GAGA mediates the enhancer blocking activity of the *eve* promoter in the *Drosophila* embryo

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Insulator DNAs and promoter competition regulate enhancer–promoter interactions within complex genetic loci. A transgenic embryo assay was used to obtain evidence that the *Drosophila eve* promoter possesses an insulator activity that can be uncoupled from the core elements that mediate competition. The *eve* promoter contains an optimal TATA element and a GAGA sequence. The analysis of various chimeric promoters provides evidence that TATA is essential for promoter competition, whereas GAGA mediates enhancer blocking. The Trithorax-like (Trl) protein interacts with GAGA, and mutations in *trl* attenuate *eve* promoter insulator activity. We suggest that Trl–GAGA increases the stability of enhancer–promoter interactions by creating an open chromatin configuration at the core promoter.

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The two major Hox gene clusters in *Drosophila*, the Antennapedia complex (ANT-C) and the Bithorax complex (BX-C), contain >100–200 kb of *cis* regulatory DNA (e.g., Gorman and Kaufman 1995; Martin et al. 1995). How do the right enhancers interact with the proper promoters? This '*cis* trafficking' depends on at least two regulatory mechanisms—insulator DNAs and promoter competition.

Insulators were first identified in the flanking regions of the *Drosophila hsp70* locus (Kellum and Schedl 1991, 1992). They are thought to organize *hsp70* within a chromatin loop so that the heat-induced activation of *hsp70* does not influence the regulation of neighboring genes and vice versa. Insulators selectively block interactions of distal, not proximal, enhancers with a target promoter (Cai and Levine 1995; Scott and Geyer 1995).

The best characterized insulator is located within the gypsy retrotransposon, which is 340 bp in length and located just downstream of the gypsy 5′ LTR. This insulator contains clustered binding sites for the Suppressor of Hairy wing [Su(Hw)] zinc finger protein, which in turn recruits Mod(mdg4), a protein that suppresses position effect variegation (Gerasimova et al. 1995; Gerasimova and Corces 1998). Insulators have been identified within the BX-C (Hagstrom et al. 1996; Zhou et al. 1996; Mihaly et al. 1997), where they have been proposed to organize the extensive *cis* regulatory DNA into a series of separate chromatin loop domains (e.g., Vasquez et al. 1993).

Promoter competition was first identified in the chicken globin gene cluster (Choi and Engel 1988; Foley and Engel 1992). In principle, a shared enhancer can activate multiple genes but selects the promoter region of just one. Activation of the preferred gene precludes expression of the neighboring genes. Promoter competition has been implicated in the regulation of the *Sex combs reduced* (*Scr*) and *fushi tarazu* (*ftz*) genes within the ANT-C (Ohtsuki et al. 1998). The *ftz* autoregulatory enhancer (AE1) is located between the divergently transcribed *Scr* and *ftz* genes but selectively interacts with *ftz* (Pick et al. 1990; Schier and Gehring 1992). This regulatory specificity depends on core promoter elements, particularly TATA. The type 1 *ftz* promoter contains TATA but lacks the downstream promoter element (Dpe) (Laughon and Scott 1984), whereas type 2 promoters contain initiator (Inr) (Smale 1997) and/or Dpe sequences (Burke and Kadonaga 1996, 1997) but lack TATA (Ohtsuki et al. 1998). Some enhancers, such as AE1, preferentially activate type 1 promoters when given a choice between linked type 1 and type 2 promoters. Others, such as the *rhomboid* (*rho*) neuroectoderm enhancer (NEE), promiscuously activate both classes of promoters (Ohtsuki et al. 1998). The regulation of mammalian Hox genes also depends on promoter competition (e.g., Herault et al. 1997; Sharpe et al. 1998).

Here we present evidence that the type 1 *even-skipped* (*eve*) promoter possesses an insulator activity, which can be uncoupled from the TATA, Inr, and Dpe core elements. Mutations in a GAGA element, located between TATA and the transcription start site, impair this insulator activity, so that genes residing 5′ of an otherwise normal *eve* promoter are now activated by a 3′ enhancer. Similar results were obtained in *trithorax-like* (*trl*) mutants that diminish the levels of the Trl protein. Mutations in GAGA do not diminish *eve* promoter function in competition assays. We suggest that Trl–GAGA traps distal enhancers by stabilizing enhancer–promoter interactions.

