Development 128, 3395-3404 (2001)
Printed in Great Britain © The Company of Biologists Limited 2001
DEV5920

Formation of the egg-laying system in *Pristionchus pacificus* requires complex interactions between gonadal, mesodermal and epidermal tissues and does not rely on single cell inductions

Benno Jungblut*, André Pires-daSilva and Ralf J. Sommer‡

Max-Planck Institut für Entwicklungsbiologie, Abteilung Evolutionsbiologie, Spemannstrasse 37-39, D-72076 Tübingen, Germany
*Present address: Department of Biochemistry and Biophysics, UC San Francisco, San Francisco, CA 94143, USA
‡Author for correspondence (e-mail: ralf.sommer@tuebingen.mpg.de)

Accepted 18 June 2001

SUMMARY

The invariant cell lineage of nematodes allows the formation of organ systems, like the egg-laying system, to be studied at a single cell level. The *Caenorhabditis elegans* egg-laying system is made up of the vulva, the mesodermal gonad and muscles and several neurons. The gonad plays a central role in patterning the underlying ectoderm to form the vulva and guiding the migration of the sex myoblasts to their final position. In *Pristionchus pacificus*, the egg-laying system is homologous to *C. elegans*, but comparative studies revealed several differences at the cellular and molecular levels during vulval formation. For example, the mesoblast M participates in lateral inhibition, a process that influences the fate of two vulval precursor cells. Here, we describe the M lineage in *Pristionchus* and show that both the dorsal and ventral M sublineages are involved in lateral inhibition. Mutations in the homeotic gene *Ppa-mab-5* cause severe misspecification of the M lineage, resembling more the *C. elegans* Twist than the *mab-5* phenotype. Ectopic differentiation of P8.p in *Ppa-mab-5* results from at least two separate interactions between M and P8.p. Thus, interactions among the *Pristionchus* egg-laying system are complex, involving multiple cells of different tissues occurring over a distance.

Key words: Evolution, M lineage, *Pristionchus pacificus*, Vulva, *mab-5*

INTRODUCTION

Cell-cell interactions among cells of different germ layers are of importance for the development and the evolution of multicellular organisms. The invariant cell lineage of free-living nematodes such as *Caenorhabditis elegans*, allows cell-cell interactions to be studied at a single cell level. One well known example in *C. elegans* is the formation of the egg-laying system, which consists of the mesodermal gonad and muscles, the epidermal vulva and some neurons. Various cell-cell interactions between groups of cells of the egg-laying system have been described and several genetically identified components have been molecularly characterized (for review see Greenwald, 1997; Moerman and Fire, 1997).

At hatching, the gonad consists of four cells, called Z(1-4) and it develops initially in an autonomous fashion. Z(2,3) make the germline, whereas Z(1,4) are the precursors of the somatic gonad. During postembryonic development, Z(1,4) divide multiple times, forming all somatic derivatives of the gonad including the uterus (Kimble and Hirsch, 1979). Early experimental studies on gonad development in *C. elegans* identified a crucial cell-cell interaction between the gonadal anchor cell (AC) and the underlying epidermis. If the AC was ablated at birth, no vulva was formed, resulting in animals that are egg-laying defective (Kimble, 1981).

The vulva itself is a derivative of the ventral epidermis, which consists of 12 precursor cells, named P(1-12).p according to their anteroposterior position (Fig. 1; Sulston and Horvitz, 1977). The six central cells, P(3-8).p, form a vulva equivalence group, because all these six cells can participate in the formation of the vulva (Sternberg and Horvitz, 1986). However, in wild-type animals only the three cells P(5-7).p form vulval tissue by adopting one of two alternative cell fates. P(5,7).p generate seven progeny each, form the outer part of the vulva and have a so-called 2° fate. P6.p generates eight progeny, forms the inner part of the vulva and has the 1° fate. P(3,4,8).p divide once, remain epidermal and have a 3° fate. A hierarchy of cell fates can be distinguished among P(3-8).p, because cells with a lower fate (i.e. 3° cells) can replace 2° or 1° cells. Similarly, 2° cells can replace the 1° cell, whereas the 1° cell P6.p does not replace any other cell (for review see Kornfeld, 1997; Greenwald, 1997).

In addition to the induction of vulva formation, the gonad is also required for the correct positioning of the eight vulval and uterine muscles, all of which derive from two sex myoblasts (SM). The SM cells are formed during postembryogenesis from the mesoblast M, a blast cell that in total gives rise to 14 body muscles, two coelomocytes and the two SM cells. The two SM cells, one on each side of the animal, are born midway between gonad and rectum and migrate to their final position

