Morphometric analysis of cellular specification in *Platynereis* and *Pomatoceros* embryogenesis (Annelida, Polychaeta)

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ABSTRACT

Despite differences in size and yolk content, the eggs of two sibling species, *Platynereis dumerilii* and *P. massiliensis*, show the same asymmetrical mode of cytoplasmic segregation. The largest part of the yolk-free cytoplasm is shunted into the D-quadrant after second cleavage. The specific cleavage behavior of the D-cell-line suggests a causal relation between cytoplasmic specification, speed of cell cycles and determination. We have expanded our morphometric analysis to the development of *Pomatoceros triqueter*, a polychaete with equal cleavage. However, we show that small though significant size difference between the quadrants may foreshadow the future dorsoventral polarity of this embryo. The largest quadrant cleaves asynchronously with respect to the other three quadrants. As in *Platynereis*, the speed of cell cycles of blastomeres are positively correlated with the volume of cytoplasm.

RÉSUMÉ

Analyse morphométrique de spécification des cellules de *Platynereis* et *Pomatoceros* (Annélide Polychète)

Malgré des différences de taille et de contenu de vitellus, les oeufs des deux espèces proches, *Platynereis dumerilii* et *P. massiliensis*, montrent le même mode de diversification cytoplasmique. La majeure partie du cytoplasme clair et sans vitellus est répartie dans le quadrant D après le deuxième clivage. Le comportement spécifique de clivage de la lignée cellulaire D suggère une relation entre la spécification cytoplasmique, la vitesse des cycles cellulaires et la détermination. Nous avons élargi l'analyse au développement de *Pomatoceros triqueter*, polychète à segmentation égale. Pourtant nous pouvons montrer que des différences minimes bien que significatives de taille entre les quadrants seraient indicatrices de la future polarité dorsoventrale de cet embryon. Le quadrant le plus large se divise asynchroniquement par rapport aux trois autres. Comme chez *Platynereis*, les vitesses des cycles cellulaires sont reliées positivement au volume du cytoplasme.

INTRODUCTION

Embryogenesis in polychaetous annelids is regarded as a textbook example of determinative development. In many such eggs, structural animal-vegetal polarity is apparent before fertilization and foreshadows the future

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antero-posterior axis of the trochophore larva. Even in seemingly apolar eggs this first body-axis is set up shortly after fertilization (KLUGE, 1991). Apart from the emergence of polar bodies which mark the animal pole, polarity is also manifested by ooplasmic segregation leading to a polar distribution of cytoplasmic components.

In nereids, the second body-axis, i.e. the dorsoventral axis, is believed to be set up at first cleavage (WILSON, 1892). In *Platynereis dumerilii*, the first cleavage produces two daughter blastomeres of unequal size: the small AB blastomere (27% of the total egg volume) and the larger CD blastomere. DORRESTEIJN (1990) was able to show that the cytoplasmic composition of these two cells differs as well. Volumetric analysis revealed that approximately 80% of the yolk-free cytoplasm, which accumulates at the animal pole prior to first cleavage, is allocated to the large CD blastomere, despite the fact that this blastomere includes only 73% of the total egg volume. Experiments in which we have equalized these volumetric proportions led to a duplication of dorsoventral polarity, and of the trunk structures of the young worm (DORRESTEIJN, BORNEWASSER & FISCHER, 1987). These data thus support the idea that the establishment of the dorsoventral axis depends on the differential distribution of both volume and cytoplasmic contents between the first two blastomeres.

In the present paper, we describe the developmental principles encountered in the unequally cleaving embryos of two sibling species of *Platynereis* and in the equally cleaving embryo of the serpulid *Pomatoceros triqueter*. Our results indicate that the creation of cytoplasmic asymmetry among the four embryonic quadrants may set up dorsoventral polarity even in "equally" cleaving polychaetes. The data also suggest a correlation between cytoplasmic distribution and cell cycle duration in individual blastomeres.

MATERIALS AND METHODS

Platynereis dumerilii and *Platynereis massiliensis* were kept in laboratory cultures (HAUENSCHILD & FISCHER, 1969) at 18°C. The serpulid, *Pomatoceros triqueter*, was collected from the seabed round Helgoland. For *Platynereis dumerilii*, the mode of fertilization was previously described by DORRESTEIJN (1990). Fertilized eggs from *P. massiliensis* were collected from brood tubes in culture dishes. To induce spawning in *Pomatoceros* the calcareous tubes in which the animals live were opened with sturdy tweezers at the narrow, rear end. Subsequent agitation of the animal's tentacles made them crawl backwards by which they exposed themselves at the artificial hatch in the tube. Males (with pale abdomen) and females (with red abdomen) were put into separate bowls of seawater and started spawning spontaneously. The eggs were washed in seawater several times and were then fertilized with diluted sperm.

