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The *pax-3* gene is involved in vulva formation in *Pristionchus pacificus* and is a target of the Hox gene *lin-39*

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The Hox gene *lin-39* plays a crucial role in the establishment of the nematode vulva equivalence group. Mutations in *lin-39* in *Caenorhabditis elegans* and *Pristionchus pacificus* result in a vulvaless phenotype because presumptive vulva precursor cells adopt non-vulval fates. Interestingly, the non-vulval fate of anterior and posterior epidermal cells differs between *Caenorhabditis* and *Pristionchus*; in *C. elegans*, non-vulval cells fuse with the hypodermis, whereas, in *P. pacificus*, they die as a result of programmed cell death. *C. elegans lin-39* (*Cel-lin-39*) indirectly controls the cell fusion gene *eff-1* by regulating the GATA transcription factors *egl-18* and *elt-6*. In *P. pacificus*, the genetic context of its *lin-39* (*Ppa-lin-39*) function was unknown. Here, we describe the isolation and characterization of *gev-2*, a second generation-vulvaless mutant in *P. pacificus.* We show that *gev-2* is the *Ppa-pax-3* gene and that it has distinct functions in the cell fate specification of epidermal cells. Whereas *Ppa-pax-3* regulates cell survival of the presumptive vulval precursor cells, it controls cell death of posterior epidermal cells. Molecular studies indicate that *Ppa-pax-3* is a direct target of *Ppa*-LIN-39. Thus, we describe the first specific developmental defect of a nematode *pax-3* gene and our data reveal different regulatory networks for the specification of the vulva equivalence group.

KEY WORDS: *P. pacificus*, Vulva development, *C. elegans*, Pax genes, *lin-39*

INTRODUCTION

In nematodes, basic principles in developmental biology and in evolutionary developmental biology can be studied at the single-cell level (Hong and Sommer, 2006). In *Caenorhabditis elegans*, detailed genetic and molecular studies of vulva formation present a framework for studying pattern formation, cell fate specification, developmental competence and induction (Sternberg, 2005). At the same time, comparative studies in other nematodes, in particular the satellite organism *Pristionchus pacificus*, provide insight into the evolutionary changes of developmental processes and mechanisms (Sommer, 2005). Nearly all aspects of vulva formation are subject to evolutionary change. *P. pacificus* is amenable to forward and reverse genetic analysis, and provides a platform to identify genetic and molecular alterations that control evolutionary changes of developmental processes. *P. pacificus* propagates as a self-fertilizing hermaphrodite and has an integrated genome map that contains a genetic linkage map of more than 600 molecular markers and a physical map of nearly 10,000 fingerprinted BAC clones (Srinivasan et al., 2002; Srinivasan et al., 2003). A whole-genome sequencing project has recently been finished (Dieterich et al., 2007).

The nematode vulva is a derivative of the ventral epidermis, which consists of 12 precursor cells, called P(1-12).p, in all nematodes studied to date (with 'Pn.p' denoting the complete group of ventral epidermal precursor cells) (Fig. 1A). These 12 Pn.p cells adopt different cell fates according to positional information provided by homeotic control genes. In the *C. elegans* hermaphrodite, the first cell fate decision in the ventral epidermis is between potential vulval precursor cells (VPCs) and 'non-vulval' cells. Cells in the anterior and posterior body region, P(1,2,9-11).p, fuse with the hypodermal syncytium hyp7 early in development. By contrast, P(3-8).p in the central body region remain non-fused, become VPCs and form a so-called vulva equivalence group (VEG) (Fig. 1A). Genetically, the Hox gene *lin-39* defines the developmental competence of P(3-8).p and establishes this group of cells as VPCs. In *C. elegans lin-39* (*Cel-lin-39*) mutants, P(3-8).p fuse with the hypodermis, resulting in a phenotype that has been designated as 'generation vulvaless' (Gev; Fig. 1B) (Clark et al., 1993; Wang et al., 1993). It has been suggested that the early role of *Cel*-LIN-39 is the indirect regulation of the cell fusion effector *eff-1*. *Cel*-LIN-39 regulates the GATA transcription factors *egl-18* and *elt-6*, which in turn might regulate *eff-1* (Fig. 2A) (Shemer and Podbilewicz, 2002; Koh et al., 2002; Cassata et al., 2005).

In *C. elegans*, P(3-8).p adopt one of three alternative fates later in development (Sternberg, 2005). P6.p generates eight progeny and forms the central part of the vulva, a fate that is designated as 1° fate. P(5,7).p have a 2° fate, generate seven progeny each and form the anterior and posterior part of the vulva, respectively. P(3,4,8).p do not participate in vulva formation in wild-type animals, remain epidermal and have the so-called 3° fate. After cell ablation of P(5-7).p, P(3,4,8).p can substitute for the other VPCs, indicating that all VPCs have the competence to form part of the vulva. Detailed genetic and molecular studies revealed a complex regulatory network involved in *C. elegans* vulva formation. An epidermal growth factor (EGF)-like molecule encoded by the gene *lin-3* is secreted from the gonadal anchor cell (AC) and induces P(5-7).p to adopt 1° and 2° vulval fates. The LIN-3 signal is transmitted within the VPCs by an EGFR/RAS pathway. Wnt signaling and DSL/Notch signaling have been shown to participate in vulva development, and various specification events involve redundant functions of different signaling cascades. The Hox gene *lin-39* is a downstream target of EGF and Wnt signaling during vulva induction (Maloof and Kenyon, 1998; Wagmaister et al., 2006). Thus, *Cel*-LIN-39 is used twice during vulva formation: first, to establish the VEG and, second, in response to vulva induction.

In *P. pacificus*, vulva formation differs substantially from that in *C. elegans*. Although, in both organisms, P(5-7).p form vulval tissue with a 2°-1°-2° fate pattern, novel cell-cell interactions that

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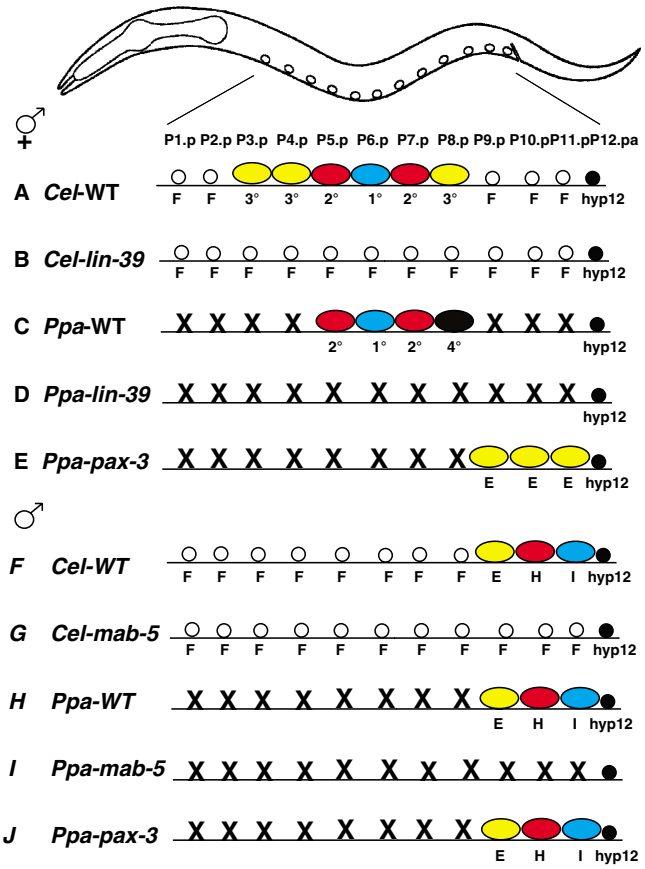