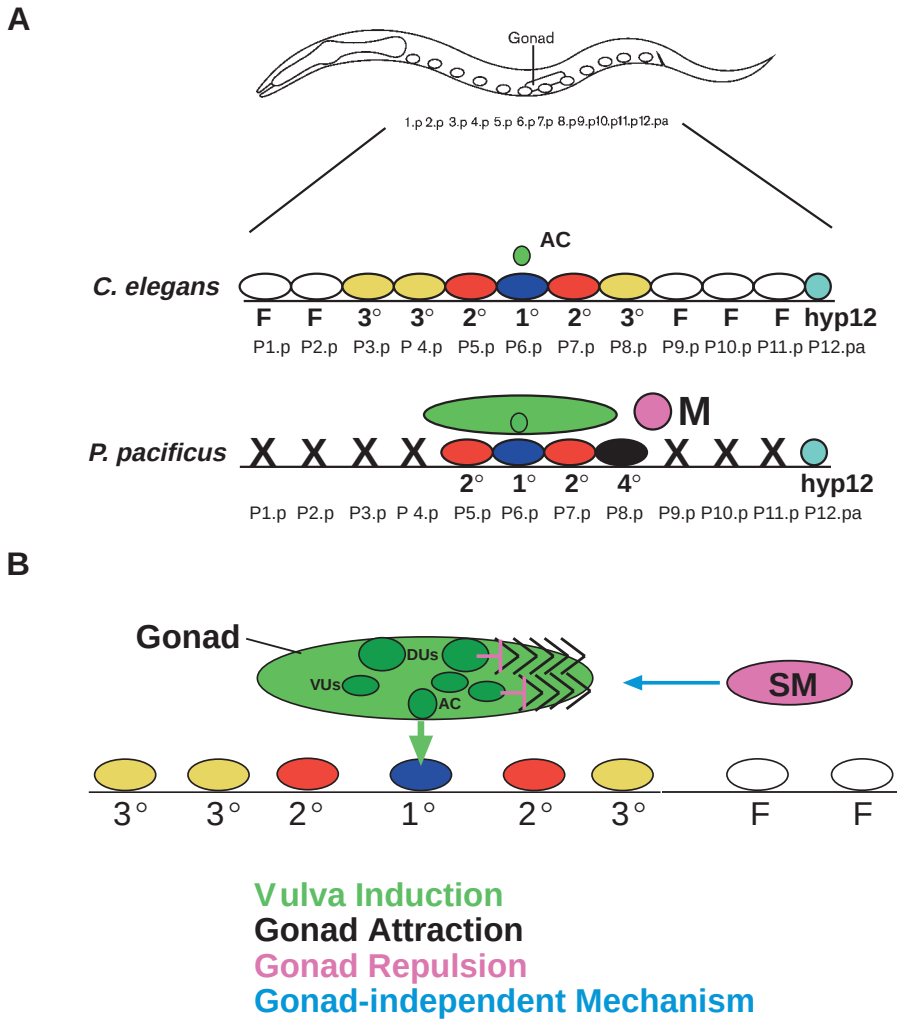

Fig. 1. Evolutionary variations in vulval cell fate specification between *C. elegans* and *P. pacificus* (A) and a schematic summary of the multiple guidance mechanisms for sex myoblast migration in *C. elegans* (B). (A) During the L1 stage in *C. elegans*, the 12 ventral epidermal cells P(1-12).p are equally distributed between pharynx and rectum. P(1,2,9-11).p fuse with the hypodermal syncytium *hyp7* (F, white ovals). P(3-8).p form the vulva equivalence group and adopt one of three alternative cell fates. P6.p has a 1° fate (blue oval), and P(5,7).p have a 2° fate (red ovals). P(3,4,8).p have a 3° fate and remain epidermal (yellow ovals). The anchor cell (AC, green circle) provides an inductive signal for vulva formation. In *P. pacificus*, P(1-4,9-11).p die by programmed cell death. P6.p and P(5,7).p have a 1° and 2° fate, respectively. P8.p has a 4° fate (black oval) and remains epidermal. Vulva induction is provided by several cells of the somatic gonad (green ellipse). (B) The SM cells are born midway between gonad and rectum and migrate anteriorly. A gonad-independent mechanism (blue arrow) guides the SMs to an anterior position. Precise positioning around the center of the gonad and vulva requires a gonadal attraction that involves an FGF-like molecule encoded by the *egl-17* gene. *egl-17* is expressed in several cells of the somatic gonad, the dorsal uterine precursors (DU), the ventral uterine precursors (VU) and the AC, but also in P6.p. A third signal influencing SM position is a gonad-dependent repulsion (pink bar). Repulsion has been identified in *egl-17* mutants, in which the SM cells stay in the posterior region rather than migrating to the central body region by the gonad-independent mechanism. The precise role of gonad repulsion is unknown.

in the center of the animal (Fig. 1B). After migration, both SM cells divide and form a total of 16 progeny, eight vulval and uterine muscles, respectively. SM migration in *C. elegans* hermaphrodites is controlled by multiple guidance mechanisms (Stern and Horvitz, 1991; DeVore et al., 1995; Burdine et al., 1998; Branda and Stern, 2000). A gonad-independent mechanism propels the SM cells anteriorly to a broad range of positions near the center of the animal. However, their precise final position flanking the center of the gonad depends on a gonad-dependent attraction. Thus, the gonad induces cell fate specification of epidermal cells to form vulva and also guides the SM cells to a precise position, thereby organizing the core of the egg-laying system.

Two different molecular pathways are defined to control the cell-cell interactions of the gonad with other tissues. The AC expresses an EGF-like protein encoded by the gene *lin-3*, which constitutes the signal for vulva induction (Hill and Sternberg, 1992). Within the VPCs, this signal is transmitted via an EGFR/RAS/MAPK signaling (Kornfeld, 1997). The anterior migration of the SM cells requires EGL-17, an FGF-like protein (Burdine et al., 1997) that is expressed in several cells of the somatic gonad (Branda and Stern, 2000). For the proper formation of the complete egg-laying system, additional reciprocal signaling between the gonad, the SMs and the vulva are required. Once P6.p has been specified to adopt the 1° fate, some of its descendants express the LIN-3 protein themselves and induce a particular cell fate in the uterus (Chang et al., 1999; Newman et al., 2000). Furthermore, P6.p expresses EGL-17 and contributes to the correct alignment of the SM cells (Burdine et al., 1998). Taken together, reciprocal signaling between cells and tissues of different germ layers are required for organogenesis of the egg-laying system.

Cell fate specification and organogenesis can be studied in several free-living nematodes providing insight into the evolution of complex developmental structures (for review see Sommer, 2000). One particularly attractive system for evolutionary studies is the development of the vulva. At the cellular level, multiple differences in cell fate specification and cell-cell interactions have been identified between various nematode species (Fig. 1A) (Sommer, 1997a; Sommer, 2000). Furthermore, detailed genetic and molecular studies in *Pristionchus pacificus* indicated that even if the cells forming the vulva are homologous, multiple changes can occur at the genetic and molecular levels. For example, the homeotic genes *lin-39* and *mab-5* or the *even-skipped* homolog *vab-7* have different functions during vulva formation in *P. pacificus* and *C. elegans* (Eizinger and Sommer, 1997; Sommer et al., 1998; Jungblut and Sommer, 1998; Jungblut and Sommer, 2001).

In particular, four important differences have been identified between vulva development in *P. pacificus* and *C. elegans*. First, non-vulval cells in the anterior and posterior body region fuse with the surrounding hypodermis in *C. elegans*, but die of programmed cell death in *P. pacificus* (Fig. 1A) (Eizinger and Sommer, 1997). Second, vulva induction relies on a continuous interaction between several cells of the somatic gonad and the vulval precursor cells (VPCs) rather than an interaction of the single AC, as in *C. elegans* (Sigrist and Sommer, 1999). Third, P8.p represents a novel cell type in *P. pacificus* and is involved in multiple cell-cell interactions during vulva formation, not known in *C. elegans* or other nematodes (Jungblut and Sommer, 2000). For instance, P8.p inhibits P5.p and P7.p to adopt the 1° cell fate, a process called 'lateral inhibition'. Additional experiments also indicated that the mesoblast M is involved in lateral inhibition and that P8.p and M interact to inhibit the fate of both VPCs (Jungblut and Sommer, 2000). In contrast, no interaction between the P8.p and the M cell has been observed in *C. elegans*.

We describe the cell lineage of the mesoblast M in *P. pacificus* and determine which cells of the M lineage interact with the VPCs in *P. pacificus*. We show that the mesoblast M has an identical cell lineage to that in *C. elegans*. Lateral inhibition of P(5,7).p requires cells of both major M sublineages, the dorsal and ventral lineage, respectively. In *Ppa-mab-5* mutants, the complete M lineage is misspecified. The first two cell divisions occur along a longitudinal axis instead of the dorsoventral and the left/right division axes, resembling a phenotype known for mutations in the *C. elegans* Twist gene. Furthermore, no proper sex myoblasts are formed in *Ppa-mab-5* mutant animals, causing a strong egg-laying defect. In contrast, *mab-5* mutants in *C. elegans* form normal SM cells indicating yet another difference between *Ppa*-MAB-5 and *Cel*-MAB-5 function. Finally, the ectopic differentiation of P8.p in *Ppa-mab-5* mutants depends on the misspecification of the M lineage and requires at least one inhibitory and one inductive interaction, both of which might be neomorphic.

MATERIALS AND METHODS

Strains and cultures

All experiments were carried out using the laboratory strain *P. pacificus* PS312, which is a derivative of a wild isolate from Pasadena, California, USA (Sommer et al., 1996). Worms were grown on *E. coli* OP50 as described elsewhere (Sommer et al., 1996).

Cell ablation experiments

Cell ablation experiments were carried out using standard techniques described for *C. elegans* (Epstein and Shakes, 1995) and using a 'Laser Science' dye laser of the type described previously (Avery and Horvitz, 1987). Animals were picked into M9 buffer placed on a pad of 5% agar in water containing 10 mM sodium azide as anaesthetic.

Cell lineage characters and cell fate terminology

The different cell fates of the VPCs are distinguished using the terminology 1°, 2°, 3° and 4° for cell fates, and T (transverse), L (longitudinal), N (non-dividing) and O (oblique) for cell division patterns (Sommer and Sternberg, 1995; Sommer and Sternberg, 1996). During normal development, P6.p has the 1° fate and generates six progeny with the cell division pattern TNNT. The two 'N' cells (P6.pap and P6.ppa), which do not divide (in contrast to *C. elegans*), attach to the AC. P(5,7).p have a 2° fate and generate seven progeny each, with a cell division pattern LLLN (for P5.p). After ablation of other VPCs, an isolated 1° and an isolated 2° cell can be distinguished from one another by several cell lineage characteristics. In the intermediate four-cell stage (after two rounds of cell divisions of a VPC) of a 1° cell, the AC moves between the two central cells P6.pap and P6.ppa. In the final six-cell stage, the cells are located symmetrically around the AC. In the four-cell stage of a 2° cell, the AC does not move between the central Pn.pxx cells. When the invagination is formed, the distribution of the seven progeny is asymmetric and variable with respect to the AC. VPCs that remain epidermal in the absence of vulva induction were designated as 3°. The fate of P8.p was designated as 4° based on the finding that this cell loses its competence to form vulval tissue during early larval development (Jungblut and Sommer, 2000).

