RESEARCH COMMUNICATION

Core promoter elements can regulate transcription on a separate chromosome in *trans*

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Transvection can cause the expression of a gene to be sensitive to the proximity of a homolog. It can account for many cases of intragenic complementation at the *Drosophila yellow* gene, where one mode of transvection involves the action of enhancers in *trans* on a promoter present on a separate chromosome. Our goal was to identify *cis*-acting elements that regulate the *trans* action of enhancers. Using gene replacement, we altered two core promoter elements at *yellow* and tested the resulting alleles for their ability to support transvection. We found that the TATA box and initiator element can regulate transvection.

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Homologous sequences can influence each other in profound ways, resulting in changes in gene structure and expression (for review, see Henikoff and Comai 1998). A good example of homolog interaction is transvection, which was defined in *Drosophila* (Lewis 1954) and is now known to occur elsewhere (for review, see Henikoff and Comai 1998). In *Drosophila*, somatic homolog pairing brings homologous sequences into proximity. The expression of some genes is sensitive to somatic pairing and these genes are said to exhibit transvection effects. One example of such a gene is *yellow* (Geyer et al. 1990). The *yellow* gene is required for cuticle pigmentation and is controlled by several independent tissue-specific enhancers. Mutations in *yellow* result in decreased pigmentation. Interestingly, some mutant *yellow* alleles that reduce pigmentation in the same cuticular structures are able to complement each other in pairwise combinations to give a nearly wild-type phenotype. This form of intragenic complementation can be explained by transvection.

One mode of transvection at *yellow* entails the action of enhancers in *trans* on the promoter of a paired homolog (Geyer et al. 1990; Morris et al. 1998). Figure 1A illustrates this mechanism with the y^2 and $y^{1\#8}$ alleles (Geyer et al. 1990). The y^2 allele results from the inser-

tion of a *gypsy* retrotransposable element between the promoter and the two enhancers directing expression in the wings and body. When *gypsy* is bound by the Suppressor of Hairy wing [Su(Hw)] protein, it establishes a chromatin insulator (for review, see Dorsett 1996; Gdula et al. 1996; Geyer 1997) that prevents the wing and body enhancers from communicating with the promoter, and therefore, y^2 flies have mutant wing and body pigmentation. The $y^{1\#8}$ allele is a null that is associated with a 780-bp deletion removing the promoter. Although homozygous y^2 and $y^{1\#8}$ flies show mutant wing and body pigmentation, $y^2/y^{1\#8}$ flies show almost wild-type pigmentation. This intragenic complementation can be explained by the action of the wing and body enhancers of $y^{1\#8}$ in *trans* on the y^2 promoter (Geyer et al. 1990).

Although the wing and body enhancers of some *yellow* alleles are able to act in *trans* on the y^2 promoter, the enhancers of other *yellow* alleles cannot. For example, the y^1 allele, which is caused by an A to C mutation in the translation initiation codon, has intact wing and body enhancers, but fails to complement y^2 (Fig. 1B; Geyer et al. 1990). This observation suggests that the action of enhancers in *trans* on a paired promoter is not always possible, that it occurs only under certain circumstances. In this way, transvection is a regulated process at *yellow*.

We are interested in the molecular basis for the regulation of transvection. Previous studies at *yellow* delineated a large region encompassing the promoter as a key player in that its presence appeared to inhibit transvection (Geyer et al. 1990; Morris et al. 1999). For example, $y^{1\#8}$, which lacks this region, can participate in transvection, while y^1 , which carries this region, cannot. The importance of the promoter region is also highlighted by findings at the *Ultrabithorax* (*Ubx*; Martínez-Laborda et al. 1992; Casares et al. 1997) and *Abdominal-B* (Hendrickson and Sakonju 1995; Sipos et al. 1998) genes. Our

