK in the noncanonical Wnt pathway should be blocked by the loss of PKCδ function. To examine this possibility, we assayed the JNK activity. GAL4 DNA-binding domain (DBD)-tagged c-Jun mRNA was injected into Xenopus embryos, and the phosphorylation level of c-Jun was assessed by Western blotting using anti-phosphorylated-c-Jun and anti-DBD antibodies. As shown in Figure 5C, the overexpression of PKCδ or Xfz7 alone slightly activated the JNK activity. However, the activity was greatly enhanced by the coexpression of Xfz7 and PKCδ. Moreover, PKCδ MO blocked the activation of JNK by Xfz7. These results indicated that PKCδ is required for the activation of JNK by Xfz7 signaling.

Activation of PKCδ is sufficient for Xdsh translocation and for activation of the JNK pathway

As described above, PKCδ and Xdsh form a complex, and both are translocated to the plasma membrane upon the activation of the noncanonical Wnt pathway. We postulated that PKCδ recruits Xdsh to the membrane in this process. If this is true, the activation of PKCδ might be sufficient for the translocation of Xdsh. To test this possibility, we injected RNAs encoding Flag-tagged PKCδ1 and myc-tagged Xdsh into Xenopus embryos, and the animal caps were explanted and treated with PMA. PMA is a functional analog of DAG that activates PKC on the plasma membrane by PMA within 15 min. In addition, PMA treatment activated the JNK in the animal cap explants [Fig. 5E]. Thus, PKCδ activation is sufficient for Xdsh translocation and the activation of downstream signaling.

The PKCδ loss-of-function phenotype is partially rescued by the overexpression of active MKK7

We have shown that PKCδ is required for the activation of JNK by Xfz7. If the gastrulation-defective phenotype caused by PKCδ MO is due to the blockade of JNK activation, it might be rescued by the overexpression of MKK7, which is known to activate JNK directly. To examine this possibility, we coinjected PKCδ Mo with constitutively active (CA) MKK7 [Yamanaka et al. 2002]. Closure of the blastopore of the injected embryos was compared at stage 14, which is when the blastopore in the control embryos was completely closed. PKCδ MO blocked the gastrulation movement in more than 90% of the injected embryos (Fig. 6A). PKCδ and CA MKK7 mRNAs rescued the phenotype completely and partially, respectively, suggesting that MKK7/JNK functions at least in part downstream of PKCδ. This result supported the idea that Xfz7 regulates JNK activity through PKCδ.

PKCδ is not essential for the canonical Wnt pathway

We next tested whether PKCδ plays a role in the canonical Wnt pathway. It is known that the ectopic expression

Figure 6. PKCδ loss-of-function is rescued by overexpression of active MKK7. (A) Twenty nanograms of PKCδ MO were coinjected with 1 ng of PKCδ1, and 200 pg of constitutively active (CA) MKK7. The closure of the blastopore of injected embryos was compared at stage 14, when the blastopore in the control embryos was completely closed. PKCδ MO blocked gastrulation movement. PKCδ mRNA rescued the phenotype completely, and CA MKK7 mRNA partially rescued it. (B) At the tadpole stage, embryos coinjected with CA MKK7 showed a partially rescued phenotype. (C) To test whether PKCδ is essential for the canonical Wnt pathway, 100 pg of PKCδΔC or 20 ng of PKCδ MO was coinjected with 10 pg of Xwnt8 into Xenopus embryos. PKCδΔC and PKCδ MO did not inhibit the induction of siamois or Xnr3, indicating that PKCδ does not affect the canonical Wnt signaling pathway.
of Xwnt8 induces secondary axis formation (Smith and Harland 1991) and marker genes such as siamois and Xnr3 by activating the canonical Wnt pathway in Xenopus embryos [Brannon and Kimelman 1996; Carnac et al. 1996; McEndry et al. 1997]. When Xwnt8 was co-injected with PKCδAC or PKCδ MO, the induction of siamois and Xnr3 by Xwnt8 was not inhibited [Fig. 6C]. Furthermore, the secondary axis formation by Xwnt8 was not inhibited by the loss of PKCδ function [data not shown]. Therefore, although PKCδ is required for the Wnt/JNK pathway, it may not be necessary for the canonical Wnt pathway, which is independent of the membrane relocalization of Dishevelled [Yang-Snyder et al. 1996; Axelrod et al. 1998; Moriguchi et al. 1999; Rothbacher et al. 2000].

