

Embryonic axis specification in nematodes: evolution of the first step in development

Bob Goldstein*[†], Linda M. Frisse[‡] and W. Kelley Thomas[‡]

In 1828, von Baer proposed that the early stages of development must be the most conserved [1]. Others have since countered that the middle stages of development are the most conserved [2]. To address whether the earliest step in pattern formation can evolve, we have examined how asymmetry along the antero–posterior (AP) axis is generated in various nematode species. AP asymmetry is specified in *Caenorhabditis elegans* at fertilization by the sperm, which directs a cytoplasmic rearrangement that segregates critical factors such as the P granules to one side of the uncleaved embryo [3,4]. We found that AP asymmetry is generated differently in another nematode species: the sperm is not used to specify AP asymmetry, there are no signs of cytoplasmic movements, and P granules are segregated differently. Despite these differences, development from the two-cell stage is remarkably similar in the two species. We have reconstructed the evolutionary history of these mechanisms by analyzing the development of 30 nematode species and mapping the results onto a molecular phylogeny of the nematodes [5]. The results suggest that a new mechanism for axis specification evolved in an ancestor of some of the relatives of *C. elegans*. We conclude that this fundamental step in development can evolve without affecting other aspects of development.

Addresses: *MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, UK. [†]School of Biological Sciences, Biological Sciences Building, University of Missouri-Kansas City, Kansas City, Missouri 64110, USA.

Present address: [‡]Department of Molecular and Cell Biology, 385 LSA, University of California, Berkeley, California 94720, USA.

Correspondence: Bob Goldstein
E-mail: bobg@uclink4.berkeley.edu

Received: 20 October 1997
Revised: 20 November 1997
Accepted: 24 November 1997

Published: 19 January 1998

Current Biology 1998, 8:157–160
<http://biomednet.com/elecref/0960982200800157>

© Current Biology Ltd ISSN 0960-9822

Results and discussion

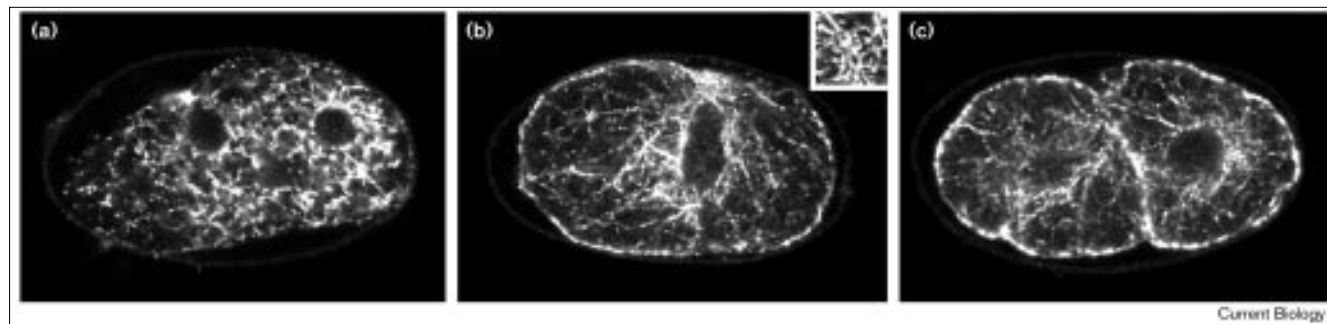
The unfertilized egg in *C. elegans* has no developmentally significant asymmetries. Instead, asymmetry is generated by the fertilizing sperm [3]. Upon entry, the sperm directs a cytoplasmic rearrangement in the uncleaved embryo: as

the sperm-associated astral microtubules begin to grow, cortical material flows away from the sperm pronucleus and central cytoplasmic material flows in the opposite direction. With the central cytoplasm move the P granules, composed of particular RNAs and proteins which become segregated to the germ-line precursors through successive cell divisions [4].

