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Phenotypic Rescue by a Bovine Transgene in a Cu/Zn Superoxide Dismutase-Null Mutant of *Drosophila melanogaster*

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Null mutants for Cu/Zn superoxide dismutase (CuZnSOD) in *Drosophila melanogaster* are male sterile, have a greatly reduced adult life span, and are hypersensitive to paraquat. We have introduced a synthetic bovine CuZnSOD transgene under the transcriptional control of the *D. melanogaster* 5C actin promoter into a CuZnSOD-null mutant of *D. melanogaster*. This was carried out by P-element-mediated transformation of the *Drosophila*-bovine CuZnSOD transgene into a CuZnSOD$^+$ recipient strain followed by genetic crossing of the transgene into a strain carrying the CuZnSOD-null mutation, *cSOD*^{n108}. The resulting transformants express bovine CuZnSOD exclusively to about 30% of normal *Drosophila* CuZnSOD levels. Expression of the *Drosophila*-bovine CuZnSOD transgene in the CuZnSOD-null mutant rescues male fertility and resistance to paraquat to apparently normal levels. However, adult life span is restored to only 30% of normal, and resistance to hyperoxia is 90% of that found in control flies. This striking differential restoration of pleiotropic phenotypes could be the result of a threshhold of CuZnSOD expression necessary for normal male fertility and resistance to the toxicity of paraquat or hyperoxia which is lower than the threshold required to sustain a normal adult life span. Alternatively, the differential rescue of fertility, resistance to active oxygen, and life span might indicate different cell-specific transcriptional requirements for these functions which are normally provided by the control elements of the native CuZnSOD gene but are only partly compensated for by the transcriptional control elements of the actin 5C promoter.

The high concentration of oxygen in the earth's atmosphere and the metabolic dependence of higher eukaryotes on the respiratory utilization of oxygen confers upon these organisms the necessity of dealing with multiple toxic species of active oxygen. Most organisms deal effectively with active oxygen through a complex system of defenses which include (i) enzymatic scavengers such as superoxide dismutases (SODs) and catalase, which in concert reduce superoxide to H_2O and O_2, (ii) small metabolite scavengers such as urate, ascorbate, and bilirubin which react stoichiometrically to convert active oxygen species to less reactive metabolites, and (iii) multiple systems for repairing radical damage to DNA and possibly proteins (11, 25). The biological consequences of imperfect detoxication of active oxygen species originate with the destructive reactivity of these agents with cellular macromolecules, including DNA, RNA, protein, lipid, and carbohydrate, and are manifested through a variety of whole-organism pathologies ranging from inflammatory and cardiovascular disease to cancer (7). Finally, a causative relationship between reactive oxygen species and aging was postulated many years ago (12). This attractive hypothesis has recruited many proponents (9, 20); however, the supporting evidence is still largely circumstantial and indirect.

We have been utilizing the genetic system of *Drosophila melanogaster* to investigate the biological consequences arising from directed genetic perturbation of specific components of oxygen defense metabolism (22). For example, null mutation for Cu/Zn SOD (CuZnSOD), one of two distinct SODs found in higher animals, confers reduced adult life span, hypersensitivity to paraquat and $CuSO_4$, and male sterility (21). In contrast, moderate overexpression of native CuZnSOD generated by chromosomal duplications of the CuZnSOD gene (28) or by native CuZnSOD transgenes (27) confers increased resistance to ionizing radiation, decreased resistance to paraquat toxicity, and marginal increase in adult life span.

A complementary genetic approach to investigating the biological role of CuZnSOD involves the use of transgenes which specify a heterologous CuZnSOD expressed under the transcriptional control of novel promoters. Reveillaud et al. (23) undertook such an approach, in which a *Drosophila*-bovine CuZnSOD transgene, consisting of a bovine CuZnSOD cDNA under the transcriptional control of the *D. melanogaster* actin 5C promoter, was integrated into CuZnSOD$^+$ recipients by P-element-mediated transformation. Such transformants, which express *D. melanogaster* and bovine homodimeric CuZnSODs as well as an active *Drosophila*-bovine heterodimeric CuZnSOD, and which exhibit up to 160% of normal total CuZnSOD levels, show increased resistance to paraquat (in contrast with finding discussed above) and hyperoxia and marginally but significantly increased adult life span.

