Prickle 1 regulates cell movements during gastrulation and neuronal migration in zebrafish

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SUMMARY

During vertebrate gastrulation, mesodermal and ectodermal cells undergo convergent extension, a process characterised by prominent cellular rearrangements in which polarised cells intercalate along the medio-lateral axis leading to elongation of the antero-posterior axis. Recently, it has become evident that a noncanonical Wnt/Frizzled (Fz)/Dishevelled (Dsh) signalling pathway, which is related to the planar-cell-polarity (PCP) pathway in flies, regulates convergent extension during vertebrate gastrulation. Here we isolate and functionally characterise a zebrafish homologue of Drosophila prickle (pk), a gene that is implicated in the regulation of PCP. Zebrafish pk1 is expressed maternally and in moving mesodermal precursors. Abrogation of Pk1 function by morpholino oligonucleotides leads to defective convergent extension movements, enhances the silberblick (slb)/wnt11 and pipetail (Ppt)/wnt5 phenotypes and suppresses the ability of Wnt11 to rescue the slb phenotype. Gain-of-function of Pk1 also inhibits convergent extension movements and enhances the slb phenotype, most likely caused by the ability of Pk1 to block the Fz7-dependent membrane localisation of Dsh by downregulating levels of Dsh protein. Furthermore, we show that pk1 interacts genetically with trilobite (tri)/strabismus to mediate the caudally directed migration of cranial motor neurons and convergent extension. These results indicate that, during zebrafish gastrulation Pk1 acts, in part, through interaction with the noncanonical Wnt11/Wnt5 pathway to regulate convergent extension cell movements, but is unlikely to simply be a linear component of this pathway. In addition, Pk1 interacts with Tri to mediate posterior migration of branchiomotor neurons, probably independent of the noncanonical Wnt pathway.

Key words: Wnt signalling, Planar cell polarity, Convergent extension, Gastrulation, Neuronal migration, Zebrafish

INTRODUCTION

Morphogenetic movements driven by co-ordinated cell-shape changes coupled with directed cell migration, lead to the establishment of the body axis during early development. During vertebrate gastrulation, the large rearrangements of cell groups that generate the three germ layers overtly shape the embryonic axis. One such movement is convergence and extension (CE) in which mesendoderm and ectoderm undergo cell intercalations along the medio-lateral axis that narrow the tissues dorsalwards (convergence) and consequently extend them along the anterior-posterior axis (extension) (Concha and Adams, 1998; Keller et al., 2000). Cells undergoing medio-lateral intercalations are elongated along their medio-lateral axis and this is closely associated with polarised protrusive activity and re-organisation of the actin cytoskeleton (Shih and Keller, 1992).

Recent functional studies in Xenopus and genetic analyses of gastrulation mutants in zebrafish have revealed that a noncanonical Wnt pathway is involved in the regulation of CE. This pathway is related to the planar cell polarity (PCP) pathway that mediates the establishment of cell polarity in the plane of epithelia in Drosophila (reviewed in Adler, 2002; Mlodzik, 2002; Tada et al., 2002; Wallingford et al., 2002a). In vertebrates, the secreted glycoproteins Wnt11/Silberblick (Sib) and Wnt5/Pipetail (Ppt) act as ligands (Heisenberg et al., 2000; Rauch et al., 1997; Tada and Smith, 2000), although a Wnt ligand mediating PCP has yet to be found in Drosophila. Shared components of these pathways include: Fz receptors; an intracellular signal transducer, Dsh; a 4-pass transmembrane protein, Van gogh/Strabismus/Trilobite (Vang/Stbm/Tri); small GTPases RhoA and Cdc42; and a RhoA effector, Rho kinase 2 (Darken et al., 2002; Djiane et al., 2000; Goto and Keller, 2002; Habas et al., 2001; Heisenberg et al., 2000; Jessen et al., 2002; Marlow et al., 2002; Park and Moon, 2002; Tada and Smith, 2000; Wallingford et al., 2000). In vertebrates, a formin-like protein, Daam1, functions between Dsh and RhoA to regulate the actin cytoskeleton (Habas et al., 2001). Moreover,
downstream of the small GTPases, the activation of Jun-N-terminal kinase (JNK) appears to be required for proper CE movements in vertebrates and for establishing PCP in Drosophila ommatidia (Park and Moon, 2002; Yamanaka et al., 2002) (reviewed in Mlodzik, 1999).