We looked for nematode species in which the sperm is not used to specify AP asymmetry. The development of various species was examined by time-lapse recordings, to determine if the position of sperm entry consistently predicts the posterior side. In *Acrobeloides* sp. PS1146 (hereafter referred to as *Acrobeloides*), the position of sperm entry bears no consistent relationship to embryonic polarity. This indicates that the sperm is not used to specify AP asymmetry in this species. We next asked to what extent these two mechanisms differ by examining *Acrobeloides* embryos between fertilization and the first cell division for three components of asymmetry — astral microtubules, the cytoplasmic rearrangement and the pattern of P granule segregation. Anti-tubulin immunocytochemistry in *Acrobeloides* revealed that, in contrast to *C. elegans*, no asters formed in association with the sperm pronucleus (Figure 1). Instead, a net of microtubules was present throughout the embryo until mitosis, when the microtubules formed asters adjacent to the two nuclei, in the center of the embryo. No gross asymmetry in microtubule distribution was apparent at any time in the first cell cycle. In *C. elegans*, sperm nucleus-associated asters have been implicated in directing the cytoplasmic rearrangement, as the rearrangement begins when asters grow, and because asters have been implicated in directing movements of an actin-rich cortex in other systems [3].

Time-lapse videomicrography was used to examine *Acrobeloides* embryos for a cytoplasmic rearrangement. This rearrangement is visible in time-lapse films of *C. elegans* as a fountain flow of cytoplasm directed toward the sperm pronucleus in the uncleaved embryo [4]. No such flows were found in *Acrobeloides* during pronuclear migration, nor at the time when P granules become asymmetrically distributed (see below), nor at any time between fertilization and the first cell division. All other signs of the flows found in *C. elegans* [4] were absent as well — ruffling of the anterior membrane, a pseudocleavage furrow, and the sperm pronucleus pushing up against the posterior cortex as the oocyte nucleus begins to migrate across the uncleaved embryo. Instead, the sperm and oocyte nuclei migrated simultaneously towards the center of the

Figure 1



Microtubule distribution in *Acrobelooides* embryos, confocal sections of immunostained [3] embryos. (a) Soon after meiosis. (b) After the pronuclei have met. The inset is a confocal section of the cortex, showing that microtubules in the cortex are in the form of a network. Microtubules become enriched in the cortex during pronuclear migration. (c) Two-cell stage.

embryo. Some membrane ruffling occurred during meiosis in *Acrobelooides*, but neither this nor the position of the meiotic spindle were predictors of embryonic polarity.

P granule distribution was examined using antibodies generated against *C. elegans* material but which cross-react with P granules in several nematode species [6]. In *Acrobelooides*, P granules did not become asymmetrically distributed during pronuclear migration, the time that they migrate in *C. elegans* [4]. Instead, most were on the surface of the pronuclei at this time; the rest were distributed through the cytoplasm (Figure 2). During metaphase, most of the P granules surrounded the chromosomes. In *Acrobelooides*, P granules first became asymmetrically distributed at anaphase, redistributing primarily around one of the two asters. Confocal microscopy after double-label immunocytochemistry showed that the P granules were not in contact with the astral microtubules (data not shown). Upon cell division, the P granules were inherited by only one of the two cells, in which they returned to the surface of the nucleus. The same pattern of P granule segregation occurred during each of the following three cell cycles. This pattern bears a superficial resemblance to that found in some of the later embryonic cells of *C. elegans*, in which the P granules are segregated by attaching to the nucleus while the nucleus moves to an asymmetric position [7]. In *Acrobelooides*, however, segregation occurs during mitosis, and does not involve asymmetric nuclear positioning.

