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The involvement of PCP proteins in radial cell intercalations during *Xenopus* embryonic development

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ABSTRACT

The planar cell polarity (PCP) pathway orients cells in diverse epithelial tissues in *Drosophila* and vertebrate embryos and has been implicated in many human congenital defects and diseases, such as ciliopathies, polycystic kidney disease and malignant cancers. During vertebrate gastrulation and neurulation, PCP signaling is required for convergent extension movements, which are primarily driven by mediolateral cell intercalations, whereas the role for PCP signaling in radial cell intercalations has been unclear. In this study, we examine the function of the core PCP proteins Vangl2, Prickle3 (Pk3) and Disheveled in the ectodermal cells, which undergo radial intercalations during *Xenopus* gastrulation and neurulation. In the epidermis, multiciliated cell (MCC) progenitors originate in the inner layer, but subsequently migrate to the embryo surface during neurulation. We find that the Vangl2/Pk protein complexes are enriched at the apical domain of intercalating MCCs and are essential for the MCC intercalatory behavior. Addressing the underlying mechanism, we identified KIF13B, as a motor protein that binds Disheveled. KIF13B is required for MCC intercalation and acts synergistically with Vangl2 and Disheveled, indicating that it may mediate microtubule-dependent trafficking of PCP proteins necessary for cell shape regulation. In the neural plate, the Vangl2/Pk complexes were also concentrated near the outermost surface of deep layer cells, suggesting a general role for PCP in radial intercalation. Consistent with this hypothesis, the ectodermal tissues deficient in Vangl2 or Disheveled functions contained more cell layers than normal tissues. We propose that PCP signaling is essential for both mediolateral and radial cell intercalations during vertebrate morphogenesis. These expanded roles underscore the significance of vertebrate PCP proteins as factors contributing to a number of diseases, including neural tube defects, tumor metastases, and various genetic syndromes characterized by abnormal migratory cell behaviors.

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1. Introduction

The planar cell polarity (PCP) pathway was originally defined as a mechanism allowing epithelial cells to polarize in a plane of a tissue perpendicular to their apicobasal axis (Adler, 2012; Peng and Axelrod, 2012; Wallingford, 2012; Zallen, 2007). Besides the organization of fly and mammalian epithelia, the conserved PCP proteins are involved in the migration of neurons and germ cells (Glasco et al., 2012; Jessen et al., 2002), asymmetric cell division (Bellaiche et al., 2004; Lake and Sokol, 2009; Vladar et al., 2009), branching morphogenesis (Miller et al., 2011; Yates et al., 2010a, 2010b), angiogenesis (Cirone et al., 2008) and ciliogenesis (Gray et al., 2011; Wallingford and Mitchell, 2011). Due to their connection to cilia functions, mutations in the corresponding PCP genes cause diverse ciliopathies. These range from a relatively common polycystic kidney disease to more rare genetic syndromes such as Meckel–Gruber syndrome, Bardet–Biedl syndrome, Oro-facio-digital syndrome and nephronophthisis. (Simons and Mlodzik, 2008; Wang and Nathans, 2007). Additionally, PCP proteins have been associated with multiple congenital abnormalities, such as neural tube and cardiac defects, and implicated in tumor invasiveness (Gray et al., 2011; Hamblet et al., 2002; Luga et al., 2012; Zhu et al., 2012).

In vertebrate early development, PCP pathway components are critical for several cell behaviors, such as cell intercalations and apical constriction during neural tube closure and mesoderm convergent extension (Ossipova et al., 2015a; Sokol, 2000, 2015; Wallingford et al., 2002a). All major components of the Wnt/PCP pathway, including Wnt11 (Heisenberg et al., 2000; Tada and Smith, 2000), Disheveled (Sokol, 1996; Wallingford et al., 2000), Vangl2/Stbm (Goto and Keller, 2002; Jessen et al., 2002; Park and Moon,

