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Two modes of transvection: Enhancer action in *trans* and bypass of a chromatin insulator in *cis*

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ABSTRACT **Ed Lewis introduced the term "transvection" in 1954 to describe mechanisms that can cause the expression of a gene to be sensitive to the proximity of its homologue. Transvection since has been reported at an increasing number of loci in *Drosophila*, where homologous chromosomes are paired in somatic tissues, as well as at loci in other organisms. At the *Drosophila yellow* gene, transvection can explain intragenic complementation involving the *yellow*^{2} allele (y^2). Here, transvection was proposed to occur by enhancers of one allele acting in *trans* on the promoter of a paired homologue. In this report, we describe two *yellow* alleles that strengthen this model and reveal an unexpected, second mechanism for transvection. Data suggest that, in addition to enhancer action in *trans*, transvection can occur by enhancer bypass of a chromatin insulator in *cis*. We propose that bypass results from the topology of paired genes. Finally, transvection at *yellow* can occur in genotypes not involving y^2, implying that it is a feature of *yellow* itself and not an attribute of one particular allele.**

Studies in a wide variety of organisms have shown that the structure and function of a segment of DNA can be profoundly affected by the presence of homologous sequences (1–13). The impact of homologous sequences can be dramatic, ranging from changes in DNA sequence and methylation to changes in chromatin structure and global chromatin architecture. In many instances, these changes are considered epigenetic. We are interested in understanding homologue interactions and epigenetic forms of regulation. Our approach has been to investigate transvection, a process that can cause genes to be sensitive to the proximity of a homologue (1, 8, 14). Transvection was first defined in *Drosophila*, where somatic homologue pairing brings homologous sequences into close proximity (1). Our studies have focused on the *yellow* gene of *Drosophila*.

At the *yellow* gene, transvection is the basis for numerous cases of intragenic complementation (15). The *yellow* gene is required for cuticle pigmentation (16). The *yellow*^{2} allele (y^2) reduces pigmentation in the wing and body but does not affect pigmentation of other tissues. It is caused by the insertion of a *gypsy* retrotransposon between the wing and body enhancers and the promoter (ref. 17; Fig. 1*A*). Gene expression is disrupted because *gypsy* is a target for the suppressor of Hairy wing [su(Hw)] protein, which, when bound, establishes a chromatin insulator that prevents the wing and body enhancers from activating transcription (18–20). Several *yellow* alleles complement y^2 (15, 21–24). One case of complementation is illustrated in Fig. 2*A*. $y^{1\#8}$ is a null allele and causes fully mutant yellow pigmentation of the wing, body, and other cuticular structures. It is a deletion removing the promoter and some transcribed sequences (ref. 15; Fig. 1*B*). Of interest, although neither y^2 nor $y^{1\#8}$ is expressed significantly in wing or body, $y^2/y^{1\#8}$ flies show nearly wild-type pigmentation in these tissues. This type of intragenic complementation at *yellow* was shown to depend on allelic pairing, and, as such, transvection was implicated as the mechanistic basis. Of importance, the paired state of y^2 is not sufficient, on its own, to induce transvection. For example, pairing of y^2 with the y^1 allele, caused by an A to C transition in the translation initiation codon, does not result in complementation. The observation that complementation of y^2 is achieved only with a specific subset of alleles indicates that complementing alleles provide input in addition to that of the paired state.

We are interested in the molecular mechanisms of transvection. One explanation for the complementation of y^2 and $y^{1\#8}$ is that, when these two alleles are paired, the wing and body enhancers of $y^{1\#8}$ act in *trans* on the promoter of y^2 (ref. 15; Fig. 2*A*, double-lined arrow). Such a model is consistent with studies pointing to the ability of regulatory elements to act in *trans* in other contexts (refs. 1, 25–29, and, most recently, refs. 30–41).

