

Differentiation markers in the *Drosophila* ovary

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SUMMARY

A library of monoclonal antibodies, raised against imaginal discs of *Drosophila melanogaster*, was screened for binding to differentiation antigens in the adult ovary by immunofluorescence. Several lectins were similarly assayed. Two antibodies, DOV 1 and DOV 2, and wheat germ agglutinin exhibited binding which was restricted to particular stages of ovarian cell differentiation. DOV 2 also showed a marked preferential binding to the cell surface of germ line cells in the ovary. A differentiation of the portion of the tunica propria covering the anterior part of the germarium was revealed by the monoclonal antibody DOV 3. Another monoclonal antibody, DOV 4, identified a molecular specialization of the chorion at the tip of the micropyle. These markers should provide tools for the molecular analysis of oogenesis.

INTRODUCTION

The development from stem line oogonium to mature egg is presented in a linear sequence in the ovarioles of *Drosophila melanogaster*. This simple arrangement has permitted the analysis of the cell types involved and the patterns of their divisions and movements (King, 1970; Wieschaus & Szabad, 1979; Mahowald & Kambyzellis, 1980). Moreover, as the end product of this process is the fertile egg, mutations which interfere with this process can be recovered as female steriles. Thus a large collection of mutations which disrupt oogenesis are available (King & Mohler, 1975; Gans, Audit & Masson, 1975). The existing wealth of descriptive knowledge combined with the ease of genetic analysis recommend the *Drosophila* ovary as a model developmental system for the study of mechanisms involved in the maintenance of the stem cell pool, the commitment to developmental fate, the differentiation of cell type and the organization of positional information in the egg.

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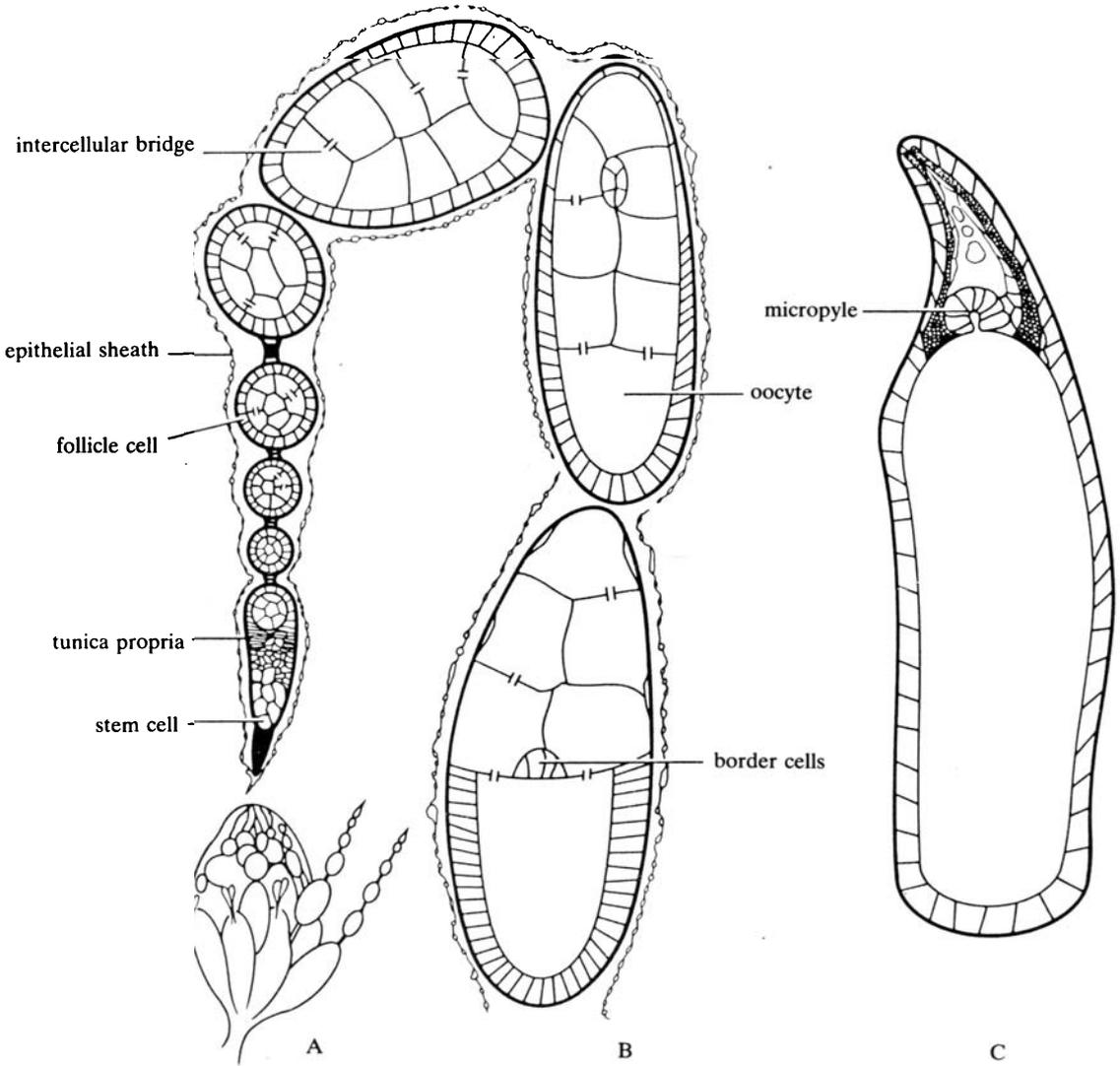


