Ultrastructural changes in the nuclear and perinuclear regions of the oogonia and primary oocytes of *Caenorhabditis elegans*, Bergerac strain

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SUMMARY

Electron microscope studies in and around the nucleus of *C. elegans* oogonia and primary oocytes showed fibro-granular formations whose presence is not always constant. The nucleolus, very large in the first oogenetic stages, disappeared during early diakinesis. In pachytene and diplotene, it underwent a vacuolization phase in its central part. Perinuclear corpuscules and groups of granules were limited to the vitellogenetic phase of meiotic I prophase : they were localized in the vicinity of the nuclear membrane and nucleolus. The perinuclear formations, detectable as early as germigene, remained until full ooplasmic growth. Their localization at the limit of the nucleus led to their involvement in the exchange processes which took place between the nucleolus and the cytoplasm, by way of the perinucleolar granules and corpuscules.

RÉSUMÉ

Modifications ultrastructurales dans les régions nucléaires et périnucléaires de l'oogonie et des oocytes primaires de Caenorhabditis elegans, *souche Bergerac.*

Au cours de l'ovogenèse de *C. elegans*, la microscopie électronique montre, dans le noyau (ou à proximité de celui-ci) des ovogonies et ovocytes de premier ordre, des formations fibro-granulaires opaques aux électrons dont la présence n'est pas constante. Le nucléole, très volumineux dans les premiers stades ovogénétiques, disparaît en début de diacinèse. Au niveau du pachytène et du diplotène, il passe par une phase de vacuolisation de sa partie centrale. Les groupements de granules et les corpuscules périnucléolaires se limitent à la phase vitellogénique de la prophase méiotique : ils sont localisés au voisinage de la membrane nucléaire et du nucléole. Les formations périnucléaires, discernables dès le germigène, subsistent jusqu'à la phase de grand accroissement. Leur localisation, à la limite du noyau, incite à les impliquer dans les processus d'échanges qui se produisent entre le nucléole et le cytoplasme, par l'intermédiaire des granules et corpuscules périnucléolaires.

Nucleocytoplasmatic interactions during oogenesis have been rarely studied in nematodes. They comprise the studies of Delavault (1952) on the synthesis of cytoplasmic RNA in the ovotestis of *Caenorhabditis elegans*, those of Wessing (1956) on the expulsion of the diakinetic nucleolus of *Rhabditis anomala* and the ultrastructural details observed by Yuen (1971) in *Aphelenchoides blastophthorus*. This lack of information has been detrimental to the understanding of the remarkable metabolic processes responsible for the surprising ovary activity of nematodes which is the source of their extraordinary proliferation ; for example, in the adult free-living soil nematode *C. elegans*, four eggs are laid every hour so that the full volume of the ovary is transformed into eggs every 6 hours! (Hirsh *et al.* 1976).

Nigon and Brun (1955) have pointed out the exceptionally important volume of the nucleolus during oogenesis and prediakinetic phases of *C. elegans* since its diameter can reach 2/3 of that of the nucleus. It is indeed well known that the nucleolus is the main source of ribosomal RNA which is the principal agent of cellular syntheses. Recently, Abirached (1974) and Starck (1977)

have shown a very large density of ribosomes during the initial phases of oogenetic growth in *C. elegans*. This led us to investigate in this animal, by electron microscopy, ribonucleoproteic material transfers from the nucleolus. In this study, we show that the presence of some perinucleolar inclusions as well as cytoplasmic formations adjacent to the nucleus membrane sustains this conception.

Material and methods

Material

Five-day-old autogamic protandrous hermaphroditic individuals of the free-living nematode *C. elegans* (Bergerac strain) were collected from xenic cultures reared at 18 ° C (Brun, 1966). Under these conditions, spermatogenesis had stopped and oogenesis was occuring (Fig. 1). This adult reproductive system is very simple for a metazoan and is a linear display of an axis of development from oogonia to mature oocyte.

Methods

As methods used have been described in detail by Abirached and Brun (1975), only most important technics are mentioned here :

— Nematodes were fixed by immersing them for two hours in 3 % glutaraldehyde in phosphate buffer or 0.1 M sodium cacodylate at pH 7.2. After rinsing, they were postfixed in a mixture of 1 % osmic acid in phosphate buffer or 0.01 M sodium cacodylate and 0.1 M saccharose. In both cases, swelling of structures was avoided by adding a small volume of 0.03 M CaC12.