Fig. 1. Schematic summary of ventral epidermal cell fate specification in *P. pacificus* and *C. elegans* wild-type and mutant animals. The ventral epidermis of hermaphrodites and males derives from 12 ectoblasts, named P(1-12).p according to their anteroposterior position. The 12 cells are equally distributed between the pharynx and rectum. (A-E) Cell fate specification in the hermaphrodite. (**A**) *C. elegans* wild-type. The vulva is formed from the progeny of the 1° (blue ovals) and 2° (red ovals) vulva precursor cells. 3° cells (yellow ovals) are competent to form vulval tissue, but remain epidermal under wild-type conditions. In *C. elegans*, P(1,2,9-11).p fuse ('F', white circles) with the hypodermis and are not competent to form part of the vulva. P12.pa is a special cell called *hyp12* and forms part of the rectum. (**B**) In *Cel-lin-39* mutants, positional information for the formation of the vulva equivalence group is missing and P(3-8).p fuse with the hypodermis, as do their lineage counterparts in the anterior and posterior body region. (**C**) In *P. pacificus*, P(1-4,9-11).p die as a result of programmed cell death (X) and reduce the size of the vulva equivalence group further. P(5-7).p have a 2°-1°-2° pattern, as is also observed in *C. elegans*, and P8.p is a special epidermal cell (black oval), which is designated as a 4° cell fate. (**D**) In *Ppa-lin-39* mutants, the vulva equivalence group is not formed and P(5-8).p die as a result of programmed cell death ('X'). (**E**) The *Ppa-pax-3* mutants have opposite effects on the Pn.p cells in the central and the posterior body region: P(5-8).p undergo ectopic cell death, whereas P(9-11).p often survive. 'E' denotes epidermal cell fate. (F-J) Cell fate specification in males. (**F**) In *C. elegans*, P(1-8).p fuse (white circles) with the hypodermis. P10.p and P11.p divide multiple times and form the hook ('H') and associated interneurons ('I'). P9.p remains epidermal, but has been shown to be able to replace P10.p after cell ablation of the latter. (**G**) In *Cel-mab-5* mutants, P(9-11).p fuse with the hypodermis, and no hook is formed. (**H**) In *P. pacificus*, P(1-8).p undergo programmed cell death and P(9-11).p development resembles the pattern observed in *C. elegans*. (**I**) In *Ppa-mab-5* mutants, P(1-11).p die as a result of programmed cell death, suggesting that *mab-5* provides positional information for P(9-11).p specification in both species. (**J**) In *Ppa-pax-3* mutants, no patterning differences are observed.

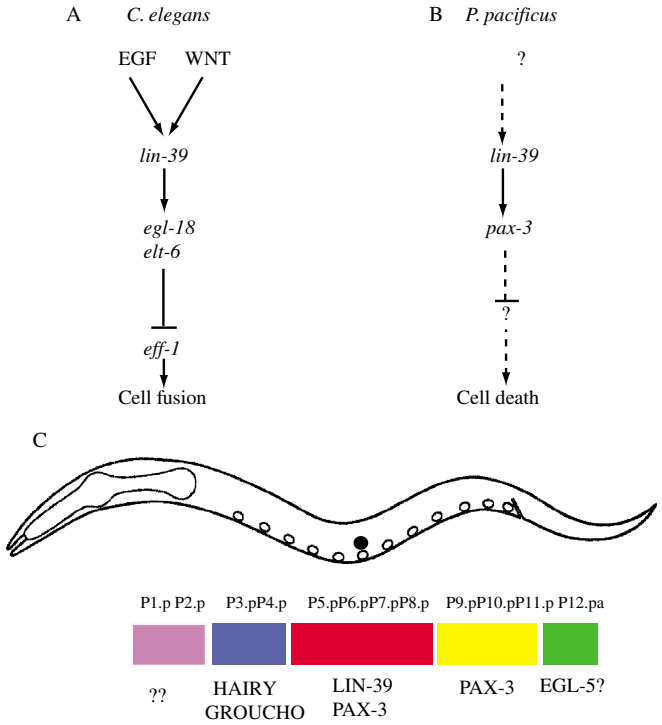

Fig. 2. Model of the regulation of cell fusion and cell death in the ventral epidermis in *C. elegans* and *P. pacificus* hermaphrodites. (**A**) Model of cell fusion regulation in the ventral epidermis of *C. elegans*. *lin-39* is transcriptionally regulated by EGF and WNT signaling. Several studies have suggested that LIN-39 regulates the expression of *egl-18* and *elt-6*, which encode GATA transcription factors, which in turn regulates expression of the cell fusion effector gene *eff-1*. (**B**) Based on data described in this study, *Ppa*-LIN-39 acts upstream of *Ppa-pax-3*. (**C**) Model of *P. pacificus* cell fate specification in the ventral epidermis of the hermaphrodite. Positional information subdivides the 12 ventral epidermal cells of *P. pacificus* into at least five distinct areas. P(5-8).p (red box) are regulated by *Ppa*-LIN-39 and *Ppa*-PAX-3, as described in this study. P(9-11).p (yellow box) die from programmed cell death (PCD) and *Ppa-pax-3* is the first gene shown to be involved in this regulatory process. P12.pa is a special cell that forms part of the rectum. In *C. elegans*, it is regulated by the posterior Hox gene *egl-5* (green box). P(3,4).p (blue box) die from PCD and are specifically regulated by *Ppa*-HAIRY and *Ppa*-GROUCHO, which downregulate *Ppa-lin-39* expression in these cells. P(1,2).p (pink box) also die from PCD, but no specific regulators have been obtained.

are unknown from *C. elegans* are crucial for vulva induction in *P. pacificus* (Sommer, 2005). Four important differences in cell fate specification are apparent between *P. pacificus* and *C. elegans* (Fig. 1C). First, in *P. pacificus*, non-vulval Pn.p cells do not fuse with hyp7 as they do in *C. elegans*; instead, they die from programmed cell death (PCD) (Sommer and Sternberg, 1996). Second, this PCD involves P(1-4).p in the anterior body region and P(9-11).p in the posterior body region, and has an influence on the size of the VEG; therefore, P(3,4).p are members of the VEG in *C. elegans* but die in *P. pacificus*. Thus, PCD limits the size of the VEG in *P. pacificus* (Eizinger and Sommer, 1997). Third, vulva induction in *P. pacificus* involves multiple cells of the somatic gonad and takes more than 10 hours, whereas the AC induces the vulva in a one-step interaction in *C. elegans* (Sigrist and Sommer, 1999). Fourth, the epidermal cell P8.p does not divide in *P. pacificus* and is not competent to adopt a vulva fate,

Table 1. Phenotype of *Ppa-pax-3*, *Ppa-lin-39* and double mutants in the hermaphrodite ventral epidermis

Genotype	P(1-4).p	P5.p	P6.p	P7.p	P8.p	P9.p	P10.p	P11.p	SI	*n*
A.Wild type PS312	0:0	100:100	100:100	100:100	100:0	0:0	0:0	0:0	4	many
B. *Ppa-lin-39(tu29)*	0:0	13:13	44:38	19:13	19:0	0:0	0:0	0:0	0.9	16
C. *Ppa-pax-3(tu214)*	0:0	34:12	48:31	48:17	57:0	16:0	14:0	22:7	1.9	50
D. *Ppa-pax-3(tu358)*	0:0	43:43	52:48	57:55	62:0	10:0	7:0	17:6	2.2	42
E. *Ppa-lin-39(tu29)/Ppa-ced-3(tu84)*	100:0	100:100	100:100	100:100	100:0	100:0	100:0	100:100*	11	15
F. *Ppa-pax-3(tu358)/Ppa-ced-4(tu324)*	100:0	100:100	100:100	100:100	100:0	100:0	100:0	100:90*	11	19
G. *Ppa-pax-3(tu214)/Ppa-ced-4(tu324)*	100:0	100:80	100:80	100:73	100:0	100:0	100:0	100:90*	11	15
H. *Ppa-pax-3(tu214)/Ppa-pax-3(tu358)*	0:0	32:21	47:32	47:32	53:0	11:0	11:0	21:5	1.8	19
I. *Ppa-pax-3* MO	0:0	100:100	100:100	100:100	100:0	0:0	0:0	0:0	4	50
J. *Ppa-pax-3(tu358)/Ppa-pax-3* MO	0:0	17:7	23:20	23:10	23:0	33:0	23:0	43:27	0.9	30
K. *Ppa-pax-3(tu214)/Ppa-lin-39(tu29)*	0:0	25:7	32:18	18:4	11:0	0:0	0:0	0:0	0.9	28

The ratios for the P5.p to P11.p cells give the percentage of surviving cells to the percentage of the cells differentiating into vulval (1° or 2°) tissue. *Note that, in the cell death defective genetic background of Ppa-ced-3 and Ppa-ced-4, P11.p undergoes differentiation and forms an invagination in the pre-anal region (see Sommer et al., 1998). SI, survival index describes the number of surviving P(5-8).p cells per animal; *n*, number of analyzed animals.

whereas it is a VPC in *C. elegans*. However, P8.p is involved in cell fate specification of the VPCs by inhibiting P(5,7).p from taking a 1° fate, an interaction that has been designated as lateral inhibition (Jungblut and Sommer, 2000).

