ALLELE SPECIFIC PATTERNS OF SUPPRESSION OF THE VERMILION LOCUS IN *DROSOPHILA MELANOGASTER*

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THE characteristic autofluorescence of kynurenine has facilitated detection of its intracellular distribution in the larval fat body of *Drosophila melanogaster* where it has been demonstrated that a specific group of larval fat cells is involved in elaborating this metabolite (RIZKI 1961). The *vermilion* (*v*) mutant which lacks tryptophan pyrrolase activity does not accumulate kynurenine in the larval fat cells. When the non-allelic mutant gene, *suppressor-of-sable* (*su²-s*) is combined with the *v* mutant gene, tryptophan pyrrolase activity is restored (BAGLIONI 1960; KAUFMAN 1962; MARZLUF 1965), normal eye pigment is formed, and kynurenine production within the larval fat cells can be verified by examination with the fluorescence microscope (RIZKI 1963). The cellular pattern of kynurenine restitution in one double mutant combination (*su²-s v¹*) examined previously indicated that a modification in the cellular distribution of enzyme activity was associated with the suppression phenomenon (RIZKI 1963). One of the purposes of the present investigation was to examine the pattern of kynurenine autofluorescence in the larval fat bodies of various combinations of *v* alleles with this same suppressor gene in order to determine whether a specific pattern of cell activity is a consistent feature of suppression. Information was also obtained on the biosynthetic activity in the fat cells of the *su²-s* strain carrying the wild-type allele at the *v* locus.

The diffusibility of kynurenine or its metabolic products from the Malpighian tubules and the fat cells to the developing eye was a valuable asset to the pioneer studies on biochemical genetics of *Drosophila* (BEADLE 1937), but this factor is one of concern to an analysis of the genetic potential of the individual fat cell. Previous investigations on the larval fat cells of *Drosophila* have demonstrated by implantation techniques as well as *in vitro* isolation that kynurenine production is a function of the fat cells (RIZKI 1964). A study of fluorescence in the fat cells in gynandromorphs has now been utilized to support the contention that the appearance of kynurenine in an individual fat cell represents the synthetic activity of that specific cell.

MATERIALS AND METHODS

The larval fat body of *D. melanogaster* is a bilaterally symmetrical, continuous tissue mass

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extending dorso-laterally from the cephalic region to the caudal end of the larva. This tissue is a single cell layer in thickness, thus providing excellent material for *in vitro* examination with the fluorescence microscope. Intact fat bodies were dissected in *Drosophila* Ringer solution and examined with the fluorescence microscope at the white puparium stage when maximum kynurenine accumulation has occurred. In order to properly classify the fluorescence pattern of the fat body, it is necessary to maintain the morphological integrity of the tissue mass as one piece while mounting it flat so that no cells overlap or disarrangement occurs. The autofluorescence pattern of the fat bodies of the following strains has been studied: v^k ; $su^2-s v^k$; v^{48a} ; $su^2-s v^{48a}$; v^{51c} ; $su^2-s v^{51c}$. These alleles were examined against a common genetic background by utilizing the method presented previously for studies on the v^l and v^{36f} strains (RIZKI 1964). Tryptophan feeding experiments were performed as reported previously (RIZKI and RIZKI 1964).

The production of genetic gynandromorphs for the fluorescence studies utilized $\gamma^2 w^a v$ flies and the $X^{c2} In(1)w^{vc}/In(1)w^{m4}$ strain which was kindly provided to us by Professor EDWARD NOVITSKI of the Biology Department, The University of Oregon for this study.

RESULTS

The fat cell as an autonomous unit of kynurenine production: The ease with which kynurenine or its metabolic products diffuse can be demonstrated by implantation of v^+ tissues containing this metabolite into v specimens lacking it (BEADLE 1937). Therefore, it must be considered that diffusion may play a role in the distribution pattern of kynurenine in the fat body despite the fact that the pattern of autofluorescence is a characteristic feature for a given strain. The behavior of individual cells in the fat body can be analyzed by examination of a mixed cell population of two genotypes within the same tissue mass, some cells containing the v^+ allele and other cells with the v allele. Fortunately, such a mixture of genotypes can be achieved in gynandromorphs rather than by resorting to external experimental manipulation with the undesirable aspects of injury and altered environment (STURTEVANT 1932).

The occurrence of gynandromorphs is low, but their frequency can be increased by utilizing the $X^{c2} In(1)w^{vc}$ strain in which the loss of the ring X chromosome is particularly high. The analysis can be further facilitated by using several additional genetic markers on the X chromosome which influence larval phenotype, in the present instance, yellow mouthparts and mottled Malpighian tubules. The details of this cross are outlined in Figure 1. Two types of female larvae can be separated on the basis of color of mouthparts: (1) larvae which are heterozygous for the ring X chromosome are heterozygous for the mutant genes *yellow* (γ), *white^{apricot}* (w^a), and *vermilion* (v) carried on the rod X chromosome. Mouthparts of these larvae are the normal black color; (2) larvae with yellow mouthparts which are homozygous γ/γ .

