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CYTOCHEMICAL STUDIES ON NUCLEOLUS AND ITS EXTRUSIONS IN *CLIBANARIUS LONGITARSUS* (ANOMURA : CRUSTACEA)

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ABSTRACT

Many nucleolar buds have been observed in the nucleus of the early oocytes of *Clibanarius*. Some of them are extruded in the cytoplasm through the nuclear membrane. They fragment into fine granules containing ribonucleoproteins. There is a decrease in pyroninophilia in the ooplasm with the advent of appearance of compound yolk. RNA present in nucleolar extrusions is utilised in protein yolk synthesis. Nucleolus and nucleolar extrusions contain proteins (-NH_2 and -SH groups; and tyrosine), RNA and lipoproteins.

INTRODUCTION

ALTHOUGH some work has been carried out on the nucleolar extrusions in crustaceans by a few workers (Raven, 1961; Nath, 1968; Norrevang, 1968), yet this type of studies have not been dealt with in anomurans. Thus the present work was undertaken to know the cytochemical nature of nucleolus and nucleolar extrusions in *Clibanarius longitarsus* and their role in yolk formation.

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MATERIAL AND METHODS

Clibanarius longitarsus the hermit crab, found in the shells of molluscs, was collected from the standing waters at Shingle and Krusadai Islands of Tamilnadu.

The female specimens were vivisected and their ovaries were fixed in Zenker, Bouin's fluid, Carnoy's fluid. These were dehydrated, cleared and embedded in paraffin wax of 60-62°.

Zenker and Bouin's fixed sections were stained in Heidenhain's haematoxylin to study the histology. Various cytochemical studies were performed according to the procedures detailed in Pearse (1968) to know the sites of proteins, lipoproteins and nucleic acids in various stages of oocytes.

OBSERVATIONS

In *Clibanarius* the nucleus in the young oocyte is large and circular, but the cytoplasm is little (Pl. I A). But when the ooplasm has grown and the yolk platelets have appeared the germinal vesicle is comparatively smaller.

The chromatin is slightly pink in Feulgen (negative after control) revealing the presence of DNA. This disappears quite early during the period of previtellogenesis. The nucleus is darkly positive in mercuric-bromphenol blue (Hg-BPB) (Pl. I A), negative in Sudan black B for lipids.

A few nucleolar buds have been observed on the nuclear periphery in the nucleoplasm in the younger oocytes. A few of them have also been observed in the cytoplasm (Pl. I A). The tendency of the nucleolus is towards the nuclear membrane in early oocytes (Pl. I A), but it starts becoming centric during vitellogenic phase (Pl. I B).