Although the model of *trans*-acting enhancers at *yellow* is attractive, it has not been proven. It is formally possible that, because the wing and body enhancers of y^2 are intact, $y^2/y^{1\#8}$ complementation actually results from the release of the blocked enhancers of y^2 to act in *cis* (Fig. 2*A*, solid arrow). In fact, as all well documented cases of complementation at *yellow* have involved y^2, no complementing genotype of *yellow* has demonstrated unambiguously enhancer action in *trans*. In this report, we address this issue by using two *yellow* alleles, y^{82f29} and y^{3c3}, which were identified in a genetic analysis of *yellow* transvection (J.R.M., J.-l.C., S. T. Filandrinos, R. C. Dunn, R. Fisk, P.K.G., and C.-t.W., unpublished work). These two alleles permitted us to test and confirm the model of *trans*-acting enhancers at *yellow* and also led to the suggestion of a second mechanism of transvection.

MATERIALS AND METHODS

***Drosophila* Stocks.** The y^2, $y^{1\#8}$, y^{82f29}, and y^{3c3} alleles are described in the text. The X chromosome bearing y^{3c3} also is marked with an allele of *echinus*. Females hemizygous for a *yellow* allele were generated by placing the *yellow* allele in *trans* to $Df(1)y^- ac^- w^{1118}$, a deficiency that removes the entire *yellow* gene (42). The phenotypes of the *echinus* allele, ac^-, and w^{1118}, described in ref. 16, are not relevant to this study and, therefore, these mutations will not be discussed further.

Culture Condition. Flies were cultured at 25 ± 1°C on standard *Drosophila* cornmeal, yeast, sugar, and agar medium

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with *p*-hydroxybenzoic acid methyl ester as a mold inhibitor. In general, three females were mated with three or more males in vials and were brooded daily. Temperature and crowding were controlled carefully because both affect pigmentation.

Scoring of Pigmentation. Pigmentation was scored in 1–3-day-old flies on a scale of 1 to 5. According to this scale, 1 represents the null or nearly null state, and 5 represents the wild-type or nearly wild-type state. The null phenotype is defined by the pigmentation seen in flies that are homozygous or hemizygous for y^1 or $Df(1)y^- ac^- w^{1118}$, and the wild-type phenotype is defined by the pigmentation seen in our wild-type Canton S strain. Body pigmentation refers to pigmentation in the abdominal stripes. At least two independent crosses were set up for each genotype, and at least 100 females were scored from each cross. Pigmentation scores were determined independently by at least two people.

Analysis of y^{82f29}. One phage was isolated from a genomic library constructed in the Lambda DASH II vector (Stratagene) and screened with *yellow* genomic sequences using standard techniques (43). DNA corresponding to the entire phage insert was subcloned as a *Not*I fragment into Bluescript (Stratagene). Restriction analyses indicated that this fragment contained 4.5 kbp of DNA upstream of the y^{82f29} deletion breakpoint, the *yellow* transcription unit, and 2.6 kbp of DNA 3′ to the poly(A) addition signal. Restriction analyses of phage DNA by using *Hin*dIII, *Pst*I, and *Bgl*II, which cut within *gypsy*, and Southern analysis (43) with a complete *gypsy* probe, revealed no *gypsy* sequences within the cloned region [hybridized in 5× standard saline citrate (SSC), 50% formamide, 5× Denhardt's solution, 50 mM sodium phosphate (pH 6.8), and 40 μg/ml calf thymus DNA at 42°C for 14 hours and washed in 2× SSC, 0.1% sodium pyrophosphate, and 0.1% SDS). A 1.2-kbp *Hin*dIII fragment containing the y^{82f29} breakpoint was subcloned and sequenced by using the primer 5′TTTCGATTGGGCGTCAC, which begins at −749. This produced ≈400 bp of sequence extending 5′ of the breakpoint. The corresponding wild-type region was cloned from a phage containing y^{3c3} genomic DNA and was sequenced. Sequence comparison demonstrated a clean deletion in y^{82f29}.

Analysis of y^{3c3}. Southern analysis (43) of y^{3c3} indicated this allele to be associated with an intragenic deletion. The deletion was confirmed by PCR amplification and sequencing of a 578-bp fragment spanning the breakpoints. The sequences of the primers were 5′ATGGATCC*TGCAGCGATCGCATCATTAG, where the C* corresponds to position −1629, and 5′GTAGGATCC*GAGTGAGACTGCAACGACCA, where the C* corresponds to position +2533. The 5′ end of both primers contains a short run of nucleotides that is not homologous to *yellow* sequence.