Results and Discussion

In the following experiments, *white*, *CAT*, and *lacZ* reporter genes were placed under the control of the type 1 *eve* promoter and type 2 *white* promoter, as well as various modified and chimeric promoter sequences. The IAB5 enhancer was used to monitor the activities of these different promoters in transgenic embryos via in situ hybridization. The 1-kb IAB5 enhancer directs expression in the presumptive abdomen of early embryos, and like AE1, it preferentially activates the *eve* promoter

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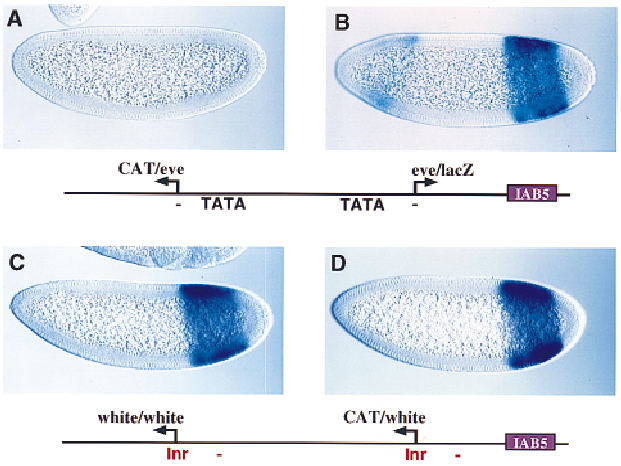

Figure 1. The *eve* promoter possesses an enhancer blocking activity. Transgenic embryos are undergoing cellularization and are oriented with anterior to the *left* and dorsal *up*. Embryos were hybridized with digoxigenin-labeled antisense *white*, *CAT*, and *lacZ* RNA probes and stained with anti-digoxigenin antibodies. (*A*) Embryo contains the *eve/CAT–eve/lacZ* P-transformation vector indicated in the diagram. It was hybridized with a *CAT* probe to monitor the expression of the distal *eve/CAT* reporter gene. Replacing the proximal *eve* promoter with the *white* promoter sequence results in the full induction of *eve/CAT* expression (see Fig. 2A). (*B*) Same as *A* except that the embryo was hybridized with a *lacZ* probe to monitor the expression of the proximal *eve/lacZ* reporter gene. The weak staining in head regions is due to the P-transformation vector used in these experiments (Small et al. 1992). (*C*) Embryo contains the *white/white–white/CAT* P-transformation vector indicated in the diagram. It was hybridized with a *white* probe to monitor expression of the distal *white/white* reporter gene. (*D*) Same as *C* except that a *CAT* probe was used to monitor the expression of the proximal *white/CAT* reporter gene.

when given a choice between *eve* and *white* (Ohtsuki et al. 1998).

When the *CAT* and *lacZ* reporter genes were both placed under the control of the *eve* promoter (Fig. 1A,B), IAB5 selectively activates the proximal *eve/lacZ* gene (Fig. 1B) but fails to activate the distal *eve/CAT* gene (Fig. 1A). In contrast, IAB5 is nearly equally effective at activating both a proximal *white/CAT* reporter gene (Fig. 1D) and a distal *white/white* reporter gene (Fig. 1C). These results indicate that the *eve* promoter, but not *white*, possesses an enhancer blocking activity.

Various *white–eve* chimeric promoters were examined to identify the sequences in *eve* that mediate enhancer blocking. One of these, $white^{eve}$, contains 5′ sequences (TATA) from *eve* and 3′ sequences (Inr) from *white*. $white^{eve}$ contains optimal TATA and Inr elements but is not only virtually inactive (Fig. 2D) but also functions in a dominant-negative fashion to attenuate the activation of a linked *eve/CAT* gene (Fig. 2C). In this experiment the wild-type *eve* promoter was placed upstream of a distal *CAT* reporter gene while the chimeric $white^{eve}$ promoter was attached to the proximal *lacZ* reporter gene. The residual staining directed by *eve/CAT* (Fig. 2C) is substantially reduced as compared with control embryos (Fig. 2A). Thus, it would appear that the chimeric $white^{eve}$ promoter uncouples enhancer looping and transcriptional activation; it possesses enhancer blocking activity, even though it is essentially inactive.

The $white^{eve}$ promoter was mutagenized to identify the sequences responsible for enhancer blocking activity. Mutations in the $white^{eve}$ TATA sequence resulted in only a slight increase in *eve/CAT* activity (data not shown). The *eve* and $white^{eve}$ promoters contain a

