Transvection at the End of the Truncated Chromosome in *Drosophila melanogaster*

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ABSTRACT

The phenomenon of transvection is well known for the Drosophila yellow locus. Thus enhancers of a promoterless yellow locus in one homologous chromosome can activate the yellow promoter in the other chromosome where the enhancers are inactive or deleted. In this report, we examined the requirements for trans-activation of the yellow promoter at the end of the deficient chromosome. A number of truncated chromosomes ending in different areas of the yellow regulatory region were examined in combination with the promoterless y alleles. We found that trans-activation of the yellow promoter at the end of a deficient chromosome required ~6 kb of an additional upstream sequence. The nature of upstream sequences affected the strength of transvection: addition of *gypsy* sequences induced stronger trans-activation than addition of *He-T-A* or yellow sequences. Only the promoter proximal region (within ~158 bp of the yellow transcription start) was essential for trans-activation; i.e., transvection did not require extensive homology in the yellow upstream region. Finally, the yellow enhancers located on the two pairing chromosomes could cooperatively activate one yellow promoter.

ENHANCERS are cis-regulatory DNA elements that can activate gene transcription irrespective of their orientation or distance relative to the transcription start site (Blackwood and Kadonaga 1998; Dorsett 1999; Dillon and Sabbattini 2000). A fundamental question in transcriptional regulation is how enhancers find and control their target promoters over long distances. The basic models proposed to explain the distal enhancer function invoke protein-protein interactions causing DNA looping, sliding of proteins along the DNA, or generation of large chromatin domains that facilitate or prohibit gene activation (Blackwood and Kadonaga 1998; Dorsett 1999; Dillon and Sabbattini 2000; Engel and Tanimoto 2000; West et al. 2002). New insight into long-distance communication between enhancer and promoter came from the finding that in some cases a promoter on one chromosome can be activated by enhancers on the paired homolog (Geyer et al. 1990; Goldsborough and Kornberg 1996; Sipos et al. 1998; Pirrotta 1999; Wu and Morris 1999). The common term for such events—transvection—was introduced by Lewis to describe mechanisms in Drosophila that underlie the sensitivity of expression of a gene to the proximity of its homolog (Lewis 1954). Examples of transvection include events of trans enhancer-promoter interactions at the Ultrabithorax (Martinez-Laborda et al. 1992), Abdominal B (Sipos et al. 1998; Zhou et al. 1999), and yellow (Geyer et al. 1990) genes.

The interchromosomal enhancer-promoter interaction was most clearly shown for the yellow gene. The latter is required for larval and adult cuticle pigmentation (Nash and Yarkin 1974; Walter et al. 1991). The enhancers controlling yellow expression in the wings and body cuticle are located in the upstream region of the gene, whereas the enhancer controlling yellow expression in bristles resides in the intron (Geyer and Corces 1987; Biessmann and Mason 1988; Martin et al. 1989). The wing and body enhancers of one allele can trans-activate the yellow promoter on the paired homologous chromosome (Geyer et al. 1990; Morris et al. 1999b). However, the cis-preference of enhancers for their own promoter precludes their action in trans (Morris et al. 1999b). These observations indicate that the yellow regulatory region is an excellent system to study the mechanisms of long-distance interaction between enhancer and promoter.

Recent studies demonstrate that yellow transvection can occur at multiple genomic locations and indicate that the Drosophila genome is generally permissive to enhancer action in trans (Chen et al. 2002). However, the features of the yellow gene required for transvection have not been fully assessed. The yellow gene is located close to the X chromosome telomere, which may play a role in chromosome pairing. It is obscure whether the yellow sequences located upstream of its promoter are required for transvection.

Here we used the yellow terminal deficiencies to examine the role of telomere sequences and the requirement of homologous sequences in the upstream yellow region. Several y alleles are known to complement y², which laid
suggests that the wing and body enhancers of
y2−− (GEYER et al. 1990; MORRIS et al. 1998). The
yellow alleles are drawn only approximately to scale. En-w, wing enhancer; En-b, body enhancer; Su(Hw)bs, Su(Hw)-binding
site. The coding yellow region is shown as a solid box. The start of yellow transcription is shown by a horizontal arrow.

(A) Transvection involving trans-acting y178 enhancers and/or cis-acting y2 enhancers. The body and wing enhancers in the y2 allele are blocked by the Su(Hw)bs. The y178 allele comple-
ments y2 to give flies nearly wild-type pigmentation. The model suggests that the wing and body enhancers of y178 act in trans on the promoter of the y2 allele. It is possible that the y2 enhancers bypass the Su(Hw) insulator and act on the y+ promoter.

(B) Transvection involving trans-activation of the y178 wing
and body enhancers.

### Figure 1

A diagram showing transvection patterns at the yellow locus (GEYER et al. 1990; MORRIS et al. 1998). The yellow alleles are drawn only approximately to scale. En-w, wing enhancer; En-b, body enhancer; Su(Hw)bs, Su(Hw)-binding site. The coding yellow region is shown as a solid box. The start of yellow transcription is shown by a horizontal arrow.

