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Immunohistology with Antibodies Specific to Test Cells in the Ascidian *Ciona intestinalis* Suggests Their Role in Larval Tunic Formation

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ABSTRACT—To investigate the differentiation of test cells and their roles during oogenesis and embryogenesis in the ascidian *Ciona intestinalis*, we prepared two monoclonal antibodies (UA165 and UA464) that specifically recognize test cells. In the ovary, these antibodies recognized different cytoplasmic contents of test cells surrounding a vitellogenic oocyte. The recognition of the antibodies was retained after they were released from the surface of the oocyte into the perivitelline space and throughout embryogenesis. After hatching, however, these antibodies also recognized the larval tunic. These findings suggested that during oogenesis, test cells synthesize antigens recognized by the antibodies and that these antigens are stored in the cytoplasm through embryogenesis, then they are used to form the larval tunic.

INTRODUCTION

The ascidian oocyte is enclosed by accessory cells (outer follicular, inner follicular and test cells). These cells, except for the outer follicular type, remain around the embryo until hatching. Test cells are unique to the ascidian egg as they are not found in eggs of other animals (cf. Satoh, 1994). Test cells are embedded in the surface of the oocyte under the chorion during oogenesis and they are released into the perivitelline space when the germinal vesicle of the oocyte breaks down. Embryos develop with test cells within the chorion until hatching and tadpole larvae swim out from the chorion into seawater. These findings suggest that test cells dynamically interact with oocytes and/or embryos during ascidian development. However, direct evidence for this interaction has not been reported.

Although several investigators have studied the origin, differentiation and function of test cells, their conclusions are inconsistent. This may be partly due to species differences, but there is a lack of multilateral understanding of test cells. The morphological changes of test cells during oogenesis have been studied by means of light-microscopy by Harvey (1927), Tucker (1942) and Cowden (1961), and by electron-microscopy by Kessel (1962), Kessel and Kemp (1962), Kalk (1963a,b), Kessel and Beams (1965), Mancuso (1965), Gianguzza and Dolcemascolo (1979) and Sugino *et al.* (1987). Some of these reports describe the characteristic cytoplasmic structures, electron-dense granules and large vacuoles. The former appears at the early stage of oogenesis and collapses during vitellogenesis, whereas the latter develops during vitellogenesis and occupies most of the cytoplasm of test cells. It was thought that the granules, due to their osmiophilic nature, include a lipid in *Ciona intestinalis* (Harvey, 1927; Mancuso, 1965) or a vanadium compound in *Ascidia pygmaea* (Kalk, 1963a,b). The inclusion in the large vacuoles is reportedly a pigment produced by the Golgi complex in *Styela* sp. (Kessel and Beams, 1965) or the granular structure called "ornament" that is required for larval tunic formation (Cavey, 1976). However, test cell-specific substances are not sufficiently understood at the molecular level to identify their function(s).

Mancuso (1965) has reported that cytoplasmic constituents of test cells dynamically change during oogenesis, especially at the stage of vitellogenesis. He considered that test cells provided yolk precursors of oocytes, from an indirect observation. In addition, several functions of test cells in oogenesis have been proposed (Harvey, 1927; Kalk, 1963a,b; Kessel and Kemp, 1962; Reverberi and Mancuso, 1960).

It has also been proposed that test cells are associated with larval tunic formation during late embryogenesis (Cloney and Cavey, 1982; Satoh *et al.*, 1982; Smith, 1967). Satoh *et al.* (1982) investigated by means of scanning electron-microscopy, the behavior of test cells during embryogenesis of *Halocynthia roretzi.* They found that the test cells migrate with extending numerous pseudopodia toward the surface of the embryo when the tunic formation is initiated, then they form a net over the entire embryo. Cloney and

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Cavey (1982) investigated the larval tunic formation of dechorionated embryos at various stages in *Corella inflata* and found that at the neurula stage, they produced all of the major components of the larval tunic but could not constitute the functional fin that normally arises from the outer cuticular layer of tunic. The tunic was also deficient in the submicroscopic granular structures called "ornaments" that are synthesized by test cells during vitellogenesis and secreted over the tunic during larval tunic formation (Cavey, 1976; Cloney and Cavey, 1982). They concluded that test cells of this species might induce or control the morphogenesis of the larval fins prior to the late tailbud stage.

All of these findings suggest dynamic changes in the ultrastructure, metabolic activity and behavior of test cells during development. The molecular processes that control these events remain unknown. Studies at the molecular level should be very helpful in directly understanding the function of test cells during oogenesis and embryogenesis. Thus, the molecules that are synthesized specifically by test cells should be identified and analyzed. In this paper, we report the isolation of antibodies that specifically detect test cells around the vitellogenic oocytes of *C. intestinalis*. In addition, we describe the distribution of these antigens during embryogenesis. The results suggested a relationship between these antigens and larval tunic formation.

MATERIALS AND METHODS

Biological materials

Ciona intestinalis was collected in the vicinity of the Onagawa Fisheries Laboratory of Tohoku University, Onagawa-bay, Miyagi, Japan and the Mukaishima Marine Biological Laboratory of Hiroshima University, the Inland Sea of Japan. Ovaries were surgically removed from adults. Eggs and sperm were obtained by dissection of the gonoducts. Embryos were obtained by means of artificially inseminating gametes from different individuals. They were raised until the appropriate stages at room temperature (16–20°C) or at 20°C in an incubator. These samples were fixed with Carnoy's solution (see below) and after dehydration, stored at –30°C in 100% ethanol until use.

Monoclonal antibodies

The immunogen was prepared as follows. Ovaries were homogenized in about 4 volumes of 10 mM phosphate-buffered saline (PBS; pH 7.2) containing 5 mM EDTA in an ice bath using a Poritron Homogenizer, then separated by centrifugation for 30 min at 1,500 × *g* at 4°C. The supernatant (S1) was mixed with activated charcoal powder (Nacalai Tesque, Kyoto, Japan; Landsteiner, 1942) and stirred overnight at 4°C.

Then charcoal was washed with PBS three times and injected intraperitoneally into female ddY mice. After 4 weeks, 0.5 ml of S1 was injected intraperionally. Three days later, the splenocytes were fused with 1×10^7 P3U1 myeloma cells (Flow Lab.) using 42.5% polyethylene glycol 1000 (Nacalai Tesque) and 15% DMSO. Antibodies in the culture medium of hybridomas were screened on sections of ovaries by immunofluorescence microscopy. Hybridomas secreting antibodies of interest were cloned several times.