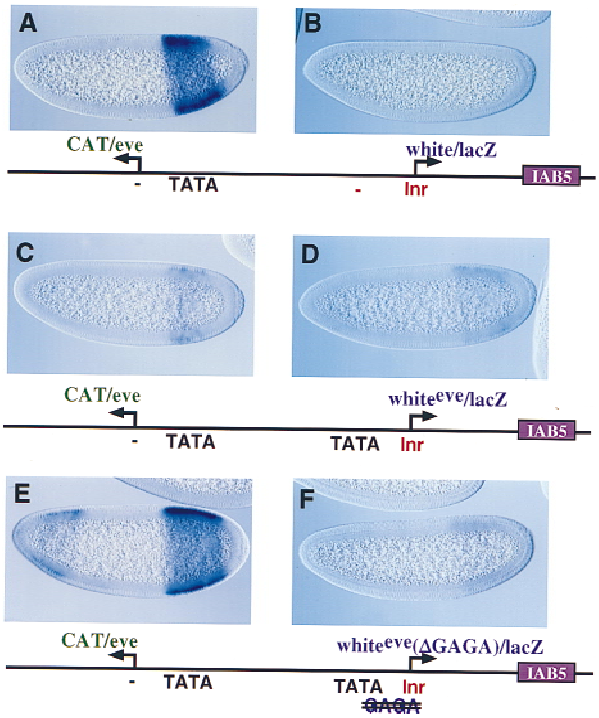

Figure 2. A chimeric *eve–white* promoter functions in a dominant-negative fashion to block linked genes. Transgenic embryos, oriented with anterior to the *left* and dorsal *up*, are at various stages of cellularization and express the transgenes shown in the diagrams beneath the photomicrographs. All embryos shown were stained in parallel, thereby permitting a direct comparison of expression levels. (*A,B*) *eve/CAT–white/lacZ* transgenic embryos with the *eve* promoter upstream of the distal *CAT* reporter gene and the *white* promoter attached to the proximal *lacZ* gene. The distal *CAT* gene is fully activated by the 3′ IAB5 enhancer (*A*) and exhibits intense expression in the presumptive abdomen. In contrast, hybridization with a *lacZ* probe indicates that the proximal *white/lacZ* gene is inactive. (*C,D*) Embryos carry a P-transformation vector that is similar to the one shown in *A* and *B* except that the proximal *white* promoter was replaced with the chimeric promoter, $white^{eve}$. This promoter contains the 5′ TATA region from *eve* and the 3′ Inr region from *white*, as indicated in the diagram. (*D*) Hybridization with the *lacZ* probe reveals that the $white^{eve}$ promoter is virtually inactive. (*C*) Hybridization with the *CAT* probe demonstrates that expression of the distal *eve/CAT* gene, which contains a completely wild-type *eve* promoter, is severely attenuated. (*E,F*) Same as *C* and *D* except that the $white^{eve}$ promoter was mutagenized to disrupt the GAGA element (TATA is intact; see diagram). The modified promoter essentially fails to direct expression of the *lacZ* reporter (*F*). However, the distal *eve/CAT* reporter gene is nearly fully active (*E*; cf *A*).

GAGA sequence located between TATA and the transcription start site. A single nucleotide substitution in the *white*^{eve} GAGA results in nearly normal levels of *eve/CAT* expression (Fig. 2E, cf. A). These results suggest that GAGA is responsible for the enhancer blocking activity of *white*^{eve}, and additional experiments were done to determine whether it has a similar role in the wild-type *eve* promoter.

Disruption of the *eve* GAGA element permits the activation of *eve/CAT* (Fig. 3A,B), without impairing the expression of the mutagenized *eve/lacZ* gene (Fig. 3B, cf. D). GAGA is bound by the Trl protein, which is maternally expressed and distributed throughout early embryos (Farkas et al. 1994; Wilkins and Lis 1997). Mutations in the GAGA element located in the *white*^{eve} (GAGAG–GAGAT) and *eve* promoters (GAGAG–CACGT) severely reduce Trl binding in gel shift assays (V. Calhoun and M. Levine, unpubl.). Additional evidence for Trl–GAGA interactions stems from gene dosage assays. Females heterozygous for the R85 mutation of *trl* were mated with wild-type males carrying the *eve/CAT–eve/lacZ* transgene (Fig. 3E,F). Normally, the proximal *eve* promoter blocks the activation of *eve/CAT* (Fig. 3C,D). However, the reduction in *trl*$^{+}$ activity allows the IAB5 enhancer to activate both the proximal *eve/lacZ* gene (Fig. 3F) and the distal *eve/CAT* reporter (Fig. 3E).