prickle (pk) is one of a core group of PCP genes that controls planar polarity in the eye, leg and wing of Drosophila (Gubb et al., 1999). pk encodes an intracellular protein containing three LIM domains and a conserved ‘PET’ domain (for Prickle, Espinas and Testin). Epistasis analyses have demonstrated thatPk is required for some aspects of Fz/Dsh-mediated PCP signalling, but is not placed in a linear cascade with Fz and Dsh. Recent studies indicate that Pk regulates the subcellular distribution of Fz through binding to Dsh, thereby localising the Fz/Dsh complex to one side of the epithelial cells (Tree et al., 2002).

Recently, it has become evident that genes involved in CE may also be involved in mediating cell migration in the CNS. For instance, the PCP gene may also be involved in mediating cell migration in the CNS. For instance, the PCP gene

For transplantations, donor embryos were injected with either rhodamine-dextran (MW 10,000, Molecular probe) plus 5 pg pk1 RNA or fluorescein-dextran (MW 10,000, Molecular probe) at the one-cell stage. Cells were taken from late-blastula host embryos and transplanted into deep regions of the germ ring of host wild-type embryos as described previously (Heisenberg et al., 2000). Tailbud-staged embryos were mounted in 1.5% methylcellulose and image analysis was performed using Openlab software.

Analyses for sub-cellular protein localisation

To monitor Dsh localisation, embryos at the one-cell stage were injected with 200 pg RNA encoding Dsh-GFP either with or without 50 pg fz7 RNA and either with or without 5 pg pk1 RNA and were mounted in 1% agarose at 40% epiboly. Image analysis of living embryos was carried out using a Leica DMLFS confocal microscope with a 63x water-immersion lens. We were unable to monitor Pk1 localisation in living embryos because expression of a GFP-tagged version of Pk1, even at moderate doses caused embryos to develop abnormally at early stages. Therefore, to analyse Pk1 localisation embryos were injected with 25 pg of RNA encoding Venus-Pk1 (Venus is an EYFP-derivative that was kindly provided by Atsushi Miyawaki) and fixed at 40% epiboly for anti-GFP antibody staining.

Western-blot analysis

To monitor the levels of Dsh protein, embryos were injected with 200 pg RNA encoding myc-Dsh either with or without 50 pg fz7 RNA and either with or without 5 pg pk1 RNA at the one-cell stage. Blastoderm from 20 embryos at 40% epiboly were collected for Western-blot analysis after removal of the yolks according to a protocol kindly provided by Carl-Philipp Heisenberg (personal communication). Protein from the equivalent of five blastoderm was subject to SDS-PAGE (8% acrylamide gel) and then blotted to a PVDF membrane (Amersham). The membrane was reacted with 9E10 anti-myc monoclonal antibody (Santa Cruz Biotechnology) and subsequently with anti-mouse IgG conjugated with HRP followed by detection with ECL (Amersham). For loading control, the membrane was counter-stained with anti-β-tubulin monoclonal antibody (Sigma) and visualised with NBT and BCIP.
RESULTS

A planar polarity gene pk1 is expressed maternally and in moving mesodermal precursors

To investigate whether homologues of the *Drosophila* PCP gene *pk* function during vertebrate gastrulation, we isolated a full-length clone of a zebrafish *pk* gene from a shield library. The predicted protein encoded by zebrafish *pk1* shares 85% and 82% amino acid identity to *Xenopus* XPk-A (Wallingford et al., 2002b) in a highly conserved PET domain of unknown function and three LIM domains, respectively (Fig. 1A). Phylogenetic analysis on the basis of the conserved PET and LIM domains shows zebrafish *pk1* is more closely related to *Drosophila pk* and *espinas* than to another closely related gene *testin* (data not shown).