Then, the worms were embedded in 3 % agar to facilitate accurate orientation (Wright & Jones, 1965). Agar blocks were dehydrated and embedded subsequently in ERL 4206 Spurr epoxy resin.

— Serial sections 500 to 1000 Å thick were first stained with saturated uranyl acetate in methylic alcohol (15 min.) and subsequently with lead citrate (6 min.).

Observations were made with a Hitachi 11 C at 75 kv or with a Philips EM 300 at 80 kv.

Results

Nucleolus evolution (Fig. 2)

Observations in vivo (Nigon & Brun, 1955) and of fixed material (Delavault, 1952) have shown that the nucleolus can be observed from the germinal zone till early diakinesis (Fig. 1). It consists of a large spherical mass dotted with a few clear granules. Electron microscopy enlarges these findings on the morphological level as well as the ultrastructural level.

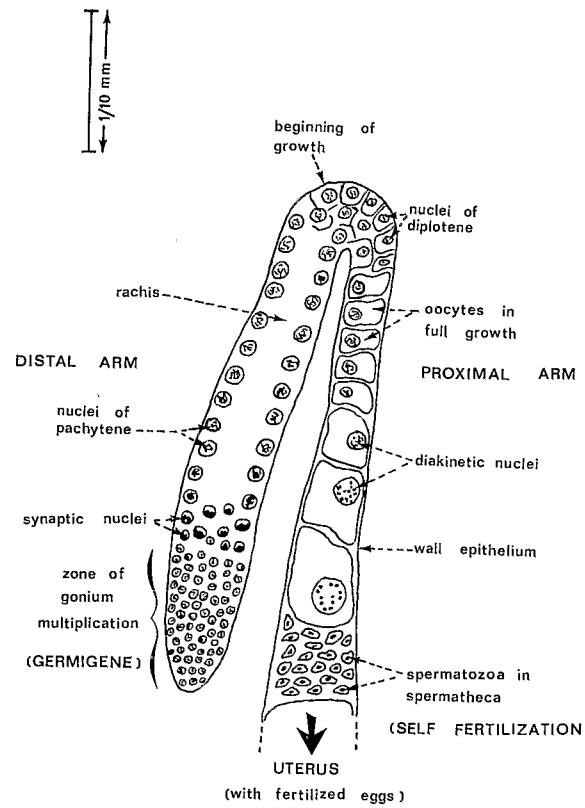

Fig. 1 : General organization of an ovarian tube in *Caenorhabditis elegans* : linear repartition of the different stages of oogenesis. (From a longitudinal section on a 5-day animal, stained with light green Feulgen, ×250).

Morphological evolution

In the germinal zone, the irregular shaped nucleolus of the oogonia, occupied half the volume of the nucleus and was situated in the

center of the nucleoplasm. It was electron dense but at times one to three empty spaces similar to vacuolar structures might be observed.

In the area where synapsis occurs (cells are in the stage of leptotene-zygotene), the nucleolus was situated at a pole of the nucleus and seemed to be in contact with the nuclear membrane (Fig. 2a). During pachytene (Fig. 2b and 3h), the nucleolus reassumed its central position. It slightly increased in size (3 μm) although the nucleus hardly increased in volume. Sometimes, the nucleolus was crown shaped and possesed one, exceptionally two, large central vacuole-like structures. In certain cases, this vacuolar structure was thrown off center and communicated directly with the nucleoplasm. Possibly this vacuolar structure results from the fusion of the empty spaces observed in the previous stages.

Near the proximal branch of the ovary, i.e. towards the end of diplotene and the early growth phase, the nucleolus was no longer central in the nucleus. Its volume remained constant whereas the nucleoplasm volume had considerably increased. The nucleolar mass, which became round and acquired regular outlines, was rarely vacuolar.

Although still present at the beginning of diakinesis (Fig. 2c), the nucleolus was no longer observed at the end of prophase I when the oocyte was situated towards the end of the growth zone. The causes of its disappearance remain obscure.

