

Review Article

Ascidian follicle cells: Multifunctional adjuncts to maturation and development

Charles C. Lambert*

University of Washington Friday Harbor Laboratories, 620 University Road, Friday Harbor, WA 98250, USA

Ascidians are primitive chordates, subphylum Tunicata, that are sessile filter-feeding hermaphrodites as adults. Released oocytes are enclosed within a monolayer of follicle cells, a non-cellular vitelline coat and a monolayer of test cells that cover the egg membrane. Follicle cell structure is distinctive in different groups. They originate from circulating hemoblasts with functional nuclei. They are necessary for germinal vesicle breakdown in several species and may secrete a meiosis-inducing substance to the oocyte. In some families the follicle cells are necessary for fertilization. Although all ascidians are hermaphrodites, many are not capable of self fertilization. The follicle cells seem to be involved in self, non-self discrimination. Attachment of sperm to egg involves a sperm surface glycosidase binding to an egg surface glycoside. The primary block to polyspermy involves a glycosidase released by the follicle cells. In one species with direct development, the follicle cells secrete a sticky substance that anchors the embryos in a wave-swept rocky area; a brooding solitary ascidian with a tadpole larva uses a sticky substance secreted by follicle cells to attach the brood to the atrial chamber. Several species have floating eggs due to buoyancy of their follicle cells, a result of ammonia sequestration in at least one species. Many other marine invertebrates release eggs with attached follicle cells, and all vertebrates ovulate oocytes covered with follicle cells. Comparisons are discussed between these groups and ascidians.

Key words: ascidian, flotation, follicle cell, germinal vesicle, oocyte.

Introduction to ascidians and their gonads

Ascidians are invertebrates from the phylum Chordata, subphylum Tunicata, class Ascidiacea, considered by many to be the closest living relatives of the vertebrates (Ruppert 2005; Delsuc *et al.* 2006; Jeffery 2007). Because of their evolutionary position, large numbers of easily fertilized eggs, invariant cleavage pattern, small genome size (16 000 protein coding genes) and known structure (Dehal *et al.* 2002), ascidians are highly studied in terms of molecular development (Sato 2001; Sato & Levine 2005). The opportunity to study ascidian oocytes is increasing rapidly as they are among the most invasive of marine organisms and have had a deleterious effect on aquaculture (Lambert 2007).

Ascidians are distinguished from other tunicates by being sessile as adults. They include three orders:

Aplousobranchia, Phlebobranchia and Stolidobranchia (Lahille 1890). The aplousobranchs are all colonial with small zooids and ovaries either in the loop of the gut or posterior to the gut. All aplousobranchs have internal fertilization from large, complex broadcast-spawned sperm. Most phlebobranchs and stolidobranchs are large (>1 cm) solitary individuals with relatively small free spawned eggs, minute simple sperm and small uncomplicated larvae. In phlebobranchs such as *Ascidia* and *Corella* the ovary is confined to the surface of the stomach or in the loop of the gut. Stolidobranchs like *Styela* and *Halocynthia* have their ovaries in the body wall where there are usually multiple hermaphroditic gonads.

Within the ascidian ovary each oocyte is enclosed within a layer of outer follicle cells left behind at ovulation, a layer of inner follicle cells (usually referred to as follicle cells) (labeled outer follicle cells in Berrill 1929), a non-cellular vitelline coat and a layer of test cells (termed inner follicle cells in Berrill 1929). The test cells function in formation of the larval tunic (Cloney 1990; Cloney & Hansson 1996). With few exceptions inner follicle cells are larger than outer follicle cells. Figure 1A,B illustrates this organization. These structures together with the oocyte constitute an ovarian

*Author to whom all correspondence should be addressed.

Email: clambert@fullerton.edu

Received 30 April 2009; revised 12 July 2009; accepted 13 July 2009.

© 2009 The Author

Journal compilation © 2009 Japanese Society of Developmental Biologists

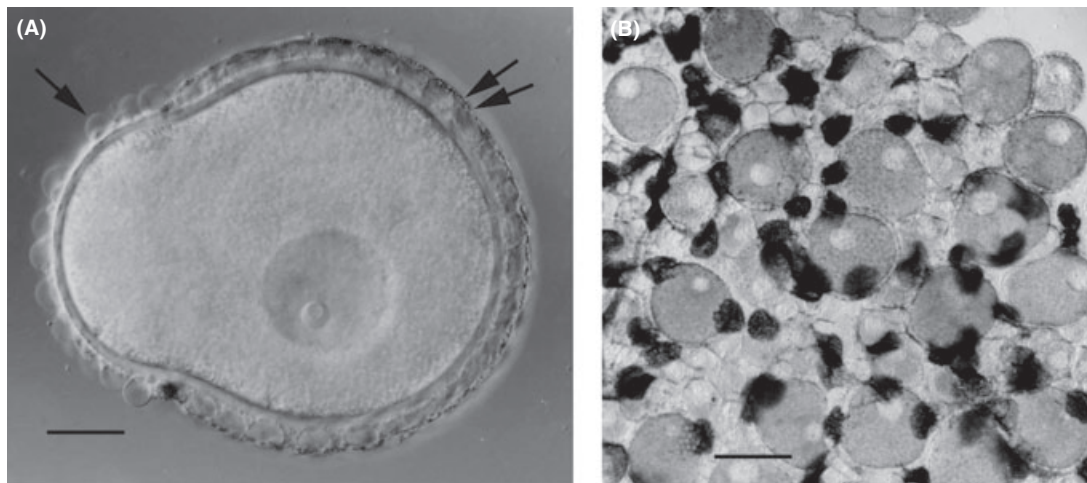


Fig. 1. (A) Ovarian follicle of *Herdmania momus*. Flat, thin red outer follicle cells (double arrows) enclose the inner follicle cells (single arrow) on most of the oocyte surface. Contraction of the outer follicle cells has resulted in partial ovulation. Bar, 50 μm (reproduced with permission of *Dev. Growth Differ.* 50 [3], cover photo.) (B) Ovarian fragment of *Herdmania momus* shortly after ovulation has resulted in expulsion of the oocytes and accumulation of contracted outer follicle cells (black irregular structures are contracted outer follicle cells, they were red in life); bar, 200 μm .