Plasmid Construction. The status of the body enhancer in y^{3c3} was determined by the analysis of transgenes, each of which contained an internal deletion of the *yellow* gene. Three constructs were made. P[SalΔ534] and P[SalΔ961] had internal deletions of 534 and 961 bp, respectively. P[5′BglΔ961] differed from P[SalΔ961] by the addition of 3.3 kbp of 5′ sequence.

The internal deletion in P[SalΔ534] was created by digesting pUC8 containing *yellow* in a 7.7-kbp *Sal*I fragment (42) with *Dra*III and *Eco*47III. The 5′ breakpoint of y^{3c3} was re-created by ligating the digestion product in the presence of the double-stranded oligonucleotide 5′GTGTTTGTTTATTTTTTCTG3′ 3′TGGCACAAACAAATAAAAAAGAC5′. The resulting plasmid was digested with *Sal*I to remove the modified *yellow* sequences, and these *yellow* sequences then were cloned into the *Sal*I site of pBSX, a modified Bluescript vector in which the *Asp*718 site was replaced with an *Xba*I site. This plasmid then was digested with *Xba*I, and the *yellow* sequences were cloned into the *Xba*I site of pCaSper3 in the reverse orientation relative to *white*.

The deletion in P[SalΔ961] was created by digesting pUC8 containing the 5′ end of the *yellow* gene in a 3.1-kbp *Sal*I/*Bam*HI fragment with *Dra*III and *Nsi*I. The 5′ breakpoint of y^{3c3} was re-created by ligating the digestion product in the presence of the double-stranded oligonucleotide 5′GTGTTTGTTTATTTTTTCTGATGCA3′ 3′TGGCACAAACAAATAAAAAAGACT5′. The resulting plasmid was digested with *Sal*I and *Bam*HI to remove the modified *yellow* sequences, which then were cloned into the *Sal*I and *Bam*HI sites of pBSXyBG. pBSXyBG is a derivative of pBSX that contains the 3′ end of the *yellow* gene in a 4.6-kbp *Bam*HI/*Bgl*II fragment inserted into the *Bam*HI site. Therefore, the insertion of the modified *yellow* gene into pBSXyBG generated an internally deleted *yellow* gene. The *yellow* gene then was cloned into pCaSper3 as described above.

P[5′BglΔ961] differs from P[SalΔ961] by an additional 5′ sequence. P[SalΔ961] was digested with *Xho*I and *Bgl*II and was religated by using the double-stranded oligonucleotide 5′TCGAGATGCTACGCATGACA 3′CTACGATGCGTACTGTCTAG to remove 1 kbp of the 5′ sequence and restore the *Bgl*II site. The resulting plasmid was digested with *Bgl*II, and a 4.3-kbp *Bgl*II fragment containing 3.3 kbp of sequence upstream of the *Sal*I site was inserted in the wild-type orientation. Deletion breakpoints of all constructs were confirmed by sequencing.

Germ-Line Transformation. *P*-element mediated germ-line transformation (44) used 0.5 mg/ml construct and 0.1 mg/ml "wings-clipped" helper DNA. The host genotype was $Df(1)y^- ac^- w^{1118}$.

RESULTS AND DISCUSSION

The goal of our studies was to understand enhancer action in transvection. We began our studies by asking whether *yellow* enhancers have the ability to act in *trans*. We addressed this issue by using the y^{82f29} allele. y^{82f29} flies show a tissue-specific alteration in pigmentation similar to that of y^2 flies, with mutant pigmentation in wing and body. On a scale of 1 to 5, where 1 represents the null or nearly null state and 5 represents the wild-type or nearly wild-type state, both alleles, when homozygous or hemizygous, give scores of 1 in wing and 1 to 1.5 in body (Table 1). We determined the structure of y^{82f29} by constructing a genomic library from y^{82f29} DNA and isolating the *yellow* gene. Restriction and sequence analysis demonstrated that y^{82f29} is caused by a 4.1-kbp deletion removing the wing enhancer and much of the region to which the body enhancer had been mapped (refs. 42 and 45; Fig. 1*C*). This structure, combined with the mutant wing and body phenotype of y^{82f29}, indicates that y^{82f29} can provide very little, if any, wing

