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Modulated expression of a surface epitope on migrating germ cells of *Manduca sexta* embryos

James B. Nardi

Department of Entomology, University of Illinois, 320 Morrill Hall, 505 S. Goodwin Avenue, Urbana, IL 61801, USA

SUMMARY

In embryos of the moth *Manduca sexta*, the first cells that immunolabel with a monoclonal antibody to a surface epitope are the primordial germ cells. With this specific immunolabel, the migratory pathways of all primordial germ cells can be readily observed in whole mounts of embryos. The germ cells begin their migration by dispersing from an aggregate that lies on the midline of the embryonic germ band. Concurrently with segmentation of the embryo several hours later, germ cells segregate to several abdominal segments. With the advent of neurulation, germ cells again segregate, this time laterally within the segments. Shortly thereafter germ cells begin moving toward the lateral margins of abdominal segments. Simultaneously, two longitudinal strips of mesodermal cells along the lateral margins in each of four abdominal segments (3-6) begin expressing the surface epitope. The four initially noncontiguous strips on each side of the embryo fuse during the next several hours and form a single immunolabeled strip that stretches over four abdominal segments. Upon contacting these two lateral strips of mesodermal cells, germ cells begin moving bidirectionally along the anteroposterior embryonic axis toward the fifth abdominal segment where gonads will complete their morphogenesis. The immunolabel is asymmetrically distributed on the surfaces of germ cells during the early stages of migration and only during the later stages of migration after neurulation does the epitope adopt a more uniform distribution on the cells' surfaces.

Key words: primordial germ cells, migration, surface protein, modulated expression, insect embryo, *Manduca sexta*

INTRODUCTION

In migrating to their final destinations in the gonads, primordial germ cells (PGCs) travel across a variety of cellular and extracellular terrains. In a number of organisms, transplantation and cell marking experiments have been used to trace these routes followed by PGCs from their sites of origin to the gonads. The PGCs of anurans and mammals may initially arise in different embryonic locations, but they all eventually move from the gut endoderm and through the dorsal mesentery before ending their migration in the gonadal mesoderm. In reptiles and birds, cells from the epiblast first migrate to a germinal crescent in the endoderm before entering blood vessels and being transported to gonadal mesoderm (Wylie et al., 1986). Far less is known about movements of germ cells in the few insects in which migration of PGCs has been studied; however, the migratory strategies seem to be as diverse for insect embryos as those observed for vertebrate embryos. In the arthropod order Collembola, the germ cells actively migrate along a route through the yolk to the presumptive gonads (Klag, 1983). In certain hemimetabolous insects, the germ cells appear to be passively carried to the primordia of the gonads during elongation of the embryonic germ band (Anderson, 1972a). The PGCs of *Drosophila* and some other holometabolous insects are first passively carried from the posterior pole of the embryos to the gonads on the surface of invaginating posterior midgut. The germ cells subsequently migrate from the inner surface of the midgut to the outer surface and into the adjacent gonadal mesoderm of the body cavity (Anderson, 1972b; Fullilove and Jacobson, 1978). Various stages in the migratory behavior for the PGCs of another holometabolous insect, *Manduca sexta*, are described for the first time in this manuscript.

The migration of PGCs in *Manduca* embryos can be followed in its entirety by immunolabeling of cells with a monoclonal antibody (mAb) to a cell surface epitope. Throughout the course of their migration, from 10% embryogenesis to 36% embryogenesis, germ cells lie at the interface between the yolk and the inner surface of the embryo. Removal of the yolk exposes the entire population of germ cells. Not only does the antibody label the germ cells but it labels small mesodermal cells that the PGCs eventually contact. With this specific immunolabel, the relationship between the global movement of PGCs and these somatic cells can be easily followed in whole mounts without sectioning and/or serial reconstructions of embryos.

MATERIALS AND METHODS

Experimental animals

All developmental stages of *Manduca sexta* were maintained in a constant temperature room (26°C) having controlled lighting conditions (18L:6D).

Eggs that had been newly deposited on tobacco plants in the *Manduca* breeding cage were collected and maintained at 26°C. Embryonic development lasts about 96 hours at this temperature. The age of an embryo is conventionally represented as the percentage of time between oviposition and hatching. Since embryogenesis lasts about 100 hours, each hour of embryogenesis represents approximately 1% development time (DT). The timetables for *Manduca* embryonic development published by Broadie et al. (1991) as well as Copenhaver and Taghert (1989) provided useful landmarks for refining the staging of embryos dissected for this study.

Generation of monoclonal antibodies

Membranes used as immunogens were prepared from wing epithelial cells of developing adult *Manduca.* Wings were dissected from *Manduca* four days after pupation and placed in Weever's saline (pH 6.6). Each wing was cut transversely into three fragments - proximal, central and distal - using wing tracheae as landmarks. The proximal fragments and distal fragments from 12 wings were collected separately. Depending on the particular immunization of a mouse, membranes from either proximal or distal fragments were used as immunogens.

Wing epithelia were homogenized on ice in phosphate-buffered saline (PBS) containing three protease inhibitors - 5 mM EDTA, 20 μg/ml phenylmethylsulfonyl fluoride (PMSF), 1 μg/ml pepstatin - as well as 80 μM phenylthiourea (PTU) to inhibit melanization. Homogenization was carried out in a glass homogenizer with a Teflon pestle. All subsequent steps of membrane preparation were carried out at 4°C. The homogenate was first centrifuged at 1,000 ***g*** for 10 minutes and the supernatant was centrifuged one final time at 200,000 ***g*** for 1 hour. The pelleted membranes were resuspended in PBS by sonication prior to mixing with adjuvant.

