Different core promoters possess distinct regulatory activities in the *Drosophila* embryo

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There are numerous examples of shared enhancers interacting with just a subset of target promoters. In some cases, specific enhancer–promoter interactions depend on promoter competition, whereby the activation of a preferred target promoter precludes expression of linked genes. Here, we employ a transgenic embryo assay to obtain evidence that promoter selection is influenced by the TATA element. Both the AE1 enhancer from the *Drosophila* Antennapedia gene complex (ANT-C) and the IAB5 enhancer from the Bithorax complex (BX-C) preferentially activate TATA-containing promoters when challenged with linked TATA-less promoters. In contrast, the *rho* neuroectoderm enhancer (NEE) does not discriminate between these two classes of promoters. Thus, certain upstream activators, such as Ftz, prefer TATA-containing promoters, whereas other activators, including Dorsal, work equally well on both classes of promoters. These results provide in vivo evidence that different core promoters possess distinct regulatory activities. We discuss the possibility that an invariant TFIID complex can adopt different conformations on the core promoter.

[*Key Words: Drosophila;* TATA box; promoter–enhancer interactions; core promoter; initiator elements]

Received October 20, 1997; revised version accepted December 19, 1997.

Complex enhancers, or *cis* regulatory modules, direct stripes, bands, and tissue-specific patterns of gene expression in the early *Drosophila* embryo. Such enhancers are typically 300–900 bp in length and contain clustered binding sites for both transcriptional activators and repressors (for review, see Gray and Levine 1996a; Rivera-Pomar 1996). Given the importance of these enhancers in development, we have become interested in the next level of *cis* organization, namely, the regulation of enhancer–promoter interactions within complex genetic loci.

Recent studies suggest that there are at least two mechanisms for regulating enhancer–promoter interactions (for summary, see Fig. 1). First, an insulator DNA can specifically block the interaction of a shared enhancer with gene B and not interfere with the activation of gene A (Fig. 1A; Hagstrom et al. 1996; Zhou et al. 1996; Mihaly et al. 1997). Second, according to a promoter competition scenario, the shared enhancer can activate both genes, but prefers the promoter region associated with gene A (Choi and Engel 1988; Foley and Engel 1992). The interaction of the enhancer with gene A precludes activation of gene B (Fig. 1B). Recent studies provide evidence for both mechanisms, as discussed below.

The major Hox gene clusters in *Drosophila*, the Bithorax complex (BX-C) and Antennapedia complex (ANT-C), contain vast arrays of tissue-specific enhancers that interact with specific target genes (e.g., Celniker et al. 1990; Gindhart et al. 1995; Lewis et al. 1995). For example, the AE1 autoregulatory element in the ANT-C specifically interacts with the *fushi tarazu* (*ftz*) promoter, but does not activate the equidistant *Sex combs reduced* (*Scr*) gene (Schier and Gehring 1992; Gindhart et al. 1995; Gorman and Kaufman 1995). Removal of the *ftz* gene and associated promoter region permits AE1 to activate inappropriate target genes within the ANT-C, including the *zen*-related gene, *z2* (Rushlow and Levine 1988). In the present study, we present evidence for promoter competition, whereby AE1–*ftz* interactions preclude the activation of the linked *Scr* gene.

We also investigate the regulation of the IAB5 enhancer, which is located ~60-kb upstream of the *abd-A* gene and ~60 kb downstream of the *Abd-B* gene within the BX-C (Busturia and Bienz 1993). IAB5 preferentially activates *Abd-B*, and is responsible for initiating *Abd-B* expression in the presumptive abdomen, particularly in parasegments (PS) 10, 12, and 14. Like AE1, IAB5 is thought to be activated by the Ftz protein. IAB5 may be inactive in PS 2, 4, 6, and 8 (Ftz stripes 1 through 4) by various gap protein repressors, including Hunchback and

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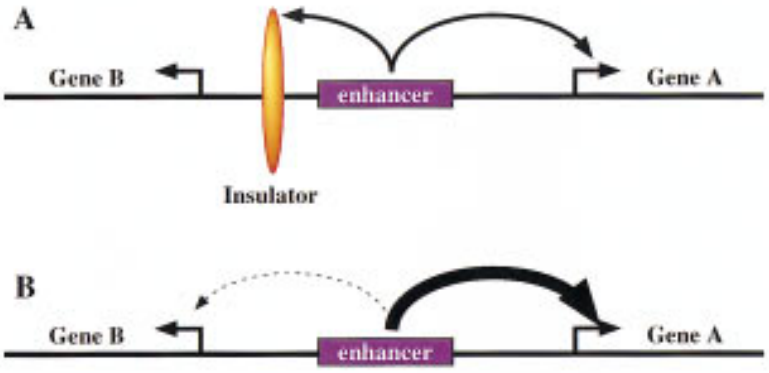

Figure 1. Regulation of enhancer–promoter interactions. The diagrams depict two divergently transcribed genes, A and B, with a common enhancer located in the intergenic region. (*A*) An insulator DNA is located between gene B and the enhancer. In principle, this blocks interactions of the enhancer with gene B, without altering the activation of gene A. (*B*) Promoter competition. In principle, the enhancer can activate both gene A and gene B, but prefers the promoter region of gene A. Enhancer–gene A interactions preclude activation of gene B.

Krüppel (see Busturia and Bienz 1993). Although IAB5 is located at comparable distances from *abd-A* and *Abd-B*, it preferentially activates the *Abd-B* promoter.

