

Studies on genetic organization in higher organisms

III. Confirmation of the single cistron-allele complementation model of organization of the maroon-like region of *Drosophila melanogaster**

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SUMMARY

A genetic fine-structure experiment on *Drosophila melanogaster* is described, which makes use of a lethal selective system which permits survival only of recombinants in the *ma-l* region, including exchanges between site mutants of *ma-l*. On the basis of prior mapping information, the experiment was designed to select for *ma-l* double mutant recombinants. Utilizing a pair of complementing *ma-l* mutants, double mutant meiotic products were recovered whose complementation properties provide independent support for the single-cistron, allele complementation model of organization of the maroon-like region inferred from other experiments described earlier.

The maroon-like mutants (*ma-l*:1-64.8) of *Drosophila melanogaster* have been under investigation as a model system for the study of a complex gene. In addition to a brownish mutant eye colour phenotype, *ma-l* mutant homozygotes and hemizygotes are characterized by the absence of activity of xanthine dehydrogenase (Glassman & Mitchell, 1959), pyridoxal oxidase (Forrest, Hanly & Lagowski, 1961), and aldehyde oxidase (Courtright, 1967), three enzyme activities which appear to be associated with distinct molecular species (Glassman, 1965; Courtright, 1967). Although several lines of evidence relate the eye colour defect solely to xanthine dehydrogenase, the relationship between these phenotypes is incompletely understood (Chovnick & Sang, 1968).

Investigation (Chovnick *et al.* 1969) of a large group of *ma-l* mutants induced on the X chromosome or a Y-borne duplication of the proximal region of the X chromosome led to their classification into two categories: (1) A group of lethal *ma-l* mutants were shown to be deficiencies which extended into the *ma-l* region. (2) A set of 19 fully viable *ma-l* mutants which fell into five complementation groups on the basis of eye colour and xanthine dehydrogenase complementation in mutant heterozygotes (Table 1). All lethal *ma-l* mutants behave as Group I non-complementers. Three possible models for the organization of this complex gene were suggested by the complementation pattern.

(1) The complementation may be taken to reflect functional distinction, and the existence of several cistrons concerned with separate steps in the production of this phenotype. Following this model, Groups III, IV and V mutants (Table 1) represent mutations in each of three adjacent cistrons which are transcribed separately. This model requires that the viable Group I mutants be deletions extending into all three cistrons, while the Group II mutants must be deletions involving two adjacent members of the cluster of three cistrons.

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(2) A variant of the first model would consider the *ma-l* region to consist of several adjacent cistrons which are cotranscribed to produce a polycistronic message. It differs from the first model in that it predicts that the viable Group I and II mutants would include site mutants as well as deficiencies, the site mutants reflecting direction of transcription and translation. Thus, Group II site mutants would be polar mutants located in the second cistron, while the Group I site mutants might include lesions at sites concerned with regulation or initiation of transcription as well as polar translational mutants in the first cistron.

Table 1. *The complementation map of fully viable maroon-like mutants*

Group III		Group IV		Group V
Group II				
Group I				
Group I	Group II	Group III	Group IV	Group V
<i>ma-l</i> ^{F2}	<i>ma-l</i> ²	<i>ma-l</i> ^{F1}	<i>ma-l</i> ¹	<i>ma-l</i> ^{F3}
<i>ma-l</i> ¹⁴	<i>ma-l</i> ²¹	<i>ma-l</i> ^{F4}		
<i>ma-l</i> ²⁰	<i>ma-l</i> ²⁶	<i>y⁺Yma-l</i> ¹¹⁶		
<i>ma-l</i> ²³	<i>ma-l</i> ²⁷			
<i>ma-l</i> ²⁴	<i>ma-l</i> ³⁰			
<i>ma-l</i> ²⁵				
<i>ma-l</i> ²⁸				
<i>ma-l</i> ²⁹				
<i>y⁺Yma-l</i> ¹⁰⁶				