PKCδ regulates the cell shape and intercalative behavior of the mesodermal cells during convergent extension movements

During convergent extension of the mesoderm, cells are polarized and aligned mediolaterally, then intercalated. To test whether PKCδ is required in this process, the convergent extension in DMZ explants was observed microscopically. The procedure was basically according to Wallingford et al. (2000). PKCδ MO, Rhodamine dextran, and mRNA for Venus (a YFP variant; Nagai et al. 2002) fused with the membrane localization signal of K-ras [mb-Venus] were co-injected into one of the two dorsal blastomeres at the four-cell stage. As a control, mb-Venus mRNA alone was injected into the other dorsal blastomere [Fig. 7A]. At the gastrula stage, dorsal marginal zone explants were cultured on a cover glass coated with fibronectin. These explants adhered to the surface, and subsequently, convergent extension movements occurred in the mesoderm. In the absence of PKCδ MO, red cells and non-red cells intercalated [Fig. 7B]. In the PKCδ MO-injected explants, the non-red cells, which were assumed to lack the MO, were polarized and showed convergent extension movements. In contrast, the red cells [MO-injected cells] were round-shaped, were not polarized, and did not participate in the intercalation, even when they were adjacent to the intercalating cells. Thus, this inhibition by PKCδ MOs appeared to be cell-autonomous. These results indicated that PKCδ is essential for the cell polarization during convergent extension movements.

To investigate the subcellular localization of PKCδ and Xdsh in the dorsal mesodermal cells, we expressed these proteins tagged with Venus. Interestingly, these were accumulated around the tips of the elongated cells [Fig. 7C]. However, Xdsh lacking the DEP domain, which is known to play an important role in the tissue polarity [Axelrod et al. 1998] was almost uniformly distributed (XdshΔDEP). In addition, Rac tagged with Venus is also localized in the same region. The finding that Rac forms a complex with Xdsh [Habas et al. 2003] suggests that Rac may be recruited by Xdsh.

Figure 7. PKCδ is required for convergent extension movements. [A] PKCδ MO, Rhodamine dextran, and mRNA for Venus fused with a membrane localization signal [mb-Venus] were co-injected into one of the two dorsal blastomeres at the four-cell stage. As a control, mb-Venus mRNA alone was injected into the other dorsal blastomere of the same embryo. [B] At the gastrula stage, dorsal marginal zone [DMZ] explants were cut and cultured on a cover glass coated with fibronectin, and the convergent extension movements were observed by laser-scanning confocal microscopy. [C] The indicated cDNAs were fused to Venus and expressed in the dorsal mesodermal cells. DMZ explants were cultured and observed as described above. Bar, 50 µm.
The process of convergent extension movements includes cell shape change and cell movements, suggesting that the regulation of actin polymerization may be crucial for the process. It is known that the Arp2/3 complex is a key component of the assembly of actin filaments and the cell motility (Suetsugu et al. 2002, Weaver et al. 2003). Thus, we tested the localization of the Arp3 tagged with Venus in these cells. As shown in Figure 7C, Arp3 is also localized around the tips of these cells. In addition, Rac and Arp3 were localized almost uniformly on the membrane or the cortical region when PKC\(_\text{H9254}\) was injected. The Arp2/3 complex may be recruited by PKC\(_\text{H9254}\) and its downstream PCP signaling and may regulate the cell polarity, bipolar protrusive activity, and cell motility in these cells.

**Discussion**

**PKC\(_\text{H9254}\) is essential for Xdsh function in the noncanonical Wnt pathway**

The membrane localization of Xdsh is thought to be an important step for Xdsh activation in the noncanonical Wnt pathway (Moriguchi et al. 1999; Rothbacher et al. 2000; Wallingford et al. 2000, Axelrod 2001). However, the molecular mechanism of this process has not been well understood. In the present study, we identified PKC\(_\text{H9254}\) in the *Xenopus* embryo and investigated its role in the Wnt/JNK pathway. These analyses revealed that PKC\(_\text{H9254}\) is essential for the translocation and activation of Xdsh in the Wnt/JNK pathway, based on the following findings: [1] PKC\(_\text{H9254}\) was translocated from the cytoplasm to the plasma membrane in response to Xfz7 signaling. [2] The loss of PKC\(_\text{H9254}\) function inhibited the membrane localization and hyperphosphorylation of Xdsh. [3] PKC\(_\text{H9254}\) was also essential for JNK activation by Xfz7. [4] PKC\(_\text{H9254}\) physically interacted with Xdsh. [5] The activation of PKC\(_\text{H9254}\) by PMA was sufficient for the translocation of Xdsh and the activation of the JNK pathway. Taking all these results together, we propose the following model of the Xdsh activation mechanism in the noncanonical Wnt/JNK pathway. In the absence of Wnt/Xfz7 signaling, Xdsh and PKC\(_\text{H9254}\) localize to the cytoplasm. It is likely that Xdsh and PKC\(_\text{H9254}\) form a complex even in the absence of Xfz7 signaling. This was suggested by our finding that the Xdsh-PKC\(_\text{H9254}\) complex formation was not dependent on Frizzled signaling or PKC\(_\text{H9254}\) activity. However, further investigations may be necessary to confirm this hypothesis. When the receptor is activated, the phosphatidylinositol pathway may be activated and produce the PKC activator, diacylglycerol (DAG). Then, PKC\(_\text{H9254}\), which has a DAG-binding C1 domain, is translocated and recruits Xdsh to the membrane. PKC\(_\text{H9254}\) may be activated by DAG, resulting in the hyperphosphorylation of Xdsh. The membrane localization and/or phosphorylation of Xdsh activate the downstream JNK pathway.