A careful estimate of cell cycle duration of individual blastomeres was achieved by evaluating several video-time-lapse recordings of the development (at 18 °C) of each of the three polychaetous species.

Procedures to collect morphometric data from serial sections (1 µm) were as described by SCHNEIDER, FISCHER & DORRESTEIJN (1992). For the small *Pomatoceros* embryos, however, we digitized every second 1 µm section in serial sections.

RESULTS AND DISCUSSION

An intricate combination of pre-cleavage cytoplasmic segregation and unequal first cleavage during normal development of *Platynereis dumerilii* creates daughter-blastomeres which differ both in size and in cytoplasmic composition. This seems an essential initial step to create and propagate cellular diversity in the nereid embryo. At second cleavage, the small AB blastomere divides equally and there is no sign of cytoplasmic diversification. The large CD blastomere which contains the bulk of yolk-free cytoplasm (see introduction), however, cleaves unequally and the largest part of this yolk-free cytoplasm is shunted into the D-quadrant. As a result of this cleavage strategy, the D-cell obtains 60 % of the yolk-free cytoplasm although it includes only 51% of the total egg volume. Again, the cleavage introduces inequality in blastomere size and simultaneously regulates the cytoplasmic composition of the daughter blastomeres with a disproportional increase of yolk-free cytoplasm in the largest cell, the D-blastomere. The cleavage pattern of the D-quadrant differs from that of the other quadrants. Two descendants of the D-cell-line, the somatoblasts 2d and 4d, are formed at fourth and sixth cleavage, respectively. Both somatoblasts are exceptionally large cells and contain yolk-free cytoplasm almost exclusively. DORRESTEIJN (1990) has shown a positive correlation between the amount of yolk-free cytoplasm and the speed of cell cycles for all blastomeres (Fig. 1). As a result of the allocation of yolk-free cytoplasm, these D-quadrant-derived cells, 2d and 4d, proliferate much more rapidly than comparable cells of other quadrants. The relatively long initial cell cycle of

2d compared to its precursor cell 1D and its progeny $2d^1$ and $2d^{11}$, despite the fact that it obtained a large amount of yolk-free cytoplasm, seems to be exceptional. DORRESTEIJN (1990) proposes that the interval between fourth and fifth cleavage may be elongated due to cellular interactions which imprint dorsoventral polarity at the 16-cell stage.

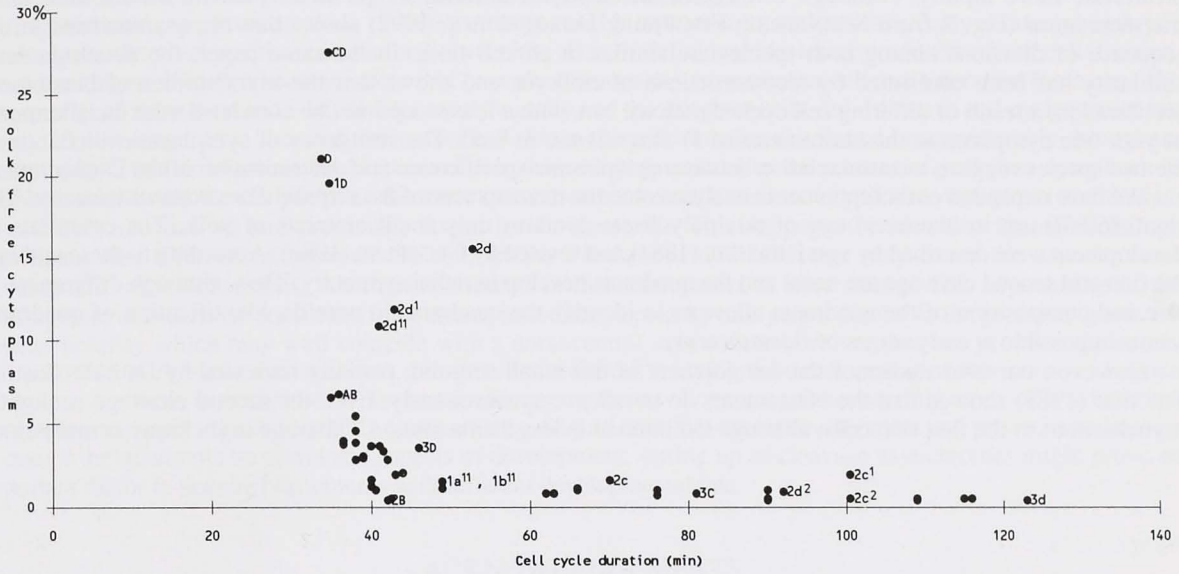

FIG. 1. — The amount of yolk-free cytoplasm in blastomeres of successive cleavage stages of *Platynereis dumerilii* is positively correlated with the rate of cell division. This diagram shows this correlation for the individual blastomeres. We have labeled some of the data points to show that CD and D-quadrant-derived cells proliferate more rapidly than other cells. However, cell cycles last longer after cells (even in the D-quadrant, e.g. $2d^2$ and 3d) have lost most of their clear cytoplasm.

