



Pergamon

Insect Biochemistry and Molecular Biology •• (2002) •••••

*Insect  
Biochemistry  
and  
Molecular  
Biology*

www.elsevier.com/locate/ibmb

# Design and function of transcriptional switches in *Drosophila*

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Received 10 May 2001; received in revised form 25 September 2001; accepted 1 October 2001

## Abstract

Extensive genetic and biochemical analysis of *Drosophila melanogaster* has made this system an important model for characterization of transcriptional regulatory elements and factors. Given the striking conservation of transcriptional controls in metazoans, general principles derived from studies of *Drosophila* are expected to continue to illuminate transcriptional regulation in other systems, including vertebrates. With improvement in technologies for genetic manipulation of insects, research in *Drosophila* will also aid the design of systems for controlled expression of genes in other hosts. This review focuses on recent advances from *Drosophila* in analysis of the functional components of transcriptional switches, including basal promoters, enhancers, boundary elements, and maintenance elements. © 2002 Published by Elsevier Science Ltd.

**Keywords:** Transcription; Enhancer; Promoter; Promoter specificity; Boundary element; Insulator; Polycomb; Trithorax

## Contents

1. Introduction . . . . .	000
1.1. Elements of transcriptional switches . . . . .	000
1.2. Analysis of transcriptional control elements . . . . .	000
2. Basal promoter elements . . . . .	000
3. Enhancers . . . . .	000
3.1. Nature of transcriptional switch action . . . . .	000
3.2. Modularity . . . . .	000
3.3. Integrative capabilities . . . . .	000
3.4. Patterning . . . . .	000
3.5. Enhancer–promoter specificity . . . . .	000
3.6. Evolution . . . . .	000
4. Boundary Elements . . . . .	000
5. Long-acting switches . . . . .	000
5.1. Polycomb/trithorax genes . . . . .	000
5.2. Enzymatic activities . . . . .	000
5.3. Establishment . . . . .	000
5.4. Targeting . . . . .	000
5.5. Future studies of transcriptional switch design . . . . .	000

## 1. Introduction

### 1.1. Elements of transcriptional switches

RNA polymerases require specific signals from transcriptional switches to initiate transcription. This review discusses the components and function of transcriptional switches required by RNA polymerase II, the enzyme responsible for transcription of protein-coding genes in eukaryotes. While many cellular processes affect transcription of genes, including signaling cascades, protein trafficking, and chromosome dynamics, I will focus on the discrete DNA elements that are directly involved in regulating the transcription of genes, and the proteins that interact specifically with these elements. The emphasis will be on research from *Drosophila*, with mention of important results from other systems as required. For information on the general transcriptional machinery, specific biochemical activities associated with transcriptional activators and repressors, chromatin structure, and chromatin modifying factors, the reader is referred to recent reviews (Berk et al., 1998; Davie and Moniwa, 2000; Henikoff, 2000; Jenuwein and Allis, 2001; Reinberg et al., 1998; Roth et al., 2001).

Transcription initiates at basal promoter regions that contain conserved sequences immediately surrounding the start site. At the promoter, the enzyme interacts with a complex of over two dozen polypeptides comprising the basal transcription machinery. In vitro, the basal machinery, including the TATA-box binding factor TFIID<sup>1</sup>, is sufficient to allow RNA polymerase II to initiate on non-chromatinized templates, but in vivo, where the template is chromatinized and presumably less accessible to DNA binding factors, additional signals are required. These signals come from DNA-binding transcriptional activators bound to *cis* elements located 5' or 3' of the initiation site. The location of these *cis* elements can be up to 100 kbp from their site of action, suggesting that loops in the DNA allow these activators to make close contact with the basal machinery, although alternative modes of action have also been suggested (Bulger and Groudine, 1999).

The term "promoter" is used in various circumstances to describe the entire regulatory region of a gene or a small area around the initiation site. In this review, I differentiate basal promoter elements from enhancers. Basal promoters are DNA elements that interact with the basal transcriptional machinery at the site of transcriptional initiation but are not active in vivo unless linked

to an enhancer. Enhancers, originally defined as sequences of DNA that stimulate transcription of genes in a distance- and orientation-independent manner, can be regarded as platforms for the assembly of sequence-specific DNA-binding transcription factors. These clusters of transcription factor binding sites can be located in both distal and promoter-proximal locations. Both activators and repressors bind to enhancers, making these elements integrators of both positive and negative transcriptional information.

The signaling between enhancers and promoters can be highly regulated to provide specificity in the communication between a gene and its control elements. This communication can be regulated by boundary elements that block enhancer-promoter interactions in a directional manner, or it can be regulated by functional properties of the basal promoter or enhancer that make certain enhancer-promoter combinations incompatible.

An additional level of transcriptional control is afforded by placement of a gene relative to heterochromatic regions; most genes are located in euchromatic domains and are inhibited by close proximity to heterochromatin. Constitutive heterochromatin, characterized by an abundance of repeat elements and a paucity of genes, possesses a distinct chromatin structure and is bound by specific types of heterochromatin-associated proteins such as HP-1. The Polycomb group proteins are suggested to control the transcription of a number of developmentally regulated genes through facultative formation of heterochromatin-like structures (Pirrotta, 1998).

### 1.2. Analysis of transcriptional control elements

Eukaryotic transcriptional elements and proteins have been studied extensively in yeast, *Drosophila*, and vertebrates, and it is clear that many aspects of the transcription machinery are highly conserved, facilitating comparisons among systems. Certain features of higher eukaryotic transcriptional switches are lacking in yeast, however, such as complex regulatory regions involving multiple enhancers and long-distance activation. The similarity of complex elements in higher metazoans and the availability of advanced genetic techniques make *Drosophila* particularly well suited to studies of transcriptional regulatory systems. Three complementary approaches have been utilized to characterize transcriptional elements in *Drosophila*: biochemical characterization of the transcriptional machinery, classical genetic analyses, such as those carried out by Lewis and colleagues on the *Bithorax Complex* (Lewis, 1978), and molecular genetic studies. This last approach involves in vivo analysis of individual *cis* elements coupled to reporter genes, permitting the analysis of native enhancer and promoter combinations in a physiologically relevant setting. Analysis of *trans* acting factors has also been

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<sup>1</sup> In this review, protein factors are indicated by non-italicized names, genes by italics. Lower case (*hairy*) indicates a recessive gene. The protein is accordingly written hairy. Definitions to terms used in this review are in Table 1.

Table 1  
Definition of terms

**General concepts**

Genomic regions rich in expressed genes, characterized by an open chromatin structure readily accessible to DNA binding proteins. Zytogically transcribed genes expressed in well-defined but broad regions of embryo, required for establishment of pair-rule gene expression patterns (see Krüppel, giant, hunchback).  
Genomic regions characterized by repeated sequences, low density of gene coding information, and compacted chromatin containing specialized histones and histone binding proteins.  
Zytogically transcribed genes expressed in circumferential stripes required for generating metameric repeat pattern of embryo (see *ftz*, hairy, *eve*)  
Functional DNA elements that coordinate activity of DNA and chromatin binding proteins, resulting in correct regulation of transcription.