Immunohistology

Fresh ovaries and embryos were fixed with Carnoy's solution (ethanol:chloroform:acetic acid, 6:3:1) at 4°C overnight. Fixed samples were dehydrated with absolute ethanol, embedded in polyester wax (BDH Chem. Ltd.; Steedman, 1957) and sectioned. After removing the wax with absolute ethanol, sectioned specimens were immersed in PBS for 10 min, then in each supernatant of the hybridoma culture medium for 1 hr at room temperature. Specimens were washed in PBS containing 0.05% Tween-20 (PBST) for 6 × 5 min, then incubated for 1 hr at room temperature with the appropriate fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse IgG or IgM serum (KPL) diluted 1:20–40 in 5% FBS/PBS containing 0.01% thimerosal. After washing in PBST as before, the specimens were mounted in 95% non-fluorescent glycerol/PBS and examined using an Olympus fluorescence microscope. If necessary, preparations were immersed in DAPI (1 μg/ml in DW) for 5 min. Some samples were stained with horseradish peroxidase (HRP)-conjugated rabbit anti-mouse IgM or IgG3 serum (ZYMED) diluted 1:800 in 5% FBS/PBS containing 0.01% thimerosal and DAB substrate (0.06% w/v diaminobenzidine, 0.015% v/v hydrogen peroxide in PBS), then observed under an Olympus light microscope equipped with Nomarski differential interference optic system. For whole-mount preparations, the same protocol was followed, except for extending the reaction period with the antibodies and the wash, from several hours to overnight at 4°C.

Western blots

Samples were prepared as follows. The S1 fraction prepared from ovaries was mixed with an equal volume of 2 x SDS-sample buffer (0.125 M Tris-HCl, pH 6.8, 2.3% SDS, 20% glycerol, 10% β-mercaptoethanol), then boiled for 2 min. Proteins in the sample were separated by SDS-PAGE (Laemmli, 1970) then transferred to a PVDF-membrane (Clear Blot Membrane-p, ATTO, Osaka, Japan). The membrane was blocked with 5% skim milk/PBS for 30 min at room temperature, then incubated in the supernatants of the UA165 and UA464 hybridoma culture media for 1 hr at room temperature. The membrane was washed six times in PBST for 5 min each, then incubated for 1 hr at room temperature with horseradish peroxidase (HRP)-conjugated rabbit anti-mouse IgM or IgG3 serum (ZYMED) diluted 1:800 in 5% FBS/PBS containing 0.01% thimerosal. After washing in PBST as before, the membrane was incubated in DAB substrate (0.06% w/v diaminobenzidine, 0.015% v/v hydrogen peroxide in PBS) until a protein band that reacted with antibody visible.

RESULTS

Staging of oogenesis in Ciona intestinalis

We staged ascidian oogenesis to define when the antigenicity first appeared. In the ovary of *Ciona intestinalis*, various follicles with oocytes at different development stages were distinguishable (Fig. 1A). Several investigators have described the staging of ascidian oogenesis (e.g., Cowden, 1961; Harvey, 1927; Mancuso, 1965). Mancuso (1965), for example, divided the ascidian oogenesis into three stages: young (or stage I oocytes), previtellogenic (or stage II oocytes) and vitellogenic oocytes (or stage III oocytes). We basically followed his criteria, although we did not study the first two stages in detail. We distinguished the latter two by means of the PAS histochemical reaction, which stained yolk granules of oocytes at the vitellogenic, but not at the previtellogenic stage. In addition, in this study, we distinguished another stage of oogenesis, referred to as the GVBD oocyte, because at this stage, the germinal vesicle of the oocyte broke down and test cells were released into the perivitelline space.