In this study we investigate the role of core promoter elements, particularly TATA, in regulating interactions between the AE1 and IAB5 enhancers with specific target promoters. There is considerable information regarding the organization and composition of the core transcription complex (for review, see Roeder 1996; Verrijzer and Tjian 1996). TFIID is a critical regulatory component of the complex. It is composed of TATA-binding protein (TBP) and associated factors (TAFs). The binding of TFIID to the core promoter appears to be a pivotal rate-limiting step in transcriptional activation (for review, see Burley and Roeder 1996; Manley et al. 1996; Stargell and Struhl 1996). Sequence-specific upstream activators have been shown to make direct contact with different components of the TFIID complex, including specific TAFs as well as TBP itself (e.g., Sauer et al. 1996; for review, see Ptashne and Gann 1997). Different core promoters appear to interact with an invariant TFIID complex, so it is unclear whether they possess distinct regulatory activities. There are numerous examples of combinatorial interactions between upstream activators. For example, the Bicoid and Hunchback transcription factors function synergistically to specify head structures and initiate the segmentation cascade (e.g., Simpson-Brose et al. 1994; Arnosti et al. 1996; Sauer et al. 1996), whereas Dorsal and bHLH activators initiate the differentiation of the embryonic mesoderm and neurogenic ectoderm (for review, see Rusch and Levine 1996). It is currently unclear whether different upstream activators collaborate with specific core promoter elements to specify cell fate during embryogenesis.

The binding of the TFIID complex to a target promoter depends on at least three different core promoter elements located within a 50- to 60-bp sequence flanking the transcription start site (e.g., Burke and Kadonaga 1996; for review, see Smale 1997), the TATA box, the initiator element (Inr), and the downstream promoter element (Dpe). In general, promoters that lack a TATA sequence must possess conserved copies of the Inr and/or Dpe. Conversely, promoters containing optimal TATA sequences do not require Inr and Dpe elements for the binding of TFIID (e.g., Burke and Kadonaga 1996). The present study provides evidence that TATA versus Inr/Dpe promoters possess distinct regulatory activities in development.

We show that both the AE1 and IAB5 enhancers preferentially activate TATA-containing promoters when challenged with linked TATA-less promoters. The analysis of chimeric core promoter sequences reveals the importance of the TATA element in these selective enhancer–promoter interactions. The *rhomboid* (*rho*) neuroectoderm enhancer (NEE) (Ip et al. 1992), however, does not discriminate between TATA-containing and TATA-less promoters, thereby providing evidence that certain upstream activators, such as Ftz, prefer TATA-containing promoters, whereas other activators, including dorsal and bHLH proteins, are promiscuous and work equally well on both classes of promoters. We propose that TFIID can adopt different conformations, and thereby expose distinct basal targets for interaction with upstream activators.

Results

This study involves the analysis of two minimal enhancers, AE1 and IAB5, which are located within the ANT-C and BX-C, respectively. The 430-bp AE1 is located in the middle of the *Scr–ftz* interval, ~7 kb from both promoters (Fig. 2; Pick et al. 1990). AE1 preferentially activates *ftz*, but not *Scr*, and a number of experiments were conducted to determine whether the distinct core promoter sequences associated with the two genes play a role in this regulatory specificity. In particular, the *ftz* promoter contains an optimal TATA sequence, but lacks both Inr and Dpe initiator elements. In contrast, *Scr* contains a potential Dpe element (Burke and Kadonaga 1996) but lacks TATA. The 1-kb IAB5 enhancer selectively activates *Abd-B*, not *abd-A*, although neither promoter contains an obvious TATA element (Martin et al. 1995; Lewis et al. 1995).

The AE1 and IAB5 enhancers were inserted into P-transformation vectors that contain at least two different reporter genes, including *lacZ* and *white*. These reporters were placed under the control of different core promoter sequences, and gene expression was monitored in transgenic embryos via in situ hybridization with digoxigenin-labeled RNA probes.

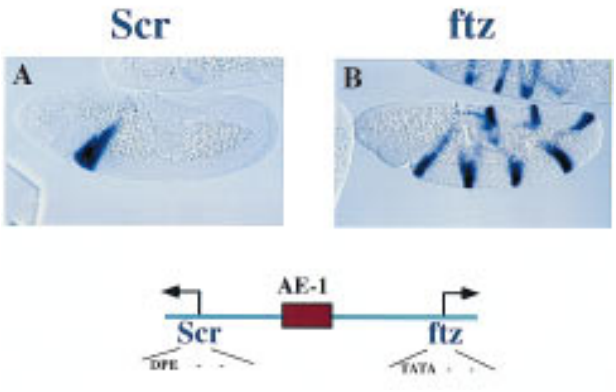

Figure 2. Regulatory specficity in the *Scr–ftz* interval of the ANT-C. Embryos were hybridized with either a dioxigenin-labeled *Scr* or *ftz* antisense RNA probe and visualized via histochemical staining. The embryos are undergoing the rapid phase of germ-band elongation (4–5 hr postfertilization) and are oriented with anterior to the left and dorsal up. *Scr* is expressed within the primordia of parasegment 2 (PS 2), which gives rise to regions of the labial and prothoracic segments. *ftz* is expressed in a series of pair–rule stripes. The diagram below the embryos shows the location of the AE1 enhancer within the *Scr–ftz* interval. AE1 specifically interacts with the *ftz* promoter to maintain the seven-stripe pattern. It does not activate the linked *Scr* gene.

AE1 can activate different classes of core promoters

Related core promoter sequences were initially used for the analysis of AE1. *ftz* and *eve* contain optimal TATA sequences, but lack Inr (INIT) and Dpe (DPE) elements (see Fig. 3A,B). Both the *eve/CAT* and *ftz/lacZ* fusion genes are expressed in a series of seven stripes in response to the endogenous *ftz* activator. Similarly, AE1 activates both *white* and *lacZ* when the two reporter genes are regulated by the *white* and Tp promoters (Fig. 3C,D). Both core promoters contain conserved copies of the INIT and DPE sequences, but either lacks a TATA sequence (*white*) or contains a suboptimal TATA (Tp). These results indicate that AE1 can simultaneously activate linked TATA-containing promoters or linked INIT/DPE-containing promoters. Additional experiments investigated the consequences of placing AE1 between different classes of promoters.