(MATERIALS AND METHODS)

**Drosophila mutations and genetic crosses:** The y, y2, y29, y178, and y+ alleles were described in GEYER et al. (1986, 1990) and MORRIS et al. (1998, 1999a,b). The genetic symbols of the yellow alleles and their origin were described elsewhere (MIKHAILOVSKY et al. 1999; KAHN et al. 2000; SAVITSKY et al. 2002). The Df(1)hw, y29321, Su(var)2-5/y+ line was ob-
tained from J. Eissenberg. All other mutant alleles and chro-
mosomes used in this work and all balancer chromosomes are described in LINDSLEY and ZIMM (1992).

To test the effect of a su(Hw) background, the su(Hw)/
su(Hw) trans-heterozygote (HARRISON et al. 1995) was combined with y alleles as described in GEORGIJEV and KOZYCINA (1996).

To balance the deficient chromosome terminating in the yellow regulatory region, we used the y ac chromosome. This chromosome underwent deletion of the yellow gene and the regulatory region of the achaete gene, but not of any vital genes, and thus allowed us to examine the behavior of the yellow gene on the homologous chromosome in the absence of other yellow sequences.

To induce HeT-A transpositions to the end of the yellow terminal deficiency, we used the Su(var)2-5 mutation as described in SAVITSKY et al. (2002). Breaks between −1200 and −140 bp result in the y-like phenotype with yellow-colored aristae. Addition of a HeT-A sequence restores aristal pigmen-
tation. HeT-A attachment to the yellow sequences upstream of −1500 bp partially restores the activity of the yellow body and wing enhancers. These observations allowed us to monitor the attachment of HeT-A to the yellow terminal sequences.

**Complementation tests:** All crosses were conducted at 25° on standard medium. Wing refers to wing blades, and body refers to pigmentation in the abdominal stripes, not in the interstripe abdominal cuticle or thoracic cuticle. Routinely, five females were mated with two males in vials and brooded every second day. Temperature and crowding were controlled.
Transvection at the End of the Truncated Chromosome

1377

carefully, because both affect pigmentation. Pigmentation in the wing and body cuticle was scored in 3- to 4-day-old females on a five-point scale (Morris et al. 1998): wild-type expression was ranked 5 while the absence of yellow expression was ranked 1. Pigmentation scores were assigned by comparing the progeny of flies obtained from parallel controls. Intermediate scores were determined relative to the pigmentation levels of y38/y48, y5/y5, z200/z200, and y79b/y79b females, which in this and previous studies (Morris et al. 1998; Chen et al. 2002) corresponded to scores of 1, 1; 1, 1–2; 3, 3; and 4, 4 in the wing and body, respectively. Pigmentation scores were determined by two people independently and represent an analysis of 30–50 female flies from each of two independent crosses. The small variations in fly pigmentation that depended on culture condition did not exceed 0.5 point. Scores listed in Tables 1–3 represent the most common phenotype observed.

Molecular methods: DNA from adult flies was isolated using the protocol described in Ashburner (1989). The genomic DNA was digested with restriction enzymes according to the supplier’s instructions and separated in standard agarose gels (Sambrook et al. 1989). The DNA was transferred to Hybond in either allele and in both heterozygous and hemizygous females (Amersham, Piscataway, NJ). Subcloning and purification of the plasmid DNA and mapping of restriction sites were performed by standard techniques (Sambrook et al. 1989).

The junctions between newly transposed mobile elements and the DNA terminus were cloned by DNA amplification with two oligonucleotide primers. The primers in the yellow gene were:

y1, 5’ act tcc act tac cat cac gcc ag 3’ (+421; +399);
y2, 5’ aag acg ggc tca cca agg tga ct 3’ (+229; +207);
y3, 5’ gta acg gct ggt gcc cat atg ag 3’ (+36; +15);
y4, 5’ cag gac gct gct gca tag aat gc 3’ (+439; +461);
y5, 5’ att gga ttt cga ttg ggc gtc ac 3’ (+744; -767);
y6, 5’ gct cta atg acc aac gcc gca gc 3’ (+978; -1100);
y7, 5’ gtt cca ctt gat ccc ttc gcg gtc gc 3’ (-1382; -1404);
y8, 5’ caa cat cag cgg agg ggc gtc cta a 3’ (-1814; -1835);
y9, 5’ tcc agg aca aag ggt gga tcc 3’ (+176; +196).

The nucleotide map positions relative to the yellow transposition start site (Geyer et al. 1986) are given in parentheses. The primers in the Hf-TA element were:

h1, 5’ tgt tgc aag tgg tgt cgc gcc tca 3’ (456–434);
h2, 5’ ggt gct ctc gta ctt cgg gcc g 3’ (359–338);
h3, 5’ ccc aaa ctc acc cca tcc aat g 3’ (141–120).

The nucleotide map positions according to the sequence of the Hf-TA element (Biesmann et al. 1992) are given in parentheses.

The amplification products were fractionated by electrophoresis in 0.8% agarose gels in TAE. The successfully amplified products were cloned in a Bluescript plasmid (Stratagene, La Jolla, CA) and sequenced using a sequence kit (Amersham).