Fig. 1. Line drawing of adult ovary of *Drosophila melanogaster* (A) Ovary: two ovarioles have been pulled out from the ovary. (B) Ovariole showing egg chambers up to stage 10. (C) Stage-13 egg chamber. (after King, 1970).

A diagram of an ovariole and its investing membranes is shown in Fig. 1. The anterior portion of the germarium contains the stem line oogonia and the mesodermal follicle cell precursors. The stem line oogonia divide asymmetrically to produce a stem cell daughter and a cystoblast. The cystoblast divides to provide a cluster of 16 cystocytes connected by cytoplasmic bridges. The follicle cell precursors migrate around this cluster and, by the time the cluster has reached the posterior end of the germarium, provide a monolayer

envelope of cuboidal epithelium. During this time the cystocyte cluster has differentiated into 15 nurse cells undergoing polyploidization and one oocyte which begins meiosis. It has been suggested (Brown & King, 1964; Koch, Smith & King, 1967) that the determination of the oocyte is controlled by the pattern of intercellular bridges resulting in pro-oocyte development in the two cells that possess four bridges. The process determining which of these two cells finally becomes the oocyte is unknown.

Subsequent stages reveal the growth of the oocyte, the onset of yolk accumulation, the migration of the follicle cells, the laying down of the embryonic membranes (the vitelline membrane and the chorion with its specialized dorsal appendages and the micropyle), the discharge of the nurse cell contents into the oocyte, the decay of the follicle cells and the remains of the nurse cells and finally the appearance of the mature stage-14 oocyte.

In order to approach a molecular understanding of oogenesis we have identified monoclonal antibodies which bind differentiation antigens in the ovary. We took a library of monoclonal antibodies with binding activity against *Drosophila* imaginal discs and screened for antibodies of interest in oogenesis on the basis of their pattern of labelling on ovarioles in an immunofluorescence assay. A series of lectins was similarly assayed. In this paper we present the initial characterization of these markers together with a consideration of their potential roles in the analysis of oogenesis.

MATERIALS AND METHODS

Flies

Drosophila melanogaster Oregon-R strain, raised at 25 °C were used throughout. Ovaries were dissected from 3- to 5-day-old adults and from third instar larvae.

Monoclonal antibodies

Monoclonal antibodies were raised according to Köhler & Milstein (1976) using mass-isolated imaginal discs as the immunogen. The myeloma cell line used for the fusion was P3X63-Ag8-653 (Kearney, Radbruch, Liesegang & Rajewsky, 1979). A library of 152 hybridoma supernatants which showed binding activity to imaginal discs either by radioimmunoassay or by immunofluorescence on sections was collected. This library was used to screen for binding activity to ovaries and interesting hybridomas were cloned by limiting dilution. Ascites fluid was prepared and used for the immunohistochemical assays. We have given these antibodies the name DOV for *Drosophila* ovary.

Immunofluorescence assay

Ovaries were dissected in *Drosophila* Ringers and settled onto slides coated with poly-L-lysine (Sigma; 1 mg/ml) and the ovarioles were separated. The

preparations were then fixed in acetone at -20°C for 10 min, washed in PBS, then incubated in PBS/0.5% BSA (PBSA) for 30 min. The incubation in Ascites Fluid, diluted 1:500 – 1:2,000 in PBSA, was for 2 h at 20°C . For a negative control, undiluted P3X63-Ag8-653 myeloma supernatant was used in this incubation. The slides were washed for 1 h at 20°C in PBSA and then incubated with OX-20-TRITC at $10\ \mu\text{g/ml}$ in PBSA for 2 h at 20°C . OX-20 is a rat anti-mouse kappa light chain monoclonal antibody and was the gift of Dr. N. Barclay (MRC Cellular Immunology Unit, Oxford). The purified IgG was coupled with tetra-methyl rhodamine isothiocyanate (TRITC) by a standard method (Amante, Ancona & Forni, 1972). After a final 2 h wash the preparations were mounted in glycerol and observed by epifluorescence on a Zeiss Universal microscope. Photographs were taken on Kodak Tri-X film.

