CHAPTER 14

Many roads lead to Rome: different ways to construct a nematode

Einhard Schierenberg and Jens Schulze

It has been well established that considerable differences exist in the developmental pattern among animal taxa, for instance with respect to how blastomeres perform their early cleavages, how they acquire different fates or how symmetry is formed (Gilbert and Raunio 1997). Even among relatively closely related species, for instance within sea urchins or tunicates, impressive differences can be found in the pattern of development (Jeffery *et al*. 1999, Raff 1999).

Nematodes appear to be excellent candidates for a comparative study of early embryogenesis (Schierenberg 2005a). The phylum Nematoda is very old, its origin dating back to the Cambrian (Douzery *et al*. 2004), and has many different species (estimates range from tens of thousands to several millions); eggs can develop outside the mother from the first cleavage onward, they are transparent (although to a variable degree), the freshly hatched juveniles appear to have essentially invariant species-specific cell numbers of around 600 cells (for those species tested so far), many strains can be cultured in the laboratory on simple agar plates, and, last but not least, one of them, *Caenorhabditis elegans*, has become one of the best-studied model systems.

In this chapter, selected aspects of the early embryogenesis of five representatives from different branches of the phylogenetic tree are compared with *C. elegans* and the impact of the observed differences for evolutionary considerations are discussed. Following a brief reference to phylogeny, basic features of early embryogenesis of *C. elegans* will be summarised to aid in appreciating the data from other nematodes reported subsequently.

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NEMATODE PHYLOGENY

Based mainly on molecular sequence data, a modern nematode phylogeny was suggested by Blaxter *et al.* (1998), extended and modified by De Ley and Blaxter (2002), with five clades in three subclasses. Recently, from a larger set of species, 339 nearly full-length small-subunit rDNA sequences were analysed and revealed a backbone of 12 consecutive dichotomies that subdivide the phylum Nematoda into 12 clades (Holterman *et al.* 2006; Figure 14.1). The clade numbers used below refer to this work.

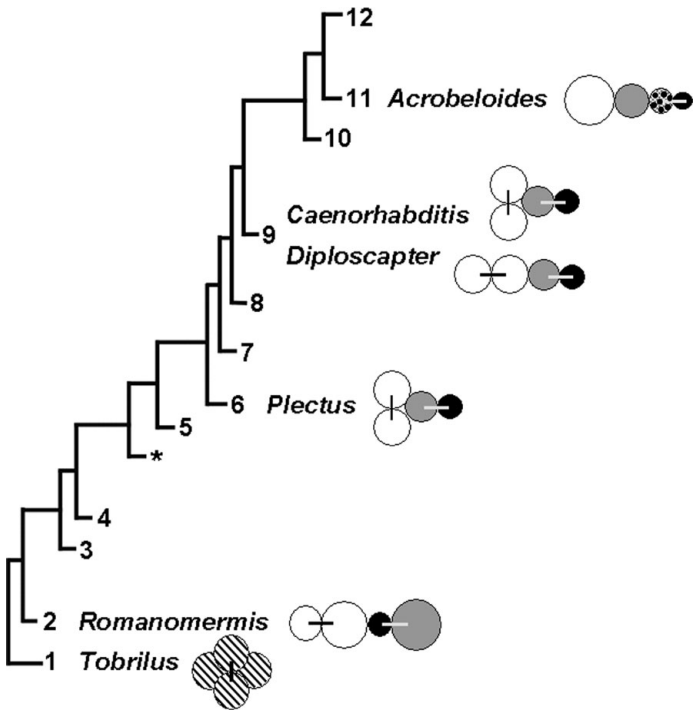

Figure 14.1 Simplified phylogenetic tree of nematodes. The tree is subdivided into 12 clades (1–12) and one unresolved branch (*) based primarily on DNA sequence data (Holterman *et al.* 2006). Branch lengths reflect substitution rates. Affiliations of the six representatives discussed here to individual clades and blastomere arrangements in four-cell stages are shown. The latter illustrate primary cell positions resulting from the orientation of cleavage spindles. Because of constraints of the egg envelope, rearrangements lead to a diamond-shaped pattern in *Caenorhabditis*, *Plectus* and *Romanomermis*. White, AB (S_1 in *Romanomermis*; for nomenclature, see legend to Figure 14.6) or AB daughters; grey, EMS (S_2); dotted, C (S_3); black, germ line (P_2; in *Acrobeloides*, P_3); striped, apparently equal cells of unknown fate. Connecting lines, sister cells.

CAENORHABDITIS ELEGANS EMBRYOGENESIS: THE REFERENCE SYSTEM

Caenorhabditis elegans is a small (about 1 mm long) hermaphroditic soil nematode, which can be easily cultured in the laboratory on agar plates. Development from first cleavage to hatching is very rapid (12 h at 25□) and eggs (size *c.* 55 × 35 μm) are remarkably transparent. The fact that rare males occur that can be mated to the hermaphrodites (male sperm is used preferentially) makes *C. elegans* a particularly amenable system for developmental geneticists (Brenner 1974). A number of scientific milestones have been reached with *C. elegans*. It was the first metazoan whose genome was completely sequenced (The *C. elegans* Genome Consortium 1998); the complete wiring diagram of the nervous system has been described (White *et al.* 1986); ground-breaking methods like gene silencing with RNAi (Fire *et al.* 1998) and visualisation of gene expression in vivo with the GFP technique (Chalfie *et al.* 1994) were originally established in this system; and, finally, cell lineages of all 558 cells present at hatching have been documented (Sulston *et al.* 1983).

