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The *Abdominal-B* Promoter Tethering Element Mediates Promoter-Enhancer Specificity at the Drosophila Bithorax Complex

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Abstract

At the Drosophila bithorax complex many distinct classes of *cis*-regulatory modules work collectively during development to control gene expression. *Abdominal-B* (*Abd-B*) is one of three homeotic genes in the BX-C and is expressed in specific presumptive abdominal segments in the embryo. The transcription of *Abd-B* is tightly controlled by an array of *cis*-regulatory modules that direct its expression over extended genomic distances. These regulatory modules include promoters, insulators, silencers, enhancers, promoter targeting sequences and the recently identified promoter tethering element (PTE). To activate gene expression at the endogenous complex, enhancers located >50 kb away must bypass intervening insulators to interact with the *Abd-B* promoter. The molecular mechanisms that allow enhancers to bypass insulators are not currently well understood. In this short article, we report on a novel mechanism for insulator bypass involving the PTE. In addition, we use bioinformatic analysis across twelve Drosophila genomes to identify putative *cis*-regulatory sequences that may be capable of facilitating specific promoter-enhancer interactions at the bithorax complex and propose a model for their molecular function during development.

Keywords

cis regulation; enhancer; promoter; Drosophila; bithorax; *Abdominal-B*

REGULATION OF GENE EXPRESSION IN THE BITHORAX COMPLEX

The organization and function of *Homeotic* (*Hox*) genes has been evolutionarily conserved across many diverse species.[1,2] These highly conserved genes are expressed during embryonic development and are responsible for governing the formation of body structures along the major body axis.[3] In Drosophila, the bithorax *Hox* cluster contains three genes, *Ultrabithorax* (*Ubx*), *abdominal-A* (*abd-A*) and *Abdominal-B* (*Abd-B*), which assign the proper identity to the posterior thoracic and abdominal segments.[4] An extensive array of *cis*-regulatory modules with diverse functions is essential to control the expression of these genes in the bithorax complex (BX-C).[5–7]

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The embryonic expression pattern of *Abd-B* is regulated in cis by a large set of *infraabdominal* (*iab*) regulatory domains, *iab-5*, *iab-6*, *iab-7* and *iab-8*, that specify abdominal segments A5–A9, respectively (Fig. 2).[6,7] Each *iab* domain contains at least one enhancer module responsible for driving expression of *Abd-B* in the corresponding presumptive abdominal segment.[8] These enhancer modules must bypass intervening insulators to activate their target promoter. For example, the IAB5 enhancer must bypass at least two characterized insulators, Fab-7 and Fab-8, to activate the *Abd-B* promoter (Fig. 2).[9,10] As we previously reported, a mechanism by which IAB5 overcomes these intervening insulators involves a 255bp promoter tethering element (PTE) located 40bp 5′ of the *Abd-B* transcriptional start site.[5] The PTE is capable of recruiting IAB5 to the *Abd-B* promoter in competition assays in transgenic embryos. The removal of the PTE results in redirection of enhancer-specific expression on transgenes. Here we report on the anti-insulator activity of the PTE. We also examine the conservation of the PTE sequence across twelve Drosophila species and speculate on a possible molecular model for the functional activity of the PTE at the BX-C.

