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Different roads to form the same gut in nematodes

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SUMMARY The morphogenesis of a gut from the endoderm has been well studied among the animal kingdom and is also well described in the nematode *Caenorhabditis elegans*. But are there other ways to build a nematode intestine? Sulston et al. (1983) described a different intestinal cell lineage in the species *Panagrellus redivivus* and *Turbatrix aceti* that includes two programmed cell deaths. However, no details are known about the three-dimensional (3D) configuration and the role of the cell deaths. Here, we describe the intestinal morphogenesis of *P. redivivus* and five other nematode species by means of four-dimensional microscopy, which gives us a 3D representation of gut formation at the cellular level. The morphological pathway of gut formation is highly conserved among these distantly related species. However, we found the *P. redivivus* pattern in another related species *Halicephalobus gingivalis*. In this pattern, the intestinal precursors migrate inward in concert with the mesoderm precursors. Based on the observations, we propose a hypothesis that could explain the differences. The positions of the mesoderm precursors create a possible spatial constraint, by which the establishment of bilateral symmetry in the intestine is delayed. This symmetry is corrected by cell migrations; other cells are eliminated and compensated by supplementary cell divisions. This pattern leads to the same result as in the other nematodes: a bilateral symmetrical intestine with nine rings. This illustrates how conserved body plans can be achieved by different developmental mechanisms.

INTRODUCTION

The formation of an epithelial gut tube from the endodermal germ layer has been well studied (reviewed by Stainier 2005). These studies revealed that the transcriptional regulators of endoderm formation are well conserved in the animal kingdom (Gata, Forkhead families), but that different signaling pathways regulate the endoderm induction between cells (Nodal in vertebrates and Wnt in ascidians, sea urchins, and *Caenorhabditis elegans*).

In the model organism *C. elegans*, the endoderm arises from a single progenitor in the eight-cell stage: the E-cell (Sulston et al. 1983). A simple epithelial tube, which consists of only 20 cells, is formed out of this E-cell and its morphogenesis is well described (Sulston et al. 1983; Leung et al. 1999). The induction of the endoderm in the four-cell stage between the two sister cells EMS and P2 was one of the first inductions demonstrated in the development of *C. elegans* (Schierenberg 1987; Goldstein 1992, 1993, 1995) in what was considered a mosaic development with specification of cells by differential segregation of developmental potential (Laufer et al. 1980; Sulston et al. 1983).

But is the endoderm formation of *C. elegans* also a good model for the other members of the phylum nematoda? Bolker (1995) mentioned that the development of model systems is biased by the way they are chosen (particularly for their ease of culture under laboratory conditions) and that these model systems have a rapid, highly canalized development. Recent research shows that there is much more diversity regarding developmental mechanisms in other nematode species than previously appreciated (Sommer and Sternberg 1996a; Félix 1999; Fitch 2000; Borgonie et al. 2000; Schierenberg 2000). A study of Wiegner and Schierenberg (1998) revealed a different specification mechanism of the endoderm in the nematode *Acrobeloides nanus*.

Is the morphogenesis of the *C. elegans* intestine conserved throughout the phylum? Or are there other ways to form an intestine and how are they related to each other in evolution? Little is known about intestinal morphogenesis in nematodes other than *C. elegans*. From the embryonic cell lineage of the nematode *Pellioditis marina*, which is closely related to *C. elegans*, it is clear that these two nematodes have the same mode of intestinal morphogenesis (Houthoofd et al. 2003). Sulston et al. (1983) described a different intestinal cell lineage in two species of the family Panagrolaimidae, *Panagrellus redivivus* and *Turbatrix aceti*, in which 18 intestinal cells are formed and two precursors undergo cell death. However, a detailed description of the organogenesis of the intestine of *P. redivivus* and *T. aceti* has not yet been described. So to what extent are the differences in E-lineage translated into

differences in spatial organization? Is this intestinal pattern common for the family of the Panagrolaimidae or higher taxa or is there a high diversity in intestinal patterns in nematodes? How do these intestinal patterns relate to each other, which intestinal pattern is the ancestral one, and which one is the derived one?

To address these questions, we describe the spatial and temporal organogenesis of the embryonic intestine of *P. redivivus* and three other members of the Panagrolaimidae and two nematode species from two adjacent families in clade IV based on the molecular phylogeny of Blaxter et al. (1998): *Rhabditophanes* sp. (Alloionematidae), sister taxon of the parasitic genus *Strongyloides* (Dorris et al. 2002), and *Cephalobus cubaensis* (Cephalobidae). Based on four-dimensional (4D) microscopy recordings, we followed the intestinal precursor cells in all these species through space and time, established their complete division pattern, and made three-dimensional (3D) reconstructions at regular time points of the intestinal morphogenesis. This gives us a powerful tool to unravel evolutionary changes at the cellular level.

