VITELLOGENESIS OF *RUGOGASTER HYDROLAGI* (TREMATODA, ASPIDOGASTREA)

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SUMMARY

Vitelline follicles are surrounded by a basal lamina and fibrous matrix. Cytoplasmic processes of peripheral perikarya extend between the vitelline cells and into them. Immature vitelline cells have relatively little cytoplasm, many mitochondria, much rough endoplasmic reticulum, Golgi complexes and none or few yolk granules. Maturing vitelline cells have relatively more cytoplasm and contain large vacuoles with many dark grana, free ribosomes, much rough endoplasmic reticulum, lipid droplets, and many mitochondria. At later stages of maturation and in mature cells dense irre-

gularly shaped inclusions and large interconnected spaces containing much α -glycogen are present. Intact vitelline cells are incorporated into the egg. Small vitellogonads are ciliated, have intraepithelial nuclei separated by lateral cell membranes, surface lamellae, and are surrounded by a basal lamina and fibrous matrix. Presence of perikarya in the vitellogonads separated by lateral cell membranes is characteristic of monogeneans and trematodes including the aspidogastreans, syncytial vitellogonads are synapomorphic for the Gyrocotylidae, Amphilinidae and Cestodea.

RÉSUMÉ : Vitellogénèse chez *Rugogaster hydrolagi* (Trematoda, Aspidogastrea).

Les follicules vitellins sont entourés par une lame basale et une matrice fibreuse. Des extensions cytoplasmiques issues de péricaryons périphériques s'étendent entre et à l'intérieur des cellules vitellines. Les cellules vitellines immatures ont relativement peu de cytoplasme, des mitochondries nombreuses, un réticulum endoplasmique granulaire abondant, des appareils de Golgi, et pas ou peu de granules de vitellus. Les cellules vitellines en maturation ont un cytoplasme relativement plus abondant et contiennent des grandes vacuoles avec de nombreux grains denses, des ribosomes libres, un réticulum endoplasmique granulaire abondant et de nombreuses gouttelettes lipidiques et mitochondries. Aux stades suivants de la maturation et dans les cellules mûres, on observe des

inclusions denses de forme irrégulière et des grands espaces interconnectés contenant beaucoup d'alpha-glycogène. Les cellules vitellines sont incorporées intactes dans l'œuf. Les petits vitellogonades sont ciliés, ont des noyaux intraépithéliaux séparés par des membranes cellulaires latérales, présentent des expansions lamellaires de surface et sont entourés par une lame basale et une matrice fibreuse. La présence de péricaryons séparés par des membranes cellulaires latérales dans les vitellogonades est caractéristique des monogènes et des trématodes y compris les aspidogastres, alors que les vitellogonades syncytiaux constituent une synapomorphie des Gyrocotylidae, Amphilinidae et Cestodea.

INTRODUCTION

Ultrastructural studies have made major contributions to our understanding of the phylogeny of Platyhelminthes (Ehlers, 1985; Rohde, 1990), but aspects mainly considered are ultrastructure of protonephridia (review by Rohde, 1991) sperm and spermatogenesis (review by Justine, 1991; Rohde *et al.*, 1991b), sensory receptors (*e. g.* Rohde and Watson, 1990b; Rohde *et al.*, 1991c) epidermis and tegument (*e. g.* Rohde and Watson, 1990a), and pharynx (Watson and Rohde, in press a, b). Relatively little attention has been paid to vitellogenesis, although a major difference between large platyhelminth taxa is the occurrence of ecto- or entolecithal eggs, *i. e.* of separate vitellaria and ovaries in the

Neophora, and of female gonads producing egg cells containing the yolk in the « lower » taxa (Ehlers, 1985).

Ultrastructural studies on the female gonads of non-parasitic taxa were reviewed by Ehlers (1985, references therein). Fine structure of vitellaria, vitellogenesis and egg shell formation (to which the vitellaria contribute) were studied by Irwin and Threadgold (1970), Halton *et al.* (1974), Swiderski and Mokhtar (1974), Erasmus (1975), Irwin and Maguire (1979), Hanna (1976), Swiderski and Mackiewicz (1976), Swiderski *et al.* (1978), Erasmus *et al.* (1982), Fukuda *et al.* (1983), Lumsden and Hildreth (1983), and Xylander (1987, 1988). The papers by Xylander cover phylogenetic aspects, whereas emphasis of the other papers is on functional and morphological aspects. In this paper, for the first time, we describe the ultrastructure of vitellogenesis in an aspidogastrea trematode, *Rugogaster hydrolagi* from the rectal glands of chimaerid fishes off the coast of south-eastern Australia (Rohde *et al.*, 1991a).