Lectins

A kit of fluorescein-coupled lectins supplied by Vector Laboratories was used. This contained the lectins Concanavalin A (ConA); Wheat Germ Agglutinin (WGA); Soybean Agglutinin (SBA); *Dolichos biflorus* Agglutinin (DBA); *Ulex europaeus* Agglutinin 1 (UEA1); Peanut Agglutinin (PNA); and *Ricinus communis* Agglutinin – 120 (RCA₁₂₀). WGA-FITC was also kindly provided by Dr. E. Hedgecock (MRC Laboratory of Molecular Biology, Cambridge). WGA-FITC was used on acetone-fixed ovaries at $15\ \mu\text{g/ml}$ in PBSA. Incubations were for 1 h followed by a 1 h wash in PBSA. The preparations were mounted in 70% glycerol 0.1 M-Tris-HCl pH9. The specificity of the WGA binding was tested in a control incubation in which N,N'-diacetylchitobiose (Sigma) was included at 0.1M.

RESULTS

In preliminary immunofluorescence experiments on unfixed whole mounts of ovaries a number of antibodies appeared to selectively label nurse cells. However, after acetone fixation of the ovary preparations, these antibodies labelled the cytoplasm of all cells in the ovary. Thus it seems that variability of penetration can produce artifactual immunofluorescence patterns in this system and that this can be overcome by the use of acetone fixation.

DOV 1: a cell surface marker for early stages of ovariolo development

Fig. 2 shows the binding of DOV 1. This antibody predominantly labels the surface membranes of the cells in the germarium and the labelling is most intense on the cells of the anterior part. The labelling can be seen to extend posteriorly as far as the stage-2 egg chamber where the cell membranes of the follicle cells exhibit weak fluorescence. The large cells at the extreme anterior of the germarium, the stem line oogonia and cystoblasts, are clearly labelled

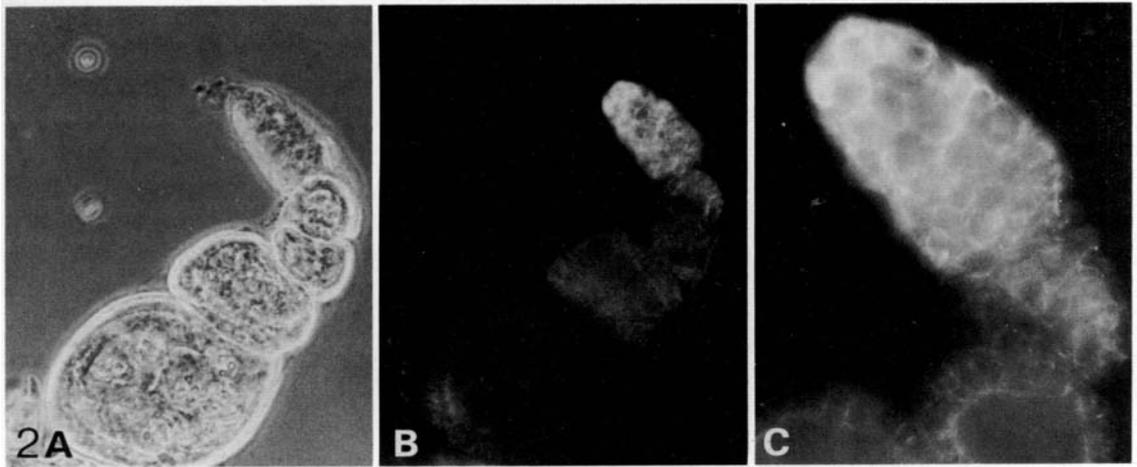


Fig. 2. Immunofluorescence on whole mounts of adult ovary: labelling with DOV 1 (A) Phase contrast ($\times 400$) (B) DOV 1/OX-20-TRITC labelling. The labelling is predominantly over the germarium. ($\times 400$) (C) DOV 1/OX-20-TRITC labelling ($\times 1000$). Note the strong cell surface labelling of the large cells at the top of the germarium. The follicular epithelium is clearly labelled in the early egg chambers.

and thus the antigen is present on the precursor cells for both the germ cell and follicle cell lineages. As DOV 1 labels follicle cells and does not appear to label other mesodermally derived cells in the adult ovary it was possible that DOV 1 might provide a marker for the localization of the pre-follicular subset of mesodermal cells in the larval ovary. However, at this stage most if not all of the cells are labelled (Fig. 3).

DOV 2: preferentially labels the cell surface of germ-line cells in the ovary

With DOV 2 there appears to be little binding to early stages however there is clear binding to the cell surface membranes of nurse cells and the oocytes from about stage 6 onwards (Fig. 4A). The level of binding increases with maturation of the egg chambers. There also appears to be some weak labelling of the follicle cell surface membranes, particularly at later stages. However, by comparison with an antibody that appears to label all cell surface membranes indiscriminately (III.24; Fig. 4B) the binding of DOV 2 is highly selective for germ-line cell surface membranes. This antibody also reveals the intercellular bridges between nurse cells and also those that connect the nurse cells to the oocyte (Fig. 4C, D).

