

## ***Drosophila* larvae deficient for super-oxide dismutase activity are thermosensitive but show normal heat shock response**

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**Effects of deficiency for Cu-Zn superoxide dismutase (SOD) enzyme (EC 1.15.1.1) activity on thermosensitivity and heat shock response in *Drosophila melanogaster* were examined using a null allele (*cSOD*<sup>n108</sup>) of the gene coding for this enzyme activity. The *cSOD*<sup>n108</sup> homozygous larvae were poorly viable at 31°C while the *cSOD*<sup>n108</sup> heterozygotes had only a slightly reduced viability when compared with that at 21°C, indicating that deficiency for SOD activity makes the larvae thermosensitive. Deficiency for Cu-Zn SOD neither affected the inducibility of heat shock genes by temperature stress nor caused heat shock genes to express constitutively. In this sense, the accumulation of superoxide ions in SOD-deficient larvae did not mimic temperature stress. Thus the observed thermosensitivity of SOD-deficient larvae does not appear to be due to any aberration in the heat shock response.**

MONOVALENT reduction of oxygen in aerobic cells generates a series of unstable and highly active intermediates which attack other cellular constituents. The most common intermediate of oxygen metabolism is the superoxide radical ( $O_2^-$ ). To protect cells from such oxygen toxicity, an oxygen defence system is present in all aerobic cells. Superoxide dismutase (SOD) enzyme plays a central role in rapid dismutation of the  $O_2^-$  radical and its protonated form, the hydroxylperoxy radical ( $HO_2^-$ ) to hydrogen peroxide which is subsequently converted by catalases and peroxidases to water<sup>1</sup>.

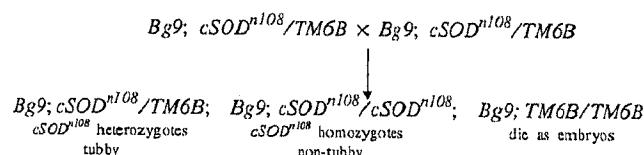
Heat shock or temperature stress (TS) is known to increase oxygen consumption in cells<sup>2-4</sup> and this leads to the possibility that TS could also damage cellular activity through oxygen toxicity. In mouse lung cells and in *E. coli*, TS was shown to induce SOD activity<sup>5,6</sup>; however in *Neurospora* and *Tetrahymena*, TS had no significant effect on cellular SOD levels<sup>7,8</sup>. The increased SOD activity due to oxidative stress induced by TS could be cell-type and/or organism-specific<sup>7</sup>. Several studies have shown that hydrogen peroxide by itself can induce the heat shock response<sup>9-11</sup>; it has also been suggested that  $O_2^-$  radicals too may be involved in its induction<sup>4</sup>. The availability of appropriate genetic systems makes it attractive to study this aspect of the heat shock response in *Drosophila*.

An EMS-induced recessive mutation that abolishes the Cu-Zn SOD (EC 1.15.1.1) activity in *Drosophila melanogaster* was discovered by Campbell *et al.*<sup>12</sup>. It was subsequently shown to be a null allele (named

*cSOD*<sup>n108</sup>) of the structural gene locus for Cu-Zn SOD and was found to be recessive semi-lethal: larvae homozygous for this mutant survived well (although slightly delayed in their development); however, the life span of adults was considerably reduced and the females were sterile<sup>13</sup>. It was further shown that the *cSOD* null condition not only caused a reduced metabolism of  $O_2^-$  generated by xenobiotic agents like paraquat but also led to a reduced capacity to dismutate metabolically-generated  $O_2^-$ . The reduced viability and sterility of the *cSOD* null flies was thus correlated with the toxicity of increased  $O_2^-$  radicals<sup>13</sup>.

In view of the possible inter-relation between heat shock genes and oxygen metabolites noted above, the following questions were asked in this study using the above null mutation for Cu-Zn SOD activity: (i) are the homozygous *cSOD*<sup>n108</sup> larvae thermosensitive? and (ii) does the absence of Cu-Zn SOD activity and consequent build-up of  $O_2^-$  radicals alter the heat shock response?

A stock of *D. melanogaster* of the following constitution was used in this study — *Bg9*; *cSOD*<sup>n108</sup> *red/TM6B*. The original stock (received from Dr. John P. Phillips, Univ. Guelph, Ontario, Canada) was *cSOD*<sup>n108</sup> *red/TM3*. The *Bg9* and *TM6B* chromosomes were introduced by appropriate crossings. *Bg9* refers to a germline transformed X-chromosome that carries a P-transposon with the *lac Z* gene of *E. coli* put under the control of *hsp70* promoter of *D. melanogaster* (the P-transposon in this line is inserted at 9B region of X-chromosome, for further details, see references 14 and 15). Cells carrying this P-transposon synthesize  $\beta$ -galactosidase when heat-shocked<sup>14,15</sup>. *cSOD*<sup>n108</sup> is the Cu-Zn SOD null allele isolated by Campbell *et al.*<sup>12</sup> while *TM6B* refers to a balancer chromosome 3 (for details of genetic symbols etc see reference 16). The *TM6B* balancer chromosome is homozygous lethal and carries a dominant marker, *Tubby*, which causes larvae and pupae to have a *tubby* phenotype<sup>16</sup>. *cSOD*<sup>n108</sup>/*TM6B* flies produce only two types of viable progeny as shown below:



The *cSOD*<sup>n108</sup>/*cSOD*<sup>n108</sup> and *cSOD*<sup>n108</sup>/*TM6B* larvae and pupae can be easily distinguished from each other due to the latter being distinctly shorter and thicker ('*tubby*' phenotype) than the former which resemble wild type in their outward appearance. All progeny in this stock carry the *hsp70-lacZ* fusion gene on X-chromosome (*Bg9*). To check thermosensitivity of SOD null (*cSOD*<sup>n108</sup> homozygous) larvae, eggs from healthy *Bg9*;