Supplemental Data

Opposing Wnt Pathways Orient

Cell Polarity during Organogenesis

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Supplemental Results

VNS::SYS-1 localization during P7.p division

Previously, divisions of P5.p and P7.p were considered asymmetric in that their daughters produce cells with different fates. However, the formal possibility that the division is symmetric and the different fates are a result of signaling immediately following the division had not been ruled out. To distinguish between these possibilities, we monitored the localization of VNS::SYS-1 during P7.p division (Figure 4B). During metaphase, VNS::SYS-1 became concentrated in spots at the anterior and posterior poles of the nuclear membrane, probably corresponding to centrosomes (Phillips et al., 2007). Five minutes after metaphase, the VNS::SYS-1 associated with the anterior daughter nucleus appeared to spread throughout the nucleus, while the VNS::SYS-1 associated with the posterior daughter nucleus disappeared. Since VNS::SYS-1 asymmetry is observed during the division, we conclude that P7.p is polarized before or during cell division. In contrast to wild-type worms, VNS::SYS-1 in *lin-17(lf)* mutants remained associated with the posterior daughter nucleus and either disappeared from the anterior daughter nucleus or persisted in both cells. Thus *lin-17(lf)* affects the polarity of the P7.p cell prior to or during division.

Heat-shock cam-1

To confirm that the phenotypes we observed upon heat-shock were not due to intracellular signaling by Ror/CAM-1, we generated a kinase-inactive version of Ror/CAM-1 by changing two conserved lysines in the kinase domain to arginine (Forrester et al., 1999). Heat-shock of transgenic worms carrying a *hs::CAM-1(kinase-dead)* transgene caused both P-Rvl and A-Rvl phenotypes (Table S1), indicating that these phenotypes are independent of Ror/CAM-1 kinase activity. As it remained possible that Ror/CAM-1 signals intracellularly despite lacking kinase activity, we also generated transgenic worms carrying heat-shock inducible Ror/CAM-1 in which most of the intracellular domain is removed (*hs::CAM-1del-intra::GFP*). While heat-shock of worms carrying this transgene did not cause a Rvl phenotype, we did observe P-Rvl worms when combined with a *Wnt/lin-44(lf)* mutation. The reduced activity of this transgene compared to the full-length *hs::CAM-1* or *hs::CAM-1(kinase-dead)* transgenes may be due to a requirement of the intracellular domain for efficient membrane localization. We also note that P5.p polarity may be less sensitive to perturbation than P7.p because the posterior-facing orientation is reinforced by multiple signaling events. Finally, overexpression of full-length *CAM-1* in the VPCs using the *sur-2* (Singh and Han, 1995), and *lst-1* (Yoo et al., 2004) 5' regulatory sequences did not cause VPC polarity defects, confirming that the CAM-1 overexpression phenotype is not due to CAM-1 signaling in the VPCs. In

addition to VPC polarity defects, *hs::CAM-1* also caused VPC induction defects (when younger worms were heat-shocked) and severe gonad migration defects, both phenotypes consistent with general loss of Wnt signaling.

The anchor cell is the relevant source of MOM-2

The following experiments demonstrate that *Pfos-1a::CAM-1::GFP* interferes specifically with *mom-2* in the AC to produce a P7.p orientation defect. Like *mom-2(rf), Pfos-1a::CAM-1::GFP* enhanced the *lin-17(lf)* P-Rvl phenotype to nearly 100% (Table S1). Because *mom-2(rf)* is the only Wnt mutant that enhances the P-Rvl phenotype of *lin-17(lf)*, this suggests that *Pfos-1a::CAM-1::GFP* interferes with *mom-2* in the AC. Furthermore, expression of *mom-2* in the AC (*Pfos1a::MOM-2::YFP*) rescued the P-Rvl phenotype of *lin-44(lf)*; *mom-2(rf)* double mutants (Table S1).