Figure 1. A model to explain complementation patterns at *yellow* (Geyer et al. 1990). (A) The $y^{1\#8}$ allele complements y^2 to give flies with nearly wild-type pigmentation. The model suggests that the wing and body enhancers of $y^{1\#8}$ act in *trans* on the promoter of the y^2 allele. (B) The y^1 allele does not complement y^2 . The intact promoter region of y^1 may preclude the action of enhancers in *trans*. (W) Wing enhancer; (B) body enhancer; (Br) bristle enhancer; (T) tarsal claw enhancer; (solid rectangle) exon; (open rectangle) *gypsy* long-terminal repeat (LTR); (large arrow above exon) transcription from *yellow* promoter; (small arrow above LTR) transcription of *gypsy*; (●) su(Hw)-binding region. There are 17 bp of P-element sequence at the breakpoints of $y^{1\#8}$ (Geyer et al. 1990).

[Key Words: Enhancer; pairing; promoter; transcription; transvection; *yellow*]

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goal has been to identify the elements that regulate transvection at *yellow*. Here, we present our studies that were prompted by the presence of the promoter within the delineated region. It has been proposed that *cis*-preference of enhancers for their own promoter precludes their action in *trans* (Geyer et al. 1990; Martínez-Laborda et al. 1992; Hendrickson and Sakonju 1995; Casares et al. 1997; Sipos et al. 1998; Morris et al. 1999). Our strategy involved the introduction of mutations into putative core promoter elements and assessment of the resulting *yellow* alleles for their ability to support transvection.

Results and Discussion

We focused first on the putative TATA box of *yellow*, extending from -32 to -24 relative to the transcription start site (Chia et al. 1986; Geyer et al. 1986). Our method of mutagenesis was targeted gene replacement at the endogenous locus. This approach does not compromise the ability of *yellow* alleles to pair and is required because ectopically located *yellow* transgenes have not been observed to pair and promote transvection with the endogenous *yellow* gene (Geyer et al. 1990).

Targeted gene replacement in *Drosophila* involves the excision of a marked P element followed by gap repair from a template (Fig. 2; Keeler et al. 1996). To generate a marked P element in *yellow*, we began with an allele, y^{h12} , that is associated with an unmarked P element at +4 relative to the transcription initiation site (Geyer et al. 1990). We then used targeted transposition (e.g., see Heslip and Hodgetts 1994) to substitute the internal sequences of the y^{h12} P element with sequences containing the *white*⁺ marker gene (Fig. 2A). The resulting *yellow* allele, called y^{h12w+} , became our target for the introduction of sequence changes from a plasmid template (Fig. 2B). Using this strategy, we replaced 6 bp of the wild-type TATA box with the 6-bp recognition site for the *Sma*I restriction enzyme (Table 1). Introduction of the *Sma*I site allowed us to use restriction analysis of PCR-amplified fragments in the identification of gene convertants. Changes were subsequently confirmed by sequencing, and the integrity of the *yellow* locus was ascertained by Southern analysis. The new *yellow* allele with a mutant TATA box is called y^{tata} (Table 1).

Homozygous y^{tata}/y^{tata} and hemizygous y^{tata}/Df flies showed a reduction in the pigmentation of all adult cuticular structures (Table 2). For example, the wings showed intermediate pigmentation, the body showed low-level pigmentation, and the bristles showed fully mutant pigmentation. These observations demonstrate that y^{tata} compromises transcription, and therefore, that the putative TATA box is functional.

We placed y^{tata} in *trans* to y^2 and discovered that y^{tata} can support transvection. The wings and body of y^2/y^{tata} flies were darker than the wings and body of flies homozygous or hemizygous for either y^2 or y^{tata} (Table 2). This finding has two implications. First, y^{tata} identifies 6 bp that, when mutant, allow transvection to proceed, and represents the most precise definition, to date, of an el-

ement that acts in *cis* to determine whether transvection can occur. Second, the 6 bp implicate a promoter element in the regulation of transvection.

