Nucleoli in the Oocytes of Some Arachnids

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Introduction

A good deal of information is available about nucleoli in various animal cells (Caspersson 1950, Vincent 1955, Brachet 1957, 1960, Swift 1959, Sirlin 1961). Nath (1925) observed an active nucleolus which emits nucleolar extrusions in young oocytes of *Buthus judaicus* and *Euscorpius napolii* and an inactive one in *Palamnaeus fulvipes* and *P. swammerdami*. Recently I have dealt with the morphology and cytochemistry of oocytes in arachnids (Sareen 1961, 1962, 1963, 1964, 1965a, b, c). The present communication gives detailed account of nucleoli in oocytes of some arachnids.

Material and technique

Ovaries of the following eleven species of arachnids belonging to eight genera have been employed in the current study. *Buthus hendersoni* Pocock, *B. macmahoni* Pocock, *B. tamulus* Pocock (Scorpionida: Buthidae), *Palamnaeus fulvipes* Koch, *P. bengalensis* Koch (Scorpionida: Scorpionidae), *Hyalomma aegyptium* (Linn.), *Argas persicus* (Oken) (Acarina: Ixodidae), *Lycosa chaperi* Simon (Araneae: Lycosidae), *Crossopriza lyoni* (Blackwall) (Araneae: Pholcidae), *Melanopa unicolor* Roewer (Opiliones: Phalangidae), *Diplotemnus insolitus* Chamberlin (Chelonethida: Atemnidae).

Fixing fluids and histochemical tests employed are summarized in Table 1.

Observations

A single nucleolus is present in *Buthus* and *Palamnaeus* oocytes (Figs. 1-5). The nucleolus in *Palamnaeus* measures about 5.0μ whereas in *Buthus* it is about 12.0μ . The yolk synthesis in *Buthus* seems to depend upon nucleolar activity (Figs. 1 to 4). In *Palamnaeus*, only Zenker/haematoxylin preparations reveal some nucleolar budding (Fig. 5).

In an oocyte (about 24.0μ) of *Argas* (Fig. 6) there is seen a single nucleolus (about 11.0μ). Occasionally there are two nucleoli, a small (about 1.5μ) and a large (about 5.6μ) in oocyte measuring about 15.0μ (Fig. 7). In *Hyalomma* oocyte (about 35.0μ) there are as many as eight nucleoli (Figs. 8, 9) out of which one or two are larger (about 3.6μ) than the others (about 1.8μ). With the advancement of vitellogenesis the nucleoli decrease in number, and one or two nucleoli are generally left in the well developed oocyte. In *Hyalomma* and *Argas*, the nucleoli in the young oocytes bud off fragments into the nucleoplasm; the fragments ultimately pass into the ooplasm (Fig. 10). The nucleolar buds in the nucleoplasm and the circumnuclear ooplasm are larger than those seen at the peripheral ooplasm.

Technique	Fixation	Reference	<i>Buthus</i>	<i>Palamnaeus</i>	<i>Hyalomma</i> & <i>Argas</i>	<i>Lycosa</i>	<i>Cros-</i> <i>sopriza</i>	<i>Melanopa</i>	<i>Diplo-</i> <i>temnus</i>	
									P	K
SBB in 70% ethanol	FCa	Baker, 1949	+	+	+	+	+	+	+	+
SBB* hot acetone	Fr	Pearse, 1960	-	-	-	-	-	-	-	-
AH	FCa	Baker, 1946	+	+	+	+	+	+	+	+
AH* PE	WB+PE	"	##	##	##	##	##	##	##	##
PAS	Z, C	Hotchkiss, 1948	+	+	+	+	+	+	+	+
PAS* acetylation	"	McManus and Cason, 1950	-	-	-	-	-	-	-	-
PAS* KOH reversal	"	"	+	+	+	+	+	+	+	+
Hg-BPB	"	Mazia and others, 1953	Bl	Bl	Bl	Bl	Bl	Bl	Bl	Bl
CTZ	"	Pearse, 1960	##	##	##	##	##	##	##	##
CTZ* benzoylation	"	"	-	+	+	+	+	+	+	+
CTZ* DNFB	"	"	-	+	+	+	+	+	+	+
DNFB, Millon	"	Pearse, 1960; Baker, 1956	+	+	##	+	+	+	+	+
CTZ* performic acid	"	Peares, 1960	+	+	+	+	+	+	+	+
Sakaguchi	"	"	+	+	+	+	+	+	+	+
FF, AT	10% F, Z	"	+	+	+	+	+	+	+	+
FF* aq. HgCl ₂	"	"	-	-	-	-	-	-	-	-
AFG	Z	Alfert and Geschwind, 1953	##	NC	+	+	+	+	##	+
MG/PG; MG/TB; TB (pH 4.2)	Z	Jordan and Baker, 1955; Korson, 1951, Gelfant and others, 1955	##	##	##	+	+	+	##	+
MG/PG*, MG/TB*, TB (pH 4.2) TCA, RNase	Z	Pearse, 1960, Bradbury, 1956	-	-	-	-	-	-	-	-
Feulgen	Z, C	Feulgen and Rossenbeck, 1924	+	+	+	+	+	+	+	+
Feulgen* TCA	"	Pearse, 1960	-	-	-	-	-	-	-	-
Alkaline phosphatase	Cold AC, W	Gomori, 1952	+	+	+	+	+	+	+	+

