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Transplantation of Posterior Polar Plasm in *Drosophila*. Induction of Germ Cells at the Anterior Pole of the Egg

(cytoplasmic determinants/transplantation of cytoplasm/germ-line mosaics/insect embryogenesis)

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ABSTRACT **In *Drosophila melanogaster* the primordial germ cells are normally formed at the posterior tip of the egg during the preblastoderm stage. In order to determine whether the posterior polar plasm is capable of inducing the formation of primordial germ cells in another region of the embryo, portions of this cytoplasm were transferred from wild-type embryos of the early cleavage stage to the anterior tip of *mwh e* embryos of the same age. At various times after the injection (15–200 min), embryos were fixed for histological analysis. Alternating thick and thin sections were examined for the presence of experimentally induced pole cells. In more than half of the embryos analyzed in this way, one to six cells were found containing the polar granules as well as round nuclear structures, both of which are characteristic of normal pole cells and are not present in blastoderm cells. In order to determine whether these "pole cells" function normally, i.e., develop further into germ cells, the cells induced at the anterior tip of *mwh e* blastoderm embryos were introduced into the posterior region of *y w sn*^{3} hosts of the same age. The flies resulting from these embryos were mated to *y w sn*^{3} partners. In addition to the expected *y w sn*^{3} progeny, wild-type flies heterozygous for *mwh e* and, therefore, descended from the experimentally induced pole cells were found in 4% of the crosses. Such flies did not appear in the control experiments after transfer of normal anterior cells from noninjected blastoderm embryos.**

These results demonstrate that the posterior polar plasm can be transferred to the anterior tip of the embryo and that in this presumptive somatic region it still retains its capacity to determine the formation of the primordial germ cells.

Differential segregation of ooplasmic factors is involved in the formation of special cell types during early development (1). The clearest examples of such segregation of cytoplasmic determinants are found in the formation of germ cells in *Ascaris* (2), some insects (3), and amphibians (4, 5). In these instances RNA-containing cytoplasmic granules are segregated into a small number of cells that have been shown to become the primordial germ cells. By utilizing procedures that remove this specific region of the egg (6, 7), destroy it with ultraviolet light (8–12), or displace it by centrifugation (13–15), many workers have been able to verify the determinative role of a specific region of the egg for forming germ cells. Furthermore, Smith (16) has been able to restore the capacity of vegetal cytoplasm of frog eggs to produce germ cells by injecting "germinal plasm" into eggs that had been sterilized by UV-irradiation.

In *Drosophila melanogaster*, the germinal plasm is found at the posterior tip of the mature egg, and during the preblastoderm stage of embryogenesis, it is incorporated into a small number of cells, the pole cells (3). Subsequently, some of these cells differentiate into primordial germ cells within the embryonic gonad, while others probably participate in forming the midgut (10). Many workers (e.g., 8, 10–12) have shown that UV-irradiation or removal of the posterior polar plasm of eggs before the presence of nuclei in this region results in embryos devoid of primordial germ cells and, consequently, in flies that are sterile. Direct confirmation of the embryological evidence that pole cells become germ cells has been achieved by transplanting genetically marked pole cells to a differently marked host embryo and obtaining offspring from the transplanted cells (17). Similarly, a direct test of the determinative quality of the posterior polar plasm is possible by transferring this particular kind of cytoplasm to the anterior pole of the egg where normally only somatic cells form (18), and then analyzing these embryos for the formation of primordial germ cells in this new region. A preliminary report of this work has been published (19).

MATERIALS AND METHODS

Drosophila melanogaster Strains. Three different genetic stocks were used in order to determine the origin of the cells derived from cytoplasmic transplantations. (*1*) Oregon R wild type as donors for posterior polar plasm; (*2*) *multiple wing hairs* (*mwh*), *ebony* (*e*) (third chromosome) as recipients for cytoplasmic injections; (*3*) *yellow* (*y*), *white* (*w*), *singed* (*sn*^{3}) (X-chromosome) as hosts for cell transfers. (For description of mutants, see ref. 20.)

