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Genetic analysis of the right (3′) end of the rosy locus in *Drosophila melanogaster*

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Summary

Prior reports from this laboratory have described the experimental basis for our understanding of the rosy locus (*ry*: 3–52·0) of *Drosophila melanogaster* as a bipartite genetic entity consisting of a structural element that codes for the xanthine dehydrogenase (XDH) peptide and a contiguous *cis*-acting control element immediately to the left of the structural element. Although the left end (5′) of the structural element has been well defined, the right boundary (3′) has been given only casual treatment in our prior reports. In our recent studies of rosy locus expression we have been concerned with the production and identification of mutations in the non-structural regions immediately flanking the structural element. An improved definition of the right end of the structural element is essential to this analysis. In addition to producing a better definition of the right boundary of the structural element, this study produced several phenotypically novel mutations. These mutations were classified initially as control element mutations, but upon analysis were found to map within the rosy structural element. *No* evidence was obtained for the existence of a control element contiguous with the right end of the structural element.

1. Introduction

The rosy locus of *Drosophila melanogaster* is essential for xanthine dehydrogenase (XDH) activity and is located on the right arm of chromosome three at map position 52·0. The immediate genetic region surrounding rosy is well defined and the locus is an independent genetic unit within that region (Hilliker *et al.* 1980). The locus was originally defined by brown eye colour mutants which were deficient in drosopterin pigment. Such mutants were subsequently shown to exhibit no detectable xanthine dehydrogenase (XDH) activity (Glassman & Mitchell, 1959). Two lines of evidence establish the rosy locus as the structural gene for XDH: (1) XDH activity increases with increasing doses of ry^+ alleles in diploid flies (Grell, 1962; Glassman, Karam & Keller, 1962) and (2) variation in electrophoretic mobility was shown to map to the vicinity of the rosy locus (Yen & Glassman, 1965), and, subsequently, to map within the rosy locus as defined by *ry* null mutants (McCarron, Gelbart & Chovnick, 1974; Gelbart *et al.* 1974).

Extensive fine structure analysis of rosy eye colour variants provided a linear array of sites associated with loss of XDH activity (Chovnick, Ballantyne & Holm, 1971). More recent experiments with a variety of unambiguous structural mutations have defined the genetic limits of the structural gene (McCarron, Gelbart & Chovnick, 1974; Gelbart *et al.* 1974; Gelbart, McCarron & Chovnick, 1976).

There is, however, an ambiguity in the genetic definition of one of the boundaries of the XDH structural element. Essentially, the outer boundaries of the structural element are defined by intragenic fine structure mapping experiments that located the relative map positions of sites associated with unambiguous XDH peptide alterations. The left boundary of the structural element, defined by the complementing rosy eye colour mutant ry^{606}, has not changed despite extensive testing in recent years. In contrast, the right boundary has been given only casual treatment in our prior reports (*loc. cit.*) which provided evidence for the rightmost sites being ry^{41}, $ry^{e\,111}$ and ry^{2}. Of these sites, only the electrophoretic site, $ry^{e\,111}$, is an unambiguous site of structural variation. The mutant ry^{2} was listed as an allele exhibiting interallelic complementation (Gelbart, McCarron & Chovnick, 1976) on the basis of a slight eye colour complementation seen in one mutant heteroallele combination. However, we are unable to confirm this diagnosis in terms of XDH activity. Moreover, recent association of this spontaneous mutant with a large insertion (W. Bender, Personal communication) further confounds this classification.

As part of our goal to explore the control of rosy locus expression, we have been concerned with the production and identification of mutations in the non-structural regions immediately flanking the structural element. An essential feature of such an analysis

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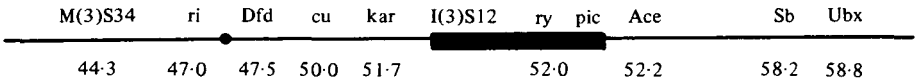

Fig. 1. Genetic map of the centromere proximal region of chromosome 3. From Hilliker & Chovnick (1981). Map positions of various mutants used in this investigation are indicated. Mutants not described in Lindsley & Grell (1968) are discussed in Chovnick *et al.* (1976), Hilliker *et al.* (1980), and Hilliker & Chovnick (1981). The $Ace^{l\,26}$ allele is abbreviated throughout the text as *l26*.

involves an improved definition of the right end of the structural element. This task assumes particular importance since Coté *et al.* (1986), utilizing strand specific M13 probes, have demonstrated that the rosy RNA is transcribed from left to right (5′ to 3′) on the genetic map (Figs. 2 and 3).