The multiple pleiotropic effects conferred by under- and overexpression of CuZnSOD confirm the many complex cellular roles of CuZnSOD in normal *D. melanogaster* biology. In a continued genetic approach to understanding the various roles of CuZnSOD in specific aspects of oxygen

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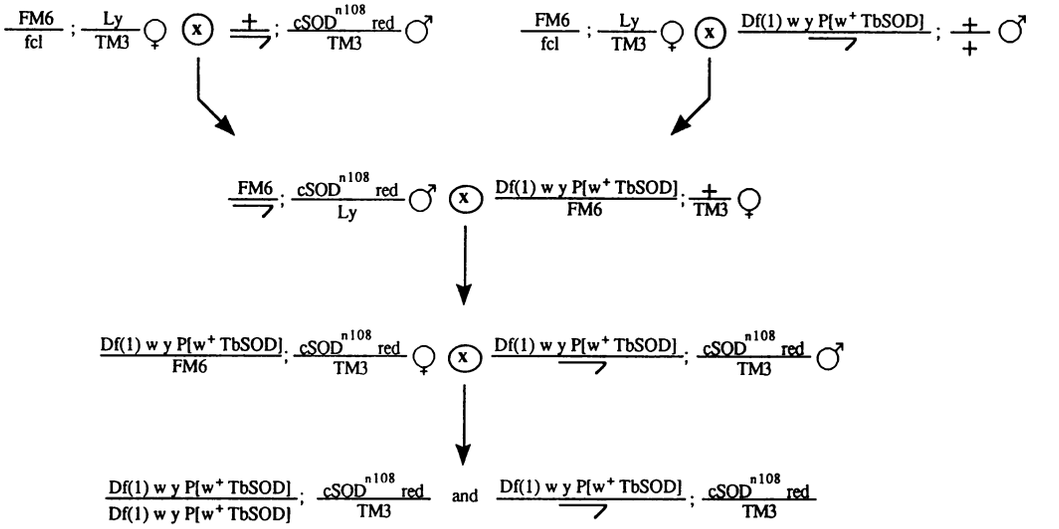

FIG. 1. Incorporation of the *Drosophila*-bovine transgene from the transgenic strains, with genotypes *Df(1)w*^{67C23} *y P[w*$^+$*, bSOD]11* and *Df(1)w*^{67C23} *y P[w*$^+$*, bSOD]12*, into the CuZnSOD-null mutant strain, *cSOD*^{n108}*, red.*

defense biology, we have investigated the extent to which a synthetic *Drosophila*-bovine transgene can compensate for the loss of native CuZnSOD through the rescue of specific pleiotropic phenotypes characteristic of a CuZnSOD-null mutant. The results show that male fertility, active oxygen toxicity, and adult life span are differentially rescued by expression of the *Drosophila*-bovine transgene. This result provides a new experimental approach for further understanding the mechanism of the diverse biological roles of CuZnSOD at the level of the whole organism.

MATERIALS AND METHODS

***Drosophila* strains.** The CuZnSOD-null (*cSOD*^{n108}) mutant strain was initially generated by ethyl methanesulfonate mutagenesis and is described by Campbell et al. (5) and Phillips et al. (21). The two bovine CuZnSOD transgenic strains, with genotypes *Df(1)w*^{67C23} *y P[w*$^+$*, bSOD]11* and *Df(1)w*^{67C23} *y P[w*$^+$*, bSOD]12*, designated *Tb11* and *Tb12*, respectively, carry single-copy transgenes on the X chromosome and were generated (23) by P-element-mediated transformation of the recipient strain, genotype *Df(1)w*^{67C23} *y*, with the P-element vector, pW8 (14), carrying a bovine CuZnSOD cDNA under the transcriptional control of the *D. melanogaster* actin 5C promoter (23). Control strains with genotypes *Df(1)w*^{67C23} *y P[w*$^+$*]3*, *Df(1)w*^{67C23} *y P[w*$^+$*]4*, and *Df(1)w*^{67C23} *y P[w*$^+$*]10*, designated *Ct3*, *Ct4*, and *Ct10*, respectively, are the same recipient strain transformed with an empty pW8 vector (23). These control strains confer w^+ function to the recipient strain in the presence of normal CuZnSOD function. All other strains and markers used are described in Lindsley and Zimm (17). Stocks were maintained on standard cornmeal agar medium at 25°C unless otherwise described.

Incorporation of the *Drosophila*-bovine transgene into the CuZnSOD-null mutant. A series of standard genetic crosses was used to incorporate the X chromosome carrying the *Drosophila*-bovine transgene from the transgenic strains *Tb11* and *Tb12* into the CuZnSOD-null mutant strain, designated *cSOD*^{n108} *red* (Fig. 1). The resulting two strains, with genotypes *Df(1)w*^{67C23} *y P[w*$^+$*, bSOD]11; cSOD*^{n108} *red/TM3* and *Df(1)w*^{67C23} *y P[w*$^+$*, bSOD]12; cSOD*^{n108}*, red/TM3* (designated *Tb11; cSOD*^{n108} *red/TM3* and *Tb12; cSOD*^{n108} *red/TM3*, respectively), are maintained as third-chromosome-balanced heterozygous stocks. CuZnSOD-null control strains, with genotypes *Df(1)w*^{67C23} *y P[w*$^+$*]3; cSOD*^{n108} *red/TM3* and *Df(1)w*^{67C23} *y P[w*$^+$*]10; cSOD*^{n108} *red/TM3* (designated *Ct3; cSOD*^{n108} *red/TM3* and *Ct10; cSOD*^{n108} *red/TM3*, respectively), were generated from *Ct3*, *Ct10*, and *cSOD*^{n108} *red* in the same manner.