The DNA attachment in the y79 allele was cloned by an invert-PCR technique. DNA isolated from y79 flies was cleaved with BamHI; 2.4 to 3.6-kb DNA fragments were isolated from the gel, purified, and ligated. The required DNA fragment was amplified between primers y4 and y9 and cloned into the pSK vector.

RESULTS

Trans-activation of the yellow promoter at the tip of the deficient chromosome requires ~/6 kb between the promoter and the end of the terminal deficiency: To assess whether the yellow promoter located at the end of the deficient chromosome could be trans-activated by the yellow enhancer located on the homologous chromosome, we selected five lines with the chromosome terminating in the region from 400 to 800 bp upstream of the yellow transcription start site. The chromosomes carrying yellow terminal deficiencies (abbreviated as y79) were balanced over the y ac w chromosomes bearing a deficiency of the yellow-achaete region. The y79/y ac w females displayed a y-like phenotype: yellow wing and body cuticle and pigmented bristles.

To test the intragenic complementation, the y79 alleles were placed in trans to y108 or y56. For the complementation tests, two alleles were considered to complement each other if the scores in the wings or body for a trans-heterozygote were at least one point darker on the pigmentation scale than those for females heterozygous in either allele and in y ac. All trans-heterozygous females obtained had pigmentation scores of 1 in wing and body tissue as control y79/y ac w females (data not shown). Thus, the body and wing enhancers located on the y108 or y56 chromosome failed to trans-activate the yellow promoter if it was located close to the end of the deficient chromosome.

To determine the distance between the end of the deficient chromosome and the yellow promoter that is required for trans-activation, we used the method of terminal chromosome elongation by gene conversion, which allows different combinations of the regulatory elements to be generated at the same genomic position (Mikhailovsky et al. 1999). As a template for terminal gene conversion, we used the y522 allele with a 4.1-kb deletion removing the wing and body enhancers (Figure 3A; Morris et al. 1998). Flies of the y522/y108 or y56 genotypes had pigmentation scores of 3 in both wing and body, suggesting trans-activation of the y522 promoter by the enhancer located on the homologous chromosome (Morris et al. 1998).

By Southern blot analysis, we selected the y79+108 line with a deficient chromosome terminating ~100 bp downstream of the yellow transcription start site. The y79+108 flies had a y-null phenotype because of deletion of the yellow promoter. To induce terminal gene conversion, the y79+108 w chromosome was paired with the y522 w chromosome (Figure 2A). The y79+108w/y522 w females were crossed to y522 w males for three subsequent generations. To select the derivative y79 alleles with terminal DNA addition, y79+108w/y522 w females were crossed to y ac w males. y79w y ac w females displaying y79-like phenotype were selected and individually crossed with y ac males. In the next generation, y79w y ac w females were crossed with y ac males. The size of terminal DNA elongation in the derivative y79 lines was examined by Southern blot analysis (Figure 3B). As a result, we selected nine derivative lines with the deficient chromosome extended to reach between ~1700 and ~6100 bp relative to the yellow transcription start site. Since the
Figure 2.—Schemes used to obtain terminal DNA elongation. (A) The genetic scheme to obtain deficient chromosomes ending in different regions of the \( y^{82f29} \) allele. (B) The genetic scheme to obtain deficient chromosomes ending in different regions of the \( y^2 \) allele. (C) The genetic scheme for isolation of individual stable lines bearing terminally truncated chromosomes with new HeT-A attachment. \( y^{TD^*} \) indicates the \( y \) allele with new phenotype (new HeT-A attachment).
The y82f29 allele was used for terminal DNA elongation, we named all newly obtained terminally deficient alleles as yTD82f. Flies bearing any yTD82f in trans to y ac were fully mutant in wings and body, with scores of 1 for both tissues (Table 1). If the distance between the yellow promoter and the end of the deficient chromosome was ≤4000 bp, flies bearing such yTD82f in trans to y ac or yzh showed no pigmentation in the body and wing (Table 1). The yTD82f allele, which had the end of the truncated chromosome at ~4800 bp, complemented weakly only with y ac but not with yzh (Table 1). The yTD82f allele produced better complementation with y18 or y59b; it had ~6000 bp between the yellow promoter and the end of truncated chromosome (Figure 3), suggesting that this distance is essential for transvection.

**Gypsy facilitates trans-activation of the yellow promoter at the end of the truncated chromosome:** Next, we examined how gypsy sequences at the end of the deficient chromosome would influence the strength of transvection. For this purpose, we generated a number of lines with the chromosomes ending at different parts of gypsy inserted in the y2 allele (Figure 2B).

