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RESEARCH ARTICLE

PLANAR CELL POLARITY PROTEINS VANG-1 AND DSH-2 CONTRIBUTE TO JNK-1 ACTIVATION IN *C. ELEGANS* EMBRYOGENESIS

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ABSTRACT

Morphogenesis requires Jun N-terminal kinase (JNK) and planar cell polarity (PCP) signaling in many organisms. However, regulation of JNK activation during morphogenesis is still elusive. Combined data from *Xenopus* and *Drosophila* indicate that JNK is activated under circumstances in which PCP is also activated. VANG-1, the ortholog of Strabismus/Van Gogh and DSH-2, one ortholog of Dishevelled, are core components of PCP and mediate intercalation of intestinal cells during morphogenesis in *C. elegans*, whereas JNK-1 expression has not been described during embryogenesis so far. Here, we demonstrate that phosphorylated JNK-1 is detectable by immunofluorescence analysis during intestinal morphogenesis. In addition, we show that JNK-1 phosphorylation is modulated by the embryonic *vang-1* and *dsh-2(RNAi)* phenotype. Notably, JNK-1 phosphorylation becomes detectable in *C. elegans* morphogenesis stages after VANG-1 and DSH-2 mediated events, suggesting a role for activated JNK subsequently to PCP signaling.

INTRODUCTION

The PCP pathway regulates orientation, arrangement and movement of cells during development, leading to proper organ shape (Wu and Mlodzik, 2009). Core components of PCP signaling pathway are the transmembrane protein Strabismus/Van Gogh (Stbm/Vang) and the cytoplasmic mediator Dishevelled (Zallen, 2007). Impairment of PCP signaling leads to changes in pattern formation, e.g. in the *Drosophila* compound eye, or lethality due to intercalation defects during neural closure in vertebrates (Wolff and Rubin, 1998; Darken *et al*., 2002). Also it has been shown in mice, one downstream factor of PCP signaling is the Jun N-terminal kinase, which is activated by Stbm/Vang (Yao *et al*., 2004). Further data describe the involvement of PCP and JNK in motoneuron migration (Vivancos *et al*., 2009), suggesting a general role for JNK during PCP signaling events. JNK activation is required for embryogenesis in most organisms (Martin and Wood, 2002; Mizuno *et al*., 2004; Xia and Karin, 2004), however in *C. elegans* JNK-1 seems to be exclusively required for postembryonic processes (Kawasaki *et al*., 1999; Wolf *et al*., 2008). In *C. elegans*, less is known about PCP signaling and the consequences of its impairment in comparison to the aforementioned model systems.

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VANG-1, the *C. elegans* ortholog of Strabismus/Van Gogh participates in ground polarity of vulva cells (Green *et al*., 2008). In addition, VANG-1 and the dishevelled ortholog DSH-2 display a dynamic subcellular expression during morphogenesis, presumably contributing to intercalation and subsequent arrangement of intestinal cells during embryonic development (Hoffmann *et al*., 2010). In this work, we found a novel intestine specific activation of JNK-1 during *C. elegans* embryogenesis. Moreover, activation of JNK-1 in *dsh-2(RNAi)* embryos appears stronger if compared to *vang-1* mutant or wild type embryos. This data suggest a role for JNK-1 depending on but after PCP has been established. Staining of phosphorylated and hence activated JNK-1 (pJNK-1) is not detectable in the *C. elegans* embryo – even not in the developing intestinal primordium – until the onset of the morphogenesis phase (Fig. 1a). In this developmental stage, elongation of the embryo starts from one-fold onwards. It has been demonstrated in *Xenopus* and *Drosophila*, that the elongation of epithelia is a process which is facilitated by cell-cell intercalation as a driving force (Wallingford *et al*., 2002; Baum, 2004; Keller *et al*., 2008). Coordinated intercalation and division of cells within a plane of an epithelium are regulated by proteins of the PCP signaling pathway (Gong *et al*., 2004), and JNK mediated MAPK signaling has been described as necessary for cells to change their shape in migration events (Harden, 2002; Ramet *et al*., 2002; Kwon *et al*., 2010).

