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Progressive loss of DNA sequences from terminal chromosome deficiences in *Drosophila melanogaster*

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Terminal deficiencies at the tip of the X chromosome can be induced at a high frequency (0.2–0.3%) by irradiating *Drosophila* females carrying a homozygous mutator (*mu-2*) with low doses of X-rays. These terminal deficiencies are unstable, since over a period of 3½ years DNA sequences were lost from their distal ends at a rate of 75 bp per generation, presumably due to the absence of a complete wild-type telomeric structure. Breakpoints of these deletions in the 5′ upstream regulatory region of the *yellow* gene, giving rise to a mosaic cuticle pigmentation pattern typical of the y^2 type, were used to define the location of tissue-specific *cis*-acting regulatory elements that are required for body, wing or bristle pigmentation.

Key words: DNA loss/*Drosophila*/ telomere/terminal deficiencies/yellow gene

Introduction

The tips of linear chromosomes are believed to contain specialized telomeric structures (Muller, 1932, 1940; Muller and Herskowitz, 1954), because broken, 'uncapped' chromosomes are recovered as rearrangements with two or more breakpoints. In maize endosperm a broken chromatid end may fuse with its sister chromatid broken end to produce a dicentric chromosome, which forms a bridge in the next anaphase. This chromosome bridge breaks at other sites, establishing a bridge–break–fusion cycle during endosperm development (McClintock, 1939, 1941, 1942). However, at the time of the first mitotic cycle in the embryo the broken chromosome 'heals' and thus can be recovered as a stable terminal deficiency. Similarly, a broken chromosome end in yeast may heal to produce a stable terminal deficiency (Haber and Thorburn, 1984; Haber *et al.*, 1984).

In *Drosophila* the recovery of stable, 'healed' broken chromosomes as terminal deficiencies is extremely rare after irradiation of sperm (Roberts, 1975). Somatic cells with broken chromosome ends divide poorly, if at all (Gatti, 1979). In the presence of a homozygous mutation at the *mu*-2 locus, however, putative terminal deficiencies of all chromosomes are recovered at a high frequency (0.2–0.3%) after irradiating females with low doses of X-rays (Mason *et al.*, 1984). These terminal deficiencies not only lack the most distal genetic marker on the X chromosome [*1(1)EC1*], but also the telomere-associated moderately repetitive sequences represented by the 3.0 kb telomere-specific DNA clone cDm 356 (Rubin, 1977; Mason *et al.*, 1984). In this paper we have examined the relative stability of terminal deficiencies on the X chromosome [Df(1)RT, henceforth abbreviated RT], and their use in the analysis of the 5′ upstream regulatory region of the *yellow* gene.

Results

Deficiencies removing the tip of the X chromosome were selected by screening for the loss of the *yellow* (y^+) gene function (located at the tip of the X chromosome in band 1B1) as apparent by the complete or partial absence of black melanin pigmentation of the adult cuticle. Of 12 putative terminal deficiencies that affected *y* but not the nearest proximal gene *achaete (ac)*, five exhibited a mosaic pigmentation pattern typical of y^2-type mutations (yellow body and wings, wild-type pigmented black bristles), while seven were fully mutant for *y* (y^1-type, all body structures lack melanin).

Whole genomic Southern blots of RT deficiencies probed with fragments immediately distal to the breakpoints revealed the absence of these DNA sequences from all deficiencies (data not shown). The positions of distal DNA breaks with respect to the *y* gene region, as mapped in November 1984, are shown in Figure 1. DNA breakpoints in the 5′ upstream region gave rise to a y^2-type phenotype (*RT653, RT276, RT741, RT473, RT394*), while breaks in the *y* transcription unit resulted in y^1-type phenotype.

To address the question of whether these deficiencies are terminal, DNA from several RT stocks was hybridized on genomic Southern blots with appropriate *y* DNA probes. Using four different restriction enzymes, the length of the terminal DNA fragments was determined with respect to a known proximal restriction site in the *y* gene region. These experiments revealed an apparent clustering of sites for three or four restriction endonucleases at the distal breakpoints of the RT deficiencies tested (Table I), suggesting that these breakpoints represent the structural end of the chromosome and indicating that the deficiencies are indeed terminal.

