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CELL DIFFERENTIATION AND PROLIFERATION

Effect of Aphidicolin on the Differentiation of Trochoblasts in Early Ontogenesis of Polychaetes

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Abstract—The differentiation of trochoblasts in *Nereis virens* and *Platynereis dumerilii* embryos was investigated during different periods of time after fertilization by means of the DNA replication block. It was shown that the quantal cycle of ciliogenesis is not connected directly with the time of primary trochoblast founder cell formation. Development of the cilia on trochoblasts in *P. dumerilii* needed normal DNA replication at the fifth cycle. Ciliogenesis in *N. virens* completely stopped after the inhibition of replication at the fourth cycle and only partly when the fifth and sixth rounds of DNA replication were affected. The data obtained are discussed from the standpoint of Newport and Kirschner's "depletion" hypothesis.

Key words: DNA replication, ciliogenesis, aphidicolin, cleavage, trochoblast, polychaete

Time is an essential parameter of embryogenesis. Precise timing of the elementary events of developments is necessary in order for normal embryogenesis to be realized. Attempts to understand the mechanisms providing for this schedule led to the concept of the "biological clock of development," i.e., a system measuring the duration and the time of developmental events. It was proposed that the schedule of early embryonic events is based on cyclic processes related to cell reproduction, rather than on chronological time counted from the time of fertilization.

According to one viewpoint based on experiments mostly carried out on lower vertebrates, the initiation of transcription in early embryogenesis is due to a certain ratio between the total nuclear DNA and cytoplasm. Newport and Kirschner (1982a, 1982b) put forward the "depletion" hypothesis, according to which a certain "key" process is inhibited in the unfertilized egg by a special repressor. This repressor has an affinity to DNA and is inactivated through binding to it. Therefore, as the nuclear DNA concentration increases during cleavage, that of the free active repressor not bound to DNA decreases. When a critical nucleocytoplasmic ratio is achieved, the repressor concentration is below the threshold one, thus leading to uncoupling of the "key" process and, hence, the simultaneous activation of diverse hitherto suppressed events (so-called "mid-blastula transition," for details see Rott, 1987).

According to another viewpoint related predominantly to the results of experiments with ascidian embryos, the clock mechanism of development is based on DNA replication cycles. It was shown with the help of a specific blocker of α-DNA-polymerase. For muscle differentiation, whose initial signs are found at the neurula stage, to be realized it is necessary that all cells, progenitors of the muscle clones, pass through eight cycles of DNA replication during the period of cleavage. The cells that passed through only seven cycles of replication are not capable of muscle differentiation, although their cytoplasm contains the corresponding determining factors (Satoh and Ikegami, 1981a, 1981b; Satoh, 1982; Mita-Miyazawa *et al*., 1985). It was also shown in the same laboratory that the "quantal cycle" necessary for the initiation of differentiation is characteristic of the expression of tyrosinase, a specific enzyme of the pigment cells of the ascidian larval brain (7th cycle); of the expression of alkaline phosphatase, a specific enzyme of the embryonic endoderm (5th cycle) (Satoh, 1982); of a specific muscle antigen interacting with monoclonal antibody *Mu*-2 (6th cycle) (Nishikata *et al*., 1987a); and of specific epidermal antigens (Nishikata *et al*., 1987b).

The effects of aphidicolin on differentiation during early embryogenesis was shown on echinoderms (Brachet and De Petrocellis, 1981). Stephens *et al*. (1986) established that in sea urchins, suppression of DNA replication by aphidicolin at the blastula stage before the vegetative plate thickening induces the developmental arrest before the gastrula stage; if a similar effect was produced after the vegetative plate thickening, development proceeded without the visual defects until the pluteus stage, although DNA synthesis was suppressed by no less than 90%.

In mammals, the fourth cell cycle after fertilization is recognized as quantal for the blastocyst formation (Alexandre, 1982; Spindle *et al*., 1985); the second cycle for compactization of the embryo, which normally takes place at the 8-cell stage (Smith and

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Johnson, 1985) and changes in the protein synthesis pattern at this stage depend on the normal course of DNA synthesis phase (Mayor and Izquierdo, 1994).