Coelomocyte staining

Coelomocytes were stained using rhodamine injections. J2 and J3 stage animals were injected into the pseudocoelomic space with rhodamine dextran (Sigma – Cat. No R-8881). 6-8 hours later, the number of coelomocytes was scored using fluorescence miscroscopy.

RESULTS

Background

Vulva development in *P. pacificus* differs from *C. elegans* with respect to several aspects of cell fate specification. First, several cells of the somatic gonad are involved in vulva induction in *P. pacificus* (Sigrist and Sommer, 1999), whereas induction in *C. elegans* relies on a signal from the single AC (Kimble, 1981). Second, new types of cell-cell interactions among the VPCs occur during vulva formation in *P. pacificus*, which also involves the mesoblast M (Jungblut and Sommer, 2000). Specifically, *P. pacificus* P8.p represents a new ventral epidermal cell type and is involved in two novel interactions, negative signaling and lateral inhibition, respectively.

Negative signaling by P8.p refers to the observation that VPCs can differentiate and form vulva-like tissue after both Z(1,4) and P8.p are ablated together. 18% of the VPCs differentiated in this experiment, whereas after Z(1,4) ablation no vulva differentiation was observed. Thus, P8.p in *P. pacificus* provides a negative signal that antagonizes inappropriate vulva differentiation (Jungblut and Sommer, 2000).

Lateral inhibition refers to the observation that P5.p and P7.p, but not P6.p, are unable to adopt the 1° cell fate in the presence of P8.p after the ablation of other VPCs (Jungblut and Sommer, 2000). For example, if P(6,7).p are ablated at hatching, P5.p has a 2° fate in the majority of ablated animals (Table 1A). In contrast, if P(6-8).p are ablated, P5.p predominantly adopts the 1° fate (Jungblut and Sommer, 2000). Further experiments had indicated that lateral inhibition was mediated by the M cell lineage. After ablation of P(6,7).p and M, P5.p had a 1° fate in the presence of P8.p indicating for the first time that an interaction between a Pn.p cell and the M cell influences vulval fate specification (Table 1B) (Jungblut and Sommer, 2000). Further evidence for an interaction between Pn.p cells and the M lineage came from the observation that the ectopic differentiation of P8.p in *Ppa-mab-5* mutants was dependent on a signal from the M lineage. In unablated *Ppa-mab-5(tu74)* mutant animals, P8.p differentiates in 80% of the animals. If M was ablated at hatching, differentiation of P8.p was strongly reduced (Jungblut and

Table 1. Ablation of the M lineage and its effect on lateral inhibition of P5.p

	Cells ablated	Time (after hatching)	P5.p	P6.p	P7.p	P8.p	No. animals (frequency)
A	P(6,7).p	0-1	2°	–	–	4°	34/45 (76%)
			1°	–	–	4°	8/45 (18%)
			1°	–	–	2°	2/45 (4%)
			3°	–	–	4°	1/45 (2%)
B	P(6,7).p	0-1	1°	–	–	4°	10/12 (84%)*
	M	0-1	1°	–	–	2°	1/12 (8%)*
			2°	–	–	4°	1/12 (8%)*
C	P(6,7).p	0-1	1°	–	–	4°	8/13 (61%)
	M.v	8-9	1°	–	–	2°	1/13 (8%)
			2°	–	–	4°	4/13 (31%)
D	P(6,7).p	0-1	1°	–	–	4°	7/18 (39%)
	M.d	8-9	1°	–	–	2°	4/18 (22%)
			2°	–	–	4°	6/18 (33%)
			2°	–	–	2°	1/18 (6%)
E	P(6,7).p	0-1	1°	–	–	4°	9/13 (69%)
	SM	17-18	2°	–	–	4°	4/13 (31%)

*Contains data from Jungblut and Sommer, 2000.

Sommer, 2000). Taken together, these results suggested the importance of interactions between the M lineage and the Pn.p cells, in particular P8.p.

The M lineage in *P. pacificus* is identical to *C. elegans*

To study the interactions between the M lineage and the Pn.p cells in more detail, we first had to carry out cell lineage analysis of the M cell in *P. pacificus* using Nomarski microscopy. No lineage differences were observed between *P. pacificus* and *C. elegans*. The M cell divides first along the dorsoventral axis, followed by a left-right division of the two daughter cells (Figs 2, 4A,C,E,F). 10 hours after hatching, these four descendants of M form the muscle precursors in the four muscle quandrants.

Within the next few hours, the Md/v,l/r cells divide again giving rise to 14 body muscles, two coelomocytes and two SM cells. Rhodamine dextran staining revealed that *P. pacificus* has a total of six coelomocytes (Fig. 4G). Thus, *P. pacificus* forms four coelomocytes during embryogenesis and two during postembryogenesis, like *C. elegans*. The two SM cells are born 18 hours after hatching, shortly before the AC is generated. At that time, the SM cells are located in the posterior body region behind P8.p (Figs 3A, 4C). Subsequently, they migrate towards the central body region and arrive at the position of the future vulva about 26 hours after hatching (Fig. 4F). Both SM cells start to divide 31 hours after hatching when the VPCs have already divided twice. Three rounds of cell divisions follow in a short time period, resulting in a pattern of vulval and uterine muscles similar to *C. elegans* (Fig. 2). Thus, the complete M cell lineage is identical in both nematodes. This observation is in agreement with the vulval cell lineages in *C. elegans* and *P. pacificus*, which also show only minor differences (Sommer and Sternberg, 1996). In contrast, other comparative studies revealed important lineage differences. For example, Sternberg and Horvitz (Sternberg and Horvitz, 1982) studied the M lineage in *Panagrellus redivivus* and observed programmed cell death of some of the intermediate precursor cells that form body muscles in *P. pacificus* and *C. elegans*. The vulval cell lineages between different species of the Rhabditidae, like *Mesorhabditis* or *Oscheius*, also revealed substantial lineage alterations (Sommer and Sternberg, 1994; Sommer and Sternberg, 1995).

Lateral inhibition of P(5,7).p requires multiple cells of the M lineage

One novel cell-cell interaction during vulva development in *P. pacificus* that is absent in *C. elegans* is lateral inhibition, which describes the influence of P8.p on the cell fate decision of P(5,7).p upon gonadal signaling (Table 1A) (Jungblut and Sommer, 2000). Previous experiments indicated the involvement of the M lineage in lateral inhibition (Table 1B). However, in all cell ablation experiments, the M cell itself was ablated immediately after hatching (Jungblut and Sommer, 2000). To study if the influence of the M lineage on lateral

