STRUCTURAL AND PHENOTYPIC DEFINITION OF THE ROSY CISTRON IN DROSOPHILA MELANOGASTER[1]

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GENETIC fine structure analysis of the rosy (*ry*) cistron in *Drosophila melanogaster* has shown that the rosy mutants can be resolved by crossing over into separable sites whose arrangement is consistent with a linear order (CHOVNICK, SCHALET, KERNAGHAN and TALSMA 1962; CHOVNICK, SCHALET, KERNAGHAN and KRAUSS 1964). Additional information concerning the rosy cistron and the region of chromosome 3 adjacent to it has been obtained by the analysis and utilization of rosy mutants associated with chromosomal changes. Such studies lead to the following generalizations: (1) In this region there is only one cistron concerned with xanthine dehydrogenase activity. (2) Mutations limited to the rosy cistron and selected on the basis of a mutant eye color phenotype are viable under routine culture conditions. (3) Lethal effects found with certain rosy mutants have proven to involve lesions outside of the rosy cistron and are associated with adjacent genetic units which are functionally and spatially distinct from the rosy cistron. A brief report of some of these results appeared earlier (SCHALET, KERNAGHAN and CHOVNICK 1963).

MATERIALS AND METHODS—GENERAL

Descriptions of the following markers and rearrangements are given in BRIDGES and BREHME (1944): curled (*cu*); Dichaete marks inversion complex, *ru h DcxF*; Deformed (*Dfd*); karmoisin (*kar*); Lyra (*Ly*); Minute-34 (*M34*); Moiré (*Mé*); Stubble (*Sb*); Ultrabithorax (*Ubx*) described under bithorax-dominant; and Xasta (*Xa*). *Mé, Ins ri Sb*^{1} and *Ubx*^{130} are inversion complexes described by LEWIS (1949, 1952). *In(3)MRS* and lethal (3) *26* (*l26*) are described in CHOVNICK *et al.*, (1962). The positions within the rosy cistron of all viable rosy mutants are given in CHOVNICK *et al.* (1964). The following mutants are described and/or located for the first time in this paper (Figure 1): *kar*^{31}, a lethal allele of kar induced in a *Ly M34 Dfd* chromosome; messy (*mes*), semilethal mutants characterized by bristle and wing abnormalities (see below); piccolo (*pic*), a visible mutant characterized by bristle and abdominal abnormalities (see below); all the rosy-lethal mutants and the unnamed non-rosy lethal mutants of Figure 1.

All of the newly induced rosy and non-rosy mutants reported in this study were obtained by X-raying adult males of laboratory stocks: (1) The 14 viable rosy mutants used in the fine-structure analysis as well as the first 14 rosy mutants with lethal effects listed in Figure 1 were induced in males carrying the chromosome-3 markers *cu* and *kar* mated to females carrying *ry*^{2}. rosy^{66} and *ry*^{70} were induced in Oregon-R males mated to females bearing *In(3)MRS, M34 ry*^{2} *Sb*. (2) In the experiment to detect rosy-like mutants complementary to rosy^{2} both Oregon-R