Transplantations. The procedures for collecting and preparing eggs and for transplanting cytoplasm and cells are basically the same as used previously (17, 21). The cytoplasmic transfers were carried out approximately 30 min after egg deposition. At this time the donors and recipients contained about four nuclei located in the central region of the egg. In order to obtain peripheral cytoplasm from the posterior pole of donor eggs, a siliconized glass capillary of 3-μm inside diameter was introduced into the antero-ventral region of the egg and then moved through the interior to the posterior pole until the tip of the micropipette touched the vitelline membrane. A volume of about 5 pl (equivalent to the content of approximately 10 pole cells) of posterior polar plasm from a wild-type embryo was drawn into the capillary and subsequently injected peripherally into the anterior region of a *mwh e* recipient egg adjacent to the micropyle.

After blastogenesis (2–2.5 hr after the cytoplasmic transfer), when cells had been formed in embryos with implanted

cytoplasm, some of these recipients were used for anterior cell transfers. Only those *mwh e* embryos in which round cells could be seen in the injection region and which had received posterior polar plasm from fertilized eggs were used for cell transfers. Whether or not the cytoplasmic donors had been fertilized could be determined 1.5 hr after the transplantation when the cytoplasmic donor embryos reached the preblastoderm stage. A siliconized glass capillary of 12-μm inside diameter was introduced into the embryo through the injection wound resulting from the former cytoplasmic implantation. Between one and five cells were drawn into the pipette from the anterior region and then implanted into the middle posterior region of a blastoderm host among its pole cells. This kind of transfer was repeated between one and five times, with the same *mwh e* embryo but different *y w sn*^{3} hosts for each transfer (Fig. 1). On the average, a total of about 11 cells was taken out of each *mwh e* embryo and distributed to an average of four hosts. In a series of control experiments, cells were taken from the anterior pole of normal, noninjected *mwh e* blastoderm embryos and were implanted into the posterior pole region of *y w sn*^{3} blastoderm embryos in the same way as for the experimental embryos.

Flies developing from the *y w sn*^{3} host embryos with implanted cells were mated to *y w sn*^{3} partners, and their progeny were scored phenotypically. Adult descendants with wild phenotype were backcrossed to *mwh e* partners in order to determine their genotype.

Histology. At various times after cytoplasmic injections (15–200 min), eggs were fixed in 2% glutaraldehyde–1% acrolein in 0.1 M cacodylate buffer at pH 7.2 supplemented with 1 mM $CaCl_2$ and 2.5% dimethylsulfoxide (modified from refs. 22 and 23) for 1 hr at 4°. They were washed in 0.1 M cacodylate buffer containing 0.2 M sucrose overnight at 4°, and then postfixed in 1% OsO_4 for 2 hr at 4°. After dehydration in ethanol the eggs were embedded in DER 732-332 plastic. Alternating sets of thick (0.5-μm) and thin (80-nm) sections were cut. The thick sections were stained with 0.5% Toluidine blue in 1% sodium borate; the thin sections were double-stained with uranyl acetate and lead citrate and examined with a Philips EM 300.

RESULTS

A brief description of the early embryology of *Drosophila melanogaster* is helpful for following these experiments (for details, see ref. 24). After the formation of the zygote nucleus, there are nine synchronous nuclear divisions without cytokinesis. After the seventh mitotic division (90 min after fertilization), the first nuclei reach the posterior polar plasm and, immediately, a small number of pole cells containing the polar granules separate from the rest of the embryo. Subsequently, these cells will divide asynchronously to produce an average of 55 cells at the blastoderm stage. Meanwhile, most of the remaining nuclei migrate to the surface and complete three more synchronous divisions, also without cytokinesis. At this time (150 min) nuclear divisions cease and cytoplasmic cleavage occurs, producing the cellular blastoderm. After this, gastrulation occurs.