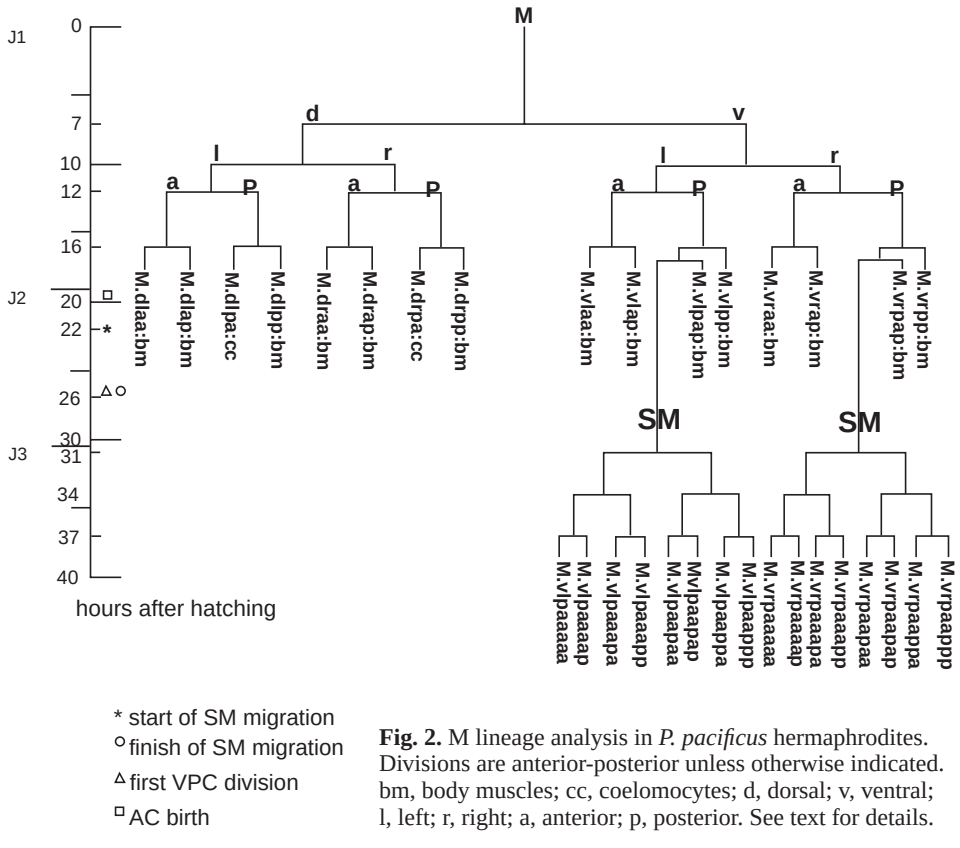

Fig. 2. M lineage analysis in *P. pacificus* hermaphrodites. Divisions are anterior-posterior unless otherwise indicated. bm, body muscles; cc, coelomocytes; d, dorsal; v, ventral; l, left; r, right; a, anterior; p, posterior. See text for details.

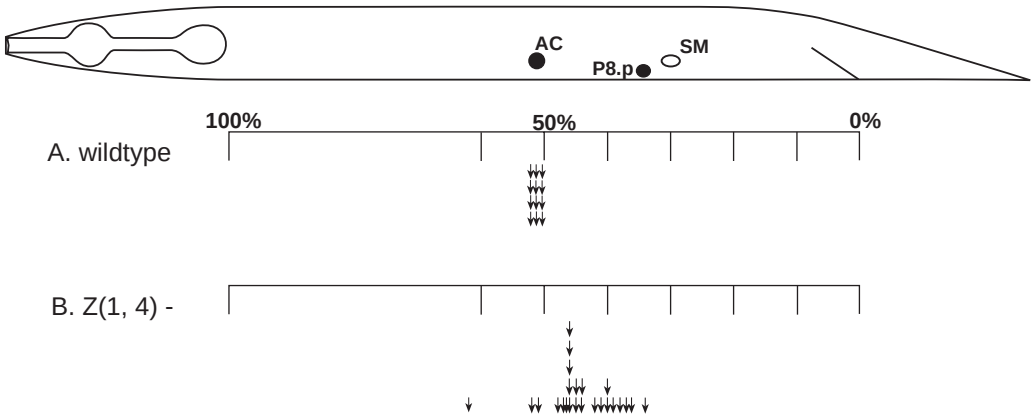

Fig. 3. Effects of gonad ablations on SM positioning in *P. pacificus* wild-type hermaphrodites. Z(1,4) were ablated at hatching. Each arrow represents the position of a single SM. SM position was scored with respect to the position of the remnants of the gonad and the position of P8.p. The position of P(5-7).p cannot be taken as a representative landmark, as these cells migrate slightly towards the posterior after gonad ablation.

inhibition is the result of a single mesodermal cell interacting with the VPCs, we ablated different M sublineages and P(6,7).p simultaneously and analyzed the presence or absence of lateral inhibition.

Our experiments show that lateral inhibition requires multiple cells of the M lineage, which derive from the two major M sublineages. When we ablated P(6,7).p and M.v or M.d, P5.p had a 1° fate in the majority of cases. Specifically, P5.p had a 1° fate in 69% and 61% of the animals (Table 1C,D). Thus, ablation of either the ventral or the dorsal M sublineage results in the elimination of lateral inhibition. These results indicate that lateral inhibition requires multiple cells and cell types of the M lineage. For example, the interaction involves the sex myoblasts as indicated by the ablation of only the SM cells (Table 1E). However, it is not restricted to the SM cells because the ablation of M.d has a similar effect (Table 1D). Together, these data suggest that the signal acts over a distance and probably in a quantitative manner.

P. pacificus SM migration depends on a gonadal signal

A *C. elegans* cell-cell interaction that involves multiple cells of various sublineages is the anterior migration of the SM cells. Several guidance mechanisms are involved in this process in *C. elegans*, viz. a gonad-dependent attraction, a gonad-independent attraction and a gonad-dependent repulsion (Fig. 1B) (Stern and Horvitz, 1991; DeVore et al., 1995; Burdine et al., 1998; Branda and Stern, 2000). If the somatic gonad is ablated in *C. elegans*, the SM cells are no longer correctly positioned in the center of the gonad. However, the SM cells still migrate anteriorly, indicating that this movement is guided by a gonad-independent attraction. The analysis of *egl-17* mutants revealed the existence of yet another gonad-dependent signal affecting SM migration. In *egl-17* mutants, the SM cells stay in the posterior body region indicating that under these mutant conditions, the gonad repels the SM cells (Stern and Horvitz, 1991). It is unknown, how the gonad-independent and the gonad-dependent attraction dominate over the gonad-dependent repulsion under wild-type conditions.

We sought to determine if SM migration in *P. pacificus* also relies on a gonad-dependent guidance mechanism and therefore, ablated Z(1,4) in wild-type animals at hatching. SM cell position is variable after gonad ablation, as in *C. elegans* (Fig. 3). Also, the SM cells divide and differentiate independently of their final position (Fig. 3). Most of the 24 SM cells studied after gonad ablation stopped migrating and started to differentiate in a region between P6.p and P7.p. Thus, the SM cells remain in a position slightly more posterior than in wild-type animals, a result that is qualitatively and quantitatively similar to observations in *C. elegans* (Thomas et al., 1990). Therefore, our results demonstrate that in wild-type animals SM migration in *P. pacificus* is influenced by a gonad-dependent and a gonad-independent guidance mechanism.

Ppa-mab-5 mutants show various M lineage defects that resemble the *C. elegans Twist* and *mab-5* phenotypes

Previous studies indicated that the mesoblast M is misspecified in *Ppa-mab-5* mutants (Jungblut and Sommer, 2000). Given the M cell lineage analysis in *P. pacificus*, we sought to determine which cellular aspects of the M lineage are altered in *Ppa-mab-5* mutants. In *C. elegans*, the requirement of MAB-5 in M lineage patterning has been studied in detail (Kenyon, 1986; Harfe et al., 1998; Corsi et al., 2000; Liu and Fire, 2000). The *Cel-mab-5* mutant exhibits, (i) variable defects in the division planes during the first divisions of M, (ii) the absence of the M-derived coelomocytes and (iii) the transformation of some body-wall muscles and the coelomocytes into sex myoblast-like cells (Harfe et al., 1998; Liu and Fire, 2000; Corsi et al., 2000).

We analyzed the role of *Ppa-mab-5* in M lineage specification by studying the strong 'reduction-of-function' alleles *tu74* and *tu31* (Jungblut and Sommer, 1998). We found that strong reduction of *mab-5* function in *P. pacificus* causes strong early and late lineage defects in the M lineage, providing a pattern of similarities and differences with respect to the *Cel-mab-5* mutant. First, a strong lineage defect was seen in the first two divisions in the M lineage. In 35 of 36 analyzed *Ppa-mab-5* animals, M divided in the anteroposterior direction instead of a dorsoventral division (Table 2A). Also, the two daughters of M divided in the anterior-posterior direction instead of a left-right division in 35 out of 36 animals (Table 2A). As a result, the four descendants of M are in the right ventral quandrant in *Ppa-mab-5* mutants (Fig. 4B). This reversal of division axes in the early M divisions is rarely seen in *Cel-mab-5(lof)* mutants, but is known from mutants in *Cel-hlh-8*, the *C. elegans* Twist gene (Corsi et al., 2000).

