

Ovulation and egg segregation in the tunic of a colonial ascidian, *Diplosoma listerianum* (Tunicata, Ascidiacea)

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Summary. The process of egg segregation in the tunic of the ovoviviparous ascidian *Diplosoma listerianum* was studied by light and electron microscopy. One egg at a time was seen to mature in each zooid. The eggs had large yolk and grew on the ovary wall enveloped in four layers: (1) outer follicle cells (OFC), long and rich in RER (rough endoplasmic reticulum) and with dense granules in the Golgi region; (2) flat inner follicle cells (IFC); (3) a loosely fibrillar vitelline coat (VC); (4) test cells encased on the egg surface. The growing egg protrudes from the ovary wall and presses on the contiguous epidermis. Granulocytes enter the space between the epidermis and the egg and insinuate cytoplasmic protrusions, disrupting the continuity of the OFC layer. At ovulation, OFC and IFC are discharged and form a post-ovulatory follicle (*corpus luteum*). The epidermis shrinks and closes, possibly by activation of microfilaments, causing the egg to be completely surrounded by the tunic. In the zooid, the wound caused by the passage of the egg is repaired both by contraction of the epidermis and by phagocytic activity. Altered spermatozoans are found in phagocytosing cells in the lumen of the ovary. These are presumably remnants of those which entered to fertilize the egg before segregation.

A. Introduction

Various modes of sexual reproduction have evolved in ascidians. Solitary ascidians are generally oviparous and produce a great quantity of eggs with relatively little yolk; fertilization is external and embryonic development is rapid. Compound ascidians are generally ovoviviparous and produce a limited number of large, yolky eggs; embryos develop over a long period of time. However, some compound ascidians are truly viviparous and have small alecithical eggs (Mukai et al. 1987) and exceptionally long periods of embryo development – as long as 5 months in *Hypsistozoa fasmariana* (see Brewin 1956).

In most ovoviviparous ascidians the embryos are maintained inside the branchial chamber, exposed to seawater. In others, they are segregated in particular regions of the oviduct or tunic (Van Name 1945; Berrill 1950; Brewin 1956).

Reproduction has been studied especially in the solitary forms of ascidians (see Kessel 1983; Monroy and Rosati

1983). In comparison, little attention has been paid to the colonial species. We recently described the mechanism of ovulation and placentation in the ovoviviparous *Botryllus schlosseri* (Zaniolo et al. 1987). In this species, the prominent outer follicle cells (OFC) are discharged at ovulation and the egg, covered by inner follicle cells (IFC), remains in the atrial chamber kept by a “placenta” (or brood pouch). The mechanism of ovulation involving discharge of the OFC is common in botryllids (Mukai 1977; Mukai et al. 1987), but it differs from that of oviparous ascidians, such as *Ciona* (Pérès 1954; Cotelli et al. 1981), in which the OFC are lacking or are poorly developed. Nevertheless, in all ascidians studied so far the ovulated eggs are enveloped by follicle cells which correspond to the IFC of *Botryllus schlosseri*.

To obtain further information regarding the reproduction of colonial ascidians, we studied ovulation and egg segregation in *Diplosoma listerianum*. In this species, embryos develop in the tunic to form tadpole larvae, which escape by swimming from the colony. Brief, preliminary reports on this topic have previously been published (Burighel et al. 1986; Martinucci et al. 1986).

B. Materials and methods

Diplosoma listerianum (Milne-Edwards, 1841) (Aplousobranchiata, Didemnidae) forms flat encrusting colonies with numerous small zooids (1–2 mm high). The colonial tunic is mainly arranged as a basal layer attached to the substrate, and an upper layer on whose surface the oral siphons of the zooids and the common cloacal apertures emerge. The zooids stand in the tunic and their short recto-oesophageal peduncle separates the thorax from the abdomen containing the gut and the hermaphroditic gonads (Fig. 1). Each colony produces a great number of tadpoles during the breeding season, which occurs when temperatures exceed 10° C (Brunetti et al. 1988).

We used some wild colonies obtained from the Lagoon of Venice and others from cultures reared in aquaria at the Stazione Idrobiologica di Chioggia. Parts of colonies, fixed in 1.5% glutaraldehyde buffered with 0.2 M sodium cacodylate and osmicated in 1% OsO₄, were processed for electron microscopy (EM) as previously described (Burighel and Schiavinato 1984). Several specimens, selected with regard to gonad development, were cut serially. Thin sections were stained with uranyl acetate and lead citrate and examined under a Hitachi H 600 electron microscope.

C. Results

In *D. listerianum* the ovary is vesicular and lies in the abdomen close to the bilobed testis. It is dorsal to the testis and sperm duct.

Eggs grow in the wall of the ovary and herniate by pressing on the contiguous epidermis (Figs. 1–3). Eggs at different stages of development occur in the same zooid but undergo maturation one at a time. Fully grown eggs possess four envelopes: the OFC, the IFC, the vitelline coat (VC), and the test cells (Figs. 3, 6). As they increase in size, the eggs come to protrude completely from the ovary wall. However, at the point of attachment the ovary wall penetrates the thickness of the OFC layer (Figs. 4, 5) and continuity is maintained between the basal membranes of the two epithelia. The ovary forms a tubular structure which extends along the recto-oesophageal peduncle flanking the sperm duct and the intestine. On the opposite side it ends in the OFC of the most developed egg, which lies at the bottom of the ovary (Figs. 4, 5).

1. Egg envelopes

In the fully grown egg the OFC are numerous and are arranged in a single continuous layer resting on a thick basal membrane. They are joined by intercellular adhering junctions and are characterized by numerous elongated cisternae of rough endoplasmic reticulum (RER). In the portion of the cell facing the egg there is an extended Golgi region and a number of round dense granules of different sizes (Fig. 6).

The IFC are flat and form a loose layer. They are connected to each other at some points and there are wide spaces through which the prolongations of the OFC can reach the underlying vitelline coat (Fig. 6). The IFC are also connected to the OFC at scattered points by adhering junctions. A few RER cisternae, vesicles and tubules of smooth endoplasmic reticulum, together with occasional lipid droplets, also occur.

The VC adheres to the basal membrane of the IFC. It consists of a loose network of filamentous material, crossed by finger-like microvillar protrusions of the oocyte. The protrusions reduce in number as the egg matures (Fig. 6).

Test cells (Figs. 3, 4, 6) are roundish and encased in

pits in the egg surface. They are characterized by the presence of round granules, the content of which is arranged in concentric rings or in an irregular filamentous network.

