CYTOGENETIC ANALYSIS OF THE *cSOD* MICROREGION IN *DROSOPHILA MELANOGASTER*

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ABSTRACT

This report describes the genetic organization of a euchromatic region on the third chromosome of *Drosophila melanogaster* extending cytologically from 68A2 to C1, an interval comprising 10 or 11 polytene chromosome bands. The gene for cytoplasmic superoxide dismutase (*cSOD*) maps within this interval, as does low xanthine dehydrogenase (*lxd*).—Recessive lethal mutations were generated within the region by ethyl methanesulfonate mutagenesis and by hybrid dysgenesis. These lethals fall into 11 functional groups, which were partially ordered by complementation with deletions having breakpoints within the region. The distribution of dysgenesis-induced mutations in the region is highly nonrandom, the majority being within a single group. The mutability of this gene is comparable to that of *singed* (*sn*), a documented “hot-spot” for *P*-element insertion.—One of the EMS-induced lethals, *l-108*, fulfills biochemical criteria expected of a hypomorphic allele of *cSOD*. To our knowledge this is the first such allele recovered of this gene, and it should prove very useful in an analysis of the *in vivo* function of cytoplasmic SOD. Indeed, it has been demonstrated that *cSOD* is almost certainly a vital gene.

THE superoxide dismutases (SOD) are metalloenzymes that catalyse the dismutation of superoxide radicals generated by a number of metabolic reactions involving molecular oxygen (reviewed by FRIDOVICH 1978) and by the interaction of ionizing radiation with water (VAN HEMMEN and MEULING 1975). Numerous observations suggest that these enzymes, which are ubiquitous among aerotolerant organisms, protect cells from the toxic effects of superoxide radicals. For example, increased levels of SOD activity are observed in a number of prokaryotes and lower eukaryotes after exposure to either hyperbaric oxygen or the radical-generating compounds paraquat and streptonigrin (reviewed by FRIDOVICH 1983). Further, the expression of SOD activity in the facultative anaerobe *Saccharomyces cerevisiae* is directly dependent on growth in aerobic conditions (GREGORY, GOSCIN and FRIDOVICH 1974). Al-

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though it is apparent that the superoxide dismutases play an important role in the metabolism of superoxide radicals, what is not apparent is the full variety and nature of the physiological roles of these enzymes, as well as the basic genetic mechanisms that control their synthesis, activity and cellular functions. To investigate phenomena such as these, we have embarked on a genetic investigation of the superoxide dismutases in *Drosophila melanogaster*.

As in most eukaryotes, *D. melanogaster* has different forms of superoxide dismutase associated with the mitochondria (mSOD) and the cytosol (cSOD) (LEE, AYALA and MISRA 1981). *Drosophila* cSOD is a homodimer of 32 kilodaltons that constitutes approximately 0.4% of the total soluble protein of adults (LEE, AYALA and MISRA 1981). Two allozyme variants of cSOD, which differ in electrophoretic mobility, thermostability and specific activity, are found in natural populations (AYALA, POWELL and DOBZHANSKY 1971; LEE, MISRA and AYALA 1981).

This report describes the analysis of a chromosomal region, hereafter referred to as the *cSOD* microregion, containing the *D. melanogaster cSOD* structural gene. Recessive lethal mutations induced by ethyl methanesulfonate (EMS) and by hybrid dysgenesis (HD) were recovered and characterized by cytological, genetic and enzymatic analyses. Our objectives were to (1) identify the vital genetic functions in the immediate vicinity of the *cSOD* gene, (2) recover hypomorphic or amorphic alleles of *cSOD* with which to investigate the physiological functions of cSOD and (3) recover *P*-element insertions in the *cSOD* microregion that could be used in a molecular characterization of the *cSOD* gene by the approach discussed by BINGHAM, LEVIS and RUBIN (1981).