Figure 14.2A depicts the generation of five somatic founder cells via a series of unequal cleavages in the germ line and fates of their decendants. Upon fertilisation, immediately after fusion of the two pronuclei, the zygote divides into two unequal cells, a larger, anterior somatic cell AB and a smaller, posterior germline cell P_1. The AB cell divides with a transverse spindle orientation into ABa and ABp (Figure 14.2A). Both AB blastomeres are initially equipotent but nevertheless execute different developmental programs owing to inductive signals that they (and at least some of their descendants) receive from neighbouring cells (see below). The P_1 cell cleaves with a longitudinal spindle orientation unequally into a somatic cell EMS and a new germline cell P_2 (Figure 14.2B). Further unequal divisions of P_2 and its daughter P_3 generate the somatic founder cells C and D, respectively. Soon after the division of P_3, leading to the 24-cell stage, the two daughters of the gut precursor E initiate gastrulation by moving into the interior of the embryo. This important process will be considered in more detail at the end of this chapter.

From this brief synopsis the central role of the germ line with its stem-cell-like character from the first division of the embryo onward becomes obvious, leading to the stepwise generation of somatic founder cells. Germline cells contain specific cytoplasmic granules ('P granules') which can be visualized with antibodies (Strome and Wood 1983).

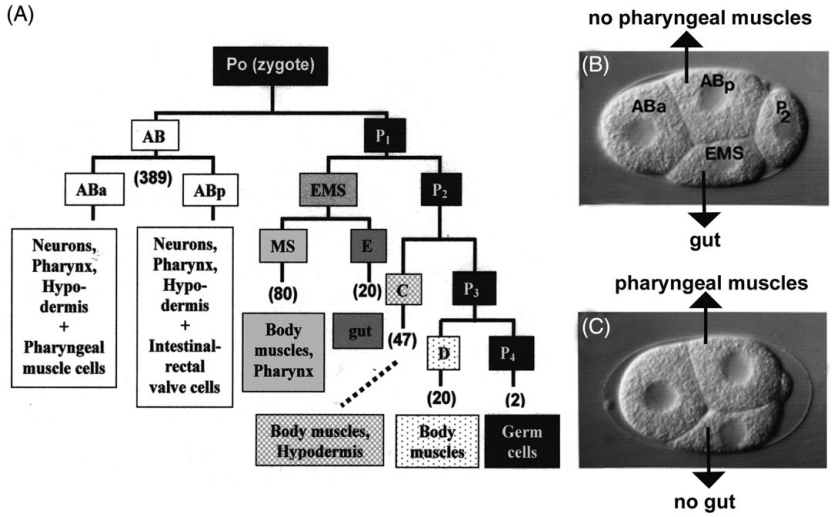

Figure 14.2 Cell lineages and inductive interactions in the early *C. elegans* embryo. A, Early cell lineage showing generation of five somatic founder cells (AB, MS, E, C, D) and the primordial germ cell P_4. Predominant or exclusive fates are given below individual lineage branches. Numbers in parentheses indicate cell numbers at hatching. B, Four-cell embryo with selected cell fates derived from ABp and EMS. C, After elimination of P_2 the developmental program of both neighbouring cells is altered because of missing inductions.

Other important features of early *C. elegans* embryogenesis require experimental interference (e.g. visualisation of gene expression with green fluorescent protein [GFP] constructs, mutant analysis, laser micromanipulation or blastomere recombination) to become obvious. These include inductive events between individual blastomeres, just two of which will be mentioned here (for a more detailed description, see reviews by Basham and Rose 2001, Edgar 2001). In the four-cell stage (Figure 14.2B), the germline cell P_2 induces both of its neighbouring cells ABp and EMS via receptor–ligand interactions to execute specific developmental programs. While in the former case (ABp) homologues of Delta/Notch are involved, in the second case (EMS) genes of the Wnt/Frizzled signal cascade are active (Kimble and Simpson 1997; Rocheleau *et al.* 1997). Thus, in both cases mechanisms that are well conserved in the animal kingdom play a central role in embryonic cell specification. If the signalling source P_2 is eliminated (Figure 14.2C), ABp and EMS generate descendants with an altered fate and embryos arrest without reaching a vermiform stage (Priess and Thomson 1987, Schierenberg 1987).

OTHER NEMATODES SELECTED FOR EMBRYONIC STUDIES: A BRIEF DESCRIPTION

Diploscapter coronatus is a close relative of *C. elegans*: both are members of clade 9 (Figure 14.1). However, *D. coronatus* is only about half the size of *C. elegans* and reproduces parthenogenetically. It lays its eggs prior to first cleavage. Eggs are only slightly smaller than those of *C. elegans* but embryogenesis takes about five times as long at room temperature.

Plectus sp. (strain ES 601; clade 6) and *Acrobeloides nanus* (clade 11) are similar to *D. coronatus* with respect to the features listed above. However, all three species can be easily distinguished on the basis of behaviour, body shape and a variety of anatomical features. While *Diploscapter* and *Acrobeloides* are cultured like *C. elegans*, all *Plectus* species we have studied require low-salt conditions and thus seem to occupy specific ecological niches.

