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Conservation and diversification of Wnt signaling function during the evolution of nematode vulva development

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Cell-fate specification and cell-cell signaling have been well studied during vulva development in *Caenorhabditis elegans* and provide a paradigm in evolutionary developmental biology[1,2]. *Pristionchus pacificus* has been developed as a 'satellite' organism with an integrated physical and genetic map that allows detailed comparisons to *C. elegans*[3–5]. A common aspect of vulva formation in both species is the polarization of the P7.p lineage, which is responsible for vulval symmetry. In *C. elegans*, Wnt signaling is crucial for P7.p cell-fate patterning[6]; nothing is known about vulval symmetry in *P. pacificus*. We isolated mutations that disrupt polarization of the P7.p lineage in *P. pacificus* and found that the corresponding gene encodes a Frizzled-like molecule. In addition, mutations in *Ppa-lin-17* (encoding Frizzled) and morpholino knock-down of *Ppa-lin-44* (encoding Wnt), *Ppa-egl-20* (encoding Wnt), *Ppa-mig-5* (encoding Dsh), *Ppa-apr-1* (encoding APC) and *Ppa-bar-1* (encoding β-catenin) results in gonad-independent vulva differentiation, indicating that these genes have a role in a negative signaling process. In contrast, in *C. elegans*, Wnt signaling has a positive role in vulva induction, and mutations in *bar-1* result in a hypoinduced phenotype[7]. Therefore, whereas the molecular mechanisms that generate vulval symmetry are conserved, the genetic control of vulva induction diversified during evolution.

Some studies suggest that there are more than 10 million nematode species and that nematodes occur in all ecosystems on earth[8]. The detailed genetic and molecular understanding of various developmental processes in *C. elegans* provides a platform for studying the plasticity of genetic mechanisms among nematode species. The ease with which *C. elegans* is cultured and used as a model system under laboratory conditions also applies to a number of other nematode species. *P. pacificus* has been developed as a 'satellite' system in evolutionary developmental biology with an integrated genome map combined with both forward and reverse genetic tools[3–5]. *P. pacificus* and *C. elegans* are separated from one another by 200–300 million years and are members of two distinct nematode families[9].

Three of the 12 ventral epidermal cells, P(5–7).p, are induced to form the vulva, and at least four different signaling systems are required for proper vulva formation in *C. elegans*[1,10–12] (**Fig. 1a**,**b**). *P. pacificus* has substantial differences in cell-fate specification and the mode of vulva induction. Of the 12 ventral epidermal cells, 7 die by programmed cell death in *P. pacificus*[13], whereas the corresponding cells fuse with the hypodermis in *C. elegans* (**Fig. 1a**,**d**). The *P. pacificus* vulva is induced by a continuous signal from multiple cells of the somatic gonad[14]; in contrast, a single anchor cell induces the vulva in *C. elegans*[15] (**Fig. 1b**,**e**). The epidermal cell P8.p in *P. pacificus* develops differently from the corresponding cell in *C.* elegans[16] (**Fig. 1d**).

Despite differences in cell-fate specification and vulva induction, the vulva in *P. pacificus* and *C. elegans* is formed by cells P(5–7).p, which adopt a 2°-1°-2° fate pattern (**Fig. 1c**,**f**). The cell lineage of the 2° vulval precursor cell (VPC) P7.p of both species is identical to that of cell P5.p but occurs in the opposite anteroposterior orientation (**Fig. 1c**,**f**). In *C. elegans*, the P5.p lineage orientation represents the default state for the P5.p and P7.p lineages[6].

To identify the genetic basis of the polarization of the P7.p lineage in *P. pacificus*, we carried out various mutagenesis screens. We isolated seven egg-laying–defective mutants in *P. pacificus* with a new vulva phenotype (**Table 1**). Complementation tests showed that all seven mutants are alleles of the same gene, which is named P-ectoblast-defective-7, *Ppa-ped-7*. Detailed cell-lineage analysis showed that *Ppa-ped-7* mutant worms have lineage defects restricted to the P7.p lineage (**Table 1**). Specifically, we analyzed the cellular anatomy of 482 mutant worms and found that 159 (33%) had a lineage reversal of P7.p, resulting in a Bivulva (Biv) phenotype. In *C. elegans*, mutations in two genes, *lin-17* and *lin-18*, cause a similar cell lineage reversal of P7.p that results in a Biv phenotype[6].