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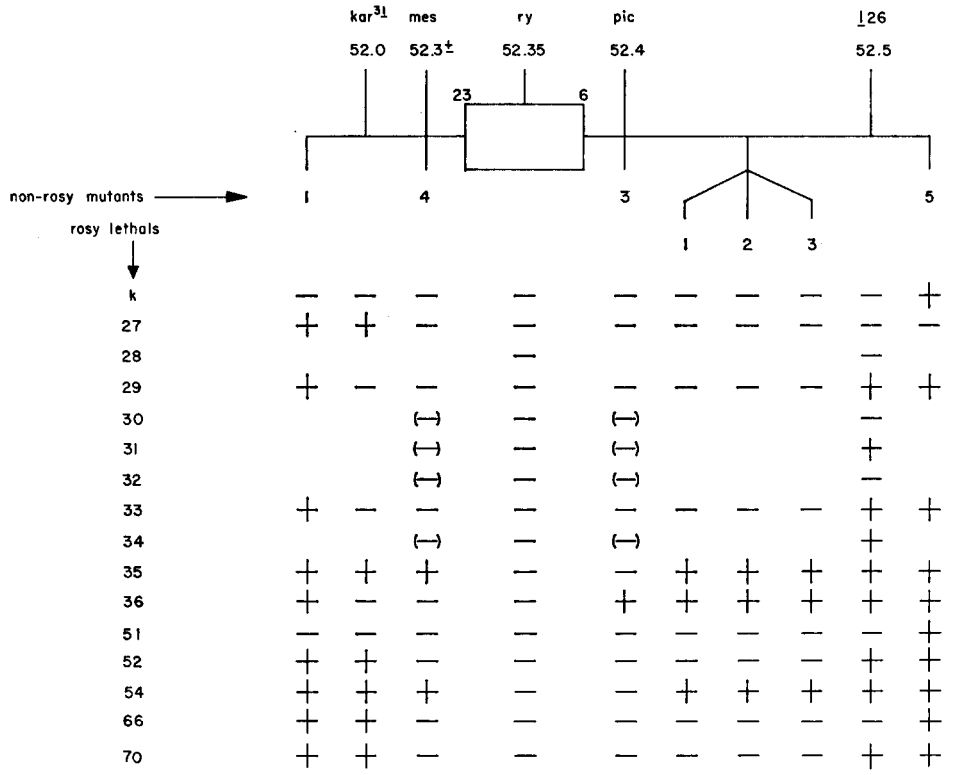

FIGURE 1.—Linkage map and complementation relations in rosy region of chromosome 3. + = normal; − = mutant (lethal or visible); (—) = mutant condition inferred from data given in text.

and *kar*^{2} treated males were mated to females with either *Df(3)ry*^{k} or *Df(3)ry*^{27}. (3) Non-rosy lethal and visible mutants in the vicinity of the rosy cistron arose in treated chromosomes carrying *kar*^{2} and tested with either *Df(3)ry*^{k} or *Df(3)ry*^{27}.

The rosy-lethals in Figure 1 represent the residue of those chromosomes carrying rosy mutants and recessive lethal effects after the elimination of chromosomes in which the lethal effect was easily separated from the rosy phenotype by a cross of the following type: *cu kar ry*^{x}*/Sb Ubx* females × *cu kar ry*^{x}*/Xa* males. When the offspring of such a cross failed to include a viable rosy homozygote, counts of up to a few thousand chromosomes were sufficient to show a marked reduction in crossover values in one or more of the intervals: *cu-kar-ry-Sb*. Most of the rosy-lethal mutants were crossed to one another and to the non-rosy lethal and visible mutants in the vicinity of the rosy cistron. The non-rosy mutants were also crossed to one another. In a few cases, *Df(3)ry*^{k}, *Df(3)ry*^{52} and T(1;3)*ry*^{35}, cytological examinations were made.

RESULTS

Analysis of rosy mutants with lethal effects: Figure 1 summarizes the results obtained in crosses of rosy-lethal mutants to non-rosy mutants in the vicinity of the rosy cistron. Eleven of the 16 rosy-lethal mutants were tested against all nine of the non-rosy genetic units, three of which are located to the left, and six to the right, of the rosy cistron. It is clear that rosy alleles *k*, *27*, *29*, *33*, *51*, *52*, *66* and

70 produce lethal or visible effects in heterozygous combinations with markers both to the left and to the right of rosy. Consequently, both the rosy and non-rosy lethal or visible effects of the rosy-lethal mutants are probably pseudodominant effects of deficiencies. Cytological examinations (RICHMOND, unpublished) of *k* and *52* show the absence of sections including at least part of 87E on BRIDGES' salivary chromosome map. GRELL (1962) has reported that a chromosome lacking *kar* and *ry* appears to be deficient for a small section in 87E.