SOD activity assay. CuZnSOD was extracted by the method of Bannister and Bannister (3), with the following modifications. Approximately 50 flies were homogenized in 300 μl of 20 mM Tris-acetate buffer (pH 7.8) containing 0.2% Triton X-100 and 1 mM phenylmethylsulfonyl fluoride. The homogenate was centrifuged at 11,000 × *g* for 15 min at 4°C, and the supernatant was collected, heated rapidly to 60°C for 10 min, acidified to pH 5.5 with acetic acid, and then centrifuged again at 11,000 × *g* for 15 min at 4°C. The resulting proteins in this preparation were separated on a 10% polyacrylamide nondenaturing gel for 18 h at 35 V. After electrophoresis, the SOD bands were identified in the gel by the Nitro Blue Tetrazolium (NBT) method at 25°C (4). The protein bands on the polyacrylamide gels were visualized by the NBT method as follows. Gels were soaked in 2.5 mM NBT for 30 min with shaking, rinsed, and immersed in a solution of 0.028 M tetramethylethylenediamine, 2.8 × 10^{-5} riboflavin, and 0.036 M potassium phosphate (pH 7.8). Gels were illuminated on a fluorescent light box for up to 20 min. The gels became uniformly blue (reduced NBT) except at the positions of the SODs. When maximum contrast was achieved, the gels were photographed. Digitization of the resulting films was performed by Kendricks Laboratories, Inc., Madison, Wis. Microdensitometry was then carried out to determine the relative quantities of SOD on these gels (16).

Life span determination. Adult flies, 0 to 24 h old, were transferred to fresh cornmeal medium and maintained at 25°C in incubators on a 12-h/12-h light cycle. Flies were transferred to fresh medium every 2 to 3 days, and the number of dead flies was recorded throughout the life span. Percent survival was calculated at the end of the experiment after correction for total loss resulting from handling. Statis-

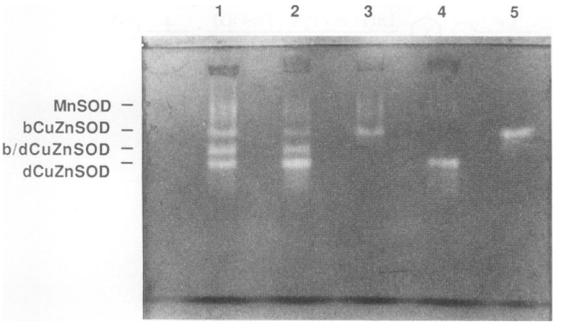

FIG. 2. Nondenaturing polyacrylamide gel analysis of CuZnSOD activity. Lanes: 1, *Tb11; cSOD^{n108}/TM3*; 2, *Tb11*; 3, *Tb11; cSOD^{n108}*; 4, *Ct4*; 5, commercial bovine CuZnSOD (bCuZnSOD). Gel procedures and strain designations are given in Materials and Methods. b/dCuZnSOD, bovine-*Drosophila* CuZnSOD; dCuZnSOD, Drosophila CuZnSOD.

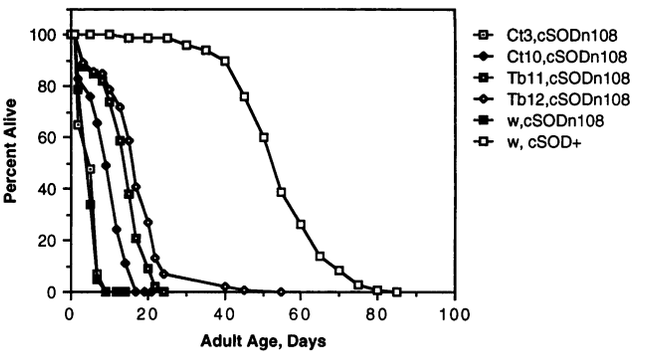

FIG. 3. Life spans of transgenic strains and their genetic controls. Methods for life span determination and strain designations are described in Materials and Methods.

tical analyses of the mortality data for each population were performed by the SAS life test procedure (24).

