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Tissue-specific transcriptional enhancers may act in *trans* on the gene located in the homologous chromosome: the molecular basis of transvection in *Drosophila*

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The y^2 mutation resulted from the insertion of the gypsy element into the X-linked *yellow* locus of *Drosophila melanogaster*. As a consequence of this insertion, transcriptional enhancers that control the expression of the *yellow* gene in the wings and body cuticle of adult flies are unable to act on the *yellow* promoter, resulting in a tissue-specific phenotype characterized by mutant coloration in these structures. Some *yellow* null alleles (y^n) are able to complement the y^2 phenotype giving rise to near wild type y^2/y^n females. The molecular structure of the *yellow* locus in complementing and non-complementing mutations was determined by cloning and sequencing the various alleles examined. From the information obtained in these studies, we propose a model suggesting that the complementing wild type phenotype of y^2/y^n flies might be due to the ability of functional wing and body cuticle transcriptional enhancers located in the y^n locus to act in *trans* on the promoter of the *yellow* gene found in the y^2-containing chromosome. Furthermore, this transactivation is abolished by the presence of an intact promoter in *cis*, suggesting that promoter competition between the *yellow* genes located on each homolog precludes the activation in *trans* by transcriptional enhancers in favour of *cis* effects on their own promoter.

Key words: Drosophila/enhancers/transcription/transposable elements/transvection

Introduction

The *yellow* gene of *Drosophila melanogaster* is involved in the establishment of proper pigmentation in various larval and adult cuticular structures. This gene encodes a 1.9 kb RNA whose expression is regulated in a developmental and tissue-specific fashion (Chia *et al.*, 1986; Geyer *et al.*, 1986; Parkhurst and Corces, 1986). Transcription of the *yellow* gene in the late embryonic-early larval developmental stages is responsible for the coloration of the larval denticle belts and mouth parts, whereas its expression in mid-pupae results in the normal pigmentation of adult cuticular structures such as wing blades, bristles and hairs, aristae, thoracic and abdominal cuticle, sex combs, and tarsal claws. This pattern of temporal and spatial expression of the *yellow* locus is controlled by tissue-specific transcriptional enhancers located in the 5′ region and in the intron of the gene. For example, a wing-specific enhancer element is located between −2873 and −1868 bp from the *yellow* transcription start site, and a second enhancer responsible for expression in the thoracic and abdominal cuticle is located between −1868 and −700 bp. In addition, sequences that control the transcription of the *yellow* gene in the adult bristles, and the larval denticle belts and mouth parts are located respectively in the intron and in the region between −225 and −91 bp (Geyer and Corces, 1987; Martin *et al.*, 1989).

In spite of the simple structure of this gene, some *yellow* null alleles produce a near wild type phenotype when compounded with y^2, while other *yellow* mutants fail to do so (Stone, 1935; Frye, 1960; Green, 1961). The existence of different sequences responsible for *yellow* gene expression in various tissues and developmental stages offers a paradigm to explain the paradox created by this pattern of interallelic complementation. The y^2 mutation is caused by the insertion of the gypsy element −700 bp from the transcription start site, separating those transcriptional enhancers that control *yellow* expression in the wing and body cuticle from the promoter, and resulting in flies that show normal coloration of larval structures and adult bristles and hairs, but mutant pigmentation of the wings and body cuticle of the adults (Geyer and Corces, 1987). This phenotype can be reversed by mutations at the unlinked *suppressor of Hairy-wing* [*su(Hw)*] gene, which encodes a protein structurally similar to some eukaryotic transcription factors and interacts with specific gypsy sequences to activate its transcription (Geyer *et al.*, 1988a; Parkhurst *et al.*, 1988; Spana *et al.*, 1988; Mazo *et al.*, 1989). The mutant phenotype of the y^2 allele arises as a consequence of the inability of the wing and body enhancers to interact with the *yellow* promoter when the *su(Hw)* protein is bound to the gypsy element (Harrison *et al.*, 1989).

Here we present results showing the structure of the *yellow* locus in complementing and non-complementing alleles. When these mutations are compounded with y^2, the resulting phenotype is respectively wild type or y^2. Given the structure of these mutants and the molecular basis of the y^2 phenotype, an explanation for the complementation pattern between *yellow* mutants is advanced based on the ability of transcriptional enhancers located on the chromosome containing the *y* null mutation to act on the intact promoter and transcription unit of the *yellow* gene in the y^2 allele contained in the homologous X chromosome.

Results

The molecular basis of complementation between y^2 and y^{59b}

The tissue-specific phenotype of the y^2 mutation can be complemented by the null allele y^{59b} to give rise to almost wild type y^2/y^{59b} females (Figure 1). To understand the molecular mechanisms underlying this process, we carried