During the 3½ year perid of this study we noticed that the terminal deficiency stocks with breakpoints in the 5′ upstream region, expressing the y^2-phenotype, converted to the more extreme y^1 phenotype pigmentation. Some mosaicism for bristle pigmentation occurred in intermediate stages, and terminal deficiencies with breakpoints closest to the first exon of the *y* gene (e.g. *RT473, RT394*) lost their y^2-type phenotype first. We also observed in October 1984 that single *RT394*/y^2 *sc* Y (y^2-type) males produced both y^1- and y^2-type daughters when crossed to y^1 females. In similar experiments in December 1985, a group of 15 *RT653*/y^2 *sc* Y males taken from a single stock vial, was mated singly to y^1 *ac v* females and their female progeny scored for the presence of a y^2 phenotype. Four of the males produced both y^2- and y^1-type daughters (Table II),

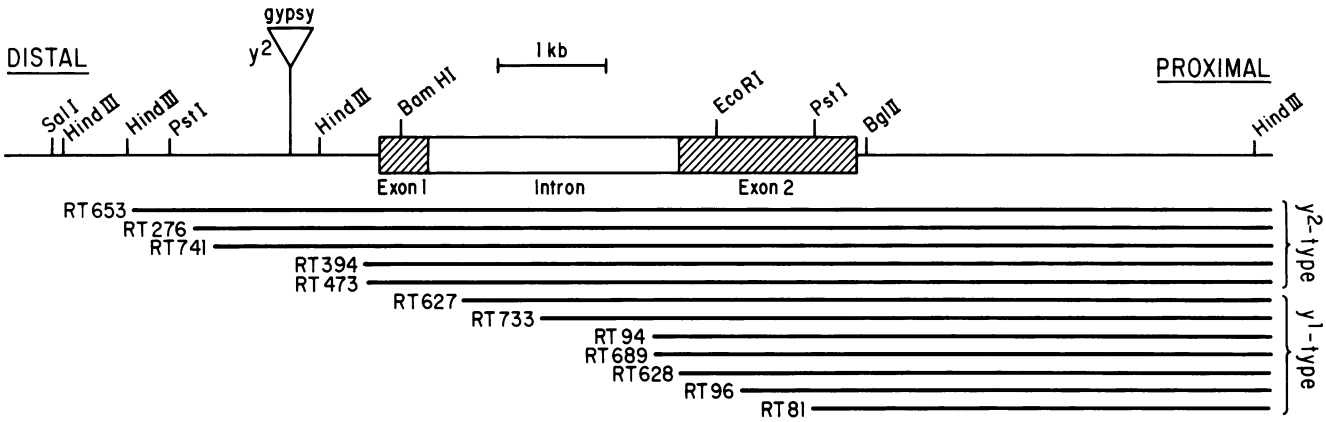

Fig. 1. Location of breakpoints in November 1984 of 12 terminal deficiencies Df(1)RT with respect to the *y* gene coding region, as determined by whole genomic Southern blot analyses. Solid bars represent DNA sequences still remaining in the terminal deficiencies. Hybridization was done with nick-translated fragments from the *y* gene region, and no signals were obtained with probes from several kb distal of the breakpoints. *RT653, RT276, RT741, RT473* and *RT394* exhibited a y^2-type phenotype, the rest were y^1. The position of the gypsy insertion element in the y^2 allele is also indicated.

indicating that the males originally carried a y^2-type RT chromosome, but that the germ line of these males was now mosaic. To confirm this, the y^2-type daughters were progeny tested to determine whether they carried the y^2 *sc* Y chromosome; none did. In fact, the y^2 segregated with the lethal-bearing ac^+ X chromosome, and away from the lethal-free *ac* chromosome. These observations suggested that the terminal deficiencies were unstable, and led us to investigate this apparent instability at the molecular level.

Genomic DNA Southern blots of flies from several RT stocks, collected at different times during a 3½ year period, and probed with a DNA fragment from the *y* gene coding region revealed that the deficiency breakpoints were receding. Two representative examples are shown in Figure 2. The distal DNA fragments of the terminal deficiencies always appeared much broader than regular restricted fragments, indicating heterogeneity at the ends of RT chromosomes. Loss of DNA sequences from the distal end of the breaks appeared to occur at a rate of about 50 bp per month at 18°C (Figure 3), which is equivalent to about 75 bp per generation. The same rate was observed in all of the four RT deficiencies analyzed. Within the limited sensitivity of our method, DNA sequences appear to be lost continuously over the 3½ year study period.