MATERIALS AND METHODS

Cultures

The soil nematodes, *Panagrobelus stammeri* PDL0024, *P. redivivus* PS1163, *Halicephalobus gingivalis* JB128, *Panagrolaimus rigidus* AF36, *Rhabditophanes* sp. KR3021, and *C. cubaensis* PS1197, are cultured on 1% agar plates with *Escherichia coli* OP50 as a food source. Culture and handling are as described by Brenner (1974).

4D microscopy and cell lineage analysis

Early-stage embryos from *Rhabditophanes,* where the embryos develop within the adult, are obtained by cutting gravid females in distilled water. Embryos from the other species, which lay their eggs in the one-cell stage, are collected by washing the eggs of the agar plate with distilled water. One-cell embryos are selected under a dissecting microscope, mounted on a 5% agar pad, covered with a coverslip, and sealed with vaseline (Sulston and Horvitz 1977).

The cell lineage of the embryo is established using 4D microscopy described in detail by Schnabel et al. (1997) and Houthoofd et al. (2003). The recordings are analyzed with the software program Simi Biocell (Simi GmbH, D-85705 Unterschleissheim, Germany) (Schnabel et al. 1997). The embryonic cell lineage is established by identifying all cells and cell divisions in space and time. By establishing the positions of all the nuclei of the cells, 3D reconstructions of the embryo are made, where the positions of the nuclei are displayed in 3Ds and can be rotated in all directions. With these reconstructions, the spatial configuration of tissues is reconstructed and cell migrations are followed.

The cells are named according to Sulston and Horvitz (1977), Deppe et al. (1978), and adapted by Sulston et al. (1983). Here, we repeat briefly the nomenclature for better readability of the study. Founder cells formed in the first division rounds are given names in capital letters according to Boveri (1899) and Deppe et al. (1978). When a founder cell divides, each daughter is named by adding to the name of the mother cell a single lowercase letter representing its position immediately after division relative to its sister cell. For divisions in the anterior–posterior direction, the anterior and posterior daughters are indicated, respectively, with an "a" and "p," dorso-ventral divisions are indicated with "d" and "v," left–right divisions are indicated with "l" and "r." For example, when founder cell E divides in the anterior–posterior direction, the daughters are named Ea and Ep and when Ep divides in the left–right direction its daughters are named Epl and Epr. A pair of cells may be designated by the use of internal parentheses, for example, Ea(l/r)aa means Ealaa and Earaa. In the cell lineage tree the "a," "d," and "l" daughters are represented by the left branches, and the "p," "v," and "r" daughters by right branches.

Phylogenetic analysis

The developmental data were mapped on a molecular phylogeny based on 18S rRNA sequences of the species treated in this study, all available in GenBank under accession numbers: *C. elegans* (X03680), *P. marina* (AF083021), *C. cubaensis* (AF202161), *Rhabditophanes* sp. (AF202151), *P. stammeri* (AF202153), *P. redivivus* (AF083007), *T. aceti* (AF202165), and *H. gingivalis* (AF202156). The sequence of 18S rRNA of *P. rigidus* was sequenced for this study and is deposited in GenBank under accession number Q285636. *Plectus aquatilis* (AF036602) and *Mononchus truncatus* (AY284762) were used as out-groups. The sequences were aligned using the program Clustal X under default settings (Thompson et al. 1997, version 1.64). Preliminary analysis demonstrated striking branch length differences within our data set. As a consequence, only model-based methods were implemented in our analyses as they incorporate better substitution bias (Swofford et al. 2001). The software Modeltest (Posada and Crandall 1998, version 3.06) was used to determine the best-fit maximum likelihood models; this was the general time-reversible model (GTR+I+G). The parameters for base frequencies, substitution rate matrix, and shape and proportion of invariant sites were allowed to vary throughout the Bayesian analysis. The total number of generations in this analysis was set to 1 million, 300 times greater than the burn-in value. Four parallel chains (one cold and three heated) were used. Trees were sampled every 100 generations. The burn-in value was set to 10,000 generations, which equated to the next 3000 generations above the level at which the log likelihood reaches a stable value in a preliminary run. Majority-rule consensus trees were reconstructed from the fundamental trees. Phylogenies were estimated under maximum likelihood criteria as implemented in PAUP (Swofford 1998, version 4b10) with bootstrap support from 100 replicates, and using Bayesian inference as implemented in MrBayes (Huelsenbeck and Ronquist 2001, version 3.0b4).

