CHARACTERIZATION OF AN AUTOSOMAL RUDIMENTARY-SHAPED WING MUTATION IN DROSOPHILA MELANOGASTER THAT AFFECTS PYRIMIDINE SYNTHESIS

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ABSTRACT

A new autosomal mutation, rudimental (ral), which causes rudimentary-shaped wings in Drosophila melanogaster, has been isolated following ethyl methanesulfonate (EMS) mutagenesis. The wing phenotype of rudimental is identical to that of the X-linked rudimentary (r) mutation, which affects the first three enzymes in the pyrimidine biosynthetic pathway. The autosomal mutant maps very close to ebony (3-70.7) at 70.42 on the right arm of chromosome 3. Analysis of the enzyme activities of orotate phosphoribosyltransferase (OPRTase) and orotidylate decarboxylase (ODCase) indicates that the rulae6a allele has less than wild-type activity for both enzymes. This result is discussed in light of the fact that the OPRTase and ODCase activities are part of an enzyme complex, as are the carbamyl phosphate synthetase (CPSase), aspartate transcarbamylase (ATCase) and dihydroorotase (DHOase) activities, which are encoded by the complex rudimentary locus. We suggest that rudimental is also a complex locus.

THE first three enzymes of the pyrimidine biosynthetic pathway have been extensively studied in Drosophila melanogaster. Flies mutant at the X-linked rudimentary locus (r, 1-55.3) have less than wild-type levels of one or more of these three enzymes, carbamyl phosphate synthetase (CPSase; EC 2.7.2.9), aspartate transcarbamylase (ATCase; EC 2.1.3.2) and dihydroorotase (DHOase; EC 3.5.2.3) (Norby 1973; Jarry and Falk 1974; Rawls and Fristrom 1975). The locus for these enzymes is a complex X-linked locus as determined by Carlson's (1971) fine-structure mapping, with DHOase alleles mapping at one end, CPSase alleles in the middle and alleles eliminating only ATCase mapping at the other end. Biochemical evidence indicates that all three of these enzymes in Drosophila are contained within a single enzyme complex (Jarry 1976; Brothers et al. 1978; Jarry 1978).

Falk and DeBoer (1980) discuss the induction of 86 new X-linked, rudimentary-shaped wing mutations in an attempt to determine the number of loci on the X chromosome that can mutate to give a rudimentary phenotype. All of these mutants were alleles of the rudimentary locus, suggesting that there are...
few, if any, other genes on the X chromosomes that play a direct role in the pyrimidine biosynthetic pathway.

To date there has been no genetic analysis of the last three enzymes in the pathway: dihydroorotate dehydrogenase (DHODehase; EC 1.3.3.1), orotate phosphoribosyltransferase (OPRTase; EC 2.4.2.10) and orotidylate decarboxylase (ODCase; EC 4.1.1.23). The screen for mutants affecting pyrimidine biosynthesis was extended to the autosomes in an attempt to recover mutants affecting the later enzymes in the pathway (Falk, DeBoer and Lastowski 1979). This paper will discuss the characterization of an autosomal mutant that exhibits a rudimentary-like phenotype. We demonstrate that this mutant affects the last two enzymes of the pyrimidine pathway, which, like the first three enzymes, comprise an enzyme complex in Drosophila (Rawls 1979). Furthermore, we suggest that this locus, the rudimental ($ral$) locus is, like the $r$ locus, a complex locus that encodes both of the last two enzymes of the pyrimidine biosynthetic pathway.

**MATERIALS AND METHODS**

**Stocks and growth conditions:** Flies were cultured on dead yeast-sucrose media (Falk and Nash 1974). Most experiments were carried out at 21$^\circ$ ± 1$^\circ$.