Histological Studies. Posterior polar plasm from 30-min-old wild-type embryos was transplanted into the anterior region of *mwh e* embryos of the same age. The fate of this particular cytoplasm was analyzed histologically during the first developmental stages up to the early gastrula. Specific components of the implanted cytoplasm, the polar granules, are easily recognizable by their characteristic structure (Figs. 2, 3, and 5) and, thus, serve as a suitable morphological marker. These subcellular organelles are present only at the posterior pole of the egg (25). During the first 30 min after fertilization, the polar granules are located in a narrow band of cortical cytoplasm approximately 5-μm thick (Fig. 2). The material used for transplantation was limited to this peripheral layer of posterior polar plasm. The protein yolk spheres, which are located 10 μm away from the tip of the egg, were not observed in the transferred cytoplasm.

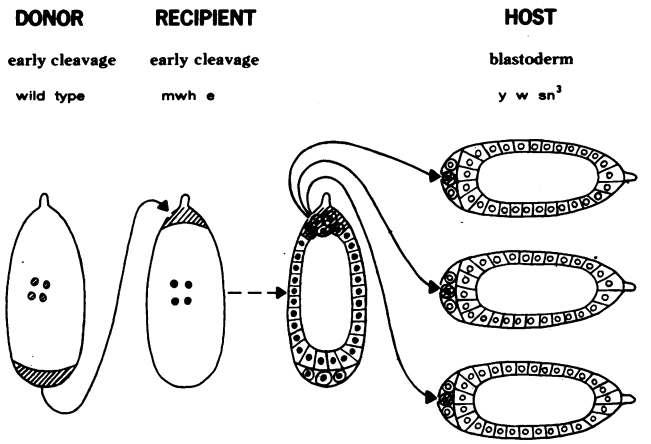
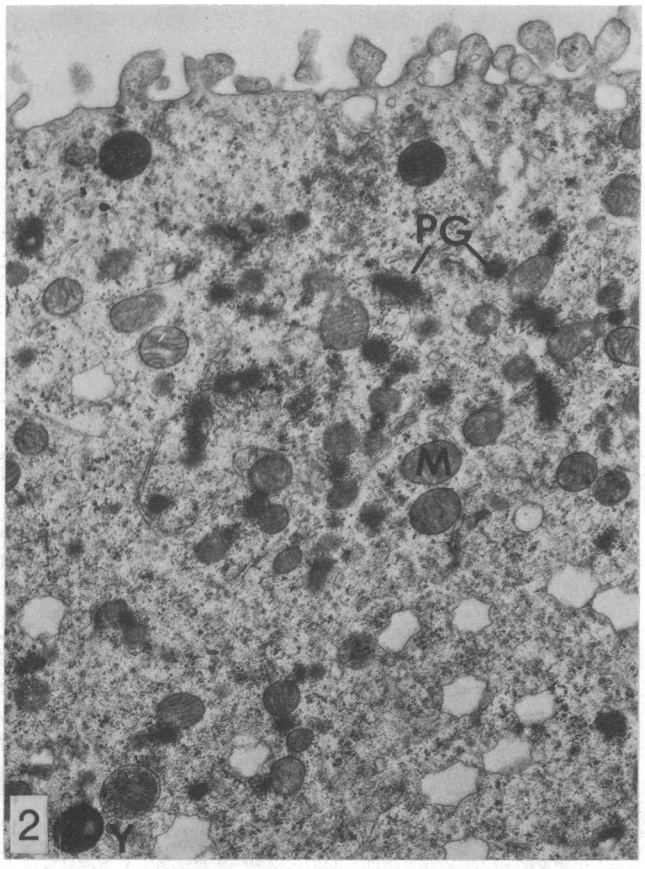
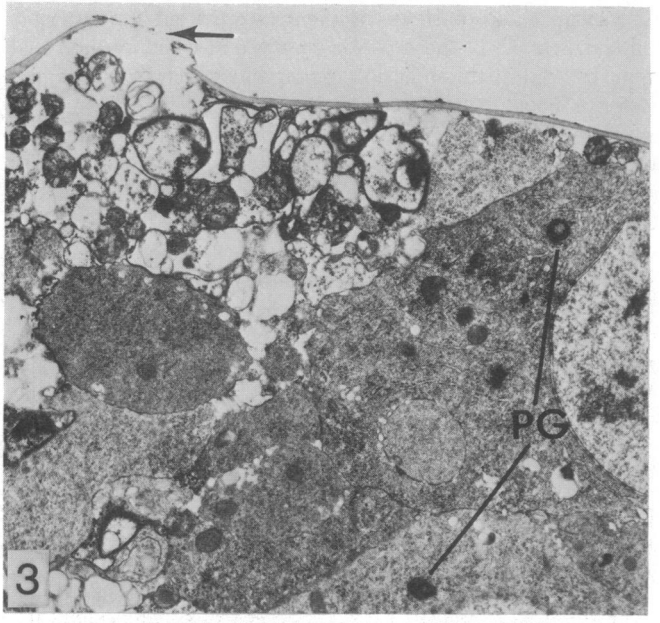