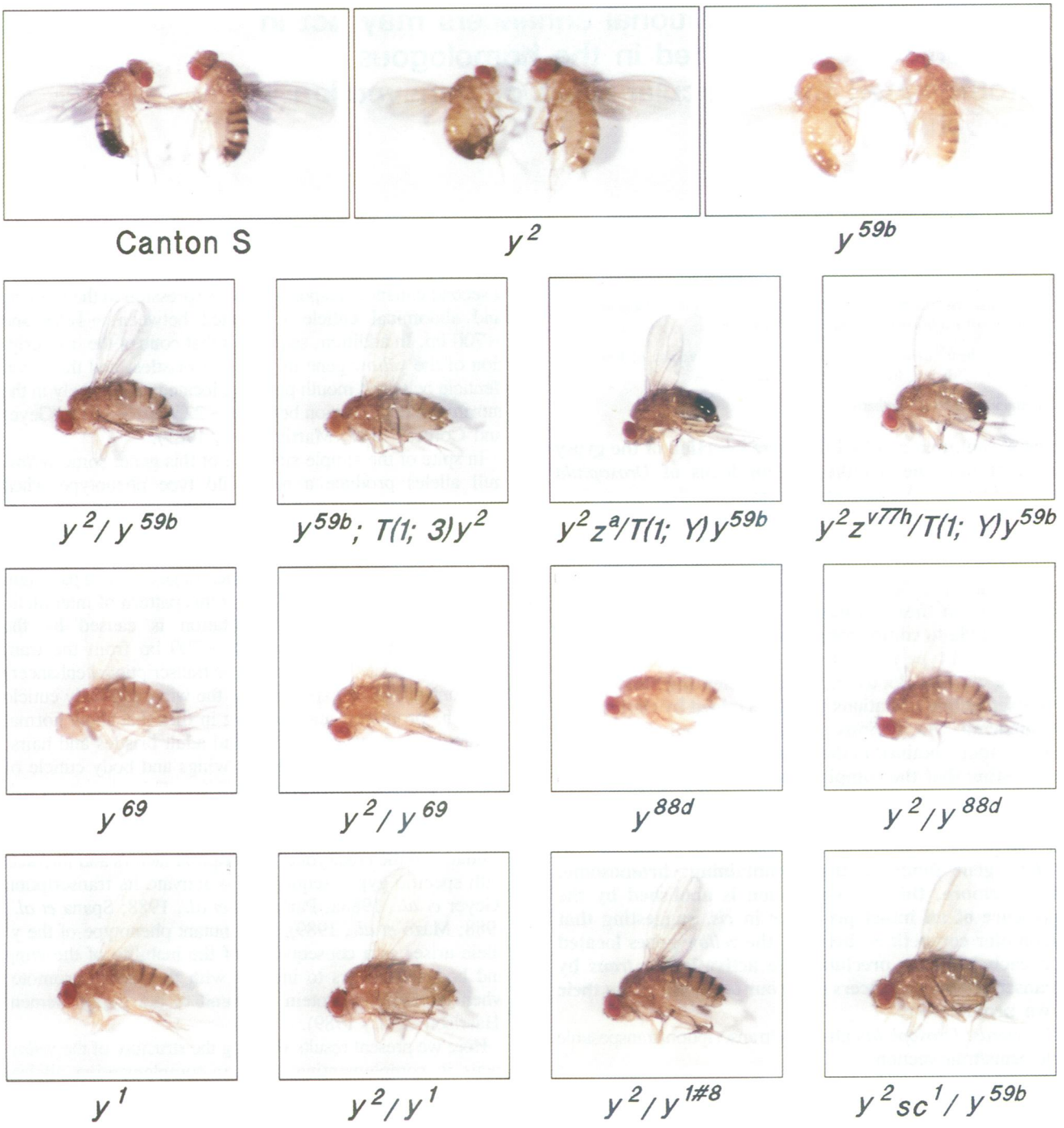

Fig. 1. Phenotypes of wild type and mutant stocks. The photographs depict the coloration of adult flies from Canton S, several *yellow* mutant stocks, and the progeny resulting from various crosses described in the text. The genotype of each fly is described under the corresponding photograph.

out a structural analysis of the *yellow* gene associated with the y^{59b} mutation. The y^{59b} mutant arose following X-irradiation in a stock carrying y^2. A λ library was constructed from genomic DNA of adult flies homozygous for y^{59b}, and the mutant *yellow* locus was isolated. Restriction and sequence analysis showed that the null phenotype of y^{59b} is the consequence of an internal deletion of both *yellow* and gypsy sequences present in the parental y^2 gene. The distal breakpoint of the deletion is in the internal portion of gypsy, at nucleotide 4230 (numbering of gypsy sequences is as described in Marlor *et al.*, 1986), whereas the proximal breakpoint is in the intron of the *yellow* gene, at nucleotide +519 (numbering of *yellow* sequences as described in Geyer *et al.*, 1986) (Figure 2A). As a consequence of this event, the gypsy 5′ LTR and the 12 copies of the octamer-like repeat that interact with the *su(Hw)* protein are deleted; in addition, *yellow* sequences located between −700 and +519, including the promoter and the first exon of the gene, are also missing (Figure 2B).

Further insight into the molecular mechanism of interallelic complementation between y^2 and y^{59b} was obtained by determining the structure and pattern of developmental

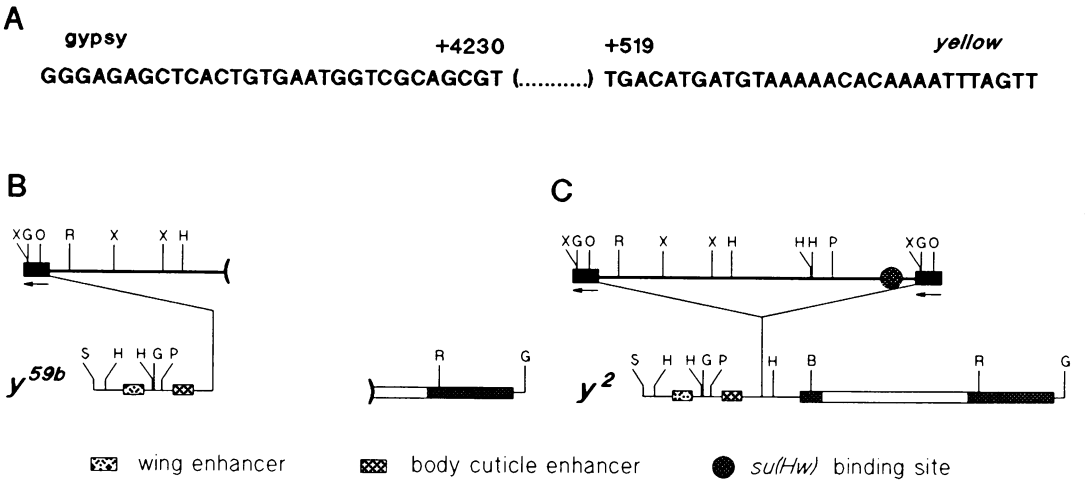

Fig. 2. DNA structure of the y^{59b} and y^2 alleles. **Panel A** shows the DNA sequence at the deletion breakpoint between gypsy and *yellow* sequences in the y^{59b} allele. **Panels B** and **C** show restriction maps of the y^{59b} and y^2 loci respectively. Cross-hatched boxes represent exons of the *yellow* gene whereas the empty box represents the intron; the location of the wing and body cuticle enhancers involved in *yellow* expression in these tissues of the adult fly is also indicated. Brackets represent deletion end-points in the y^{59b} allele. Gypsy sequences are shown inserted in the 5′ region of the *yellow* gene; filled-in boxes represent the LTRs of the element and the filled-in circle depicts bound *su(Hw)* protein. Symbols for restriction enzymes are as follows: B, *Bam*HI; G, *Bgl*II; H, *Hin*dIII; P, *Pst*I; O, *Xho*I; R, *Eco*RI; S, *Sal*I; X, *Xba*I. Transcription of gypsy is from right to left whereas *yellow* transcription takes place from left to right.

expression of the *yellow* RNA present in y^2/y^{59b} transheterozygotes. Figure 3 shows a Northern analysis of poly A^+ RNA obtained from these flies. Whereas *yellow* transcription is undetectable in pupae of a y^2 stock (Parkhurst and Corces, 1986) and is absent in y^{59b} (data not shown), y^2/y^{59b} transheterozygotes accumulate a substantial amount of mRNA in agreement with their almost wild type phenotype, although it is lower than in Canton S. The size of this RNA is 1.9 kb, suggesting that it is transcribed from the normal *yellow* promoter. In addition, its temporal expression is the same in y^2/y^{59b} as in wild type flies, with decreased accumulation in late pupal stages of development, indicating that the normal transcriptional enhancers present in the *yellow* locus must be responsible for this pattern of expression. Since the wing and body cuticle enhancers present in the y^2 gene are inactive, and the coding region of the y^{59b} gene is deleted, the pattern of *yellow* transcription in y^2/y^{59b} could be interpreted as the result of the activation of the y^2 promoter by enhancers located on the other chromosome in the y^{59b} locus. The rest of the results presented below will be discussed in the framework of this hypothesis.