RESULTS

In all the nematode species studied so far, the intestinal cells are exclusively derived from the founder cell E, which does not give rise to any other tissue. The daughter cells Ea and Ep enter the body ventrally during gastrulation, except for *Rhabditophanes* sp., where gastrulation occurs in the 4E cell stage. The bilateral symmetry of the intestine in all the studied

species is established in two different ways, giving rise to two distinct patterns, named after the species where the pattern was first described (Table 1). In *H. gingivalis*, the E-lineage is identical to the one of *P. redivivus* (Sulston et al. 1983) (Figs. 1A, 2, and 3A–F, Supplementary Animation S-1A). Eighteen intestinal cells are formed from the E blastomere and two precursors undergo programmed cell death. The 3D cellular patterning during organogenesis of the intestine of *P. redivivus* and *H. gingivalis* has not been described before and will be called the *P. redivivus* pattern. In *Rhabditophanes* sp., *C. cubaensis*, *P. rigidus*, and *P. stammeri*, the embryonic intestine consists of 20 cells according to a pattern like in *C. elegans* (Sulston et al. 1983) and *P. marina* (Houthoofd et al. 2003) and will be called the *C. elegans* pattern (Figs. 1B and 3, G–K, Supplementary Animation S-1B).

Two distinct patterns lead to the same result

In the *P. redivivus* pattern (Figs. 2 and 3 A–F), the two daughters of E, Ea, and Ep, divide in perpendicular division planes: Ea divides anteroposteriorly, and Ep divides left–right (Figs. 2B and 3B). In the next division round, the two daughters of Ea, Eaa, and Eap, divide asymmetrically in an anteroposterior plane (Figs. 2C and 3C). The two smaller posterior daughters Eaap and Eapp undergo programmed cell death. The larger anterior daughters migrate, respectively, to the left and right sides of the intestinal primordium (Figs. 2D and 3D). In this way, a bilateral symmetric gut primordium of six cells arises. In the next division round, all remaining cells divide anteroposteriorly to form a 12-cell primordium of six pairs of cells (Figs. 2E and 3E). The cells of the first, fifth, and sixth pair divide once more to form three additional pairs. Each pair of cells will undergo morphogenesis to form a ring in the hollow tube. So, the *P. redivivus* intestine consists of 18 cells in two rows and nine rings (Figs. 2F and 3F).

Table 1. List of studied species grouped per family and their intestinal pattern like in *Panagrellus redivivus* (P) or in *Caenorhabditis elegans* (C)

Family	Species	Pattern	Remarks
Panagrolaimidae	*Panagrobelus stammeri*	C	
	Panagrellus redivivus	P	
	Halicephalobus gingivalis	P	
	Panagrolaimus rigidus	C	
Alloionematidae	*Rhabditophanes* sp.	C	Gastrulation at 4E cell stage Ep(l/r)a divides instead of Ep(l/r)p
Cephalobidae	*Cephalobus cubaensis*	C	
Rhabditidae	*Pellioditis marina*	C	No rotation of anterior rings
	Caenorhabditis elegans	C	

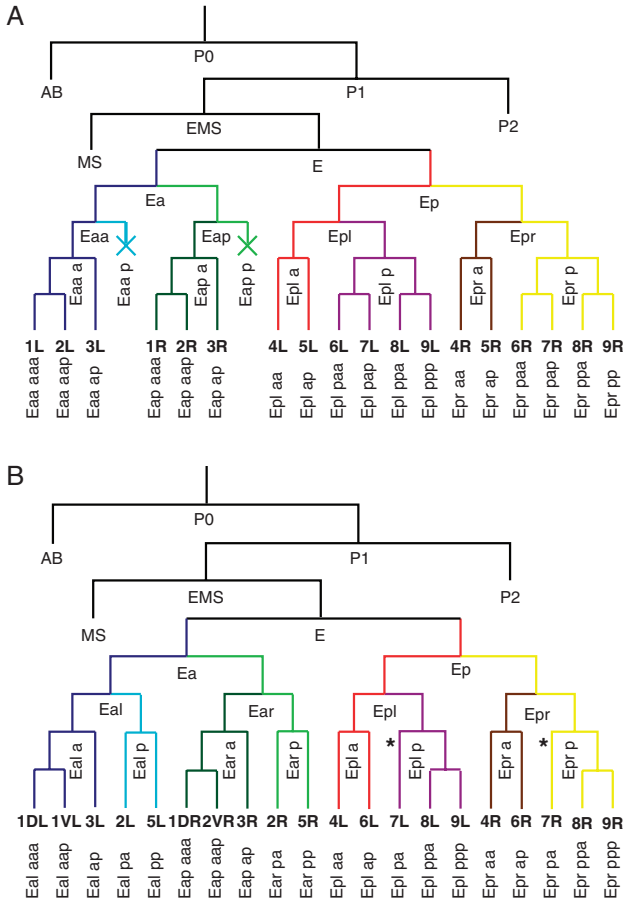

Fig. 1. Schematic reconstruction of the embryonic cell lineage of the intestinal precursor cell E in (A) *Panagrellus redivivus* and *Halicephalobus gingivalis* and in (B) *Caenorhabditis elegans*, *Cephalobus cubaensis*, *Panagrobelus stammeri*, and *Panagrolaimus rigidus*. The left branch is anterior (a) or left (l) daughter; the right branch is posterior (p) or right (r) daughter. X, programmed cell death. Numbers mark the subsequent intestinal rings in the embryonic intestine. L, left cell; R, right cell of the ring. The asterisks mark the cells Ep(l/r)pa that divide in *Rhabditophanes* sp. instead of their posterior daughter cells Ep(l/r)pp.