**Cis-regulatory elements**

DNA sequences surrounding start site of transcription with which the general transcriptional machinery interacts prior to initiation of transcription, usually containing a TATA box, DPE, and/or Initiator sequence (see below).  
Conserved promoter element G(A)TCG located at +30, contacted by TAF<sub>II</sub>80.  
Cis-regulatory element containing multiple sites for sequence-specific DNA binding proteins, classically defined as working in an orientation- and distance-independent manner.  
*Fab-7* and *Fab-8* DNA elements act as insulators to separate the *iab-6,7*, and *8* enhancer regions within the *Bithorax* complex.  
Retroviral element containing binding sites for the *su(Hw)* boundary element protein.  
Parasegment- or segment-specific enhancers controlling expression of transcripts of the *Bithorax* complex.  
Conserved sequence (TCAGT) flanking the +1 site of transcriptional initiation.  
DNA element that can block enhancer/basal promoter communication and prevent heterochromatic silencing of genes.  
General term for a PRE or TRE; element that locks in an initially set pattern of transcriptional activation or repression.  
Specific sequence element of several hundred bp that serves as a nucleation site for action of *Polycomb* group proteins. Establishes long-acting repression of transcription.  
A DNA sequence element found within the *iab-7* enhancer that allows distal enhancers to overcome boundary element blocking effects.  
Boundary elements flanking the 87A7 *hsp70* genes.  
Basal promoter element at -30 nt that interacts with the basal transcription factor TATA binding protein, TBP.  
Specific sequence element of several hundred bp that serves as a nucleation site for action of *trithorax* group proteins. Establishes long-acting activation of transcription.

**Examples of regulated genes**

HOX gene cluster containing homeotic transcription factor genes *lab*, *pb*, *Dfd*, *Scr* and *Antp*, specifying developmental fates of anterior structures. The cluster also contains additional nonhomeotic transcriptional factor genes *ftz*, *bcd*, and *zen*.  
HOX gene cluster containing homeotic genes *abdA*, *AbdB*, and *Ubx*, specifying developmental fates of posterior regions. Regulatory elements included within this 300 kbp region include the *iab* enhancers and *Fab* boundary elements.  
Transforming growth factor  $\beta$  (TGF- $\beta$ ) homolog expressed in complex patterns in embryo and larval imaginal disks under the control of a series of distant 3' enhancers.  
Pair-rule gene encoding a homeodomain transcriptional repressor that is important for segmentation. Expressed initially in a seven-stripe blastoderm pattern. Modular enhancers located 5' and 3' of the transcription unit specify expression in blastoderm embryo, in neuronal cells, and mesoderm.  
Gene encoding a secreted protein ligand of the patched receptor; expressed in embryo and in larval imaginal disks.

(continued on next page)

Table 1 (continued)

HOX genes  
Linked homeotic genes found in two clusters (*ANTP* and *BXC*) in *Drosophila* that specify anterior/posterior developmental identities. The genes encode transcription factors containing DNA binding homeodomains.  
*Iroquois* Complex (*Iro-C*)  
Gene cluster of three related homeobox-containing transcription factors important for regional specification of eye, head and mesothorax. Regulated by Pc-G genes.  
*rhomboid* (*rho*)  
Gene encoding a transmembrane protein important for modulating EGF receptor signaling. Expressed in the embryonic neuroectoderm and larval imaginal disks.  
*wingless* (*wg*)  
Segment polarity gene encoding a secreted protein, ligand of the Frizzled and Frizzled2 transmembrane receptors. Expressed in embryo and in larval imaginal disks.

#### Sequence-specific DNA-binding transcription factors

*abdominal-A, B* (*abd-A, Abd-B*)  
Homeodomain proteins of the *BXC* important for thoracic and abdominal development.  
Adult enhancer factor 1 (*AEF-1*)  
Zn finger transcriptional repressor; negatively regulates *yp1*, *yp2*, *Adh*, *Fbp-1* genes.  
Antennapedia (*Antp*)  
Transcription unit of the *ANTC* encoding a homeodomain protein important for head and thorax development.  
bicoid (*bcd*)  
Maternally expressed, anteriorly localized, homeodomain protein that activates gap and pair-rule genes. Important for establishment of anterior–posterior polarity in the embryo.  
cut (*ct*)  
Widely expressed homeodomain transcription factor. Enhancers up to 85 kbp 5' of promoter control expression of gene.  
dorsal (*dl*)  
Rel-domain transcriptional factor translocated to nucleus in ventral regions of the embryo. Functions as activator or repressor, depending on gene context.  
eyeless (*ey*)  
Paired domain/homeodomain transcription factor important for regulation of eye development in *Drosophila*; homolog of vertebrate Pax-6.  
engrailed (*en*)  
Segment polarity gene product; long-range transcriptional repressor containing homeodomain.  
extradenticle (*exd*)  
Homeodomain containing protein; acts as a cofactor in transcriptional activation with homeotic gene products.  
fushi-tarazu (*ftz*)  
Pair-rule gene product; homeodomain transcriptional activator expressed in stripe pattern in blastoderm embryo.  
GAGA (*Trl*)  
trx-G protein; also required for activity of some PREs; product of *Trithorax-like*. Binds to GAGA sequences in *eye*, *hsp70* promoters and recruits SWI/SNF activity to remodel chromatin.  
giant (*gt*)  
Gap gene product; short-range transcriptional repressor required for head and abdominal patterning in the blastoderm embryo.  
hairy (*h*)  
Pair-rule gene long-range transcriptional repressor involved in segmental patterning in the embryo.  
hunchback (*hb*)  
Gap gene product; transcription factor expressed maternally and zygotically; zinc finger protein functions as transcriptional activator or repressor depending on gene context.  
Krüppel (*Kr*)  
Gap gene product; short-range transcriptional repressor required for abdominal patterning in the embryo.  
scalloped (*sd*)  
DNA binding transcription factor; cooperates with other DNA binding factors to mediate signaling from the Notch and wingless signaling pathways.  
snail (*snai*)  
Mesoderm-specific zinc finger protein; short-range transcriptional repressor.  
Suppressor of Hairless *Su(H)*  
DNA binding transcription factor; effector of the Notch signaling pathway.  
Suppressor of Hairy wing *su(Hw)*  
Cellular protein that binds to su(Hw) sites within the *gypsy* retroviral element, allowing it to function as an insulator.  
Ultrathorax (*Ubx*)  
Homeodomain protein of the *BXC* important for thorax and abdominal development.  
zeste (*z*)  
trx-G protein thought to recruit brahma remodeling complexes to TRE to activate gene expression.

