J. mar. biol. Ass. India 1981, 23 (1 & 2) : 36-38

YOLK NUCLEUS DURING OOGENESIS IN *NEPTUNUS PELAGICUS* (BRACHYURA, CRUSTACEA)

P. K. MITTAL

Department of Zoology, Punjab University, Chandigarh-160 014

ABSTRACT

The yolk nucleus of *Neptunus* consists of three concentric zones. It begins to divide in the circumnuclear region with its contents (proteins, nucleolar extrusions, mitochondria and phospholipids) spread over in the ooplasm. Its indirect role in vitellogenesis has been discussed.

INTRODUCTION

HEBERER (1930) and Anteunis *et al.* (1964, 1966) have described the yolk nucleus in crustaceans in the past. The cytochemical studies, however, remained neglected from their accounts. Mittal (1976) has though described cytochemistry of yolk nucleus in *Panulirus homarus* and now observes the morphology and cytochemistry of the yolk nucleus and its role in yolk formation in *Neptunus pelagicus*.

The author is thankful to the Head, Department of Zoology. Punjab University, Chandigarh for providing necessary laboratory facilities.

MATERIAL AND METHODS

Specimens of *N. pelagicus* were collected from the beach of Pamban (Tamil Nadu). The animals were dissected after decapitation and the ovaries were taken out in normal saline. These were fixed in Zenker's fluid (3-6h). Lewitsky saline, formaldehyde calcium (postchromed) and weak Bouin. Following the methods of Himes and Moriber (1956) and Pearse (1968), the material was then processed to localize the sites of carbohydrates, proteins, nucleic acids and lipids in different stages of oocytes.

OBSERVATIONS

The yolk nucleus is round, single and juxtanuclear in position, and is an aggregation of nucleolar extrusions, mitochondria and the phospholipid bodies in the youngest oocytes (PI. I A-D).

The nucleolar extrusions are concentrated in the juxtanuclear position in oogonia. In oocytes they are mainly concentrated in the yolk nucleus and a few are also seen in the ooplasm (PI. I A, B). The yolk nucleus is deeply stained pink in MG/PG for RNA (PI.I B; blue in Hg-BPB, and yellow in Himes and Moriber's technique (HM) (PI. I A) for proteins; and blue in acetone-Sudan black B (Ac-SBB) for lipoproteins. The yolk nucleus is negative in periodic-acid-Schiff (PAS) for 1 : 2 glycol groups.

The phospholipid bodies and mitochondria are also concentrated in the yolk nucleus and lie in ooplasm in oogonia as revealed by LS/IH, SBB, acid haematein (AH) (-ive after pyridine extraction). They are blue in AH (PI IC) and Nile blue sulphate (NBS) revealing the presence of phospholipids in them. The mitochondria are also blue in Hg-BPG for proteins, and Ac-SBB for lipoproteins whereas the phospholipid bodies are -ive for both the tests.

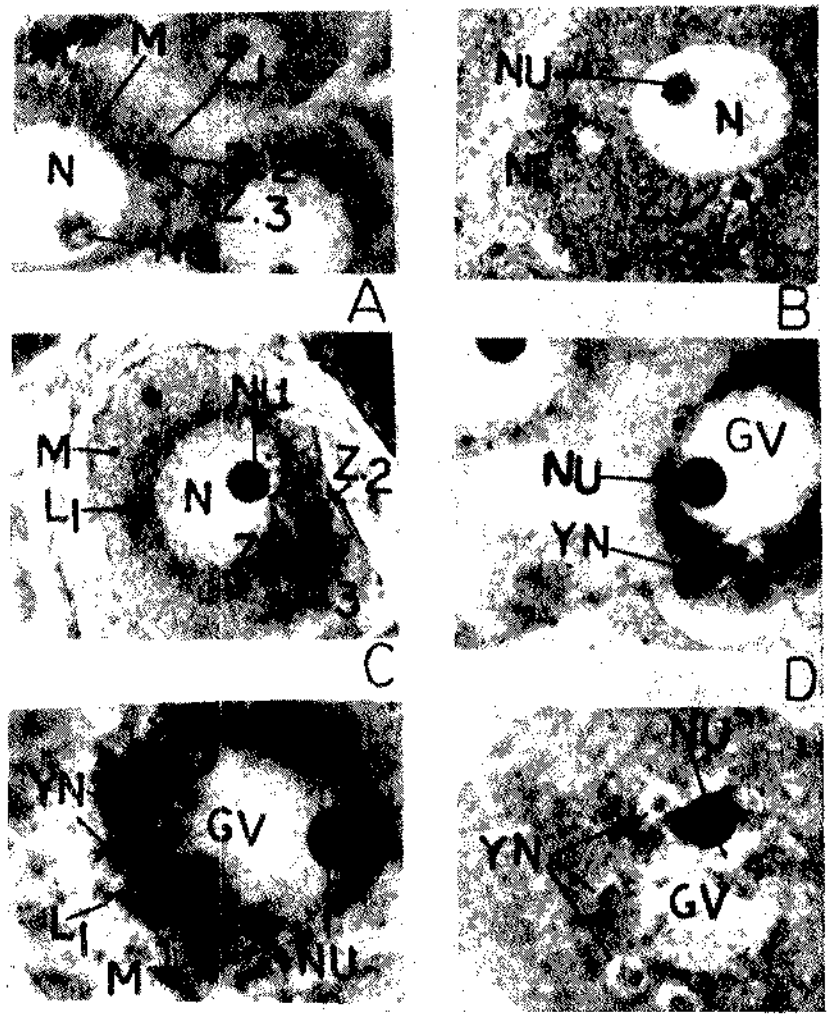

PLATE I A. Young oocyte showing protein rich yolk nucleus (Zenker/Himes and Moriber's test, X 1250). B. Yolk nucleus in juxtanuclear position containing RNA (Z/MG-PG, X 1250). C. Developing oocyte showing yolk nucleur rich in phospholipids in the juxtanuclear position containing mitochondria and phospholipids (FCa/PC-AH, X 1250). D. Dividing yolk nucleus in the circumnuclear region (FCa/PC-AH, X 1250). E. Dividing yolk nuclei rich in phospholipids in the circumnuclear region (FCa-PC/AH, X 1250) and F. Yolk nuclei scattered in the circumnuclear region containing proteins (Z/Hg-BPB). (DB: Duplex bodies; N: Nucleus; NU: Nucleolus; PL: Phospholipid bodies; Zones 1, 2, 3 yolk nucleus).

