INTERSPECIFIC TRANSPANTATION OF POLAR PLASM BETWEEN DROSOPHILA EMBRYOS

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ABSTRACT

Posterior polar plasm of the Drosophila egg has been shown to function autonomously in germ cell determination after transplantation to either the anterior or mid-ventral region of the early embryo. By means of similar transplantations, we have tested the ability of polar plasm of Drosophila immigrans to induce the formation of pole cells in a Drosophila melanogaster embryo. After the transplantation of polar plasm, “hybrid” pole cells were found in which both pole cell-specific organelles, the polar granules and nuclear body, were structurally similar to those characteristic of the transplanted cytoplasm.

In order to determine whether these hybrid pole cells can function as germ cell precursors, these cells were transplanted to the posterior tip of genetically marked embryos. Approximately 5% of the flies obtained from embryos receiving potential pole cells produce offspring derived from the induced pole cells. This result demonstrates that polar plasm can function in interspecific species combinations and indicates that the molecular mechanisms of germ cell determination are conservative in evolution. Finally, in order to test whether there is any evidence for cytoplasmic inheritance of polar granules, embryos derived from hybrid pole cells were examined for their polar granule morphology. The fine structure of the granules conformed to that of the nucleus. Thus, no evidence was found for the cytoplasmic inheritance of these particular organelles.

A distinctive cytoplasmic region is located at the posterior tip of dipteran, coleopteran, and hymenopteran eggs which is responsible for the formation of pole cells. These cells are the precursors of the primordial germ cells (reviewed in references 3, 7). In recent studies, utilizing the technique of transplantation of polar plasm, it has been shown that this posterior polar plasm can function to induce pole cell formation at the anterior (11), mid-ventral (12), and posterior (25, 31) regions of the early cleavage stage embryo of Drosophila melanogaster. We have also found that the polar plasm from unfertilized eggs and oocytes at terminal stages of oogenesis was functional in germ cell determination (13). In each of our studies the induced pole cells were shown to produce offspring, thus demonstrating that germ cell determinants are localized in the posterior polar cytoplasm of Drosophila eggs.

Characteristic subcellular organelles or polar granules, which are found only in the posterior polar plasm and which segregate into the pole cells (18), served as morphological markers to follow the injected cytoplasm. The ultrastructural charac-
teristics of polar granules are distinctive for each Drosophila species (19). For example, there are interspecific similarities as well as species-specific differences in the structure and morphological changes characteristic of polar granules during the stages leading to pole cell formation (18, 19). In every species examined, the polar granule is a fibrous structure, usually less than 0.5 μm in diameter, not bounded by a membrane, with many granules attached end-to-end in the mature egg. After fertilization, the granules fragment and disperse in the posterior polar plasm. After pole cell formation, the granules reaggregate into large granules. Both the degree of disaggregation at fertilization and the manner of reaggregation have many species-specific characteristics. For example, in Drosophila immigrans, the granules reaggregate into one large plaque per cell, 2–5 μm in diameter, and the substructure of the granule changes from an interwoven meshwork of fibers to a semicrystalline array of short, 0.5 μm rods. In D. melanogaster, however, a number of large, 0.75 μm to 1.0 μm in size, spherical polar granules form in each pole cell without any appreciable change in substructure.

Because of these clearly recognizable differences between species, a number of interesting questions can be asked by means of interspecific transplantation of polar plasm. We can determine whether the cytoplasmic germ cell determinants can function in a “hybrid” situation, i.e., in a pole cell whose nucleus is of one species and the polar granules and polar plasm from a different one. Furthermore, we can determine whether a species-specific series of polar granule changes will occur autonomously after transplantation to another species; and finally, by obtaining eggs produced by a hybrid pole cell, we can determine whether or not the polar granules are inherited as an autonomous cytoplasmic organelle.

MATERIALS AND METHODS

The procedures utilized in this study have been described previously (11–13). Only changes or additional techniques will be described here.

Drosophila Strains

For ultrastructural studies of polar granules and pole cells of normal embryos, the multiple wing hair, ebony (mwh e4) mutant stock of D. melanogaster (no differences from the Oregon R wild-type strain used previously [18, 19] have been detected) and a wild-type strain of D. immigrans collected locally by Dr. R. Richmond (Indiana University) were used. Cleavage embryos as recipients for polar plasm transplants were obtained from the same mwh e4 stock. Blastoderm embryos which served as hosts for cell implantations were collected from yellow, singed, maroon-like (y sn3 mal) stock of D. melanogaster. (For detailed description of the mutants, see reference 17.)

Ultraviolet Irradiation

The procedure for irradiation was similar to that described previously. Approximately 30 min after fertilization, embryos obtained from a y sn3 mal stock were irradiated with 7,200 ergs/mm² at a dose rate of 2.0 × 10⁶ ergs/cm²/s (Ultra-Violet Products Inc., San Gabriel, Calif.). Before each irradiation the dosage was controlled with a dosimeter (Model 225, Ultra-Violet Products). After UV treatment, the eggs were transferred to paraffin oil and kept in the dark up to the blastoderm stage. Only those embryos that showed normal blastoderm formation were selected as suitable recipients for cell implantation. This UV dose was sufficient to prevent the formation of pole cells (Fig. 1 a, b). Approximately 40% of the irradiated embryos developed to adult flies of which 95% were sterile.