Ultrastructural evolution

During premeiotic stages and prophase I, the nucleolus continuously had a fibro-granular appearance (Fig. 2b and 2d). This granular structure was most conspicuous and constituted of fine particles of about 150 Å, i.e. the size of a ribosome. In the early stages (germigene), these granules were generally closely associated. However, at some levels, their association loosened and the particles were scattered in the empty spaces (Fig. 2b). During pachytene, these large vacuole-like areas were almost transparent to electrons. During diakinesis, the dispersion of the granules coincided with the disappearance of the periphery of the nucleolus. In those conditions, the irregular outlines of the nucleolus became blurred, suggesting the progressive dissolution of the nucleolus (Fig. 2d).

The nucleolar granules were generally associated with a weft possessing extremely thin fibrils which did not show clearly when photographed (Fig. 2d). Considering the generally close association of fibrils and granules, one might question whether ribonucleic component would be obscured by an amorphous matrix. The latter is most likely of protein nature because electron density of the nucleolus was higher after fixation with aldehyde than after fixation with osmium. This also would explain that nucleolar fibrils were better visualized after fixation with osmium.

Evolution of perinucleolar structures (Fig. 2 and 3)

Although granules similar to nucleolar granules (diameter 150 Å), scattered in the nucleoplasm, might be observed during premeiotic and synapsis stages, it was only during pachytene and more particularly during diplotene that groups of granules were visible.

During pachytene, these groups were still not clearly individualized (Fig. 2b), but during diplotene, they appeared as rings or aggregates composed of about fifteen granules ranging from 300 to 500 Å. These groups were not distributed at random. Their presence were noticed in 2 places mainly : either very near the nuclear envelope (Fig. 3i) or near the nucleolus (Fig. 3e). In the latter case, the granular group could often be found in the vicinity of a contact zone of chromatin and the nucleolus (Fig. 3f).

Small perinucleolar bodies were observed only in early diakinesis. During this stage, in the vicinity of the nuclear membrane, a spherule with a diameter of 0.15 to 0.5 μm consisting of amorphous electron dense material was often observed (Fig. 2c). Small fibrillar extrusions could come into contact with the inner membrane of the nuclear envelope.