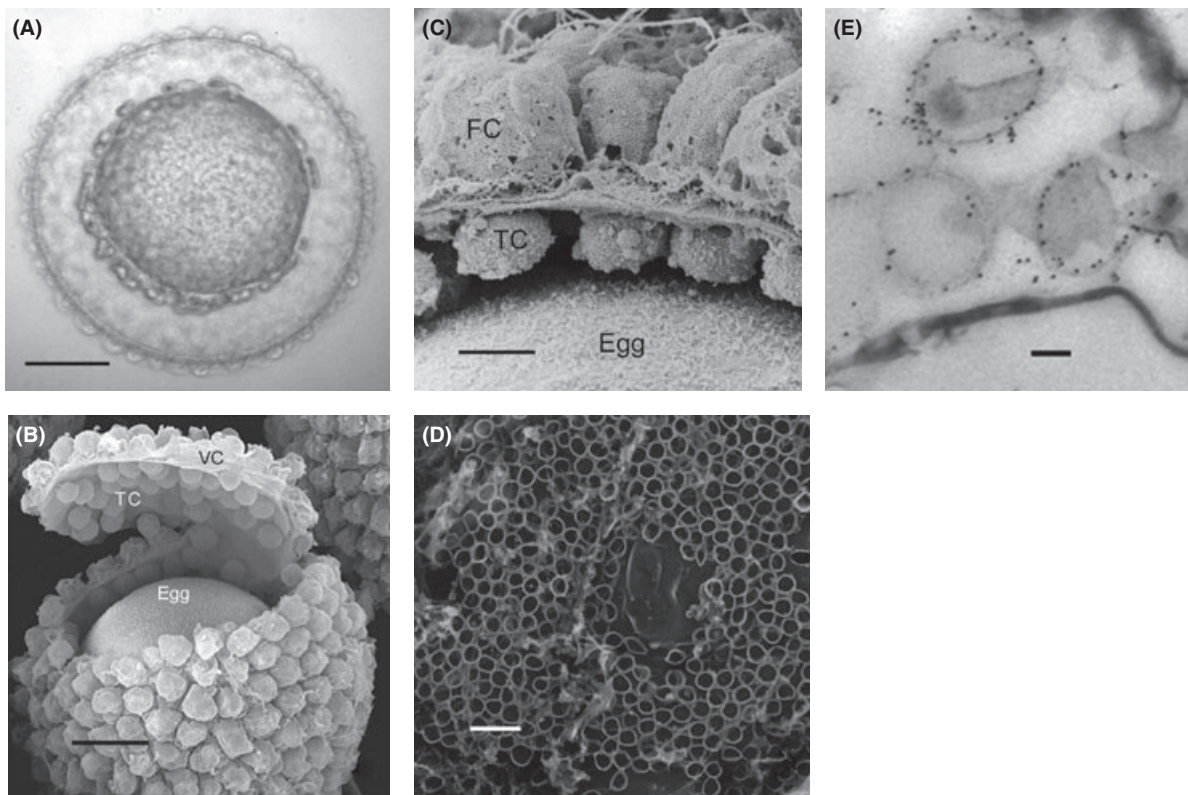


Fig. 2. (A) *Phallusia mammillata* egg. Bar, 50 μm . (B) Scanning electron micrograph of *Phallusia mammillata* egg showing follicle and test cells (Courtesy of T. Honegger, reproduced with permission of *Int. J. Dev. Biol.*). Bar, 50 μm . (C) *Ascidia ceratodes* egg. FC, follicle cells; TC, test cells; bar, 5 μm . (D) Microannuli on follicle cells of *Ascidia ceratodes* (quick freeze, fractured, deep etched, rotary shadowed preparation); bar, 100 nm. (E) Transmission electron micrograph of three microannuli binding succinylated WGA 1 nm colloidal gold particles (wheat germ agglutinin [WGA] is a lectin specific for glucosamine); bar, 10 nm. (C, D and E courtesy of R. Koch and J. Johnson).

follicle (Fig. 1A). Since the ovulated oocyte lacks the outer follicle cells, in this review, when not otherwise specified I will refer to the inner follicle cells as follicle cells but recognize that this is only strictly correct after ovulation has removed the outer follicle cells (Fig. 2A–C).

“Extensive evidence, both direct and indirect, demonstrates that somatic follicle cells, particularly those enveloping and directly attached to the oocyte, execute multiple and variable functions throughout the life and at distinct stages of gamete and follicle development in most animals” (Schuetz 1985). The follicle cells are involved in all aspects of oogenesis but have specific functions during maturation and early development. This review is concerned mainly with these events and does not deal with other earlier functions of follicle cells during oogenesis.

Ovulation and structure of follicle cells in solitary ascidians

Contraction of ovarian cells overlying the oocyte results in ovulation throughout the animal kingdom. This has been studied in many vertebrates and invertebrates, but not in ascidians (Schroeder & Talbot 1985). Oocytes of *Pyura haustor*, *Herdmania pallida* and *H. momus* assume a dumbbell shape during ovulation (C. Lambert, unpubl. data, 1985) which is indicative of contraction by overlying cells (Schroeder & Talbot 1985). Furthermore the heavily pigmented, contracted outer follicle cells are clearly visible after ovulation in *Herdmania momus* (Fig. 1B). Therefore it is reasonable to conclude that contraction of the outer follicle cells causes ovulation with the expulsion of the oocyte with its remaining accessory cells into the lumen of the follicle stalk. The contracted outer follicle cells remain behind in the ovary (Fig. 1B). It is not known if they persist as a corpus luteum after ovulation. In most species the outer follicle cells are transparent and difficult to see in living ovarian follicles (Villa & Patricolo 2001) but in *Herdmania momus* the outer follicle cells are pigmented red, which makes them readily visible especially during and after contraction and ovulation (Fig. 1A,B). The epithelium and lumen of the follicle stalk is continuous with the epithelium and lumen of the ovary. Thus the ovulated oocyte covered by its inner follicle cells arrives in the lumen of the ovary. Ovulated oocytes may be stored in the lumen of the ovary or in the oviduct depending upon the species (Burighel & Cloney 1997). Ascidian oocytes are ovulated with intact germinal vesicles, a situation quite different from brachiopod (Stricker & Folsom 1997) and mammalian oocytes, which initiate germinal vesicle breakdown (GVBD) at ovulation. In

the phlebobranchs *Ascidia*, *Phallusia* and *Ciona* mature eggs are stored in the oviduct; in *Corella inflata* they are stored in the ovary and pass into the ciliated oviduct during spawning (C. Lambert, unpub. data, 1967). Stolidobranchs store eggs in the ovary or multiple oviducts.

Structure and interspecific variation in follicle cells

Follicle cells vary in size and shape but most are characterized by numerous vacuoles and other often granular inclusions. In scanning electron micrographs the surface of *Phallusia* and *Ascidia* follicle cells is covered by microannuli (Fig. 2D,E) or membrane protrusions varying in size between 1.0 and 2.5 μm (Villa & Patricolo 1993; Honegger & Füglistner 2001; Honegger & Koyanagi 2008) which seem to be absent in stolidid follicle cells (Villa & Patricolo 2000). These surface ornaments preferentially bind wheat germ agglutinin (WGA) in *Ascidia ceratodes* (Fig. 2E) and *Phallusia mammillata* (Honegger & Füglistner 2001; Honegger & Koyanagi 2008). According to Honegger & Füglistner (2001) they are the remnants of secretory activity.

Follicle cell size and shape varies between species especially in phlebobranchs. They are more uniform in stolidobranchs. Probably the greatest differences in size are seen in phlebobranchs where one species such as *Ascidella scabra* or *Corella eumyota* produces eggs that sink in still water, while the sister species *Ascidella aspersa* and *Corella inflata* produce floating eggs with large follicle cells (Fig. 3A–C,E) (Lambert *et al.* 1995). Follicle cells in the genus *Ciona* are coniform and of considerable length, an adaptation that retards sinking (Fig. 3F). Each *Ciona* follicle cell contains one or more refringent granules. The granules are terminal and single in *Ciona intestinalis* and *Ciona roulei* (Lambert *et al.* 1990) but sub-terminal and multiple in *Ciona edwardsi* and *Ciona savignyi* (Byrd & Lambert 2000).

