# ORGANIZATION OF A GENE IN DROSOPHILA: A PROGRESS REPORT

(Drosophila melanogaster, *rosy locus, XDH, genetic fine structure, peptide coding region,* cis-*acting control element*)

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## SUMMARY

*The present report summarizes our progress in the genetic dissection of an elementary genetic unit in a higher organism. The rosy locus (*ry: *3-52.0) in* Drosophila melanogaster *codes for xanthine dehydrogenase, and is characterized by several classes of induced mutants as well as naturally occurring variants. Pursuing the hypothesis that the rosy locus includes a non-coding, control region as well as a structural element coding for the XDH peptide, experiments have been carried out which place genetic boundaries to the structural element in terms of a map of unambiguous structural element variants. More than sixty sites have been mapped within the boundaries of the structural element. Presently, our research is largely concerned with the elaboration of a* cis-*acting control element located adjacent to the structural element.*

## INTRODUCTION

In recent years, increasing attention has been directed to questions concerning the structure, function and regulation of elementary genetic units in higher organisms. Much of this interest derives from inferences drawn from cytogenetic data (JUDD, SHEN and KAUFMAN 1972; HOCHMAN 1973; LEFEVRE 1974) as well as studies of whole genome DNA, RNA and chromatin (See NIERLICH, RUTTER and FOX 1976; BRADBURY and JAVAHERIAN 1977; WILCOX, ABELSON and FOX 1977 for extensive reviews of this subject). Emerging from these studies is the proposition that the genetic unit in higher organisms is a much larger entity than its prokaryote counterpart. Since eukaryotic mRNA is monocistronic, and the size range of polypeptide gene products of higher organisms is similar to that of prokaryotes, attention is drawn to the non-coding portion(s) of the higher organism gene which we infer to be quite large. Until very recently, conventional wisdom focused upon the notion that this large excess of non-coding DNA might serve a control function and would be located adjacent to and contiguous with the 5' end of the coding section. Now, studies of several

higher organism gene sequences have revealed that the structural information, in these cases, is not continuous, but rather is interrupted by non-coding segments which are removed at one or more steps leading to the production of a continuous messenger sequence. These intriguing observations have already generated far-reaching speculation (GILBERT 1978).

It is our conviction that the study of these and other questions concerning the structure, function and regulation of higher organism genes will require specific genetic units whose DNA is marked and genetically mapped in order to permit identification and ordering of structural and regulatory components, and to relate these components to their specific functions. For some years, major emphasis in this laboratory has been directed towards the development of such an experimental system.

## THE GENETIC SYSTEM

The rosy locus in *Drosophila melanogaster* (*ry*:3-52.0) is a genetic unit controlling xanthine dehydrogenase activity, located on the right arm of chromosome 3 (Fig. 1) within polytene chromosome section 87D. The locus was originally defined by a set of brownish eye color mutants deficient in drosopterin pigment. Such mutants were shown subsequently to exhibit no detectable XDH activity (GLASSMAN and MITCHELL 1959). Figure 2 summarizes reactions used in this laboratory to assay Drosophila XDH (FORREST, GLASSMAN and MITCHELL 1956; GLASSMAN and MITCHELL 1959). Zygotes possessing little or no XDH activity are unable to complete development and die before eclosion on standard Drosophila culture medium supplemented with purine. This fact serves as the basis for a nutritional selective procedure which has made large scale fine structure mapping within the rosy locus a routine laboratory exercise (CHOVNICK, BALLANTYNE and HOLM 1971; CHOVNICK 1973).

It is now clear that Drosophila XDH is a homodimer (GELBART *et al.* 1974), with a subunit molecular weight of 150,000 daltons (EDWARDS, CANDIDO and CHOVNICK 1977). Two observations serve to demonstrate that the structural information for XDH is encoded by the rosy locus: (1) Variation in dosage of $ry^+$ alleles, from 0-3 doses, appears to be the limiting factor in determining level of XDH activity/fly in otherwise wild-type flies (GRELL 1962; GLASSMAN, KARAM and KELLER 1962). (2) The genetic basis for variation in XDH electrophoretic mobility seen in wild-type strains maps to sites within the rosy locus map of $XDH^-$, rosy eye color mutants (GELBART *et al.* 1974; GELBART, MCCARRON and CHOVNICK 1976).