MATERIALS AND METHODS

Generation of recessive lethals in the *cSOD* microregion by EMS: Males homozygous for the third chromosome markers *sr e^s ca* were mutagenized with EMS according to the method of LEWIS and BACHER (1968). Further information on genetic markers not described for the first time in this report may be found in LINDSLEY and GRELL (1968), with the exception of deletion stocks provided by V. FINNERTY. Treated males were mated to *Df(3L)lxd-9/In(3LR)TM3*, *Sb Ser ri p^b sep bx^{34e} e^s* females. *Df(3L)lxd-9* uncovers an interval of 10 or 11 polytene chromosome bands, within which map *cSOD* and *lxd* (V. FINNERTY, personal communication). Male F₁ progeny heterozygous for the treated paternal chromosome and the *TM3* balancer chromosome were mated singly to *Df(3L)lxd-9/TM3* females. F₂ cultures in which all progeny were *Sb Ser* (or in which the numbers of *Sb⁺Ser⁺* progeny were significantly less than one-third) were scored as putative lethal (or semilethal) alleles of *Df(3L)lxd-9*. In such cultures, the presence of the *e^s* marker carried by the treated chromosome permitted the establishment of a stock heterozygous for the putative lethal-bearing chromosome and *TM3*.

Putative lethals were subsequently tested for complementation with *Df(3L)vin-2*, *e*, a larger deletion encompassing the *cSOD* microregion as defined by *Df(3L)lxd⁹*, to confirm that lethality was specific to the region. Lethals that did complement *Df(3L)vin-2*, *e* were presumed to be lethal alleles of secondary mutations accumulated by the *Df(3L)lxd-9* chromosome. Such mutations are known to accumulate on permanently heterozygous autosomes (MUKAI 1964), and alleles have been recovered in other screens involving specific deletion-bearing chromosomes (e.g., HILLIKER 1976). Putative semilethal alleles of *Df(3L)lxd-9* were tested for complementation with *Df(3L)lxd-2* (another deletion en-

compassing the *cSOD* microregion) to determine whether or not they were specific to the *cSOD* microregion.

EMS-induced lethals specific to the *cSOD* microregion were tested for complementation in all *inter se* combinations. Since all lethal-bearing chromosomes were maintained as balanced stocks with the *TM3* balancer chromosome, complementation is indicated by the presence of *Sb⁺Ser⁺* progeny in a testcross. All progeny were scored before noncomplementation was inferred. In cases where there was reason to doubt the interpretation of a particular cross due to low progeny numbers (<50), the cross was repeated. All experiments were performed at 22–26°, and flies were grown on standard cornmeal-agar-yeast-sucrose-dextrose medium.

Generation of recessive lethals by hybrid dysgenesis: Several *P* strains (Cranston, Kerbinou and Harwich) and two *M* strains (Canton-S and Ottawa), all provided by M. G. KIDWELL, were tested by assaying *F*₁ hybrid female sterility to ascertain which *P-M* combination would show maximal dysgenic sterility. The combination of Kerbinou (*P* strain) and Canton-S (*M* strain) gave consistently high levels of dysgenic sterility; therefore, these stocks were used for the generation of HD-induced lethals. Dysgenic hybrids were produced by crossing Kerbinou (*P* strain) males with Canton-S (*M* strain) females *en masse*. *F*₁ hybrid females were mated *en masse* with *In(3L)P+In(3R)C Sb Tb/Ubx*¹⁰⁹ males. Single dysgenic third chromosomes were recovered from this cross, the maternal or paternal origin of which could not be distinguished because the stocks used to generate dysgenic hybrids were not marked. Male progeny heterozygous for a treated chromosome and *In(3L)P+In(3R)C Sb Tb* were singly mated to *Df(3L)lxd-9/TM3* females. Cultures in which all progeny had an *Sb* phenotype were scored as putative lethal alleles of *Df(3L)lxd-9*. Several *Sb Ser* male progeny from such cultures were then tested for complementation with *Df(3L)vin-2, e/TM3*. This cross simultaneously established whether putative lethal-bearing chromosomes were specific to the *cSOD* microregion, and it permitted the establishment of a balanced stock heterozygous for the lethal-bearing chromosome and the *TM3* balancer chromosome.

Initially, all lethal alleles of the *cSOD* microregion generated by hybrid dysgenesis were to be tested for complementation in all *inter se* combinations. After several hundred crosses had been performed, however, it became apparent that most lethals fell into a single complementation group. Thereafter, all members of this class of lethals were tested against all other HD-induced lethal alleles, but all *inter se* combinations within the class were not tested. Representatives of each complementation group identified were later tested for complementation with all EMS-induced lethals.