Intraspecific variation: Incipient speciation

Currently there is a great deal of interest in speciation in the genus *Ciona*. *Ciona intestinalis* has historically been considered to have a world-wide distribution, but recently it has been shown that the species by which classical morphology has been identified as *C. intestinalis* actually includes at least two genetic clades: A and B (Nydam & Harrison 2007). In 2005 I examined oocytes of *C. intestinalis* from Gamagori Harbor in Ise Bay, Japan and found that their follicle cells were very different from those collected in California. Rather than the “normal” California organization with a single

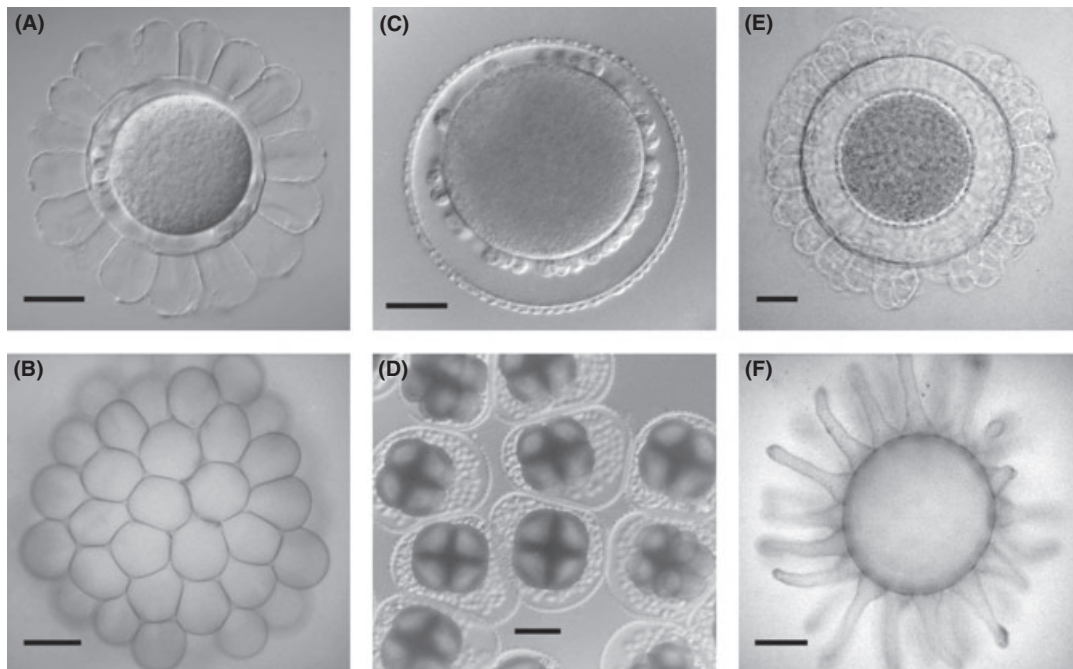


Fig. 3. (A) *Corella inflata* egg showing large floating follicle cells; bar, 50 μ m. (B) Surface view of *Corella inflata* follicle cells; bar, 50 μ m. (C) *Corella eumyota* egg with small non-floating follicle cells; bar, 50 μ m. (D) *Corella eumyota* brooded embryos adherent by sticky follicle cell mucus; bar, 100 μ m. (E) *Ascidiella aspersa* egg with large floating follicle cells; bar, 50 μ m. (F) *Ciona intestinalis* egg with long follicle cells that slow sinking rate in still water; bar, 50 μ m. (C and D reproduced with permission of *Canadian Journal of Zoology*).

terminal refringent drop (Byrd & Lambert 2000), most had a single drop midway along the length of the follicle cell, while some individuals lacked the refringent drop. I call this difference to the attention of ascidiologists because differences in follicle cell structure easily seen in a conventional light microscope may very well mirror differences in molecular makeup.

Origin of follicle cells

The origin of follicle cells is a contentious issue with well reasoned hypotheses supporting two quite divergent views. In one model the inner follicle cells are thought to have arisen by division of cells of the outer follicular envelope (Tucker 1942; Sugino *et al.* 1987; Manni *et al.* 1993). In the other model, undifferentiated hemoblasts (stem cells) infiltrate the outer follicular epithelium to take up their definitive positions and undergo differentiation (Mukai & Watanabe 1976; Gianguzza & Dolcemascio 1978; Mancuso 1994; Cloney 1995). Thorough searches of sections have not been successful in either detecting mitosis of outer follicle cells or migration of hemoblasts into ovarian follicles (Cloney 1995). What is needed to clarify this long-standing issue is studies on cell surface markers, either naturally occurring glycosides or antibodies to cell surface markers. A monoclonal antibody that

recognizes certain hemoblast cells of *Halocynthia roretzi* also binds to follicle cells (Arai *et al.* 2001). Moreover, Lorian Ballarin* (pers. comm., 2008) isolated a monoclonal antibody to a cell surface marker that labeled one type of circulating blood cell in *Botryllus schlosseri*, which also labeled follicle cells. These data support the hemoblast origin of follicle cells, but leave us with the problem of trans-epithelial emigration of hemoblasts which has not been seen in gonads but occurs in other tissues of ascidians and many other organisms (Cloney & Grimm 1970).

Synthetic properties of follicle cells

Electron microscopy of ascidian follicle cells discloses multiple signs of protein synthesis including a well developed rough endoplasmic reticulum (Cloney 1995; Burighel & Cloney 1997) and secretory granules (Cloney 1995). In the colonial stolidobranch *Botryllus schlosseri* the outer follicle cells are much larger than the inner follicle cells; these outer follicle cells secrete protein yolk during oogenesis (Zaniolo *et al.* 1986; Martinucci *et al.* 1988) which is incorporated into growing oocytes. Direct autoradiographic

*Correction added after online publication 07 September 2009: Fabio Gasparini was corrected to Lorian Ballarin.

data on synthesis by follicle cells by Jeffery (1980) demonstrated nuclear incorporation of radioactive uridine and cytoplasmic incorporation of radioactive leucine. He showed that the follicle cells synthesize a 42 000 MW polypeptide of unknown function. Isolated follicle cells release a protease (Lambert 2008) and a glycosidase (Lambert *et al.* 1997; Honegger & Koyanagi 2008), which were presumably synthesized by the follicle cells.

Role in GVBD

In stolidobranch ascidians, oocytes with intact germinal vesicles are stored in the ovary. Transfer of oocytes to ordinary sea water results in breakdown of the germinal vesicle in a few minutes. GVBD occurs very rapidly after placing oocytes of the stolidobranchs *Halocynthia roretzi* (Hirai 1941), *Molgula manhattensis* (Sawada & Schatten 1989), *Herdmania pallida* and *Boltenia villosa* (Lambert 2005, 2008) in pH 8.2 sea water (SW). Oocytes kept at low pH fail to undergo GVBD unless stimulated (Sakairi & Shirai 1991; Lambert 2005, 2008).