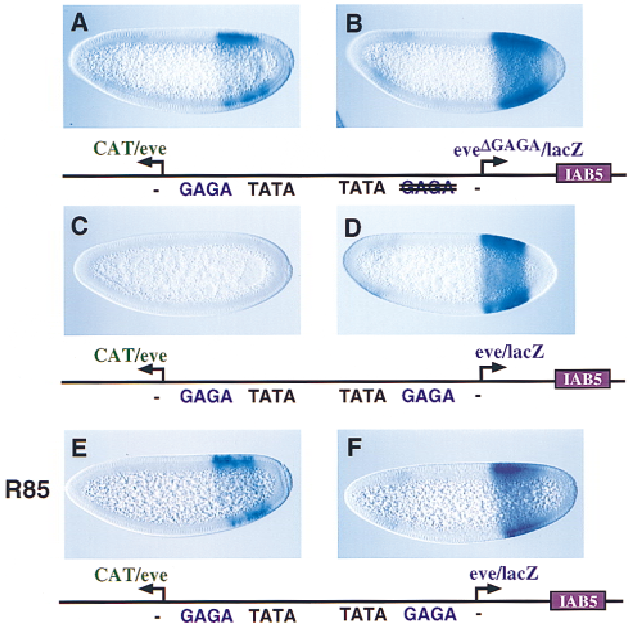

Figure 3. GAGA–Trl interactions mediate the enhancer blocking activity of the *eve* promoter. Transgenic embryos carry the indicated P-transformation vectors and were hybridized and oriented as described. (*A*,*B*) Embryos express *CAT* and *lacZ* transgenes that are both driven by the *eve* promoter, except that the proximal *eve/lacZ* reporter gene was mutagenized to disrupt the GAGA element (see diagram). The distal *eve/CAT* gene exhibits moderate levels of expression (*A*, cf. *C*); the proximal *eve/lacZ* gene is expressed at normal levels (*B*, cf. *D*). It is conceivable that the residual enhancer blocking activity is due to a 'cryptic' GAGA element located in the transcribed region. (*C*,*D*) Same as *A* and *B* except that both the proximal *lacZ* and distal *CAT* genes are attached to normal *eve* promoter sequences (same as Fig. 1A,B). The proximal, wild-type *eve* promoter (*D*) has full enhancer blocking activity so that the distal *eve/CAT* gene is silent (*C*). (*E*,*F*) Same as *C* and *D* except that the transgene was crossed into an embryo derived from an R85/+ heterozygous female. R85 is a null mutation in the *trl* gene, so that these embryos contain half the normal dose of *trl*$^{+}$ gene activity. This reduction in Trl attenuates the enhancer blocking activity of the proximal, wild-type *eve* promoter (*F*), so that the distal *eve/CAT* gene is active (*E*). The levels of *eve/CAT* expression are similar to those obtained when the proximal GAGA element was mutagenized (*A*). These results suggest that Trl–GAGA interactions are critical for the enhancer blocking activity of the *eve* promoter.

The IAB5 enhancer preferentially interacts with the TATA-containing *eve* promoter, even when the TATA-less *white* promoter is inserted between IAB5 and *eve* (Ohtsuki et al. 1998; see Fig. 2A). Additional assays were conducted to determine whether GAGA participates in this promoter competition process. The IAB5 enhancer was placed 5′ of the divergently transcribed *CAT* and *lacZ* reporter genes (Fig. 4). It strongly activates the rightward *eve/lacZ* gene (Fig. 4B) but only weakly activates *white/CAT* (Fig. 4A). A mutagenized form of the *eve* promoter, which lacks the GAGA element, is equally effective in mediating IAB5 activity (Fig. 4D) and attenuating *white/CAT* expression (Fig. 4C). The *white* promoter used in these assays is fully active, replacing IAB5 with the promiscuous *rho* NEE leads to lateral stripes of both *white/CAT* and *eve/lacZ* expression (data not shown). Moreover, the *white/CAT* reporter gene is fully active in the absence of the *eve* promoter (see Fig. 1D).

These results suggest that GAGA is not essential for *eve* versus *white* promoter competition. However, it is conceivable that the *white* promoter is inherently 'weak;' perhaps GAGA is required for competition between equally 'strong' promoters. This was tested by placing IAB5 5′ of *eve/CAT* and *eve/lacZ* reporter genes. The genes are expressed at similar levels (Fig. 4E,F), even when the GAGA element is mutagenized in the rightward *eve* promoter (Fig. 4G,H). Thus, the mutagenized *eve*$^{\Delta GAGA}$ promoter is not generally weakened or impaired in promoter competition but is specifically defective in enhancer blocking activity (Fig. 3A,B).

The *eve* promoter contains TATA and GAGA elements. We have presented evidence that GAGA is essential for enhancer trapping, whereas TATA mediates promoter competition (Ohtsuki et al. 1998). Several observations suggest that these activities can be uncoupled. The *eve*$^{\Delta GAGA}$ promoter is impaired in enhancer blocking (Fig. 3) but is just as effective as the wild-type *eve* promoter in competition assays (Fig. 4). A modified *white* promoter containing a synthetic TATA element is nearly as active as a linked *eve* promoter but lacks enhancer trapping activity (Fig. 5A,B; Ohtsuki et al. 1998). This *white*^{TATA} promoter acquires enhancer blocking activity upon insertion of a GAGA sequence (Fig. 5C,D), as judged by the diminished expression of the distal *eve/CAT* gene (Fig. 5C, cf. A). In this experiment, GAGA was

