Characterization of third chromosome dominant α-methyl dopa resistant mutants (Tcr) and their interactions with l(2)amd α-methyl dopa hypersensitive alleles in Drosophila melanogaster

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Summary

In Drosophila melanogaster two alleles at the Third chromosome resistance locus (Tcr; 3-396) were isolated in a screen of EMS mutagenized third chromosomes for dominant resistance to dietary α-methyl dopa, α-MD, a structural analogue of DOPA. Both alleles of Tcr are recessive lethals exhibiting partial complementation. Almost half (48.3%) of the Tcr40/Tcr45 heterozygotes die as embryos but some survive past adult eclosion. Both the embryonic lethal phenotype and the adult phenotype suggest that Tcr is involved in cuticle synthesis. Tcr mutants suppress the lethality of partially complementing alleles at the α-MD hypersensitive locus, l(2)amd. The viability of Tcr40/Tcr45, however, is not increased by the presence of a l(2)amd allele. The possibility that the Tcr and l(2)amd mutations reveal a catecholamine metabolic pathway involved in cuticle structure is discussed.

1. Introduction

The metabolism of biogenic amines in insects is an intriguing example of evolutionary conservatism. Not only do these compounds act as neurotransmitters and modulators of adenylate cyclase activity, but they are also essential for the formation and stabilization (sclerotization) of the insect cuticle.

Studies of these compounds and the enzymes involved in their synthesis and degradation, especially in a genetically well characterized organism such as Drosophila melanogaster, should contribute not only to our understanding of this complex developmental process but also to our knowledge of catecholamine metabolism.

Numerous enzymes have been implicated in the complex events involved in formation and sclerotization of the insect cuticle (for reviews see Brunet, 1980; Wright, 1987). The best characterized genetically and biochemically is dopa decarboxylase (DDC, EC 4.1.26, 3-4-dihydroxy-L-phenylalanine-carboxylase; for reviews see Wright, 1977, 1987). DDC converts DOPA (3,4-dihydroxy-L-phenylalanine) to dopamine, a precursor of N-acetyldopamine and N-β-alanyldopamine, two compounds which appear to play a major role in sclerotization (Karlson & Sekeris, 1976; Hopkins et al. 1982).

α-methyl dopa (α-MD, α-methyl dihydroxy-phenylalanine) is a structural analog of DOPA. When fed to developing larvae of both D. melanogaster (Sparrow & Wright, 1974) and Lucilia cuprina (Turnbull & Howells, 1980), α-MD is toxic, causing death at the next molt. Both the time of death and the abnormal outer epicuticle in L. cuprina fed the inhibitor (Turnbull et al. 1980) suggest that lethality is the result of abnormal sclerotization.

Initially it was assumed that α-MD induced mortality was due to inhibition of DDC. Surprisingly, however, screens to recover D. melanogaster mutants hypersensitive to α-MD toxicity produced no mutations affecting the Dopa decarboxylase (Ddc; 2-539) locus. Instead they revealed another locus, amd, which maps directly adjacent to Ddc at 539 on the second chromosome (Sparrow & Wright, 1974; Wright et al. 1976). Mutants of the amd locus, l(2)amd alleles, exhibit dominant lethality on low concentrations of α-MD and recessive lethality on media without the inhibitor. Lethality of the homozygotes occurs late in

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embryogenesis and the cuticles of those larvae which hatch have no structural integrity. Some \( l(2)amd \) heterozygotes are partially complementary, producing morphologically normal adults, but at a lower frequency than expected.

Analysis of the two loci in \( D. \) melanogaster indicates that the toxic effect of \( \alpha \)-MD is due to its interaction with the product of the \( amd \) locus. The \( l(2)amd \) mutations have absolutely no effect on the activity or amount of DDC (Sparrow & Wright, 1974; Clark et al. 1978). Furthermore, flies with 150% DDC activity not only fail to exhibit increased resistance to the inhibitor but actually have an increased sensitivity to \( \alpha \)-MD (Marsh & Wright, 1986). Recent studies (Black et al. 1987) have shown that \( l(2)amd \) mutants lack a compound identified as a catecholamine by low resolution mass spectroscopy and other criteria.