The mutant $rpyr^{11}$ was isolated by Falk and Nash (1974) and has wings that are slightly defective due to reduced pyrimidine synthesis. The markers shibire ($shi$) and forked ($f$) are described in Grigliatti et al. (1973) and Lindsley and Grell (1968). $FNC4$ is an X chromosome that contains an unmapped temperature-sensitive lethal isolated by Falk and Nash (1972), and was used as a virginator stock. The chromosome 2 balancer, $SM5$, carries the dominant mutation $Cy$, and the chromosome 3 balancer, $TM3$, carries $Sb$ and $Ser$ (Lindsley and Grell 1968). A multiply marked chromosome 3, $ru h th st cu sr e^8 ca$, with markers spaced at 2 to 20 map-unit intervals (Lindsley and Grell 1968) was used to obtain preliminary mapping data. Two mutants, $g^B$ (3-63.1) and $e^8$ (3-70.7) (Lindsley and Grell 1968), were used for the final mapping study.

**Mutagenesis:** Ethyl methanesulfonate (EMS) mutagenesis was performed using 1- to 3-day-old $shi$ $rpyr^{11}$ $f$ males, according to the method of Lewis and Bacher (1968). The $shi$ $rpyr^{11}$ $f$ chromosome was used since $rpyr^{11}$ already results in decreased pyrimidine synthesis. This had two potential advantages: first, it increased the probability of detecting a leaky mutant; second, it reduced the possibility of a toxic accumulation of intermediates that might result in the lethality of mutants with a block late in the pathway. The screen for autosomal mutants is detailed in Figure 1. Putative autosomal mutants obtained in the $F_2$ generation in Figure 1 were mated to attached-X females. If the males in the $F_2$ generation of this next cross were not mutant but some of those in the $F_1$ were, then the mutation was confirmed as autosomal. Furthermore, the presence of some mutant attached-X females in the $F_2$ generation indicated that the mutation can express itself independently of $rpyr^{11}$.

**Mapping:** Determination of linkage group was carried out using $SM5$ and $TM3$. $SM5$ carries $Cy$ and $TM3$ carries $Sb$ and $Ser$. If the mutant always segregated from $Sb$ and $Ser$ and not from $Cy$, then the mutant is on chromosome 3. On the other hand, consistent segregation from $Cy$ but not from $Sb$ and $Ser$ indicated that the mutation maps to chromosome 2. Two different chromosome 2 and 3 markers, $Pm$ and $H_8$, were also used for linkage group determination.

Preliminary mapping with the $ru h th st cu sr e^8 ca$ chromosome involved crossing multiply marked females with homozygous $ral^{{ala}}$ males. The $F_1$ generation was crossed inter se, and the resulting progeny were screened for recombinants. Representatives of most of the 7 recombinant classes and their reciprocals were then checked to see if they carried $ral^{{ala}}$ by mating them to attached-X $shi/Y$; $TM3/ral^{{ala}}$ females. Further preliminary localization of the mutant em-
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played a deficiency of the ebony region, Df(3R)eR8, with breakpoints at 93B4–5 and 94A5–16, that was isolated by BRUCE BAKER.

The markers glb (3–63.1) and e4 (3–70.7) were used for accurate mapping of rudimental. Males that were homozygous for ral^neu were mated to the homozygous glb^e4 females. Virgin glb^e4/ral females were back-crossed to homozygous glb^e4 males, and the progeny of this cross were screened for recombinants. Male recombinants were subsequently mated to attached-X shi/Y; TM3/ral females to see if ral had been incorporated into the recombinant chromosome. The 95% confidence level was determined by the method described by O'BRIEN and MACINTYRE (1978).

Female sterility test: Females that are homozygous for r are female sterile and are sometimes rescuable at a low frequency by wild-type sperm (Morgan 1915; Falk et al., 1980). To determine whether this was also true for ral, a total of 20 attached-X shi/Y; ral/ral females were crossed to 20 +/+Y; TM3/ral males.

Enzyme assays: Materials include DEAE-cellulose paper, grade DE-81, from Whatman, Inc., formic acid and ammonium formate from Fisher Scientific, Omnifluor from New England Nuclear, [6-14C] orotic acid (59 mCi/mmole) from Amersham and orotidine from CalBiochem. All enzymes, the Dowex resins and all other biochemicals were purchased from Sigma Chemical Company.