Genetic and molecular studies revealed that the VEG in *P. pacificus* is also specified by the Hox gene *lin-39* (Eizinger and Sommer, 1997). In *P. pacificus lin-39* (*Ppa-lin-39*) mutants, P(5-8).p die from PCD like their anterior and posterior lineage counterparts, indicating that the ground state of ventral epidermal cells in *P. pacificus* is PCD (Fig. 1D, Table 1B). Thus, *Ppa*-LIN-39 prevents PCD, whereas *Cel*-LIN-39 prevents cell fusion, indicating that *Ppa*-LIN-39 must be part of a different regulatory network than *Cel*-LIN-39 during VEG formation.

Proteins encoded by the Pax gene family play a crucial role in animal development (for a review, see Chi and Epstein, 2002). Pax proteins contain up to three highly conserved protein domains: an N-terminal paired domain (PD) and a C-terminal homeodomain (HD), both of which are involved in DNA-binding, as well as an octapeptide. Pax proteins were shown to function as both transcriptional activators and repressors. For example, in mammals, Pax5 (also known as BSAP in humans) is converted from a transcriptional activator to a repressor via interactions with the co-repressors of the Groucho family in an octapeptide-dependent manner (Eberhard et al., 2000). At the sequence level, the presence or absence of the HD and/or the octapeptide, and the sequence similarities within the PD, allow the subdivision of Pax proteins into specific subfamilies (Chi and Epstein, 2002). Although there is a general conservation of Pax genes throughout the animal kingdom, many differences can be found in individual taxa. For example, the genome of the nematode *C. elegans* contains five bona-fide Pax genes (Hobert and Ruvkun, 1999). *vab-3* belongs to the *Pax-6* subfamily and is involved in head and tail development (Chisholm and Horvitz, 1995; Zhang and Emmons, 1995). *egl-38* is a member of the *Pax-2/5* subfamily and regulates uterine and tail development (Chamberlin et al., 1997). The highly related gene *K06B9.5* results from a recent gene duplication. Interestingly, the gene *F27E5.2* is the only gene present in the *C. elegans* genome that contains all three protein domains, the PD, HD and octapeptide.

Here, we describe, by mutant analysis, the first specific postembryonic developmental function of a nematode *Pax-3*-type gene. We show that *Ppa-pax-3* is involved in the formation of the VEG and that *Ppa-pax-3* has distinct functions in cell fate specification of epidermal cells. Whereas *Ppa-pax-3* regulates cell survival of the central Pn.p cells, it regulates cell death of posterior epidermal cells. Additional data indicate that *Ppa-pax-3* is a direct target of *Ppa*-LIN-39. Together, these data show a function for a nematode *Pax-3*-type gene during vulva development and indicate a different regulatory network for the formation of the VEG.

MATERIALS AND METHODS

Nematode strains and cultures

Worms were grown on 5 cm NGM agar plates seeded with OP50, a uracil-requiring mutant of *Escherichia coli* (Sommer and Sternberg, 1996). The following strains were used in this study: *P. pacificus* PS312 (the wild-type strain) is a derivative of an isolate from Pasadena, California and represents the wild-type strain used for genetic analysis; *P. pacificus* PS1843, isolated from Port Angeles, Washington represents the polymorphic reference strain for mapping (Srinivasan et al., 2002). Other mutant strains used in this study were *Ppa-lin-39(tu29)* (Eizinger and Sommer, 1997) and *Ppa-mab-5(tu357)* (Jungblut and Sommer, 1998).

Mutagenesis

TMP/UV mutagenesis was carried out as described elsewhere (Jungblut and Sommer, 2001). In short, mixed-stage animals were washed off the plates in M9 buffer and were incubated for 20 minutes with 33 μg/ml TMP and then UV irradiated for 50 seconds with an intensity of 500 μW/cm^2. In the F2 generation, egg-laying defective mutants were isolated and their progeny were reanalyzed for vulva defects using Nomarski microscopy. Mutant hermaphrodites were backcrossed multiple times using wild-type males. Complementation tests were carried out using *dpy*-marked Gev mutants (Kenning et al., 2004).

Mapping and SSCP detection

For mapping, mutant hermaphrodites in the California background were crossed with males of the Washington strain. To extract genomic DNA, F2 mutant animals were picked 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% NP-40; 0.45% Tween; 0.01% gelatin; 5 μg/ml Proteinase K) and incubated for 1 hour at 65°C, followed by inactivation of the Proteinase K at 95°C for 10 minutes. To assign linkage of a mutation to a certain chromosome, two representative single-strand conformational polymorphism (SSCP) markers per chromosome were tested against 42 Washington-backcrossed mutant animals. For SSCP detection, PCR samples were diluted 1:1 in denaturing solution (95% formamide, 0.1% xylene cyanol, 0.1% bromophenol blue), denatured at 95°C for 5 minutes and loaded onto a GeneGel Excel prepoured 6% acrylamide gel (PharmaciaBiotech, Piscataway, NJ). Gels were fixed and silver stained to detect the DNA.

Morpholino experiments

Oligonucleotides (Gene Tools) were dissolved in water and subsequently diluted to a concentration of 100 μM. Primer sequences are available on request.

Quantitative PCR experiments

A total of 120 J1 animals were picked into 15 μl of 1:10 diluted single worm lysis buffer. RNA was extracted with 100 μl of TRIZOL using a repeated freeze-thaw protocol in liquid nitrogen. RNA was reverse transcribed in 20 μl total volume reaction (Invitrogen) with negative controls without reverse transcriptase included for each sample. Quantitative PCR was performed on a Roche LC480 LightCycler using the manufacturer's SYBR green PCR mix. Primer concentrations were 0.5 mM.

Electrophoretic mobility shift assay

Ppa-LIN-39 and *Ppa*-CEH-20 proteins were expressed by coupled in vitro transcription/translation (Promega) using plasmids pBY01 and pBY02. Complementary oligonucleotides were annealed and end-labeled with [γ32P]-ATP using T4 polynucleotide kinase (NEB, Beverly, MA). Labeled oligonucleotides (1.5 pmol) were used in bandshift experiments. Electrophoresis was carried out at 4°C on a 6% non-denaturing polyacrylamide gel.

Bioinformatic analysis of Hox-binding sites

We used the SITEBLAST software (Michael et al., 2005) and consensus sequences from the JASPAR database to identify conserved putative Hox target enhancers in the *P. pacificus* genome.

RESULTS

P. pacificus gev-2 defines a genetic locus with distinct functions in ventral epidermal cell fate specification

In various TMP/UV mutagenesis screens, we isolated two new mutants (*tu214* and *tu358*) with a Gev phenotype. Complementation tests revealed that *tu214* and *tu358* were not allelic to *Ppa-lin-39*, but were allelic to one another (data not shown). Therefore, we have named this novel gene *gev-2* for *generation vulvaless-2*.

gev-2 mutant animals were strongly egg-laying defective. More-detailed cell lineage analysis in the ventral epidermis of *gev-2* mutants revealed two independent defects in Pn.p cell fate specification (Table 1). First, the central cells, P(5-8).p, died from PCD in many *gev-2* mutant animals, resulting in the absence of a vulva (Fig. 3). Although both alleles had a similar PCD phenotype, they showed a slight posterior bias in that P8.p survived with a higher frequency than P5.p (Table 1C,D). Second, mutations in *gev-2* resulted in a distinct phenotype in the posterior body region. In wild-type and *Ppa-lin-39* mutant hermaphrodites P(9-11).p die from PCD (Table 1A,B) (Sommer and Sternberg, 1996). However, P(9-11).p survive in *gev-2* mutant animals with an frequency of 10-20% (Table 1C,D). Thus, *gev-2* has different functions in Pn.p cell fate specification in the central and the posterior body region: it positively regulates cell survival of P(5-8).p and induces the PCD of P(9-11).p (Fig. 1E).

gev-2 is required for vulva induction

Mutations in *lin-39* result in a Gev phenotype in both *P. pacificus* and *C. elegans* (Clark et al., 1993; Wang et al., 1993; Eizinger and Sommer, 1997). However, *Ppa-lin-39* and *Cel-lin-39* differ with regard to their involvement in vulva induction. Whereas *Cel-lin-39* is crucial for vulva induction and was shown to represent a downstream target of EGF/RAS and WNT signaling (Maloof and Kenyon, 1998; Wagmaister et al., 2006), *Ppa-lin-39* is dispensable for vulva induction: in *Ppa-lin-39; Ppa-ced-3* double mutants, the PCD of Pn.p cells is rescued and the surviving VPCs undergo normal vulva differentiation (Table 1E) (Sommer et al., 1998).

