J. Embryol. exp. Morph. 88, 249–263 (1985)
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Autoradiographic analysis of RNA synthesis in the oocyte–nurse cell complex of the polychaete *Ophryotrocha labronica*

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SUMMARY

Females of the polychaete *Ophryotrocha labronica* have been pulse labelled with [5-^{3}H] uridine, and the incorporation of label into the RNA of the oocyte–nurse cell complex was followed by light and electron microscope autoradiography. Up to its regression the polyploid nurse cell displays an intense synthesis of rRNA and mRNA, which sustains an extensive production of electron-dense protein granules in it. Concomitantly rRNA and mRNA are synthesized also in the oocyte. Short-term treatment (7 h) of polychaete females with α-amanitin provokes serious disturbances of oogenesis and subsequent embryonic development, irrespective of when it is applied during oogenesis. In contrast actinomycin gives such effects only when it is applied at the onset of oogenesis.

A previous investigation has demonstrated that nurse cell granules are gradually exported through an intercellular canal to the oocyte, where a fraction of them is incorporated into the typical yolk granules. The present labelling experiments indicate that nurse cell RNA is associated with the exported yolk precursor material. From inhibition experiments with α-amanitin and from *in situ* hybridizations with a poly(U)probe it appears that mRNA is particularly involved.

At the final collapse of the nurse cell practically all its contents are transferred to the oocyte. From that time the ooplasm is found to contain nuage-like RNA aggregates, which in contrast to other [5-^{3}H]uridine-labelled ooplasmic structures (yolk granules, and minor granules and aggregates) have a non-uniform distribution. The possible origin and function of these aggregates is discussed. The investigation indicates that the nurse cell has a significant export of RNA essentially similar to that from insect nurse cells.

INTRODUCTION

In the polychaete *Ophryotrocha labronica* rapid oocyte growth is made possible by an intimate cooperation between the oocyte and a single associated nurse cell. At the onset of the oocyte growth phase the polyploid nurse cell is larger than the oocyte, but degenerates in the final phase of oocyte maturation (Korschelt, 1894; Ruthmann, 1964; Pfannenstiel & Grünig, 1982). During oogenesis preformed materials from the nurse cell are transferred to the growing oocyte by way of a specific canal, a fusome, connecting the two cells (Emanuelsson, 1969). This

Key words: polychaete, oogenesis, nurse cell, RNA synthesis, yolk granules, autoradiography, *Ophryotrocha labronica*.

activity presupposes an intense nurse cell protein synthesis, which has also been demonstrated in labelling experiments (Emanuelsson & Anehus, 1985).

In the polytrophic and teloptrophic insect ovaries, which are also connected with nurse cells, much of the RNA in the oocyte is of nurse cell origin, whereas little or no RNA synthesis is undertaken by the oocyte during the growth phase (Bier, 1965; King, 1970; Davenport, 1976; Capco & Jefferey, 1979).

It seems likely that a similar transfer of nurse cell RNA into the oocyte should exist also in *Ophryotrocha*, but the existence of it was disputed after autoradiographic analysis of RNA synthesis in the polychaete germ cells (Ruthmann, 1964). However, [5-^{3}H]uridine labelling experiments, performed on *Ophryotrocha* females in mid-oogenesis, have demonstrated that RNA becomes associated with the yolk precursors present in the nurse cell cytoplasm (Emanuelsson & Heby, 1983). Therefore it has become necessary to re-examine the pattern of RNA synthesis in the polychaete germ cell complex, taking into special account the fact that the nurse cell material is successively exported to the oocyte, and ultimately in its entirety transferred to it.

The synthesis of RNA in the oocyte–nurse cell complex has been studied in [5-^{3}H]uridine labelling experiments, using light and electron microscope autoradiography. The experiments also included treatment with the RNA synthesis inhibitors α-amanitin and actinomycin D, which made possible a rough discrimination between synthesized mRNA and rRNA. Moreover, mRNA was identified and localized in the germ cells by *in situ* hybridization with [^{3}H]poly(U).

MATERIALS AND METHODS

Animals

The polychaete *Ophryotrocha labronica* La Greca and Bacci from the Mediterranean (Naples) is kept in sea-water cultures (20°C) at our institute. It breeds all through the year, and the interval between successive generations is about 30 days. At the age of 20–22 days the females show the first obvious signs of oocyte growth and are then kept separated from the males. At about 30 days of age the females are mated, and within 24 h they produce egg packs containing approximately 300 eggs.

Pulse labelling with [5-^{3}H]uridine

Polychaete females aged 20–22, 24–26, 28–30, and 30–32 days were pulsed in sea water with [5-^{3}H]uridine (The Radiochemical Centre, Amersham, specific activity 1·11 GBq mmol^{-1}; final activity 1·85 KBq ml^{-1}) for 3 h, followed by a 1 h chase with cold uridine. They were fixed for light and electron microscope autoradiography, either immediately after the chase or 1–2 days later.

Inhibition of RNA synthesis

Females 20–22, 24–26, 28–30 and 30–32 days old were exposed for 7 h to sea water containing α-amanitin (0·03 mM). Other females of the same age were given a 7 h exposure to actinomycin D (0·04 mM). In both cases the females were thoroughly washed in sea water after the treatment and were then, supplied with food, kept in pure sea water until maturation (32 days old). At this time they were mated. The resulting eggs were counted and their development up to normal hatching time was controlled. In some of the 24- to 26-day-old females, exposed for 7 h to sea

water containing α-amanitin (0·03 mM) the concomitant RNA synthesis was monitored at the end of the treatment period by pulsing with [5-^{3}H]uridine (3 h, 1·85 KBq ml^{-1}) followed by a 1 h chase with cold uridine. Immediately after the chase the animals were fixed for light and electron microscope autoradiography.