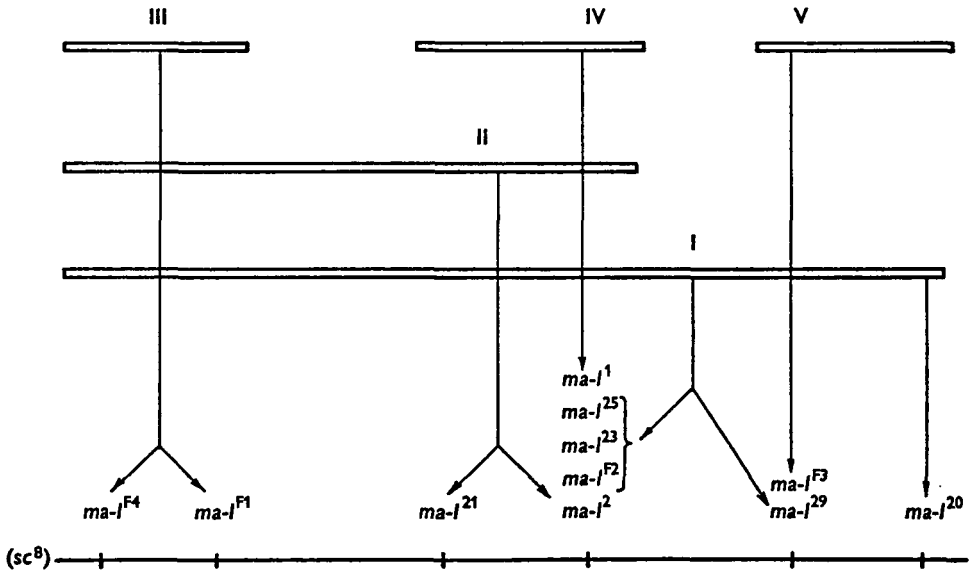


Fig. 1. Co-linearity of genetic and complementation maps.

(3) A third model would consider maroon-like as a single cistron exhibiting allele complementation. The model argues that maroon-like is a structural gene whose biologically active product is a dimer or higher multiple aggregate of a single polypeptide.

Complementation between different mutants then would reflect the production of hybrid aggregates which possess some biological activity.

Mutants of *ma-l*, representative of the five known complementation classes, were subjected to fine structure mapping experiments utilizing a nutritional selective procedure which permits the survival of rare *ma-l*⁺ progeny from large scale crosses (Finnerty, Duck & Chovnick, 1970). A summary of the genetic map and its relationship to the complementation map is presented in Fig. 1. It should be noted that the genetic map presents an inverted order of *ma-l* mutant sites, which merely reflects the fact that the fine structure experiments were carried out on *In(1)sc*⁸ and *In(1)sc*^{S1Lsc^{8R}} chromosomes. Two points from this study are pertinent to the question concerning the genetic organization of the *ma-l* region. (1) Groups III, IV and V mutants map as site mutants in an order consistent with the complementation map, thus permitting the possibility that they are mutants in adjacent cistrons. (2) The tested Group I and Group II mutants map in positions consistent with the polar nature of their complementation, and the recombination data serve to eliminate Model 1 which would require that all of the Group I and II mutants be deletions.

While the fine structure analysis is unable to distinguish between Models 2 and 3, strong support of the single cistron-allele complementation model emerged from quantitative examination of xanthine dehydrogenase and pyridoxal oxidase activities in various mutant heterozygote combinations. Moreover, experiments which examined enzyme activity levels in genotypes carrying three doses of various specific *ma-l* mutant combinations provided striking support for the single cistron model (Chovnick *et al.* 1969). The present report describes an experiment which derives from the fine structure analysis, and which provides an independent confirmation of the single cistron-allele complementation model.

Consider the mutants *ma-l*^{F4} (Group III), *ma-l*¹ (Group IV) and *ma-l*^{F3} (Group V) which complement in all single mutant combinations. If it were possible to recover a double mutant (i.e. *ma-l*^{F4} *ma-l*^{F3}) one would expect that such a double mutant chromosome would be mutant in heterozygotes with *ma-l*^{F4} or *ma-l*^{F3}. However, consider the heterozygote *ma-l*^{F4} *ma-l*^{F3}/*ma-l*¹. On the three-cistron model, one would expect complementation, since we would be dealing with a triple heterozygote involving three different cistrons. In contrast, the allele complementation model forces no prediction of complementation. Indeed, one might reasonably expect that a doubly altered polypeptide, such as that produced by the double mutant, *ma-l*^{F4} *ma-l*^{F3}, might not produce active hybrid aggregates with the *ma-l*¹ product. Following this logic, a large scale recombination experiment was carried out designed to recover double mutant recombinants. The experiment makes use of a lethal selective system which selects for survival only male progeny of certain crossover classes involving the *ma-l* region, and assumes the correctness of the map order of *ma-l* sites established earlier (Fig. 1). Females of the genotype