The organogenesis of the intestine of *Rhabditophanes* sp., *C. cubaensis*, *P. rigidus*, and *P. stammeri* follows the *C. elegans* pattern (Table 1, Fig. 3, G–K). The two daughter cells Ea and Ep divide left–right (Fig. 3H) and thereby establish the bilateral symmetry of the primordial intestine: Eal and Epl form the left row, and Ear and Epr the right row. After the next two division rounds the intestinal tube is elongated and consists of two rows of eight cells (Fig. 3, I and J). Ealp and Earp (respectively, the light blue and light green cells in Fig. 3I) lie more ventrally according to the other intestinal precursors. Their anterior daughters Ea(l/r)pa migrate between Ea(l/r)aa and Ea(l/r)ap and its posterior daughter Ea(l/r)pp between Ep(l/r)aa and Ep(l/r)ap (Fig. 3, J and K). The first pair of cells divides dorso-ventrally to form a ring of

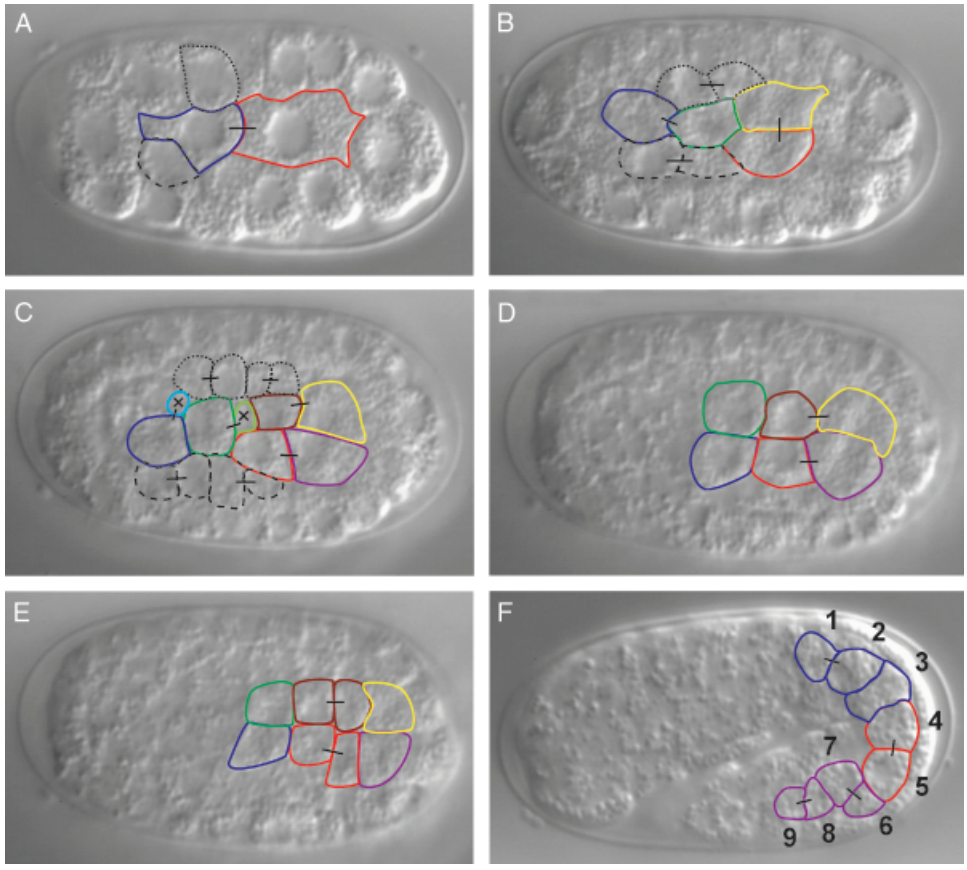

Fig. 2. DIC photographs of the subsequent stages of the *Panagrellus redivivus* pattern of intestinal development in the *Halicephalobus gingivalis* embryo. Ventral view, anterior to the left; except F: left-lateral view. Cells are outlined according to colors in Fig. 1. Black, MSap descendants; gray, MSpp descendants. Visible nuclei are marked with dashed circles. Time (*t*) is indicated as minutes after first division; optical section (*l*) as a number from 01 to 25, with 01 as the most ventral level and 25 the most dorsal level. (A) 2E stage ($t = 113$ min; level = 04); note that Ea (blue) is positioned in between MSap (black) and MSpp (gray). (B) 4E stage ($t = 160$ min; level = 07); here, Eaa and Eap are positioned in between MSap and MSpp daughters. (C) 8E stage ($t = 270$ min; level = 12). (D) 6E-stage ($t = 360$ min; level = 14); Bilateral symmetry is established by migration of Eaaa (blue to the left side of the primordium). (E) 12E stage ($t = 430$ min; level = 15); second pair Ea(a/p)ap and last pair Ep(l/r)pp lie more ventrally than other cells and are not visible in this focal plane. (F) 18E-stage ($t = 635$ min; level = 08), left lateral view of comma stage embryo; nine subsequent rings are numbered.