## NOMENCLATURE

Electrophoretic mobility variants of XDH are readily isolated from laboratory stocks and natural populations of *Drosophila melanogaster*. From these sources, we have established a number of wild-type isoalleles of the rosy locus. These are maintained as stable lines which possess XDH molecules with distinctive electrophoretic mobilities and thermal properties. Moreover, the XDH enzyme activity level associated with each of these wild-type alleles is also a distinctive, stable phenotypic character. Table 1 summarizes our present array of $ry^+$ isoalleles. The XDH produced by $ry^{+0}$ serves as a mobility standard, and is designated $XDH^{1.00}$. Under standard conditions of electrophoresis (MCCARRON, GELBART and CHOVNICK 1974), all variant XDHs that are slower are designated by relative mobility values < 1.00, while faster XDHs are designated by super-

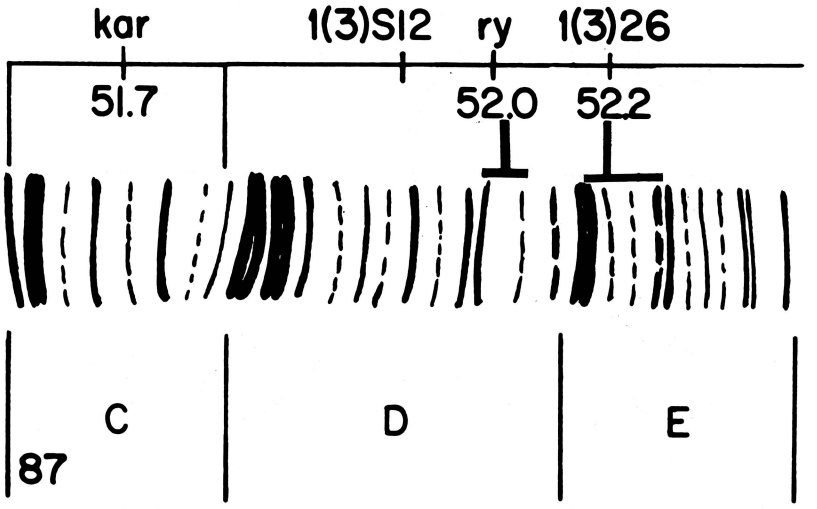


Figure 1. Polytene chromosome map of the rosy region of chromosome three in *Drosophila melanogaster* (BRIDGES 1941).

scripts > 1.00. The $ry^{+4}$ allele is associated with sharply higher XDH activity than all others and is classified in Table 1 as high (H), while $ry^{+10}$ exhibits much lower activity than all others and is classified as low (L). The activity levels of the remaining alleles are representative of intermediate levels which we presently classify as normal (N).

Enzymatically inactive rosy mutants are readily selected by virtue of their visibly mutant eye color phenotype. Over a period of many years, X-ray, γ-ray and EMS mutagenesis experiments have provided us with a large number of such mutants. We have adopted the convention of labeling each mutant with a superscript which identifies the $ry^{+}$ isoallele from which the mutant was derived. Thus, the $XDH^{-}$, rosy eye color mutant, $ry^{402}$, is the second variant isolated from $ry^{+4}$, and $ry^{1201}$ is the first mutant derivative of the $ry^{+12}$ allele. Another class of rosy locus mutants exhibits normal or near-normal eye color in homozygotes, possesses low levels of XDH activity, and dies on purine supplemented media (GELBART, MCCARRON and CHOVNICK 1976). These mutants are designated by the prefix *ps* (purine sensitive). Thus, $ry^{ps214}$, the $14^{th}$ identified variant of $ry^{+2}$, is a purine sensitive mutant associated with a low level of XDH activity and a wild-type eye color. Electrophoretic sites are noted with the prefix *e*. The site responsible for the mobility difference between the $ry^{+4}$ product ($XDH^{1.02}$) and the $ry^{+0}$ enzyme ($XDH^{1.00}$) is designated *e408*. Thus, $ry^{+0}$ possesses *e408s* (slow), while $ry^{+4}$ carries the *e408f* (fast) alternative. The difference in intensity or level of XDH activity associated with the $ry^{+4}$ isoallele as compared to other wild type isoalleles (Table 1) has been localized to a site designated *i409*, with $ry^{+4}$ possessing