*pk1* is expressed maternally (Fig. 1B), and zygotic expression is initiated on the dorsal side of the embryo at pregastrula stages (Fig. 1C) and spreads throughout the germ ring by the shield stage (Fig. 1D). As gastrulation proceeds, expression becomes restricted to dorsal involuted cells and to overlying ectodermal cells, predominantly in axial tissues (Fig. 1E,F). By the tail-bud stage, expression is downregulated in the anterior ectoderm and becomes restricted to presomitic mesoderm, the posterior neuroectoderm and the lateral edge of the neural plate (Fig. 1G). The expression in the mesoderm and ectoderm is conserved in *Xenopus* (Wallingford et al., 2002b) and this prompted us to investigate whether *Pk1* is involved in the regulation of gastrulation movements.

Interfering with Pk1 function disrupts CE during gastrulation

To analyse the function of Pk1, we first employed an antisense approach using morpholino oligonucleotides against *pk1* (*pk1*-Mo) to reduce the level of endogenous Pk1 protein (Nasevicius and Ekker, 2000). Injection of 3 ng *pk1*-Mo led to a shorter body axis with a curled down tail at pharyngula stage (99%, n>500) (Fig. 2A,B). At higher doses, injected embryos exhibited a more severe phenotype with shorter trunk and tail, but occasionally this was associated with cell death in the brain at later stages (data not shown). Thereafter, we used a moderate dose (3 ng) for further analyses of the *pk1* morphant phenotype.

Although the curly tail phenotype of *pk1* morphants is not obviously indicative of CE defects, analyses with various markers revealed that gastrulation cell movements were affected. At tailbud to 2-somite stages, *pk1* morphants showed a slightly posteriorly located prechordal plate (hgg1), wider neural plate (*dlx3* and *pax2.1*), a shorter, wider notochord (ntl) and laterally expanded presomitic and head mesoderm (*papc* and *snail2*) (Fig. 2C-J). This phenotype is reminiscent of the CE mutants *slb*, *ppt*, *knypek* (*knv*) and *tri* in which gastrulation cell movements are disrupted but dorso-ventral and antero-posterior patterning remains unaffected (Hammerschmidt et al., 1996; Heisenberg and Nusslein-Volhard, 1997; Heisenberg et al., 2000; Jessen et al., 2002; Kilian et al., 2003; Rauch et al., 1997; Solnica-Krezel et al., 1996; Topczewski et al., 2001). Consistent with this, in *pk1* morphant embryos, the dorsal marker *chd* and ventral marker *bmp2b* are unchanged (Fig. 2K-N) and the telenephalic marker *enmsl* and midbrain marker *pax2.1* are expressed in the correct positions, although their expression domains are laterally expanded (Fig. 2O-R), reflecting reduced convergence of the neural plate (Fig. 2G,H). These results indicate that *pk1* is required for proper gastrulation CE movements of both mesendoderm and ectoderm but that it has no obvious role in the specifying cell fates.

**Fig. 1.** *pk1* is expressed maternally and in migrating mesodermal precursors in zebrafish. (A) Homology of Prickle proteins between zebrafish (*Pk1*), *Xenopus* (XPk-A) (Wallingford et al., 2002b) and human (BAB71198). The numbers refer to the percentage amino-acid identities between the different orthologues. (B-E) Expression of *pk1* at early stages. *pk1* is expressed maternally at the eight-cell stage (B, animal view), on the dorsal side at ~40% epiboly (C, lateral view with dorsal to the right), and around the germ ring at 50% epiboly (D, animal view with dorsal to the right). At 90% epiboly (E, dorsal view, F, lateral view, anterior is up), expression is in dorsal involuted cells and in overlying ectodermal cells and highlighted in the axial cells (arrowheads). At tailbud stage (G, dorsal view, anterior is up), expression is restricted to the presomitic mesoderm, posterior neuroectoderm, the lateral edge of neural plate (arrows) and anterior axial mesodermal cells (black arrowhead), but slightly downregulated in the posterior axial mesodermal cells (red arrowhead). d, dorsal; s, shield.