Paraquat toxicity studies. To determine sensitivity of flies to paraquat, groups of 30 male flies were placed in 35-ml glass vials with a 3- by 3-cm piece of Whatman no. 1 filter paper wetted with a 15% sucrose solution containing either 0, 1, 5, or 10 mM paraquat, made fresh daily. The number of dead flies was counted at 16 h as described previously (23). Flies were 5 to 7 days old.

Hyperoxia. For determination of sensitivity to hyperoxia, groups of approximately 100 male adult flies, 1 to 5 days old, were placed in 1,000-ml sealed conical side arm flasks containing instant *Drosophila* medium 4-24 (Carolina Biological Supply Co.). Flasks were then flushed for 20 min with 100% medical-grade oxygen each day, and the flasks were sealed. The number of dead flies was recorded each day following the exposure period.

Fertility determination. Male fertility for each genotype was determined by setting up 50 single-pair matings in vials for each genotype. The percentage of vials which produce adult progeny was taken as the fertility value for males of that genotype.

RESULTS

CuZnSOD expression in *Tb11; cSOD^{n108}* homozygotes. It was previously shown (23) that *Drosophila* and bovine CuZnSODs are electrophoretically distinguishable. *Drosophila* CuZnSOD migrates faster than bovine CuZnSOD in nondenaturing polyacrylamide gels. Furthermore, *Drosophila* and bovine CuZnSOD polypeptides associate in enzymatically functional heterodimers. As shown in Fig. 2, adult *Drosophila* CuZnSOD-null homozygotes selected from the *Tb11; cSOD^{n108}/TM3* stock express only dimeric bovine CuZnSOD, while *TM3* heterozygotes express the expected homodimeric bovine and *D. melanogaster* CuZnSODs and a heterodimeric *Drosophila*-bovine CuZnSOD. The same results were obtained for *Tb12; cSOD^{n108}* (data not shown). Note that, as expected, the level of *D. melanogaster* homodimeric CuZnSOD in *Tb11; cSOD^{n108}/TM3* is approximately half of that in *Tb11*. Note also that homozygous expression of the *D. melanogaster* CuZnSOD polypeptide in *Tb11* traps a greater proportion of the bovine CuZnSOD polypeptide into the *Drosophila*-bovine CuZnSOD heterodimer than in *Tb11; cSOD^{n108}/TM3*. Total CuZnSOD activities in *Tb11; cSOD^{n108}* and *Tb12; cSOD^{n108}* homozygotes are 42 and 39%, respectively, of CuZnSOD activity in normal control adults. Homozygous stocks of *Tb11; cSOD^{n108}* and *Tb12; cSOD^{n108}* can be established but are quite weak.

Adult life span of *Tb11; cSOD^{n108}* and *Tb12; cSOD^{n108}*. The effect of expression of the *Drosophila*-bovine CuZnSOD transgene on restoration of adult life span in the *cSOD^{n108}* mutant was examined (Fig. 3). The control strains, *Ct3; cSOD^{n108} red* and *Ct10; cSOD^{n108} red*, which are devoid of detectable CuZnSOD activity, show median adult life spans of approximately 4.5 and 9 days, respectively. Because reduced w^+ gene expression can markedly reduce adult life span, the shorter life span of these control strains than of the original *cSOD^{n108} red* strain may be attributable to underexpression of the w^+ minigene carried on the pW8 transformation vector. In contrast, strains *Tb11; cSOD^{n108} red* and *Tb12; cSOD^{n108} red*, which express bovine CuZnSOD in the absence of endogenous *D. melanogaster* CuZnSOD, show median adult life spans of 14 and 16 days, respectively (Fig. 3). Thus, expression of the *Drosophila*-bovine CuZnSOD transgene in the CuZnSOD-null mutant at most restores adult life span from 9 days (*Ct10; cSOD^{n108} red*) to approximately 16 days (*Tb12; cSOD^{n108} red*). This represents only a partial restoration of life span from 16.7 to 29.6% of the 54-day median life span of the CuZnSOD$^+$ control strain.

Sensitivity of *Tb11; cSOD^{n108}* and *Tb12; cSOD^{n108}* to paraquat. The effect of expression of the *Drosophila*-bovine CuZnSOD transgene on restoration of resistance of the *cSOD^{n108}* mutant to dietary paraquat is shown in Fig. 4. The control strains, *Ct3; cSOD^{n108} red* and *Ct10; cSOD^{n108} red*, which are devoid of detectable CuZnSOD activity, both show dietary LD_{50} values (concentration in the medium required to kill 50% of the population in the designated time of 16 h) of less than 1.0 mM paraquat. In contrast, strains *Tb11; cSOD^{n108}* and *Tb12; cSOD^{n108}*, which express bovine CuZnSOD in the absence of endogenous *D. melanogaster* CuZnSOD, both show enhanced resistance to paraquat with dietary LD_{50} values of greater than 5.0 mM. This level of resistance is comparable to that of the CuZnSOD$^+$ control strain. Note that the differential resistance of *Tb12; cSOD^{n108} red* compared with *Tb11; cSOD^{n108} red* correlates with the level of expression of the transgene.

