PTK7 recruits dsh to regulate neural crest migration

Iryna Shnitsar and Annette Borchers*

PTK7 regulates planar cell polarity (PCP) signaling during vertebrate neural tube closure and establishment of inner ear hair cell polarity; however, its signaling mechanism is unknown. Here, we demonstrate a new function for PTK7 in Xenopus neural crest migration and use this system in combination with in vitro assays to define the intersection of PTK7 with the non-canonical Wnt signaling pathway that regulates PCP. In vitro, using Xenopus ectodermal explants, we show that PTK7 recruits dishevelled (dsh) to the plasma membrane, a function that is dependent on the PDZ domain of dsh, as well as on the conserved kinase domain of PTK7. Furthermore, endogenous PTK7 is required for frizzled7-mediated dsh localization. Immunoprecipitation experiments confirm that PTK7 can be found in a complex with dsh and frizzled7, suggesting that it cooperates with frizzled to localize dsh. To evaluate the in vivo relevance of the PTK7-mediated dsh localization, we analyzed Xenopus neural crest migration, as loss-of-function of PTK7 inhibits neural crest migration in whole embryos as well as in transplanted neural crest cells. Supporting the in vivo role of PTK7 in the localization of dsh, a PTK7 deletion construct deficient in dsh binding inhibits neural crest migration. Furthermore, the PTK7-mediated membrane localization of a dsh deletion mutant lacking PCP activity inhibits neural crest migration. Thus, PTK7 regulates neural crest migration by recruiting dsh, providing molecular evidence of how PTK7 intersects with the PCP signaling pathway to regulate vertebrate cell movements.

KEY WORDS: Planar cell polarity, Neural crest migration, Xenopus, dsh, fz7

INTRODUCTION

Wnt signaling controls diverse developmental processes such as axis formation, anteroposterior patterning and the development of the neural crest (Moon et al., 1997; Raible and Ragland, 2005; Wodarz and Nusse, 1998). Precise regulation is necessary for many developmental processes and misregulation of several components of the canonical Wnt signaling pathway has been implicated in cancer formation (Polakis, 2007). Conversely, reduction or loss of function of Wnt signaling leads to general developmental defects or loss of organs, demonstrating the need for a tight regulation of the levels of Wnt signaling (Logan and Nusse, 2004).

The Wnt signaling pathway is highly conserved among all metazoans. Wnt ligands bind to frizzled (fz) transmembrane receptors leading to accumulation and nuclear localization of β-catenin, which serves as a transcriptional co-activator for TCF/Lef transcription factors (Logan and Nusse, 2004; Wodarz and Nusse, 1998). In addition to this so-called ‘canonical’ Wnt signaling pathway, fz receptors also activate alternative signaling pathways like the planar cell polarity (PCP) pathway, which defines the orientation of cells in the plane of an epithelium (Klein and Mlodzik, 2005; Seifert and Mlodzik, 2007). PCP signaling has been best characterized in Drosophila, where it determines for example the ommatidia organization in the eye and the bristle hair orientation in the wing (Axelrod and McNeill, 2002; Klein and Mlodzik, 2005). In vertebrates, PCP signaling is necessary for the orientation of the stereocilia bundles in the neurosensory epithelium of the inner ear, and dynamic convergent extension movements during gastrulation and neurulation (Wallingford et al., 2002; Wang and Nathans, 2007).

Dishevelled (dsh) is a key regulator of both the canonical as well as the PCP signaling pathway and regulates cell fate specification as well as cell movements such as convergent extension. Dsh consists of three major conserved domains, the DIX, PDZ and DEP domain, that have been implicated in different downstream signaling events (Boutros and Mlodzik, 1999; Wallingford and Habas, 2005). The DIX domain is used for canonical Wnt signaling, whereas the DEP domain is involved in PCP signaling (Boutros et al., 1998; Habas et al., 2003; Itoh et al., 2000; Rothbacher et al., 2000). By contrast, the PDZ domain is shared by both pathways. As downstream effectors of dsh are distinct for canonical and PCP signaling, it remains unclear how dsh selectively activates one or the other pathway. In the vertebrate PCP pathway, the signaling mechanisms affecting the subcellular localization and choice of effectors proteins of dsh are not well defined.