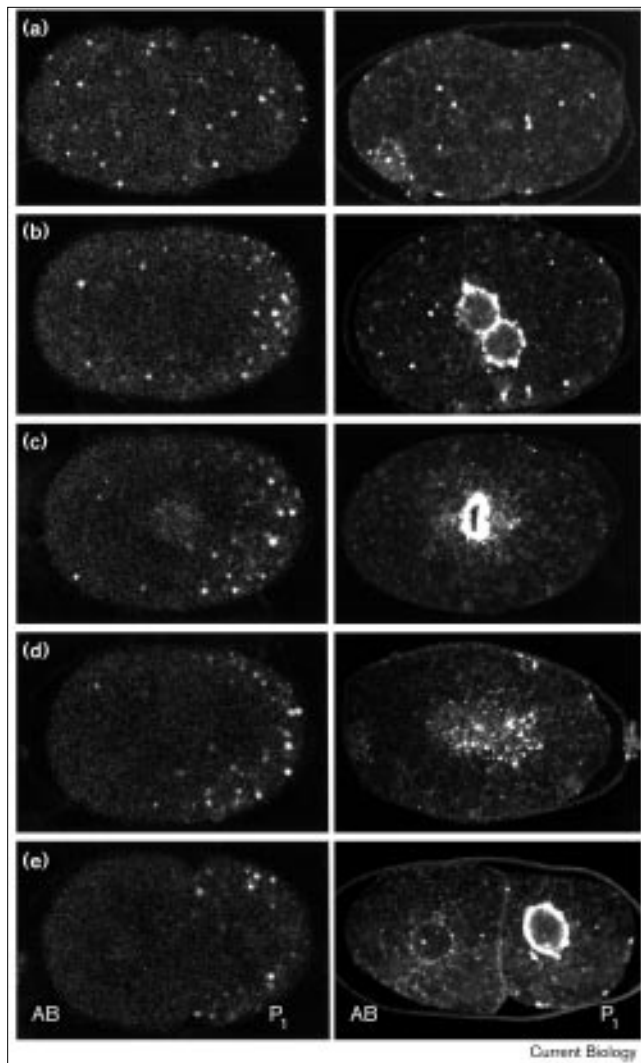
If the sperm does not specify AP asymmetry in *Acrobelooides*, what does? One possibility is that polarity is induced by a component of the mother's reproductive tract: *Acrobelooides* embryos are normally laid before AP orientation is apparent, but by anesthetizing worms (in 180 μ g/ml Tetramisole) to prevent egg laying, we found that the orientation of embryos inside their mothers' reproductive tracts is an early indicator of AP polarity: the side of the embryo facing the vulva consistently developed as the anterior ($n = 21/21$). Similar results have been found in three other species in

the same suborder as *Acrobelooides* (*Cephalobus oryzae* and *Zeldia punctata*, B.G., unpublished observations; *Acrobelooides* sp. ES501, M. Kutzowitz and E. Schierenberg, personal communication). This is not the case in *C. elegans* [3].

As the sperm is not used to specify asymmetry in *Acrobelooides*, and each of the components of asymmetry differs markedly from *C. elegans*, we conclude that the mechanism for generating asymmetry has evolved within the nematodes. Despite this, development from the two-cell stage proceeds similarly in *C. elegans* and *Acrobelooides*. In both species, P granules are inherited by just one cell of the two-cell stage. This cell continues asymmetric and asynchronous divisions and gives rise to all of the endoderm, the bulk of the mesoderm and some ectoderm. The other cell goes through much more symmetric and synchronous divisions and produces mostly ectoderm. With the exception of the orientation of certain germ-cell divisions (which occurred as in [6]), cell lineages and cell positions through the beginning of gastrulation were nearly identical in these two species (B.G., unpublished observations). Similar conclusions have been made previously for several closely related species [6,8]. Hence the mechanism for axis specification has evolved without significant effect on ensuing development.

In order to reconstruct the evolutionary history of these two mechanisms, we examined 28 other nematode species by time-lapse videomicrography. We determined whether the site of sperm entry develops as the posterior and whether a cytoplasmic rearrangement occurs. In some species, P granules and microtubules could also be examined by immunocytochemistry. The species fell almost exclusively into two groups, one like *C. elegans* and the other like *Acrobelooides* (Table 1). We mapped the data onto a nematode phylogeny [5], using the presence or absence of a cytoplasmic rearrangement as an indicator of which mechanism is used. The results suggest that the *C. elegans*-type mechanism is ancestral for this group,