Romanomermis culicivorax (clade 2) is a gonochoristic (male/female) parasitoid in mosquitos which leaves its host in the pre-reproductive phase and can then be kept in distilled water without food, where the animals copulate while forming prominent and permanent aggregates. Females grow to more than 2 cm in length and can lay more than 2000 one-cell stage eggs with a diameter of 80–90 μm. Embryogenesis takes about 10 times as long as in *C. elegans*.

Tobrilus diversipapillatus (clade 1) was found on the shores of lakes and small river banks. Although specimens can be kept in the laboratory for several weeks, we are not yet able to culture them. Adults are about twice as long as *C. elegans* but eggs only about 30% longer than those of *C. elegans*. Compared with other representatives of this clade embryos are rather transparent and develop fast, i.e. only about half the rate of *C. elegans*.

MODE OF REPRODUCTION AND ESTABLISHMENT OF THE PRIMARY EMBRYONIC AXIS

Most higher organisms follow a gonochoristic mode of reproduction, which is thought to give at least long-term advantages because of the continuous recombination of alleles, resulting for instance in the loss of lethal mutations (Maynard-Smith 1978) and a better resistance to parasites (Hamilton *et al.* 1990). However, the advantages of sex are counterbalanced by at least short-term advantages of parthenogenetic species where each individual can reproduce and where the costs of mate search, courtship, intraspecific competition etc. can be saved. It is generally agreed that the gonochoristic mode is original and other variants like hermaphroditism and parthenogenesis are derived forms.

Parthenogenesis is frequently observed in certain free-living nematode taxa. Several such species are being cultured and studied in our laboratory (Skiba and Schierenberg 1992, Lahl *et al*. 2003, 2006), including the *Diploscapter*, *Acrobeloides* and *Plectus* species introduced above. This offers the opportunity to analyse in detail developmental peculiarities that accompany the parthenogenetic type of reproduction.

During oogenesis in the internally self-fertilising hermaphrodite *C. elegans*, oocytes arrest during meiosis and need to be induced by a sperm-derived signal to resume their meiotic program (Miller *et al*. 2001, Hajnal and Berset 2002) in order to become haploid and be ready for fertilisation. Egg cells lose their centrioles, and meiotic divisions take place without them (Albertson and Thomson 1993). The sperm then delivers the centriole necessary to generate embryonic cleavage spindles. In *C. elegans* it is also the sperm that induces formation of the primary embryonic axis: the area of its entrance into the egg defines the posterior pole (Goldstein and Hird 1996, Cowan and Hyman 2004).

These findings make clear that development of parthenogenetic nematodes must require certain modifications during oogenesis and/or early embryogenesis. These include: (1) establishment of egg polarity without fertilisation, i.e. either by random chance processes or via polarising cues acting in the mother; (2) preservation or restoration of diploidy without paternal contribution, either through absent or incomplete meiosis or via compensating postmeiotic processes; and (3) formation of cleavage spindles despite the absence of a sperm-derived centriole requiring either survival of the original centriole, a *de novo* synthesis in the egg cell, or formation of centrosomes without centrioles.

Here, we want to point out some peculiarities concerning aspects (1) and (2). By experimentally inhibiting egg-laying we determined the orientation of early-stage embryos within the uterus relative to the vulva (Figure 14.3; Lahl *et al*. 2006). In *C. elegans* oocytes are fertilised at the pole that enters the spermatheca first and thus embryos cleaving in the uterus point with their posterior pole toward the vulva. In *A. nanus* we found that embryos also showed a preferred orientation in the gonadal tube, but with opposite orientation to *C. elegans*. Thus, it appears that in *A. nanus* some external cue other than the one from sperm induces the direction of egg polarity. In eggs of *D. coronatus* we found that half of them point with their anterior pole and half with their posterior pole toward the vulva. Here, the fixation of anterior–posterior polarity seems to be independent of an external signal and determined randomly by chance.

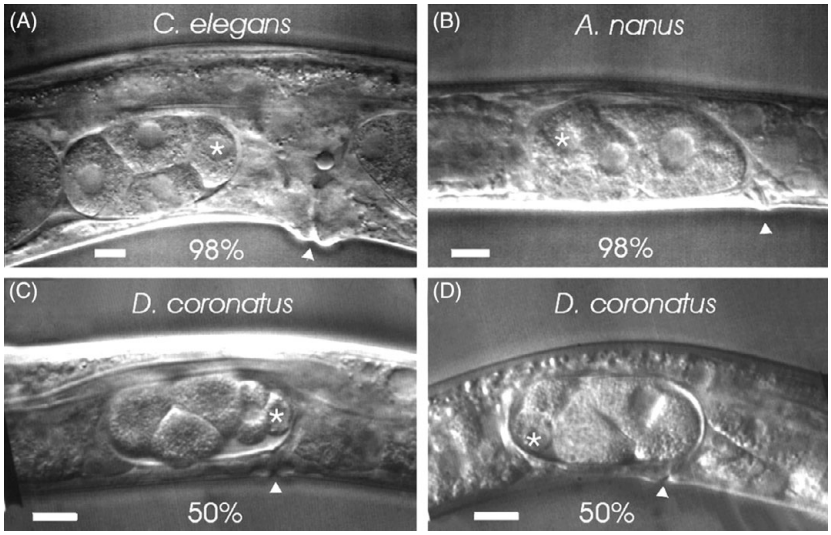

Figure 14.3 Establishment of axis polarity in parthenogenetic nematodes. Variations in the establishment of embryonic polarity. A, *C. elegans*, 98% of all embryos point with their posterior pole toward the vulva; B, *A. nanus*, 98% of all embryos point with their anterior pole toward the vulva; C and D, *D. coronatus*, equal proportions of embryos point with their anterior or posterior pole toward the vulva; arrowheads, position of the vulva; asterisk, germline cells P_2 (A, B) or P_3 (C, D). Scale bars, 10μm. From Lahl *et al.* (2006).