Key: AC=acetone, AFG=alkaline fast green, AT=alkaline tetrazolium, Bl=blue, C=Carnoy's fixative, CTZ=coupled tetrazonium, FF=ferric ferricyanide, FCa=formaldehyde-calcium, Fr=fresh, Hg-BPB=mercuric bromophenol blue, MG/PG=methyl green pyronin G, NC=not clear, PAS=periodic acid-Schiff, TB=toluidine blue, TCA=trichloroacetic acid, W=Wolman's fixative: WB+PE=weak Bouin followed by pyridine extraction, Z=Zenker's fluid, *=after treatment with, +=moderate reaction, +=strong reaction, ##=very strong reaction.

Figs. 1-4. 1, *Buthus hendersoni*, Rossman/PAS. 2, *B. hendersoni*, Carnoy/haematoxylin. 3, *B. macmahoni*, Carnoy/toluidine blue at pH 4.2. 4, *B. tamulus*, WB+PE/AH.

Figs. 5-15. 5, *Palamnaeus fulvipes*, Zenker/haematoxylin. 6, *Argas persicus*, FCa+PC/SBB. 7, *A. persicus*, Zenker/toluidine blue, pH 4.2. 8-9, *H. aegyptium*, FCa+PC/AH. 10, *H. aegyptium*, Canroy/haematoxylin. 11, *Lycosa chaperi*, Zenker/CTZ. 12, *Crossopriza lyoni*, Zenker/haematoxylin. 13, *Melanopa unicolor*, FCa+PC/AH. 14, *Diplotennus insolitus*, Carnoy/Feulgen. 15, Zenker MG-PG. Abbreviations: en, extruded nucleolus. l, lipids. n, nucleolus. ne, nucleolar extrusions. v, vacuole.

The nucleolar extrusions disappear at the beginning of yolk formation which first appears at the peripheral ooplasm.

In *Lycosa* the nucleoli vary in size from 2.7 to 11.5 μ in the developing oocytes measuring 23.0 to 54.5 μ . In young oocytes five to six small nucleoli are usually observed. In a slightly grown up oocyte (Fig. 11) there is left a single large heterogeneous nucleolus which is of irregular shape. It seems to be formed of aggregation of small and large spherules.

In *Crossopriza* there is a single nucleolus measuring 4.0 to 14.0 μ in the developing oocytes measuring 120.0 to 410.0 μ . The nucleolus buds off extrusions (Fig. 12) which remain in the vicinity of the germinal vesicle in the ooplasm and disappear as soon as the yolk globules have appeared.

In *Melanopa*, the single nucleolus measures 4.2 to 10.0 μ in the developing oocytes measuring 14.4 to 57.6 μ (Fig. 13). The nucleolus buds off small granules in the young oocytes. The granular buds disappear when the yolk spheres appear in the ooplasm.

In *Diplotemnus* five to six nucleoli measuring 2.0 to 3.7 μ have been observed in the developing oocytes measuring 20.0 to 80.0 μ (Figs. 14, 15). Two types of nucleoli have been identified, i) Feulgen positive (Karyosomes of other workers) and ii) Feulgen negative (plasmosomes of other workers) (Wilson 1928, Duryee 1950).