four cells, connecting the intestine with the pharynx (Fig. 3K). The last pair of cells Ep(l/r)pp divide once more to form a ninth row of intestinal cells that make contact with the rectum (Fig. 3K). In *Rhabditophanes* sp., it is the last but one pair of cells Ep(l/r)a that divides to form a seventh and eighth ring. Eventually, in all the studied species, the embryonic intestine consists of 18–20 cells in two rows and nine rings.

The embryonic intestine of *C. cubaensis*, *P. rigidus*, and *P. stammeri* is formed completely identically as in the embryos of *C. elegans* (Sulston et al. 1983, Leung et al. 1999) and *P. marina* (Houthoofd et al. 2003). The cell lineage of E is identical; also, the division axes of every division and the 3D configuration are the same. For example, in those three species, Eal and Ear divide obliquely like in *C. elegans*, so that the posterior daughters lie more ventrally and that their daughters migrate between the other intestinal cells. The intestine of *P. marina* is built identically as in the *C. elegans* embryo, except for the orientation of the second to the fourth ring (Houthoofd et al. 2003). In *C. elegans*, those rings rotate 90° counter clockwise immediately after the division of the first pair of cells and before attachment to the pharyngo-intestinal valves. This twist is not observed in the analogous elongation period until the start of muscle contraction in the *P. marina* embryo.

The most important difference between the two patterns is the establishment of the bilateral symmetry in the primordial intestine. In the *C. elegans* pattern, this symmetry is established early in the 4E cell stage (Fig. 3H) by the left–right division of the two daughters of E, Ea, and Ep. From this stage on, Ea(l/r) and Ep(l/r) have an identical division pattern. In the *P. redivivus* pattern, the bilateral symmetry is established later after the third division round of E when Eaaa migrates to the left side of the primordial intestine (Figs. 2D and 3D).