The presence of a critical cycle of DNA replication has been proposed in *Caenorhabditis elegans*: the results of studies of the aphidicolin effect on gene expression suggest that appearance of specific granules in the intestine cells and synthesis of carboxyl esterase, a specific marker of the intestine cells, are somehow due to DNA synthesis during the first cell cycle after separation of the intestine stem cell, since suppression of DNA replication at this time leads to the loss of capacity for expressing the above markers (Edgar and McGhee, 1988).

The necessity of normal DNA replication during cleavage for development of cilia in the *Chaetopterus* larvae was first found by Brachet *et al*. (1981). The same model, ciliogenesis, was studied in detail on the embryos of *Patella* (Mollusca) (Janssen-Dommerholt *et al*., 1983), in which the prototroph ciliary cells are formed at the 64-cell stage. Those authors have shown that differentiation of the cilia is due to a cytoplasmic factor entering the cells, which are precursors of trophoblasts, during cleavage. Therefore isolation of the corresponding blastomeres or trochoblasts does not affect the development of cilia. Segregation of the ciliogenesis factor appears to take place during the third cell cycle, since suppression of cytokinesis by cytochalasin B before the third cleavage division blocks ciliogenesis, while later this inhibitor does not prevent the development of cilia. At the same time, aphidicolin at a concentration markedly suppressing DNA replication blocks ciliogenesis, when applied at the *S*-phase of the third cell cycle, but does not prevent it when applied at later stages. The role of this "critical" cycle of replication is not quite clear, since practically full suppression of RNA synthesis by actinomycin D does not block development of the cilia in the cells forming the larval prototroch (Janssen-Dommerholt *et al*., 1983).

We are forced to state that the role of DNA replication in cytodifferentiation and morphogenesis in molluscs and polychaetes has been little studied. At the same time, these animals with well studied cell lineage and pronounced determinative developmental traits present a special interest for studies of the mechanisms underlying the temporal control of morphogenesis in animals.

This study was aimed at the investigation of the critical cycle of DNA replication in early embryogenesis of the polychaetes *Nereis virens* and *Platynereis dumerilii* as a factor of differentiation of the trophoblasts.

MATERIALS AND METHODS

Experiments on the *Nereis virens* embryos were carried out at the Marine Biological Station, St. Petersburg State University, on the White Sea in the second half of June–beginning of July. Germ cells were obtained separately from the males and females caught during spawning. Artificial insemination was performed in the laboratory (Dondua, 1975). The embryos were cultivated in a thermoregulated room at 10.5–11.0°C.

Experiments on the embryos of the Mediterranean polychaete *Platynereis dumerilii* were carried out at the Zoological Institute, University of Mainz (Germany), with the help of the laboratory culture of polychaetes (Hauenschild and Fisher, 1969). The fertilized eggs were obtained by placing a sexually mature male and female in a small vessel, where they released gametes almost simultaneously within a short time interval. The fertilized eggs were washed with sea water (Dorresteijn, 1990). The polychaetes were kept and experiments were carried out in the thermoregulated room at 17–18°C.

Experiments with aphidicolin. In order to prepare aphidicolin solutions, 1 mg aphidicolin (Sigma, USA) was dissolved in 1 ml DMSO (Serva, Germany). For the stock solution to be prepared, preliminarily filtered sea water heated at 18°C for 15 min was used. The stock solution had 10 μg/ml aphidicolin and 1% DMSO (v/v). In the experiments on *N. virens*, 9.5 ml of suspension of the fertilized eggs (about 500/ml sea water) were mixed with 0.5 ml stock solution of aphidicolin to obtain 10 ml of working solution at 0.5 μg/ml (0.05% DMSO v/v). In the control, the egg suspension was complemented with a necessary amount of DMSO to obtain the same DMSO concentration as in the experiment.

Experiments with the *P. dumerilii* embryos differed in that a drop of suspension of the embryos was added to a microvessel with 5 ml aphidicolin at 0.5 μg/ml.

Constant and transient preparations. For total preparations, Zenker and Bouin fixatives were used (the latter for *N. virens*). The embryos were stained with gallocyanin (Fluka, Switzerland) at pH 0.85. Paraffin sections 5 μm thick were stained with Mauer hematoxylin. For serial semithin sections to be obtained, the materials fixed by the Zenker fluid were stained by gallocyanin (see above) and oriented in agar plates (Dorresteijn, 1990), and then the plates were dehydrated in alcohols of increasing concentrations and embedded in araldite. The sections were made on a LKB-Nova ultratome and post-stained by methylene blue. For transient preparations, fixation after Haller was used.