In situ *hybridization with [^{3}H]poly(A)*

The hybridization technique according to Capco & Jefferey (1978) was followed. Polychaete females, 20 to 32 days old were fixed for 15 min in absolute alcohol: glacial acetic acid and after rinsing in ethanol embedded in paraffin and sectioned at 6 μm. Slides with sections were first rinsed in buffer (100 mM-Tris: HCl (pH 7·6) – 3 mM-$MgCl_2$) and then treated for 1 h at 37 °C with deoxyribonuclease I (Sigma Chemical Co.), dissolved in the same buffer (100 μg ml^{-1}). After that they were rinsed once in this buffer and once in the buffer used at the subsequent hybridization (10 mM-Tris: HCl (pH 7·6) – 200 mM-NaCl – 5 mM-$MgCl_2$). The annealing solution used in the hybridization process contained [^{3}H]poly(U) (The Radiochemical Centre, Amersham; specific activity 0·74–2·66 TBq mmol^{-1}; 41–147 nucleoside residues), dissolved in hybridization buffer. Each slide was supplied with 100 μl of the annealing solution (370 KBq ml^{-1}), and was then sealed with an acid-washed coverslip and incubated for 4 h at 50 °C in a moist chamber, equilibrated with hybridization buffer. After the incubation the coverslips were removed and the slides were washed once in hybridization buffer and once in ribonuclease digestion buffer (50 mM-Tris: HCl (pH 7·6) – 100 mM-KCl – 1 mM-$MgCl_2$). Ribonuclease A (Sigma Chemical Co.) dissolved in this buffer (50 μg ml^{-1}) was used to remove uncomplexed [^{3}H]poly(U). The slides were treated for 1 h at 37 °C and were then rinsed once in buffer alone and twice in distilled water. After immersion in ice-cold 5 % trichloroacetic acid for 15 min the slides were rinsed in distilled water and air dried. Slides covered with autoradiographic film were exposed for light microscope autoradiography for 14 days. More details about the autoradiography are given below.

Controls have included slides pretreated with 0·01 N-KOH for 30 min at 20 °C, and slides pretreated with ribonuclease A for 16 h at 37 °C in a buffer, consisting of 10 mM-Tris: HCl (pH 7·6) – 10 mM-KCl – 1 mM-$MgCl_2$. The mean grain density over the oocyte nucleus in untreated slides (30-day females) was 55·9 ± 6·3. For slides pretreated with ribonuclease A it was 11·6 ± 2·4 (20·7 %), and for slides pretreated with KOH 6·0 ± 2·6 (10·7 %).

To increase resolution some slide-mounted sections after hybridization and subsequent rinsing and drying were embedded in Vestopal W on the slide, and afterwards sectioned for electron microscope autoradiography. The ultrathin sections, which comprised the superficial layer only of the original tissue section, were autoradiographed as described below.

Autoradiography

Females labelled with [5-^{3}H]uridine were fixed in 1 % osmium tetroxide according to Millonig (1961) (pH 7·4, 1 h, 4 °C). After rinsing in buffer they were dehydrated, stained in ethanol containing 1 % phosphotungstic acid and 0·5 % uranylacetate, and embedded in Vestopal W. They were sectioned for light microscope autoradiography (1 μm) and electron microscope autoradiography.

All sections for light microscope autoradiography were covered with Ilford K 2 liquid nuclear emulsion according to the dipping method. Exposure: 1 or 2 weeks at 4 °C. The autoradiographs were developed in Kodak D 19 (5 min, 18 °C), briefly rinsed in distilled water, and fixed in Kodak F 24 (6 min, 18 °C). After a final rinsing they were stained through the film with Richardson's azure II and methylene blue and mounted in DePeX.

The ultrathin sections for electron microscope autoradiography were first coated with a protective carbon layer and then covered with a monolayer of Ilford L 4 liquid nuclear emulsion according to the loop method. Exposure: 1–3 months at 4 °C. The autoradiographs were developed in Kodak D 19 (2 min, 20 °C), rinsed in distilled water (30 s, 20 °C), fixed in newly made 15 % $Na_2S_2O_3$ (3 min, 20 °C), and finally washed in distilled water. They were examined in a Jeol 100 CX electron microscope at the Unit of Electron Microscopy, Department of Zoology, University of Lund.

RESULTS

Light microscope autoradiographs

The four age groups of autoradiographed females cover the major part of oogenesis, including the whole period of vitellogenesis and the final phase in oocyte maturation. In females of the first age group, 20–22 days old, the nurse cell is still larger or equal in size with the attached oocyte. At this early stage the oocyte contains only a few yolk granules. The rapid growth of the germ cells has the effect, however, that in 24- to 26-day-old females the nurse cell has already reached its maximum size, and the oocyte has accumulated a considerable amount of yolk granules. In the 28- to 30-day-old females the nurse cell has diminished in size and its contents of cytoplasmic granules have decreased, whereas the oocyte has expanded further in consequence of the continued accumulation of yolk. In the last age group, 30- to 32-day females, the nurse cell has entirely disappeared and the oocyte has gained its final size.

At the light microscope level the intercellular canal between the nurse cell and the oocyte is not discernible. In light microscope sections clear morphological indications of a transfer of nurse cell material into the oocyte are observed first in 28- to 30-day-old females. In these an accumulation in the oocyte region near the nurse cell border of typical nurse cell granules and of basophile cytoplasm, characteristic of nurse cells, signifies such a transfer.

From the light microscope autoradiographs it appears that as long as the germ cells form an intact oocyte–nurse cell complex there is substantial labelling of the nucleolus and of the chromatin in both cell types. It is obvious, however, that until the decline of the nurse cell, nuclear labelling in it is more intense than in the oocyte. Also the cytoplasmic labelling is initially higher in the nurse cell. Labelled structures in the nurse cell cytoplasm are notably the endoplasmic reticulum, and the small cytoplasmic granules, characteristic of the nurse cell. In the oocyte cytoplasm most of the labelling is localized to the yolk granules, but silver grains also appear over small granules, similar to those in the nurse cell.