By Southern blot analysis, two yTDw lines were selected carrying deficient chromosomes terminating ~400 bp downstream of the yellow transcription start site. To select a proper combination of the y alleles, we used the z mutation as a marker. To induce terminal gene conversion, the yTDw chromosomes were paired with the y2z homologs containing the y2 allele as a template for terminal gene conversion (Figure 2B). In the next generation, yTDw/y2z females were crossed to y2 males. The yTDw/y2z females had red eyes, while homozygous y2z/y2z females had yellow eyes. After three generations, in which terminal DNA elongation could occur, yTDw/y2z females were individually crossed to y59b males to select yTDw/y59b females with darker wing and body pigmentation (partial or complete complementation). In the next generation, we selected six independent yTDw/y59b females (red eyes) that had pigmented wing and body cuticle. Flies with the same yTD alleles balanced over y2 produced better complementation with y18 or y59b; it had ~4000 bp between the yellow transcription start site. Flies of genotypes yTD2-3, yTD2-4, or yTD2-5 vs. y1#8 or y59b had pigmentation scores 1-2 in body and wings (Table 2), suggesting that pairing with the y59b allele is required for terminal gene conversion, the terminally deficient alleles obtained were named yTD2.

To determine the end of the deficient chromosome, DNA samples isolated from yTD lines were studied by Southern blot analysis (Figure 4C). The yTD2, yTD2.4, and yTD2.5 had the end of the truncated chromosome between ~4200 and ~3800 bp relative to the yellow transcription start site. Flies of genotypes yTD2.1, yTD2.4, or yTD2.5 vs. y2z or y59b had pigmentation scores of 2–3 in body and 3 in wings (Table 2), suggesting that ~4000 bp from the yellow promoter is enough for trans-activation of the yellow promoter in this case. However, flies with yTD2.1 or
**TABLE 1**
Complementation data for terminally deficient derivatives of the \( y^{5929} \) allele

<table>
<thead>
<tr>
<th>Allele</th>
<th>Distance(^b) (bp)</th>
<th>( y^{ac} ) (W, B)</th>
<th>( y^{88} ) (W, B)</th>
<th>( y^{88} ) (W, B)</th>
</tr>
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<tbody>
<tr>
<td>( y^{5929} )</td>
<td></td>
<td>1, 1</td>
<td>3, 3</td>
<td>3, 3</td>
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<tr>
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<td>–1700</td>
<td>1, 1</td>
<td>1, 1</td>
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<td>1, 1</td>
<td>1, 1</td>
</tr>
<tr>
<td>( y^{5929} )</td>
<td>–2200</td>
<td>1, 1</td>
<td>1, 1</td>
<td>1, 1</td>
</tr>
<tr>
<td>( y^{5929} )</td>
<td>–2500</td>
<td>1, 1</td>
<td>1, 1</td>
<td>1, 1</td>
</tr>
<tr>
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<td>–3000</td>
<td>1, 1</td>
<td>1, 1</td>
<td>1, 1</td>
</tr>
<tr>
<td>( y^{5929} )</td>
<td>–3800</td>
<td>1, 1</td>
<td>1, 1</td>
<td>1, 1</td>
</tr>
<tr>
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<td>1, 1</td>
<td>1, 1</td>
<td>1, 1</td>
</tr>
<tr>
<td>( y^{5929} )</td>
<td>–4800</td>
<td>1, 1</td>
<td>1, 2</td>
<td>1, 1</td>
</tr>
<tr>
<td>( y^{5929} )</td>
<td>–6100</td>
<td>1, 1</td>
<td>2, 2</td>
<td>2, 2</td>
</tr>
</tbody>
</table>

Pigmentation scores in body (B) and wings (W) when alleles are in trans to one of the alleles listed across the top of the table. Italic numbers indicate complementation between \( y \) alleles.

\(^a\) Complementation with the \( y^{5929} \) allele was described in Morris et al. (1998).

\(^b\) Distance between the end of the truncated chromosome and the yellow transcription start site.

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**TABLE 2**
Complementation data for the terminally truncated derivatives of the \( y^{82f} \) allele

<table>
<thead>
<tr>
<th>Allele</th>
<th>Distance(^b) (bp)</th>
<th>( y^{ac} ) (W, B)</th>
<th>( y^{88} ) (W, B)</th>
<th>( y^{88} ) (W, B)</th>
</tr>
</thead>
<tbody>
<tr>
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<td>1, 1</td>
<td>4, 4</td>
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<tr>
<td>( y^{82f} )</td>
<td>–1300</td>
<td>1, 1</td>
<td>1, 1</td>
<td>1, 1</td>
</tr>
<tr>
<td>( y^{82f} )</td>
<td>–1500</td>
<td>1, 1</td>
<td>1, 1</td>
<td>1, 1</td>
</tr>
<tr>
<td>( y^{82f} )</td>
<td>–1700</td>
<td>1, 1</td>
<td>1, 1</td>
<td>1, 1</td>
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<tr>
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<td>1, 1</td>
<td>1, 1</td>
<td>1, 1</td>
</tr>
<tr>
<td>( y^{82f} )</td>
<td>–3800</td>
<td>1, 1</td>
<td>3, 2–3</td>
<td>3, 2–3</td>
</tr>
<tr>
<td>( y^{82f} )</td>
<td>–4000</td>
<td>1, 1</td>
<td>3, 2–3</td>
<td>3, 2–3</td>
</tr>
<tr>
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<td>3, 2–3</td>
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<td>1, 1</td>
<td>4, 2</td>
<td>4, 4</td>
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<tr>
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<td>–10000</td>
<td>1, 1</td>
<td>4, 2</td>
<td>4, 4</td>
</tr>
<tr>
<td>( y^{82f} )</td>
<td>&gt; –10000</td>
<td>1, 1</td>
<td>4, 4</td>
<td>4, 4</td>
</tr>
<tr>
<td>( y^{82f} )</td>
<td>–2400</td>
<td>1, 1</td>
<td>1, 1</td>
<td>1, 1</td>
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<tr>
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<td>–3100</td>
<td>1, 1</td>
<td>1, 1</td>
<td>1, 1</td>
</tr>
</tbody>
</table>

\(^a\) Complementation with the \( y^{82f} \) allele was described in Morris et al. (1998). Other designations are as in Table 1.