2. Ovulation

In *D. listerianum*, ovulation and the almost concurrent segregation of the egg in the tunic occur according to a sequence of events summarized in Fig. 22. The fully grown egg protrudes into the neighbouring tunic and is progressively enveloped by the epidermis (Fig. 3), while blood cells, especially granulocytes, move into the space between the egg and the epidermis (Figs. 6, 7). Granulocytes possess numerous dense granules, mostly of two types. The smaller have a homogeneous, moderately dense content resembling that of contiguous RER cisternae, while the others are of various size and have a strongly dense core often surrounded by a light halo (Fig. 8). Granulocytes aggregate in a cap-like structure encrusting the OFC on the opposite side to that of the egg-ovary attachment (Fig. 7). Some of them emit cytoplasmic protrusions which are inserted progressively between the OFC (Figs. 9, 10). The infiltrating cytoplasmic portion lacks granules and is rich in filamentous material (Figs. 9, 10). Occasionally entire granulocytes are seen to penetrate the OFC layer.

Between the egg and the zooid the epidermis shrinks in a collar, which becomes increasingly narrow. The OFC break at the level of the cap and, together with the IFC, slide onto the surface of the oocyte, retracting towards the zooid. They form a plug-like structure which occludes the lumen of the epidermal collar (Fig. 11). Near the egg, the epidermis breaks all around the collar forming a provisional wound, but it rapidly extends to close the edges of the wound so that the egg becomes completely enveloped and segregated in the tunic. On the zooid side the wound is initially closed by the discharged OFC and then by the epidermal cells which press the follicle cells into the zooid (Figs. 11, 12). Some of the OFC remain in the tunic (Fig. 12) until they degenerate and are engulfed by granulocytes or epidermal cells functioning as macrophages (Buriqhel et al. 1987).

The epidermis undergoes changes during egg segregation. In the collar, its cells become narrower and increase in height, while cytoplasmic protrusions extend, accompanied by the basal membrane which becomes folded

Fig. 1. Detail of a zooid of *Diplosoma listerianum*, seen in vivo, showing abdomen connected to branchial basket (*br*) by peduncle (*arrow*). One egg (*o*) protrudes from the abdomen

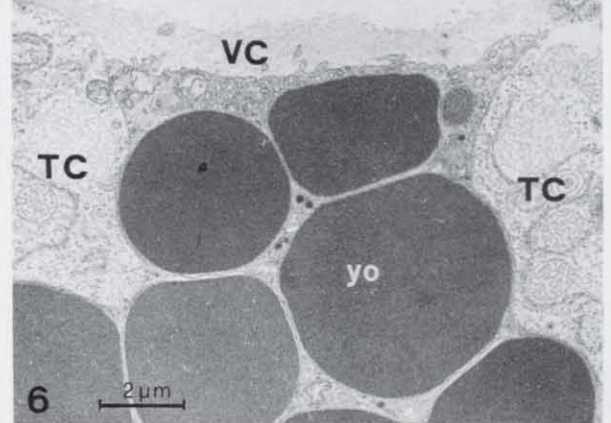
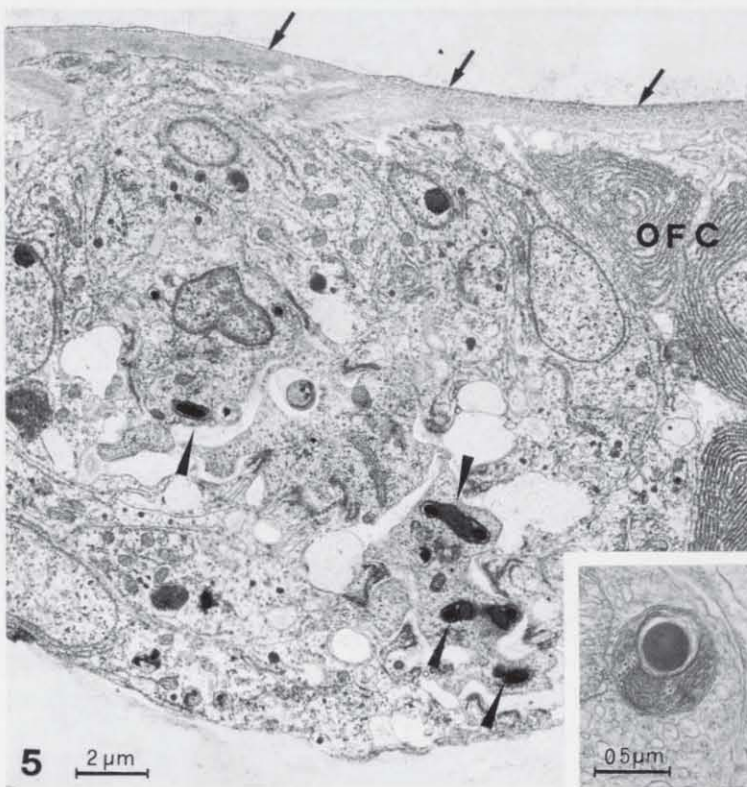
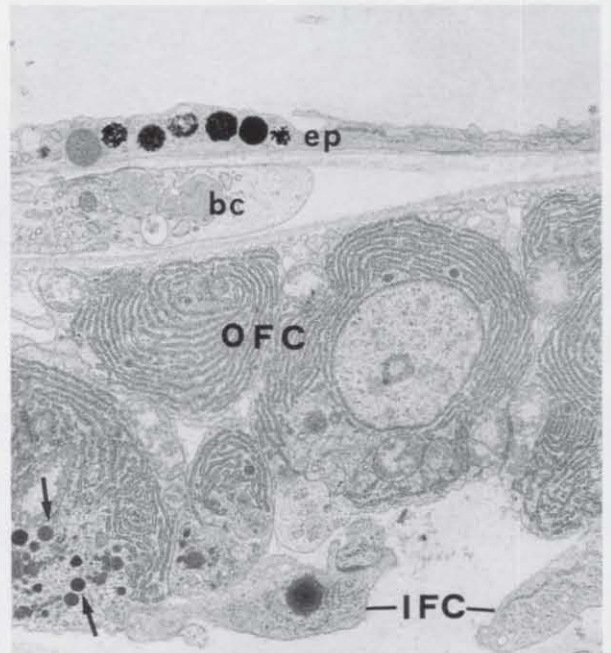
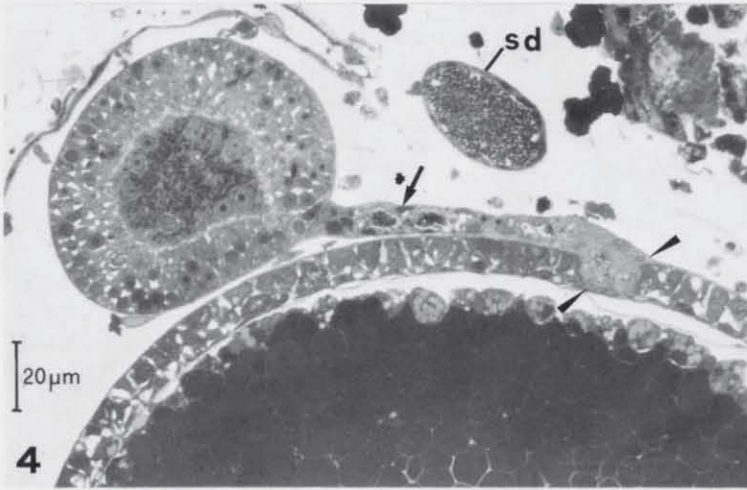
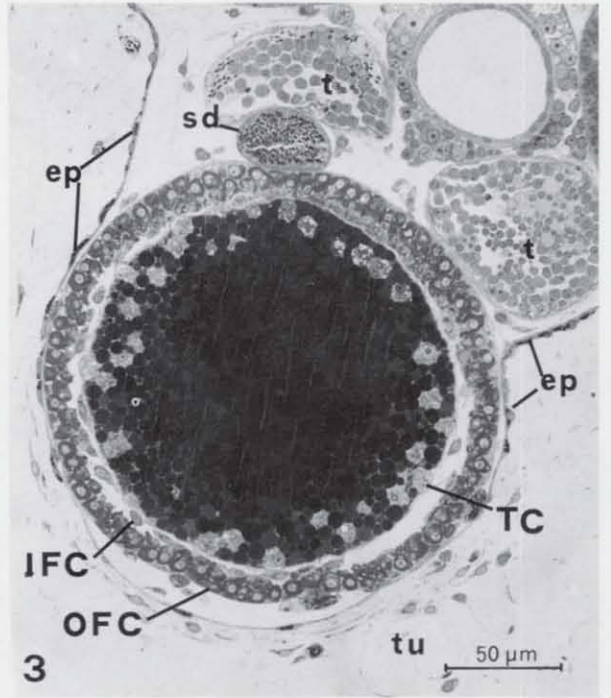
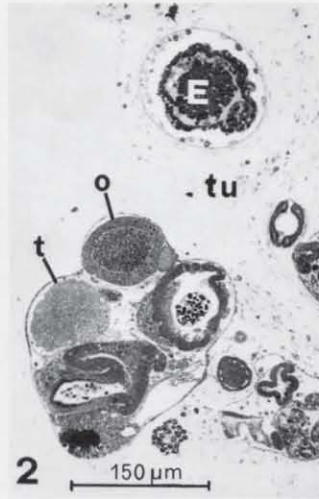
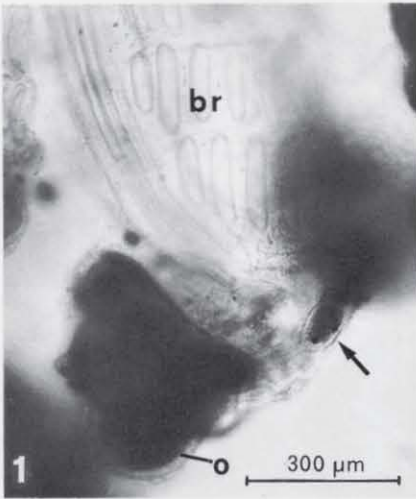
Fig. 2. Thick section showing one embryo (*E*) segregated in the tunic (*tu*) and one egg (*o*) in the abdomen, close to testis (*t*) and gut. Toluidine blue stained

