Cell lineage analysis of germ cells of *Drosophila melanogaster*

THE initial events of differentiation of genetically identical nuclei within the eggs of higher organisms are generally believed to be triggered by a heterogeneous distribution of substances within the egg[1,2]. One of the clearest examples of such differential distribution of determinative substances concerns the posterior polar plasm of some insects. Morphological observations suggest that all cleavage nuclei in such insects have identical developmental potential, and that only the few nuclei that chance to migrate to the posterior polar region and thus to include a sufficient quantity of the morphologically distinguishable polar plasm, acquire the capacity to give rise to the germ line[3]. Various experimental approaches[4–8] have provided very strong, although not compelling [9] evidence for such a causal relationship between the polar plasm and germ line differentiation. Genetic fate mapping experiments described here strongly suggest that the germ cells of *Drosophila melanogaster* originate from the posterior-most region of the blastoderm, and they unequivocally rule out the possibility that the germ cells originate from a mid-dorsal position[10]. The posterior-most location of the germ cells supports, or at least is consistent with, the notion that the polar plasm determines whether or not a given cleavage nucleus will acquire the capacity to differentiate into a germ cell.

A priori, two possibilities can be distinguished. The first, as outlined above, states that "any nucleus may become a polar nucleus if it happens to move into the region of the posterior polar plasm, regardless of cell lineage" (ref. 3). The second assumes that the prospective germ cells are set aside during early cleavage before their interaction with the posterior polar plasm or with any other cytoplasmic region. One critical test between these two alternatives involves genetic determination of cell lineage relationships of the primordial germinal nuclei: only the first requires that they are mapped to the area of the blastoderm that contains the posterior polar plasm. The reason for this prediction is apparent, for example, from the observation that one ancestral nucleus in the posterior pole region can give rise to one daughter cell which contains the visible polar granules and one which does not[3], and hence that presumably only the former is likely to contribute to the germ line.

The technique of genetic fate mapping[11,12] provides one approach by which cell lineage relationships of various anatomical regions can be determined[8]. Using this technique, Falk *et al.*[10] located the gonads on the genetic fate map of *D. melanogaster* "about half way along the blastoderm near the mid-ventral line". They suggested therefore that "the assumption that the site at which a nucleus lands in the periplasm decides its fate has to be modified, at least for nuclei destined to give the gonads" (ref. 10).

Both the observations that some pole cells in *D. melanogaster* give rise to somatic cells rather than primordial germ cells[13], and the observation that in the wasp *Pimpla turionellae*, although characteristic pole cell formation and migration occur, germ cells are found in the absence of pole cells[14,15], seem to be compatible with this suggestion. But two other attempts[16,17] have been made to determine the developmental origin of the gonads by means of genetic fate mapping, and these gave a posterior position compatible with the hupothesis that the site at which a nucleus lands in the cytoplasm decides its fate. These inconsistent results are surprising in view of the reproducible interrelationships between cuticular landmarks that are reflected by many independently derived maps[11,12,16-23] and in view of the strong evidence for the validity of the fate mapping procedure[12,21,24].

All three investigations mapped whole gonads either at the larval stage[10,17] or at the adult stage[16]. It is well established that the gonads of *Drosophila* derive from two developmentally unrelated components: the germ cells which give rise to the germ line and mesodermal cells which give rise to the gonadal sheath and interstitial cells[8,25]. The nature of the interaction between these two components is unclear[13,25]. In addition, the attachment of testes in adult gynandromorphs to the developmentally distinct female oviducts causes degeneration of the testes[26]. These theoretical considerations, coupled with the technical difficulties encountered in fate mapping of internal organs and the need to use a functional, rather than morphological criterion for the germ nuclei, make it very difficult to resolve these contradictory results and their bearing on the hypothesis that the posterior polar plasm contains germ cell determinants. A clearcut resolution of this problem is therefore likely to emerge only from functional mapping of the germinal nuclei themselves, irrespective of other elements of the reproductive system[8]. The experiments described here constitute the first clearcut genetic evidence for the developmental origin of the germ cells, and they show that the germ cells of *Drosophila* do indeed originate from the general area that has been traditionally assigned to them by cytologists—the posterior tip of the egg.