DOV 3: reveals basal lamina differentiation over the germarium

The labelling pattern of DOV 3 is shown in Fig. 5. The binding is clearly restricted to the part of the tunica propria (the innermost basal lamina)

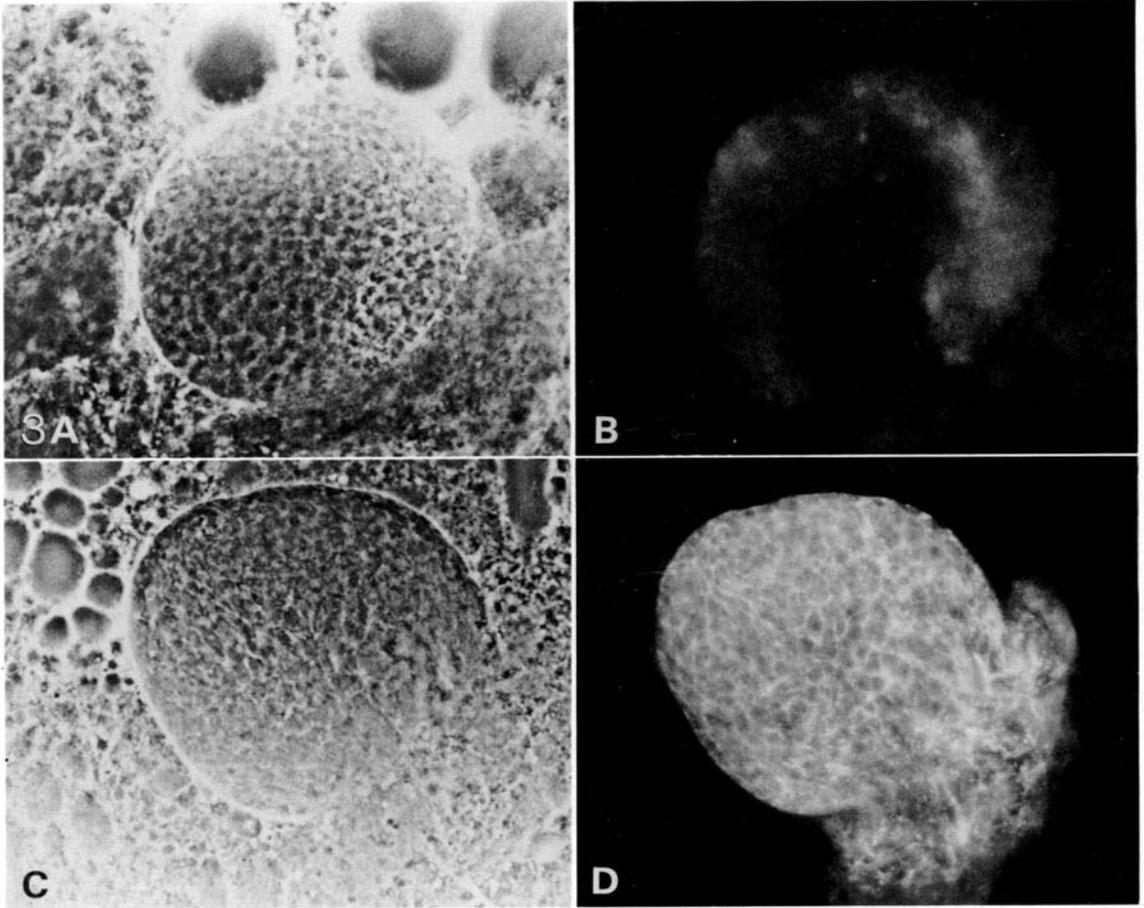