Fig. 3. Fully grown egg protrudes towards tunic (*tu*), pressing on contiguous epidermis (*ep*). Outer follicle (*OFC*), inner follicle (*IFC*) and test cells (*TC*) are recognizable. *sd* sperm duct; *t* testis

Fig. 4. Micrograph shows two eggs at different stages of development, connected to the ovary wall (*arrow*), which appears to end in outer follicle layer of more mature egg (*arrowheads*). *sd* sperm duct

Fig. 5. Electronmicrograph showing point of connection of ovary wall in *OFC* layer. Basal membranes of the two epithelia are continuous with each other (*arrows*). Several macrophages containing degenerating spermatozoans (*arrowheads*) occupy lumen of ovary. *Inset*: transverse section of a spermatozoon engulfed in a macrophage

Fig. 6. Electronmicrograph corresponding to peripheral region of egg shown in Figure 3. *OFC* are high in number, rich in *RER* cisternae, and have dense granules (*arrows*) in the Golgi region towards the oocyte. *IFC* layer is flattened and discontinuous. A wandering blood cell (*bc*) is between the epidermis (*ep*) and the *OFC*. *TC*: test cells, *VC*: vitelline coat, *yo*: yolk



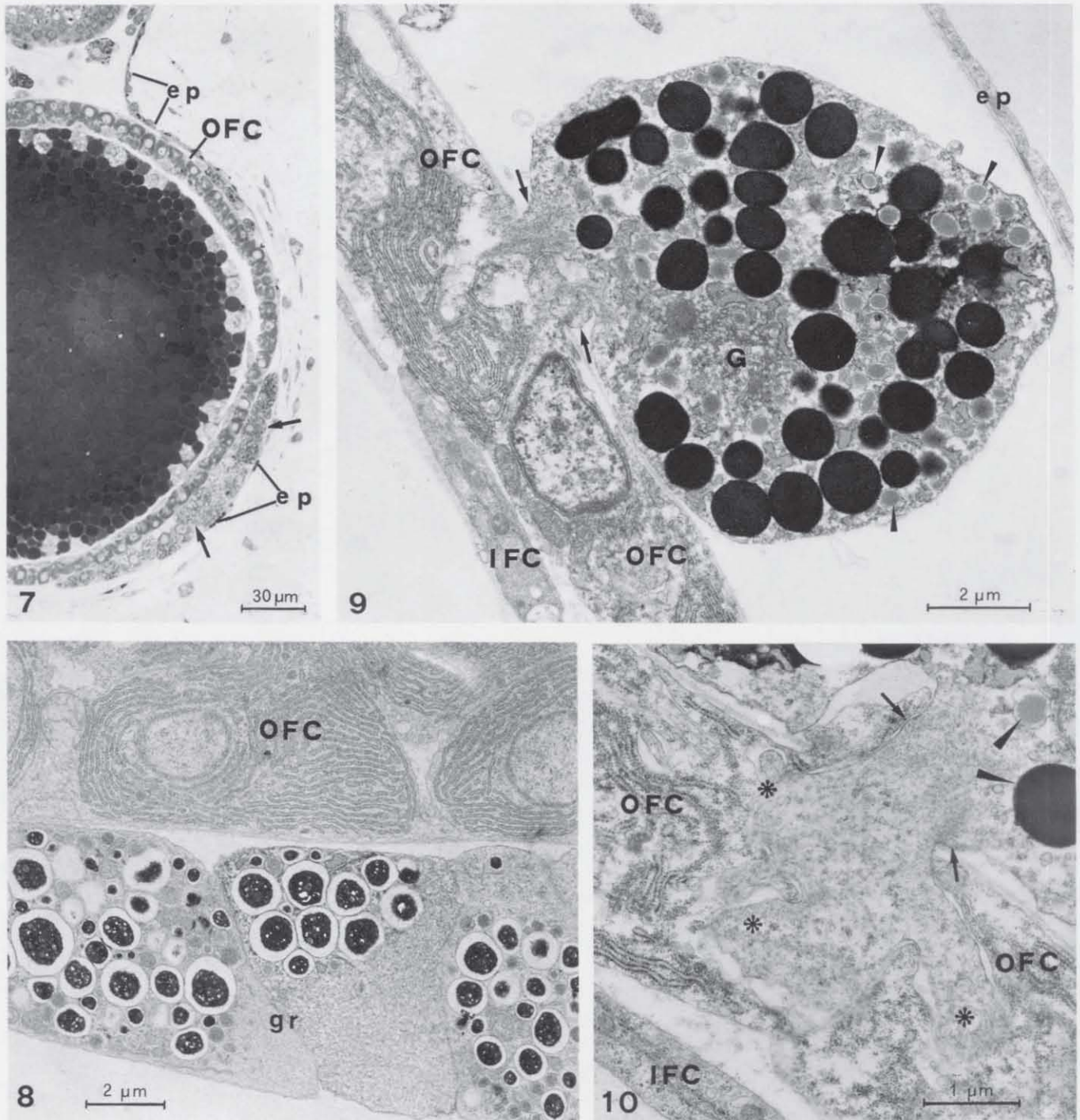


Fig. 7. With approaching ovulation, numerous granulocytes infiltrate from the blood into the space between *OFC* and epidermis (*ep*). Several of them (*arrows*) accumulate in a cap on opposite side from zooid

Fig. 8. Detail of a thin section of cap in Fig. 7 showing granulocytes (*gr*) adhering to *OFC* rich in *RER*. Granulocytes contain numerous round granules, except in some regions where undifferentiated cytoplasm appears

Fig. 9. Granulocyte containing black, strongly electron-dense granules and others, smaller and less dense (*arrowheads*), adheres to *OFC* and inserts cell protrusions (*arrows*) devoid of granules between them. *ep*: epidermis, *G*: Golgi field, *IFC*: inner follicle cells

Fig. 10. Detail of a granulocyte, labelled by its granules (*arrowheads*), penetrating among *OFC*. Invading cytoplasmic portion (*asterisks*) has no organelles, but fibrillar material is recognizable (*arrows*). *IFC*: inner follicle cells

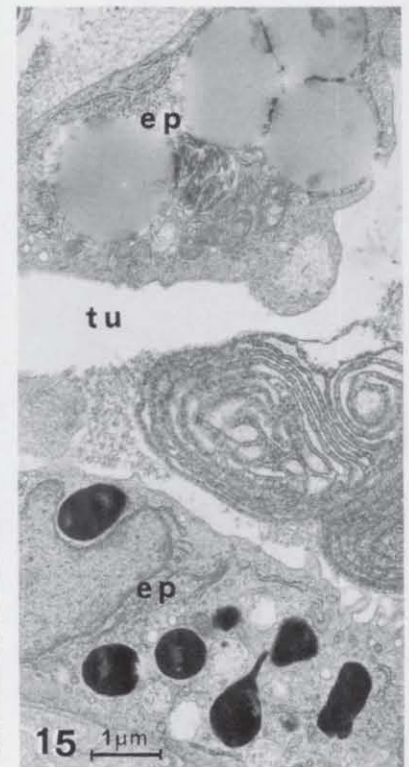
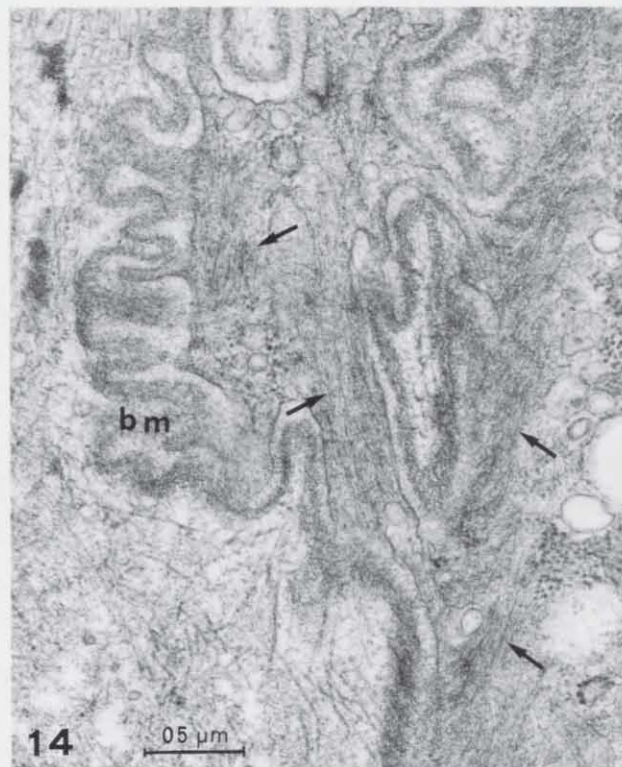
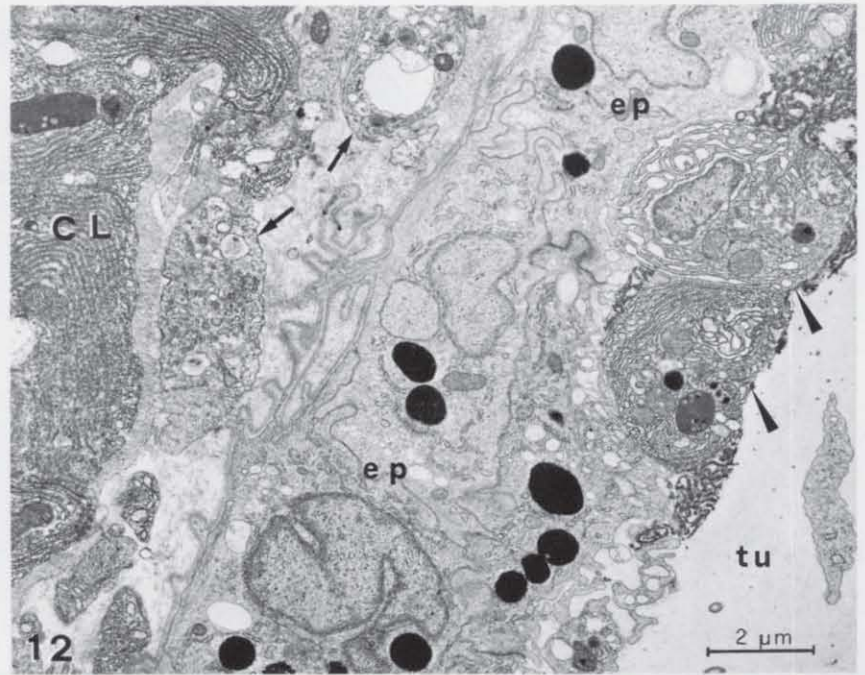
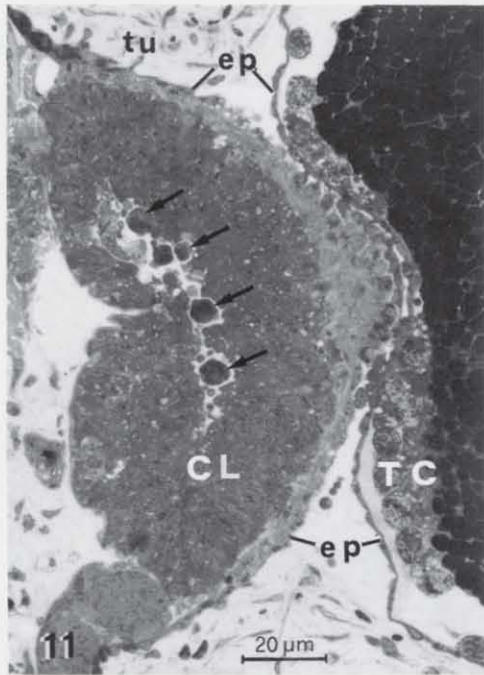