PTK7 (protein tyrosine kinase 7) is a regulator of PCP signaling that could modulate the dsh localization as well as the interaction with pathway-specific effector proteins. PTK7 regulates PCP in the inner ear hair cells and during neural tube closure in mice (Lu et al., 2004). In Xenopus, PTK7 is required for neural convergent extension (Lu et al., 2004). PTK7 is a transmembrane protein containing seven extracellular immunoglobulin domains and a kinase homology domain. Although the kinase domain lacks the DFG triplet necessary for catalytic activity, its overall structure is evolutionary conserved from Hydra to humans (Krohmer et al., 2001; Miller and Steele, 2000). The signaling mechanism of PTK7 has not been characterized, but considering the structure and function of PTK7, it could affect the localization of dsh as well as its downstream signaling.

Neural crest migration is a new system to analyze the mechanistic role of PTK7 in vertebrate PCP signaling. Neural crest cells are induced at the border region of the neural plate and migrate on defined routes throughout the embryo, where they give rise to a variety of derivatives ranging from neurons and glia cells of the peripheral nervous system, to cartilage and pigment cells. Although canonical Wnt signaling plays a role in the induction, delamination and differentiation of neural crest cells (Schmidt and Patel, 2005;
Yanfeng et al., 2003), PCP signaling seems to be required for neural crest migration. De Calisto et al. have shown that a dsh mutant lacking PCP activity inhibits neural crest migration. Conversely, inhibition of neural crest migration by loss of Wnt11 function can be rescued by expressing a dsh mutant, which activates PCP signaling in neural crest cells (De Calisto et al., 2005). Furthermore, ‘core-PCP signals’ such as Van Gogh/strabismus, prickle and daam are expressed in cranial neural crest cells (Bekman and Henrique, 2002; Darken et al., 2002; Goto and Keller, 2002; Nakaya et al., 2004), indicating that the PCP signaling cascade is active during neural crest migration.

Here, we identify a new function for PTK7 in neural crest migration. By analyzing the signaling mechanism of Xenopus PTK7 in vitro in animal cap explants and in vivo in migrating neural crest cells, we provide evidence that PTK7 localizes dsh to the plasma membrane and that this function is required for neural crest migration.

MATERIALS AND METHODS

Construct design

Myc-tagged PTK7 (PTK7-myc) was amplified by PCR (forward primers 5'GGGACCATGCTGGGCGATTGTC3'c, reverse primers 5'CATTCTGATACCCCTTGCTTGCTGC3'), cut with BamHI and ClaI, and ligated into the respective restriction sites of pCS2-MT. HA-tagged PTK7 (PTK7-HA) was obtained from PTK7-myc by removing the myc-tag with ClaI and XbaI, and inserting an HA-tag generating SalI/BspI site of a700BA-GFP (Vallin et al., 2001) and inserted into the slug promoter and the GFP-coding sequence were excised from a700BA-GFP (Vallin et al., 2001) and ligated into the SpeI/BamHI site of a700BA-GFP (Vallin et al., 2001) and inserted into the Spec/BamHI site of a700BA-GFP (Vallin et al., 2001) and inserted into the slug promoter, full-length PTK7 was excised using Rsr II and Not I from a700BA-GFP (Vallin et al., 2001) and inserted into the slug promoter and the GFP-coding sequence were excised from a700BA-GFP (Vallin et al., 2001) and inserted into the slug promoter.