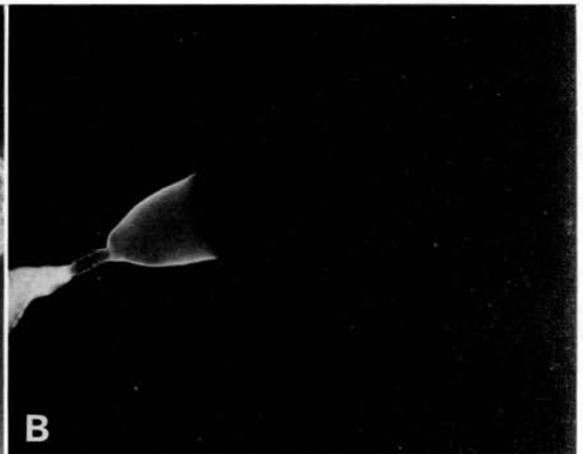
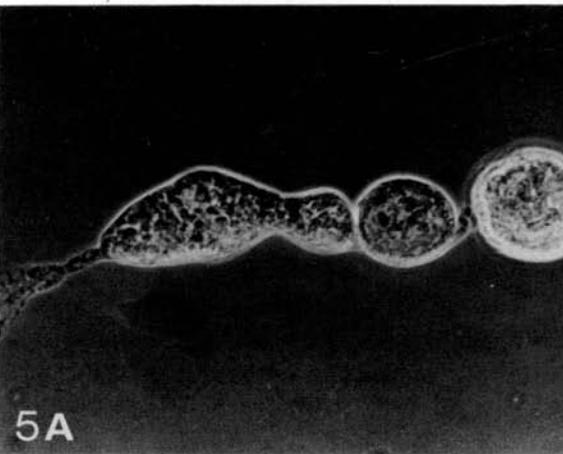
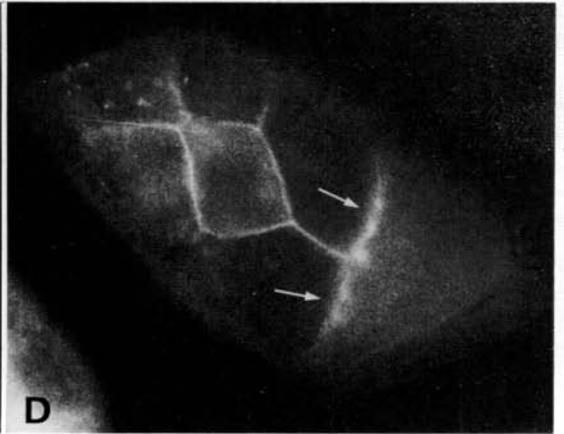
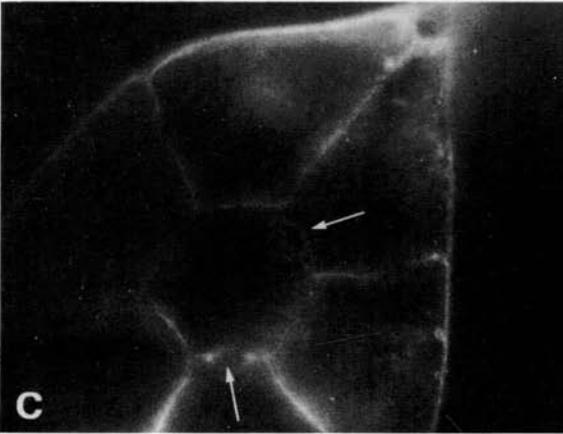
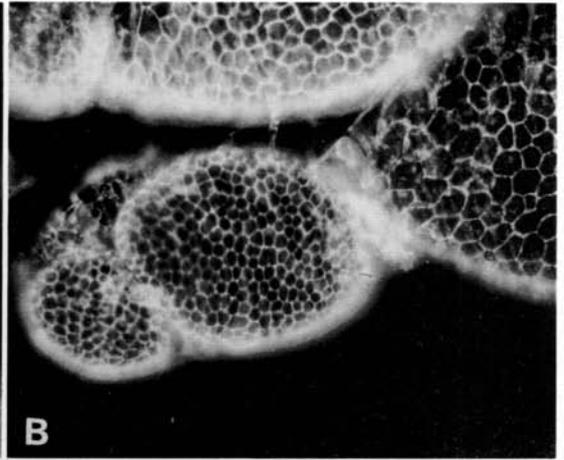
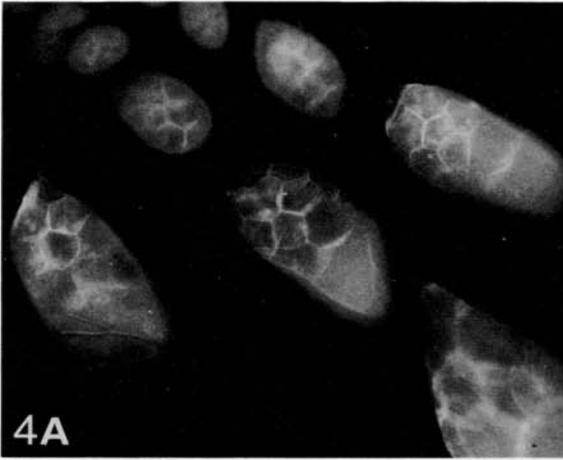