To determine whether *gev-2* has a role in *P. pacificus* vulva induction, we generated similar double mutants with a cell-death-defective mutant to overcome the PCD of P(5-8).p. Because genetic mapping of *gev-2* revealed that it is located on chromosome II in close proximity to the *Ppa-ced-3* gene (see below for details), we generated double mutants containing *gev-2* with a second cell-death-defective mutant previously described as *ipa-2* (Sommer et al., 1998). *ipa-2* shows complete cell survival of all Pn.p cells, similar to *Ppa-ced-3*. *ipa-2* maps to chromosome III and has been shown to be the *Ppa-ced-4* gene, which encodes an ortholog of the cell-death adaptor CED-4/APAF-1 (Dinkelacker, Lee and Sommer, unpublished information). Interestingly, the phenotype of the *Ppa-gev-2; Ppa-ced-4* double mutant was allele-specific: whereas *Ppa-gev-2(tu358); Ppa-ced-4(tu324)* mutant animals mostly formed a normal vulva (Table 1F), *Ppa-gev-2(tu214); Ppa-ced-4(tu324)* double mutants were slightly induction vulvaless. Specifically, 20-30% of the VPCs remained un-induced in *Ppa-gev-2(tu214); Ppa-ced-4(tu324)* double-mutant animals (Table 1G). This is consistent with findings in the *gev-2(tu214)* single mutant, in which the majority of the surviving VPCs

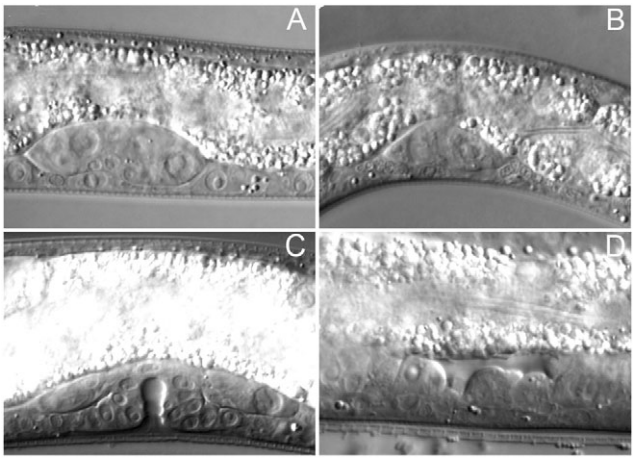

Fig. 3. *Ppa-pax-3* mutant animals are generation vulvaless. Nomarski photomicrographs of early (A,B) and late (C,D) stages of the vulva in *P. pacificus* wild-type (A,C) and *Ppa-pax-3* mutant (B,D) animals. (**A**) *P. pacificus* wild-type animal in the early J3 stage showing P(5-8).p before the onset of cell divisions. (**B**) *Ppa-pax-3* mutants in a similar developmental stage. P(5-8).p are absent. (**C**) *P. pacificus* wild-type animal in the mid J4 stage. A vulva has been formed. In this plane of focus, the progeny of the 2° cells P(5,7).p are visible. (**D**) *Ppa-pax-3* mutants in a similar developmental stage. No vulva was formed.

Table 2. Phenotype of *Ppa-pax-3*, *Ppa-mab-5* and double mutants in the male ventral epidermis

Genotype	P(1-4).p	P(5-8).p	P9.p	P10.p	P11.p	*n*
A. Wild type PS312	X	X	E	H	I	many
B. *Ppa-pax-3(tu214); Ppa-him-2(tu95)*	X	X	E	H	I	10
C. *Ppa-mab-5(tu357)*	X	X	X	X	X	13
D. *Ppa-pax-3(tu214); Ppa-mab-5(tu357)*	X	X	X	X	X	10

Like for the hermaphrodite P8.p cell, P9.p in males does not divide. X, programmed cell death; E, epidermal cell fate of P9.p; H, cell fate of P10.p, which generates the hook and its associate sensillum; I, cell fate of P11.p, which generates interneurons and epidermis; *n*, number of analyzed animals.

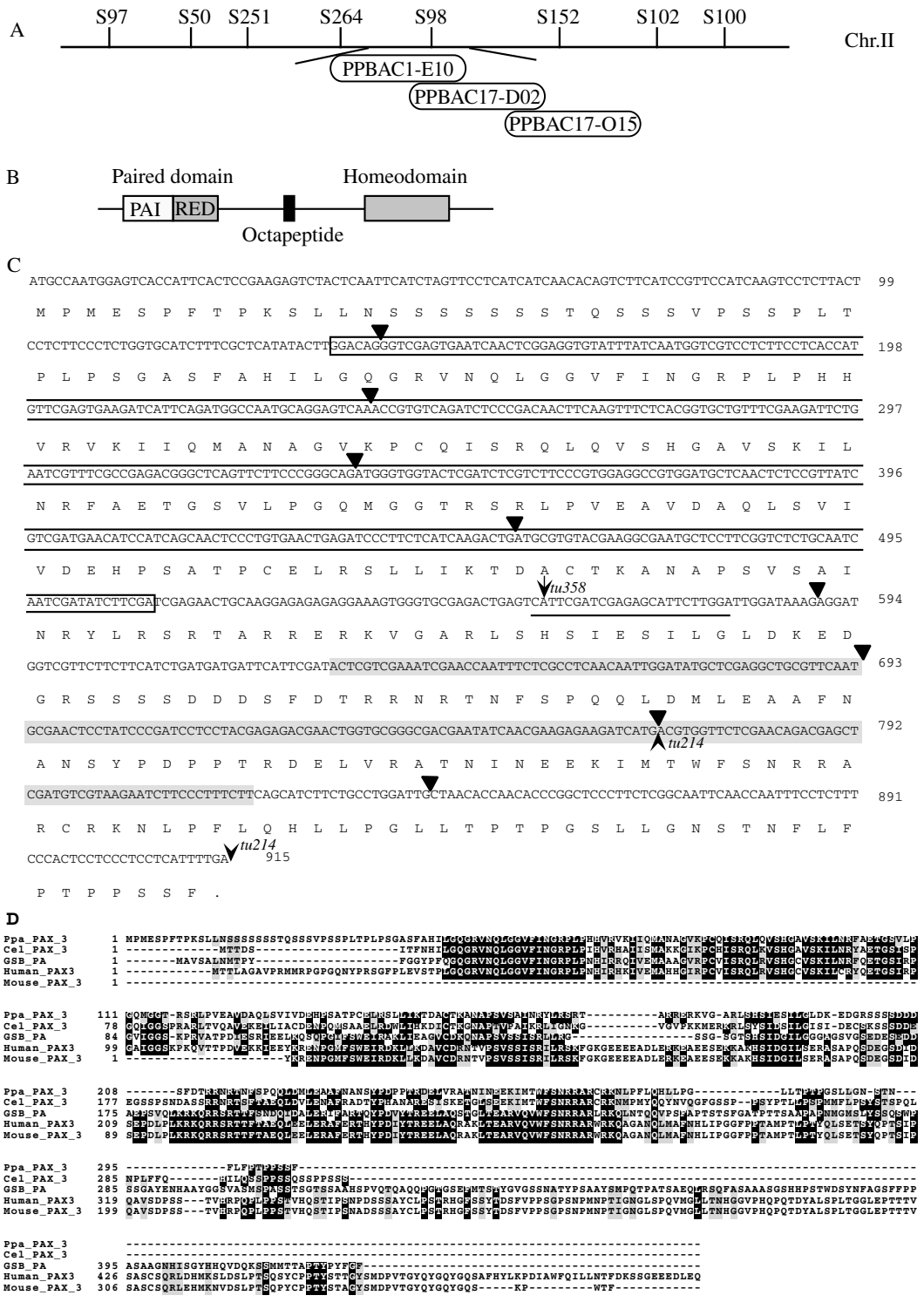

Fig. 4. Molecular cloning and gene structure of *Ppa-pax-3*, and the alignment of PAX-3-type proteins. (**A**) Map position of *Ppa-gev-2* (*Ppa-pax-3*) on chromosome II close to the molecular marker S98. The three BAC clones 1-E10, 17-D02 and 17-O15 define a physical interval for the gene. (**B**) Protein domain structure of *Ppa-pax-3*. *Ppa-pax-3* contains a bona-fide paired domain (PD) with the PAI and RED subdomains, as well as an octapeptide and a homeodomain (HD). (**C**) *Ppa-pax-3* cDNA sequence as obtained from 5′ and 3′ RACE experiments. Conceptual translation starts with the first in-frame ATG codon after the SL1 splice acceptor site. Introns are indicated by black triangles. The breakpoints of the C-terminal deletion of *tu214* are indicated by arrowheads. The mutation of *tu358* results in an amino acid replacement from His to Arg and is indicated by an arrow. The PD domain is boxed, the octapeptide is underlined and the HD is highlighted. (**D**) Amino acid comparison between *P. pacificus*, *C. elegans*, mouse and human PAX-3-type proteins, indicating a strong amino acid sequence conservation of all protein domains.