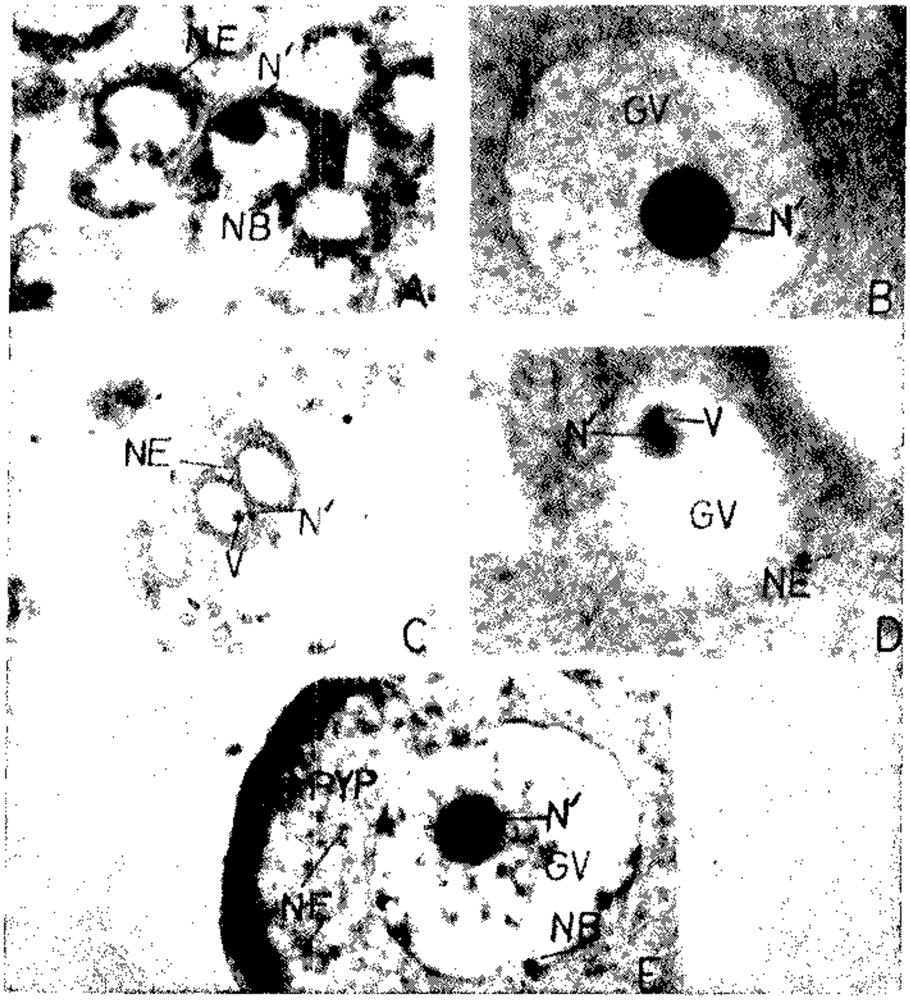

PLATE I A. Very young oocytes. Zenker/Mercuricbromophenol blue. X 1250, B. Advancing oocyte. Zenker/Methyl green-pyronin G. X 1250, C. Young oocytes showing vacuolar nucleolus. Zenker/Methyl green-pyronin G. X 1250, D. Advancing oocyte. Zenker/Methyl green-pyronin G. X 1250 and E. Advanced oocyte showing origin of protein yolk. Zenker/Mercuric-bromophenol blue (X 1250). (GV: Germinal vesicle; N': Nucleolus. NB: Nucleolar buds; NE : Nucleolar extrusions; PYP : Protein yolk precursors; V: Vacuole).

To begin withthe nucleolus stains uniformly in haematoxylin and Hg-BPB (P. I A), but it shows vacuoles in methyl green/pyronin G (MG/PG) (Pl. I C). During growth period of the oocyte it develops vacuoles which are negative in MG/PG and Hg-BPB)Pl. I) B, E).

The nucleolar emissions in the anomuran are large granules which seem to pierce the nuclear membrane (Pl. I A-E). They lie in the perinuclear region and are strongly pryroninophil (Pl. I C) (negative after control). The nucleolar bodies start fragmenting and dispersing towards the cortical ooplasm and are ultimately found evenly distributed in the ooplasm (Pl. I B, D). At this stage the ooplasm shows much less of basophilia. The Protein synthesis starts in the cortical ooplasm and the nucleolar emissions start showing decrease in their basophilia in this region. As the protein yolk starts appearing in the whole ooplasm, the ooplasm becomes less basophilic and ultimately the basophilia disappears completely with the advent of yolk bodies. No basophilic granules (nucleolar extrusions) have been traced in the mature oocyte.

The nucleolus and nucleolar extrusions are positive in acetone-Sudan black B, revealing the presence of lipoproteins in them; positive in ninhydrin-Schiff, ferric-ferricyanide, blue in alkaline terazolium, pink in Sakaguchi, revealing the presence of $-NH_2$, -SH groups of proteins; and arginine respectively; positive in Millon and coupled tetrazonium (positive after performic acid and negative after benzoylation and di-nitrofluorobenzene controls), revealing the presence of amino acid, tyrosine.

Discussion

During the present observations many nucleolar buds have been observed in the younger oocytes. These observations are in conformity with those of Bhatia and Nath (1931) in *Paratelphusa and Palaemon*. Dense bodies which stain similar to the nucleolus have been found in the ooplasm along the nuclear periphery in *Clibanarius*. Such bodies have also been reported by Hilton (1931) in *Calanus* and Heberer (1930) in *Eucalanus*.