To determine whether *Ppa-ped-7* mutants have other vulval defects, we carried out cell ablation and genetic experiments. The results of these experiments indicate that *Ppa-ped-7* has an additional function in a negative signaling event that prevents vulva formation. When

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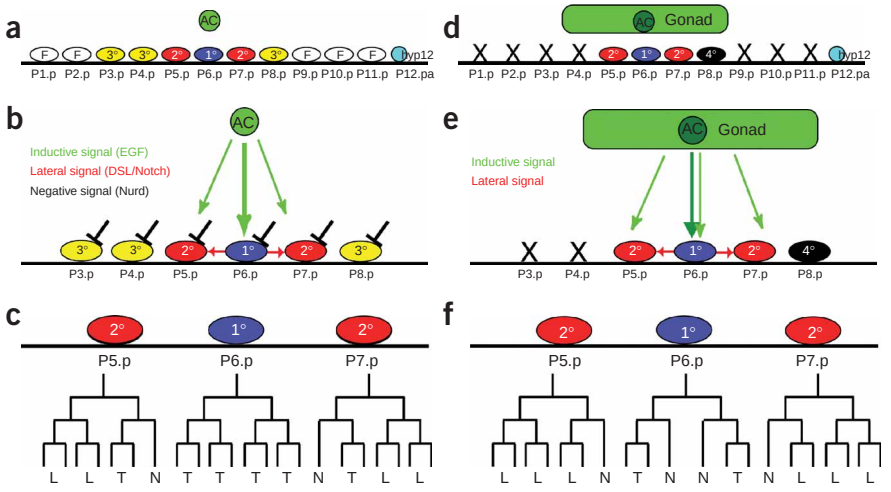

Figure 1 Schematic summary of the position, fate and cell lineage of ventral epidermal cells in *C. elegans* (**a**–**c**) and *P. pacificus* (**d**–**f**). (**a**) Cell-fate specification in *C. elegans*. Cells P(1,2,9–11).p fuse with the hypodermal syncytium *hyp7* (F, white ovals). Cells P(3–8).p form the vulva equivalence group and adopt one of three alternative cell fates. Cells P(3,4,8).p remain epidermal and have a 3° fate (yellow ovals). Cells P(5,7).p have a 2° fate and form the anterior and posterior part of the vulva (red ovals). Cell P6.p has a 1° fate and forms the central part of the vulva (blue oval). The anchor cell (AC, green circle) is born dorsally to cell P6.p, induces vulva formation and makes contact with the progeny of cell P6.p. (**b**) Schematic summary of signaling interactions during vulva formation in *C. elegans*. An inductive EGF-like signal originates from the anchor cell (AC, green arrows). Cell P6.p signals its neighbors to adopt a 2° fate by 'lateral signaling' (red arrows). Negative signaling (black bars) prevents inappropriate vulva differentiation. (**c**) Cell lineage diagram of *C. elegans* P(5–7).p. The two 2° VPCs P5.p and P7.p have identical lineages with different anteroposterior orientations. Division plane of the last round of cell divisions of the VPCs is indicated: L, longitudinal; T, transversal; N, nondividing. (**d**) Cell-fate specification in *P. pacificus*. Cells P(1–4,9–11).p die by programmed cell death (X). Cells P(5–7).p form the vulva with a 2°-1°-2° pattern. Cell P8.p does not form part of the vulva and has a special fate, designated 4° (black oval). Cell ablation experiments indicate that *P. pacificus* cell P8.p has distinct developmental properties. Cell P8.p cannot respond to a signal from the gonad after ablation of cells P(5–7).p but responds to lateral signaling after ablation of cell P7.p[16]. (**e**) Model for cell-cell interactions during vulva development in *P. pacificus*. Vulva induction involves multiple cells of the somatic gonad. Lateral signaling occurs between cell P6.p and cells P(5,7).p (red arrows). (**f**) Cell lineage diagram of *P. pacificus* P(5–7).p. The two 2° VPCs P5.p and P7.p have identical lineages with different anteroposterior orientations.