The present studies produced several novel and unanticipated classes of mutations. One class, involving heterochromatic position effects on rosy locus expression, is discussed elsewhere (Chovnick *et al.* 1980; Rushlow & Chovnick, 1984; Rushlow, Bender & Chovnick, 1984). The present report describes several additional mutations, each representing a phenotypic class whose initial biochemical characterization led to its designation as a putative, *cis*-acting control mutant. However, upon further study, each was found to be a rosy locus structural element mutation of a class heretofore unseen in this system.

2. Materials and Methods

(i) *The genetic system*

Fig. 1 presents a genetic map of the centromere proximal region of chromosome 3 noting the rosy region and closely linked gene markers used in this investigation.

Rosy locus variants have been the subject of an intensive and continuing intragenic mapping analysis. Fig. 2 summarizes the state of this effort prior to the present report. Fig. 2A presents a map of XDH⁻,

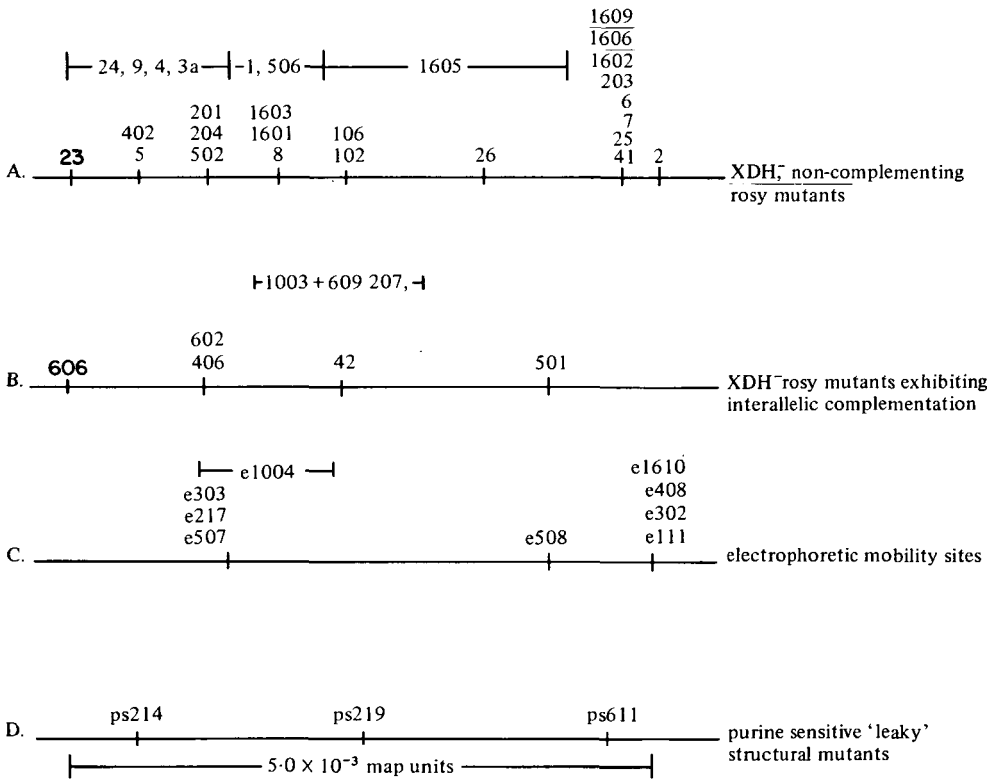

Fig. 2. Genetic fine structure maps of the rosy locus. Map locations of unambiguous structural element variants (B, C, and D) are positioned relative to the map of XDH-non-complementing mutants (A).

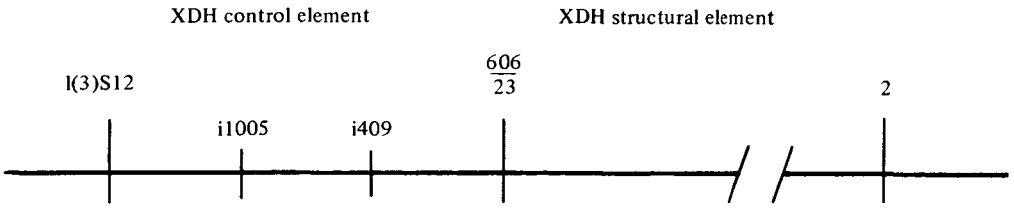

Fig. 3. The rosy locus control and structural elements.

