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Embryonic lineage evolution in nematodes

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Summary – Because of the high number of species and its ancient roots in evolution, the phylum Nematoda is well suited for comparative embryonic study. Using 4D microscopy we have reconstructed the embryonic lineages of several nematodes. This allows us to identify changing developmental strategies in the phylum Nematoda. Generally there has been a shift in the phylum from a non-determined, non-strict development to a faster, highly determined embryonic development.

Résumé – ***Évolution du lignage embryonnaire chez les nématodes –*** En raison du nombre élevé d'espèces et de ses racines anciennes dans l'évolution, le phylum Nematoda est bien approprié à des études d'embryologie comparée. A l'aide d'un microscope 4D, les lignages embryonnaires de plusieurs nématodes ont été reconstruits. Cela nous a permis d'identifier les modifications de stratégie développementales dans le phylum. Généralement, il y a eu un changement dans le phylum depuis un développement non déterminé et non précis jusqu'à un développement embryonnaire très rapide et hautement déterminé.

Keywords – 4D microscopy, Cephalobidae, embryo, Mononchida, Panagrolaimidae, Rhabditida.

The phylum Nematoda has ancient roots (Vanfleteren *et al.*, 1990), large number of species (Lambshead, 1993) and sufficiently large numbers of nematodes can be cultured with relative ease. As a result, the phylum Nematoda lends itself ideally to the study of comparative embryonic development. Here we present the work underway at the University of Gent and the preliminary data on the developmental strategies in the phylum, based on the nearly complete lineage of several nematode species other than *Caenorhabditis elegans* (Sulston *et al.*, 1983). Considering the limited number of species investigated the conclusions can but be preliminary, however we believe significant insights in developmental strategies are emerging.

Material and methods

Embryonic cell lineaging of nematode embryos is achieved using 4D microscopy (Fig. 1). This time-lapse recording device allows the recording of the entire embryonic development for replay afterwards. Using the SIMI Biocell software (SIMI Biocell, Unterschleissheim, 85705, Germany) especially developed for this work, cells can subsequently be followed and the lineage tree and embryo reconstructed (Schnabel *et al.*, 1997).

Unlike the lineage determined for *C. elegans*, we currently do not follow the cells until hatching. Efficient tracing occurs up to the moment the embryo starts moving (depending on the species, around 500 cells). This lineaging is repeated completely three times for each species. Transmission electron microscopy is needed to reconstruct the remainder of the embryo beyond the point where somatic muscle contraction begins. However, since by the time of the first contraction most of the embryonic cells have reached their destination, the amount of data not recovered does not, in the species studied so far, hamper the interpretation. Additional information/confirmation of the reconstructed lineage can come from antibody labelling of tissues. However, most of the currently used antibodies have been raised against *C. elegans* tissue and not all of these antibodies cross react with other nematode species.

To date the lineage of following nematodes has been partially or completely determined: *Halicephalobus* sp. (Panagrolaimidae), *Cephalobus cubaensis* (Cephalobidae), *Rhabditophanes* sp. (Rhabditidae) and *Prionchulus punctatus* (Mononchidae).