Sensitivity of *Tb11; cSOD^{n108}* and *Tb12; cSOD^{n108}* to hyperoxia. The effect of expression of the *Drosophila*-bovine CuZnSOD transgene on restoration of resistance of the *cSOD^{n108}* mutant to hyperoxia is shown in Fig. 5. The control strains, *Ct3; cSOD^{n108} red* and *Ct10; cSOD^{n108} red*,

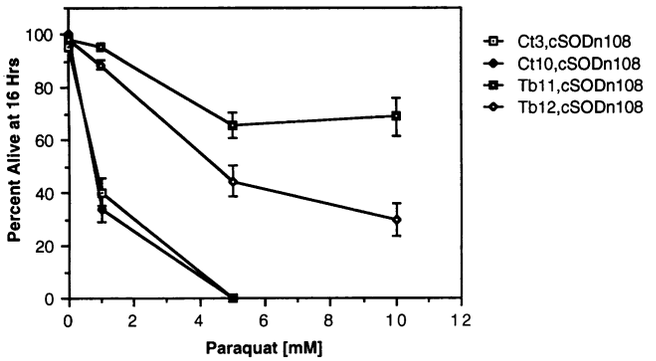

FIG. 4. Resistance of transgenic strains *Tb11; cSOD^{n108}* and *Tb12; cSOD^{n108}* to dietary paraquat. The method of paraquat administration and strain designations are described in Materials and Methods.

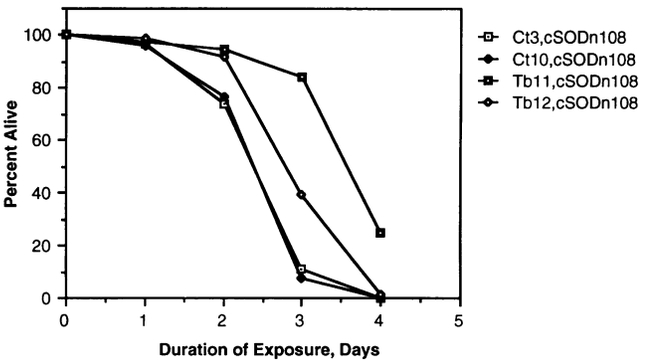

FIG. 5. Resistance of transgenic strains *Tb11; cSOD^{n108}* and *Tb12; cSOD^{n108}* to hyperoxia. The method of hyperoxia administration and strain designations are described in Materials and Methods.

which are devoid of detectable CuZnSOD activity, both show Kt_{50} values (time required to knock down 50% of the population) of approximately 2.4 days. In contrast, strains *Tb11; cSOD^{n108}* and *Tb12; cSOD^{n108}*, which express bovine CuZnSOD in the absence of endogenous *D. melanogaster* CuZnSOD, show enhanced resistance to hyperoxia, with Kt_{50} values of 3.6 and 2.8 days, respectively. This level of resistance is comparable to that of the CuZnSOD$^+$ control strain. As with paraquat resistance (see above), the differential resistance to hyperoxia of *Tb12; cSOD^{n108} red* and *Tb11; cSOD^{n108} red* correlates with the level of expression of the transgene.

Male fertility of *Tb11; cSOD^{n108} red* and *Tb12; cSOD^{n108} red*. The effect of expression of the *Drosophila*-bovine CuZnSOD transgene on restoration of male fertility in the *cSOD^{n108}* mutant is shown in Table 1. As reported earlier (21), *cSOD^{n108} red* is devoid of detectable CuZnSOD activity and shows complete male sterility. In contrast, strains *Tb11; cSOD^{n108} red* and *Tb12; cSOD^{n108} red*, which express bovine CuZnSOD in the absence of endogenous *D. melanogaster* CuZnSOD, show fertility levels of 72 and 58%, respectively. As with resistance to paraquat and hyperoxia noted above, the differential restoration of male fertility in *Tb12; cSOD^{n108} red* and *Tb11; cSOD^{n108} red* correlates with the level of expression of the transgene. It should be born in mind that these data on fertility reflect the proportion of males of a given genotype which can sire offspring and not the relative fecundity of individual males. Active sperm counts, for example, could vary widely within the cohort of males scored as fertile.