We did observe a new, 3.0 kb bigger *Pst*I band in *RT473* in December 1986 (marked with an asterisk in Figure 2) that might reflect the spontaneous addition of new sequences to the breakpoint and represent a 'healing' event. The nature of the added DNA sequences and the mechanism of the addition will have to be determined. This reflects the coexistence of two different X chromosomes in the *RT473* stock in December 1986, and future analyses will be performed to study their fates in the following generations.

We used the receding terminal deficiencies to analyze *cis*-acting elements in the 5′ regulatory region of the *y* gene. While DNA breakpoints in the 5′ upstream region always gave rise to a y^2-type phenotype (*RT653, RT276, RT741, RT473, RT394*), breaks in the *y* transcription unit resulted in y^1-type phenotype. These findings support the notion that the mosaic pigmentation pattern of these y^2-type alleles is caused by disturbing the regulation of *y* gene expression in a way that is different from that associated with the insertion of transposable elements (see Parkhurst and Corces, 1985, 1986). Our data suggest that the terminal deficiencies exert the y^2-type phenotype by removing *cis*-acting elements from the *y* regulatory region. Because the terminal deficiency *RT653* produces yellow body and wings but black

Table I. Sizes of terminal DNA restriction fragments of RT deficiencies in November 1984

Deficiency stock	*Eco*RI	*Pst*I		*Bgl*II		*Hin*dIII	
RT653	5.5	–[a]		7.0	(5.7)	10.5[b]	(5.5)
RT276	4.7	5.7	(4.75)	6.5	(5.2)	10.0[b]	(5.0)
RT741	4.4	5.5	(4.55)	5.9	(4.6)	–[a]	
RT473	2.9	4.1	(3.15)	4.3	(3.0)	8.3	(3.3)
RT627	2.2	3.2	(2.25)	3.6	(2.3)	7.2	(2.2)
RT733	1.4	2.5	(1.55)	2.9	(1.6)	6.6	(1.6)
RT94	n.d.[c]	1.5	(0.55)	1.8	(0.5)	5.6	(0.6)
RT689	n.d.	1.5	(0.55)	1.8	(0.5)	5.6	(0.6)
RT628	n.d.	1.4	(0.45)	1.6	(0.3)	5.6	(0.6)
RT96	n.d.	0.7	(–0.25)	0.9	(–0.4)	4.7	(–0.3)
RT81	n.d.	n.d.		n.d.		4.0	(–1.0)

The sizes in (kb) of the terminal DNA fragments of the RT deficiencies were determined by hybridization with various *Eco*RI fragments of the *y* gene, subcloned in pBR329, to whole genomic DNA Southern blots. Their map positions are given in kb along the chromosomal walk, with values increasing from proximal to distal (Biessmann, 1985). For reference, the *Eco*RI site in the second exon of the *y* gene is located at position 33.5. Fragments used as probes: 14a3B (33.5–38.0), 14b15B (31.7–33.5), 14a3A (27.8–33.5). Four restriction sites in the *y* gene region were used as reference points, their positions are indicated in Figure 1. The *Eco*RI site used here is located at position 33.5, the *Pst*I site lies 0.95 (pos. 32.55), the *Bgl*II site 1.3 (pos. 32.2) and the *Hin*dIII site 5.0 kb (pos. 28.5) proximal to this *Eco*RI site. Using these positions, the calculated distances of the chromosome end to the *Eco*RI site for each deficiency are given in parentheses. An apparently close clustering for three or four restriction enzymes at the distal breakpoints of all RT deficiencies is observed, indicating the end of the chromosome.

[a]Size of terminal fragment could not be determined because a *Pst*I or *Hin*dIII site was present very close to the chromosome end at the proximal side.

[b]Length of the terminal fragment with respect to the *Hin*dIII site at position 28.5 was determined by adding the sizes of the two fragments that hybridize to probe 14a3B (see map Figure 1).

[c]n.d. = fragment too small to be detectable on genomic Southern blot.