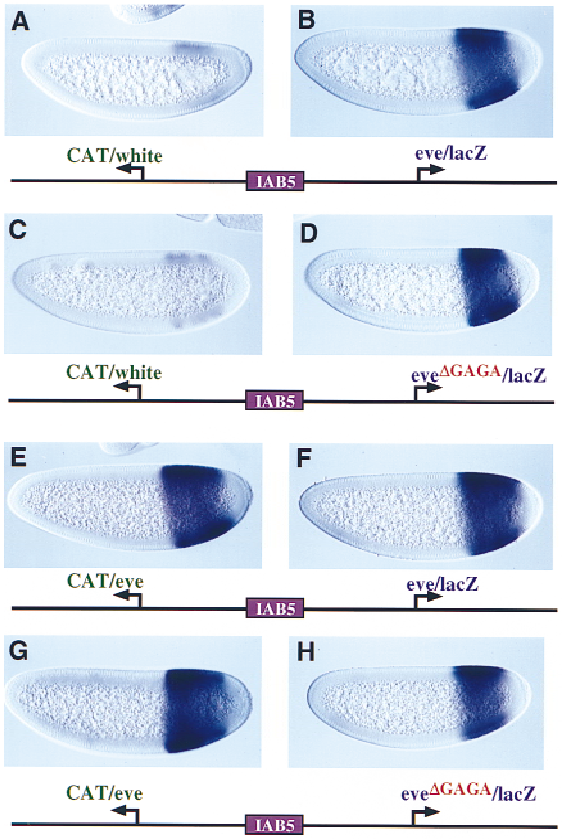

Figure 4. Uncoupling competition and enhancer trapping. The IAB5 enhancer was placed 5′ of the divergently transcribed *CAT* and *lacZ* reporter genes. Transgenic embryos were oriented as described and stained with *CAT* (*left*) or *lacZ* (*right*) probes. (*A*,*B*) The transgene contains the *white* and *eve* promoters. As shown previously (Ohtsuki et al. 1998), IAB5 preferentially interacts with the TATA-containing *eve* promoter (*B*) and only weakly activates the TATA-less *white* promoter (*A*). (*C*,*D*) Same as *A* and *B* except that the rightward *eve* promoter was mutagenized to eliminate the GAGA element. The resulting promoter, $eve^{\Delta GAGA}$, contains TATA and continues to be the preferred target of the IAB5 enhancer (*D*). (*E*,*F*) The *CAT* and *lacZ* reporter genes are regulated by the wild-type *eve* promoter. Both reporter genes are strongly activated by IAB5 and exhibit intense staining in the presumptive abdomen. (*G*,*H*) Same as *E* and *F* except that the rightward *eve* promoter was mutagenized to eliminate the GAGA element. The resulting $eve^{\Delta GAGA}$ promoter continues to direct intense expression of *lacZ* (*H*) and is essentially as active as the distal *eve* promoter (*G*).

inserted between the TATA and Inr elements, and the resulting promoter ($white^{TATA+GAGA}$) functions in a dominant-negative fashion, as seen for $white^{eve}$ (Fig. 2). However, the insertion of GAGA 5′ of TATA results in a modified promoter ($white^{GAGA+TATA}$) that possesses enhancer trapping activity (Fig. 5E, cf. A) but is fully active (Fig. 5F, cf. D). These results suggest that the dominant-negative activities of the $white^{eve}$ and $white^{TATA+GAGA}$ promoters depend on the positioning of GAGA between optimal TATA and Inr elements.

Promoters that possess enhancer blocking activities should facilitate the orderly trafficking of *cis*-regulatory elements. For example, *eve* stripe enhancers located 3′ of the transcription unit should be unable to interact with neighboring genes located 5′ of *eve*. Similarly, the *ftz* promoter contains a GAGA element located 5′ of TATA. Based on our analysis of $white^{eve}$, $white^{TATA+GAGA}$, and $white^{GAGA+TATA}$, this configuration of core elements should allow the *ftz* promoter to be both transcriptionally active and able to block distal enhancers. Perhaps the *ftz* promoter helps inhibit interactions between 3′ *Antp* enhancers and 5′ homeotic genes [*Dfd* (*Deformed*) and *Scr*] within the ANT-C. It is conceivable that many promoters possess an intrinsic enhancer blocking activity. Inspection of ~250 *Drosophila* promoter sequences (Arkhipova 1995) reveals that ~15% contain at least one optimal GAGA element within 50 bp 5′ of the transcrip-