ANTI-INSULATOR ACTIVITY

A major functional question is whether the PTE is necessary for IAB5 to bypass the Fab-8 insulator and activate the *Abd-B* promoter at the BX-C. In a control experiment, the Fab-8 insulator was positioned between a minimal *Abd-B* promoter, lacking the adjacent PTE sequence, driving *CAT* expression and the *abd-A* promoter driving *lacZ* expression. On this construct, the IAB5 enhancer was inserted distal to *Abd-B* ($B^{\Delta PTE}$-Fab8-A-5 in Fig. 1). In this configuration the IAB5 enhancer was directed to the proximal *abd-A* promoter, as strong *lacZ* expression was detected in transgenic embryos (Fig. 1B), while *CAT* expression was absent (Fig. 1A). Based on this result, we wanted to test if the interaction between the *Abd-B* promoter and the IAB5 enhancer seen at the endogenous BX-C is dependent on the presence of the PTE. To test this we inserted the 255bp PTE adjacent to the *Abd-B* promoter (B-Fab8-A-5 in Fig. 1). Interestingly, in this configuration the Fab-8 insulator was not capable of disrupting the interaction between IAB5 and the distal *Abd-B* promoter, as strong *CAT* expression was detected in posterior stripes in blastoderm stage embryos (Fig. 1C), while no *lacZ* expression was detected (Fig. 1D). These results suggest that in the presence of the PTE the intervening Fab-8 insulator no longer disrupts the interaction between IAB5 and the *Abd-B* promoter. The insertion of the PTE confirms that this *cis*-regulatory module is necessary for the recruitment of IAB5 to the *Abd-B* promoter on transgenes. The anti-insulator activity of the PTE is critically important in the context of the endogenous BX-C, where IAB5 must bypass at least two insulators, including Fab-8, to interact with the *Abd-B* promoter.

EVOLUTIONARY CONSERVATION OF SEQUENCES IN THE PTE

The PTE contains two highly conserved sequence stretches, a 24-mer near the 3′ end and a 27-mer near the 5′ end, as identified using the genomes from seven Drosophila species.[5] Comparative genomics from all twelve sequenced Drosophila species[11] reveals that the 24-mer is very strongly conserved across all species (Fig. 2). The conservation occurs at a hexamer motif, TGGT(T/C)(C/T), which is present as four tandem copies in the 24 bp window. In many species the last two base pairs in some of the hexamer motifs are inverted (CT vs. TC, see Fig. 2). The genomic location of this motif cluster is also conserved across many of the Drosophila species, as it remains adjacent to the predicted *Abd-B* transcription site. Computational analysis does not strongly predict the binding of any currently known Drosophila transcription factors to the TGGT(T/C)(C/T) motif. A bioinformatic survey using the Fly Enhancer search engine[12] of the genomic interval between the *Abd-B* and *abd-A* genes in the BX-C identifies only seven high-stringency clusters of the TGGT(T/C)(C/T) motif. One of these clusters lies in the PTE and an additional four clusters are found in the approximately 65 kb *Abd-B* 3′ *cis*-regulatory region (Fig. 2). In contrast, only two clusters are present in the adjacent and

equivalently sized *abd-A* 5′ regulatory regions. Two of the clusters found in the *Abd-B* regulatory region are themselves also very highly conserved across the Drosophila species (Fig. 2). Intriguingly, each of the three distal *iab* regions that are known to regulate *Abd-B* expression (*iab-5, iab-6* and *iab-7*) contains at least one cluster of the conserved motif. In most instances these clusters are located close to defined enhancers (Fig. 2). A less stringent search for clusters of the hexamer motif identifies the sequence TGGTTC GGTTC within the defined 1 kb IAB5 enhancer (Fig. 2) which we have shown to be capable of tethering to the PTE sequence on transgenes.[5]

TOWARDS A MECHANISM OF PTE FUNCTION—A MOLECULAR BRIDGE?