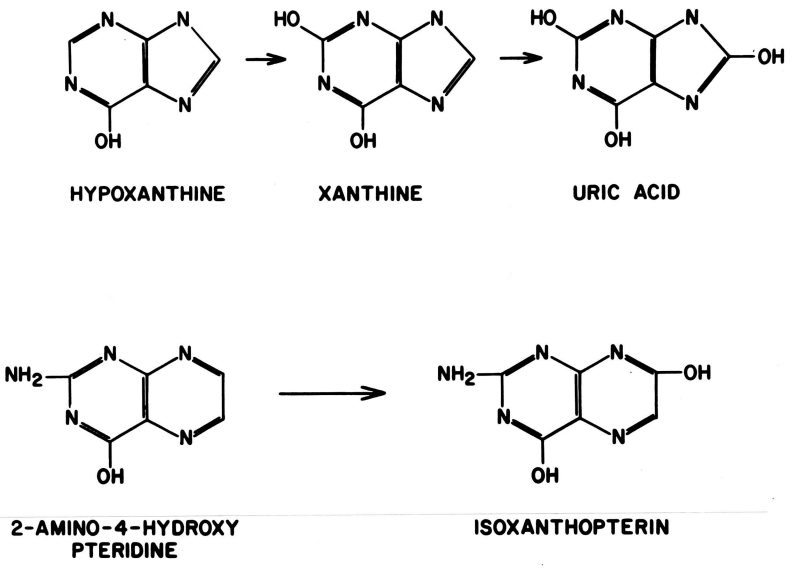


Figure 2. Reactions of Drosophila xanthine dehydrogenase.

Table 1. Wild-type isoalleles of rosy

| $ry^+$ alleles | Mobility | XDH Activity |
|---|---|---|
| +12, +13 | 0.90 | N |
| +14 | 0.94 | N |
| +10 | 0.97 | L |
| +0, +6, +7, +8 | 1.00 | N |
| +1, +11, +16 | 1.02 | N |
| +4 | 1.02 | H |
| +2 | 1.03 | N |
| +3, +5 | 1.05 | N |

*i409H* (High) while $ry^{+0}$ and other alleles possess *i409N* (Normal)

(CHOVNICK *et al.* 1976).

## GENETIC DISSECTION OF THE *ROSY* REGION

The rosy region of chromosome 3 has been the subject of extensive cytogenetic analysis in our laboratory. The precise segment which has been the major focus of this analysis extends from 87D7-9 to 87E12-F1, a chromosomal section of 18 to 19 polytene chromosome bands (Fig. 1). A detailed description of this work will be reported elsewhere.

The raw material of this study is a group of 137 non-rosy, lethal and semi-lethal, apparent site mutants, induced largely with EMS, but including some radiation-induced mutants. These were selected, originally, as being lethal with one or another of several chromosomes possessing deletions of part or all of this chromosomal segment. Then from a series of experiments involving: (1) *inter se* complementation; (2) tests for lethality with chromosomes carrying smaller overlapping deletions of this region; and (3) some recombination tests, we have been able to identify and order a total of 16 lethal complementation groups within the 87D7-9 to 87E12-F1 segment of 3R (Fig. 1). Thus, with the addition of the rosy locus, a total of 17 complementation groups have been identified in this segment of 18 to 19 polytene bands, a result entirely consistent with the one gene:one chromomere hypothesis (BRIDGES 1935; JUDD *et al.* 1972; HOCHMAN 1973). Moreover, if we assume saturation of the map of this region and the validity of the one gene:one chromomere hypothesis, then we are able to further define the rosy locus to 87D12-13 (Fig. 1).

Another point of interest bearing upon the organization of the rosy locus derives from the following observations: (1) The analysis of a large number of EMS and radiation-induced rosy mutants has revealed that none are recessive lethals under standard nutritional conditions, with the exception of *bona fide* deletions which involve adjacent genetic units. (2) All of the lethal site mutants in the rosy region belong to complementation groups that are functionally and spatially distinct from the rosy locus. Taken together, these points support the contention previously drawn (SCHALET *et al.* 1964; CHOVNICK 1966) that rosy is a discrete genetic unit, and not part of a functional complex involving any of the adjacent genetic units.

Figure 3 presents a photograph of polytene chromosome segment 87D provided by Dr. George Lefevre. Estimate may be made of the haploid DNA content of bands 87D12-13 by relating them to the hybrid DNA content of bands measured by Rudkin (1965). The bands that might be associated with rosy are much finer than the average band (30kB), but are certainly greater than the finest bands that were measured by Rudkin (5kB).

## ORGANIZATION OF THE *ROSY* LOCUS

Figure 4A presents a summary map of separable $XDH^-$ mutant sites within the rosy locus resulting from fine structure recombination experiments. Since none of these mutant alleles has been associated with a detectable, altered XDH product, this map provides no information about the organization of the locus. However, tentative boundaries of the XDH structural element may be defined from experiments involving several classes of unambiguous structural variants. Figure 4B summarizes the map of mutants which exhibit allele complementation. Figure 4C positions electrophoretic sites and Figure 4D locates sites associated with purine sensitive "leaky"