To explore further the \emph{in vivo} effect of \( \alpha \)-MD, we have isolated two third chromosome mutations resistant to the inhibitor, \emph{Third chromosome resistant} (\( Tcr \) alleles), and investigated their interactions with the \( amd \) locus. The results reported below suggest that \( Tcr \) and \( l(2)amd \) mutations may define a previously unrecognized pathway involved in cuticle structure and catecholamine metabolism.

2. Materials and methods

Except where otherwise noted, all gene and chromosome designations are those of Lindsley & Grell (1968).

Media to which \( \alpha \)-MD was added was similar to that described by Sparrow & Wright (1974).

(i) Isolation of third chromosome mutations resistant to \( \alpha \)-MD

\( bw; TM3, Ser/Sb \) virgins were mass mated to lethal free \( bw; st \) males which had been mutagenized with ethyl methanesulphonate according to the method of Lewis & Bacher (1968). \( bw; TM3, Ser \) males carrying mutagenized \( bw; st \) chromosomes were mated individually to \( bw/bw \) pr. \( TM3, Sb Ser/st \) virgins, and the progeny generated on media containing a lethal concentration (0.8 mM) of \( D, L-\alpha \)-MD. In 674 of the 1715 crosses, \( F_2 \) males were used to resolve for possible mosaics (Jenkins, 1967). All putative mutations were re-tested on the same concentration of the inhibitor.

(ii) Mapping of two \( \alpha \)-MD resistant mutations

Resistance to lethal concentrations of \( \alpha \)-MD of the two strains tested segregated with the mutagenized third chromosomes. A preliminary mapping of \( Tcr^{45} \) with a multiply marked third chromosome placed the resistant locus between \emph{hairy} (\( h; 3-26-5 \)) and \emph{thread} (\( th; 3-43-2 \)). A more precise mapping for the resistance of \( Tcr^{45} \) was performed using the multiply marked third chromosome \emph{ru th st cu sr e' ca}. Progeny of the mapping cross were raised on media containing 0.7 mM \( D, L-\alpha \)-MD. As a control, wild-type flies (Oregon-R and Canton-S) were raised on the same media.

(iii) Effective lethal phase and mutant phenotypes

In order to determine the effective lethal phase and observe any lethal phenotypes of \( Tcr \), reciprocal matings of \( Tcr^{45} st/+ \) with \( Tcr^{45} st/+ \) were made. Eggs were collected and placed on square pieces of blotting paper (50 eggs/square). One square was placed in each vial. After 48 h the unhatched eggs were removed and transferred to another piece of blotting paper in a new vial. This procedure allowed more time for the embryos to hatch and prevent other larvae from moving the eggs off the paper. After another 24 h the chorion of the eggs was removed using double stick tape, the eggs mounted and scored for fertilization. The vials were all placed at 25 °C. The number of pupae was counted and this number subtracted from the number of hatched eggs to determine how many died in the larval stages. Adults were scored for \( st \) eyes. Dead pupae were dissected from their pupal cases and examined for phenotype.