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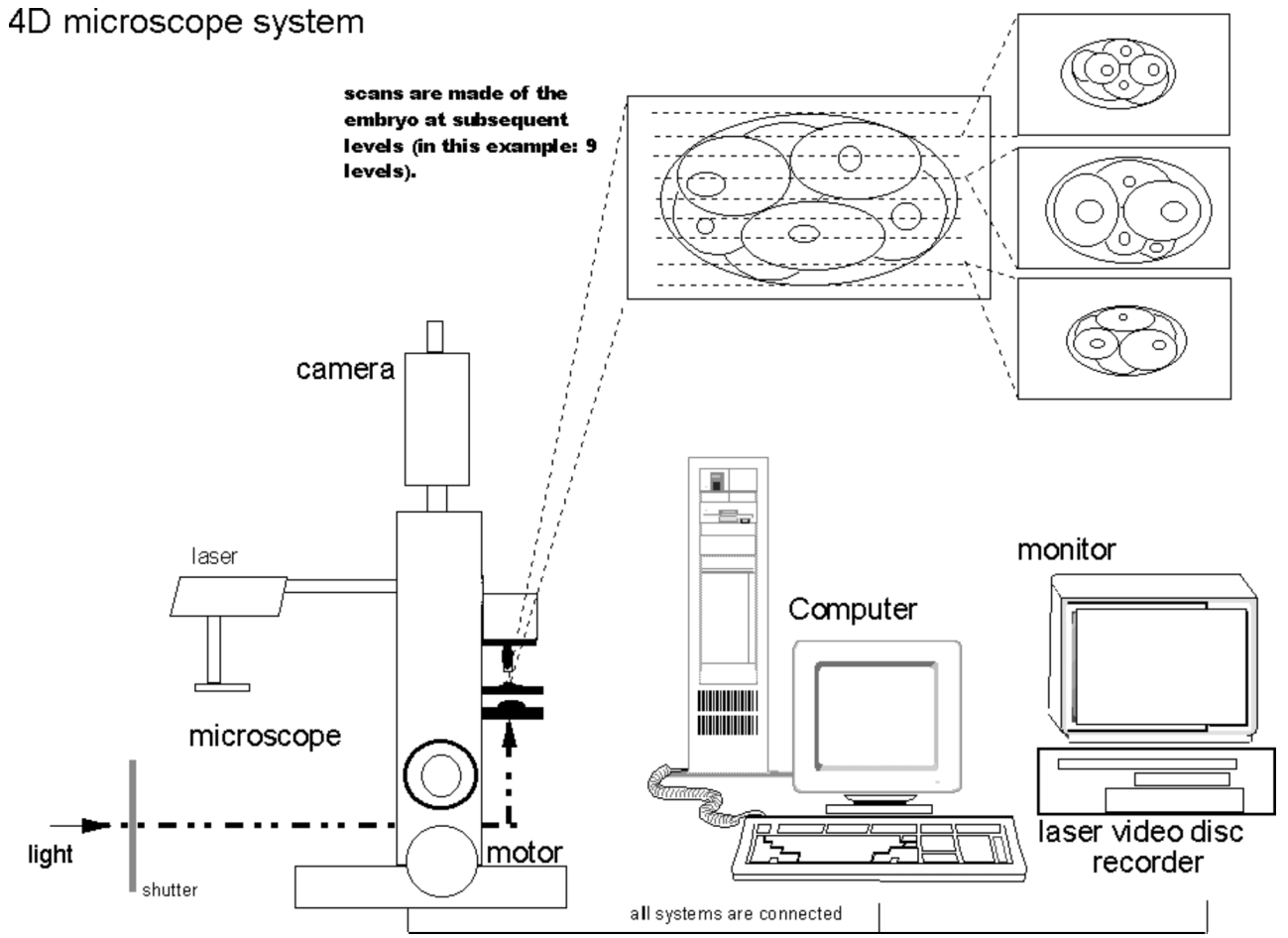

Fig. 1. *Schematic presentation of a 4D microscope system.*

Results

Halicephalobus sp., *Rhabditophanes* sp. and *C. cubaensis*

Early development. The early development of *Halicephalobus* sp., *C. cubaensis* and *Rhabditophanes* sp. is very similar in comparison to *C. elegans.* All major founder blastomeres are established and positioned very much like *C. elegans.* The major difference is in timing with *C. elegans* and *Rhabditophanes* sp. being fastest, *Halicephalobus* sp. requiring approximately twice as long and *C. cubaensis* requiring 21 h from single cell to first muscle contraction.

Cell deaths. The major cell deaths early in development in the C, MS and AB lineage are not always present in the other species. In *Halicephalobus* sp. two cell deaths occur in the Ea. All species studied have large number of cell deaths in the AB lineage later in development. However, these cell deaths occur at widely varying positions between species and often later in development than in *C. elegans.* Furthermore, the differences in cell deaths between species concern predominantly nerve cells. We therefore believe that one major reason for this variability in cell death position is that it reflects adaptations (among others) to different pharyngeal shapes requiring different routing.