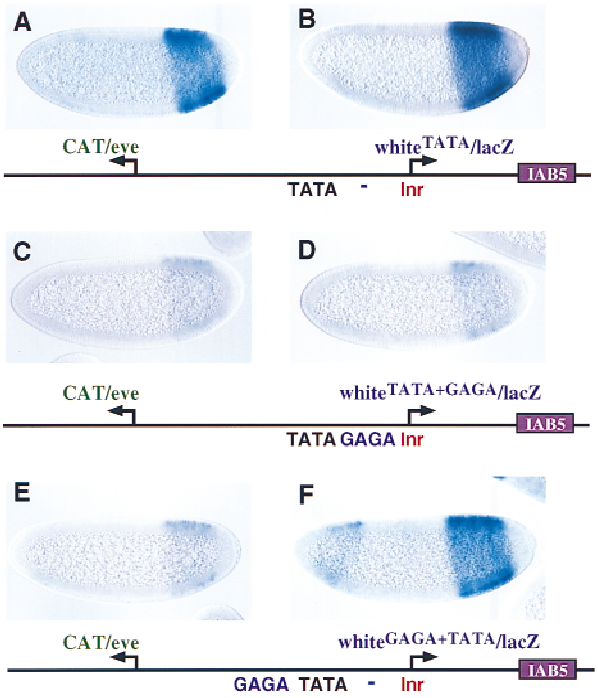

Figure 5. Changing the location of GAGA. Transgenic embryos carry the indicated P-transformation vectors and were hybridized and oriented as described. Embryos were stained in parallel, thereby alowing direct comparison of relative expression levels. (*A*,*B*) The distal *CAT* gene is under the control of the normal *eve* promoter; the proximal *lacZ* gene was attached to a modified *white* promoter containing an optimal TATA element (see Ohtsuki et al. 1998). The 3′ IAB5 enhancer interacts with both promoters to direct nearly equal levels of *CAT* (*A*) and *lacZ* (*B*) expression. The normal *white* promoter, lacking TATA, is not activated by IAB5 in similar transgenes (e.g., Fig. 2B). (*C*,*D*) Same as *A* and *B* except that the proximal $white^{TATA}$ promoter was further modified to include a GAGA element between TATA and the Inr. This promoter exhibits the same type of dominant-negative activity as $white^{eve}$ (see Fig. 2D). (*E*,*F*) Same as *C* and *D* except that the GAGA element was placed 28 bp 5′ of TATA. The resulting promoter, $white^{GAGA+TATA}$, traps the IAB5 enhancer so that *CAT* expression (*E*) is weak (cf. *A*). However, unlike the $white^{TATA+GAGA}$ promoter (*D*), this promoter is fully active (*F*).

tion start site. An earlier analysis of one of these promoters, *α1-tubulin*, indicates that GAGA helps insulate tubulin expression from position effects (O'Donnell et al. 1994).

The enhancer blocking activity of the *eve* promoter appears to be mediated by interactions of Trl with GAGA. Trl has been shown to recruit the NURF protein complex, which facilitates the binding of upstream activators or core polymerase II components by decondensing chromatin (Tsukiyama and Wu 1997; Wilkins and Lis 1997). Trl–GAGA might trap distal enhancers by increasing the stability of enhancer–promoter interactions through the creation of an open chromatin configuration or by increasing the occupancy of core Pol II components such as TFIID.

Materials and methods

P-transformation assays and genetic crosses

yw^{67} flies were used for all P-transformation assays. Fusion genes were introduced into the *Drosophila* germ line as described by Small et al. (1992). Between three and seven independent transformed lines were generated for each construct, and at least three separate lines were examined by in situ hybridization. Embryos were collected, fixed, and hybridized with digoxigenin-labeled *white*, *CAT*, and *lacZ* antisense RNA probes exactly as described (Tautz and Pfeifle 1989; Small et al. 1992) .

The genetic cross used in the experiment presented in Figure 3 (E,F) was done as follows. Females heterozygous for the R85 *trl* mutant (Bhat et al. 1996) were mated with yw^{67} transgenic males carrying the *eve/CAT–eve/lacZ* transgene. F_1 embryos were collected and fixed, and hybridized as described previously (Tautz and Pfeifle 1989; Jiang et al. 1991). The reciprocal cross, yw^{67} transgenic females mated with R85/+ males, does not impair the enhancer blocking activity of the proximal *eve* promoter (S. Ohtsuki, unpubl.). This observation suggests that maternal Trl products interact with *eve*.

Preparation of P-transformation vectors

The mini-*white* promoter region that was used in this study extends from –316 bp upstream of the transcription start site to +174 bp. The *eve* promoter extends from –34 bp to +166 bp. The chimeric $white^{eve}$ promoter was prepared by fusing 5′ *eve* promoter sequences, from –31 to –1 bp, with 3′ *white* sequences (–1 to +174 bp). This fusion was made by PCR and confirmed by DNA sequence analysis. The mutagenized $white^{eve}$ promoter lacking TATA was prepared with a mutagenic oligonucleotide that converts the sequence GTATAAAAG into GGAGCAAAG. The mutagenized $white^{eve}$ promoter lacking GAGA was prepared with a mutagenic oligonucleotide that converts the sequence TGAGAGCAGTT into TGAGATCAGTT (the +1 site is underlined). This mutation converts the G residue at position 6 of the GAGAG motif into T. Previous studies have shown that this substitution markedly reduces the binding of the Trl protein (Omichinski et al. 1997). The GAGA element was disrupted in the *eve* promoter with a mutagenic oligonucleotide that converts the sequence TGAGAGCA into TCACTGCA (the +1 site is underlined).