Table 1. Pigmentation scores for control (homozygous and hemizygous) and complementing genotypes

Genotypes*	Pigmentation wing, body
Control	
y^2/y^2	1, 1.5
y^2/Df	1, 1
y^{82f29}/y^{82f29}	1, 1-1.5
y^{82f29}/Df	1, 1
$y^{1\#8}/y^{1\#8}$	1, 1
$y^{1\#8}/Df$	1, 1
y^{3c3}/y^{3c3}	1, 1
y^{3c3}/Df	1, 1
Complementing	
$y^2/y^{1\#8}$	4, 4
$y^{82f29}/y^{1\#8}$	3, 3
y^2/y^{3c3}	4, 4
y^{82f29}/y^{3c3}	3, 1

*Df is used here to mean $Df(1)y^-ac^-w^{1118}$.

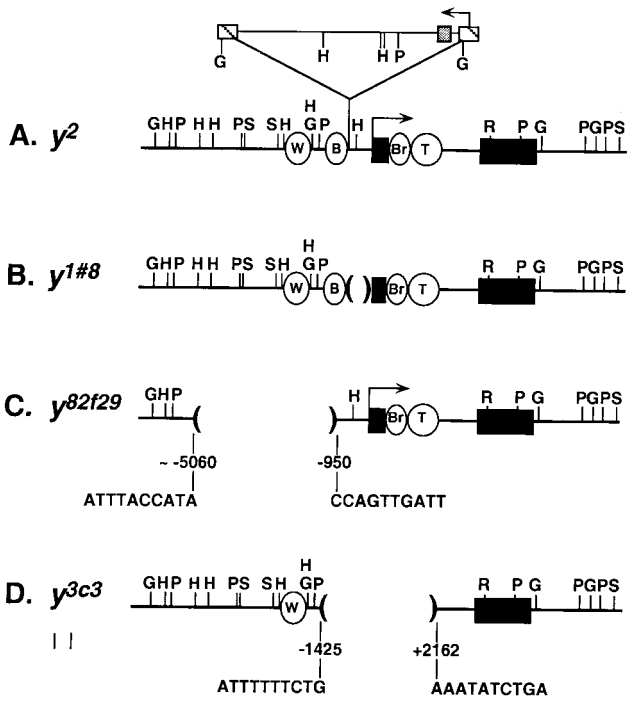

FIG. 1. *yellow* alleles. y^2 is caused by a *gypsy* insertion (raised triangle) at −700. $y^{1\#8}$, y^{82f29}, and y^{3c3} are deletions of 0.8, 4.1, and 3.6 kbp, respectively. $y^{1\#8}$ has 17 bp of *P*-element sequence at its breakpoints (15). Extent of tarsal claw enhancer remaining in y^{3c3} is unknown. Arrows, promoters; W, wing enhancer, −2873 to −1868; B, body enhancer, −1425 to −700; Br, bristle enhancer; T, tarsal claw enhancer; +1, transcription start; black rectangles, exons; striped square, long terminal repeats; gray square, su(Hw) binding region; G, *Bgl*II; H, *Hin*dIII; P, *Pst*I; S, *Sal*I; R, *Eco*RI.

and body enhancer activity. We tested the ability of y^{82f29} to participate in intragenic complementation by placing it in *trans* to $y^{1\#8}$. As mentioned above, $y^{1\#8}$ is a null allele. It produces pigmentation scores of 1 in wing and body tissue when homozygous or hemizygous (Table 1). Of significance, y^{82f29} complements $y^{1\#8}$; flies of the genotype $y^{82f29}/y^{1\#8}$ produce pigmentation scores of 3 in both wing and body (Table 1). Although the complementation is not as strong as that seen in $y^2/y^{1\#8}$ flies, which produce scores of 4, wing and body pigmentation are notably greater than that seen in the parental y^{82f29} and $y^{1\#8}$ lines. We conclude that complementation arises from the wing and body enhancers of $y^{1\#8}$ acting in *trans* on the promoter of y^{82f29} because y^{82f29} cannot contribute the complementing levels of wing or body enhancer activity (Fig. 2*B*). These data demonstrate that transvection at *yellow* can occur by the action of enhancers in *trans*.

