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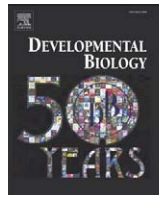

The N- or C-terminal domains of DSH-2 can activate the *C. elegans* Wnt/β-catenin asymmetry pathway

Ryan S. King [a], Stephanie L. Maiden [b], Nancy C. Hawkins [c], Ambrose R. Kidd III [a], Judith Kimble [d,e], Jeff Hardin [f,*], Timothy D. Walston [g]

[a] *Cellular and Molecular Biology Program, University of Wisconsin, USA*
[b] *Molecular and Cellular Pharmacology Program, University of Wisconsin, USA*
[c] *Department of Molecular Biology and Biochemistry, Simon Fraser University, Canada*
[d] *Howard Hughes Medical Institute, University of Wisconsin, USA*
[e] *Department of Biochemistry, University of Wisconsin, USA*
[f] *Department of Zoology, University of Wisconsin, USA*
[g] *Department of Biology, Truman State University, USA*

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ABSTRACT

Dishevelleds are modular proteins that lie at the crossroads of divergent Wnt signaling pathways. The DIX domain of dishevelleds modulates a β-catenin destruction complex, and thereby mediates cell fate decisions through differential activation of Tcf transcription factors. The DEP domain of dishevelleds mediates planar polarity of cells within a sheet through regulation of actin modulators. In *Caenorhabditis elegans* asymmetric cell fate decisions are regulated by asymmetric localization of signaling components in a pathway termed the Wnt/β-catenin asymmetry pathway. Which domain(s) of Disheveled regulate this pathway is unknown. We show that *C. elegans* embryos from *dsh-2*(*or302*) mutant mothers fail to successfully undergo morphogenesis, but transgenes containing either the DIX or the DEP domain of DSH-2 are sufficient to rescue the mutant phenotype. Embryos lacking zygotic function of SYS-1/β-catenin, WRM-1/β-catenin, or POP-1/Tcf show defects similar to *dsh-2* mutants, including a loss of asymmetry in some cell fate decisions. Removal of two dishevelleds (*dsh-2* and *mig-5*) leads to a global loss of POP-1 asymmetry, which can be rescued by addition of transgenes containing either the DIX or DEP domain of DSH-2. These results indicate that either the DIX or DEP domain of DSH-2 is capable of activating the Wnt/β-catenin asymmetry pathway and regulating anterior–posterior fate decisions required for proper morphogenesis.
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Introduction

Throughout development, cells communicate with each other using intricate signal transduction pathways. One key group of such signaling pathways are those regulated by Wnt ligands, including the Wnt/β−catenin, planar cell polarity (PCP) and Wnt/Ca^{2+} pathways (reviewed in Copp et al., 2003; Herman and Wu, 2004; Kohn and Moon, 2005; Mlodzik, 2002; Sokol, 2000; Wallingford and Habas, 2005).

The activity of the Dishevelled (Dsh) proteins partially determines which Wnt pathway is activated. Dsh family proteins have three major conserved domains: an N-terminal DIX, a central PDZ, and a C-terminal DEP domain (reviewed in Wallingford and Habas, 2005; Wharton, 2003). The DIX and PDZ domains primarily interact with proteins that lead to activation of the Wnt/β−catenin pathway, which has many roles during development, including axis specification and cell fate determination (reviewed in Cadigan and Nusse, 1997; Moon et al., 1997; Weaver and Kimelman, 2004; Yamaguchi, 2001). In contrast, the DEP domain is responsible for both PCP and Wnt/Ca^{2+} signaling, which may act cooperatively through similar or identical pathways (reviewed in Wharton, 2003). These pathways control basic morphogenetic processes, such as the polarization of epithelial sheets and convergent extension movements (reviewed in Mlodzik, 2002; Veeman et al., 2003; Wallingford and Habas, 2005).

In *Caenorhabditis elegans*, Wnt signaling acts at many points throughout embryonic and larval stages of development to specify cell fate, to polarize cells in asymmetric divisions, and to control cell migrations (reviewed in Eisenmann, 2005; Herman and Wu, 2004; Korswagen, 2002). Blastomere polarity in the early embryo is controlled by a Wnt/spindle alignment pathway that does not require transcription (Schlesinger et al., 1999; Walston et al., 2004). Many asymmetric cell fate decisions in *C. elegans* are controlled through asymmetric localization of the Tcf transcription factor POP-1 and the β-catenins SYS-1 and WRM-1 in a pathway termed the Wnt/β-catenin asymmetry pathway (reviewed in Mizumoto and Sawa, 2007b). WRM-1, in association with the Nemo-like kinase LIT-1, targets POP-1 for nuclear export, leading to its asymmetric nuclear localization (Lo

* Corresponding author.
E-mail address: jdhardin@wisc.edu (J. Hardin).