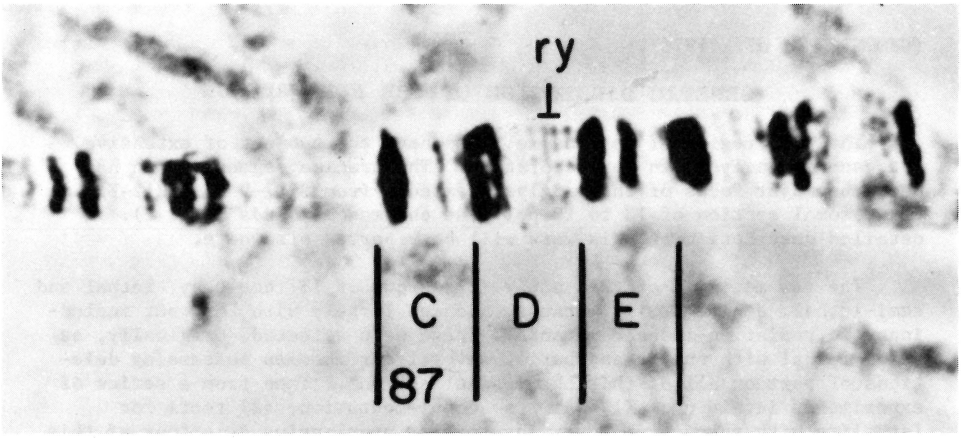


Figure 3. The rosy region of polytene section 87D of chromosome 3R.

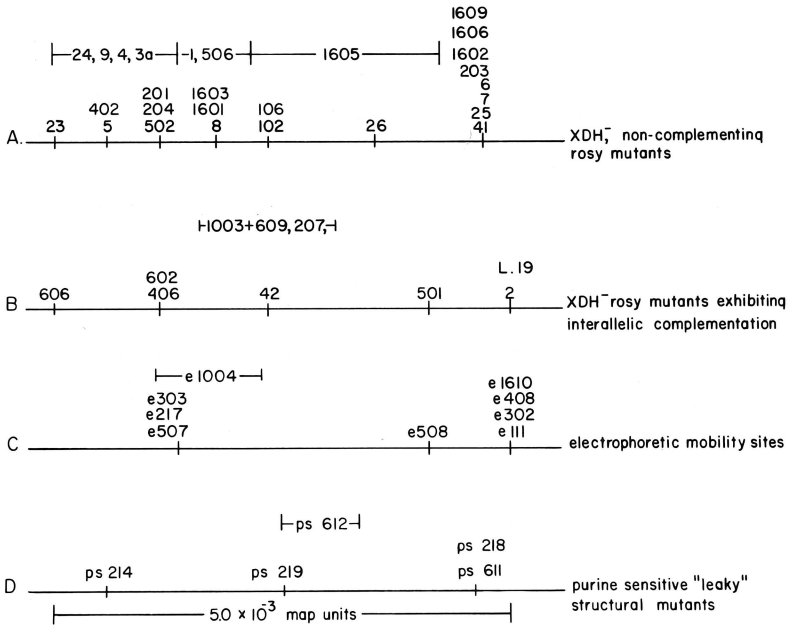


Figure 4. Genetic fine structure maps of rosy locus sites. Map locations of unambiguous structural element variants (B, C, and D) are positioned relative to XDH⁻ non-complementing mutants (A).

mutants that possess normal or near-normal levels of a protein that reacts with XDH specific antibodies (CRM). At the right end of the map, several electrophoretic sites, and the complementing mutants, $ry^{2}$ and $ry^{L\cdot 19}$, identify the right border of the structural element with no known XDH structural variants beyond them. The complementing rosy eye color mutant, $ry^{606}$, and the purine sensitive variant, $ry^{ps214}$, are the leftmost unambiguous structural variants, with $ry^{606}$ definitely to the left of $ry^{ps214}$. On the basis of comparative recombination data and the failure of large-scale tests with $ry^{606}$ to produce recombinants, we conclude that the leftmost member of the standard map, $ry^{23}$, must also mark the left border of the structural element.

The maps of Figure 4 position 51 sites to the right of our present left boundary of the XDH structural element. Moreover, an additional ten sites not indicated, in fact, map in the structural element. Eventually, we hope to relate the genetic boundaries described in Figure 4 to the amino and carboxy termini of the XDH polypeptide chain. For the present, however, the genetic boundaries must suffice.