Table II. Recovery of y^2 females from *RT653*/y^2 *sc* Y males crossed with *y ac v* females

Male no.	y^2 male	y^1 female	y^2 female	y^1 male
1	15	14	3	0
2	22	25	0	0
3	23	21	0	0
4	19	27	0	0
5	15	25	0	1(*ac v*)
6	11	24	0	0
7	21	37	0	0
8	29	20	6	0
9	15	32	0	0
10	24	28	5	0
11	11	18	7	0
12	15	24	0	0
13	9	24	0	0
14	5	17	0	0
15	7	14	0	0

bristles, a DNA element required for proper pigmentation of adult body and wing cuticle must be localized at least 1.75 kb upstream of the start of transcription. However, 2.8 kb of the 5′ upstream region are sufficient to produce a wild-type phenotype in P-element mediated transformants (Chia *et al.*, 1986). More distal breakpoints would escape detection because we selected for a visible *yellow* phenotype. The deficiencies *RT473* and *RT394* gave the same black bristle phenotype as *RT653*, suggesting that the 250 bp of upstream region remaining in these deficiencies are sufficient for proper bristle pigmentation.

Discussion

Our results demonstrate that terminal deficiencies generated by X-rays in the presence of a homozygous *mu-2* mutation are not stable over extended periods of time, which suggests that they do not harbor a fully functional telomere. The fact that such deficiencies were recovered at all may be due to the absence of a fully functional *mu-2* gene product. The function of *mu-2* is not known, but since the terminal deficiencies did not show the typical properties of broken chromosome ends, Mason *et al.* (1984) hypothesized that *mu-2* stocks may be defective in a mechanism involved in repairing X-ray induced DNA breaks. To maintain the deficiency stocks, however, the *mu-2* mutation is no longer required (Mason *et al.*, 1984). Until the DNA fragments from the putative chromosomal end have been isolated by molecular cloning, we cannot tell whether the newly created ends are devoid of any telomere-like DNA sequences such as the ones that have been described in natural telomeres from several species (reviewed by Blackburn and Szostak, 1984), or whether some 'healing' event has occurred, producing a defective or incomplete telomeric structure. At least in the case of *RT394* and *RT473* which exhibit a y^2-phenotype, yet, in November 1984, have a terminal breakpoint located only about 250 bp upstream from the start of *y* gene transcription, we can conclude that no extensive telomeric structure exceeding about 100 bp could have been added to their distal breakpoints.

There are two possible explanations for the loss of DNA sequences from the terminal deficiencies. Either terminal sequences are lost and there are at least some periods during which no telomeric DNA sequences are present on the end of the chromosome, or some unique sequence DNA is lost proximal to the telomere without disturbing the telomere itself. If the former is true, and the deficiencies are not recognized as having broken ends, there may be other non-DNA components at the terminus that confer telomere function. Telomere-binding proteins have been identified from yeast (Berman *et al.*, 1986) and *Oxytricha* (Gottschling and Zakian, 1986). Such proteins may also exist in *Drosophila* and might function to identify a telomere as distinct from a broken end in the absence of telomere-associated DNA sequences.