Our analysis of y^{82f29} resolved another issue regarding transvection at *yellow*. All well documented cases of *yellow* transvection have involved y^2, raising the possibility that y^2 and/or *gypsy* is required. The complementing $y^{82f29}/y^{1\#8}$ genotype demonstrates that transvection at *yellow* does not require y^2 and therefore does not depend on any particular allele. Furthermore, sequence analysis of the breakpoints, followed by restriction and Southern analyses, revealed no *gypsy* sequences within the cloned y^{82f29} region (Fig. 1*C*). Therefore, transvection at *yellow* also does not require a *gypsy* element in either participating allele, a finding that is in line with observations at other loci (46, 47). We conclude that transvection at *yellow* is a feature of the locus and not an attribute of an extraordinary allele.

Although our studies of y^{82f29} demonstrate that enhancers are capable of acting in *trans* at *yellow*, they leave unresolved the question of whether the wing and body enhancers of y^2 also

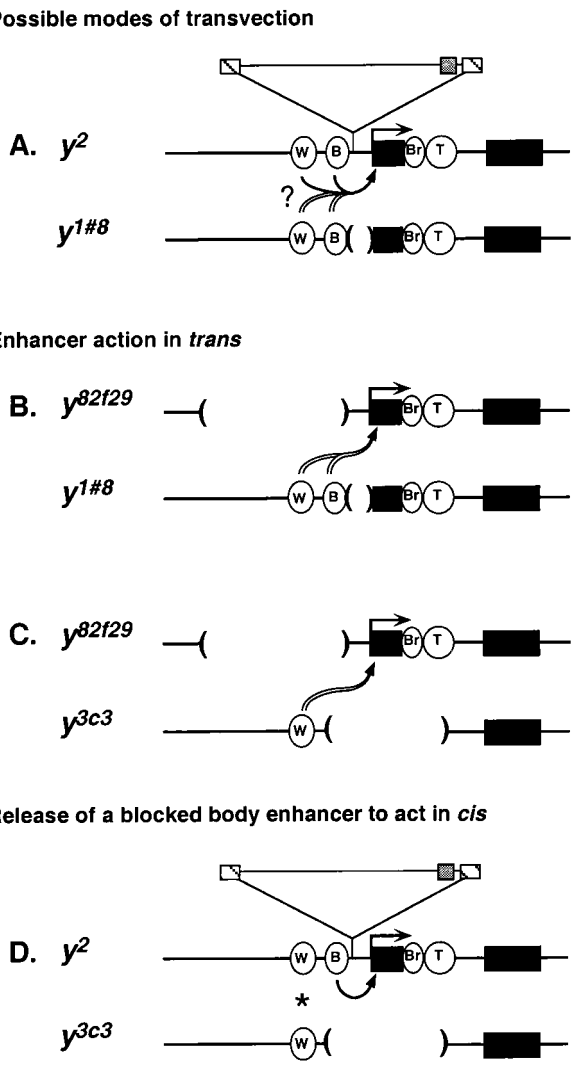

FIG. 2. Models. The *yellow* alleles are drawn only approximately to scale. Symbols are used as in Fig. 1. (*A*) Transvection involving *trans*-acting $y^{1\#8}$ enhancers and/or *cis*-acting y^2 enhancers. (*B*) Transvection involving *trans* action of the $y^{1\#8}$ wing and body enhancers. (*C*) Transvection involving *trans* action of the y^{3c3} wing enhancer. (*D*) The y^2 body enhancer bypassing the insulator and acting on the y^2 promoter. Wing complementation (*) may involve the y^{3c3} wing enhancer acting in *trans* and/or the y^2 wing enhancer acting in *cis*.