Since gynandromorphs are produced by the elimination of the w^{vc} ring chromosome, a small fraction of the gynandromorph larvae will also have yellow mouthparts. However, such cases can be further differentiated on the basis of the mottling effect on the pigmentation of the Malpighian tubules ($In(1)w^{m4}$). Larval genotype can thus be unambiguously recognized before the fat body is subjected to microscopic examination for determination of the autofluorescence pattern. Female larvae were separated into two groups and all those with normal

LARVAL PHENOTYPES CORRESPONDING TO THE MARKERS UTILIZED
FOR SCREENING GYNANDROMORPHS

$$\text{♀♀ } \text{In}(1)w^{vc}/\text{In}(1)w^{m4}, yw^{m4}X \times \text{♂♂ } y^2w^av$$

$F_1 \text{ ♀♀ larvae:}$	$\text{In}(1)w^{vc}/y^2w^av$	$\&$	$\text{In}(1)w^{m4}, yw^{m4}/y^2w^av$
Phenotypic contrasts:-			
mouth parts	normal		yellow
malpighian tubules	normal pigment		mottled due to w^{m4} effect
gynandromorph	mosaic tissue:- yellow mouthparts, malpighian tube cells pigmented & non- pigmented.		

FIGURE 1.—The scheme of the crosses used to obtain the gynandromorph-generating genotype and the corresponding phenotypes which facilitated selection of the appropriate female larvae $X^{C2} \text{In}(1)w^{vc}/y^2w^av$. See text for explanation.

colored mouthparts were dissected and examined with the fluorescence microscope. Among the female larvae with yellow mouthparts, only the specimens which did not show mottling of the Malpighian tubules were examined with the fluorescence microscope, for yellow mouthparts could also result from the elimination of the ring chromosome w^{vc} .

From this cross, 47 female larvae showed normal pigmentation in the Malpighian tubules as well as the normal wild-type fluorescence pattern in the fat body (see Figure 4 for normal pattern of kynurenine distribution). Seven larvae were classified as gynandromorphs: among these seven gynandromorphs, three specimens showed the normal bilateral fluorescence pattern in the fat body indicating that elimination of the w^{vc} chromosome had not occurred in any of the anterior fat cells of these larvae; three specimens lacked kynurenine fluorescence in the entire fat body and presumably the w^{vc} ring chromosome was eliminated during early embryonic development before the fat cells segregated from other cells in their cell lineage so that all prospective kynurenine-producing cells contained the v gene in hemizygous condition; one specimen was a partial bilateral gynandromorph in terms of the kynurenine-producing cells. In this case, kynurenine cells were found in regions 1 through 3 in the normal pattern on one side of the body while the other side of the fat body contained kynurenine in the cells of region 1 and extending into the ventral commissure. Thus approximately

215 cells on the one side of the body were of the v type since the w^{ec} ring chromosome must have been eliminated from these cells (see Figure 4: the stippled area indicates fluorescence in the fat body of this individual and Figure 2 is a photomicrograph of this specific region).

Two conclusions can be drawn from these observations: first, the occurrence of the three specimens which lacked kynurenine in the fat cells but contained gynandromorph-mosaic Malpighian tubules demonstrates that there is no other site in the body which can provide kynurenine to the fat cells prior to the puparium stage. Second, the pattern of autofluorescence in the specimen which was a gynandromorph-mosaic for kynurenine in the fat cells indicates that kynurenine synthesis in the fat cells at this stage of development represents the expression of the genes within each individual cell and is the autonomous property of each cell.

Contribution of the suppressor gene to the fluorescence pattern of the fat cells: In order to assess the contribution of the su^2-s gene to the fluorescence pattern in the fat body, information is needed concerning the fat cell population of larvae carrying the su^2-s gene in the absence of any mutant allele of v . Analysis of the autofluorescence pattern of the su^2-s allele was achieved by examining the F_2 male larval progeny of a cross between a homozygous $su^2-s v^{36f}$ female and an *Ore-R* male (Figure 3). Among the offspring from this mating, the non-recombinant classes in terms of fluorescence pattern in the fat body would be of two types; autofluorescence restricted to regions 1 through 3 in the pattern typical for $su^2-s^+ v^+$ fat body, and the lack of kynurenine autofluorescence in regions 2 and 3 in the $su^2-s v^{36f}$ larvae. The su^2-s gene is located at the left tip of the X chromosome (0.0) and v is located in the middle of the X chromosome at 33

FIGURE 2.—Photomicrograph of the ventral commissure of the fat body of the gynandromorph-mosaic showing 27 fluorescent cells at the point where the kynurenine autofluorescence ceases. Refer to Figure 4 for the complete pattern of autofluorescence as indicated by the stippled area. This photograph is a copy from an Ektachrome high speed color transparency; technical limitations permit photography of only a few cells in one field at this magnification ($\times 250$).