**Fig. 2.** *pk1*-Mo led to a shorter body axis with a curled down tail at pharyngula stage (99%, n>500) (A, B). At higher doses, injected embryos exhibited a more severe phenotype with shorter trunk and tail (C, D). As gastrulation proceeds, expression becomes restricted to dorsal involuted cells and to overlying ectodermal cells, predominantly in axial tissues (E, F). By the tail-bud stage, expression is downregulated in the anterior ectoderm and becomes restricted to presomitic mesoderm, the posterior neuroectoderm and the lateral edge of the neural plate (G). The expression in the mesoderm and ectoderm is conserved in *Xenopus* (Wallingford et al., 2002b) and this prompted us to investigate whether Pk1 is involved in the regulation of gastrulation movements. (A) Homology of Prickle proteins between zebrafish (*Pk1*), *Xenopus* (XPk-A) (Wallingford et al., 2002b) and human (BAB71198). The numbers refer to the percentage amino-acid identities between the different orthologues. (B-E) Expression of *pk1* at early stages. *pk1* is expressed maternally at the eight-cell stage (B, animal view), on the dorsal side at ~40% epiboly (C, lateral view with dorsal to the right), and around the germ ring at 50% epiboly (D, animal view with dorsal to the right). At 90% epiboly (E, dorsal view, F, lateral view, anterior is up), expression is in dorsal involuted cells and in overlying ectodermal cells and highlighted in the axial cells (arrowheads). At tailbud stage (G, dorsal view, anterior is up), expression is restricted to the presomitic mesoderm, posterior neuroectoderm, the lateral edge of neural plate (arrows) and anterior axial mesodermal cells (black arrowhead), but slightly downregulated in the posterior axial mesodermal cells (red arrowhead). d, dorsal; s, shield.

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slb and pk1. Injection of pk1-Mo in slb embryos enhanced the CE defect compared to either mutant/morphant alone, with the consequence that the prechordal plate remained posteriorly located beneath the neural plate (50%, n=92) (Fig. 3A,B). This result indicated that Pk1 may function in the Wnt/PCP pathway or in parallel to this pathway.

Given that Pk is an intracellular protein, it might act downstream of Wnt signals. To test this possibility, we assessed whether elevated Wnt11 activity can be suppressed by interfering with Pk1 function. Injection of slb−/− embryos with a low dose of wnt11 RNA (10 pg) either fully rescued the phenotype (Heisenberg et al., 2000; and data not shown) or occasionally leads to lateral mispositioning of prechordal plate (Fig. 3C) (91%, n=45), indicating that Ppt and Pk1 function redundantly in regulating CE in the posterior region.

Together, these results support the notion that Pk1 modulates the activity of the Wnt/PCP pathway, thereby influencing regulation of CE movements during gastrulation.

**Gain-of-function of pk1 causes defective CE movements by modulating the Wnt/PCP pathway**

To complement our analysis of cell movements in embryos with reduced Pk1 activity, we investigated the consequences of increased levels of Pk1. Ubiquitous over-expression of pk1 RNA, even at low dose (5 pg), caused abnormal cell aggregation at blastula stages precluding analysis of cell migration during gastrulation (data not shown). To overcome these early defects, we assayed the behaviour of small groups of cells over-expressing Pk1 in a wild-type environment. To achieve this, differentially labelled wild-type and Pk1-overexpressing cells were transplanted into the germ rings of wild-type host embryos (Fig. 5A). Following transplantation, convergence and extension movements redistribute the wild-type cells along the antero-posterior axis by tailbud stage (Fig. 5B) (Heisenberg et al., 2000). In comparison to wild-type, cells overexpressing Pk1 show less dorsal convergence and less spread along the antero-posterior axis (70%, n=40) (Fig. 5B). This indicates that elevated Pk1 activity inhibits cells from undergoing proper gastrulation movements.