The monoclonal antibody (mAb 3B11) that labels surfaces of *Manduca* germ cells was derived from a BALB/c mouse that had been immunized with membranes from wing epithelial cells. This mouse was first tolerized to cell membranes from distal wing fragments. The membranes suspended in PBS were mixed in a ratio of 1:1 v/v with Freund's adjuvant. The mouse was immunized with the resulting emulsion (~ 1.5 mg protein/injection) and then immediately immunosuppressed with cyclophosphamide (3 mg/injection) in PBS. Two additional intraperitoneal (IP) injections of cyclophosphamide followed at 24 and 48 hours after the immunization. Three weeks later the mouse was again immunized with distal wing membranes mixed with incomplete Freund's adjuvant and immunosuppressed (at 0, 24 and 48 hours after immunization) using the same concentrations of immunogen and cyclophosphamide that were used in the first immunization. The last two immunizations with membranes from proximal wing fragments were not accompanied by immunosuppression with cyclophosphamide. Proximal membranes suspended in PBS were mixed 1:1 with incomplete Freund's adjuvant and used as immunogens 3 weeks, 6 weeks and 9 weeks after the last immunosuppression.

Three days after the last immunization with proximal membranes, the mouse's spleen was removed aseptically and spleen cells were isolated for cell fusion. Spleen cells were fused with SP2/O-Ag14 myeloma cells using polyethylene glycol (Kennett et al., 1982). Fused cells were then plated in hypoxanthine, aminopterin, thymidine (HAT) selection medium from which HAT-resistant clones were picked after 2-3 weeks. Culture supernatants from these clones were screened on whole mounts of wing epithelia (4 days postpupation).

Screening of hybridoma supernatants

Wings for screening of hybridoma supernatants were fixed in 4% paraformaldehyde dissolved in PBS (room temperature, 30 minutes) and then rinsed three times in PBS. Before being incubated with supernatants, fixed wing tissues were first preincubated with PBS containing 3% normal goat serum (NGS) and 0.1% Triton X-100 for 12 hours at 4°C. Tissues were then incubated with supernatants for 24 hours at 4°C. Three rinses with PBS + 3% NGS + 0.1% Triton X-100 followed. A secondary goat anti-mouse IgMAG antibody (Zymed) labeled with fluorescein isothiocyanate (FITC) was prepared at a dilution of 1:50 in PBS + 3% NGS + 0.1% Triton X-100 and added to wing tissues. Wing tissues remained in the secondary antibody solution for 2 hours at room temperature and then were rinsed three times with PBS + 3% NGS + 0.1% Triton X-100. For viewing with fluorescent optics, immunolabeled tissues were mounted on glass slides using medium containing 70% glycerin, 30% Tris (0.1 M, pH 9.0) and 0.35% *n*-propyl gallate.

Immunolabeling of embryos

Staged embryos of *Manduca sexta* (192 embryos between 10% and 36% DT) were carefully removed from their chorions and vitelline membranes using finely sharpened forceps. Yolk adhering to the embryos was gently removed with tungsten needles. All dissections were performed in Grace's insect tissue culture medium (GIBCO). As embryos were freed of egg membranes and yolk, they were transferred to PBS containing 4% paraformaldehyde for 30 minutes at room temperature and then rinsed several times in PBS before being immunolabeled.

Following an overnight incubation in PBS containing 3% normal horse serum (NHS) and 0.1% Triton X-100 (hereafter referred to as PHT), fixed embryos were exposed to mAb 3B11 ascites fluid diluted 1:10,000 in PHT. After a 24-hour exposure to the primary antibody, embryos were rinsed three times with PHT and then placed in the secondary antibody diluted 1:200 with PHT. The secondary antibody was biotinylated horse anti-mouse (Vector). Exposure to this antibody for 24 hours was followed by three rinses in PBS + 0.05% Tween-20. A 2-hour incubation with avidin and horseradish peroxidase (HRP) coupled HRP to the antibody complex. This incubation was first followed by three rinses with PBS + 0.05% Tween-20 and then by the addition of diaminobenzidine (1 mg/ml) and an equal volume of 0.02% H_2O_2 in PBS for 5 minutes. Several rinses in PBS removed any excess substrate from the embryos. Prior to mounting the embryos in glycerin, they were partially dehydrated in graded concentrations of ethanol (10%-90%).

All incubations with antibodies were carried out at 4°C; all rinses and other steps in the immunolabeling procedure were performed at room temperature.

Thin sections of embryos were prepared from tissues that had been processed as described above. Immediately after rinsing with PBS to remove excess diaminobenzidine and H_2O_2, however, embryos were rinsed in 0.1 M cacodylate buffer (pH 7.3) containing 0.58 mM sucrose and 0.18 mM $CaCl_2$. Next embryos were placed in the same buffer containing 2.5% glutaraldehyde and 0.5% paraformaldehyde for 2 hours at 4°C (Tolbert and Hildebrand, 1981). The embryos were rinsed three times with the cacodylate buffer and then postfixed for 3 hours at 4°C with 2% OsO_4 dissolved in this buffer. Several rinses with cacodylate buffer removed excess osmium. Dehydration of embryos in a graded ethanol series (10%-100%) and propylene oxide preceded embedding in Epon 812. Sections ranging in thickness from 0.5 μm to 1.0 μm were cut on a Reichert OMU2 ultramicrotome and

serially arranged on glass slides. Sections were examined without the addition of stains.