Fig. 11. Ovulation has just occurred. Egg (right) is in the process of being completely enveloped by epidermis (*ep*) which is closing lip of wound (cfr. Fig. 22). *OFC*, discharged from egg during ovulation, accumulate in wound region to form a post-ovulatory follicle (*corpus luteum*: *CL*). Some granulocytes are visible among them (*arrows*). *tu* tunic; *TC* test cells

Figs. 12–14. Details of zooid epidermis in region involved in egg extrusion

Fig. 12. Epidermal cells (*ep*), marked by round dense bodies, are thick and deeply interdigitated. Most of the discharged *OFC*, still rich in *RER*, are in the post-ovulatory follicle (*CL*), but some (*arrowheads*) are in the tunic (*tu*) and will be engulfed by macrophages and epidermal cells. Some macrophages (*arrows*) are under pleated basal membrane of epidermis

Figs. 13, 14. Basal regions of epidermal cells (*ep*) showing interdigitation and several deep protrusions accompanied by basal membrane (*bm*). Bundles of microfilaments (*arrows*) are visible in basal cytoplasm, attached to baso-lateral plasmalemma. Basal membrane is connected to a well developed underlying layer of filamentous material

Fig. 15. The epidermis (*ep*) of the zooid (*below*) had dark granules. In the epidermis surrounding the egg (*above*) the granules enlarge, becoming less dense. They are then extruded into the tunic (*tu*) where residues of discharged *OFC* are also visible

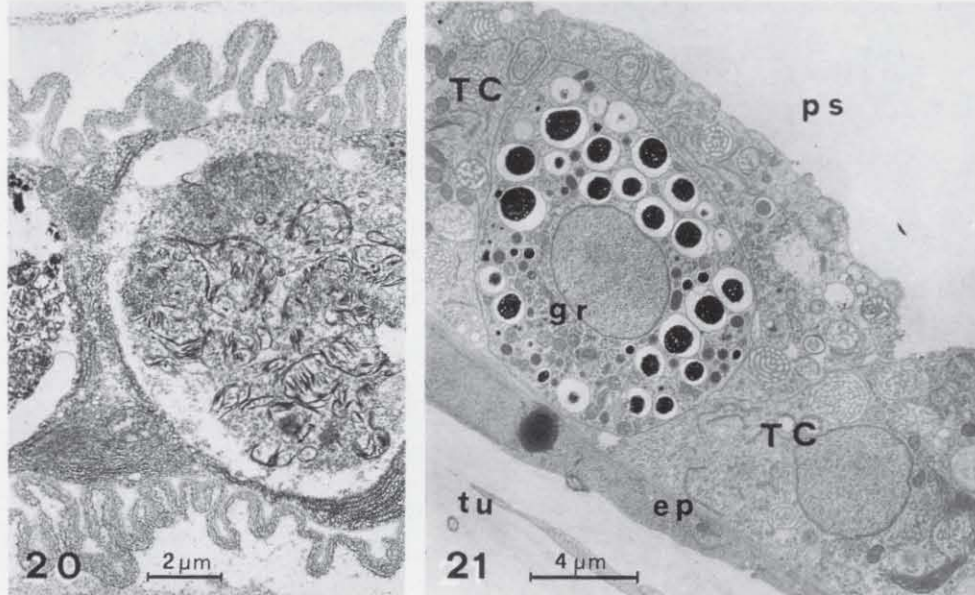
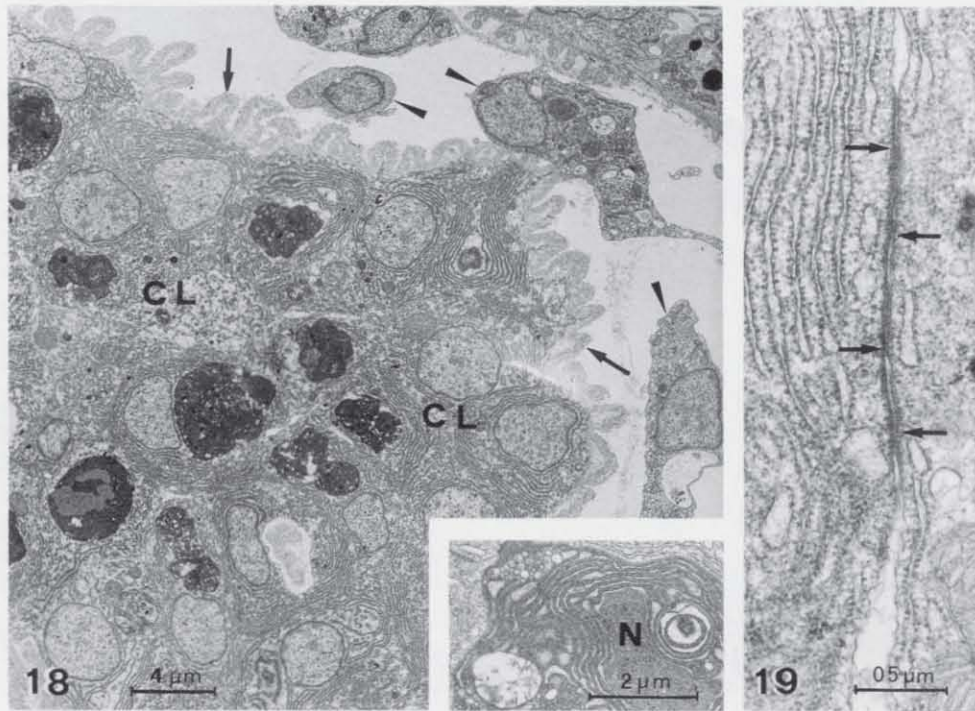
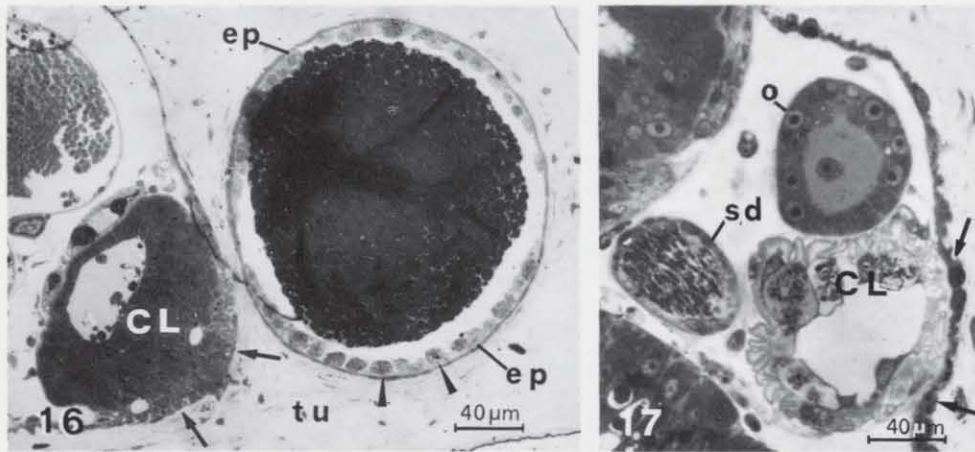