Hypodermis. The origin of the hypodermis is very much like that in *C. elegans* but more hypodermal cells are formed outside AB in *Halicephalobus* sp. and *C. cubaensis.*

Body muscle. In *C. elegans* one somatic body muscle originates in the AB lineage, while the remaining muscles come from the MS, C and D blastomere. *Halicephalobus* sp. and *C. cubaensis* have exactly the same number of

muscle cells but all somatic body muscles originate from MS, C and D.

Intestine. The intestine originates in all species from a single precursor E which forms the entire intestine.

Nervous system. In *C. elegans* a minor part of the nervous system is formed by MS and C. This is not so for *Halicephalobus* sp. and *C. cubaensis* where all of the nervous system originates from AB and migrates to its final destination.

Germ line precursors. As in *C. elegans* a small cell P4 is quickly separated and becomes the germline precursor. The only difference is the amount of cytoplasm that this cell contains. In *C. elegans* the cell does contain clearly visible cytoplasm; however, in *Halicephalobus* sp. and *C. cubaensis* the P4 cell is smaller and other than the nucleus no granular cytoplasm can be seen.

P. PUNCTATUS

The embryonic development of *P. punctatus* cannot be determined using 4D microscopy as the embryo is not transparent enough to allow visualisation of deeper lying cells. Divisions were followed under Nomarski for the first few division rounds and subsequently tracer dyes were injected to map further development.

In the mononchid *P. punctatus*, the early development is fundamentally different from that of the other nematodes described here. In *P. punctatus* the first division is equal and is followed by wave after wave of equal divisions. No body axes can be determined at that stage. Microinjection with tracer dyes confirms largely the findings for the marine nematode *Enoplus* (Voronov & Panchin, 1998). The intestine is formed by a single precursor, but the other blastomeres give rise to the other different cell types but in a non-repetitive manner. This indicates that in *P. punctatus* the fates of the blastomeres are not determined initially and that this species lacks a precise cell lineage. Furthermore, it appears that cells become determined *en bloc* and migrate to their final destination afterwards. Moreover, experiments in which up to half the embryo was ablated still led to a normal, reproducing adult *P. punctatus.* However, shortly before the onset of elongation, the embryo of *P. punctatus* is very similar to that of the other nematodes at a similar stage.

In *P. punctatus* no germline precursor can be identified during early development although TEM analysis of single cell embryos has shown the presence of the P granule-like structures in the cytoplasm of *P. punctatus.* However, since half of the embryo can be ablated and a fecund worm is still formed it raises the question whether in *P. punctatus* a similar early separation of germline precursors strategy is present.

Discussion

Although the number of nematodes analysed in detail is limited so far, some assumptions can be made concerning the trends in developmental evolution within nematodes.

C. elegans and *Rhabditophanes* sp. have, in comparison to other nematodes, a rather 'chaotic' lineage in that several founder blastomeres give rise to cells with different fates close to one another in the lineage. Except for the AB lineage this phenomenon is absent in all the other nematodes. On the contrary there the lineage is made up of blocks of cells giving rise to cells with the same fate. Furthermore, in several blastomeres the anterior and posterior lineages are identical copies of each other. This is most marked in *P. punctatus* where division round after division round gives rise to an embryo with seemingly identical large blocks of cells.

We believe that in more derived forms of nematode embryonic development (*e.g.*, *C. elegans*; Fig. 2) a major evolutionary trend has been that cells originate close to where these are needed rather than be determined *en bloc* and migrate afterwards. Data to support this hypothesis come from comparing the Caa lineage in *Halicephalobus* sp. and *C. elegans* (Fig. 2). In *Halicephalobus* sp. the C lineage only gives rise to hypodermis and somatic body muscle. In *C. elegans* there is one major cell death and close neighbours form hypodermis and nerve cells (DVC and PVR). When comparing their final position these cells remain in both species more or less in the same position but their fate has changed to nerve cells in *C. elegans*, and they undergo only minor migration post-embryonically. As such they originate close to their final destination and migration is reduced.

We believe that in the ancestral state, embryos developed much like that of *P. punctatus*, with rounds of equal divisions giving rise to a large number of cells. These seemingly remain undetermined until much later in development where in *P. punctatus* and *Enoplus* (Voronov & Panchin, 1998) there are indications that large numbers of blastomeres become determined *en masse* and migrate extensively to their final destination. In the most primitive state inductions will have been very dominant in determining cell fate and most likely occurred on a large scale. This inductive regulation allowed a large degree of flexibility including the ability to compensate for loss of large parts of the embryo.