(iv) Interaction between the \( Tcr \) and \( amd \) loci

Interaction between \( Tcr \) and \( amd \) was determined by examining the effects of a mutant allele of one locus on partially complementing alleles at the other locus. Males of the following genotypes: \( l(2)amd^{111} en \) \( bw/SM5; TM3, Sb Ser/Tcr^{40} st \) or \( l(2)amd^{111} en \) \( bw/SM5; Gl/Tcr^{40} st \) were mated to virgin females that were either \( SM5 \) or \( CyO \) and heterozygous for another \( l(2)amd \) allele at 3–5 pairs per vial. Partially complementing \( l(2)amd \) heterozygotes could be identified by either the absence of \( Cy \) (from \( SM5 \) or \( CyO \)) or by homozygous \( cn \) \( bw \) eyes. The presence of a \( Tcr \) null allele was indicated by the absence of dominant markers \( Gl \) or \( Sb Ser \). The effects of \( l(2)amd \) alleles on \( Tcr^{40}/Tcr^{45} \) complementation were tested with \( l(2)amd^{111} \) \( Gl^{120} Bl/+; Tcr^{40} st/+ \) crossed to \( bw/+; Tcr^{45} st/+ \). Reciprocal crosses were used to look for any maternal effects. Eggs from this cross were picked (50 eggs/vial), allowed to develop at 25 °C and scored as for the effective lethal phase egg pick. This protocol was followed to insure uncrowded conditions since the only \( Tcr^{40}/Tcr^{45} \) adults recovered have come from uncrowded cultures. The presence of \( l(2)amd^{111} \) was ascertained by the presence of \( Bl \) since they are closely linked (0.9 map units apart) and \( Tcr^{40}/Tcr^{45} \) heterozygotes were scored by their rough eyes; a characteristic of the \( Tcr \) mutant phenotype.

To determine if any \( Tcr \) allele induced increase in complementation was dependent upon at least partially functional \( amd \) gene product, \( l(2)amd^{111} en \) \( bw/Sp bw \); \( Tcr^{40} st/TM3, Ser \) males were crossed to \( CyO \) females to score for \( st \) eyes; a characteristic of the \( Tcr \) mutant phenotype.
Table 1. Segregation of resistance to L-α-MD with the third chromosomes of Tcr⁴⁰ and Tcr⁴⁵

| α-MD (mM) | No. of eggs | Adult genotypes |
|-----------|-------------|-----------------|
|           |             | TM₃, Ser/Tcr⁴⁰ st × TM₃, Sh Ser/Tcr⁺ st | TM₃, Sh Ser/Tcr⁴⁰ st/Tcr⁺ st | TM₃, Ser/Tcr⁺ st |
| 0         | 249         | 63              | 53              | 62              |
| 0-1       | 276         | 78              | 61              | 2               |
| 0-2       | 286         | 56              | 37              | 0               |
| 0-3       | 287         | 41              | 12              | 0               |
| 0-4       | 121         | 8               | 0               | 0               |

Table 2. Tcr⁴⁰ st/Tcr⁴⁵ st effective lethal phase

| Cross*: Tcr⁴⁰ st/+ x Tcr⁴⁵ st/+ | Dead or eclosed |
|---------------------------------|-----------------|
|                                 | Embryos (480)   |
|                                 | Pupae (366)     |
|                                 | Adults (352)    |
| Wild-type                       | 2               | 343             |
| Expected (%) (Expected = 75% of 480 = 360) | 0.6% | 95.2% |
| Tcr⁴⁰/Tcr⁴⁵                     | 58%             | 11              | 9               |
| Expected (%) (Expected = 25% of 480 = 120) | 48.3% | 9.2% | 7.5% |
| Unknown                         | 0               | 1               | 0               |

* Data from reciprocal crosses were pooled since no maternal effect was found.
* Tcr embryos have a pale cephalopharyngeal apparatus.

3. Results
(i) Recovery of mutations

Eighty putative, α-MD resistant mutations were found among 1715 ethyl methanesulphonate mutagenized chromosomes. Interestingly, all were recovered from those crosses in which the extra generation to resolve for mosaicism was not included and, as expected, most failed to maintain the resistant phenotype in subsequent tests. Two, Tcr⁴⁰ and Tcr⁴⁵, were chosen for further investigation as they consistently demonstrated high viability on 0.8 mM-D,L-α-MD, a concentration well above the LD₁₀₀ for wild type.

Table 1 shows the resistance of the two strains to α-MD and the segregation of this phenotype with the mutagenized third chromosomes. The L form of the inhibitor, which is approximately twice as toxic as the D,L compound (Sparrow & Wright, 1974), was used in this experiment. The LD₅₀ for both mutations was found to be above 0.3 mM. The LD₅₀ for wild type is less than 0.1 mM.