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2002), Prickle (Pk) (Carreira-Barbosa et al., 2003; Takeuchi et al., 2003; Wallingford et al., 2002b), Fmi/Celsr1 (Formstone and Mason, 2005), have been shown to modulate convergent extension movements during gastrulation and neurulation. As convergent extension is driven largely by mediolateral cell intercalations (Keller, 2002; Shih and Keller, 1992), PCP components were proposed to stabilize mediolateral cell protrusions (Jessen et al., 2002; Wallingford et al., 2000) and/or promote actomyosin contractility at mediolaterally oriented cell junctions (Shindo and Wallingford, 2014). By contrast, the involvement of PCP signaling in radial cell intercalation has been less clear.

During radial intercalation, cells change their position between different tissue layers, along the apical-basal embryonic axis. As a result, the tissue becomes thinner and contains fewer cell layers. In lower vertebrates, radial intercalations are the predominant cellular mechanism of tissue spreading before and during gastrulation, and contribute to the development of the neural tube and epidermis (Keller, 1980; Solnica-Krezel and Sepich, 2012; Walck-Shannon and Hardin, 2014). Specifically, during *Xenopus* neurulation, the neural plate, consisting of multiple cell layers, is converted into the single-cell-layered neural tube (Hartenstein, 1989; Keller, 1991). Similarly, the two-layered embryonic skin develops from multi-layered epidermal ectoderm (Deblandre et al., 1999; Drysdale, 1992; Stubbs et al., 2006). These processes involve diverse molecular events including changes in cell adhesion and microtubule-dependent vesicular trafficking (Itoh et al., 2014; Kim et al., 2012; Lepage et al., 2014; Marsden and DeSimone, 2001; Solnica-Krezel and Sepich, 2012; Song et al., 2013; Werner et al., 2014).

Evidence has been accumulating that the Wnt/PCP pathway may be involved not only in mediolateral cell intercalations, but also in radial intercalations. Mouse epiblast cells reveal Pk1-dependent apical-basal polarity, an observation possibly relevant to radial intercalations (Tao et al., 2009). Consistent with abnormal radial intercalatory behavior, intestine does not properly elongate in Wnt5-deficient mouse embryos (Cervantes et al., 2009). This has been explained as a cell proliferation defect, but cell intercalations have not been examined. Furthermore, the interference with the activity of Celsr in zebrafish embryos results in epiboly defects, although this role has been attributed to the modulation of cell adhesion rather than PCP signaling (Carreira-Barbosa et al., 2009). Together, these observations suggest that PCP proteins might function in radial cell intercalation.

Our study has addressed this possibility in *Xenopus* embryonic epidermis, in which some cell types, including multiciliated cells (MCCs), intercalate into the superficial cell layer. Due to tissue-targeted gene manipulation, *Xenopus* skin has become an established *in vivo* model for other mucociliary epithelia containing MCCs, such as those of human airways or reproductive tract (Brooks and Wallingford, 2014; Dubaissi and Papalopulu, 2011). A specific technical advantage of this system is to unilaterally manipulate protein function by targeted microinjections, with the uninjected side serving as an internal control. Our analysis of *Xenopus* epidermal ectoderm revealed an enrichment of the core PCP component Vangl2 at the apical surface of MCCs. We also identified Prickle3 (Pk3), a member of the Prickle family that is mainly expressed in the embryonic skin, and demonstrated the requirement of Vangl2, Pk3 and Disheveled for the radial intercalation of MCC precursors into the superficial cell layer of the skin. Additionally, interference with PCP signaling inhibited radial intercalation of inner layer cells in the neural plate and non-neural ectoderm. To further address the underlying mechanism, we identified the motor kinesin KIF13B as a Disheveled-interacting protein. KIF13B has been previously implicated in cell polarity and cell migration (Horiguchi et al., 2006; Tarbashevich et al., 2011). We demonstrate that KIF13B physically associates with Disheveled and synergizes with PCP signaling to regulate cell intercalatory behavior. Collectively, our data support a general role for PCP signaling in radial cell intercalations during *Xenopus* gastrulation and neurulation.