The four descendants of M in *Ppa-mab-5* mutants divide irregularly during the next few hours. 22 hours after hatching (20°C), a group of 7-14 cells is formed, most of which are

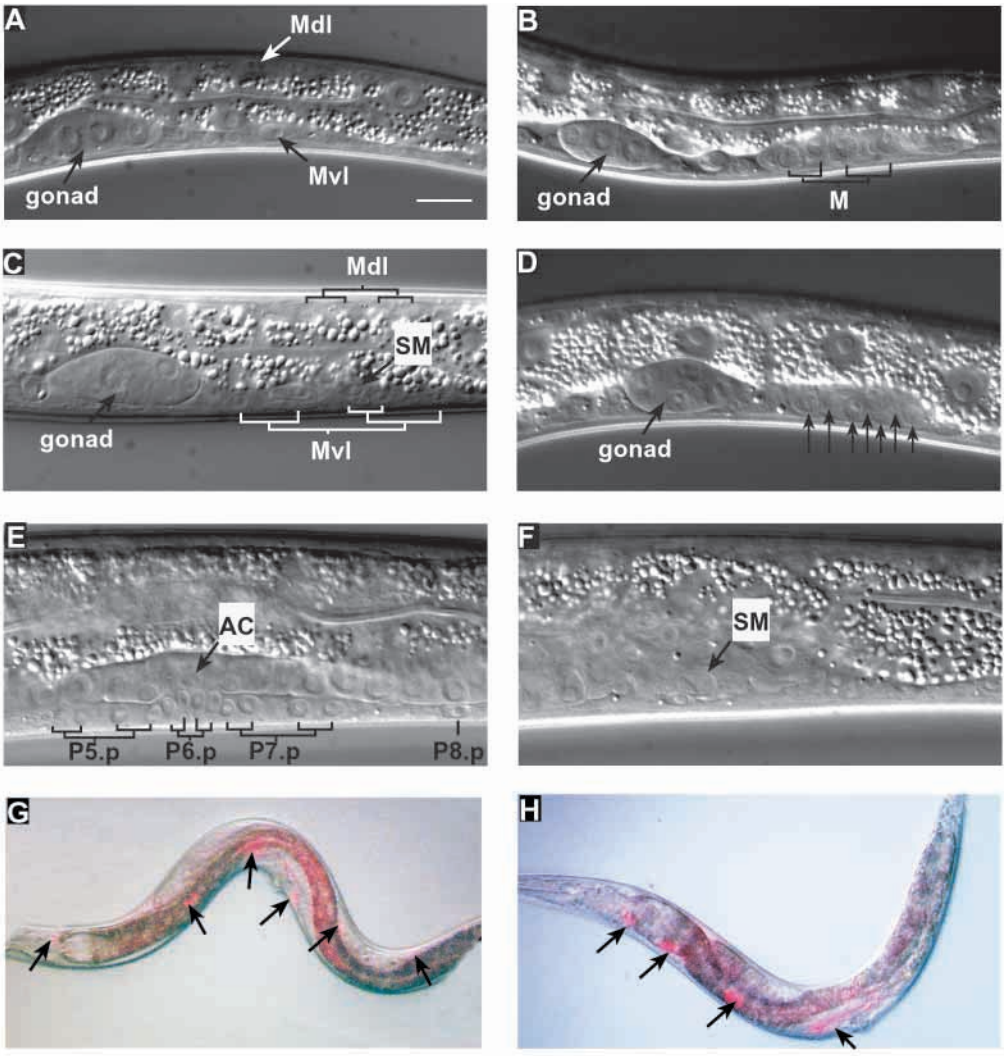

Fig. 4. Nomarski photomicrographs of various stages of the M lineage and rhodamine dextran injected *P. pacificus* wild-type and *Ppa-mab-5* mutant animals. (A) Wild-type animal, 11 hours after hatching showing two of the four intermediate muscle precursors in the muscle quadrants. In this plane of focus, the ventral (M.vl) and dorsal (M.dl) cell in the left focal plane are visible. (B) *Ppa-mab-5* mutant animals, 11 hours after hatching, showing all four progeny of M in the ventral region on the right side of the animal after both divisions occurred longitudinally. (C) Wild-type animal 19 hours after hatching showing the four and five progeny of M.dl and M.vl, respectively. Note that similar muscle lineages exist on the right side of the animal. The SM cell (SM) is already morphologically distinct from its sisters. (D) *Ppa-mab-5* mutant animals 19 hours after hatching, showing a group of disorganized descendants of M. In this plane of focus, seven cells are visible (arrows) none of which has specific characters of SM-like or coelomocyte-like cells. (E) Wild-type animal 30 hours after hatching showing the AC and the progeny of P(5-7).p after two rounds of cell division. (F) The same animal in a left focal plane showing the undivided SM cell in the region of the vulva. (G) Rhodamine dextran injected wild-type animal with six coelomocytes. (H) Rhodamine dextran injected *Ppa-mab-5* mutant animals, where the postembryonic coelomocytes are absent.

located in the right ventral quandrant of the animal (Table 2B) (Fig. 4D). These cells differ in shape and their division behaviour is variable so that the exact pattern varies between mutant animals. In wild-type animals, 18 progeny, including the SM cells and the coelomocytes, are formed at the corresponding stage (Figs 2, 4C).

To study if coelomocytes and SM cells are formed at all in *Ppa-mab-5* mutants we looked specifically for these two cell types at later stages of development. We found that only one out of 24 analyzed *Ppa-mab-5* animals had a SM cell in the central body, which formed vulval and uterine muscles later in development (Table 2C). In the other 23 animals, no SM-like cells where present in the central or the more posterior region. Thus, in contrast to *Cel-mab-5* animals, which show extra SM-like cells, SM cells are absent in *Ppa-mab-5* mutants. This observation also explains why *Ppa-mab-5* animals are egg-laying defective, whereas *Cel-mab-5* animals are egg-laying positive. However, the egg-laying defect of *Ppa-mab-5* mutants is not as penetrant as the SM defect as only 13 of 23 animals that were shown by lineage analysis to lack SM cells were egg-laying defective (Table 2C). Thus, *P. pacificus* hermaphrodites are, to a certain degree, able to lay eggs in the absence of functional vulval and uterine muscles. This result has been confirmed by ablating the M or SM cells in wild-type animals, which results in approximately 20% of animals that are egg-laying positive (data not shown).

Though the SM cell phenotypes of *Ppa-mab-5* and *Cel-mab-5* mutants are different, both mutants have a similar coelomocyte phenotype. We counted the number of coelomocytes by rhodamine dextran injection of young mutant and wild-type animals. In 10 out of 11 wild-type animals (91%) six coelomocytes were observed, however only 12% had more than four coelomocytes in *Ppa-mab-5* mutant animals (Table 2D; Fig. 4G,H). Taken together, the misspecifation of the M lineage in *Ppa-mab-5* animals is much stronger than in *Cel-mab-5* mutants.

The M lineage influences P8.p differentiation in at least two distinct interactions in *Ppa-mab-5* mutants

Previous cell ablation studies showed that the ectopic differentiation of P8.p in *Ppa-mab-5* mutants relies on an induction by the M lineage. After ablation of Z(1,4) in *Ppa-*

Table 2. M lineage defects in *Ppa-mab-5(tu74)* mutants

		Wild-type	*Ppa-mab-5(tu74)*
A	Early M division planes		
	1. division d/v	100%	3%
	1. division a/p	0%	97%
	2. division l/r	100%	3%
	2. division a/p	0%	97%
	n	Many	36
B	M lineage progeny 22 hours after hatching		
	7	0%	33%
	8	0%	13%
	9	0%	27%
	10	0%	20%
	14	0%	7%
	18	100%	0%
	n	Many	15
C	Number of SM cells per animal (at vulval 4-cell stage, 30 hours after hatching)		
	0	0%	98%
	1	0%	2%
	2	100%	0%
	Egg-laying defective	0%	54%
	n	Many	24
D	Number of coelomocytes per animal		
	3	0%	22%
	4	0%	66%
	5	9%	6%
	6	91%	6%
	n	11	18

Table 3. Ablation of the M lineage in *Ppa-mab-5(tu74)* mutants and its effect on the ectopic differentiation of P8.p

	Cells ablated	Time (hours after hatching)	P5.p	P6.p	P7.p	P8.p	No. animals (frequency)
A	Unablated		2°	1°	2°	D	19/25 (76%)*
			2°	1°	2°	4°	5/25 (20%)*
			3°	2°	1°	D	1/25 (4%)*
B	M	0-1	2°	1°	2°	4°	20/27 (74%)
			2°	1°	D	4°	1/27 (4%)
			2°	1°	2°	D	4/27 (15%)
			2°	1°	2°	2°	2/27 (7%)
C	M.x	7-8	2°	1°	2°	4°	20/20 (100%)
D	M.xx	12-13	2°	1°	2°	4°	8/15 (53%)
			2°	1°	2°	D	7/15 (47%)

*Data from Jungblut and Sommer, 1998. The fate designation 'D' refers to Pn.p cells that differentiate ectopically. Cells with a 'D'-fate have a 2°-type or hybrid lineage and generate between six and eight progeny.