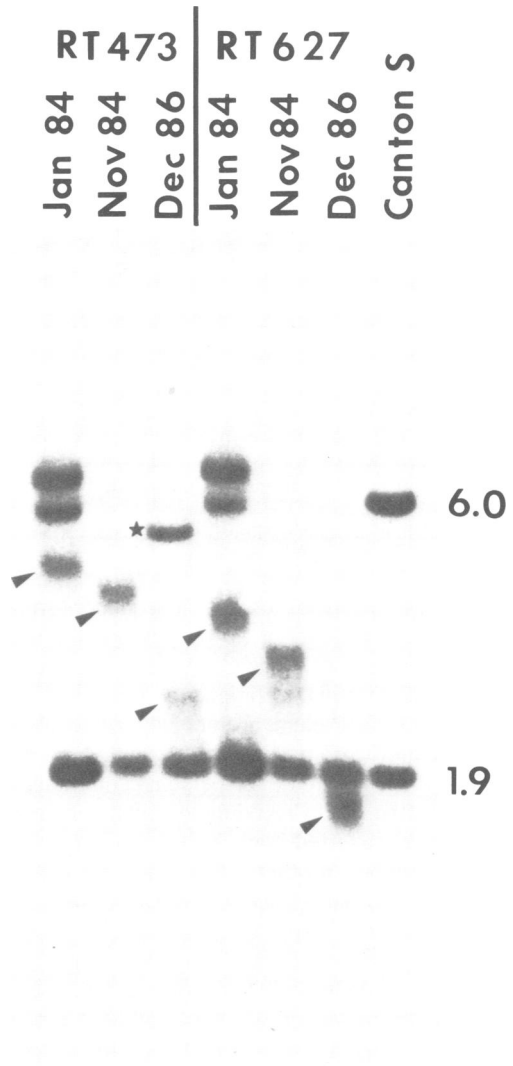

Fig. 2. Whole genomic Southern blot of DNA from terminal deficiencies *RT473* and *RT627*, and Canton S wild-type, hybridized to a 2 kb DNA fragment, beginning at the *Eco*RI site in the second exon of the *y* gene and extending proximally (see Figure 1). DNA was isolated from flies collected at times indicated above and digested with *Pst*I. Wild-type fragments hybridizing with this probe are 6.0 and 1.9 kb in size. The *Pst*I fragments in the RT deficiencies, marked by arrowheads, decreased in size during the three year period tested, and a new band, marked with an asterisk, appeared in *RT473* in December 1986. This band is 3.0 kb longer than the 2.7 kb fragment in the same lane, which represents the terminal fragment of the *RT473* deficiency. The higher mol. wt bands in DNA preparations made in January 1984 are due to the y^1 and y^2 alleles in the balancer chromosomes y^2 *sc* Y and *C(1)DX, y f.* Later, flies were isolated over *Df(1) y ac*, which does not hybridize with the labeled DNA probe.

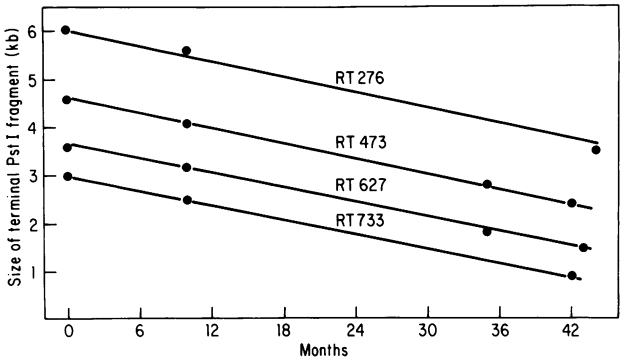

Fig. 3. Loss of DNA from the terminal deficiencies *RT276, RT473, RT627* and *RT733*. *Pst*I fragment sizes representing the terminal DNA fragments of the four deficiencies, as determined by whole genomic Southern blotting, were plotted against the time at which flies were collected for DNA preparation. The first analysis ($t = 0$) was made in January 1984. All four deficiencies lose DNA sequences at an apparently constant rate of ~50 bp per month.

The terminal deficiencies lose DNA sequences from their distal ends with a rate about 50 bp per month, which is ~75 bp per generation at 18°C. A very similar loss of nucleotides from terminal deficiencies, generated in quite a different way by a P-element under dysgenic conditions at the tip of the third chromosome, has recently been reported (Levis *et al.*, 1987; and R.Levis, personal communication). Moreover, our findings of receding terminal deficiencies in the *y* gene region reported here, have been confirmed and extended to a number of other RT deficiencies with breakpoints in the proximal adjacent *achaete–scute* complex (M.Ruiz Gomez and J.Modolell, manuscript submitted). Since DNA polymerase will always leave a single-stranded gap at the ends of a linear DNA molecule after the RNA primer has been removed, it seems plausible to explain this loss of sequences by the inability of the DNA replication machinery to completely replicate the ends of linear chromosomes. In *Drosophila*, RNA primers are octanucleotides (Kitani *et al.*, 1984), but the loss of 75 bp per generation cannot simply be explained by a loss of 8 bp from the terminal deficiency for every round of DNA replication, because DNA is certainly replicated more than ten times per generation. More likely, losses of this order of magnitude occur normally at the ends of chromosomes but are balanced by the action of a terminal transferase-like activity that adds nucleotides to the telomeric repeats (Bernards *et al.*, 1983; Pays *et al.*, 1983; Van der Ploeg *et al.*, 1984; Shampay *et al.*, 1984; Walmsley *et al.*, 1984; Blackburn, 1984; Larson *et al.*, 1987). This balance can be altered as evidenced by mutations that affect the length of these terminal sequences (Larson and Hartwell, 1985; Lustig and Petes, 1986). In addition, some if not all tandemly repeated telomere-associated DNA sequences (Rubin, 1977; Bedbrook *et al.*, 1980; Jones and Flavell, 1983; Chan and Tye, 1983; Young *et al.*, 1983; Renkawitz-Pohl and Bialojan, 1984; Steinemann, 1984; Saiga and Edström, 1985) that could function as a 'buffer' between chromosome tips and single-copy DNA, are apparently absent from the RT deficiencies (Mason *et al.*, 1984). In our case the loss of 75 bp per generation observed in four different terminal deficiencies may thus reflect an imbalance of the two competing reactions, caused by the absence of crucial telomeric components.