Fig. 16. Egg just segregated in the tunic (*tu*) is enveloped by epidermis (*ep*) under which test cells and granulocytes (*arrowheads*) are scattered in the perivitelline space. The epidermis is thick in the wound region through which the egg passed (*arrows*). *CL*: corpus luteum

Fig. 17. Corpus luteum (*CL*) almost completely reabsorbed; only residual scattered cell debris and basal membrane are visible. One young oocyte (*o*) and sperm duct (*sd*) flank it. The epidermis is flat, close to the corpus luteum (*arrows*)

Fig. 18. Just after ovulation, the corpus luteum (*CL*) is externally covered by discrete basal membrane (*arrows*). Cells still possess *RER* cisterns although several of them are undergoing involution, producing dense bodies. Several blood macrophages (*arrowheads*) are near the corpus luteum. *Inset*: a dense body, with a condensed nucleus (*N*) and vacuolated cytoplasm, is visible

Fig. 19. Detail of two discharged *OFC* connected by possible tight junction (*arrows*)

Fig. 20. Residual part of corpus luteum, defined by pleated basal membrane, shows necrotic cells containing large heterophagic vacuoles

Fig. 21. Test cells (*TC*) and one granulocyte (*gr*) close to epidermis (*ep*), in the perivitelline space (*ps*) of the egg, which is completely segregated in the tunic (*tu*)