It is possible that the conserved TGGT(T/C)(C/T) motif represents a protein factor-binding site. We propose a model in which proteins bind the clustered motif in the PTE and a cluster in the *Abd-B* 3′ regulatory region. Interactions between these factors may facilitate the formation of a chromatin loop, which mediates specific promoter-enhancer interactions (Fig. 3). At the endogenous BX-C, there are several known enhancers capable of directing expression of *Abd-B*, but only one enhancer is active in a given spatial region of the developing embryo.[6,7] Our model is therefore that the tethering of a specific enhancer to the *Abd-B* promoter serves two critical functions: (1) it prevents promiscuous enhancer activity by physically restricting interactions at the PTE to a single enhancer and (2) it ensures that as the genomic DNA of the BX-C is subjected to rearrangement over evolutionary time,[13] enhancers can still function properly even if they become distally located relative to their target promoter (Fig. 3). This model is consistent with data from the Antennapedia complex (ANT-C), which contains the only other cluster of *Hox* genes in *Drosophila melanogaster*.[13] In the ANT-C, the T1 enhancer preferentially activates the distal *Scr* gene over the proximal *ftz* gene. This activity is dependent on a 450bp tethering element located upstream of the *Scr* promoter, which recruits the T1 enhancer.[14] Moreover, this tethering element contains a cluster of the hexamer motif TTCGAA, and a second cluster is also found near the T1 enhancer.[15] This led to speculation that proteins binding to the clustered motif facilitate the formation of a chromatin loop.[15] We believe, therefore, that the molecular bridge mediated by promoter tethering elements represents a general transcription mechanism in the regulation of *Hox* genes. In order to more fully address this issue it will be critically important to investigate the functional activity of the PTE in the context of the endogenous BX-C.

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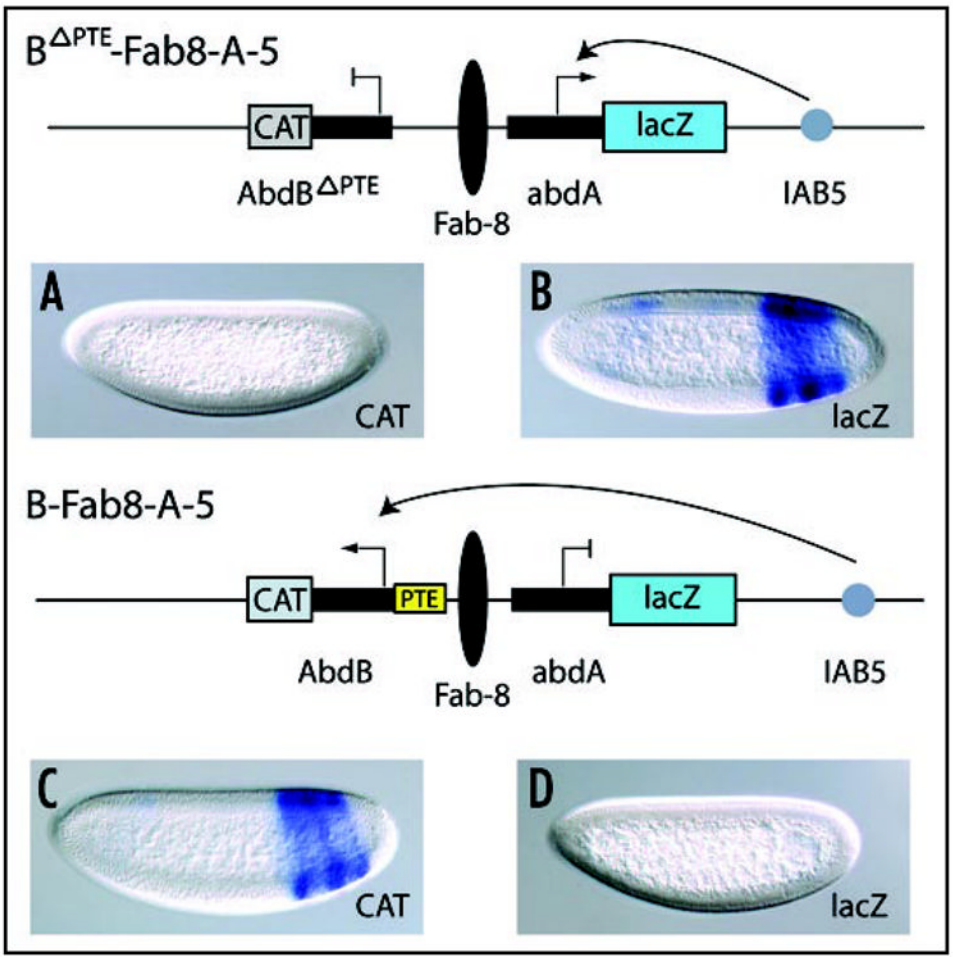

Figure 1.