Histology

Some of the mwh e4 embryos injected with polar plasm from D. immigrans embryos were prepared for electron microscopy either at the end of blastoderm formation or during gastrulation as previously described (11–13). Embryos derived from hybrid pole cells were fixed in a trialdehyde fixative (16), postfixed with osmium tetroxide and uranyl acetate, and embedded in DER 732-332 plastic. Several of the y sn3 mal larvae and flies developing from embryos with injected hybrid pole cells were analyzed histochemically for aldehyde oxidase activity (6, 14). Cells containing this enzyme (mwh e4) become stained blue due to the reduction of nitro blue tetrazolium in the presence of benzaldehyde as a substrate whereas the cells lacking the enzyme (y sn3 mal) remain unstained.

RESULTS

A brief description of the early events in pole cell formation in Drosophila embryos will assist in understanding these experiments. After fertilization, a series of 13 synchronous nuclear divisions (30, 33) occur approximately every 10 min at 25°C without intervening cytokinesis. At the time of the eighth division, the first nuclei reach the posterior polar plasm and, immediately, 10 or more pole cells containing the polar granules bud off from the remainder of the embryo. These cells continue to divide until there are approximately 40 pole cells. During gastrulation, the pole cells

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are included within the posterior midgut invagination. Subsequently, they migrate through the midgut layer of cells and reach the embryonic gonad where they establish the germ line. (For further details, consult reference 29.)

**Ultrastructure of Normal Pole Cells**

The ultrastructure of polar granules and pole cells during embryonic development has been described previously (18-20). In order to assist in understanding the unusual interactions between nucleus and cytoplasm in the hybrid cells, it is necessary to describe briefly the species-specific features of pole cells at the blastoderm stage. The pole cells at this stage have completed their initial series of cell divisions. In *D. melanogaster*, many small (0.25 μm) polar granules aggregate into a number of clusters of spherical granules (Fig. 2). The electron-dense fibrous component forms the outer rim around a central region which is similar in structure to the adjoining cytoplasm (Fig. 3). Frequently, small chains of granules appear (Fig. 3), but a number of discrete polar granules, 0.5 to 0.75 μm in diameter, remain in each cell. The polar granules retain these ultrastructural features until the time that pole cells migrate from the posterior midgut when most of the polar granules become associated with the outer nuclear envelope as fibrous bodies or “nuage”-like material (5, 20).

At the blastoderm stage, another organelle unique to the pole cells appears in their nuclei. It is a spherical structure with an electron-lucid central core (Fig. 2). Fine nuclear fibers appear to connect to the periphery of this nuclear body. These structures are distinct from the nucleolus which forms in association with the chromocenter containing the nucleolar-organizer regions (2). Although we have not obtained complete serial sections of pole cell nuclei, we estimate that there are about four nuclear bodies per nucleus. We have not been able to discern any regular substructure to the body with high resolution microscopy. These nuclear bodies have been found in pole cells during gastrulation without any changes in their morphology. However, they are no longer evident at the time the pole cells migrate from the gut to the embryonic gonad.

The polar granules in *D. immigrans* pole cells are larger and more abundant. Before the aggregation of polar granules at the blastoderm stage, their substructure is similar to that seen in *D. melanogaster* (19). After the last pole cell division, clear differences appear. First, the substructure of the granule rapidly changes (Fig. 4). Instead of an interwoven meshwork, a dense rod-like structure appears which is 25 nm in thickness and rarely
FlcurtE 2 Survey of a pole cell in *D. melanogaster* to show the structure of polar granules (P) during gastrulation. Occasionally the polar granules reaggregate into large granules but they are always dispersed in the cytoplasm. The nucleus contains two nuclear bodies (N). × 11,000. Insert: nuclear body. × 37,000.

FIGURE 3 Higher magnification of polar granules in *D. melanogaster* during gastrulation. The substructure of the granules remains an interwoven mat of fibrils. Small electron-dense particles (arrows), approximately 50 nm in diameter, are found adjacent to the granules during gastrulation. Possibly they are early stages in the fragmentation of the granules, which result in the granules becoming associated with the nuclear envelope (20). × 37,000.

A fourth major difference concerns the pole cell-specific nuclear body. Four or more spherical structures appear in the pole cell nucleus after the last division. Initially, they are smaller and much denser than in *D. melanogaster* (cf. Figs. 8 and 9). They quickly become large, possibly by fusion (Fig. 6a, b; Fig. 7), and develop an irregular peripheral and central region (Fig. 6b, c). At late blastoderm and early gastrulation, the nuclear body breaks up into small electron-dense masses (Fig. 6d). Occasionally, nuclear bodies are found during early blastoderm stages (Fig. 7) which resemble those found in *D. melanogaster*. This type of nuclear body has never been found in pole cells of late blastoderm or gastrulation stages of *D. immigrans*. Furthermore, even in those bodies found at the early blastoderm stage, there is some indication of the type of discontinuities in structure characteristic of *D. immigrans* (Fig. 7 insert). Nuclear bodies of the *D. immigrans* type (Fig. 6) have never been found in *D. melanogaster* embryos.