Although the model for transvection shown in Figure 1 predicts that complementation between y^2 and y^{tata} results from the y^{tata} enhancers activating transcription from the y^2 promoter, it is formally possible that complementation arises from pairing-mediated activation of the y^{tata} promoter. For instance, pairing-mediated activation of transcription has been reported for *Ubx*

A Generation of target

B Gene replacement

Figure 2. Scheme for targeted gene replacement. (A) Generation of y^{h12w+} by targeted transposition (Heslip and Hodgetts 1994). Males carrying $y^{h12} w^{1118}$, $P[w^+]$, and a source of transposase ($\Delta 2-3$) were mated to attached-X (>) females. Of the 1500 progeny, 106 males were selected as candidates for targeted transposition events by the indicated phenotype. Candidates were singly mated to attached-X females, and two were shown to be true examples of targeted transposition by (1) demonstration of X-linkage of the w^+ , (2) Southern analysis of genomic DNA digested separately with *Bam*HI–*Bgl*II, *Hind*III–*Bam*HI, and *Pst*I (Boehringer-Mannheim) and probed with full-length *yellow* sequence, and (3) PCR analysis with primers in *yellow* and *white* (not shown), and primers 1 and 2, which flank the insertion site. (B) Gene replacement (Keeler et al. 1996). $y^{h12w+} w^{1118}$ embryos were injected with template sequences and the transposase-encoding helper plasmid (W. Bender, unpubl.) at concentrations of 1 and 0.25 mg/ml, respectively. Surviving males were crossed to attached-X females and male progeny with altered pigmentation and/or eye color were singly mated to attached-X females to establish lines. These lines were screened by single-fly PCR (Czank 1996) with primers 1 and 2, which gives a 519-bp PCR product, followed by digestion of the product with restriction enzymes diagnostic of the desired changes (Table 1). Candidate lines were verified by sequence and Southern analyses. Sequence analyses were carried out on a 973-bp PCR product generated from genomic DNA with primers 3 and 4. Southern analyses of genomic DNA digested separately with *Hind*III–*Bam*HI, *Pst*I, and *Eco*RI (Boehringer-Mannheim) and hybridized to a full-length *yellow* probe gave patterns identical to those of wild-type flies (data not shown). Symbols are as in Fig. 1; (triangle) P-element insertion; (xxx) mutated sequence; (B) *Bam*HI; (G) *Bgl*II; (E) *Eag*I; (H) *Hind*III; (K) *Kpn*I; (P) *Pst*I; (R) *Eco*RI; (S) *Sal*I.

Table 1. Targeted gene replacement at yellow

A. Alleles generated by targeted gene replacement						
Allele	Motif ^a			Number ^b		
	TATA	Inr	ATG	embryos	males	convertants ^c
y^{tata}	m	+	+	300	51	2
y^{inr}	+	m	+	600	96	5
$y^{tata-inr}$	m	m	+	600	104	5
y^{tata-1}	m	+	m	550	112	5 ^d
y^{inr-1}	+	m	m	600	92	8
$y^{tata-inr-1}$	m	m	m	900	102	10

B. Consensus and mutant sequences						
	TATA box		Inr		ATG	
	C		T			
Consensus ^e	A	GG	G	T T		ATG
Wild-type ^f	<u>GTATAAAAC</u>		<u>ATCAGT</u> CG			<u>ATG</u>
Mutant ^g	<u>ACCCGGGAC</u>		<u>ACATCGAT</u>			<u>GTGCACTG</u>
	<i>SmaI</i>		<i>ClaI</i>			<i>Alw44I</i>

^aThe TATA box, Inr, and ATG translation initiation codon were mutated; (+) wild-type; (m) mutant.

^bShown are the numbers of embryos injected, fertile males obtained, and independent conversion events generated.

^cIn some cases, Southern analysis showed duplicated conversion tracts. Convertants of this type were not used in our complementation studies.