The mutagenized third chromosomes of both strains were found to behave as recessive lethals on media with or without the inhibitor. Crosses between Tcr⁴⁰ and Tcr⁴⁵ stocks showed that the heteroallelic combination (Tcr⁴⁰/Tcr⁴⁵) are poor partial complementors (less than 20% complementation, see Tables 2 and 4) indicating that they are alleles and that recessive lethality is an additional phenotype.

(ii) Genetic localization

Preliminary mapping of Tcr⁴⁵ with a multiply marked third chromosome placed the locus between h and th. A finer mapping was performed for Tcr⁴⁰ by crossing Tcr⁴⁰/ru h th st cu sr e ca virgins with ru h th st cu sr e ca males on 0.7 mM-DL-α-MD. Control bottles: Oregon R; 10 females and 7 males, and Canton S; 7 pairs of parents, failed to produce progeny at this inhibitor concentration. The mapping cross was set in a total of 12 bottles; one with seven pairs of parents, the remainder with 10 females and 5-10 males, and the progeny scored for h and th. A total of 1790...
alleles partially complementing l(2)amd alleles

Table 3. Effects of Tcr\textsuperscript{−} alleles on viability of partially complementing l(2)amd alleles

| Basic cross: Virgins l(2)amd\textsuperscript{−}/Cy balancer \times l(2)amd\textsuperscript{+} en bw/Cy balancer; Tcr\textsuperscript{−} st/ marker males | Expected (%) |
|---|---|
| Control without Tcr\textsuperscript{−} | Experimental with Tcr\textsuperscript{−} |
| l(2)amd\textsuperscript{+}+ /l(2)amd\textsuperscript{−}; Tcr\textsuperscript{−}/+ | 12 | 111-0 |
| l(2)amd\textsuperscript{+}+ /l(2)amd\textsuperscript{−}; Tcr\textsuperscript{−}/+ | 24 | 74-1 |
| l(2)amd\textsuperscript{+}+ /l(2)amd\textsuperscript{−}; Tcr\textsuperscript{−}/+ | 84-0 | 124-0 |
| l(2)amd\textsuperscript{+}+ /l(2)amd\textsuperscript{−}; Tcr\textsuperscript{−}/+ | 64-7 | 69-6 |
| l(2)amd\textsuperscript{+}+ /Df{2L)TW130; Tcr/ + } | 22-3 | 110-9 |
| l(2)amd\textsuperscript{+}+ /Df{2L)TW130; Tcr/ + } | 0 | 0 |

Progeny were obtained: 1392 h\textsuperscript{+} th\textsuperscript{+}, 310 h\textsuperscript{+} th\textsuperscript{−}, 82 h\textsuperscript{+} th and 6 h th. The position of the Tcr locus was calculated to be approximately 39-6 by determining the percentage of crossovers between h and Tcr and multiplying by the standard map distance between h (3-295) and th (3-432).

(iii) Effective lethal phase and mutant phenotype

The results of the lethal phase experiment indicated that slightly less than half (48-3\%) of the Tcr\textsuperscript{+} st/Tcr\textsuperscript{−} st individuals die during embryogenesis (Table 2). These heterozygotes lived for 48 or more hours after egg deposition but failed to escape from the egg. They appeared to be mature first instar larvae except that their cephalopharyngeal apparatus was usually underpigmented and they were very slow to darken after death compared to other embryonic lethals.

Those Tcr\textsuperscript{+} st/Tcr\textsuperscript{−} st heterozygotes which completed pupariation survived to become pharate adults with 45\% of these eclosing from their pupal cases. The adults which eclosed were 7-5\% of the expected and usually found dead or dying in the vial food. Adults which were not in the food were usually unable to walk on their tarsi and walked instead on their metatarsi or tibia with uncoordinated leg movements.