Superoxide dismutase assay: All lethal-bearing chromosomes were derived from stocks that carried the *cSOD^F* allele (AYALA, POWELL and DOBZHANSKY 1971), as does the *TM3* balancer chromosome (data not shown). Balanced lethal stocks to be tested for cSOD activity were crossed with a stock that is homozygous for the *cSOD^S* allele, and *Sb⁺Ser⁺* progeny were assayed for SOD as described below. Flies (10–20) were homogenized in distilled water (2 µl per fly), and the homogenate was microfuged for 2 min to pellet particulate matter. Supernatant (10–15 µl) was applied on a paper wick to starch gels made with 11% starch (Sigma electrophoresis grade) and 8% sucrose in a 20 mM Tris, 6 mM citrate, 0.2 mM EDTA buffer (pH 7.0). Gels were run in buffer comprised of 200 mM Tris, 60 mM citrate, 2 mM EDTA (pH 7.0) at 200 V (30–35 mA) for 5–6 h in a 4° cold room, during which time they were also cooled by circulating ice water. After electrophoresis, the gel was cut out of the apparatus and was sliced horizontally. SOD activity was assayed according to the procedure of BEAUCHAMP and FRIDOVICH (1971), staining first in 2.45 mM Nitroblue tetrazolium (Sigma), then in 28 mM TEMED-0.028 mM riboflavin (Sigma) in 36 mM potassium phosphate buffer (pH 7.8). Regions of the gel containing SOD activity remain white in an otherwise purple background, which develops after exposure to a 150-watt tungsten light at 10 cm for 15–25 min.

Flies which are heterozygous for the two allozyme variants of *cSOD* show three strong bands, corresponding to dimers composed of fast/fast, slow/fast and slow/slow subunits.

Null alleles of *cSOD* in heterozygous combination with a tester chromosome show only one strong band corresponding to the allele carried by the tester.

A solution assay for total SOD activity in crude fly homogenates (McCord and Fridovich 1969) was used to compare the *cSOD*⁺ genotype (Canton-S) with *cSOD* mutant genotypes. Adult flies were homogenized in 50 mM potassium phosphate, 0.1 mM EDTA (pH 7.8) and were then microfuged for 2 min to pellet cell debris. Supernatant was added to a reaction mixture consisting of the same buffer plus 50 mM xanthine and 10 mM cytochrome C (Type III Sigma). The addition of xanthine oxidase (Boehringer Mannheim) to this reaction mixture generates O₂⁻ radicals that reduce ferricytochrome C to ferrocycytochrome C, as measured by an increase in absorbance at 550 nm. The decrease in rate of cytochrome C reduction relative to reactions with no fly extract is used to calculate the units of SOD activity (McCord and Fridovich 1969). Total protein content of fly extracts was determined by the method of (Lowry 1951), using bovine serum albumin as a standard.

Rosy phenocopy assay for new alleles of *lxd*: The low xanthine dehydrogenase gene (*lxd*) is characterized by lowered levels of xanthine dehydrogenase (XDH) activity. Flies which are homozygous or hemizygous for *lxd* phenocopy *rosy* (*ry*) when grown on allopurinol (HPP) containing medium (Keller and Glassman 1965). It was determined empirically that *lxd* hemizygotes could reliably be identified on this basis at a concentration of 10 mg of HPP (Sigma)/100 ml of medium.

Lethal-bearing stocks were crossed with a stock homozygous for *ru lxd by* to generate progeny heterozygous for *lxd* and the lethal-bearing chromosome. Allelism with *lxd* was indicated by such progeny phenocopying *rosy* when raised on medium containing HPP.

Cytological analysis of deficiencies: Genetically defined deficiencies recovered in this study were crossed with a stock homozygous for the larval marker *red*. F₁ Sb⁺Ser⁺ progeny were backcrossed to *red* to facilitate selection of the desired heterozygote for analysis. Larvae were raised under normal conditions on standard cornmeal medium. Polytene chromosomes were examined from salivary glands that were dissected in 45% acetic acid and stained in 2% orcein in lactoacetic acid. Temporary squash preparations were examined on a Zeiss microscope equipped with phase contrast optics.

RESULTS

A total of 28 new EMS-induced lethals and 5 semilethals of the *SOD* micro-region were identified on the basis of failure to complement both *Df(3L)lxd-9* and *Df(3L)vin-2*, *e* from the total of 3496 chromosomes tested. An additional 13 putative second-site lethals were recovered that failed to complement *Df(3L)lxd-9* but did complement *Df(3L)vin-2*. A total of 73 HD-induced lethals were identified by the same criteria from a total of 4741 chromosomes tested. No HD-induced semilethals were recovered, and only one putative second-site lethal that complemented *Df(3L)vin-2* was recovered.