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Table 1 (continued)

**Basal transcriptional machinery**

TBP associated factors; cofactors that bind to and modulate the activity of TBP, forming RNA polymerase II-specific TFIID complex.  
 TATA binding protein; binds to TATA element within basal promoter.  
 General transcription factor that associates with TFIID and TFIIB to initiate preinitiation complex formation.  
 General transcription factor that associates with TFIID and TFIIA to initiate preinitiation complex formation; contacts DNA adjacent to TATA box.  
 General transcription factor complex, consisting of TBP and ~10 distinct TAF<sub>II</sub>s, that initiates preinitiation complex formation by binding to TATA box.

**Sequence-specific DNA binding domains**

ARID  
 homeodomain  
 AT-rich interacting domain; DNA binding domain found in SWII and *osa* proteins.  
 Helix-turn-helix DNA interacting domain found in a number of homeotic genes and other transcription factors.

**Chromatin remodeling/modifying proteins**

Polycomb group (*PcG*)  
 trithorax group (*trxG*)  
 brahma (*brm*)  
 extra sex combs (*esc*)  
 Enhancer of zeste (*E(z)*)  
 histone deacetylase (HDAC)  
 moira (*mor*)  
*osa* (*osa*)  
 pleiohomeotic (*pho*)  
 Polycomb (*Pc*)  
 SWI/SNF  
 trithorax (*trx*)  
 A group of proteins that associates as a complex and stably represses developmentally regulated genes, including HOX genes. Histone deacetylase activity has been associated with some PcG proteins.  
 A group of proteins that counteract PcG protein activity and positively regulate HOX genes. A subcomplex of *trxG* proteins forms the brahma chromatin remodeling complex.  
*trxG* protein; ATPase, helicase; component of SWI/SNF related chromatin remodeling complex required for proper expression of HOX genes of BXC and ANTP. Homologous to yeast SWI2.  
 PcG protein; contains a WD-40 protein-protein interaction domain. Present in a complex containing HDAC activity.  
 PcG protein; contains myb DNA binding domain. Present in a complex containing HDAC activity.  
 Enzymes that catalyze the removal of acetyl groups from lysine residues of histone N-terminal regions, generally associated with repression of transcription.  
*trxG* protein; component of brahma chromatin remodeling complex; homologous to yeast SWI3.  
*trxG* protein; component of brahma chromatin remodeling complex; homologous to yeast SWII protein. Contains ARID DNA binding domain.  
 PcG protein; homologous to mammalian Zn finger YY1 transcription factor.  
 PcG protein; contains chromodomain thought to be involved in binding to methylated histone tails.  
 Yeast protein complex with ATPase activity involved in regulation of gene expression via chromatin remodeling; homologous to *Drosophila* brahma complex.  
*trxG* protein; contains a SET domain that may be involved in association with histone tails.

**Additional terms**

Chip (*Chl*)  
 HP-1 (*Su(var)205*)  
 Nipped-B (*Nipped-B*)  
 Notch (*N*)  
 Regulator of enhancer-promoter interactions, suggested to facilitate binding of homeodomain proteins between promoter and enhancer.  
 Mutant identified as an enhancer of *su(Hw)* boundary element function on the *cut* gene.  
 Heterochromatin-associated protein; contains methyllysine binding chromodomains.  
 Regulator of enhancer-promoter interactions. Homologous to fungal adherin proteins that play a role in chromosome structure and condensation, and DNA repair. Identified in same screen as Chip.  
 Transmembrane protein that functions as receptor for cell-cell communication in numerous developmental pathways. Proteolytic product enters nucleus as co-activator with Su(H) protein.

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possible using tissue- and stage-specific misexpression systems.

Large-scale sequencing projects have yielded comprehensive information about the protein coding capacity of entire genomes, however, transcriptional regulatory elements are considerably more difficult to identify from primary sequence alone. Two current approaches to identify regulatory regions from genomic data involve bioinformatic methods to find clusters of putative binding sites of transcription factors and phylogenetic comparisons to identify evolutionarily conserved sequences. Identifying the function of such putative regulatory regions requires direct experimental testing, however.

## 2. Basal promoter elements

The basal transcriptional machinery interacts with at least three regions near the transcriptional initiation site (Fig. 1). A TATA element located at  $-30$  bp is recognized by the TATA-binding protein (TBP)-containing complex, TFIID. The Initiator is a pentameric sequence (consensus TCAGT) surrounding the transcriptional initiation site (usually "A") that makes close contact with the TBP-associated factor TAF<sub>II</sub>230 and the catalytic site of RNA polymerase II (Cherbas and Cherbas, 1993; Wu et al., 2001). A more recently identified tetrameric sequence G(A/T)CG located at  $+30$ , the Downstream Promoter Element (DPE), is recognized by TAF<sub>II</sub>40 and TAF<sub>II</sub>60, and may function as an alternative to a TATA box to anchor TFIID (Burke and Kadonaga, 1997; Kutach and Kadonaga, 2000). Work from mammalian systems indicates that other basal transcription factors make additional nonspecific DNA contacts throughout the promoter region (Douziech et al., 2000; Forget et al., 1997). In addition, sequences flanking the TATA box can affect TBP and TFIIB interactions (Wolner and Gralla, 2000). Not all promoters contain TATA boxes and DPEs; a recent survey indicated that approximately 14% of 205 well-characterized promoters from *Drosophila* contain both TATA and DPE elements, 55% contained only a TATA box or a DPE element, and 31% contained neither (Kutach and Kadonaga, 2000). In vertebrate cells, house-keeping genes are often associated with basal promoter

elements that are rich in CpG sequences and lack canonical TATA boxes and DPE elements. It is not known whether *Drosophila* contains similar basal promoters, however.

A similar or identical complement of basal transcription factors is involved in initiation at most promoters, and in transfection assays, many basal promoter elements are functionally interchangeable (Kermekchiev et al., 1991). Not all basal promoters have identical properties, however. Some germline-specific gene expression may involve distinct basal promoter elements and tissue-specific components of the basal transcriptional machinery (Rorth, 1998; Hiller et al., 2001). In addition, there are clear differences in activities of basal promoter elements. Transcriptional initiation is the rate-limiting step for utilization of most promoters, but heat-shock promoters are also limited at a later step, promoter escape. Sequences responsible for this post-initiation effect have been mapped to regions around  $+30$  and  $5'$  of the TATA box for the hsp70 promoter (Lee et al., 1992; Tang et al., 2000). In transfection studies, certain mammalian promoters respond selectively to distinct classes of transcriptional activators, suggesting that distinct promoter architecture may dictate different requirements for activation (Das et al., 1995). Recent studies demonstrate that the presence or absence of a DPE element dictates whether the DRAP1 repressor protein activates or represses transcription in *in vitro* transcription assays (Willy et al., 2000). These functional differences in basal promoter elements are sufficient in some cases to create enhancer-promoter specificity, as described below.