EGL-20 gradient

We attempted to completely reverse the EGL-20 gradient such that the EGL-20 source would also be anterior to P5.p. Although we observed GFP expression from our transgenes, EGL-20 expressed in the head using *Pmyo-2* (pharyngeal muscle) or *Plim-4* (few head neurons) promoters in *lin-17*; *egl-20* double mutant worms neither rescued the P-Rvl phenotype nor caused any A-Rvl phenotype (not shown), suggesting that sufficient EGL-20 failed to reach the VPCs. The *Pmyo-2::EGL-20::GFP* construct we used was previously shown to rescue Q cell migration, a process that occurs in the first larval stage, in a dose-dependent manner (Whangbo and Kenyon, 1999). However, VPC division occurs in the third larval stage when the worms are much larger and the Wnts have both a greater distance to travel and a greater volume to diffuse into. It is possible that the cells expressing EGL-20 using *Pmyo-2* are less efficient than the endogenous EGL-20 source and that the amount of functional EGL-20 reaching the VPCs is not sufficient to orient their polarity. The *Plim-4::EGL-20::GFP* construct we used (Pan et al., 2006) was previously shown to partially rescue the HSN overmigration phenotype of *vab-8* and *ceh-10* mutants and to repel growth cones of the AVM and PVM neurons. However, these experiments have not been demonstrated to convey long-range *egl-20* activity because: 1) HSN migration occurs during embryogenesis and 2) AVM and PVM growth cones did not turn away until they were extremely close to the *egl-20* source. Therefore, our results are inconclusive as it is unknown whether we generated an EGL-20 gradient that reached the VPCs.

POPTOP

Seven copies of the TCF binding site, AGATCAAAGG, were transferred from Super8XTOPflash (plasmid M50) (Veeman et al., 2003) into Fire lab vector L3135 to place them downstream of the *pes-10* minimal promoter. The seven TCF sites and the *pes-10* minimal promoter were amplified using forward primer AAGCTTGGTACCGAGCTCGG and reverse primer ATGCCTAGGCAATCAATGCCTGAAAGTTAAAAATTAC. The product was then cloned into mCherry plasmid (PJIM20) with *let-858* 3' UTR (kind gift from Jon Audhya) using sites SpeI and AvrII. We also generated a control reporter, POPFOP; POP-1 Far from Optimal Promoter, that contains mutated Tcf/POP-1 binding sites. POPFOP was

made by a similar strategy as POPTOP using mutated TCF binding sites from plasmid Super8xFOPflash (plasmid M51).

Worms carrying an integrated POPTOP transgene display a dynamic expression pattern in many cells affected by Wnt signaling as well as cells in which Wnt has not been shown to function (Table S2). It is likely that POPTOP exhibits some background expression due to activity of the minimal promoter, the sequence linking or flanking the Tcf/POP-1 sites, or the 3' UTR.

To determine in which cases POPTOP represents a true readout of canonical Wnt pathway activity, we compared the POPTOP expression pattern to the that of POPFOP and to worms carrying *pop-1(q645)*, a mutation that disrupts the transactivation (β-catenin-binding) domain of Tcf/POP-1 (Siegfried and Kimble, 2002). In some cases, we also compared the POPTOP expression pattern to that in worms carrying a null mutation in *Axin/pry-1*, a negative regulator of Wnt signaling (Korswagen et al., 2002). We considered expression present in wild type worms carrying POPTOP and absent both in worms carrying POPFOP and in worms mutant for *Tcf/pop-1* to be a valid occurrence of Wnt activity. Elevated POPTOP expression in *Axin/pry-1* mutant worms further validated this conclusion and identified potential sites, such as body wall muscle, where Wnt activity is suppressed. A review by M. Herman (Herman, 2002), delineates the known examples of Wnt signaling in *C. elegans*. Canonical Wnt signaling influences QL.d migration, P12 and VPC fate specification. Non-canonical Wnt signaling controls cell polarity and has been reported to regulate the T and B cells, Z1/Z4 somatic gonadal precursors (SGPs), V5 and the EMS blastomere. We observed POPTOP expression in many of these cells including the Q cells and their descendents, the T cell, the B cell, V5 and the SGPs. We also observed POPTOP expression in the male hook precursor cells, whose division is regulated by *Fz*/*lin-17* (Sternberg and Horvitz, 1988). We did not detect POPTOP expression in the embryo until the gastrulation stage. This could be because POPTOP expression is too weak to be detected earlier, or it could be due to the common phenomenon of germline silencing of transgenes (Kelly et al., 1997). POPTOP is expressed in the P cells and the Pn.p cells, however, this expression was also present in *Tcf/pop-1* mutant worms and in POPFOP negative control worms and was thus considered to be background expression. This background expression prevented any analysis of P12 specification or VPC induction. Background Pn.p expression vanished at the L3 stage allowing us to analyze POPTOP expression in the VPC daughters and granddaughters. Besides the known sites of Wnt activity, we also observed non-background expression in the distal tip cells (DTCs) and uterine cells (Table S2). Between strains, POPTOP expression often differed in intensity rather than "on" or "off." Thus, to compare expression between strains, we selected conditions (Texas Red filter, one-second exposure, contrast set to zero, Openlab by Improvision version 5.0.2 software) where we regularly observed expression in wild type worms, but not in *pop-1(q645)* mutants, and held these conditions constant during our analysis of different strains. At these conditions, POPTOP expression in the VPC progeny was scored as detectable or not detectable (Table S3).