we ablated Z(1,4), the precursors of the somatic gonad, gonad-independent vulva differentiation (Gid) was observed for all seven *Ppa-ped-7* alleles (**Table 1**). For four of the seven alleles, cells P5.p, P6.p and P7.p differentiated in 100% of the gonad-ablated worms. In contrast, cells P(5–7).p remained epidermal after ablation of Z(1,4) in wild-type worms[16] (**Table 1**). Additional evidence supporting the idea that *Ppa-ped-7* is part of a negative signaling system comes from analysis of *Ppa-ped-5 Ppa-ped-7* double mutants (**Table 2**). In wild-type worms, the four anterior cells P(1–4).p die by programmed cell death (**Fig. 1d**). Therefore, there are no extra cells in *P. pacificus* that could form an ectopic vulva-like structure in the anterior body region. In *Ppa-ped-5* mutant worms, ventral epidermal patterning is partially converted to that of *C. elegans*[13]. Cells P(3,4).p survive and have an epidermal (3°) cell fate, thereby reconstituting the vulva equivalence group of *C. elegans* (**Table 2**). Cell P4.p formed ectopic vulval tissue in 47% of *Ppa-ped-5 Ppa-ped-7* double mutant worms, reminiscent of the multivulva phenotype in *C. elegans*[1] (**Table 2**). Taken together, these results indicate that *Ppa-ped-7* has two distinct functions in *P. pacificus* vulva formation: it polarizes the P7.p lineage and is involved in a negative signaling process that inhibits ectopic vulva formation.

To identify the molecular nature of *Ppa-ped-7*, we mapped the locus in the polymorphic strain *P. pacificus* var. Washington[5]. *Ppa-ped-7* is located on chromosome V and maps in the central region between the single-stranded conformation polymorphism (SSCP) markers S13 and S59 (**Fig. 2a**). The *P. pacificus* physical map indicates that several BAC clones are located in this region of chromosome V[5]. When we analyzed the end sequences of these BAC clones, we identified a sequence with strong similarity to the gene *lin-17* (ref. 17) in the PPBAC-39-F14 clone and obtained full-length cDNA and genomic clones. *Ppa*-LIN-17 and LIN-17 are quite similar structurally, with an overall amino acid identity of 46% (**Fig. 2b,c**). The highest amino acid similarity is in the cysteine-rich domain in the N-terminal part of the protein, which interacts directly with Wnt ligands. To determine whether *Ppa-lin-17* corresponds to *Ppa-ped-7*, we sequenced *Ppa-lin-17* in six *Ppa-ped-7*

Table 1 Penetrance of *Ppa-ped-7* alleles

Allele	Molecular lesion	P7.p lineage reversal, % (*n*)	Gid, % (*n*)
	Wild-type	0 (100)	0 (93)
tu33	Splice	27 (132)	64 (75)
tu66	Nonsense	25 (61)	67 (36)
tu76	Splice	17 (101)	82 (72)
tu106	Deletion	39 (46)	100 (24)
tu62	Nonsense	48 (64)	100 (30)
tu108	Deletion	51 (37)	100 (36)
tu107	Unknown	59 (41)	100 (18)

The P7.p lineage reversal is scored as the percentage of worms with a Biv phenotype. Cellular anatomy was scored in the late J3 and early J4 stages. Gid is scored as the percentage of P(5–7).p VPCs adopting vulval fates after the ablation of the somatic gonad at hatching. The Gid phenotype is scored on per cell (rather than per worm).