The fact that Pk1 may act downstream of the Wnt/PCP pathway to regulate CE movements during gastrulation led us to test whether the slb phenotype could be rescued by overexpression of pk1 RNA. Given the problems associated with pkl overexpression, we injected pk1 RNA at a dose (0.5
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that has no effect in wild-type embryos \( (n=30) \) (Fig. 5C). Surprisingly, rather than rescue, this enhanced the severity of the \( \text{slb} \) phenotype \( (27\%, n=107) \) (Fig. 5D,E), implying that excess Pk1 compromises Wnt/PCP-pathway-dependent cell movements. Taken together, the loss-of-function and gain-of-function studies with Pk1 indicate that Pk1 regulates CE movements by modulating the Wnt/PCP pathway, but that it is not simply a positive or negative linear component of this pathway.

Next, we attempted to assay if Pk1 modulates the Wnt/PCP pathway by regulating the subcellular localisation of components of this pathway. In the \textit{Drosophila} wing, asymmetric localisation of the Fz-Dsh complex at the distal edge of each cell determines cell polarity within the plane of the epithelia (Axelrod, 2001; Strutt, 2001). During this process, Pk regulates localisation of Fz/Dsh by inhibiting the complex from forming at the proximal edges of the cells (Tree et al., 2002). In vertebrates, membrane localisation of Dsh in cells undergoing CE (Wallingford et al., 2000) is dependent on Fz (Axelrod et al., 1998; Rothbacher et al., 2000; Umbhauer et al., 2000). Therefore, we examined whether Pk1 affects the localisation of the Fz/Dsh complex in zebrafish embryos. When Dsh-GFP is expressed in animal pole blastomeres, it predominantly localises to the cytoplasm, sometimes associated with vesicles-like structures \( (100\%, n=10) \) (Fig. 5F). This presumably reflects the requirement of Dsh to localise to vesicles for canonical Wnt signalling (Capelluto et al., 2002). In response to Fz7, Dsh is targeted to the membrane \( (90\%, n=30) \) (Fig. 5G), but this is inhibited by increasing Pk1 activity \( (100\%, n=21) \) (Fig. 5H). The predominantly cytoplasmic localisation of Pk1 (Fig. 5I) remains unchanged in the presence of Fz7 (data not shown). These observations, together with the fact that cytoplasmic Dsh-GFP becomes faint and hazy when Pk activity is increased (Fig. 5H), raised the possibility that Pk1 activity may destabilise Dsh, thereby blocking Fz7-mediated membrane localisation of Dsh.

To test if Pk1 affects the levels of Dsh protein, we quantified myc-tagged Dsh in the presence of Fz7 with or without Pk1. Western blot analysis revealed that the levels of Dsh are significantly lower in the presence of Pk1 (Fig. 5J). These data indicate that the disruption/degradation of the Fz/Dsh complex by Pk1 may contribute to the ability of exogenous Pk1 to negatively regulate the Wnt/PCP pathway.

**pk1 genetically interacts with tri/stbm**

The ability of Pk1 to regulate CE by modulating the Wnt/PCP pathway is similar to that of Tri/Stbm (Jessen et al., 2002). We therefore investigated whether there is any genetic interaction between \( \text{pk}1 \) and \( \text{tri/stbm} \) in regulating CE movements. In the progeny of crosses between heterozygous \( \text{tri} \) carriers, approximately one quarter of embryos injected with 3 ng \( \text{pk}1\)-Mo showed a more severely compressed body axis as compared to \( \text{tri} \) homozygotes (Fig. 6A,B,G-J; Table 1). In addition, a further half of the injected population exhibited a phenotype indistinguishable from homozygous \( \text{tri} \) embryos. To confirm that the \( \text{tri} \)-like phenotype arose from abrogation of Pk1 activity in heterozygous \( \text{tri}^{+/−} \) embryos, we injected \( \text{pk}1\)-Mo in embryos from crosses between heterozygous \( \text{tri} \) female and wild-type

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**Fig. 4.** Abrogation of Pk1 function enhances the \( \text{ppt} \) phenotype. Lateral (A,C,E,G) and dorsal (B,D,F,H) views of 14-somite embryos with anterior to the left. \( \text{myoD} \) expression shows the shape of the somites in (B,D,F,H). Arrowheads indicate the most anterior and posterior extent of the axis. (A,B) Wild-type embryos. (C,D) Wild-type embryos injected with 3 ng of \( \text{pk}1\)-Mo. (E,F) \( \text{ppt}^{+/−} \) embryos. (G,H) \( \text{ppt}^{+/−} \) embryos injected with 3 ng of \( \text{pk}1\)-Mo.
male fish. About 40% of injected embryos showed a tri-like phenotype, more severe than wild-type embryos injected with pk1-Mo (Fig. 6C-F, Table 1). These results suggest that Pk1 and Tri function in the same genetic pathway.