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Table 1. *XDH activity and CRM levels of extracts of adults homozygous for the indicated mutant alleles relative to their respective wild-type isoalleles*

Allele	XDH activity (%)	XDH CRM (%)
$ry^{ps\,223}$	44·5	55·1
$ry^{ps\,228}$	15·6	10·9
$ry^{ps\,612}$	13·9	132·8

non-complementing, rosy eye colour mutant sites. Estimation of the boundaries of the XDH coding element is provided by the maps of three classes of unambiguous coding element site variants presented in Fig. 2B (XDH^-, allele-complementing, rosy eye colour mutant sites), Fig. 2C (electrophoretic mobility sites) and Fig. 2D (purine sensitive 'leaky' structural mutant sites). Additionally, experiments from this laboratory have elaborated a *cis*-acting control element which maps between the left, centromere proximal boundary of the XDH structural element and the immediately adjacent vital gene, *l(3)S12* (Fig. 3). The control element is defined by two recombinationally separable site variants. Thus, the $ry^{i\,409H}$ variant is associated with a tissue specific increase in rosy locus expression in contrast to the normal, $ry^{i\,409N}$, and $ry^{i\,1005L}$ is associated with a non-tissue specific reduction in expression in contrast to the normal, $ry^{i\,1005N}$ (Clark *et al.* 1984).

(ii) *Genetic fine structure analysis*

Large scale fine structure recombination tests were carried out making use of the purine selective system described in McCarron *et al.* (1979).

(iii) *Mutants used in the analysis*

The allele-complementing rosy eye colour mutation, $ry^{L.\,19}$, is an ethyl methanesulphonate (EMS) induced mutation provided by E. B. Lewis. The purine sensitive, 'leaky' mutations $ry^{ps\,218}$, $ry^{ps\,223}$ and $ry^{ps\,228}$ are derived from the $ry^{+\,2}$ allele while $ry^{ps\,612}$ is derived from the $ry^{+\,6}$ allele. These resulted from EMS mutagenesis experiments described earlier (Gelbart, McCarron & Chovnick, 1976). The $ry^{ps\,5205}$ mutation resulted from a 1-ethyl-1-nitrosouracil (ENU) mutagenesis of the $ry^{+\,5}$ allele (McCarron & Chovnick, 1981).

(iv) *XDH tests*

Electrophoresis procedures are those of McCarron *et al.* (1979), modified only in that the gel is 6% acrylamide and 0·3% *N,N'*-methylenebisacrylamide. Enzyme activity is measured by the fluorometric procedure described by Chovnick *et al.* (1970) as modified in McCarron *et al.* (1979). The rocket immunoelectrophoresis procedure is described by McCarron *et al.* (1979).

3. Results

A number of purine sensitive, 'leaky' mutant alleles of the rosy locus were characterized with respect to XDH CRM (cross-reacting material) and XDH enzyme activity in extracts of mutant homozygotes relative to extracts of their respective parental wild-type isoallele homozygotes. Those purine sensitive mutations associations with normal CRM levels and reduced enzyme activity were assigned as structural element variants. However, several additional classes of mutants, associated with both altered CRM and enzyme activity were subjected to further analysis as putative control element variants. The XDH activity and CRM levels associated with representative members of these classes of variants is presented in Table 1. Further genetic and biochemical analyses of these variants is discussed below.