The terminal DNA fragments on Southern blots always appear somewhat diffuse, as expected if they were associated with telomeric sequences of varying length. This explanation is not adequate, however, because we have been unable to establish the presence of such telomeric sequences on these terminal fragments. Two other sources of fragment-length heterogeneity are obvious. First, the existence of germ-line and somatic mosaics indicate heterogeneity within an individual. Second, the loss of sequences may not occur at the same rate in all individuals, suggesting heterogeneity within a line. The latter was minimized in the following ways. Vial stocks were used to keep the population size small, and were kept at 18°C to extend the length of a generation to ~6 weeks. When flies were collected for sampling, 5–10 males from a single stock vial were outcrossed to remove the y^2 *sc* Y, and the F1 females were frozen. Given these sources of fragment-length heterogeneity, we would expect the terminal fragment to show some diffusion on a Southern blot, but it is difficult at present to say how diffuse we would expect the terminal fragments to be. The 5–10 males taken from the stock vial to generate females for sampling are probably closely related, but not necessarily siblings. This raises the further possibility that two or more different terminal bands might appear on a Southern blot that reflect the positions of the terminus in two different sublines within the stock (see Figure 2).

The *y* gene is transcribed in a distal to proximal direction, and thus the terminal deficiencies have been very useful in analyzing its 5′ upstream regulatory region. Since all RT deficiencies described here have been selected on the basis of their *y* phenotype, it was to be expected that they affected the *y* gene in some way. We have shown here that deficiencies with breakpoints within the transcribed region of the *y* gene result in a y^1-type (null) phenotype, but that upstream breakpoints give rise to y^2-type mosaic phenotypes. No breakpoint located more than about 2.5 kb away from the start of transcription was found, suggesting that more distal breaks have no effect on *y* gene expression. However, when originally recovered in September 1983, *RT653*/y^1 females had black bristles but body cuticles intermediate in color between *y* and wild-type, especially on the abdomen and legs. Extrapolating from the position of the breakpoint in November 1984 at about −1.75 kb upstream of the *y* gene, it may have resided at −2.45 kb in September 1983. This is consistent with results from P-element mediated transformation experiments that showed complete rescue of the *y* phenotype with DNA fragments containing 2.8 kb of upstream sequence (Chia *et al.*, 1986).

It has been proposed that the phenotype of the original y^2 mutation (yellow body and wings, normally pigmented bristles), that is associated with the insertion of a gypsy element 700 bp upstream of the start of transcription (Biessmann, 1985; Parkhurst and Corces, 1985), may be caused by transcriptional interactions of the inserted element with the *y* gene promoter (Parkhurst and Corces, 1985, 1986; Geyer *et al.*, 1986). While this model may explain the mosaic pigmentation pattern of some y^2-type alleles, we offer an alternative view, based on our deficiency data. We postulate the existence of *cis*-acting control elements in the upstream region that specify spatial and/or temporal expression of the *y* gene promoter. Evidence for *cis*-acting tissue-specific elements have recently been discovered in the *Drosophila white* gene (Levis *et al.*, 1985; Pirrotta *et al.*, 1985; Davison *et al.*, 1985), in the yolk protein genes (Garbedian *et al.*,

1985) and in the *fushi tarazu* gene (Hiromi *et al.*, 1985). There are some 40 cuticle structures that show independent coloration, yet some correlation exists between the A-type (sex combs, body and wing bristles) and the B-type (general body cuticle) structures (Nash and Yarkin, 1974; Nash, 1976). Consistent with those observations, our results suggest that a regulatory element responsible for correct expression of the *y* gene in the epidermis of body and wings is located between −1.75 and −2.8 kb upstream of the start of transcription, and that a short 250 bp flanking region is sufficient for proper bristle pigmentation. These hypothetical *cis*-acting elements could either confer tissue-specificity or proper temporal expression. Deposition of black melanin occurs after 70 h of pupal development and proceeds from anterior to posterior for the next 20 h in a defined sequence (Nash, 1976). Thus, the *y* gene also has to be expressed at the correct time, which often precedes the visible pigmentation (Nash, 1976). Moreover, expression of the *y* gene has to be co-ordinated with the tyrosine metabolism that generates the substrates for melanin, and any disturbance in *y* gene timing could result in a mutant phenotype in those cells where *y* responds inappropriately. It is, therefore, conceivable that y^2-type pattern mosaics could be caused by mutations that either alter timing or tissue specificity of *y* gene expression.