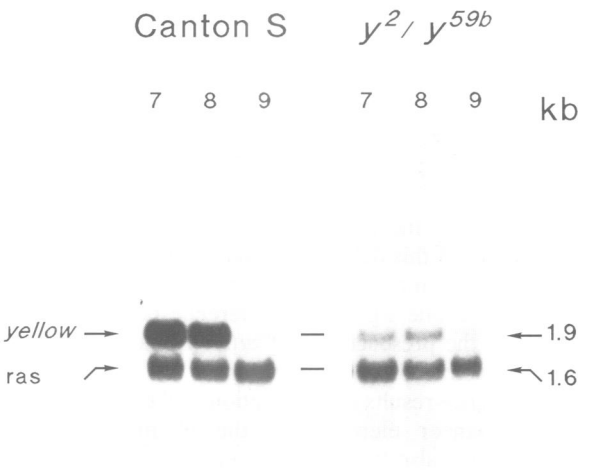

Fig. 3. Transcription analysis of y^2/y^{59b} transheterozygotes. Five micrograms of poly A^+ RNA from Canton S and y^2/y^{59b} flies were obtained from different pupal stages of development and electrophoresed on a 1% agarose−formaldehyde gel. The RNAs were then transferred to a nitrocellulose filter and simultaneously hybridized with ^{32}P-labeled DNAs containing the *Sal*I−*Eco*RI fragment of the *Drosophila yellow* gene (Geyer *et al.*, 1986) and a *Pst*I fragment of the *Dras2* gene (Mozer *et al.*, 1985). Numbers on top of each lane denote days of development at 22.5°C; days 7 and 8 correspond to mid-pupae stages and day 9 is the last day of pupal development at this temperature. Levels of *ras* transcript control for the amount of RNA loaded per lane.

Complementation by other y^2-derived alleles

In addition to y^{59b}, two other *yellow* alleles also derived from y^2, y^{69} and y^{88d}, were tested for their ability to complement the tissue-specific y^2 phenotype. y^{69} is a null allele that was induced by the mutator *mu-1* in a y^2 chromosome and is unable to complement this mutation, i.e. the phenotype of y^2/y^{69} females is the same as y^2 (Figure 1). On the other hand, a second spontaneous null allele y^{88d} partially complements y^2, such that the phenotype of y^2/y^{88d} is intermediate between wild type and y^2 flies (Figure 1).

Clones containing the *yellow* gene from the y^{69} and y^{88d} mutations were isolated from genomic λ libraries and their structure was determined by restriction mapping and sequence analysis. Figure 4A shows that y^{69} contains a deletion of DNA sequences corresponding to gypsy and the *yellow* gene. One of the breakpoints is located in the internal region of the gypsy element, at nucleotide 653, such that the 12 copies of the octamer-like repeat that interact with the *su(Hw)* protein are still present; the second breakpoint is located immediately 3′ to the *yellow* locus, although its exact position has not been determined, and it contains sequences corresponding to the jockey element. As a consequence of this deletion, both the promoter and the

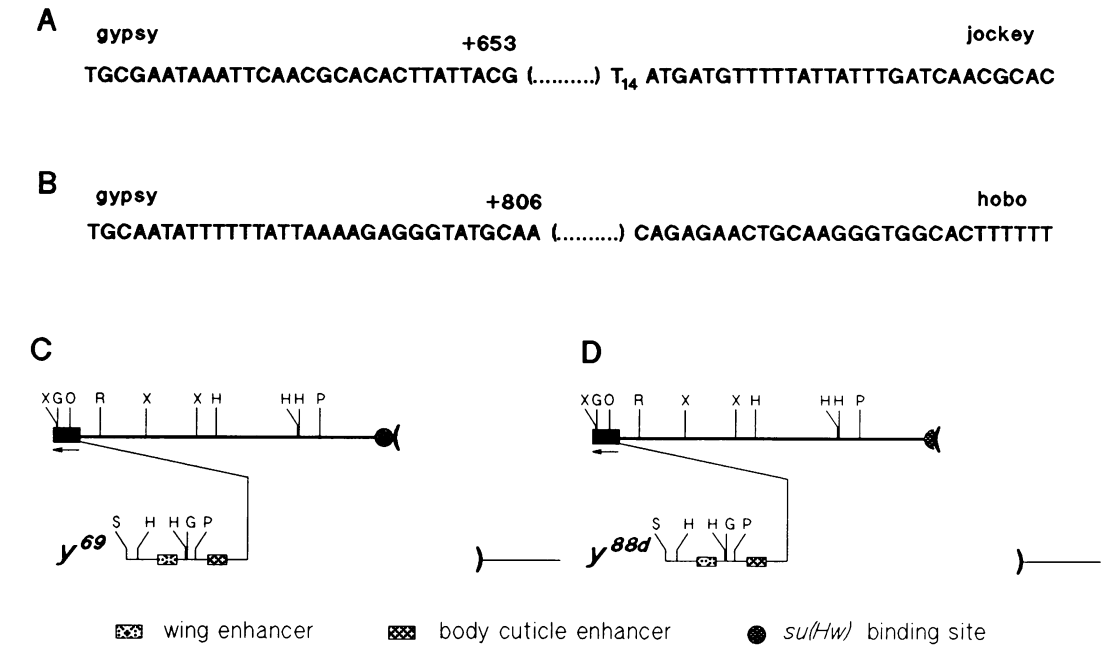

Fig. 4. DNA structure of the y^{69} and y^{88d} alleles. **Panel A** indicates the DNA sequence of the deletion breakpoint in the y^{69} locus; this deletion juxtaposes gypsy sequences with the 3′ region of a copy of the jockey element located 3′ to the *yellow* gene. **Panel B** shows sequences at the deletion breakpoint in the y^{88d} allele. In this case, the deletion event brings gypsy sequences adjacent to a copy of the hobo element located in the *achaete-scute* complex region. **Panels C** and **D** show restriction maps of the y^{69} and y^{88d} alleles respectively. Gypsy sequences are shown inserted in the 5′ region of the *yellow* gene. Brackets represent deletion end-points in the y^{69} and y^{88d} alleles; filled-in boxes represent the LTRs of the element and the filled-in circle depicts bound *su(Hw)* protein. Symbols for restriction enzymes are as follows: B, *Bam*HI; G, *Bgl*II; H, *Hin*dIII; P, *Pst*I; O, *Xho*I; R, *Eco*RI; S, *Sal*I; X, *Xba*I. Transcription of gypsy is from right to left whereas *yellow* transcription takes place from left to right in the figure.

coding region of the *yellow* gene are missing (Figure 4C). The structure of this deletion is quite similar to that of y^{59b}, but while y^{59b} complements the y^2 phenotype, y^{69} is unable to do so. The one significant difference between the two deletions is the presence of the *su(Hw)* binding site in the y^{69} chromosome. Since the binding of the *su(Hw)* protein to this sequence results in inactivation of the wing and body cuticle enhancer elements in the y^2 mutation, these enhancers may also be non-functional in y^{69} and therefore unable to act in *trans* on the y^2 promoter, producing the y^2 phenotype of y^2/y^{69} flies. An alternative possibility is that sequences deleted in the 3′ region of *yellow* in y^{69} are important for this interaction. Nevertheless, the deletion of 3′ sequences extends even further in y^{88d} but this mutant can still partially complement y^2 (see below), suggesting that sequences 3′ to the *yellow* gene are not involved in the complementation phenomenon.