(i) *Analysis of* ry^{ps223}

Enzyme assays of extracts of homozygous $ry^{ps\,223}$ adults indicated an approximate 50% decrease in

Table 2. *Fine structure localization of* ry^{ps223}: *number and classes of* ry^+ *chromosomes recovered from progeny of crosses of* ry^x/ry^y *females to tester males of the genotype* Dfd Df(3R) kar^{31} ry^{60}/kar^2 Df(3R) ry^{75}

Expt	ry^x/ry^y	Crossovers		Conv. ry^x	Conv. ry^y	Zygotes sampled ($\times 10^6$)
		kar ry⁺ 126⁺	*kar⁺ ry⁺ 126*	*kar ry⁺ 126*	*kar⁺ ry⁺ 126⁺*	
1.	*kar2 ry⁶⁰⁶ 126* / + *ry^ps223* +	0	15 [1·00] 28 [1·03]	2 [1·00]	16 [1·03] 1 [1·00]	2·05
2.	*kar² ry¹⁰⁶ 126 Sb Ubx* / + ry^ps223 + + +	0	4 [1·05]	1 [1·02]	3 [1·03] 1 [1·05]	2·75
3.	*cu kar ry⁴¹ 126 Sb Ubx* / + + *ry^ps223* + + +	0	0	0	0	3·57
4.	*kar² ry^ps223 126 Sb Ubx* / + *ry²* + + +	0	0	1 [1·03]	0	2·65

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XDH activity, and this was clearly correlated with reduced amounts of XDH CRM (Table 1). We were led by these observations to consider $ry^{ps\ 223}$ as a putative control variant subject to fine structure mapping studies to determine its location within the rosy locus.

Table 2 summarizes results of those mapping studies. The presentation in this and subsequent tables follows a classification of recombinants as crossovers or conversions based upon flanking markers (reviewed in Hilliker & Chovnick, 1981). The phenotypic classification of recombinants with respect to electrophoretic mobility relates to unselected electrophoretic site heterozygosity (Fig. 2C). The classification is consistent with prior reports from this laboratory and is discussed at length by Gelbert, McCarron & Chovnick (1976). The crossover class of recombinants in the first experiment (Table 2) establishes the position of $ry^{ps\ 223}$ to the right of ry^{606} and hence to the right of the previously described control element (Fig. 3). Prior study established the existence and location of an electrophoretic site difference (*e217*) between the parental wild-type alleles that is responsible for their XDH mobility difference (Gelbart *et al.* 1974; Gelbart, McCarron & Chovnick, 1976). Thus, ry^{+6}, which produces an $XDH^{1\cdot00}$, carries *e217S* while ry^{+2}, associated with an $XDH^{1\cdot03}$, is *e217F*. The presence of both possible electrophoretic mobilities (1·00 and 1·03) among the crossover class further localizes $ry^{ps\ 223}$ to the right of the unselected electrophoretic site, *e217*. The crossovers of Expt. 1 (Table 2) establish the order of sites to be *606–e217–ps223*.

Expt. 2 (Table 2) was designed to further probe the location of $ry^{ps\ 223}$ utilizing the mutation ry^{106}, which is centrally located in the XDH structural element. An important feature of our choice of ry^{106} for this test is the fact (*loc. cit.*) that the heterozygote $ry^{106}/ry^{ps\ 223}$ would have two non-selective electrophoretic markers in the cross. Thus, the ry^{106} allele is *e217S 106 e111F* while $ry^{ps\ 223}$ is marked by *e217F* and *e111S*. Expt. 1 (Table 2) places *ps223* to the right of *e217*. Expt. 2 questions its location with respect to *106* and *e111*. Since all of the crossovers (Expt. 2, Table 2) exhibited an electrophoretic mobility of 1·05 (*e217F, e111F*), we are able to place *ps223* to the right of *106*. However, these data are ambiguous with respect to the placement of *ps223* relative to the *e111* site. Since one of the four conversions of *ps223* exhibited a co-conversion for the *e111* site, we conclude that these sites are in close proximity to each other. The failure to recover crossovers in the large scale recombination experiment between $ry^{ps\ 223}$ and ry^{41} (Expt. 3 of Table 2) implies a close proximity of these mutations. Since the ry^{41} allele is associated with a very high conversion frequency (Chovnick, Ballantyne & Holm, 1971; Hilliker & Chovnick, 1981), the apparent absence of ry^{41} conversions in this experiment provides additional evidence for the immediate proximity of $ry^{ps\ 223}$.

On the basis of its complementation with ry^{406} and its map position to the right of ry^{41}, the ry^{2} allele is considered to be the rightmost known structural variant. Consequently, we assayed for recombination between $ry^{ps\ 223}$ and ry^{2}, and failed to observe crossovers in a very large scale experiment (Expt. 4, Table 2).

At this point in the study, it *appeared* that $ry^{ps\ 223}$ was a mutation within a control element region located at the right end of the structural element of the rosy locus, and thus identified a new control element region for this gene.