Figure 2



Asymmetric redistribution of P granules occurs during pronuclear migration in *C. elegans* (left), and during anaphase in *Acrobeloides* (right). Stages shown for both species are (a) soon after meiosis, (b) after the pronuclei have met, (c) metaphase, (d) anaphase, and (e) two-cell stage. AB and P₁ are the names of the cells at the two-cell stage. *C. elegans* pictures are derived from a film of a single embryo (by permission of S. Hird). *Acrobeloides* pictures are confocal sections of fixed, immunostained [3] embryos.

and that the *Acrobeloides*-type mechanism is a derived mechanism, which may have arisen just once, in an ancestor to the suborder Cephalobina (Figure 3). One species is of particular interest: *Meloidogyne incognita* was traditionally considered a member of a separate order (branching outside of these two suborders), but was significantly placed within the suborder Cephalobina by the molecular phylogeny. This species was examined as a test of our hypothesis and was found to share the *Acrobeloides*-type mechanism, consistent with a single derivation of this mechanism.

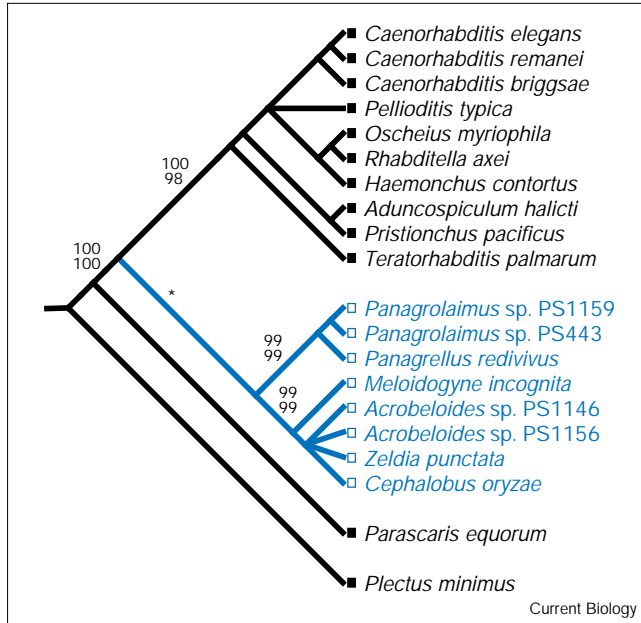
Table 1

Data from 30 nematode species.

Species (strain number)	Sex	Tubulin	P granules
No cytoplasmic rearrangement, and site of sperm entry does not predict posterior			
<i>Acrobeloides nanus</i> (S. Bostrom)	f		
<i>Acrobeloides</i> sp. (ES501)	f	Acr	Acr
<i>Acrobeloides</i> sp. (PS1146)	m/h	Acr	Acr
<i>Acrobeloides</i> sp. (PS1156)*	m/f		
<i>Cephalobus oryzae</i> (PS1165)	f	Acr	Acr
<i>Cephalobus</i> sp. (PS1215)	f		
<i>Chilopacus minimus</i> (S. Bostrom)	f		
<i>Meloidogyne incognita</i> (N. von Mende)	m/f		
<i>Panagrellus redivivus</i> (PS1163)	m/f		
<i>Panagrolaimus</i> sp. (PS1159)	f		
<i>Panagrolaimus</i> sp. (PS443)	m/f		
<i>Turbatrix acetii</i> (Carolina Biol. Supply)	m/f		
<i>Zeldia punctata</i> (PS1153)	f		
Cytoplasmic rearrangement, and site of sperm entry predicts posterior (observed in at least 6/6 cases)			
<i>Aduncospiculum halicti</i> (JB120)	m/f		
<i>Caenorhabditis briggsae</i> (G16)	m/h		
<i>Caenorhabditis elegans</i> (N2)	m/h	Ce	Ce
<i>Caenorhabditis remanei</i> (EM464)	m/f		
<i>Dolichorhabditis dolichura</i> (DF5033)	m/f		
<i>Dol. dolichuroides</i> (DF5018)	m/f		
<i>Haemonchus contortus</i> (E. Munn) [†]	m/f		
<i>Oscheius myriophila</i> (DF5020)	m/h	Ce	Ce
<i>Pellioditis</i> sp. (PS1191)	m/h		
<i>Pellioditis typica</i> (DF5025)	m/f		
<i>Pelodera strongyloides</i> (DF5022)	m/f		
<i>Plectus aquatilis</i> (PDL0018) [‡]	f		
<i>Plectus minimus</i> (PDL0012) [§]	f	Ce	
<i>Pristionchus pacificus</i> (PS312)	m/h	Ce	
<i>Rhabditella axei</i> (DF5006)	m/f		
<i>Rhabditoides regina</i> (DF5012)	m/f		
<i>Teratorhabditis palmarum</i> (DF5019)	m/f	Ce	