The case of y^{88d} is interesting and further supports both ideas, the inactivation of upstream enhancer function by binding of *su(Hw)* product, and the ability of these enhancers to act in *trans*. The y^{88d} mutant partially complements y^2, giving rise to y^2/y^{88d} females that show pigmentation of the wing and body cuticle elevated relative to y^- and y^2 (Figure 1). The ability of y^{88d} to partially complement y^2 is more apparent in y^{88d} males which carry a transposition of the 1A-2B region including y^2 to the Y chromosome (data not shown); this transposition does not interfere with normal complementation (see below). The y^{88d} null mutation arose spontaneously from a partial revertant of y^2 (called y^{PR2}) that in turn had resulted from the insertion of the hobo element into the *su(Hw)* binding domain of the gypsy element. This insertion took place at nucleotide 805, such that 5 of the octamer-like repeats are present between the gypsy 5′ LTR and the insertion point of the element (Geyer *et al.*, 1988a). Cloning and sequence analysis of the *yellow* locus in y^{88d} indicate that this mutation arose by homologous recombination between the copy of hobo located in gypsy and a second copy located in the *achaete-scute* complex, resulting in deletion of DNA sequences located between both copies of hobo. One of the breakpoints of the deletion is at nucleotide 806 of gypsy, leaving 7 copies of the octamer repeat (Figure 4B,D). It is known that deletion of some of these repeats results in a partial reversion of the phenotype of gypsy-induced *bithoraxoid* and *yellow* mutations (Peifer and Bender, 1988; P.A.Smith and V.G.Corces, unpublished), suggesting that the presence of a partial binding site decreases the affinity of the *su(Hw)* protein for these sequences, leading to only incomplete inactivation of the upstream enhancers. These results can be interpreted in the light of the hypothesis we have developed above to explain the complementation phenomenon. The partially active enhancers from the y^{88d} chromosome may then be able to act in *trans* on the y^2 gene, resulting in only partial rescue of the mutant phenotype.

The molecular structure of other non-complementing alleles

Three additional *yellow* mutations y^1, y^{76d28}, and $y^{1\#7}$ were tested for their ability to complement y^2. The y^1 and $y^{1\#7}$ alleles have the same null *yellow* phenotype in which all larval and adult tissues are mutant (Figure 1), whereas

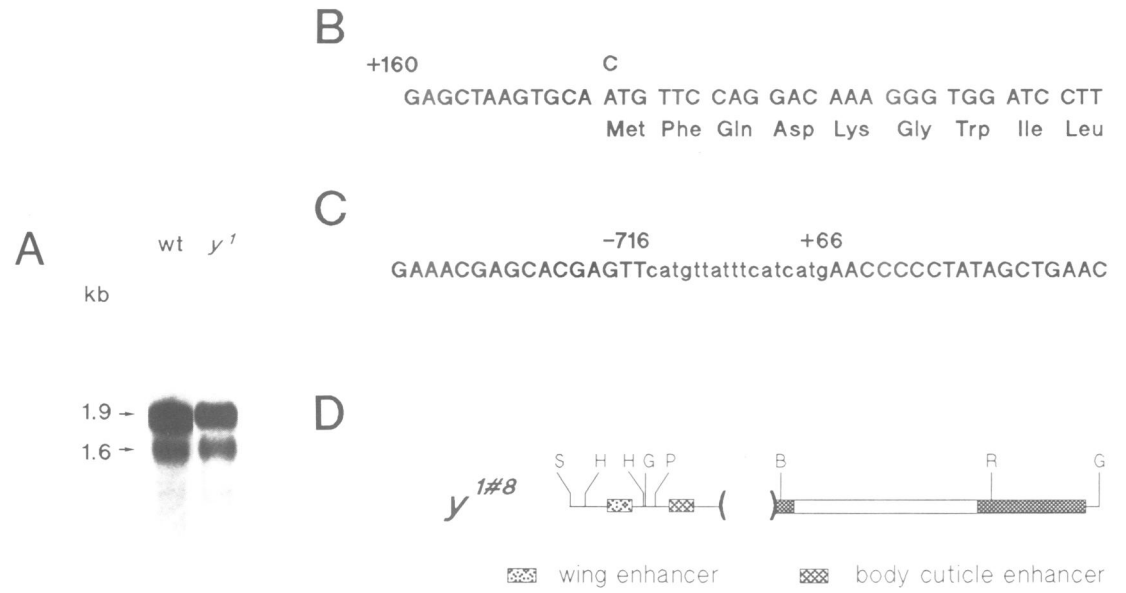

Fig. 5. Structure and expression of mutant null *yellow* alleles. **Panel A** shows a Northern analysis of Canton S (wt) and y^1 RNA. Five micrograms of poly A^+ RNA obtained from pupal stages of development were electrophoresed on an agarose–formaldehyde gel, blotted onto a nylon membrane, and probed with ^{32}P-labeled DNA fragments containing the *yellow* and *Dras2* genes. The *yellow* gene gives rise to a 1.9 kb transcript, whereas *ras2* hybridizes to a 1.6 kb RNA that serves as a control for the amount of RNA loaded on each lane (Mozer *et al.*, 1985). **Panel B** shows the DNA sequence of the *yellow* gene around the ATG protein initiation codon. The y^1 mutation is a consequence of an A to C transversion in the ATG initiation codon, indicated on top of the wild type sequences. **Panel C** shows the DNA sequence around the deletion breakpoint that gives rise to the $y^{1\#8}$ mutation. Capital letters indicate *yellow* sequences, whereas lower case letters denote sequences of the P element inserted in the *yellow* gene of the parental stock from which $y^{1\#8}$ arose. **Panel D** represents a restriction map of the *yellow* locus in the $y^{1\#8}$ mutant. Cross-hatched boxes represent exons of the *yellow* gene whereas the empty box represents the intron; the location of the wing and body cuticle enhancers involved in *yellow* expression in these tissues of the adult fly is also indicated. Brackets represent deletion end-points. Symbols for restriction enzymes are as follows: B, *Bam*HI; G, *Bgl*II; H, *Hin*dIII; P, *Pst*I; R, *Eco*RI; S, *Sal*I. Transcription of the *yellow* gene takes place from left to right.

y^{76d28} has a tan phenotype characterized by a slight coloration in all larval and adult pigmented structures (Geyer *et al.*, 1988b). All three alleles were tested for their ability to complement y^2 by examining the phenotype of females carrying y^2 in one X chromosome and one of these alleles in the other homolog. In all three cases, the transheterozygous females displayed a y^2 phenotype identical to that of y^2/y^1 shown in Figure 1, indicating that none of these alleles could complement y^2.

To understand the basis for this lack of complementation, we determined the structure of the *yellow* locus in these three mutations. Southern analysis of genomic DNA failed to reveal any abnormalities in the restriction pattern of the y^1 gene (data not shown), and Northern analysis of poly A^+ RNA indicates that y^1 flies produce a wild type sized RNA that is expressed at normal levels and at the appropriate times of development (Figure 5A). In order to understand the molecular basis for the y^1 phenotype, DNA corresponding to this mutation was cloned using the polymerase chain reaction technique and sequenced. The only difference found between wild type and y^1 is an A to C transversion in the ATG initiation codon (Figure 5B). In the event that a mutant protein could be made initiating at the next downstream ATG, it would lack the signal peptide necessary for membrane targeting and export of the *yellow* protein (Geyer and Corces, 1987), producing a non-functional product and a corresponding null phenotype. Nevertheless, the promoter and transcriptional enhancers that control *yellow* expression in the wings and body cuticle are intact and functional, since this mutation accumulates normal levels of RNA during the pupal stages of development.