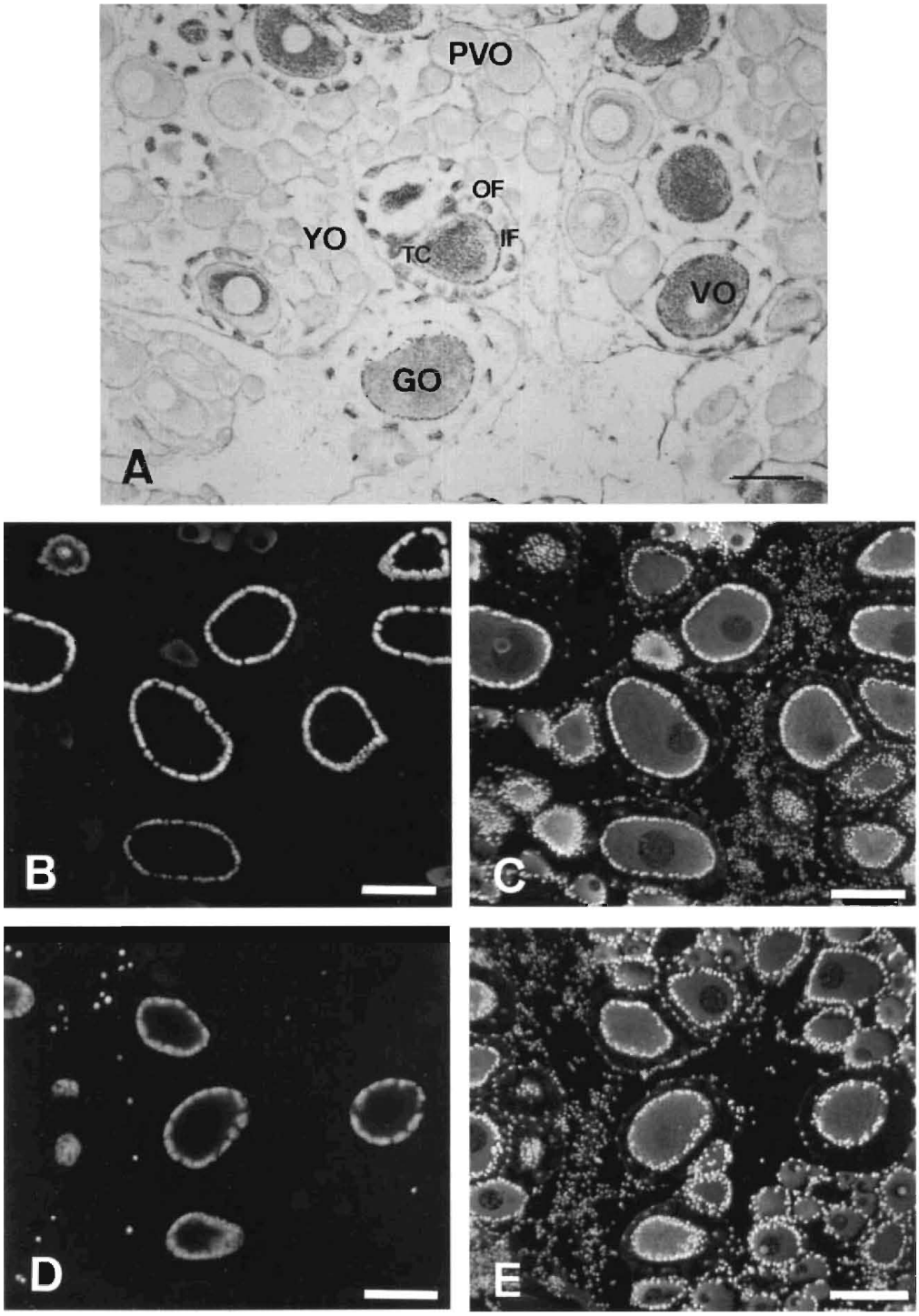

Fig. 1. Sections of *Ciona intestinalis* ovary stained (A) histochemically with PAS and Alcian blue, (B, D) immunohistochemically with UA165 (B) or UA464 (D) antibodies, and (C, E) with DAPI. (C) and (E) are the same sections as (B) and (D), respectively. Scale bar represents 100 μm for all photographs. There are many follicles at various oogenic stages, including young (YO), previtellogenic (PVO), vitellogenic (VO) and GVBD oocytes (GO). Yolk granules of vitellogenic and GVBD oocytes were stained by the PAS reaction. Alcian blue stained outer follicle cells (OF), inner follicle cells (IF) and test cells (TC). Test cells are embedded on the surface of the oocyte until the vitellogenic oocyte stage then released into the perivitelline space at the GVBD oocyte stage (A). Both UA165 (B) and UA464 (D) reacted with vitellogenic and GVBD oocytes.

Two monoclonal antibodies specific to test cells and their immunoblot profiles of the antigens

By immunizing mice with homogenates of the *C. intestinalis* ovary, we obtained various monoclonal antibodies that specifically recognized oocytes, follicle and test cells, respectively. Here we describe the monoclonal antibodies, UA165 and UA464, that specifically reacted with test cells. Both recognized the cytoplasmic components of test cells that surround the oocyte at a later stage of oogenesis, namely the vitellogenic stage (Fig. 1B-E). In addition, UA165, but not UA464, reacted also test cells in the ovary of *Ciona savignyi* (data not shown).

To ascertain whether or not the antigens recognized by the antibodies are proteins, we immunoblotted the S1 fraction (see Materials and Methods) from ovaries with UA165 antibody (data not shown). A smear band above 100 kDa was evident in S1 fraction.

The UA464 antibody recognized a smear band with a high molecular weight and sometimes did not react with any bands in Western blots from S1 fraction under our conditions (data not shown).

Distribution of UA165 antigen in the ovary, embryos, larvae and juveniles

Staining with the UA165 antibody was undetectable in the ovary with oocytes at the earlier stages of oogenesis. The staining was first detected in cells in the ovary with oocytes at the early vitellogenic stage (Fig. 2A). Positive cells

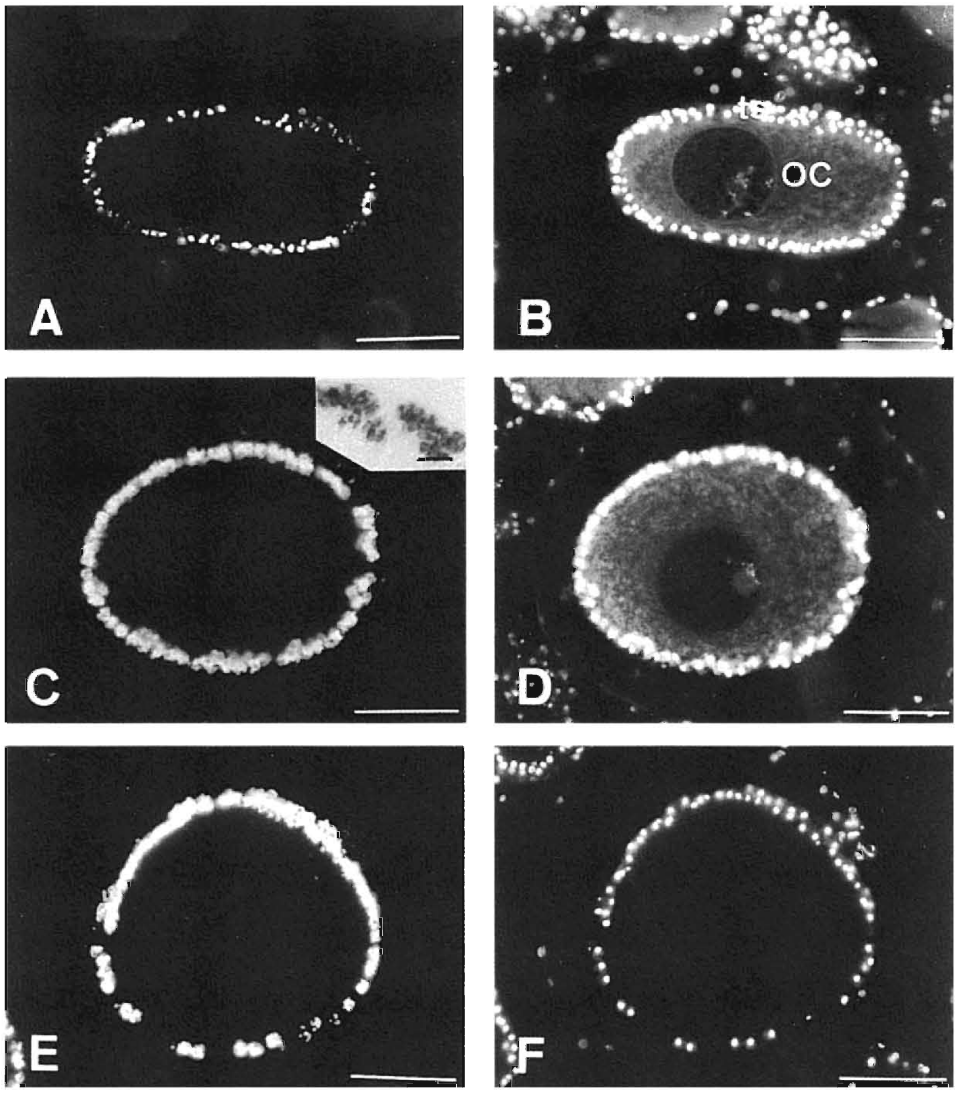

Fig. 2. Distribution of UA165 antigen in the ovary of *Ciona intestinalis*. Staining with (A, C, E) the UA165 antibody and (B, D, F) DAPI (controls). Scale bar represents 50 μm for all photographs. (A) Staining with the antibody was first detected in cells surrounding an oocyte at the early stage of vitellogenesis. (B) A control (the same section as A) stained with DAPI showing the many cells constituting the ovary. OC, oocyte; ts, test cells. (C) Staining of a vitellogenic oocyte showing the distribution of UA165 antigen in the test cells. The inset at higher magnification shows enzymatic staining of vacuoles of the test cells. (Scale bar, 10 μm) (D) The same section as C stained with DAPI. (E) Staining of GVBD-oocyte with the antibody showing positive cells within the perivitelline space. (F) The same section as E stained with DAPI.