Electrophoresis and western blotting

Lysates were prepared from embryos (20-30% DT) as well as wing epithelia of developing adult *Manduca* (4 days and 7 days postpupation). Proteins from lysates were separated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on nonreducing 8% gels (Laemmli, 1970) and subsequently transferred to nitrocellulose (Towbin et al., 1979). Approximately 10 μg total protein were applied to each lane. Nitrocellulose filters containing proteins from lysates were incubated for 6 hours at 37°C in PBS and 5% nonfat dry milk (blot buffer) to reduce nonspecific binding of antibodies. This incubation was followed by several rinses with PBS + 0.05% Tween-20 prior to transferring the nitrocellulose to blot buffer with 3% NHS and a 1:5,000 dilution of mAb 3B11 ascites fluid. The nitrocellulose incubated for 24 hours at 4°C with the primary antibody. As a control, an identical nitrocellulose filter with bound proteins was incubated in blot buffer + 3% NHS without any primary antibody. In subsequent steps, this filter was processed exactly as all other filters to determine if any proteins in the lysates are recognized by the secondary antibody. After incubation with the primary antibody solution, filters were washed several times with PBS + 0.05% Tween-20. Biotinylated horse anti-mouse antibody (Vector) diluted 1:200 in blot buffer + 3% NHS served as the secondary antibody. After incubating filters in the anti-mouse antibody for 24 hours at 4°C and removing excess antibody with several rinses of PBS + 0.05% Tween-20, they were transferred to a solution of avidin-HRP (Vector) in PBS + 0.05% Tween-20. After several washes with PBS + 0.05% Tween-20, immunolabeled bands on the nitrocellulose were then visualized by placing the filters in a solution of diaminobenzidine (0.5 mg/ml) and 0.01% H_2O_2 in PBS.

RESULTS

The epitope recognized by mAb 3B11

Western blotting of proteins from lysates of wing epithelial cells revealed that mAb 3B11 recognizes a single protein with a relative molecular mass of 156,000 (Fig. 1, arrowhead). mAb 3B11 immunolabels proteins that have been fractionated under nonreducing conditions using SDS-PAGE. The only band that is immunolabeled after fractionation of proteins under reducing conditions is the same band that labels on control filters exposed to the secondary horse anti-mouse antibody in the absence of mAb 3B11. The secondary antibody from horse always recognizes a *Manduca* polypeptide in wing lysates that has a relative molecular mass of 125,000 (Nardi, 1990). Since the antigenicity of the protein recognized by mAb 3B11 is destroyed under reducing conditions, immunoblotting of reduced proteins did not provide any information about the number of polypeptides represented by this protein. However, the relative molecular mass of the protein purified on a mAb 3B11 affinity column is 156,000 on both reducing and nonreducing gels (Nardi, unpublished results). Apparently the protein from wing epithelial cells represents a single polypeptide.

Although mAb 3B11 was obtained from a screening of hybridomas in which membranes from wing epithelial cells were used as immunogens, the first immunolabeling of cells with mAb 3B11 occurs early in embryogenesis (10% DT).

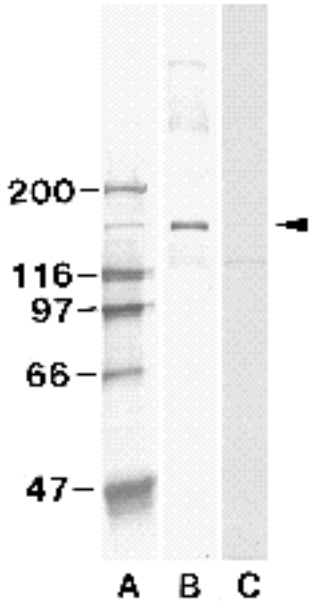


Fig. 1. Immunoblotting of *Manduca* wing lysates with mAb 3B11. Proteins were fractionated with SDS-PAGE on 8% gels and electrophoretically transferred to nitrocellulose. Positions of biotinylated protein standards (Bio-Rad) are indicated ($M_r \times 10^{-3}$). (A) Molecular weight standards; (B) nonreducing gel labeled with mAb 3B11; (C) reducing gel labeled with mAb 3B11.

Repeated attempts to immunolabel lysates of embryos (20-30% DT) that had been fractionated with SDS-PAGE have failed, suggesting that either (1) the embryonic epitope is present in amounts too low to detect with our immunoblotting technique or that (2) the epitope recognized by mAb 3B11 is a carbohydrate moiety found on a glycolipid(s) in embryos as well as on a protein with relative molecular mass of 156,000 from wing epithelium.

As judged from its immunolabeling pattern, the 3B11 epitope is exclusively expressed on PGCs prior to neurulation. At neurulation, neuroblasts begin to express the epitope and, as germ cells migrate laterally within the abdominal segments, a few other cells of the embryo immunolabel with mAb 3B11. The 3B11 epitope is expressed by germ cells not only throughout their migration but also after the cessation of their migration. Neurons and a few other cell types that immunolabel with mAb 3B11 during embryogenesis retain their cell surface labeling throughout postembryonic development.

Early stages of primordial germ cell migration

Primordial germ cells are first detected with mAb 3B11 at the time of germ band formation (10% DT). These are relatively large cells that lie as a loose aggregate on the midline of the embryo near its posterior pole (Figs 2A, 3A). By the germ band stage most of the germ cells have segregated from the epithelium of the germ band and lie on its inner surface (Fig. 4A,B). The germ band quickly elongates between 10% DT and 15% DT (Fig. 2A-C). Concomitant with elongation of the embryo, invagination of a contiguous strip of cells proceeds along the length of the ventral midline. As the embryo elongates, the cells of the aggregate