Each strain number identifies one isolate. Sources are listed for species that lack strain numbers. Some data is from [3]. m/f, male/female; m/h, male/hermaphrodite; f, parthenogenetic females. Parthenogenetic females produce progeny in the absence of males and sperm and have no second pronucleus in the first cell cycle. Some data on sexual mode is from L. Carta (personal communication). Ce, *C. elegans*-type tubulin and P granule rearrangements; Acr, *Acrobeloides*-type. Antibodies used were KMX-1 (Boehringer) for PS1146 and YL1/2 (gift of J. Kilmartin) for all others to visualize tubulin; OIC1D4 for *O. myriophila* and L416.10 for all others to visualize P granules (gifts of S. Strome). *Sperm entry position coincided with the future posterior pole in 6/6 cases, but might not have a role in generating polarity as no signs of a cytoplasmic rearrangement occurred. [†]*H. contortus* embryos were available in limited amounts. Cytoplasmic flows were not observed but all the other signs of a reorganization were. [‡]*P. aquatilis* embryos rotate within their eggshell during the first cell cycle, which would have obscured a cytoplasmic rearrangement occurring during rotation. [§]In *P. minimus*, the oocyte nucleus appears to take the role of the sperm nucleus in *C. elegans*: *P. minimus* develops parthenogenetically and has a cytoplasmic reorganization (after a period of embryo rotation) directed toward the oocyte pronucleus. As *Plectus* is the outgroup to all others examined here (Figure 3), we cannot conclude whether the rearrangement in the ancestor to all these nematodes was directed by the sperm or the oocyte nucleus. Some strains might be synonymous species, as 7 strains have not yet been identified.

Figure 3



Mechanisms for axis specification mapped onto a nematode molecular phylogeny. Species with no visible cytoplasmic rearrangement before first cleavage are drawn in blue; those with the rearrangement are in black. The top clade of ten species represents the suborder Rhabditina, the next clade of eight species (in blue) represents the suborder Cephalobina. We have not excluded the possibility that members of the Cephalobina actually use diverse mechanisms, which all differ from the *C. elegans*-type mechanism. The phylogeny was determined for the 20 taxa in which developmental mechanism can be assigned and 18S sequence data is also available. Character states were reconstructed on the phylogeny by parsimony. *Plectus* was used as an outgroup based on larger-scale phylogenetic analysis [5]. Sequences for *P. minimus* (GenBank accession no. U61578), *C. oryzae* (AF034390), *Acrobelooides* sp. PS1146 (AF034391), *Acrobelooides* sp. PS1156 (U81576), *O. myriophila* (U81588) and *M. incognita* (U81578) were determined as in [5]. The *C. remanei* sequence was from [10]. The remaining sequences are from [5]. The 18S sequence from *Ascaris suum* [11] was used in place of *Parascaris equorum* as these two species are sister taxa among eight species of ascaridoid nematodes [11]: a cytoplasmic rearrangement has been observed in *Parascaris* before first cleavage [12]. Bootstrap values for the relevant branches are based on maximum parsimony (top) and neighbor joining (bottom) analyses. The Cephalobina consist of two distinct groups by 18S sequence, with *Panagrolaimus* and *Panagrellus* (family Panagrolaimidae) in one group, and the other five species (family Cephalobidae) in the other. Morphological and molecular evidence supports the monophyly of these two groups, suggesting that the *Acrobelooides*-type mechanism is derived and arose once. However, we cannot significantly rule out one other possibility – the existence of two separate clades representing the two families, both branching basally to the Rhabditina: bootstrap percentages at the asterisk are 54/57. Such a result would suggest that the *Acrobelooides*-type mechanism is derived twice.