\(^b\) Distance between the end of the truncated chromosome and the yellow transcription start site.

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\( y^{592} \) in trans to \( y^{88} \) or \( y^{88} \) had the maximum pigmentation scores of 4 in both wing and body, as did \( y^{2} / y^{88} \) flies (Table 2). This result shows that strong transvection can be reproduced at the end of a deficient chromosome, and the telomere sequences are not permanently required for the pairing between the \( y \) alleles. The \( y^{592} \) and \( y^{592} \) alleles had the ends of the truncated chromosomes at \(-10,000\) and \(-6000\) bp, respectively (Figure 4, A and C). Thus, an \sim 6000-bp distance between the promoter and the end of the truncated chromosome is required for strong transvection.

As found previously (Briessmann and Mason 1988, 1997), chromosomes lose DNA sequences from the broken end at the same rate of 70–80 bp/generation. After maintaining \( y^{592} / y^{88} \) flies for several months, we found females with less pigmented or nonpigmented wing and body cuticle. Southern blot analysis showed that in the new \( y^{592} \) derivatives truncated chromosomes ended in the region between \(-2800\) bp (\( y^{592} \)) and \(-1300\) bp (\( y^{592} \)) relative to the yellow transcription start site (Figure 4, A and C; Table 2). Thus, there is no transactivation if the distance between the promoter and the end of the truncated chromosome is <3 kb.

One of the \( y^{592} \) alleles had a large DNA addition with a restriction map unrelated to the gypsy or yellow sequences (Figure 4D). Southern blot analysis showed that the new DNA is attached to the 5’ LTR of gypsy: the XhoI and Xbal restriction sites located in the LTR were retained, while the Ncol site following the Su(Hw)-binding site was removed (Figure 4D). To elucidate the nature of the new DNA attachment, the DNA fragment adjacent to the gypsy LTR was cloned by the inverse PCR technique with the y4 and y9 primers shown in Figure 4B. Sequencing of the cloned DNA showed that the newly added DNA sequence was derived from an F element attached to the twelfth Su(Hw)-binding site of gypsy.
gypsy (Figure 4B). Hence this $y^{TDF2}$ allele was renamed as $y^{TDF}$. Flies of the genotype $y^{TDF}/y^{TDF}$ or $y^{TDF}/y^{TDF}$ had scores of 4 in both wing and body (Table 2). Upon observing the $y^{TDF}/y^{TDF}$ line for several generations, two females were isolated that had no pigmentation (score 1) in the body and wings. By Southern blot analysis, the ends of the truncated chromosomes in these $y^{TDF}$ derivatives were mapped at approximately $-2400$ bp ($y^{TDF1}$) and $-3100$ bp ($y^{TDF2}$) relative to the yellow transcription start site (Figure 4, B and D). Thus, these results again show that the distance between the yellow promoter and the end of the deficient chromosome has to be $>3.5$ kb to provide any transvection.

The $y^{TDF2}$ and $y^{TDF}$ alleles have the Su(Hw) insulator that might be responsible for the effective transcription. The Su(Hw) protein binds to the Su(Hw) insulator and is responsible for its activity (Dorsett 1990; Spana and Corces 1990; Holdridge and Dorsett 1991; Scott et al. 1999). To find out the role of the Su(Hw) insulator in transvection between the $y$ alleles, we obtained the $y^{TDF}/y^{TDF}$ and $y^{TDF2}/y^{TDF}$ trans-heterozygotes on the $su(Hw)^{3100}$ background, which proved to have no effect on fly pigmentation (data not shown). Thus, the Su(Hw) insulator is not required to facilitate trans-activation of the yellow promoter at the end of the truncated chromosome.

Role of the yellow upstream regulatory sequences in transvection between the yellow alleles: The existence of transvection at the end of the truncated chromosome offers an opportunity to assess the role of the upstream yellow sequences in trans-activation of the yellow promoter. Savitsky et al. (2002) described a number of truncated chromosomes carrying HeT-A attachments to the yellow regulatory region. For the complementation test, we selected the $y^{D}$ alleles that had HeT-A attachments $>7$ kb (Figure 5B). To distinguish the $y^{D}$ alleles generated by attachment of HeT-A to the yellow $5'$ regulatory region, we named them $y^{TDF}$.