surrounded the oocyte (Fig. 2A, C), suggesting that they are accessories of the oocyte. The UA165 antibody recognized some granular components in the cytoplasm of the test cell at early vitellogenic stage (Fig. 2A). As vitellogenesis advanced in the oocyte, granular components became larger and vacuolated, and occupied most of the cytoplasm in the cell (Fig. 2C). These structures were inclusion of the test cells, since their shape and size were irregular and there was more of them than nuclei as judged by DAPI staining (Fig. 2C, D). This conclusion was supported by observations at higher magnification with HRP enzymatic immunostaining (Fig. 2C, insert). At the GVBD-oocyte stage, test cells moved into the perivitelline space, while follicle cells were evident on the outer surface of the chorion. The UA165 antibody only stained the test cells (Fig. 2E).

During embryogenesis, the UA165 antibody stained test cells in the perivitelline space (Fig. 3). Until at least the early late-tailbud stage (11 hr after fertilization at 20°C), no significant change was detected in the distribution of the antigen. This antibody also recognized the surface of the embryos (Fig. 3A-E). At 14 hr (20°C), which was about 1 hr before hatching and after ocellus pigmentation was complete, test cells migrated around the surface of the embryo (Fig. 3F).

When the larva hatched from the chorion, some test cells scattered off from the larva, whereas the others stayed within the new larval tunic. In hatched tadpole larvae, the antibody recognized the larval tunic as well as test cells (Fig. 4A). At this stage the reaction site seemed to be around the

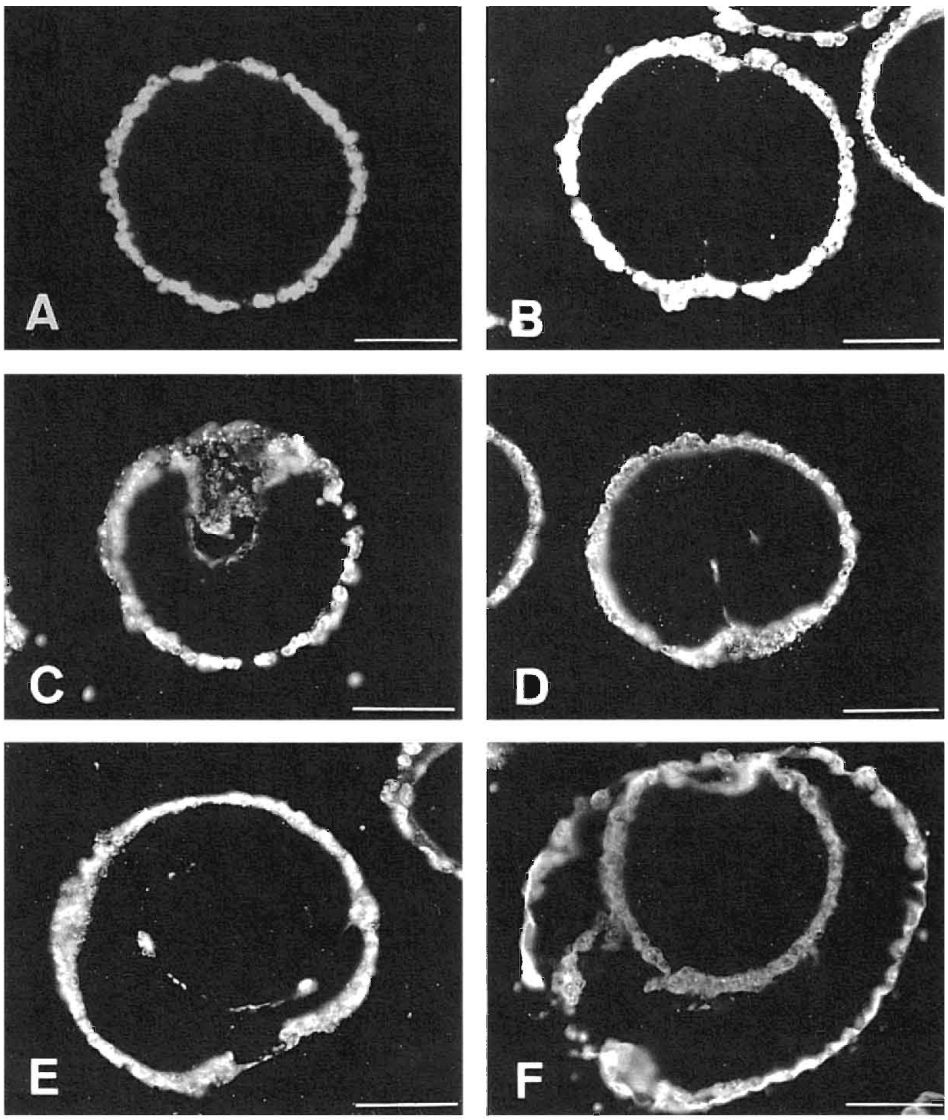

Fig. 3. Distribution of UA165 antigen in embryos of *Ciona intestinalis*. Scale bar represents 50 μm for all photographs. Staining with the UA165 antibody of (A) an unfertilized egg, (B) 2-cell embryo, (C) gastrula, (D) mid-tailbud embryo, (E) late-tailbud embryo (11 hr at 20°C), and (F) late-tailbud embryo (14 hr at 20°C). The antigen distribution did not largely change until the tailbud stage. Test cells surrounding embryos are intensely stained with the antibody and reaction of the surface of embryos is also seen (A-F). In the 14-hr embryo, when the larval tunic formation advances, test cells crowd around the whole embryo (F).

surface of test cells rather than in the vacuoles within them. This antibody also stained larvae of *Halocynthia roretzi* (data not shown). The entire tunic was stained evenly (Fig. 4A). This was evident in sagittal (Fig. 4B) and cross sections (Fig. 4C) of the larva. The major site of the staining was the larval tunic (Fig. 4A-C). In a recently metamorphosed juvenile, the tunic staining was very weak except at the root (Fig. 4D). In addition, there was secondary staining in part of the stomach, oesophagus, intestine (Fig. 4D, E) and in other individuals, the heart.