can participate in intragenic complementation. Our analysis of y^{3c3} addressed this issue and led us to propose a second mechanism for transvection. The y^{3c3} allele is a null; when homozygous or hemizygous, it results in fully mutant pigmentation of wing, body, and other cuticular structures (Table 1). Molecular analysis showed that y^{3c3} is a 3.6-kbp deletion that removes promoter, 5′ regulatory, and transcribed sequences (Fig. 1*D*). Like the $y^{1\#8}$ allele, y^{3c3} complements y^2, producing scores of 4 in wing and body (Table 1). For this reason, we expected y^{3c3} to carry intact wing and body enhancers. The coordinates of the breakpoints confirmed the presence of the wing enhancer. However, the 5′ deletion breakpoint was within the region to which the body enhancer had been mapped in earlier studies (42, 45), making it unclear whether y^{3c3} carries the body enhancer.

To determine whether y^{3c3} has a body enhancer, we carried out germ-line transformation studies with three deletion constructs of *yellow* (Fig. 3). Each construct carried an internal deletion whose 5′ breakpoint was identical to that of y^{3c3} and whose 3′ breakpoint was upstream of the promoter. Two of the constructs, P[SalΔ534] and P[SalΔ961], carried the same

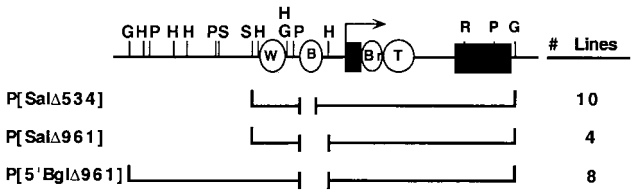

FIG. 3. Transgenic lines. Wild-type *yellow* gene and constructs are depicted by using symbols as shown in Fig. 1.

amount of 5′ and 3′ flanking DNA that was shown previously by germ-line transformation to be sufficient to produce wild-type levels of pigmentation in all tissues (42, 45). The third construct, P[5′BglΔ961], differed from the other two by an additional 3.3 kbp of 5′ genomic sequence, placing the 5′ endpoint of P[5′BglΔ961] upstream of the 5′ deletion breakpoint present in y^{82f29}. As y^{82f29} produces a null or nearly null phenotype in the wing and body, its deletion breakpoints are a good indicator of the boundaries of wing and body enhancer function. Therefore, our constructs should reveal whether y^{3c3} retains any body enhancer function.

The three constructs were used separately to transform a line lacking the endogenous *yellow* gene. We obtained 22 independent transgenic lines. All except one showed wild-type pigmentation in the wing, indicating that the promoter and wing enhancer were functional. The exceptional line showed reduced wing pigmentation, most likely reflecting a repressive position effect. Of importance, all lines had a low level of body pigmentation, corresponding to scores of 1 to 2, which was considerably less than that seen in wild-type or complementing y^2/y^{3c3} flies. From these data, we conclude that body enhancer function is disrupted severely in y^{3c3}. Therefore, it is unlikely that complementation in the body of y^2/y^{3c3} flies results from a body enhancer of y^{3c3} acting in *trans* on the y^2 promoter.

The simplest interpretation of our data is that body pigmentation of y^2/y^{3c3} flies arises from the body enhancer of y^2 bypassing the su(Hw) chromatin insulator to act on its own promoter in *cis* (Fig. 2*D*). Bypass of the su(Hw) chromatin insulator is surprising considering that this insulator provides a versatile and general block of enhancer-activated transcription (18–20). In fact, current models for insulator function do not predict that the blocked body enhancer can bypass the insulator in the absence of secondary changes in y^2 (48–50) or the factors that generate the insulator, such as the su(Hw) protein (18–20). A potential explanation for bypass is suggested by the structure of y^{3c3} and rests on what we call a pairing-mediated topology effect (TOPE). In this view, bypass results from conformational changes in the gene caused by homologue pairing. The ability of structures arising from homologue pairing to influence gene expression was predicted in 1935 by H. J. Muller (ref. 51; cited by ref. 52) and since has been considered by others (14, 31, 36, 39, 41, 50, 52–62). The body complementation in y^2/y^{3c3} flies advances this concept by providing a compelling example in which the structure of paired alleles can explain the induction of gene expression.