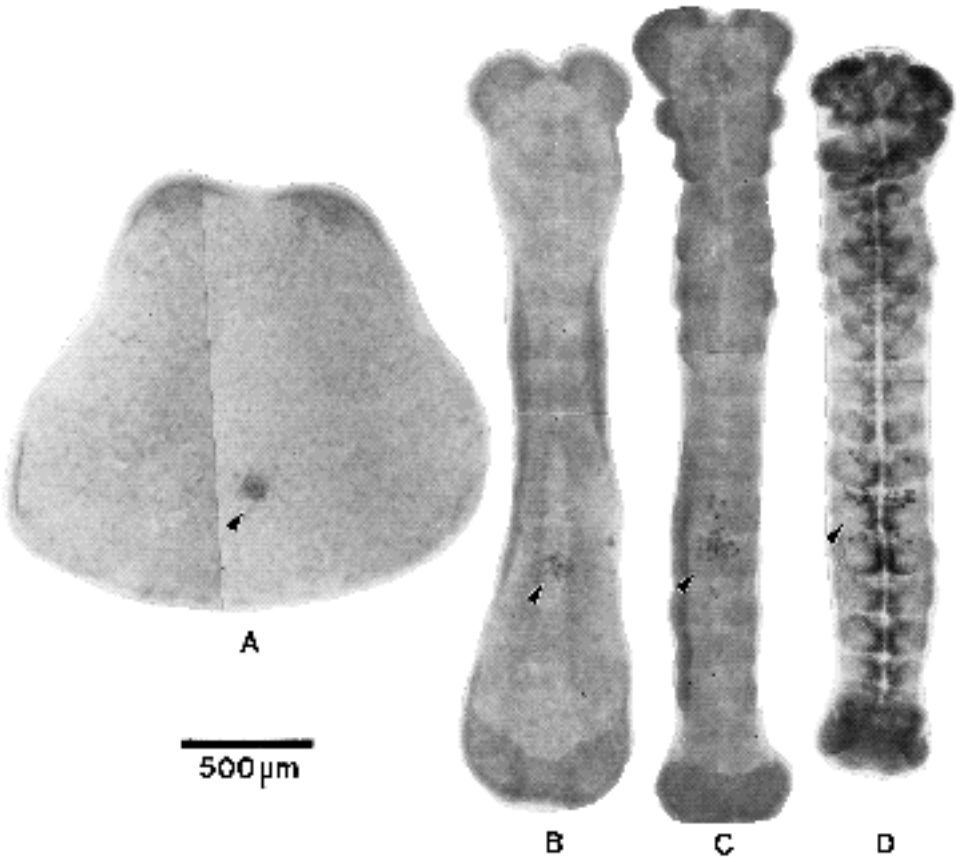
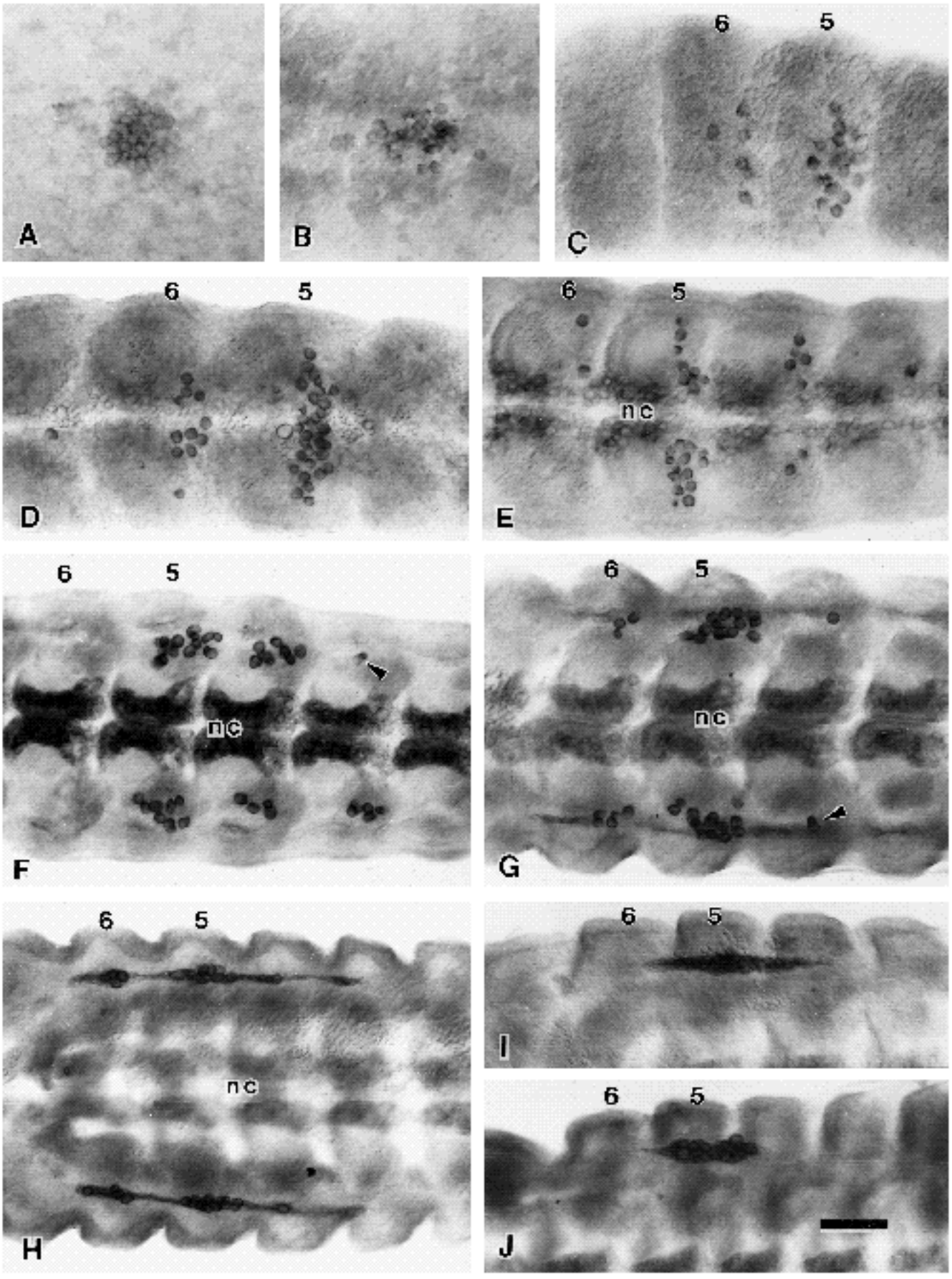