Parthenogenetic species not only differ from *C. elegans* with respect to how diploidy is established but also differ among themselves. In *A. nanus* only one polar body (PB) is formed because the products of the second meiotic division fuse again. In *D. coronatus* two PBs are found, but these result from a cleavage of the first PB while the second meiotic divison is suppressed. In *Plectus* sp. two PBs are generated as well, but here in conjunction with two regular meiotic divisions. Circumstantial evidence suggests that *Plectus* restores its diploid status via an additional DNA replication round (Lahl *et al.* 2006).

Our preliminary studies on three *Acrobeloides* species with different modes of reproduction indicate that embryonic variances beyond meiosis and fertilisation are not correlated to parthenogenesis.

VARIATIONS IN EARLY LINEAGE AND PATTERN FORMATION

It is remarkable that the species considered here form a variety of different spatial patterns already from the four-cell stage onwards. However, even

before or during gastrulation they all merge into a similar scheme (Schierenberg 2001, 2005a).

Diploscapter: a close relative of *C. elegans* with different early cell patterning

Instead of a diamond-shaped blastomere arrangement in the four-celled embryo, some nematode species show a linear grouping along the anterior-posterior axis (Malakhov 1994; Dolinski *et al.* 2001). Such an arrangement is also found in *D. coronatus* where not only P_1 but also the AB cell forms a longitudinally oriented cleavage spindle (Figure 14.1). This means much more than just a minor variation of a common pattern, as consequently P_2 never contacts ABp and contacts P_3 in only 50% of all embryos. Thus, an induction as found in *C. elegans* requiring cell–cell contacts (Priess and Thomson 1987) cannot take place here. Physical removal of P_1 through a laser-induced hole in the eggshell reveals that the unusual spindle orientation in AB is cell-autonomous. Cell lineage studies show that despite the absence of induction, like in *C. elegans* ABp descendants execute different fates from those of ABa descendants (V. Lahl, J. Schulze and E. Schierenberg, manuscript in preparation). Later, cells rearrange and reach a *C. elegans*-like pattern. Cell ablation experiments show that it is the EMS cell that takes the leading function in this process.

In conclusion, even close relatives of *C. elegans* may show considerable deviations during early development. In the case of *Diploscapter* it has been speculated that the differences may reflect a simplification of the developmental program (reduction of cell–cell interactions) at the cost of speed (necessary cell rearrangements). In addition, the linear array of blastomeres accompanied by an elongated eggshell may allow even a small species with a little vulva to produce relatively large eggs with an increased amount of nutritive or other maternal gene products (V. Lahl, J. Schulze and E. Schierenberg, manuscript in preparation).

Acrobeloides: an example for early embryonic plasticity

Developmental studies in *A. nanus* led to some unexpected findings (Wiegner and Schierenberg 1998, 1999). Overall embryogenesis proceeds about five times slower than in *C. elegans*, whereby initial cell cycles are particularly long. Inhibiting transcription shows that early cleavage requires zygotic gene activity while the *C. elegans* embryo reaches more than 100

cells under these conditions because of a generous maternal supply. Like in *C. elegans*, five somatic founder cells and a primordial germ cell are generated during early embryogenesis. However, the sequence of cleavages is different in that divisions in the germ line occur prematurely relative to mitoses in somatic cells (Figure 14.1). Thus, the primordial germ cell P_4 is already present in the six-cell stage while in *C. elegans* this occurs much later, at the 24-cell stage. In contrast to *C. elegans* no indication of germline-induced induction was found in *A. nanus*. For instance, any blastomere in the neighbourhood of the gut precursor cell can be removed and the remainder of the embryo will nevertheless form differentiated gut cells (Figure 14.4). However, the story goes further. Even when the gut precursor itself is eliminated the embryo compensates for this loss and partial embryos

