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Evidence for endocytotic incorporation of nutrients from the haemal sinus by the oocytes of the brittlestar *Ophiolepis paucispina*

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ABSTRACT: Ophiolepis paucispina is a small brittlestar that produces large yolky eggs and has ovoviviparous development. Ultrastructural examination suggests that oogenesis is supported by exogenously derived nutrients. During oogenesis, oocytes evaginate into the genital haemal sinus. As the oocytes grow, they are surrounded by the haemal sinus, although they remain separated from the haemal fluid by a basal lamina. Endocytotic activity is prevalent throughout oogenesis, indicating uptake of exogenous material. The ER and Golgi complex appear to be involved in elaboration of the yolk bodies. It is proposed that yolk precursors formed outside the oocyte are delivered to the oocytes by the genital haemal sinus and that this sinus functions as an intragonadal nutrient storage organ.

1 INTRODUCTION

There is a growing body of evidence supporting the contention that the echinoderm haemal system plays a role in gametogenesis as a source of nutrients (Walker 1982; Ferguson 1982; Kanatani & Nagahama 1983). As shown in several ultrastructural studies, the haemal sinus is closely associated with the developing oocytes of asteroids (Walker 1979; Schoenmakers et al.1981; Beijnink et al. 1984), holothuroids (Davis 1971;Smiley & Cloney 1985), crinoids (Davis 1971; Holland 1971) and ophiuroids (Davis 1971). For these echinoderms, the haemal fluid or cells contained within the haemal sinus are a potential source of nutrients. Additional evidence for a role of the haemal system in providing nutrient material for gametogenesis comes from autoradiographic, immunocytochemical and histochemical investigations of sea stars (Beijnink et al. 1984; Ferguson 1984; Voogt et al. 1985; Beijnink & Voogt 1986). Although it appears that vitellogenesis in many echinoderms is at least partially dependent on yolk precursors present in the genital haemal sinus (Walker 1982; Kanatani & Nagahama 1983; Voogt et al. 1985), for the most part it is not known how the oocytes internalize these precursors (Ferrand 1984; Beijnink et al. 1984). Endocytosis of nutritive material is reported for the oocytes of several echinoids (Bal 1970; Tsukahara 1970) and for a crinoid (Holland 1971). Recent biochemical investigations with echinoids provides clear evidence for the somatic origin of yolk precursors (Harrington & Ozaki 1986; Shyu et al. 1986), but it is not known if the haemal system is a source of nutrients for echinoid oogenesis.

Ophiolepis paucispina broods its young in the genital bursae and as for other brooding ophiuroids (Hendler 1975; Strathmann & Strathmann 1982), this species is small (5-7 mm disc diameter), is a simultaneous hermaphrodite and has a low fecundity (2-70 oocytes). Development of O. paucispina is ovoviviparous and embryogenesis appears to be completely supported by the nutrient reserves present in the large yolky oocyte (400 µm diameter). There is a paucity of information on oogenesis in ophiuroids, especially at the ultrastructural level (Walker 1982). The question of vitellogenesis is particularly interesting for diminutive species where size-associated energetic constraints may limit oocyte production (Chia 1974; Stearns 1976). An ultrastructural investigation of oogenesis of O. paucispina was undertaken to determine the vitellogenic mechanisms involved with the production of the large oocytes and the role of the genital haemal sinus.

2. MATERIALS AND METHODS

Specimens of Ophiolepis paucispina were

collected from Conch Key, Florida, U.S.A. and from Southwater and Carrie Bow Cays, on the Belize Barrier Reef. The yolky eggs of O. paucispina were difficult to preserve for ultrastructural examination. and several fixation methods were employed, including the prodedures detailed in Walker (1979) and Eckelbarger (1979). Fixation however, was poor. Satisfactory fixation was obtained with the method of Buckland-Nicks et al. (1984). Freshly collected O. paucispina were placed directly in 2.5% glutaraldehyde in 0.45µm Millipore filtered natural sea water without prior anaesthesia. The ovaries were dissected and placed in fresh fixative for 1 hr at room temperature. This was followed by a rinse in 2.5% sodium bicarbonate (pH 7.2) and secondary fixation in 2% osmium tetroxide in 1.25% sodium bicarbonate for 1 hr at room temperature. Following fixation, the tissue was rinsed in distilled water and dehydrated in increasing concentrations of ethanol, transferred through two changes of propylene oxide and embedded in Epon 812. Thin sections were stained with 2% uranyl acetate followed by 2% lead citrate and viewed with a Zeiss EM9-S2 electron microscope.