remained epidermal. Only 12% of P5.p cells, 31% of P6.p cells and 17% of P7.p cells, respectively, formed vulva tissue in *tu214* mutant animals, although a higher percentage of cells did survive (Table 1C). By contrast, nearly all of the surviving VPCs underwent vulva differentiation in the *tu358* allele (Table 1D). When we scored vulva induction in transheterozygous *tu214/tu358* animals, we found vulva differentiation in most of the surviving VPCs (Table 1H). Together, these results suggest that *gev-2(tu214)* has an allele-specific effect indicating a function of *gev-2* during vulva induction.

gev-2 is dispensable for P(9-11).p development in males

Pn.p cell fate specification differs between *P. pacificus* hermaphrodites and males. Whereas the anterior cells P(1-4).p died in both sexes, P(5-8).p survived only in hermaphrodites and P(9-11).p survived only in males (Fig. 1; Tables 1, 2). In *P. pacificus* males, P10.p and P11.p formed the hook and various neural structures, respectively, and thus have cell fates similar to their counterparts in *C. elegans* (Fig. 1F,H). P9.p did not divide in *P.*

pacificus and remained epidermal, a fate pattern that is also identical to P9.p in *C. elegans*. Given that mutations in *gev-2* result in distinct phenotypes in P(5-8).p and P(9-11).p in hermaphrodites, we wanted to know whether this phenotype is sex-specific. We generated *gev-2; Ppa-him-2* double mutants, which result in a high incidence of males, and found that P(9-11).p survived and differentiated normally, as in wild-type animals (Table 2B). Similarly, in the occasionally observed spontaneous *gev-2* males in an otherwise wild-type background, P(9-11).p differentiated normally (Fig. 1J).

In *C. elegans*, the Hox gene *mab-5* is crucial for P(9-11).p development in males and has a function similar to the Hox gene *Cel-lin-39* in hermaphrodites (Fig. 1G) (Kenyon, 1986). To study whether *Ppa-mab-5* is involved in P(9-11).p determination in males, we investigated *Ppa-mab-5(tu357)* mutant males. Indeed, P(9-11).p underwent PCD in *Ppa-mab-5(tu357)* males, indicating that the role of MAB-5 in P(9-11).p male-specification is conserved between *P. pacificus* and *C. elegans* (Fig. 1G,I; Table 2C). Similarly, P(9-11).p also underwent PCD in *gev-2(tu214); Ppa-mab-5(tu357)* double mutants (Table 2D). Taken together, the analysis of P(9-11).p determination in *P. pacificus* males suggests strong similarities to *C. elegans*, but does not provide any evidence for a role of *gev-2*. Thus, *gev-2* represents a hermaphrodite-specific regulator of Pn.p cell fate specification with distinct functions in the central and the posterior body region.

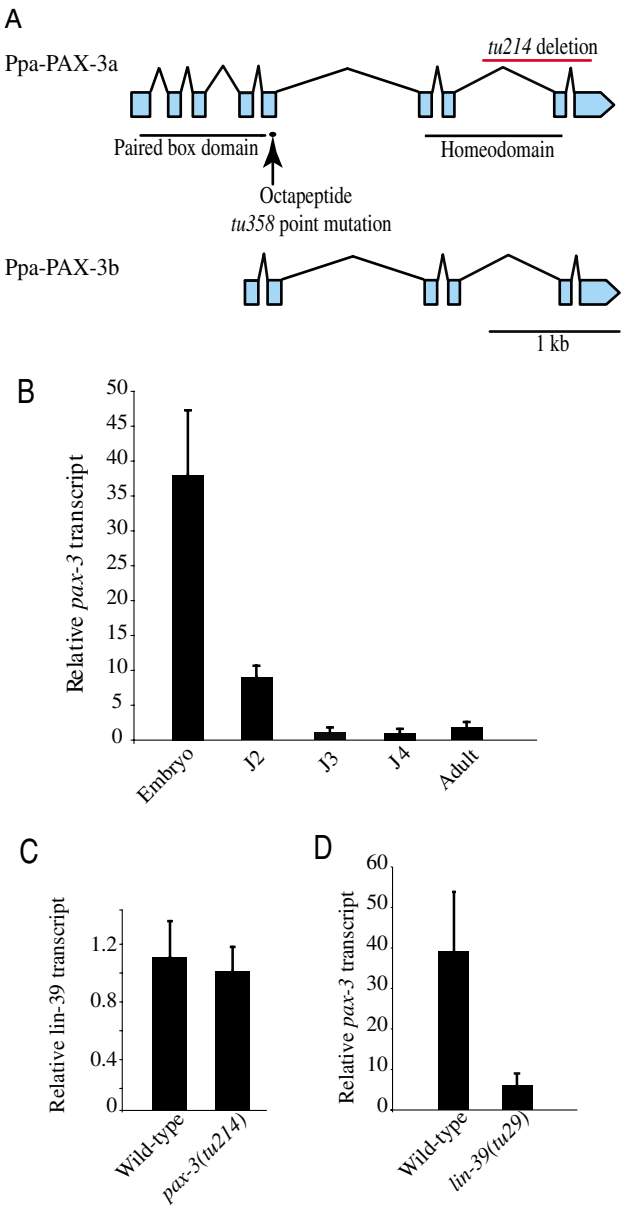

Fig. 5. *Ppa-pax-3* transcript levels are reduced in *Ppa-lin-39* mutants compared with *P. pacificus* wild type. (**A**) PCR amplification and sequence analysis of *Ppa-pax-3* cDNA clones reveals the existence of two isoforms. The *Ppa-pax-3b* isoform misses the N-terminal PAI subdomain of the paired domain (PD), but contains the RED subdomain, the octapeptide and the homeodomain (HD). Both isoforms are trans-spliced to an SL1 leader sequence. (**B**) *Ppa-pax-3* transcript levels in staged *P. pacificus* wild-type animals. Transcript levels are given as arbitrary concentration unit ratios between *Ppa-pax-3* and *Ppa*-β-*tubulin* as an internal standard. RNA was prepared from 100 animals and experiments were carried out in triplicate. Error bars represent standard deviations. (**C**) Relative *Ppa-lin-39* transcript levels in wild-type and *Ppa-pax-3(tu214)* mutant animals. *Ppa-lin-39* transcript levels are unchanged. (**D**) Relative *Ppa-pax-3* transcript levels in wild-type and *Ppa-lin-39(tu29)* mutant animals. *Ppa-pax-3* transcript levels are decreased from approximately 40 to 5 concentration units.

gev-2 is the *Ppa-pax-3* gene

To identify the molecular nature of *gev-2*, we mapped the locus using the polymorphic reference strain *P. pacificus var.* Washington (Srinivasan et al., 2002). *gev-2* maps to chromosome II between the molecular markers S264 and S152, and is most closely linked to the marker S98, which is associated with the BAC clone BACPP17D-2 (Fig. 4A). Fine mapping determined recombination breakpoints to the left and right of S98, and established an interval of three BAC clones. Light shotgun sequencing of all three BAC clones and BLAST searches of these sequences with the draft of the *P. pacificus* genome sequence identified the *pax-3* gene as a potential candidate gene for *gev-2* (Fig. 4B). When we sequenced the *pax-3* gene as a candidate for *gev-2*, we found that it is mutated in both alleles of *gev-2* (Fig. 4C). We identified a deletion of 830 bp from intron 7 to exon 9 in *tu214*. Sequence analysis of mutant cDNA clones revealed the use of a novel splice acceptor site, which caused skipping of the complete exon 9, resulting in a deletion of the HD and the 3′ UTR (Fig. 4C, Fig. 5A). *tu358* results in an amino acid replacement of His to Arg, which is one of the highly conserved residues in the octapeptide (Fig. 4C,D). We conclude that *gev-2* is *Ppa-pax-3*.