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**Figure 1.**—General scheme for isolation of autosomal mutants. (* indicates EMS-treated chromosome.)
Due to the commercial inavailability of $^{14}$C-orotidine 5'-monophosphate (OMP) labelled in the pyrimidine ring, the compound was synthesized from [6-$^{14}$C] orotic acid (OA) using a modification of the method of Rawls (1978). After termination, the reaction mixture was loaded onto a column of Dowex 1-X8, 100 to 200 mesh, formate form. Isolation of $^{14}$C-OMP from other products and unreacted $^{14}$C-OA was accomplished by washing the column with a 15 ml aliquot of 0.1 M ammonium formate, pH 4.5, and then eluting with a linear gradient from 0.1 to 0.6 M ammonium formate, pH 4.5. The rest of the procedure followed the protocol of Rawls (1978).

Flies for the assay of OPRTase and ODCase were cultured at 21° ± 1°C. Homozygous $r a l$ males and control $g l^P e^4$ males were collected and aged so that they were 1 to 3 days old when they were frozen at −70°. Freshly frozen flies were homogenized in cold potassium morpholinopanesulfonate (MOPS) buffer (10 mM, pH 7.2) at a concentration of 25 flies/0.5 ml of buffer. The OPRTase and ODCase activities were assayed simultaneously according to the method of Rawls (1978; 1979) in which radioactive OMP is converted to $^{14}$C-orotic acid and $^{14}$C-uridine 5'-monophosphate (UMP). The 25 μl reaction tube contained 5 μl extract, MOPS-KOH buffer (50 mM, pH 7.2), tetrasodium pyrophosphate (2.5 mM), MgSO₄ (1mM) and [6-$^{14}$C] OMP (0.088 mM, 59 mCi/mmole). Control reaction mixtures consisted of heat-denatured enzyme. After incubation for 15 min at 30°, the reaction was terminated by addition of 5 μl of 10 M formic acid. A 5μl aliquot of the reaction mixture was spotted on DEAE-cellulose paper to separate the radioactive substrate and products. Chromatograms were developed in a methanol-formic acid-water (1:1:1,v:v:v) solvent (Solvent I), and spots corresponding to OMP, UMP, OA and uridine were excised and counted. To determine orotidine formation, a Solvent II consisting of ammonium formate (0.075 M, pH 4.5) was used.

Protein determinations were made using the BioRad Protein Assay Kit, using bovine plasma gamma globulin as a standard.

**RESULTS**

The screen for autosomal mutants affecting pyrimidine biosynthesis yielded several rudimentary-shaped wing mutations. Of these typical rudimentary-like mutants, all except one, $r a l^{a r e 6}$, were X linked. The mutant $r a l^{a r e 6}$ was determined to be autosomal by the attached-X test, as discussed in MATERIALS AND METHODS. Moreover, attached-X females in the F₂ generation showed the mutant phenotype, indicating that $r a l^{a r e 6}$ is not dependent on $r a l^{r e 11}$. The wing phenotype of the new mutation appears to be identical to that of rudimentary. The wings are short and truncated, and the marginal wing hairs are abnormally distributed with some hairs missing.

Linkage group information was obtained in the crosses with SM5 and TM3. The mutant $r a l^{a r e 6}$ consistently segregated from $S b$ and Ser, the two dominant markers on TM3, indicating that $r a l^{a r e 6}$ is on chromosome 3. Crosses with $P m$ and $H$ further indicated that $r a l^{a r e 6}$ maps to chromosome 3. Preliminary mapping using the $r u h t h s t c u s r e^s c a$ chromosome indicated that the $r a l$ mutation is situated in the interval between $c u$ (3–50.0) and $e$ (3–70.7) on the right arm of chromosome 3. Crossing the mutation to the deficiency $D f(3R)e^{R6}$, whose approximate breakpoints are 93B4–5 and 94A5–16, yielded $D f(3R)e^{R6}/r a l$ progeny that had an extreme rudimentary-like phenotype, placing the $r a l$ mutation within the interval uncovered by the deficiency.