For light microscopy, specimens of O. paucispina were relaxed in a 1:1 mixture of 7% magnesium chloride and sea water for 10 min. The discs were fixed and decalcified in Bouin's fixative. the tissues were then dehydrated with ethanol, transferred to xylene, embedded in paraffin and sectioned at 7µm thickness. The sections were stained with the alcian blue/periodic acid Schiff's method (AB/PAS) for carbohydrates.

3 RESULTS

3.1 Structure of the ovary

The ovary wall consists of two parts, the inner and outer sac, and these are separated by the genital coelom (Figs. 1,2,4). Each sac is composed of three layers of tissue. The outer sac consists of the visceral peritoneum, a central connective tissue layer and the genital coelomic epithelium. The inner sac is composed of the internal coelomic epithelium, a connective tissue layer that contains the haemal sinus and the germinal epithelium.

The genital haemal sinus is an extracellular matrix consisting of granules, small diameter striated collagen fibrils and unstriated fibrils (Fig. 5). Amoebocytes and phagocytes are occasionally

FIGURE LEGENDS

Fig. 1. Cross section of the ovary of Ophiolepis paucispina stained with AB/PAS. The previtellogenic oocytes (PVO) stain blue and early vitellogenic oocytes (EVO) stain pink. PAS+ yolk bodies are apparent in the mid-vitellogenic oocyte (MVO) and late vitellogenic oocytes (LVO) are intensely PAS+. The oocytes have a PAS+ halo (arrow) and the haemal sinus (H) is filled with PAS+ fluid. Paraffin section. Scale = 50 µm.

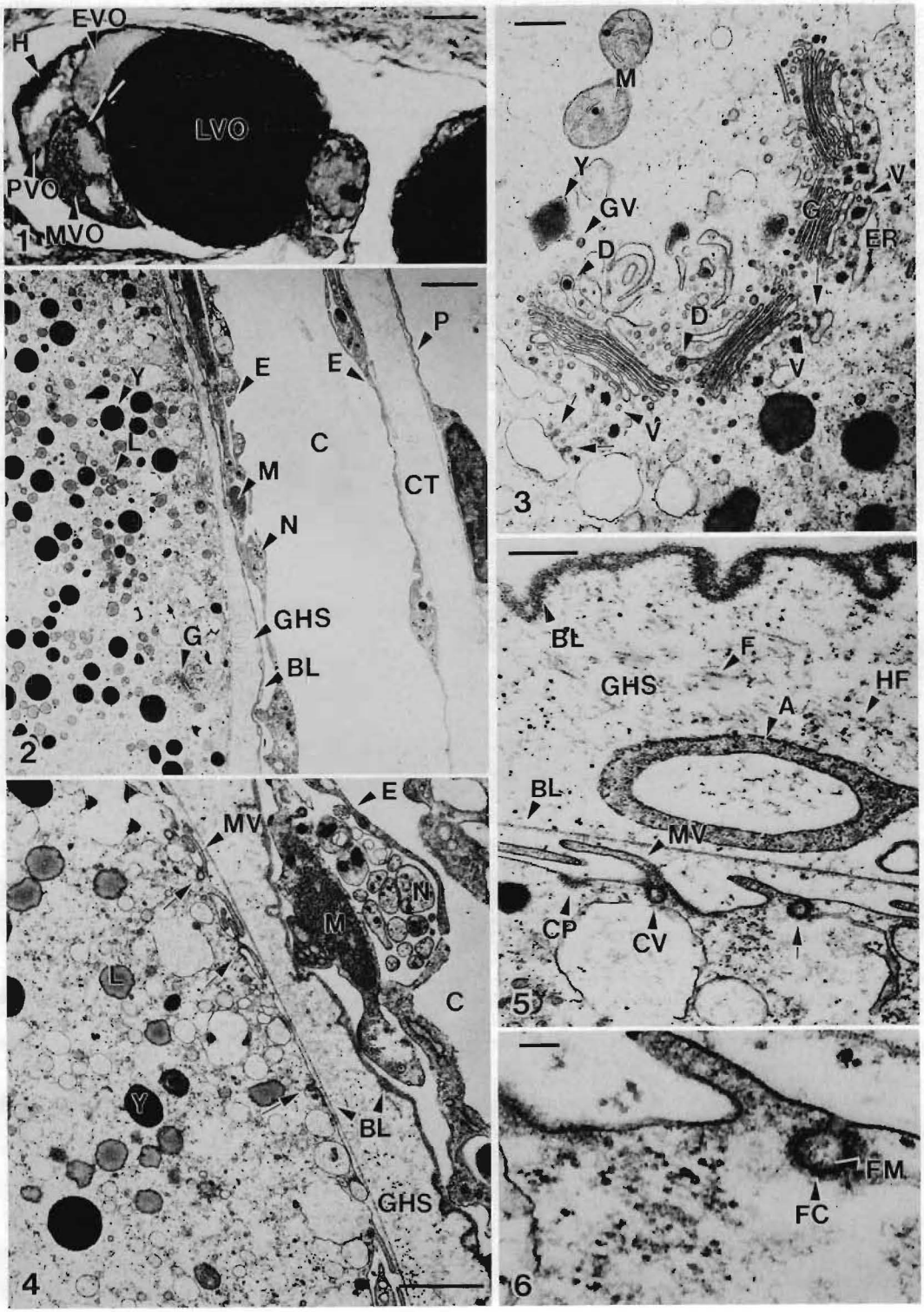
Fig. 2. Low power cross section of the ovary showing the outer and inner sacs separated by the genital coelom (C). The outer sac consists of the coelomic peritoneum (P), connective tissue (CT) and the genital coelomic epithelium (E). The inner sac sonsists of epithelium (E) and connective tissue containing the genital haemal sinus (GHS). The sinus surrounds a vitellogenic oocyte. BL, basal lamina: G, Golgi complex; L, lipid; M, muscle; N, nerves; Y, yolk. Scale=3 µm.

