# *Drosophila* larvae deficient for superoxide 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 ($cSOD^{n108}$) of the gene coding for this enzyme activity. The $cSOD^{n108}$ homozygous larvae were poorly viable at 31°C while the $cSOD^{n108}$ 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 hydroxyperoxy radical ($HO_2^-$) to hydrogen peroxide which is subsequently converted by catalases and peroxidases to water[1].

Heat shock or temperature stress (TS) is known to increase oxygen consumption in cells[2-4] 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[5,6]; however in *Neurospora* and *Tetrahymena*, TS had no significant effect on cellular SOD levels[7,8]. The increased SOD activity due to oxidative stress induced by TS could be cell-type and/or organism-specific[7]. Several studies have shown that hydrogen peroxide by itself can induce the heat shock response[9-11]; it has also been suggested that $O_2^-$ radicals too may be involved in its induction[4]. 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.*[12]. It was subsequently shown to be a null allele (named $cSOD^{n108}$) 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[13]. 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[13].

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 $cSOD^{n108}$ 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*; $cSOD^{n108}$ *red/TM6B*. The original stock (received from Dr. John P. Phillips, Univ. Guelph, Ontario, Canada) was $cSOD^{n108}$ *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 β-galactosidase when heat-shocked[14,15]. $cSOD^{n108}$ is the Cu–Zn SOD null allele isolated by Campbell *et al.*[12] 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[16]. $cSOD^{n108}$/*TM6B* flies produce only two types of viable progeny as shown below:

*Bg9*; $cSOD^{n108}$/*TM6B* × *Bg9*; $cSOD^{n108}$/*TM6B*

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*Bg9*; $cSOD^{n108}$/*TM6B*; $cSOD^{n108}$ heterozygotes, tubby — *Bg9*; $cSOD^{n108}$/$cSOD^{n108}$; $cSOD^{n108}$ homozygotes, non-tubby — *Bg9*; *TM6B*/*TM6B*, die as embryos

The $cSOD^{n108}$/$cSOD^{n108}$ and $cSOD^{n108}$/*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 ($cSOD^{n108}$ homo zygous) larvae, eggs from healthy *Bg9*;