However, our next experiment, to determine if the $ry^{ps\ 223}$ lesion was a *cis*-acting control element variant, provided strong, and unexpected, evidence that $ry^{ps\ 223}$ is a structural element alteration! To test for *cis* action of the $ry^{ps\ 223}$ lesion on XDH levels, electrophoresis was performed on extracts of flies heterozygous for $ry^{ps\ 223}$ [1·03] and ry^{+13} [0·90]. Control extracts were prepared from flies heterozygous for the parental wild-type isoalleles ry^{+2}[1·03] and ry^{+13}[0·90]. Since XDH is a homodimer, extracts of heterozygotes would exhibit three classes of dimers: Fast homodimers [1·03]; Intermediate hybrid dimers [0·97]; and Slow homodimers [0·90]. If the heterozygote produces monomers in equal numbers (or their steady state levels are equal), then the three dimer classes will exhibit a 1:2:1 pattern upon electrophoresis. Indeed, the control heterozygote, ry^{+2}/ry^{+13}, exhibits just such an electrophoretic pattern (Lane 2, Fig. 4). If $ry^{ps\ 223}$ were, in fact, a *cis*-acting, 'underproducer' control variant like $ry^{i\ 1005L}$ (McCarron *et al.* 1979; Clark *et al.* 1984), then the heterozygote $ry^{ps\ 223}/ry^{+13}$ would exhibit a dimer pattern that would reflect a significantly reduced availability of fast monomers for dimerization. On the basis of 50% CRM in $ry^{ps\ 223}$ homozygotes (Table 1), one expects a 2:1 ratio of slow:fast monomer availability for dimer formation in the heterozygote $ry^{ps\ 223}/ry^{+13}$ if $ry^{ps\ 223}$ is a *cis*-acting control variant. Such a monomer distribution would produce a 4:4:1 pattern of slow:intermediate:fast dimers. However, a very different result is obtained (Lane 1, Fig. 4). Essentially, a truncated normal pattern of slow:intermediate dimers is seen, with a very faintly staining fast dimer band. Such a distribution implies a normal production of monomers by the $ry^{ps\ 223}$ allele, but that mutant, fast homodimers are unstable, and that this instability is attenuated in the hybrid dimer. Therefore,

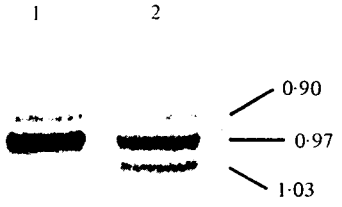

Fig. 4. XDH electropherogram indicates the relative accumulation of slow homodimers, fast homodimers and intermediate hybrid dimers present in extracts of adult flies. Lane 1, $ry^{ps\ 233}$ *(1·03)*/ry^{+13} *(0·90)*; lane 2, ry^{+2} *(1·03)*/ry^{+13} *(0·90)*.

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Table 3. *Fine structure localization of* ry^{ps228}: *number and classes of* ry^+ *chromosomes recovered from progeny of crosses of* ry^x/ry^y *females to tester males of the genotype* Dfd Df(3R) kar^{31} ry^{60}/kar^2 Df(3R) ry^{75}

Expt	ry^x/ry^y	Crossovers		Conv. ry^x	Conv. ry^y	Zygotes sampled ($\times 10^6$)
		kar ry⁺ 126⁺	*kar⁺ ry⁺ 126*	*kar ry⁺ 126⁺*	*kar⁺ ry⁺ 126⁺*	
1.	*kar² ry⁶⁰⁶ 126* / + *ry^ps228* +	0	7 [1·00]	2 [1·00]	1 [1·00]	0·72
2.	*cu kar ry⁴¹ 126 Sb Ubx* / + + *ry^ps228* + + +	5 [1·00]	0	0	1 [1·03]	0·40

Table 4. *Number and classes of* ry^+ *chromosomes recovered from progeny of* cu kar ry^{41} 126 Sb Ubx/+ + ry^{ps612} + + + *females crossed to tester males of the genotype* Dfd Df(3R) kar^{31} ry^{60}/kar^2 Df(3R) ry^{75}

Crossovers		Conv. ry^{41}	Conv. ry^{ps612}	Zygotes sampled ($\times 10^6$)
kar ry⁺ 126⁺	*kar⁺ ry⁺ 126*	*kar ry⁺ 126*	*kar⁺ ry⁺ 126⁺*	
2 [1·00]	0	3 [1·00]	3 [1·00]	1·11

$ry^{ps\,223}$ is not a *cis*-acting control variant, but rather an unusual structural element variant with superficial control element characteristics.