^dTwo of these convertants had changes in the TATA box only.

^eConsensus sequences according to Arkhipova (1995).

^fUnderlined bases in the TATA box, Inr, and ATG motifs correspond to positions -32, +1, and +172.

^gMutated sequences are underlined. The *SmaI* and *ClaI* recognition sites are the same as the mutated sequences. The *Alw44I* recognition site is GTGCAC.

(Goldsborough and Kornberg 1996). To address this issue, we used targeted gene replacement to generate a derivative of y^{tata} that is disrupted post-transcriptionally and, therefore, unable to contribute to a complementing phenotype. This derivative, called y^{tata-1} , bears the A → C change in the translation initiation codon that is found in y^1 (Table 1). As anticipated, homozygous and hemizygous y^{tata-1} flies showed fully mutant pigmentation in all cuticular structures (Table 2). The ability of y^{tata-1} to support transvection was determined by placing it in *trans* to y^2 . We found that y^{tata-1} complements y^2 , with wing and body pigmentation of y^2/y^{tata-1} flies significantly darker than that of either parental line (Table 2). This result demonstrates that when y^{tata-1} complements y^2 , the promoter of y^2 is activated in *trans*.

We asked whether y^{tata-1} could complement alleles other than y^2 by placing y^{tata-1} in *trans* to three additional alleles: y^{62a} , y^{82f29} , and y^{2374} . These alleles are similar to y^2 in that they complement $y^{1\#8}$ and fail to complement y^1 (Morris et al. 1998, 1999), and together with y^2 will be referred to as tester alleles. The y^{62a} allele is structurally disrupted in the vicinity of the wing and body enhancers and, although it produces significant lev-

els of pigmentation in the body, pigmentation in the wings is minimal (Chia et al. 1986; Morris et al. 1999). The y^{82f29} allele is caused by a deletion of the wing and body enhancers and produces very little, if any, pigmentation in the wings and body (Morris et al. 1998, 1999). The y^{2374} allele carries a large insertion between the promoter and the wing and body enhancers, and also produces very little, if any, pigmentation in the wings and body (Morris et al. 1999). We placed y^{tata-1} in *trans* to y^{62a} , y^{82f29} , and y^{2374} and found that it complements each allele (Table 2). These findings demonstrate that the ability of y^{tata-1} to activate a promoter in *trans* is not specific for the y^2 allele.

Our data regarding the TATA box suggest that core promoter elements can participate in the regulation of transvection at *yellow*. To pursue this idea, we determined the role of another potential *yellow* core promoter element, the putative initiator (Inr) that encompasses the transcription start site (Arkhipova 1995; Emami et al. 1997). We replaced 6 bp of the putative Inr with the 6 bp recognition site for the *ClaI* restriction enzyme and called the new allele y^{inr} (Table 1). In contrast to the mutation in the TATA box, mutation of the Inr caused only a slight reduction in *yellow* expression as judged by pigmentation levels (Table 2). Homozygous y^{inr}/y^{inr} flies showed wild-type wing, body, and bristle pigmentation, while hemizygous y^{inr}/Df flies showed only a slight reduction in body pigmentation as compared to pigmentation levels seen in y^+/Df flies.

To determine the effect of the Inr mutation on transvection, we made a companion allele, as was done for y^{tata} . This allele, y^{inr-1} , carried an A → C change in the translation initiation codon in addition to the changes in the Inr and produced a fully mutant pigmentation phenotype. We placed y^{inr-1} in *trans* to the four tester alleles and found that it supports complementation (Table 2). The level of complementation was similar to that seen with y^{tata-1} .

