Proceedings of the National Academy of Sciences
Vol. 65, No. 3, pp. 633–637, March 1970

Stability of a Position-Effect Variegation in Normal and Transdetermined Larval Blastemas from *Drosophila melanogaster*

Ernst Hadorn, Robert Gsell, and Jack Schultz*

ZOOLOGISCH-VERGL. ANATOMISCHES INSTITUT, UNIVERSITÄT ZÜRICH, SWITZERLAND, AND THE INSTITUTE FOR CANCER RESEARCH, PHILADELPHIA, PA.

Communicated December 22, 1969

Abstract. Male genital disks of a yellow-variegated genotype were implanted into the abdomens of adult females to test the stability of variegated clones in the blastemas formed by the implants. Upon reimplantation into metamorphosing larval hosts, test fragments of the proliferating blastemas differentiated into variegated organs, with *yellow* and wild-type areas. In later transfer generations clones were separated, which appeared stable for either wild type or *yellow;* variegation was no longer occurring. In all the lines differentiation occurred also into other organs (allotypic) than those characteristically formed by the genital disc (transdetermination). The absence of new variegation in these transdetermined organs is discussed as evidence against a reversal to the embryonic state in the cells of the transdetermining blastema. The variegation process seems not to be affected by, nor does it in this case influence, the process of transdetermination.

The controls of determination in embryonic development and the influences of the genes on differentiation, become more accessible to analysis when techniques of cell culture are applied to genetically appropriate material. Many opportunities in these directions are afforded in *Drosophila melanogaster*. We here report the application of the techniques of continuous culture of larval blastemas[1, 2] to a stock showing varying activities of genes among the somatic cells (socalled position-effect variegation). The results have bearing, we believe, not only on the mechanism of variegation as an aspect of developmental programming,[3, 4] but also relate to the process of "transdetermination," the formation by the larval blastema of "allotypic" organs[1, 2] normally not produced by the originally implanted imaginal disk. When cells from individuals susceptible to the variegation process as embryos proliferate in the female abdomen, they form stable clones. No new variegation occurs in the larval blastema. Yet in the same tests, allotypic organs are formed, indicating separate controls for the organ determination and the variegation process.

Materials and Methods. In these initial experiments, we have used a strain of *Drosophila melanogaster* showing extensive variegation at the *yellow* locus, which can be detected in all parts of the integument. The Y chromosome responsible, designated y^+Y (ref. 5, p. 418), carries a small fragment of the X, containing the terminal segment from *yellow* through *achaete*, on the long arm of the Y chromosome by transfer from the

inversion *scute*.[8] In the usual genotype, variegation in this fragment is slight; only occasional yellow patches are detectable. To increase the frequency and extent of the variegation, two enhancers were introduced into the stock: *E(Var) 7*, located in the left arm of the second chromosome; and *E(Var) 21*, whose locus is not determined.[6] Their total effect in this genotype is to produce flies with large yellow patches on the head, thorax, wing, and to a lesser degree, on the abdomen. Also, the stock is homozygous for *multiple wing hairs* (*mwh*). The formula[5] for the genotype, is y/y^+Y; *E(Var) 7; mwh; E(Var) 21*. Variegation for other genes, such as *achaete* and the several lethals also included in the fragment, is not detected in this system. Also, the presence of the fragment as a duplication shields the cells from adverse effects of variegational changes at lethal loci, since their normal alleles exist in the X chromosome. A detailed study of the cell lineage has not yet been made. Noujdin[7] did make such a study in *scute*,[8] from which the variegational fragment in y^+Y was derived, and made an analysis of cell lineage which compared well with the data from gynandromorphs.

The procedures for continuous culture of larval blastemas in female abdomens have been described in detail elsewhere.[1] Male genital disks from 96-hr-old larvae were the source of the cultures in these experiments. The disks were cut in half, one half serving to test its phenotype when implanted into an 80-hr-old larua, which then proceeded through metamorphosis. The other half, placed in the adult female abdomen, continued proliferation forming a blastema during a two-week period before transfer to a new host. At this time, the implant was divided into two parts, one of which continued the stem line in a new adult host, the other being allowed to metamorphose and display its phenotype. Thus a test of the potential for differentiation, and of the variegated phenotype, was made at a desired transfer generation.

Results and Discussion. The results of this first series of experiments, performed with a transfer every two weeks over a period of seven months, are shown in Figure 1. The initial implant has a variegated phenotype; it is a mosaic of yellow and wild-type anal plates and claspers, the organs normal (autotypic) for the genital disk. From transfer generations 0 to 3, mosaic areas continue in the tests. The implants varied between extremes ranging from almost completely wild type, to almost completely yellow. At transfer generation 4, it was possible, with the continued growth of the stem line, to establish a series of sublines, labelled *a–m*.