Promoter tethering element contains anti-insulator activity. On the $B^{\Delta PTE}$-Fab8-A-5 construct (top diagram), the Fab-8 insulator was placed between two homeotic promoters, *Abd-B* and *abd-A*. In this arrangement, the IAB5 enhancer is directed to the *abd-A* promoter. The abd-A-lacZ reporter gene exhibits a three stripe IAB5 expression pattern in presumptive abdominal segments 5, 7 and 9 that is readily detected in blastoderm-stage embryos (B), while no *CAT* expression is detected (A). When the 255bp promoter tethering element is inserted upstream of the *Abd-B* promoter (B-Fab- 8-A5 construct, bottom diagram) the IAB5 enhancer bypasses the proximal *abd-A* promoter and is now redirected to the *Abd-B* promoter, as strong *CAT* expression is detected (C), while no *lacZ* expression is detected (D).

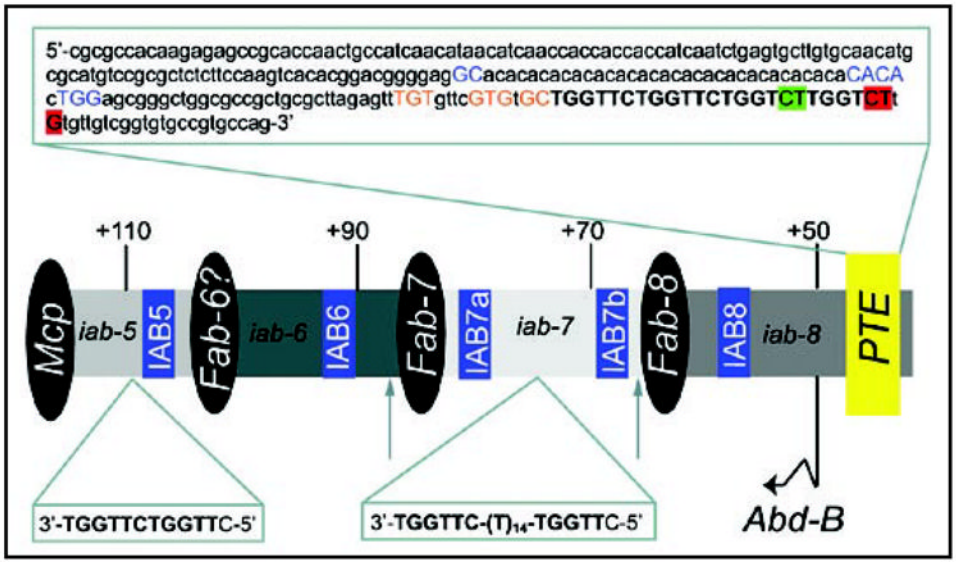

Figure 2.

Conservation of a clustered hexamer sequence in *Abd-B* regulatory regions. The 3′ regulatory region that controls *Abd-B* expression is shown with the genomic locations of the enhancers (blue boxes), insulators (black ellipses) and PTE (yellow box). The PTE contains a highly conserved 24-mer (49112–49135) that is a tandem array of four TGGT(T/C)(C/T) motifs, that was identified using *cis*-Decoder software (http://evoprinter.ninds.nih.gov/cisdecoder/index.htm). In the PTE sequence (top box) the nucleotides in lowercase are not conserved, while the nucleotides in bold caps are conserved across all twelve Drosophila species. The colored nucleotides represent sequences conserved in all species except; *D. willistoni* (orange), *D. pseudoobscura* (blue), *D. mojavensis* (green), *D. grimshawi* (red). Fly Enhancer software (http://genomeenhancer.org/fly) was used to search for clusters of the hexamer motif within 150 kb of the *Abd-B* transcription start site. This program allowed for a stringent search for the occurrence of at least two of the (TGGTCT) or (TGGTTC) motifs in a 50 bp window. In addition to the cluster in the PTE, there are four other locations in the *Abd-B* 3′ regulatory region that met this stringent criterion. One highly conserved cluster is located 5 kb 3′ of the IAB5 enhancer (109929–109940) and contains two tandem conserved hexamer sequences. A second highly conserved cluster is located between the IAB7a and IAB7b enhancers (75042–75067). Two other clusters (gray arrows) are found in the *Abd-B* 3′ regulatory regions, one in the iab-6 region (86869–86901) and one in the *iab-7* region close to the IAB7b enhancer (66397–66417). All genomic co-ordinates refer to Genbank sequence DM31961.