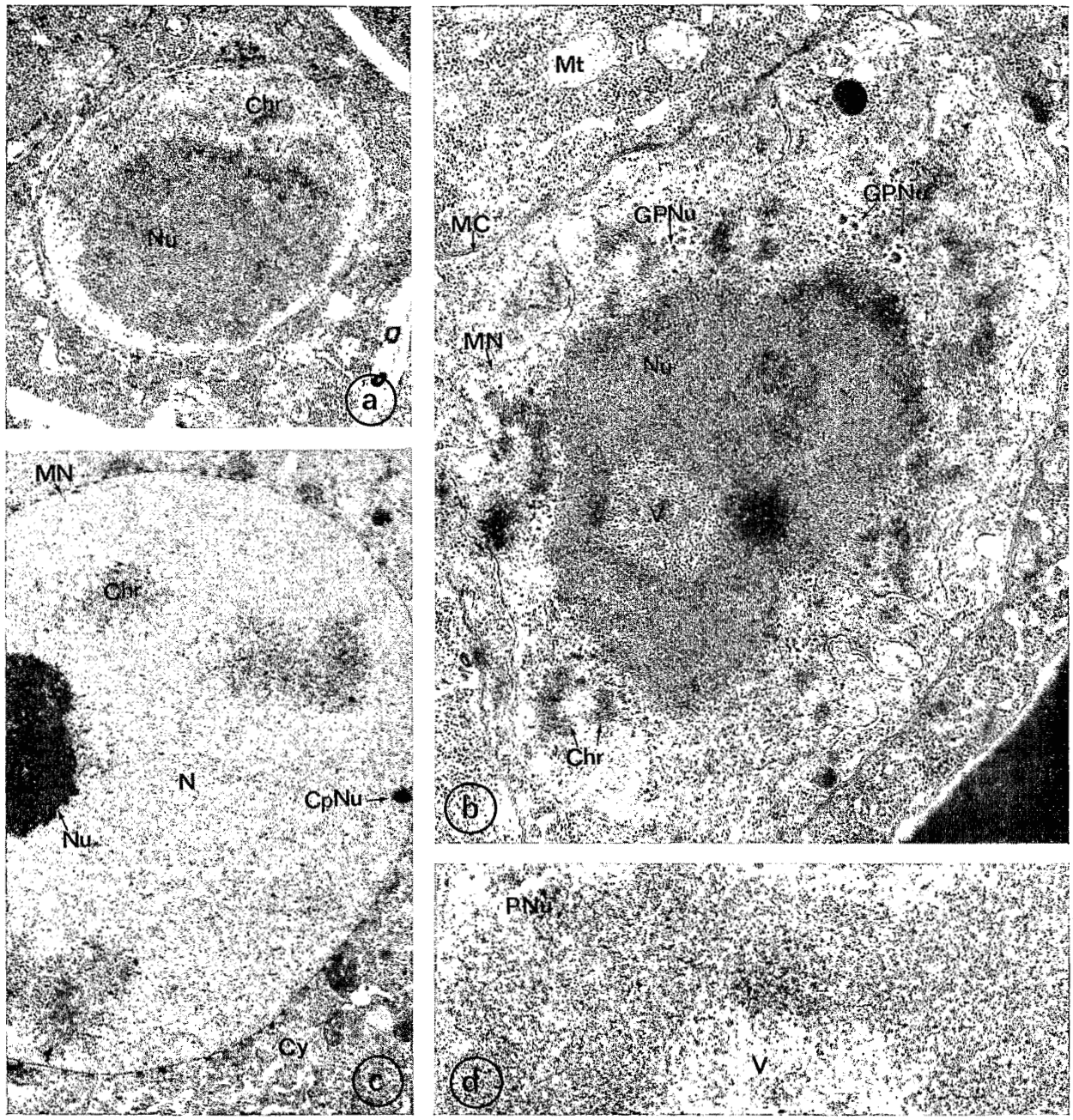

Fig. 2 : Nucleolar evolution in the meiotic I prophase.
a : Synapsis (× 22000) ; b : Pachytene (× 31000) ; c : early diakinesis (× 12000) ; d : nucleolar vacuolization (V) during diakinesis (× 48000).
Chr : chromatine ; CpNu : perinucleolar body ; Cy : cytoplasm ; GpNu : group of perinucleolar granules ; Mc : membrane of the cell ; Mn : membrane of the nucleus ; Mt : mitochondria ; N : nucleus ; Nu : nucleolus ; PNu : periphery of the nucleolus ; V : vacuolization of the nucleolus.

Changes in the interphase of nucleus and cytoplasm (Fig. 3)

The nuclear membrane

The nuclear envelope was formed by the classical close association of two membranes limiting an area transparent to electrons. Independant of the developmental stage and kind of fixation, this envelope was interrupted by numerous pores always exhibiting a granular deposit (Fig. 3h). In transversal sections, each pore appears to be a circle, almost always opaque to electrons (Fig. 3g). Sometimes however, a central granule might be clearly observed (Fig. 3g). This granule has been reported in other organisms during oogenesis : for instance in amphibians (Franke & Scheer, 1970) and birds (Schjeide, 1970).

Perinuclear deposits

During all stages of premeiosis and prophase I (except at the end of diakinesis), masses of dense fibrogranular cytoplasmic material was observed in contact with the nucleus of germ cells or in its close vicinity. It is proposed to name these structures : perinuclear deposits. During germigene, they were not easily distinguished from the rest of the electrodense granular cytoplasm, owing to ribosome accumulation. As early as the beginning of vitellogenesis, they clearly individualized, forming 5 to 7 fibro-granular stains joined to the nuclear envelope and in the invaginations that were shown when sections from whole organisms were observed (Fig. 3i and 3j). These deposits often seemed to possess a heterogeneous structure. In contact with the nucleus, they showed a dense fibro-granular structure becoming progressively looser as the distance from the surface of the nucleus increased (Fig. 3j). Finally, the granules were very dispersed, which created a light area in the vicinity of fundamental cytoplasm (Fig. 3i). At the pachytene stage only, it was possible to observe clearly the relationships between the nuclear membrane and the perinuclear deposit. They corresponded to granular rows denser in the immediate extension of the nuclear pores (Fig. 3h).

Conclusions

The electron microscopy observation of oogonia and primary oocytes during oogenesis in *C. elegans* shows the existence, in the nucleus or in its vicinity, of fibro-granular electron dense formations, the presence of which, contrarily to chromatin structures, is not constant : the single nucleolus, groups of perinucleolar granules, perinucleolar bodies and perinuclear deposits.