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et al., 2004; Rocheleau et al., 1999). SYS-1 then functions as a limiting co-activator for POP-1 (Huang et al., 2007; Kidd et al., 2005; Phillips et al., 2007).

Although many developmental events in *C. elegans* require Wnt signaling, the roles of specific Dshs in these processes vary. *C. elegans* has three conserved Dsh genes, *dsh-1*, *dsh-2*, and *mig-5*. In the early embryo, all three Dshs function to varying degrees in orienting the mitotic spindle of the EMS blastomere in the four-cell embryo and the ABar blastomere in the eight-cell embryo (Walston et al., 2004). The Dshs also transduce the Wnt signal that specifies the fate of the EMS daughter cells, E (endoderm) and MS (mesoderm), and regulate myosin phosphorylation and contractility to drive the initial ingression of Ea and Ep during *C. elegans* gastrulation (Lee et al., 2006).

The Dshs also regulate several asymmetric cell divisions. These include those in the PHA neuronal lineage (Hawkins et al., 2005), the somatic gonad precursor lineage (Chang et al., 2005; Walston et al., 2006), the P11/P12 lineage, and the male-specific B cell lineages (Walston et al., 2006; Wu and Herman, 2007). In addition, MIG-5 controls polarity of the hermaphrodite B cell lineage through a PCP-like pathway involving RHO-1/RhoA and LET-502/ROCK (Wu and Herman, 2007). Asymmetric division in the B cell lineage and the PHA neuronal lineage requires the DEP but not the DIX domain (Hingwing et al., 2009; Wu and Herman, 2007). MIG-5 is also necessary for proper hypodermal morphogenesis (Walston et al., 2006). How the Dshs regulate hypodermal morphogenesis and how they activate the Wnt/β-catenin asymmetry pathway is unclear.

Here we describe the Wnt signaling pathways that act through DSH-2 to control hypodermal morphogenesis. Embryos from *dsh-2* (*or302*) mutant mothers have disorganized hypodermal cells that migrate incorrectly. Removing conserved components of PCP signaling does not reveal a role for these proteins in hypodermal morphogenesis. However, removing zygotic function of POP-1, SYS-1, or WRM-1 using mutants characterized here for the first time results in defects in hypodermal morphogenesis similar to *dsh-2* mutants. Removal of both DSH-2 and MIG-5 results in global loss of POP-1 asymmetry that can be rescued to wild-type levels by *dsh-2* constructs lacking either the DIX or the DEP domain. These same constructs rescue morphogenetic defects in *dsh-2* mutants. These results suggest that both the DIX and DEP domains of DSH-2 function in the Wnt/β-catenin asymmetry pathway to regulate cell fates prior to hypodermal morphogenesis.

Materials and methods

Strains and alleles

The Bristol strain N2 was used as wild-type. Strains were maintained and cultured as previously described (Brenner, 1974). A deletion allele of *pop-1* was isolated from a UV-TMP mutagenized library of worms (Chase et al., 2001). Sequencing confirmed the presence of a 334 bp deletion extending from the first exon into the first intron, as well as a 22 bp insertion in the first intron of *pop-1*. The following mutations and transgenes were used for analysis or balancing. *LGI*: *pop-1*(*q772*), *sys-1*(*q736*) (Kidd et al., 2005), *fog-1* (*q325*), *fog-3*(*q520*); *LGII*: *dsh-2*(*or302*) (Hawkins et al., 2005), *mIn1* [*dpy-10*(*e128*); *mIs14*], *eff-1*(*oj55*) (Mohler et al., 2002); *LGIII*: *frl-1* (*ok460*), *wrm-1*(*ne1982ts*) (Nakamura et al., 2005), *wrm-1*(*ok738*), *dpy-1*(*e164*), *let-727*(*s2589*), *unc-32*(*e189*); *LGIV*: *jnk-1*(*gk7*); *LGX*: *mig-15*(*rh148*), *mig-15*(*rh80*) (Poinat et al., 2002), *mig-15*(*rh326*), *jkk-1*(*km2*); transgenes: *jcIs1*[AJM-1::GFP] (Köppen et al., 2001), *jcEx68* [*nhr-73*::*gfp*, *rol-6*(*su1006*)], *jcEx76*[*nhr-73*::*gfp*, *rol-6*(*su1006*)], *qIs74* [*pJK908*(*sys-1p*::*GFP*::*POP-1*)] (Siegfried et al., 2004), *jcIs26*[*pRF4*(*rol-6*(*su1006*), *pJS191*(*AJM-1*::*GFP*), (*CEH-16*::*GFP*)].