As shown in Table 1, we also examined female fertility. Only 20% of *cSOD^{n108} red* females produce progeny. In contrast, 75 and 78% of *Tb12; cSOD^{n108} red* and *Tb11; cSOD^{n108} red* females, respectively, produce progeny. Thus, the partial loss of fertility in *cSOD^{n108} red* females is restored by the *Drosophila*-bovine CuZnSOD transgene.

DISCUSSION

CuZnSOD is found in virtually all aerobic organisms (6, 18, 19), but how the radical-scavenging activity of this enzyme contributes to the diverse functions of specific cells and tissues of higher organisms is virtually unknown. Genetic manipulation of CuZnSOD is one route to identifying different specific roles of CuZnSOD which are important at the level of the whole organism. For example, null mutation for CuZnSOD in *D. melanogaster* interferes with a variety of normal functions, including resistance to active oxygen, fertility, and adult life span (21). Some of these phenotypes, e.g., infertility, probably arise from the absence of CuZnSOD activity in specific tissues. For others, the origins of the effects are less clear. We have used a *Drosophila*-bovine transgene to begin to investigate the origin of specific pleiotropic phenotypes of CuZnSOD-null mutants of *D. melanogaster*. We find that the expression of this transgene in an otherwise CuZnSOD-null mutant variably rescues the phenotypes of hypersensitivity to active oxygen, infertility, and reduced adult life span. This differential restoration of pleiotropic phenotypes could merely result from different thresholds of CuZnSOD activity necessary to support normal expression of these functions. Alternatively, it might indicate different cell-specific transcriptional requirements for these functions which are normally provided by the control elements of the native CuZnSOD gene but which are only spuriously and inadequately compensated for by the transcriptional control elements of the actin 5C promoter.

The tissue-specific distribution of CuZnSOD activity is not yet known for *D. melanogaster*. However, levels of CuZnSOD activity in some insects are known to vary extensively from tissue to tissue (1). In light of this, we think it likely that the variable rescue of different pleiotropic phenotypes of the CuZnSOD-null mutant by the *Drosophila*-bovine transgene reflects a strict requirement for tissue-specific expression of CuZnSOD. Further support for this interpretation comes from the results of experiments on CuZnSOD overexpression. As indicated in the introduction, overexpression of native CuZnSOD generated either by chromosomal duplication of the CuZnSOD gene (28) or by native CuZnSOD transgenes (27) confers decreased resistance to paraquat toxicity while at the same time conferring increased resistance to ionizing radiation and marginal increase in adult life span. In contrast, CuZnSOD$^+$ transformants which carry the *Drosophila*-bovine transgene and which express both *Drosophila* and bovine CuZnSODs show increased resistance to paraquat toxicity. This finding suggests to us that overexpression of CuZnSOD in cells or tissues in which it is normally found, such as by chromosomal duplication of the CuZnSOD gene (28) or by native CuZnSOD transgenes, is deleterious. This is similar to the consequences of transient or stable overexpression of CuZnSOD in transfected mammalian cells (8, 15) in culture or transformed bacteria (13, 26) and may arise from the generation of hydroxyl radicals from the interaction of increased levels of unmetabolized H_2O_2

TABLE 1. Effects of *Tb11* and *Tb12* on the fertility of *cSOD^{n108} red*

Cross	No. of matings of type[a]:					% Fertility[b]
	A	B	C	D	E	
cSOD^{n108} red × *cSOD^{n108} red*	50	50	50	0	0	0
cSOD^{n108} red × Ore-R	50	50	50	0	0	0
cSOD^{n108} red × Ore-R	47	41	36	9	8	19.5
Tb11; cSOD^{n108} red × *Tb11, cSOD^{n108} red*	50	45	39	23	22	48.9
Tb11; cSOD^{n108} red × Ore-R	50	50	50	36	36	72.0
Tb11; cSOD^{n108} red × Ore-R	50	44	44	33	33	75.0
Tb12; cSOD^{n108} red × *Tb12; cSOD^{n108} red*	50	36	34	9	4	11.1
Tb12; cSOD^{n108} red × Ore-R	50	50	50	29	29	58.0
Tb12; cSOD^{n108} red × Ore-R	50	40	39	31	31	77.5

[a] A, single pair; B, female parent surviving for at least 48 h; C, producing eggs; D, producing larvae; E, producing adults.
[b] % fertility = (number of matings of type E/number of matings of type B) × 100.

with the overly abundant CuZnSOD (29). On the other hand, the actin 5C promoter of the *Drosophila*-bovine transgene may direct the expression of CuZnSOD primarily in cells in which it is otherwise present in low levels or not present at all. Such ectopic expression could augment the overall resistance of transformants to increased flux of superoxide radicals generated by paraquat without the deleterious consequences of CuZnSOD hyperactivity.