**Ultrastructure of Hybrid Cells**

Polar plasm from early cleavage embryos of *D. immigrans* was transplanted to the anterior tip of
FiGuve 4 Polar granules (P) of *D. immigrans* embryos following the last cell division at the blastoderm stage. The substructure of the granule becomes changed to a rodlike component (arrows). The granules are associated with the centrioles (C) of the cell and have begun to reaggregate into large granules which subsequently will fuse together. × 25,000.

FiGuve 5 Cross section through the reaggregated polar granules of *D. immigrans* embryo at an early gastrula stage. The large granule is closely associated with the nucleus (N). Individual granules are still evident within the aggregate because of the retention of the electron-lucid central areas (arrow). The substructure of the granule is composed of rods as in Fig. 4. The centrioles are no longer associated with the polar granules at this stage. × 16,000.

*mwh* e4 embryos of *D. melanogaster* of the same age. When these embryos reached the blastoderm or early gastrula stage, six embryos were selected for serial thick and thin sections. The observations are summarized in Table I and illustrated in Figs. 8-10. In every embryo, we found hybrid pole cells (i.e., pole cells with a *D. melanogaster* nucleus and polar granules and possibly other cytoplasmic constituents from *D. immigrans*) which were identifiable by the presence of polar granules and nuclear bodies. The number of pole cells found varied from two to at least 15, and they were located either between the blastoderm layer and the vitelline membrane (Fig. 9) or deeper in the blastoderm layer (Fig. 8).

A number of unusual features were found in these induced hybrid pole cells. Although the polar granules of normal *D. immigrans* embryos at the end of blastoderm formation have reaggregated into a large plaque (Figs. 4, 5, and 11) and show the "crystalline" substructure, the polar granules of the six embryos examined, three of which had begun gastrulation, retained the interwoven fibrous structure characteristic of earlier stages. Furthermore, they had aggregated into large polar granular masses (Figs. 8-10) which were dispersed throughout the cell in a manner resembling *D. melanogaster*. Third, the nuclear bodies were either small and very dense (Figs. 8 and 9), frequently with two fused together as in *D. immigrans*, or they resembled the form found in *D. melanogaster* and also the early blastoderm stage of *D. immigrans* (compare Fig. 10 with Fig. 7). This latter type of nuclear body was found in three embryos (embryos 2, 3 and 5 in Table I); the remaining embryos showed dense bodies resem-
FIGURE 6 Distinctive nuclear bodies in *D. immigrans* embryos at the blastoderm (*a, b, c*) and gastrula stage (*d*). (*a*) × 35,000; (*b, c*) × 30,000; (*d*) × 60,000.

FIGURE 7 Pole cells of *D. immigrans* during an early stage of pole cell formation to show that at this time some nuclear bodies (*nb*) have a structure similar to that found in *D. melanogaster*. However, even in these nuclear bodies, there is some indication of the discontinuities characteristic of *D. immigrans* (insert: arrow). × 8,000. *Insert*: × 75,000.

blinking those found at early blastoderm stages of *D. immigrans*. A fourth difference was the observation that pole cells were still dividing at the end of blastoderm formation (Table I). We have never found a pole cell dividing at this time at the posterior tip of the *D. melanogaster* embryo, and in only one instance has an induced pole cell been found in division [in an embryo that received polar plasm from a stage 14 oocyte (Illmensee and Mahowald, unpublished observation)]. In *D. immigrans* embryos, however, pole cells at the posterior tip occasionally divide during the period of blastoderm formation. The failure of the polar granules derived from *D. immigrans* to undergo the species-specific structural changes when they were transplanted to
TABLE I
Summary of Ultrastructural Studies of the Anterior Tip of D. melanogaster Embryos Which Had Received Transplants of Posterior Polar Plasm from D. immigrans

| Embryo no. | Age at fixation (min) | No. of hybrid pole cells | Characteristics of polar granules | Nuclear bodies | Nuage |
|------------|----------------------|--------------------------|----------------------------------|----------------|-------|
| 1          | 150                  | At least 10; one dividing | Large, individual granules, dispersed in cell; fibrous | Solid, fused, similar to D. immigrans | Absent |
| 2          | 170                  | Six-seven                | Large, individual granules, dispersed in cell; fibrous | Solid, fused, similar to D. immigrans; one similar to D. melanogaster | Absent |
| 3          | 170                  | 8-10; one dividing       | Large, individual granules, dispersed in cell; fibrous | Solid as in D. immigrans; one similar to D. melanogaster | Absent |
| 4          | 190                  | Six-seven; one dividing  | Large, individual granules, dispersed in cell; fibrous | Solid and very dense as in D. immigrans | Absent |
| 5          | 190                  | 15                       | Large, individual granules, dispersed in cell; fibrous | Many hollow nuclear bodies similar to D. melanogaster; some fused. | Absent |
| 6          | 190                  | Two                      | Large, individual granules, dispersed in cell; fibrous | Only one found: solid and very dense as in D. immigrans | Absent |
| 7          | 210                  | Three                    | Many per cell; adjacent to nucleus; crystalline | All like D. immigrans | Only a few present |
| 8          | 240                  | 10                       | Many per cell; adjacent to nucleus; crystalline | All like D. immigrans | Many present |
| 9          | 240                  | 11; one dividing         | One or small number per cell; crystalline | All except two are clearly D. immigrans; two similar to D. melanogaster | Many present |
| 10         | 240                  | Six                      | Small number per cell; adjacent to nucleus; crystalline | All like D. immigrans | Very abundant |