The chromosome carrying ry^{35} produces a nonmutant phenotype in heterozygous combination with chromosomes carrying each of the non-rosy outside markers with the exception of a single locus, *pic*, located to the right of rosy. $rosy^{35}$ is lethal with each of the three alleles of this locus. Genetic evidence indicates that 35 arose simultaneously with an X–3 translocation in which a piece of chromosome 3 was inserted into the X. Cytological examination by LINDSLEY shows that a segment of chromosome 3 with a left break at 87C–E and a right break at 91B–C on the salivary chromosome map has been inserted into the base of the X chromosome. The mutant eye color and lethal expression of ry^{35} when made heterozygous with chromosomes carrying *ry* and *pic* respectively may be position effects produced by the left breakage point of the X–3 translocation.

The behavior of ry^{54} in crosses to chromosomes carrying outside markers is similar to that of ry^{35} in that mutant phenotypes are produced only with *ry* and *pic* mutants. However ry^{54} in combination with the *pic* alleles results in a visible phenotype which involves the reduction or absence of bristles on the head and/or thorax. Occasionally homozygous ry^{54} survivors appear in our ry^{54}/Xa stock, and these are invariably females which have the mutant bristles phenotype of the ry^{54}/pic heterozygotes. Yet the ry^{54} bearing third chromosome appears to segregate independently from the X chromosome in breeding tests. No conclusive salivary gland examinations of ry^{54} have been made.

$rosy^{36}$ appears normal in heterozygous combinations with all of the outside markers to the right of the rosy cistron and mutant with kar^{31} and mes just to the left of rosy.

The remaining five rosy-lethal chromosomes, *28*, *30*, *31*, *32* and *34* were all lost after they had been tested with *l26*. Of these, *30* and *32* were lethal with *l26*. Each was also lethal when made heterozygous with *35* and *36*. Since subsequent crosses of ry^{35} and ry^{36} to outside markers, as shown above and in the figure, demonstrated that the lethality of ry^{35} is associated with a locus to the right of the rosy cistron and the lethality of ry^{36} is associated with a locus (loci?) to the left of the rosy cistron, it is likely that *30* and *32* were deficiencies extending on either side of the rosy cistron. Although *31* and *34* were viable with *l26*, they were lethal with *35* and *36* and therefore, they too were probably deficiencies which included the rosy cistron. As for *28*, it was lethal with *l26*, but was not tested with *35* or *36*. Consequently, it appears to have a lesion to the right of rosy as well as a rosy mutation.

Evidence for a single cistron in the rosy region concerned with xanthine dehydrogenase activity: In the accompanying paper (CHOVNICK *et al.*, 1964) it is shown that 16 rosy mutants of independent origin, two spontaneous and 14

X-ray induced, have been resolved by recombination into at least six separable sites. The induced mutants were all selected over the spontaneous ry^2 allele. All 16 mutants are members of a single complementation group on the basis of phenotypic tests and all homozygous and heterozygous combinations lack the activity of the enzyme xanthine dehydrogenase as shown by chromatographic tests (CHOVNICK *et al.*, 1962). Furthermore, we have had occasion to make crosses involving at least a dozen additional viable rosy mutants originally selected over ry^2. In none of these combinations, usually with ry^{26} and/or ry^1, have we observed any visible allelic complementation. On the other hand, we may ask whether there is more than one cistron in the rosy region concerned with xanthine dehydrogenase activity.

Adult males from an Oregon-R stock were X-rayed and crossed to females that were genotypically *cu kar Df(3)*ry^{27}*/In(3)MRS, M34* ry^2 *Sb* or *cu Df(3)*ry^k*/ In(3)MRS, M34* ry^2 *Sb*. In another cross, the treated males were kar^2 and were mated to *cu Df(3)*ry^k*/Ubx*^{130} females. From these experiments, we selected F_1 individuals that were rosy-like in phenotype, and had received the deficiency bearing chromosome from the mother. Subsequently, the chromosome bearing the rosy appearing mutant was rendered heterozygous with ry^2. If the region of chromosome 3 adjacent to the rosy cistron contained additional functional units concerned with xanthine dehydrogenase activity, these units would be expected to have mutable sites capable of exhibiting a rosy-like phenotype when they are made heterozygous with a chromosome deficient for these units. Such mutants would complement mutant sites in the rosy cistron. All of the 24 rosy-like mutants isolated in the above manner were noncomplementary when heterozygous with ry^2 and must have been alleles of the rosy cistron.