Although *Ppa-pax-3(tu214)* represents a strong reduction-of-function allele, the available alleles do not necessarily indicate the null phenotype of *Ppa-pax-3*. To identify a potential null allele of *Ppa-pax-3*, we used reverse genetic deletion library screens, but were unable to identify a third allele after screening more than 4 million gametes (data not shown). To study the function of *Ppa-pax-3* further, we used morpholino (MO) knockdown experiments, which were previously shown to function in sensitized genetic backgrounds (Zheng et al., 2005). Consistent with these previous findings, we saw no effect of a *Ppa-pax-3* MO when applied in wild-type animals (Table 1I). However, a *Ppa-pax-3* MO in a *Ppa-pax-3(tu358)* mutant background showed a strong enhancement of the cell-death and the vulva-differentiation phenotype (Table 1J), further indicating that the observed effects are due to a reduction of *Ppa-pax-3* function.

gev-2 is expressed in the embryo and in the early larval stages

Ppa-PAX-3 has bona-fide PD and HD domains and an octapeptide, as known from other PAX-3-type proteins (Fig. 4B) (Chi and Epstein, 2002). The overall amino acid sequence identity between *Ppa*-PAX-

3 and *Cel*-PAX-3 is 49%, and is highest in the three conserved domains (Fig. 4D). Interestingly, *Ppa-pax-3* and *Cel-pax-3* differ in gene structure and isoform formation. Whereas *Cel-pax-3* has six exons and forms a single isoform, *Ppa-pax-3* contains nine exons and two isoforms were isolated in reverse transcriptase (RT)-PCR experiments. Using the spliced leader SL1 as the forward primer in RT-PCR experiments, two alternatively spliced forms were obtained (Fig. 5A). The second *Ppa-pax-3* isoform misses the N-terminal part of the PD, but contains the octapeptide and the HD (Fig. 5A).

To study the expression of *Ppa-pax-3*, we used quantitative RT-PCR experiments with synchronized embryos, J2, J3, J4 and adult animals. We found *Ppa-pax-3* expression in only embryos and young larvae up to the J2 stage (Fig. 5B). *Ppa-pax-3* was not expressed in *P. pacificus* adults, which, unlike *C. elegans*, do not retain many eggs. This expression coincides with the generation of the VPCs and the PCD of their anterior and posterior sister cells. PCR primers in the N-terminal part of the cDNA resulted in a similar expression profile, suggesting that the two isoforms are regulated in a similar way (data not shown). These results suggest that *Ppa-pax-3* is primarily expressed in the embryo and early larval stages during the generation of the Pn.p cells, but prior to vulva induction.

Ppa-lin-39 acts upstream of *Ppa-pax-3*, and *Ppa*-LIN-39 binds to the *Ppa-pax-3* promoter in vitro

Given that mutations in *Ppa-pax-3* and *Ppa-lin-39* have a similar PCD phenotype in P(5-8).p, we wanted to know whether these two loci interact with one another. First, we generated a *Ppa-pax-3(tu214); Ppa-lin-39(tu29)* double mutant and analyzed the cell survival frequencies of P(5-8).p. *Ppa-pax-3(tu214); Ppa-lin-39(tu29)* double mutants had a cell survival index of 0.9 cells per animal, which is similar to the survival index of the *Ppa-lin-39(tu29)* single mutant (Table 1B,K). This result indicates that the frequency of cell survival in *Ppa-pax-3; Ppa-lin-39* double mutants is not additive, providing a first indication that these genes might act in a linear pathway.

To obtain further evidence, we analyzed the expression of *Ppa-pax-3* in *Ppa-lin-39* mutant and wild-type animals, and the expression of *Ppa-lin-39* in *Ppa-pax-3* mutant and wild-type animals, by quantitative RT-PCR. Whereas the expression of *Ppa-lin-39* was unchanged between wild-type and *Ppa-pax-3(tu214)* mutant animals, *Ppa-pax-3* expression was drastically reduced in *Ppa-lin-39(tu29)* mutant animals when compared with wild-type (Fig. 5C,D). Specifically, the relative *pax-3* transcript level was reduced eightfold in *Ppa-lin-39* mutants. These results suggest that *Ppa-lin-39* might act upstream of *Ppa-pax-3*.

To test whether *Ppa-pax-3* is directly regulated by *Ppa*-LIN-39, we searched for Hox-binding sites in the *Ppa-pax-3* promoter. Hox-binding sites have been studied intensively by in vivo and in vitro studies, and Hox proteins are known to interact with cofactors, such as Extradenticle-like proteins of the PBC class in *Drosophila* (Pearson et al., 2005). A common property of Hox target enhancers is the requirement for multiple Hox-monomer-binding sites, most of which possess an ATTA (or TAAT) core sequence (Pearson et al., 2005). In addition, many Hox-response elements require Hox-PBC heterodimer binding sites. We searched for potential binding sites in the *Ppa-pax-3* promoter using SITEBLAST software (Michael et al., 2005) and found one putative Hox–PBC-binding site, which conformed to the sequence TGATNNAT (Fig. 6A).

Next, we tested whether *Ppa*-LIN-39 could bind this putative binding site by using electrophoretic mobility shift assays (EMSA) (Fig. 6B). *Ppa*-LIN-39 caused a shift in mobility of an oligonucleotide containing a high-affinity binding site for *Drosophila Antennapedia*, but not for an oligonucleotide containing the binding site of the *Ppa-pax-3* promoter (Fig. 6B). However, when we added the PBC-type cofactor *Ppa*-CEH-20 to *Ppa*-LIN-39, the oligonucleotide with the binding site of the *Ppa-pax-3* promoter was shifted in a similar way as the *Drosophila Antennapedia* control oligonucleotide (Fig. 6B). At the same time, *Ppa*-CEH-20 alone did not cause a shift of the oligonucleotide (Fig. 6B). When the binding site of the *Ppa-pax-3* promoter had been mutated, the shift of the oligonucleotide by *Ppa*-CEH-20 and *Ppa*-LIN-39 was abolished. We conclude that a heterodimer of *Ppa*-LIN-39 and *Ppa*-CEH-20 can bind to the *Ppa-pax-3* promoter in vitro.

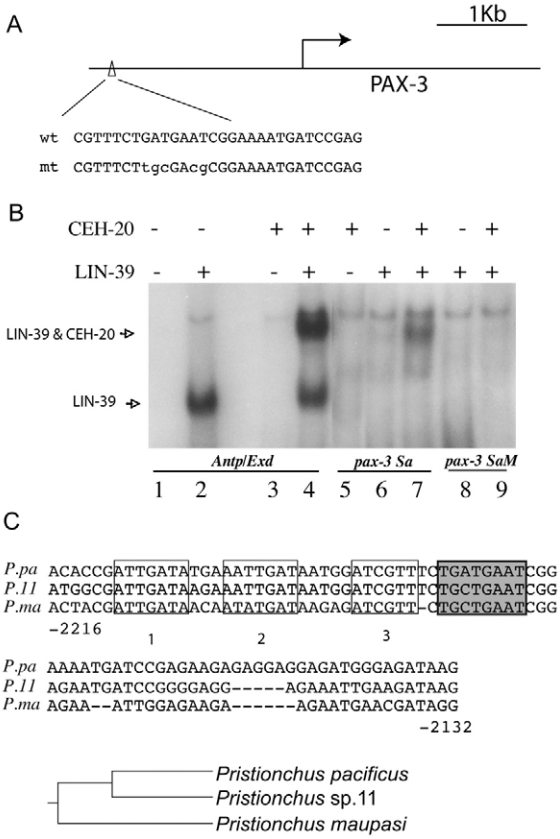

Fig. 6. *Ppa*-LIN-39 and its Extradenticle-like cofactor *Ppa*-CEH-20 can bind to the *Ppa-pax-3* promoter in vitro. (**A**) Putative HOX–PBC-binding sites in the *Ppa-pax-3* promoter. SITEBLAST analysis of the *Ppa-pax-3* promoter including 3 kb of upstream sequence revealed one putative binding site (arrowhead). Beneath, we show the nucleotide sequence of the predicted binding site 'a' with the core binding site TGATGAATCG (wild type, wt). For electrophoretic mobility shift assays this core binding site has been mutated (mt) to TtgcGAcgCG. (**B**) In electrophoretic mobility shift assays, *Ppa*-LIN-39 binds to a *Drosophila Antennapedia* control oligonucleotide alone and in conjunction with *Ppa*-CEH-20. *Ppa*-LIN-39 does not bind *Ppa-pax-3* oligonucleotides on its own (lane 6), but strongly binds together with *Ppa*-CEH-20 (lane 7). Ppa-LIN-39 and *Ppa*-CEH-20 do not bind to the mutated site (SaM, lane 9). (**C**) Phylogenetic footprint of the HOX–PBC-binding site in the *Ppa-pax-3* promoter. The sequence from position –2216 to –2132 of *Ppa-pax-3* is shown. Comparison of *P. pacificus* (*P.pa*), *Pristionchus* sp. 11 (*P.11*) and *Pristionchus maupasi* (*P.ma*). The consensus HOX–PBC-binding site is indicated by a shaded box, other types of consensus binding sites are boxed. Boxes 1 and 2 are HOX monomer binding sites; box 3 is a HMG-binding site. (Bottom) The tree shows the phylogenetic relationship of these three species.