Recently, we were able to show experimentally that the cleavage characteristics normal to a D-quadrant can also be observed in more than one quadrant if such quadrants contain sufficient yolk-free cytoplasm (DORRESTEIJN & EICH, 1991).

The eggs of the sibling species, *P. massiliensis*, are structurally polar from the start and extremely yolky. They have more than 10 times the volume and develop nearly four times slower than the eggs of *P. dumerilii*. Should the mode of cytoplasmic diversification found and documented by morphometric data for *P. dumerilii* be essential for determination of blastomeres, one would expect the development of this closely related species to follow essentially the same mode, even though the initial properties of such eggs are not the same. We have compared our data (SCHNEIDER, FISCHER & DORRESTEIJN, 1992) with early light microscope observations on the development of this species (WISTINGHAUSEN, 1891), which was then called the "nereidogenic form of *Nereis dumerilii*". Unfortunately, W failed to observe certain cleavages which makes the comparison of his proposed cell lineage tree with our cell-lineage results impossible.

Our comparative analysis of early development gave interesting insights into the way blastomeres become cytoplasmically diverse. The difference in size between the AB and CD blastomeres of *P. massiliensis* is less than in *P. dumerilii*. The CD blastomere of *P. massiliensis* measures 65 % of the total egg volume, yet contains approximately 73 % of the total amount of yolk-free cytoplasm. Thus, cytoplasmic diversification at first cleavage is a feature common to both species. Unlike the development of *P. dumerilii*, second cleavage of *P. massiliensis* is unequal in both blastomeres. The allocation of the yolk-free cytoplasm to the daughter blastomeres of AB is

proportional to the volumes of these cells. Although A- and B-blastomeres differ in size, there is no cytoplasmic diversification between these blastomeres. The cleavage in CD, however, allocates the yolk-free cytoplasm in proportions deviating from the relative volumes of the daughter cells with the largest amount (54 % of the total) ending up in the D-blastomere, which represents only 45 % of the total egg volume. Again, the cytoplasmic diversification of the D-quadrant is accomplished by both sibling species. The cytoplasmic specification of the D-cell-line is followed by a specific cleavage behavior differing in many respects from the other three quadrants. As in *P. dumerilii*, the somatoblasts 2d and 4d are large cells, receive almost exclusively yolk-free cytoplasm and proliferate more rapidly. Although cell cycles are nearly four times longer in *P. massiliensis*, a time scale transformation (Fig. 3 from SCHNEIDER, FISCHER & DORRESTEIJN, 1992) shows that the asymmetries in the sequence of divisions among both species are similar in all cell lines. In the same paper, the developmental similarity has been confirmed by reconstructions of embryos and shows that the asynchronies of blastomere proliferation, a result of differing cell cycle durations, can, with a few exceptions, be correlated with the allotments of yolk-free cytoplasm to the blastomeres of *P. massiliensis* as well. The stringency of cytoplasmic allocation in the two species suggests a causal relation between cytoplasmic specification and determination of the D-quadrant.

We have expanded our morphometric analyses onto the development of the serpulid *Pomatoceros triqueter*. The small (60-70 µm in diameter) egg of this polychaete contains only small amounts of yolk. The essentials of development were described by von DRASCHE (1884) and revised by GROEPLER (1986). According to these authors, the first and second cleavage are equal and the quadrants develop in radial symmetry. Thus, although differences in size and composition of the quadrants allow us to identify the quadrants in nereids, identification of quadrants seems impossible at early stages of *Pomatoceros*.