The cytoplasm of younger oocytes of *Clibanarius* shows a few nucleolar extrusions. In the developing occytes the cytoplasm is invaded by nucleolar extrusions but the nucleolus shows vacuoles which are not stainable with MG/PG. The vacuoles in nucleolus have also been observed by Fautrez and Fauterz-Firlefyn (1951) in *Daphnia*. This reveals that the nucleolar extrusions carrying ribonucleoproteins are extruded into the cytoplasm through the nuclear membrane. These observations are in agreement with Fautrez-Firlefyn (1957) in *Artemia;* Montefoschi and Magaldi (1953) in *Asellus;* and Fautrez (1958) in *Cyclops;* Bhatia and Nath (1931) in *Paratelphusa* and *Palaemon;* Sareen and Monga (1970) in *Porcellionides* and *Cubaris;* and Bonina (1974) in *Palaemon adspersus*. With electron microscopy, it has been confirmed that the nucleolar extrusions migrate from the nucleus into the cytoplasm through the pores present in nuclear membrane (Vincent, 1955; Brachet, 1957; Sirlin, 1960; Wischnitzer, 1960; King, 1960). A few workers (Beams and Kessel, 1962 a, b; Kessel and Beams, 1963; Anteunis *et al;* 1964 in crustaceans) concluded from their ultrastructural studies that the nucleolar extrusions mainly serve to transport nucleoproteins from the nucleus to the cytoplasm for building up the machinery of protein synthesis.

There is a decrease in pyroninophilia in the cortical region where the protein yolk spheres make their appearance with the advent of decrease of pyroninophilia due to RNA. This reveals that RNA present in nucleolar extrusions is utilised in protein synthesis. These observation are in conformity with those of light microscopists (Bhatia and Nath,

1931) in *Paratelphusa* and *Palaemon;* Sareen and Monga, 1970 in *Porcellionides* and *Cubaris*). Electron microscopists (Beams and Kessel, 1962 b in *Cambarus*, Kessel and Beams, 1963 in *Homarus;* Anteunis *et al;* 1964 in *Artemia;* and Hinsch and Cone, 1969 in *Libinia*) have described the mechanism by which the intracisternal granules give rise to the protein yolk bodies. Broek and Tates (1961) and Labordus (1968) in *Asellus* with autoradiographic techniques; and Kessel (1968) in crustaceans with autoradiographic and chromatographic techniques concluded that RNA present in ribosomes is utilised in yolk formation.

The presence of sulphydryl group, tyrosine and poproteins in nucleolus and nucleolar extrusions is in agreement with Fautrez and Fautrez-Firlefyn (1951) in *Artemia*, Montefoschi and Magaldi (1953) in *Asellus*, Fautrez (1959) in *Cyclops*, Fautrez and Fautrez-Firlefyn (1955) in *Artemia*, Albanese *et al.* (1973) in *Maja*, and Bonina (1974) *in Palaemon.*

References

ALBANESE, M. P., A. BOLOGARIN AND G. ZACCONE 1973. Morphological study on the yolk globules in the growing oocytes of *Maja verrucosa* Edw. (Crustacea, Brachyura). *Acta Histochem.*, **46**: 195-201.

ANTEUNIS, A., N. FAUTREZ-FIRLEFYN, FAUTREZ, J. AND A. LAGASSE 1964. L'ultrastructure du noyan vitellin de l'oeuf d'*Artemia salina. Exptl. Cell. Res.*, **35**: 239-247.

BEAMS, H.W. AND R.G. KESSEL 1962 a. Fine structure of the developing crayfish egg with special reference to the origin of yolk. *Anat. Rec.*, **142**: 214 (Abs.).

———, 1962 b. Intracisternal granules of the endoplasmic reticulum in the crayfish oocyte. *J. Cell Biol.*, **13**: 158-162.

BHATIA, D. R. AND V. NATH 1931. Studies in the origin of yolk. VI. The crustaceans oogenesis. *Quart. J. micr. Sci.*, **74**: 669-699.

BONINA, M. T. 1974. Cytochemical observations on the vitellogenetic oocytes of *Palaemon adspersus* Rathke (Crus. Dec.) *Acta Histochem.*, **50**: 116-123.