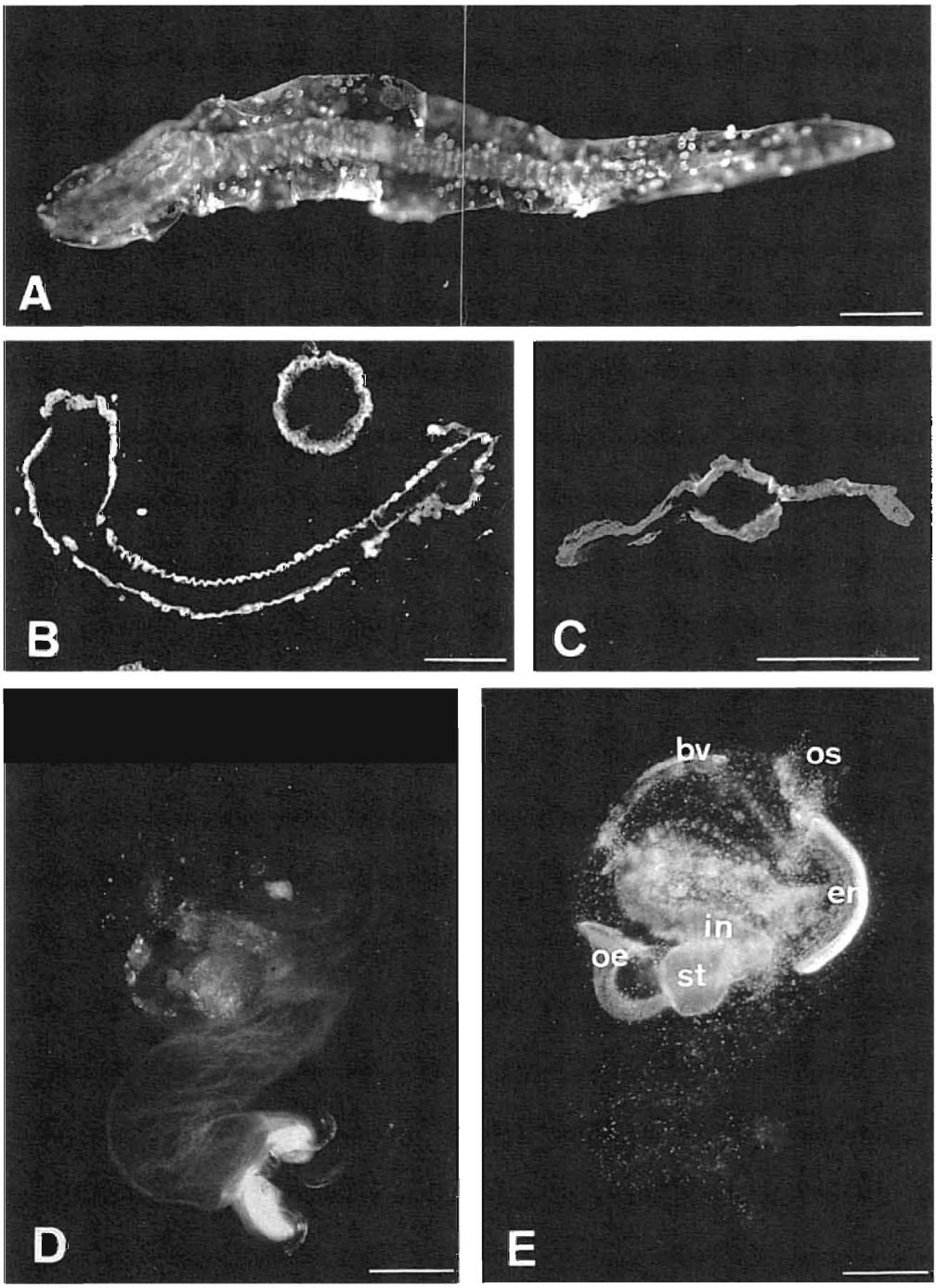

Fig. 4. Distribution of UA165 antigen in larvae and juveniles of *Ciona intestinalis*. (A-D) Staining with the UA165 antibody and (E) DAPI (controls). Scale bar represents 100 μm for all photographs. (A, D, E) Whole mount preparations, (B) a sagittal section of a larva and (C) a cross section of the larval tail. The larval tunic as well as the test cell is intensely stained (A). The surface of test cells is stained rather than the vacuoles within the cell. The larval body itself is not stained (B, C). (D, E) Juvenile (one week after hatching). Staining of the tunic is weak. Secondary staining is evident in the stomach, oesophagus and intestine. bv: brain vesicle. en: endostyle. in: intestine. oe: oesophagus. os: oral siphon. st: stomach.

Distribution of UA464 antigen in the ovary, embryos, larvae and juveniles

Similar to the UA165 antibody, reactivity with the UA464 antibody was not detected at the previtellogenic and earlier stages of oocytes. The reactivity first appeared in follicles with vitellogenic oocytes (Fig. 5A). Positive cells were embedded in the peripheral cytoplasm of the oocyte, indicating that they were test cells (Fig. 5C). At first, the UA464 antibody stained small granules, but soon the staining became homogeneous over the cytoplasm of the test cell. Finally, the peripheral regions of test cells were intensely stained (Fig. 5A, C). This staining site seemed to be the membrane of test cells (Fig. 5C, insert). In addition, the peripheral cytoplasm of the oocyte was also weakly reactive (Fig. 5C). After the test cells moved into the perivitelline space, this staining was retained (Fig. 5E). The UA464 antibody also reacted with some types of blood cells (Fig. 5A, arrows).