However, our own studies of the development of this small serpulid, partially reviewed by DORRESTEIJN & FISCHER (1988) showed that the blastomeres do not cleave synchronously. Even the second cleavage is slightly asynchronous in the first two cells, although the interval is less than a minute. Cleavage asynchrony is maintained

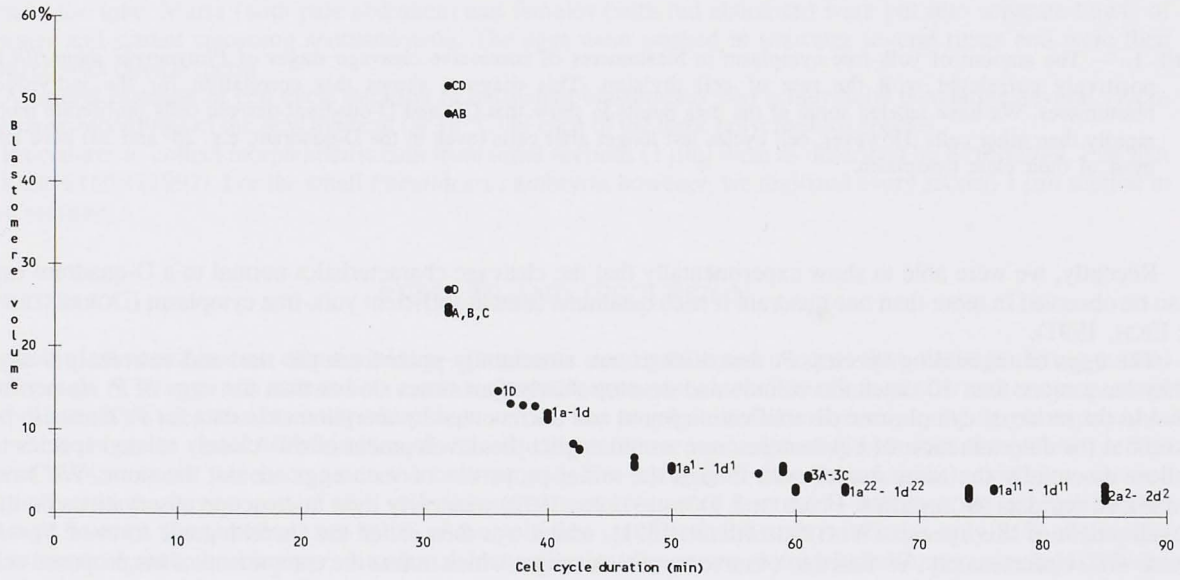

FIG. 2. — In *Pomatoceros triqueter*, blastomeres of successive cleavage stages which were studied up the 32-cell-stage show a positive correlation between the amount of cytoplasm and the rate of cell division. Since the yolk is distributed homogeneously within the cytoplasm, the blastomere volumes can be used as a measure of the amount of yolk-free cytoplasm.

and becomes more pronounced at later stages, especially among the macromeres and the different quartets of cells along the animal-vegetal axis. Morphometry of sectioned and reconstructed embryos shows small, yet statistically significant size differences between the blastomeres as early as at the two cell stage. The largest cell, which we shall call CD (according to the rules for the majority of unequally cleaving spiralians), is larger than AB by 3.5% (SD=1.3%; n=9) of the total egg volume. At the four cell stage, one of the daughter cells of CD is larger than the other daughter cell by little more than 2%, and larger than the daughter cells of AB by 3% (SD=1.9%; n=9). The size differences are maintained in the vegetal macromeres, but eliminated in the quartets of micromeres which are formed towards the animal pole during the third and subsequent cleavages.

Light microscope investigations of living embryos and morphometric analysis of serially sectioned embryos resulted in a clear and positive correlation between the sizes of individual blastomeres and the rate of cell division. From the diagram Fig. 2 it becomes clear that once the blastomeres grow smaller cell cycle duration increases. The first quartet of micromeres (1a-1d) divides nearly equally and the daughter cells, $1a^1$-$1d^1$, have cell cycles which are nearly 20 min longer than those of the mother cell. Since the yolk remains distributed homogeneously throughout the cytoplasm of the blastomeres, blastomere size must be proportional to the content of yolk-free cytoplasm in the blastomeres, and we can postulate the same correlation between the amount of yolk-free cytoplasm and the rate of cell division that we had previously found for *P. dumerilii*. This supports the idea that the small though significant size differences between the cells of the *Pomatoceros* 4-cell stage may cause the asynchronous development of its quadrants, which is reinforced in the macromeres at later stages. This asynchrony in the development of otherwise equivalent blastomeres introduces a polarity axis perpendicular to the preexisting animal-vegetal polarity which may well coincide with a dorsoventral axis which, however, has not yet been rigorously demonstrated in *Pomatoceros* embryos. For this reason we are studying the development of *Pomatoceros* beyond the stages we have investigated so far and focussing on the development of mesodermal stem cells which is a developmental aspect limited to the D-quadrant only. Should both mesoderm development and precocious cleavage in one of the quadrants be correlated aspects of development, setting up of cleavage asynchronies might prove an important factor to acquire blastomeres with different developmental fate.

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