The *white/CAT/lacZ* P-transformation vector that was used for all the experiments presented in this study is a modification of pCasPer, which contains divergently transcribed *white* and *lacZ* reporter genes (Small et al. 1992). It was modified by insertion of a *CAT* reporter gene between *white* and *lacZ*, as described by Ohtsuki et al. (1998).

The *eve*, *white*, $white^{eve}$, and various modified promoters were isolated as *Asc*I–*Bam*HI fragments and cloned into a unique *Bam*HI site located at the 5′ end of either the *CAT* or *lacZ* coding sequence present in pBluescript vectors. The *CAT* fusion genes were subsequently isolated as *Asc*I–*Not*I fragments and used to replace the *Asc*I–*Not*I *CAT* fragment in the pCasPer vector. The *lacZ* fusion genes were isolated as *Asc*I–*Xba*I fragments and used to replace the *Asc*I–*Xba*I *lacZ* fragment in the pCasPer vector. For most of the experiments, the IAB5 enhancer was isolated as a 1-kb *Pst*I–*Pst*I fragment and cloned into a unique *Pst*I site located 3′ of the *lacZ* reporter gene. IAB5 was placed in a 5′ position of the vector (Fig. 4) by isolating it as a 1-kb *Asc*I–*Asc*I fragment and cloning it into the unique *Asc*I site located between the divergently transcribed *CAT* and *lacZ* genes located in the pCasPer vector.

The *eve/CAT–*$white^{TATA}$*/lacZ* transgene used in Figure 5 (A,B) is described in Ohtsuki et al. (1998). GAGA was inserted either 3′ (Fig. 5C,D) or 5′ (Fig. 5E,F) of TATA by PCR mutagenesis. The 3′ GAGA was created by altering nucleotides between –7 and –3 bp upstream of the transcription start site (CGCCT–GAGAG). The 5′ GAGA was made by altering nucleotides between –61 and –57 bp upstream of the start site (CTGCG–GAGAG).

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References

Arkhipova, I.R. 1995. Promoter elements in Drosophila melanogaster revealed by sequence analysis. *Genetics* **139:** 1359–1369.

Bhat, K.M., G. Farkas, F. Karch, H. Gyurkovics, J. Gausz, and P. Schedl. 1996. The GAGA factor is required in the early Drosophila embryo not only for transcriptional regulation but also for nuclear division. *Development* **122:** 1113–1124.

Burke, T.W. and J.T. Kadonaga. 1996. Drosophila TFIID binds to a conserved downsteam basal promoter element that is present in many TAT-box-deficient promoters. *Genes & Dev.* **10:** 711–724.

———. 1997. The downstream core promoter element, DPE, is conserved from *Drosophila* to humans and is recognized by TAFII60 of *Drosophila. Genes & Dev.* **11:** 3020–3031.

Cai, H. and M. Levine. 1995. Modulation of enhancer-promoter interactions by insulators in the Drosophila embryo. *Nature* **376:** 533–536.

Choi, O.R. and J.D. Engel. 1988. Developmental regulation of beta-globin gene switching. *Cell* **55:** 17–26.

Dorsett, D. 1993. Distance-independent inactivation of an enhancer by the suppressor of Hairy-wing DNA-binding protein of Drosophila. *Genetics* **134:** 1135–1144.

Farkas, G., J. Gausz, M. Galloni, G. Reuter, H. Gyurkovics, and F. Karch. 1994. The Trithorax-like gene encodes the Drosophila GAGA factor. *Nature* **371:** 806–808.

Foley, K.P. and J.D. Engel. 1992. Individual stage selector element mutations lead to reciprocal changes in β vs. ϵ-globin gene transcription: Genetic confirmation of promoter competition during globin gene switching. *Genes & Dev.* **6:** 730–744.

Gerasimova, T.I. and V.G. Corces. 1998. Polycomb and trithorax group proteins mediate the function of a chromatin insulator. *Cell* **92:** 511–521.

Gerasimova, T.I., D.A. Gdula, D.V. Gerasimov, O. Simonova, and V.G. Corces. 1995. A Drosophila protein that imparts directionality on a chromatin insulator is an enhancer of position-effect variegation. *Cell* **82:** 587–597.

Geyer, P.K. 1997. The role of insulator elements in defining domains of gene expression. *Curr. Opin. Genet. Dev.* **7:** 242–248.

Geyer, P.K. and V.G. Corces. 1992. DNA position-specific repression of transcription by a *Drosophila* zinc finger protein. *Genes & Dev.* **6:** 1865–1873.

Gorman, M.J. and T.C. Kaufman. 1995. Genetic analysis of embryonic cis-acting regulatory elements of the Drosophila homeotic gene sex combs reduced. *Genetics* **140:** 557–572.

Hagstrom, K., M. Muller, and P. Schedl. 1996. Fab-7 functions as a chromatin domain boundary to ensure proper segment specification by the *Drosophila* bithorax complex. *Genes & Dev.* **10:** 3202–3215.