Removal of the follicle cells of *Halocynthia roretzi* oocytes blocks GVBD in response to normal pH 8.2 SW (Sakairi & Shirai 1991). The opposite was concluded in other studies with *Halocynthia roretzi* (Bates & Nishida 1998; Prodon *et al.* 2008) and *Ciona intestinalis* (Prodon *et al.* 2006), though it is possible that the follicle cells had already triggered GVBD before removal (Bates & Nishida 1998; Prodon *et al.* 2006, 2008). Boiling or addition of protease inhibitors to a supernatant obtained from *Halocynthia* oocytes undergoing GVBD suggested to Sakairi & Shirai (1991) that a protease was produced from the follicle cells that induced GVBD. Ascidians, like several other invertebrates, use an increase in cytoplasmic cAMP levels to induce GVBD (Lambert 2008). Removal of the follicle cells of *Boltenia villosa* oocytes abolished GVBD in response to pH but not to the adenylyl cyclase stimulator forskolin (Lambert 2008). In addition, soybean trypsin inhibitor inhibited GVBD in response to increased pH but not forskolin. I then isolated *Boltenia villosa* follicle cells in pH 4.0 SW, raised the pH to 8.2 and found that protease activity was released, supporting the Sakairi & Shirai (1991) hypothesis. However, other compounds such as serotonin and progesterone also trigger GVBD (Lambert 2008). Therefore, clearly the follicle cells are involved in GVBD in *Ciona intestinalis* (Prodon *et al.* 2006), *Molgula manhattensis* (Sawada & Schatten 1989), *Halocynthia roretzi* (Hirai 1941) and *Boltenia villosa* (Lambert 2008), and perhaps other species. Protease inhibitors block GVBD, and isolated follicle cells release protease

activity, but the actual nature of the meiosis inducing substance remains to be elucidated.

Function in fertilization

Most ascidian eggs except for a few species have been reported to undergo fertilization after removal of the follicle cells. Possibly in some other cases where attempts were made to fertilize supposedly completely defolliculated eggs, there remained a few follicle cells attached to the vitelline coat or suspended in solution with the eggs (Honegger & Koyanagi 2008). *Halocynthia roretzi* (Fuke 1983), *Boltenia villosa* (Hice & Moody 1988) and *Phallusia mammillata* (Honegger & Füglistner 2001) cannot be fertilized with either heterologous or homologous sperm without follicle cells. Follicle cells may facilitate fertilization at least in some species (Lambert & Lambert 1981; Kawamura *et al.* 1988; Villa & Patricolo 1993; Fukumoto 2009). They seem to be involved in triggering the sperm reaction (Villa & Patricolo 1993; Honegger & Koyanagi 2008) and sperm capacitation in *Asciidiella aspersa* (Villa & Patricolo 1992).

Self-discrimination

Although nearly all ascidians are simultaneous hermaphrodites, many species are unable to fertilize their own eggs (Taneda *et al.* 1985; De Santis & Pinto 1991; Murabe & Hoshi 2002). This block to self-fertilization is very complex and genetically controlled (Murabe & Hoshi 2002) but is in part at the level of the follicle cells (De Santis & Pinto 1991; Pinto *et al.* 1995; Fuke & Numakunai 1996, 1999; Arai *et al.* 2001). In another case, self discrimination is the result of a protein added to the vitelline coat but from an unknown source (Sawada & Yokosawa 2001). Self/non-self discrimination appears to have evolved multiple times among the ascidians. Many phlebobranchs such as several from the genera *Ascidia*, *Phallusia* and *Corella* can fertilize their own eggs but the situation varies among populations and seasons in *Ciona* (De Santis *et al.* 2001; Jiang & Smith 2005). Among the stolidobranchs self fertilization seems to be possible with most molgulids but only with some species of *Styela* such as *S. canopus* (= *S. partita*) (Morgan 1942; C. Lambert, unpubl. data, 1978) but not *S. clava* (C. Lambert, unpubl. data, 2002) or *S. plicata* (Villa & Patricolo 2001). In the family Pyuridae, self fertilization is possible in *Pyura chilensis* (Manríquez & Castilla 2005) and *Halocynthia hilgendorfi* (Fuke & Numakunai 1999). But *Halocynthia roretzi* (Fuke 1983; Arai *et al.* 2001) and *Boltenia villosa* (Hice & Moody 1988) are not capable of self fertilization.

In *Ciona intestinalis* heat shock protein and proteosomes derived from follicle cells contribute to the block to self fertilization (Pinto *et al.* 1995; Marino *et al.* 1998, 1999). In *Halocynthia roretzi* (Fuke & Numakunai 1996, 1999) self sterility results, in part, from protease activity released from follicle cells.

Primary block to polyspermy

Ascidians have an invariant cleavage pattern so that any error in early cleavage is lethal (Satoh 1994). To avoid polyspermy and the attendant embryonic wastage, ascidians have evolved at least two blocks to polyspermy (Lambert *et al.* 1997). Part of the binding of sperm to the ascidian vitelline coat involves a sperm surface glycosidase (Godknecht & Honegger 1991) forming an enzyme/substrate complex with a vitelline coat glycoside (Hoshi *et al.* 1985; Honegger 1992; Bossi & Honegger 1997; Honegger & Koyanagi 2008). This enzyme/substrate complex is stable because the pH optimum of the enzyme is far below that of sea water. Ascidian eggs prevent polyspermy by first releasing a glycosidase that binds to vitelline coat glycosides to prevent supernumerary sperm binding (Lambert 1986, 1989; Litscher & Honegger 1991; Koyanagi & Honegger 2003) followed by an electrical modification of the egg plasma membrane (Lambert *et al.* 1997). The egg glycosidase is a product of the follicle cells (Lambert *et al.* 1997; Koyanagi & Honegger 2003) attached to the cell surface via a phosphatidyl inositol anchor (Lambert & Goode 1992). A follicle cell surface phospholipase releases the glycosidase (Goode *et al.* 1997; Robert *et al.* 1999). Sperm cause release of follicle surface glycosidase activity from isolated follicle cells (Lambert *et al.* 1997). Although fertilization is species-specific in ascidians, glycosidase release is not (Lambert 2000).

Embryo localization

Egg adhesion

In the colonial stolidobranch *Botryllus schlosseri*, follicle cells secrete a glue-like substance, which anchors the brooded embryo to its somatic attachment site (Zaniolo *et al.* 1986). *Molgula pacifica* is a direct developing solitary stolidobranch that frequents high current, wave swept surfaces (Bates 2002). Spawned eggs adhere to rock surfaces by means of a sticky substance secreted by the follicle cells (Young *et al.* 1988; Bates & Mallett 1991). A single large vacuole in the follicle cell secretes the sticky substance by holocrine secretion in response to lowered salinity (Young *et al.* 1988).

Several *Corella* species produce eggs with large follicle cells that float in still water (Fig. 3A,B). However, in the brooder *C. eumyota* (Brewin 1946; Lambert *et al.* 1995), the embryos adhere to one another and to the atrial wall in the vicinity of the gonoduct opening (Lambert 2004) by means of a sticky mucus secreted by the follicle cells (Fig. 3C,D). Eggs removed directly from the ovary into pH 6.0 sea water are not initially sticky but become sticky upon transfer to pH 8.0 sea water (Lambert *et al.* 1995). South African *C. eumyota* exhibit a high degree of self-fertilization (Dupont *et al.* 2007) as do those from New Zealand (Mackie G. pers. comm. quoted in Lambert 2004).