The nucleolus, very large in the first oogenetic stages, maintains, until its diakinetic disappearance, a dense fibro-granular structure apart from a quasi-central area of transitory vacuolization, during synapsis and pachytene. This evolution differs from that described for the oogenesis of *Ascaris lumbricoides* (Foor, 1972) and *Aphelenchoides blastophthorus* (Yuen, 1971). Indeed in *Ascaris*, the nucleolus has an essentially vesicular structure. In *Aphelenchoides*, the nucleolus has a double structure : a compact part always associated with a vacuolar part. The vacuolization mechanism, although observed in oocytes from varied animals has never received any satisfying explanation (Bernhard, 1968 ; Callan, 1966 ; Johnson, 1969 ; Lane, 1967 ; Smetana & Busch, 1974).

The perinucleolar structures are limited to the vitellogenetic phase of meiotic I prophase of *C. elegans* oogenesis. They seem to be analogous with the nucleoplasmic granules described in the primary oocytes of numerous amphibians : Axolotl (Callan, 1966), *Rana* (Massover, 1968), *Salamandra* (Franke & Scheer, 1970), *Triturus* (Lane, 1967). Their localization in the vicinity of nuclear membrane and nucleolus gives rise to the question of their origin as well as their future (see below).

The fibro-granular perinuclear deposits represent typical structures of the *C. elegans* initial oogenetic evolution. Their presence has been mentioned in oocytes I from various other organisms such as the nematode *Aphelenchoides blastophthorus* (Yuen, 1971), echinoderms and crustaceans (Kessel & Beams, 1963, 1968), prochordate (Kessel, 1966), fishes (Anderson & Beams, 1968) and mammals (Szollozi, 1965). However, our observations appear to be original on the following two points :