Complementation analysis of EMS and HD lethals: Figure 1 summarizes the data compiled from the complementation analysis of *cSOD* microregion lethals. The 28 EMS-induced lethals were divided into ten groups by *inter se* complementation. One lethal-bearing chromosome *Df(3L)l-3* failed to complement five of the ten groups, by which criterion it was classified as a deletion, and was confirmed as such by cytological analysis.

The 73 HD-induced lethal alleles of the *cSOD* microregion were divided by complementation analysis into four single-site groups. Five lethals failed to complement members of more than one group, by which criterion they were classified as deletions. These deletions will be discussed in more detail later. A highly nonrandom distribution of HD-induced lethals among the four single-

FIGURE 1.—Complementation map of the *cSOD* microregion. Region 68A2 to C1 of polytene chromosome 3L is redrawn after BRIDGES (1941). Complementation groups within brackets were not separated by deletion breakpoints. Each group is represented by the indicated number of EMS- and HD-induced alleles. The extent of the deletions was assessed by genetic and cytological analysis. The complementation data are ambiguous with respect to the relative positioning of group H; although it is placed to the left of the region in this map, it may well lie to the right.

site groups were observed. One group (A) comprises 85% of all HD-lethal alleles recovered, and the remainder were distributed between three of the nine remaining groups or were identified as deletions.

All EMS-induced lethal alleles were tested for complementation with representatives of all HD-induced lethal groups to establish the identity of alleles of common groups. All EMS-induced lethal alleles were also tested for complementation with all HD-induced lethal alleles were also tested for complementation with all HD-induced deletions. By this means breakpoints of the new deletions which fell within the *cSOD* microregion were genetically defined. The recovery of deletions with breakpoints within the *cSOD* microregion has allowed the partial ordering of the lethal complementation groups. Adjacent complementation groups illustrated within brackets in Figure 1 have not been separated from each other by a deletion, hence their relative order is unknown. Although the phenomenon of interallelic complementation can confuse attempts to define adjacent nonallelic complementation groups (FINNERTY 1976; HILLIKER 1976), we assume, in the absence of evidence to the contrary, that each complementation group identified does, in fact, represent a separate genetic unit. Each complementation group identified in this study will now be discussed separately with reference to the alleles available.

Complementation group H has one EMS-induced allele, *l-104*, which fails to

complement *Df(3L)h-1* and is separated from all other complementation groups in the *SOD* microregion by *Df(3L)h-2*, *Df(3L)h-5* and *Df(3L)h-8*.

Complementation group D is defined by two HD-induced alleles, *h-9* and *h-23*, which completely fail to complement each other and four EMS-induced alleles, *l-55*, *l-57*, *l-92*, *l-97*, all heterozygous combinations of which also fail to complement each other. Lethal allele *h-9* fails to complement all EMS-induced group D alleles, whereas *h-23* fails to complement *l-57* and *l-97*, but does complement *l-55* and *l-92*. Interallelic complementation would seem to be the most likely explanation of this data. To our knowledge there have been no previously published reports of interallelic complementation involving HD-induced mutations. Unlike other group D alleles, all of which were tested, lethal *h-9* has been identified by *rosy* phenocopy (see MATERIALS AND METHODS) as deficient for the *lxd*⁺ function. Thus, *h-9* may be a small deletion extending from *lxd* to the group D locus (or, alternatively, group D may be a complex locus displaying interallelic complementation with regards to both lethal and visible phenotype). Every allele of group D fails to complement *Df(3L)h-1*, *Df(3L)h-5* and *Df(3L)h-8*, all of which uncover *lxd*, as defined by the *rosy* phenocopy test.

Complementation group J is defined by one EMS-induced allele, *l-58*.

Complementation group G (designated as *cSOD* in Figure 1) is represented by one EMS-induced allele, *l-108*. In heterozygous combination with *Df(3L)h-2*, *Df(3L)h-5*, *Df(3L)h-76* and *Df(3L)h-8*, less than 10% of the expected *Sb*⁺*Ser*⁺ progeny are recovered. These progeny are very weak and usually die within a few hours of eclosion, although a few individuals occasionally survive longer. It also was noted that this class of progeny was associated with slightly uplifted wings. The majority of such *l-108*/deficiency heterozygotes progressed through metamorphosis only to die during eclosion. The gross morphology of these imagoes appeared normal when such pupae were dissected. Lethal *l-108* has been identified biochemically as an allele of *cSOD* and will be discussed in more detail presently.