Staining by bisbenzimide. The embryos were fixed by formaldehyde on sea water containing bisbenzimide (44.5 ml sea water, 5 ml 37% formaldehyde, 0.5 ml "Hoechst 33342" at 1 mg/ml distilled water). After washing twice with sea water, the materials were stored in DABCO-glycerol at –20°C.

Autoradiography. The 32-hour *N. virens* embryos were placed in 0.5 μg/ml aphidicolin and, within 13 h, transferred to a medium with ^{3}H-thymidine (specific activity 740 TBq/mmol) at 148×10^4 Bq/ml. The 45-hour embryos served as the control. The embryos

were incubated in the medium with ^{3}H-thymidine for 2 h; by the end of incubation, the experimental and control embryos were at the beginning of the rotation stage. The embryos were fixed in Bouin fluid and embedded in paraffin. After removal of paraffin, sections 5 μm thick were covered with emulsion and exposed for 24 h. The intensity of ^{3}H-thymidine incorporation in the nuclear DNA was estimated by counting the number of silver grains on autographs.

Vital observations over development of *P. dumerilii* were carried out by videography with the help of a Panasonic WV-BL6000 device with a Panasonic AG6720 recorder and an Axioskop microscope (Zeiss, Oberkochen, Germany) using the differential interference contrast optics at ×20.

RESULTS

Experiments on *N. virens*. In order to determine the minimal effective concentration, aphidicolin solutions at 0.1, 0.5, and 1 μg/ml were used. A distinct morphogenetic effect was obtained in the two latter cases. Correspondingly, the concentration of 0.5 μg/ml was used in further experiments. Starting from the 8-cell stage (11 h after fertilization), the embryos obtained from the same pair of polychaetes were placed in aphidicolin at 0.5 μg/ml successively with 1-hour interval for 15 h.

Vital observations over the appearance of cilia and motor activity of the embryos in the presence of aphidicolin gave the following results. The embryos placed in the aphidicolin solution at 11 h after fertilization did not show motor activity within five days of observations and started to die at the end of this period. In the control, the embryos reached the metatrochophore stage by this time. The embryos placed in the aphidicolin solution by 1 h later remained immobile for 2.5 days. Within 63 h after the beginning of the experiment, the embryos without prototrochs but with some cells carrying cilia could be found among immobile embryos. Short-term beating of these cilia was interrupted by a long pause. Similar pictures were observed in the variants, where treatment with aphidicolin began 13 or 14 h after fertilization. Similarly, a weak, "flickering" movement was also observed in the embryos placed in the medium with aphidicolin 15 h after fertilization. In this case, weak beating of the cilia insufficient for the larval rotation started somewhat earlier: within two days after the beginning of development. These larvae amounted to approximately 5% of the total amount of aphidicolin-treated embryos. In the variants when the embryos were treated with aphidicolin 16 h after fertilization, the mobility of individual embryos (up to 6%) was observed simultaneously with the beginning of rotation of the protrochophores in the control. The sensitivity of the embryos to aphidicolin sharply changed 1 h later: in six variants, where the embryos were taken 16, 18, 19, 20, 21, or 22 h after fertilization, their movement started simultaneously with that in the control. Just as in the control, after a certain period of rotation (protrochophore), the larvae acquired (although with a certain delay) the capacity for progressive movement (trochophore). However, in these variants, unlike those when the embryos at the age of 23, 24, and 25 h were treated with aphidicolin, the development was stopped at the stage of non-pigmented trochophore. In any case within five days when the control embryos reached the metatrochophore stage, the embryos treated at the age of 18–22 h developed into actively swimming but non pigmented ball-shaped larvae. At the same time, when the embryos were treated with aphidicolin at the age of 23–25 h, pigmented trochophores appeared.

In another experiment, the threshold of resistance to aphidicolin was observed at an earlier age: the 15-hour embryos treated with aphidicolin had only individual separated trochophore cells and the beating of their cilia could not provide for the rotation of the embryo, while the embryos treated at the age of 16 h formed a prototroch.