**How does Frizzled activate PKC\(_\text{H9254}\)?**

*Xenopus* PKC\(_\text{H9254}\) has a highly conserved C1 domain, which binds to DAG and phorbol esters such as PMA, a functional analog of DAG. PKC\(_\text{H9254}\) was translocated to the plasma membrane in animal cap cells in response to both Xfz7 and PMA. These results and other observations suggested that Xfz7 might activate PKC\(_\text{H9254}\) through DAG on the plasma membrane, although there is no direct evidence that activation of the Wnt/Frizzled pathway produces DAG. However, heterotrimeric G proteins have been implicated in the Wnt/Frizzled pathway [Liu et al. 1999, 2001]. It has been shown that certain heterotrimeric G proteins coupled with seven-transmembrane receptors activate phospholipase C-\(\beta\), which hydrolyzes phosphatidylinositol phosphate to produce DAG and inositol triphosphate. In addition, Xfz7 function is blocked by pertussis toxin, which inhibits the G\(\alpha\) family [Sheldahl et al. 1999; Winkbauer et al. 2001]. Taken together, these findings suggest that Xfz7 probably activates PKC\(_\text{H9254}\) through a heterotrimeric G protein that produces DAG. It will be important to determine which G protein is involved in this pathway and whether DAG is produced by G protein function.

**Mechanism of activation of the Xdsh/JNK pathway**

In this study, we showed that Xdsh and PKC\(_\text{H9254}\) form a complex and that the complex formation is not dependent on PKC\(_\text{H9254}\) activity. In addition, the activation of PKC\(_\text{H9254}\) is sufficient and necessary for the membrane localization of Xdsh in response to Xfz7. These findings suggest that Xfz7 may be involved in the translocation of the PKC\(_\text{H9254}\)-Xdsh complex to the plasma membrane through the production of DAG. In other words, PKC\(_\text{H9254}\) recruits Xdsh to the membrane in response to Xfz7 signaling. It will be necessary to determine which domain of Xdsh interacts with PKC\(_\text{H9254}\) and vice versa. Our preliminary work showed that a C-terminal fragment including the DEP domain of Xdsh coimmunoprecipitated with PKC\(_\text{H9254}\) as well as the full-length Xdsh protein [A. Miyakoshi, unpubl.]. This is consistent with the fact that this domain of Dishevelled is sufficient for its membrane translocation and function in the PCP pathway [Axelrod et al. 1998; Boutros et al. 1998, Moriguchi et al. 1999; Rothbacher et al. 2000, Wallingford et al. 2000].

The Dishevelled protein is known to be hyperphosphorylated in response to Wnt and Frizzled [Yanagawa et al. 1995, Willert et al. 1997, Rothbacher et al. 2000, Tada and Smith 2000]. We showed that the loss of PKC\(_\text{H9254}\) function blocked this phosphorylation of Xdsh. It has been shown that the phosphorylation and membrane localization of Xdsh are closely related [Rothbacher et al. 2000]. The simplest model is that DAG activates PKC\(_\text{H9254}\) on the membrane, and PKC\(_\text{H9254}\) phosphorylates Xdsh directly. Kuhl et al. (2001) showed that PKC\(_\text{x}\) phosphorylates Xdsh in vitro. PKC\(_\text{H9254}\) may have the similar activity. However, Dishevelled is known to interact with other kinases, such as casein kinases 1 and 2, Par-1, and PAK1/MuSK [Willert et al. 1997; Sun et al. 2001; Vielhaber and Virshup 2001, Luo et al. 2002]. PKC\(_\text{H9254}\) may regulate such protein kinases and thus indirectly regulate Xdsh phosphorylation. It would be interesting to examine whether PKC\(_\text{H9254}\) phosphorylates Xdsh directly, and to elucidate the
Role of Xdsh phosphorylation in its localization and in the activation of downstream signaling. Determination of the sites in Xdsh that are phosphorylated by Xfz7 signaling awaits further study.