The molecular structure of the y^{76d28}, and $y^{1\#7}$ alleles has been previously reported (Geyer *et al.*, 1988b) and is discussed here only for the sake of comparison with y^1. Both mutations are caused by the insertion of a P element at +76 bp in the transcribed untranslated region of the *yellow* gene, but the orientation of the P element in y^{76d28} is opposite to that in $y^{1\#7}$. As a consequence of this insertion, both mutants accumulate an RNA that includes the P element, but whereas y^{76d28} is able to splice out P element sequences with low efficiency, $y^{1\#7}$ is unable to do so (Geyer *et al.*, 1988b). Therefore, the structural defect associated with these two non-complementing alleles has something in common with that responsible for the y^1 phenotype, i.e. in all three alleles the promoter and enhancer sequences responsible for wing and body cuticle *yellow* expression are functional, and the mutations affect processes downstream from the transcription initiation step.

Mutations that affect the structure or function of the yellow promoter can complement y^2

Evidence suggesting that the lack of a functional promoter is a prerequisite for interallelic complementation was obtained from the molecular analysis of *yellow* alleles associated with various types of defects affecting the promoter region of the *yellow* gene. The $y^{1\#8}$ mutation arose from the imprecise excision of a P element inserted in the *yellow* promoter and it has a null phenotype identical to that shown for y^1 in Figure 1; sequence analysis of the *yellow* locus in this mutation shows a deletion of DNA sequences located between −716 and +66 including the TATA box (Figure 5C,D). Phenotypic analysis of $y^2/y^{1\#8}$

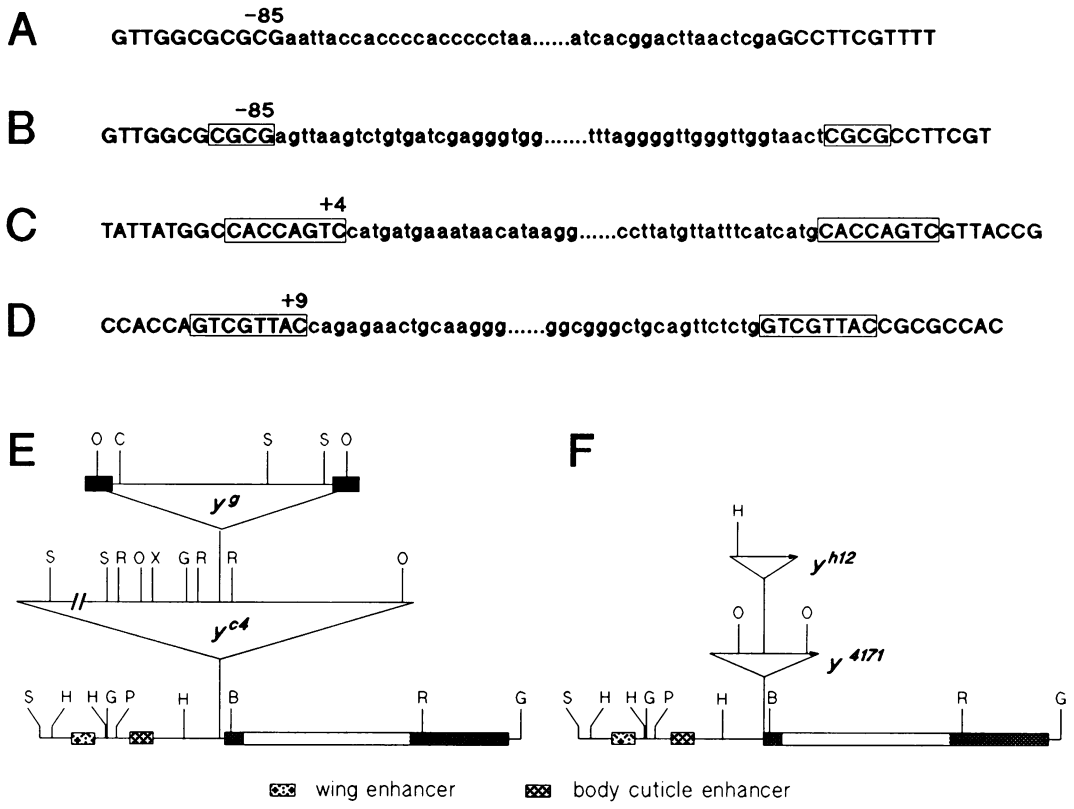

Fig. 6. Structure of *yellow* mutant alleles. Numbers on top of each sequence denote the last base of the *yellow* gene before the insertion of foreign DNA. **Panel A** shows the DNA structure of the region where the insertion of undefined DNA sequences results in the y^{c4} mutation; capital letters indicate *yellow* DNA, whereas lower case letters indicate insertion sequences. **Panels B, C,** and **D** show the structure of the insertion point of the pregun, P, and hobo elements in the y^{g},y^{h12} and y^{4171} mutations respectively. Following the same convention as above, inserted sequences are indicated by lower case letters, *yellow* sequence is in capitals, and nucleotides repeated upon insertion are boxed. **Panels E** and **F** show restriction maps for these four alleles. Cross-hatched boxes represent exons of the *yellow* gene whereas the empty box represents the intron; the location of the wing and body cuticle enhancers involved in *yellow* expression in these tissues of the adult fly is also indicated. Symbols for restriction enzymes are as follows: B, *Bam*HI; C, *Cla*I; G, *Bgl*II; H, *Hin*dIII; P, *Pst*I; R, *Eco*RI; S, *Sal*I; X, *Xba*I. Transcription of the *yellow* gene takes place from left to right, and transcription of the P and hobo elements is indicated by arrows.

females indicates that $y^{1\#8}$ can complement the y^{2} phenotype to give wild type transheterozygotes (Figure 1). One obvious difference that sets apart the complementing $y^{1\#8}$ mutation from the non-complementing y^{1} is the lack of a functional promoter in the former. This difference could be rationalized into the working hypothesis we have used to explain the structure and patterns of complementation between various *yellow* alleles. In reference to this framework, one could suggest that the wing and body cuticle enhancers might act preferentially on the *yellow* promoter located in *cis*, allowing *trans* effects only when this promoter is non-functional or missing as is the case of $y^{1\#8}$.

Additional evidence supporting this hypothesis was obtained by analyzing spontaneous mutations caused by the insertion of various transposable elements into the *yellow* gene. The alleles y^{h12}, and y^{4171} are mutations in which all tissues at all developmental stages show null pigmentation (see the y^{1} phenotype in Figure 1), whereas y^{c4} and y^{g} show a tan phenotype, similar to that of y^{76d28}, in which all tissues have a slightly darker coloration. These four alleles were tested for their ability to complement y^{2} by examining the phenotype of transheterozygous females. In all cases these females had an almost wild type phenotype indistinguishable from that of y^{2}/y^{59b} shown in Figure 1, indicating that all four mutations are able to complement y^{2}. In order to understand the basis of this complementation, we cloned and sequenced the *yellow* locus from these four alleles. y^{c4} is associated with the insertion into the promoter at position −85 bp of DNA sequences not yet characterized (Figure 6A,E), whereas y^{g} is caused by the insertion, also at −85 bp, of a new LTR-containing retrotransposon we have named pregun (the Russian word for jumper) (Figure 6B,E). The other two complementing alleles, y^{h12} and y^{4171}, result from the insertion of a P element at +4 bp (Figure 6C,F) and a hobo element at +9 bp (Figures 6D,F) respectively. In agreement with the null or tan phenotypes of these mutations, the 1.9 kb *yellow* RNA is absent or present at low levels in flies carrying each of these alleles (Figure 7).