Promoter competition

There is a substantial reduction in the *white* staining pattern when the Tp promoter (Fig. 3D) is replaced with the core *eve* promoter sequence (Fig. 4A,B). The *eve/lacZ* reporter gene is expressed in a series of seven stripes in response to the AE1 enhancer (Fig. 4B). This AE1–*eve* interaction appears to block the expression of the linked *white* gene (Fig. 4A). In the absence of *eve*, *white* is fully active (Fig. 3C). These observations are compatible with a promoter-competition mechanism whereby AE1–*eve* interactions inhibit *white* (see Discussion).

Previous studies have shown that the gypsy retrotransposon contains an insulator DNA that blocks the interactions of distal, not proximal, enhancers with a target promoter (Geyer and Corces 1992; Dorsett 1993; Cai and Levine 1995; Scott and Geyer 1995). The *lacZ* reporter gene is blocked when the gypsy insulator is placed between AE1 and the *eve* promoter (Fig. 4D). The insulator redirects AE1 to the less preferred *white* gene, which is now expressed in a series of stripes (Fig. 4C). These results raise the possibility that a combination of promoter competition and insulator DNAs regulates enhancer–promoter interactions within complex loci.

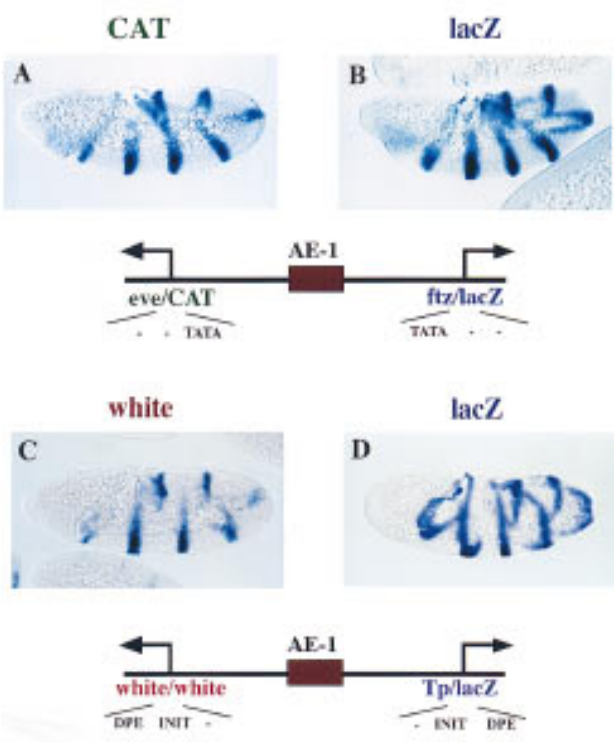

Figure 3. AE1 can coactivate linked reporter genes. Transgenic embryos carry fusion genes containing the 430-bp AE1 enhancer placed between divergently transcribed reporter genes that can be independently assayed. Embryos are undergoing the rapid phase of germ-band elongation. (*A*,*B*) Transgenic embryos carry a fusion gene with linked *CAT* and *lacZ* reporter genes. The arrows indicate the location and orientation of the transcription start sites. The leftward *CAT* gene was linked to the *eve* promoter, whereas the rightward *lacZ* reporter gene was attached to the *ftz* promoter. *A* was hybridized with a *CAT* antisense RNA probe; *B* was hybridized with a *lacZ* probe. Both reporter genes are expressed in a series of seven stripes, indicating that AE1 activates both the *ftz* and *eve* promoters. The diagrams indicate that the promoters contain TATA sequences, but lack optimal Inr (INIT) and Dpe (DPE) sequences. (*C*,*D*) Transgenic embryos carry a fusion gene with linked *white* and *lacZ* reporter genes. The *white* gene contains a mini-*white* promoter sequence, whereas *lacZ* was placed under the control of the core promoter sequence from the transposase gene (Tp) located within the P-element vector. *C* was hybridized with a white antisense RNA probe; *D* was hybridized with a *lacZ* probe. Both reporter genes are activated by AE1 and expressed in a series of stripes. The diagrams indicate that the promoters lack TATA sequences, but contain INIT and DPE elements.

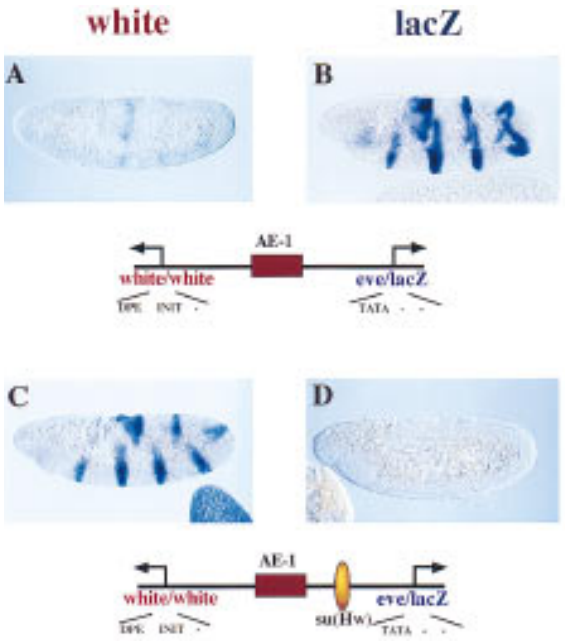

Figure 4. Promoter competition influences AE1 activity. Transgenic embryos contain a P transposon with divergently transcribed *white* and *lacZ* reporter genes that are under the control of different core promoter sequences. The AE1 enhancer was placed between the linked genes, as summarized in the diagrams below the stained embryos. The embryos were hybridized with digoxigenin-labeled *white* or *lacZ* antisense RNA probes. (*A*,*B*) The *white* and *lacZ* reporter genes are driven by minimal *white* and *eve* promoter sequences, respectively. The *eve*/*lacZ* gene is expressed in a series of seven stripes, but the *white* gene exhibits just residual staining. It would appear that AE1-*eve* interactions preclude activation of the linked *white* gene, because AE1 can activate *white* in the absence of *eve* (e.g., see Fig. 3C). (*C*,*D*) Same as *A* and *B* except that the 340-bp su(Hw) insulator DNA from the gypsy retrotransposon was placed between the AE1 enhancer and *eve*/*lacZ* fusion gene. This silences *lacZ* staining and results in the activation of *white*.