D. melanogaster embryos was unexpected. A possible cause for our failure to detect these changes might be a difference in developmental time between the embryos of these two species. Embryos of D. immigrans at 25°C require almost 4 h to reach gastrulation compared to 3.5 h for D. melanogaster. The structural change in polar granules of D. immigrans occurs during blastoderm formation (3-3.75 h), and gastrulation in D. immigrans occurs at 4 h. Consequently, four additional embryos, which had received anterior transplants of D. immigrans polar plasm, were examined 30-60 min after gastrulation had begun (Table I, embryos 7-10). Between 3 and 11 pole cells were found, and in each embryo the polar granules were clearly "crystalline" (Figs. 11 and 13). In three of the embryos there were many polar granules in each cell, which indicates that the granules had not reaggregated in a manner typical of D. immigrans, thus conforming to the observations made of embryos 1 to 6. However, in embryo 9 the granules in some cells had reaggregated to form one large granule (Fig. 13) while in the other hybrid pole cells of this embryo there were one or two large granules and a few smaller ones.

The pole cell-specific nuclear bodies from these older embryos were clearly of the D. immigrans type (Fig. 12). There is also some indication that these structures follow a timetable for structural changes which corresponds to that of the cytoplasmic polar granules. The nuclear bodies found during gastrulation (embryos 7-10, Table I) are similar to those found in normal D. immigrans embryos during blastoderm formation (compare Fig. 12 to Fig. 10a-c). Nuclear bodies of the type characteristic of gastrulation (Fig. 6d) were not found in any of the hybrid cells, possibly because hybrid cells were not examined at a sufficiently late developmental stage.

The final difference we discovered in the induced hybrid pole cells was in the premature transformation of the polar granules to form fibrous bodies or "nuage" (7, 20). Normally, polar granules undergo this transition after the pole cells have been enclosed in the posterior midgut rudiment in both species. However, in the embryos examined during late gastrulation stages (embryos 7-10, Table I), fibrous bodies were present along the nuclear envelope. We also were able to find transition stages between the structure of the polar granule and these fibrous bodies (Fig. 11), which indicates that these structures probably originate from polar granules. This confirms earlier observations made on normal D. immigrans pole cells (20).

Test for the Function of Hybrid Pole Cells

Previously, we have found that pole cells induced at the anterior and the ventral surface of
FIGURE 8 Survey electron micrograph of embryo 3 showing some of the morphology of the induced pole cells at the injection site (arrow). Cleavage furrows (f) have invaginated from the surface and are near the base of the blastoderm nuclei (N). A region of membrane whorls and extracellular cytoplasmic debris (D) mark the region where the injection was made. Approximately 10 pole cells (PC) could be identified as a cluster of cells below the blastoderm nuclei. Polar granules (P) are large and compact and are composed of a dense interwoven mat of fibers. One pole cell is in division (arrow). Small dense nuclear bodies (nb) can be seen in two pole cells. \( \times 3,500 \).

FIGURE 9 Low magnification of embryo 6 in which one of the two pole cells (PC) is found between the vitelline membrane (v) and the blastoderm cells (BC). The polar granules (P) are large and scattered in the cytoplasm. A small dense nuclear body (nb) is present at this stage in pole cells. \( \times 16,000 \).
Drosophila embryos were able to function as germ cells if they were transplanted to the pole cell region of a blastoderm embryo (11-13). The use of this test for the function of induced pole cells is especially essential in the case of hybrid cells because of the many unusual interactions between the D. immigrans polar plasm and the D. melanogaster nuclei and cytoplasm. Thus, before we can conclude that polar plasm can function as germ cell determinants in interspecific combination, it is necessary to show that these cells can produce gametes. In conjunction with this test, a further observation is possible. With the availability of nuclear-cytoplasmic hybrid germ cells (provided these cells function as germ cells), we can test for cytoplasmic inheritance of polar granules by analyzing ultrastructurally the eggs and embryos originating from these cells for the morphology of their polar granules. Since this analysis requires electron microscopy, it is essential to obtain flies which produce eggs derived only from a hybrid pole cell.

Hence, we used an UV dose of 7,200 ergs/mm² which produced 95% sterility. Pole cells did not form in most embryos after this irradiation (Fig. 1). The survival to adulthood was approximately 40%. This is probably the upper limit of usable UV dosages since a dose that produced 98% sterility gave only 14% survival (25).

Since UV irradiation of the posterior tip of early cleavage stages clearly affects other developmental processes besides germ cell formation, it is important to show that the posterior tip of irradiated embryos is suitable for the integration of transplanted pole cells. Thus, pole cells from mwh e¹ embryos at the blastoderm stage were transplanted to irradiated y sn² mal hosts at the same stage. The composition of the progeny derived from these 10 flies indicated that three types of germ lines were present: two flies produced gametes only from the host (y sn² mal); seven produced gametes from the transplanted cells only (mwh e¹); and one produced gametes from both
Figure 11. Polar granule (P) in an induced pole cell from embryo 8 at the mid-gastrula stage. The fibrous body or nuage (arrow) is adjacent to the pole cell nucleus (N) and retains some of the substructure of the polar granule. × 38,000.