Males of the constitution y–y^+ *Y; pal–pal* were crossed to XY, *y B* females[20,27]. The sons from this cross were screened for *pal*-induced somatic mosaicism for the extra Y chromosome; this can be detected by the appearance of yellow and non-yellow cuticular patches in the same male. The frequency of mosaicism among the F_1 males has not been calculated directly, but ranged approximately from 0.001 to 0.002. On average, each cuticular region was yellow in 40% of the mosaic males, non-yellow in 55% and mixed in the remaining 5%. The mosaic distribution of the entire cuticle of each rare mosaic male was recorded. Subsequently, each mosaic male was crossed to five to nine *yellow* females. These *yellow* females were kept in a stock in which all males carried the B^sY chromosome and thus any non-virgin yellow female could be detected from a phenotypic examination of her sons. The constitution of the germ cells of each 0–14-d old mosaic male was inferred from the phenotype of his sons: the germ cells of a male that only gave rise to non-yellow and fertile sons must have contained the extra marked Y chromosome; yellow and sterile sons indicate that they did not contain this extra Y chromosome, and when both phenotypes were found among the male progeny of a single male it could be assumed that such a phenotypically mosaic male was also germinally mosaic.

Out of a total of 260 phenotypically mosaic males obtained, six died shortly after their isolation and gave no offspring, 17 were sterile, six gave rise to less than 62 sons and 16 gave rise to some haplo-4 progeny. Of the remaining 215 mosaic males, 85 gave rise to y sons, 94 gave rise to y^+ sons and 36 to both y and y^+ sons. An average of 312 sons was observed per mosaic male and the smallest number of sons scored per male was 62. The data obtained from these 215 males made it possible to correlate the constitution of the germ cells to various cuticular landmarks and thus to determine their position on the genetic fate map of *D. melanogaster* (Fig. 1).

To rule out any sex-specific differences in the developmental origin of the germ cells and to obtain an independent corroboration of their posterior-most position (Fig. 1), an analogous

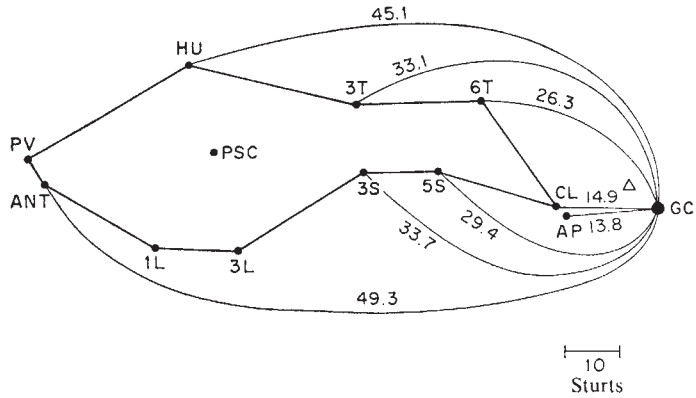

Fig. 1 Genetic fate map of the right half of *D. melanogaster* blastoderm, with special reference to the position of the germ cells. Because it was not possible to rule out *a priori* a bilateral symmetrical origin of the germ cells, data from germinally mosaic males were not included in the calculations of distances between the germ cells and various cuticular landmarks. But the general outline of the map, and, in particular, the posterior position of the germ cells, is preserved when the data from germinally mosaic males are also included or when the map distances are computed according to the suggested algebraic correction[19,22] for systems in which the mean proportion of mutant tissue is significantly different from 0.5. The open triangle represents the position of the germ cells triangulated on a map derived from 105 mosaic females and then superimposed on the map of 215 phenotypically mosaic males shown here. ANT, antenna; AP, anal plate; CL, clasper; GC, germ cells; HU, humeral bristles; PSC, posterior scutellar bristle; PV, post-vertical bristle; 1L, first leg; 3S, third hemisternite; 3T, third hemitergite.

investigation has been carried out with mosaic females. Males of the constitution y/y^+Y; *pal/pal* were crossed to attached X *C(1)RM*, *y pn v*/0 females. The automatic virgin daughters obtained from this cross were screened for somatic mosaicism and their pattern of mosaicism was recorded. Subsequently, these mosaic females were crossed to *yellow* males. A total of 116 Y-chromosome mosaic females was obtained. The analysis, however, was based only on 105 mosaic females that gave rise to a minimum of 22 sons. The mean number of sons per female in this group was 98. Again, a fate map of cuticular landmarks was constructed and the location of the germ nuclei on this map was determined. Although there were some differences between this map and the map derived from mosaic males (Fig. 1), they were very similar in general outline and hence the map obtained from these 105 mosaic females is not reproduced here. Instead, the position of the germ cells in this map is superimposed on the map shown in Fig. 1.

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