IAB5 prefers the eve *promoter*

The 1-kb IAB5 enhancer also exhibits a preference for TATA-containing promoters. IAB5 was placed downstream of an *eve*/*lacZ* fusion gene; the linked *CAT* reporter gene was placed under the control of the mini-*white* promoter (Fig. 5). There is strong expression of the *lacZ* reporter gene in the presumptive abdomen (Fig. 5B), whereas *CAT* is not expressed above background levels (Fig. 5A). This result suggests that IAB5 prefers the *eve* promoter over *white*. As shown below, IAB5 continues to select the *eve* promoter even when it is attached to the distal *CAT* reporter gene (see Fig. 6).

An *eve*–*white* chimeric promoter was analyzed in an effort to assess the importance of the core elements, particularly the TATA sequence. An ~20-bp region of the *eve* sequence (the TATA region) was replaced with the corresponding region of *white*. This modified *eve* promoter (eve^{white}) is attenuated and mediates only weak expression of *lacZ* in the presumptive abdomen (Fig. 5D). In contrast, the linked *white* promoter directs strong

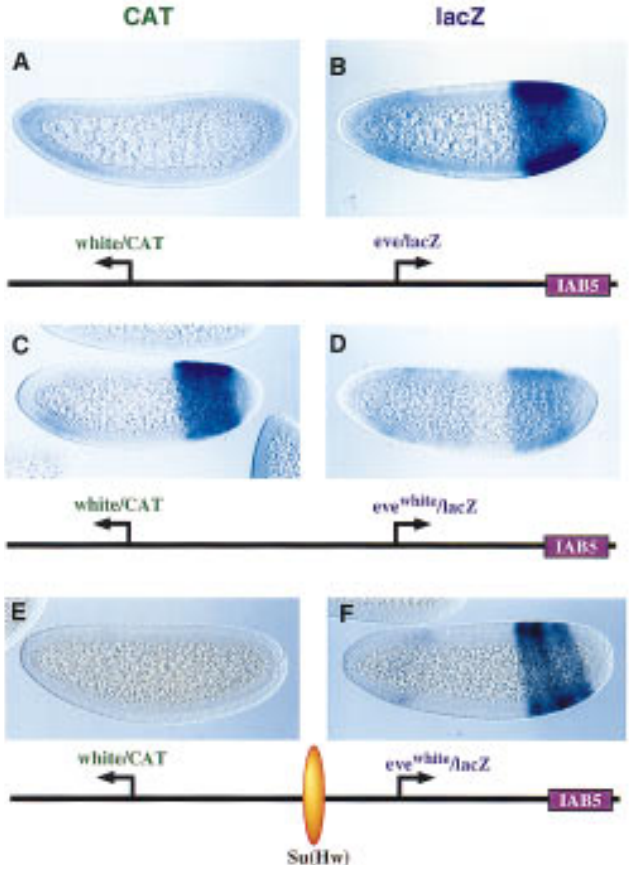

Figure 5. The IAB5 enhancer prefers TATA-containing promoters. Transgenic embryos were stained and oriented as described in the previous figure legends, except that these are younger embryos (between cellularization and the onset of gastrulation). The IAB5 enhancer was placed downstream of the rightward *lacZ* reporter gene. The distal *CAT* gene is under the control of the *white* promoter. The proximal *lacZ* gene is driven by *eve* (*B*) or an eve^{white} chimeric promoter (*D*,*F*) whereby the *eve* TATA region was replaced with the corresponding sequences in white. (*A*,*B*) *CAT* (*A*) and *lacZ* (*B*) staining patterns obtained with linked *white*/*CAT* and *eve*/*lacZ* genes. The IAB5 enhancer selects *eve* over *white*, so that the *eve*/*lacZ* reporter gene exhibits strong expression whereas *white*/*CAT* is silent. (*C*,*D*) Same as *A* and *B* except that the proximal *lacZ* gene is under the control of the eve^{white} chimeric promoter (*D*). There is only weak expression of the *lacZ* reporter in the presumptive abdomen (*D*). IAB5 now mediates strong expression of the distal *white*/*CAT* fusion gene (*C*). The residual *lacZ* staining observed in anterior regions (*D*) may be a position effect resulting from the site of P insertion. (*E*,*F*) Same as *C* and *D* except that the 340-bp gypsy insulator DNA [su(Hw)] was placed between the leftward *CAT* gene and rightward *lacZ* reporter. The insulator blocks IAB5–*white* interactions, so that *CAT* is not expressed above backround levels. Instead, IAB5 directs strong expression of the eve^{white}/*lacZ* in the presumptive abdomen, indicating that the chimeric promoter is not defective. The weak staining seen in head regions is caused by sequences contained within the P-transformation vector (Small et al. 1992).