Egg flotation

Density (sinking/floating rate) of ascidian eggs and embryos varies strikingly between species; some float in still water while most sink rapidly. Density of many species is determined by the follicle cells (Child 1927; Berrill 1929; Lambert & Lambert 1978; Epel *et al.* 1999). Some species such as *Corella inflata* (Fig. 3A,B), *C. willmeriana*, *C. japonica*, *C. parallelogramma* and *Ascidella aspersa* (Fig. 3E) have large buoyant follicle cells that cause the eggs to float in still water (Lambert & Lambert 1978; Lambert *et al.* 1995) while other species such as *Ascidia ceratodes* have intermediate-sized follicle cells that slow the sinking rate (Epel *et al.* 1999). Also the elongate follicle cells of *Ciona* species (Fig. 3F) decrease the sinking rate by increasing the frictional drag. These decreases in egg sinking rate subject the eggs to increased danger of solar UV damage, which is to a major degree inhibited by UV absorbing mycosporine-like amino acids contained in the test cell/follicle cell layer in *A. ceratodes*, the only ascidian species so far examined for such molecules (Epel *et al.* 1999; Epel 2003). Flotation in *C. inflata* is the result of a decrease in follicle cell density by the accumulation of ammonium ions that replace more dense ones. This is an energy dependent process which uses glycolysis rather than oxidative phosphorylation (Lambert & Lambert 1978). In *C. inflata* the floating eggs and embryos are held in a special atrial brood chamber until well after hatching (Lambert *et al.* 1995) which diminishes the dangers of UV present in free-spawned eggs.

Comparison with follicle cells in other animals

Other invertebrate chordates

While oogenesis in nearly all animals involves various functions of the follicle cells, in only a few species do

they function in maturation and development. Little is known about the follicle cells of the pelagic tunicates. Doliolids have scattered follicle cells that persist through development while the follicular layer is so dense in salps that they obscure development (Godeaux 1990). Nothing is known about their function. In the Appendicularia, some species such as *Oikopleura dioica* produce afolliculate oocytes, while others such as *Oikopleura fusiformis*, *O. longicauda* and *O. labradoriensis* produce oocytes with a follicular envelope. Their function in the folliculate species remains a mystery (Ganot *et al.* 2006).

Other invertebrate phyla

With relatively recent re-alignments of invertebrate relationships, the only other common invertebrate deuterostomes besides tunicates are the cephalochordates, hemichordates and echinoderms (Swalla 2001; Winchell *et al.* 2002). Cephalochordates and hemichordates ovulate oocytes without follicle cells and there is nothing known about their possible functions during maturation.

In the phylum Echinodermata follicle cells are usually left behind in the ovary at ovulation (Smiley 1990) but are on the oocyte surface in artificially extracted oocytes. Asteroid oocytes are stimulated to undergo GVBD by radial nerve factor, which causes the follicle cells to release 1-methyladenine, the asteroid meiosis inducing substance (MIS) (Kanatani 1975). Unlike ascidians and several other invertebrates, cAMP inhibits GVBD in asteroid oocytes (Stricker & Smythe 2001 for review). In the closely related ophiuroids an increase in cytoplasmic cAMP induces GVBD without follicle cells (Yamashita 1988). Holothurian oocytes are also stimulated to undergo GVBD by an MIS released from follicle cells in response to radial nerve factor. The MIS appears to be 2-methyl 8-amino adenine or some other di-substituted adenine (Smiley 1990). Echinoids are quite different from asteroids, holothurians, crinoids and ophiuroids as they undergo ovulation and GVBD weeks or months before spawning (Smiley 1990). However, pigmented follicle cells of sand dollars produce an MIS that causes GVBD in asteroid oocytes (Burke & Bouland 1989).

In most other invertebrate phyla, follicle cells are important in oocyte growth in oogenesis, but with little evidence for involvement in GVBD or development. In many invertebrates, the function of the follicle cells is completed much before ovulation. Insect oocytes are surrounded by follicle cells during GVBD but their function in this process is unknown. Articulate brachiopod oocytes undergo maturation shortly after detachment of the follicle cells and ovulation (Stricker & Folsom 1997) but in an inarticulate brachiopod, *Glottidea*,

detachment of the follicle cells does not lead to GVBD (Freeman 1994). *Glottidea* oocytes resume meiosis upon incubation in membrane-permeant forms of cAMP, suggesting that a rise in oocyte cAMP is instrumental in GVBD (Freeman 1994). Perhaps the follicle cells either trigger this process by releasing an MIS or directly release cAMP into the oocyte.

Vertebrates

Generally vertebrate oocytes are enclosed by a follicular layer which like the follicle cells of ascidians and echinoderms secretes an MIS, in vertebrates usually designated a maturation inducing hormone (MIH) (Yamashita *et al.* 2000 for review). In fish the MIH is 17α , 20β -dihydroxy-4-pregnen-3-one (Nagahama *et al.* 1995). Most studies on vertebrate follicle cell function involve amphibians in which the natural MIH appears to be progesterone produced by the follicle cells, although insulin and growth factors can also induce GVBD (Ferrell 1999). Mammalian oocytes are stimulated to undergo GVBD by other steroids specific to each group (Byskov *et al.* 1999). In most vertebrate and starfish oocytes, increased cAMP levels in the oocyte inhibits GVBD (Ferrell 1999). In contrast, several marine invertebrates including nemerteans, hydrozoans, molluscs and ascidians are stimulated to undergo GVBD by an increase in intracellular cAMP levels (Lambert 2008). Oocytes of a few mammals are also stimulated to undergo GVBD by an increase in intracellular cAMP (Stricker & Smythe 2001 for review).

Rabbit follicle cells and their matrix are responsible for ciliary transport on the fimbriae of the ampulla of the oviduct (Blandau 1969); adhesion of the oocyte complex to the oviductal cilia is mediated by the follicle cell secretions. Similarly, egg pickup in other mammals also requires follicle cell adhesion to oviductal cilia (Lam *et al.* 2000; Talbot *et al.* 2003 for review). Adhesion of the oocyte to the oviduct requires the follicular matrix to bind to the cilia. This is reminiscent of the situation in the ascidians *Corella eumyota* and *Molgula pacifica* where follicle cell secretions are responsible for adhering the eggs to the atrial chamber in *C. eumyota* (Lambert *et al.* 1995) or substrate in *M. pacifica* (Young *et al.* 1988; Bates & Mallett 1991). The ascidian lineage diverged from the lineage that led to mammals millions of years ago, yet when adhesion of oocytes was required in mammalian reproduction similar mechanisms were deployed.

Acknowledgments

Gretchen Lambert contributed to many of the experiments. I am also indebted to her for help with this man-

uscript and figures. My experiments were carried out in laboratories around the world; the directors and staffs gave considerable help with development of ideas, collecting, and carrying out experiments. I owe thanks to T. Honegger, and the *International Journal of Developmental Biology* for permission to use a micrograph from Honegger & Koyanagi 2008. *Development, Growth & Differentiation* and the *Canadian Journal of Zoology* also allowed use of previously published figures. R. A. Koch and J. S. Johnson provided the unpublished micrographs used in Fig. 2C–E.