Fig. 3. Immunofluorescence on whole mounts of larval ovary: labelling with DOV 1. (A) Phase contrast, (B) Negative control with P3X63.Ag8.653 myeloma supernatant/OX-20-TRITC labelling. (C) Phase contrast, (D) DOV 1/OX-20-TRITC labelling. Most if not all cells are labelled. ($\times 530$).

Fig. 4. Immunofluorescence on whole mounts of adult ovary: labelling with DOV 2 and III.24. (A) DOV 2/OX-20-TRITC labelling. The cell surface of nurse cells and oocytes is predominantly labelled. ($\times 150$). (B) III.24/OX-20-TRITC labelling. An indiscriminate cell surface marker which displays here the cell surface membranes of the follicle cells. ($\times 330$). (C) DOV 2/OX-20-TRITC labelling. Arrows indicate intercellular bridges between nurse cells. ($\times 480$). (D) DOV 2/OX-20-TRITC labelling. Arrows indicate intercellular bridges between nurse cells and the oocyte. ($\times 400$).

Fig. 5. Immunofluorescence on whole mounts of adult ovary: labelling with DOV 3. (A) Phase contrast, (B) DOV 3/OX-20-TRITC labelling. The antibody labels the tunica propria over the anterior part of the germarium. In this preparation the epithelial sheath (which is also labelled by this antibody) has been stripped off the ovariole and is visible on the extreme left. ($\times 125$).



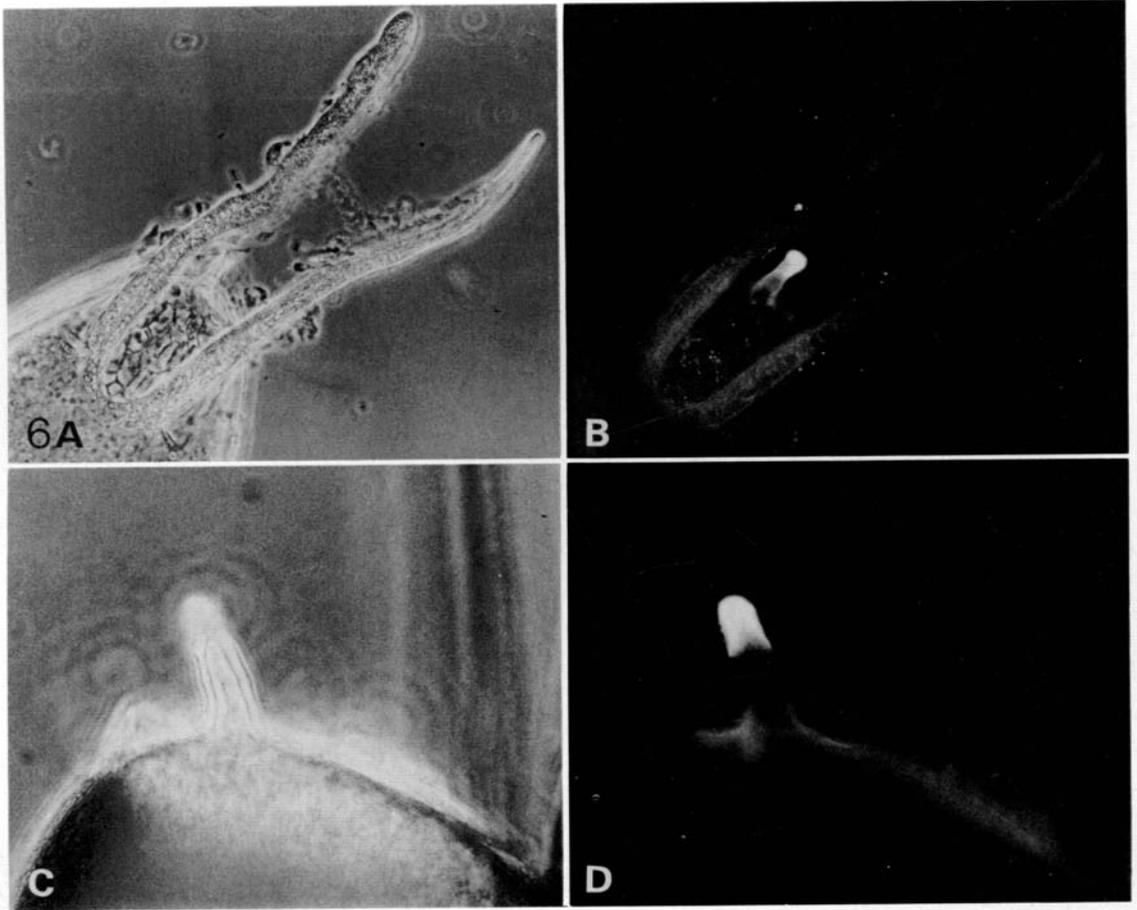


Fig. 6. Immunofluorescence on whole mounts of adult ovary: labelling with DOV 4. (A and C) phase contrast of stage-14 oocyte. (B and D) DOV 4/OX-20-TRITC labelling. The micropyle is strongly labelled. The dorsal appendages seen flanking the micropyle in A and B, exhibit weak autofluorescence ($\times 320$ A, B). ($\times 700$ C, D).

covering the anterior portion of the germarium. (We attribute this binding to the basal lamina because of its position and the sheet-like appearance of the fluorescence. It is, however, possible that a similar appearance would be produced by a cell surface antigen restricted to basal cell surfaces.) The basal lamina of the epithelial sheath is uniformly weakly labelled with this antibody. Six other monoclonal antibodies that bind to basal lamina were tested in this system and all bound uniformly along the whole length of the tunica propria and the basal lamina of the epithelial sheath (data not shown).

DOV 4: reveals molecular specialization of the micropyle

Labelling with DOV 4 produces a ring of fluorescence around the tip of the micropyle (Fig. 6). The micropyle is a tube through which sperm enter the egg.