Fig. 2. Whole mounts of *Manduca* embryos that have been immunolabeled with mAb 3B11. Anterior is at the top. Arrowheads point to embryonic regions occupied by germ cells. (A) 10% DT; (B) 13% DT; (C) 15% DT; (D) 20% DT.

begin to disperse. The dispersal of germ cells can probably be attributed to active movement of these cells as well as elongation of their cellular substratum. The rapid rearrangement and elongation of the cellular substratum to which the PGCs adhere may be, at least partially, responsible for the displacement of PGCs along the anteroposterior (AP) axis of the embryo. Since elongation of the embryo does not occur laterally, the lateral movement of germ cells is most likely an active process. Short processes extending from the surfaces of germ cells (Fig. 4C, arrowheads) are probably involved in locomotion of the cells.

As soon as abdominal segmentation of the embryo occurs (~13% DT), the germ cells are partitioned into two, three, or four segments (Figs 2C, 3C). In the majority of embryos examined, germ cells are segregated to three abdominal segments. The individual differences in segmental distributions of germ cells from the time of abdominal segmentation to the cessation of germ cell migration do not seem to display a particular pattern related to sex of the embryo. A bimodal distribution for segmental location of germ cells is not obvious from a survey of thirty-three embryos examined between 13% DT and 20% DT. Differences in the number of abdominal segments occupied by germ cells may simply reflect individual differences in germ cell dispersal behavior that are independent of sex. The fifth abdominal segment, without exception, always contains more germ cells than any of the other segments. Germ cells have not been found anterior to the third abdominal segment nor posterior to the seventh

Fig. 3. Whole mounts of *Manduca* embryos showing the stereotypic migration of immunolabeled germ cells between 10% and 36% DT. In all photographs, anterior is to the right. The fifth (5) and sixth (6) abdominal segments have been marked in each specimen older than 12% DT. The scale bar represents 100 µm in each photograph. nc, nerve cord. (A) 10% DT (germ band). Germ cells form a loose aggregate on the embryonic midline. (B) 12% DT. Germ cells are beginning to disperse. (C) 15% DT. Concomitant with embryonic segmentation, germ cells become segregated to different abdominal segments. (D) 19% DT. Lateral segregation of germ cells begins as neurulation commences along the embryonic midline. (E) 21% DT. Germ cells have segregated laterally and neuroblasts now express the 3B11 epitope along the entire nerve cord (nc). (F) 25% DT. Germ cells are moving to lateral margins of abdominal segments. Short strips of cells along the lateral margins of abdominal segments 3-6 first begin to immunolabel with mAb 3B11. Arrowhead points to a cell that asymmetrically labels with antibody and that is extending a process. (G) 28% DT. The four strips of small, immunolabeled mesodermal cells lying along each lateral margin of abdominal segments 3-6 are still noncontiguous. Many PGCs have established contacts with these mesodermal cells. Arrowhead points to a cell that has extended a process. (H) 32% DT. The immunolabeled mesodermal cells now form a contiguous strip along each lateral margin of the four abdominal segments (3-6). PGCs now lie along the length of each mesodermal strip. (I) 34% DT and (J) 36% DT. Germ cells and mesodermal cells migrate concurrently toward the fifth abdominal segment. The embryonic midline and nerve cord lie at the bottom edge of each photograph.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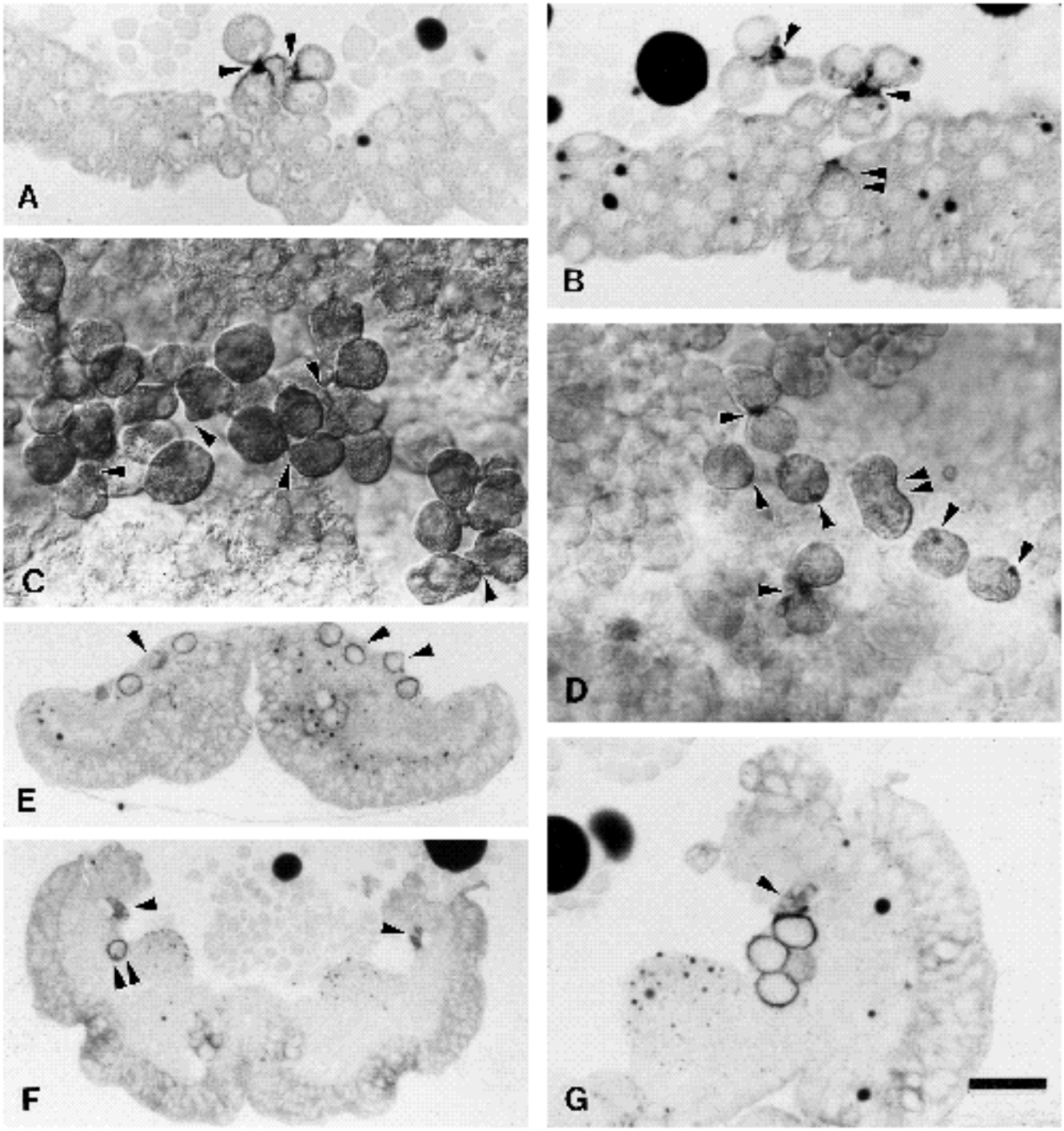