Binding sites for sequence-specific transcription factors are sometimes found within the basal promoter element, leading to greater functional complexity of these elements. The *even-skipped* (*eve*) basal promoter contains a GAGA factor site, which confers boundary element function on this promoter (Ohtsuki and Levine, 1998). The *ovarian tumor* basal promoter is bound by the ovo zinc-finger protein, which is required for promoter function *in vivo* (Lu and Oliver, 2001). As described below, one of the tandem promoters for *Adh* is bound by a sequence-specific transcriptional repressor, providing developmental timing control (Ren and Maniatis, 1998).

TATAAA	TCAGT	GA/TCG
<b>TATA box</b>	<b>Inr</b>	<b>DPE</b>
<b>-30</b>	<b>+1</b>	<b>+30</b>

Fig. 1. Elements of basal promoters for RNA polymerase II. Shown are TATA box at  $-30$  bp, the site of TATA binding protein (TBP) interaction, Initiator (Inr) surrounding the transcriptional initiation site (most commonly "A" – in boldface), and Downstream Promoter Element (DPE) at  $+30$ . Most promoters contain only a subset of these elements.

## 3. Enhancers

### 3.1. Nature of transcriptional switch action

Two general pathways by which enhancers are thought to drive transcription are by direct engagement of the basal machinery or by modification of the local chromatin environment. These activities are mediated by enhancer-binding transcription factors that contact the

basal machinery or that recruit chromatin-modifying activities. In some cases, the functional result of enhancer action is an increase in the likelihood that a gene will be activated, without influencing the rate of initiation transcriptional (on/off model), while in other cases enhancers increase the rate of transcriptional initiation (rheostat model) (Blackwood and Kadonaga, 1998). Both of these effects are observed with reporter genes in the *Drosophila* embryo (Fig. 2). A weak activator drives *lacZ* reporter gene expression in a punctate pattern in which some nuclei fail to show any expression at all. In contrast, a strong activator drives expression in a more uniform pattern, with overall higher levels of expression. This result suggests that a minimal threshold for activation must be crossed to ensure consistent transcriptional activity, and that the on/off and rheostat effects can be manifestations of the same phenomenon.

### 3.2. Modularity

The complex temporal and spatial expression patterns of developmentally regulated genes often reflect the action of modular regulatory elements, generally 300–700 bp in size, that can be located up to 100 kbp from the promoter. Some of the best-studied complex loci in *Drosophila* are the pair-rule genes *hairy* and *eve* that are expressed in a seven-stripe pattern in the blastoderm embryo. The first indication that this complex pattern represented inputs from separable elements came from studies of *hairy* mutants with chromosomal rearrangements 5' of the transcription unit. These mutants showed loss of individual stripes, not uniform attenuation of all stripes, indicating that autonomously acting elements direct individual stripe formation (Hooper et al., 1989).

Subsequent comprehensive analysis of the *eve* locus

identified within a 16 kbp locus five separable elements 5' and 3' of the transcription unit, that together create the seven-stripe *eve* pattern (Fujioka et al., 1999). The general strategy for pair-rule stripe gene expression is an initial widespread activation under the influence of generally distributed activators, followed shortly afterwards by repression in the interstripe regions (Frasch et al., 1987). The initial repression patterns are established by gap gene products, including Krüppel, giant, hunchback and knirps, which are regionally distributed transcriptional repressors (Small et al., 1992). A key feature of the stripe enhancers' activities is that their action is autonomous, so that repression of one element does not lead to general repression of the entire locus. This autonomy depends on spaces between elements (Fig. 3A). Repression of the *eve* stripe 2 enhancer in central regions of the embryo by Krüppel does not prevent the more distal *eve* stripe 3 enhancer from activating the promoter, but when the spacing between the enhancers was artificially reduced, repression signals from one enhancer interfered with the activity of the adjacent enhancer (Small et al., 1993).

These initial findings indicated that short-range repression plays an important role in enhancer autonomy. In direct tests, the exact ranges of repressor activity were measured in transgenic embryos. Zygotically active repressors such as Krüppel, knirps, giant, and snail were shown to repress either activators within enhancers or basal promoter elements when bound within ~100 bp of their apparent targets (Gray et al., 1994; Arnosti et al., 1996a). In contrast, long-range repressors such as hairy can repress enhancer elements over distances of >500 bp, leading to dominant repression of multiple enhancer complexes (Barolo and Levine, 1997).

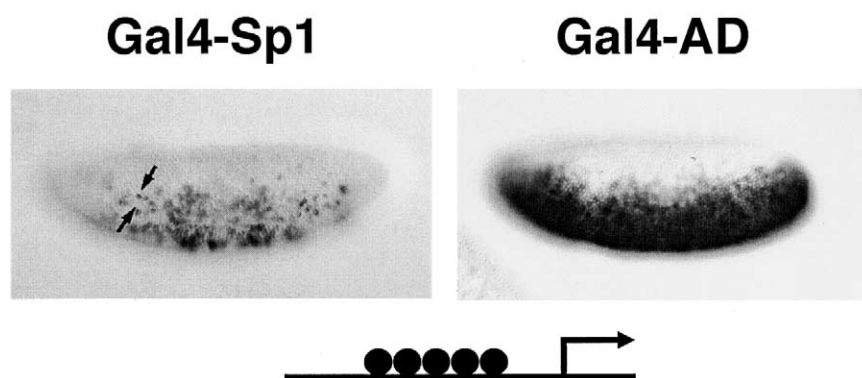


Fig. 2. Comparison of on/off vs. rheostat function of enhancer elements. A transgene containing a cluster of GAL4 binding sites (bottom) is activated by a Gal4-Sp1 (left) or a minimal activation domain from the Gal4 activator (AD, right). A punctate pattern of expression is observed with the Gal4-Sp1 activator, indicating an on/off effect (arrows show adjacent nuclei, one in which the reporter gene is expressed). A much more intense expression is obtained with the Gal4-AD activator, indicating that the promoter can be driven at higher levels than with the weaker Sp1 activator, thus, a rheostat effect is seen. However, a punctate pattern is also observed in lateral regions where limiting amounts of Gal4-AD are present, suggesting that at low concentrations, a stochastic on/off effect is observed. *lacZ* expression was analyzed by in situ hybridization as described in Small et al., 1992. Expression is restricted to ventral regions of the embryo where the Gal4-chimeric proteins are expressed. Embryos are oriented anterior to the left, dorsal surface up.