**Pk1 functions with Tri to mediate tangential migration of motor neurons**

In addition to the regulation of CE, Tri function is required for tangential migration of facial (nVII) branchiomotor neurons (Bingham et al., 2002). This activity of Tri appears to be independent of the Wnt/PCP pathway (Jessen et al., 2002). Our demonstration of a strong genetic interaction between pk1 and tri during CE prompted us to test the involvement of pk1 in the regulation of neuronal migration. Initially we examined the temporal and spatial expression of pk1 in relation to the

**Table 1. Genetic interaction between pk1 and tri in the regulation of CE**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>pk1-Mo (ng)</th>
<th>Wild type</th>
<th>pk1-Mo (ng)</th>
<th>tri-like (severe)</th>
<th>n1</th>
</tr>
</thead>
<tbody>
<tr>
<td>tri+/–×tri+/–</td>
<td>3±0.0</td>
<td>76±2</td>
<td>24±1</td>
<td>226</td>
<td></td>
</tr>
<tr>
<td>tri+/–×tri–TL</td>
<td>3±0.0</td>
<td>100±2</td>
<td>–</td>
<td>76±1</td>
<td></td>
</tr>
<tr>
<td>tri–×TL</td>
<td>3±0.0</td>
<td>–</td>
<td>51±2</td>
<td>226</td>
<td></td>
</tr>
<tr>
<td>tri–×TL</td>
<td>3±0.0</td>
<td>–</td>
<td>49±2</td>
<td>226</td>
<td></td>
</tr>
</tbody>
</table>

*Phenotypes were scored at 30 hpf according to the definition in Fig. 5 [wild type (A); pk1-Mo (C); tri-like (E,G); tri (severe) (I)].

†Number of embryos.

position of branchiomotor neurons. pk1 is expressed strongly in ventro-lateral regions of the hindbrain except at the level of rhombomere 4 (r4)-r6, where weaker expression is detected more medially, in the vicinity of migration pathway of the new-born facial motor neurons (Fig. 7A,B and data not shown). Thus pk1 is expressed in the right place and at the right time to locally influence branchiomotor-neuron migration.

In embryos injected with pk1-Mo, nVII motor neurons accumulated in r4 and failed to migrate to r6-r7 (Fig. 7C,D, Table 2), confirming a requirement for Pk1 function in branchiomotor-neuron migration. Next, we tested for genetic interaction between pk1 and tri during branchiomotor-neuron migration. To do this, embryos from crosses between heterozygous tri+/– females and males carrying the Isl1-GFP transgene were injected with a dose of pk1-Mo (1 ng), which does not affect neuronal migration in wild-type embryos (data not shown and Table 2). About 35% of embryos (the majority of presumed tri+/– heterozygotes) injected with the low dose of pk1-Mo (Fig. 7D,F, Table 2) showed defects in neuronal migration. Consistent with the fact that pk1 is not required for correct regional patterning of the neuroectoderm (Fig. 2O-R), krox20 and hoxb1a were still expressed appropriately in rhombomeres 3, 4 and 5 in pk1 morphants (Fig. 7G-J). These results show that Pk1 and Tri function together to mediate the tangential migration of facial motor neurons.

**DISCUSSION**

In this study, we have shown that Pk1 function is required, together with Slb/Wnt11 and Ppt/Wnt5, to regulate mesendodermal and ectodermal CE movements. Furthermore,
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Gain-of-function of Pk1 also impairs CE movements and enhances the slb loss-of-function phenotype, probably because Pk1 can block the Fz7-induced membrane localisation of Dsh. Finally, we showed that pk1 genetically interacts with tri/stbm to mediate both tangential neuronal migration and CE. These results reveal Pk1 to be a key player in mediating cell movements in the vertebrate embryo.