Further localization of $r a l^{a r e 6}$ involved mapping it with respect to two chromosome 3 markers, $g l^Z$ and $e^s$. These results are presented in Table 1. A total of 240 recombinants between $g l^Z$ and $e^s$ were checked to see if the recombinant chromo-
Mapping of the ral mutation*

<table>
<thead>
<tr>
<th>Genotype of recombinants between the outside markers</th>
<th>Number scored</th>
<th>Number of recombinants between ral and e</th>
<th>Number of recombinants between ral and gl</th>
</tr>
</thead>
<tbody>
<tr>
<td>gP e+</td>
<td>120</td>
<td>4</td>
<td>116</td>
</tr>
<tr>
<td>gl+ e+</td>
<td>120</td>
<td>5</td>
<td>115</td>
</tr>
<tr>
<td>Total</td>
<td>240</td>
<td>9</td>
<td>231</td>
</tr>
</tbody>
</table>

Distance from e: 9/240 \( \times 7.6 = 0.29 \text{ m.u.} \)

Distance from gl: 231/240 \( \times 7.6 = 7.32 \text{ m.u.} \)

* Virgin gP e+/ral female progeny from the cross ral/ral 8 8 \( \times \) gP e+/gl e+ 0 0 were back-crossed to homozygous gP e+ males. The resulting progeny were screened for recombinants. Male recombinants were then crossed to attached-X shi/Y; TM3/ral females.

some had incorporated ral<sup>aza</sup>. In nine of the 240 recombinants the recombinational event took place between ral<sup>aza</sup> and e<sup>+</sup>, whereas in 231 of the 240 recombinants the event occurred between gl<sup>2</sup> and ral<sup>aza</sup>. Therefore, ral<sup>aza</sup> maps at 70.42 ± 0.19 map units. The map interval is at the 95% confidence level.

Since the visible phenotype of ral<sup>aza</sup> is like that of r, we determined whether ral<sup>aza</sup> was also female sterile. Homozygous ral<sup>aza</sup> females were crossed to TM3/ral<sup>aza</sup> males, as outlined in MATERIALS AND METHODS. The results of this cross are shown in Table 2. From the data, it is clear that ral<sup>aza</sup> exhibits female sterility when homozygous; 20 ral/ral mothers produced only six homozygous mutant progeny. The fact that homozygous mutant progeny were recovered at such a low frequency from this cross indicates that the female sterility caused by this allele of the ral locus is incompletely penetrant. The 132 TM3/ral<sup>aza</sup> progeny demonstrate that ral<sup>aza</sup> is resuable by wild-type sperm at a low frequency. The sex ratio obtained in Table 2 may possibly be due to the debilitating effect of the shi attached-X chromosome.

The enzyme activities of OPRTase and ODCase were measured in a common reaction mixture. Since the reaction catalyzed by OPRTase is reversible, the OPRTase activity was determined by the amount of <sup>14</sup>C-OMP that was converted to <sup>14</sup>C-orotic acid. The activity of ODCase was measured by the amount of <sup>14</sup>C-UMP and <sup>14</sup>C-uridine. The values for <sup>14</sup>C-UMP and <sup>14</sup>C-uridine were

Female sterility test of ral<sup>aza</sup>*

<table>
<thead>
<tr>
<th>Genotype of progeny</th>
<th>Number</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>TM3/ral&lt;sup&gt;aza&lt;/sup&gt; 8</td>
<td>95{</td>
<td>132</td>
</tr>
<tr>
<td>TM3/ral&lt;sup&gt;aza&lt;/sup&gt; 0</td>
<td>37}</td>
<td></td>
</tr>
<tr>
<td>ral&lt;sup&gt;aza&lt;/sup&gt;/ral&lt;sup&gt;aza&lt;/sup&gt; 8</td>
<td>6{</td>
<td>6</td>
</tr>
<tr>
<td>ral&lt;sup&gt;aza&lt;/sup&gt;/ral&lt;sup&gt;aza&lt;/sup&gt; 0</td>
<td>0}</td>
<td></td>
</tr>
</tbody>
</table>

* Twenty attached-X shi/Y; ral<sup>aza</sup>/ral<sup>aza</sup> females were crossed to 20 +/Y; TM3/ral<sup>aza</sup> males.
taken from chromatograms developed in Solvent II in order to separate orotidine from UMP, both of which chromatograph together in Solvent I (RAWLS 1978). The data in Table 3 indicate that the OPRTase and ODCase activities in \( rala^{ase} \) are much lower than the \( gl^{e} e^{e} \) control. The OPRTase activity of \( rala^{ase} \) relative to that of the control is 2.28%, whereas ODCase in the mutant is 1.48% of the control.