## VARIATION IN INTENSITY OF XDH ACTIVITY

In addition to electrophoretic mobility differences, we have already noted that the various $ry^{+}$ isoalleles are associated with variation in level of XDH activity, which also behaves as a stable phenotypic character. Consider the $ry^{+4}$ and $ry^{+10}$ lines which exhibit much greater and much less activity, respectively, than all of our other wild-type lines (Table 1). These differences are observed by following either the purine or pteridine reaction (Fig. 2), and are readily classified in cuvette assays (spectrophotometry or fluorimetry) or upon gel electrophoresis. Figure 5 illustrates typical fluorimetric assays of XDH activity in matched, partially purified extracts of several $ry^{+}$ isoallelic stocks including both $ry^{+4}$ and $ry^{+10}$. Measurements of XDH activity/mg protein, activity/fly and activity/mg wet weight invariably yield similar relationships. Activity levels associated with $ry^{+2}$, $ry^{+6}$ and $ry^{+11}$ exhibit a range of variation (Fig. 5) which we classify as normal (N) in Table 1, and never overlaps the $ry^{+4}$ and $ry^{+10}$ extremes. Indeed, $ry^{+10}$ homozygotes exhibit such low activity, that they may be distinguished from other wild-type strains by virtue of their purine sensitivity. A detailed analysis of the basis for the $ry^{+4}$ phenotype is presented elsewhere (CHOVNICK *et al.* 1976), and a report on the $ry^{+10}$ character is now in preparation. Together, these studies identify a *cis*-acting control element located adjacent to the left (centromere proximal) side of the XDH structural element. The following sections outline the experimental basis for this conclusion.

## FURTHER CHARACTERIZATION OF THE XDH ACTIVITY LEVEL VARIANTS

That variation in level of XDH activity (Table 1 and Fig. 5) is a property of the rosy locus is derived from conventional genetic analysis involving standard mapping procedures (CHOVNICK *et al.* 1976). However, the task of distinguishing whether the genetic bases for the level of activity differences reside in the XDH structural element, or possibly in a control element is not as simply resolved. Based upon fine structure recombination analysis, some of our $ry^{+}$ isoalleles differ by as many as five or six structural element sites. Independent studies (COYNE 1976; SINGH *et al.* 1976) of XDH structural gene polymorphism in natural populations additionally support the view that any two isolated lines will

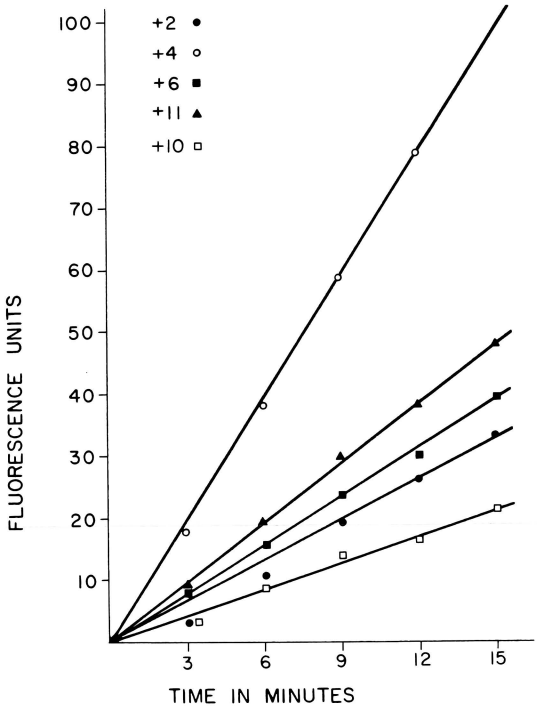


Figure 5. Fluorimetric assay of XDH activities of matched extracts of strains homozygous for the indicated wild-type isoalleles.

very likely possess structural element differences. Obviously, the genetic polymorphism exhibited by the XDH structural element might also extend into an adjacent control element. Certainly, level of enzyme activity is a superficial phenotypic character that might reflect either structural or control element variation. It thus behooves us to further define this character, and to carry out high resolution fine structure mapping experiments.

We have considered the possibility that there are structural differences between the XDH molecules produced by $ry^{+4}$ and those produced by other $ry^{+}$ isoalleles that are responsible for the sharply increased activity of $ry^{+4}$ preparations. However, we have been unable to associate this activity difference in any systematic manner with electrophoretic, thermolability or kinetic differences (CHOVNICK *et al.* 1976; EDWARDS *et al.* 1977). Similar comparisons involving XDH molecules produced by $ry^{+10}$ and those produced by other $ry^{+}$ isoalleles also have failed.

On the other hand, immunological experiments clearly support the notion that the activity differences reflect differences in number of XDH molecules. One approach has used standard antiserum titration experi-

ments. A series of dilutions of an antibody preparation against Drosophila XDH is tested for ability to remove XDH activity from matched extracts of the various $ry^{+}$ isoallele stocks. Still another approach has used the method of quantitative "rocket electrophoresis" (LAURELL 1966; WEEKE 1973) to compare these extracts. Both kinds of experiments indicate that $ry^{+4}$ preparations contain more XDH molecules than do matched preparations from normal activity strains, and these, in turn, have more molecules of XDH than does $ry^{+10}$.