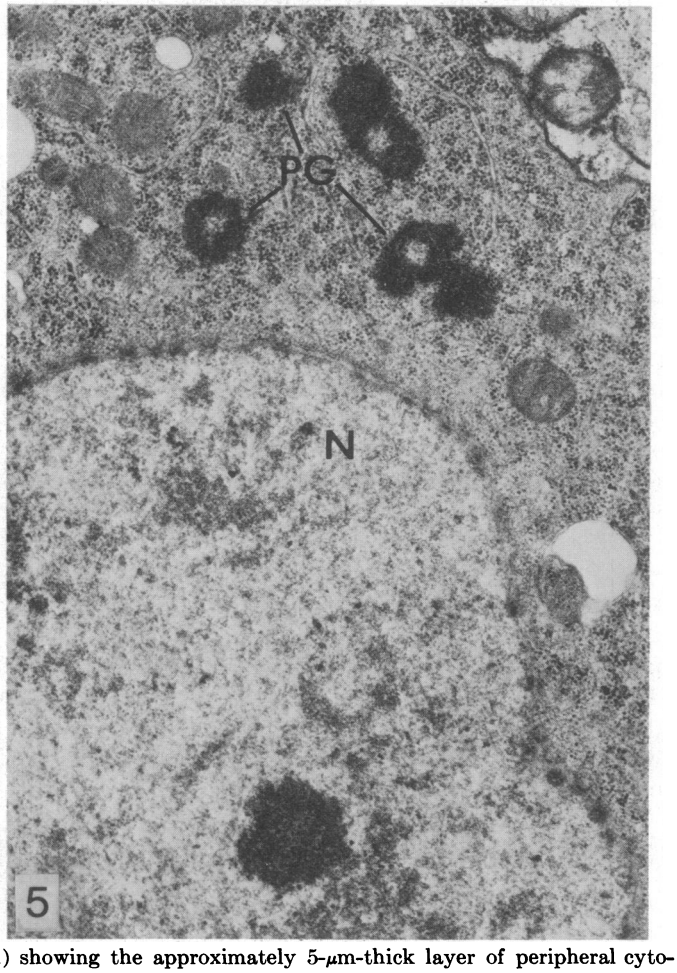
FIG. 1. Schematic illustration of the procedure for transplanting posterior polar plasm and anterior cells.

Seven embryos were studied histologically at the end of blastogenesis when fully formed cells are first present. In three of the cases, polar granules could not be recognized in the transplant area. In these instances a thin layer of dense cytoplasm was found adjacent to the vitelline membrane. This material is presumed to be portions of injected cytoplasm. In the other four cases, polar granules could clearly be identified in one to six cells, indicating that in these embryos some of the transplanted cytoplasm had been incorporated into cells. They were located at the periphery of the embryo, either directly underlying the vitelline membrane (Fig. 3) or with some remaining cytoplasmic material between the cell and the membrane of the egg (Fig. 4). Three additional embryos were analyzed at the early gastrula stage, and in two of these, cells containing polar granules were found between blastoderm cells or located more internally as a cluster of cells that bordered the yolk. In the third case there was no indication that the injected cytoplasm had been integrated into the embryo. The cells containing polar granules resembled the typical pole cells of the posterior tip, not only by the presence of these organelles, but also by their round shape and by the formation of dense, round nuclear structures (Fig. 4) that are characteristic of pole cells at this stage (Mahowald, unpublished observation). Between one and three of these structures could be observed in a single nucleus. Their biological function is unknown, but their appearance is a further confirmation at the morphological level that these anterior cells are "pole cells."