A hypothetical paired structure for the y^2/y^{3c3} genotype is shown in Fig. 4*A*. In this model, the promoter and body enhancer of y^2 are unpaired and looped out because y^{3c3} lacks these elements. A correlation between the unpaired state and increased accessibility to regulatory factors has been proposed as a contributing factor in transvection-related phenotypes elsewhere (14, 57, 59). In the case of y^2/y^{3c3}, it may be that the unpaired state of genetic elements and their presence in a looped structure make them unusually accessible. For example, increased accessibility of the promoter may make it responsive to enhancers that normally are blocked by the insulator or, in an extreme case, cause it to be constitutive. Recently, the unpaired looped state also has been proposed to augment accessibility and facilitate transvection at the *Abdominal-B* gene (41). Alternatively, inclusion of the promoter and body enhancer of y^2 within the same loop may lead to the apposition and interaction of these two elements. Finally, the insulator may be compromised by constraints imposed by the looped structure. This interpretation is in line with proposals that binding of su(Hw) protein to *gypsy* is sensitive to DNA topology (63) and that insulator function entails an increase in DNA flexibility (64). Although our models focus on the promoter, enhancer, and insulator elements and on loop formation, they are also compatible with pairing exerting its effects on other genetic elements or via other topologies, such as the structural alterations of chromatin and DNA that accompany the transcriptionally active state (65). All of these possibilities share the common theme that the structure of paired homologues plays an important role in regulating transvection and gene expression.

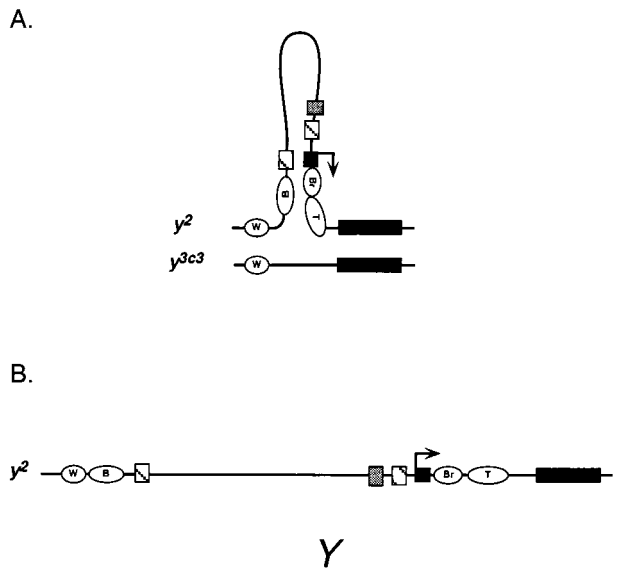

FIG. 4. Model for insulator bypass. The *yellow* alleles are drawn only approximately to scale. Symbols are used as in Fig. 1. (*A*) Homologue pairing in y^2/y^{3c3} flies generates an unpaired loop in regions of heterogeneity between the alleles. This topology allows for bypass of the su(Hw) chromatin insulator by the y^2 body enhancer to act on its own promoter in *cis*. (*B*) The mutant phenotype of y^2/Y males and hemizygous females (not shown) demonstrates that simple unpairing of *yellow* sequences is not sufficient to activate the y^2 promoter.

Although the most conspicuous aspect of our model is the unpaired state of the y^2 promoter and body enhancer, we emphasize that simple unpairing of elements is not sufficient to explain y^2 expression in body tissue. Evidence comes from females that carry y^2 in *trans* to a deficiency of the entire *yellow* gene and from y^2/Y males that are hemizygous for *yellow*, which is present on the X chromosome (Fig. 4*B*). In both genotypes, the body enhancer and promoter of y^2 are unpaired, yet the unpaired state has no effect on the y^2 phenotype.