As was shown by analysis of the total preparations and sections, the *N. virens* embryos placed in the solution of aphidicolin within 11 h after fertilization were at the 8-cell stage. Within 1 h, the fourth cleavage division was completed. Within 13 h, the embryos were at the 16-cell stage and within 14 h the beginning of the fifth cleavage division was noted, and within 1 h (15 h of development) it was completed. Within 16 h after fertilization, the embryos passed through the sixth cycle and had more than 40 cells and within 17 h about 60 cells. In the second experiment, the development proceeded at a higher rate, so that the embryos after 15 h of development were at the end of the sixth cycle (57 cells, on the average) and after 16 h had about 80 cells, which corresponded to the seventh cell cycle.

Experiments on *P. dumerilii*. In order to determine a minimum concentration, at which the ciliogenesis-inhibiting effect is preserved, the embryos were treated with aphidicolin at 0.1 and 0.5 μg/ml at various stages of embryogenesis (2 to 12 h after fertilization). The observations have shown that the former concentration was ineffective, while the latter exerted a certain morphogenetic effect.

Six experiments were carried out to determine the stage critical for ciliogenesis, which gave largely similar results. A graph plotted from the results of one experiment is given as an example (Fig. 1). In the first three variants, when continuous incubation in the presence of aphidicolin began within 2 h 20 min, 3 h 20 min, or 4 h 15 min, the development was blocked and the embryos did not show any signs of motor activity within 21 h after the beginning of development, while in the control, early trochophores appeared by this time. In the variants, where the embryos within were treated with aphidicolin 5 h 20 min, the embryos were mobile, although the beating of cilia was periodic, with long pauses between short-term periods of motor activ-

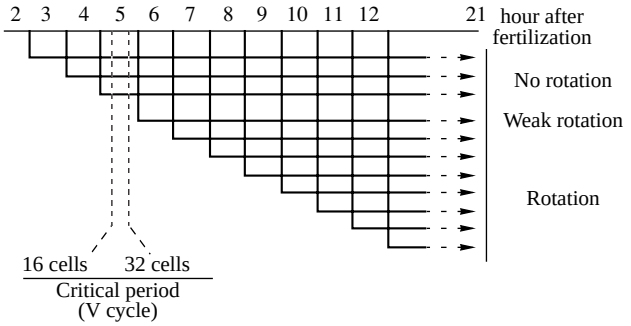

Fig. 1. Schematic diagram of experiment with the *P. dumerilii* embryos. The embryos were placed in an aphidicolin solution (0.5 μg/ml) at different moments of time (upper scale). The results of the experiments were estimated within 21 h after the beginning of development, when the control embryos reached the early trochophore stage. The fifth cycle is critical for the formation of the cilia.