Electron microscope autoradiographs

To form a clear opinion of the association of label with the various types of cytoplasmic granules in the oocyte–nurse cell complex electron microscope autoradiographs proved necessary. These autoradiographs demonstrate that in the nurse cell of 20- to 22-day-old females the small (1 μm) electron-dense granules characteristic of the nurse cell cytoplasm are still comparatively few, as are also the yolk granules in the attached oocyte. Most of the nurse cell granules are associated with silver grains, indicating the presence of RNA. In many cases the site of the silver grains is clearly within the granules.

In the electron microscope autoradiographs of 24- to 26-day-old females the numerous electron-dense (1 μm) granules in the nurse cell cytoplasm are labelled to a great extent as are also the oocyte yolk granules, which from this stage on are the dominant structural component in the ooplasm. Morphologically the electron-

dense granules in the nurse cell differ in having either an amorphous or a granular content (Fig. 1A,B), but transitional forms exist as well. Labelling mainly occurred in those with granular content. Both types are observed also in the attached oocyte, but here the granules with an amorphous content are dominating.

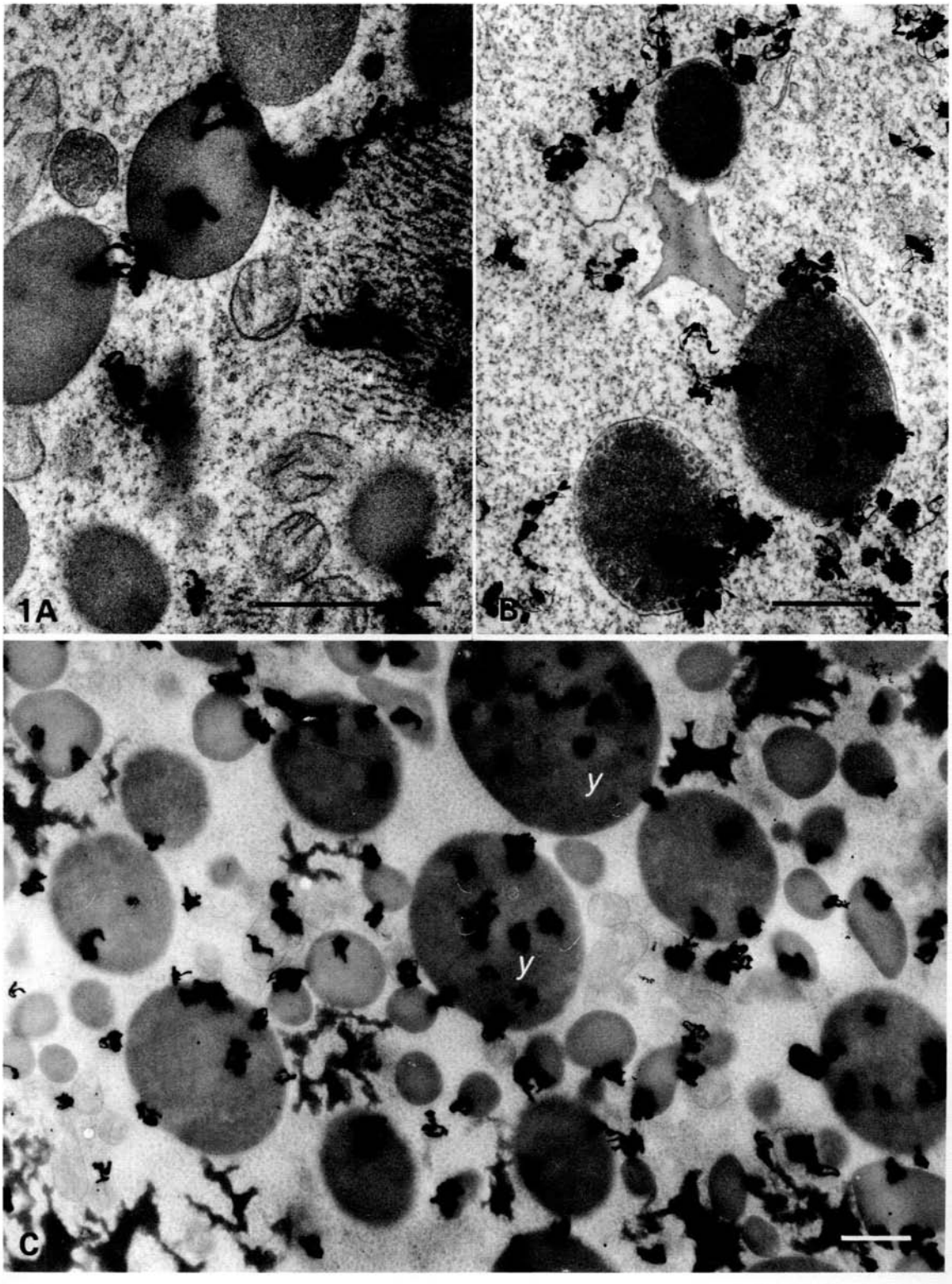
In 24- to 26-day-old females, which were fixed one day after the [5-^{3}H]uridine pulse, cytoplasmic labelling in the nurse cell is considerably lower than in females fixed immediately after the pulse. On the other hand there is substantial labelling of the oocyte with most of the label found in the yolk granules. The percentage of maturing and mature oocyte yolk granules significantly labelled has in fact increased during this interval, as seen from Table 1. Within the yolk granules labelling was evenly distributed, Fig. 1C.

Nurse cells in 28- to 30-day-old females display a decreasing number of labelled electron-dense granules, and the dominating labelled structure in the cytoplasm is rather the endoplasmic reticulum. In the attached oocyte the labelling pattern of the cytoplasm is similar to that in 24- to 26-day females, but in addition there appear two labelled structural elements not observed at the earlier stages. The first one constitutes electron-dense masses, encircled by a ring of mitochondria, and with a diameter of about 1 μm, Fig. 2A. During oogenesis mitochondria in the oocyte–nurse cell complex regularly show incorporation of [5-^{3}H]uridine, but in this case it is the extrinsically located material that is predominantly labelled. The other new element appears as circular aggregates of fibrillar structure, about 5 μm in diameter, Fig. 2B. These aggregates show a strong labelling; they are only observed in that half of the oocyte remote from the nurse cell.