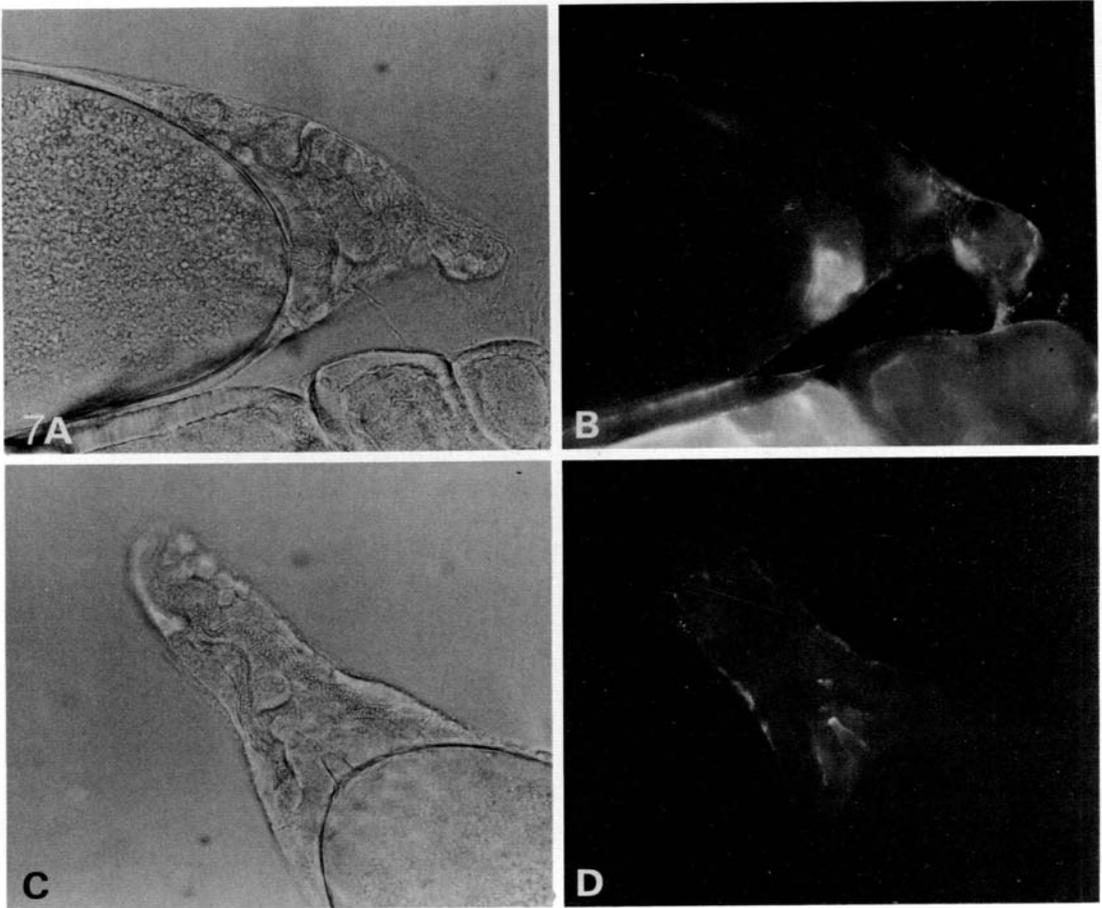


Fig. 7. Developmental stages of expression of the DOV 4 antigen: whole mounts of adult ovary. (A) Phase contrast; stage 12. Labelling first appears in the cells surrounding the developing micropyle. (B) DOV 4/OX-20-TRITC labelling; stage 12. (C) Phase contrast; stage 13. (D) DOV 4/OX-20-TRITC labelling; stage 13. At this stage the labelling becomes concentrated at the tip of the micropyle. ($\times 250$).

Antibody DOV 4 also shows weak labelling of basal lamina. However, the labelling of the micropyle does not appear to be due to the exposure of basal lamina antigenic determinants in this structure as six monoclonal antibodies which bind to basal lamina showed no labelling of the micropyle (data not shown). With DOV 4, labelling in the anterior end of the egg chamber first appears at stage 12 as a nebulous patch surrounding the developing micropyle (Fig. 7A, B). This may represent the synthesis of this antigen by the border cell cluster of follicle cells. By stage 13 the labelling on the tip of the micropyle is visible (Fig. 7C, D). In a preliminary experiment there appeared to be no change in the labelling pattern after fertilization (data not shown).