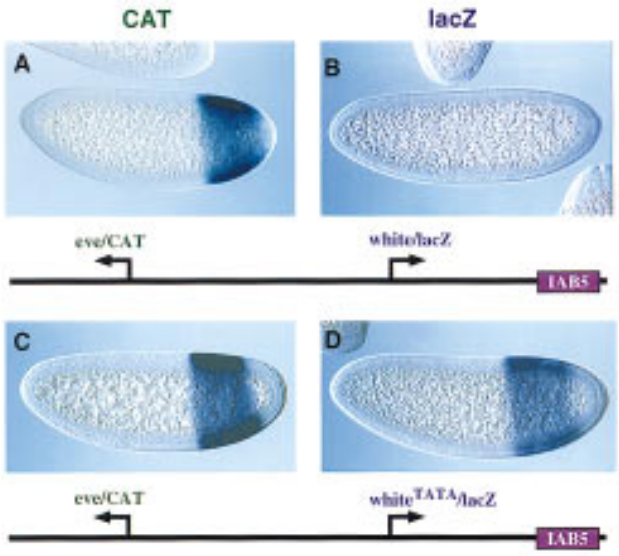

Figure 6. TATA is an important determinant of IAB5–*eve* interactions. Transgenic embryos carry the indicated P transposons and are oriented as described in the legend to Fig. 5. (*A*,*B*) Nuclear cleavage 14 embryos that carry a P transposon with the distal *CAT* gene driven by the core *eve* promoter and the rightward *lacZ* gene driven by mini-*white*. The IAB5 enhancer selectively interacts with the *eve* promoter, and directs intense expression of the *CAT* reporter in the presumptive abdomen (*A*). In contrast, the *white/lacZ* reporter gene is not expressed above background levels (*B*). (*C*,*D*) Cellularizing embryos carrying the same P transposon as *A* and *B* except that a synthetic TATA sequence was inserted into the mini-*white* promoter. IAB5 activates the *lacZ* reporter gene in the presumptive abdomen (*D*). The *eve/CAT* fusion gene is also activated by IAB5 (*C*). These results suggest that the $white^{TATA}$ promoter is almost as active as *eve*.

expression of *CAT* (Fig. 5C). These results suggest that the removal of the *eve* TATA releases the IAB5 enhancer so that it can now interact with the *white* promoter.

The selection of the *white* promoter over eve^{white} might reflect differences in the remaining core elements. The *white* promoter contains both Inr and Dpe sequences, whereas eve^{white} contains an Inr element that possesses just a 4 of 6 match with the consensus sequence (Smale 1997).

Additional experiments were done to determine whether the eve^{white} chimeric promoter possesses normal activity. The gypsy insulator was inserted between the distal *white/CAT* gene and proximal eve^{white}/*lacZ* gene (see diagram below Fig. 5E,F). The *lacZ* reporter gene is strongly activated in the abdomen (Fig. 5F). This level of expression is comparable to that obtained with the normal *eve* promoter, suggesting that the eve^{white} promoter is fully functional.

TATA is a prime determinant of IAB5 specificity

The preceding results suggest that the *eve* TATA region is important for selective interactions with the IAB5 enhancer. Additional evidence was obtained by analysis of the activities of a synthetic *white* promoter ($white^{TATA}$), which contains just 9 nucleotide substitutions between −29 and −21 bp upstream of the transcription start site. These changes create an optimal TATA box (GTATAAAAG) that is identical in sequence to the *eve* TATA (see Materials and Methods). The $white^{TATA}$ promoter was attached to the proximal *lacZ* reporter gene, whereas the distal *CAT* gene was placed under the control of the normal *eve* promoter sequence (Fig. 6). As seen previously, the 3′ IAB5 enhancer selects the distal *eve/CAT* fusion gene, and ignores the more proximal *white/lacZ* reporter (Fig. 6A,B). The $white^{TATA}$ promoter, however, mediates strong induction of *lacZ* expression (Fig. 6, cf. D and B), which is nearly comparable to that obtained with the normal *eve* promoter (Fig. 6C).

Promoter specificity

Several different embryonic enhancers were challenged with linked TATA and TATA-less promoters, and the 300-bp *rho* NEE was found to be equally effective in activating the two classes of promoters (data not shown; see below). Additional experiments were done to determine whether the targeting of IAB5 to *eve* influences the activities of the nonspecific *rho* NEE. The latter enhancer is activated by the maternal dorsal gradient in lateral stripes within the neurogenic ectoderm (Ip et al. 1992).

A synthetic gene complex was prepared that contains both the NEE and IAB5 enhancers. *white* and *CAT* reporter genes were attached to the mini-*white* promoter, whereas *lacZ* is driven by *eve* (Fig. 7). The *rho* NEE activates all three reporter genes, so that *white*, *CAT*, and

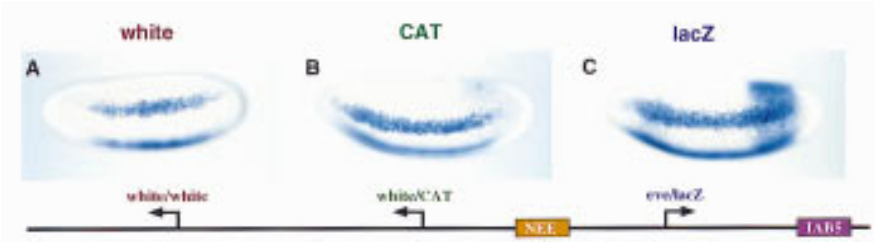

Figure 7. Independent activities of the IAB5 and NEE enhancers. Transgenic embryos carry the P-transformation vector shown in the diagram and are oriented as described in the previous legends to the figures. This synthetic gene complex contains three different reporter genes, *white*, *CAT*, and *lacZ*. The *white* and *CAT* genes are driven by the mini-*white* promoter, whereas *lacZ* contains the *eve* promoter. All three reporter genes exhibit robust expression in the lateral neurogenic ectoderm, indicating that the NEE enhancer interacts equally well with the mini-*white* and *eve* promoters. In contrast, *lacZ* is strongly activated in the presumptive abdomen (*C*), whereas *white* and *CAT* exhibit little or no expression in this region (*A*,*B*).

lacZ are all expressed in lateral stripes. In contrast, IAB5 primarily activates the *eve* promoter, so that only *lacZ* exhibits strong expression within the presumptive abdomen (Fig. 7C). The *white* reporter gene is not expressed in the abdomen (Fig. 7A), whereas *CAT* exhibits only residual staining in this region (Fig. 7B). These results suggest that IAB5–*eve* interactions do not influence the nonspecific activities of the *rho* NEE.