Analysis of non-rosy mutants in the rosy region: Using chromosomes deficient for the rosy cistron and the regions immediately adjacent, it was possible to identify genetic units which are functionally and spatially distinct from the rosy cistron, and to place more precise limits on the rosy cistron.

The genetic scheme employed is illustrated in Figure 2. Males of the genotype kar^2/kar^2 or *kar*^{2}*/Ubx*^{130} were irradiated and crossed to virgin females, *M34 Dfd kar* ry^{26} *l26/Mé, Ins ri* Sb^1. F_1 males, *kar*^{2}*/M34 Dfd kar* ry^{26} *l26*, were individually crossed to virgin females (1), *In(3)MRS, M34* ry^2 *Sb/cu kar Df(3)*ry^{27} or (2), *In(3)MRS, M34* ry^2 *Sb/cu Df(3)*$/ry^k$.

A. F_2 zygotes carrying the unirradiated paternal chromosome, *M34 Dfd kar* ry^{26} *l26* will fail to survive in combination with either maternally contributed chromosome because they will be homozygous for the recessive lethal of *M34* or they will die because both *Df(3)*ry^{27} and *Df(3)*ry^k include the locus of *l26*.

B. F_2 offspring carrying the irradiated kar^2 chromosome will appear *Sb* with the *MRS* chromosome or *kar* when in combination with either of the deficiency chromosomes (*Df(3)*ry^k is deficient for *kar*).

C. If a recessive lethal mutation is produced within the irradiated chromosomal segment corresponding to the region of the deficiency in the homologous chromosome, there will be no F_2 offspring phenotypically *kar*. Similarly, a recessive visible in the region will appear in all the *kar* offspring.

From these radiation experiments we have obtained at least 24 non-rosy recessive visibles and lethal mutations located within the region uncovered by the ry^k and/or ry^{27} deficiencies. Nineteen of the new mutations have been crossed to one

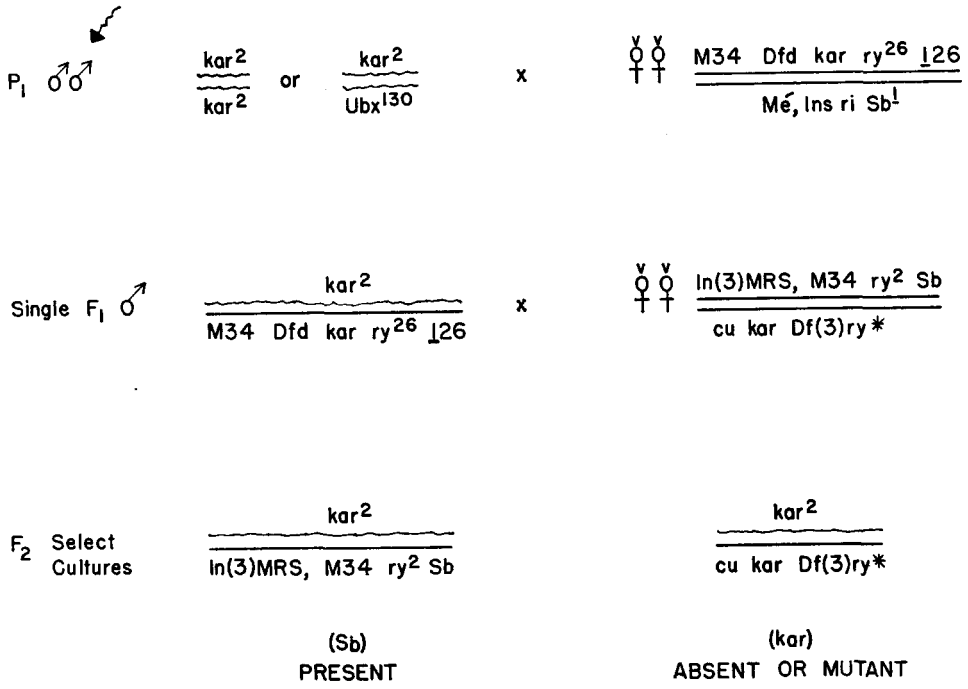

FIGURE 2.—Scheme to detect recessive mutations in the rosy region. The asterisk denotes *Df(3)ry*^{k} or *Df(3)ry*^{27}.

another and to all of the extant rosy-lethal aberration stocks listed in Figure 1. The results of these complementation tests have been consistent so as to place the 19 mutants into seven positions. These are indicated at the top of Figure 1 by the extensions of the vertical lines below the horizontal line. Of special interest are those mutants apparently located immediately to the left and to the right of the rosy cistron.