The following three results indicate that PKCδ mediates the activation of JNK by Xfz7: (1) JNK activation by Xfz7 was inhibited by the loss of PKCδ function. (2) The activation of PKCδ by PMA was sufficient for JNK activation. (3) The gastrulation-defective phenotype of PKCδ MO was rescued by active MKK7, which activates JNK. JNK has been implicated in the noncanonical Wnt pathway (Boutros et al. 1998; Yamanaka et al. 2002), but it is still unknown how Xdsh activates the JNK pathway. The membrane localization and/or phosphorylation of Xdsh may enable other proteins such as Rho to interact with Xdsh to activate the JNK cascade. It will be interesting and important to learn how JNK regulates convergent extension movements during gastrulation.

Specificity of PKC in the Wnt pathway

The PKC family is comprised of three subfamilies, cPKC, nPKC, and aPKC. cPKC and nPKC can be activated by DAG and/or Ca²⁺, both of which may increase upon activation of the Wnt signaling pathway. In addition to PKCδ, which belongs to the nPKC subfamily, two cPKCs, PKCo and PKCb, are expressed during Xenopus gastrulation. We decided to focus on PKCδ for the following reason. Truncated PKCo, PKCb, and PKCδ lacking the catalytic domain were constructed and overexpressed in Xenopus embryos, with the assumption that they would function as dominant-negative mutants. PKCδΔC seemed to function as a dominant-negative mutant because the phenotype of Xenopus embryos injected with PKCδΔC was very similar to that caused by the injection of PKCδ MO and was rescued by full-length PKCδ. Of these three mutants, PKCδΔC blocked gastrulation movements very effectively, whereas PKCoΔC or PKCbΔC had little effect, although comparable levels of protein expression were detected by Western blotting. We also made constructs with point mutations at the lysine residue in the ATP-binding domain, which are generally used as dominant-negative mutants for PKCs and other kinases (e.g., see Li et al. 1996). When these forms were overexpressed, the PKCo and PKCb mutants had no effect on gastrulation [data not shown]. These results indicated that PKCo and PKCb might not play a crucial role in this process. Although PKCδ regulates the Wnt/JNK pathway, as we have shown, it is possible that PKCo and/or PKCb may be mainly involved in the Wnt/Ca²⁺ pathway, although we cannot completely exclude the possibility that PKCo and/or PKCb may also be involved in the Wnt/JNK pathway.

Role of PKCδ in gastrulation movements

Convergent extension is comprised of several steps involving changes in cell morphology and movements. As convergent extension begins, cells extend lamellipodia in random directions. The cells are then polarized and become narrow along the mediolateral direction, followed by the intercalation of these cells. Xdsh function is required for this regulation of cell polarity (Wallingford et al. 2000). We clearly showed that PKCδ MO-injected cells were not polarized, nor did they participate in the intercalation, indicating that PKCδ is essential for controlling cell polarity and the change in cell shape during convergent extension movements.

Interestingly, PKCδ and Xdsh tagged with Venus were localized around the tips of the elongated cells. In addition, Rac and Arp3 are also localized in the same regions. It is known that these regions have a lamellipodial protrusive activity. Arp3 is one of Arp2/3 complex components, which is a key regulator of the actin polymerization in lamellipodial protrusion of membranes (Suetsugu et al. 2002; Weaver et al. 2003). The Arp2/3 complex is also known to be regulated by Rac (Suetsugu et al. 2002). It is strongly suggested that the proper localization of Rac, Arp3, and other cytoskeletal regulators may be important for cell elongation and intercalative movements. Without PKCδ function, cells were round-shaped, and Rac and Arp3 did not localize in the specific region. This suggests that PKCδ, Xdsh and the downstream PCP signaling may be required for the localization of such machinery, including the Arp2/3 complex.

Materials and methods

Plasmids, RNA synthesis, and morpholino oligos

Our Xenopus EST database [NIBB XDB, http://Xenopus.nibb.ac.jp] was searched with the cDNA sequences of mammalian nPKC family members using BLAST. Full-length cDNA clones, XL011f02 and XL066d07, were identified. These clones were sequenced and designated PKCδ1 and PKCδ2, respectively. GenBank accession numbers are AB109739 and AB109740, respectively. Plasmids for the expression in Xenopus embryos were constructed with PCR products inserted into the expression vector pCS2+. Capped mRNAs were synthesized using the mMESSAGE mMACHINE kit (Ambion). PKCδΔC contained the N-terminal regulatory domain [1–347 amino acids] of PKCδ1. A plasmid bearing the gene for constitutively active MKK7 [MKK7 DED] was a kind gift from Dr. E. Nishida (Kyoto University, Japan). The plasmid bearing the gene for GAL4[DBD]-tagged c-Jun was a kind gift from Dr. M. Tada (National Institute for Medical Research, UK). The myc-Xdsh was a kind gift from Dr. R. Harland (University of California, Berkeley). For the Venus-tagged constructs, the indicated fragments were amplified with PCR, fused to Venus gene (Nagai et al. 2002), and sequenced. For mb-Venus, a cDNA fragment of mb-Venus was cloned by PCR using the plasmid XL213p09 (NIBB XDB) as a template. PKCδΔC was fused to the N terminus of Venus, and Xdsh and XdshADEP were fused to the C terminus.