During embryogenesis, the UA464 antibody stained test cells. Moreover the surface of the embryo was stained more intensely than that of the oocyte (Fig. 6A). This antigen distribution was retained during embryogenesis (Fig. 6B-F). In the 14-hr embryo, test cells formed a thick layer around the

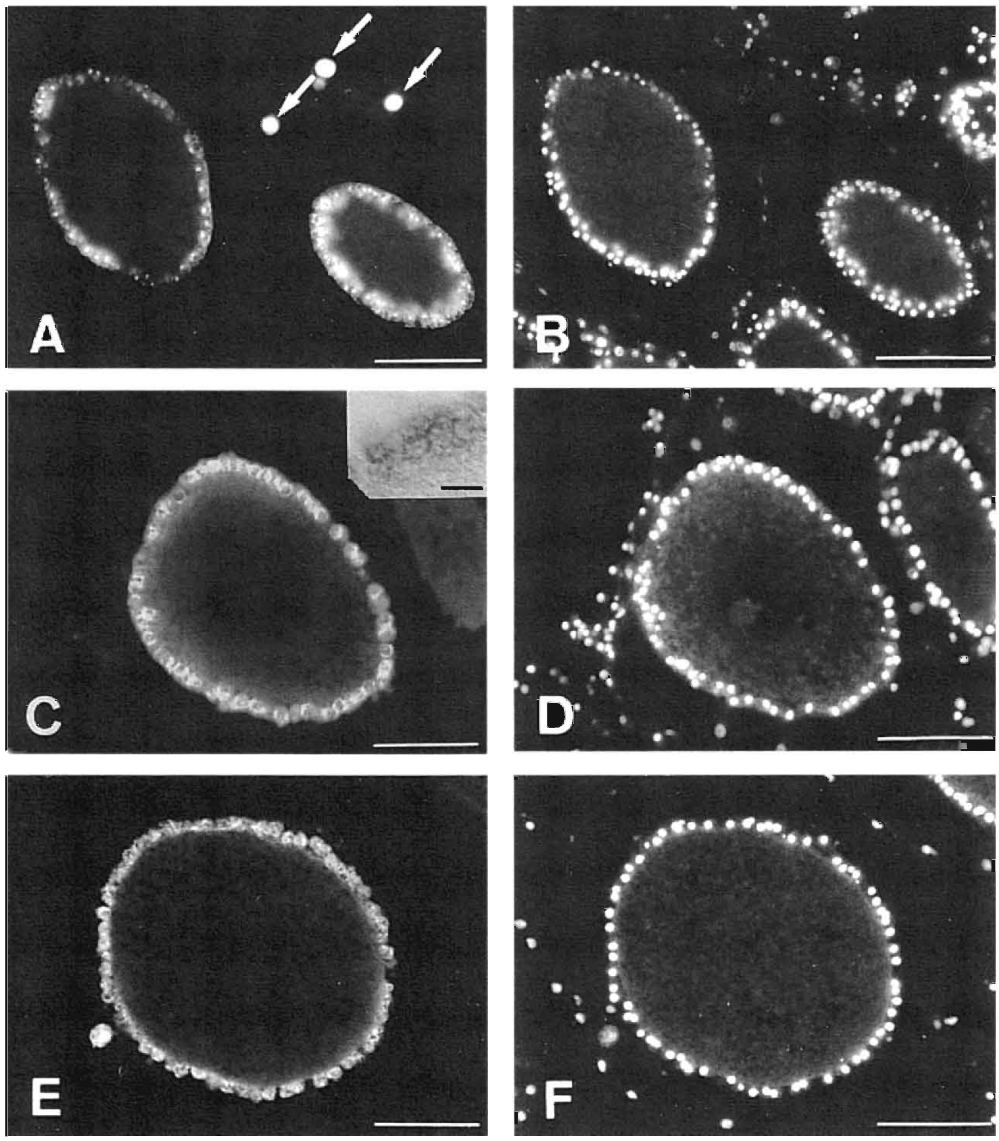

Fig. 5. Distribution of UA464 antigen in the ovary of *Ciona intestinalis*. (A, C, E) Staining with the UA464 antibody and (B, D, F) DAP (controls). Scale bar represents 50 μm for all photographs. (A) Staining with the antibody was first detected in cells surrounding an oocyte at the early stage of vitellogenesis. Some blood cells (arrows) are also stained. (B) A control (the same section as A) stained with DAPI. (C) Staining of a vitellogenic oocyte showing the distribution of UA464 antigen in the test cells. The antigen seemed to localize at the peripheral more than at other parts of test cells. Additionally, the peripheral cytoplasm of the oocytes is weakly stained. The inset at higher magnification showing enzymatic staining of the membrane of the test cells. (Scale bar, 10 μm) (D) The same section as C stained with DAPI. (E) Staining of GVBD-oocyte with the antibody showing positive regions within the perivitelline space. (F) The same section as E stained with DAPI.

embryo, which was intensely stained (Fig. 6F).

In the tadpole larva, the antibody recognized the larval tunic and some cells in the larval trunk as well as test cells (Fig. 7A-D). The staining in sections of the larva indicated that these cells in the larval trunk were some mesenchymal cells (Fig. 7C, arrowhead). This antigen distribution in mesenchymal cells was detected in the 11-hr embryos (Fig. 6E, arrows), but not all of these cells were stained (Fig. 6F, arrows). In some embryos and larvae, test cells and tunic were very weakly stained, but that of mesenchymal and ancestor cells was intense (Fig. 7B). In a freshly metamorphosed juvenile, the tunic was still intensely stained. Moreover, some coelomic cells reacted with this antibody. Most of them were localized near the gill slits and under the brain vesicle, and others seemed to be between the tunic and the body of juveniles (Fig. 7E, F).