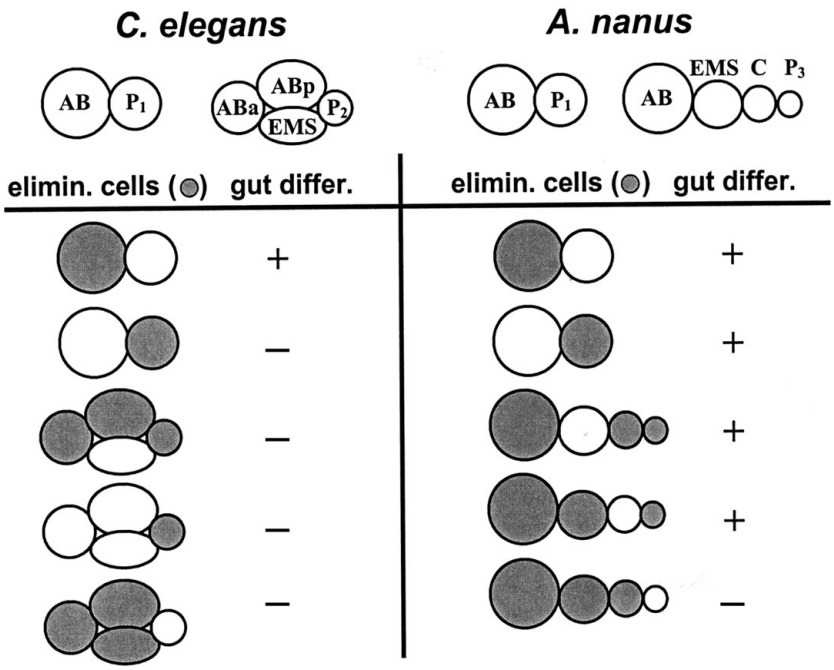

Figure 14.4 Differences in regulative behaviour between early *A. nanus* and *C. elegans* embryos. Top, intact two- and four-cell arrangements. Eliminated cells are marked in grey. '+', development of differentiated gut cells; '–', absence of differentiated gut cells at the terminal phenotype.

can even develop into hatching juveniles. This demonstrates that *A. nanus* carries a regulative potential absent in *C. elegans*.

Based on these data a model has been suggested according to which early blastomeres in *A. nanus* are multipotent and compete for a primary fate (Figure 14.5).

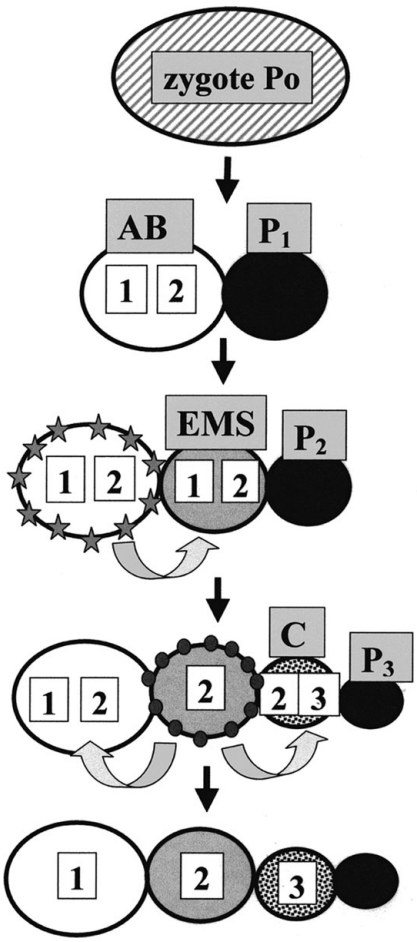

Figure 14.5 Model for cell specification in *A. nanus*. Early blastomeres can execute two alternative developmental programs (1 + 2 or 2 + 3; 1 = AB, 2 = EMS, 3 = C). Competing for a primary fate, inhibiting interactions (curved arrows) transmitted by specific cell surface molecules (stars and circles) between neighbouring cells lead to the restriction of developmental potential in a hierarchical manner. At least between AB and EMS, reciprocal interactions take place (after Schierenberg 2005a, modified).

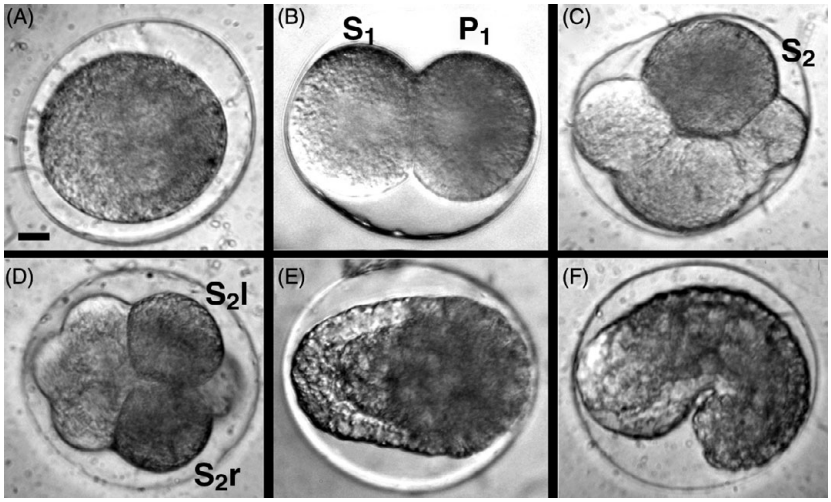

Figure 14.6 Segregation of coloured cytoplasm in *Romanomermis*. Translocation of brownish cytoplasm to the posterior pole prior to first cleavage (A) and consequent segregation into P_1 and later into S_2 (B, C). With the next division both daughter cells (S_2l and S_2r) receive the coloured components. During further development S_2 descendants expand from posterior to anterior (E, F). Note that nomenclature differs from *C. elegans* because of differences in cell position and fate. Formally, S_1 corresponds to AB and S_2 to EMS in *C. elegans*. A–C, F, Lateral view; D, E, dorsal view. Scale bar, 10μm.

Plectus sp.: differences in symmetry formation and gastrulation

Although the four-cell stage of *Plectus* looks similar to that of *C. elegans* (Figure 14.1), soon afterwards peculiarities arise that appear to be typical for the whole family Plectidae.