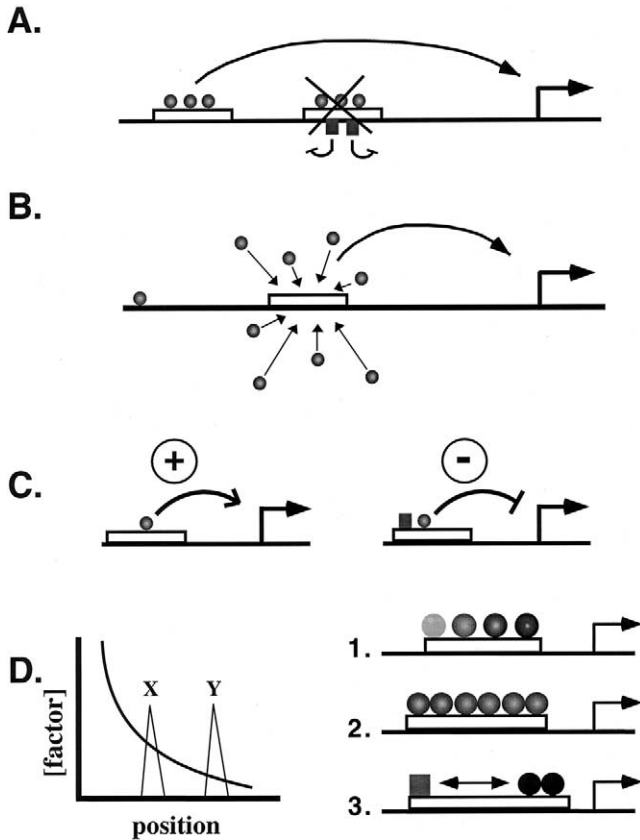


Fig. 3. Activities of enhancers. **A.** Autonomous functioning of modular enhancers; a distal enhancer is free to interact with a promoter while a proximal enhancer is locally inhibited by short-range repressors acting within the element, as is seen with modular enhancers within the *eve* gene. When the distance between the *eve* stripe 2 and 3 enhancers was reduced, improper cross-regulation resulted (Small et al., 1993). **B.** Concentration of transcription factors by an enhancer containing several high-affinity binding sites, leading to a functional output. A non-specifically bound factor, incapable of activation by itself, is shown at a distal position. **C.** Programming of transcription factor activity by enhancer; left, a simple element binds a transcriptional activator, such as the dorsal protein, and transmits an activating signal, as on the *twist* promoter (Jiang and Levine, 1993); right, a complex element brings together multiple proteins, assembling a complex in which the erstwhile activator functions as a repressor, as on the *zen* gene, where the dorsal protein functions as part of a repression complex (Valentine et al., 1998). **D.** Conversion of positional information into gene activity by enhancers. Left, a gradient of a transcriptional activator, such as dorsal, is differentially interpreted by genes X and Y, causing them to be activated at different levels of the gradient, as is seen with dorsal target genes *twist* and *rhomboid* (Jiang and Levine, 1993). Right, transcription switch design mechanisms that allow enhancers to convert the gradient information into differential gene activity: 1. Differences in binding site affinity, symbolized by shading, allows a gene to become active at higher or lower levels of transcription factor concentration, as has been demonstrated for the bicoid activator acting on the *hunchback* promoter (Driever et al., 1989). 2. The number of binding sites plays a similar role, with a higher number of binding sites making the gene more sensitive. 3. Spacing between short-range repressors and targets sensitizes a transcriptional switch to respond to lower or higher levels of the repressor, as has been demonstrated for the giant repressor (Hewitt et al., 1999).

The use of different modes of repression may serve specific gene regulatory needs. Clearly, short-range repressors can be useful for creating precise, tunable repression, while long-range repressors impose a dominant regulatory scheme on a locus. The autonomy of many enhancer elements, such as those operating on the *eve* gene, would be compromised if long-range repressors interacting with one enhancer cross-regulated other enhancers. It is not clear whether these functional differences between short-range and long-range repressors are fully exploited in the design of most transcriptional switches, or whether on most genes, either type of repression would be sufficient.

### 3.3. Integrative capabilities

Enhancer sequences serve as binding platforms for regulatory proteins, concentrating and localizing factors that affect expression of linked genes. In addition to this passive role as a transcription factor collection point, enhancers can in effect perform computational functions, dictating that nature of the signal sent by the arrangement of *cis* elements. These functions include signal amplification, conversion of potential activation signals to repression signals, and tuning to affect a gene's sensitivity to gradients of regulators (Fig. 3B–D).

Signal amplification is an inherent property of enhancer sequences through the clustering of transcription factors on a specific genomic sequence. This grouping provides a strong signal above the noise of non-specifically bound factors that may be of no functional consequence (Biggin and McGinnis, 1997). The specific placement of adjacent binding sites can also foster cooperative binding between factors, providing more effective occupancy and a greater signal, as has been observed for the bicoid activator (Hanes et al., 1994).

Conversion of intrinsic activators to repressors by specific regulatory elements is seen in the case of the dorsal protein, a *rel* homology domain factor that functions as a transcriptional activator in numerous contexts but is a necessary part of a repression complex within the silencing elements of the *decapentaplegic* (*dpp*), *zen* and *tld* regulatory regions. On these specific promoter elements, neighboring sites for other DNA binding factors permit the assembly of a complex that interacts with the groucho co-repressor, effectively overriding the endogenous activation domain of dorsal (Valentine et al., 1998). In the absence of these additional binding sites, dorsal reverts to an activator (Jiang et al., 1992). Similar context-dependent repression by a number of transcription factors has been observed in mammalian systems as well (Fry and Farnham, 1999).

Transcription factors can establish “morphogen” (developmental field-determining) gradients that dictate differential transcriptional responses at different levels of the factor (Jiang and Levine, 1993). Three features of

enhancers have been identified that allow genes to respond to different levels of a given transcription factor (Fig. 3D). A gene can respond to the lowest levels of an activator if the enhancer contains either high-affinity binding sites or numerous lower-affinity sites (Struhl et al., 1989; Driever et al., 1989). For the giant short-range repressor, it was found that a third, novel feature applied; because of the extreme distance-dependence of this protein, subtle changes in the spacing of giant binding sites endowed a promoter with the ability to discriminate between less than twofold differences in the level of this factor in the embryo (Hewitt et al., 1999).

Many genes are regulated by small modular elements, but the generality of this conclusion must be tempered by the realization that large and distributed regulatory elements are much harder to characterize and will tend to be underreported (Klingler et al., 1996). Davidson and colleagues have documented one example of a gene containing multiple, interacting signaling modules: the *endo16* gene of the sea urchin *Strongylocentrotus purpuratus* is regulated by a 2.3 kbp segment that can be experimentally subdivided into at least 6 regions binding 13 different factors. These separable regions are interconnected into what has been termed a *cis* regulatory logic device, where positive and negative signals from upstream regions are interpreted by an integrative unit, until a final output reaches the basal promoter (Yuh et al., 1998).