Table 2 *Ppa-ped-7* is a multivulva mutant

Organism and background	P3.p	P4.p	P5.p	P6.p	P7.p	P8.p	*n*
C. elegans wild-type	3°	3°	2°	1°	2°	3°	Many
P. pacificus wild-type	X	X	2°	1°	2°	4°	Many
P. pacificus ped-7(tu108)	X	X	2°	1°	2°	4°	19/37
P. pacificus ped-5	3°	3°	2°	1°	2°	4°	30/30
P. pacificus ped-5 ped-7	3°	2°	2°	1°	2°	4°	16/53
	3°	2°	2°	1°	2°	4°	7/53
	3°	3°	2°	1°	2°	4°	14/53
	2°	2°	2°	1°	2°	4°	2/53
	3°	3°	2°	1°	2°	4°	14/53

X, programmed cell death; 1°, vulval cell fate; 2°, vulval cell fate; 3°, epidermal cell fate of uninduced VPCs; 4°, specific epidermal fate of *P. pacificus* P8.p. The lineage reversal of P7.p is underlined.

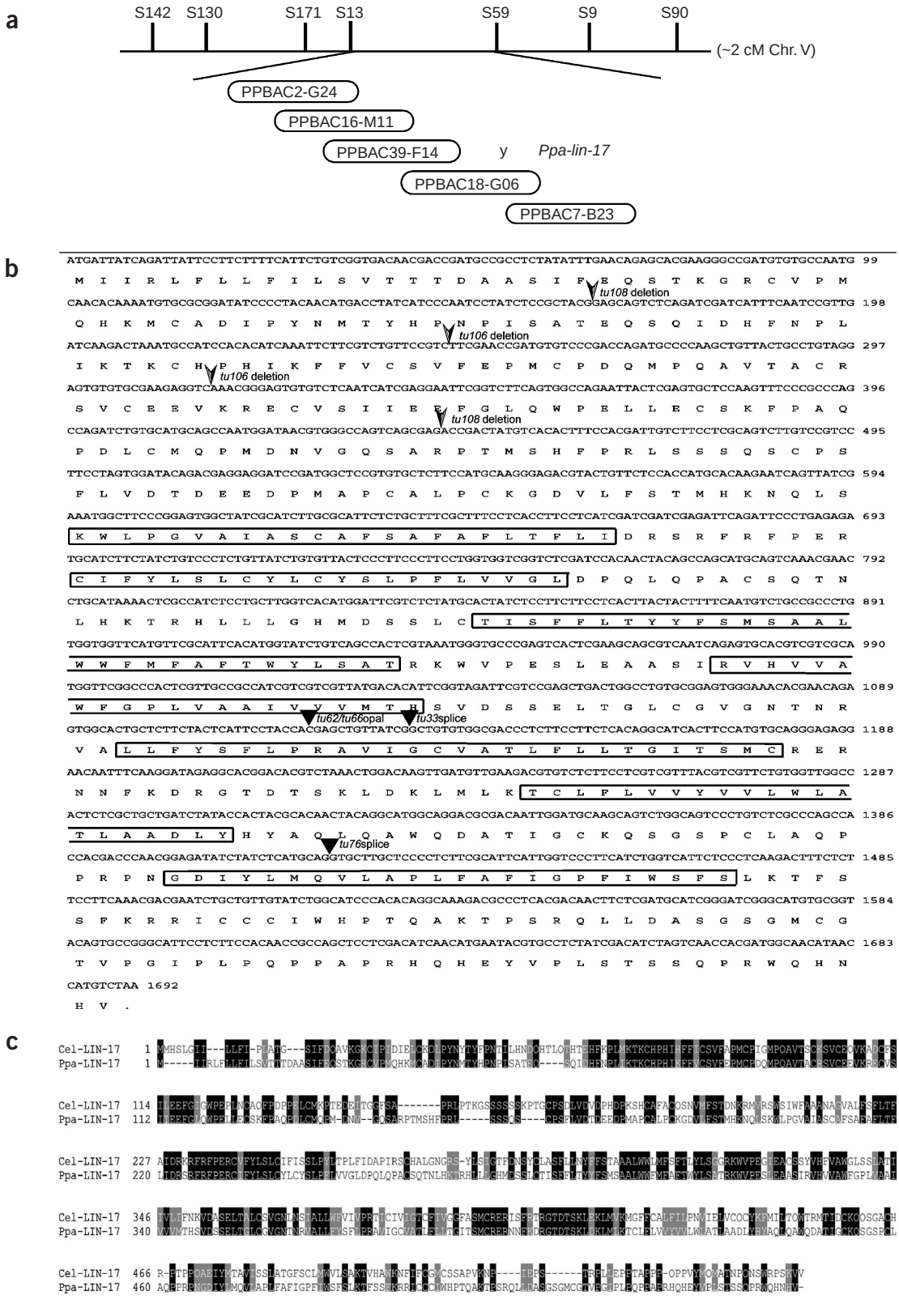

Figure 2 Molecular characterization of *Ppa-lin-17*. (**a**) Map position of *Ppa-lin-17* on chromosome V between the SSCP markers S13 and S59. Several BAC clones span the region between S13 and S59. The PPBAC-39-F14y end contains a strong prediction for the gene *Ppa-lin-17*. (**b**) Conceptual translation of *Ppa-lin-17*. The seven transmembrane domains are boxed. The margins of the deleted regions in the *tu106* and *tu108* alleles are indicated by arrowheads, and the mutations of the four ethyl methanesulphonate alleles are indicated by triangles. *tu62* and *tu66* are opal mutations and *tu33* and *tu76* are splice-site mutations. (**c**) Amino acid sequence comparison of LIN-17 between *P. pacificus* (Ppa) and *C. elegans* (Cel). Identical amino acids are shown in black; similar amino acids, in gray.

Table 3 Gid and P7.p lineage reversal phenotype of morpholino-treated *Ppa-lin-17(tu33)* mutant worms

Genetic background	Gid, % (*n*)	*P* value	P7.p lineage reversal, % (*n*)	*P* value
Wild-type	0 (93)		0 (100)	
Ppa-lin-17(tu33)	64 (75)		27 (132)	
Ppa-lin-17(tu33) Dr-rx3	59 (27)		26 (283)	