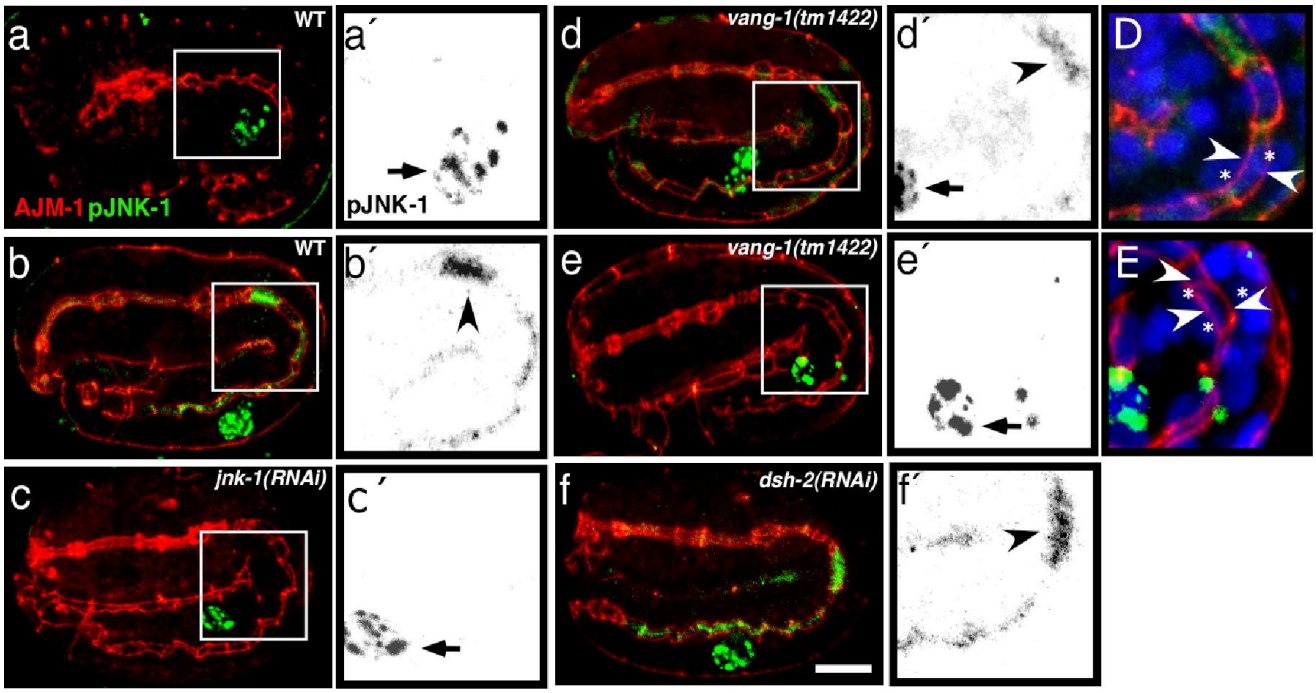

Fig. 1. JNK-1 is phosphorylated specifically in the *C. elegans* intestine during mid morphogenesis and is altered in *vang-1(tm1422)* and *dsh-2(RNAi)* embryos

(**a-f**) Double immunofluorescence labelings against the apical junction protein AJM-1 (red) and phosphorylated JNK-1 (pJNK-1, green) in WT (**a,b**), *jnk-1(RNAi)* (**c**), *vang-1(tm1422)* embryos (**d, e**) and *dsh-2(RNAi)* embryos (**f**). Black arrowheads indicate staining of pJNK-1 in the intestine and arrows indicate staining of P-granules, respectively. (**E, F**) To better visualize intercalation defects, same embryos as displayed in d,e were stained with YoYo-1 (green), anti-AJM-1 (red) and anti-pJNK-1 (blue). Asterisks and arrowheads indicate nuclei of intestinal ring IV and *C. elegans* apical junction, respectively. (**a**) In early morphogenesis pJNK-1 is not detectable (n=49/49). (**b**) In two fold stage embryos, pJNK-1 specifically localizes subapically to intestinal cells (n=56/56). (**c**) Note that stainings of P-Granules might be unspecific as it is not removed by *jnk-1(RNAi)* (n=25/25). (**d, D**) In total, 154 *vang-1* embryos and 91 *dsh-2(RNAi)* embryos were analysed with respect to intercalation defects and JNK-1 activation, respectively. Activated JNK-1 is detectable in most *vang-1* mutant embryos, however display a diminished signal (n=115/154, 65%). (**e, E**) Activated JNK-1 is occasionally absent in *vang-1* mutant embryos (n=17/154, 11%). (**f, f´**) In *dsh-2(RNAi)* activated JNK-1 is detectable in the majority of analysed embryos (n=75/91, 82%) and display an enhanced signal intensity. Orientation: anterior left, dorsal top; bar 10 µm.