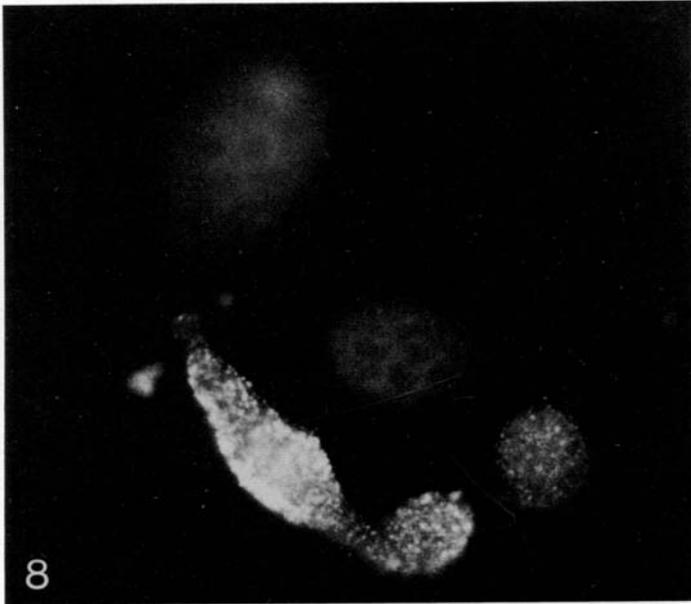


Fig. 8. Whole mount of adult ovary: labelling with WGA-FITC. The labelling is restricted to the germarium and the first two egg chambers. ($\times 300$).

*Wheat Germ Agglutinin: a marker for early stages of development
in the ovariole*

Of the lectins tested (Con A, WGA, SBA, DBA, UEA 1, PNA, RCA₁₂₀) only WGA appeared to give a readily interpretable pattern of labelling. Fig. 8 shows that WGA appears predominantly to label cells of the germarium and of the first two egg chambers. As a specificity control 0.1M-N, N'-diacetylchitobiose was included in the incubation and this abolished the binding of WGA-FITC. Further experiments will be required to elucidate which cells are labelled by wheat germ agglutinin.

DISCUSSION

In comparison to the wealth of information on the morphological description of oogenesis and the perturbation of this process caused by mutations, little is known of the molecular basis of ovarian differentiation. This study represents an initial characterization of molecular changes during oogenesis and we report the description of three classes of markers: those specific for particular stages of oogenesis; those specific for particular cell types and, lastly, those specific for particular specialized structures.

The monoclonal antibodies used in this study were initially identified on the

basis of their binding to imaginal discs where they did not reveal any striking differentiations. It may be interesting to investigate whether these antibodies recognize the same antigens in both tissues. However, at present the fact that these antibodies reveal differentiation antigens in the ovary probably merely indicates that the ovary is antigenically as well as morphologically more highly differentiated than the imaginal discs.

The value of these markers is first: they allow a description of molecular events in oogenesis and permit an analysis of stages of cell differentiation; second: they allow the analysis of mutant phenotypes and this may allow the implication of certain molecules in developmental events affected by these mutations and third: they suggest experiments which may determine the roles played by these molecules in developmental events.

Two antibodies, DOV 1 and DOV 2, and WGA provide stage-specific markers for the analysis of oogenesis. WGA and DOV 1 predominantly label early stages of oogenesis whereas DOV 2 appears not to label cells in the germarium and binds to the cell membrane of nurse cells and the oocyte only at later stages.

DOV 2 shows clear preference for the cells of the germ line. It is also interesting as it allows the visualization of the intercellular bridges connecting the nurse cells and also those connecting the nurse cells to the oocyte. These bridges have been implicated in the process of determination of the oocytes and this has involved their identification in wild-type and mutant ovaries by means of serial reconstruction from light microscope and EM sections (Brown & King, 1964; Johnson & King, 1972). The facility to locate these bridges by immunofluorescence on whole mounts may prove useful.

The differentiation of the tunica propria over the germarium revealed by DOV 3 suggests a functional correlation. From EM sections it has been observed that in the anterior portion of the germarium the follicle cells are tightly apposed to the tunica propria whereas more posteriorly they detach from the tunica propria and move inwards to surround the 16-cell cluster (Koch & King, 1966). The restriction of the DOV 3 antigen to the portion of the tunica propria covering the anterior portion of the germarium prompts the hypothesis that this antigen may be involved in the control of follicle cell migration. It is unfortunate that whereas mutants have been isolated which specifically affect subsequent migrations of follicle cells (King & Mohler, 1975), to our knowledge, no mutants have yet been isolated which principally affect this initial migration.

In a study of the binding of a monoclonal antibody, DA.1B6, to the adult ovary Brower and colleagues reported that the binding to the follicular epithelium begins in the posterior region of the germarium (Brower, Smith & Wilcox, 1981). It may be interesting to compare the position of onset of DA.1B6 binding with the posterior limit of the labelling of DOV 3 on the tunica propria.

As the laying down of the tunica propria constitutes a dramatic and early step in the morphogenesis of the ovary in the pupal stage, DOV 3 may provide a useful marker for the study of the complex process of the division of the ovary into ovarioles.

The antibody DOV 4 reveals a specialization of the chorion at the micropyle. While this may be due to a structural component required for the formation of the micropylar apparatus, it is tempting to suggest that this antigen may play a role in the process of sperm entry. It will be interesting to investigate whether the DOV 4 antigen will specifically bind to sperm.

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