Fig. 3. Golgi complex (G) and ER in the cortical ooplasm of an early vitellogenic oocyte. ER-projections (arrows) give rise to vesicles (V) which fuse with the forming face of the Golgi complex. Dilations of the Golgi complex (D) give rise to vesicles (GV) that fuse to form yolk bodies (Y). M, mitochondrion. Scale=0.5 µm.

Fig. 4. The oolemma of late vitellogenic oocytes have numerous microvilli (MV) and endocytotic invaginations (arrows). BL, basal lamina; E, epithelium; C, genital coelom; GHS, genital haemal sinus; L, lipid; M, muscle; N, nerve; Y, yolk body. Scale=2 µm.

Fig. 5. Endocytosis: The process starts a coated pit (CP) which is invaginated to form an omega figure (arrow) and finally internalized forming a coated vesicle (CV). The genital haemal sinus (GHS) contains granulated haemal fluid (HF) and fibrils (F). A, amoebocyte; BL, basal lamina; MV, microvillus. Scale=0.5 µm.

Fig. 6. Detail of the endocytotic invagination showing the fuzzy coat along the cytoplasmic surface (FC) and the flocculent material attached to the extracellular surface (FM) of the coated pit. Scale= 0.1 µm.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encountered in the haemal sinus. The haemal sinus is delimited on either side by a basal lamina that is often tortuous in outline, although the inner basal lamina is straight when adjacent to vitellogenic oocytes (Figs. 5,6). The germinal epithelium lies adjacent to the inner basal lamina and usually contains oocytes at different stages of oogenesis and oosorption.

3.2 Histochemistry of vitellogenesis

The tissue layers comprising the inner and outer sacs of the ovary cannot be discerned with the light microscope. However, part of the ovarian wall and a layer adjacent to the oocytes corresponding to the location of the haemal sinus, exhibits a PAS+ tinctoral response (Fig. 1). Oogenesis is continuous with oocytes at different stages of development present in the ovary (Fig. 1). Because the yolk bodies are PAS+, the process of yolk deposition can be followed with the AB/PAS method. Previtellogenic oocytes (10-50 µm diameter) stain blue with AB/PAS and the onset of the vitellogenic period is marked by the presence of pink yolk bodies in the ooplasm (Fig. 1). Early vitellogenic eggs (50-100 µm diameter) stain pale pink and as yolk accumulates, the colour deepens. Late vitellogenic oocytes are characterised by their intense and homogeneous PAS+ response, staining a deep magenta. At the end of oogenesis the oocytes are 400 µm in diameter.