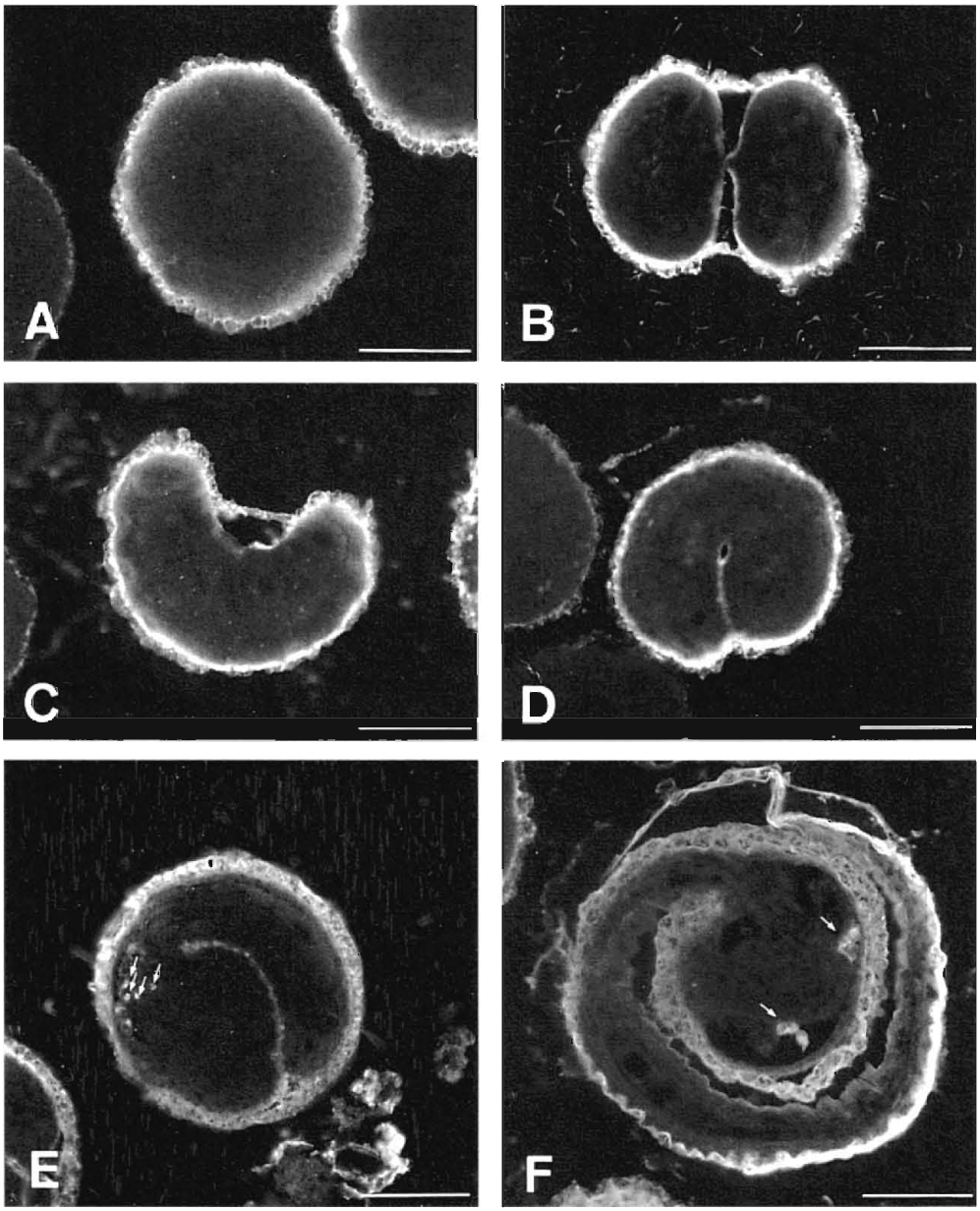

Fig. 6. Distribution of UA464 antigen in embryos of *Ciona intestinalis.* Scale bar represents 50 μm for all photographs. Staining with the UA464 antibody of (A) unfertilized egg, (B) 2-cell embryo, (C) gastrula, (D) mid-tailbud embryo, (E) late-tailbud embryo (11 hr at 20°C), and (F) late-tailbud embryo (14 hr at 20°C). The antigen distribution did not markedly change until the tailbud stage. Test cells surrounding the embryos as well as the surface of the embryo are stained with the antibody (A-F). The region between the test cells and the embryo is stained more intensely until mid-tailbud stage (A-D). At the late-tailbud stage the stained region surrounding the embryo becomes a thick layer, in which test cells seems to be embedded (E, F). In addition, some mesenchymal precursor cells are stained at the late-tailbud stage (E and F, arrows).