Cell shape changes are often based upon reorganization of the actin cytoskeleton, and under certain conditions JNK signaling is activated in response to a PCP signal (Boutros *et al*., 1998; Axelrod and McNeill, 2002). In *C. elegans*, mechanisms that lead to elongation of the embryo are not completely understood, but intercalation of hypodermal cells and stretching of intestinal cells occur isochronic, and both might provide the force necessary for elongation (Piekny and Mains, 2003). Staining of pJNK-1 arises in mid to late morphogenesis specifically in intestinal primordial cells (Fig. 1b). Because in *jnk-1(gk7)* deletion allele the antibody epitope is still present, we performed *jnk-1(RNAi)*. In *jnk-1(RNAi)* embryos, pJNK-1 staining is absent (Fig. 1c), suggesting that the intestinal immunofluorescence signal is specific to pJNK-1, whereas staining of P-granules might be not (Fig. 1c´, arrow). In wild type embryos activated JNK-1 is detectable in mid to late morphogenesis, hence chronological subsequent to PCP events, which are mediated by VANG-1 and DSH-2 in early to mid morphogenesis to regulate intercalation of intestinal cells (Hoffmann *et al*., 2010). Depletion of JNK-1 by RNAi or in *jnk-1(gk7)* did not result in an embryonic PCP phenotype that is characterized by intercalation defect of intestinal primordial cells (Segbert *et al*., 2004; Hoffmann *et al*., 2010). So far, neither impairment of cell shape changes nor elongation defects have been described for *jnk-1(RNAi)* or *gk7* embryos. In line with our findings, previous data by Green *et al*. show that JNK-1 signaling is not required for establishing ground polarity in vulva cells, whereas signaling via VANG-1 participate in this process (Green *et al*., 2008).

To analyse whether JNK-1 activation in the intestine depends on VANG-1 signaling, we stained *vang-1(tm1422)* embryos against pJNK-1. The *vang-1* mutant allele *tm1422* encodes for a truncated protein, missing three of four transmembrane domains (Hoffmann *et al*., 2010). The localization pattern of truncated VANG-1^{TM1422} is comparable to wild type but the missing transmembrane domains may influence VANG-1 mediated signaling. Intestinal intercalation defects observed in *vang-1(tm1422)* embryos (34%; (Hoffmann *et al*., 2010) may reflect correct localization of VANG-1^{TM1422} but could also indicate impaired function. PCP signaling in general is required to establish polarity inside cells within an epithelium, and to activate downstream effectors at the proper site, e.g. actin reorganization at the posterior cell cortex. This mechanism leads to formation of extrusions at the posterior cortex only to regulate well ordered intercalation, or to place other active signaling complexes to the correct site.

We detect phosphorylated JNK-1 in *tm1422* embryos, hence suggesting that activation of JNK-1 is independent of VANG-1^{TM1422} (Fig. 1d, D). Certainly, in addition and in contrast to wild type embryos, we observed that in *vang-1* embryos the signal of activated pJNK-1 is reduced, or even absent (Fig. 1e, E). However, we were not able to find a direct correlation between the incidences of VANG-1/PCP phenotype, which appears in approx. 34% and can be visualized by immunofluorescence stainings of the *C. elegans* apical junction, and the reduced or missing JNK-1 activation in the intestine (Hoffmann *et al*., 2010). Because PCP signaling in

most organisms is required for asymmetric distribution of downstream effector proteins or complexes, our result suggests that downstream effectors of VANG-1^{TM1422} might be necessary for JNK-1 activation in the *C. elegans* intestine. The only PCP homolog known to resemble the *vang-1* intestinal phenotype so far is *dsh-2*. Depletion of DSH-2 by RNAi leads to an impaired localization pattern of VANG-1, thus interfering with proper PCP signaling required for intestinal cell intercalation (Hoffmann *et al*., 2010). We therefore analysed pJNK-1 pattern in morphogenesis stage embryos of *dsh-2(RNAi)* (Fig. 1f). In general, pJNK-1 signal intensity in *dsh-2(RNAi)* appears to be stronger than in wild type or pJNK-1 positive *vang-1* embryos, as judged independently by M.H. and S.H. We also found that pJNK-1 is still detectable in a majority of *dsh-2(RNAi)* embryos (n=72, 93%), independent to a PCP phenotype in the intestine. However, the authors were not able to quantify differences in signal intensity. In summary, our data show that JNK-1 is detectable in the *C. elegans* embryo. JNK-1 is phosphorylated during *C. elegans* morphogenesis phase in which the embryo elongates. Phosphorylation, and thus activation of JNK-1 seems to occur chronologically subsequent to VANG-1/DSH-2 mediated processes, which are required for correct arrangement of intestinal cells. Hence, *jnk-1(RNAi)* or *jnk-1(gk7)* embryos do not display an intestinal intercalation phenotype.