(ii) *Analysis of* ry^{ps228}

XDH activity and XDH CRM in $ry^{ps\,228}$ homozygotes showed parallel decreases relative to ry^{+2} homozygotes (Table 1), suggesting the 'underproduction' of XDH monomers by the $ry^{ps\,228}$ allele. Electrophoretic analysis of XDH in $ry^{ps\,228}$ [1·03]/ry^{+13} [0·90] heterozygotes (in contrast to $ry^{ps\,223}$) were consistent with this hypothesis (data not shown). Thus, by biochemical and immunological criteria, $ry^{ps\,228}$ may be classified as a *cis*-acting control element variant.

However, $ry^{ps\,228}$ was found to map within the XDH structural element. In the first recombination experiment (Table 3), $ry^{ps\,228}$ was mapped against the leftmost known structural variant, ry^{606}. The crossover class of recombinants indicated that $ry^{ps\,228}$ was to the right of ry^{606}. Moreover, all crossover chromosomes had an electrophoretic mobility of 1·00 indicating that all crossovers were between ry^{606} and *e217*, implying that $ry^{ps\,228}$ is in the vicinity of *e217*, well within the XDH structural element. (Indeed, the single conversion of $ry^{ps\,228}$ observed was a co-conversion for *e217*). Expt. 2 (Table 3) tests this interpretation by examining recombination in the heterozygote $ry^{ps\,228}/ry^{41}$. Since ry^{41} lies within the right boundary of the structural element, the recovered crossovers (Expt. 2, Table 3) confirm that $ry^{ps\,228}$ lies within the XDH structural element boundaries.

(iii) *Analysis of* ry^{ps612}

A third EMS induced purine sensitive allele, $ry^{ps\,612}$ (derived from ry^{+6}), had unusual properties. Enzyme activity assays of $ry^{ps\,612}$ homozygotes indicated that the variant was associated with an estimated 14% of the parental XDH activity; however, numerous immunoelectrophoresis experiments demonstrated consistently higher levels of XDH CRM relative to homozygotes for the parental allele, ry^{+6} (Table 1). Prior fine structure mapping had positioned $ry^{ps\,612}$ to the right of ry^8 (Gelbart, McCarron & Chovnick, 1976). Recombination was assayed between $ry^{ps\,612}$ and ry^{41}, and the results definitely place $ry^{ps\,612}$ within the XDH structural element. We infer that the $ry^{ps\,612}$ lesion produces a protein with a much reduced catalytic ability. Its excess accumulation suggests several alternative models discussed below (see Discussion).

(iv) *The right end of the XDH structural element*

As noted above (see Introduction), ry^2 is not entirely suitable as a marker for the right end of the structural element. It is associated with reduced crossing over in its vicinity, and exhibits an exceedingly low conversion frequency (Chovnick, Ballantyne & Holm, 1971). Indeed, it has recently been shown to be associated with a large insertion of extraneous DNA at the right end of the locus (W. Bender in Hilliker & Chovnick, 1981) which may severely limit the recovery of intragenic recombinants in its vicinity.

At this juncture, it became apparent that mutants were available to permit further experimental definition of the right end of the XDH structural element. Moreover, such effort is essential for the eventual analysis of non-coding variants at this end of the locus. Several known structural variants (rosy complementing alleles and purine sensitive structural variants) were mapped against ry^{41} (a defined right end null mutation) in an effort to extend and further define the right structural terminus of the rosy locus.