We also obtained additional HeT-A attachments by crossing the $y^{D}$ alleles with the Su(var)/2-5' end that strongly induced HeT-A transposition to the chromosome end (Figure 2C). Eight $y^{D}/y^{ac}$; $If/CyO$ lines carrying deficiencies ending at $-2800$ to $-300$ bp were selected by the Southern blot protocol. The $y^{D}/y^{ac}$; $If/CyO$ males were crossed to $y$ or $Su(var)/2-5'/CyO$ males as shown in Figure 2C. In the offspring, $y^{D}/y^{ac}$; $Su(var)/2-5'/CyO$ females were crossed to $y$; $Su(var)/2-5'/CyO$ males for three to seven generations to determine the appearance of flies with the new $y$ phenotype. To establish stable $y^{D}$ lines, the $Su(var)/2-5^{D2}$ mutation was crossed out from the individual $y^{D}/y^{ac}$; $Su(var)/2-5^{D2}/CyO$ females. As a result, new HeT-A additions were selected in the $y^{D}/y^{ac}$; $If/CyO$ progeny.

To find out the precise site of HeT-A attachments to the yellow sequences, the junctions between terminal yellow sequences and new DNA attachments were cloned by PCR and sequenced (Figure 5A; Table 3). The PCR primers were located in the yellow gene and in the conserved regions from the $3'$ ends of HeT-A. A number of new HeT-A attachments were obtained in the region of the wing and body enhancers. As shown previously (Mikhailovsky et al. 1999), the wing and body enhancers are inactive at the end of the deficient chromosome. Large HeT-A attachments restored activation of the yellow promoter by the enhancers (Table 3, classes D and E).

The ability of each $y^{D}$ allele to support transvection was assessed in combination with $y^{108}$ or $y^{97}$. The complementation test defined five classes of the $y^{D}$ alleles. No transvection was determined in the $y^{D}$ alleles that have HeT-A attachments in the region between $-80$ and $+165$ bp around the yellow transcription start site (Table 3, class A). The promoter located at the $3'$ end of the HeT-A element (Danilevskaya et al. 1997) can substitute for the deleted yellow promoter and drive yellow expression in bristles (Biessmann et al. 1990b; Kahn et al. 2000). However, lack of complementation suggests that the yellow enhancers are not able to trans-activate the HeT-A or yellow promoter in the absence of upstream yellow sequences.

In class B, flies of genotype $y^{D}/y^{108}$ or $y^{D}/y^{97}$ had pigmentation scores of 1–2 for the wing and 1 for the body. These $y^{D}$ alleles had HeT-A attachments in the small region between $-98$ and $-116$ bp. In class C, flies of genotype $y^{D}/y^{108}$ or $y^{D}/y^{97}$ had pigmentation scores of 2 or 2–3 for the wing and 1–2 or 2 for the body. Class C includes the $y^{D}$ alleles with the HeT-A attachments in the region between $-158$ and $-1142$ bp. All the $y^{D}$ alleles of classes A, B, and C in heterozygous $y^{TDF}/y^{ac}$ flies had pigmentation scores of 1 for both the wing and the body cuticle (Table 3). These results suggest that the promoter proximal region between the TATA box and $-158$ bp is essential for trans-activation of the yellow promoter by the wing and body enhancers located on the homologous chromosome, whereas the yellow upstream region between $-158$ and $-1142$ bp has no effect on transvection efficiency.

Cooperation between enhancers located on the pairing chromosomes in activation of the yellow promoter: In class D, flies of genotype $y^{D}/y^{ac}$ displayed different levels of yellow activation in the wing and body (Table 3). The addition of the sequences corresponding to the body and wing enhancers gradually increased the level of pigmentation. The $y^{D}/y^{ac}$ flies had pigmentation scores of 1 for the wing and 2 for the body, while the $y^{D}/y^{ac}$ flies had scores of 2–3 for the wing and 5 for the body. Other $y^{D}/y^{ac}$ flies of class D displayed the pigmentation level of intermediate scores. The $y^{D}/y^{ac}$ and $y^{D}/y^{ac}$ flies produced a wild-type level of pigmentation, suggesting that both body and wing enhancers are localized downstream of $-2491$ bp (class E). The complementation analysis with each of the class D $y^{D}$ alleles showed that $y^{D}/y^{ac}$ or $y^{D}/y^{ac}$ flies had scores of 1 or 2 points higher than $y^{D}/y^{ac}$ flies. This result may
be explained by coactivation of the yellow promoter in the y\textsuperscript{TDL}/y\textsuperscript{108} or y\textsuperscript{59b} flies by the enhancers located on the paired chromosomes.