In contrast to all other taxa mentioned here, gastrulation starts as early as the eight-cell stage (Figure 14.8A–C). This can be interpreted as a heterochronic shift giving the gut founder cell the premature ability to ingress while in other nematodes only its daughters or even granddaughters can do so. The migration of the EMS cell in *Diploscapter* (see above) can be understood along the same lines: that is, even another cell generation earlier the gut precursor cell becomes competent to migrate and/or neighbouring cells exhibit necessary cell surface molecules to do so. A second characteristic feature of *Plectus* is its early prominent bilateral symmetry which is formed within individual lineages via cell divisons with strict left–right spindle orientations (Lahl *et al*. 2003; Figure 14.8A, B).

Romanomermis: visible cytoplasmic segregation and different fate assignments

Embryonic cell lineage analyses have been performed in a variety of species from clades 6–12, while for the the remaining clades only a few lineage studies exist (Malakhov 1994, Voronov 1999). Reasons are that only some of the latter are being cultured in the laboratory, development is slow and embryos are insufficiently transparent. One exception is *Romanomermis culicivorax* (Figure 14.1), whose development proceeds reasonably rapidly and in which a moderate density of yolk granules allows detailed cell lineage studies (J. Schulze and E. Schierenberg, unpublished data).

In several respects *Romanomermis* differs from the species introduced above. The embryo contains – so far uniquely among nematodes – coloured cytoplasm segregated to the somatic founder cell S_2 (Figure 14.6), reminiscent of coloured myoplasm in some ascidian embryos (Jeffery 2001). However, here this blastomere appears to give rise to the complete hypodermis which eventually overgrows the remainder of the embryo. This process with the repeated duplication of cell groups (Figure 14.7) seems fundamentally different from the way in which hypodermis is generated in *C. elegans* (see concluding remarks). As another major difference to representatives of clades 6–12, we find that in *Romanomermis*, another early blastomere generates the complete alimentary tract, i.e. pharynx and gut. However, this is obviously true for other members of clades 1 and 2 as well (Malakhov 1994). In summary, our observations indicate that cell lineages and fate assignment in *Romanomermis* follow a less complex scheme than in *C. elegans*.

Tobrilus*: a nematode with unusual gastrulation*

Gastrulation, the most dramatic process of reorganisation in the embryo, results in the formation of distinct germ layers. The classical type of gastrulation and probably the archaic one (Technau and Scholz 2003) starts with the formation of a hollow sphere (coeloblastula) and subsequent invagination of endo- and mesodermal precursors. However, major variations exist even within the same phylum (Gilbert and Raunio 1997).

The nematodes studied in the past show a unique pattern of gastrulation not found elsewhere in the animal kingdom. Some key features of *C. elegans* gastrulation (Bucher and Seydoux 1994, Nance and Priess 2002) are briefly summarised here (Figure 14.8A–C). Soon after the primordial germ cell P_4 has been generated in the 24-cell stage, the two daughters of the gut precursor cell E, lying at a posterior-ventral position, start to ingress. Instead of a

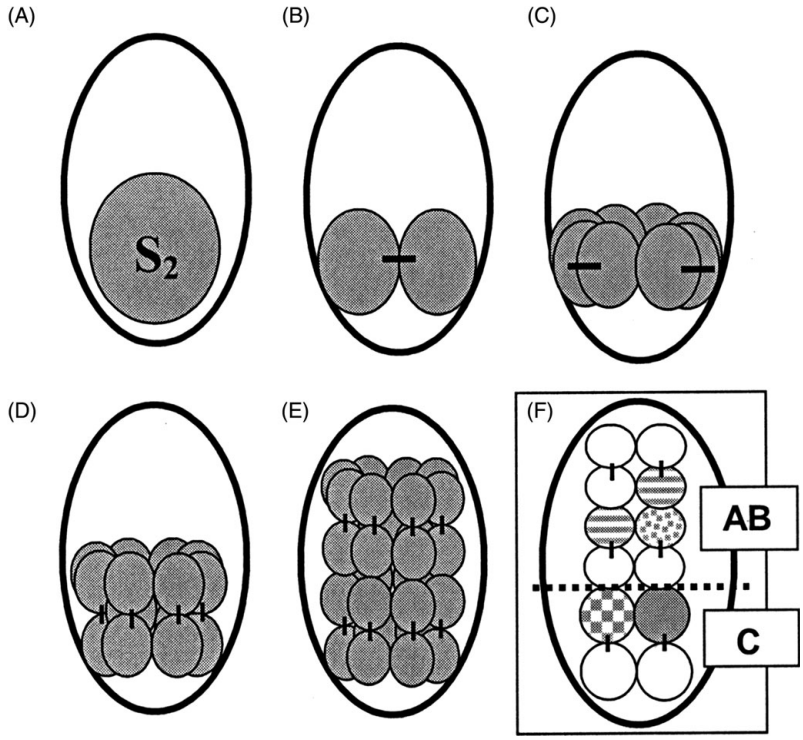

Figure 14.7 Hypodermis formation in *R. culicivorax* and *C. elegans*. Dorsal view. A–E, *Romanomermis*; owing repeated divisions with transverse spindle orientation (B, C), descendants of S_2 (carrying brownish cytoplasm, see Figure 14.6), form a ring-like structure (C). As a result of consecutive divisions with longitudinal spindle orientations, repetitive units form that extend from posterior to anterior (D, E). F, *C. elegans*. Hypodermis is derived from two different lineages, AB (eight anterior cells) and C (four posterior cells). Colour code indicates to what extent the descendants of the blastomere shown differentiate into hypodermis (Sulston *et al.* 1983). Grey, 100%; checkered, 50–60%; dotted, 20–30%; white, 0–10%. Note that in reality already 16 AB cells are already present when four C cells are formed.