Fig. 4. Sections and whole mounts of embryos immunolabeled with mAb 3B11 show the distribution of the 3B11 epitope on surfaces of individual cells. The scale bar represents 25 μm in A-D,G and 50 μm in E,F. The solid black circles of various diameters are sections of lipid droplets from the yolk that have been fixed with osmium. (A) In this section of a germ band (10% DT), the 3B11 epitope is localized on these surfaces of germ cells that are in contact with each other (arrowheads). (B) This section taken at 12% DT shows that caps of surface epitope expression occur on germ cells as they leave the germ band epithelium (double arrowhead) and at surfaces of mutual contact (arrowheads). (C) Germ cells are dispersing at 13% DT. Several germ cells are extending short processes (arrowheads). (D) At 15% DT germ cells are scattered and some are isolated. The 3B11 epitope is asymmetrically expressed in both isolated cells as well as germ cells that are in contact with other germ cells (arrowheads). One germ cell is dividing (double arrowheads). (E) By 22% DT germ cells are migrating toward the lateral margins of the embryo and the 3B11 epitope is more symmetrically expressed on germ cells. Some cells (arrowheads) still show an asymmetry in their immunolabeling. (F) This section clearly shows the two symmetrically placed populations of lateral mesodermal cells (arrowheads) that lie in abdominal segments 3-6. One PGC (double arrowhead) is moving toward the mesodermal cells on the left side of the photograph. (G) Several PGCs have aggregated and are in contact with the mesodermal cells (arrowhead) that stretch along the AP axis of the segment. Note the relatively uniform distribution of the 3B11 epitope on surfaces of the PGCs.

abdominal segment. The presence of germ cells in the seventh abdominal segment is evidently a rare occurrence. Of the almost two hundred embryos examined during the period of germ cell migration, in only one embryo (Fig. 3D) was a germ cell found in the seventh abdominal segment. In all other embryos examined, the germ cells occupied abdominal segments 3-6.

With the onset of neurulation at about 19% DT, lateral segregation of germ cells within abdominal segments 3-6 is observed (Figs 2D, 3E). As neural epithelium invaginates along the ventral midline, mesodermal cells and germ cells move laterally. This displacement of germ cells to their new positions may be, at least in part, a passive process. However, the extension of processes (Figs 3F,G, 4E) by cells that are moving laterally suggest that lateral displacement may also be to some extent an active process.

During their initial dispersal and segregation to different abdominal segments, PGCs establish only tenuous connections with their cellular substratum (Fig. 4A,B). During their lateral movement within a given segment, however, germ cells become embedded among adjacent mesodermal cells (Fig. 4E,F). This temporal difference in the interaction of germ cells with their cellular substrata is also reflected in a temporal difference in the spatial distribution of the 3B11 epitope on surfaces of individual germ cells.

Distribution of the 3B11 epitope on surfaces of individual germ cells

As PGCs leave the epithelium of the germ band to form an aggregate on the basal surface of the epithelium, these cells asymmetrically express the 3B11 epitope on their surfaces. This distribution is suggested by the immunolabeling of whole embryos (Figs 2A, 3A), but can be most clearly seen in sections of labeled germ bands. In Fig. 4B, a germ cell that is separating from the epithelium immunolabels at only one pole (double arrowhead). Germ cells that still lie within the germ band epithelium most strongly express 3B11 on their free surfaces. Germ cells that have segregated from the epithelium primarily express 3B11 on cell surfaces that contact other germ cells (Fig. 4A,B). This polarized expression of 3B11 on the cell surface is maintained throughout the early movements of germ cells. Anisotropic expression of this surface epitope is particularly evident prior to neurulation and lateral segregation of germ cells. Regional expression of 3B11 is observed wherever two or more germ cells contact, but caps of 3B11 are also frequently observed on individual, isolated germ cells (Fig. 4D). As germ cells move laterally within a segment, the