Transgenics

Extrachromosomal arrays were generated by co-injecting a transgene with *unc-119(+)* [60ng/μL] into *unc-119(ed4)* hermaphrodites or with *pha-1(+)* [90 ng/μL] into *pha-*

1(e2123ts) hermaphrodites as described (Mello et al., 1991) except for *vang-1::YFP*, which was generated using bombardment (Praitis et al., 2001). Construction of transgenes *syEx710*, *syEx777*, *syEx780*, *syEx864* and *syIs198* was described previously (Green et al., 2007). *syIs202 [vang-1::YFP]* contains *vang-1* genomic DNA amplified with forward primer TTCTACCGGTGTGGAATAGGAAACCTGAAATTATGAATTATG and reverse primer CCAATCGTATGGCCGTTAATTAAGATACGCTTAAAGCTGG and includes coding sequence up to the beginning of the 5th exon and 3kb of sequence 5' of ATG. *Pfos-1a::EGL-20::GFP* was made by replacing the *myo-2* promoter of pJW33 (Whangbo and Kenyon, 1999) with the *fos-1a* promoter amplified using primer CGCGGATCCTGGGCAGCTGTAAAACGTCTTTAC (Bam HI site engineered 5') and reverse primer GCAGCTAGCTCCACTCTCTTATATAGCAGAGGTG (Nhe I site engineered 3'). To make *Pfos-1a::MOM-2::YFP*, the above *fos-1a* promoter was transferred into Fire vector L4817 using BglII and NheI. *mom-2* cDNA, amplified by forward primer AGCATGCTAGCCATGCACATCAACACGCCAGTTC and reverse primer CTACCGGTACCAAACAGTAGTTTCTTTCTACTAACTTCTT, was then introduced using sites NheI and AgeI. *syEx1005[Pheat-shock::CAM-1(del-intra)]* was made by switching the DNA encoding the carboxy-terminus of *syEx710[Pheat-shock::CAM-1]* with *syEx814[Pmyo-3::CAM-1(del-intra)]* (Green et al., 2007) using NotI and SbfI. To make *Pheat-shock::CAM-1(kinase-dead)*, we started with *syEx710* and changed codons 624 and 625 from encoding lysines to encoding arginines, as previously done to inactivate the CAM-1 kinase domain (Forrester et al., 1999). Because worms carrying *Pmyo-3::CAM-1::GFP* and *Psnb-1::CAM-1::GFP* as extrachromosomal arrays (Green et al., 2007) did not perform well in crosses, these plasmid were injected into *lin-17(n671); cam-1(gm122)* double mutants and progeny that carried the arrays were scored. We did not obtain stable lines for either of these transgenes. To make *Pbar-1::4XNLS::GFP*, we amplified the 5' regulatory region using forward primer GCTCTAGACTTCATTCGATAGCAGATACAAC (introducess XbaI) and reverse primer TCAGATCTCCCAGTTTTCTGAAAAAAAAAGCCAAA (introduces BglII). The product was cloned into PGEM T-Easy (Promega) and then transferred into Fire vector L4018 using PstI and BglII/BamHI. BAR-1::GFP (*gaIs45*) (Eisenmann et al., 1998), GFP::POP-1 (*qIs74)* (Siegfried et al., 2004), VNS::SYS-1 (*qIs95*) (Phillips et al., 2007), WRM-1::GFP *(osEx158)* (Takeshita and Sawa, 2005), GFP::LIT-1 (Rocheleau et al., 1999) and *Pheat-shock::EGL-20* (pJW30) (Whangbo and Kenyon, 1999) were previously described. Our attempts to reverse the EGL-20 gradient utilized plasmids pJW33 [*Pmyo-2::EGL-20::GFP*] (Whangbo and Kenyon, 1999) injected at 15ng/µl, 20ng/µL and 80ng/µL and [*Plim-4::EGL-20::GFP*] (Pan et al., 2006) injected at 20ng/µl.