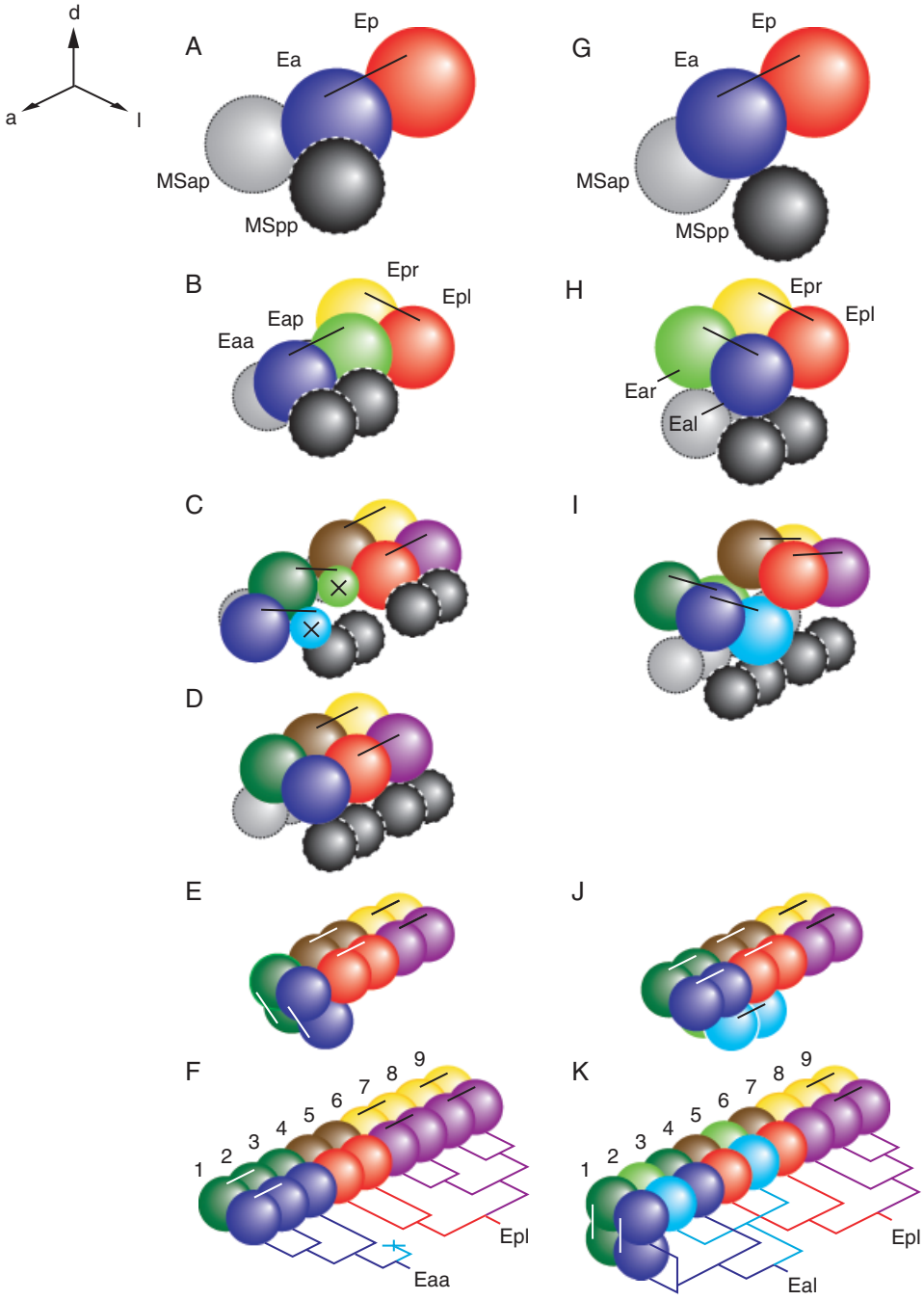

Fig. 3. Schematic three-dimensional (3D) reconstruction of the development of the embryonic intestine in *Panagrellus redivivus* (A–F) and of *Caenorhabditis elegans* (G–K). Dorsal lateral view from the left. Spheres represent the individual cells, based on the 3D positions marked during lineage analysis. Colored cells are E descendants, colored according to the cell lineages shown in Fig. 1. Black, MSap descendants; gray, MSpp descendants. The lines in 3D reconstructions connect daughter cells. In F and K, the nine rings in the intestine are numbered with their corresponding lineage, shown in Fig. 1. **Animation 1** (available at: http: www.nematology.ugent.be)**.** Time-lapse animation of gastrulation and intestinal morphogenesis from the 2E stage until muscle contraction. Animations are based on the three-dimensional reconstructions of the nuclei of the embryo. Left lateral view. Colored cells are E descendants, colored according to the cell lineages shown in Fig. 1. Black, MSap descendants; gray: MSpp descendants. Other cells are colored in white in the embryo, to emphasize the size of the embryo. Lines connect daughter cells after cell division. At the end of the animation, the nine rings in the intestine are numbered with their corresponding lineage in Fig 1. Time in the left upper corner is the time after the first division of the zygote. (A) *Panagrellus redivivus*. Note that Ea and its descendants remain in between MSap (black) and MSpp (gray) during gastrulation. Eaap and Eapp undergo programmed cell death (marked with+) and Eaaa migrates to the left side to restore the bilateral symmetry (marked with arrow). (B) *Panagrobelus stammeri*, which has a *Caenorhabditis elegans* intestinal pattern. Here, Ea migrates inwards before MSap and MSpp and divides left–right to establish early bilateral symmetry in the intestine. The daughters of Ealp (light blue) and Earp (light green) are situated more ventrally and migrate between the other cells to form the second and fifth intestinal ring (marked by arrows).

Differences in *P. redivivus* and *C. elegans* patterns are correlated with the timing of gastrulation of mesodermal precursors

The difference in establishment of bilateral symmetry between the *P. redivivus* and *C. elegans* pattern is correlated with the timing of gastrulation of the two daughters of E in relation to the MS descendants MSap and MSpp (mainly mesoderm precursors). In the *C. elegans* pattern, Ea and Ep migrate inward earlier than MSap and MSpp. Ea and Ep are therefore positioned more dorsally than the MS descendants during their division in left–right orientation (Fig. 3G). Similarly,

during their subsequent division, the Ea daughters are positioned dorsally of the MSap and MSpp descendants in the *C. elegans* pattern (Fig. 3H). In contrast, in the *P. redivivus* pattern, Ea and Ep migrate inwards together with MSap and MSpp, so Ea is positioned between MSap and MSpp during its anterior–posterior division (Figs. 2A and 3A). The daughter cells Eaa and Eap are still positioned between the MSap and MSpp descendants during their gastrulation (Figs. 2B and 3B). After the subsequent division round, the daughters of Eaa and Eap lie more dorsally than the mesodermal precursors, derived from MSap and MSpp (Figs. 2C and 3C). The bilateral symmetry is re-established by the anterior daughters with the migration of Eaaa to the left side (Figs. 2D and 3D). The posterior daughters Ea(a/p)p undergo apoptosis.