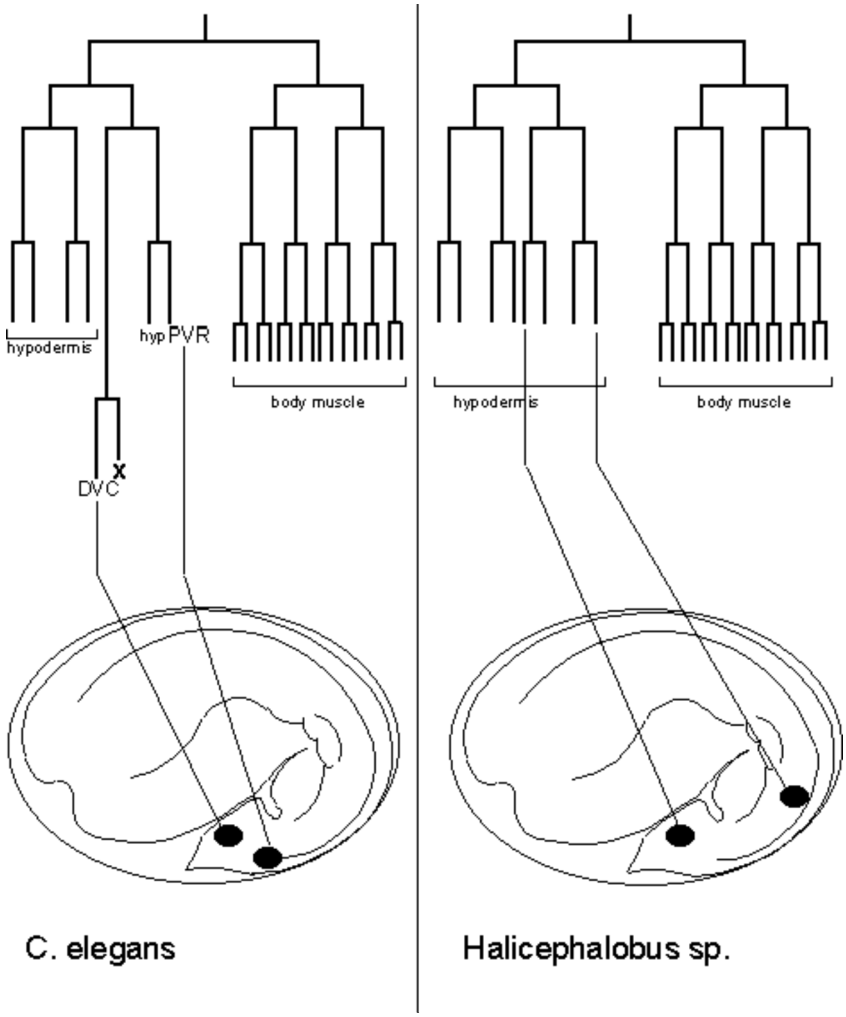

Fig. 2. *Example of change in fate of identical blastomeres in two different species. The Ca lineage of both species is shown. In* Caenorhabditis elegans *the two cells originate close to their final post embryonic position. In* Halicephalobus *sp. all nerve cells are made by the AB blastomere and migrate extensively to their final destination.*

Inductions in more derived developmental strategies became less prevalent and were replaced with more intrinsic determining factors making blastomeres more independent but less flexible, in the sense that loss of blastomeres resulted in embryonic arrest. These intrinsic factors removed the need for large blocks of cells and reduced the number of division rounds allowing embryonic development with a limited number of founder blastomeres and as such allowed faster development.

Finally, in some parts of the lineage there are strong indications that a further step in speeding up development was achieved by producing the different cells close to their final position. This latter fact may explain the somewhat illogical and chaotic lineage of *C. elegans.*

Based on the results obtained so far it becomes clear that the model system *C. elegans* has a highly derived form of embryonic development which seems to be somewhat atypical for the phylum Nematoda.

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