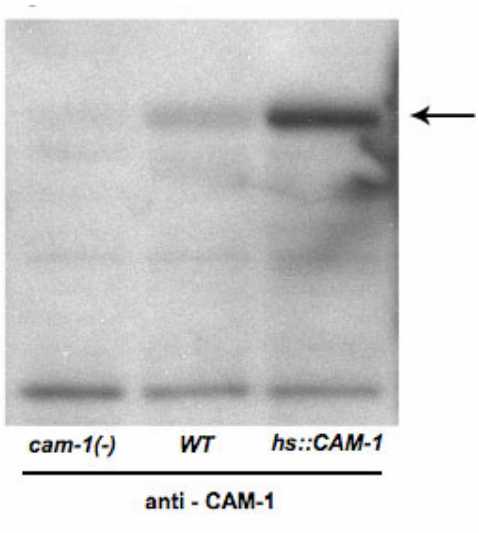

Figure S1. *hs::CAM-1* Western blot
Worms carrying *syEx710[hs::CAM-1]* were heat-shocked for 45 min. at 33˚C. This resulted in elevated CAM-1 levels (arrow) compared to wild type and *cam-1(lf)* mutants. Total worm lysates were probed with an anti-CAM-1 polyclonal antibody.

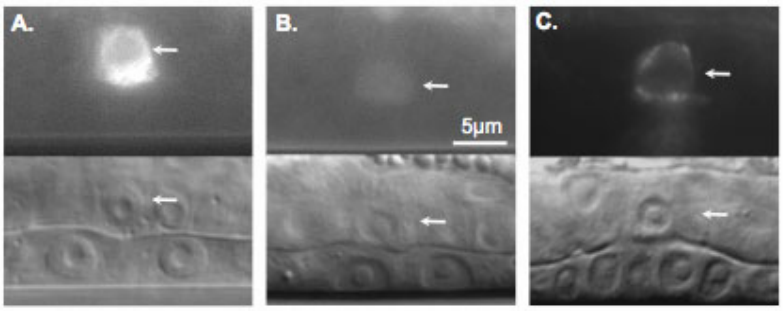

Figure S2. Anchor cell expression of *egl-20, lin-44* and *cam-1*
Fluorescence (top) and Nomarski (bottom) images of A) *Pfos-1a::EGL-20::GFP*, B) *Plin-44::GFP*, and B) *Pfos-1a::CAM-1::GFP* transgenes expressed in the AC (arrow).