Effects of RNA synthesis inhibitors on oogenesis and subsequent embryonic development

The impressive [5-^{3}H]uridine labelling of the nurse cell chromatin indicates a substantial mRNA production in the nurse cell during vitellogenesis. This activity is apparently connected with the intense protein synthesis going on in the nurse cell. However, there are reasons to suspect that much of this mRNA is intended for transport into the oocyte, to be utilized by the future embryo. The autoradiographs described above have indicated the presence of RNA in the electron-dense granules of the nurse cell and in the yolk granules, but whether the silver grains in the autoradiographs signify mRNA or rRNA is of course uncertain.

To clarify this point experiments were performed, to find out how inhibition of RNA synthesis in the oocyte–nurse cell complex would affect oocyte maturation and future embryo development. In the experiments polychaete females were exposed for 7 h to 0·03 mM-α-amanitin and 0·04 mM-actinomycin D at different stages during oogenesis. After the treatment they were kept in pure sea water until maturation (32 days of age) when they were mated. The number of eggs in the resulting egg packs was recorded, as was also the developmental ability of the embryos. The result is presented in Table 2. The latter shows that exposure of the females to α-amanitin, an inhibitor of mRNA synthesis, has a deleterious effect on the germ cells at all stages of maturation. When α-amanitin was administered


1A
B
y
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C

Table 1. *The percentage of labelled oocyte yolk granules, observed in autoradiographed* Ophryotrocha *females, pulsed with [5-^{3}H]uridine for 3 h, chased with cold uridine for 1 h, and fixed for autoradiography either immediately after the chase (day 0), next day (day 1), or two days later (day 2)*

	Days of fixation		
Age of females (days)	0	1	2
20–22	51·4 ± 6·9	72·5 ± 5·0	58·7 ± 6·0
24–26	58·2 ± 7·9	75·2 ± 4·7	70·0 ± 2·6
28–30	31·8 ± 7·2	90·2 ± 3·1	76·2 ± 7·9

The observations were made on light microscope autoradiographs (1 μm sections), exposed for 1 week at 4°C. The countings have included full-grown (4 μm) and almost full-grown (2–4 μm) yolk granules. Granules were considered as labelled when displaying three or more silver grains. Each value is a mean from ten different oocytes in two separate females.

during the first half of oogenesis, i.e. during the period when the nurse cells are still in the expansive phase, the treated females produced egg packs containing markedly fewer eggs than in the egg packs from control animals. Thus significant numbers of the oocyte–nurse cell complexes must have been completely arrested in their development. In fact extended exposure (48 h) to α-amanitin was found to suppress germ cell development totally. Interestingly the eggs actually produced by 20- to 26-day-old females, exposed for 7 h have the same ability to fulfil embryonic development as the eggs from control animals. In contrast eggs from females exposed to α-amanitin during the second half of oogenesis, were less able to develop than the controls, but the number of eggs laid was not influenced.

Actinomycin D, an inhibitor of rRNA synthesis, was found to affect germ cell development in the youngest (20- to 22-day-old) polychaete females only. Females exposed at that age, i.e. at the onset of growth of the oocyte–nurse cell complexes, produced egg packs with fewer eggs than in egg packs from control animals. Furthermore, a substantial amount of the eggs from treated animals were arrested in development before gastrulation.

Suppression of RNA labelling in the oocyte–nurse cell complex by α-amanitin

These inhibition experiments were followed by an autoradiographic analysis. Females, aged 24–26 days, were exposed to α-amanitin for 7 h, and during the last 4 h pulsed with [5-^{3}H]uridine and chased with cold uridine. Fig. 3B shows an electron microscope autoradiograph of an oocyte–nurse cell complex from a 24- to 26-day-old female, exposed to α-amanitin and [5-^{3}H]uridine. An autoradiograph from an untreated control female, subjected to the same pulse and chase of label,

Fig. 1. Electron microscope autoradiographs of labelled nurse cell and oocyte granules in a 24- to 26-day-old *Ophryotrocha* female, pulse labelled with [5-^{3}H]uridine. (A) Nurse cell granules with amorphous content. (B) Nurse cell granules with granular content. Of the two types of granules the latter appears to be more frequently labelled. (C) Labelled yolk granules (*y*) in the oocyte. Bars, 1 μm.