Both the pharate adults which failed to eclose and the eclosed adults had a pleiotrophic phenotype. In addition to abnormal walking behaviour, these phenotypes were observed: dark, rough often reduced eyes with black spots; missing bristles, especially from the head; one to all of the ocelli missing; notching of the wings; reduced scutellum; Minute-like bristles mixed with wild-type bristles; unexpanded ptilinum; reduced sex combs and a greyish abdomen. The expressivity of these traits was variable and only the rough eyes had full penetrance. Individuals unable to eclose were sometimes successful in splitting open their operculum but unable to crawl out of the pupal case. Others could not open their operculum either because they were too far away from it or because they lacked an adequate ptilinum. A few seemed to completely lack a ptilinum. The pupal cases all had normal coloration and appeared to be appropriately hardened. One female survived to produce two progeny. All but two of the 20 adults and dead pupae were st and these two had a dark eye phenotype. Tcr\textsuperscript{−} flies have dark eyes except when they are also homozygous st, in which case they have st eyes, indicating that st is epistatic to Tcr for eye colour.

(iv) Interaction between Tcr\textsuperscript{+} and the amd locus

All partially complementing l(2)amd heterozygotes tested showed an increase in viability when either Tcr\textsuperscript{+} or Tcr\textsuperscript{−} was present (Table 3). The failure to produce any progeny hemizygous for a l(2)amd allele (l(2)amd/Df{2L)TW130; Tcr/ + ) when either Tcr allele was present indicates that at least a partially functional amd protein is required before a Tcr allele can suppress the lethality caused by l(2)amd.

No increase in complementation between the two Tcr alleles was seen when a l(2)amd allele was also present. l(2)amd\textsuperscript{−} failed to increase the number of Tcr\textsuperscript{+}/Tcr\textsuperscript{−} progeny produced (data not shown) when the crosses were done with 3–5 pairs per vial. Also,

Table 4. Effects of l(2)amd\textsuperscript{+26} on the viability of Tcr\textsuperscript{+}/Tcr\textsuperscript{−}

| Fertile eggs | Adult phenotypes\textsuperscript{b} |
|---|---|
| Dead\textsuperscript{a} Tcr\textsuperscript{−} embryos | wild type and l(2)amd\textsuperscript{+26} Bl |
| Cross: Virgins l(2)amd\textsuperscript{+26} Bl/ + ; Tcr\textsuperscript{+} st/ + \times bw/ + ; Tcr\textsuperscript{−} st/+ Males | Tcr\textsuperscript{−} |
| 294 | 15 (19\%)\textsuperscript{c} |
| Cross: Virgins bw/ + ; Tcr\textsuperscript{−} st/+ \times l(2)amd\textsuperscript{+26} Bl/ + ; Tcr\textsuperscript{+} st/+ Males | 315 | 17 (8\%) \times 7 (17\%) |

\textsuperscript{a} Tcr\textsuperscript{−} embryos have a pale cephalopharyngeal apparatus.

\textsuperscript{b} Adults in this table include those individuals that died as pharate adults and those which eclosed from the pupal case.

\textsuperscript{c} % of expected.
$l(2)amd^{H26}$ did not increase complementation between $Tcr^{40}$ and $Tcr^{45}$ in uncrowded (50 eggs/vial) conditions (Table 4). $l(2)amd$ alleles have a maternal effect on $Tcr^{40}/*$ embryos, causing an increase in embryonic mortality. The results in Table 4 show that a maternally derived $l(2)amd^{H26}$ chromosome increased embryonic mortality approximately three-fold (63.9% versus 19% embryonic mortality) and in another cross (data not shown) using $Sco$ as a dominant marker, embryonic lethality was also increased approximately three-fold (81.2% versus 29%) due to a maternally derived $l(2)amd^{H26}$ chromosome.