To further study the cooperation between the yellow enhancers located on the homologous chromosomes, we used y\textsuperscript{2}. In this y allele the Su(Hw) insulator blocks the wing and body enhancers (GEYER et al. 1986). Flies of the y\textsuperscript{2}/y\textsuperscript{59b} genotype have pigmentation scores of 1 and 1–2 for wing and body tissues, respectively (MORRIS et al. 1998, 1999a). Thus, the wing and body enhancers located on the y\textsuperscript{2} chromosome fail to activate the yellow promoter on the pairing y\textsuperscript{59b} chromosome. The same results were obtained in intragenic complementation tests between class C y\textsuperscript{TDL} alleles and y\textsuperscript{2} (Table 3). These observations can be explained assuming that the Su(Hw) insulator blocks cis and trans interactions between the yellow enhancers and promoter. However, the y\textsuperscript{TDL} alleles of class D complement with y\textsuperscript{2}. Flies of the y\textsuperscript{TDL}/y\textsuperscript{2} genotype had wing and body pigmentation similar to that of y\textsuperscript{TDL}/y\textsuperscript{59b} flies (Table 3), suggesting that the y\textsuperscript{2} promoter is not involved in yellow activation. Hence, again the cooperation in the promoter activation between the yellow enhancers located on the y\textsuperscript{2} and y\textsuperscript{TDL}/y\textsuperscript{59b} paired chromosomes can explain the complementation between y\textsuperscript{2} or y\textsuperscript{59b} and y\textsuperscript{TDL} alleles.

To confirm the role of pairing between y alleles in this complementation, we made use of the previous observation that insertion of another gypsy into the neighboring scute locus (sd\textsuperscript{D1} mutation) partially hinders transvection between y\textsuperscript{2} and y\textsuperscript{59b} or y\textsuperscript{108}, presumably because of the disruption of proper chromosomal synapsis between the yellow alleles (GEYER et al. 1990; GAUSE and GEORGIEV 2000). It is also possible that the interaction between the Su(Hw) insulators located in the yellow (y\textsuperscript{2}) and scute (sd\textsuperscript{D1}) loci partially blocks the ability of the wing and body enhancers to activate the yellow promoter in trans (GAUSE and GEORGIEV 2000). In this study we used the y\textsuperscript{2}sd\textsuperscript{D1}D\textsuperscript{108} and the derivative line y\textsuperscript{2}sd\textsuperscript{D1}D\textsuperscript{108}. The null y\textsuperscript{D1} allele was generated by insertion of the
The transposons inserted in TAS of different telomeres yellow karyotes, and of a relatively short subtelomeric satellite were established when a sequence of 

This result supports the assumption that the retrotransposons, which perform the elongation further addition of upstream TART y2

Telomeres in chromosome: Stalker retrotransposon yTDH hancers are involved in activation of the complementation between the y2 enhancers to efficiently act for establishing the allele proximity necessary for trans-regulatory interactions.

DISCUSSION

Transvection can occur at the end of the truncated chromosome: Telomeres in Drosophila melanogaster are very different from those in other eukaryotes (Biessmann and Mason 1997; Pardue and DeBaryshe 1999, 2000). They consist of a terminal array of HeT-A and TART retrotransposons, which perform the elongation function that is performed by telomerase in other eu- karyotes, and of a relatively short subtelomeric satellite, called TAS (Mason et al. 2000). Corepression of the transposons inserted in TAS of different telomeres suggests interaction between telomeres in Drosophila (Golubovsky et al. 2001). The yellow gene resides ~100 kb from the X chromosome telomere (Adams et al. 2000). Thus, the presence of a telomere may be important for establishing the allele proximity necessary for trans-regulatory interactions.

Terminal deletions in Drosophila have been obtained (Biessmann and Mason 1988; Traverse and Pardue 1988; Levis 1989; Biessmann et al. 1990a; Mikhailovsky et al. 1999; Golubovsky et al. 2001). Drosophila broken chromosomes behave as capped ones: they are stably transmitted through many generations (Levis 1989; Biessmann et al. 1990a). One protein that is required for capping is HP1, which binds to the telomere and terminal deficiencies (Fanti et al. 1998).

Our previous results indicate that the yellow enhancers do not function properly when located very near the chromosome end (Mikhailovsky et al. 1999). However, further addition of upstream yellow sequences gradually increased the level of pigmentation, and wild-type levels were established when a sequence of ~4 kb distal to the yellow enhancers was added. Here we found that the yellow enhancers are not able to trans-activate the yellow

### TABLE 3

Complementation data for the y^{sol} alleles

<table>
<thead>
<tr>
<th>Allele</th>
<th>Distancea</th>
<th>y ac</th>
<th>y^{18s}</th>
<th>y^{18b}</th>
<th>y^{12}</th>
<th>y^{13}</th>
<th>y^{SC^{b1}}</th>
<th>y^{tSC^{b1}}</th>
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</thead>
<tbody>
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<td>y^{52y}</td>
<td>1, 1</td>
<td>1, 1</td>
<td>3, 3</td>
<td>3, 3</td>
<td>1, 1–2</td>
<td>1, 1</td>
<td>1, 1–2</td>
<td>1, 1</td>
</tr>
<tr>
<td>Class A</td>
<td>+165, +161, +105, +51, +48, +36, +1, −4b, −28c, −46, −55, −59, −80</td>
<td>1, 1</td>
<td>1, 1</td>
<td>1, 1</td>
<td>1, 1–2</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Class B</td>
<td>−98, −99, −108, −116</td>
<td>1, 1</td>
<td>1–2, 1</td>
<td>1–2, 1</td>
<td>1, 1–2</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Class C</td>
<td>−162, −163, −166, −169, −172, −231, −244b, −288, −321, −363, −370, −376, −385, −442, −476, −479b, −490, −499, −586, −743a−d</td>
<td>1, 1</td>
<td>2(3), 2(2–2), 1–2(2)</td>
<td>1–2, 1–2</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
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<td>1, 3</td>
<td>1, 2–3</td>
<td>1, 2–3</td>
<td></td>
</tr>
<tr>
<td>Class E</td>
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<td>5, 5</td>
<td>5, 5</td>
<td>5, 5</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

