

## *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<sup>1</sup>.

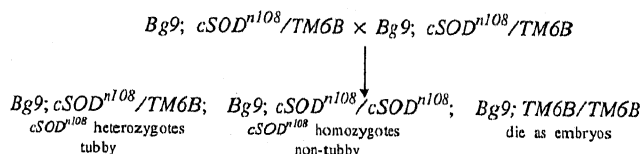
Heat shock or temperature stress (TS) is known to increase oxygen consumption in cells<sup>2-4</sup> and this leads to the possibility that TS could also damage cellular activity through oxygen toxicity. In mouse lung cells and in *E. coli*, TS was shown to induce SOD activity<sup>5,6</sup>; however 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

$cSOD^{n108}$ ) 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<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  $cSOD^{n108}$  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  $\beta$ -galactosidase when heat-shocked<sup>14, 15</sup>.  $cSOD^{n108}$  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>.  $cSOD^{n108}/TM6B$  flies produce only two types of viable progeny as shown below:



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 ( $cSOD^{n108}$  homozygous) larvae, eggs from healthy  $Bg9;$

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**Table 1.** Relative survival of *cSOD<sup>n108</sup>* homo- (non-tubby) and heterozygous (tubby) larvae when grown at 21°C or at 31°C

Growth temperature	Total pupae*	Relative proportion (% of Total) (Mean ± S.D.)*	
		Tubby pupae ( <i>cSOD<sup>n108</sup>/TM6B</i> )	Non-tubby pupae ( <i>cSOD<sup>n108</sup>/cSOD<sup>n108</sup></i> )
21°C	692	70.7 ± 3.6	29.3 ± 3.6
31°C	395	95.5 ± 5.4	4.5 ± 5.4

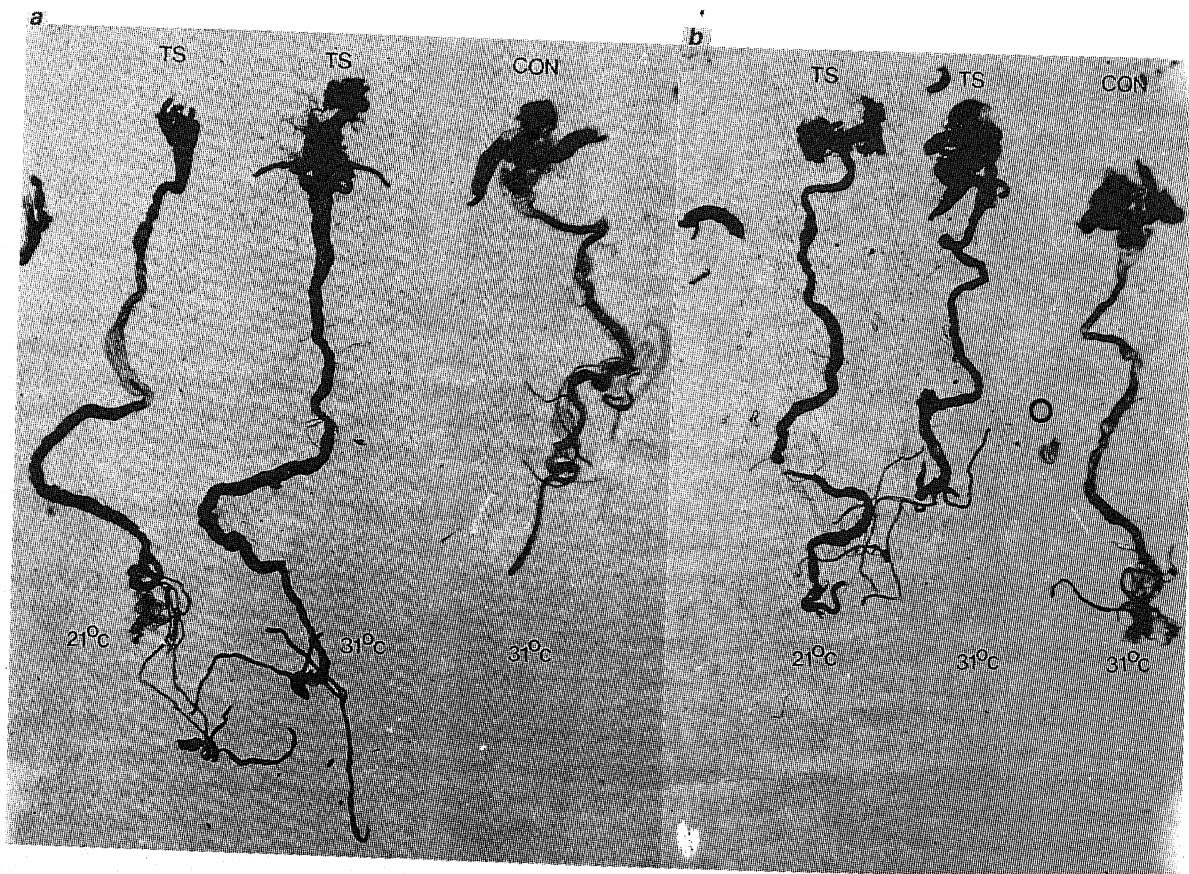
(\*Total and means of four replicates).