Antisense morpholinos were obtained from Gene Tools. The morpholino oligo sequences were as follows: PKCδ1 MO, 5′-AGGATATGGCTTAGGAAAGGACATG-3′; PKCδ2 MO, 5′-AGGATATGGCTTAGGAAAGGACATG-3′; PKCδ3 MO, 5′-AGGATATGGCTTAGGAAAGGACATG-3′.
AGGATAAGCGTAGGAAAGGAGCCAT-3’; Control MO, 5’-CCTCTTACCTCAGTTACAATTTATA-3’.

In situ hybridization and RT–PCR analysis

In situ hybridization in *Xenopus* Ueno (2003). For RT–PCR analyses, RNA from *Xenopus* tracing cell lineage was carried out as described by Kurata and Harland (1991). The detection of synthesized with Reverse Transcriptase (#TRT-101, Toyobo). cDNA was described in Yamamoto et al. (2001) and those for *TAGC*-3 were prepared with Trizol (Life Technologies). cDNA was described in Yamamoto et al. (2001) and those for *PKC* were as described in Dr. De Robertis’ home page [http://www.hhmi.edu/derobertis/index.html]. Primers for *Xmyf5* were 5’-CACAGATGGA GATGG TAGATAGC-3’; those for *Xwnt11* were 5’-AAGT-GCCACGGAGT GTCT GG-3’ and 5’-CTCAACTTCTCCTAGGCC-3’; and those for *PKCβ* were 5’-TTTATTACACCCACAGATTGAGCCG-3’ and 5’-AACTACATTCAGTAAACCAG-3’.

Whole-mount immunostaining and immunocytochemistry of *Xenopus* embryos

The procedure for whole-mount immunostaining was as described in Kurata et al. (2001). The antibodies were MZ15 for notochord [a kind gift from Dr. F. Watt; Smith and Watt (1985) and 12/101 for somites (Development Studies Hybridoma Bank, Kintner and Brockes 1984). As secondary antibodies, horseradish peroxidase-conjugated and alkaline phosphatase-conjugated antibodies were used for MZ15 and 12/101, respectively.

For immunocytochemistry, each epitope-tagged mRNA was injected into the animal pole of two-cell embryos. The animal caps were dissected from stage 9–10 embryos and fixed with MEMFA, followed by immunostaining by a standard method using a fluorescence-labeled secondary antibody. The localizations were determined by laser-scanning confocal microscopy, using a Carl Zeiss LSM510 microscope. The antibodies for immunocytochemistry were anti-myc 9E1 [Boehringer Mannheim] and rabbit polyclonal anti-Flag (Sigma) antibodies. For PMA treatment, phorbol 12-myristate 13-acetate (#P1585, Sigma) was used.

Elongation assay in *Xenopus* animal cap and DMZ explants

For the animal cap explants, mRNAs or a morpholino oligonucleotide were coinjected with 0.5 pg activin mRNA into the animal pole of two-cell embryos. The animal cap was dissected manually from stage 9 embryos. For DMZ explants, mRNA or a morpholino oligonucleotide were injected into the two dorsal blastomeres of four-cell embryos. Explants were isolated at stage 10+. These explants were cultured in 0.1% BSA/1× Steinberg’s solution until sibling embryos reached stage 17. The procedure for observing cells during convergent extension movements was basically according to Wallingford et al. (2000) with some modifications. Explants were isolated at stage 10+ and cultured in 1× Steinberg’s solution on a cover glass coated with fibronectin. The explants were observed by laser-scanning confocal microscopy.

Immunoprecipitation and Western blotting

HEK293T cells were transiently transfected with the indicated constructs using Lipofectamine Plus (Invitrogen). Cell lysates were prepared in PBS containing 0.1% Triton-X100, 20 mM NaF, 0.5 mM PMSF, and a 1/200 volume of protease inhibitor cocktail (#P8340, Sigma), and spun at 15,000g for 10 min. The indicated antibodies were added to the supernatants, and incubated at 4°C overnight. Protein A/G agarose (#SC-2003, Santa Cruz Biotechnology) was added, and the mixture was incubated for 1 h in a tumbling mixer. The agarose beads were washed five times with the lysis buffer. The antibodies used for immunoprecipitation and Western blotting were anti-myc 9E1 [Boehringer Mannheim], anti-Flag monoclonal M2 (Sigma), and anti-GFP (#598, Molecular Biological Laboratories) antibodies.