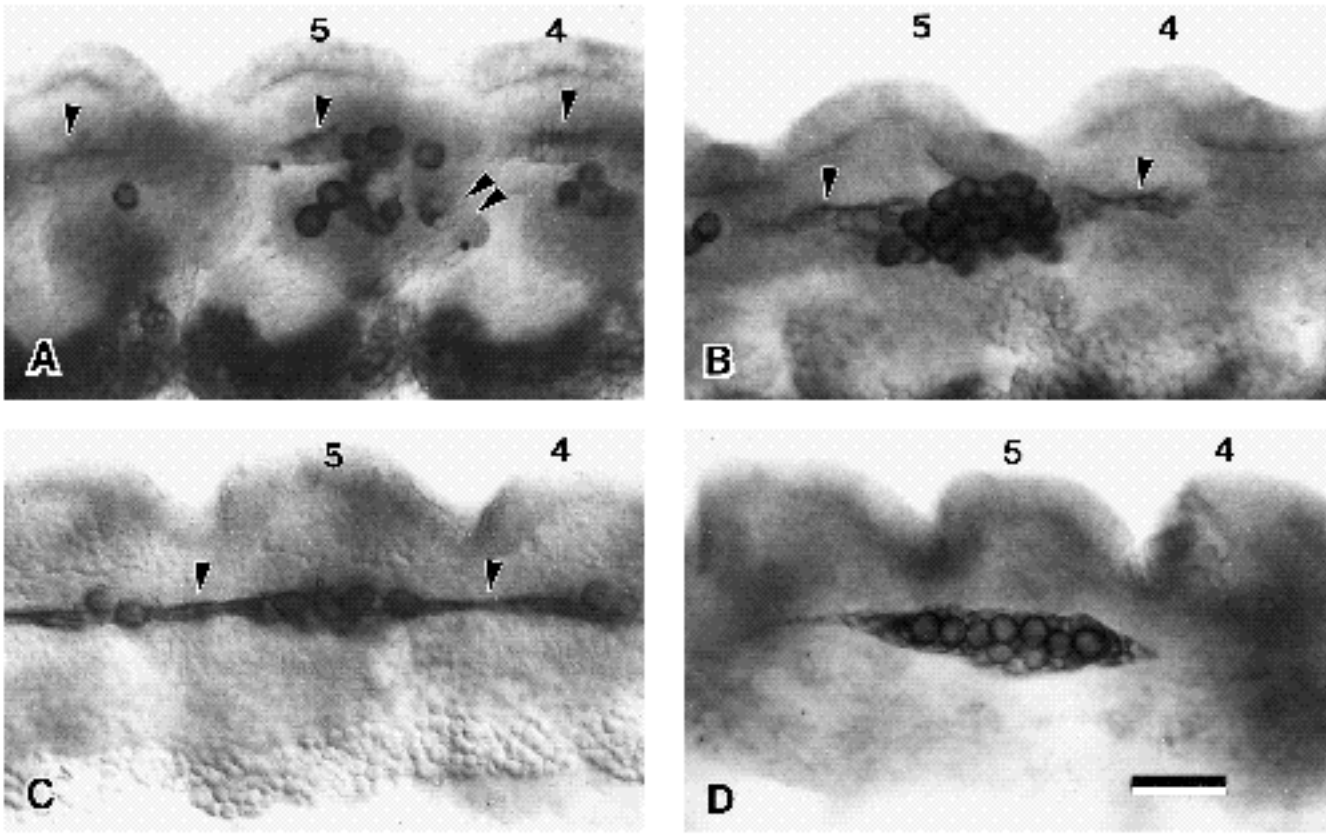

Fig. 5. The left abdominal hemisegments of four embryos. Anterior is to the right and the nerve cord lies at the bottom edge of each photograph. This series of photographs shows the interactions between PGCs and mesodermal cells (arrowheads) that occupy the lateral margins of the segments. The fourth (4) and fifth (5) abdominal segments are marked. The scale bar represents 50 μm. (A) 25% DT. Note that some germ cells (double arrowhead) still show a capped distribution of the 3B11 epitope. (B) 28% DT. (C) 31% DT. (D) 35% DT.

surface epitope is asymmetrically expressed on some cells (Figs 3F,G, 4E), but most germ cells at this stage isotropically express the epitope on their surfaces (Fig. 4E-G).

Aggregation of germ cells in the 5th abdominal segment

During the lateral movement of PGCs in the abdominal segments, small, discrete groups of mesodermal cells begin expressing 3B11 at around 25% DT. Eight groups of these mesodermal cells are found in each embryo at this stage. From the third to the sixth abdominal segment, two elongated groups of cells are found near the lateral margins of each of the four segments and are aligned along the AP axis of the embryo (Figs 3F,G, 5A,B). Although expression of the 3B11 epitope by these small mesodermal cells initially occurs in eight noncontiguous strips, over the next few hours of development, these strips of expression fuse between abdominal segments to form two long strips, one on each side of the embryo (Figs 3H, 5C). The switch from separate to contiguous expression of 3B11 by these lateral mesodermal cells could be a consequence of (1) movement along the AP axis of cells already expressing the protein or (2) the later initiation of expression in mesodermal cells that lie between those strips of mesodermal cells first labeling with mAb 3B11 at around 25% DT.