Located to the left of rosy and to the right of *kar*^{3l} there is a region capable of giving rise to a number of recessive semilethal *mes* mutants. Homozygous *mes* individuals in each of the four mutant stocks are quite similar in phenotype and display to a varying degree an assortment of abnormalities including extra head and thoracic bristles, abnormal wing (shape, size, position), and partial or complete absence of posterior crossveins. When the mutants were crossed to one another, heterozygous combinations ranged from apparently completely normal individuals that were fertile, to one combination which was semilethal yet fertile, and another combination which was definitely mutant in appearance but the females were sterile.

To test whether *mes* is in fact located to the left of the rosy cistron the four mutants were tested against the leftmost rosy site, *ry*^{23}, in the following cross: *kar*^{2} *mes/cu kar ry*^{23} *l26 Sb Ubx* female × *cu kar Df(3)ry*^{27} *Ubx/ru h DcxF* male.

Since *l26* and each of the *mes* mutants are included within the *ry*^{27} deficiency,

the only D^+ survivors of the cross will be the relatively few noncrossover chromosomes carrying *mes* and the even rarer crossovers between *mes* and *l26*. However, the latter class will be phenotypically *cu*, and individuals in which the crossover has occurred between *mes* and ry^{23} will be ry^+ in phenotype. The frequency of crossovers between *mes* and $ry^{23}(D^+$ *cu kar* mes^+ ry^+ *Ubx*)/total (*D*) for each of the four *mes* mutants was: (1)—1/5,900; (2)—1/6,700; (3)—1/4,400; (4) 0/60,000. Progeny tests of each exceptional fly confirmed the observation that the appearance of mes^+ was associated with a crossover between *cu* and *ry*.

To the right of the rosy cistron, between *ry* and *l26* there appear nine mutants (8 lethals and 1 visible) which clearly fall into four functional groups by our complementation tests. Three of these groups, consisting of one, two and three lethals respectively, have not been ordered with respect to one another. As yet we have no rosy deficiencies with a right breakage point between any of the three loci. The fourth functional group consists of one visible mutant, *pic*, which has small, fine bristles and tergite abnormalities similar in appearance to bobbed, and two other alleles which are lethal with one another and with *pic*. As previously mentioned, alleles of the *pic* locus are the only mutants to the right of rosy which fail to complement the nonrosy lethal and visible effects of ry^{35} and ry^{54}. Therefore, we believe that the *pic* locus is the leftmost of the four functional groups between *ry* and *l26*. A recombination test to demonstrate this point was made by crosses of ry^6, the rightmost of the rosy sites to the various *pic* alleles: kar^2 *pic/cu kar* ry^6 *l26 Sb Ubx* female × *cu kar* $Df(3)ry^{52}$/*Ly Dfd kar* ry^{26} *l26* male. Since the *pic* locus is included within $Df(3)ry^{52}$, and each of the *pic* alleles is lethal with the deficiency, offspring which receive the deficiency chromosome from their father and a chromosome from the mother in which a crossover has occurred between ry^6 and *pic* will show a unique phenotype. The frequency of such individuals, Ly^+ Dfd^+ cu^+ *kar* ry^+ *Sb Ubx*, over the total number of survivors in crosses of each of the *pic* alleles was: pic^1—4/4,200; pic^{2l}—3/10,400; and pic^{3l}—1/7,600. In each of the cases where progeny tests were completed, (three exceptional flies of the pic^1 series and the single exception of the pic^{3l} series), the appearance of pic^+ individuals was associated with a crossover between ry^6 and *l26*.