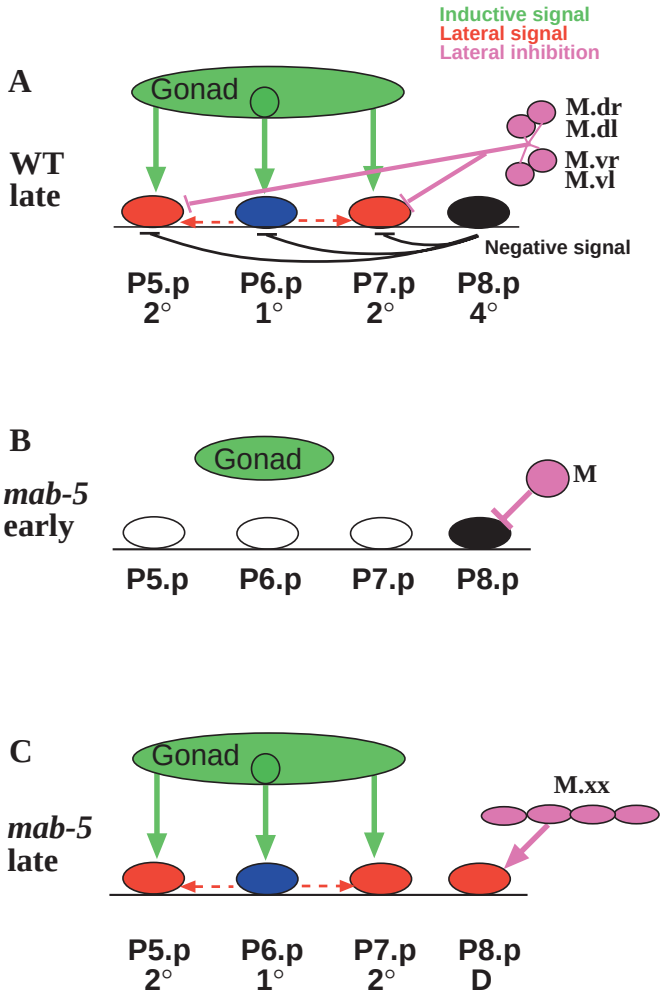

Fig. 5. Model of the cell-cell interactions between the mesodermal and the epidermal parts of the egg-laying system in *P. pacificus* wild-type (A) and *Ppa-mab-5* mutant (B,C) animals. (A) In wild-type animals, several cells of the somatic gonad induce P(5-7).p to form vulval tissue in a continuous interaction. P8.p and the descendants of M, here indicated at the level of the intermediate precursors Md/v,l/r, influence the cell fate decision of P(5,7).p upon gonadal signaling (lateral inhibition). Negative signaling by P8.p prevents the gonad-independent differentiation of P(5-7).p. (B) Cell-cell interaction during early development in *Ppa-mab-5* mutants. Early in larval development, the M cell itself inhibits the ectopic differentiation of P8.p. The inductive source for this differentiation remains unknown and might even be P8.p itself. (C) Cell-cell interactions during late development in *Ppa-mab-5* mutants. The gonad induces vulva formation, as in wild-type animals. The descendants of M induce the ectopic differentiation of P8.p. This interaction is most likely neomorphic.

mab-5 mutants, P8.p and P7.p were able to differentiate in a gonad-independent manner (Jungblut and Sommer, 1998; Jungblut and Sommer, 2000). However, the differentiation of P8.p decreased from 80% to 22% after the ablation of M at hatching in *Ppa-mab-5(tu74)* mutant animals (Table 3A,B; Jungblut and Sommer, 2000). Given the M lineage analysis in *P. pacificus* wild-type and the various M lineage defects in *Ppa-mab-5* animals, the question arises of which cells of the M lineage are involved in the ectopic induction of P8.p. To address this question, we ablated the M cell or all of its descendants at various time points in development in *Ppa-mab-5(tu74)* animals.

Ablation experiments at different time points revealed that the M lineage provides at least two signals to P8.p, an inhibitory and an inductive signal. Surprisingly, P8.p differentiation was completely abolished when M.d and M.v were ablated 7-8 hours after hatching, which is shortly after the first division of M (Table 3C). In all 20 ablated animals, P8.p had an epidermal fate, which is in contrast to the result of M ablation at hatching ($P<0.027$, χ^2-test; Table 3B,C). To

confirm our previous results (Jungblut and Sommer, 2000), we repeated the original ablation experiment and again observed a differentiation of P8.p in approximately 20% of the animals (Table 3B). These results suggest that M inhibits vulval differentiation of P8.p in the first few hours after hatching in *Ppa-mab-5* mutants.

When we ablated the descendants of M later in development, we found that they provide an inductive signal for P8.p differentiation. After ablation of the four progeny of M, 12-13 hours after hatching, P8.p differentiation was observed in approximately 50% of the animals (Table 3D). Specifically, P8.p differentiated in seven out of 15 animals ($P<0.0006$, χ^2-test; Table 3C,D). These results suggest that the misspecified M lineage in *Ppa-mab-5* mutants provides an inhibitory signal early in development and an inductive signal later in vulva development. Most likely, several of the descendants of M are involved in this induction. However, as the exact cell lineage of M is misspecified and variable in these mutants, we were unable to assign inductive activity to single cells.

DISCUSSION

The present work describes the cell lineage of the postembryonic mesoblast M and identifies complex interactions of the muscle lineages with other parts of the egg-laying system, the mesodermal gonad and the epidermal vulva, respectively. Although the blast cells from which the individual parts of the egg-laying system are derived, are clearly homologous between *P. pacificus* and *C. elegans*, cellular and molecular functions and interactions have changed during evolution (Figs 1, 5). This study provides the basis for a more comprehensive understanding of the development of the egg-laying apparatus in *P. pacificus* and the evolution of the structure between two related but distinct nematode species.

The evolution of cell fate specification and patterning mechanisms is uncoupled from the evolution of cell lineages

Comparative cell lineages analysis has been carried out for vulva development and in part also for gonad development by studying more than 50 species of seven different nematode families (Sommer and Sternberg, 1994; Sommer and Sternberg, 1995; Sommer and Sternberg, 1996; Sommer, 1997b; Félix and Sternberg, 1997; Félix and Sternberg, 1998; Félix et al., 2000). These studies indicated that during the evolution of developmental processes, cell fate specification and patterning mechanisms are uncoupled from the evolution of the underlying cell lineages. Vulval cell lineages are constant in all studied species of the Diplogastridae (the family that *P. pacificus* belongs to; Sommer, 1997b) and also between most studied species of the Cephalobina (the family that *Panagrellus redivivus* belongs to; Félix et al., 2000). In contrast, species of the Rhabditidae, with *C. elegans* as the most prominent member, differ tremendously in their vulval cell lineage, whether or not vulva position differs between species (Sommer and Sternberg, 1994; Sommer and Sternberg, 1995). Irrespective of the vulval lineages however, the interactions among cells of different tissues evolved substantially between species of the same family. The best example for this are the various cellular mechanisms underlying vulva induction by the somatic gonad (Sommer, 2000).