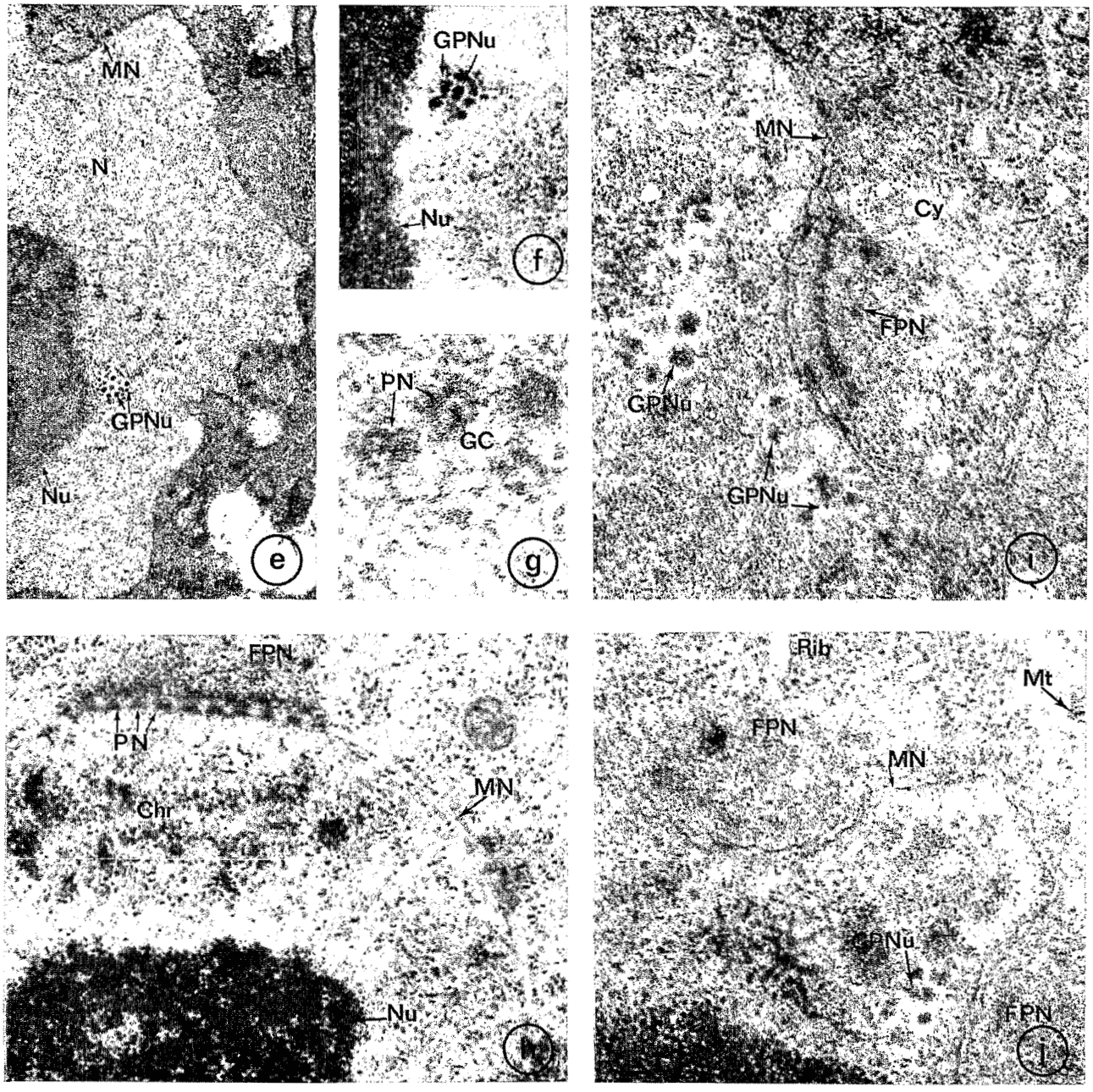

Fig. 3. Nucleocytoplasmic relationships during meiotic I prophase.
e : (× 15000) and f (× 32000) : diplotene nucleolus (Nu) and a group of perinucleolar granules (GpNu) ; g : transversal section of nuclear pores (PN) in pachytene (× 100000) ; h : nucleolus (Nu) and perinuclear deposits (FPN) in pachytene (× 40000) ; i and j : nucleolus (Nu) group of perinucleolar granules (GPNu) and perinuclear deposits (FPN) in diplotene (× 61000).
Chr : chromatine ; Cy : cytoplasm ; FPN : perinuclear deposit ; GC : central granule ; GPNu : group of perinucleolar granules ; MN : membrane of the nucleus ; Mt : mitochondria ; N : nucleus ; Nu : nucleolus ; PN : pore of the nucleus ; Rib : ribosomes.

1. Perinuclear deposits had never been observed in nematodes oogonia. Moreover, they have been very rarely observed in other organisms, since only Mahowald (1971) and Clerot (1968) described them in *Drosophila melanogaster* and in *Rana cabestiana* respectively ;

2. In *C. elegans*, they are rarely in contact and, anyhow, never systematically associated with mitochondria. It differs from the great majority of organisms, where they have been observed including the nematode *Aphelenchoides blastophthorus*. In these species, mitochondria tend to gather in perinuclear formations : the so-called mitochondrial cement.

Discussion

Role of perinuclear and perinucleolar structures in nucleocytoplasmic interactions

Nucleoplasmic interactions, which differ according to type of concerned cells, are equally variable within a same cell with respect to its physiological state. They are particularly intense in cells possessing a great anabolic activity which manifests itself by perinuclear accumulations. They have been described for somatic cells as well as for some oocytes (Goldstein, 1974 ; Sidebottom-Deak, 1976). Generally, these structures are involved in processes of ribonucleoproteins transfers. Indeed, the cytoplasmic RNA synthesis, which occurs inside the nucleus, supposes its ulterior transport into the cytoplasm. Nevertheless, a small number of authors, among whom nematologist Wessing (1954) considers that they are a particular manner of chromatin elimination from the nucleus. The concept of DNA extrusion has been the subject of much controversy : Hadek and Swift (1962) even considered it the likely manifestation of a degeneration. Anyhow, this cannot be the case for the oogenesis of *C. elegans* because the cytoplasm is never stained by the Feulgen reaction. The hypothesis of the interference of perinuclear formations in transfer of RNA from nucleus into cytoplasm can be justified by three arguments :

1. The similarity of ultrastructures of nucleolus and perinuclear deposits on one hand, and ribosomes constituting the major part of cytoplasmic RNA on the other hand.

In *C. elegans*, the female germ cell is always very rich in ribosomal particles which are a sign of a large quantity of rRNA. Moreover, this granular ultrastructure also characterizes the perinuclear formations and the nucleolus. Besides, the nucleolar and perinuclear granules have the same size as the ribosomes (about 150 Å).