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MATERIALS AND METHODS

Specimens of *Rugogaster hydrolagi* were collected from the rectal glands of chimaerid hosts during an expedition of the CSIRO Research vessel, FRV « Southern Surveyor », off the north-eastern coast of Tasmania during June-July 1991. They were fixed for 2 h in 3 % glutaraldehyde in 0.1 M sodium cacodylate buffer, made with filtered sea-water, at pH 7.3 and 4° C. After four washings in buffer they were stored in buffer at 4° C for 21 days, post-fixed in 1% O_3O_4 in buffer for 1 h at room temperature, dehydrated in ethanol, embedded in Spurr resin and polymerized at 65° C for 20 h. Ultrathin sections were stained with uranyl acetate and lead citrate and examined under a Jeol 1,200 Ex microscope at 60 kV.

RESULTS

Yolk follicles extend in two lateral bands along the body of *Rugogaster*. Follicles are surrounded by a basal

lamina and a fibrous matrix underneath it (Fig. 1). Thin cytoplasmic processes extend from the periphery between the yolk cells, and — as indicated by sections through cytoplasmic protusions in the periphery of the cells — into the cells (Figs. 1, 6, 7). Perikarya belonging to these processes were not seen with the electron microscope, but were visible in semithin sections stained with toluidine blue. Immature vitelline cells, with relatively little cytoplasm, many mitochondria, abundant rough endoplasmic reticulum, Golgi complexes and no or few yolk granules (Figs. 1-4) are located in the periphery of the follicles (Figs. 1, 2). Maturing vitelline cells have relatively more cytoplasm and contain varying numbers of large round vacuoles filled with round dense droplets, free ribosomes, much rough endoplasmic reticulum, lipid droplets and many mitochon-

FIG. 1-2. — Sections through yolk follicle. Note follicle surrounded by basal lamina (b) and fibrous matrix (f), cytoplasmic processes between developing yolk cells (asterisks), immature yolk cells (1), maturing yolk cells (2), and large space filled with electron dense material. Note two immature yolk cells and yolk filling much of the lumen of the follicle in Figure 2. li - lipid droplets (?). Scale bars 2 μ m.

FIG. 3-4. — Immature yolk cells of Figure 2 at higher magnification. Note nucleus (n), numerous mitochondria (m), abundant rough endoplasmic reticulum (r), golgi bodies (go), and secretory droplets (s). Scale bars 2 μ m.

dria (Figs. 1, 6, 7). In vitellocytes at more advanced stages of maturation, some dense irregularly shaped inclusions may be present (Fig. 1, top left). Mature vitelline cells, in addition to the vacuoles with dark droplets and lipid droplets, possess large numbers of irregularly shaped dense inclusions (Figs. 5, 6, 8-10), as well as large interconnected spaces containing numerous granules of medium electron density, apparently glycogen (Figs. 5, 6, 8). Intact mature vitelline cells may be found in large spaces of the yolk follicles (Fig. 6), which may also contain large amounts of yolk grana of various types (Fig. 2) indicating that some yolk cells disintegrate in the yolk follicles. In some instances, large spaces partly filled with an electron-dense substance, apparently fused yolk granules, were seen in the follicles (Fig. 1). But typically, intact mature vitelline cells migrate into the yolk ducts (Figs. 9-11) and are incorporated intact into the egg (Fig. 12). Vitelline cells in the egg

contain lipid droplets, dark bodies and spaces with glycogen, but vacuoles with round dark granules are absent (Fig. 12).

The vitellogenic duct is ciliated, has intraepithelial nuclei (Figs. 9, 11), lamellae extending into the duct lumen, and many mitochondria (Figs. 9-11). The duct, like the yolk follicles, is surrounded by a basal lamina and fibrous matrix (Figs. 9-11). Presence of lateral cell membranes connected by apical septate junctions (Figs. 10, 11) indicates that the duct epithelium is not syncytial. Some apposed membranes are broken up into short pieces (Fig. 10).

DISCUSSION

Structure of vitelline follicles and yolk cells in various stages of development closely resembles that in other parasitic Platyhelminthes examined to date (Trematoda: Irwin

FIG. 5-8. — Maturing and mature yolk cells. Mature yolk cell with nucleus (n), large lipid droplets (li), vacuoles with many round dense secretory droplets (s), large irregularly shaped dense inclusions, interconnected spaces containing many bodies of medium electron density. Fig. 6. Yolk follicles with many maturing (2) and two mature yolk cells (3), the latter in a large liquid filled space. Also note cytoplasmic processes (asterisks) between maturing yolk cells. Fig. 8. Nucleus (n) and inclusions of mature yolk cell (vacuoles with dark round droplets, large irregularly shaped dense inclusions, and interconnected spaces containing many bodies of medium density = glycogen?). Scale bars 2 μ m (Figs. 5, 7, 8), 5 μ m (Fig. 6).