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Table 5. *Analysis of the right end of the XDH structural element. Number and classes of* ry$^+$ *chromosomes recovered from progeny of crosses of* ry^x/ry^y *females to tester males of the genotype* Dfd Df(3R) kar^{31} ry^{60}/kar^2

Females (ry^x/ry^y)			Crossovers		Conversions ry^x	*Conversions* ry^y	Zygotes sampled
			kar ry$^+$ 126$^+$	*kar$^+$ ry$^+$ 126*	*kar ry$^+$ 126*	*kar$^+$ ry$^+$ 126$^+$*	($\times 10^6$)
kar^2	*ry$^{L.19}$*	*126*	1 [0·90]	0	1 [0·90]	3 [1·00]	1·26
+	*ry^{41}*	+			1 [0·94]	1 [1·02]	
kar	*ry^{41}*	*126*	0	0	0	1 [1·03]	0·41
+	*ry$^{ps\,218}$*	+					
kar^2	*ry$^{L.19}$*	*126*	1 [0·94]	0	4 [0·94]	5 [1·03]	1·93
+	*ry$^{ps\,218}$*	+			1 [0·90]		
kar^2	*ry$^{ps\,223}$*	*126*	0	0	1 [1·03]	0	1·39
+	*ry$^{L.19}$*	+					

Table 6. *Fine structure localization of* ry^{ps5205}: *number and classes of* ry$^+$ *chromosomes recovered from progeny of crosses of* ry^x/ry^y *females to tester males of the genotype* Df(3R) ry^{36}/Tp(3)MKRS M(3)S34 kar ry^2 Sb

Females (ry^x/ry^y)			Crossovers		Conversions ry^x	Conversions ry^y	Zygotes sampled
			kar ry$^+$ 126$^+$	*kar$^+$ ry$^+$ 126*	*kar ry$^+$ 126*	*kar$^+$ ry$^+$ 126$^+$*	($\times 10^6$)
kar^2	*ry^{606}*	*126*	0	16	1	1	0·26
+	*ry$^{ps\,5205}$*	+					
kar^2	*ry$^{L.19}$*	*126*	0	1	1	1	1·20
+	*ry^{ps5205}*	+					

The *ry$^{L.19}$* allele proved to be a useful right end marker. Unlike *ry^2*, *ry$^{L.19}$* is an excellent complementing allele of the locus restoring full wild-type eye colour and biochemically detectable XDH activity in several complementing heterozygotes with other *ry* complementing alleles (Gelbart, McCarron & Chovnick, 1976), and recombination analysis indicated that *ry$^{L.19}$* was to the right of *ry^{41}* (Row 1, Table 5). The appearance of two electrophoretic mobility classes among the *ry$^{L.19}$* conversions indicates the existence of an electrophoretic site heterozygosity to the right of *ry^{41}* in the immediate vicinity of, but separable from, *ry$^{L.19}$*. Thus, the *ry$^{L.19}$* mutation, and an electrophoretic site in its immediate vicinity define the right end of the XDH structural element.

Several experiments were conducted to map the location of the structural element mutation *ry$^{ps\,218}$* to the right end of the XDH structural element. Two experiments are summarized (Table 5, Rows 2 and 3) which position *ry$^{ps\,218}$* to the left of *ry$^{L.19}$* in the vicinity of *ry^{41}*. Of greater interest is the fact that the conversions of *ry$^{L.19}$* in the *ry$^{L.19}$/ry$^{ps\,218}$* test (Row 3, Table 5) serve to position the electrophoretic site (discussed above in the analysis of the *ry$^{L.19}$/ry^{41}* cross) to the left of *ry$^{L.19}$*. This site, *e1407*, is present as *e1407S* in *ry^{+0}*, *ry^{+2}* and their mutant derivatives such as *ry^{41}* and *ry$^{ps\,218}$*, while *ry$^{L.19}$* carries *e1407F*. It is quite possible that *e1407* and *e111* are synonymous.

The final experiment of Table 5 (Row 4) summarizes and effort to map *ry$^{ps\,223}$* against *ry$^{L.19}$*. The failure to obtain crossovers indicates that *ry$^{ps\,223}$* maps near the right end of the structural element.

(v) *Analysis of* ry^{ps5205}

The *ry$^{ps\,5205}$* allele and the experiments described below derive from studies carried out independent of those described in prior sections. Their inclusion in the present report relates to the identification of the right boundary of the XDH structural element. The *ry$^{ps\,5205}$* allele is associated with a borderline mutant eye colour, and very low XDH activity. Extracts do not stain for activity on routine polyacrylamide electrophoretic gels, but do stain faintly on immunoelectrophoretic gels. Extracts of homozygous adults have approximately 25% of the XDH CRM relative to the extracts of *ry^{+5}*, the parent wild-type isoallele. Furthermore, *ry$^{ps\,5205}$* exhibits allelic complementation in mutant heteroallele tests with other low XDH 'leaky' mutants. The complementation tests (data not shown) involve titration experiments for purine resistance and phenocopy induction by allopurinol. In such experiments, complementation is indicated when the mutant heterozygote exhibits significantly greater resistance to purine killing and phenocopy induction by allopurinol than the mutant heteroallele homozygotes. Complementation in these