### 3.4. Patterning

Two extremes describe the computational function of regulatory regions or enhancers: at one end of the spectrum, an enhancer can simply bind a tissue-specific transcription factor and provide the output in the same tissue-specific fashion (Fig. 4A), and at the other, the enhancer can integrate multiple positive and negative signals to generate a pattern different from each of the individual inputs (Fig. 4B, C). Three examples show the range of activities lying within this spectrum. At the simplest level, a transgene with multimerized sites for the homeodomain Pax-6/eyeless factor is expressed in a pattern directly recapitulating the expression of Pax-6/eyeless protein. In this case, tissue-specificity lies upstream in the regulatory network, with the control of *Pax-6/ey* expression (Sheng et al., 1997).

A more complex situation is seen with wing imaginal disk expression of *cut*, where the scalloped (*sd*) transcription factor potentiates transcriptional activation of this gene throughout the disk. Specific Notch signaling along the dorsal/ventral boundary of the disk activates the Su(H) transcription factor, and Su(H) together with *sd* binds to the *cut* gene and activates transcription. A simple cluster of *sd* and Su(H) sites is sufficient to recapitulate this pattern of expression (Guss et al., 2001). The pattern of *cut* activation reproduces the dorsal/ventral

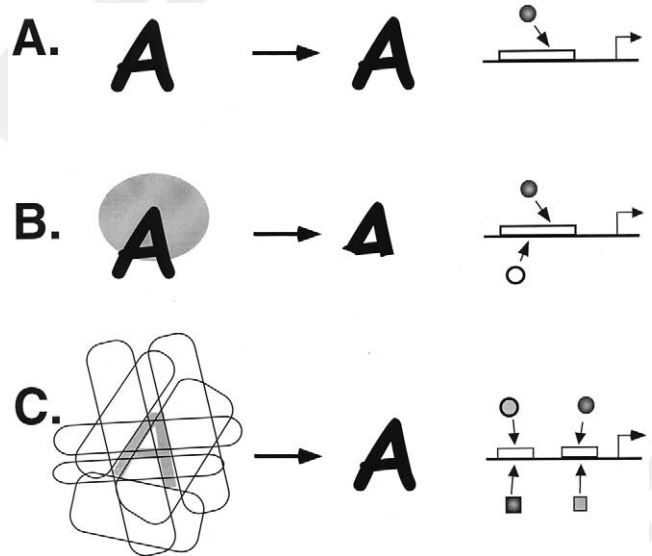


Fig. 4. Three modes of pattern processing by enhancers. Enhancer function is represented by the horizontal arrows at left, examples of transcriptional switch structure represented at right. **A.** In the simplest case, an enhancer faithfully recapitulates another pattern of gene expression (symbolized by the letter 'A'). The binding of a single tissue-specific transcription factor to a gene is in some cases sufficient to create this effect, as seen with the eye-specific Pax-6/eyeless protein binding to cognate sites (Sheng et al., 1997). **B.** An enhancer can integrate two types of patterning information to reproduce a subset of one of the patterns; such switches can involve the binding of factors responding to multiple signaling pathways, as has been noted with the cooperative action of the scalloped and Su(H) proteins on the *cut* promoter (Guss et al., 2001). **C.** An enhancer can create novel gene expression patterns that resemble none of the original inputs, integrating information from multiple sources. At left, gray 'A' represents boundary areas between domains of expression of regulatory factors (rounded rectangles), which is transformed into the signal (black 'A'). In the case of the pair-rule genes such as *even-skipped*, such integration is accomplished by multiple factors interacting with separate modular enhancers (Small et al., 1992).

boundary pattern of *Notch* signaling specified by prior regulatory events, but the additional requirement for *sd* specifies the developmental field in which activation can take place. A similar situation applies to the action of regulatory proteins encoded by HOX genes that bind to DNA via the low-specificity homeodomain. These proteins may bind to numerous sites on the genome that may be of no regulatory consequence, as has been observed for another homeodomain protein, *even-skipped*. A higher level of regulatory specificity is derived from additional proteins, such as extradenticle, that interact with the homeodomain partners in permissive or non-permissive fashions, either enhancing binding affinity, or synergistically activating transcription (Biggin and McGinnis, 1997). In both of these circumstances, the action of one factor is to potentiate gene transcription in a wide field that is narrowed by combinatorial interaction of other factors expressed in a more limited pattern (Fig. 4B).

The segmentally repeating stripe expression of pair-

rule genes in the blastoderm embryo represents a more involved process, in which an elaborate iterated pattern is generated that does not closely resemble any of the underlying positive and negative inputs but rather represents a synthesis of this information. In this case, enhancers integrate both positive and negative inputs from regionally expressed gap genes, producing the seven stripes of primary pair rule genes such as *hairy* and *eve*. In contrast to earlier suggestions that such a pattern might be generated by a global morphogenic field with differentially diffusing activators and repressors, the solution for each stripe of expression appears to consist of a customized combination of activators and repressors (Turing, 1952; Small et al., 1992; Hartmann et al., 1994; Small et al., 1996). Stripes form at edges where expression of one gap gene tails off and another begins (Fig. 4C). The highly heterogeneous design of the individual *eve* stripe enhancers indicates the opportunistic nature of transcriptional switch design.

### 3.5. Enhancer–promoter specificity

As described in the previous section, basal promoter elements are heterogeneous in structure, leading to distinct functional properties that permit enhancer–promoter specificity. This specificity allows enhancers to target a particular gene in a complex locus, even if its initiation site is not the closest one (Fig. 5). The *dpp* locus provides an example of this enhancer–promoter specificity (Schwyter et al., 1995; Merli et al., 1996). Enhancer elements that drive expression of *dpp* in the imaginal discs are located 20–35 kbp 3' of the *dpp* transcriptional initiation site. Two genes located only 5–10 kbp 3' of the disk enhancers, *SLY1 homologous (Slh)* and *out at first (oaf)*, are not normally activated by the *dpp* disk enhancers, but these enhancers can activate *oaf* when this gene's basal promoter was substituted with –50 to +65 bp fragment containing the *hsp70* promoter

(Merli et al., 1996). Similar basal-promoter-driven enhancer specificity has been observed with the tissue-specific expression of the divergently transcribed *gooseberry (gsb)* and *gooseberry neuro* genes (Li and Noll, 1994).