**JNK assay**

mRNA encoding GAL4 (DBD)-tagged c-Jun [100 pg] was injected into two-cell embryos. The animal caps were isolated at stage 10 and smashed by pipetting in sample buffer for SDS-PAGE. These samples were boiled and fractionated by SDS-PAGE. Western blotting was performed using anti-GAL4 (DBD, #SC-510, Santa Cruz Biotechnology) and anti-phospho-c-jun (#9261S, Cell Signaling) antibodies.

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References

Adler, P.N. 2002. Planar signaling and morphogenesis in *Drosophila*. Dev. Cell 2: 525–535.

Axelrod, J.D. 2001. Unipolar membrane association of Dishevelled mediates planar cell polarity signaling. *Genes & Dev.* 15: 1182–1187.

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: 2610–2622.

Baum, E.Z. and Bebermitz, G.A. 1990. K-ras oncogene expression in *Xenopus laevis*. Oncogene 5: 763–767.

Bell, R.M. and Burns, D.J. 1991. Lipid activation of protein kinase C. *J. Biol. Chem.* 266: 4661–4664.

Boutros, M. and Mlodzik, M. 1999. Dishevelled: At the crossroads of divergent intracellular signaling pathways. *Mech. Dev.* 83: 27–37.

Boutros, M., Paricio, N., Strutt, D.I., and Mlodzik, M. 1998. Dishevelled activates JNK and discriminates between JNK pathways in planar polarity and wingless signaling. *Cell* 94: 109–118.

Brannon, M. and Kimelman, D. 1996. Activation of Siamois by the Wnt pathway. *Dev. Biol.* 180: 344–347.

Cadijan, K.M. and Nusse, R. 1997. Wnt signaling: A common theme in animal development. *Genes & Dev.* 11: 3286–3305.

Carnac, G., Kodjabachian, L., Gurdon, J.B., and Lemaire, P.
Role of PKCδ in *Xenopus* gastrulation

1996. The homeobox gene *Siamois* is a target of the Wnt dorsalisation pathway and triggers organiser activity in the absence of mesoderm. *Development* **122**: 3055–3065.

Chen, K.H., Peng, Z.G., Lauv, S., and Kung, H.F. 1988. Molecular cloning and sequence analysis of two distinct types of *Xenopus laevis* protein kinase C. *Second Messengers Phosphoproteins* **12**: 251–260.

Cohen, E.D., Mariol, M.C., Wallace, R.M., Weyers, J., Kamberov, Y.G., Pradel, J., and Wilder, E.L. 2002. DWnt4 regulates cell movement and local adhesion kinase during *Drosophila* ovarian morphogenesis. *Dev. Cell* **2**: 437–448.

Djiane, A., Roujou, I., Umhauer, M., Boucaut, J., and Shi, D. 2000. Role of frizzled 7 in the regulation of convergent extension movements during gastrulation in *Xenopus laevis*. *Development* **127**: 3091–3100.

Habas, R., David, I.B., and He, X. 2003. Coactivation of Rac and rho by Wnt/Frizzled signaling is required for vertebrate gastrulation. *Genes & Dev.* **17**: 295–309.

Harland, R.M. 1991. In situ hybridization: An improved whole-mount method for *Xenopus* embryos. *Methods Cell Biol.* **36**: 685–695.

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. Silverblick/Wnt11 mediates convergent extension movements during zebrafish gastrulation. *Nature* **405**: 76–81.

Kemp, R.E., Parker, M.W., Hu, S., Tiganis, T., and House, C. 1994. Substrate and pseudosubstrate interactions with protein kinases: Determinants of specificity. *Trends Biochem. Sci.* **19**: 440–444.

Kikkawa, U., Kishimoto, A., and Nishizuka, Y. 1989. The protein kinase C family: Heterogeneity and its implications. *Annu. Rev. Biochem.* **58**: 31–44.

Kintner, C. and Brockes, J. P. 1984. Monoclonal antibodies identify blastema cells derived from differentiating muscle in newt limb regeneration. *Nature* **308**: 67–69.

Kuhl, M. 2002. Noncanonical Wnt signaling in *Xenopus*: Regulation of axis formation and gastrulation. *Semin. Cell Dev. Biol.* **13**: 243–249.