Which pattern is the ancestral state within clade IV?

In order to reconstruct the evolutionary history of these two patterns, we calculated a molecular phylogeny of the studied species based on 18S rDNA sequences and plotted these two patterns on this phylogeny (Fig. 4). The results indicate that the *C. elegans* pattern is the ancestral state for clade IV and that the *P. redivivus* pattern has evolved from the *C. elegans* pattern. Secondly, the *P. redivivus* pattern has evolved within the family Panagrolaimidae. According to the 18S rDNA sequences, the *P. redivivus* pattern is a homoplastic trait within the clade (*P. redivivus*, *P. rigidus; H. gingivalis, T. aceti*), as the *P. rigidus* intestine has the *C. elegans* pattern. Based on the relationships in this phylogeny, there are two possible explanations. Either the *P. redivivus* pattern arose twice independently in *P. redivivus* and the (*H. gingivalis*, *T. aceti*) clade, or there was a reversal in *P. rigidus* back to the ancestral *C. elegans* pattern. Another possibility would be that the homoplasy of this trait plotted by this phylogeny is caused by homoplasy in the molecular characters of the 18S rDNA sequences that were used to reconstruct the phylogeny. This possibility can be tested in the future with different genes.

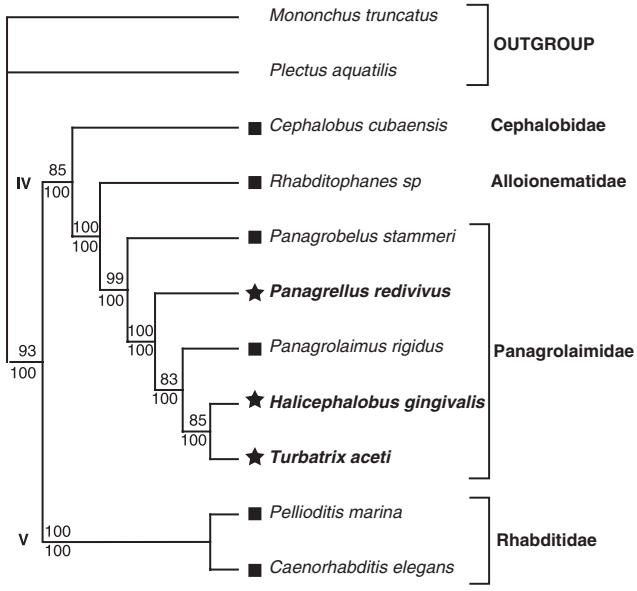

Fig. 4. The species with known intestinal lineage and three-dimensional arrangement were mapped onto a molecular phylogeny based on aligned 18S rDNA. Summary cladogram obtained from maximum likelihood (ML) and Bayesian analysis. Branch support indicated by bootstrap values (calculated by 100 replicates) for ML (above branch), and Bayesian probability values (below branch), all expressed as percentage. Species with the *Caenorhabditis elegans* pattern are indicated with a square; species with the *Panagrellus redivivus* intestinal pattern are indicated with a star. Out-group: *Plectus aquatilis* and *Mononchus truncatus*. Corresponding families are to the right. Roman numbers IV and V indicate clades according to the classification of Blaxter et al. (1998).

DISCUSSION

Position of the mesoderm precursors may constrain the establishment of bilateral symmetry in the *P. redivivus* pattern

According to the phylogenetic analysis, a new variant of gut formation evolved within the family of the Panagrolaimidae. However, these two distinct patterns lead to the same result: an embryonic bilateral symmetric intestine, built up by nine consecutive rings.

Here, we propose a hypothesis based on the observations made in this study that can explain the difference between the two intestinal patterns. The establishment of bilateral symmetry in the Ea lineage in the *P. redivivus* pattern is possibly constrained by the position of the MSap and MSpp descendants during their concerted gastrulation. As Ea is positioned in between MSap and MSpp during its gastrulation (Figs. 2A and 3A), no left–right division of Ea would be possible. This is in contrast with its posterior daughter Ep, which has no extra neighbors, but the lateral AB cells, during its gastrulation. For the same reason, the bilateral symmetry could be established secondarily by migration of the daughters to the left and right position or by a subsequent left–right division of the daughters. Instead, Eaa and Eap divide once more asymmetrically in the anterior–posterior direction (Figs. 2C and 3C). The bilateral symmetry is established by migration of the anterior daughter of Eaa, Eaaa, to the left side of the embryo, when the E descendants have reached a more dorsal position than the MS descendants (Figs. 2D and 3D). In the *C. elegans* pattern, Ea migrates inward before MSap and MSpp, so there would be no such constraint of MSap and MSpp on the division of Ea. Ea is positioned dorsally of the MS descendants and therefore can divide left–right to establish bilateral symmetry in the four-cell intestinal primordium (Fig. 3H).