Transplantation Studies. The important question—whether these experimentally induced pole cells can produce functional germ cells—was tested by transplanting them to the posterior tip of *y w sn*^{3} host embryos of the same age. From 184 *mwh e* embryos that had received posterior polar plasm, 53 embryos







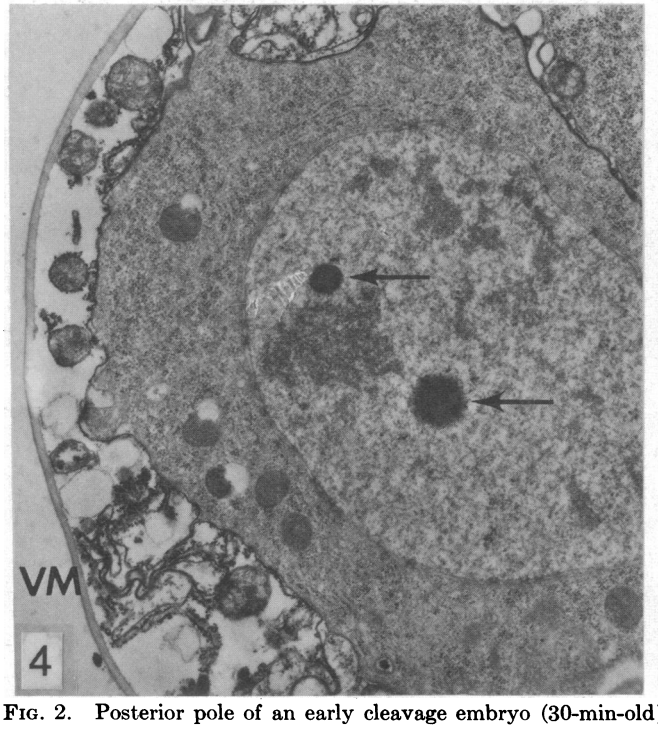


FIG. 2. Posterior pole of an early cleavage embryo (30-min-old) showing the approximately 5-μm-thick layer of peripheral cytoplasm that was collected for transplantation. The polar granules (*PG*) are located about 1 μm away from the periphery and concentrated in a 3-μm layer. Yolk (*Y*); mitochondrion (*M*). (×114,500).

FIG. 3. Anterior pole of a blastoderm embryo (170-min-old) into which posterior polar plasm has been injected during early cleavage. Two experimentally induced cells containing polar granules can be identified. Some transferred cytoplasm lies near the injection wound (*arrow*) and is not integrated into cells. (×8250).

FIG. 4. An induced "pole cell" with two round nuclear structures (*arrows*). Part of the injected cytoplasm remains between this cell and the vitelline membrane (*VM*). (×12,500).

FIG. 5. Cluster of polar granules located near the nucleus (*N*) of a "pole cell" induced at the anterior tip. (×24,000).

TABLE 1. *Transfer of anterior cells to the posterior pole of blastoderm embryos*

	mwh e embryos from which cells were taken	*y w sn*³ embryos into which cells were implanted	Flies developed		
			Total	Fertile	Germ-line mosaics
Experiment	53*	228	125	92	4
Control	61†	179	83	67	—

* Selected from a total of 184 cytoplasmic transfers. Cytoplasm was taken from the posterior pole of wild-type embryos at the early cleavage stage and was injected into the anterior pole of *mwh e* embryos at the same age. After blastogenesis, anterior cells were transferred from these *mwh e* embryos to the posterior region of *y w sn*³ blastoderm hosts. Flies developing from these embryos were mated to *y w sn*³ partners, and their offspring were analyzed with respect to their phenotype and genotype (Table 2).