### 4. Discussion

Two third-chromosome mutations, $Tcr^{40}$ and $Tcr^{45}$, conferring dominant resistance to dietary concentrations of the enzyme inhibitor $\alpha$-MD lethal to control stocks were isolated and characterized. The mutations were found to be recessive lethal alleles, defining the $Tcr$ locus which was mapped between $h$ and $th$ at approximately 39.6 on the third chromosome. The effective lethal phase and lethal phenotypes at two stages have been reported as well as the interaction between $Tcr$ and a locus which affects sensitivity to $\alpha$-MD, $l(2)amd$. $l(2)amd$ alleles cause a dominant hypersensitivity to $\alpha$-MD whereas mutations at $Tcr$ confer dominant resistance.

A critical period for $Tcr$ expression is during embryogenesis. This was determined by the lethal phase experiment from a mating of $Tcr^{40}/*$ with $Tcr^{45}/*$. It was assumed that all progeny with at least one wild type $Tcr$ allele survived to the adult stage (75% of the progeny), while those which died were heterozygous for the two $Tcr$ alleles. 95.8% of the expected wild-type progeny were recovered as either one wild type $Tcr$. 95.8% of the expected wild-type progeny were recovered as either one wild type $Tcr$ allele survived to the adult stage (95.8%) or eclosed adults (95.2%), indicating that almost all of the dead progeny could be accounted for by the expected number of $Tcr^{40}/Tcr^{45}$ heterozygotes. The greatest lethality of $Tcr^{40}/Tcr^{45}$ individuals occurred during embryogenesis when 48.3% of the expected number of $Tcr^{40}/Tcr^{45}$ heterozygotes died. Most of the dead embryos had an underpigmented cephalopharyngeal apparatus (mouthparts) and were slow to darken after death when compared to other embryonic lethals. An examination of the eggs 48 h after laying showed that the unhatched larvae were active and looked like wild-type mature first instar larvae except for the pale mouthparts. One can infer that the failure of the larvae to hatch is a result of incompletely sclerotized mouth hooks as indicated by the lack of pigmentation.

Lethality during the pupal stage occurs because the $Tcr^{40}/Tcr^{45}$ heterozygotes cannot eclose. In some instances they were unable to split open their operculum, either because they were too far from it, or because their ptilinum did not expand enough to force it open. Other individuals could open the operculum but were unable to escape from the pupal case.

Whether this latter failure is due to a lack of coordinated movement or to a cuticle which is not hard enough to permit them to crawl out could not be determined. When removed from the pupal case, these individuals were capable of movement but it was very spastic. This abnormality may be the result of uncoordinated muscular movements or inability of their legs to support their body.

The adult $Tcr^{40}/Tcr^{45}$ heterozygotes that did eclose were usually found stuck in the food, dead or dying, but one female lived to produce two offspring indicating that the females are at least partially fertile. The adults as well as the pupae have a pleiotropic phenotype. Although most of the phenotypes might be due to cell death (missing ocelli, missing bristles, notching of the wings, dark spots in the eyes, reduced eyes, reduced scutellum, reduced ptilinum and reduced sex combs), not all can be easily explained that way (dark eyes and the soft cuticle).

The soft adult cuticle and underpigmented embryos whose mouthparts are probably not hard enough to rip through the egg membrane, together with the fact that these $Tcr$ mutants were isolated by their dominant resistance to a compound ($\alpha$-MD) known to interfere with cuticle synthesis (Turnbull et al. 1980), strongly suggests that the $Tcr$ gene is required for normal cuticle production. The two other loci known to be affected by dietary $\alpha$-MD, $Ddc$ and $l(2)amd$, are apparently also involved in cuticle synthesis (Wright et al. 1981; Wright, 1987).

Alleles at both the $Ddc$ and $l(2)amd$ loci exhibit intracistronic complementation, and the $Ddc$ locus has been identified as the structural locus for DDC (Wright et al. 1976). In the case of the $amd$ locus, the enzyme produced by the wild type allele is necessary for the synthesis of an, as yet, unidentified catecholamine (Black et al. 1987).