Figure 12. Nuclear bodies in pole cells induced with *D. immigrans* polar plasm. All of the nuclear bodies clearly resemble those found in pole cells of *D. immigrans* (cf. Fig. 6). (a) × 55,000; (b, c) × 50,000; (d) × 35,000.

Figure 13. Large polar granule from embryo 9. In this cell nearly all of the polar granules were aggregated into this large structure. In the other hybrid pole cells of this embryo, similar large granules were found. × 38,000.
host and transplanted cells. Thus, these control transplantations show that it is possible to reconstitute the germ line of UV-sterilized embryos by implanted pole cells. Consequently, this method should be suitable for testing the function of induced hybrid pole cells and for assaying cytoplasmic vs. nuclear inheritance of polar granules as well.

The results of transfers of anterior cells are summarized in Table II. From the 388 y sn3 mal embryos which received anterior cells, 152 mature flies developed, of which 16 were fertile in test matings with mwh e4 partners. 10 flies produced offspring which were wild in phenotype, which indicates that their germ lines were populated with host germ cells. The wild phenotype results from the heterozygous situation of the recessive genes located on both the X (y sn3 mal) and third (mwh e4) chromosomes. The progeny of six flies, however, showed homozygous mwh e4 offspring (Table III). One of these flies, a male, produced progeny that were both wild in phenotype (derived from gametes produced by surviving host germ cells) and homozygous for mwh e4. The remaining five flies produced only mwh e4 offspring, indicating that their germ lines had been exclusively repopulated from the transplanted anterior cells.

In addition to progeny tests for transferred cells, histochemical analyses were made on all flies with offspring from hybrid pole cells as well as on eight of the 10 flies with progeny from their own germ line only and on 89 of the 136 sterile flies. Cells with the mwh e4 genotype contain the aldehyde oxidase activity (6, 14). Two of the three males (Table III) showed weak staining for this enzyme in one of the testes. Since reactions with control mwh e4 males are also weak, this result is not unexpected. All three females (Table III) showed clear staining in only one ovary whereas the other ovary had neither staining nor germ cells (Fig. 16a). The enzyme tests of both the sterile flies and the flies with progeny derived from host germ cells only, showed no staining except for one sterile female which had two stained ovarioles and three unstained ones in one ovary and no ovarioles in the other. Although this fly was mated to several males over a 2-wk period, no offspring were obtained.

Three flies (the germ line mosaic male no. 2 [Table III], one of the sterile flies, and one of the fertile flies with host germ cells only) showed a cluster of stained cells in the midgut (Fig. 16b), and one sterile fly had stained cells in the midgut. Since more than one cell was implanted in each instance, we do not know whether these cells originated from anterior blastoderm cells or from hybrid pole cells. This latter possibility has been found previously with an implanted normal pole cell (13).

Since induced pole cells cannot be unambiguously distinguished at the time of transfer from other anterior cells, it is necessary to show that anterior cells are not able to participate in germ

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**Table II**

| Injection of polar cytoplasm | mwh e4 embryos from which cells were taken | y sn3 mal embryos* into which cells were implanted | Total | Fertile | Host origin only | Mosaic origin only | Total |
|-----------------------------|------------------------------------------|-----------------------------------------------|-------|---------|-----------------|-----------------|-------|
| Control transfers           |                                         |                                               |       |         |                 |                 |       |
| 1‡                          | 85                                      | 150                                           | 56    | 10      | 2               | 1               | 7     | 14.3  |
| 2§                          | 42                                      | 103                                           | 49    | 4       |                 |                 |       |       |

* The posterior pole of these host embryos had been treated with UV radiation during early cleavage in order to prevent pole cell formation.
‡ Pole cells from the posterior region of normal blastoderm embryos.
§ Anterior blastoderm cells from noninjected embryos.
TABLE III
Progeny of y sn3 mal Flies* Mated to mwh e4 Partners

| Sex of mosaic fly | Wild type | mwh e4 |
|------------------|-----------|--------|
|                  | v         | d      | v     | d     |
| ♂                | 239       | 238    | 36    | 89    |
| ♀                | 73        | 91     | 184   | 156   |
| ♀♂               | 52        | 11     | 177   | 203   |

* From Table II.

† After giving rise to a small number of progeny, this female was sacrificed for histochemical analysis (Fig. 24 a).

cell formation after being transferred to the posterior tip, even after extensive UV irradiation of the host. Consequently, anterior cells were transferred to the posterior tip of 103 UV-irradiated embryos (Table II, control 2). No gametes were formed from the transplanted cells. However, somatic anterior cuticular structures differentiated autonomously in the abdomen of four of the adults, producing one head, one antenna, and two mouth structures. These results are comparable to those obtained earlier in similar anterior blastoderm cell transfers (11, 13). Thus, we can conclude that the germ cells of the six flies in Table III were derived from the hybrid pole cells. This result clearly demonstrates that the polar plasm of D. immigrans is able to induce the formation of functional germ cells in D. melanogaster embryos.