BRACHET, J. 1957. *Bilohemical cytology.* Academic Press. NEW YORK.

BROEK, C. J. H. VAN DEN AND A. D. TATES 1961. The incorporation of ^{14}C-adenine into the oocytes of *Asellus aquaticus* as studied by autoradiography. *Exptl. Cell Res.*, **24**: 201-219.

FAUTREZ, J. 1958. Etude histochemique de l'oocyte de premier ordre. *C.R. Assic. Anat.*, **45**: 41 (Quoted from Raven, 1961).

——— 1959. La nucleoli et l'anabolisme proteique. *Biol. Jaarb.*, **27**: 17-20.

——— AND N. FAUTREZ-FIRLEFYN, 1951. A propos de la chromatine et des nucleoles dans la vesicule germinative de l'oocyte de quelques crustaces. *Biol. Jaarb.*, **18**: 27-40.

——— AND ——— 1955. Sur le presence de phospho lipines dans les nucleoles de l'oocyte d'*Artemia salina C.R. Assoc. Anat.*, **42**: 506 (Quoted from Raven, 1961)

FAUTREZ-FIRLEFYN, N. 1957. Proteines, lipides et glucides dans l'oeful d'*Artemia salina. Arch Biol.*, **68**: 249-296.

HEBERER, G. 1930. Die strukture der Oocyten von *Eucalanus elongattus* Dana mit bemerkungen uber dan bau des weiblichen genitalapparates (Cytologische Mittellungen I.). *Z. Wiss. Zool.*, **136**: 155 (Quoted from Raven, 1961).

HILTON, I.F. 1931. The oogenesis of *Calanus finmarchicus Quart. J. Micr. Sci.*, **74**: 193-222.

HINSCH, G.W. AND M.V. CONE 1969. Ultrastructural observations of vitellogenesis in the spider crab, *Libinia emarginata. J. Cell Biol.*, **40**: 336-342.

KESSEL, R. G. 1968. Mechanisms of protein yolk synthesis and deposition in crustacean oocytes. *Z. Zellforsch.*, **89**: 17-38.

——— and H. W. Beams 1963. Electron microscopic studies on developing oocytes of the lobster, *Homarus* with special reference to the method of yolk formation. *J. Cell Biol.*, 19: 87 A (Abst. 209).

King, R. C. 1960. Oogenesis in adult *Drosophila melanogaster*. IX. Studies on the cytochemistry and ultrastructure of developing oocytes. *Growth*, 24: 265-323.

Labordus, W. J. - Van Breukelen 1968. Op Zoek naar Reserve DNA en RNA Een autoradiografisch onderzoek van oocytes eieren en embryonen van *Asellus aquaticus*. Wiegertje Johana Labordus van Breukelen geboren 15 November, 1936 te Soest (*Personal communication.*)

Montefoschi, S. and A. Magaldi 1953. Compartmento degli acidi nucleonici nella ovogenese di *Asellus aquaticus Pubbl. Staz. Zool. Napoli*, 24: 167 (Quoted from Raven, 1961).

Nath, V. 1968. *Animal Gametes(Female)*. A morphological and cytochemical account of yolk formation in oogenesis. Asia Publishing House. Bombay.

Norrevang, A. 1968. Electron microscopical morphology of oogenesis. *Int. Rev. Cytol.*, 23: 114-186.

Pearse, A. G. E. 1968. *Histochemistry: Theoretical and Applied.* J. & A. Churchill. London.

Raven, C. P. 1961. *Oogenesis: The storage of Developmental Information.*

Sareen, M. L. and D. Monga 1970. Morphology and cytochemistry of vitellogenesis in *Cubaris robusta* and *Porcellionides* sp. (Isopoda: Crustacea). *Res. Bull. Punjab University*, 21: 135-143.

Sirlin, J. L. 1960. The nucleolus of the cell nucleus. *Endeavour*, 20: 146.

Vincent, W. S. 1955. Structure and chemistry of nucleoli. *Int. Rev. Cytol.* 4: 269-298.

Wischnitzer, S. 1960. The ultrastructural of the nucleus and nucleo-cytoplasmic relations. *Ibid.*, 10: 137.