The interactions between $Tcr$ and $l(2)amd$ were examined by looking at the effects of a mutant allele at one of the loci on the viability of partially complementing alleles at the other locus. Either allele of $Tcr$ caused an increase in the viability of partially complementing $l(2)amd$ alleles. At least some functional $amd$ gene product is necessary for this interaction to occur since, even in the presence of $Tcr$ mutants, $l(2)amd$ hemizygotes remain completely lethal. On the other hand, not only did two different alleles of $l(2)amd$ fail to increase the viability of $Tcr^{40}/Tcr^{45}$ heterozygotes, but if the chromosome bearing the $l(2)amd$ allele was maternally derived, the viability of $Tcr^{40}/Tcr^{45}$ embryos was decreased. It is clear from these results that the products of the two loci are not competing for the same substrate because a reduction in either enzyme would increase the amount of substrate available to the other and that should increase the viability of partially complementing alleles at the second locus.

$D. melanogaster$ DDC appears not to be significantly inhibited by $\alpha$-MD in vitro (Black &
Smarrelli, 1986). Furthermore, genetic results indicate that in *D. melanogaster* inhibition of DDC is not the major cause of α-MD induced lethality (Wright et al. 1981). If lethality were caused by a failure to produce enough dopamine, anything which reduced the amount of DDC present should decrease resistance to α-MD. Just the opposite occurs. Individuals with a Ddc<sup>-</sup> allele are more resistant to α-MD, while individuals with an extra dose of the Ddc gene are more sensitive (Marsh & Wright, 1986). Thus the actual in vivo effect of α-MD appears to result from inhibition of another enzyme which also uses DOPA as its substrate. Increased DDC removes more DOPA from the pool, thus reducing the substrate available to this enzyme and enhancing the sensitivity of the organism to the inhibitor.

The data obtained so far strongly suggest that toxicity of α-MD is due to inhibition of the protein produced by the *amd* locus. This locus is directly adjacent to *Ddc* and exhibits an extensive sequence homology with *Ddc* (Eveleth et al. 1986; Marsh et al. 1986). Comparison of the deduced primary and secondary structures of the products of these loci (Eveleth & Marsh, 1986) suggest that they resulted from gene duplication. Thus it is quite likely that both share DOPA as a substrate, and possibly their subsequent divergence has rendered the *amd* product more sensitive to α-MD. The genetic evidence supports this view. Thus far, mutations to hypersensitivity have been recovered only at *amd*. Furthermore, three wild type doses of the *amd* locus increase resistance to α-MD (Marsh & Wright, 1986).

The observed interaction between the *amd* and *Tcr* loci has provided evidence for another metabolic pathway in *Drosophila* which appears to be involved in the formation of the cuticle. The data available thus far can be explained by the simple scheme presented in Fig. 1. The substrate, probably DOPA, is converted by the *amd* enzyme to a catecholamine, compound A, which is then converted to B<sup>1</sup> by an enzyme produced by the *Tcr* locus, and to B by the enzymatic product of an as yet unidentified gene. The recessive lethality of mutations at the *Tcr* locus indicates that some B<sup>1</sup> is necessary for viability, but B is the more vital of the two compounds. A reduction in the activity of the *amd* enzyme, either by α-MD inhibition or by incomplete complementation among *l(2)amd* alleles results in complete or partial lethality by reduction in B. In either case, heterozygosity for the *Tcr* mutation would increase the amount of A available resulting in an elevation of B compatible with increased viability. The *amd* protein is placed first in the pathway because at least some activity of this enzyme is necessary for these interactions to occur: the *Tcr* mutations do not confer absolute resistance to the inhibitor (Table 1) and a genotype which provides little or no *amd* protein shows no response to *Tcr* (Table 3). While other, more complicated models can be presented, such as *Tcr* coding for a protein required to produce an enzyme co-factor or a transport protein, this simple scheme can account for all of the observed data. The fact that the *l(2)amd* adult escaper phenotype is different from the *Tcr* escaper phenotype would be due to the increased need of compound B over compound B<sup>1</sup>. Whenever enough A is present to permit adequate B to be produced, more than enough B<sup>1</sup> will be made. The *l(2)amd* escapers probably have just enough B but ample B<sup>1</sup> from the *Tcr* enzyme so that no *Tcr* phenotype is produced. A similar argument can be used to explain the difference in the embryonic phenotype.