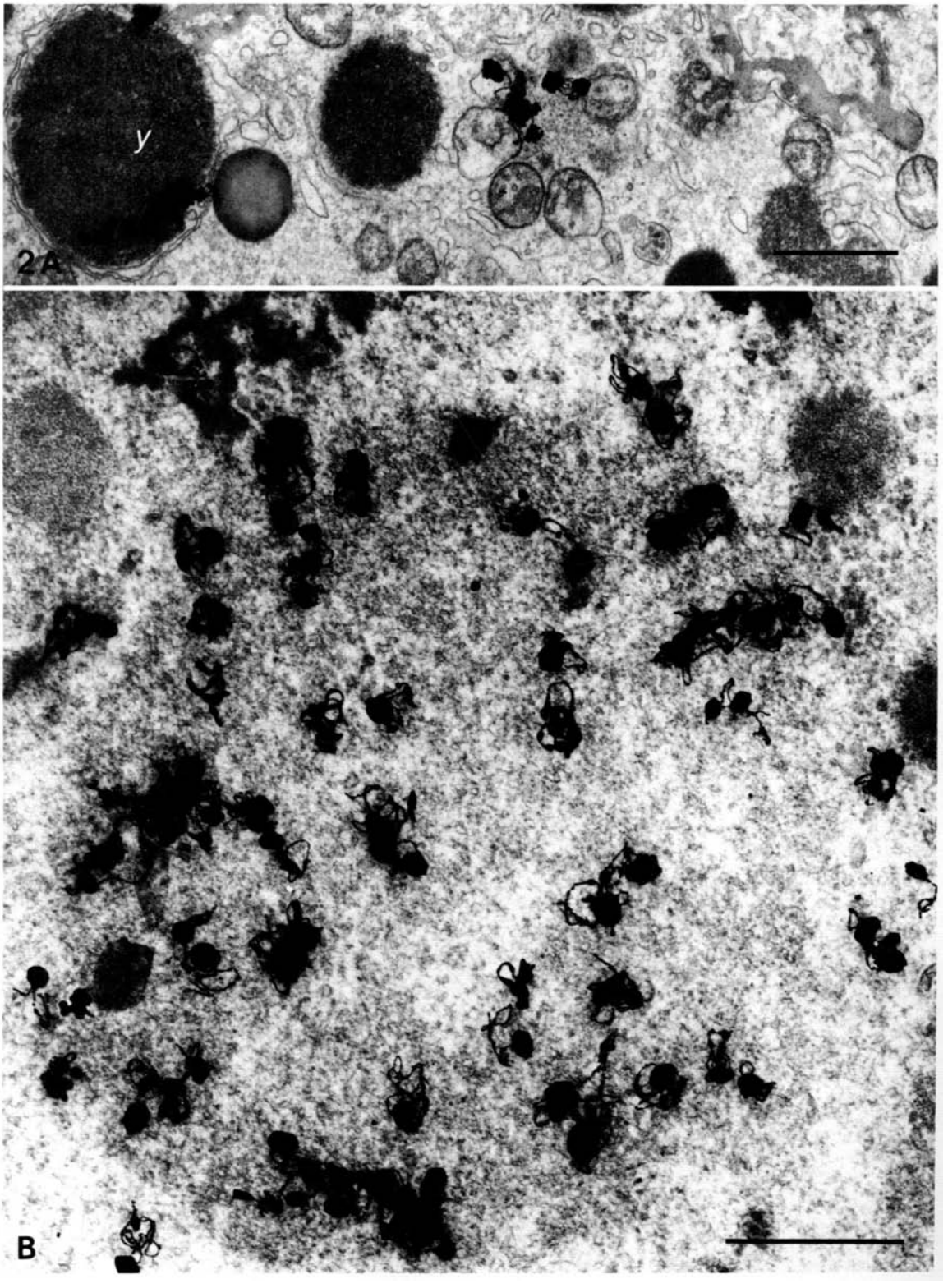

y
2A
B

Table 2. *Developmental ability of oocytes and eggs of* Ophryotrocha *females treated with inhibitors of RNA synthesis at various stages of oogenesis*

Treatment	Dose	Duration of treatment (h)	Age of females (days) at treatment	Amount of eggs in egg packs from treated females (egg packs from untreated controls: 100 %)*	Percentage of eggs from treated females reaching hatching stage (eggs from untreated controls: 100 %)
α-amanitin	0·03 mM	7	20–22	61·0 ± 4·5	80–100
			24–26	76·3 ± 7·0	80–100
			28–30	103·6 ± 4·7	30–50
			30–32	105·6 ± 6·0	30–50
actinomycin D	0·04 mM	7	20–22	67·2 ± 6·1	50–70
			24–26	102·0 ± 7·2	80–100
			28–30	105·8 ± 8·1	80–100
			30–32	95·0 ± 7·1	80–100

* The percentages represent means from 10–12 egg packs.

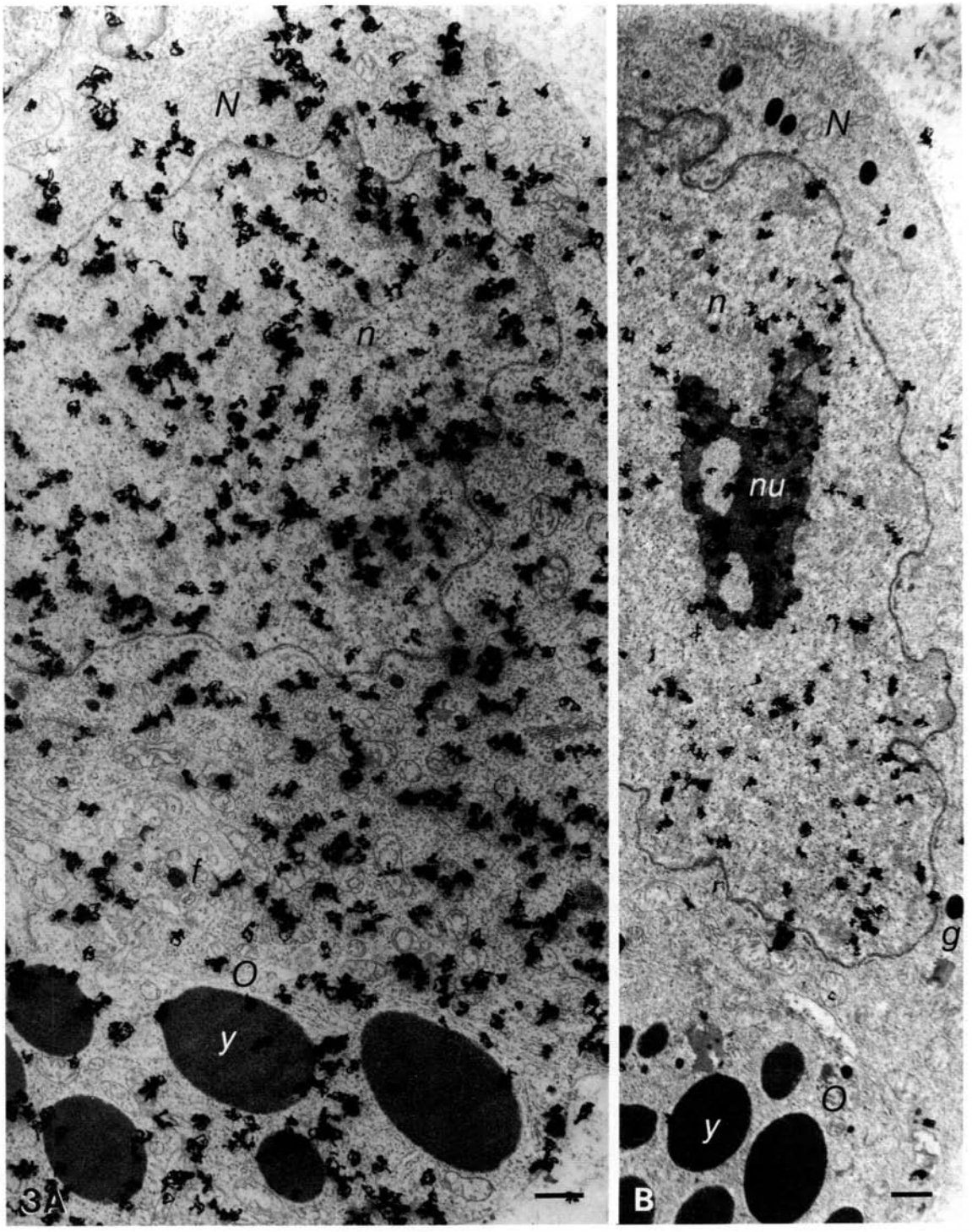
is demonstrated in Fig. 3A. In the nurse cell of the untreated animal the nucleolus and the nucleoplasm are substantially labelled. Label is also abundant in the cytoplasm, associated with the endoplasmic reticulum, mitochondria and electron-dense granules. In the nurse cell of the animal, treated with α-amanitin, the nucleolus is substantially labelled, but in the nucleoplasm and in the cytoplasm label is sparse. Notably the electron-dense granules are almost completely unlabelled.