Finally, we made two additional alleles, $y^{tata-inr}$ and $y^{tata-inr-1}$, in which both the TATA box and Inr had been mutated and where $y^{tata-inr-1}$ also carried a mutated translation initiation codon (Table 1). The doubly mutant $y^{tata-inr}/y^{tata-inr}$ flies showed a reduction in wing, body, and bristle pigmentation that was more severe than that seen in flies homozygous for either y^{tata} or y^{inr} (Table 2). These phenotypes are consistent with studies showing functional synergy between the TATA box and Inr (Emami et al. 1997). We placed $y^{tata-inr-1}$ in *trans* to all four tester alleles and observed complementation in each case. The level of complementation was greater than levels seen with either y^{tata-1} or y^{inr-1} (Table 2).

In short, our data show that mutations in the TATA box and Inr induce transvection and that they do so by activating gene expression from a promoter in *trans*. How might promoter mutations in one allele induce expression from the promoter of a paired allele? One attractive explanation proposes that y^{tata} and y^{inr} influence transvection as a direct consequence of their effects on the transcriptional process. As noted earlier, transvection may be regulated by *cis* preference of enhancers for

Table 2. Complementation data

	<i>Df</i>	Homozygous	2	62a	82f29	2374
<i>Df</i>	1, 1	—	1, 1	1, 4	1, 1	1, 1
Homozygous	—	—	1, 1–2	1, 3	1, 1 ^a	1, 1–2
<i>tata</i>	2–3, 1	3, 2	4, 3	4, 4	3, 1–2	4, 3
<i>inr</i>	5, 4	5, 5	5, 5	5, 5	5, 5	5, 5
<i>tata-inr</i>	2, 1	2, 1	4, 3–4	4, 4	3, 1–2	4, 3
<i>tata-1</i>	1, 1	1, 1	3, 2–3	2, 4	2, 1–2	3, 2–3
<i>inr-1</i>	1, 1	1, 1	3, 2–3	2, 4	2, 1–2	3, 2–3
<i>tata-inr-1</i>	1, 1	1, 1	4, 3–4	3, 4	3, 1–2	4, 3
1#8	1, 1	1, 1	4, 4	4, 4	3, 3	4, 4

Pigmentation scores for wings and body (wings, body) when alleles are in *trans* to a deficiency (*Df*), homozygous, or in *trans* to one of the alleles listed at the top. Scores in boldface type indicate complementation. The y^1 allele behaved like *Df* in complementation tests involving each promoter mutation, as did $y^{1\#8}$ in *trans* to y^{tata-1} , y^{inr-1} , and $y^{tata-inr-1}$. Flies bearing $y^{1\#8}$ in *trans* to y^{tata} , y^{inr} , or $y^{tata-inr}$ showed slightly darker pigmentation compared to flies bearing *Df* in *trans* to these three promoter mutations, suggesting that these altered promoters can receive enhancer input in *trans*.

^aThe scores given were all obtained in the laboratory of C.-t.W. We note that y^{82f29}/y^{82f29} flies give a score of 1, 1–2 in the laboratory of P.K.G. The difference may be due to culture condition.

their own promoter. If so, y^{tata} and y^{inr} may induce transvection because disruption of transcription leads to the release of enhancers from *cis* preference. In this way, our observations parallel studies addressing the mechanism by which enhancers select among multiple *cis*-linked promoters (Blackwood and Kadonaga 1998) and indicate that the parameters governing promoter choice in *cis* may also apply in *trans*. Specifically, the commitment of an enhancer for a particular promoter may reflect promoter strength, accessibility (e.g., see Geyer et al. 1990; Martínez-Laborda et al. 1992; Cai and Levine 1995; Hendrickson and Sakonju 1995; Scott and Geyer 1995; Casares et al. 1997; Dillon et al. 1997; Krebs and Dunaway 1998; Sharpe et al. 1998; Sipos et al. 1998; Tilghman et al. 1998; Morris et al. 1999) and/or identity as determined by sequence or associated factors (e.g., see Hirschman et al. 1988; Li and Noll 1994; Hansen and Tjian 1995; Merli et al. 1996; Ohtsuki et al. 1998; Sharpe et al. 1998). Likewise, at *yellow*, mutations in core promoter elements may release *cis*-acting enhancers to act on a promoter in *trans* because they compromise promoter strength through reduction in transcriptional efficiency, decrease promoter accessibility through alterations in chromatin structure or DNA topology, or change promoter identity through disruption of the TATA box or *Inr*. The consequence of such events could be that the intact promoter in *trans* becomes more competitive for the input of the enhancers in *cis* to the promoter mutations. The weakening of a promoter such that it cannot retain transcription factors could also lead to an increased concentration of transcription factors at a paired and functional second promoter, again, causing enhancers to be drawn to the second promoter.