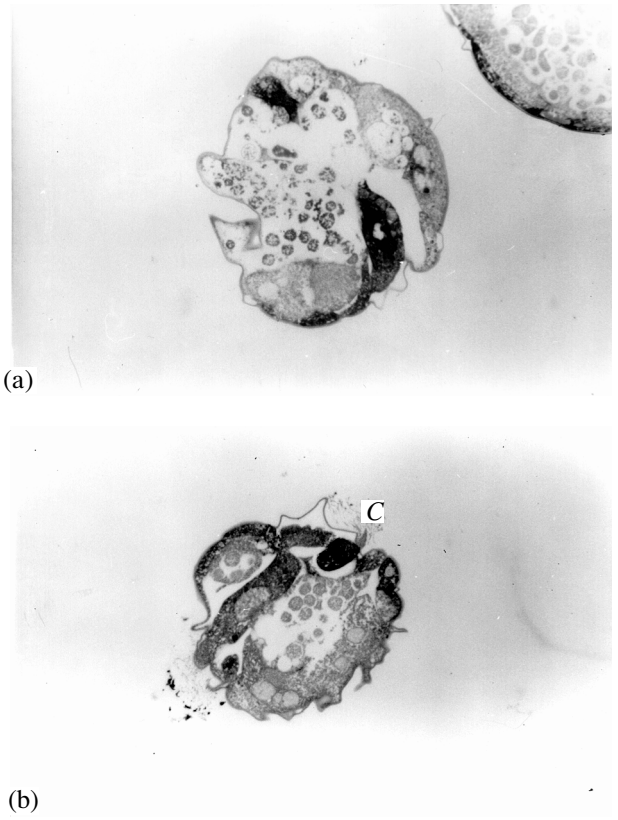

Fig. 2. Development of the trochoblast cilia in the *Platynereis* embryos, treated with aphidicolin within 30 h after the beginning of experiment: (a) the experiment was started at the 16-cell stage (4.5 h after fertilization); (b) the experiment was started at the fifth cleavage division stage (5 h after fertilization); *C*, cilia. Staining by gallocyanin and methylene blue, magnification 40 × 10.

ity. The variants with later embryos did not differ externally from the control, the rotating larvae appeared already within 21 h. Later (52.5 h after fertilization), the embryos remained immobile in the first three variants, the development stopped at the stage of rotating larvae in the fourth and fifth variants (beginning of incubation 5 h 20 min and 6 h 20 min after fertilization, respectively), while in the variants with later developmental stages, there were ball-shaped trochophore capable of progressive development. At this time in the control, the larvae reached the stage of cone-shaped trochophore. It follows from these experiments that the period between 4 h 20 min and 5 h 20 min is critical for the development of trochoblast cilia.

In the experiments, where the time interval between successive variants was reduced to 30 min, the critical period was timed between 4.5 and 5 h after fertilization (Fig. 2), although in some experiments the embryos placed in the aphidicolin solution within 4.5 h after fertilization showed weak rotation within 24 h.

In order to study the reversibility of the aphidicolin effect, the 4-cell embryos were placed in the aphidicolin solution (0.5 μg/ml). Within 0.5, 1, and 1.5 h, the embryos were washed twice with sea water. The effect of aphidicolin became irreversible already within 1.5 h after the beginning of experiment according to the presence or absence of the embryo rotation.

Since the aphidicolin concentration used (0.5 μg/ml) was close to the limiting effective concentration (the concentration 0.1 μg/ml is ineffective) and specific aphidicolin concentration per nuclear DNA in the experiments with advanced embryos was less than that in the experiments with small-cellular early embryos, we should have tested whether the absence of inhibitory aphidicolin effect on ciliogenesis is due to "depletion" of the aphidicolin solution. With this in view, the aphidicolin solutions, where the embryos were placed 3.5 and 5 h after fertilization, and then filtered within 24 h of incubation and used again. The 4-cell embryos (3.5 h of development) placed in these solutions did not show any signs of trochoblast differentiation, thus excluding the suggestion about depletion of the aphidicolin solution in variants with multicellular embryos.

Vital observations and analyses of total preparations, semithin, and paraffin sections suggest that the period critical for ciliogenesis in *P. dumerilii* is related to the fifth cell cycle. In our experiments, the embryos were at the 16-cell stage within 4.5 h after fertilization and completed the fifth cleavage cycle within 5 h after fertilization. During this latter cycle, the first division of the cells, founders of the primary trochoblasts, occurred and formed eight trochoblasts ($1a^{21}$–$1d^{21}$ and $1a^{22}$–$1d^{22}$) and separate secondary trochoblasts ($1a^{12}$–$1d^{12}$).

DISCUSSION

Cell reproduction and differentiation are sufficiently autonomous processes of embryogenesis, but nevertheless they have diverse forms of coupling, which some-

times appear to acquire strict features of cause–effect relations. This can be illustrated by the relationship between the initiation of cytodifferentiation and the number of cell cycles. In some cases, this dependence is explained by the restoration of the "somatic" nucleocytoplasmic ratio during egg cleavage, which often takes place at the midblastula stage (Newport and Kirschner, 1982a, 1982b; Newport and Dasso, 1989). Another possible explanation is the presence of a quantal or critical cell cycle possibly determined by some functional changes of DNP in a sequence of DNA replication cycles during cleavage divisions (Satoh and Ikegami, 1981a, 1981b; Satoh, 1982; Alexandre, 1982; Petzold, 1984; Mita-Miyazawa *et al*., 1985; Nishikata *et al*., 1987a; Edgar and McGhee, 1988; Zagris and Matthopoulos, 1989).