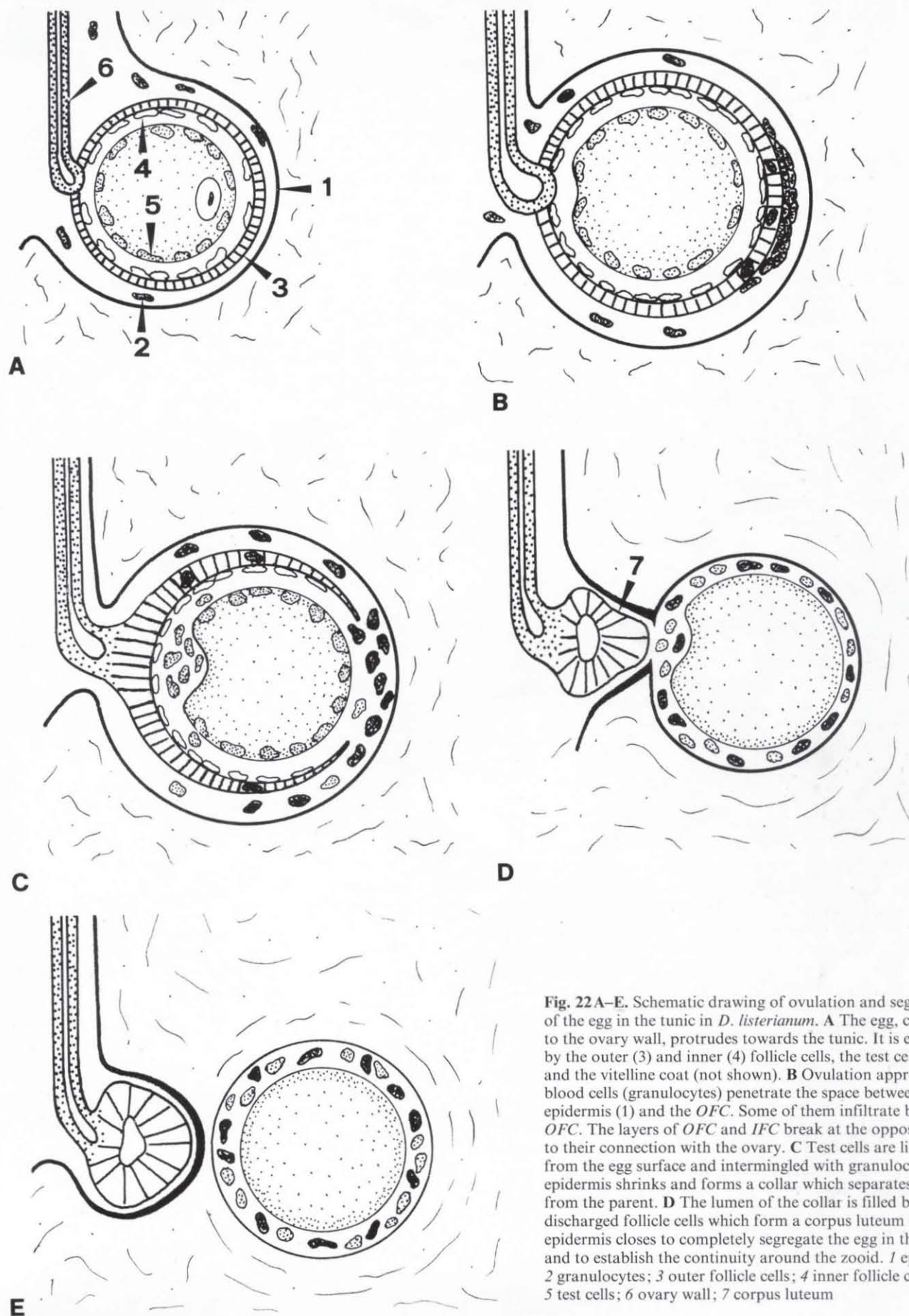


Fig. 22A–E. Schematic drawing of ovulation and segregation of the egg in the tunic in *D. listerianum*. **A** The egg, connected to the ovary wall, protrudes towards the tunic. It is enveloped by the outer (3) and inner (4) follicle cells, the test cells (5) and the vitelline coat (not shown). **B** Ovulation approaching, blood cells (granulocytes) penetrate the space between the epidermis (1) and the OFC. Some of them infiltrate between OFC. The layers of OFC and IFC break at the opposite side to their connection with the ovary. **C** Test cells are liberated from the egg surface and intermingled with granulocytes. The epidermis shrinks and forms a collar which separates the egg from the parent. **D** The lumen of the collar is filled by the discharged follicle cells which form a corpus luteum (7). **E** The epidermis closes to completely segregate the egg in the tunic and to establish the continuity around the zooid. 1 epidermis; 2 granulocytes; 3 outer follicle cells; 4 inner follicle cells; 5 test cells; 6 ovary wall; 7 corpus luteum

(Figs. 12, 13). In the basal cytoplasm bundles of microfilaments, arranged parallel to the axis of cell shortening, are recognizable. They are inserted onto the baso-lateral plasmalemma of the cells (Figs. 13, 14). All these changes occur in the epidermis of the collar close to the zooid. In contrast, the epidermis enveloping the egg appears to be flat, with no evidence of bundles of microfilaments or folds of the basal membrane. Nevertheless, this epidermis does show changes in its granules. These become less dense and are later extruded into the tunic (Fig. 15).

3. *Corpus luteum*

In the zooid the OFC (and IFC) discharged from the egg form a provisional post-ovulatory follicle, resembling a sort of corpus luteum (Figs. 16–18). The OFC maintain intercellular junctions (Fig. 19) and delimit a cavity, the presumed residue of the space occupied by the egg (Fig. 16). The surface of the corpus luteum is coated by the original OFC basal membrane, which is thick and pleated (Figs. 17, 18). Many macrophages are near the corpus luteum and some also adhere to it, but they do not penetrate to the inside (Fig. 18). Cell degeneration occurs in the corpus luteum by both necrosis and formation of autophagic bodies with a heterogeneous content (Fig. 18). As degeneration proceeds, the corpus luteum is reduced in volume, large vacuoles are formed, and the products of digestion are dispersed through the basal membrane, which persists as the last indication of ovulation (Figs. 17, 20).

During corpus luteum regression the epidermis of the wound region becomes flat. In the tunic, the epidermis delimits a large perivitelline space around the egg, where the test cells are liberated from the egg surface and several granulocytes entered during ovulation (Figs. 16, 21). As the egg begins cleavage, it moves away from the zooid toward the basal layer of the tunic, where it completes development.

Figure 22 illustrates ovulation and egg segregation in *D. listerianum* based on the present study.

D. Discussion

In most ascidians the oviduct or the analogous follicle stalk is the path through which the ripe egg reaches the site of fertilization. In some compound ascidians the oviduct plays another role in addition to simple transport, becoming modified to form special structures which allow the egg to settle. This occurs, for example, in *Botryllus* (Mukai 1977; Zaniolo et al. 1987), where the oviducal epithelium participates in the formation of the brood pouch, and in several families of Aplousobranchiata, both ovoviviparous and viviparous, in which the oviduct forms a distal arm having the function of a brood pouch (see Van Name 1945; Brewin 1956; Kott 1969). In all these cases, ovulated eggs are exposed to seawater and sperms reach the eggs either in the atrial chamber or in the oviduct.

In *D. listerianum*, the ovulated egg is segregated in the tunic and is not exposed to seawater, so the question of which pathway the sperm follows in order to reach and fertilize the ripe egg is one of great interest. However, wandering cells containing degenerating spermatozoans in heterophagic vacuoles were sometimes seen in the lumen of the ovary. This strongly suggests that spermatozoans are able to enter the ovary to fertilize eggs. We have recently obtained evidence that this actually occurs and that it is

made possible by the presence of a type of oviduct (Burighel et al. 1986 and unpublished data).

1. *Outer follicle cells*

The ovarian egg of *D. listerianum* has thick OFC which show signs of intense protein synthesis (numerous long RER cisternae, Golgi region with secretory granules). Similar features were observed in *B. schlosseri*, where the OFC were reported to be engaged in processing material to be passed to the oocyte (Zaniolo et al. 1987). This may also be the case for *D. listerianum*. Moreover, the hypothesis that the OFC layer is involved in yolk formation agrees with the evidence that the oviparous species with small eggs have a very thin OFC layer (Cotelli et al. 1981; Kessel 1983; Sugino et al. 1987) and with the evidence that in *Botrylloides*, the species with large, yolky eggs have a thicker OFC layer than the species with eggs devoid of yolk granules (Mukai et al. 1987).

As in botryllids (Mukai 1977; Mukai et al. 1987; Zaniolo et al. 1987), the discharged OFC of *D. listerianum* form a provisional post-ovulatory follicle (corpus luteum), whose possible influence on other tissues is not clarified.