Henikoff, S. 1996. Dosage-dependent modification of position-effect variegation in Drosophila. *BioEssays* **18:** 401–409.

Herault, Y., N. Fraudeau, J. Zakany, and D. Duboule. 1997. Ulnaless (Ul), a regulatory mutation inducing both loss-of-function and gain-of-

function of posterior Hoxd genes. *Development* **124:** 3493–3500.
Jiang, J., D. Kosman, Y.T. Ip, and M. Levine. 1991. The dorsal morphogen gradient regulates the mesoderm determinant *twist* in early *Drosophila* embryos. *Genes & Dev.* **5:** 1881–1891.
Kellum, R. and P. Schedl. 1991. A position-effect assay for boundaries of higher order chromosomal domains. *Cell* **72:** 741–752.
———. 1992. A group of scs elements function as domain boundaries in an enhancer-blocking assay. *Mol. Cell. Biol.* **12:** 2424–2431.
Laughon, A. and M.P. Scott. 1984. Sequence of a Drosophila segmentation gene: protein structure homology with DNA-binding proteins. *Nature* **310:** 25–31.
Martin, C.H., C.A. Mayeda, C.A. Davis, C.L. Ericsson, J.D. Knafels, D.R. Mathog, S.E. Celniker, E.B. Lewis, M.J. Palazzolo. 1995. Complete sequence of the bithorax complex of Drosophila. *Proc. Natl. Acad. Sci.* **92:** 8398–8402.
Mihaly, J., J. Hogga, I. Gausz, H. Gyurkovics, and F. Karch. 1997. In situ dissection of the Fab-7 region of the bithorax complex into a chromatin domain boundary and a Polycomb-response element. *Development* **124:** 1809–1820.
O'Donnell, K.H., C.T. Chen, and P.C. Wensink. 1994. Insulating DNA directs ubiquitous transcription of the Drosophila melanogaster alpha 1-tubulin gene. *Mol. Cell. Biol.* **14:** 6398–6408.
Ohtsuki, S., M. Levine, and H.N. Cai. 1998. Different core promoters possess distinct regulatory activities in the *Drosophila* embryo. *Genes & Dev.* **12:** 547–556.
Omichinski, J.G., P.V. Pedone, G. Felsenfeld, A.M. Gronenborn, and G.M. Clore. 1997. The solution structure of a specific GAGA factor-DNA complex reveals a modular binding mode. *Nat. Struct. Biol.* **4:** 122–132.
Pick, L., A. Schier, M. Affolter, T. Schmidt-Glenewinkel, and W.J. Gehring. 1990. Analysis of the ftz upstream element: Germ layer-specific enhancers are independently autoregulated. *Genes & Dev.* **4:** 1224–1239.
Schier, A.F. and W.J. Gehring. 1992. Direct homeodomain-DNA interaction in the autoregulation of the *fushi tarazu* gene. *Nature* **356:** 804–807.
Scott, K.S. and P.K. Geyer. 1995. Effects of the su(Hw) insulator protein on the expression of the divergently transcribed Drosophila yolk protein genes. *EMBO J.* **14:** 6258–6267.
Sharpe, J., S. Nonchev, A. Gould, J. Whiting, and R. Krumlauf. 1998. Selectivity, sharing and competitive interactions in the regulation of Hoxb genes. *EMBO J.* **17:** 1788–1798.
Smale, S.T. 1997. Transcription initiation from TATA-less promoters within eukaryotic protein-coding genes. *Biochim. Biophys. Acta.* **1351:** 73–88.
Small, S., A. Blair, and M. Levine. 1992. Regulation of *even-skipped* stripe 2 in the Drosophila embryo. *EMBO J.* **11:** 4047–4057.
Tautz, D. and C. Pfeifle. 1989. A non-radioactive in situ hybridization method for the localization of specific RNAs in Drosophila embryos reveals translational control of the segmentation gene hunchback. *Chromosoma* **98:** 81–85.
Tsukiyama, T. and C. Wu. 1997. Chromatin remodeling and transcription. *Curr. Opin. Genet. Dev.* **7:** 182–191.
Vasquez, J., G. Farkas, M. Gaszner, A. Udvardy, H. Muller, K. Hagstrom, H. Gyurkovics, L. Sipos, J. Gausz, M. Galloni, I. Hogga, F. Karch, and P. Schedl. 1993. Genetic and molecular analysis of chromatin domains. *Cold Spring Harbor Symp. Quant. Biol.* **58:** 45–54.
Wilkins, R.C. and J.T. Lis. 1997. Dynamics of potentiation and activation: GAGA factor and its role in heat shock gene regulation. *Nucleic Acids Res.* **25:** 3963–3968.
Zhou, J., S. Barolo, P. Szymanski, and M. Levine. 1996. The Fab-7 element of the bithorax complex attenuates enhancer-promoter interactions in the *Drosophila* embryo. *Genes & Dev.* **10:** 3195–3201.