Our observation that the *Drosophila*-bovine transgene does not completely restore adult life span may be related to the inability of the actin 5C promoter to restore CuZnSOD expression in those cells and tissues which are life span limiting in the CuZnSOD-null mutant. Although the whole-body level of CuZnSOD in the transgenically rescued CuZnSOD-null mutant is elevated by 30%, the adult life span is restored to only 30% of normal. CuZnSOD-null heterozygotes which have 50% of normal CuZnSOD activity have a normal adult life span (21). Moreover, a mutant recovered from natural populations which has only 3% of normal whole-body CuZnSOD activity (10) has a life span nearly 70% of normal (27). Since actin is one of the most abundant transcripts in the cell, it is uncertain why its expression in the null mutants does not restore life span to normal levels. It may be that the particular promoter used, the actin 5C promoter, is not particularly active. Moreover, it has been noted that actin 5C is not expressed uniformly. The regulation of the CuZnSOD gene can be investigated by the construction and transformation of CuZnSOD transgenes carrying different sequence elements of the 5′ upstream region of the CuZnSOD gene, to the bovine CuZnSOD cDNA, or to a histochemical reporter gene.

The complete sterility of CuZnSOD-null mutant males and its rescue by the *Drosophila*-bovine transgene indicates an important biological role of CuZnSOD in spermatogenesis and in sperm function. It has been reported, for example, that the generation of reactive oxygen species can directly affect human sperm function (2). *cSOD^{n108} red* males produce cytologically abnormal spermatozoa which are kinked and twisted and few in number (20a). These cytological abnormalities are not detected in spermatozoa produced by *cSOD^{n108}/cSOD^{+}* heterozygous males or by *Tb11; cSOD^{n108} red* males (20a), which is consistent with their normal fertility. Whether these abnormalities arise from the cytotoxic effects initiated by unscavenged superoxide generated in developing spermatocytes, spermatids, and spermatozoa or in surrounding somatic cells is at present unknown. One might expect that the high respiratory activity of mature spermatozoa might render them acutely dependent on CuZnSOD activity. But whether male germ cells of *D. melanogaster* normally contain CuZnSOD has not yet been determined.

The effect of the CuZnSOD-null condition on fertility and its rescue by the *Drosophila*-bovine transgene is not male specific. Fertility of *cSOD^{n108} red* females is only 10 to 20% of the wild-type level and, as in males, is restored to approximately normal levels by the *Drosophila*-bovine transgene. However, the intense respiratory activity of mature spermatozoa, as opposed to mature eggs, may place additional oxygen stress on spermatozoa, which in turn could account for the complete sterility and the cytologically abnormal spermatozoa in CuZnSOD-null mutant males.

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REFERENCES

1. **Ahmad, S., D. Duval, L. C. Weinhold, and R. S. Pardini.** 1991. Cabbage loper antioxidant enzymes: tissue specificity. Insect Biochem. **21**:563–572.
2. **Aitken, R. J., J. S. Clarkson, and S. Fishel.** 1989. Generation of reactive oxygen species, lipid peroxidation and human sperm function. Biol. Reprod. **41**:183–197.
3. **Bannister, J., and W. Bannister.** 1985. Purification of copper/zinc superoxide dismutase, p. 5–7. *In* R. A. Greenwald (ed.), Handbook of methods for oxygen radical research. CRC Press, Boca Raton, Fla.
4. **Beauchamp, C., and I. Fridovich.** 1971. Superoxide dismutase: improved assays and an assay applicable to polyacrylamide gels. Anal. Biochem. **44**:276–287.
5. **Campbell, S. D., A. J. Hilliker, and J. P. Phillips.** 1986. Cytogenetic analysis of the cSOD microregion in *Drosophila melanogaster*. Genetics **112**:205–215.
6. **Carlioz, A., and D. Touati.** 1986. Isolation of superoxide dismutase mutants in *E. coli*: is superoxide dismutase necessary for aerobic life? EMBO J. **5**:623–630.
7. **Chow, C. K.** 1988. Cellular antioxidant defense mechanisms, vol. I to III. CRC Press, Boca Raton, Fla.
8. **Elroy-Stein, O., Y. Bernstein, and Y. Groner.** 1986. Overproduction of human Cu/Zn-superoxide dismutase in transfected cells: extenuation of paraquat-mediated cytotoxicity and enhancement of lipid peroxidation. EMBO J. **5**:615–622.
9. **Fleming, J. E., J. Miquel, S. F. Cottrell, L. S. Yengoyan, and A. C. Economos.** 1982. Is cell aging caused by respiration-dependent injury to the mitochondrial genome? Gerontology **28**:44–53.