The problem of relationships between cell reproduction and differentiation in the *Spiralia* is of special interest, since in the embryogenesis of these animals, early segregation of cell clones, with a role and spatial position strictly determined by the type of cleavage, takes place. The determined state of primary trochoblasts was found in experiments with isolated blastomeres in molluscs *Dentalium* and *Patella* (Wilson, 1904; Janssen-Dommerholt *et al*., 1983) and polychaete *Nereis* (Costello, 1945). The incubation results of the molluscan and polychaete embryos in the presence of actinomycin D suggest that the prototroch formation may proceed, despite the RNA synthesis suppression, from the earliest stages of embryogenesis (*Nereis virens*—Dondua, 1975; *Patella*—Janssen-Dommerholt *et al*., 1983). This suggests the presence of the corresponding cytoplasmic factors of differentiation in the mature egg.

Janssen-Dommerholt *et al*. (1983) suggest on the basis of the results of experiments with cytochalasin that the cytoplasmic factor of ciliogenesis in the *Patella* embryos is segregated during the third cleavage division into the first quartet of micromeres. These authors consider the third cycle of DNA replication as critical ciliogenesis in *Patella*, since when the embryos were placed in a medium with aphidicolin (0.1 μg/ml) "shortly before the second cleavage," the development of cilia was fully suppressed. If incubation in the aphidicolin solution was started directly before the third cleavage division, the development of cilia was only delayed, although considerably. In the variants, when the treatment began before the fourth, fifth, or sixth cleavage division, the cilia were formed without visual deviations from the norm.

Aphidicolin induced the fragmentation of the nuclear material (Janssen-Dommerholt *et al*., 1983). We also observed chromatin fragmentation in *P. dumerilii* after staining with bisbenzimide.

The principal scheme of formation of the primary and accessory trochoblasts in the studied polychaetes is given in Fig. 3. In the *Nereis* embryos, the maternal cells of primary trochoblasts ($1m^2$) appear in the end of

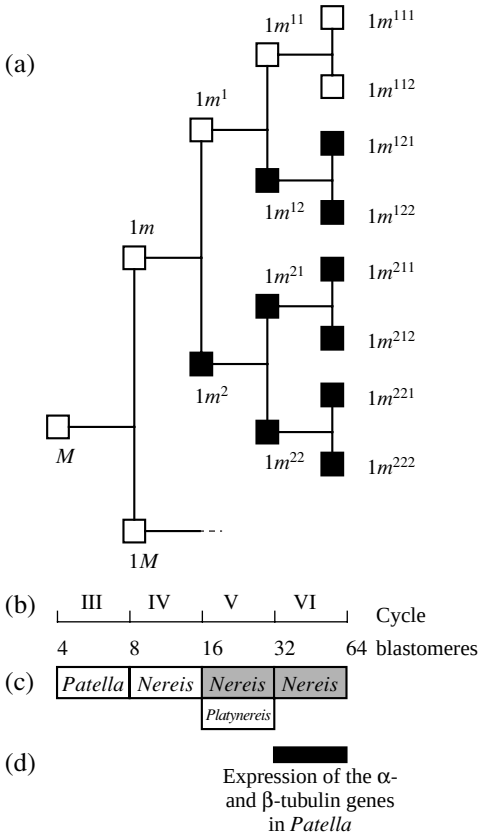

Fig. 3. Critical cycles in development of trochoblasts: (a) scheme of formation of cell lineages of the primary and accessory trochoblasts (darkened squares); (b) scale of cycles and number of blastomeres; (c) critical cycles in *Nereis*, *Platynereis*, and *Patella* (the latter from Janssen-Dommerholt *et al*., 1983); (d) beginning of expression of the α- and β-tubulin genes in the *Patella* embryos (Damen *et al*., 1995)

the fourth cycle as a result of division of the first quartet of micromeres (Wilson, 1892; current nomenclature). These cells are precursors of primary trochoblasts in *P. dumerilii*. Accessory trochoblasts are formed during the fifth cycle in *P. dumerilii* ($1m^{12}$) (Dorresteijn, 1990; Schneider *et al*., 1992). A similar picture is also observed in the *Patella* embryos (Janssen-Dommerholt *et al*., 1983).