Ppa-lin-17(tu33) Ppa-dnmt	63 (54)		22 (100)	
Ppa-lin-17(tu33) Ppa-lin-17	88 (102)	2×10^{-4}	46 (411)	1×10^{-4}
Ppa-lin-17(tu33) Ppa-lin-44	94 (84)	6×10^{-6}	50 (458)	2×10^{-6}
Ppa-lin-17(tu33) Ppa-egl-20	91 (66)	3×10^{-4}	52 (138)	2×10^{-5}
Ppa-lin-17(tu33) Ppa-mig-5	90 (60)	1×10^{-3}	60 (129)	1×10^{-7}
Ppa-lin-17(tu33) Ppa-apr-1	93 (90)	7×10^{-6}	27 (212)	0.9605
Ppa-lin-17(tu33) Ppa-bar-1	91 (54)	1×10^{-3}	26 (132)	1
Ppa-lin-17(tu33) Ppa-lin-18	63 (96)	0.97	50 (139)	0.014

See legend of **Table 1** for test of Gid and lineage reversal phenotypes. Statistical tests are pairwise comparisons between mutant-morpholino combinations and the *tu33* allele alone and were done using the χ^2 test.

alleles and identified mutations in all of them (**Fig. 2b**). Taken together, these results indicate that *Ppa-ped-7* is *Ppa-lin-17*.

We further assessed the mechanisms of negative signaling and the polarization of the P7.p lineage by reducing the activity of individual genes using the morpholino technology[9]. Injection of a *Ppa-lin-17* morpholino into wild-type worms resulted in progeny with only a weak Biv and Gid phenotype. But RNA interference often has little effect on vulva development in a wild-type *C. elegans* background as well[11]. Therefore, we sensitized the system by using a weak *Ppa-lin-17* mutated allele as genetic background. The *Ppa-lin-17* morpholino strongly enhanced both aspects of the phenotype for the *Ppa-lin-17(tu33)* allele (**Table 3**). Specifically, Gid was enhanced from 64% to 88% and P7.p lineage reversal from 27% to 46% (**Table 3**). In contrast, two morpholino control experiments with a heterologous zebrafish gene *Dr-rx-3* and the *P. pacificus* gene *Ppa-dnmt* showed no effect in a *Ppa-lin-17(tu33)* mutant background (**Table 3**).