HOX–PBC-binding sites in the *Ppa-pax-3* promoter are phylogenetically conserved

Finally, we wanted to know whether the HOX–PBC-binding site in the *Ppa-pax-3* promoter is evolutionarily conserved. *P. pacificus* and *C. elegans* are members of different nematode families, and promoter elements cannot be compared over such evolutionary distances (Grandien and Sommer, 2001). Therefore, we cloned the *pax-3* gene, including the 5′ regulatory regions, from *Pristionchus* sp. 11 and *Pristionchus maupasi*, two additional members of the genus *Pristionchus* (Herrmann et al., 2006; Herrmann et al., 2007). In these species, cell fate specification of the VPCs is identical to *P. pacificus* (data not shown). Although the *pax-3* coding regions were found to be highly conserved between all three *Pristionchus* species, the 5′ regulatory regions were much less conserved. We found that the HOX–PBC-binding site that caused the shift in the EMSA assay is nearly completely conserved between all three *Pristionchus* species, further supporting the significance of the *Ppa-pax-3* promoter element for regulation by *Ppa*-LIN-39 (Fig. 6C).

DISCUSSION

In this study, we provide the genetic and molecular characterization of a genetic locus involved in cell fate specification of the ventral epidermis of *P. pacificus* hermaphrodites. *Ppa-pax-3*, together with *Ppa-lin-39*, is involved in the formation of the *P. pacificus* VEG and in the regulation of the survival of P(5-8).p. In contrast to *Ppa-lin-39*, *Ppa-pax-3* has an additional function in the posterior body region and regulates the PCD of P(9-11).p. We show that *Ppa-pax-3* is expressed in the embryo and early larval stages, and we provide molecular evidence that *Ppa*-LIN-39 regulates *Ppa-pax-3* and that Hox–PBC-binding sites in the *Ppa-pax-3* promoter are evolutionarily conserved.

This study provides the first detailed analysis of the function of a Pax-3-type gene in nematode postembryogenesis and indicates a role for *Ppa-pax-3* in a Hox gene-regulated developmental process, namely the establishment of the VEG. *Ppa-pax-3* mutants are Gev and represent the second complementation group besides *Ppa-lin-39* with such a phenotype. Also, in *C. elegans*, two complementation groups with a Gev phenotype have been identified – *Cel-lin-39* (Wang et al., 1993; Clark et al., 1993) and *Cel-ceh-20*, the latter of which encodes a PBC-like gene (Liu and Fire, 2000; Takacs-Vellai et al., 2007). Although *Ppa-ceh-20* was originally a candidate for the genetic locus described in this study, further mapping analysis indicated that *Ppa-ceh-20* and *gev-2* are located on different chromosomes.

pax-3 is unique among the nematode Pax genes with regard to the presence of all three functional domains: the PD, the HD and the octapeptide. Our mutant analysis revealed that *Ppa-pax-3(tu358)* represents a point mutation in the octapeptide, resulting in an amino acid substitution of the first amino acid from histidine to arginine. This histidine is highly conserved in PAX-3-type proteins throughout the animal kingdom. The fact that the cell-survival phenotype of *tu358* is nearly identical to the one of the deletion mutant *tu214*, which eliminates part of the HD, supports the idea that the octapeptide is of functional importance for the repression of PCD. Interestingly, studies of the mammalian Pax5 protein BSAP indicate that the octapeptide is only required for the repressive function of BSAP, whereas it is dispensable for the activator function (Eberhard et al., 2000). Given that surviving VPCs have no vulva phenotype in the *tu358* allele, whereas mutant animals of the deletion allele *tu214* result in vulva differentiation defects, we speculate that the octapeptide is dispensable for the role of *Ppa-pax-3* in vulva induction, which is most probably involved in transcriptional activation.

Our analysis of *Ppa-pax-3* indicates significant differences in the regulation of the VEG between *P. pacificus* and *C. elegans*. Whereas studies in *C. elegans* suggest an indirect role of LIN-39 in the regulation of the cell fusion effector *eff-1* via the GATA transcription factors *egl-18* and *elt-6* (Shemer and Podbilewicz, 2002; Koh et al., 2002; Cassata et al., 2005), our studies show that *Ppa*-LIN-39 is part of a different regulatory network. Our genetic and molecular results suggest that *Ppa*-LIN-39 acts upstream of the *Ppa-pax-3* gene (Fig. 2B). We speculate that *Ppa*-PAX-3 is directly involved in the suppression of PCD of P(5-8).p. However, the exact molecular mechanism of this suppression remains to be identified and several distinct mechanisms might be at work. One potential target for *Ppa*-PAX-3 might be the ortholog of *egl-1*, a key regulator of PCD in *C. elegans* (for a review, see Yuan, 2006). So far, we have been unable to detect an ortholog of *egl-1*, which encodes a small peptide, in the *P. pacificus* genome assembly. However, alternative mechanisms could also be involved in the regulation of PCD. Park et al. have recently shown that the Pax2/5/8 member *egl-38* and *pax-2* promote cell survival in *C. elegans* (Park et al., 2006). These studies suggest a role for *egl-38* and *pax-2* in the regulation of *ced-9* (Park et al., 2006). Future work in *P. pacificus* will indicate whether, and how, *Ppa-pax-3* is coupled to the PCD machinery.

Besides its role in vulva formation, *Ppa*-PAX-3 plays a role during the regulation of the PCD of P(9-11).p. This finding indicates for the first time that P(9-11).p are regulated in a different way than the ventral epidermal cells in the anterior body region. Interestingly, the function of *Ppa-pax-3* in P(9-11).p specification is independent of *Ppa*-LIN-39, indicating that *Ppa-pax-3* is regulated differently in these cells. Indications about the mechanism of this regulation might come from the analysis of a second gene, *ped-9*, which, when mutated, causes the survival of P(9-11).p, and which is currently being analyzed further (R. Molnar and R.J.S., unpublished observation). With regard to P(1-4).p, recent studies have shown that P(3,4).p are regulated differently from P(1,2).p; mutations in *Ppa-hairy*, a gene that has no counterpart in the *C. elegans* genome, or in *Ppa-groucho*, the ortholog of *C. elegans unc-37*, result in the survival of P(3,4).p, but not of P(1,2).p (Schlager et al., 2006). Genetic and biochemical studies revealed that *Ppa*-HAIRY and *Ppa*-GROUCHO downregulate the activity of *Ppa-lin-39* and thereby restrict the size of the VEG to P(5-8).p. The finding that *Ppa-pax-3* can rescue P(9-11).p, but not P(1,2).p, from PCD indicates that at least five different types of positional information subdivide the 12 ventral epidermal cells of *P. pacificus* (Fig. 2C): in the central body region, P(5-8).p survive based on positional information provided by *Ppa*-LIN-39 and *Ppa*-PAX-3 (Eizinger and Sommer, 1997) (this study). In the posterior body region, P(9-11).p die from PCD and *Ppa*-PAX-3 has a role distinct from the one described for P(5-8).p. P12.pa is a special cell that might be regulated by the Hox gene *Ppa-egl-5*, as occurs in *C. elegans*. However, a *Ppa-egl-5* mutant has not been identified yet. In the anterior region, P(3,4).p die because of the downregulation of *Ppa-lin-39* by *Ppa*-HAIRY and *Ppa*-GROUCHO (Schlager et al., 2006). The regulation of PCD in P(1,2).p is distinct from the PCD of P(3,4).p and P(9-11).p, but no specific mutants have been isolated that rescue P(1,2).p in a specific manner. Because all Pn.p cells survive in *Ppa-ced-3* and *Ppa-ced-4* mutants, the PCD of P(1,2).p is dependent on the normal PCD machinery (Sommer et al., 1998). One might speculate that the fate of P(1,2).p represents the ground state of Pn.p cells and that no specific positional information is required for the determination of this fate. Taken together, the seven cell-death events in the ventral epidermis of the *P. pacificus* hermaphrodite are highly regulated and require three distinct genetic mechanisms in the different regions of the animal

(Fig. 2C). In *C. elegans*, the distinct cell fates of Pn.p cells in the hermaphrodite are also regulated in a region-specific manner. In particular, cell fusion of P(1,2).p and P(9-11).p are regulated by distinct genetic programs (Alper and Kenyon, 2001).