DISCUSSION

The lethal effect of each of the 16 rosy-lethal mutants analyzed can be attributed to a lesion in an adjacent or neighboring cistron. It is probable that no alterations of the rosy cistron (including complete losses) are capable of producing a lethal phenotype. This is not surprising in view of the fact that the mutants used in the fine structure analysis, although completely lacking in xanthine dehydrogenase activity, nevertheless are viable. With the classical example at hand of a single enzyme, tryptophan synthetase in *E. coli*, controlled by two contiguous cistrons (CRAWFORD and YANOFSKY 1958; YANOFSKY and CRAWFORD 1959), we have attempted to elicit the rosy phenotype by selecting for mutations within a small chromosomal region that included the rosy cistron. Good evidence

that there is only one cistron in the rosy region concerned with xanthine dehydrogenase activity, is provided by the failure to complement ry^2 of the 24 rosy-like mutants selected over a deficiency for the rosy cistron and the contiguous chromosomal region.

In fact, the 24 mutants must have been alleles of rosy. They provided additional examples of our failure to detect interallelic complementation between rosy alleles. Such complementation does occur between mutant alleles of another locus concerned with xanthine dehydrogenase activity. GLASSMAN (1959) has shown that two mutants of the X-chromosome locus maroon-like, each of which lacks enzymatic activity and has a mutant eye color, when made heterozygous with one another produce an eye color which approaches the wild type. Actually, very low levels of enzyme activity are sufficient to produce a wild-type eye color (URSPRUNG 1961). Consequently, lesions in the rosy cistron that alter but do not abolish enzyme action, i.e. "leaky" mutants, are undetected by our methods.

If lack of enzyme activity is not lethal, and the presence of some activity produces a wild-type eye, then point mutants selected on a basis other than the eye color phenotype would be expected to be located outside the rosy cistron. The analysis of the non-rosy mutants found in the rosy region of chromosome 3 bears out this expectation. Complementation tests had indicated that *pic* was the closest of the newly detected loci lying to the right of the rosy cistron. Recombination tests of each of the three *pic* alleles places them to the right of the rightmost rosy site, ry^6. A similar methodology leads us to place the *mes* locus to the left of the leftmost site in the rosy cistron, ry^{23}. However, one of the *mes* alleles did fail to yield a crossover between *mes* and ry^{23}. If the average frequency of crossovers between *mes* and ry^{23}, 1/5,600, found among the progeny in the crosses involving the other *mes* alleles is representative of the interval between *mes* and ry^{23}, then the failure is significant. Moreover, other crosses using the "aberrant" *mes* mutant show no apparent reduction in crossing over in the *kar* to *ry* interval, and we have even obtained crossovers between this *mes* mutant and ry^2. Since the functional relationships among the *mes* mutants are not simple (see above) and the spatial relationships have not been investigated, we do not consider this crossover failure to prejudice our conclusion that *mes* is functionally and spatially removed from the rosy cistron.

All of the evidence cited above and in CHOVNICK *et al.*, (1964) strengthens the conclusion that the rosy locus is a single functional unit quite comparable to the cistrons of microorganisms. We contrast the rosy locus with other complex loci in Drosophila where we agree with LEWIS (1963) "that it seems best . . . to consider a pseudoallelic series as a set of cistrons rather than one cistron." The selective methods we have employed have proven sufficiently sensitive to resolve the closest known separable sites in Drosophila, and to demonstrate with some assurance the occurrence of an intracistronic rearrangement. Further refinement of the selective procedures may provide tools with which more meaningful answers can be obtained concerning the nature and mechanism of spontaneous and induced forward and reverse gene mutations in Drosophila.

SUMMARY

Studies of 73 mutants with genetic changes involving the rosy cistron, as judged by their eye color phenotype, and 24 non-rosy visible or lethal mutants selected within a small chromosome segment including the rosy cistron, permit the following generalizations: There is only one cistron concerned with xanthine dehydrogenase activity in this region. There is no allelic complementation in the rosy cistron. Mutations restricted to the rosy cistron are viable under routine conditions. Lethal effects found with certain rosy mutants involve lesions within adjacent genetic units which are functionally and spatially distinct from the rosy cistron.

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