† In the controls, anterior cells from noninjected, normal *mwh e* blastoderm embryos were implanted into the posterior pole of *y w sn*³ blastoderm hosts.

were chosen as cell donors because of the visible presence of round cells located peripherally to the blastoderm. Several transfers were made from each embryo to different hosts in order to ensure that any "pole cell" formed would be transferred (Fig. 1). Among the fertile flies derived from these host embryos, four cases (4%) were found to be germ-line mosaics (Tables 1 and 2). Flies with mosaic gonads produce gametes carrying either the *y w sn*³ or *mwh e* genotype. Consequently, after the cross to *y w sn*³ partners, some of the offspring are hetero- or hemizygous for *y w sn*³ and heterozygous for *mwh e*. In Table 2 the progeny of these four flies are recorded. Two of the flies produced phenotypically wild-type females and males. Fly no. 2, a male, lacked sons with wild phenotype due to the use of the sex-linked marker genes *y w sn*³. Female no. 4 had only males wild in phenotype. This probably resulted from the relatively low number of descendants arising from this female. With the exception of this latter case, the percentage of the progeny originating from the experimentally induced cells ranged from 12 to 18%, similar to the values obtained in earlier experiments with normal pole cells (17). In order to demonstrate that the phenotypically wild-type flies had derived from *mwh e* cells containing transferred posterior polar plasm, these flies were mated to *mwh e* partners. In each instance, homozygous *mwh e* descendants were found after such a backcross.

Because of the close association between the experimentally induced pole cells and the anterior blastoderm cells, some of these latter cells were also transferred to the posterior pole. In order to determine whether such blastoderm cells might also become germ cells, anterior cells from noninjected *mwh e* embryos were transferred to the posterior pole of *y w sn*³ hosts. In these controls, germ-line mosaics did not appear (Table 1). However, imaginal structures, all of *mwh e* phenotype, were found in the body cavity of five out of the 83 *y w sn*³ control flies. In three cases these cuticular structures could be identified as labial derivatives, whereas the other two pieces of cuticle could not be recognized because of their small size. In two of the 125 experimental flies adult structures, identified as eye and wing, also occurred.

TABLE 2. *Progeny of the four germ-line mosaic flies mated to* y w sn³ *partners*

Germ-line mosaic flies		Phenotype of progeny				
		*y w sn*³		Wild type		
No.	Sex	♀	♂	♀	♂	%
1	♀	115	98	19	28	18.1
2	♂	298	365*	46	—	6.5†
3	♀	173	159	10	34	11.7
4	♀	62	39	—	2	1.9

* Thirty-seven of these males were mated to *mwh e* females; three of them gave progeny that included homozygous *mwh e* descendants. This result indicates that about 10% of the 365 *y w sn*³ males carry the *mwh e* genes from the transplanted anterior germ cells, and thus belong to the missing wild-type class for the germ-line mosaic male.

† Theoretical value should be approximately 13% because of the failure to detect male progeny. Sperm derived from the transplanted anterior germ cells would not be expected to produce wild-type male offspring in test matings with *y w sn*³ females because the Y-bearing sperm do not carry the wild-type alleles for *y w sn*³. However, those *y w sn*³ sons, which are derived from the transplanted germ cell(s) and are therefore heterozygous for the third chromosomal genes *mwh e*, are present among the *y w sn*³ male progeny.

DISCUSSION

During the early embryonic period of *Drosophila melanogaster*, before the blastoderm formation, the posterior polar plasm of the egg becomes incorporated into several cells known as pole cells, some of which become primordial germ cells. Although several attempts have been made to prove the existence of germ-cell determinants in the polar plasm in an *indirect* way by removing or destroying them (e.g., 8, 10–12), we have *directly* shown their presence by transferring this particular kind of cytoplasm to a new region of the embryo. Not only is the posterior polar plasm able to induce the formation of morphologically recognizable "pole cells," but in four instances, at least, these cells could function as primordial germ cells. The anterior tip of the egg was chosen as the site for the cytoplasmic transfer because of its exclusively somatic fate (18) and its similar morphology to the posterior tip.