However, pJNK-1 displays altered immunofluorescence in *vang-1(tm1422)* and *dsh-2(RNAi)* embryos, suggesting a direct regulation of JNK-1 activation by PCP signaling. In other organisms orthologs of VANG-1 are capable to activate JNK. In line with these findings we have shown that *vang-1(tm1422)* embryos display reduced pJNK-1 signal intensity, suggesting that VANG-1 and/or its downstream components are required for JNK-1 phosphorylation in *C. elegans* embryogenesis, similar to the situation in other model organisms. However, DSH-2 seems to negatively modulate the activity of JNK-1, because *dsh-2(RNAi)* embryos display enhanced immunofluorescence signal of pJNK-1. One hypothesis might be that VANG-1 is required for asymmetric clustering of effectors, which activate JNK-1 if properly distributed or concentrated. Supporting this hypothesis depletion of DSH-2, which alters VANG-1 subcellular distribution (Hoffmann *et al*., 2010), leads to a stronger activation of JNK-1. We also found that in *vang-1(tm1422)* embryos the activation of JNK-1 is frequently diminished or absent. This observation suggests that intestinal cell intercalation and activation of JNK-1 are two temporally separated events, which may require a similar set of VANG-1 downstream effectors. Identification of physical interacting partners of VANG-1 and the analysis of specific *C. elegans* PCP mutants may help to identify these effectors and to better understand the processes required for JNK-1 activation by PCP components.

MATERIALS AND METHODS

C. elegans strains and culture

Maintenance and handling of *C. elegans* were carried out as described previously (Brenner, 1974). Bristol N2 was used as the wild type (WT) strain. The following mutant alleles were used: *jnk-1 (gk7), vang-1 (tm1422)*. *tm1422* was out crossed two times. For further detailed information for the mutant alleles visit www.wormbase.org.

Immunostaining, microscopy, and image processing

Antibody staining, microscopy and image processing was essentielly performed as described previously (Van Furden *et al*., 2004). Morphogenesis stage embryos were analysed with respect to the apical junctional pattern and JNK-1 activation (pJNK-1) in the intestine. The following primary and secondary antibodies were diluted in blocking buffer: anti-AJM-1 (mabMH27, mouse, hybridoma supernatant, diluted 1:1500), anti-DLG-1 (rabbit, 1:100; (Bossinger *et al*., 2001)), anti-pJNK-1 (SC-12882, goat, 1:5, Santa Cruz Biotechnology). Secondary antibodies were Cy2, Cy-3 (1:200; Jackson Immunoresearch Laboratories, West Grove, PA) or Alexa 647 (1:200; Molecular Probes) conjugated.

RNA mediated interference

RNAi by feeding was performed as described by others (Kamath *et al*., 2001). For *jnk-1(RNAi)* and *dsh-2(RNAi)*, full length cDNA generated by RT-PCR was cloned into feeding vector pPD129.36, respectively.

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REFERENCES

Axelrod JD, McNeill H. 2002. Coupling planar cell polarity signaling to morphogenesis. ScientificWorldJournal 2:434-454.

Barr RK, Bogoyevitch MA. 2001. The c-Jun N-terminal protein kinase family of mitogen-activated protein kinases (JNK MAPKs). Int J Biochem Cell Biol 33:1047-1063.

Baum B. 2004. Animal development: crowd control. Curr Biol 14:R716-718.

Bossinger O, Klebes A, Segbert C, Theres C, Knust E. 2001. Zonula adherens formation in Caenorhabditis elegans requires dlg-1, the homologue of the Drosophila gene discs large. Dev Biol 230:29-42.

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

Brenner S. 1974. The genetics of Caenorhabditis elegans. Genetics 77:71-94.

Darken RS, Scola AM, Rakeman AS, Das G, Mlodzik M, Wilson PA. 2002. The planar polarity gene strabismus regulates convergent extension movements in Xenopus. EMBO J 21:976-985.