FIGURE 3.—Outline of the crosses made to examine the phenotypic effect of the *su*²-*s* gene. *fl*⁺, fluorescence; *fl*⁻, absence of fluorescence; *q*, frequency.

Centimorgan units from the *su*²-*s* locus. Therefore, it is expected that 0.335 of the male progeny of females heterozygous for these markers (*cis*-heterozygotes) will be carrying the *su*²-*s*⁺ *v*⁺ parental combination and 0.335, the *su*²-*s* *v*^{sef} combination. Recombinants would include *su*²-*s*⁺ *v*^{sef} larvae and *su*²-*s* *v*⁺ larvae, each with a frequency of 0.165. The group *su*²-*s*⁺ *v*^{sef} will lack kynurenine fluorescence in all of the fat cells; therefore, in classifying the autofluorescence distribution in regions 2 to 4, these larvae are pooled with the *su*²-*s* *v*^{sef} larvae, and the expected fraction of larvae lacking fluorescence in these regions is represented by 0.50 of the male offspring. The remaining recombinant class, *su*²-*s* *v*⁺ would be recognizable as distinct only if its fluorescence pattern differs from the preceding descriptions. Adult flies from the cross would be vermilion and normal eye color in an expected 1:1 ratio.

The male larvae were clearly separable into three classes when their fat bodies were examined with the fluorescence microscope. In addition to the expected groups, one with the normal fluorescence pattern in regions 2 and 3, and one lacking kynurenine extension into this region, 15 larvae out of a total of 63 examined showed an increased autofluorescence of kynurenine extending into region 4. These larvae must represent the *su*²-*s* *v*⁺ recombinant group, and the distinct appearance of this group lends support to the hypothesis that the *su*²-*s* gene has a recognizable phenotypic action of its own.

The observed distribution of cell patterns in the three categories are within the statistical limits of error of sampling according to the segregation hypothesis

for two linked genes. The remaining male larvae from this mating were allowed to pupate and were classified in the adult stage (Figure 3). It is clearly evident that the v^+ and v^{36f} alleles are segregating in regular Mendelian fashion and that the larval sample examined for autofluorescence is not a biased sample of the total male progeny.

Allele specific pattern of suppression: In the wild-type *Ore-R* strain, light blue autofluorescence characteristic of kynurenine is found in the cells of the fat body regions designated 1 through 3 (Figure 4). Larvae which are homozygous or

FIGURE 4—Diagrammatic reconstruction from a camera-lucida drawing of a larval fat body of *D. melanogaster*. The stippled area of the fat body indicates the extent of kynurenine autofluorescence in a gynandromorph-mosaic described in the text. The solid line following each genotype corresponds to the regions included in the kynurenine autofluorescence pattern. The cells of region 4 of $su^2-s v^1$ and $su^2-s v^2$ show a heterogeneity in degree of autofluorescence as indicated by the broken line. The dotted line represents the extension of the fluorescence pattern induced by tryptophan administration.

hemizygous for the su^2-s gene and the suppressible v^1 (or homo-allelic v^2) allele contain kynurenine in region 4 as well as regions 1 through 3. The $su^2-s v^{36f}$ mutant larvae contain a small amount of kynurenine in the anterior-most fat cells designated region 1 and in this sense v^{36f} is not an unsuppressible allele as has been indicated by examination of eye color (GREEN 1954). On the other hand, the unsuppressible alleles, v^{48a} and v^{51c} show no evidence of kynurenine production in the fat body in the presence of the su^2-s gene.

The v^k suppressible allele in combination with su^2-s shows the same cellular distribution pattern of kynurenine as the *Ore-R* wild-type strain. It is interesting that this allelic combination ($su^2-s v^k$) also resembles the *Ore-R* wild-type strain in its response to high levels of dietary tryptophan. When 0.03M tryptophan is fed to *Ore-R* larvae beginning at approximately 68 hours of age, kynurenine synthesis is induced in the posterior fat cells in regions in which it does not occur under normal feeding conditions (RIZKI and RIZKI 1964). A similar extension of kynurenine-synthesizing cells is found in $su^2-s v^k$ larvae which have been fed tryptophan. When this same quantity of tryptophan is given to $su^2-s v^{36f}$, $su^2-s v^1$, or $su^2-s v^2$ larvae, kynurenine autofluorescence is intensified in the cells in which it normally appears in each of these strains, but it does not appear in any additional groups of cells.