The complementation between y^{2} and these different alleles can be rationalized on the basis of the molecular defects associated with them. The insertion of DNA sequences within the promoter of the *yellow* gene in y^{c4},y^{g},y^{h12} and y^{4171} might interfere with the ability of TATA binding factors and/or RNA polymerase to interact with the promoter causing a mutant phenotype that affects gene expression in every tissue at all stages of development. Therefore, the low levels or lack of RNA in these mutants may result from a decrease in the rate of transcription initiation and thus, the four alleles that complement y^{2}

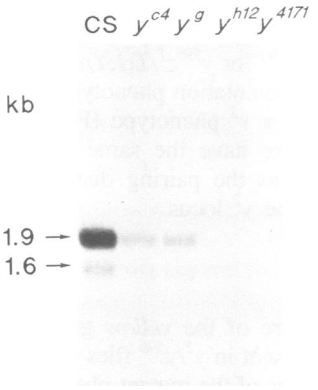

Fig. 7. RNA Northern analysis of wild type and *yellow* mutants. Two micrograms of poly A$^+$ RNA from mid pupal developmental stages was obtained from Canton S (CS), y^{c4},y^{g},y^{h12} and y^{4171} stocks, electrophoresed on an agarose–formaldehyde gel, and blotted onto nitrocellulose. The filter was then probed with ^{32}P-labeled DNA containing copies of the *yellow* and *Dras2* genes. The *yellow* locus gives rise to a 1.9 kb RNA, whereas *Dras2* encodes a 1.6 kb transcript that serves as control for the amount of RNA loaded in each lane (Mozer *et al.*, 1985).

contain defects that influence the function of the *yellow* promoter. This is specially obvious in the y^{c4} and y^{g} alleles, since the insertion that causes the mutation took place upstream from the TATA box. Therefore, $y^{1\#8}$,y^{c4},y^{g},y^{h12} and y^{4171} whose promoter is missing or not functional, are able to complement y^{2}, while y^{1},$y^{1\#7}$, and y^{76d28}, whose TATA box is functional, are unable to do so. This suggests that the wing and body cuticle enhancers might act preferentially on the *yellow* promoter located in *cis*, and that the *cis* action prevents *trans* effects.

The gypsy element might be involved in the interallelic complementation at yellow

In all *yellow* mutants tested so far, at least one allele of each pair-wise complementing combination contains a full or deleted copy of the gypsy element. The question then arises as to whether gypsy mediates the interallelic complementation, and whether the presence of this mobile element in at least one in each complementing gene pair is required for this phenomenon.

The involvement of gypsy in interallelic complementation at *yellow* was tested by examining the effect of a gypsy insertion at the *scute* locus on the complementation between y^{2} and y^{59b}. Surprisingly, although y^{2}/y^{59b} females are wild type in phenotype, the presence of the sc^{1} mutation in $y^{2}sc^{1}/y^{59b}$ flies results in a y^{2} phenotype (Figure 1). These results suggest that the existence of a copy of gypsy in the *scute* locus, approximately 70 kb from the gypsy present in y^{2} (Campuzano *et al.*, 1985), interferes with complementation, probably due to gypsy–gypsy interactions that disrupt synapsis at the *yellow* locus. The existence of these interactions is supported by recombination events previously observed between the y^{2} and sc^{1} loci (Geyer *et al.*, 1988c). To test the putative role of gypsy in this phenomenon and rule out the possibility that other genetic factors in the $y^{2}sc^{1}$ strain were responsible for the abnormal pattern of complementation, we analyzed the ability of a sc^{+} revertant, obtained from the $y^{2}sc^{1}$ stock, to complement y^{59b}. This $y^{2}sc^{+}$ revertant arose spontaneously and should be isogenic with the parental $y^{2}sc^{1}$ except for the changes that have taken place at the *sc* locus. DNA from the *sc* region in the revertant strain was isolated and sequenced. This analysis indicated that revertant flies have lost the gypsy element located at the *sc* locus by recombination between the LTRs (data not shown). When compounded with y^{59b}, the $y^{2}sc^{+}$ revertant gave rise to $y^{2}sc^{+}/y^{59b}$ females phenotypically identical to y^{2}/y^{59b} shown in Figure 1. This result indicates that the presence of gypsy at the *sc* locus was responsible for the inability of the parental $y^{2}sc^{1}$ stock to complement y^{59b}. A similar effect of the gypsy element on interallelic complementation has been also noted at the *bithorax* complex. For example, the presence of a gypsy-induced bxd^{1} allele on the Ubx^{1} chromosome enhances the *bx* phenotype of bx^{3}/Ubx^{1} flies (Lewis, 1955); similar suppression of complementation is observed when a $Cbx^{1}Ubx^{1}$ chromosome is paired with a *bx bxd*-containing chromosome, in which *bx* and *bxd* are both gypsy insertions (Babu *et al.*, 1987).

Is complementation at the yellow locus dependent on chromosome pairing?

The complementation of the tissue-specific y^{2} phenotype by the null allele y^{59b} to give rise to almost wild type flies that we have examined takes place in females when each of the mutant alleles is located at its normal position on the X chromosome (Figure 1). To determine whether the alignment of the two genes that results from chromosome pairing of the two X homologs is important for the interallelic complementation we tested whether the disruption of pairing affects this phenomenon. Our first approach to test synapsis effects involved the targeting of the y^{2} locus to a chromosomal location other than that normally occupied by the *yellow* gene at the tip of the X chromosome in subdivision 1B. To this end, a 15.3 kb DNA fragment encompassing the entire *yellow* locus from the y^{2} allele, including the gypsy element and all DNA sequences required for proper *yellow* gene expression (Geyer and Corces, 1987), was inserted into the transformation vector Carnegie 20. The resulting plasmid was injected into preblastoderm embryos of the genotype $y^{-}ac^{-}$; ry^{506} which contain a deletion of the *yellow* and *achaete* loci and therefore exhibit a null *yellow* phenotype. One ry^{+} transformant was obtained and this transformant also showed the characteristic y^{2} phenotype (see Figure 1); genetic analysis established that the transposed y^{2} was linked to the third chromosome. Additional insertions of y^{2} were generated by transposing the original transformant with a third chromosome MR element which regulates P element mobility (Hiraizumi, 1971; Green, 1977, 1986). Six transpositions to an X chromosome containing y^{59b} were recovered, as well as two transpositions to the second chromosome and two to the homologous third chromosome, among a total 4523 progeny scored. In each case, the transposed y^{2} locus exhibits the same y^{2} phenotype described above, indicating that the different chromosomal environments of the various insertions have no repercussion on the transcription of the *yellow* gene or the mutagenic effect of the gypsy element. Interestingly, seven independent reversions of the transformed y^{2} to y^{+} were also recovered during these experiments. Genomic Southern analysis revealed that the molecular basis of reversion in five of seven independent strains studied resulted from excision of the gypsy element leaving behind a single intact LTR (data not

shown). The y^+ revertants rescue a *y* deletion producing a wild type phenotype, substantiating that all the sequences necessary for the expression of the *yellow* gene are included within the transformed DNA.