For immunoprecipitation, 50 stage 11 embryos were homogenized in 500 μl NOP buffer (10 mM Tris-HCl pH 7.8, 150 mM NaCl, 5% NP40) supplemented with protease and phosphatase inhibitors to a final concentration of 1 mM PMSF, 0.1 mM NaVO₄, 1 mM NaF and 1 mM β-glycerophosphate. Inhibitor tablets (Roche) were added according to the manufacturer’s instructions. Lysates were centrifuged at 16,000 g for 20 minutes at 4°C and the supernatant was pre-cleaned by a 30 minute incubation with Protein A sepharose (Amersham). After incubation with anti-myc antibodies (9E10, Sigma) for 1.5 hours at 4°C, protein A sepharose (Amersham) was washed three times with NOP buffer, boiled in Laemmli sample buffer and analyzed by western blotting using anti-HA antibodies (Covance).

RESULTS

PTK7 leads to membrane localization of dsh

PTK7 is necessary for disparate processes regulated by PCP signaling such as neural tube closure and hair cell polarity in the inner ear (Lu et al., 2004); however, its precise signaling mechanism is unknown. Although the genetic interaction of PTK7 with Vangl2 (Lu et al., 2004) suggests that PTK7 is an integral part of the PCP signaling cascade, so far its intersection with it has not been defined. One possibility is that PTK7 signals via dsh, which like PTK7 regulates neural convergent extension (Wallingford and Harland, 2001; Wang et al., 2006). A prerequisite for this would be that dsh co-localizes with PTK7 at the plasma membrane. To analyze whether this is the case, we employed the Xenopus animal cap assay, which has previously been used to demonstrate the interaction and co-localization of dsh with fz at the plasma membrane (Axelrod et al., 1998; Medina et al., 2000; Medina and Steinbeisser, 2000;
of wild-type PTK7, this deletion mutant (ΔPTK7) abolishes translocation of dsh. Indeed, in contrast to overexpression of animal caps. As the intracellular domain of PTK7 contains a kinase domain of PTK7 and the PDZ domain of dsh, deletion mutants were tested for co-localization in To identify the molecular domains necessary for the interaction of PTK7 with dsh, deletion mutants of the DIX, the PDZ and the DEP domain of dsh were required for dsh recruitment to the plasma membrane. Thus, the animal cap localization assay as well as the glycerol gradient density centrifugation indicate that PTK7 connects with the Wnt signaling pathway at the level of dsh.

**The kinase domain of PTK7 and the PDZ domain of dsh are required for co-localization**

To identify the molecular domains necessary for the interaction of PTK7 with dsh, deletion mutants were tested for co-localization in animal caps. As the intracellular domain of PTK7 contains a conserved tyrosine kinase motif, we analyzed whether its deletion abolishes translocation of dsh. Indeed, in contrast to overexpression of wild-type PTK7, this deletion mutant (ΔPTK7) was not able to translocate dsh to the plasma membrane (Fig. 1F,G). Furthermore, this mutant also failed to shift dsh to higher molecular weight fractions in *Xenopus* lysates separated by glycerol gradient centrifugation (see Fig. S1 in the supplementary material). Taken together, this indicates that the kinase motif of PTK7 is required for dsh recruitment to the plasma membrane.

Dsh contains different functional domains that are involved in canonical and non-canonical Wnt signaling, respectively (Wallingford and Harba, 2005). To identify which of these domains are necessary for the PTK7-dependent dsh-translocation, we expressed GFP-tagged deletion mutants of the DIX, the PDZ and the DEP domain of dsh (Fig. 2A) in animal caps. In the absence of PTK7 expression, all mutant dsh proteins were mainly localized in the cytoplasm (Fig. 2B,D,F,H). However, co-expression of PTK7 transferred ΔDIX- as well as ΔDEP-dsh to the membrane (Fig. 2C,G,H). In the case of the ΔDIX-dsh injected caps, a residual cytoplasmic staining remained that was not apparent in ΔDEP-dsh injected caps. By contrast, ΔPDZ-dsh was not translocated to the plasma membrane in the presence of PTK7 (Fig. 2E,H), indicating that the PDZ domain is required for function. In summary, these data show that the tyrosine kinase domain of PTK7 as well as the PDZ domain of dsh are necessary for the translocation of dsh to the plasma membrane.