If promoter choice reflects relative promoter appeal, how do *yellow* enhancers act when paired promoters are identical? The inability of y^1 to complement the four tester alleles is informative. As the promoters of the

tester alleles are intact and, at this level, identical to the promoter of y^1 , lack of complementation indicates that *cis* preference dominates. It may be that mechanisms of enhancer–promoter interaction (Blackwood and Kadonaga 1998) favor interactions between *cis*-linked, over unlinked, elements. We note, however, one caveat. The lesions of the tester alleles, while outside the promoter region, may compromise the promoters of the tester alleles such that they are unable to compete effectively with the promoter of y^1 . That is, *cis* preference of y^1 enhancers may be accentuated by a reduced potential of the promoter in *trans*.

How, then, might *yellow* enhancers act when the paired alleles are identical throughout, such as in the case of paired wild-type genes? It is plausible that *cis* action will prevail. Again, arguments of linkage may favor *cis* action. *Trans* action might also be inhibited because a wild-

type promoter that is fully engaged with its *cis* enhancers might not be able to receive input in *trans* (see also Sipos et al. 1998), although this does not appear to be the case at *Ubx* (Martínez-Laborda et al. 1992). It is equally plausible that when faced with identical promoters, enhancers will be free to act in *cis* or *trans*, as has been demonstrated with catenated plasmids in a *Xenopus* oocyte system (Krebs and Dunaway 1998). While our data do not address the situation where genes are identical, they do suggest that enhancers that are able to act in *trans* may still be able to act in *cis*, consistent with the behavior of regulatory elements at the bithorax gene complex (Lewis 1955; and most recently, Goldsborough and Kornberg 1996; Casares et al. 1997; Sipos et al. 1998). In the case of *yellow*, the pigmentation seen in flies that bear a tester allele in *trans* to y^{tata} or y^{inr} is darker than that of flies bearing the tester allele in *trans* to y^{tata-1} or y^{inr-1} (Table 2). This observation indicates that y^{tata} and y^{inr} are contributing directly to the pigmentation levels and, therefore, are transcriptionally productive even as they participate in transvection.

The higher degree of transvection supported by $y^{tata-inr}$ as compared with that supported by y^{tata} or y^{inr} is consistent with transcription-based interpretations because, by pigmentation levels, $y^{tata-inr}$ is more compromised than y^{tata} and y^{inr} (Table 2). The comparable levels of transvection supported by y^{tata} and y^{inr} , though, were not expected as y^{inr} flies are much more pigmented than y^{tata} flies. If pigmentation levels are a good indicator of transcriptional competency, then the similar levels of transvection seen with y^{tata} and y^{inr} may indicate that degree of transvection does not directly correlate with degree of transcription. The actual relationship between levels of transcription and pigmentation, however, is not known for *yellow*.