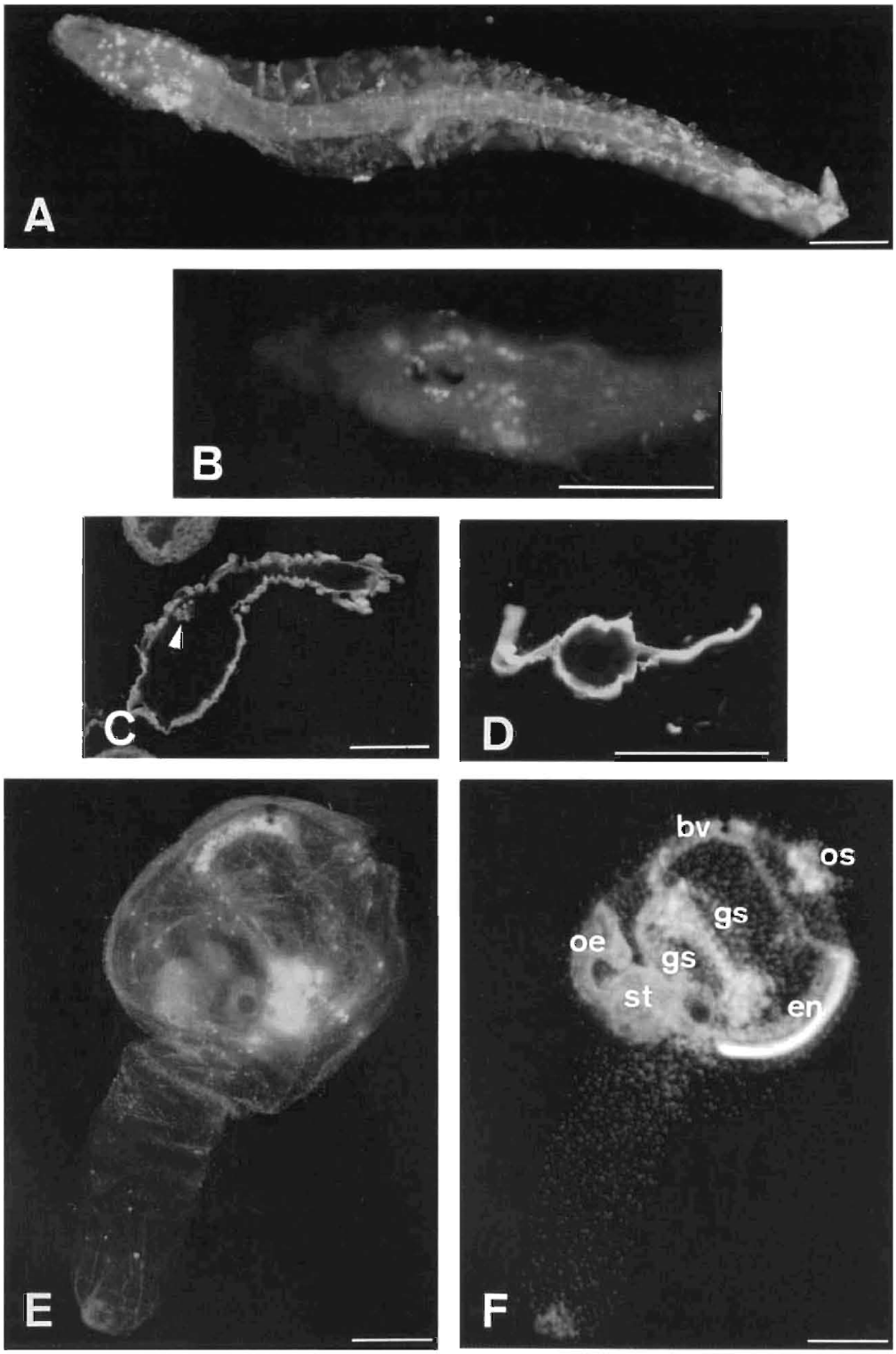

Fig. 7. Distribution of UA464 antigen in larvae and juveniles of *Ciona intestinalis.* (A-E) Staining with the UA165 antibody and (F) DAPI (controls). Scale bar represents 100 μm for all photographs. (A, B, E, F) Whole mount preparations, (C) a sagittal section of a larval trunk and (D) a cross section of the larval tail. The larval tunic is evenly stained intensely (A, C, D). In the other larva, the staining of the tunic and test cells is very weak but that of some cells in the trunk is still intense (B). These cells are mesenchymal cells (C, arrowhead). (E, F) Juvenile (one week after hatching). The tunic is intensely stained and some coelomic cells are also stained. Most of them localize under the brain cavity and near gill slits. Other positive cells are present between the body and the tunic. bv: brain vesicle. en: endostyle. gs: gill slit. oe: oesophagus. os: oral siphon. st: stomach.

DISCUSSION

Functions of test cells during oogenesis

In this study we produced two monoclonal antibodies specific to test cells in *C. intestinalis*. We showed for the first time, that there are specific antigens in test cells. It is likely that they are synthesized by the test cell itself during vitellogenic stages of oogenesis because they were initially detected at this stage in the test cell only, not at earlier stages and never in other locations.

Test cells at this stage change dramatically with respect to cytoplasmic components and metabolic activity. Mancuso (1965) observed ultrastructural changes at this stage in test cells of *C. intestinalis*. He reported that in the cytoplasm of the test cell at the vitellogenic stage, the amount of ribosomes and rough-surfaced endoplasmic reticulum that had been rich at earlier stages, decreased. He also reported that the electron-dense granules became collapsed and at the same time, the Golgi complex developed and large vacuoles appeared. These findings suggested that the metabolism of test cells changes from the synthesis of proteins to the modification and storage of proteins. Although he regarded these changes as being related to yolk formation, we propose that these events are also associated with the synthesis and storage of some materials such as UA165 and UA464 antigens. In fact most of the cytoplasm at later stages becomes occupied by many large vacuoles and probably UA165 antigen is included within them, although this requires confirmation by immuno-electron microscopy.

UA165 antigen may not be associated with oogenesis, because this antigen localized only in vacuoles of the test cell during oogenesis and early embryogenesis. On the other hand, UA464 antigen is synthesized in the cytoplasm of the test cell at the same time as UA165 antigen and then it migrates to the cell surface. Moreover, the peripheral cytoplasm of the oocyte reacted with UA464 antibody. This suggested that this antigen is secreted from test cells to the oocyte during the late vitellogenic stage, when these two types of cells interact. This antigen may not be a yolk precursor, since its distribution was not exclusive to yolk granules.

The possibility cannot be excluded that test cells have functions such as providing oocytes with yolk precursors, nutrients and other factors during oogenesis, because we could not obtain an antibody against the electron-dense or test granules that Mancuso (1965) regarded as the source of the yolk precursor. However, we obtained some antibodies that might react to the yolk granules; none of them recognized test cells at any oogenic stage (Takamura *et al.*, unpublished data). It remains unknown whether or not this finding resulted from a lack of antigens such as yolk precursor synthesized in test cells or inadequate preparation of immunogens.

Functions of UA165 antigen during embryogenesis

Because UA165 antigen was localized in the larval tunic but neither in embryos, nor in the larval body during development, we propose that this antigen moves from test cells into the larval tunic before hatching and thus plays an important role in larval tunic formation, as pointed out by others. Satoh *et al.* (1982) observed the behavior of live test cells during the embryogenesis of *H. roretzi* and reported that the behavioral activity of test cells reached a maximum at the time of larval tunic formation. Cloney and Cavey (1982) examined the significance of test cells in larval tunic formation by mechanically removing extraembryonic structures including test cells at various developmental stages. Their results showed that the larval fin could not be formed normally when test cells were removed at the neurula stage. In addition, they found that the tunic produced by this deficient embryo lacked the granular "ornament" structure (Cavey, 1976; Cloney and Cavey, 1982). They concluded that test cells play a role in larval fin formation before the late tailbud stage. Our results support this view, since UA165 antigen shows the same temporal and spatial localization as the ornament structural materials. However it is unknown whether or not this antigen is included in this structure. Repeated studies using our antibodies to identify the antigen in the structure should confirm this hypothesis.

In a freshly metamorphosed juvenile, the staining of tunic was very weak except at the root. The larval tunic is composed of two cuticle layers: outer C1 and inner C2 (Cloney and Cavey, 1982). C1 is exclusive to larval tunic and discarded during metamorphosis, while C2 continues to surround the juvenile body. One possibility is that UA165 antigen, unlike UA464 antigen, localizes only in C1 and therefore it is specific to the larval tunic. Immuno-electronmicroscopic studies are necessary to confirm this notion. In addition, there was secondary staining in the stomach, oesophagus, intestine and heart. So far, we have no appropriate explanation for this observation.

Functions of UA464 antigen during embryogenesis

During embryogenesis, UA464 antibody stained the area between the surface of the oocyte and test cells more intensely than test cells. These results suggest that the antigen is secreted on the surface of the oocyte and/or into the perivitelline space from test cells at very early stage of embryogenesis. Because in the 14-hr embryo, test cells seemed to be embedded in the region around embryos, and afterwards the tunic of larvae and juveniles was intensely stained, this antigen may also be related to tunic formation. One possible function of this antigen is that of an adhesive between the oocyte and test cells. To confirm this, this antigen must be identified. We obtained some plaques that reacted with UA464 as well as UA165 antibody from an ovary cDNA library, and we are now analyzing them.

This antigen was also detected in some mesenchymal cells in the larval trunk. This antigenicity had appeared in some of mesenchymal cells, though not all, by the late tailbud stage. In a freshly metamorphosed juvenile, some coelomic cells which localize in the particular region of juveniles were stained. Some blood cells were also recognized

by this antibody in the ovary. These results suggested that some larval mesenchymal cells are the precursors of some blood cells, although we can not identify which type.

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