In short, y^{3c3} plays a key role in inducing y^2 transcription, and we suggest that it acts by promoting bypass of the insulator when it is paired with y^2. One model for bypass, discussed above, proposes that it results from the topology of paired alleles. We also are considering alternative explanations. For example, y^{3c3} may potentiate y^2 transcription by altering local concentrations of transcription factors. One possibility is that the lack of a body enhancer and promoter in y^{3c3} causes an increase in the local concentration of transcription factors in the vicinity of the y^2 body enhancer. This change may strengthen the y^2 body enhancer, making it more difficult to be blocked by the insulator (66). Of importance, regulated transcription in this case still would call for insulator bypass. Alternatively, y^{3c3} may retain some body enhancer activity. If

so, our transgene studies indicate that the level of body pigmentation it directs is significantly below that seen in complementing y^2/y^{3c3} flies. Therefore, should residual body enhancer activity of y^{3c3} be responsible for complementation, we would need to postulate that it is induced by pairing with y^2 to act more strongly in *trans* than it does in *cis*. That genetic elements may be strengthened when paired has been proposed elsewhere (14, 36, 55). We also have considered the possibility of a dosage-sensitive *trans*-acting repressor of y^2 transcription contained within the region absent from y^{3c3}. We do not favor this model because it invokes a genetic element for which we have no evidence.

The data in this paper suggest two mechanisms for transvection: enhancer action in *trans* and bypass of a chromatin insulator in *cis*. A good test of the models comes from the placement of y^{82f29} in *trans* to y^{3c3} (Fig. 2*C*). Our models predict that y^{82f29}/y^{3c3} flies should show complementation in the wing, because of *trans* action of the y^{3c3} wing enhancer on the y^{82f29} promoter, but not in the body, because neither y^{3c3} nor y^{82f29} can provide significant body enhancer activity. This is indeed the case, with wing complementation reaching a level similar to that seen in $y^{82f29}/y^{1\#8}$ flies (Table 1). These results also demonstrate that y^{3c3} can contribute enhancer action in *trans* and, combined with the absence of body complementation in y^{82f29}/y^{3c3} flies, confirm that y^{3c3} is deficient in body enhancer function. These observations support our proposal that body complementation in y^2/y^{3c3} flies arises from the y^2 body enhancer bypassing the insulator. The tissue-specific phenotype of y^{82f29}/y^{3c3} flies has a further implication. It reinforces the idea that promoter unpairing, by itself, is not sufficient to explain complementation. If unpairing were sufficient, the hypothetical paired structure for y^{82f29}/y^{3c3}, which leaves the y^{82f29} promoter unpaired, would predict the body to be pigmented. Instead, pigmentation remains minimal in the body. These observations argue that the unpaired state of the promoter is also not sufficient to explain complementation in the $y^{82f29}/y^{1\#8}$ and y^2/y^{3c3} genotypes and that models invoking enhancer action and pairing-mediated topologies are a more likely explanation.

Comparison of the four complementing genotypes shows that complementation is stronger in the wing and, where appropriate, in the body for the two genotypes involving y^2 as compared with the two genotypes involving y^{82f29} (Table 1). The different degrees of complementation may be caused by modifiers extragenic to *yellow* or the abnormal juxtaposition of sequences at the deletion breakpoints of y^{82f29}. On the other hand, the differences may indicate intricacies of transvection at *yellow*. For example, the deletion nature of y^{82f29} may disrupt pairing and compromise transvection in a manner that has been proposed to influence pairing-mediated processes elsewhere (54, 55). Alternatively, if wing and body enhancers are more potent when paired, their absence from y^{82f29} may compromise the ability of the enhancers of the paired allele to act in *trans*. It is also possible that, although *gypsy* sequences are not necessary for transvection, their presence in y^2 facilitates transvection. A positive role for *gypsy* in transvection has been suggested (15, 17, 67), and there is mounting evidence for the ability of the su(Hw) protein to exert its influence in *trans* on a paired homologue (68–71). Finally, the stronger complementation seen in genotypes involving y^2 may arise from the promoter of y^2 receiving contribution both from enhancers located in *cis*, because of insulator bypass, as well as from enhancers located in *trans*.

In conclusion, we propose that homologue pairing promotes at least two forms of transvection at *yellow*, the mechanism or mechanisms used being determined by the features of the alleles involved. In one case, gene expression is directed by the *trans* action of genetic elements. In the other, gene expression is induced by the presence of a structurally dissimilar homologue where an obvious input from the homologue is its extent of homology as translated by the forces of pairing.

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