**PTK7 is part of a fz7/dsh complex and is required for fz7-mediated dsh localization**

The ability of PTK7 to control dsh localization suggests that the two proteins might interact. We tested for binding by co-expressing HA-tagged PTK7 with myc-tagged dsh and immunoprecipitating with

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**Fig. 1. PTK7 recruits dsh to the plasma membrane.** Animal caps were injected with different tagged RNAs, and protein localization was analyzed by confocal microscopy. The GFP-tagged dsh (green, left), the co-expressed myc-tagged protein (red, middle) and the merged images (right) are shown. (A) GFP-tagged dsh is localized to the cytoplasm of animal caps injected with 100 pg *dsh*-GFP RNA. (B) Myc-tagged fz7 is predominantly membrane localized in animal caps injected with 100 pg *fz7*-myc RNA. (C) Co-injection of 100 pg *dsh*-GFP and 100 pg *fz7*-myc RNA leads to membrane recruitment of dsh. (D) PTK7 is membrane localized in animal caps injected with 500 pg myc-tagged PTK7 RNA. (E) Animal caps co-injected with 100 pg *dsh*-GFP RNA and 500 pg *PTK7*-myc RNA show membrane-recruitment of dsh. Cells that do not express PTK7 in the membrane do not show membrane-localization of dsh (white arrowhead). (F) Animal caps injected with 250 pg RNA coding for a PTK7 mutant lacking the conserved kinase domain (ΔPTK7) and 100 pg *dsh*-GFP RNA do not show membrane localization of dsh. (G) Graph summarizing the percentage of cells with cytoplasmic dsh localization DAPI co-staining was used. The total number of cells is indicated above each column.
either myc- or HA-antibodies. Independent of the antibodies used, we could not detect co-immunoprecipitation of PTK7 and dsh (data not shown), indicating that additional molecules are required for PTK7-mediated dsh localization. A likely candidate is fz7, which, like PTK7, is also able to recruit dsh to the plasma membrane. To test whether PTK7 forms a complex with fz7 and dsh, we expressed HA-tagged PTK7 with either myc-tagged dsh or myc-tagged fz7, or a combination of the two (see Fig. S2 in the supplementary material). Although HA-tagged PTK7 was co-precipitated with myc-tagged fz7 in one out of three experiments, we detected only robust co-precipitation in combination with myc-tagged dsh and myc-tagged fz7 (see Fig. S2 in the supplementary material), indicating that PTK7 is part of a protein complex that includes fz7 and dsh.

If PTK7 forms a complex with fz7 and dsh, PTK7 could affect the fz7-mediated dsh recruitment. To test this, we analyzed animal caps, which endogenously express PTK7 (see Fig. S3 in the supplementary material), how loss of PTK7 function interferes with the fz7-mediated dsh recruitment. In the presence of control morpholino oligonucleotides (MOs), fz7 recruits GFP-tagged dsh to the plasma membrane (Fig. 3A,D). However, the PTK7 MO abolished the fz7-mediated dsh translocation (Fig. 3B,D). This could be rescued by co-injection of PTK7 RNA lacking the MO binding site (Fig. 3C,D).

As fz-mediated dsh membrane localization correlates with hyperphosphorylation of dsh (Rothbacher et al., 2000), we further examined whether PTK7 loss of function also affects the phosphorylation status of dsh. Animal caps expressing myc-tagged dsh alone or in combination with PTK7 show only a single band in western blots using anti-myc antibodies. However, an additional high molecular weight band representing hyperphosphorylated dsh is detected in lysates expressing dsh with fz7 (Fig. 3E). Interestingly, this fz7-mediated hyperphosphorylation of dsh is inhibited by the PTK7 MO, indicating that PTK7 is required for the fz7-mediated dsh hyperphosphorylation. Thus, these data support that PTK7 is part of a dsh-fz7 complex required for dsh localization and phosphorylation.