Next, we wanted to determine which protein acts as a potential ligand in the negative signaling system. In *C. elegans*, two Wnt-like molecules, encoded by the genes *lin-44* and *egl-20*, interact with *lin-17* in a variety of postembryonic processes[17]. We cloned the genes *Ppa-lin-44* and *Ppa-egl-20* and showed that the encoded proteins are highly similar to the orthologous proteins in *C. elegans* (**Supplementary Fig. 1** online). We generated morpholino oligonucleotides for *Ppa-lin-44* and *Ppa-egl-20* and tested them in the sensitized genetic background of *Ppa-lin-17(tu33)*. Morpholino knock-down of each gene has a similar effect and results in an increase of Gid (**Table 3**). These results suggest that *Ppa-lin-44* and *Ppa-egl-20* both have a role during negative signaling and that they may act as redundant ligands for *Ppa-lin-17*.

To determine the specificities of Wnt signaling during vulva formation in *P. pacificus*, we tested three of the cytoplasmic components of Wnt signaling, *Ppa-mig-5* (encoding Dsh), *Ppa-apr-1* (encoding APC) and *Ppa-bar-1* (encoding β-catenin), in the morpholino assay. We identified *P. pacificus* orthologs of *mig-5*, *apr-1* and *bar-1* in the available BAC end and fosmid end sequences and obtained full-length cDNA clones (**Supplementary Fig. 1** online). Gid was enhanced to 90%, 93% and 91% in worms treated with *Ppa-mig-5*, *Ppa-apr-1* and *Ppa-bar-1* morpholinos, respectively (**Table 3**). These results suggest that negative signaling in *P. pacificus* requires a Wnt pathway that contains many of the known components of Wnt signaling pathways in other organisms.

To test which components of the Wnt signaling pathway have a role in the polarization of the P7.p lineage in *P. pacificus*, we also used the morpholino assay. Results of these experiments suggest that the polarization of the P7.p lineage and negative signaling are regulated by distinct Wnt pathways. *Ppa-egl-20*, *Ppa-lin-44* and *Ppa-mig-5* morpholinos caused similar enhancement of the lineage reversal of P7.p (**Table 3**). In contrast, *Ppa-apr-1* and *Ppa-bar-1* morpholinos did not result in an increase of P7.p lineage reversal (**Table 3**). In addition to *lin-17*, *lin-18* (encoding Ryk) is also involved in P7.p polarization in *C. elegans*[6]. We cloned the *P. pacificus* ortholog of *lin-18* (**Supplementary Fig. 1** online) and tested it in the morpholino assay. *Ppa-lin-18* morpholino resulted in an increase of P7.p lineage reversal but did not cause an enhancement of the Gid phenotype (**Table 3**). Taken together, these results indicate that *Ppa-apr-1* and *Ppa-bar-1* do not have a role in the lineage reversal of P7.p, whereas *Ppa-lin-18* has a role in P7.p polarization but not in negative signaling. These results support the idea that distinct Wnt pathways act during *P. pacificus* postembryonic development.

We showed that a Wnt signaling pathway has a new role in providing a negative signal during vulva formation in *P. pacificus* that inhibits ectopic vulva differentiation. No such negative signaling function is known from the Wnt pathway during *C. elegans* vulva development. Specifically, when we ablated the somatic gonad in *lin-17(n671)* mutant *C. elegans*, no vulva differentiation was observed in 43 tested worms. Another negative signaling system does exist in *C. elegans* vulva formation and requires the nucleosome remodeling and histone deacetylase complex[18]. We speculate that *P. pacificus* negative signaling is coupled to the mode of vulva induction. A continuous inductive signal from the somatic gonad, as occurs in *P. pacificus*, has to be balanced to prevent precocious vulva differentiation[14] (**Fig. 1d**). The most notable result of our analysis of *P. pacificus* vulva formation is that Wnt signaling has opposite roles in *P. pacificus* and *C. elegans*. In *C. elegans*, mutations in *bar-1* result in hypoinduction of the vulva[7], whereas mutations and morpholino knock-down in *Ppa-bar-1* in *P. pacificus* result in gonad-independent (multivulva) phenotypes. These results suggest that the genetic control of vulva development differs between nematode species, with Wnt signaling function evolving towards activation and repression of vulval cell fates in independent evolutionary lineages. Consistent with our findings, the only identified downstream target of Wnt signaling during *C. elegans* vulva induction, the homeotic gene *lin-39*, is not required in *P. pacificus* vulva induction[19,20]. Given the opposite function of Wnt signaling in two present-day species, it remains unknown how Wnt signaling evolved in nematodes and which function Wnt signaling might have had in the last common ancestor of *Pristionchus* and *Caenorhabditis*.