The yolk nucleus is made up of 3 zones, *viz.* outer, middle and the innermost zones (Pl. I A-C). The outermost zone forms a ring around the middle clear zone. The outer zone is made up of mitochondria and ribonucleoproteins (Pl. I A-C) and lipoproteins. The innermost zone is deeply stained and is the region of mitochondria and phospholipids. This region contains lipoproteins, phospholipids (Pl. I C-E), RNA (Pl. I B), and proteins (Pl. I A-F). The carbohydrates and DNA are not present in either of the zones. The yolk nucleus is positive in ninhydrin-Schiff for $-NH_2$ bound proteins; Millon for tyrosine; Sakaguchi for arginine; and coupled tetrazonium (+ive after dinitrofluorobenzene and performic acid-Schiff, and –ive after benzoylation) for tyrosine and arginine.

The yolk nucleus begins to divide into further yolk nuclei which surround the nucleus (Pl. I D-F). They fragment and the phospholipid bodies, mitochondria and RNA particles get loosely scattered, arranging in the circumnuclear regions and other parts of the ooplasm (Pl. I E, F). Later these organelles get scattered in whole of the ooplasm when the yolk nuclei are completely broken.

The protein synthesis takes place under the influence of RNA present in nucleolar extrusions and protein yolk globules arise in the cortical ooplasm. Subsequently, the phospholipid bodies transform into the spheroids made up of phospholipids and triglycerides. These do not transform into triglycerides, but contribute lipids to compound yolk globules as these are absent in mature oocytes. In mature oocyte only one type of yolk containing carbohydrates, proteins, phospholipids and triglycerides is present.

By comparing the metabolites of the yolk nucleus with the composition of yolk, it has been observed that the yolk nucleus plays an indirect role in the initiation of yolk formation in *N. pelagicus*.

Discussion

The yolk nucleus of *N. pelagicus* differs from that of *Panulirus homarus* already described by Mittal (1976), in that as it is long and compact; and does not have any zonations and is more active as the nucleolar extrusions, phospholipids andmi tochondria get first concentrated and then scatter in the ooplasm of the developing oocytes.

The yolk nucleus in *N. pelagicus* is rich in RNA which has also been reported in *Eucalanus* by Heberer (1930) and many animals (Raven, 1961; Nath, 1968). The ribosomes in yolk nucleus have been observed on the endoplasmic reticulum in the ooplasm of certain animals by many workers (for references see Norrevang, 1968). Anteunis *et al.* (1956), Malaviya (1973), Norrevang (1968) and Nath (1968) have also reported the presence of mitochondria in the yolk nucleus. In fishes, reptiles, birds, mammals and spiders, various workers as quoted by Nath (1968) have reported the presence of phospholipids in the yolk nucleus of young oocytes; and phospholipids and triglycerides in that of the developing oocytes.

The three zones described in the yolk nucleus during present study have not been observed by any other worker in crustaceans.

Raven (1961) and Nath (1968) have summarized the light and electron microscopical studies of the previous workers; and stated that ribonucleoproteins arise from or in close association with the nucleus. As it grows, the mitochondria and the phospholipids are seen very closely associated with it. The present study reveals that the yolk nucleus comprises these metabolites since the onset of the same. These later disperse throughout the ooplasm and contribute to the synthesis of proteins and lipids of the yolk. Thus yolk nucleus plays an indirect role in the initiation of yolk formation in *N. pelagicus*.

References

ANTEUNIS, A., N. FAUTREZ-FIRLEFYN AND J. FAUTREZ 1966. A propos d'un complexe tubulo-mitochondrial ordonné dans le jeune oocyte d' *Artemia salina*. *J. Ultrast. Res.*, **15**: 122-130.

HEBERER, G. 1930. Die struktur der oocyten von *Eucalanus elongatus* Dana mit bemerkungen uber den bau des weiblichen genitalapparates (Cytologische Mitteilungen I). *Z. Wiss. Zool.*, **136**: 155.

HIMES, M. AND L. MORIBER 1956. A triple stain for deoxyribonucleic acid, polysaccharides and proteins. *Stain Tech.*, **31**: 67-70.

MALAVIYA, B. B. 1973. The yolk nucleus in the oocytes of *Clarias batrachus* (Linn.). *Zool. Pol.*, **22**: 189-196.

MITTAL, P. K. 1976. Morphological and cytochemical studies on yolk nucleus during vitellogenesis in a macruran *Panulirus homarus* (Macrura: Crustacea). *J. mar. biol. Ass. India*, **18**: (1) : 165-168.

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

NORREVANG, A. 1968. Electron microscopic morphology of oogenesis. *Int. Rev. Cytol.*, **23**: 113-186.

PEARSE, A. G. E. 1968. *Histochemistry: Theoretical and Applied*. J. and A. Churchill, London. Vol. I.

RAVEN, C. P. 1961. *Oogenesis: The Storage of Developmental Information*. Pergamon Press, New York.