The oocytes display corresponding labelling patterns. Thus in the oocyte of an untreated animal the nucleolus, the nucleoplasm and the cytoplasm are substantially labelled. In the amanitin-treated animal, nucleolar labelling in the oocyte is considerable, but nucleoplasm labelling is markedly reduced compared with that observed in oocytes of the untreated control. Moreover, the labelling of the oocyte yolk granules is significantly lower than in the control.

Identification and localization of mRNA in polychaete germ cells by in situ *hybridization with [^{3}H]poly(U)*

The conspicuous suppression by α-amanitin of [5-^{3}H]uridine incorporation in the nucleoplasm and cytoplasmic granules of the nurse cells indicates that a considerable part of the [5-^{3}H]uridine label observed in untreated nurse cells

Fig. 2. Electron microscope autoradiographs of labelled intracellular structures of non-granule type, observed in the maturing oocyte of a 28- to 30-day-old *Ophryotrocha* female, pulse labelled with [5-^{3}H]uridine. (A) Labelled intermitochondrial material. *y*, yolk granule. (B) Circular aggregates of fibrillar material. So far these aggregates have only been observed in the oocyte region distally from the degenerated nurse cell. Bars, 1 μm.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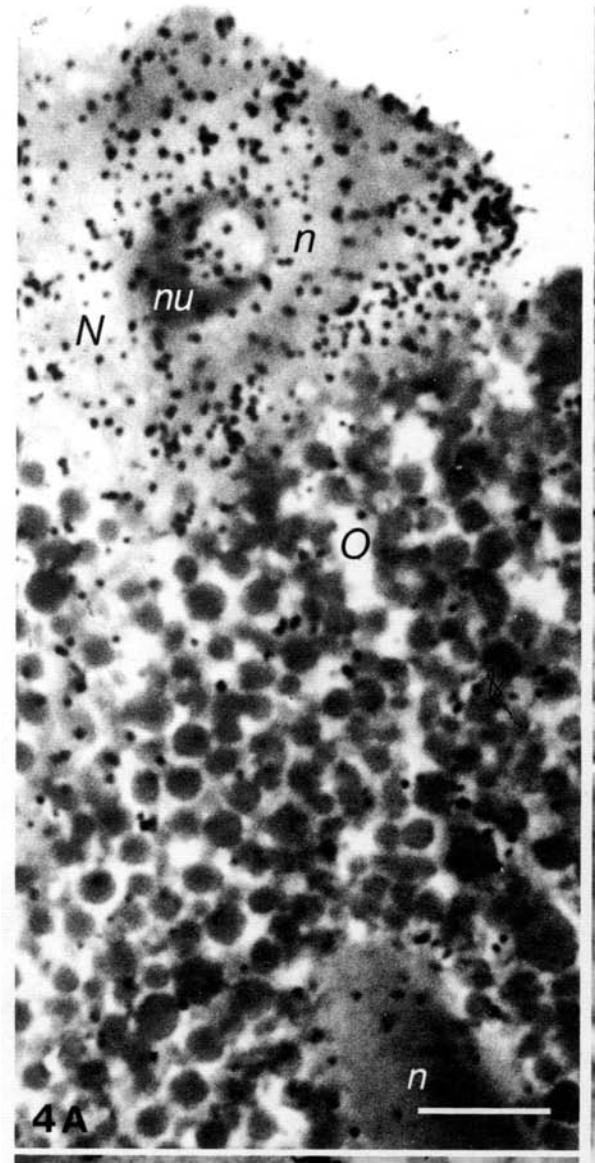
represents mRNA. This indication of substantial amounts of mRNA in the nurse cell cytoplasm was confirmed with another independent method in which poly(A)-containing RNA is detected in histological sections by *in situ* hybridization with a [^{3}H]poly(U) probe (Capco & Jefferey, 1978). The probe interacts specifically with poly(A) sequences in the section and the complexes formed are localized by autoradiography. The analyses were performed on deparaffinized sections (6 μm) from polychaete females, 20 to 32 days old.

A representative light microscope autoradiograph of a sectioned oocyte–nurse cell complex from a 24- to 26-day-old female is shown in Fig. 4A. In the oocyte–nurse cell complex silver grains, revealing the presence of mRNA, are preferentially located in the nurse cell. In the latter the majority of the grains are found in the peripheral cytoplasm. Furthermore, the grains are markedly concentrated to the cytoplasm, adjacent to the oocyte. In the nurse cell nucleus there are numerous grains in the nucleoplasm. Some grains even appear associated with the nucleolus, but closer inspection has revealed their perinucleolar location. In the oocyte there is a comparatively low number of grains both in the nucleus and in the cytoplasm. In the latter the grains are associated with 1 μm granules, occasionally also with the full-sized yolk granules. In Fig. 4B part of the ooplasm is shown in an electron microscope autoradiograph of a preparation, which after hybridization was embedded and sectioned for electron microscopy. In spite of the poor fixation it is seen that label is preferentially associated with 1 μm granules with a granular composition. Fig. 4C demonstrates localization of mRNA in the oocyte of a 28- to 30-day-old female. The nurse cell has disappeared shortly before, leaving an accumulation of mRNA just inside the oocyte wall. Fig. 4D shows mRNA distribution in and around the nucleus in a mature oocyte.