DISCUSSION

The mutant effects of a number of non-allelic loci are suppressed by the su^2-s gene in *Drosophila*. Among these mutants are *vermillion* and *sable* on the X chromosome, and *speck* and *purple* on the second chromosome. The same suppressor gene also acts as an enhancer of the *Ultrabithorax* complex in *trans*-heterozygous condition (E. B. LEWIS, personal communication). An allele of su^2-s has been reported which suppresses the mutant *black* on the second chromosome. There are no known biochemical relationships between the pathways affected by these mutant genes, and although the action of the su^2-s gene is not locus specific, an allele-specific suppression pattern is achieved at the v locus. Such a distinction in interaction with the su gene was noted by GREEN (1954) who classified the v alleles into two functional groups, suppressible and unsuppressible, and demonstrated that v^1 and v^{36f} are separable by crossing over. Recombination between the v^{48a} allele and v^{36f} was later reported by BARISH and FOX (1956), but attempts to separate v^{48a} from v^1 were unsuccessful. Immunological studies have revealed antigenic dissimilarities between the v^1 , v^{48a} , and v^{36f} alleles (BARISH and FOX 1956), and the present report extends the analysis of physiological characteristics of the v alleles to the level of cellular suppression pattern. Restoration of kynurenine autofluorescence in the fat bodies of $su^2-s v^{36f}$ larvae is distinct in pattern from that of $su^2-s v^1$, and both differ from the fluorescence pattern found in $su^2-s v^k$ larvae. It is interesting that the latter mutant combination represents a case of suppression which imitates that of the *Ore-R* normal strain with regard to the cell population involved in kynurenine production.

A number of mechanisms by which one gene may suppress the action of another non-allelic gene have been demonstrated in microbial systems, but infor-

mation on the action of the suppressor gene in *Drosophila* is insufficient to render any hypothesis feasible at the present time. Tryptophan pyrrolase activity in suppressed-vermilion flies approximates 7% of that found in wild-type flies (MARZLUF 1965). Apparently this restoration of enzyme synthesis by the suppressor gene is not achieved through the removal of a metabolic inhibitor produced by the *v* locus (BAGLIONI 1960; MARZLUF 1965). MARZLUF was unable to detect any qualitative differences between the enzymes produced by suppressed-vermilion flies and wild-type flies and concluded that suppressor leads to the formation of a small amount of normal enzyme. He accordingly noted the operational similarity between this case of suppressor action in *Drosophila* and suppression in the tryptophan synthetase system of *E. coli* (CARBON, BERG, and YANOFSKY 1966) where the molecular basis for suppression resides in an altered t-RNA or activating enzyme. In reporting a 13-fold redundancy for each of the t-RNA species in *Drosophila*, RITOSSA, ATWOOD, and SPIEGELMAN (1966) have suggested that suppression effected via t-RNA mutations in this organism will be limited to instances of low level suppression. It is difficult to include variability in the cellular suppression patterns in such considerations; however, a codon restriction hypothesis of cellular differentiation as proposed by STREHLER, HENDLEY and HIRSCH (1967) provides an interesting possibility. Alanine t-RNA synthetases are differentially distributed among rabbit tissues and hence which of the alternate sets of t-RNA in a given tissue will be utilized by the cell machinery in translation is restricted to the available activating enzyme sets. Suppression by a mutation in either an activating enzyme cistron or t-RNA could be confined to certain cell populations by this hypothesis. However, the allele specific patterns of suppression observed in the fat body appear to be a property of the mutated heteroalleles of the vermilion gene rather than the action of the suppressor mutation alone. In a study of misreading of codons induced by streptomycin, DAVIES, JONES and KHORANA (1966) have noted that misreading of a base is influenced considerably by the nature of neighboring bases, and it seems reasonable to expect that a codon position effect may be a contributory factor to efficiency of suppression of a series of heteroalleles.

SUMMARY

A study of gynandromorphs for *vermilion* and *vermilion*⁺ tissues has been utilized to demonstrate that kynurenine synthesis is an autonomous property of the fat cell. The autofluorescence pattern of kynurenine synthesis in the fat bodies of a series of suppressed vermilion strains of *Drosophila melanogaster* has been examined and an allele-specific pattern noted. The kynurenine cellular distribution pattern in *su*²-*s v*^k fat bodies resembles that of the Ore-R wild-type strain in which approximately one third of the fat cells synthesize kynurenine; additional fat cells participate in this function in the *su*²-*s v*¹ strain while this activity is limited to approximately one tenth of the fat tissue in *su*²-*s v*^{36f} larvae. The unsuppressible alleles, *v*^{48a} and *v*^{51c}, do not show any kynurenine autofluorescence when combined with the suppressor gene. Genetic analysis indicates

that the suppressor gene influences the autofluorescence pattern in the fat body and thus has a recognizable phenotypic characteristic in addition to its action in suppressing the mutant vermilion.

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