Ultrastructure of Pole Cells of F1 Generation

A possible continuity of germ plasm granules in the germ cells has been raised on purely morphological grounds in dipterans (20, 22, 23), and, recently, in amphibians (10, 15, 24). A critical feature of the evidence is the observation that polar granules can be shown to become transformed into perinuclear fibrous bodies or “nuage” (10, 20) (Fig. 11). Nuage can be found in the germ line of both dipterans, amphibians, and possibly mammals (7) throughout most of the life cycle of the organism. Consequently, these studies suggest that polar granules or their derivatives are always present in the germ line of these organisms. Because of this continuity, cytoplasmic inheritance appears reasonable. The availability of a hybrid pole cell containing the nuclear genome of one species and the cytoplasmic granules of a different species provides the unique opportunity to test this possibility. The polar granules present in the eggs derived from hybrid pole cells should be of either the D. immigrans or D. melanogaster type. In order to ascertain that an egg originated from the hybrid cell lineage, we first determined by progeny tests that all eggs produced by the fly were derived from hybrid cells. Then, we obtained embryos that were prepared for electron microscopy. Finally, histochemical tests were performed on the ovaries in order to determine whether any host oocytes were present.

Fortunately, three of the flies whose germ lines were repopulated by descendants of the hybrid pole cells were females. This has made possible an ultrastructural analysis of the F1 generation since all of the eggs produced by these flies are derived from the hybrid pole cells. Six embryos were analyzed at the late blastoderm or mid-gastrula stage, and in every instance the morphology of the polar granules and nuclear bodies was unmistakably typical of D. melanogaster. The polar granules (Fig. 14) reaggregated into a number of polar granules scattered around the cell with the characteristic hollow spherical structure. Two embryos were examined at mid-gastrula (the stage comparable to the time the transplanted polar granules of D. immigrans acquired their “crystalline” substructure) and both the granules and the nuclear bodies possessed the structure characteristic of D. melanogaster (Figs. 14 and 15).

DISCUSSION

In this study, we have been able to show conclusively that the posterior polar plasm of D. immigrans embryos is able to function as a germ cell determinant in D. melanogaster embryos. After transplantation of polar plasm to the anterior tip of D. melanogaster embryos, we found cells in all 10 embryos examined that were pole cells by ultrastructural characteristics i.e., both polar granules and the specific nuclear body were present. We further showed that induced hybrid pole cells were capable of functioning as germ cells and gave rise to offspring. Whatever the nature of germ cell determinants is, these experiments on interspecific transplantation indicate that these components are conservative in evolution since D. melanogaster and D. immigrans are from different portions of the genus (26). A similar conclusion is suggested by preliminary results of Okada et al. (5) in which...
the percentage of flies containing gametes after the UV-irradiation of *D. melanogaster* embryos was increased by the transplantation of polar plasm from *D. immigrans* or *D. hydei*.

Except for chloroplasts (9), mitochondria (27), some cytoplasmic symbiotic microorganism (32), viruses (1), and cortical inheritance in ciliates (4), there has been little conclusive evidence that any other cytoplasmic organelle displays inheritance independently of the nucleus. Centrioles and basal bodies may be candidates, but there is no conclusive evidence yet that they have independent inheritance (8). Since they may possess some nucleic acid (R. Dippell of this institution, personal communication), this possibility has not yet been excluded. Polar or germ plasm granules are another candidate because they are present in the germ line throughout the life of the organism (20, 23), and at least at one stage they contain RNA (21). A critical test of cytoplasmic inheritance requires distinctive features that can be followed during progeny analyses and the opportunity to observe whether or not the cytoplasmic organelle retains its features independently of the nuclear genome. The availability of easily distinguishable morphological features of both polar granules and the pole-cell specific nuclear body and the creation of the hybrid pole cell containing polar granules only of *D. immigrans* with a *D. melanogaster* nucleus are the necessary prerequisites for such an analysis. Inasmuch as the progeny analysis requires electron microscope observations, it was also essential to obtain female flies, all of whose gametes were derived from such a hybrid cell. Fortunately, three of the flies that contained gonads populated solely by cells derived from a hybrid pole cell were females. Our analysis showed that the polar granules of the embryos derived from these three flies were of the *D. melanogaster* type. Consequently, we can conclude that the structural features of polar granules are dependent upon the nuclear genome.

In addition to these primary observations, these experiments provide the opportunity to discover a
FIGURE 16 Histochemical detection of implanted mwh e4 cells after integration into the germ line (a) or soma (b) of y snb mal hosts. In both instances, three cells from the anterior region of two different D. melanogaster blastoderm embryos, into which D. immigrans polar plasm had been injected during cleavage, were transplanted to the posterior pole of D. melanogaster blastoderm embryos which had been UV-irradiated during cleavage. (a). Reproductive tract of adult female no. 3 (from Table III). This female was derived from a UV-sterilized embryo whose germ line had been reconstituted with pole cell(s) experimentally induced with D. immigrans polar plasm. One of the two ovaries contains eight ovarioles with stained oocytes (o) and nurse cells (nc), all of which originated from the "hybrid" pole cell(s). The unstained follicle cells (fc) are of host origin. The other ovary (arrow) lacks any germ cells as a result of UV irradiation. × 45. (b). Middle midgut of adult male no. 2 (from Table III). A cluster of approximately 20 cells is integrated into the midgut near a characteristic enlargement (arrow). × 30.