Kuhl, M., Sheldahl, L.C., Park, M., Miller, J.R., and Moon, R.T. 2000. The Wnt/Ca2+ pathway: A new vertebrate Wnt signaling pathway takes shape. *Trends Genet.* **16**: 279–283.

Kuhl, M., Geis, K., Sheldahl, L.C., Pukrov, T., Moon, R.T., and Wedlich, D. 2001. Antagonistic regulation of convergent extension movements in *Xenopus* by Wnt/β-catenin and Wnt/Ca2+ signaling. *Mech. Dev.* **106**: 61–76.

Kurata, T. and Ueno, N. 2003. *Xenopus* Nbx, a novel NK-1 related gene essential for neural crest formation. *Dev. Biol.* **257**: 30–40.

Kurata, T., Nakabayashi, Y., Yamamoto, T.S., Mochii, M., and Ueno, N. 2001. Visualization of endogenous BMP signaling during *Xenopus* development. *Differentiation* **67**: 33–40.

Li, W., Miichi, P., Aliamandi, M., Lorenzi, M.V., Wu, Y., Wang, L.H., Heidaran, M.A., and Pierce, J.H. 1996. Expression of an activated rat frizzled-1 to the *Xenopus* embryo and its role in early embryogenesis. *J. Biol. Chem.* **271**: 93057–93062.

Luo, Z.G., Wang, Q., Zhou, J.Z., Wang, J., Luo, Z., Liu, M., He, X., Wynshaw-Boris, A., Xiong, W.C., Lu, B., et al. 2002. Regulation of AChR clustering by Dishevelled interacting with MuSK and PAK1. *Neuron* **35**: 489–505.

McKendry, R., Hsu, S.C., Harland, R.M., and Grosschedl, R. 1997. LEF-1/TCF proteins mediate Wnt-inducible transcription from the *Xenopus* nodal-related 3 promoter. *Dev. Biol.* **192**: 420–431.

Medina, A. and Steinbeisser, H. 2000. Interaction of Frizzled 7 and Dishevelled in *Xenopus*. *Dev. Dyn.* **218**: 671–680.

Medina, A., Reintsch, W., and Steinbeisser, H. 2000. *Xenopus* frizzled 7 can act in canonical and noncanonical Wnt signaling pathways: Implications on early patterning and morphogenesis. *Meth. Dev.* **92**: 227–237.

Moriguchi, T., Kawachi, K., Kamakura, S., Masuyama, N., Yamakana, H., Matsumoto, K., Kikuchi, A., and Nishida, E. 1999. Distinct domains of mouse dishevelled are responsible for the c-Jun N-terminal kinase/stress-activated protein kinase activation and the axis formation in vertebrates. *J. Biol. Chem.* **274**: 30957–30962.

Nagai, T., Ibata, K., Park, E.S., Kubota, M., Mikoshika, K., and Miyawaki, A. 2002. A variant of yellow fluorescent protein with fast and efficient maturation for cell-biological applications. *Nat. Biotechnol.* **20**: 87–90.

Newton, A.C. 1997. Regulation of protein kinase C. *Curr. Opin. Cell Biol.* **9**: 161–167.

Nieuwoop, P.D. and Faber, J. 1994. Normal Table of *Xenopus laevis* (Daudin), pp. 163–188. Garland Publishing Inc., New York.

Nishizuka, Y. 1995. Protein kinase C and lipid signaling for sustained cellular responses. *FASEB J.* **9**: 484–496.

Ort, J.W. and Newton, A.C. 1994. Intrapeptide regulation of protein kinase C. *J. Biol. Chem.* **269**: 8383–8387.

Otte, A.P. and Moon, R.T. 1992. Protein kinase C isoforms have distinct roles in neural induction and competence in *Xenopus*. *Cell Biol.* **68**: 1021–1029.

Otto, A.P., Koster, C.H., Snoek, G.T., and Durston, A.J. 1988. Protein kinase C mediates neural induction in *Xenopus laevis*. *Nature* **334**: 618–620.

Pandur, P., Lasche, M., Eisenberg, L.M., and Kuhl, M. 2002. Wnt-11 activation of a noncanonical Wnt signalling pathway is required for cardiogenesis. *Nature* **418**: 636–641.

Rauch, G.J., Hammerschmidt, M., Blader, P., Schauerte, H.E., Strahle, U., Ingham, P.W., McManus, A.P., and Halfter, P. 1997. Wnt5 is required for tail formation in the zebrafish embryo. *Cold Spring Harb. Symp. Quant. Biol.* **62**: 237–234.