Critical cycles for the differentiation of trochoblasts in different representatives of the *Spiralia* are presented in Fig. 3. It follows from this scheme that the cycles are not directly related to the separation of the maternal cells of primary trochoblasts $1m^2$. In *Patella*, the third cycle is critical (Janssen-Dommerholt *et al*., 1983), in *P. dumerilii* the fifth cycle is critical, and in *N. virens* the fourth cycle is critical, if the critical cycle is defined as the last cycle fully block differentiation during the suppression of DNA synthesis. This, however, is complicated by the fact that disturbed replication during the fifth and sixth cycles in *N. virens* also suppresses ciliogenesis in most embryos. While ciliogenesis takes

place, individual ciliary cells are formed instead of prototroch. From this viewpoint, both the fifth and sixth cycles can also be considered as critical for the normal development of trochoblasts.

A question arises about the relationship between the suppression of DNA replication and the blocked or disturbed development of the trochoblast cilia in the *N. virens* and *P. dumerilii* embryos. The critical cycle may be related to the expression of the specific genes of ciliogenesis. In this case, the aphidicolin-induced underreplication of DNA should have inevitably led to the disturbed function of these genes and blocked differentiation of trochoblasts. The involvement of zygotic genes in the differentiation of trochoblasts is favored by the studies on the *Patella* embryos, which suggest that the total activity of the genome is observed during the sixth cycle (van Loon *et al*., 1995). Specifically, expression of the α- and β-tubulin genes is markedly enhanced, which control synthesis of the main component of the cilia (van Loon *et al*., 1995). Moreover, expression of these genes is indeed localized in the trochoblast line age (Damen and Dictus, 1994).

It should be stressed, however, that specific expression begin when the 32-cell stage is attained and is timed to the sixth cell cycle after fertilization (Damen and Dictus, 1994), i.e., two cycles after the critical cycle, as shown in the experiments with aphidicolin (Janssen-Dommerholt *et al*., 1983).

The conclusion that the observed expression of the tubulin genes is a prerequisite of differentiation of the trochoblasts is questioned also with reference to the data that, in the *Patella* embryos, actinomycin D at 5 μg/ml suppresses the uridine incorporation in RNA of the trochoblasts by more than 90% but, nevertheless, does not prevent ciliogenesis. The trochoblast cilia develop normally even when the embryos were continuously incubated in the presence of actinomycin D at 50 μg/ml (they were placed in the inhibitor solution 1 h before the first cleavage division) (Janssen-Dommerholt *et al*., 1983). Similar data about transcription-independent development of the prototroch were also obtained for the *N. virens* embryos (Dondua, 1975). With an account of these data, the question whether expression of the tubulin genes in the *Patella* trochoblasts is an element of the mechanism of double provision, which is realized during normal development, but is not strictly obligatory and can be substituted under conditions of limited transcription, remains open and requires special analysis.

At present, the following working hypothesis can be proposed for the mechanism underlying the temporal control of differentiation of the trochoblasts. The critical cycle in the differentiation of the trochoblasts can be considered in terms of the "depletion" hypothesis (Newport and Kirschner, 1982a, 1982b). It can be proposed that the initiation of differentiation of the trochoblasts is due, first, to segregation of maternal cytoplasmic determinants of ciliogenesis and, second, to activation of these determinants when a certain critical ratio between nuclear DNA and the factor both blocking this determinant and having an affinity to DNA is achieved. Hence, ciliogenesis can be realized, at least, on two conditions: first, the ciliogenesis factor should be segregated in special cells, e.g., in micromeres at the 8-cell stage in *Patella*, in maternal cells of the primary trochoblasts in *N. virens*, and in cells of the first generation of primary trochoblasts in *P. dumerilii*; second, the trochoblasts, where this material comes to, should contain the normal (diploid) amount of DNA necessary for the blocker binding. Under the conditions of aphidicolin-induced underreplication of DNA and chromatin pigmentation, the permissible concentration of the blocker is not achieved and differentiation is suppressed. In this way, partial formation of ciliary cells in *N. virens* in the presence of aphidicolin and starting from the fifth or sixth cycle can be explained by the blocker binding in some cells.

In future studies, we propose to reveal the actual mechanisms of the delay and arrest of differentiation of the trochoblasts in the embryogenesis of polychaetes under conditions of blocked DNA replication.

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