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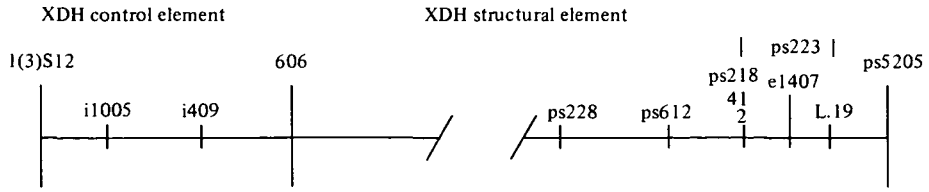

Fig. 5. Summary map of the rosy locus. Emphasis is placed upon the relative position of sites located at the right end of the locus.

titration experiments has been correlated with increased enzyme activity in the heterozygotes. On the basis of these observations, $ry^{ps\,5205}$ is classified as a structural element mutation.

Fine structure analysis demonstrated that $ry^{ps\,5205}$ marked the genetic right end of the rosy structural element. When mapped relative to ry^{606}, at the genetic left end of the structural element, $ry^{ps\,5205}$ was clearly far to the right of ry^{606} (Row 1, Table 6). Indeed, the crossover distance observed, 0·0123 map units, indicated that it lay near the extreme right end of the rosy locus. Accordingly, it was mapped against $ry^{L.\,19}$, the right end structural boundary marker, and proved to be just to the right of $ry^{L.\,19}$ (Row 2, Table 6). The map distance between $ry^{L.\,19}$ and $ry^{ps\,5205}$, $1{\cdot}67 \times 10^{-4}$ map units, indicates that $ry^{ps\,5205}$ is very close to $ry^{L.\,19}$. Thus, $ry^{ps\,5205}$ defines the right end of the XDH structural element.

4. Discussion

Although the present study failed to discern control element function adjacent to the right end of the rosy locus structural element, we were successful in providing a genetic definition of the right end of the XDH structural element. Fig. 5 summarizes the current genetic map of the rosy locus.

Several mutations within the structural element exhibit unusual properties. Two alleles, $ry^{ps\,223}$ and $ry^{ps\,228}$ were initially considered as possible control variants on the basis of their biochemical and immunological characteristics (low XDH activity and correspondingly reduced levels of XDH CRM relative to their parental $ry^{+\,2}$ isoallele). One of these mutations, $ry^{ps\,223}$, is clearly a structural variant. The low CRM level of mutant homozygotes, which led to its putative classification as an 'underproducer', was due to the fact that the mutant lesion produces unstable homozygous XDH dimers. Examination of the XDH dimer array produced by the heterozygote involving $ry^{ps\,223}$ and a very different wild-type electromorph ($ry^{+\,13}$) provides opportunity to estimate the available peptide monomers produced by the $ry^{ps\,223}$ allele by virtue of the fact that they form stable hybrid dimers. We infer from the low level of CRM in $ry^{ps\,223}$ homozygotes that XDH monomers must disappear rapidly unless 'rescued' by dimer formation as in the hybrid dimer experiment. Perhaps monomeric forms of the peptide are subject to rapid proteolysis.

The basis of the biochemical phenotype associated with $ry^{ps\,228}$ is unknown. It may produce an XDH peptide which is of low stability in both homozygous and hybrid dimers. The $ry^{ps\,612}$ allele is also unusual in that it exhibits lower XDH activity but elevated levels of XDH CRM. It may be a structural mutation that produces an XDH enzyme of lowered catalytic ability and greater molecular stability (or possibly altered affinity for XDH antibody).

An entirely different category of interpretation is raised by the recent discovery of a small intron within the previously defined structural region of the rosy locus (W. Bender and D. Curtis, Personal communication). These mutations might be lesions in a DNA sequence which may be important in RNA processing. Thus, a splice site lesion could explain the biochemical phenotype of $ry^{ps\,228}$, while $ry^{ps\,612}$ may be a coding element lesion in the vicinity of an intron that enhances splicing.

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