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References

Alper, S. and Kenyon, C. (2001). REF-1, a protein with two bHLH domains, alters the pattern of cell fusion in *C. elegans* by regulating Hox protein activity. *Development* **128**, 1793-1804.
Cassata, G., Shemer, G., Morandi, P., Donhauser, R., Podbilewicz, B. and Baumeister, R. (2005). *ceh-16/engrailed* patterns the embryonic epidermis of *Caenorhabditis elegans*. *Development* **132**, 739-749.
Chamberlin, H. M., Palmer, R. E., Newman, A. P., Sternberg, P. W., Baillie, D. L. and Thomas, J. H. (1997). The PAX gene *egl-38* mediates developmental patterning in *Caenorhabditis elegans*. *Development* **124**, 3919-3928.
Chi, N. and Epstein, J. A. (2002). Getting your Pax straight: Pax proteins in development and disease. *Trends Genet.* **18**, 41-47.
Chisholm, A. D. and Horvitz, H. R. (1995). Patterning of the *Caenorhabditis elegans* head region by the Pax-6 family member *vab-3*. *Nature* **377**, 52-55.
Clark, S. G., Chisholm, A. D. and Horvitz, H. R. (1993). Control of cell fates in the central body region of *C. elegans* by the homeobox gene *lin-39*. *Cell* **74**, 43-55.
Dieterich, C., Roeseler, W., Sobetzko, P. and Sommer, R. J. (2007). Pristionchus.org: a genome-centric database of the nematode satellite species *Pristionchus pacificus*. *Nucleic Acids Res.* **35**, D498-D502.
Eberhard, D., Jimenez, G., Heavey, B. and Busslinger, M. (2000). Transcriptional repression by Pax5 (BSAP) through interaction with corepressors of the Groucho family. *EMBO J.* **19**, 2292-2303.
Eizinger, A. and Sommer, R. J. (1997). The homeotic gene *lin-39* and the evolution of nematode epidermal cell fates. *Science* **278**, 452-455.
Grandien, K. and Sommer, R. J. (2001). Functional comparison of the nematode Hox gene *lin-39* in *C. elegans* and *P. pacificus* reveals evolutionary conservation of protein function despite divergence of primary sequences. *Genes Dev.* **15**, 2161-2172.
Herrmann, M., Mayer, W. E. and Sommer, R. J. (2006). Nematodes of the genus *Pristionchus* are closely associated with scarab beetles and the Colorado potato beetle in Western Europe. *Zoology Jena* **109**, 96-108.
Herrmann, M., Mayer, W., Hong, R., Kienle, S., Minasaki, R. and Sommer, R. J. (2007). The nematode *Pristionchus pacificus* (Nematoda: Diplogastridae) is associated with the Oriental beetle *Exomala orientalis* (Coleoptera: Scarabaeidae) in Japan. *Zool. Sci.* In press.
Hobert, O. and Ruvkun, G. (1999). Pax genes in *Caenorhabditis elegans*: a new twist. *Trends Genet.* **15**, 214-216.
Hong, R. L. and Sommer, R. J. (2006). *Pristionchus pacificus*: a well-rounded nematode. *BioEssays* **28**, 651-659.
Jungblut, B. and Sommer, R. J. (1998). The *Pristionchus pacificus mab-5* gene is involved in the regulation of ventral epidermal cell fates. *Curr. Biol.* **8**, 775-778.
Jungblut, B. and Sommer, R. J. (2000). Novel cell-cell interactions during vulva development in *Pristionchus pacificus*. *Development* **127**, 3295-3303.
Jungblut, B. and Sommer, R. J. (2001). The nematode *even-skipped* homolog *vab-7* regulates gonad and vulva position in *Pristionchus pacificus*. *Development* **128**, 253-261.
Kenning, C., Kipping, I. and Sommer, R. J. (2004). Isolation of mutations with dumpy-like phenotypes and of collagen genes in the nematode *Pristionchus pacificus*. *Genesis* **40**, 176-183.
Kenyon, C. (1986). A gene involved in the development of the posterior body region of *C. elegans*. *Cell* **46**, 477-487.
Koh, K., Peyrot, S. M., Wood, C. G., Wagmaister, J. A., Maduro, M. F., Eisenmann, D. M. and Rothman, J. H. (2002). Cell fates and fusion in the *C. elegans* vulval primordium are regulated by the EGL-18 and ELT-6 GATA factors – apparent direct targets of the LIN-39 Hox protein. *Development* **129**, 5171-5180.
Liu, J. and Fire, A. (2000). Overlapping roles of two Hox genes and the exd ortholog *ceh-20* in diversification of the *C. elegans* postembryonic mesoderm. *Development* **127**, 5179-5190.
Maloof, J. N. and Kenyon, C. (1998). The Hox gene *lin-39* is required during *C. elegans* vulval induction to select the outcome of Ras signaling. *Development* **125**, 181-190.
Michael, M., Dieterich, C. and Vingron, M. (2005). SITEBLAST–rapid and sensitive local alignment of genomic sequences employing motif anchors. *Bioinformatics* **21**, 2093-2094.
Park, D., Jia, H., Rajakumar, V. and Chamberlin, H. M. (2006). Pax2/5/8 proteins promote cell survival in *C. elegans*. *Development* **133**, 4193-4202.
Pearson, J. C., Lemons, D. and McGinnis, W. (2005). Modulating Hox gene functions during animal body patterning. *Nat. Rev. Genet.* **6**, 893-904.
Schlager, B., Roseler, W., Zheng, M., Gutierrez, A. and Sommer, R. J. (2006). HAIRY-like transcription factors and the evolution of the nematode vulva equivalence group. *Curr. Biol.* **16**, 1386-1394.
Shemer, G. and Podbilewicz, B. (2002). LIN-39/Hox triggers cell division and represses EFF-1/fusogen-dependent vulval cell fusion. *Genes Dev.* **16**, 3136-3141.
Sigrist, C. B. and Sommer, R. J. (1999). Vulva formation in *Pristionchus pacificus* relies on continuous gonadal induction. *Dev. Genes Evol.* **209**, 451-459.
Sommer, R. J. (2005). Evolution of development in nematodes related to *C. elegans*. In *WormBook* (ed. The *C. elegans* Research Community), WormBook, doi/10.1895/wormbook.1.46.1, http://www.wormbook.org.
Sommer, R. J. and Sternberg, P. W. (1996). Apoptosis and change of competence limit the size of the vulva equivalence group in *Pristionchus pacificus*: a genetic analysis. *Curr. Biol.* **6**, 52-59.
Sommer, R. J., Eizinger, A., Lee, K. Z., Jungblut, B., Bubeck, A. and Schlak, I. (1998). 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.
Srinivasan, J., Sinz, W., Lanz, C., Brand, A., Nandakumar, R., Raddatz, G., Witte, H., Keller, H., Kipping, I., Pires-daSilva, A. et al. (2002). A bacterial artificial chromosome-based genetic linkage map of the nematode *Pristionchus pacificus*. *Genetics* **162**, 129-134.
Srinivasan, J., Sinz, W., Jesse, T., Wiggers-Perebolte, L., Jansen, K., Buntjer, J., van der Meulen, M. and Sommer, R. J. (2003). An integrated physical and genetic map of the nematode *Pristionchus pacificus*. *Mol. Genet. Genomics* **269**, 715-722.
Sternberg, P. W. (2005). Vulval development. In *WormBook* (ed. The *C. elegans* Research Community), WormBook, doi/10.1895/wormbook.1.6.1, http://www.wormbook.org.
Takacs-Vellai, K., Vellai, T., Chen, E. B., Zhang, Y., Guerry, F., Stern, M. J. and Muller, F. (2007). Transcriptional control of Notch signaling by a HOX and a PBX/EXD protein during vulval development in *C. elegans*. *Dev. Biol.* **302**, 661-669.
Wagmaister, J. A., Gleason, J. E. and Eisenmann, D. M. (2006). Transcriptional upregulation of the *C. elegans* Hox gene *lin-39* during vulval cell fate specification. *Mech. Dev.* **123**, 135-150.
Wang, B. B., Muller-Immergluck, M. M., Austin, J., Robinson, N. T., Chisholm, A. and Kenyon, C. (1993). A homeotic gene cluster patterns the anteroposterior body axis of *C. elegans*. *Cell* **74**, 29-42.
Yuan, J. (2006). Divergence from a dedicated cellular suicide mechanism: exploring the evolution of cell death. *Mol. Cell* **23**, 1-12.
Zhang, Y. and Emmons, S. W. (1995). Specification of sense-organ identity by a *Caenorhabditis elegans* Pax-6 homologue. *Nature* **377**, 55-59.
Zheng, M., Messerschmidt, D., Jungblut, B. and Sommer, R. J. (2005). Conservation and diversification of Wnt signaling function during the evolution of nematode vulva development. *Nat. Genet.* **37**, 300-304.