typical blastocoel, only a few small extracellular spaces are present at any time (von Ehrenstein and Schierenberg 1980, Nance and Priess 2002). Cells forming the mesoderm (i.e. body muscles and part of the pharynx) are derived from four different lineages. They immigrate in a piecemeal fashion at different times and places (von Ehrenstein and Schierenberg 1980, Sulston *et al*. 1983).

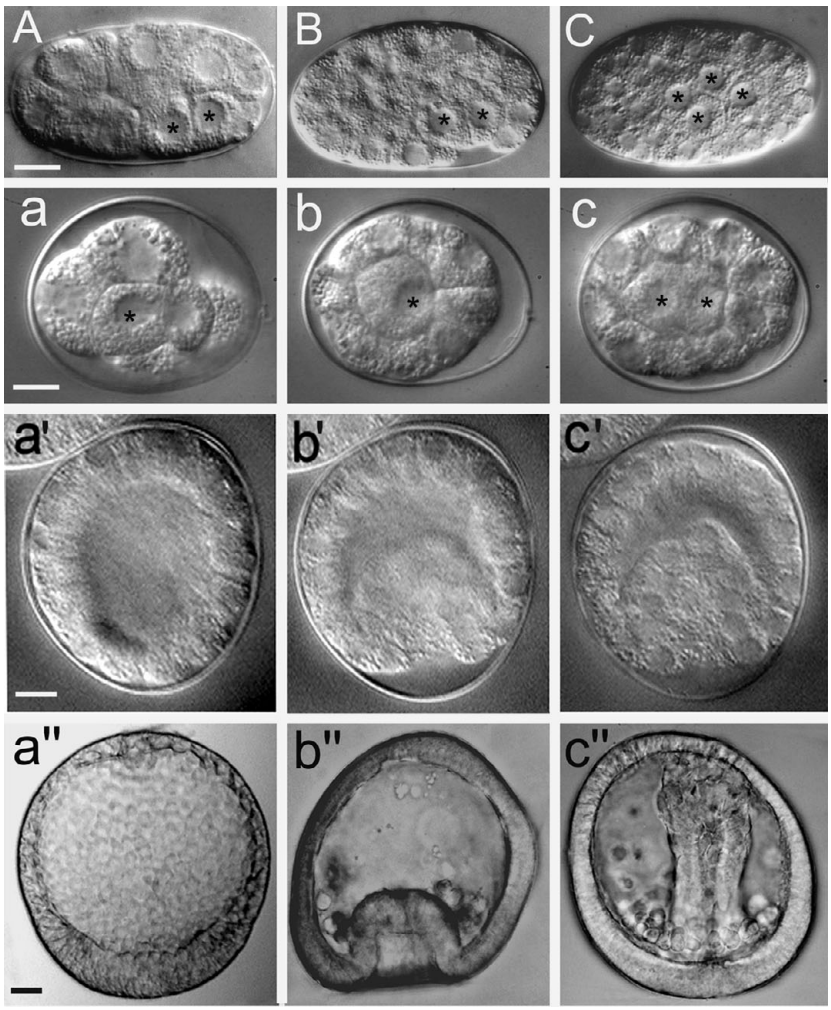

Figure 14.8 Gastrulation in nematodes and sea urchin. A–C, *C. elegans*, immigration of two gut precursors and subsequent division into four cells; blastocoel essentially absent. a–c, *Plectus* sp., immigration of one gut precursor and subsequent division into two cells, dorsal view. Note strict bilateral symmetry (b, c). a′–c′, *Tobrilus diversipapillatus*, large blastocoel, invagination of multiple cells. After formation of a large blastocoel (a′) invagination of endoderm (b′, c′) takes place. a⊠–c⊠, *Psammechinus miliaris* (sea urchin). Asterisks, gut precursors. Orientation: lateral view except *Plectus* (dorsal view). Scale bars, 10 μm.

In contrast to all nematodes described so far, in *Tobrilus* a large blastocoel surrounded by a single layer of blastomeres forms (Figure 14.8a′), similar to blastula stages in other invertebrates (such as sea urchins; Figure 14.8a″) and also vertebrates. Around the 64-cell stage a small number of cells start to invaginate into the blastocoel (similar to sea urchin; Figure 14.8b′, b″). Movement and division of the internalised blastomeres result in their continuous extension and a corresponding decrease in blastocoel size (Figure 14.8c′, c″). It seems that these invaginated cells form not only intestine but also pharynx. From the 128-cell stage onward, a third layer of blastomeres invaginates and extends between the compact mass of central cells and the surrounding ectoderm (Schierenberg 2005b).

The observations reported here demonstrate that a change took place within the phylum Nematoda in how three different germ layers are generated. It appears likely that gastrulation as seen in *Tobrilus* represents the original (plesiomorphic) state and that the standard nematode pattern is a derived condition.