FIG. 9-10. — Vitelloduct with mature yolk cell. Note nucleus (n) in wall of duct, invagination of surface membrane (arrows), some incomplete (double arrows), apical septate junctions (arrowheads), surface lamellae (l), numerous cilia (c), mitochondria (m), basal lamina (b) and fibrous matrix (f) surrounding vitelloduct, and mature yolk cell (3) with nucleus (n), lipid droplets (li), vacuoles with secretory droplets (s) and large irregularly shaped dense bodies. Scale bars 2 μ m.

FIG. 11-12. — Part of vitelloduct of *Figure 9* at higher magnification (*Fig. 11*) and young egg with yolk cells in uterus (u). Note basal lamina (b) and fibrous matrix around vitelloduct, nucleus (n), surface lamellae (l), cilia (c), and mature yolk cell (3) in vitelloduct. g, glycogen; li, lipid droplet; n, nucleus; s, secretory droplet in vacuole of yolk cell. Scale bars 2 μ m (*Fig. 11*) and 10 μ m (*Fig. 12*).

and Threadgold, 1970; Erasmus, 1975; Irwin and Maguire, 1979; Erasmus *et al.*, 1982; Fukuda *et al.*, 1983; Monogenea: Halton *et al.*, 1974; Cestoidea: Swiderski and Mokhtar, 1974; Swiderski and Mackiewicz, 1976; Swiderski *et al.*, 1978; Lumsden and Hildreth, 1983; Amphilinidea: Xylander, 1988; Gyrocotylidea: Xylander, 1987). For example, in the recent study of *Gyrocotyle urna*, Xylander (1987) described yolk follicles as being surrounded by an « extracellular lamina » and a nucleated cytoplasmic sheath, the latter also enveloping the peripheral vitelline cells. Similar sheaths surrounding the follicles were also described for *Amphilina foliacea* (Xylander, 1988), although nuclei belonging to the sheaths were not found. Cytoplasmic outgrowths of these « nurse cells » extend between the vitelline cells. Swiderski and Mackiewicz (1976) refer to an « interstitial syncytium » with cytoplasmic extensions that envelope the vitelline cells in the cestode *Glaridacris catostomi*. In some species of Monogenea, Halton *et al.* (1974) observed fingerlike insertions of nurse cells, referred to as parenchyma cells, into the vitelline cells, similar to those observed by us. Nurse cells are also known from trematodes (Irwin and Maguire, 1979). « Central cells », large single cells usually located in the centre of each follicle of the cestode *Glaridacris catostomi* described by Swiderski and Mackiewicz (1976), were not seen by us in ultrathin and semithin sections.

In the various species examined, all stages of the maturation of vitelline cells can (at least usually) be found in a single yolk follicle, from immature to mature. During maturation, vitelline cells form Golgi complexes (dictyosomes), abundant endoplasmic reticulum, lipid droplets and egg-shell granules. In mature cells, numbers of ribosomes, endoplasmic reticula and Golgi complexes are reduced (*e. g.* Xylander, 1987, 1988). Egg shell granules correspond to the vacuoles containing dense grana, in *Rugogaster*. In some species, *e. g.* the caryophyllidean cestodes *Glaridacris catostomi*, abundant glycogen as in *Rugogaster* is present, but such glycogen rosettes are, for instance, not obvious in *Amphilina* and *Gyrocotyle*, judged from the illustrations in Xylander (1987, 1988). Much glycogen, however, is present in large cellular spaces of the Monogenea examined by Halton *et al.* (1974). In the cestode *Glaridacris*, Swiderski and Mackiewicz (1976) found massive accumulations of glycogen even in the nuclei of yolk cells.

Erasmus *et al.* (1982) described calcareous bodies in mature yolk cells of three species of *Schistosoma*, but such bodies were absent in the fourth schistosome species examined, *S. japonicum*, and we did not find them in *Rugogaster*, although histochemical tests were not made and it cannot be excluded that the irregularly-shaped dense bodies, for example, are calcareous. In *S. japonicum*, irregularly shaped dense bodies were found in the mature vitelline cells, perhaps corresponding to similar bodies in *Rugogaster*.

Xylander (1988) drew attention to differences in the structure of the vitellogens in cestodes on the one hand and the monogeneans and trematodes on the other. Whereas cestodes have a syncytial wall lining the duct (without lateral cell membranes and junctions), the duct walls in the monogeneans and trematodes consist of discrete cells separated by lateral cell membranes. *Rugogaster* has vitellogens like the digenean trematodes and monogeneans examined to date. Following Xylander we consider syncytial vitellogens to be a synapomorphy of the cestodes (Cestoidea, Gyrocotylidea and Amphilinidea). Correspondingly, cestodes have syncytial protonephridial capillaries, whereas capillaries containing septate junctions occur in the Monogenea/Trematoda (Rohde, 1990).

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