References

- Arai, M., Suzuki-Koike, M., Ohtake, S., Ohba, H., Tanaka, K. & Chiba, J. 2001. Common cell-surface antigens functioning in self-recognition reactions by both somatic cells and gametes in the solitary ascidian *Halocynthia roretzi*. *Microbiol. Immunol.* **45**, 857–866.
- Bates, W. R. & Mallett, J. E. 1991. Ultrastructural and histochemical study of anural development in the ascidian *Molgula pacifica* (Huntsman). *Roux Arch. Dev. Biol.* **200**, 193–201.
- Bates, W. R. & Nishida, H. 1998. Developmental roles of nuclear complex factors released during oocyte maturation in the ascidians *Halocynthia roretzi* and *Boltenia villosa*. *Zool. Sci.* **15**, 69–76.
- Bates, W. R. 2002. The phylogenetic significance of maximum direct development in the ascidian, *Molgula pacifica*. *Invertebr. Reprod. Dev.* **41**, 185–192.
- Berrill, N. J. 1929. Studies in tunicate development. Part I. General physiology of development of simple ascidians. *Phil. Trans. R. Soc. Lond. B* **218**, 37–78.
- Blandau, R. J. 1969. Gamete transport-comparative aspects. In: *The Mammalian Oviduct*, (eds E. S. E. Hafez & R. J. Blandau), pp. 129–162. Univ. Chicago Press, Chicago.
- Bossi, T. & Honegger, T. G. 1997. Identification of sperm plasma membrane proteins exhibiting binding affinity for the ascidian egg coat. *Dev. Growth Differ.* **39**, 551–561.
- Brewin, B. I. 1946. Ascidians in the vicinity of the Portobello Marine Biological Station, Otago Harbour. *Trans. Roy. Soc. NZ* **76**, 87–131.
- Burighel, P. & Cloney, R. A. 1997. Urochordata: Ascidiacea. In: *Microscopic Anatomy of Invertebrates*, (eds F. W. Harrison & E. E. Ruppert), pp. 221–347. Wiley-Liss, Inc., NY.
- Burke, R. D. & Bouland, C. 1989. Pigmented follicle cells and the maturation of oocytes in the sand dollar, *Dendraster excentricus*. *Dev. Growth Differ.* **31**, 431–437.
- Byrd, J. & Lambert, C. C. 2000. Mechanism of the block to hybridization and selfing between the sympatric ascidians *Ciona intestinalis* and *Ciona savignyi*. *Mol. Reprod. Dev.* **55**, 109–116.
- Byskov, A. G., Andersen, C. Y., Leonardsen, L. & Baltsen, M. 1999. Meiosis activating sterols (MAS) and fertility in mammals and man. *J. Exp. Zool.* **285**, 237–242.
- Child, C. M. 1927. Developmental modification and elimination of the larval stage in the ascidian, *Corella willmeriana*. *J. Morphol. Physiol.* **44**, 467–514.
- Cloney, R. A. 1990. Larval tunic and the function of the test cells in ascidians. *Acta Zool.* **71**, 151–159.
- Cloney, R. A. 1995. Origin and differentiation of the inner follicular cells during oogenesis in *Molgula pacifica* (Urochordata), an ascidian without test cells. *Acta Zool.* **76**, 89–104.
- Cloney, R. A. & Grimm, L. 1970. Transcellular emigration of blood cells during ascidian metamorphosis. *Z. Zellforsch.* **107**, 157–173.
- Cloney, R. A. & Hansson, L. J. 1996. Ascidian larvae: the role of test cells in preventing hydrophobicity. *Acta Zool.* **77**, 73–78.
- De Santis, R. & Pinto, M. R. 1991. Gamete self-discrimination in ascidians: a role for the follicle cells. *Mol. Reprod. Dev.* **29**, 47–50.
- De Santis, R., Marino, R. & Pinto, M. R. 2001. Further observations on the molecular bases of gamete self-discrimination in *Ciona intestinalis*: seasonal variation of self sterility rate. In: *The Biology of Ascidians*, (eds H. Sawada, H. Yokosawa & C. C. Lambert), pp. 14–17. Springer, Tokyo.
- Dehal, P., Satou, Y., Campbell, R. K., Chapman, J., Degnan, B., De Tomaso, A., Davidson, B., Di Gregorio, A. et al. 2002. The draft genome of *Ciona intestinalis*: insights into chordate and vertebrate origins. *Science USA* **298**, 2157–2167.
- Delsuc, F., Brinkmann, H., Chourrout, D. & Philippe, H. 2006. Tunicates and not cephalochordates are the closest living relatives of vertebrates. *Nature* **439**, 965–968.
- Dupont, L., Viard, F., David, P. & Bishop, J. D. D. 2007. Combined effects of bottlenecks and selfing in populations of *Corella eumyota*, a recently introduced sea squirt in the English Channel. *Divers. Distrib.* **13**, 808–817.
- Epel, D., Hemela, K., Shick, M. & Patton, C. 1999. Development in the floating world: defenses of eggs and larvae against damage from UV radiation. *Am. Zool.* **39**, 271–278.
- Epel, D. 2003. Protection of DNA during early development: adaptations and evolutionary consequences. *Evol. Dev.* **5**, 83–88.
- Ferrell, J. E. 1999. *Xenopus* oocyte maturation: new lessons from a good egg. *BioEssays* **21**, 833–842.
- Freeman, G. 1994. The endocrine pathway responsible for oocyte maturation in the inarticulate brachiopod *Glottidia*. *Biol. Bull.* **186**, 263–270.
- Fuke, M. 1983. Self and non-self recognition between gametes of the ascidian, *Halocynthia roretzi*. *Roux Arch. Dev. Biol.* **192**, 347–352.
- Fuke, M. & Numakunai, T. 1996. Establishment of self-sterility of eggs in the ovary of the solitary ascidian, *Halocynthia roretzi*. *Roux Arch. Dev. Biol.* **205**, 391–400.
- Fuke, M. & Numakunai, T. 1999. Self-sterility of eggs induced by exogenous and endogenous protease in the solitary ascidian, *Halocynthia roretzi*. *Mol. Reprod. Dev.* **52**, 99–106.
- Fukumoto, M. 2009. Mitochondrial translocation in ascidian spermatozoa and its role in reproduction. *Bull. Gifu Univ. Med. Sci.* **3**, 101–108.
- Ganot, P., Bouquet, J. M. & Thompson, E. M. 2006. Comparative organization of follicle, accessory cells and spawning Anlagen in dynamic semelparous clutch manipulators, the urochordate Oikopleuridae. *Biol. Cell* **98**, 389–401.
- Gianguzza, M. & Dolcemascolo, G. 1978. On the ultrastructure of the follicle cells of *Ascidia malaca* during oogenesis. *Acta Embryol. Exp.* **2**, 197–211.
- Godeaux, J. E. A. 1990. Urochordata – Thaliacea. In: *Reproductive Biology of Invertebrates*, (eds K. G. Adiyodi & R. G. Adiyodi), pp. 453–469. Oxford & IBH Publ. Co, New Delhi.
- Godknecht, A. & Honegger, T. G. 1991. Isolation characterization and localization of a sperm-bound *N*-acetylglucosaminidase that is indispensable for fertilization in the ascidian *Phallusia mammillata*. *Dev. Biol.* **143**, 398–407.