DISCUSSION

The pulse-labelling experiments with [5-^{3}H]uridine demonstrate a considerable synthesis of nucleolar RNA (rRNA) in the polychaete nurse cell and a no less extensive synthesis of mRNA, judging from the labelling of the nurse cell chromatin. The indicated mRNA synthesis in the nurse cell appears high all the time, and it is particularly noteworthy that even the regressing nurse cell maintains a high chromatin labelling. From the autoradiographs of pulse-labelled 20- to 26-day-old females an export of nurse cell RNA is scarcely suspected unless one is aware of the yolk formation process in *Ophryotrocha* and of the intercellular connection between the germ cells. Not until the onset of shrinkage of the nurse

Fig. 3. Electron microscope autoradiographs showing incorporation of [5-^{3}H]uridine in oocyte–nurse cell complexes of 24- to 26-day-old *Ophryotrocha* females in the absence (A) and presence (B) of α-amanitin. In the latter case the females were treated with 0·03 mM-α-amanitin for 7 h, and during the final hours they were pulse labelled with [5-^{3}H]uridine. There is marked labelling of the nucleolus in B, whereas the labelling of nurse cell granules and yolk granules is lacking or insignificant. *N*, nurse cell; *O*, oocyte; *n*, nucleus; *nu*, nucleolus; *g*, nurse cell granule; *y*, yolk granule. Bars, 1 μm.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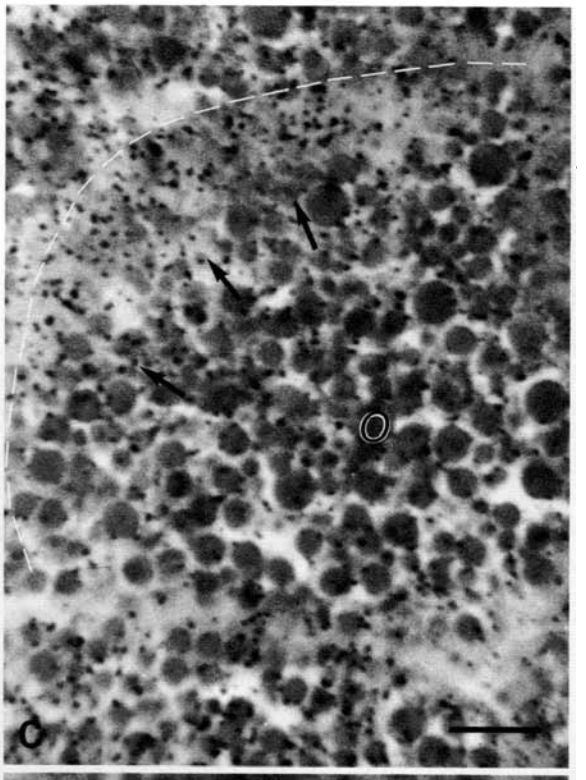
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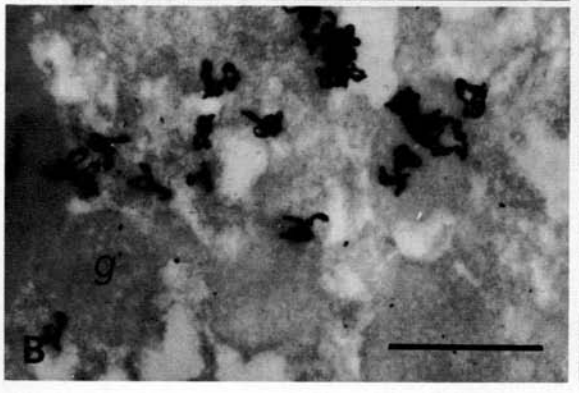
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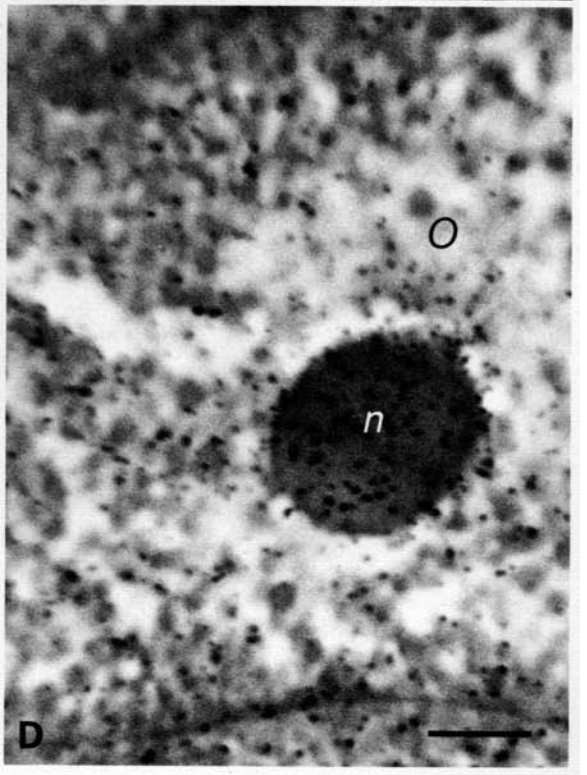
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cell (in 28- to 30-day-old females) is there a clear but moderate increase of silver grains over the oocyte region nearest the nurse cell, intimating an inflow of labelled material.