The time course of appearance of enzyme during development has been determined for a number of our wild-type isoallelic lines, as well as for the activity level variants. Synchronous populations from the various lines are raised to the appropriate developmental stages, at which time extracts are examined for XDH activity, total protein and response to XDH antibody (CRM). Thus, we are able to examine and compare XDH activity/individual, XDH activity/mg protein or in terms of CRM levels throughout development. No gross differences have yet been detected among the various strains. While $ry^{+4}$ exhibits relatively increased amounts of enzyme throughout development, and $ry^{+10}$ is associated with reduced enzyme, their developmental profiles are otherwise normal. Similarly, tissue distribution studies have failed to identify qualitative differences that might be associated with the activity level variants.

## GENETIC FINE STRUCTURE EXPERIMENTS

Let us now consider localization of the genetic bases for the level of enzyme activity differences described above. Fine structure recombination experiments were carried out that were designed to pursue the difference between the $ry^{+4}$ allele (associated with high activity) and other wild-type alleles which exhibit normal levels of activity (CHOVNICK *et al.* 1976). These studies led to the identification of a site, *i409*, with $ry^{+4}$ possessing *i409H* (associated with high activity) while such alleles as $ry^{+0}$, $ry^{+2}$, $ry^{+6}$ and $ry^{+11}$ carry *i409N* (associated with normal levels of activity). Moreover, these experiments localized this site to the left, but definitely outside of the genetic boundaries of the XDH structural element. Figure 6 illustrates this localization of *i409* between the left end of the XDH structural element and *l(3)S12*, a site mutant in the very next genetic unit to the left of rosy.

Now, let us turn to the low level of XDH activity associated with the $ry^{+10}$ isoallele. A series of fine structure recombination experiments were carried out that parallel, in many respects, the $ry^{+4}$ experiments. From these studies we identify a site, designated *i1005*, with $ry^{+10}$ carrying *i1005L* (Low), and our normal wild-type alleles carrying *i1005N* (Normal). Additionally, these crosses position *i1005* to the right of *l(3)S12* and to the left of the XDH structural element. Clearly, these experiments localize *i1005* to the same region as *i409* (Fig. 6).

What is the relationship between *i1005* and *i409*? Might they be synonyms designating the same site, or might they mark separable sites? Heterozygotes of the composition $\frac{+\ \ i1005L\ \ +}{kar\ \ i409H\ \ ry^{406}}$ were mated to an appropriate tester strain, and their progeny reared on medium containing a level of purine sufficient to kill *i1005L* or $ry^{406}$ bearing zygotes. [There were additional markers in the cross to facilitate diagnosis of all survivors.] In a total of $1.25 \times 10^{6}$ progeny sampled, there were 16 surviving $ry^{+}$ recombinants. Three were conversions of $ry^{406}$ and exhibited

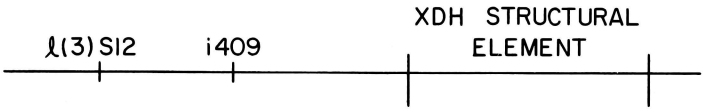


Figure 6. Genetic map location of *i409* site relative to *l(3)S12* and the XDH structural element.

the *high* level of XDH activity associated with *i409H*. One was a conversion of *i1005L* and possessed a *normal* level of XDH activity. Finally, there were twelve $ry^+$ progeny associated with exchange for the flanking markers. Nine of these possessed *high* activity, while the remaining three exhibited *normal* XDH activity. The results of this cross indicate that *i1005* and *i409* mark separable sites. However, in the absence of additional information, this experiment is unable to determine the relative position of the two sites. Figure 7 illustrates the ambiguity inherent in this experiment. Heterozygote (A) of Figure 7 illustrates surviving crossovers in appropriate selective system matings given that the order of the intensity sites is *i1005 - i409*. Crossover (1) then would represent the $ry^+$ progeny exhibiting high levels of activity, while crossover (2) would comprise the $ry^+$ survivors with normal levels of activity. Heterozygote (B) of Figure 7 illustrates surviving crossovers given that the order of intensity sites is *i409 - i1005*. On this model, crossover (3) would produce $ry^+$ progeny exhibiting high levels of activity, while crossover (4) would exhibit normal levels of activity. It should be noted that the results of this cross are ambiguous only if the double variant, *i1005L, i409H*, leads to the production of normal levels of XDH. If the double variant exhibits activity levels distinguishable from normal, then only the order illustrated by heterozygote (A) of Figure 7 will satisfactorily explain the observed results. Experiments designed to resolve this ambiguity are presently in progress.