PTK7 functions in cranial neural crest migration

To further evaluate the in vivo relevance of the PTK7-mediated dsh membrane localization, we focused on Xenopus neural crest migration. Recently, PCP signaling has been implicated in the regulation of neural crest migration (De Calisto et al., 2005). As PTK7 is expressed in premigratory (Fig. 4A,B) as well as migratory cranial neural crest cells (Fig. 4C), we analyzed whether PTK7 also functions in neural crest development. Xenopus laevis embryos were injected with PTK7 MO and GFP RNA as a lineage tracer in one blastomere at the two-cell stage and neural crest migration was analyzed at neurula and tadpole stages using in situ hybridization for different neural crest markers. Starting at the neurula stage, injection of the PTK7 MO inhibited neural crest migration. Although twist-positive cells were induced, they failed to migrate in PTK7 MO-
PTK7 recruits dsh

(A-D) PTK7 is required for fz7-mediated dsh recruitment and phosphorylation. (A) GFP-tagged dsh is localized to the plasma membrane in animal caps injected with 100 pg dsh-GFP RNA, 100 pg fz7-myc RNA and 20 ng control MO. (B) GFP-tagged dsh is not recruited to the plasma membrane in animal caps injected with 100 pg dsh-GFP and 100 pg fz7-myc RNA and 20 ng PTK7 MO. (C) Co-injection of 100 pg wild-type PTK7 RNA lacking the MO binding site rescues dsh-localization of animal caps injected with 100 pg dsh-GFP RNA, 100 pg fz7-myc RNA and 20 ng PTK7 MO. (D) Graph summarizing the percentage of cells with simultaneously membrane-localized dsh and fz7. The total number of cells is indicated on each column. (E) PTK7 is required for fz7-dependent hyperphosphorylation of dsh. Embryos were injected with 100 pg dsh-myc RNA, 100 pg fz7 RNA, 500 pg PTK7-myc RNA, 20 ng control MO or 20 ng PTK7 MO in the combinations indicated. Animal caps were cut at stage 9 and their lysates were analyzed by western blotting using anti-myc antibodies. Hyperphosphorylated dsh is detected as a second high molecular weight band. One representative experiment of three independent experiments is shown.

Injected embryos (Fig. 4H), whereas induction and migration of twist-positive cells was normal in embryos injected with the control MO (Fig. 4F). At tadpole stages, a few migrating neural crest cells are found in the PTK7 MO-injected embryos; however, their number as well as their migration distance is dramatically reduced compared with the control (Fig. 4J,K). Similar migration defects are also seen with other neural crest markers such as AP-2 (Fig. 4L,M) and Sox10 (data not shown), whereas the expression of the midbrain-hindbrain marker engrailed is not affected at these MO concentrations (Fig. 4I, right embryo). Thus, the PTK7 MO does not lead to morphological changes, but rather seems to specifically affect neural crest development.

To verify that the neural crest migration defect is caused by loss of PTK7 function, we performed rescue experiments. Embryos were injected with PTK7 MO in combination with increasing concentrations of wild-type PTK7 RNA lacking the MO-binding sites. Embryos were injected in one blastomere at the two-cell stage using lacZ RNA as a lineage tracer and neural crest migration was analyzed by twist in situ hybridization at tadpole stages. Fig. 3O summarizes a total of three injection experiments showing a concentration-dependent rescue effect of the PTK7 MO phenotype by wild-type PTK7 RNA. This demonstrates that the MO phenotype is specific to loss of PTK7 function.

In contrast to loss of PTK7 function, overexpression of PTK7 did not affect neural crest migration (Fig. 5). Different concentrations of myc-tagged and untagged PTK7 RNA were injected in one blastomere at the two-cell stage and neural crest migration was analyzed at neurula and tadpole stages. Neither neural crest induction nor neural crest migration were affected by overexpression of PTK7 (Fig. 5A-D), although the tagged PTK7 protein was detected by western blotting and immunostaining (Fig. 5E,F). Thus, PTK7 is required for neural crest migration; however, an excess of the protein seems not to disturb neural crest development. In summary, these data show that PTK7 is expressed and probably directly required in neural crest cells to enable their migration.