*cSOD<sup>n108</sup>/TM6B* flies were collected at hourly intervals at 22°C: each batch of eggs (about 250 to 350 eggs) was divided into two nearly equal parts and grown till pupation either at 21 ± 0.5°C or at 31 ± 0.5°C respectively. Four replicates were made. When all larvae had pupated, the numbers of tubby (*cSOD<sup>n108</sup>/TM6B*) and non-tubby (*cSOD<sup>n108</sup>/cSOD<sup>n108</sup>*) pupae in each case were counted. The results are presented in Table 1.

It is clear from these results that the ratio of tubby (*cSOD<sup>n108</sup>/TM6B* heterozygotes) to non-tubby (*cSOD<sup>n108</sup>/cSOD<sup>n108</sup>* homozygotes) larvae pupating at 21°C was close to 2:1 as expected (on  $\chi^2$ -test,  $P > 0.01$ ). However, very few

*cSOD<sup>n108</sup>* mutant (only ~ 5% instead of the expected 33%) larvae survived at 31°C. Thus *Drosophila* larvae devoid of Cu-Zn superoxide dismutase activity (due to *cSOD<sup>n108</sup>* mutation), and consequently having increased levels of O<sub>2</sub><sup>-</sup> radicals<sup>13</sup>, were thermosensitive. The results further showed that the viability of *cSOD<sup>n108</sup>/TM6B* heterozygotes too was partially affected at 31°C: starting with nearly equal numbers (about 800) of eggs grown at the two temperatures, the total number of tubby pupae at 31°C was 315 while that at 21°C was 489. Since the *cSOD<sup>n108</sup>* heterozygotes have a reduced Cu-Zn SOD activity in comparison with that of the wild type<sup>13</sup>, their somewhat reduced viability at 31°C is also suggestive of the possibility that loss of SOD activity is responsible for thermosensitivity.

To examine the effect of SOD activity levels on constitutive expression of heat shock genes, the entire gut and associated structures from the tubby and non-tubby late third instar larvae grown as above at 21°C or at 31°C were processed for  $\beta$ -galactosidase activity staining using the chromogenic X-gal substrate as described earlier<sup>14</sup>. Likewise, to examine the TS-induced expression of hsp70 promoter, Tubby and non-tubby



**Figure 1.** *Bg9*-dependent X-gal staining of larval gut and associated structures with (TS) or without (CON) heat shock in *cSOD<sup>n108</sup>* mutant (a, nontubby) and *cSOD<sup>n108</sup>/TM6B* (b, tubby) larvae grown at 21°C or at 31°C: hsp70 promoter-driven  $\beta$ -galactosidase activity of *Bg9* chromosome is seen as dark-blue staining (X-gal staining in non-heat shocked larvae grown at 21°C was similar to that in 31°C grown larvae and is, therefore, not shown).

late third instar larvae grown at 21°C or at 31°C were transferred to 37°C for 1 h (heat shock) and then allowed to recover from the heat shock at 24°C for 1 h following which their gut and associated structures were processed for X-gal staining as above.