## RELATIONSHIP BETWEEN i409, il005 AND THE STRUCTURAL ELEMENT

Might *i409H* represent a tandem duplication of the XDH structural element? On this notion the $ry^{+4}$ allele would be considered to possess two functional XDH structural elements in tandem, presumably resulting from an unequal exchange event. Such a model is precluded on several counts:

1. EMS mutagenesis of $ry^{+4}$ has produced rosy eye color mutations at

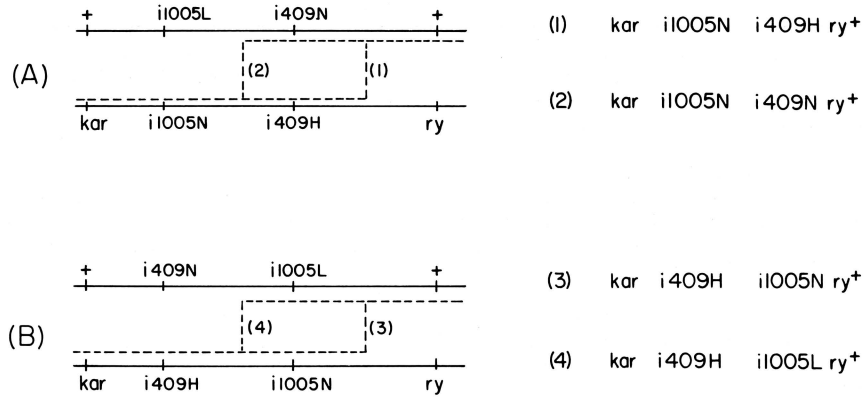


Figure 7. Relative map positions of *i1005* and *i409*. Ambiguity inherent in random strand mapping experiment.

a frequency that does not distinguish this allele from other $ry^+$ isoalleles.

2. The $ry^{+4}$ allele is associated with a single XDH electrophoretic band of the mobility class, $XDH^{1.02}$ (Table 1). XDH is a homodimer, and the presence of two electrophoretically distinct structural elements will produce individuals possessing three XDH moieties. The tandem duplication model then requires that the $ry^{+4}$ allele possess two XDH structural elements whose peptide products are indistinguishable, and of the mobility class, $XDH^{1.02}$. Thus, *i409H* should be associated with an $XDH^{1.02}$. On this point, the tandem duplication model fails. In all experiments which recombine *i409H* with other electrophoretically distinct structural elements, there is no evidence of the production of an $XDH^{1.02}$ moiety.

3. Tandem duplications are characterized by instability in homozygotes due to increased incidence of unequal exchange events. The $ry^{+4}$ stock has been quite stable. Moreover, fine structure recombination experiments involving tests of $ry^{400}$ series mutants against other $XDH^-$ mutants have been characterized by regular exchange events, and the complete absence of unequal crossing over.

4. Cytological examination of polytene chromosomes reveals no such tandem duplication.

Now let us consider *i1005L*. While the association of *i1005L* with a reduction in the number of XDH molecules/fly does not seemingly lend itself to the tandem duplication model, precedence for such consideration exists in the case of the Bar duplication in *Drosophila melanogaster* and its associated position effect (STURTEVANT 1925; MULLER *et al.* 1936; BRIDGES 1936; MULLER 1936). On such a notion, the $ry^{+10}$ isoallele would be considered to possess two XDH structural elements in tandem. Moreover, by virtue of the resulting change in position of each member of the duplex relative to some adjacent genetic element(s), a disturbed function of both XDH structural elements results. Such a model is precluded by the same arguments described above for *i409H*.

Having eliminated the possibility that either *i409* or *i1005* might be associated with a tandem duplication of the XDH structural element, it is appropriate at this point to consider that these separable sites mark one or more genetic elements that serve to regulate XDH. Arguments in support of this point are:

1. We have succeeded in mapping more than 60 sites within a region clearly marked by unambiguous XDH structural variants (Fig. 4). The *i409* and *i1005* sites are the only sites which have mapped outside these boundaries.

2. Variation at *i409* and *i1005* leads to alteration in number of molecules of XDH/fly.

3. We now possess stocks carrying *i409N* and *i409H* with structural elements producing $XDH^{0.97}$, $XDH^{1.00}$, $XDH^{1.02}$, $XDH^{1.03}$ and $XDH^{1.05}$. Examination of these lines has failed to associate *i409* with any XDH structural characteristic.

4. On a more limited scale, we have similarly produced stocks carrying *i1005N* and *i1005L* with structural elements producing $XDH^{0.97}$, $XDH^{1.00}$ and $XDH^{1.05}$. Similarly, we are unable to associate *i1005* with any structural characteristic.