- Goode, C. A., Gamboa-Pinto, A. J., Cruz, R., Gough, L. L., Lund, C. V. & Lambert, C. C. 1997. Evidence for cell surface and internal phospholipase activity in ascidian eggs. *Dev. Growth Differ.* **39**, 655–660.
- Hice, R. E. & Moody, W. J. 1988. Fertilization alters the spatial distribution and the density of voltage-dependent sodium current in the egg of the ascidian *Boltenia villosa*. *Dev. Biol.* **127**, 408–420.
- Hirai, E. 1941. The early development of *Cynthia roretzi*. *Sci. Rep. Tohoku Imp. Univ., Biol.* **16**, 217–232.
- Honegger, T. G. 1992. The involvement of sperm and egg glycosidases in animal fertilization. *Trends Glycosci. Glycotechnol.* **4**, 437–444.
- Honegger, T. & Füglistner, M. 2001. Structural and molecular investigations on the egg coat in *Phallusia mammillata*. In: *The Biology of Ascidians*, (eds H. Sawada, H. Yokosawa & C. C. Lambert), pp. 3–8. Springer, Tokyo.
- Honegger, T. G. & Koyanagi, R. 2008. The ascidian egg envelope in fertilization: structural and molecular features. *Int. J. Dev. Biol.* **52**, 527–533.
- Hoshi, M., De Santis, R., Pinto, R. M., Cotelli, F. & Rosati, F. 1985. Sperm glycosidases as mediators of sperm-egg binding in the ascidians. *Zool. Sci.* **2**, 65–69.
- Jeffery, W. R. 1980. The follicular envelope of ascidian eggs: a site of messenger RNA and protein synthesis during early embryogenesis. *J. Exp. Zool.* **212**, 279–289.
- Jeffery, W. R. 2007. Chordate ancestry of the neural crest: new insights from ascidians. *Semin. Cell Dev. Biol.* **18**, 481–491.
- Jiang, D. & Smith, W. C. 2005. Self- and cross-fertilization in the solitary ascidian *Ciona savignyi*. *Biol. Bull.* **209**, 107–112.
- Kanatani, H. 1975. Maturation-inducing substances in asteroid and echinoid oocytes. *Am. Zool.* **15**, 493–505.
- Kawamura, K., Fujita, H. & Nakauchi, M. 1988. Helper function of follicle cells in sperm-egg interactions of the ascidian, *Ciona intestinalis*. *Dev. Growth Differ.* **30**, 693–703.
- Koyanagi, R. & Honegger, T. G. 2003. Molecular cloning and sequence analysis of an ascidian egg β -N-acetylhexosaminidase with a potential role in fertilization. *Dev. Growth Differ.* **45**, 209–218.
- Lahille, F. 1890. *Contributions a l'Etude Anatomique et Taxonomique des Tuniciers*. pp. 328, Imprimerie Lagarde et Sebillé, Toulouse.
- Lam, X., Giesecke, C., Knoll, M. & Talbot, T. 2000. Assay and importance of adhesive interaction between hamster (*Mesocricetus auratus*) oocytes-cumulus complexes and the oviductal epithelium. *Biol. Reprod.* **62**, 579–588.
- Lambert, C. C. 1986. Fertilization-induced modification of chorion N-acetylglucosamine groups in the ascidian chorion, decreasing sperm binding and polyspermy. *Dev. Biol.* **116**, 168–173.
- Lambert, C. C. 1989. Ascidian eggs release glycosidase activity which aids in the block against polyspermy. *Development* **105**, 415–420.
- Lambert, C. C. 2000. Germ-cell warfare in ascidians: sperm from one species can interfere with the fertilization of a second species. *Biol. Bull.* **198**, 22–25.
- Lambert, C. C. 2005. Signaling pathways in ascidian oocyte maturation: effects of various inhibitors and activators on germinal vesicle breakdown. *Dev. Growth Differ.* **47**, 265–272.
- Lambert, C. C. 2008. Signaling pathways in ascidian oocyte maturation: the role of cAMP and follicle cells in germinal vesicle breakdown. *Dev. Growth Differ.* **50**, 181–188.
- Lambert, C. C. & Goode, C. A. 1992. Glycolipid linkage of a polyspermy blocking glycosidase to the ascidian egg surface. *Dev. Biol.* **153**, 95–100.
- Lambert, C. C., Goudeau, H., Franchet, C., Lambert, G. & Goudeau, M. 1997. Ascidian eggs block polyspermy by two independent mechanisms: one at the plasma membrane, the other involving the follicle cells. *Mol. Reprod. Dev.* **48**, 137–143.
- Lambert, C., Lafargue, F. & Lambert, G. 1990. Preliminary note on the genetic isolation of *Ciona* species (Ascidacea, Urochordata). *Vie Mil.* **40**, 293–295.
- Lambert, C. C. & Lambert, G. 1978. Tunicate eggs utilize ammonium ions for flotation. *Science USA* **200**, 64–65.
- Lambert, C. C. & Lambert, G. 1981. Formation of the block to polyspermy in ascidian eggs: time course, ion requirements, and role of the accessory cells. *J. Exp. Zool.* **217**, 261–270.
- Lambert, C. C., Lambert, I. M. & Lambert, G. 1995. Brooding strategies in solitary ascidians: *Corella* species from north and south temperate waters. *Can. J. Zool.* **73**, 1666–1671.
- Lambert, G. 2004. The south temperate and antarctic ascidian *Corella eumyota* reported in two harbors in northwestern France. *J. Mar. Biol. Assoc. UK* **84**, 239–241.
- Lambert, G. 2007. Invasive sea squirts: a growing global problem. *J. Exp. Mar. Biol. Ecol.* **342**, 3–4.
- Litscher, E. & Honegger, T. G. 1991. Glycoprotein constituents of the vitelline coat of *Phallusia mammillata* (Ascidacea) with fertilization inhibiting activity. *Dev. Biol.* **148**, 536–551.
- Mancuso, V. 1994. Folliculum formation in the ascidian *Phallusia mammillata* ovary. *Anim. Biol.* **3**, 3–14.
- Manni, L., Zaniolo, G. & Burighel, P. 1993. Egg envelope cytodifferentiation in the colonial ascidian *Botryllus schlosseri* (Tunicata). *Acta Zool.* **74**, 103–114.
- Manríquez, P. H. & Castilla, J. C. 2005. Self-fertilization as an alternative mode of reproduction in the solitary tunicate *Pyura chilensis*. *Mar. Ecol. Prog. Ser.* **305**, 113–125.
- Marino, R., Pinto, M. R., Cotelli, F., Lamia, C. L. & De Santis, R. 1998. The hsp70 protein is involved in the acquisition of gamete self-sterility in the ascidian *Ciona intestinalis*. *Development* **125**, 899–907.
- Marino, R., De Santis, R., Giuliano, P. & Pinto, M. R. 1999. Follicle cell proteasome activity and acid extract from the egg vitelline coat prompt the onset of self-sterility in *Ciona intestinalis* oocytes. *Proc. Natl. Acad. Sci. U.S.A.* **96**, 9633–9636.
- Martinucci, G. B., Burighel, P., Zaniolo, G. & Brunetti, R. 1988. Ovulation and egg segregation in the tunic of a colonial ascidian, *Diplosoma listerianum* (Tunicata, Ascidacea). *Zoomorphology* **108**, 219–227.
- Morgan, T. H. 1942. Cross- and self-fertilization in the ascidian *Styela*. *Biol. Bull.* **82**, 161–171.
- Mukai, H. & Watanabe, H. 1976. Studies on the formation of germ cells in a compound ascidian *Botryllus primigenus* Oka. *J. Morphol.* **148**, 337–362.
- Murabe, N. & Hoshi, M. 2002. Re-examination of sibling cross-sterility in the ascidian, *Ciona intestinalis*: genetic background of the self-sterility. *Zool. Sci.* **19**, 527–538.
- Nagahama, Y., Yoshikuni, M., Yamashita, M., Tokumoto, T. & Katsu, Y. 1995. Regulation of oocyte growth and maturation in fish. *Curr. Top. Dev. Biol.* **30**, 103–145.
- Nydam, M. L. & Harrison, R. G. 2007. Genealogical relationships within and among shallow-water *Ciona* species (Ascidacea). *Mar. Biol.* **151**, 1839–1847.
- Pinto, M. R., De Santis, R., Marino, R. & Usui, N. 1995. Specific induction of self-discrimination by follicle cells in *Ciona intestinalis* oocytes. *Dev. Growth Differ.* **37**, 287–291.