3.3 Ultrastructure of vitellogenesis

The oocytes of O. paucispina undergo vitellogenesis unaccompanied by somatic cells. Each oocyte is surrounded by the haemal sinus throughout vitellogenesis, although is separated from the haemal fluid by the inner basal lamina (Figs. 2,4,5). This investment of haemal fluid is formed by evagination of the oocytes from the germinal layer into the haemal sinus. It appears that the oocytes bulge into the sinus as they enlarge and thereby develop an evagination in the sinus. Their growth causes the inner basal lamina to unfold and attenuate, thereby following the contours of the oocyte. In contrast, the outer basal lamina is thicker and plicated (Figs. 4,5).

The vitellogenic oocytes contain numerous Golgi complexes in the cortical ooplasm composed of 7-9 cisternae (Fig. 3). A single cisterna of ER is usually associated with the forming face of the Golgi complex. All the ER in the oocyte appears to be smooth, perhaps due to loss of attached ribosomes during fixation. The oocytes posess a large germinal vesicle and the ooplasm contains free ribosomes, mitochondria, lipid droplets and microvilli are elaborated along the oolemma (Figs. 2-5).

The yolk bodies of early vitellogenic oocytes are positioned near a Golgi complex. It appears that this organelle and the ER are involved in the elaboration of the yolk bodies (Fig. 3). The process starts with small expansions of the ER cisternae that project towards the Golgi complex. These projections often have an external coat and their detachment gives rise to coated vesicles. A flocculent material is contained within the vesicles and they appear to fuse with the forming face of the Golgi complex. An electron-dense material is evident within dilations of the mature Golgi cisternae and these dilations give rise to vesicles containing proteid yolk. The Golgi vesicles fuse to form yolk bodies and throughout vitellogenesis add to existing yolk bodies by accretion. Newly formed and small yolk bodies have a granular appearance and are variable in their electron-density. The yolk material condenses and the cytoplasm of the mature oocyte is dominated by yolk bodies that are uniformly electron-dense and 2-5 µm in diameter (Figs. 2,4).

Endocytotic activity is conspicuous at the surface of vitellogenic oocytes throughout vitellogenesis (Figs. 4,5). Coated pits (150-200 nm in diameter) are abundant at the bases of microvilli with up to 3 coated pits per µm of oocyte surface. The cytoplasmic surface of the pits has a fuzzy appearance and flocculent material adheres to the extracellular surface (Fig. 5). The pit and adherent material appear to invaginate to form an omega figure and are then internalized forming a coated vesicle (Fig. 6). Coated vesicles are present near the oolemma. They appear to loose their fuzzy coat soon after internalization and their fate could not be followed.

4 DISCUSSION

The organization of the ovary of O. paucispina is similar to that described for other ophiuroids (Davis 1971; Patent 1976). Somatic cells do not accompany the oocytes of O. paucispina during vitellogenesis; unlike the ophiuroid Gorgonocephalus caryi in which the vitellogenic oocytes are surrounded by follicle cells (Patent 1976).

As found for other echinoderms (Walker 1982; Beijnink & Voogt 1986), the haemal fluid of O. paucispina is PAS+ and vitellogenesis involves the accumulation of PAS+ yolk granules in the oocytes. This accumulation is thought to be due to transfer of nutritive PAS+ material stored in the sinus

to the developing oocytes (Walker 1982). During vitellogenesis, the oocytes of O. paucispina develop in an evagination of the haemal sinus and are surrounded by haemal fluid throughout vitellogenesis. For O. paucispina, it appears that yolk precursors are largely extraovarian in origin and appear to be a component of the haemal fluid. During oogenesis potential yolk precursors present in the haemal sinus need only to pass through the inner basal lamina and the prevalence of endocytotic activity suggests that the oolemma of O. paucispina is well equipped with receptor sites to internalize the precursor material. From ultrastructural and histological evidence, it appears that the oocytes of Ophioderma panamensis and Gorgonocephalus caryi are also surrounded by haemal fluid (Davis 1971; Patent 1976), however this is not the case for Ophiopteris papillosa (Davis 1971). As for O. pausispina, crinoid oocytes also develop in an evagination of the genital haemal sinus (Davis 1971; Holland 1971).