In all the species investigated the nucleoli show intense positive reactions for proteins and RNA. They also show positive tests for carbohydrates, DNA and phospholipids. The nucleoli in *Buthus*, *Hyalomma*, *Argas*, *Lycosa*, *Crossopriza* and *Melanopa*, show slight positive test for alkaline phosphatase. The nucleoli in *Buthus* contain tyrosine, arginine, SH groups; in *Palamnaeus* and *Lycosa*, tryosine, tryptophan, SH groups; in *Crossopriza*, tyrosine, tryptophan, histidine, arginine, SH groups; in *Hyalomma* and *Argas*, tyrosine, SH groups; in *Melanopa*, tyrosine, tryptophan, histidine, SH groups. In *Diplotemnus* both the types of nucleoli show positive tests for RNA, proteins (tyrosine, arginine, SH groups) and give slightly positive tests for carbohydrates. In almost all the species the nucleoli are positive to CTZ reaction even after benzylation control. According to Mitchell (1942) and Danielli (1947, 1950) the positive tetrazonium reaction of benzyolated tissues is due to purine and pyrimidine containing compounds (For detailed reactions refer to the table).

In all the species the nucleoli appear to be active only in the early stages of vitellogenesis. The nucleoli are vacuolated in the young oocytes. The vacuoles are negative to all the histochemical tests. Often the nucleolus takes its position close to the nuclear membrane in the germinal vesicle (Fig. 4). In *Buthus*, *Hyalomma*, and *Diplotemnus*, sometimes, the nucleolus has been seen lying in the ooplasm close to the nuclear membrane (Figs. 9, 10, 15). There is no direct connection between the nucleolar extrusions and the yolk globules.

Discussion

The nucleoli in the oocytes of *Buthus*, *Hyalomma*, *Argas*, *Lycosa*, *Crossopriza*, *Melanopa* and *Diplotemnus* are quite large and play an active part in the growth of young oocytes. They emit nucleolar extrusions. In *Palamnaeus*, however, the small nucleolus rarely shows nucleolar extrusions. There is no direct evidence of the nucleolar extrusions giving rise to yolk bodies; nevertheless there is evidence that nucleolar extrusions, rich in RNA and proteins appear at an earlier stage than the yolk bodies. Recently, Gresson and Threadgold (1962) have demonstrated the extrusion of nuclear material during oogenesis in *Blatta orientalis*. They have concluded that 'the emission of material from the nucleolus and the passage of vesicles from the nuclear membrane to the cytoplasm are necessary prerequisites for the process of vitellogenesis'. In all the species the nucleoli show positive reactions for proteins and RNA. In certain cases, they also show positive tests for carbohydrates, phospholipids, DNA and alkaline phosphatase. This is in conformity with other workers (Vincent 1955, Brachet 1961, Sirlin 1961). Nucleoli in frog oocytes, occasionally when they are in close contact with the nuclear membrane evert their contents, which in turn pass into the cytoplasm (Brachet 1957). Sirlin (1961) has postulated the nucleolar contribution in RNA, proteins and enzymes to the cell metabolism. According to Brachet (1957) often the extrusion of whole nucleolus is the result of a pinching off of the nuclear membrane. Wischnitzer (1960) remarks that the extrusion of nucleoli may be specialized mechanisms called into action only at times of great metabolic activity.

Two types of nucleoli, i) Feulgen positive and ii) Feulgen negative, have been identified in *Diplotemnus* oocytes. The Feulgen positive nucleoli contain DNA, proteins (tyrosine, arginine, SH groups), RNA and traces of carbohydrates. The Feulgen negative nucleoli have a similar chemical composition as the Feulgen positive ones, except that the former do not contain DNA. Kobayashi (1954) identified two types of nucleoli, 1) Karyosomes (K) and 2) Plasmosomes (P) in the eggs of *Ostrea*. Kobayashi observed that K includes DNA and P includes RNA, K contains much volume of arginine but P does not; P contains some of histones type proteins. He remarks that the substances which are called nucleolus in general are not the same one from chemical view points but differ according to the kind of the cell and to the species of the animals.

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Summary

1. The nucleoli in arachnids play an important role in the growth of young oocytes.
2. In *Buthus*, *Hyalomma*, *Argas*, *Crossopriza* and *Melanopa* they emit

prominent nucleolar extrusions rich in ribonucleoproteins.

3. The nucleolar extrusions disappear before the appearance of yolk globules. However, the indirect participation of nucleolar extrusions in the yolk formation is not ruled out.

4. The nucleoli show intense positive test for RNA and proteins, and in some cases show slightly positive reaction for carbohydrates, phospholipids, DNA and alkaline phosphatase.

5. In *Diplotemnus* there are two types of nucleoli: i) Feulgen positive (Karyosomes of other authors) and ii) Feulgen negative (plasmosomes of other authors).

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