What are the characteristics of these sublines in which both yellow variegation and the appearance of allotypic organs (transdetermination) were studied? First, the sublines vary in the degree of yellow variegation manifested. Clones were apparently sorted out during the successive transfer generations. Most remarkable, stable wild-type lines and also some yellow lines were obtained in these transfers. Thus, lines, *c*, *d*, *g*, and *h* showed no yellow mosaicism; in cultures *g* and *h*, during 15 transfer generations (a duration of 7 months) the wild-type condition was stable. The evidence for the yellow lines is not as complete. In some tests no wild-type areas could be observed; unfortunately the lines died out too soon for a satisfactory demonstration of their stability on a large scale. But it appears safe to conclude that under the conditions of proliferation in the female abdomen, wild-type cells do not eventually produce yellow daughters; nor do the yellow cells revert to wild type.

In the different lines, both wild-type and yellow, the phenomenon of transdetermination occurred, as it has in other experiments with larval blastemas.[1, 2, 8–13] Instead of the normal derivatives of genital disks (anal plates, claspers, and penis), test implants often metamorphose into organs normally

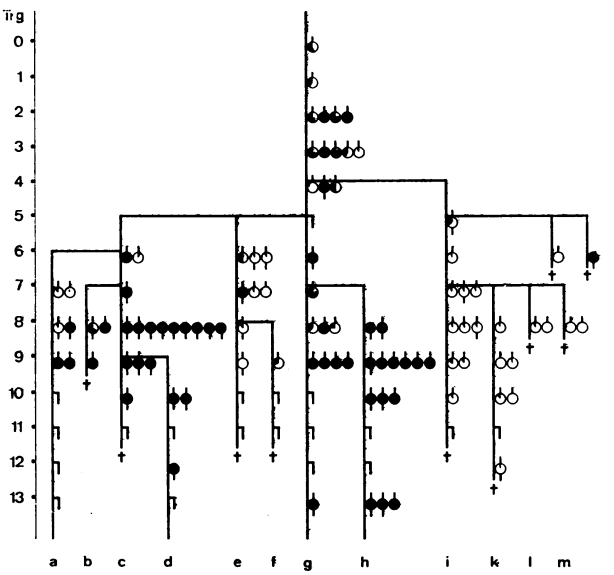

FIG. 1.—Pedigree of yellow variegation in 13 transfer generations (Trg) of larval blastemas carried in the abdomens of wild-type females. Each circle stands for a metamorphosed test implant; different sublines are designated *a–m*. Black portions of the circles correspond to the relative extent of wild-type tissue in the implant, white sectors are the yellow portions. Autotypic structures—derivatives of the genital disk—are represented by a short upward line at each generation; a downward line designates allotypic structures (head, wing, and thorax). The absence of a circle at a Trg indicates no available test information. A cross indicates the death of a subline.

derived from other disks: head, wings, and legs. These occur not only in the wild-type and yellow lines, but also in the implants containing both yellow and wild-type areas. Since it has already been shown that transdetermination may occur in groups of cells,[1, 12, 13] it is not surprising that some transdetermined organs were variegated—an independent confirmation of the earlier results.

It is important that the full range of allotypic organs occurs in all three types of lines. Apparently the variegational rearrangement does not suppress the possibility of transdetermination, nor does the switch in developmental pathway reopen the choices available to the variegating region earlier in development. What is the meaning of this independence of the two processes?

It will be useful first to recall the characteristics of the variegation process. In position-effect variegation, genes are transposed from their normal situation to become neighbors of the late-replicating heterochromatic regions. The phenotypes of the mosaic areas range from expressions like those of extreme mutant alleles of the transposed genes, to slight changes from the wild type. There is a rough correlation between the time at which an organ appears in development, and the extent of variegation. The germ cells, earliest to appear, seem exempt; the larval tissues (salivary glands and malpighian tubules) are affected to a lesser degree than the later appearing imaginal disks.

The underlying processes suggested by these correlations are those which select the constellation of genes active for each cell type.[3, 4]

Analysis of the mosaic areas show that variegation affects clones of cells. We have already noted Noujdin's analysis, pertinent since it concerns the same loci as those dealt with in our experiments. Also, more recent analyses of eye-color variegation show a similar parallelism between the cell lineage of the eye and the pattern of position-effect variegation in this organ.[15, 16]

The essential process is connected with the events of replication.[2, 14] Single white cells in the yellow malpighian tubules of white-variegated strains give evidence that only one of the daughter cells of a mitosis is altered. After this last division (during the embryological period), the malpighian tubule cells continue to grow in the larva and the white gene functions in producing more pigment. The pattern for gene activity is set, and the later replications of the polytene chromosomes during growth do not change the stable pattern. This stability of functional pattern is maintained even if larval growth proceeds under conditions (low temperature) favorable to variegation (ref. 14, p. 322).