**pk1 regulates CE movements by modulating the noncanonical Wnt/PCP pathway**

Our analysis of Pk1 function adds to a growing body of evidence that a molecular pathway involving noncanonical Wnts and homologues of fly PCP genes (Adler, 2002; Mlodzik, 2002) regulates cell movements underlying CE. Although Pk1 acts together with Wnt11 and Wnt5, it is not simply a linear
component of the Wnt/PCP pathway because both loss- and gain-of-functions of Pk1 enhance the slb mutant phenotype. Consistent with this conclusion, in Drosophila PCP, Pk is a context-dependent positive or negative modulator of Fz/PCP signalling, rather than just a downstream component of the pathway (Adler et al., 2000; Gubb et al., 1999; Tree et al., 2002).

In the Drosophila wing, Pk inhibits the Fz/Dsh complex from forming on the proximal edges of the epithelial cells, thereby functioning in a feedback loop that amplifies differences between Fz/Dsh levels on adjacent cells (Tree et al., 2002). Similarly, we have shown that vertebrate Pk1 can disrupt the Fz7-dependent membrane localisation of Dsh, despite the fact that Pk1 is neither localised to the membrane nor recruited to the membrane by Fz7. In addition, increasing Pk1 activity alters the stability of exogenous Dsh. Considering that Pk binds directly to Dsh in vitro (Tree et al., 2002), Pk might dissociate Dsh from the membrane to the cytoplasm by direct binding and, subsequently, mediate the degradation of Dsh by unknown mechanisms. Alternatively, Pk1 might destabilise Dsh at the membrane through an indirect mechanism. In this scenario, Pk1 might co-operate with a factor at the membrane that, in turn, binds to Dsh and leads to dissociation from Fz.

One such candidate is the four-pass transmembrane protein Tri/Stbm/Van Gogh (Jessen et al., 2002; Taylor et al., 1998; Wolff and Rubin, 1998). In Drosophila stbm mutants, like pk mutants, Fz is symmetrically localised in the membrane (Strutt, 2001), and stbm interacts genetically with pk (Taylor et al., 1998). These observations indicate that, in flies, Stbm functions with Pk to establish PCP. Supporting a similar interaction in vertebrates, we show that heterozygous tri+/− embryos injected with pk1-Mo exhibit a tri-like phenotype. This reveals a strong genetic interaction between pk1 and tri in the regulation of CE. Taken together with evidence that both Stbm and Pk1 can bind to Dsh and activate JNK in cultured cells (Park and Moon, 2002; Tree et al., 2002), it seems likely that Pk and Stbm function by similar mechanisms in the regulation of vertebrate CE and in the establishment of PCP in Drosophila.

pk1 and tri/stbm regulate neuronal migration independently of the Wnt/PCP pathway

In addition to disrupting CE, abrogation of Pk1 activity disrupts the tangential migration of hindbrain branchiomotor neurons. As during CE, there is a strong genetic interaction between Pk1 and Tri in the regulation of neuronal migration. However, unlike in tri and pk1 mutants/morphants, branchiomotor neuron migration is unaffected by either slb, ppt and kny mutations or by overexpression of a dominant-negative form of Dsh which efficiently suppresses the Wnt/PCP-mediated CE movements (Bingham et al., 2002; Jessen et al., 2002). These observations raise the intriguing possibility that, regardless of the presence or absence of Wnt/PCP pathway signalling, Pk might act in the same molecular pathway as Tri to regulate cell behaviours that underlie CE and tangential neuronal migration.