A sample of injected eggs that had reached the blastoderm or early gastrula stage was studied histologically in order to determine whether the transferred cytoplasm could be found at the anterior region. In six out of 10 of these embryos the implanted posterior cytoplasm could be recognized in several anterior cells by the presence of polar granules that are normally localized only in the posterior polar plasm of the egg (25). Since one to six "pole cells" were found at the anterior tip of these embryos, and a volume of cytoplasm equivalent to the content of 10 pole cells was injected, we estimate that, at maximum, one-half of the transferred polar plasm could be incorporated into "pole cells." We cannot determine by the morphological criterion used whether part of the implanted cytoplasm is included within other cells, or whether some of the experimentally induced cells contain anterior host cytoplasm, thus forming "hybrid" cells.

The presence of cells that contain transferred polar plasm and that appear morphologically like pole cells is not in itself sufficient evidence for the determinative qualities of this par-

ticular cytoplasm. The critical test is to ascertain whether or not an experimentally induced pole cell is capable of developing into functional germ cells. Inasmuch as "pole cells" at the anterior pole would not be expected to reach the embryonic gonad, it is necessary to insert them among the posterior pole cells of a genetically different host embryo and then to search for germ-line mosaics (17). About 4% of the fertile flies resulting from this test had mosaic gonads. This result demonstrates that at least in these four instances anterior cells with transferred polar plasm differentiated into germ cells, producing either mature eggs or spermatozoa. Germ-line mosaics were not found after the transplantation of normal anterior cells of noninjected embryos (Table 1). A possible objection—that the transferred anterior cells might degenerate in the abnormal position among the pole cells of the host—can be answered by the fact that imaginal structures originating from the anterior cells were found in both the control and experimental series of cell transfers. Thus, it is clear that anterior blastoderm cells are able to survive in the posterior region of the embryo and differentiate autonomously during metamorphosis into anterior structures of the adult cuticle. Recently, it was shown by *in vivo* culturing of portions of blastoderm embryos that the anterior and posterior halves at this stage are already restricted in their developmental capacities for forming either anterior or posterior adult structures (26). In our experiments reported here the anterior imaginal structures originated from at most two to five anterior blastoderm cells. Since single cells can also be transplanted, it should be possible to discover the developmental fate of individual blastoderm cells.

In recent experiments 20% of transplanted posterior pole cells produced germ-line mosaics (17). Possible explanations of the failure to obtain a higher frequency of mosaic gonads have been previously discussed. By utilizing these results, we can estimate the theoretical number of germ-line mosaics if all experimentally produced anterior pole cells were functioning normally. Since one to six "pole cells" were found in the embryos analyzed histologically, we estimate that about 150 cells with polar cytoplasm should have been formed in the 53 embryos used as cytoplasmic recipients (Table 1). Inasmuch as the four germ-line mosaics originated from the first three successive transfers, most if not all of the pole cells were probably contained in these transfers. From the 152 embryos receiving cells in these first three transfers, 74 gave rise to fertile flies. Thus, only one-half or 75 of the transferred "pole cells" could be genetically tested. Since 20% of transplanted normal pole cells produce germ-line mosaics, we should expect about 15 germ-line mosaics in our experiments as the theoretical number. The actual four germ-line mosaics obtained indicate that approximately one-fourth of the anterior "pole cells" functioned to produce germ cells.

Our results show that the property of the posterior polar plasm concerned with germ-cell formation can be transplanted to a presumptive somatic region of the egg and still retain its ability to produce germ cells. Since about a 5-μm-thick layer of peripheral cytoplasm including cortical material has been transferred, we cannot determine whether the cytoplasm *in toto* or a distinct cytoplasmic component(s) (e.g., polar granules) is responsible for this specific developmental effect. Because the polar granules are localized exclusively in the posterior polar plasm of the egg, they can be reasonably considered as involved in the process of germ-cell determination, as has earlier been postulated (27). By isolating these granules and testing their activity in a bioassay similar to the one used in these experiments, it should be possible to identify their biological function.

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