2. Methods

2.1. Plasmid constructs and morpholinos

Plasmids encoding GFP-C1 in pXT7, GFP-CAAX in pCS2+ (Kim et al., 2012); nGFP in pCS2+ (Dollar et al., 2005), CFP-Vangl2 in pCS105 (Stbm) (Itoh et al., 2009), mouse HA-Vangl2 (Gao et al., 2011), Mig12-GFP in pCS2+ (Yasunaga et al., 2011) and the Myc-tagged Disheveled constructs Xdsh (Myc-Dvl2), Xdd1 and Xdd2 (Sokol, 1996) have been described. The plasmid encoding *Drosophila* Pk in pCS105 was a gift from A. Jenny. A cDNA encoding Prickle3 (Pk3) protein missing five aminoacids from the N-terminus (GenBank accession number: BC154995) was amplified by RT-PCR from *Xenopus* neurula RNA and subcloned into pCS2-Flag. In Flag-Pk3ΔPET, the PET domain has been deleted by PCR according to what was described in Takeuchi et al. (2003).

A cDNA encoding a C-terminal CAP-GLY-domain containing fragment of *Xenopus* KIF13B was isolated in a yeast-two-hybrid screen from a *Xenopus* gastrula cDNA library (Brott and Sokol, 2005; Itoh et al., 2000), using the DIX domain of Dvl2 as a bait. pCMVtag2-FlagKIF13B was a gift from A. Chishti. For RNA injections, the FlagKIF13B insert has been subcloned into pCS2-Myc. Details of cloning are available upon request. For lineage tracing, GFP RNA (50–100 pg) was injected along with morpholino antisense oligonucleotides (MOs) or RNAs. Capped mRNA was made by *in vitro* transcription with T7 or SP6 promoter using mMessage mMachine kit (Ambion).

The following morpholino oligonucleotides (MOs) have been used: Vangl2/Stbm MO 5′-GAG TAC CGG CTT TTG TGG CGA TCCA-3′ (Ossipova et al., 2015a), KIF13B MO, 5′-ATCTTGCACAGCGAGCTCCCCTAAC-3′ (Tarbashevich et al., 2011); Pk3 MO1, 5′-GGATGCCGCCCGCTCTCTCCCTTA-3′. Pk3 MO2, 5′-CTCCTCCTGGAAT-TACGGAACATCC-3′. MO to the Fz8-associated protein phosphatase FRIED (Itoh et al., 2005) with the sequence 5′-GCTTCAGCTAGTGACA-CATGCAT-3′ has been used as a negative control and produced no detectable phenotype (Itoh et al., 2014). MOs were purchased from Gene Tools (Philomath, OR).

2.2. Xenopus embryos, RNA and morpholino microinjections, in situ hybridization

In vitro fertilization, culture and staging of *Xenopus laevis* embryos were carried out as previously described (Dollar et al., 2005). For microinjections, four-eight cell embryos were transferred into 2–3% Ficoll in 0.3× MMR buffer and 5–10 nl of mRNAs or MO solution was injected into one or more blastomeres. Amounts of injected mRNA or MO per embryo have been optimized in preliminary dose–response experiments (data not shown) and are indicated in figure legends. Whole-mount *in situ* hybridization was carried out using standard techniques (Harland, 1991) with the digoxigenin-labeled antisense and sense RNA probes for Pk3.

2.3. Immunostaining and image analysis

Embryos were fixed at desired stages with Dent's fixative (80% Methanol: 20% DMSO) for 3 h at RT followed by overnight incubation at −20 °C and rehydrated with 1xPBS washing. For cryosectioning, the embryos were embedded in the solution containing 15% cold fish gelatin and 15% sucrose, sectioned at 10–20 μm, and immunostained overnight essentially as described (Dollar et al., 2005). Antibodies

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