pk is expressed in the local environment through which the nVII branchiomotor neurons migrate while tri is expressed more broadly in the hindbrain (Park and Moon, 2002). It is intriguing that pk1 expression is relatively low in r4 and r5, whereas the nVII neurons undergo tangentially oriented caudal migrations, but higher in lateral regions of r6 (and more caudal rhombomeres), where the neurons change from tangential to laterally-directed radial migration (Chandrasekhar et al., 1997; Higashijima et al., 2000). The fact that even a subtle increase or decrease in Pk1 activity affects cell movements in pregastrula and gastrula embryos raises the intriguing possibility that changes in Pk1 activity could influence different aspects of neuronal movement. For instance, a low level of Pk1 between r4 and r6 might function together with cues (e.g. Studer, 2001) that facilitate tangential cell migration whereas high levels of Pk1 activity in r6 might modulate cues that inhibit further tangential migration and/or promote radial migration. We suggest that Pk1 might act as an intracellular sensor that mediates attractive/repulsive cues in a manner dependent on ubiquitously expressed Tri.

Possible interactions between genes involved in PCP/CE and neuronal migration

How might Stbm and Pk regulate tangential migration of facial motor neurons independent of the Wnt/PCP pathway? The Robo/Slit pathway is a candidate for exhibiting functional interaction with Stbm/Pk. Slit guides the migration of axons and neurons through its receptor Robo in both Drosophila and vertebrates (Brose et al., 1999; Hutson and Chien, 2002; Kidd et al., 1999; Wu et al., 1999; Zhu et al., 1999). Indeed, in the zebrafish hindbrain, slit2 is expressed in the midline floor plate, slit3 is expressed in branchiomotor neurons (Yeo et al., 2001) and three robo genes are expressed, overlapping with pk1, in the environment through which the nVII neurons migrate (Lee et al., 2001). Moreover, overexpression studies (Yeo et al., 2001) indicate that Slit/Robo signalling might influence CE movements through acting as a repulsive cue that modulates cell behaviour at the midline of the gastrula. Indeed, as cells approach the midline they lose bipolar promotive activity and adopt monopolar cell morphology (Elul and Keller, 2000). Given that Stbm/Pk and Slit/Robo could both be involved in the same discrete cell migrations, it will be interesting to test the possibility that these genes interact to influence cell behaviour.

Finally, flamingo (fmi), a core PCP gene that encodes a seven-pass transmembrane protein with extracellular cadherin repeats, can also function independent of Fz/Dsh signalling. Although the role of fmi in the establishment of PCP is

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Table 2. Genetic interaction between pk1 and tri in the regulation of neuronal migration

<table>
<thead>
<tr>
<th>Genotype</th>
<th>pk1-Mo (ng)</th>
<th>Position of nVII neurons (%)</th>
<th>n¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>TLisl1-GFP</td>
<td>3</td>
<td>100</td>
<td>36</td>
</tr>
<tr>
<td>TLisl1-GFP</td>
<td>1</td>
<td>60</td>
<td>51</td>
</tr>
<tr>
<td>tri+/-xisl1-GFP*</td>
<td>1</td>
<td>20</td>
<td>99</td>
</tr>
<tr>
<td>tri+/-xisl1-GFP*</td>
<td>1</td>
<td>10</td>
<td>99</td>
</tr>
</tbody>
</table>

Embryos either uninjected or injected with indicated amounts of pk1-Mo were fixed for anti-GFP staining at 36 hpf and were scored based on the final position of nVII branchiomotor neurons.

Assuming that 50% of these embryos are heterozygous tri carriers.

Number of embryos.
dependent on Fz and Dsh (Shimada et al., 2001; Usui et al., 1999), fmi also regulates dendrite outgrowth independent of the Fz/Dsh pathway (Gao et al., 2000). The Fz/Dsh-dependent and independent activities of Fmi, Stbm and Pk lead us to speculate that PCP genes might co-ordinate the behaviour of large populations of cells in a Wnt/PCP-dependent fashion, whereas they might confer directionality to either migration or process outgrowth of small groups of cells independent of the Wnt/PCP pathway. As yet, there is little data on the roles of vertebrate fmi genes and it will be interesting to determine if they do function in the same pathways as Stbm and Pk during CE and neuronal migration.

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Note added in proof
Moon’s and Ueno’s groups have also recently reported that prickle is required for CE cell movements during gastrulation in zebrafish and Xenopus (Veeman et al., 2003; Takeuchi et al., 2003).

REFERENCES