Studies of *gsb* and *dpp* did not identify particular features of the basal promoter that are correlated with enhancer discrimination. However, subsequent work indicates that the TATA box can play an important role. In a study of the promoter-specific enhancers from the *fushi tarazu* and *Abd-B* genes, respectively, basal promoters containing a TATA box were strongly preferred over similar promoters that lack this motif, suggesting that different interactions with TATA binding protein complexes might influence enhancer specificity (Ohtsuki et al., 1998). A contribution to enhancer specificity by specific TATA sequences was also reported in vertebrate cells (Wefald et al., 1990). The TATA box effect suggests that differences in promoter interactions by components of the basal machinery may influence enhancer signaling. Along these lines, a role for the basal machinery in developmentally controlled promoter utilization was suggested by biochemical reconstitution of transcription of the *Adh* gene. Here, TFIIA and TAF<sub>II</sub>150 were found to be important for the differential activation of the *Adh* tandem promoters (Hansen and Tjian, 1995). Subsequent studies found that a transcriptional repressor, AEF-1, binds directly to the initiator element of one of the promoters. Therefore, this developmental switch may actually be controlled by a sequence-specific regulatory factor (Ren and Maniatis, 1998). Despite the well-established role of enhancer–promoter specificity in a number of systems, some enhancers do not exhibit promoter preference, suggesting that the nature of the transcription factors bound to the enhancers also contributes to enhancer–promoter specificity (Ohtsuki et al., 1998; Das et al., 1995).

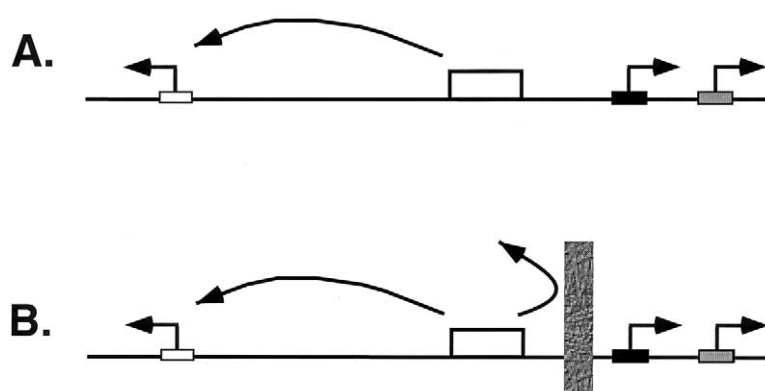


Fig. 5. Mechanisms for enhancer–promoter specificity. **A.** Enhancer interaction with specific basal promoter element reflects functional compatibility of enhancer with only one type of basal promoter, as has been demonstrated for the *dpp* gene (Merli et al., 1996). **B.** Enhancer specificity for a basal promoter directed by a boundary element (vertical rectangle) that prevents interactions with distal basal promoters, as has been demonstrated with *su(Hw)* boundary elements (Cai and Levine, 1995).

### 3.6. Evolution

Enhancer sequences are assemblages of binding sites subject to evolutionary forces, and molecular dissection of the *eve* stripe 2 enhancer suggests that there seem to be numerous routes to achieve proper regulatory results. Phylogenetic comparisons among *eve* stripe 2 enhancer elements in disparate *Drosophilids* indicate that this enhancer element has undergone some genetic drift or redesign, resulting in enhancers with similar outputs, but internally flexible architecture. Thus, the exact placement of individual factors within the element appears to be only loosely constrained (Kreitman and Ludwig, 1996; Ludwig et al., 2000). Although the *eve* stripe 2 element from *D. pseudoobscura* has functionally the same properties as the enhancer from *D. melanogaster*, a chimeric enhancer containing the 5' half of the *D. melanogaster* fused to the 3' half of the *D. pseudoobscura* sequence caused misexpression of the gene. This result indicates that the net output of this element has been conserved, but individual binding site contributions have undergone some change (Ludwig et al., 2000). One of the alterations appears to be appearance of a new, functional binding site for the bicoid activator (Ludwig and Kreitman, 1998). This increase in activator function appears to have been internally compensated for by a small deletion that moves the neighboring giant repressor site about 30 bp closer to the bicoid site (Hewitt et al., 1999).

Functional studies have also contributed to our understanding of the flexibility in enhancer design. The *eve* stripe 2 enhancer binds two types of activator proteins, bicoid and hunchback, that contribute to transcriptional activation. Are both activators utilized because of the unique properties that each contribute to activation of this element? In vitro transcriptional reactions suggested that these proteins synergize strongly to activate transcription by binding distinct TAF<sub>II</sub>s in the basal transcription machinery (Sauer et al., 1995). These activators show strong synergistic activity in vivo, suggesting that the enhancer was designed to exploit unique functional properties of both factors (Simpson-Brose et al., 1994). However, the unique hunchback site within *eve* stripe 2 could be replaced with additional bicoid binding sites or any of a variety of activation domains, suggesting that such synergy is not a requisite part of this enhancer's activity (Arnosti et al., 1996b). These results, in combination with phylogenetic studies, suggest that existing enhancers represent one of many possible solutions for achieving particular patterns of transcriptional activation.

## 4. Boundary Elements

The interaction of enhancers and promoters is regulated at an additional level, beyond that of compatibility

or specificity. Boundary elements or insulators are defined as elements that block interactions when inserted between promoters and enhancers; when placed within a gene complex, boundary elements can limit enhancer activity to a subset of promoter elements (Cai and Levine, 1995; Bell et al., 2001). A transgene that is flanked by these elements is also insulated against the repressive effect of heterochromatic sequences, conferring position-independent expression (Roseman et al., 1993).

Boundary elements that have been well studied in *Drosophila* include the specialized chromatin sequences (*scs* and *scs'*) flanking the *hsp70* genes, *Fab-7*, *Fab-8* and *Mcp* elements from the *Bithorax Complex*, and the *suppressor of hairy-wing* (*su(Hw)*) binding sites located within the *gypsy* transposon (reviewed in Bell et al. (2001). The *su(Hw)* zinc-finger protein binds directly to 12 sites within a 340 bp region of *gypsy*, establishing a functional boundary. *su(Hw)* also associates with hundreds of other chromosomal locations that may represent endogenous boundary elements (Gerasimova and Corces, 1998). An additional factor, *mod(mdg4)*, associates with *su(Hw)* and modifies its activity. Loss of *su(Hw)* always abolishes boundary element activity, but loss of *mod(mdg4)* has variable effects, inactivating the boundary element in some contexts and converting the boundary to a silencing element in others (Cai and Shen, 2001; Gerasimova et al., 1995).

All boundary elements can block enhancers, but there are differences in activities and composition of distinct elements (Fig. 6). In a direct comparison of activity, the *Fab-7* element was found to be less effective at blocking *ftz* enhancers than were *su(Hw)* sites (Hagstrom et al., 1996). *scs* and *su(Hw)* elements behave differently on episomal elements, where *scs* can act as a silencer, a property that is not been observed with *su(Hw)* sites (Parnell and Geyer, 2000). Unique proteins are found on distinct boundary elements; while *su(Hw)* and *mod(mdg4)* proteins function on *gypsy* boundary elements, different proteins, *zeste-white 5* and *BEAF*, bind to the *scs* and *scs'* elements, respectively (Gaszner et al., 1999; Zhao et al., 1995). The factors binding to *Fab-7* are not known, but this element does not require *su(Hw)* or *mod(mdg4)* for activity (Hagstrom et al., 1996; Cuvier et al., 1998; Gaszner et al., 1999).