Complementation group E has four EMS-induced alleles, *l-1*, *l-13*, *l-99* and *l-101*, all heterozygous combinations of which are completely lethal.

Complementation group A is defined by a total of 64 HD-induced alleles, of which 677 heterozygous combinations tested were completely lethal. Group A is also represented by two EMS-induced lethals, *l-4* and *l-23*, which fail to complement each other and are lethal in heterozygous combinations with four representative HD-induced alleles.

Complementation group B is defined by three HD-induced alleles, *h-11*, *h-14* and *h-19* and four EMS-induced alleles, *l-46*, *l-98*, *l-100* and *l-106*, all heterozygous combinations of which are completely lethal.

Complementation group I has two EMS-induced alleles, *l-41* and *l-53*, which completely fail to complement each other.

Complementation group F has four EMS-induced alleles, *l-17*, *l-44*, *l-103* and *l-105*, all of which fail to complement each other.

Complementation group C is defined by one HD-induced allele, *h-7*, and four EMS-induced alleles, *l-8*, *l-25*, *l-35* and *l-107*, all heterozygous combina-

FIGURE 2.—Starch electropherogram of representative EMS-induced lethals. Matched crude extracts of flies heterozygous for *cSOD* microregion lethals and a *cSOD^S* tester were assayed. Lethal alleles were tested in the following order: a, *l-4* (group A); b, *l-46* (group B); c, *l-25* (group C); d, *l-55* (group D); e, *l-1* (group E); f, *l-17* (group F); g, *l-108* (group G); h, *l-104* (group H); i, *l-53* (group I); and j, *l-58* (group J).

FIGURE 3.—Starch electropherogram of *cSOD* microregion deletions. Matched crude extracts of flies heterozygous for a deletion and a *cSOD^S* tester were assayed. Deletions were tested in the following order: a, *Df(3L)l-3*; b, *Df(3L)h-76*; c, *Df(3L)h-8*; d, *Df(3L)h-5*; e, *Df(3L)h-2*; and f, *Df(3L)h-1*.

tions of which are completely lethal. Alleles of group C are separated from the cluster of four adjacent complementation groups by *Df(3L)h-2*.

Analysis of *cSOD*: Representatives of all complementation groups identified in the *cSOD* microregion and all new deletions were assayed for SOD activity by starch gel electrophoresis using a chromosome bearing the *cSOD^S* allele as a tester. Mutations of *cSOD* are identified by their failure to show the expected heterozygote enzyme pattern. Photographs of starch gels in which representative EMS-induced lethals and new deletions were assayed for *cSOD* activity are shown in Figures 2 and 3, respectively. Of the ten complementation groups with EMS-induced alleles shown in Figure 2, only one group, represented by a single allele, *l-108*, fails to show the expected heterozygote pattern, indicating that this mutant is an allele of *cSOD*. The demonstration that *l-108* causes no *trans*-acting reduction in *cSOD^S* activity argues against the possibility of it being a *trans*-acting regulatory mutant. All new deletions recovered except *Df(3L)l-3* were scored as *cSOD* null, as shown in Figure 3. It is significant that *Df(3L)l-3* was the only deletion that was not allelic with *l-108* by genetic criteria.

FIGURE 4.—Starch electropherogram of *l-108/h8* and *l-108/h5* heterozygotes with no detectable *cSOD* activity. Rare *trans*-heterozygote progeny (identified by *Sb*⁺*Ser*⁺ phenotype), were assayed in lane (b) *l-108/h5* and lane (c) *l-108/h8*, while a *cSOD*^S/*cSOD*^F control was run in lanes (a) and (d).

Df(3L)h-2, *Df(3L)h-5*, *Df(3L)h-76* and *Df(3L)h-8* are able to weakly complement *l-108*. *cSOD* activity in such hemizygotes is undetectable, as illustrated in Figure 4, an observation that was repeated several times.

A solution assay for total SOD activity was used to compare crude extracts from the *l-108* hemizygotes with wild type (Canton-S), as described in MATERIALS AND METHODS. The specific activity of such extracts was found to be quite variable, but in 17 of 17 determinations from two independent sets of paired extracts, there was 30–40% lower total SOD activity in the *l-108* hemizygotes relative to wild type (data not shown). If one assumed that there is no real difference in total SOD activity between these genotypes, the probability that the *l-108/h-76* genotype would have a consistently lower SOD activity than Canton S is $1/2^{17}$ or 0.00001 as assessed by application of the nonparametric sign test.