5. In previous reports (CHOVNICK *et al.* 1976; CHOVNICK *et al.* 1977), evidence was presented that demonstrated the *cis*-acting nature of *i409*. Thus, in the heterozygote $\frac{i409H\ ry^{+4}}{i409N\ ry^{+12}}$, there is an increased number of $XDH^{1.02}$ molecules ($ry^{+4}$ product) and not an increase in the $XDH^{0.90}$ molecules ($ry^{+12}$ product). Parallel experiments involving *i1005* similarly reveal the *cis*-acting nature of *i1005*.

On the basis of these observations, we are drawn to the possibility that *i409* and *i1005* mark the 5' control element of the rosy locus. While it would be premature to specify the nature of the control function associated with each of these sites, it would be reasonable to expect that variants of such a control element might exhibit alterations in DNA sequences which serve as binding sites for regulatory signal(s), sites for RNA polymerase binding and initiation of transcription, transcript processing sites, ribosome binding and initiation of translation.

## STRUCTURAL AND CONTROL ELEMENT SIZE ESTIMATES

Figure 8 summarizes our present map length estimates for the rosy locus structural and control elements. Extensive mapping experiments have been carried out which have placed more than 60 sites within the structural element boundaries. Yet, both left and right borders of the structural element have not changed significantly from our earlier map (CHOVNICK 1973). The size of the control element is quite tentative. At present, we suggest that the distance from *i409* to the structural element (0.0034 map units) serve as a minimal estimate, while the distance from *l(3)S12* to the XDH structural element (0.0054 map units) be taken as a maximum estimate of the control element size (CHOVNICK *et al.* 1976).

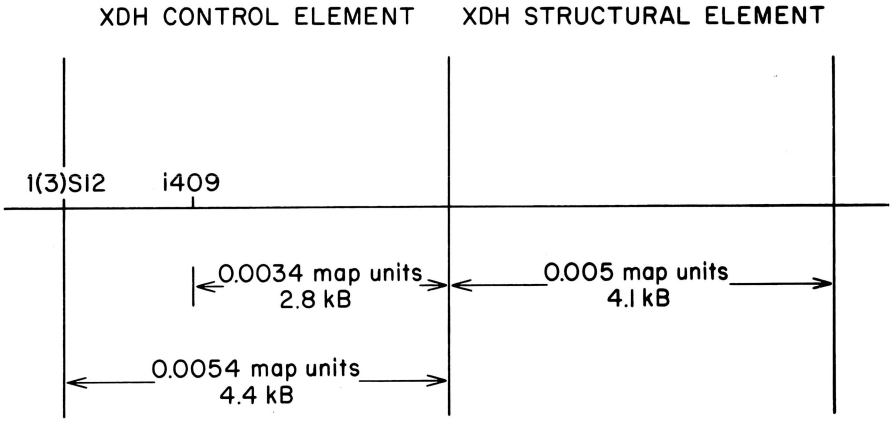


Figure 8. The rosy locus. Size estimates for the structural and control elements.

Translation of these estimates into DNA base lengths proceeds from the XDH peptide molecular weight of 150,000 daltons. Assuming an average amino acid molecular weight (adjusted for peptide linkage) to be 110, then the length of DNA in the XDH structural element responsible for such a peptide is approximately 4.1 kB (150,000 x 3/110). Then, from the recombination map length of the structural element (0.005 map units), we may relate map length to physical length (0.01 map unit = 8.2 kB), which should be directly applicable to the adjacent control element. Thus, the size of the control element is estimated to be 2.8 - 4.4 kB, and the total length of the rosy locus from 6.9 - 8.5 kB.

Another estimate of the length of DNA in the rosy locus may be derived directly from its genetic length (0.0084-0.010 map units). With

$1.6 \times 10^5$ kB as the total genome DNA represented as single copy and middle repetitive sequences (RASCH *et al.* 1971; MANNING *et al.* 1975), and 275 map units as the total map length, then 0.01 map unit represents 5.8 kB. By this method, the rosy locus DNA length is estimated at 4.87 - 5.8 kB. The reader should note that this method ignores regional differences in recombination frequency, and that the rosy locus is subject to reduced recombination due to its centromere proximal position (GELBART *et al.* 1976). In this context, we view the former method to yield a better estimate.

Models of gene organization proposed as solutions of the chromomere paradox in higher organisms postulate control elements an order of magnitude larger than their structural components (BEERMANN 1972). An alternative solution would have a discontinuous structural element interrupted by extensive non-coding segments which may or may not serve regulatory functions. At the present level of analysis, there is little support for either model. We note that the present data are consistent with the notion of a continuous coding element. Moreover, the size estimate for the rosy locus is entirely consistent with its polytene band size and DNA content estimates (See Fig. 3 and discussion above).

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