This study provides the first ultrastructural evidence of endocytosis of yolk precursors from the genital haemal sinus by an echinoderm oocyte. Similar selective uptake of yolk precursors from a circulatory system is reported for the oocytes of insects and polychaetes (Anderson 1974; Eckelbarger 1979,1984). Although the growing body of evidence suggests that vitellogenesis in many echinoderms depends on exogenously derived yolk precursors (Walker 1982; Kanatani & Nagahama 1983; Voogt et al. 1985; Harrington & Ozaki 1986; Shyu et al. 1986), the apparent absence of endocytosis is noted for the oocytes of several asteroids (Beijnink et al. 1984; Ferrand 1984; Aisenshadt & Vassetzky 1986). Based on evidence from autoradiographic and immunocytochemical investigations, vitellogenesis of the asteroid Asterias rubens is considered to be supported by nutrients stored in the genital haemal sinus (Voogt et al. 1985). In a recent ultrastructural investigation of the oocytes of A. rubens, the apparent absence of endocytosis is suggested to be due to the low frequency of coated pit formation (Beijnink et al. 1984).

Digestive processes are the ultimate source of nutrients for oogenesis but, the origin of the yolk precursors and the pathway of their delivery to the genital haemal sinus of O. paucispina are not known. The vitellogenic material may be taken from the gut and delivered to the genital haemal sinus through haemal pathways. The haemal system however, with its gelatinous haemal fluid appears poorly adapted for translocation of nutrients. On the other hand, given the unusual properties of echinoderm extracellular matrices (Wilkie 1984), it is possible that the viscosity of the haemal fluid may decrease to assist local translocation. Alternatively, the coelomic fluid may be the transport medium, whereby nutritive material present in the coelomic fluid or in coelomocytes is delivered to the gonad through the genital coelom (Ferguson 1982). The material is then taken from the coelom by the genital haemal sinus and stored for use during vitellogenesis. Recent work on echinoid vitellogenin synthesis provides evidence for the somatic origin and coelomic translocation of yolk precursors. In Dendraster excentricus, vitellogenin is synthesised by coelomocytes and secreted into the coelomic fluid by these cells (Harrington & Ozaki 1986) and in Strongylocentrotus purpuratus, vitellogenin synthesis occurs in the intestine and gonads (Shyu et al. 1986).

For O. paucispina it appears that yolk precursors are probably delivered to the ovary by the coelomic system and the genital haemal system serves as an intragonadal nutrient storage site. That the haemal system functions as an insect fat body absorbing nutritive material from the coleomic fluid is suggested by the results of several investigations (Ferguson 1984; Jackson & Fontaine 1984; Beijnink & Voogt 1986). The genital haemal sinus of O. paucispina appears to function primarily to support oogenesis, and as emphasised by Walker (1982), this sinus should be regarded as a unique componenet of the haemal system. For O. paucispina, the relationship between the oocyte and the genital haemal sinus may be enhanced to facilitate the production of large yolky oocytes.

The fate of the coated vesicles formed by endocytosis could not be followed, but it is presumed that they contain yolk precursors that are encorporated into the yolk bodies. It is not known if the uptake material is delivered to the ER and Golgi complex for processing, or if they add their contents directly to the yolk bodies. Although the ER and Golgi complex appear to be involved in formation of the yolk bodies, their contribution to the yolk material is not known. A strikingly similar relationship between rough ER and Golgi complex is described for the oocytes of the ophiuroid Ophioderma panamensis (Kessell 1968) and also for holothuroid and asteroid oogenesis (Kessell 1966; Aisenshtadt & Vassetzky 1986). This suggests that the prevalence of smooth ER in the oocytes of O. paucispina may be caused by the loss of attached ribosomes during fixation, as noted for some polychaete oocytes (Eckelbarger pers. comm.).

Endocytosis of proteins is a rapid series

of events and coated pits are usually short-lived (Dautry-Varsat 1984). For fibroblasts, coated pits have a life time estimated to be one minute (Bretscher 1987). If the coated pits of O. paucispina oocytes are also short-lived, then the prevalence of endocytotic profiles along the oolemma suggests that incorporation of material from the genital haemal sinus is intense throughout vitellogenesis. This makes the oocyte of O. paucispina a good subject with which to study the process of endocytosis.

In conclusion, the large oocyte of O. paucispina is produced through endocytotic incorporation of yolk precursors derived from a somatic source and the genital haemal sinus functions as the proximate source and store of these precursors. The results of this investigation together with the results of other studies (Walker 1982; Beijnink et al. 1984; Smiley & Cloney 1985; Beijnink & Voogt 1986; Harrington & Ozaki 1986; Shyu et al. 1986), suggests that extraovarian vitellogenesis may be common in echinoderms.

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