The X-gal staining patterns (reflecting activity of the hsp70 promoter in this case) in non-heat shocked and heat-shocked *cSOD<sup>n108</sup>/cSOD<sup>n108</sup>* homozygous (non-tubby) and *cSOD<sup>n108</sup>/TM6B* heterozygous (tubby) larvae grown at 21°C or at 31°C are shown in Figure 1. The absence of any significant X-gal staining in non-heat shocked tubby as well as non-tubby larvae showed that the hsp70 promoter was not constitutively activated in *cSOD<sup>n108</sup>* homozygous larvae, neither in those grown at 21°C nor in those grown at 31°C. After exposure to 37°C, the X-gal staining in *cSOD<sup>n108</sup>* homo- as well as heterozygotes (see Figure 1) indicated a typical induction of the hsp70 promoter. It may be noted here that the *Bg9*-dependent X-gal staining patterns (in non-heat shocked as well as heat shock conditions) seen in the present study with *cSOD<sup>n108</sup>* homo- and heterozygotes did not differ from those seen with *Bg9* in wild type genetic background (not shown, but see Figure 2 reference 14). These results thus showed that the expression pattern of hsp70 promoter (absence of constitutive expression but strongly inducible by TS) was not affected by SOD deficiency.

It may be inferred from these results that although H<sub>2</sub>O<sub>2</sub> is known to induce heat shock genes<sup>9-11</sup>, the excess of O<sub>2</sub><sup>-</sup> ions that accumulate in *cSOD<sup>n108</sup>* null mutant larvae<sup>13</sup> does not induce heat shock genes; nor does this excess of O<sub>2</sub><sup>-</sup> ions affect the inducibility of heat shock genes by TS. Therefore, the observed thermosensitivity of the *cSOD<sup>n108</sup>* mutant larvae is not because of a failure of the heat shock response but probably due to an increased oxygen toxicity at elevated temperature, analogous to the paraquat sensitivity of the *cSOD* null larvae<sup>13</sup>.

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## Oviducal gland of planktonic copepod, *Heliodiaptomus viduus* Gurney—a new report

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The occurrence of a distinct oviducal gland at the posterior end of the oviduct in the freshwater planktonic copepod, *Heliodiaptomus viduus* is reported for the first time. This gland forms an elastic sac within itself, which is filled with secretory materials. The elastic sac and its secretion subsequently form the ovisac. This paper reports on the histology and histochemistry of this gland. The oviducal gland of *H. viduus* resembles that of cirripedes in its structure and function. This gland performs the functions of a crustacean spermatheca by holding the released eggs for internal fertilization as well as the embryonic development till naupliar stage.

THE secretory epithelial cells lining the oviduct as well as accessory sex glands provide materials for the extracellular envelopes of the released eggs to serve a protective and nutritive role to the female gametes. Among female crustaceans information on the occurrence of such glands, their physiological and biochemical aspects is still scarce. Occurrence of such a gland is reported so far only in some cirripedes<sup>1</sup>. We report here, for the first time, the presence of an oviducal gland in the freshwater planktonic copepod *Heliodiaptomus viduus*. The histology and histochemistry of this gland have also been investigated in detail.

In *H. viduus* a pair of oviducts originate from the anterolateral aspects of the ovary and after giving rise to anterior diverticulae, run posteriorly and at the end of the prosome lead into oviducal glands (Figure 1). This gland which occupies the lateral aspects of the posterior segment of the prosome measures 126 ± 7 and 76 ± 3 μ in length and width respectively and continues as the distal part of the oviduct to open to the exterior by the common female reproductive pore.