$$y\ sc^{S1}\ l54\ ma-l^{F3}\ l-t2-4a^+\ f^+\ sc^8/y\ sc^{S1}\ l54^+\ ma-l^{F4}\ l-t2-4a\ f\ sc^8;$$

$$Ubx^{130}/In(3)MRS,M34\ ry^2\ Sb$$

were crossed on standard *Drosophila* medium to males carrying a *sc*⁸*Y* and *T(1;3)9*, a hitherto unreported translocation involving an unmarked chromosome 1, and a chromosome 3 carrying the right arm markers *l26 Sb Ubx*. The mutant, *l54* is a hitherto undescribed lethal located to the right of *ma-l* (standard order), and behaves as a deficiency for the region from *glufal-2* through *l114* inclusive. All other mutants and chromosomes used in this experiment are described elsewhere (Lindsley & Grell, 1967; Schalet & Finnerty, 1968; Chovnick *et al.* 1969). In order to increase recombination in the *ma-l* region, the experiment was carried out on the *In(1)sc*^{S1Lsc^{8R}} chromosome which moves

the *ma-l* region from a position proximal to the centromere to the distal end of the X chromosome.

Most of the progeny of this cross die during development due to: (1) Chromosomal imbalance due to the translocation present in the male parent. (2) All chromosomally balanced female zygotes die due to homozygous lethality of the third chromosome marker *Sb* or the lethality of *Ubx/Ubx*¹³⁰. (3) In the absence of recombination in the short region from *l54* to *l-t2-4a*, all male zygotes die. Three recombination classes of *ma-l* mutant male progeny are expected to survive: (1) crossovers between *l54* and the *ma-l* region should survive and carry the allele, *ma-l*^{F3}. (2) Crossovers between the *ma-l* region and *l-t2-4a* should survive, and these will carry the allele *ma-l*^{F4}. (3) Crossovers between the *ma-l* site mutants will be double mutants, *ma-l*^{F4} *ma-l*^{F3}. From this cross, 1778 *ma-l* mutant male progeny were recovered and successfully reproduced. They were mated singly to tester females of the genotype,

$$y\ sc^{S1}\ ma-l^1\ l-t2-4a\ f\ sc^8/yIn(1)49,sn^{X2}\ In(1)B^{M1},\ Df(1)ma-l^6.$$

In addition to testing each surviving *ma-l* mutant X chromosome for complementation in heterozygotes with *ma-l*¹, this cross retests for the presence of the adjacent markers *l-2t-4a* and *l54*. With the exception of four tests, all of the *ma-l* bearing chromosomes showed complementation with *ma-l*¹, as expected for *ma-l*^{F4} and *ma-l*^{F3}. The four exceptions which did not complement *ma-l*¹ were confirmed to be double mutants, *ma-l*^{F4} *ma-l*^{F3} by their failure in retest to complement *ma-l*^{F4} or *ma-l*^{F3} as well as *ma-l*¹. Moreover, in subsequent complementation tests they behaved as Group I non-complementers.

In addition to providing successful recovery of double mutant recombinant chromosomes, this experiment provides independent support for the allele complementation model inferred from other experiments described earlier (Chovnick *et al.* 1969). It should be noted that the experimental system described in the present report is easily adapted for study of genetic fine structure of any sex-linked gene in *Drosophila melanogaster*. Thus, given a pair of sex-linked recessive alleles, *a*¹ and *a*², with closely linked flanking recessive lethals *l*¹ and *l*², females of the constitution,

$$l^1\ a^1\ +\ +\ a^2\ l^2;\ Ubx^{130}/In(3)MRS, M34\ ry^2\ Sb$$

mated to males carrying *T(1,3) 9* will produce only male progeny whose X chromosomes are recombinants and free of the lethals. In order to maintain such lethal bearing X chromosomes in stock males, a duplication for the region is required. However, simple procedures for the construction of such duplications for any region of the X-chromosome of *Drosophila melanogaster* are available (Brosseau, Nicoletti, Grell & Lindsley, 1961).

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