number of new features of the distinctive organelles of the germ plasm, the polar granules. In the hybrid pole cells, a complex interrelationship of species characteristics was found. A number of these deserve special consideration. Normally, the substructure of polar granules in D. immigrans becomes transformed from the interwoven fibrous mat to a regular array of rods at the same time that the granules are aggregating into a single large granule per cell. In the hybrid cells containing D. immigrans polar granules and a D. melanogaster nucleus, the granules in nine out of the 10 embryos analyzed aggregate into a number of polar granules per cell, and these granules initially retain their fibrous substructure. Only during gastrulation does the granule acquire the rod-substructure characteristic of D. immigrans, and at that time there is no evidence of further fusion. Thus, the connection between aggregation and the transformation to rods, which occurs in the normal pole cell, is disrupted. The simplest explanation for this unusual interaction may be found in the differences in developmental time between D. melanogaster and D. immigrans. If the structural transition of the D. immigrans polar granule occurs according to an autonomous timing within the granule itself, then this transition in the hybrid cell would occur during gastrulation because of the more rapid development of D. melanogaster relative to that of D. immigrans. This explanation is supported by observations of Schwalm (28), who has described similar structural changes in the polar granules of Coelopa frigida at gastrulation, and he has found that these changes occur in

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unfertilized eggs of Coelopa at the exact time they would have occurred after fertilization. On the other hand, the reaggregation of polar granules appears to depend upon the age of the host embryo; that is, the pattern of reaggregation is established during pregastrula stages. In one exceptional embryo, the reaggregation pattern was that of D. immigrans. A possible explanation for this may lie in the potential variation in age of hosts and donors for transplantation. Since these embryos may vary by ±10 min, it is possible that D. immigrans polar plasm was taken from a slightly older embryo than usual and transplanted to a host that was younger than usual. The result would be that the transition to the rod-substructure would occur at the time of reaggregation of polar granules during blastoderm formation. If this explanation is correct, it suggests that the reaggregation of D. immigrans polar granules into one large granule requires the transition in substructure. This transition, in turn, requires a set amount of time, possibly for the molecular rearrangements requisite for this transition.

The occurrence of mitotic hybrid pole cells is unusual. In Drosophila, pole cells divide two to three times after their formation, and then there is little mitotic activity until the pole cells have been included in the embryonic gonad (29). Counce (5) has detected some mitosis in pole cells of D. americana, D. repleta, D. hydei, and D. willistoni embryos between the blastoderm and gonadal stages. However, during gastrulation, pole cell mitoses are rare in most species and possibly nonexistent in D. melanogaster. Nevertheless, in the embryos receiving polar plasm transplants from D. immigrans, a pole cell was found in division in two of the three pregastrula embryos and in two of the seven postgastrula embryos examined. Thus, it appears that the process that restricts mitosis in pole cells is dependent upon the cytoplasm, and the active components are not as effective in interspecific interactions as in intraspecific ones. Since at least some hybrid pole cells are functional, this faulty nucleo-cytoplasmic interaction may not interfere with germ cell formation.

Finally, the morphology of the pole cell-specific nuclear body in the hybrid pole cells resembled that found in pole cells of D. immigrans even though they formed in the D. melanogaster nuclei of the host embryo. If we assume that the morphology of this organelle is a property of the molecules constituting it, then the evidence suggests that the nuclear body derives from components contributed by the transplanted cytoplasm. At present, we do not know which component(s) of the polar plasm might migrate into the nucleus. It might be some unknown cytoplasmic constituent. Or, possibly, a component of the granules, which must be lost for reaggregation and reorganization to occur, migrates into the nucleus to form the nuclear body. Alternatively, if the polar granule contains maternal mRNA (21), then it is possible that the proteins synthesized from polar granular RNA become located in the nucleus. Whatever the actual source of the protein components of the nuclear body is, the appearance of the body clearly depends upon the polar plasm. Although a function for this nuclear body is unknown, it may be related to determination of these cells as germ cells since the nuclear body is only found in pole cells and is dependent upon the polar plasm for its formation.

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REFERENCES

1. Akai, H., E. Gateff, L. E. Davis, and H. A. Schneiderman. 1967. Virus-like particles in normal and tumorous tissues of Drosophila. Science (Wash. D. C.). 157:810-813.

2. Ashton, F. T., and J. Schultz. 1964. Stereoscopic analysis of the fine structure of chromosomes in diploid Drosophila nuclei. J. Cell Biol. 23:7a (abstr.).

3. Beams, H. W., and R. G. Kessel. 1975. The problem of germ cell determinants. Int. Rev. Cytol. 49:413-479.

4. Beisson, J., and T. M. Sonneborn. 1965. Cytoplasmic inheritance of the organization of the cell cortex in Paramecium aurelia. Proc. Natl. Acad. Sci. U. S. A. 53:275-282.

5. Counge, S. J. 1963. Developmental morphology of polar granules in Drosophila including observations on pole cell behavior and distribution during embryogenesis. J. Morphol. 112:129-145.