In this study, we provide the complete M lineage from *P. pacificus* hermaphrodites. Surprisingly, the lineage is identical to *C. elegans* hermaphrodites, which also generate 14 body muscles, two coelomocytes and a total of 16 vulval and uterine muscles. Nonetheless, some of these cells play a role during vulval fate specification, a function unknown from *C. elegans*. The observed similarity of the M lineage between *P. pacificus* and *C. elegans* is in contrast to a previous study in *Panagrellus redivivus*. Sternberg and Horvitz (Sternberg and Horvitz, 1982) showed that females of *P. redivivus* generate only eight body muscles, two coelomocytes and 12 sex muscles. The reduced number of muscle progeny results from the programmed cell death of six intermediate precursor cells (Sternberg and Horvitz, 1982). It should be noted however, that there are only quantitative differences with respect to the number of cells generated, whereas the same cell types are formed by M in all three studied species.

The mesodermal lineage influences vulval cell fates by complex interactions involving multiple cells

In *C. elegans*, interactions between different parts of the egg-laying system can occur between single cells, as in the case of vulva induction by the gonadal AC (Kimble, 1981) or between multiple cells as indicated for the role of the somatic gonad in guiding SM migration (Branda and Stern, 2000). Given the new interaction of M with the VPCs, the question arises of whether these interactions rely on single or multiple cells. We found that both major sublineages, the ventral and the dorsal M lineage, are involved in lateral inhibition of P5.p. If P(6,7).p and M.d or M.v were ablated, P5.p had a 1° fate in the majority of cases, whereas P5.p had a 2° fate after the ablation of P(6,7).p alone (Table 1). This finding allows two major conclusions. First, the elimination of one half of the system abolishes the complete interaction. Several mechanisms could account for this observation. For example, lateral inhibition might require a certain amount of a secreted substance. The ablation of M.d or M.v, could reduce the quantity of this substance below a critical threshold, as a result of which lateral inhibition no longer occurs. Second, lateral inhibition most likely acts over a distance as none of the cells of the M.d lineage is in direct contact with P(5,7).p. It remains unknown however, how many cells in each sublineage are involved in lateral inhibition. Also, the exact time point cannot be identified as with the ongoing cell divisions in the M.d and M.v lineage, the number of cells increases over time and the cell division patterns become irregular.

The finding that multiple cells of the M.d and M.v sublineage are involved in lateral inhibition is in agreement with the cellular mechanism of vulva induction in *P. pacificus*. Several cells of the somatic gonad of different sublineages are required for proper vulva induction to take place (Sigrist and Sommer, 1999).

The gonad guides the migration of the sex myoblasts

SM migration in *C. elegans* hermaphrodites is controlled by the interaction of at least three guidance mechanisms involving a gonad-dependent and a gonad-independent attraction (Stern and Horvitz, 1991; DeVore et al., 1995; Burdine et al., 1998;

Table 4. Comparison of *mab-5* mutant phenotypes in *P. pacificus* and *C. elegans*

	Ppa-mab-5	*Cel-mab-5*	*Cel-hlh-8*
M lineage defects			
Early lineage defects	>90% defects	Variable defects	Variable defects
cc vs. SM specification	No cc, no SM	No cc, extra SM	Most have cc, often extra SM
SM migration	Cells not formed	Normal	Normal
Egg-laying defect	Strong defect	No defect	Strong defect
Pn.p lineage defects			
P8.p differentiation	+++	–	–

Cel-mab-5 and *Cel-hlh-8* data (Kenyon, 1986; Corsi et al., 2000), respectively.
Only postembryonic cc (coelomocytes) are considered.

Branda and Stern, 2000). We found that the SMs cells in *P. pacificus* migrate anteriorly in gonad-ablated animals suggesting that wild-type animals contain both a gonad-dependent and a gonad-independent guidance mechanism. These experiments, however, do not indicate if a gonad-dependent repulsion also exists, as in *C. elegans*. To obtain further insight, SM migration-defective mutants have to be isolated in *P. pacificus*. In *C. elegans*, SM migration-defective mutants have been isolated as egg-laying defective mutants. Unfortunately, work described here indicates that a substantial amount of *Ppa-mab-5* mutant animals are egg-laying positive although no SM cells are generated. Thus, at least partial egg-laying can occur in *P. pacificus* in the absence of a complete egg-laying apparatus, which complicates the genetic isolation of SM-defective mutants.

Novel functions of *Ppa*-MAB-5 in mesodermal patterning

Mesodermal patterning in *P. pacificus* and *C. elegans* is specified during embryonic and postembryonic development. In recent years, several genes involved in postembryonic mesodermal patterning have been identified in *C. elegans*, involving several transcription factors: the homeodomain factor MAB-5 (Kenyon, 1986; Costa et al., 1998), HLH-8, a Twist homolog (Harfe et al., 1998), the *C. elegans* E/Daughterless homolog (Krause et al., 1997) and CEH-20, the extradenticle homolog (Liu and Fire, 2000).

Genetic and molecular studies of *P. pacificus* vulva development identified *Ppa-mab-5* as an important patterning gene, the absence of which results in ectopic vulva differentiation of P8.p (Jungblut and Sommer, 1998). In this study we show that *Ppa-mab-5* mutants have multiple defects in the M lineage resembling both the *Cel-mab-5* and the *Cel-hlh-8* genes (Table 4). For example, the division axes of M and Mx are strongly altered from wild-type in *Ppa-mab-5* mutants. The penetrance of this defect is much stronger in *Ppa-mab-5* than in *Cel-hlh-8* and *Cel-mab-5* mutants (Table 4).

With regard to later M lineage defects, *Ppa-mab-5* resembles some but not all known phenotypes of *Cel-mab-5*. For example, *Ppa-mab-5* and *Cel-mab-5* have similar coelomocyte defects, indicating that the role of MAB-5 during coelomocyte specification is conserved. In contrast, based on the strong early M lineage defect in *Ppa-mab-5* mutants, no proper SM cells are formed leading to the absence of sex muscles. In *Cel-mab-5* extra SM-like cells are observed and SM cells migrate and divide, as in wild type. As a result, *Cel-mab-5* animals are not egg-laying defective, whereas the *Ppa-mab-5* mutant was originally isolated based on its egg-laying defective phenotype (Jungblut and Sommer, 1998). Thus, the role of MAB-5 in SM specification has changed during nematode evolution. The detailed comparison of M lineage patterning in *mab-5* mutants between *P. pacificus* and *C. elegans* indicates both conservation and change of gene function. Similar results have been obtained for the homeotic gene *lin-39* and the *even-skipped* homolog *vab-7* during vulva development (Eizinger and Sommer, 1997; Sommer et al., 1998; Jungblut and Sommer, 2001).

The interaction between M and P8.p is complex and involves multiple cells

The ectopic differentiation of P8.p in *Ppa-mab-5* animals represents another novel aspect of MAB-5 function, which is not present in *Cel-mab-5*. Previous studies have indicated that the M lineage is involved in the induction of P8.p differentiation in the *Ppa-mab-5* mutant (Jungblut and Sommer, 2000). However, more detailed cell ablation studies of the M lineage at different time points during development suggest a more complex interaction between M and P8.p (Table 3). During early larval development, the M lineage provides an inhibitory signal that antagonizes P8.p differentiation, whereas later in development, M and its descendants induce P8.p differentiation in the absence of MAB-5 function (Fig. 5). It remains unknown, which tissue could be the target for the inhibition by the M lineage early in larval development. One candidate could be the gonad. To test this hypothesis, we ablated Z(1-4) and M simultaneously in *Ppa-mab-5* mutants, but observed P8.p differentiation to a similar degree as in M ablated animals alone (data not shown). Another hypothesis is, that complex interactions among the VPCs are of importance, and cell ablation studies to address this question have been initiated (M. Zheng and R. J. S., unpublished observation). Finally, it should be noted that we cannot rule out that the observed interaction between P8.p and the M lineage in *Ppa-mab-5* mutants is neomorphic and that no similar interactions exist in wild-type animals. If M or P8.p are ablated in wild-type animals, normal vulval patterning is observed as a result of the redundant nature of several overlapping specification mechanisms (Jungblut and Sommer, 2000).