Gong Y, Mo C, Fraser SE. 2004. Planar cell polarity signalling controls cell division orientation during zebrafish gastrulation. Nature 430:689-693.

Green JL, Inoue T, Sternberg PW. 2008. Opposing Wnt pathways orient cell polarity during organogenesis. *Cell* 134:646-656.

Harden N. 2002. Signaling pathways directing the movement and fusion of epithelial sheets: lessons from dorsal closure in Drosophila. Differentiation 70:181-203.

Hoffmann M, Segbert C, Helbig G, Bossinger O. 2010. Intestinal tube formation in Caenorhabditis elegans requires vang-1 and egl-15 signaling. *Dev Biol* 339:268-279.

Kamath RS, Martinez-Campos M, Zipperlen P, Fraser AG, Ahringer J. 2001. Effectiveness of specific RNA-mediated interference through ingested double-stranded RNA in Caenorhabditis elegans. *Genome Biol* 2:RESEARCH0002.

Kawasaki M, Hisamoto N, Iino Y, Yamamoto M, Ninomiya-Tsuji J, Matsumoto K. 1999. A Caenorhabditis elegans JNK signal transduction pathway regulates coordinated movement via type-D GABAergic motor neurons. *EMBO J* 18:3604-3615.

Keller R, Shook D, Skoglund P. 2008. The forces that shape embryos: physical aspects of convergent extension by cell intercalation. *Phys Biol* 5:015007.

Kwon YC, Baek SH, Lee H, Choe KM. 2010. Nonmuscle myosin II localization is regulated by JNK during Drosophila larval wound healing. *Biochem Biophys Res Commun* 393:656-661.

Martin P, Wood W. 2002. Epithelial fusions in the embryo. *Curr Opin Cell Biol* 14:569-574.

Mizuno T, Hisamoto N, Terada T, Kondo T, Adachi M, Nishida E, Kim DH, Ausubel FM, Matsumoto K. 2004. The Caenorhabditis elegans MAPK phosphatase VHP-1 mediates a novel JNK-like signaling pathway in stress response. *EMBO J* 23:2226-2234.

Piekny AJ, Mains PE. 2003. Squeezing an egg into a worm: C. elegans embryonic morphogenesis. *ScientificWorldJournal* 3:1370-1381.

Ramet M, Lanot R, Zachary D, Manfruelli P. 2002. JNK signaling pathway is required for efficient wound healing in Drosophila. *Dev Biol* 241:145-156.

Segbert C, Johnson K, Theres C, van Furden D, Bossinger O. 2004. Molecular and functional analysis of apical junction formation in the gut epithelium of Caenorhabditis elegans. *Dev Biol* 266:17-26.

Van Furden D, Johnson K, Segbert C, Bossinger O. 2004. The C. elegans ezrin-radixin-moesin protein ERM-1 is necessary for apical junction remodelling and tubulogenesis in the intestine. *Dev Biol* 272:262-276.

Vivancos V, Chen P, Spassky N, Qian D, Dabdoub A, Kelley M, Studer M, Guthrie S. 2009. Wnt activity guides facial branchiomotor neuron migration, and involves the PCP pathway and JNK and ROCK kinases. *Neural Dev* 4:7.

Wallingford JB, Fraser SE, Harland RM. 2002. Convergent extension: the molecular control of polarized cell movement during embryonic development. *Dev Cell* 2:695-706.

Wolf M, Nunes F, Henkel A, Heinick A, Paul RJ. 2008. The MAP kinase JNK-1 of Caenorhabditis elegans: location, activation, and influences over temperature-dependent insulin-like signaling, stress responses, and fitness. *J Cell Physiol* 214:721-729.

Wolff T, Rubin GM. 1998. Strabismus, a novel gene that regulates tissue polarity and cell fate decisions in Drosophila. *Development* 125:1149-1159.

Wu J, Mlodzik M. 2009. A quest for the mechanism regulating global planar cell polarity of tissues. *Trends Cell Biol* 19:295-305.

Xia Y, Karin M. 2004. The control of cell motility and epithelial morphogenesis by Jun kinases. *Trends Cell Biol* 14:94-101.

Yao R, Natsume Y, Noda T. 2004. MAGI-3 is involved in the regulation of the JNK signaling pathway as a scaffold protein for frizzled and Ltap. Oncogene 23:6023-6030.

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