2. Parallel evolution of nucleolus and perinuclear structures.

In *C. elegans*, perinuclear structures are still persistent in cytoplasm in contact with the nuclear membrane, as long as the nucleolus is present. This tends to prove a relationship between these accumulations and the nucleolus before the latter disappears during diakinesis. It should be noted that these relationships seem rather typical of this nematode, because it would be different for the crab (Kessel & Beams, 1968) and *Rana* (Eddy & Ito, 1971) in which oocytes involved in vitellogenetic synthesis, no longer show any sign of perinuclear formations.

3. Existence of perinucleolar components susceptible to represent structures intermediate between those formed by nucleolus and perinuclear deposits.

Granular groups observed in nucleoplasm around nucleolus are often present in oocytes (cf. supra). They have also been described in cells possessing a biosynthetic activity such as interphase cells of mammals (Monneron & Bernhard, 1969), salivary glands cells of dipterans (Stevens & Swift, 1966) as well as liver cells of mice and rats (Watson, 1967). They have always been identified as some ribonucleoproteic material. Yet, in the case of *C. elegans*, their localization in the immediate vicinity of the nucleolus is the only argument in favour of their nucleolar source.

Possible modalities of RNA transfer from nucleolus to cytoplasm during initial phases of oogenesis in *C. elegans*

During oogenesis of *C. elegans*, the nucleolus really seems to be the main center of ribosomal

RNA release. However, usually, this biosynthetic activity is admitted to occur during interphase (Ghosh, 1976 ; Perry, 1969). But this idea, based on the study of mitosis can, in the case of oocyte cells, most likely be extended to prophase I during which we can observe the persistence of a large nucleolus. In the case of *C. elegans*, this is confirmed by various morphological and ultrastructural characteristics of the nucleolus. First of all, it is the large diameter of the nucleolus compared to that of the nucleus, which is observable as early as the beginning of prophase I. Then, during pachytene, the transitory vacuolization of the nucleolus and mostly its ring-like aspect which can be observed in most developing cells. Finally, the predominance of granules in the nucleolus is significant in itself. Indeed, in amphibians, only aged oocytes possess nucleoli with granules and fibrils, the young oocytes having only fibrils (Lane, 1967).

These fibrils, according to Bernhard and Granboulan (1968), would be at the origin of the granules which would represent a more advanced form of RNA synthesis intented to be eliminated from the nucleolus.

The existence of the nucleolar source of ribosomal precursors implies the presence of substances released by the nucleus. This should correspond to perinuclear deposits which have at time been called pseudo-nucleoli, particularly when their outline and their fine structure closely resembles those of the nucleolus : this is the case of the oocytes of a trematode (Koulish, 1965), and of crikets. In *C. elegans*, the development of these perinuclear structures in a same oocyte, shows that, as the distance from the nuclear membrane increases, their aspect becomes progressively less dense, less granular and ends, during pachytene, with a reticulated structure. It seems as if a constitutive element, probably RNA, is released in the cytoplasm, while separating from the protein material which served as its vehicle. Stevens and Swift (1966) and Clever (1967) have already considered this process.

As far as the origin of these perinuclear ribonucleoproteic granules is concerned, they would originate from nucleolar granules moving through nucleoplasm towards the nuclear membrane, under the form of perinuclear bodies. The warped position of the nucleolus which progressively approaches the nuclear membrane would favour the acceleration of the extranuclear release of its components. This elimination of nucleolar material during oogenesis is nothing exceptional. Indeed, in nematodes, Wessing (1956) mentions the extrusion of the nucleolus in *Rhabditis anomala*. It has also been described in an echinoderm where the dissociated fragments of nucleolus are released after a rupture of the nuclear membrane (Kessel & Beams, 1963). This transfer of material seems to occur very rapidly, hence the lack of nuclear deposits is parallel to the cytoplasmic deposit. Nevertheless, one wonders whether a certain accumulation in the form of an amorphous body of 0.05 μm observed in contact with the nuclear membrane could not result from the fusion of the elements of a group of perinucleolar granules. The last argument in favour of our hypothesis is constituted by the fact that the pores of the nuclear membrane sometimes shows, in tangential section, a central granule : this could be an image of the passage of material through the nuclear membrane (Franke & Scheer, 1970).

Our hypothesis that nucleolar RNA is transfered into cytoplasm to constitute ribosomes, via intermediate forms : perinucleolar granular structures and perinuclear deposits, is actually subjected to kinetic studies with radioautographic technics. First results (Starck, 1977) supporte this view.

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