- Prodon, F., Chenevert, J. & Sardet, C. 2006. Establishment of animal-vegetal polarity during maturation in ascidian oocytes. *Dev. Biol.* **290**, 297–311.
- Prodon, F., Sardet, C. & Nishida, H. 2008. Cortical and cytoplasmic flows driven by actin microfilaments polarize the cortical ER-mRNA domain along the a-v axis in ascidian oocytes. *Dev. Biol.* **313**, 682–699.
- Robert, L. K., Lucio-Gough, L. M., Goode, C. A., McKinney, K. & Lambert, C. C. 1999. Activation of follicle cell surface phospholipase by tyrosine kinase dependent pathway is an essential event in ascidian fertilization. *Mol. Reprod. Dev.* **54**, 69–75.
- Ruppert, E. E. 2005. Key characters uniting hemichordates and chordates: homologies or homoplasies? *Can. J. Zool.* **83**, 8–23.
- Sakairi, K. & Shirai, H. 1991. Possible MS production by follicle cells in spontaneous oocyte maturation of the ascidian, *Halocynthia roretzi*. *Dev. Growth Differ.* **33**, 155–162.
- Satoh, N. 1994. *Developmental Biology of Ascidians*. pp. 234. Cambridge Univ. Press, Cambridge.
- Satoh, N. 2001. Ascidian embryos as a model system to analyze expression and function of developmental genes. *Differentiation* **68**, 1–12.
- Satoh, N. & Levine, M. 2005. Surfing with the tunicates into the post-genome era. *Genes Dev.* **19**, 2407–2411.
- Sawada, H. & Yokosawa, H. 2001. Self-nonsel self recognition and lysin system in fertilization of the ascidian *Halocynthia roretzi*. In: *The Biology of Ascidians*, (eds H. Sawada, H. Yokosawa & C. C. Lambert), pp. 18–23. Springer, Tokyo.
- Sawada, T.-O. & Schatten, G. 1989. Effects of cytoskeletal inhibitors on ooplasmic segregation and microtubule organization during fertilization and early development in the ascidian *Molgula occidentalis*. *Dev. Biol.* **132**, 331–342.
- Schroeder, P. C. & Talbot, P. 1985. Ovulation in the animal kingdom: a review with an emphasis on the contractile process. *Gamete Res.* **11**, 191–221.
- Schuetz, A. W. 1985. Local control mechanisms during oogenesis and folliculogenesis. In: *Developmental Biology: A Comprehensive Synthesis*, (ed. L. W. Browder), pp. 3–83. Plenum, New York.
- Smiley, S. 1990. A review of echinoderm oogenesis. *J. Electron Microsc. Tech.* **16**, 94–114.
- Stricker, S. A. & Folsom, M. W. 1997. Oocyte maturation in the brachiopod *Terebratalia transversa*: role of follicle cell-oocyte attachments during ovulation and germinal vesicle breakdown. *Biol. Bull.* **193**, 324–340.
- Stricker, S. A. & Smythe, T. L. 2001. 5-HT causes an increase in cAMP that stimulates, rather than inhibits maturation in marine nemertean worms. *Development* **128**, 1415–1427.
- Sugino, Y., 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.
- Swalla, B. J. 2001. Phylogeny of the urochordates: implications for chordate evolution. In: *The Biology of Ascidians*, (eds H. Sawada, H. Yokosawa & C. C. Lambert), pp. 219–224. Springer-Verlag, Tokyo.
- Talbot, P., Shur, D. & Myles, D. 2003. Cell adhesion and fertilization: steps in oocyte transport, sperm-zona pellucida interactions, and sperm-egg fusion. *Biol. Reprod.* **68**, 1–9.
- Taneda, Y., Saito, Y. & Watanabe, H. 1985. Self or non-self discrimination in ascidians. *Zool. Sci.* **2**, 433–442.
- Tucker, G. H. 1942. The histology of the gonads and development of the egg envelopes of an ascidian (*Styela plicata* Lesueur). *J. Morphol.* **70**, 81–108.
- Villa, L. & Patricolo, E. 1992. Ascidian interspecific fertilization I. Preliminary data on the involvement of the follicle cell layer. *Eur. Arch. Biol.* **103**, 25–30.
- Villa, L. & Patricolo, E. 1993. Role of the follicle cells in ascidian sperm-egg interaction. *Anim. Biol.* **2**, 175–184.
- Villa, L. A. & Patricolo, E. 2000. The follicle cells of *Styela plicata* (Ascidacea, tunicata): a sem study. *Zool. Sci.* **17**, 1115–1121.
- Villa, L. & Patricolo, E. 2001. Follicle cells of *Styela plicata* eggs (Ascidacea). In: *The Biology of Ascidians*, (eds H. Sawada, H. Yokosawa & C. C. Lambert), pp. 67–73. Springer-Verlag, Tokyo.
- Winchell, C. J., Sullivan, J., Cameron, C. B., Swalla, B. J. & Mallett, J. 2002. Evaluating hypotheses of deuterostome phylogeny and chordate evolution with new LSU and SSU ribosomal DNA data. *Mol. Biol. Evol.* **19**, 762–776.
- Yamashita, M. 1988. Involvement of cAMP in initiating maturation of the brittle star *Amphipholis kochi* oocytes: induction of oocyte maturation by inhibitors of cyclic nucleotide phosphodiesterase and activators of adenylate cyclase. *Dev. Biol.* **125**, 109–114.
- Yamashita, M., Mita, K., Yoshida, N. & Kondo, T. 2000. Molecular mechanisms of the initiation of oocyte maturation: general and species-specific aspects. *Prog. Cell Cycle Res.* **4**, 115–129.
- Young, C. M., Gowan, R. F., Dalby, J. J., Pennachetti, C. A. & Gagliardi, D. 1988. Distributional consequences of adhesive eggs and anural development in the ascidian *Molgula pacifica* (Huntsman, 1912). *Biol. Bull.* **174**, 39–46.
- Zaniolo, G., Burighel, P. & Martinucci, G. 1986. Ovulation and placentation in *Botryllus schlosseri* (Ascidacea): an ultrastructural study. *Can. J. Zool.* **65**, 1181–1190.