This whole complex of phenomena has been interpreted as resulting from the imposition by the late replicating regions of their cycle of activity on the genes transposed to their neighborhood.[4, 14] Inhibitors of DNA synthesis enhance variegation (ref. 14, p. 315). The association of compacted regions with late replication, and with repressed transcription, is striking in the giant chromosomes where, in an appropriately situated rearrangement, variegation for a puff has been shown in the rearranged segment, correlated with variegation for the expression of those genes in expected characters of the adult fly.[4]

It is to be emphasized that within the general framework ordering the position effect of heterochromatic regions in variegation, there are many specific interrelations involving differences in time of occurrence in development in response to a wide variety of controlling factors. Indeed, observation of two different rearrangements simultaneously, both affecting the same organ, reveals cases where one acts in the embryo, the other very much later during larval development.[6]

With these considerations in mind, we are prepared to ask the meaning of the stability of the yellow variegation in the larval blastemas maintained in adult abdomens. The variegation in the males of the $y:y^{+}Y$; $E(Var)$ 7 genotype shows a pattern of large yellow areas, hence the event occurs relatively early in development, often involving the whole of a imaginal disk. On this basis it is reasonable to assume that the conditions for replication at the later stages are such that the initial and early fixation of the clonal type is maintained. This view is consistent with the behavior of the white variegation in the malpighian tubules, already mentioned. The argument has considerable force applied to the wild-type lines. The yellow lines are subject to different judgments: if a replication mechanism is involved, the stability of the yellow line might in the extreme case result from a loss consequent on a failure of replication. But the simplest interpretation of the results, given early action of the enhancers, is that clonal fixation is maintained in all the later mitotic generations of the larval blastema implants.

We may now examine the impact of these results on the nature of the transdetermination process. This occurs in both wild-type, yellow, and mixed clones, and whatever the nature of the process, it apparently is not necessarily concerned with the establishment of clones, but may, judging by its relation to proliferation rate, result from more complicated processes. It is, for example, uncertain how many cells are involved in a single transdetermination. Nevertheless, one could raise the question of whether the process of transdetermination in itself might not restore the developmental condition for the appearance of new yellow clones in the wild-type lines. This would mean the restoration of conditions like those in the embryo, where the enhancer normally operates. It would also imply that a reversal of the initial genital disk determination must occur, following which the new organ is determined. None of the transdetermined organs showed any evidence of variegation at the rate (30–70% of the cells in this genotype) expected if indeed there were a reversal of determination to the embryonic cell. For example, in subline *d* (Fig. 1), four independent transdeterminations were observed: to head, leg, thorax, and wing. Allotypic structures thus occur in all test implants of transfer generation 8; and the estimate of four events must be a minimum, since coincidental switches to the same allotypic structure cannot be evaluated. Since no variegation was observed in these lines, the evidence clearly does not favor a return to the embryo in the transdetermination process—a conclusion previously reached on other grounds.[1, 8–11]

These studies have involved a single variegated genotype under the standard conditions (hormonal, nutritional, etc.) in the female abdomen. It is obvious that exploration in a variety of directions, both genetic (diverse rearrangements, controls of different types) and environmental (nutrition, temperature, hormonal agents) is possible. The interpretations here presented would be tested by determining the conditions for clonal fixation and their relation to organogenesis.

* This work was supported by the Swiss National Science Foundation and by USPHS grants CA-01613, CA-06927, and FR-05539 from the National Institutes of Health and by an appropriation from the Commonwealth of Pennsylvania.

[1] Hadorn, E., *Develop. Biol.*, **13**, 424 (1966).
[2] Hadorn, E., in *Major Problems in Developmental Biology* (New York: Academic Press, 1967), p. 85.
[3] Schultz, J., *Proceedings of the 7th International Congress of Genetics, J. Genet. Suppl.*, 257 (1941).
[4] Schultz, J., *Brookhaven Symposia in Biology*, no. 18 (1965), p. 116.
[5] Lindsley, Dan L., and E. H. Grell, "Genetic Variations of *Drosophila melanogaster*," *Carnegie Institution of Washington Publ. 627* (1967).
[6] Schultz, J., unpublished data.
[7] Noujdin, N. I., *Biol. Zhurn.*, **4**, 571 (1936).
[8] Tobler, H., *J. Embryol. Exptl. Morph.*, **16**, 609 (1966).
[9] Garcia-Bellido, A., *Devel. Biol.*, **14**, 278 (1966).
[10] Hadorn, E., R. Hurliman, G. Mindek, and G. Schubiger, *Rev. Suisse Zool.*, **75**, 557 (1968).
[11] Schubiger, G., M. Schubiger-Staub, and E. Hadorn, *Wilhelm Roux' Archiv.*, **163**, 33 (1969).
[12] Gehring, W., *Devel. Biol.*, **16**, 438 (1967).
[13] Schubiger, G., and E. Hadorn, *Devel. Biol.*, **17**, 584 (1968).
[14] Schultz, J., *Cold Spring Harbor Symposia on Quantitative Biology*, vol. 21 (1956), p. 307.
[15] Becker, H. J., *Current Topics Devel. Biol.*, **1**, 155 (1969).
[16] Baker, W. K., *Adv. Genetics*, **14**, 133 (1968).