Why did the Cephalobina evolve a new mechanism for generating AP asymmetry? As the change had no noticeable effect on ensuing development, it probably conferred no selective advantage on the organisms that inherited it. Neutral changes to early development might

be considered to have little evolutionary consequence. But it remains possible that they could have an overlooked role in evolution: that of altering a species' 'adaptive potential', allowing additional exploration of fitness landscapes. For example, dispensing with the sperm's role in axis specification might have permitted an easy route to parthenogenesis in the Cephalobina; in fact, many species in the Cephalobina were found to be parthenogens (Table 1).

Understanding to what extent particular developmental stages are conserved in the various animal phyla will require much more examination of developmental mechanisms in non-model organisms. Data thus far suggest that early steps in development have the potential to evolve independently [9]. Our results extend these findings by showing that even the initial step in embryonic pattern formation can evolve without substantial alterations to ensuing development.

Acknowledgements

We thank L. Carta, P. De Ley, D. Fitch, J. Baldwin, S. Bostrom, R. Giblin-Davis, M. Graham, N. von Mende, E. Munn, E. Schierenberg, T. Smith, R. Sommer and P. Sternberg for worms, J. Kilmartin and S. Strome for antibodies, E. Schierenberg and L. Carta for sharing unpublished results, S. Hird for part of Figure 1, and M-A. Felix, F. Piano, E. Schierenberg, D. Weisblat and J. White for comments on the manuscript. B.G. was supported by postdoctoral funding from the Human Frontiers Science Program and the Medical Research Council. W.K.T. and L.M.F. were supported by NSF grant DEB-9318249 (to W.K.T.).

References

1. von Baer KE: *Entwicklungsgeschichte der Thiere: Beobachtung und Reflexion*. Königsberg: Bornträger; 1828.
2. Duboule D: Temporal colinearity and the phylotypic progression: a basis for the stability of a vertebrate Bauplan and the evolution of morphologies through heterochrony. *Development* 1994, (suppl):135-142.
3. Goldstein B, Hird SN: Specification of the anteroposterior axis in *Caenorhabditis elegans*. *Development* 1996, 122:1467-1474.
4. Kempthues KJ, Strome S: In *C. elegans II*. Edited by Riddle DL, et al. Plainview, NY: Cold Spring Harbor Laboratory Press; 1997:335-359.
5. Blaxter ML, De Ley P, Garey JR, Liu LX, Scheldeman P, Vierstraete A, et al.: A molecular evolutionary framework for the phylum Nematoda. *Nature* 1998, in press.
6. Skiba F, Schierenberg E: Cell lineages, developmental timing, and spatial pattern formation in embryos of free-living soil nematodes. *Dev Biol* 1992, 151:597-610.
7. Hird SN, Paulsen JE, Strome S: Segregation of germ granules in living *Caenorhabditis elegans* embryos: cell-type-specific mechanisms for cytoplasmic localisation. *Development* 1996, 122:1303-1312.
8. Malakhov VV: *Nematodes: Structure, Development, Classification and Phylogeny*. Washington, DC: Smithsonian Institution Press; 1994.
9. Raff RA: *The Shape of Life: Genes, Development, and the Evolution of Animal Form*. Chicago: University of Chicago Press; 1996.
10. Fitch DHA, Bugaj-Gaweda B, Emmons SW: 18S Ribosomal RNA gene phylogeny for some Rhabditidae related to *Caenorhabditis*. *Mol Biol Evol* 1995, 12:346-358.
11. Nadler SA: Phylogeny of some ascaridoid nematodes, inferred from comparison of 18S and 28S rRNA sequences. *Mol Biol Evol* 1992, 9:932-944.
12. Nigon V, Guerrier P, Monin H: L'architecture polaire de l'oeuf et les mouvements des constituants cellulaires au cours des premières étapes du développement chez quelques nématodes. *Bull Biol Fr Belg* 1960, 94:131-202.