For analyzing rescue of mutant phenotypes, the *dsh-2* constructs (0.5 ng/μl) and the co-injection marker *sur-5*::*gfp* (80 ng/μl) (Gu et al., 1998) were injected into the gonads of NG3124 (*dsh-2*(*or302*)/*mIn1*(*dpy-10*(*e128*);*mIs14*)II) worms and maintained as extrachromosomal arrays in the following strains: full length *dsh-2*, SU302 (*jcEx87*), SU296 (*jcEx82*), SU297 (*jcIs1*, *jcEx82*); ΔDIX, SU303 (*jcEx88*), SU304 (*jcEx89*), SU321 (*jcIs1*, *jcEx89*); ΔPDZ, SU298 (*jcEx83*); ΔDEP, SU299 (*jcEx84*), SU300 (*jcEx85*), SU314 (*jcIs1*, *jcEx85*).

For analyzing rescue of POP-1 asymmetry, *dsh-2* constructs (0.5 ng/μl) were transformed with *rol-6*(*su1006D*) (80ng/μl) as a co-injection marker into SU350 (*dsh-2*(*or302*)/*mIn1*II; *qIs74*III) and maintained as extrachromosomal arrays in the following strains: full length *dsh-2*, SU369 (*jcEx100*), ΔDIX, SU378 (*jcEx103*), SU379 (*jcEx105*), SU380 (*jcEx106*), SU381 (*jcEx107*), ΔDEP, SU371 (*jcEx101*), SU372 (*jcEx108*).

RNAi

For cDNAs, PCR amplification using T3 and T7 primers produced RNA templates. For cosmid DNA, nested gene-specific primers, with T3 or T7 added to the 5′ end of the inner pair, were amplified by PCR. Ambion Megascript T3 and T7 kits (Ambion, Austin, Texas) were used for *in vitro* transcription. Transcription products were purified, mixed in equimolar concentrations and double-stranded. Y. Kohara (Gene Network Lab, NIG, Japan) kindly provided the following cDNA clones: yk55h11 (*dsh-2*), yk216a12 (*mig-5*), yk291a11 (*dsh-1*), yk397b6 (*dsh-1*), yk653h6 (*dsh-1*), yk579a6 (*mig-15*), yk369b7 (*jkk-1*), yk1117g4 (*jkk-1*), yk1092h4 (*jnk-1*), yk385e6 (*frl-1*), yk539h3 (*frl-1*), yk626c12 (*frl-1*). RNA templates for *fhod-2* were made from cosmid DNA (F56E10, obtained from Alan Coulson, Sanger Institute, England).

Double-stranded RNA was injected at a concentration of μg/μl into young adult hermaphrodites. Injected adults were allowed to lay embryos for at least 20 h prior to collection of embryos for imaging. RNAi efficacy was verified by comparison with published RNAi phenotypes available on Wormbase (www.wormbase.org).

Nomarski and fluorescent imaging of morphogenesis

Nomarski microscopy was used to collect 4D data sets as previously described (Raich et al., 1998). Typically, twenty optical sections with 1.5 μm spacing were collected at two- or three-minute intervals. Presence of the extrachromosomal array following 4D Nomarski imaging was determined by visualization of the coinjection marker SUR-5::GFP using epifluorescence microscopy.

For temperature shift experiments twenty-five optical sections with 1 μm spacing were collected at one-minute intervals on a custom made temperature controlled stage capable of shifting 10 °C/min. Embryos were shifted from the non-permissive temperature (25 °C) to the permissive temperature (15 °C; downshifts), or from the permissive temperature to the non-permissive temperature (upshift) at various times and analyzed for defects during morphogenesis.

Fluorescent images were collected with a Perkin-Elmer UltraVIEW spinning disk confocal microscope, using a Hamamatsu ORCA-ER CCD camera and Perkin Elmer UltraVIEW Imaging Suite software. For analysis of morphogenesis phenotypes twenty to thirty optical sections with 0.75 μm spacing were captured at two-minute intervals. Nomarski and fluorescent images were analyzed using macros and plugins for the public domain applications NIH Image and ImageJ (available at http://worms.zoology.wisc.edu/research/4d/4d.html). Pair-wise statistical tests between treatments were performed using Fisher's Exact Test (Zar, 1999); *p* values represent two-tailed probabilities.

Imaging and analysis of POP-1 asymmetry

GFP::POP-1 asymmetry between sister cells was assessed by confocal microscopy using twenty-two optical sections with 0.5 μm spacing and one-minute intervals at the approximately 100-cell stage. Sister pairs were identified by direct observations of cell division

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