The NEE was used as an internal control to show that the selection of *eve* over *white* by IAB5 is not caused by differences in promoter strength. The NEE was placed between an *eve/CAT* fusion gene and *lacZ* reporter, whereas IAB5 was placed 3′ of *lacZ* (see diagrams in Fig. 8). The NEE activates *eve* and the chimeric eve^{white} promoter equally well, so that both the *CAT* and *lacZ* reporter genes exhibit lateral stripes of gene expression (Fig. 8A,B). In contrast, IAB5 mediates strong expression of the distal *eve/CAT* fusion gene in the presumptive abdomen (Fig. 8A), but only weakly activates the proximal eve^{white} promoter (Fig. 8B), which lacks both the TATA and Dpe sequences (see Fig. 5). The introduction of a synthetic TATA sequence in the *white* promoter ($white^{TATA}$) results in strong IAB5–*lacZ* interactions (Fig. 8D), so that the *eve/CAT* and $white^{TATA}$/*lacZ* fusion genes are expressed at comparable levels in the presumptive abdomen (Fig. 8C,D). The NEE continues to activate both reporter genes and is not influenced by changes in the *lacZ* promoter that control IAB5 interactions.

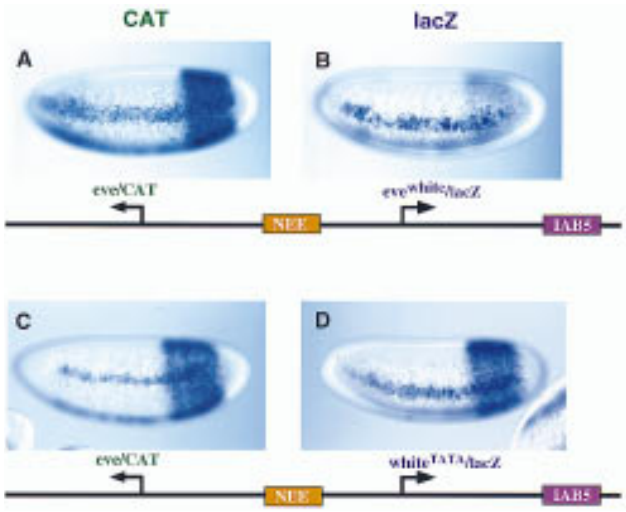

Figure 8. Independent activities of NEE and IAB5 on chimeric promoters. Transgenic embryos express the indicated P-transformation vectors, and are oriented as described previously. The *rho* NEE was placed in the intergenic region between the reporters, whereas IAB5 is located 3′ of the *lacZ* gene. The leftward *CAT* gene contains the *eve* promoter, whereas *lacZ* is driven by different chimeric promoters. (*A*,*B*) *CAT* and *lacZ* staining patterns obtained with the eve^{white} promoter, which contains an Inr element but lacks TATA and Dpe sequences. CAT transcripts are detected in lateral stripes and the presumptive abdomen (*A*), indicating activation of the *eve/CAT* gene by both the NEE and IAB5 enhancers. In contrast, *lacZ* is expressed primarily in lateral stripes; there is only residual staining in the abdomen (*B*). This staining pattern indicates that the eve^{white} promoter is strongly activated by the NEE enhancer, but only weakly interacts with IAB5. (*C*,*D*) *CAT* and *lacZ* staining patterns obtained with a synthetic *white* promoter ($white^{TATA}$) that contains a TATA sequence. Both reporter genes exhibit robust expression in lateral stripes and the abdomen, indicating that the NEE and IAB5 enhancers work equally well on the *eve* and $white^{TATA}$ promoters.

Discussion

This study provides evidence for promoter competition within the ANT-C. Preferential interactions between AE1 and the *ftz* promoter may preclude activation of the linked *Scr* gene. Previous studies on the chicken globin cluster showed that promoter competition is an important regulatory strategy for gene switching during hematopoiesis (Choi and Engel 1988; Foley and Engel 1992; Foley et al. 1994). The analysis of AE1 and IAB5 suggest that promoter competition depends, at least in part, on core promoter elements, particularly TATA. Both AE1 and IAB5 prefer the *eve* promoter, which contains an optimal TATA sequence, and fail to activate a linked mini-*white* promoter that lacks TATA but contains both Inr and Dpe sequences. In contrast, the NEE enhancer indiscriminately activates core promoters that contain either TATA or Inr/Dpe elements. These studies suggest that TATA-containing and TATA-less core promoters possess distinct regulatory activities.

Diverse core promoters

Previous studies have identified instances of specific enhancer–promoter interactions in the *Drosophila* embryo (Li and Noll 1994; Merli et al. 1996). In particular, shared enhancers located between the divergently transcribed *gooseberry* genes interact with just one of the promoters (Li and Noll 1994), whereas 3′ *decapentaplegic* (*dpp*) enhancers fail to activate neighboring genes, such as *out at first* (*oaf;* Merli et al. 1996). These studies, however, failed to determine whether promoter competition precluded inappropriate enhancer–promoter interactions and also failed to distinguish between promoter–proximal elements or core promoter elements in the regulatory specificity. The present study provides evidence that core promoter elements influence specific enhancer–promoter interactions and suggests that there are at least two classes of core promoters (for summary, see Fig. 9).

