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

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**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.
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 *mah-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.

**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 Sommer, 2000).
differences were observed between Nomarski microscopy. No lineage using P. pacificus the M cell in of six coelomocytes (Fig. 4G). Thus, during embryogenesis and two

P. pacificus revealed that Rhodamine dextran staining rises to 14 body muscles, two

Md/v,l/r cells divide again giving rise to 14 body muscles, two intermediate precursor cells that form body muscles in P. pacificus and C. elegans. The M lineage in P. pacificus 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

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

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<td>C P(6,7).p</td>
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<td>D P(6,7).p</td>
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*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 quadrants.

Within the next few hours, the Md vl lr 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
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 quadrant in Ppa-mab-5 mutants (Fig. 4B). This reversal of division axes in the early M divisions is rarely seen in Cel-mab-5(lef) 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...
located in the right ventral quadrant 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 were 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 misspecification 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-
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). 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, χ²-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-nab-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 ($P<0.0006$, $\chi^2$-test; Table 3C,D). These results suggest that the misspecified M lineage in Ppa-nab-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 different nematode families (Somer 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 (Somer 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. Sterngberg and Horvitz (Sterngberg 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 (Sterngberg 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 may 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;
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 gene, the absence of which results in ectopic vulva 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 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).

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

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

<table>
<thead>
<tr>
<th>M lineage defects</th>
<th><em>Ppa-mab-5</em></th>
<th><em>Cel-mab-5</em></th>
<th><em>Cel-hlh-8</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Early lineage defects</td>
<td>&gt;90% defects</td>
<td>Variable defects</td>
<td>Variable defects</td>
</tr>
<tr>
<td>cc vs. SM specification</td>
<td>No cc, no SM</td>
<td>No cc, extra SM</td>
<td>Most have cc, often extra SM</td>
</tr>
<tr>
<td>SM migration</td>
<td>Cells not formed</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>Egg-laying defect</td>
<td>Strong defect</td>
<td>No defect</td>
<td>Strong defect</td>
</tr>
</tbody>
</table>

**Pn.p lineage defects**

| | +++,-- | -- | -- |

*Cel-mab-5* and *Cel-hlh-8* data (Kenyon, 1986; Corsi et al., 2000), respectively.

Only postembryonic cc (coelomocytes) are considered.
and the small cell number it was a general belief 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.

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REFERENCES