Materials and methods

Recovery of RT deficiencies

Deficiencies were recovered from several different experiments between October 1980 and April 1984 in the course of the genetic analysis of the mutator *mu-2*. The basic mating scheme is described by Mason *et al.* (1984). In a variation of this approach, homozygous *mu-2* females were generated using an automatic virgin mating scheme that employs the dominant conditional lethal mutation *Killer of prune (K-pn)*. Females homozygous for the X-linked mutation *pn* and the third chromosome mutations *mu-2 st* were mated with males homozygous for the mutations *mu-2*, *ca* and *K-pn* on chromosome 3. Two types of male progeny are possible from such a mating. The regular males are hemizygous for the maternal X chromosome bearing *pn* and heterozygous for *K-pn*, and die during the larval period. Exceptional males resulting from X chromosome nondisjunction carry the paternal X chromosome and no Y chromosome, and are sterile. The regular daughters from this cross have the genotype *pn/+; mu-2 st/mu-2 ca K-pn*. They live because they are not phenotypically *pn*, and they are homozygous for the mutation *mu-2*. Exceptional daughters resulting from nondisjunction are homozygous for *pn* and die.

Homozygous *mu-2* females were collected every 24 h and transferred to vials with a heavy yeast paste. One to three days later they were given fresh yeast paste for 24 h, then irradiated with 500 roentgen of γ-rays or X-rays (except for one set of experiments described by Mason *et al.*, 1984, in which the dose of X-rays varied), and mated to *y w/*y^2 *sc* Y males. Mutations involving the *yellow (y)* gene were identified as *y*(y^{RT}/*y w*) females or y^2(y^{RT}/y^2*sc* Y) males. Two of the deficiencies (*RT276* and *RT653*), when originally recovered, complemented y^2, i.e. y^{RT}/y^2 *sc* Y males were phenotypically y^+. The *y* females were crossed to *y w/*y^2*sc* Y males and their y^2 w^+ sons were collected. The y^{RT} mutations were kept in stock at 18°C as y^{RT}/y^2 *sc* Y males crossed with *C(1)DX,y f/*y^2 *sc* Y females.

Collection of flies for analysis

For stocks analyzed after January 1984, y^{RT}/y^2 *sc* Y males were collected and mated with homozygous *Df(1) y ac* virgin females, where *Df(1) y ac* is a small, homozygous viable deficiency that uncovers the *y* and *ac* genes. Its breakpoints as determined by genomic Southern blot analysis, are between coordinates 43.4−42.5 kb (distal) and 30.3−28.3 kb (proximal), according to our map positions of the isolated *y ac* region (Biessmann, 1985). The entire *y* transcription unit resides between coordinates 36.7 and 32.1 kb. The y^{RT}*/Df(1) y ac* daughters were collected and frozen at −80°C.

DNA preparation and Southern blot hybridization

Genomic DNA was isolated from frozen females of y^{RT}*/Df(1)y ac* (November 1984 and later) or from both sexes of y^{RT}(y^2 *sc Y/C(1)DX, y f* (January, 1984) as described by Bingham *et al.* (1981). After incubation with the desired restriction enzymes, 4 μg of DNA per lane were electrophoresed on 1% agarose gels. After transfer to BA85 nitrocellulose sheets (Schleicher and Schull), hybridization was done at 42°C for 36 h with ^{32}P-nick-translated probes in 50% formamide, 5 × SSC, 50 mM Tris/HCl, pH 7.6, 10 × Denhardt's (0.2% each of Ficoll, polyvinyl pyrrolidone, and bovine serum albumin), 0.1% sodium dodecylsulfate, 2.5 mM EDTA, 0.1 mg/ml salmon sperm DNA. Filters were washed at 50°C in 0.1% SSC, 0.1% SDS for 1−2 h, dried, and exposed on Kodak X-Omat AR film with Quanta III intensifying screens (DuPont).

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Our mapping by receding terminal deficiencies of upstream regulatory elements of the *yellow* gene is consistent with recent results obtained by P-element transformations [Geyer,P.K. and Corces,V.G. (1987) *Genes and Development,* **1**, 996–1004].