The HeT-A attachments described in Savitsky et al. (2002) are underlined. Other designations are as in Table 1.

a Distance between HeT-A attachment and the yellow transcription start site.
Transvection at the End of the Truncated Chromosome

Figure 6.—Models. The yellow alleles are drawn only approximately to scale. En-w, wing enhancer; En-b, body enhancer; Su(Hw)bs, Su(Hw)-binding site. The coding yellow region is shown as a solid box. The start of yellow transcription is shown by a horizontal arrow. The upstream promoter region required for transvection is shown as an open bar.

promoter if the latter is located close to the end of the deficient chromosome. To explain these results, we propose that the hypothetical end-binding complex occupies ~4–6 kb of terminal DNA and interferes with long-distance communication between the yellow enhancers and promoter.

Addition of HeT-A or the upstream yellow sequences only partially restored the transvection as compared with the y^{527} allele generated by deletion of the upstream yellow enhancers. This observation supports the significance of the telomere sequences deleted from the terminally truncated chromosome for chromosome pairing that is required for trans-activation of the yellow promoter. However, the presence of gypsy sequences at the end of the truncated chromosome restores efficient transvection. Interestingly, the Su(Hw) protein, the major functional component of the insulator, is apparently not required to facilitate transvection. The gypsy sequences, but not the Su(Hw) insulator, negatively influence yellow transcription (Belenkaya et al. 1998), excluding the possibility of direct positive action of the gypsy sequences on the yellow promoter. The presence of the gypsy sequences only in the terminally deficient chromosome argues against the possibility that the pairing between gypsy sequences facilitates transvection. The possible explanation of the gypsy activity comes from the recent
finding that gypsy is frequently located at the nuclear envelope (Gerasimova et al. 2000). Thus, the gypsy sequences may stabilize interaction between the end of the terminally truncated chromosome and the nuclear envelope. It was shown that the yeast telomeres are positioned in clusters adjacent to the nuclear envelope (Hediger and Gasser 2002). The interaction between the regulatory elements located on the different Drosophila telomeres suggests that telomeres interact with each other to assess their integrity (Golubovsky et al. 2001). This interaction might stabilize pairing between homologous chromosomes that reinforces the transvection.

Trans-interaction between the yellow enhancers and promoter: Here we found that only ~130 bp upstream of TATA is significant for trans-activation of the yellow promoter (Figure 6, A and B). Interestingly, the upstream yellow sequences distal to ~158 bp do not substantially influence the efficiency of trans-activation of the yellow promoter. Thus, no extensive homology upstream to the yellow promoter is required for the transvection.

Our results suggest that the yellow enhancers located on the two pairing chromosomes can cooperatively activate one yellow promoter (Figure 6C). Previous studies (Geyer and Corces 1987; Martin et al. 1989; Morris et al. 1998) and our unpublished results (M. Savitsky, O. Kravchuk and P. Georgiev) suggest that the wing and body enhancers spreading over a 2-kb region consist of many separate modules, which cooperatively activate the yellow promoter. Hence it is not surprising that the enhancer modules can cooperate in trans in the case of pairing between yellow alleles located on the homologous chromosomes. Alternatively, the increase of yellow expression might be explained by the suggestion that the yellow promoter is activated by the enhancers located in cis in some cells and by the enhancers located in trans in other cells. Therefore the presence of the additional enhancers in trans increases the probability of yellow activation.

The complementation between the yTDH (class D) alleles and y+ (Figure 6, D and E) may also be explained by the interaction between the y+ and yTDH yellow enhancers that helps to bypass the gypsy insulator. However, the complementation between these alleles can also be explained by the model of Morris et al. (1998), on the basis of the striking complementation between y+ and yTDH alleles. The y+ allele, in which a deletion removes the body enhancer and promoter, complements y+ for both wing and body expression, demonstrating that the body enhancer in y+ is not insulated by Su(Hw). The simplest interpretation is that homologous chromosome pairing forces gypsy and other unpaired sequences out in a loop, thereby bringing the body enhancer in physical proximity to the promoter. Complementation between the yTDH (class D) alleles and y+ may also be explained by the suggestion that pairing between the yellow sequences extending from ~700 bp, the site of gypsy insertion in y+, to the HeT-A attachment helps in looping out the gypsy, which facilitates the trans-interaction between the y+ enhancers and the yTDH promoter. We think that the last mechanism is unlikely, because it implies strong pairing between relatively short yellow sequences. However, at this moment it is impossible to discriminate between these two models.

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LITERATURE CITED


Transvection at the End of the Truncated Chromosome


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