The translocated y^2 gene was then tested for its ability to complement y^{59b} by examining the phenotype of y^{59b}; *T(1;X or A)*y^2 males, where A represents an autosome. This type of analysis indicated that males carrying y^{59b} at the *yellow* locus and y^2 elsewhere in the X, second, or third chromosomes, are y^2 in phenotype (Figure 1). The outcome of these experiments suggests that the complementation between y^2 and y^{59b} is pairing-dependent, since it is only observed when both are located at their normal chromosomal position.

Additional circumstantial evidence suggesting that chromosome pairing is involved in the complementation phenomenon at *yellow* was obtained from the analysis of the phenotype of y^-/y^{59b}; *T(1;Y)*y^2 females, where y^- represents a deletion of the 1A-1B region of the X chromosome and *T(1;Y)*y^2 is a translocation of the 1A-2B interval to the Y chromosome. This translocation does not interfere with chromosome pairing as judged by analysis of polytene chromosomes from y^{59b}; *T(1;Y)*y^2 males in which homologous regions of the X and Y chromosomes can be seen paired (M.Yamamoto, personal communication). In agreement with the pairing ability of the two *yellow* loci, y^{59b}; *T(1;Y)*y^2 males show a complementary almost wild type phenotype. If chromosome pairing is a requisite for complementation, it could be predicted that the pairing of the y^{59b} locus to its homolog in y^{59b}/y^{59b}; *T(1;Y)*y^2 females would interfere with its pairing to the translocated y^2 gene. This was found to be indeed the case when the phenotype of y^{59b}/y^{59b}; *T(1;Y)*y^2 females was examined; to the contrary of y^{59b}; *T(1;Y)*y^2 males, these females do not show a complementary phenotype rather they display the light coloration of y^2. The inability of y^{59b} to complement the translocated y^2 in y^{59b}/y^{59b}; *T(1;Y)*y^2 females can be restored by deleting the tip of one of the X homologs up to the location of the *achaete* locus. The phenotype of y^-/y^{59b}; *T(1;Y)*y^2 females is almost wild type, suggesting that y^{59b} is again able to pair with *T(1;Y)*y^2 and give a complementary phenotype. These results, taken together with the conclusions from the analysis of the interactions between y^{59b} and the transformed y^2 locus, suggest that chromosomal pairing may be necessary for interallelic complementation at *yellow*.

This synapsis-dependent complementation at the *yellow* locus is reminiscent of the transvection phenomenon first described by Lewis in the *bithorax* complex (Lewis, 1954). In *bithorax* as well as in *white* and *decapentaplegic*, the phenotypes of certain allelic combinations are affected by rearrangements that disrupt chromosome pairing and by the allelic state of the *zeste* (*z*) locus. In general, hypomorphic alleles of the *zeste* gene represented by mutants such as z^a, have the same effect as chromosomal rearrangements, and reverse the partial complementation between selected alleles at all three loci (Kaufman *et al.*, 1973; Jack and Judd, 1979; Gelbart and Wu, 1982). In order to test whether this is also true for the complementation between y^2 and y^{59b}, we studied the effect of three different *zeste* alleles, z^1,z^a, and z^{v77h}, on the coloration of $y^2/$*T(1;Y)*y^{59b} males by examining the phenotype of the various genotypic combinations. These three *zeste* mutations have varied effects on the pattern of complementation between *white, bithorax* and *decapentaplegic* alleles (Gelbart and Wu, 1982) but only z^{v77h} had an effect on the interallelic complementation at *yellow*. Thus males y^2z^1*(T(1;Y)*y^{59b} [or $y^{59b}z^1$*/T(1;Y)*y^2] and y^2z^a*/T(1;(Y)*y^{59b} manifest a complementation phenotype, but males y^2v^{v77h}/ *T(1;Y)*y^{59b} exhibit a y^2 phenotype (Figure 1). Some *zeste* mutations therefore have the same effect on interallelic complementation as the pairing disruption induced by a transposition of the y^2 locus.

Discussion

Given the structure of the *yellow* gene in each of the X chromosomes present in y^2/y^{59b} flies and our knowledge of the molecular basis of the mutant phenotype in each allele, an explanation can be advanced to account for the resulting wild type phenotype. The *yellow* gene in the y^2 mutation is intact but interrupted by the gypsy element; only the coloration of the wings and the thoracic and abdominal cuticle are mutant in this allele due to the inability of the transcriptional enhancers controlling *yellow* expression in these tissues to act on the *yellow* promoter. This inability is in turn a consequence of the binding of the *su(Hw)* protein to specific gypsy sequences, namely the 12 copies of the octamer-like repeat. On the other hand, both the promoter and the coding region of the *yellow* gene are missing in the case of y^{59b}, thereby creating a null phenotype. But also missing are the gypsy sequences that interact with *su(Hw)*, and thus the two upstream enhancers affected in y^2 should be functional in y^{59b}. In spite of the functional and structural defects associated with y^2 and y^{59b}, transheterozygous females containing one copy of each of these genes produce a full length 1.9 kb transcript, suggesting that this RNA must originate from the intact promoter and coding region of the y^2 gene. In addition, expression of this RNA is turned off at the end of pupal development, suggesting that the expression of this transcript is under the control of the wing and body cuticle enhancers responsible for this pattern of expression. The level of RNA accumulation in y^2/y^{59b} is lower than the wild type; this is not unexpected since at most only one *yellow* gene could be active in these flies. In order to explain these results we propose that the enhancers from the y^{59b} chromosome might be able to act in *trans* on the promoter of the y^2 gene located in the homologous chromosome.

This hypothesis explains the pattern of complementation between y^2 and the y^{69} and y^{88d} mutations. The y^{69} allele differs from y^{59b} in that the gypsy element present in y^{69} still contains the sequences that interact with *su(Hw)*. Since the binding of this protein results in inactivation of the wing and body cuticle enhancers, these may now be incapable of acting in *trans* on the coding region of y^2 and produce a y^2 phenotype in y^2/y^{69} flies. The case of y^{88d} presents a situation intermediate between y^{59b} and y^{69}; because only half of the *su(Hw)* binding site is present in this allele, the affinity of this protein for gypsy DNA is lower than in the case of y^2, leading to only incomplete inactivation of the upstream enhancers. These partially active enhancers from the y^{88d} chromosome may then be able to act in *trans* on the y^2 promoter resulting in only partial rescue of the mutant phenotype as determined by the intermediate coloration of y^2/y^{88d} flies.

Alternative hypotheses to explain the interallelic complementation are also possible. For example, the *su(Hw)* bound

to the gypsy element in y^2 could interact with transcription factors present in the y^{59b} chromosome and thus release its effect on the y^2 enhancers that could then act on their own promoter to activate *yellow* transcription. This possibility would still have the same implications, i.e. that transcription factors, in this case *su(Hw)*, bound to one chromosome can exert an effect on proteins present in the other homolog. Nevertheless, the first explanation is supported by recent findings indicating that an enhancer can stimulate transcription from a promoter *in vitro* when both are linked via a protein bridge (Müller *et al.*, 1989). A similar ability of enhancer-like elements located in the *white-spotted* region to act in *trans* on the promoter of a different *white* allele has been implicated in the transvection phenomena at the white locus (Davison *et al.*, 1985; Zachar *et al.*, 1985).