6. Dickinson, W. J. 1971. Alddehyde oxidase in Drosophila melanogaster: a system for genetic studies on developmental regulation. Dev. Biol. 26:77-86.
7. Eddy, E. M. 1975. Germ plasm and the differentiation of the germ cell line. *Int. Rev. Cytol.* 43:229-280.
8. Fulton, C. 1971. Centrioles. In *Origin and Continuity of Cell Organelles*. J. Reinitert and H. Ursprung, editors. Springer-Verlag New York, Inc., New York. 170-221.
9. Giber, A., and S. Granick. 1964. Plastids and mitochondria: inheritable systems. *Science* (Wash. D. C.). 148:890-897.
10. Ikenishi, K., and M. Koyami. 1975. Ultrastructure of the "germinal plasm" in *Xenopus* embryos after cleavage. *Dev. Growth Differ.* 17:101-110.
11. Illmensee, K., and A. P. Mahowald. 1974. Transplantation of posterior polar plasm in *Drosophila*. Induction of germ cells at the anterior pole of the egg. *Proc. Natl. Acad. Sci. U. S. A.* 71:1016-1020.
12. Illmensee, K., and A. P. Mahowald. 1976. The autonomous function of germ plasm in a somatic region of the *Drosophila* egg. *Exp. Cell Res.* 97:127-140.
13. Illmensee, K., A. P. Mahowald, and M. R. Loos. 1976. The ontogeny of germ plasm during oogenesis in *Drosophila*. *Dev. Biol.* 49:40-65.
14. Janning, W. 1973. Entwicklungsgenetische Untersuchungen an Gynandern von *Drosophila melanogaster*. I. Die inneren Organe der Imago. *Wilhelm Roux' Arch. Entwicklungsmech. Org.* 174:313-332.
15. Kalt, M. R. 1973. Ultrastructural observations on the germ line of *Xenopus laevis*. *Z. Zellforsch. Mikrosk. Anat.* 138:41-62.
16. Kalt, M. R., and B. Tandler. 1971. A study of fixation of early Amphibian embryos for electron microscopy. *J. Ultrastruct. Res.* 36:633-645.
17. Lindsley, D. L., and E. H. Grell. 1968. Genetic Variations of *Drosophila melanogaster*. Carnegie Institute Publication No. 627. Washington, D. C.
18. Mahowald, A. P. 1962. Fine structure of pole cells and polar granules in *Drosophila melanogaster*. *J. Exp. Zool.* 151:201-215.
19. Mahowald, A. P. 1968. Polar Granules of *Drosophila*. II. Ultrastructural changes during early embryogenesis. *J. Exp. Zool.* 167:237-262.
20. Mahowald, A. P. 1971. Polar granules of *Drosophila*. III. The continuity of polar granules during the life cycle of *Drosophila*. *J. Exp. Zool.* 176:329-344.
21. Mahowald, A. P. 1971. Polar granules of *Drosophila*. IV. Loss of RNA from polar granules during early stages of embryogenesis. *J. Exp. Zool.* 176:345-352.
22. Mahowald, A. P. 1971. Origin and Continuity of Polar Granules. In *Origin and Continuity of Cell Organelles*. J. Reinitert and H. Ursprung, editors. Springer-Verlag New York, Inc., New York. 158-169.
23. Mahowald, A. P. 1975. Ultrastructural changes in the germ plasm during the life cycle of *Miastor* (Cecidomyiidae, Diptera). *Wilhelm Roux' Arch. Entwicklungsmech. Org.* 176:223-240.
24. Mahowald, A. P., and S. Hennen. 1971. Ultrastructure of the "germ plasm" in eggs and embryos of *Rana pipiens*. *Dev. Biol.* 24:37-53.
25. Okada, M., I. A. Kleimman, and H. A. Schneiderman. 1974. Restoration of fertility in sterilized *Drosophila* eggs by transplantation of polar cytoplasm. *Dev. Biol.* 37:43-54.
26. Patterson, J. T., and W. L. Stone. 1952. Evolution in the Genus *Drosophila*. The Macmillan Co., New York.
27. Roodyn, D. B., and D. Wilkie. 1968. The Biogenesis of Mitochondria. Methuen and Co. Ltd., London.
28. Schwalm, F. E. 1974. Autonomous structural changes in polar granules of unfertilized eggs of *Coelopa frigida* (Diptera). *Wilhelm Roux' Arch. Entwicklungsmech. Org.* 175:129-133.
29. Sonnenblick, B. P. (1950). The early embryology of *Drosophila melanogaster*. In *Biology of Drosophila*. M. Demerec, editor. John Wiley and Sons, New York. 62-167.
30. Turner, F. R., and A. P. Mahowald. 1976. Scanning electron microscopy of *Drosophila* embryogenesis. I. The structure of the egg envelopes and the formation of the cellular blastoderm. *Dev. Biol.* 50:95-108.
31. Warn, R. 1975. Restoration of the capacity to form pole cells in UV-irradiated *Drosophila* embryos. *J. Embryol. Exp. Morphol.* 33:1003-1011.
32. Wolstenholme, D. R. 1965. A DNA and RNA-containing cytoplasmic body in *Drosophila melanogaster* and its relation to flies. *Genetics.* 52:949-975.
33. Zalokar, M. 1976. Autoradiographic study of protein and RNA formation during early development of *Drosophila* eggs. *Dev. Biol.* 49:425-437.