10. **Graf, J., and F. J. Ayala.** 1986. Genetic variation for superoxide dismutase level in *Drosophila melanogaster*. Biochem. Genet. **24:**153–168.
11. **Gutteridge, J. M. C., T. Westermarck, and B. Halliwell.** 1986. Oxygen radical damage in biological systems, p. 99–140. *In* J. E. Johnson, Jr. (ed.), Free radicals in biology and medicine. Alan R. Liss, New York.
12. **Harman, D.** 1956. Aging: a theory based on free radical and radiation chemistry. J. Gerontol. **11:**298–300.
13. **Hassan, H. M., and I. Fridovich.** 1978. Superoxide radical and the oxygen enhancement of the toxicity of paraquat in E. coli. J. Biol. Chem. **253:**8143–8148.
14. **Klemenz, R., V. Weber, and W. J. Gehring.** 1987. The white gene as a marker in a new P-element vector for gene transfer to *Drosophila*. Nucleic Acids Res. **15:**3947–3959.
15. **Krall, J., A. C. Bagley, G. T. Mullenbach, R. A. Hallewell, and R. E. Lynch.** 1988. Superoxide mediates the toxicity of paraquat for cultured mammalian cells. J. Biol. Chem. **263:**1910–1914.
16. **Latter, G. I., S. Burbeck, J. Fleming, and J. Leavitt.** 1984. Identification of polypeptides on two-dimensional electrophoresis gels by amino acid composition. Clin. Chem. **30:**1925–1932.
17. **Lindsley, D. L., and G. Zimm.** 1992. The genome of *Drosophila melanogaster*. Academic Press, New York.
18. **Marklund, S. L.** 1986. Superoxide dismutase in human tissues, cells and extracellular fluids: clinical implications, p. 509–526. *In* J. E. Johnson, Jr. (ed.), Free radicals, aging and degenerative diseases. Alan R. Liss, New York.
19. **McCord, J. M., and I. Fridovich.** 1969. Superoxide dismutase. An enzymatic function for erythrocuprein (hemocuprein). J. Biol. Chem. **24:**6045–6055.
20. **Miquel, J., and J. E. Fleming.** 1986. Theoretical and experimental support for an oxygen-radical-mitochondrial injury hypothesis of cell aging, p. 51–74. *In* J. E. Johnson, Jr. (ed.), Free radicals, aging and degenerative diseases. Alan R. Liss, New York.

20a. **Phillips, J. P.** Unpublished data.

21. **Phillips, J. P., S. D. Campbell, D. Michard, M. Charbonneau, and A. J. Hilliker.** 1989. Null mutation of copper zinc superoxide dismutase in *Drosophila* confers hypersensitivity to paraquat and reduced longevity. Proc. Natl. Acad. Sci. USA **86:** 2761–2765.
22. **Phillips, J. P., and A. J. Hilliker.** 1990. Genetic analysis of oxygen defense mechanisms in *Drosophila*. Adv. Genet. **28:**43–71.
23. **Reveillaud, I., A. Niedzwiecki, K. G. Bensch, and J. E. Fleming.** 1991. Expression of bovine superoxide dismutase in *Drosophila melanogaster* augments resistance to oxidative stress. Mol. Cell. Biol. **11:**632–640.
24. **SAS Institute, Inc.** 1988. SAS technical report P-179. SAS Institute, Inc., Cary, N.C.
25. **Saul, R. L., P. Gee, and B. N. Ames.** 1987. Free radicals, DNA damage, and aging, p. 113–129. *In* H. R. Warner et al. (ed.), Modern biological theories of aging. Raven Press, New York.
26. **Scott, M. D., S. R. Meschnick, and J. W. Eaton.** 1987. Superoxide dismutase-rich bacteria: paradoxical increase in oxidant toxicity. J. Biol. Chem. **262:**3640–3645.
27. **Seto, N. O. L., S. Hayashi, and G. M. Tener.** 1991. Overexpression of Cu-Zn superoxide dismutase in *Drosophila* does not affect lifespan. Proc. Natl. Acad. Sci. USA **87:**4270–4274.
28. **Staveley, B. E., J. P. Phillips, and A. J. Hilliker.** 1990. Phenotypic consequences of copper/zinc superoxide dismutase overexpression in *Drosophila melanogaster*. Genome **33:**867–872.
29. **Yim, M. B., P. B. Chock, and E. R. Stadtman.** 1990. Copper, zinc superoxide dismutase catalyzes hydroxyl radical production from hydrogen peroxide. Proc. Natl. Acad. Sci. USA **87:**5006–5010.