Type I promoters contain TATA, whereas type II promoters contain Inr and Dpe sequences. AE1 and IAB5 activators, presumbably including Ftz, preferentially activate type I promoters, whereas NEE activators, such as Dorsal, do not discriminate between the two classes of promoters. Future studies will determine whether there are upstream activators that selectively activate type II promoters. Possible candidates include activators that bind the T1 and VM enhancers in the ANT-C (Ginhart et al. 1995; Gorman and Kaufman 1995), which preferentially activate the TATA-less *Scr* promoter, but do not

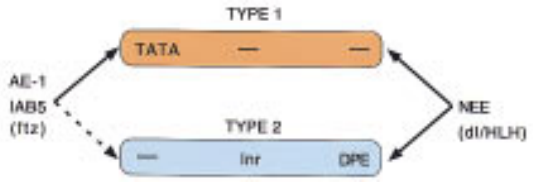

Figure 9. Different core promoters possess distinct regulatory activities. The IAB5 and AE1 enhancers preferentially activate TATA-containing promoters (type I) when given a choice between linked TATA and Inr/Dpe (type II) promoters. In contrast, the NEE activates both classes of promoters. These results suggest that the IAB5 and AE1 activators, particularly ftz, prefer type I promoters. NEE activators, including Dorsal (dl) and bHLH proteins, appear to be promiscuous and work equally well on both classes of core promoters. We propose that the TFIID complex adopts different conformations on type I and type II promoters. Basal targets for the Ftz activator may be displayed in a more accessible conformation when TFIID binds TATA. In contrast, basal targets for the Dorsal and bHLH activators may be equally accessible whether TFIID binds TATA or Inr/Dpe elements.

influence the expression of the neighboring TATA-containing *ftz* gene. Previous tissue culture and in vitro assays suggest that Sp1 may be more effective at activating Inr versus TATA promoters (Colgan and Manley 1995; Das et al. 1995; Emami et al. 1995).

It is unclear whether there are additional classes of core promoters. In this study we have investigated many, but not all possible combinations of the three TFIID core promoter elements. For example, the $white^{TATA}$ promoter contains TATA, Inr, and Dpe, but behaves like the *eve* promoter, which contains TATA but lacks Inr/Dpe sequences (see Fig. 6C,D). Perhaps TATA is dominant to Inr/Dpe in promoters that contain both types of core elements, although recent in vitro studies suggest that TATA and Inr elements work synergistically to activate transcription (Emami et al. 1997). It is also conceivable that these elements are antagonistic in transgenic embryos. For example, the proximal $white^{TATA}$/*lacZ* fusion gene does not preclude the activation of a distal *eve*/*CAT* reporter (Fig. 6). In contrast, a proximal *eve* promoter completely blocks activation of the distal *eve*/*CAT* reporter gene (S. Ohtsuki, unpubl.). TATA may be more effective at trapping the IAB5 enhancer in the absence of Inr/Dpe elements.

It has been suggested that TATA-containing promoters are intrinsically stronger than TATA-less promoters, possibly because of higher affinity interactions with the TFIID complex (e.g., Zenzie-Gregory et al. 1993). The divergent activities of the IAB5 and NEE enhancers, however, are most easily interpreted on the basis of qualitative, not quantitative, differences in type I and type II core promoter sequences. For example, the insertion of a TATA sequence in the *white* promoter allows it to compete with a linked *eve* promoter, whereas the removal of TATA from *eve* permits activation of *white*. These alterations in the *white* and *eve* promoters, the insertion and removal of TATA, dramatically alter the activities of IAB5, but have virtually no effect on the NEE enhancer (Fig. 8). NEE is equally effective in activating the *eve*, *white*, eve^{white}, and $white^{TATA}$ promoters, and thereby serves as an internal control for normal promoter function.

Mechanisms of core specificity

We propose that different core promoter sequences induce distinct conformations of the basal transcription complex. The TFIID complex is thought to bind the core promoter through direct interactions between TBP and TATA (e.g., Burley and Roeder 1996). In the absence of TATA, TFIID appears to make alternate contacts with the promoter, at least in part, through interactions between TAF150/TAF60 and Inr/Dpe sequences (Burke and Kadonaga 1996, 1997; Kaufmann et al. 1996). Previous studies have shown that the binding of TFIID induces substantial changes in DNA structure (Oelgeschlager et al. 1996). Perhaps TFIID adopts different conformations when bound to TATA versus Inr/Dpe sequences. Ftz activators bound to AE1 and IAB5 might prefer the conformation of the TFIID complex on TATA. In contrast, basal targets for NEE activators (e.g., Dorsal) might be equally accessible when TFIID is bound to either type I (TATA) or type II (Dpe/Inr) promoters.

An alternative model is that the TFIID complex is not invariant. Instead, there may be different forms of TFIID that interact with distinct core promoters. This possibility is suggested by the recent demonstration of a tissue-specific form of human $TAF_{II}130$ (Dikstein et al. 1996) and by the identification of a variant TATA-binding protein, TRF, that is expressed in just a subset of tissues (Hansen et al. 1997). Perhaps distinct, but related, TFIID complexes interact with type I and type II promoters, and the Ftz activator preferentially interacts with the type I complex.

Maintaining the integrity of Hox complexes

It is conceivable that the evolutionary conservation of Hox gene clusters stems, at least in part, from the promoter-competition mechanisms that appear to be employed for the orderly trafficking of *cis* regulatory elements in the ANT-C. Deletions and translocations within Hox complexes may be only rarely tolerated because of inappropriate enhancer–promoter interactions. For example, a deletion in the *ftz* promoter region might unlock AE1–*ftz* interactions, so that AE1 is now able to activate the linked *Scr* gene in ectopic tissues, thereby causing homeotic transformations and dominant lethality. In general, proper enhancer–promoter interactions in Hox gene complexes might depend on a combinatorial code that links specific upstream activators with particular core promoters.

Materials and methods

P-transformation assays

yw^{67} flies were used for all P-transformation assays. Fusion genes were introduced into the *Drosophila* germ line as de-

scribed in Small et al. (1992). Multiple transformants were generated for each construct, and at least three independent lines were examined. Embryos were collected, fixed, and hybridized with digoxigenin-labeled *white*, *CAT*, and *lacZ* antisense RNA probes exactly as described by Tautz and Pfeifle (1989) and Jiang et al. (1991).