Transplanted neural crest cells require PTK7 for migration

To address the function of PTK7 specifically in migrating neural crest cells, we used neural crest transplantation assays (Borchers et al., 2000). Embryos were co-injected with PTK7 MO and GFP RNA in one blastomere at the two-cell stage. At early neurula stages (14-16), the fluorescent neural crest was removed and transplanted into control embryos from which the local neural crest had been removed (Fig. 6A). To distinguish ‘non-migrating’ grafts (Fig. 6D) from ‘migrating’ grafts (Fig. 6B) the GFP fluorescence of the transplanted cells was monitored at different time points. Five hours after transplantation, grafts injected with the control MO showed streams of migrating neural crest cells (Fig. 6B,F). However, most grafts injected with the PTK7 MO did not migrate (Fig. 6D,F). Twelve hours after grafting, the number of migrating PTK7 MO grafts (Fig. 6E) increased, but was still significantly lower compared with control MO grafts (Fig. 6F). Furthermore, even if PTK7 MO grafts migrated, they showed fewer migrating cells and did not migrate as far to the ventral side as did control grafts (Fig. 6C,E). The PTK7 MO phenotype is rescued by co-injection of wild-type PTK7 RNA lacking the MO binding sites (Fig. 6F), although the rescue effect is not as pronounced as in whole embryo injection experiments, which is probably due to the more challenging experimental procedure. In summary, the transplantation assay shows that transplanted neural crest cells require PTK7 for migration.

Fig. 3. PTK7 is required for fz7-mediated dsh recruitment and phosphorylation. (A-D) PTK7 is required for fz7-mediated dsh recruitment to the plasma membrane. GFP-tagged dsh is shown in green, myc-tagged fz7 in red, HA-tagged PTK7 in blue. (A) GFP-tagged dsh is localized to the plasma membrane in animal caps injected with 100 pg dsh-GFP RNA, 100 pg fz7-myc RNA and 20 ng control MO. (B) GFP-tagged dsh is not recruited to the plasma membrane in animal caps injected with 100 pg dsh-GFP and 100 pg fz7-myc RNA and 20 ng PTK7 MO. (C) Co-injection of 100 pg wild-type PTK7 RNA lacking the MO binding site rescues dsh-localization of animal caps injected with 100 pg dsh-GFP RNA, 100 pg fz7-myc RNA and 20 ng PTK7 MO. (D) Graph summarizing the percentage of cells with simultaneously membrane-localized dsh and fz7. The total number of cells is indicated on each column. (E) PTK7 is required for fz7-dependent hyperphosphorylation of dsh. Embryos were injected with 100 pg dsh-myc RNA, 100 pg fz7 RNA, 500 pg PTK7-myc RNA, 20 ng control MO or 20 ng PTK7 MO in the combinations indicated. Animal caps were cut at stage 9 and their lysates were analyzed by western blotting using anti-myc antibodies. Hyperphosphorylated dsh is detected as a second high molecular weight band. One representative experiment of three independent experiments is shown.
Neural-crest-specific expression of ΔkPTK7 inhibits neural crest migration

To study the function of PTK7 in neural crest migration isolated from earlier developmental defects, we targeted the expression of PTK7 to neural crest cells. PTK7 constructs were expressed under the control of the neural crest-specific slug promoter, so that only the cells that are already specified to become neural crest cells are affected. Regions of the cells that are already specified to become neural crest cells are the control of the neural crest-specific PTK7 to neural crest cells. PTK7 constructs were expressed under from earlier developmental defects, we targeted the expression of To study the function of PTK7 in neural crest migration isolated

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PTK7 recruits dsh

DISCUSSION
PCP signaling in vertebrates regulates complex cell movements, but the molecular mechanisms that guide these processes are not well understood. Here, we show that PTK7, a regulator of vertebrate PCP signaling, is required for neural crest migration, and we provide in vitro as well as in vivo evidence that PTK7 regulates PCP signaling by recruiting dsh to the plasma membrane.