**DISCUSSION**

The discovery of the rudimental locus occurred during a genomic screen of *Drosophila melanogaster* that was undertaken to determine the number of loci that can mutate to give a rudimentary phenotype. We have demonstrated (FALK and DeBoer 1980) that the rudimentary locus is probably the only X-linked locus that exhibits such a phenotype when mutated. Now, we have described a new locus, rudimental, that is autosomal and mutates to a typical rudimentary phenotype. Our results, together with the report of a dosage-sensitive region for DHOdehase within the 84D-85E area of chromosome 3 (RAWLS et al. 1980), suggest that there are at least three different loci whose genes affect the pyrimidine biosynthetic pathway.

A mutant of the \( ral \) locus exhibits an extreme rudimentary phenotype when it is heterozygous with \( Df(3R)e^{R6} \), a deficiency of the ebony region whose approximate breakpoints are 93B4-5 and 94A5-16. Furthermore, the map position of \( rala^{ase} \) is 70.42 ± 0.19 map units, which puts it very close to ebony (70.7). According to D’Alessandro, Ritossa and Scangelhe (1977), the ebony locus resides within the 93D1; 93D6-7 region. Assuming that 0.065 map units correspond to one band of the polytene chromosome (Lefevre 1971), then the \( ral \) locus probably resides about five bands to the left of ebony in the region of 93C.

Enzyme activity determinations indicate that \( rala^{ase} \) has lower than wild-type activity for both the OPRTase and ODCase enzymes. The leakiness of the female sterility phenotype agrees with the fact that this mutant is not totally null. Furthermore, the variability of the wing phenotype also supports this result.

Since \( rala^{ase} \) is deficient in both enzyme activities, we suggest that the rudimental locus is a complex locus encoding the OPRTase and ODCase enzymes.

**TABLE 3**

Enzyme activities of adult males*

<table>
<thead>
<tr>
<th>Strain</th>
<th>Specific activity (pmoles/mg protein/min)</th>
<th>Standard deviation (pmoles/mg protein/min)</th>
<th>% of wild type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>OPRTase†</td>
<td>130.1</td>
<td>±13.6</td>
</tr>
<tr>
<td>( (gl^{e} e^{e}) )</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( rala^{ase} )</td>
<td>OPRTase‡</td>
<td>212.7</td>
<td>±49.8</td>
</tr>
<tr>
<td></td>
<td>ODCase‡</td>
<td>3.0</td>
<td>±0.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.1</td>
<td>±1.5</td>
</tr>
</tbody>
</table>

* Each value is the mean of three separate samples.
† OPRTase activity was determined from the orotic acid spot on solvent I chromatograms.
‡ ODCase activity is the sum of the UMP and uridine spots on solvent II chromatograms.
These enzymes exist in a cytoplasmic enzyme complex in mammals (Jones 1972; Shoaf and Jones 1973) and in Drosophila (Rawls 1979). Thus, it may be that the \textit{ral} locus is similar to the \textit{r} locus, which codes for three enzyme activities possibly in the form of a single multi-functional polypeptide (Brothers et al. 1978; Mehl and Jarry 1978). The possible existence of a second complex locus within a single biochemical pathway may provide a unique basis for future studies of gene regulation.

We would like to extend special thanks to David T. Sullivan for many helpful discussions throughout this investigation. We would also like to thank John M. Rawls for providing us with the deficiency stock. This research was supported by Public Health Service research grant GM24049 and grant No. PCM 77-15490 from the National Science Foundation.

\textit{Note added in proof:} Since submitting this manuscript, a paper, “Identification of a genetic region that encodes orotate phosphoribosyltransferase and orotidylate decarboxylase in Drosophila melanogaster,” by John Rawls has appeared (Molec. Gen. Genet. 178: 43-49 (1980). This paper describes a similar autosomal mutation (\textit{r-I}) which, based on cytological data, has been tentatively placed to the right of \textit{e}. Despite this discrepancy with our mapping data, we believe that these mutants affect the same locus.

\section*{LITERATURE CITED}


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