Preparation of enhancers and promoters

The AE1 enhancer is located between −2574 and −2145 bp upstream of the *ftz* transcription start site (Schier and Gehring 1992). This 430-bp DNA fragment was synthesized from the genomic DNA of the yw^{67} strain by use of conventional PCR methods. This genomic DNA was also used for the PCR amplification of the *ftz* promoter region. The *ftz* DNA fragment extends from −100 bp upstream of the transcription start site to +91 bp and includes the untranslated leader sequence and first seven codons of the protein coding region (Laughon and Scott 1984). The mini-*white* promoter region used in this study extends from −316 bp upstream of the start site and extends to +174 bp. The Tp promoter sequence extends from −48 bp to +502 bp and includes coding sequences in both reading frames (for review, see Kaufman and Rio 1991).

The *eve* promoter sequence used in these studies is ~200 bp in length and includes just 34 bp of 5′-flanking sequence. The core *eve* promoter contains an optimal TATA sequence (TATAAAA) but lacks both Inr and Dpe sequences (Macdonald et al. 1986; Frasch et al. 1987). In contrast, *white* lacks a TATA sequence, but contains conserved copies of the Inr and Dpe sequences (Pirrotta et al. 1985). The Inr encompasses a 6-bp consensus sequence: TCAG/TTT/C (for review, see Smale 1997). The central A corresponds to the transcription start site (+1). The Dpe is located downstream of the transcription start site, between +1 and +35 (for review, see Burke and Kadonaga 1996). It includes a 7-bp consensus sequence: A/GGA/TCGTG; the central GA/TCG motif is particularly well conserved among TATA-less promoters. The core *white* promoter contains a 6 of 6 match to the Inr consensus and a 4 of 4 match to the central Dpe sequence.

The chimeric eve^{white} promoter is 202 bp in length and includes *white* promoter sequences from −36 bp to +2 bp and *eve* sequences from +3 bp to +166 bp. It lacks TATA and Dpe sequences and contains a chimeric Inr (TCAGCA) that shares only 4 of 6 matches with the consensus sequence (Smale 1997). The $white^{TATA}$ promoter is identical to the wild-type sequence (−315 bp to +174 bp) except that in vitro mutagenesis was done to create the optimal *eve* TATA sequence (GTATAAAAG) from −29 to −21 bp.

Construction of P-element transposons

The *eve/CAT*–AE1–*ftz/lacZ* fusion gene shown in Figure 3 (A,B) was prepared by insertion of the *eve* promoter region into a pBluescript plasmid containing the CAT coding region (Barolo and Levine 1997). The *eve/CAT* recombinant was cloned into the *Not*I–*Asc*I sites of the pCasPer P transposon (Small et al. 1992). The AE1 DNA fragment was cloned into the *Asc*I site of this P element vector, and the *ftz* promoter region was cloned into the *Asc*I–*Xba*I sites.

The *white*–AE1–Tp fusion gene shown in Figure 3 (C,D) was made by cloning the 430-bp AE1 genomic DNA fragment into the unique *Eco*RI site of the C4PLZ P-transformation vector (Gray and Levine 1996b; Zhou et al. 1996).

The *white*–AE1–*eve* fusion shown shown in Figure 4 was made by cloning of AE1 into the unique *Eco*RI site of the pEb vector (Cai and Levine 1997). A derivative of this P transposon was prepared by insertion of the 340-bp su(Hw) insulator DNA (Cai and Levine 1995) into the unique *Not*I site of the pEb vector described above. The *white/CAT/lacZ* P-transformation vector that was used for all of 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 between *white* and *lacZ* (Barolo and Levine 1997). The *white* reporter gene includes the 500-bp mini-*white* promoter sequence. Various *eve*, *white*, and chimeric promoter sequences were placed upstream of the *CAT* and *lacZ* reporters. This was done by insertion of promoter sequences into the unique *Asc*I and *Bam*HI sites of pBluescript plasmids containing either the *CAT* or *lacZ* coding region. *CAT* gene fusions were then excised from the plasmid by the combination of *Not*I and *Asc*I. The resulting DNA fragment was then inserted into the unique *Not*I and *Asc*I sites in the pCasPer vector. Similarly, promoter sequences were placed upstream of the *lacZ* coding region by use of the unique *Asc*I and *Bam*HI sites of a pBluescript plasmid. The *lacZ* fusion genes were excised from the plasmid with *Xba*I and *Asc*I, and inserted into these unique sites within the pCasPer vector.

A 1-kb genomic DNA fragment containing the IAB5 enhancer (Zhou et al. 1996) was modified to include *Pst*I restriction sites and inserted into the unique *Pst*I site of the P-transformation vector, which is located 3′ of the *lacZ* coding region. Various DNA fragments were placed between the *CAT* and *lacZ* reporter genes. The P-transformation vectors used in Figure 5A–D contain a 1.6-kb spacer DNA from λ (Zhou et al. 1996). It was inserted into the unique *Asc*I site located between *CAT* and *lacZ*. The vector used in Figure 5 (E and F) contains the 340-bp gypsy insulator DNA at this *Asc*I site, in place of the λ spacer (Cai and Levine 1995). Finally, the vectors used in Figures 7 and 8 contain the 300-bp *rho* NEE enhancer (Ip et al. 1992) at the unique *Asc*I site instead of the λ spacer DNA or gypsy insulator.

Acknowledgments

We thank James Sharpe and Robb Krumlauf for sharing unpublished results, and Jim Kadonaga and Jim Manley for helpful discussions. We also thank Anna Di Gregorio, Jumin Zhou, and Hailan Zhang for comments on the manuscript. This work was funded by a grant from the National Institutes of Health (GM34431). S.O. is a fellow of the Human Frontiers Science Program.

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Genes Dev. 1998, **12:**