However, one cannot exclude another possibility: that an intrinsic change in the lineage and division axes of the intes-

tinal precursors is the cause of the change in the spatial configuration of the embryonic intestine. A more experimental setup could elucidate this problem. For example, one can test the possible spatial constraint of the MSap, and MSpp and their descendants on the development of the intestine, by killing the precursor cells MSa and MSp in a species with the *P. redivivus* pattern with laser ablation. If after ablation of MSa and MSp, the lineage of E transforms again into the *C. elegans* pattern, then MSap and MSpp are the cause of the transformation in the intestinal pattern. If no change in the intestinal pattern is observed, then there is no spatial constraint of MSap and MSpp that must be an intrinsic cue in the E-lineage.

Programmed cell deaths are indications of a reprogramming of the intestinal cell lineage

Also, the occurrence of the cell deaths in the *P. redivivus* pattern can be explained by our hypothesis. The two subsequent anteroposterior divisions in the Ea lineage result in a row of four anteroposteriorly orientated cells. The asymmetrical division and subsequent cell deaths of the posterior daughters of Eaa and Eap probably prevent the primordial intestine from becoming too long, and may facilitate the secondary establishment of symmetry. The loss of Ea(a/p)p, which produces four cells or two rings in the *C. elegans* pattern, is compensated by additional cell divisions of Ep(l/r)pa, and the anteroposterior orientation of the division of Ea(a/p)aa.

The use of programmed cell deaths in reprogramming cell lineages of nematodes is widely spread. For example, cell deaths are used to refine stereotypical sublineages that are used repeatedly, for example in the ventral nerve cord of *C. elegans* (Sulston and Horvitz, 1977, White et al. 1986) and vulval cell lineages in *P. pacificus* (Sommer and Sternberg 1996b). In nematodes with a monodelphic female gonad (only one anterior arm), the monodelphy results from the programmed cell death of one cell, the posterior distal tip cell, that induces the growth of the posterior gonadal arm in didelphic species (Sternberg and Horvitz 1981, Félix and Sternberg, 1996).

Evolutionary modification that leads to the same result

Gould (2002) mentioned that oddities or imperfections, making no sense as an optimal design in a current context, could be explained as holdovers from the past state. He makes the analogy with letters in a word that are still retained in the spelling, but become useless in the pronunciation. These can serve as a clue in seeking for its derivation. Here, the cell deaths in the intestinal cell lineages of *P. redivivus* and *H. gingivalis* can be considered as developmental anomalies and betray evolutionary signs from the past, more precisely the modification of the establishment of the bilateral symmetry in the intestine. Why are these cells generated and discarded immediately after their birth? Another solution would be discarding the preceding cell division. However, these cell divisions and generation of these cells are retained in evolution, but become useless in the development of the intestine.

The evolutionary modification of the intestinal lineage, however, has no effect on the ultimate design of the embryonic intestine, a bilateral symmetric intestine of nine rings. Other examples of how developmental mechanisms can evolve without affecting other aspects of development have already been described in nematodes and other animals. Goldstein et al. (1998) showed that a new axis specification mechanism has evolved in the ancestors of relatives of *C. elegans* without affecting further development. Comparative analysis showed that the fate specification of early blastomeres in different nematodes varies considerably without influencing the resultant structure of the nematode (Schierenberg 2001). Another class of examples is the modification of larval growth in closely related species of sea urchins without affecting the adult morphology (Wray 1994). This can be found throughout the animal kingdom, in sea urchins, molluscs, ascidia, and vertebrates (reviewed in Raff 1996).

This article is focused on the intestinal development of species from clades IV and V according to the molecular phylogeny of Blaxter et al. (1998). Observations of the early development of species from clades I and II also indicate the presence of a single intestinal precursor cell (Malakhov 1994, Voronov and Panchin 1998). Despite this generality in nematodes, important differences indicate different specification mechanisms of this single precursor. In species of clade I, this precursor cell arises from the anterior blastomere in the two-cell stage, instead of the posterior cell in clades IV and V (Malakhov 1994). In *Enoplus brevis* (Enoplidae, clade II), a single intestinal precursor arises in the eight-cell stage in an otherwise indeterminate cleavage pattern where the other cells remain undetermined (Voronov and Panchin 1998). And Schierenberg (2005) observed a different mode of gastrulation in *Tobrilus diversipapillatus* (Tobrilidae, clade II), where a large blastocoel is formed and cells migrate into this cavity after the 64-cell stage. Further investigations into the developmental mechanisms of species in these clades would help us understand fully the diversity of the developmental mechanisms of the nematode intestine and more experimental studies, like cell ablation experiments, would enable us to learn more about how these mechanisms work.

This study illustrates that the detailed description of developmental events in nonmodel organisms can help to understand the diversity of those events and how they have evolved.

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