Transcription-based mechanisms for promoter choice are attractive because the mutations we made are in core

promoter elements. Nevertheless, we are considering the possibility that promoter elements also influence transvection via routes that are independent of the transcriptional process. For instance, transvection might normally be inhibited by the packaging of homologous chromosomes in a way that favors intramolecular over intermolecular interactions (Martínez-Laborda et al. 1992; Wu and Howe 1995; Sipos et al. 1998). If so, small sequence changes, such as y^{tata} and y^{inr} , might alter relative homolog accessibilities or, if sequences within a wild-type promoter are important for homolog sequestration, y^{tata} and y^{inr} might act by disrupting the insular state. Alternatively, the changes in y^{tata} and y^{inr} may influence a promoter in *trans* by virtue of their effect on pairing. For example, if sequences in, or factors associated with, the promoter region are essential for homolog pairing (Jorgensen 1990; Cook 1997; McKee 1998), y^{tata} and y^{inr} may lead to local unpairing of the promoter in *trans*, resulting in its greater accessibility and ultimate activation. Intriguingly, this structural interpretation can explain why y^{tata} and y^{inr} support transvection equally; the magnitude of their lesions is the same. Also consistent with this view are studies suggesting that *yellow* alleles bearing deletions or insertions in the promoter region induce transvection by causing pairing-mediated changes in gene topology (Morris et al. 1998, 1999; Sipos et al. 1998). In fact, in genotypes involving y^2 , such changes may be responsible for a second mode of transvection in which the y^2 wing and body enhancers bypass the *gypsy* insulator to activate the y^2 promoter (Morris et al. 1998). If y^{tata} and y^{inr} can cause this mode of transvection, then insulator bypass may contribute to the complementation observed when these alleles are paired with y^2 .

Finally, we note that none of our promoter mutations achieves the uniformly high level of transvection that is seen with $y^{1\#8}$ (Table 2). It may be that stronger degrees of transvection require maximal disruption of transcription and/or severe structural disruption of the promoter, both of which characterize $y^{1\#8}$. For example, *yellow* may carry promoter elements, in addition to the TATA box and Inr, whose presence inhibits transvection. Alternatively, there may be as-yet-unidentified elements present in our mutant promoter alleles, but absent from $y^{1\#8}$, that are independent of promoter function but which down-regulate transvection. In fact, there is evidence for the ability of special elements, in particular insulators, to confer *cis* preference on enhancer-promoter interactions in plasmid-based assays (Krebs and Dunaway 1998) and for the existence of nonpromoter elements regulating transvection at *Abdominal-B* (Hendrickson and Sakonju 1995; Hopmann et al. 1995; Sipos et al. 1998).

In summary, our data demonstrate that the TATA and Inr promoter elements play key roles in the regulation of transvection at *yellow*. These results are particularly exciting in light of suggestions that the promoter may participate in other pairing-mediated processes (Jorgensen 1990; Lichten and Goldman 1995; Cook 1997; McKee 1998). Specifically, promoter elements have been implicated in meiotic recombination in yeast (Lichten and

Goldman 1995) and in meiotic pairing and segregation of the X and Y chromosomes in *Drosophila* (McKee 1998). The importance of promoter elements in these meiotic events, as well as in transvection-modulated gene expression in nonmeiotic tissues, argues strongly that transcriptional regulatory elements and pairing-mediated processes, while distinct, are intimately linked.

Materials and methods

Drosophila stocks

Df represents the *Df(1)y⁻ ac⁻ w¹¹¹⁸* chromosome, which lacks the entire *yellow* gene (Morris et al. 1998). The *w¹¹¹⁸* marker is a null allele of the *white* eye color gene, and the *Cy* wing and *Sb* bristle mutations are dominant markers on the *CyO* second chromosome balancer and *TMS* third chromosome balancer, respectively (Lindsley and Zimm 1992). For simplicity, Figure 2 notes the dominant markers but does not give the names of the balancer chromosomes. The attached X chromosome we used was *FMA3* (Lindsley and Zimm 1992). *P[w⁺]* is a transposon carrying a wild-type *w⁺* gene, and *P[ry⁺ Δ2-3]99B*, abbreviated Δ2-3, is a transposon encoding transposase (Keeler et al. 1996 and references within).