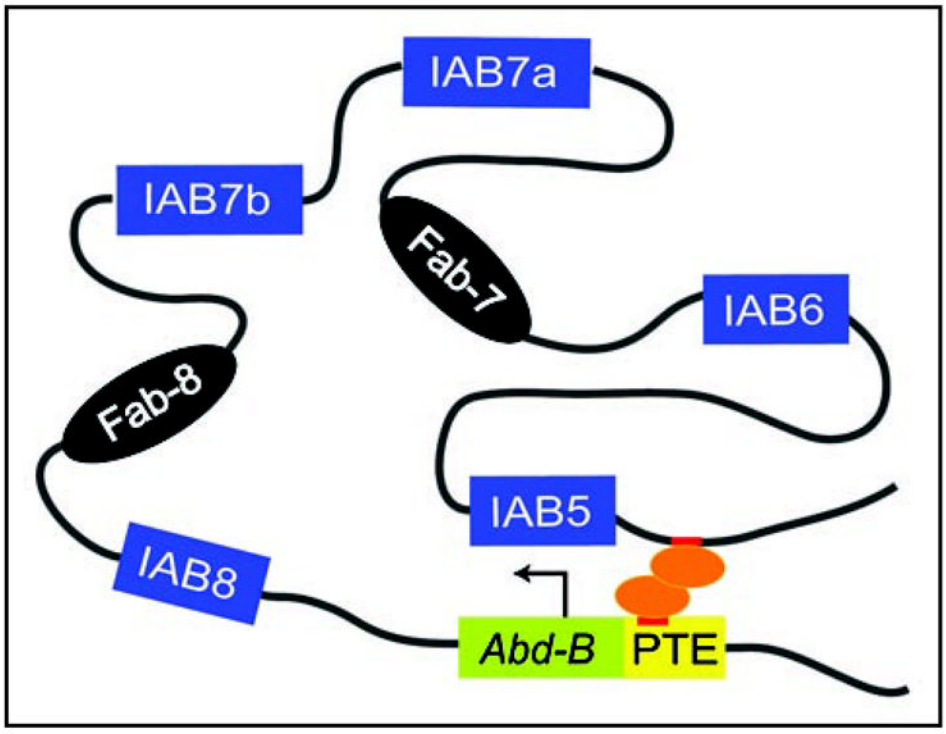

Figure 3.

Promoter tethering element regulates long-range promoter-enhancer interactions. Model for the molecular mechanism for the function of the *Abd-B* PTE involves the homomeric interaction of protein factors (orange ellipses) bound to conserved clusters of hexamer motif sequences (red line) within the PTE (yellow box) and close to the IAB5 enhancer (blue box). The interactions of these regulatory proteins facilitate a chromatin loop configuration that brings the IAB5 enhancer to the *Abd-B* promoter. The interactions shown here represent the situation in cells expressing *Abd-B* in the presumptive fifth abdominal segment in the embryo, where transcription is activated by the IAB5 enhancer. In cells from different segments the enhancer(s) specific to that segment will be tethered to the promoter; eighth segment (IAB8), seventh segment (IAB7a and IAB7b) and sixth segment (IAB6).