Neural crest cells migrate extensively on defined routes throughout the embryo to give rise to a range of derivatives. However, the signaling mechanisms guiding these organized cell movements are not well characterized. As overexpression of dsh mutants, which are defective in PCP signaling, inhibits neural crest migration in *Xenopus* (De Calisto et al., 2005), this indicates that PCP signaling plays a role (De Calisto et al., 2005). Although, most PCP effectors are expressed at the right time and location for a function in neural crest migration (Bekman and Henrique, 2002; Darken et al., 2002; Goto and Keller, 2002; Nakaya et al., 2004), their function in this process has so far not been analyzed. Here, we identify PTK7 as the first regulator of PCP with a function in neural crest migration. Loss of function of PTK7 inhibits neural crest migration, and transplantation assays show that neural crest cells directly require PTK7 for...
migration. Furthermore, this function is independent of the role of PTK7 in neural tube closure, as demonstrated by neural crest-specific expression.

The data presented here, suggests that PTK7 regulates neural crest migration by recruiting dsh to the plasma membrane. Dsh occupies a key position at the crossroad of all branches of the Wnt signaling cascade and several lines of evidence indicate that PTK7 channels dsh signaling towards PCP signaling. First, PTK7 affects processes regulated by PCP signaling such as convergent extension/neural tube closure and inner ear hair cell polarity in vertebrates (Lu et al., 2004). Second, PTK7 displays heteroallelic interaction with another regulator of PCP signaling, Vangl2 (Looptail mice) (Lu et al., 2004). Third, PTK7 does not activate β-catenin signaling, but does activate JNK signaling. Neither in TOPFLASH reporter assays (data not shown) nor in Xenopus laevis double axis assays (0% in n=168) does PTK7 activate canonical Wnt signaling. However, PTK7 overexpression in Xenopus animal caps activates the phosphorylation of JNK (see Fig. S4 in the supplementary material), indicating PCP activity (Boutros et al., 1998; Habas et al., 2003; Li et al., 1999; Yamanaka et al., 2002). Thus, these data suggest that PTK7-mediated dsh recruitment activates PCP signaling.

In vivo evidence supports a model in which PTK7 recruits dsh to the plasma membrane, thereby regulating neural crest migration (Fig. 8A). This is experimentally supported by two observations. First, the PTK7 MO as well as ΔkPTK7, which both abolish PTK7-dependent dsh-recruitment, inhibit neural crest migration (Fig. 8B). Second, overexpression of wild-type slug-PTK7 together with slug-ΔDEP (J,K) or 50 pg slug-dsh (LM) or 50 pg slug-ΔPDZ (N,O). (P) Graph summarizing three dsh and PTK7 co-injection experiments. The number of injected embryos is indicated on each column.
implicated in homophilic binding (Lu et al., 2004; Pulido et al., 1992), PTK7 may affect cell clustering or cell contact persistence. The latter was shown for fz7, which modulates local cell contact persistence to coordinate cell movements during zebrafish gastrulation (Witzel et al., 2006). Furthermore, PTK7 could also function as a receptor for signals that guide migrating neural crest cells. Indeed, functional analyses of the Drosophila PTK7 ortholog (otk, off-track) indicate that PTK7 is a co-receptor for semaphorin/plexins (Winberg et al., 2001), which are guidance cues for migrating neural crest cells in vertebrates (Gammill et al., 2007; Gammill et al., 2006; Yu and Moens, 2005). The next challenge will be to further dissect the signaling mechanism of vertebrate PTK7 and analyze whether it can also respond to extracellular signals to guide neural crest migration.

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**Supplementary material**

Supplementary material for this article is available at http://dev.biologists.org/cgi/content/full/135/24/4015/DC1

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