Rothbacher, U., Laurent, M.N., Deardorff, M.A., Klein, P.S., Cho, K.W., and Fraser, S.E. 2000. Dishevelled phosphorylation, subcellular localization and multimerization regulate its role in early embryogenesis. *EMBO J.* **19**: 1010–1022.

Sheldahl, L.C., Park, M., Malbon, C.C., and Moon, R.T. 1999. Protein kinase C is differentially stimulated by Wnt and Frizzled homologs in a G-protein-dependent manner. *Curr. Biol.* **9**: 695–698.

Shih, J. and Keller, R. 1992. Cell motility driving mediodiateral intercalation in explants of *Xenopus laevis*. *Development* **116**: 901–914.

Slusarski, D.C., Corces, V.G., and Moon, R.T. 1997a. Interaction of Wnt and a Frizzled homologue triggers G-protein-linked phosphatidylinositol signalling. *Nature* **390**: 410–413.

Slusarski, D.C., Yang-Nsne, J., Busa, W.B., and Moon, R.T. 1997b. Modulation of embryonic intracellular Ca2+ signaling by Wnt-5A. *Dev. Biol.* **182**: 114–120.

Smith, J.C. and Watt, F.M. 1985. Biochemical specificity of *Xenopus* notochord. *Differentiation* **29**: 109–115.

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**: 753–765.

Suetsugu, S., Miki, H., and Takenawa, T. 2002. Spatial and temporal regulation of actin polymerization for cytoskeleton formation through Arp2/3 complex and WASP/WAVE proteins. *Cell Motil. Cytoskeleton* **51**: 113–122.

Sun, T.Q., Lu, B., Feng, J.J., Reinhard, C., Jan, Y.N., Fantl, W.J., and Williams, L.T. 2001. PAR-1 is a Dishevelled-associated kinase and a positive regulator of Wnt signalling. *Nat. Cell Biol.* **3**: 628–636.

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.

Tada, M., Concha, M.L., and Heisenberg, C.P. 2002. Noncanonical Wnt signalling and regulation of gastrulation movements. *Semin. Cell Dev. Biol.* **13**: 251–260.

Vielhaber, E. and Virshup, D.M. 2001. Casein kinase I: From obscurity to center stage. *IUBMB Life* **51**: 73–78.

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

Wallingford, J.B., Fraser, S.E., and Harland, R.M. 2002. Convergent extension: The molecular control of polarized cell movement during embryonic development. *Dev. Cell* **2**: 695–706.

Weaver, A.M., Young, M.E., Lee, W.-L., and Cooper, J.A. 2003. Integration of signals to the Arp2/3 complex. *Curr. Op. Cell Biol.* **15**: 23–30.

Wharton Jr., K.A. 2003. Runnin’ with the Dvl: Proteins that associate with Dsh/Dvl and their significance to Wnt signal transduction. *Dev. Biol.* **253**: 1–17.

Willert, K., Brink, M., Wodarz, A., Varmus, H., and Nusse, R. 1997. Casein kinase 2 associates with and phosphorylates dishevelled. *EMBO J.* **16**: 3089–3096.

Wilson, P. and Keller, R. 1991. Cell rearrangement during gastrulation of *Xenopus*: Direct observation of cultured explants. *Development* **112**: 289–300.

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

Yamamoto, T.S., Takagi, C., Hyodo, A.C., and Ueno, N. 2001. Suppression of head formation by Xmsx-1 through the inhibition of intracellular nodal signaling. *Development* **128**: 2769–2779.

Yamanaka, H., Moriguichi, T., Masuyama, N., Kusakabe, M., Hanafusa, H., Takada, R., Takada, S., and Nishida, E. 2002. JNK functions in the noncanonical Wnt pathway to regulate convergent extension movements in vertebrates. *EMBO Rep.* **3**: 69–75.

Yanagawa, S., van Leeuwen, F., Wodarz, A., Klingensmith, J., and Nusse, R. 1995. The dishevelled protein is modified by wingless signaling in *Drosophila*. *Genes & Dev.* **9**: 1087–1097.

Yang-Snyder, J., Miller, J.R., Brown, J.D., Lai, C.J., and Moon, R.T. 1996. A frizzled homolog functions in a vertebrate Wnt signaling pathway. *Curr. Biol.* **6**: 1302–1306.

Zhang, G., Kazanietz, M.G., Blumberg, P.M., and Hurley, J.H. 1995. Crystal structure of the cys2 activator-binding domain of protein kinase C δ in complex with phorbol ester. *Cell* **81**: 917–924.
PKCδ is essential for Dishevelled function in a noncanonical Wnt pathway that regulates *Xenopus* convergent extension movements

Noriyuki Kinoshita, Hidekazu Iioka, Akira Miyakoshi, et al.

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