CONCLUDING REMARKS

The data summarised in this chapter document that embryogenesis in nematodes is more variable than the final product, the hatching juvenile, would predict (for detailed lineage studies in addition to *C. elegans*, see Houthoofd *et al.* 2003, 2006). It has been suggested that the unexpectedly large genetic differences even between closely related nematode species (Fitch and Thomas 1997) is due to a 2–3 times higher nucleotide substitution rate compared with most other Metazoa (Aguinaldo *et al.* 1997). In addition, clades 8–12 (formerly indicated as Secernentea; including *Caenorhabditis, Diploscapter* and *Acrobeloides*) seem to have evolved considerably faster than clades 1–7 (formerly indicated as Adenophorea; including *Tobrilus*, *Romanomermis* and *Plectus*), possibly owing to higher metabolic rates and shortened generation times (Holterman *et al.* 2006). The special body plan of nematodes apparently prevented a corresponding degree of morphological diversification as found in other phyla like arthropods or vertebrates.

The wealth of early developmental variations appears paradoxical in a way, as these do not have any obvious impact on structure or performance of the resulting worms. Why then are there different ways to reach essentially the same goal? Two explanations can be offered. It could either be a result of neutral evolution, in which variations are due to system-inherent plasticity without any adaptive value, or the different ways may reflect

alternative developmental strategies to increase fitness, for instance by making production faster or cheaper (Schierenberg 2001). Furthermore, it remains to be determined how dramatic the changes in the underlying genetic control must be to achieve apparently massive modifications on the cellular level. It appears rather difficult to imagine in terms of lineage transformations how the two variants of hypodermis formation as found in *Caenorhabditis* and *Romanomermis* (Figure 14.8) arose from a common pattern during evolution. However, if cell specification involves a position-based mechanism (e.g. 'all peripheral cells with no contact to the elementary tract shall form hypodermis') both species may only differ in the timing of when such a decision is made.

In addition to the different timing of gastrulation specified above, a number of other early embryonic peculiarities can be interpreted as heterochronic shifts (V. Lahl, J. Schulze and E. Schirenberg, manuscripit in preparation). As heterochrony is often considered the single most important process of evolutionary change (Raff 1996) it would be interesting to pinpoint which of the numerous developmental variances among nematodes *cannot* be explained with such a mechanism.

The model of 'cell focusing' suggested by Schnabel *et al*. (2006) to illuminate the movement of blastomeres to specific embryonic regions in *C. elegans* according to their identity may also be helpful in imagining how species-specific modifications may have arisen during evolution.

It is not immediately obvious why early embryogenesis should be more variable than later phases. One argument has been that development is modular and integration of the emerging modules increases over time, putting fewer constraints on early development (Raff 1996). This seems reasonable for organisms where cells are specified relatively late, like vertebrates and possibly very slow-developing nematodes as found in clades 1 and 2 (Voronov and Panchin 1998). However, for the fast *C. elegans*-type of development, where essential decisions going along with specific cell–cell interactions take place in a very early phase, it must be questioned whether this argument is valid. Another reason for extended early variability could be the different role of maternal gene products during that period. As model systems have usually been selected because of their rapid development (Bolker 1995) maternal gene expression may be disproportionately high there. The huge differences between *C. elegans* and *A. nanus* with respect to maternal contribution during the early cleavage phase (Wiegner and Schierenberg 1998) support such a view.

In order to correlate ontogeny and phylogeny, embryonic variations may be useful heuristically as independent phylogenetic markers in addition to

morphology and molecules. By looking at processes such as axis specification (Goldstein *et al.* 1998), cleavage pattern, arrangement of blastomeres (Dolinski *et al.* 2001, Houthoofd *et al.* 2003), germline behaviour and gastrulation (Schierenberg and Lahl 2004, Schierenberg 2005b), attempts have been made to trace the evolution of embryonic diversity in nematodes.

According to the Ecdysozoa hypothesis, nematodes are a neighbouring taxon to arthropods (Aguinaldo *et al.* 1997). Although we do not know what a last common ancestor of nematodes and arthropods might have looked like, it appears not unlikely that it was already segmented (or at least possessed some repetitive body elements) and that this feature was secondarily lost in conjunction with the reduction in cell numbers. It may therefore be attractive to look for potential remnants (or precursors) of segmentation in representatives positioned close to the basis of the nematode branch. Hypodermis formation in *Romanomermis* via generation of repetitive ring structures (Figure 14.7) is as close as we can come so far to something that is reminiscent of segmentation (J. Schulze and E. Schierenberg, unpublished results). The search for genes involved in segmentation (like *engrailed*) and their expression pattern in archaic nematodes may be helpful in determining whether such similarities are more than analogies and in general for the ongoing dispute about the phylogenetic position of nematodes.

Our studies have shown that different roads lead to Rome, i.e. to a juvenile ready to compete in the struggle for life. By extending comparative studies to a larger number of species and by identifying relevant genes, we should learn more about the intrinsic prerequisites for the implementation of embryonic novelty. In addition, we may better understand to what extent the interplay between the genetic program and external conditions (inside or outside the organism) determines the chance for deviations from an original developmental pattern to arise and to succeed. Finally, the question can be addressed of whether the establishment of modified embryonic cell behaviour as described here follows similar rules of variation and selection as assumed for so many morphological and physiological traits.

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