In addition to the divergence in the molecular mechanisms of vulva induction, this study suggests that Wnt signaling is conserved in the polarization of the P7.p cell lineage in *P. pacificus* and *C. elegans*. *lin-17* and *Ppa-lin-17* mutants have a similar P7.p lineage defect, resulting in a Biv phenotype. Recent studies in *C. elegans* provide further molecular insight into the mechanism of Wnt signaling during P7.p polarization[6]. The morpholino knockdown experiments described here support the idea that a similar Wnt pathway is required for P7.p polarization in *P. pacificus*. Therefore, the molecular mechanisms generating vulval symmetry are stable over long evolutionary time scales. Conservation and diversification of Wnt signaling function are observed in the formation of the same organ. Given the genetic and genomic tool kit available in *P. pacificus*, future work will investigate the connectivity of Wnt signaling and vulva induction.

METHODS

Cell ablation experiments and cell lineage analysis. For cell ablation experiments, we picked worms into M9 buffer placed on a pad of 5% agar in water containing 10 mM sodium azide as an anesthetic. All ablation experiments were carried out 0–1 h after hatching of the larvae (20 °C). The cell ablation data are calculated as percentage frequency and were statistically tested using the χ^2 test. The lineage reversal of P7.p was inferred based on observation of the cellular anatomy using Nomarski microscopy.

Mutagenesis. We washed mixed-stage worms off the plates in M9 buffer and added ethyl methanesulphonate to a final concentration of 50 mM for 4 h at 20 °C. After washing them in M9 five times, we spotted the worms onto the surface of NG plates. After 1 h, excess liquid had been absorbed and we picked individual motile L4 hermaphrodites to plates. In case of the trimethylpsoralen-ultraviolet mutagenesis, we incubated worms for 20 min with 33 μg ml^{-1} of trimethylpsoralen and then exposed them to 500 μW cm^{-2} of ultraviolet irradiation for 50 s. In the F_2 generation, we isolated egg-laying-defective mutants and reanalyzed their progeny for vulva defects using Nomarski microscopy.

Mapping and SSCP detection. For mapping, we crossed mutant hermaphrodites in the California background with males of the Washington strain. To extract genomic DNA, we picked F_2 mutant worms to single tubes containing 2.5 μl of lysis buffer (50 mM KCl, 10 mM Tris-HCl (pH 8.3), 2.5 mM $MgCl_2$, 0.45% Nonidet P-40, 0.45% Tween, 0.01% gelatin and 5 μg ml^{-1} proteinase K), incubated them for 1 h at 65 °C and then inactivated the proteinase K at 95 °C for 10 min. To assign linkage of a mutation to a certain chromosome, we tested two representative SSCP markers per chromosome against 42 Washington-backcrossed mutant worms. For SSCP detection, we diluted PCR samples 1:1 in denaturing solution (95% formamide, 0.1% xylene cyanol and 0.1% bromophenol blue), denatured them at 95 °C for 5 min and loaded them onto a GeneGel Excel prepoured 6% acrylamide gel (PharmaciaBiotech). We fixed gels and stained them with silver to detect the DNA.

Morpholino technology. We dissolved oligonucleotides (Gene Tools) in water and then diluted them to a concentration of 100 μM. We used morpholino oligonucleotides against *Ppa-lin-17*, *Ppa-lin-44*, *Ppa-egl-20*, *Ppa-mig-5*, *Ppa-apr-1*, *Ppa-bar-1*, *Ppa-lin-18*, *Dr-rx-3* and *Ppa-dnmt*. Primer sequences are available on request.

GenBank accession number. *Ppa-lin-17*, AY562975.

Note: Supplementary information is available on the Nature Genetics website.