Nematode developmental evolution and the *C. elegans* anchor cell

The first inductive interaction discovered in nematode postembryonic development was the induction of the vulva by the anchor cell (Kimble, 1981). Given the invariant cell lineage

and the small cell number it was a general believe that cell-cell interactions in nematodes are simple, involving only a small number of cells. However, during the last 10 years, evidence from two different research fields argue against this observation. First, the analysis of other postembryonic processes in *C. elegans* showed the involvement of multiple cells in cell-cell interactions (Newmann et al., 2000). Second, independent evidence comes from the evolutionary analysis of vulva formation. From the study of the evolution of vulva formation in more than 50 species of seven families, it is obvious that the case of the AC is specific for the genus *Caenorhabditis* (Sommer, 2000). In other nematodes, vulva development might depend on two separate inductions, on a continuous induction or might occur in a gonad-independent way. Thus, the textbook example of vulva induction by a single cell is an exception. Also, phylogenetic projection of vulval character states indicated that vulva induction by the *C. elegans* AC represents a derived character (Sommer, 2000). In summary, it seems that cell-cell interactions during postembryonic development in nematodes are mostly complex, involving multiple cells, often of different sublineages within one tissue.

We thank members of our lab for discussion and J. Srinivasan for critically reading the manuscript.

REFERENCES

Avery, L. and Horvitz, H. R. (1987). A cell that dies during wild-*type C. elegans* development can function as a neuron in a *ced-3* mutant. *Cell* **51**, 1071-1078.

Branda, C. S. and Stern, M. J. (2000). Mechanisms controlling sex myoblast migration in *Caenorhabditis elegans* hermaphrodites. *Dev. Biol.* **226**, 137-151.

Burdine, R. D., Branda, C. S. and Stern, M. J. (1998). EGL-17(FGF) expression coordinates the attraction of the migrating sex myoblasts with vulval induction in *C.elegans. Development* **125**, 1083-1093.

Chang, C., Newman, A. P. and Sternberg, P. W. (1999). Reciprocal EGF signaling back to the uterus from the induced *C. elegans* vulva coordinates morphogenesis of epithelia. *Curr. Biol*. **9**, 237-246.

Corsi, A. K., Kostas, S. A., Fire, A. and Krause, M. (2000). *Caenorhabditis elegans* twist plays an essential role in non-striated muscle development. *Development* **127**, 2041-2051.

Costa, M., Weir, M., Coulson, A., Sulston, J. and Kenyon, C. (1998). Posterior pattern formation in *C. elegans* involves posterior specific expression of a gene containing a homeobox. *Cell* **55**, 747-756.

DeVore, D. L., Horvitz, H. R. and Stern, M. (1995). An FGF receptor signaling pathway is required for the normal cell migrations of the sex myoblasts in *C.elegans* hermaphrodites. *Cell **83***, 611-620.

Eizinger, A. and Sommer, R. J. (1997). The homeotic gene *lin-39* and the evolution and nematode epidermal cell fates. *Science* **278**, 452-455.

Epstein, H. F. and Shakes, D. C. (1995). *Caenorhabditis elegans: Modern Biological Analysis of an Organism*. Methods in Cell Biology, Vol. 48 San Diego: Academic Press.

Félix, M. A. and Sternberg, P. W. (1997). Two nested gonadal inductions of the vulva in nematodes. *Development* **124**, 253-259.

Félix, M. A. and Sternberg, P. W. (1998). A gonad-derived survival signal for vulval precursor cells in two nematode species. *Curr. Biol*. **8**, 287-290.

Félix, M. A., De Ley, P., Sommer, R. J., Frisse, L., Nadler, S. A., Thomas, K., Vanfleteren, J. and Sternberg, P. W. (2000). Evolution of vulva development in the Cephalobina (Nematoda). *Dev. Biol*. **221**, 68-86.

Greenwald, I. (1997). Development of the vulva. In *C. elegans* II (ed. D. L. Riddle, T. Blumenthal, B. J. Meyer and J. R. Priess), pp. 519-542. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

Harfe, B. D., Gomes, A. V., Kenyon, C., Liu, J., Krause, M. and Fire, A. (1998). Analysis of a *Caenorhabditis elegans* twist homolog identifies conserved and divergent aspects of mesodermal patterning. *Genes Dev.* **12**, 2623-2635.

Hill, R. J. and Sternberg, P. W. (1992). The gene *lin-3* encodes an inductive signal for vulval development in *C. elegans. Nature* **358**, 470-476.

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

Kenyon, C. (1986). A gene involved in the development of the posterior body region of *C. elegans*. *Cell* **46**, 477-487.

Kimble, J. (1981). Lineage alterations after ablation of cells of the somatic gonad of Caenorhabditis elegans. *Dev. Biol.* **87**, 286-300.

Kimble, J. and Hirsh, D. (1979). Postembryonic cell lineages of the hermaphrodite and male gonads in *Caenorhabditis elegans*. *Dev. Biol.* **70**, 396-417.

Kornfeld, K. (1997). Vulva development in *Caenorhabditis elegans*. *Trends Genet*. **13**, 55-61.

Krause, M., Park, M., Zhang, J.-M., Yuan, J., Harfe, B., Xu, S.-Q., Greeenwald, I., Cole, M., Paterson, B. and Fire, A. (1997). A *C. elegans* E/Daughterless bHLH protein marks neuronal but not striated muscle development. *Development* **124**, 2179-2189.

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.

Moerman, D. G. and Fire A. (1997). Muscle: Structure, Function, and Development. In *C. elegans* II (ed. D. L. Riddle, T. Blumenthal, B. J. Meyer and J. R. Priess), pp. 417-470. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

Newman, A. P., Inoue, T., Wang, M. and Sternberg, P. W. (2000). The *Caenorhabditis elegans* heterochronic gene *lin-29* coordinates the vulval-uterine-epidermal connections. *Curr. Biol*. **10**, 1479-1488.

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. (1997a). Evolution and development – the nematode vulva as a case study. *BioEssays* **19**, 225-231.

Sommer, R. J. (1997b). Evolutionary change of developmental mechanisms in the absence of cell lineage alterations during vulva formation in the Diplogastridae. *Development* **124**, 243-251.

Sommer, R. J. (2000). Evolution of nematode development. *Curr. Opin. Genet. Dev.* **10**, 443-448.

Sommer, R. J. and Sternberg, P. W. (1994). Changes of induction and competence during the evolution of vulva development in nematodes. *Science* **265**, 114-118.

Sommer, R. J. and Sternberg, P. W. (1995). Evolution of cell lineage and pattern formation in the vulval equivalence group of rhabditid nematodes. *Dev. Biol.* **167**, 61-74.

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., Carta, L. K., Kim, S. Y. and Sternberg, P. W. (1996). Morphological, genetic and molecular description of *Pristionchus pacificus* sp. n. (Nematoda: Neodiplogastridae). *Fund. Appl. Nemat.* **19**, 511-521.

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 specifiy the vulva equivalence group and is not required during vulval induction. *Development* **125**, 3865-3873.

Sulston, J. E. and Horvitz, H. R. (1977). Postembryonic cell lineages of the nematode *Caenorhabditis elegans*. *Dev. Biol.* **56**, 110-156.

Stern, M. J. and Horvitz, H. R. (1991). A normally attractive cell interaction is repulsive in two *C. elegans* mesodermal cell migration mutants. *Development* **113**, 797-803.

Sternberg, P. W. and Horvitz, H. R. (1982). Postembryonic non-gonadal cell lineage of the nematode *Panagrellus redivivus*: Description and comparison with those of *Caenorhabditis elegans*. *Dev. Biol.* **93**, 181-205.

Sternberg, P. W. and Horvitz, H. R. (1986). Pattern formation during vulval development in *C. elegans*. *Cell* **44**, 761-772.

Thomas, J. H., Stern, M. J. and Horvitz, H. R. (1990). Cell interactions coordinate the development of the *C.elegans* egg-laying system. *Cell* **62**, 1041-1052.