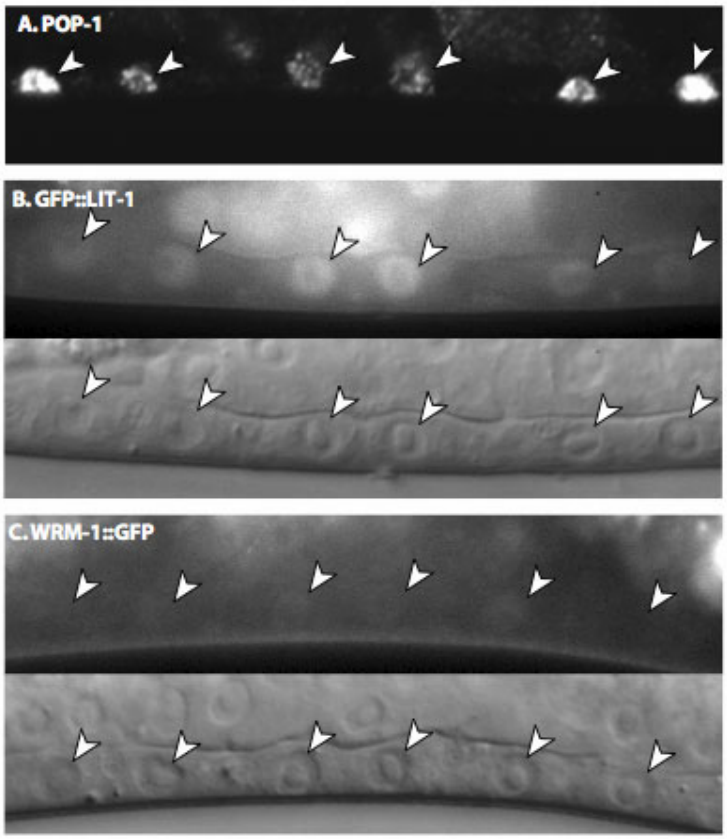

Figure S3. Localization of POP-1, GFP::LIT-1 and WRM-1::GFP in VPC progeny
A) POP-1 expression in VPC daughter nuclei (arrowheads). POP-1 antibody staining was performed as described (Deshpande et al., 2005). B) GFP::LIT-1 (Rocheleau et al., 1999) was consistently observed in the VPC daughter nuclei (arrowheads). Asymmetric localization of GFP::LIT-1 among the secondary daughter nuclei was subtle and often not detectable by eye. To analyze the expression pattern we captured still images and used Openlab software to measure the mean pixel intensity of each nucleus. If the difference between two secondary sisters was greater than two standard deviations of the mean difference between the primary sisters, the pair was classified at unequal. In 16/22 wild type worms, GFP::LIT-1 levels were higher in the proximal daughters nucleus of P5.p and were equal between P5.p daughter nuclei in 6/22 worms. GFP::LIT-1 levels were higher in the proximal daughters nucleus of P7.p in 19/22 wild type worms, were equal between the P7.p daughter nuclei in 2/22 worms, and were higher in the distal daughter nucleus in 1/22 worms. C) WRM-1::GFP (*osEx158*, (Takeshita and Sawa, 2005)), is expressed in the VPC daughter nuclei (arrowheads) at extremely low levels. The fluorescence image shown here was exposed for 10 sec. and represents one of the rare cases expression was detectable. In each of the few cases where expression was detectable, it appeared higher in the proximal daughter nuclei of P5.p and P7.p.

Table S1. CAM-1 over-expression to inhibit Wnts

Relevant Genotype	% P-Rvl	% A-Rvl	% AP-Rvl[^]	n	*P* value
parent strain[#]	0	0	0	40	
Ex[Pheat-shock::CAM-1(kinase-dead)][#]	5	8	0	39	0.026[a]
syEx1005[Pheat-shock::CAM-1(del-intra)::GFP][#]	0	0	0	20	
lin-44(n1792); syEx1005[Pheat-shock::CAM-1(del-intra)::GFP][#]	16	0	0	69	<.0001[b]
syIs198[Plst-1::CAM-1::GFP]	0	5	0	22	
syEx864[Psur-2::CAM-1::GFP]	0	0	0	38	
lin-17(n671)	74	0	0	113	
*lin-17(n671); lin-44(n1792)**	58	0	0	186	
lin-17(n671); cwn-1(ok546)	52	0	0	54	
lin-17(n671); cwn-2(ok895)	75	0	0	40	
*lin-17(n671); mom-2(or42)**	100	0	0	103	<.0001[c]
lin-17(n671); syEx780[Pfos-1a::CAM-1::GFP]	98	0	0	41	.0009[c]
*lin-44(n1792); mom-2(or42)**	59	0	0	127	
lin-44(n1792); mom-2(or42); syEx[Pfos-1a::MOM-2::GFP]	33	0	0	24	.0254[d]