2. *Granulocytes and inner follicle cells*

With approaching ovulation, a noteworthy relationship is established between granulocytes arriving from the blood stream and the egg. The granulocytes adhere to the OFC and insert cytoplasmic protrusions through the basal membrane to separate the follicle cells from each other. This results in a loss of continuity in the OFC layer, which slides onto the oocyte together with the IFC. The granulocytes seem to also have other functions, since they remain in great numbers in the perivitelline space of the ovulated egg, intermingled with the test cells. Unlike those found in other ascidians, these eggs are surrounded by the maternal epidermis and not by the follicle layer corresponding to the IFC of the ovarian egg. This situation may require the persistence of the granulocytes around the developing embryo, either as a defence or in order to prepare the hatching of the larva.

The role of the IFC in ascidians has often been debated; they were considered to be involved in fertilization (De Santis et al. 1980; Honegger 1986), in egg flotation (Lambert and Lambert 1978), and in anchoring the embryo to the parent (Zaniolo et al. 1987). In *D. listerianum*, whatever the function of the IFC, it must occur in the phase before segregation of the egg in the tunic.

3. *Microfilaments*

In the reproductive modality of *D. listerianum* the epidermis of the parent displays some special functions. Not only, as seen above, does it replace the IFC which surround the embryo in other ascidians, but it also causes segregation of the egg in the tunic. The process occurs by shrinkage of the epidermis at the boundary between egg and parent. The epidermis shows differentiated features at this point – increase in cell height and cytoplasmic extrusion in the basal region – reminiscent of the situation of tissue shrinkage seen in ascidians, all caused by activation of cytoplasmic microfilaments (Schiaffino et al. 1974; Cloney 1978; Burighel and Schiavinato 1984). Both in the latter cases and

in *D. listerianum*, bundles of microfilaments, recognizable in the basal cytoplasm and connected with the baso-lateral plasmalemma, may be responsible for cell shortening in the region of the collar.

Strict synchronism exists between egg segregation and the discharge of the OFC and IFC layers. The reason for this synchronism is not obvious, since it would seem to be simpler first to discharge the follicle cells and then to segregate the egg without forming an effective wound. Indeed, in *D. listerianum* a wound on the side of the parent is formed, through which exogenous matter may enter. However, coordination between cells involving the discharge of follicle cells, contraction of the epidermis, and the activation of phagocytes, all help to maintain the characteristics of the internal fluid and repair the wound (Burighel et al. 1987).

Recently, Mackie and Singla (1987) presented evidence that the tunic of *Diplosoma* is contractile owing to the presence of a network of monocytes rich in microfilaments and myocytes, a system which is excitable to the touch and also spontaneously active. We presume that the release of the egg excites the network of myocytes, causing contractions so that the egg is forced to move into, and eventually settle in, the basal layer of the tunic. Here the embryo undergoes its long and complex development (Brunetti et al. 1988).

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References

- Berrill NJ (1950) The Tunicata, with an account of the British species. Ray Society, London
- Brewin BI (1956) The growth and development of a viviparous compound ascidian, *Hypsistozoa fasmiana*. Q J Microsc Sci 97:435-454
- Brunetti R, Bressan M, Marin M, Libralato M (1988) On the ecology and biology of *Diplosoma listerianum* (Milne Edwards 1841) (Ascidacea, Didemnidae). Vie Milieu (in press)
- Burighel P, Schiavinato A (1984) Degenerative regression of the digestive tract in the colonial ascidian *Botryllus schlosseri* (Pallas). Cell Tissue Res 235:309-318
- Burighel P, Martinucci GB, Zaniolo G (1986) Ovulation in the ovoviviparous ascidian, *Diplosoma listerianum*. Acta Embryol Morphol Exp NS 7:102-103
- Burighel P, Martinucci GB, Zaniolo G (1987) Tissue repair during egg segregation in tunic of the compound ascidian *Diplosoma listerianum*. Acta Embryol Morphol Exp NS 8 (in press)
- Cloney RA (1978) Ascidian metamorphosis: review and analysis. In: Chia F-S, Rice ME (eds) Settlement and metamorphosis of marine invertebrate larvae. Elsevier North Holland Biomedical Press, New York, pp 255-282
- Cotelli F, Andronico F, De Santis R, Monroy A, Rosati F (1981) Differentiation of the vitelline coat in the ascidian *Ciona intestinalis*: an ultrastructural study. Wilhelm Roux' Arch Dev Biol 190:252-258
- De Santis R, Jamunno G, Rosati F (1980) A study of the chorion and follicle cells in relation to sperm-egg interaction in the ascidian, *Ciona intestinalis*. Dev Biol 74:490-499
- Honegger TG (1986) Fertilization in ascidians: studies on the egg envelope, sperm and gamete interactions in *Phallusia mammillata*. Dev Biol 118:118-128
- Kessel RG (1983) Urochordata-Ascidacea. In: Adiyodi KG, Adiyodi RG (eds) Reproductive biology of invertebrates. Vol I Oogenesis, oviposition and oosorption. Wiley, Chichester New York, pp 655-734
- Kott P (1969) Antarctic ascidiacea. Antarctic Res Ser 13:1-239
- Lambert CC, Lambert G (1978) Tunicate eggs utilize ammonium ions for flotation. Science 200:64-65
- Mackie GO, Singla CL (1987) Impulse propagation and contraction in the tunic of a compound ascidian. Biol Bull 173:188-204
- Martinucci GB, Zaniolo G, Burighel P (1986) Ovulazione nelle ascidie coloniali *Botryllus schlosseri* e *Diplosoma listerianum*. Boll Zool 53 [Suppl]:22
- Monroy A, Rosati F (1983) Review articles: a comparative analysis of sperm-egg interaction. Gam Res 7:85-102
- Mukai H (1977) Comparative studies on the structure of reproductive organs of four botryllid ascidians. J Morphol 152:363-379
- Mukai H, Saito Y, Watanabe H (1987) Viviparous development in *Botrylloides* (compound ascidians). J Morphol 193:263-276
- Pérès JM (1954) Considérations sur le fonctionnement ovarien chez *Ciona intestinalis* (L.). Arch Anat Microsc Morphol Exp 43:58-78
- Schiaffino S, Burighel P, Nunzi MG (1974) Involution of the caudal musculature during metamorphosis in the ascidian *Botryllus schlosseri*. Cell Tissue Res 153:293-305
- Sugino YM, Tominaga A, Takashima Y (1987) Differentiation of the accessory cells and structural regionalization of the oocyte in the ascidian *Ciona savignyi* during early oogenesis. J Exp Zool 242:205-214
- Van Name WG (1945) The North and South American ascidians. Bull Am Mus Nat Hist 84:1-476
- Zaniolo G, Burighel P, Martinucci GB (1987) Ovulation and placentalization in *Botryllus schlosseri* (Ascidacea): an ultrastructural study. Can J Zool 65:1181-1190

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