We have recovered mutants at a new locus which is involved in cuticle synthesis. A scheme has been presented in which all of the observations on α-MD toxicity and mutant interactions can be accounted for by placing the catecholamine produced by the *amd* enzyme before a branch point in a metabolic pathway with the *Tcr* gene product being an enzyme in one of the branches. The substrate of the *amd* enzyme, in this model, appears to be the catecholamine DOPA. The evidence supports *amd* and *Tcr* being in a previously uncharacterized branch of a catecholamine metabolic pathway involved in cuticle synthesis, and if our scheme is correct there is at least one other enzyme in the pathway.

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### References

Black, B. C. & Smarrelli, J., Jr. (1986). A kinetic analysis of *Drosophila melanogaster* Dopa decarboxylase. *Biochimica et Biophysica Acta* **870**, 31-40.

Black, B. C., Pentz, E. S. & Wright, T. R. F. (1987). The alpha methyl dopa hypersensitive gene, *l(2)amd*, and two adjacent genes in *Drosophila melanogaster*: Physical location and direct effects of *amd* on catecholamine metabolism. *Molecular and General Genetics* **209**, 306-312.

Brunet, P. C. J. (1980). The metabolism of the aromatic amino acids involved in the cross-linking of insect cuticle. *Insect Biochemistry* **10**, 467-500.

Clark, W. C., Pass, P. S., Benkataaraman, B. & Hodgetts, R. B. (1978). Dopa decarboxylase from *Drosophila*...
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Eveleth, D. D. & Marsh, J. L. (1986). Evidence for evolutionary duplication of genes in the dopa decarboxylase region of Drosophila. Genetics 114, 469–483.

Eveleth, D. D., Gietz, R. D., Spencer, C. A., Nargang, F. E., Hodggetts, R. B. & Marsh, J. L. (1986). Sequence and structure of the dopa decarboxylase gene of Drosophila: Evidence for novel RNA splicing variants. EMBO J. 5, 2663–2672.

Hodgetts, R. B., Nargang, F. E., Gietz, R. D., Eveleth, D. D. & Marsh, J. L. (1986). Sequence and structure of the dopa decarboxylase gene of Drosophila: Evidence for novel RNA splicing variants. EMBO J. 5, 2663–2672.

Karlson, P. & Sekeris, C. E. (1976). Control of tyrosine metabolism and cuticle sclerotization by ecdysone. In: The Insect Integument (ed. H. R. Hepburn), pp. 145–156. Elsevier: Amsterdam.

Lewis, E. B. & Bacher, F. (1968). Method of feeding ethyl methanesulfonate (EMS) to Drosophila males. Drosophila Information Service 43, 193.

Lindsey, D. L. & Grell, E. H. (1968). Genetic variations of Drosophila melanogaster. Carnegie Institution of Washington Publication 627.

Marsh, J. L. & Wright, T. R. F. (1979). Control of dopa decarboxylase expression during development in Drosophila. In: Eukaryotic Gene Regulation, ICN-UCLA Symposia on Molecular and Cell Biology, vol 14 (ed. R. Axel, T. Maniatis and C. F. Fox), pp. 183–194. New York: Academic Press.

Marsh, J. L. & Wright, T. R. F. (1986). Evidence for regulatory variants of the dopa decarboxylase and alpha-methyl dopa hypersensitive loci in Drosophila. Genetics 112, 249–265.

Marsh, J. L., Erfl, M. P. & Leeds, C. A. (1986). Molecular localization, developmental expression and nucleotide sequence of the alpha methyl dopa hypersensitive gene of Drosophila. Genetics 114, 453–467.

Sparrow, J. C. & Wright, T. R. F. (1974). The selection for mutants in Drosophila melanogaster hypersensitive to alpha-methyl dopa, a dopa decarboxylase inhibitor. Molecular and General Genetics 130, 127–141.