A New Mutant Affecting Aldehyde Oxidase in *Drosophila melanogaster*

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A new locus, *Aldox-2*, which affects the activity and heat stability of aldehyde oxidase in *D. melanogaster* is described. The *Aldox-2* locus is localized to map position 86 on chromosome 2, between *c* and *px*. Aldehyde oxidase activity in *Aldox-2* homozygotes is approximately 25–30% that of the Oregon-R wild-type control strain. The enzyme from the mutant stock is much more heat labile than is the enzyme from the wild-type strain. Both the activity and heat phenotypes are completely recessive.

The molybdenum hydroxylases in *Drosophila melanogaster* comprise one of the most complex gene-enzyme systems known in multicellular eukaryotes. The structural genes for aldehyde oxidase, xanthine dehydrogenase and pyridoxal oxidase are *Aldox* (3-57), *ry* (3-52) and *lpo* (3-56.9), respectively. Strains carrying the mutants *cin* (1-0.0) and *mal* (1-64.8) lack significant levels of these three enzymes as adults, while *lxd* (3-33) homozygotes possess approximately 25% of normal levels of xanthine dehydrogenase and lack the other two enzyme activities completely [1, 2]. Similar systems of molybdenum hydroxylases are known in *Aspergillus* and *Neurospora* [3, 4]. In each of these fungal systems several structural genes have been defined, as well as several regulatory genes which control enzyme activities. With these systems as models we chose to search for additional loci which are involved in the control of molybdenum hydroxylases in *Drosophila*.

In our initial screen we tested more than 1000 ethyl methanesulfonate treated X chromosomes for the "loss" of aldehyde oxidase activity. All of the mutants recovered in this screen (16) were subsequently determined to be alleles of *cin* or *mal* [5, 6]. These results indicate that, in addition to *cin* and *mal*, the X chromosome carries few if any genes that directly affect the activities of molybdenum hydroxylases. Four loci on chromosome 3 (*ry*, *Aldox*, *lpo*, *lxd*) are already known to affect the activities of these enzymes and, *a priori*, chromosome 3 was considered to be a poor choice to use in a screen for additional autosomal loci affecting molybdenum hydroxylases. We therefore focused our attention on chromosome 2 and acquired collections of stocks homozygous for second chromosomes initially derived from natural populations from Dr. Glenn Bewley, North Carolina State University, Raleigh, and Dr. George Carmody, Carleton University, Ottawa.

To test for aldehyde oxidase activity, ten individuals less than 24 hours old and all of the same sex were taken from each stock culture, homogenized in 0.3 ml of cold buffer (0.1 M Tris-HCl 0.001 M EDTA, pH 7.6) in a 1.5 ml plastic centrifuge tube and centrifuged at $8000 \times g$ for 10 minutes at 4 °C. Three 0.01 ml aliquots of the resulting supernatant were used to measure aldehyde oxidase activity by following the change in absorbance at 600 nm of dichloroindophenol on a recording spectrophotometer [7]. Three 0.005 ml aliquots were used to determine protein concentrations using bovine serum albumen as a standard [8]. The results of these assays are summarized in Table I for one of the stocks, WGM-93. Adults of this stock possess approximately 28% of the levels of aldehyde oxidase activity found in OR adults. The progeny of crosses of WGM-93 and OR flies had wild-type levels of aldehyde oxidase activity.

To confirm that WGM-93 carries a mutant on chromosome 2 which reduces aldehyde oxidase activity, we crossed WGM-93 males to *Cy/Pm; Sb/Ubx*^{130} females (all mutants are fully described in Lindsley and Grell [9]). *Cy Sb* and *Pm Sb* F_1 males of this cross were mated individually to WGM-93 females. The progenies of these matings were

Table I. Aldehyde oxidase activity in WGM-93 and OR flies.

Genotype	N	Aldehyde oxidase activity *	% OR
WGM-93	62	40.8	28.4
WGM-93/+	63	149.8	104
OR	10	143.6	100

* Nanomol of dichloroindophenol reduced per min per mg protein.