During the transition from disjunct to contiguous expression of the surface epitope on these lateral strips of mesoderm, all PGCs continue to move laterally until they contact these strips of mesoderm that eventually extend from the anterior edge of the third abdominal segment to the anterior edge of the seventh abdominal segment (Fig. 3G,H). Once all the PGCs are arrayed along these longitudinal strips, movement of cells that label with mAb 3B11 - both large germ cells and small mesodermal cells - proceeds toward the fifth abdominal segment. The movement is aligned along the AP axis and is bidirectional, with cells moving posteriorly from segments 3 and 4 as well as anteriorly from segment 6 (Fig. 3I). By 35% DT, immunolabeling of presumptive gonadal cells is confined primarily to the fifth abdominal segment (Figs 3J, 5D).

The three-dimensional arrangement of cells along the lateral margins of abdominal segments 3-6 could create an illusion of early immunolabeling in mesodermal strips. This possibility was discounted by examining sections of immunolabeled embryos at developmental stages when germ cells are moving toward the lateral margins of the abdominal segments. Immunolabel is clearly present on surfaces of these small mesodermal cells (Fig. 4F, arrowheads).

DISCUSSION

Primordial germ cells of animal embryos arise independently of the gonads and then migrate along particular pathways to the gonadal primordia. While migration of germ cells has been well studied in a number of vertebrate embryos (Wylie et al., 1986), relatively little is known about migration of these cells in insects (Anderson, 1972a,b). As might be expected, the movements of germ cells are known in most detail for *Drosophila* embryos (Fullilove and Jacobson, 1978). What is known about PGCs from other insects is based mainly on examination of sections in which germ cells are identified from their distinctively spherical shapes rather than from specific cell markers. This study of *Manduca* germ cells is unique in that it describes the stereotypic movements of immunolabeled cells in whole mounts of insect embryos. The immunolabeling of *Manduca* germ cells is well-defined and the embryonic territory traversed by the germ cells can be viewed globally as well as in sectioned specimens.

All germ cells migrate to their final destinations; but to what extent this movement is dependent on intrinsic migratory properties of PGCs rather than extrinsic forces is difficult to ascertain. During their migration, insect PGCs exhibit features of active movement as well as passive displacement (Anderson, 1972a,b; Klag, 1983). Postmigratory PGCs from mouse embryos neither spread nor move in vitro; but under identical culture conditions, migratory germ cells of mice spread, migrate and display invasive activity (Donovan et al., 1986; Stott and Wylie, 1986). Using heterochronic grafts of germ cells transplanted to irradiated, sterile hosts, Subtelny and Penkala (1984) were able to study the movement of PGCs from endoderm to the dorsal root of the mesentery in *Rana* embryos. These authors found that PGCs did not migrate out of the endoderm prior to the onset of mesentery formation. Apparently some phases of germ cell migration, at least in certain species, are dependent on morphogenetic events in adjacent embryonic tissues rather than on the intrinsic migratory ability of the germ cells.

The directed migration of germ cells - whether active or passive - implies the presence of adhesion/recognition molecules on the surfaces of PGCs and the substrate that they traverse. In *Xenopus* embryos, fibronectin is found at interfaces between migrating germ cells and the mesentery cells over which they migrate. Adhesion of germ cells to mesentery cell layers in vitro is actually inhibited by $F(ab)_2$ fragments of antibodies to fibronectin (Heasman et al., 1981). Some fibronectin is also found in the basement membranes of epithelial cells that lie along the migratory route. In addition to containing certain adhesion molecules, these basement membranes also appear to act as physical barriers, restricting the movements of germ cells to particular pathways (Heasman et al., 1985). Although fibronectin and other extracellular matrix molecules - laminin, chondroitin sulfate proteoglycan and collagen type IV - are distributed along the pathway followed by chick PGCs, these molecules are also found in areas beyond the germ cell pathway (Urven et al., 1989). Molecules of the extracellular matrix may act as cues for the migration of PGCs, but they are apparently not the only cues. Cell surface or matrix molecules whose distributions are limited to the migratory pathways for PGCs have not been found. However, the expression of certain molecules on surfaces of mammalian germ cells coincides exactly with the period of germ cell migration (Fazel et al., 1987; Wylie et al., 1986). All these molecules are lost from the surfaces of germ cells once their migration has ceased. Unlike the molecules on surfaces of vertebrate PGCs that immunolabel only during the period of germ cell migration, the epitope recognized by mAb 3B11 on *Manduca* germ cells continues to be expressed after germ cells have reached the gonadal primordia. Migration of germ

cells is obviously associated with several changes at the level of surface molecules, but the roles of these surface molecules (with the exception of fibronectin) in directing movement of germ cells remain untested.

The expression of fibronectin on PGCs of *Xenopus* embryos and the expression of the 3B11 epitope on *Manduca* PGCs share a striking, yet unexplained, feature. Heasman et al. (1981) reported that PGCs of *Xenopus* only express fibronectin on cell surfaces that interact with their mesentery substratum. This asymmetric expression of fibronectin on germ cells contrasts with the uniform expression of the same molecule on surfaces of mesentery cells. The expression of 3B11 is likewise modulated spatially on cell surfaces of PGCs during the course of cell migration. Following an early period of asymmetric expression on the cell surface, the epitope becomes uniformly distributed over the surfaces of germ cells during the later stages of their migration. The reason for this modulated expression of the surface epitope on *Manduca* PGCs is unclear, but its asymmetric distribution clearly coincides with the phase of migration prior to the lateral aggregation of PGCs (Figs 3A-C, 4A,B,D, 5A). One can hypothesize that the polar expression of 3B11 on migrating germ cells may preclude their aggregating prematurely during the segmental and lateral dispersal of the germ cells. Further characterization of the protein from wing epithelium recognized by mAb 3B11 may disclose features of the surface molecule(s) on germ cells that are responsible for this phenomenon of modulated expression.

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