Cytology of *cSOD* microregion deficiencies: As had been determined earlier (V. FINNERTY, personal communication), *Df(3L)lxd-9* is deficient for the region extending from 68A2 to 68C1 exclusive. *Df(3L)h-1* resembles *Df(3L)lxd-9* both genetically and cytologically.

Df(3L)h-5 and *Df(3L)h-8* resemble *Df(3L)lxd-9* cytologically, but are distinct from it by complementation analysis (neither includes group H).

Df(3L)l-3 (EMS-induced) was found to be deficient for most or all of 68B. The deficiency does not encompass either 68A7-8 or 68C1-2; however, 68B1-2 is definitely missing. The status of 68A9 is unclear, but 68B4 does not appear to be present.

Df(3L)h-76 is clearly a deletion in 68A, but we were not able to make a precise determination of breakpoints.

Lethal *h-9* was examined cytologically for clues to the origin of its peculiar complementation pattern. There appear to be no major rearrangements of 68A-C, although there is definitely a small aberration, possibly a deletion, in 68A proximal to 68A2. 68B1-2 is undisturbed.

In order to address the possibility that *l-108* is a deletion for *cSOD* and a flanking vital group, it too was examined. No evidence was observed for *l-108* being associated with a visible cytological aberration.

DISCUSSION

The genetic organization of the *cSOD* microregion has been investigated, utilizing recessive lethal mutations induced by hybrid dysgenesis and by EMS. We were successful in recovering a new allele of *cSOD*, and an additional nine complementation groups were identified. Evidence has also been presented that suggests that the genetic unit corresponding to *lxd* was uncovered by several newly induced deletions, bringing the total number of genetic units identified in the region to eleven. These results are consistent with other intensive studies of small genetic intervals in showing a high degree of correlation between the number of polytene chromosome bands and the number of genetic units identified by complementation (JUDD, SHEN and KAUFMAN 1972; HOCHMAN 1973; LIM and SNYDER 1974; HILLIKER *et al.* 1980).

A strikingly nonrandom distribution of HD-induced mutations of the *cSOD* microregion was observed. Eighty-five percent of all single-site HD-induced alleles recovered fell into a single complementation group, whereas no HD-induced alleles were recovered from six groups identified by EMS-induced alleles. This finding is consistent with previous studies indicating that HD-induced mutations show a high degree of site specificity (GREEN 1977; ENGELS 1979; SIMMONS and LIM 1980; SIMMONS *et al.* 1984). It is noteworthy that the mutability of the group A locus is of the same order of magnitude as that reported for the *singed* locus (ENGELS 1979), indicating that the group A locus may be "hot spot" for *P* element insertion.

A single EMS-induced lethal, *l-108*, was identified as a possible allele of *cSOD*. The analysis of this mutant was facilitated by the recovery of *l-108* hemizygote "leakers" that show no cSOD activity when assessed electrophoretically. That this genotype is also associated with lower total SOD activity (assayed in solution) further supports our conclusion that *l-108* represents a hypomorphic allele of *cSOD*. Since only one allele of *cSOD* has been recovered, the possibility that *l-108* may involve both *cSOD* and an adjacent vital gene remains unresolved. Given that there is no cytological evidence for *l-108* being a deletion, the semilethal phenotype associated with *l-108* is strongly suggestive of *cSOD* indeed representing a vital genetic locus. It is interesting to note that the onset of *cSOD* mutant lethality at the time of eclosion is coincident with an increase in oxygen metabolism by *Drosophila* (BODINE and ORR 1925).

Despite extensive investigation of the enzymatic properties of the superoxide dismutases from a variety of organisms, the *in vivo* functions of the enzymes remain speculative. The suggestion that these potentially vital enzymes protect cells against physiological or genetic toxicity of superoxide radicals generated through endogenous metabolic reactions or by ionizing radiation is based largely on indirect experiments and observations. The mutant, *l-108*, and deletions of *cSOD* produced in this study now provide the means to examine directly the physiological functions of cSOD in a higher eukaryote, and they may offer a starting point for ultimately pursuing the structure of the *cSOD* gene and the molecular mechanism of its regulation.

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