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COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

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1. Wang, M. & Sternberg, P.W. Pattern formation during *C. elegans* vulval induction. *Curr. Top. Dev. Biol.* **51**, 189–220 (2001).
2. Sommer, R.J. As good as they get: cells in nematode vulva development and evolution. *Curr. Opin. Cell. Biol.* **13**, 715–720 (2001).
3. Eizinger, A. & Sommer, R.J. The homeotic gene *lin-39* and the evolution of nematode epidermal cell fates. *Science* **278**, 452–455 (1997).
4. Simpson, P. Evolution of development in closely related species of flies and worms. *Nat. Rev. Genet.* **3**, 907–917 (2002).
5. Srinivasan, J. *et al.* An integrated physical and genetic map of the nematode *Pristionchus pacificus*. *Mol. Genet. Genomics* **269**, 715–722 (2003).
6. Inoue, T. *et al. C. elegans* LIN-18 is a Ryk ortholog and functions in parallel to LIN-17/Frizzled in Wnt signaling. *Cell* **118**, 795–806 (2004).
7. Eisenmann, D.M., Maloof, J.N., Simske, J.S., Kenyon, C. & Kim, S.K. The beta-catenin homolog BAR-1 and LET-60 Ras coordinately regulate the Hox gene *lin-39* during *Caenorhabditis elegans* vulval development. *Development* **125**, 3667–3680 (1998).
8. Lambshead, P.J.D. Recent developments in marine benthic biodiversity research. *Oceanis* **19**, 5–24 (1993).
9. Pires-DaSilva, A. & Sommer, R J. Conservation of the global sex determination gene *tra-1* in distantly related nematodes. *Genes Dev.* **18**, 1198–1208 (2004).
10. Hill, R.J. & Sternberg, P.W. The gene *lin-3* encodes an inductive signal for vulval development in *C. elegans*. *Nature* **358**, 470–476 (1992).
11. Chen, N. & Greenwald, I. The lateral signal for LIN-12/Notch in *C. elegans* vulval development comprises redundant secreted and transmembrane DSL proteins. *Dev. Cell* **6**, 183–192 (2004).
12. Ceol, C.J. & Horvitz, H.R. *dpl-1* DP and *efl-1* E2F act with *lin-35* Rb to antagonize Ras signaling in *C. elegans* vulval development. *Mol. Cell* **7**, 461–473 (2001).
13. Sommer, R.J. & Sternberg, P.W. Apoptosis and change of competence limit the size of the vulva equivalence group in *Pristionchus pacificus*: a genetic analysis. *Curr. Biol.* **6**, 52–59 (1996).
14. Sigrist, C.B. & Sommer, R.J. Vulva formation in *Pristionchus pacificus* relies on continuous gonadal induction. *Dev. Genes Evol.* **209**, 451–459 (1999).
15. Kimble, J. Alterations in cell lineage following laser ablation of cells in the somatic gonad of *Caenorhabditis elegans*. *Dev. Biol.* **87**, 286–300 (1981).
16. Jungblut, B. & Sommer, R.J. Novel cell-cell interactions during vulva development in *Pristionchus pacificus*. *Development* **127**, 3295–3303 (2000).
17. Sawa, H., Lobel, L. & Horvitz, H.R. The *Caenorhabditis elegans* gene *lin-17*, which is required for certain asymmetric cell divisions, encodes a putative seven-transmembrane protein similar to the Drosophila frizzled protein. *Genes Dev.* **10**, 2189–2197 (1996).
18. Ceol, C.J. & Horvitz, H.R. A new class of *C. elegans* synMuv genes implicates a Tip60/NuA4-like HAT complex as a negative regulator of Ras signaling. *Dev. Cell* **6**, 563–576 (2004).
19. Maloof, J.N., Whangbo, J., Harris, J.M., Jongeward, G.D. & Kenyon, C. A Wnt signaling pathway controls hox gene expression and neuroblast migration in *C. elegans*. *Development* **126**, 37–49 (1999).
20. Sommer, R.J. *et al.* The *Pristionchus* HOX gene *Ppa-lin-39* inhibits programmed cell death to specify the vulva equivalence group and is not required during vulval induction. *Development* **125**, 3865–3873 (1998).