Culture condition

Flies were cultured at 25 ± 1°C on standard *Drosophila* cornmeal, yeast, sugar, and agar medium with *p*-hydroxybenzoic acid methyl ester added as a mold inhibitor. Three females were mated with three or more males in vials and brooded daily. Temperature and crowding were carefully controlled as both affect pigmentation.

Scoring of pigmentation

Pigmentation was scored in 1- to 3-day-old flies on a scale of 1–5, where 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⁻ w¹¹¹⁸*, and the wild-type phenotype is defined by the pigmentation seen in our wild-type Canton S strain. The wing pigmentation score refers to pigmentation of the wing blade but not the wing bristles, and body pigmentation refers to pigmentation of the abdominal stripes. At least two independent crosses were set up for each genotype. Complementation between two alleles was judged to occur when wing or body pigmentation was at least one point darker on the pigmentation scale than the pigmentation of analogous tissues in females that were homozygous for either allele and females that were heterozygous for either allele and *Df*.

Template construction

The templates used for gene replacement were full-length *yellow* genes, carrying appropriate sequence changes, cloned into a Bluescript plasmid (Stratagene). The following constructs are named according to the *yellow* allele that they were used to generate. pUC8ySB is a pUC8 plasmid containing the 5' 3.1-kb *SalI*–*Bam*HI *yellow* fragment, pBSyBG is a Bluescript plasmid containing the 3' 4.7-kb *Bam*HI–*Bgl*II *yellow* fragment, and pUC8ySG is a pUC8 plasmid containing the entire *yellow* gene in a 7.8-kb *SalI*–*Bgl*II fragment. For the *tata* construct, primers 3 and 5 (see below) were used with pUC8ySG to PCR amplify a product that was digested with *Kpn*I and *Eag*I and then cloned into the *Kpn*I and *Eag*I sites of pUC8ySB to make pUC8ySB_{tata}. The *SalI*–*Bam*HI *yellow* fragment of pUC8ySB_{tata} was then cloned into the *Sal*I and *Bam*HI sites of pBSyBG to generate a full-length *yellow* gene. For the remaining constructs, the following strategy was followed. Primers were used to PCR amplify regions of pUC8ySG, and the products were digested with *Eag*I and *Bam*HI and cloned into those sites of either pUC8ySB or pUC8ySB_{tata}. The resulting plasmids were digested with *Sal*I and *Bam*HI and the *yellow* sequences cloned into those sites of pBSyBG. For the *tata*–1 construct, primers 3 and 6 were used, and the digested PCR product was cloned into pUC8ySB_{tata}. For the *inr* and *tata*–*inr* constructs, primers 7 and 8 were used, and the digested PCR products were cloned into pUC8ySB and pUC8ySB_{tata}, respectively. For the *inr*–1 and *tata*–*inr*–1 constructs, primers 7 and 6 were used, and the digested PCR products were cloned into pUC8ySB and pUC8ySB_{tata}, respectively.

Primer sequences

The primer sequences used in this study are indicated below. Mutant nucleotides are noted in boldface type and the position of the 5' base is noted in parentheses after the primer number: 1 (–448) 5'-GAGCCTCCTGGCCTTACAATTAC; 2 (+71) 5'-GTTCTTCTCTAGCCTTCGGCTGTG; 3 (–547) 5'-GCAGTCGCCGATAAAGATGAACACAG; 4 (+426) 5'-ATTAACTCCACTTACCATCACGCC; 5 (–7) 5'-CATAATATGTCGGCCGCGT**CCCGGGT**GAAAGTTTCTCCGAAGACGAAGACAG; 6 (+206) 5'-GTCACAAGGATCCACCCCTTGTCTCTGGAACAGTGCACCTAGCTCTAAGCTGACAATCAC; 7 (–30) 5'-ATAAAACGCGCCGACATATTATGGCCAC**ATCGATT**TACCGCGCCACGGTCCACAGAAG; 8 (+478) 5'-TCTGATGCCCTAAGTAAGAATGTCCC.

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