For each genotype, only worms with wild-type vulval induction, i.e. 3.0, were scored.
mom-2(or42) are homozygous progeny from heterozygous mothers.
*values originally reported in Inoue et al., 2004. [^]AP-Rvl worms are also included in A-Rvl and P-Rvl categories.
[#] Mixed stage worms were heat-shocked 45 min. (CAM-1) or 20 min. (EGL-20) at 33^{o}, mid-L4 animals were scored 16 hours later.
[a] compared to *pha-1(e2123);him-5(e1490)*, [b] compared to *lin-44(n1792)*, [c] compared to *lin-17(n671)*, [d] compared *to lin-44(n1792); mom-2(or42)* using Fisher's Exact Test.

Table S2. Partial expression pattern and validation of POPTOP

cells (stage)	*Wild type* POPTOP	*Wild type* POPFOP	*pop-1(q645)* POPTOP	*pry-1(mu38)* POPTOP
P cells (L1)	+[a]	+	+[b]	ND
QL and QR cells (L1-L2)	+[c]	-	-[d]	ND
SGPs (L1)	+	-	-	ND
V cells (L1)	+	-	ND	ND
B cell (L1)	+	ND	ND	ND
T cell (L1)	+	-	-	ND
VCNs (L1-L4)	+	+	+	+
Pn.ps (L1-late L2)	+	+	+	+
Pn.p (early L3)	-	-	-	ND
Pn.px (mid L3)	-	-	-	+++
Pn.pxx (mid L3)	+	-	-	+++
male hook precursors (L1-L4)	+	ND	ND	+
DTCs (L2-L3)	+	-	NA	++
vulval cells (L3-L4)	+	-	-	+++
uterine cells (L4)	+	-	-	+++
body wall muscle (L1-L4)	-	-	-	+++
vulval muscle (adult)	+	-	-	ND
many unidentified cells in head (all)	+	+	+	ND
many unidentified cells in tail (all)	+	+	+	ND

QL, QR: left and right Q neuroblasts. SGP: somatic goandal precursor. VCN: ventral cord neuron. DTC: distal tip cell. [a]P cell expression in 100% of worms n=34. [b]P cell expression in 100% of worms n=37. *P*<0001 using Fisher's Exact Test. [c]QL expression in 91% of worms n=34. [d]QL expression in 27% of worms n=37. *P*<0001 using Fisher's Exact Test. ND= not determined

Table S3. POPTOP expression in VPC granddaughters

genotype:	[#]% reporter expression in both daughters of (*P* value): P5.pa	P5.pp	P6.pa	P6.pp	P7.pa	P7.pp	n
POPTOP syIs186	5	24	10	10	33	5	21
POPTOP syIs187	0	38	5	5	29	0	21
POPTOP syIs188	0	43	4	11	36	0	28
pop-1(q645); syIs187*	0	0(.001)	0	0	0(.009)	0	22
pry-1(mu38); syIs188	30	95(<.001)	100	100	80(.003)	40	20
lin-17(n671); syIs187	0	0(.003)	0	0	0(.021)	0	20
lin-18(e620); syIs186	0	13(.457)	0	0	0(.003)	13	24
sys-1(q544); syIs187*	0	0(.003)	0	0	10(.238)	10	20
bar-1(ga80); syIs188	0	15(.060)	0	0	20(.338)	10	20
egl-20(hu120); syIs186	0	48(.197)	0	5	48(.530)	5	21
lin-17(n671); egl-20(hu120); syIs188	0	5(.003)	0	0	5(.014)	0	21

POPTOP expression was scored as positive if it was detectable in a one-second exposure.
[#]Percentages are of worms with wild-type vulval induction (3.0). *P* values represent a comparison to the corresponding transgenic strains in the wild-type background and were calculated using Fisher's Exact Test.
[^]Temperature sensitive allele; L1 worms were raised at 25C.
[*]Homozygous progeny from heterozygous mothers.

Supplemental References

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