The inhibition experiments above were primarily intended to reveal how suppression of RNA synthesis at various periods of germ cell maturation affects oogenesis and early embryonic development, and they give no clear indications of the transfer of nurse cell RNA. However, they seem to confirm the temporal pattern of RNA synthesis in the germ cell complex. Thus treatment of polychaete females with actinomycin does not affect germ cell maturation or subsequent embryo development, if applied at the peak of the growth phase (24- to 26-day stage) or later. This may suggest that rRNA synthesis in the oocyte–nurse cell complex at that time is not so intense as at the 20- to 22-day stage, when actinomycin has a strong adverse effect. The serious disturbances of oogenesis and embryogenesis, which occur, whenever α-amanitin treatment was applied during the investigation period, imply a continuous, indispensable mRNA production, and the autoradiographs from these experiments confirm the great extent of the mRNA synthesis in the nurse cell. They also demonstrate that [5-^{3}H]uridine labelling of yolk granules precursors in the nurse cell, and of yolk granules in the oocyte, is strongly suppressed by α-amanitin. This finding which emphasizes the connection between the nurse cell granules and the mature yolk granules in the oocyte, also intimates that significant amounts of mRNA are normally associated with and incorporated in the yolk granules. From the α-amanitin experiments one could argue that the nurse cell contribution to the maternal mRNA store appears to be synthesized mainly from 28 days on. However, such a conclusion is then based on the unconfirmed assumption that all the eggs in egg packs from females, treated at 28 days or later, actually are alive when deposited.

Specific information about the mRNA present in the germ cells is provided by the poly(U) hybridization experiments. They support the impression of an intense mRNA synthesis in the nurse cell and verify a substantial inflow of mRNA into the oocyte from the collapsed nurse cell. However, they do not indicate presence of

Fig. 4. (A) Light microscope autoradiograph showing the localization of mRNA in an oocyte–nurse cell complex of a 24- to 26-day-old *Ophryotrocha* female. Prior to autoradiography *in situ* hybridization with [^{3}H]poly(U) was performed on the sectioned germ cell complex, resulting in labelling of the poly(A) sequences in the preparation. Most of the mRNA is localized in the nurse cell cytoplasm, particularly along the oocyte border. Due to the thickness of the section perinucleolar grains appear located in the nucleolus. *N*, nurse cell; *O*, oocyte; *n*, nucleus; *nu*, nucleolus. Bar, 10 μm. (B) Electron microscope autoradiograph of a paraffin-sectioned preparation which after *in situ* hybridization with [^{3}H]poly(U) was embedded and sectioned for electron microscopy. The preparation is of the same type as in A, and shows a central portion of the oocyte with 1 μm granules (*g*). Bar, 1 μm. (C,D) Light microscope autoradiographs of maturing oocytes of 28- to 30-day-old *Ophryotrocha* females showing the localization of mRNA after *in situ* hybridization with [^{3}H]poly(U). (C) Maturing oocyte (*O*) in which the cell material received from the degenerated nurse cell is still accumulated in the fusome region (arrows). The dotted line marks the cell border to the left. (D) Maturing oocyte (*O*) shortly before germinal vesicle (*n*) breakdown. Notice the manifest mRNA synthesis at this stage. Bars, 10 μm.

mRNA in the mature yolk granules, but that is not conclusive, as any poly(A)RNA incorporated into the yolk granules is likely to be inaccessible to hybridization.

An unequivocal statement of the kind of RNA contained in the polychaete yolk granules cannot be made on the basis of present observations. It is usually assumed that RNA contained in typical (lipoprotein) yolk granules represents rRNA. The present α-inhibition experiments have rather pointed to a mRNA content. Other indications hereof, e.g. the fact that yolk granule formation in the oocyte attains a maximum, when the nurse cell is already regressing and apparently has shut off its RNA synthesis, cannot, however, exclude a contribution of oocyte rRNA. It seems necessary to suspend judgement on this question, particularly when delayed fixation after a [5-^{3}H]uridine pulse actually reveals, that incorporation of label into yolk granules is a rather time-consuming process.

That portion of the labelled material which is associated with the numerous, scattered 1 μm granules in the ooplasm apparently represents mRNA, but rRNA is probably also present. Moreover, small aggregates of granular material, conceived as ribosomes, are frequent throughout the ooplasm.

The nature of the [5-^{3}H]uridine-labelled material, associated with mitochondria in the mature oocyte, is so far unknown. Similar aggregates have actually been reported in germ cells of other animals, e.g. teleosts (Toury, Clérot & André, 1977) and have been found to represent rRNA and tRNA.

In the mature oocyte the labelled material (RNA), whether bound to yolk granules, to 1 μm granules or to minor aggregates, seems to be rather uniformly distributed in the ooplasm. The only [5-^{3}H]uridine-labelled structures which so far have shown a non-uniform distribution in the ooplasm are the rounded heterogeneous aggregates, seen in Fig. 2B. Up to now they have only been observed in that part of the oocyte distally from the nurse cell. Since the polychaete egg cleaves spirally, and is accordingly thought to be subjected to very early determination, a localized accumulation of RNA material of this kind inspires speculations regarding a relationship between ooplasmic organization and developmental capacities. In fact the aggregates have a certain likeness with the 'nuage', observed in many invertebrates (Eddy, 1975), and which is thought to correspond to germ plasm. Moreover, the spatial location of it, i.e. in that part of the oocyte which becomes the vegetative region of the future embryo, is also consistent with such an interpretation. So far the aggregates have only been found in the mature or maturing oocyte devoid of the nurse cell.

This investigation does not support the idea put forward by Ruthmann (1964) that the polychaete nurse cell has a RNA synthesis different in principle from that in insect nurse cells. Instead it reveals obvious similarities between the two materials. Generally a nurse cell is conceived as a supporting unit only, designed to speed up oogenesis. However, the fact that the entire nurse cell cytoplasm ultimately is incorporated in its accompanying oocyte leaves the impression that nurse cells are possibly instrumental also in producing ooplasmic differentiation.

This investigation was supported by the Swedish Natural Science Research Council. The excellent technical assistance by Mrs Lena Olsson and Mrs Annagreta Petersen is gratefully acknowledged.

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(*Accepted 18 March 1985*)