The ability of transcriptional enhancers to act in *trans* would presumably require close proximity between both copies of the gene. This is in principle possible in *Drosophila* since this organism, as well as other dipterans, displays pairing between chromosomes in interphase somatic nuclei (Lifschytz and Hareven, 1982). In addition, a wealth of genetic data has accumulated that indicates the existence of synapsis-dependent interallelic complementation in several *Drosophila* loci, most notably *bithorax* (Lewis, 1954; Kaufman *et al.*, 1973; Babu *et al.*, 1987), *white* (Green, 1959; Babu and Bhat, 1980), and *decapentaplegic* (Gelbart, 1982). In all three cases, the ability of two alleles to complement each other's phenotype to give almost wild type transheterozygotes is abolished by chromosomal rearrangements that disrupt synapsis of homologous genes, suggesting that pairing between the two copies of the gene is necessary for the complementation. The results presented here suggest that both properties of transvection, synapsis-dependence and *zeste* effects, are also exhibited by the interallelic complementation at the *yellow* locus. The classical approach to study the effect of pairing on the complementation pattern at various loci has been to induce chromosomal translocations or other rearrangements using X-rays. This type of strategy is more difficult to implement with the *yellow* locus. Because of its location at the tip of the X chromosome, the target size for distal breakpoints is very small, and insertional translocations of this gene are therefore difficult to obtain. Efforts to make this type of rearrangements have failed; a few insertional translocations that resulted in male sterility were obtained, but they were lost before they could be tested for complementation. Only reciprocal translocations in which the distal end of another chromosome is exchanged for the tip of the X chromosome have been readily isolated (M.M.Green, unpublished results). Reciprocal translocations such as $T(1:Y)y^2$ do not disrupt synapsis between *yellow* alleles due to the close proximity of the *yellow* locus to telomeres that tend to favor pairing, and thus they have no influence on the complementation pattern. To circumvent this problem we have used P element-mediated germline transformation as a method to obtain *Drosophila* strains in which the mutant y^2 locus has been inserted into different chromosomal locations. The transformed y^2 gene is phenotypically indistinguishable from the original one. The only test in which its behavior is different is its failure to complement a copy of the y^{59b} mutation located in the normal site of the X chromosome. This inability to complement y^{59b} probably reflects the disruption of pairing between the two *yellow* alleles due to the new chromosomal location of the transformed copy of y^2. Although these results do not unequivocally demonstrate that interallelic complementation at *yellow* is the same as the previously described transvection phenomena, and further genetic experiments are necessary to prove this point, the fact that this complementation is also affected by mutations at the *zeste* locus adds to the circumstantial evidence suggesting that this may be the case. Taken together, these results suggest that pairing might be a prerequisite for interallelic complementation at *yellow*, and support the possibility that enhancers may be able to act in *trans*.

The presence of functional enhancers in one of the complementing alleles is necessary but it is not sufficient for *yellow* interallelic complementation to take place. A second prerequisite is the lack of a functional promoter in the mutation complementing y^2, suggesting that the wing and body cuticle enhancers will preferentially act on the promoter located in *cis*. This conclusion is supported by the different behavior exhibited during complementation tests by mutations such as y^1, $y^{1\#7}$, and y^{76d28} which are unable to complement y^2 and are caused by defects that influence gene expression at the post-transcriptional level, whereas complementing alleles such as $y^{1\#8}$,y^{c4},y^{8},y^{h12} and y^{4171} display promoter defects that influence the initiation of transcription. Only when the *cis* promoter is missing or functionally inactive will the enhancers act on the promoter located in *trans* to give a complementing phenotype. A last requirement for interallelic complementation at *yellow*, may be the presence of gypsy sequences in at least one of the complementing alleles; these sequences may be involved in mediating pairing effects.

The ability of transcriptional enhancers to act in *trans* on a promoter located in a tightly synapsed chromosome might shed some light on the mechanisms by which enhancers affect gene expression and could afford discrimination among various models of enhancer action currently under consideration (see Atchison, 1988 for a recent review). The *trans* effects described above may be difficult to accommodate with models that base enhancer action on changes in torsional stress induced as a consequence of alterations in the superhelical conformation of the DNA (Ryoji and Worcel, 1984; Hann *et al.*, 1985), since supercoiling induced in one DNA molecule cannot be transferred to the DNA in a chromosome that is physically linked but topologically separated (Plon and Wang, 1986). Both gypsy-induced mutagenesis and interallelic complementation can be better explained by models that regard enhancers as binding sites for transcription factors that can then interact with RNA polymerase or other promoter-bound proteins either by tracking along the DNA until they encounter the promoter (Wasylyk *et al.*, 1983; Courey *et al.*, 1986) or through looping of the intervening DNA sequences (Dunn *et al.*, 1984; Ptashne, 1986). Tissue-specific factors bound to the wing and body cuticle enhancers in y^2 probably interact with the gypsy-attached *su(Hw)* protein, preempting their effect on the *yellow* promoter. Indeed, the *su(Hw)* product contains acidic domains similar to those present in other factors which have been implicated in protein–protein interactions (Ma and Ptashne, 1987; Struhl, 1987; Hope *et al.*, 1988; Ptashne, 1988). In our model, we suggest that the negative interactions of the *su(Hw)* protein are not operative in *trans* and do not affect transcription factors bound to the enhancers in a complementing allele such as y^{59b}. These factors could track the DNA until in close proximity with the *yellow* promoter in y^2 and then

interact with RNA polymerase or other proteins present in this promoter. Alternatively, DNA sequences located downstream of the enhancers in y^{59b} could loop out and serve as an arm to bring bound transcription factors in the vicinity of the y^2 promoter. These interactions might be mediated through binding to gypsy sequences by the product of the *zeste* locus which is itself a transcription factor (Biggin *et al.*, 1988). Further studies now in progress on the structure of other transvecting alleles and the protein factors involved in this phenomenon will give insights into the molecular mechanisms of enhancer effects on gene expression.

Materials and methods

Procedures

Flies were reared at 22.5°C and 75% humidity. The stocks y^{4171} and y^{c4} were obtained from Dr Dawson Mohler; y^{8} was obtained from Dr K.G.Gazaryan, and the origin of the mutations $y^{1\#8}$, $y^{1\#7}$, y^{76d28} and y^{h12} has been previously reported (Geyer *et al.*, 1988b). Construction of DNA libraries and other routine molecular biology techniques such as digestions with restriction enzymes, DNA labeling, etc., were carried out as described in Maniatis *et al.* (1982). RNA isolation and Northern analysis were done as previously reported (Geyer *et al.*, 1988b). P element-mediated transformation and vectors utilized were as reported by Rubin and Spradling, 1982, 1983. DNA sequencing was carried out by the chemical (Maxam and Gilbert, 1980) and chain termination (Sanger *et al.*, 1977) procedures, using sequenase (United States Biochemical, Cleveland) in the latter.

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