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scored for visible phenotypes and were assayed for aldehyde oxidase activity. The aldehyde oxidase assay for single flies consisted of homogenization in 0.03 ml of Tris-HCl EDTA buffer, centrifugation and use of 0.01 ml of supernatant for an aldehyde oxidase assay and two 0.005 ml aliquots for protein determinations. The results of these analyses are summarized in Table II. These data demonstrate that the WGM-93 strain carries a mutant on chromosome 2 that affects aldehyde oxidase activity when homozygous.

To further localize this new mutant, designated *Aldox-2*, WGM-93 females were mated to "all" males. The second chromosome of the latter stock carries 7 recessive markers *(al, dp, b, pr, c, px, sp)*. Individual females, heterozygous for the "all" markers and *Aldox-2* were crossed to *"all Bl"/Cy0* males. (The "all" markers plus the dominant *Bl* balanced with *Cy0*.) The *Bl* male progeny of these crosses were scored for visible markers to determine recombination of the "all" markers and individual males mated to single *Aldox-2* females (WGM-93). At least two Bl^+ progeny from each of these crosses were assayed for aldehyde oxidase activity. Two of the seven visible markers on chromosome 2, *c* (75.5) and *px* (100.5), demonstrated relatively close linkage with *Aldox-2*. These observations are summarized in Table III. Ten recombination events occurred between *c* and *Aldox-2* while 14 occurred between *Aldox-2* and *px*. Thus, the tentative map position of *Aldox-2* is 2-86.

Table II. Segregation of low aldehyde oxidase activity and 2nd chromosome markers.

Phenotype	N	Aldehyde oxidase activity *
Cy (or Pm); Sb	46	201.1
Cy (or Pm); +	25	173.2
+; Sb	37	61.4
+; +	18	54.4

* Nanomol of dichloroindophenol reduced per min per mg protein.

Table III. Linkage relationships of *Aldox-2* with *c* and *px*.

Phenotype	N	Aldehyde oxidase activity	
		Normal	Low
c px	21	21	0
c +	3	1	2
+ px	21	8	13
++	39	0	39

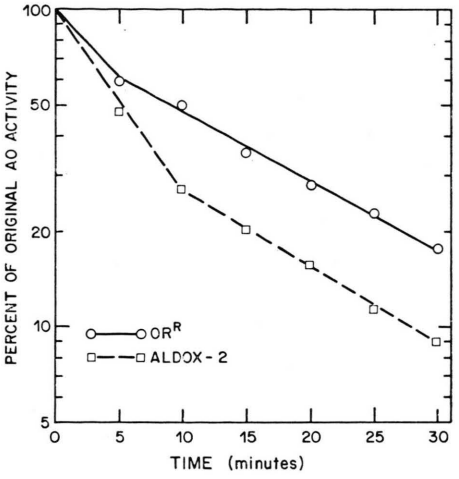

Fig. 1. Percent of original aldehyde oxidase activity remaining after heating (65 °C) crude homogenates of Oregon-R and *Aldox-2* adults less than 24 hours old.

We have previously demonstrated that mutants at the *Aldox* locus on chromosome 3 affect the thermostability of aldehyde oxidase [10]. We have also shown that alleles of *cin* can affect this property of aldehyde oxidase [5]. For comparative purposes we tested thermostability of aldehyde oxidase from the *Aldox-2* stock (Fig. 1). Obviously, the *Aldox-2* strain possesses a form of aldehyde oxidase that is sensitive to elevated temperatures.

The addition of yet another gene to the gene-enzyme system of molybdenum hydroxylases in *D. melanogaster* makes this system even more complex. However, we believe that the more we know of the genetic components of this system the better we can describe the enzyme components of the system. We also believe that additional loci affecting molybdenum hydroxylases in *Drosophila* will be uncovered by screening wild-type populations for altered levels of enzyme activity.

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