Initial suggestions that boundary elements might initiate directional inactivation of enhancers was convincingly disproved when it was shown that enhancers blocked by boundary elements were still able to activate distal promoters (Cai and Levine, 1995; Scott and Geyer, 1995). The mode of action of these elements is still not well understood, but recent work involving the *su(Hw)* system suggests that these elements can self-associate, possibly forming chromatin loops, and target the element to the nuclear periphery (Gerasimova et al., 2000; Cai and Shen, 2001; Muravyova et al., 2001). Boundary elements can interfere with gene activity on small epi-

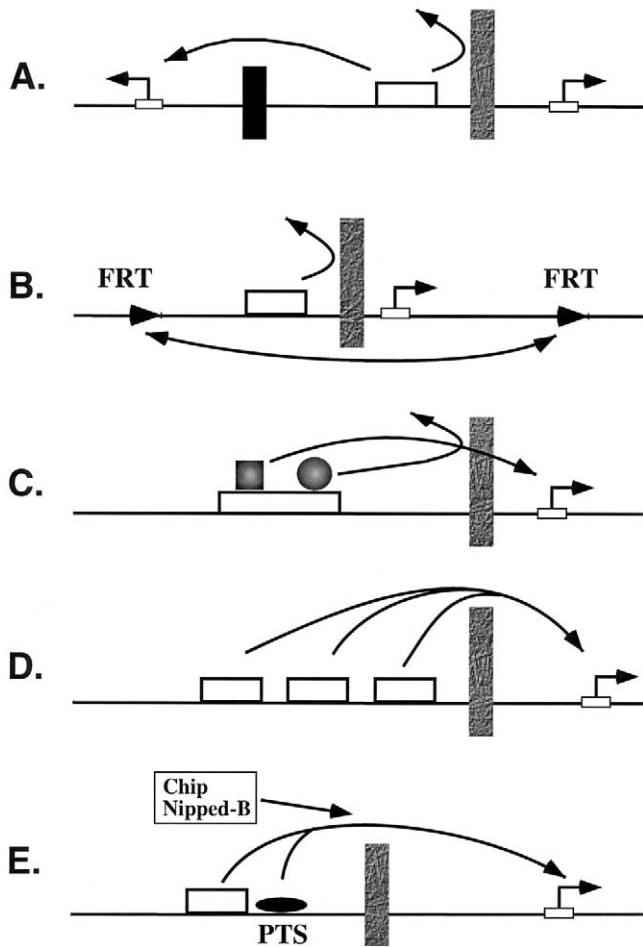


Fig. 6. Functional properties of boundary elements. **A.** Different boundary elements (represented by vertical rectangles) have different intrinsic levels of blocking activity, as is seen with direct comparisons of the *Fab-7* and *su(Hw)* elements (Hagstrom et al., 1996). **B.** Boundary elements exhibit specificity for blocking enhancer–promoter interactions, while permitting recombination between sites (FRT) for the yeast FLP recombinase (Parnell and Geyer, 2000). **C.** Boundary elements can exhibit specificity for different transcription factors, blocking some, while allowing others to interact with a distal promoter, as is seen where *su(Hw)* blocks hairy repression, while allowing dorsal activator action (Barolo and Levine, 1997). **D.** Enhancer blocking function can be influenced by enhancer strength; when multimerized, an enhancer blocked in **A.** can signal to a distal promoter (Scott et al., 1999). **E.** Cellular DNA elements and proteins can stimulate enhancer–promoter interactions, affecting boundary element effectiveness. A *cis*-acting element from the *Fab-8* element (promoter targeting sequence, PTS) facilitates enhancer communication across a boundary element (Zhou and Levine, 1999). Chip and Nipped-B proteins antagonize enhancer blocking activity and also play general roles in transcriptional regulation (Morcillo et al., 1997; Rollins et al., 1999).

some created by recombination *in vivo* and on plasmids in transient assays, suggesting that the location on a chromosome is not critical for functioning of these elements (Parnell and Geyer, 2000; Wei and Brennan, 2000). The exact placement of a boundary element can also critically influence its ability to regulate enhancer signaling. When two *su(Hw)* elements are located close to one another on transgenes, they functionally interact,

perhaps by self-association, leading to a surprising loss of boundary element activity. In some cases, the proposed association facilitates, rather than abolishes, enhancer/promoter interactions (Cai and Shen, 2001; Muravyova et al., 2001). The location and activity of other endogenous boundary elements may therefore influence the function of a given boundary element.

Despite their effectiveness at preventing most enhancer–promoter communication, boundary elements cannot be viewed as monolithic walls that block all communication between protein complexes on either side (Fig. 6B–E). The FLP recombinase protein is capable of promoting recombination between FRT sites separated by *su(Hw)* or *scs* boundary elements, suggesting that it is some particular property of transcriptional regulators that is sensitive to blocking (Parnell and Geyer, 2000). Even among transcription factors, boundary elements have also been found to be variable in their blocking specificity. The *su(Hw)* boundary element selectively blocks the hairy repressor, while allowing activators from the *rhomboid* enhancer to communicate with a promoter (Barolo and Levine, 1997). The opposite effect is seen with the *zen* ventral repression element, where a *su(Hw)* element can block activators within this element but not repressors (Cai and Levine, 1995). The balance of activators and boundary activity also appears to be critical in determining the extent of blocking: a stronger, multimerized fat body-specific enhancer was less effectively blocked by *su(Hw)* elements than was a single enhancer element (Scott et al., 1999).

The view that boundary elements are essentially static speedbumps on the road to gene expression has given way to an understanding that the activity of these elements can be modulated. The most illustrative example comes from mammalian imprinted *H19/Igf2* locus, where differential methylation appears to regulate binding of the CTCF boundary element factor, permitting distal enhancers to activate the *Igf2* gene on the paternal chromosome (Bell et al., 2001). In *Drosophila*, no examples are known of boundary elements that are modulated on a temporal or cell-specific level, but studies of boundary elements have identified *cis* acting elements and protein factors that appear to facilitate enhancer–promoter interactions, affecting the intrinsic properties of boundary elements (Fig. 6). A region associated with the *Fab-8* boundary element, the Promoter Targeting Sequence (PTS), permits enhancers distal to the *Fab-8* boundary element to communicate effectively with the *Abd-B* promoter, while apparently leaving intact the ability of the boundary element to block spreading of a heterochromatin-like state from nearby regions. The PTS is able to regulate a heterologous boundary element as well (Zhou and Levine, 1999). Analysis of the *Fab-7* and *Fab-8* boundary elements has been complicated by the presence of closely linked repressive Polycomb response elements (PREs, discussed below) (Cavalli and

Paro, 1998, 1999), but fine dissection of these elements has distinguished the boundary element from PRE activities (Hagstrom et al., 1996; Mihaly et al., 1997; Zhou et al., 1999; Barges et al., 2000).

Enhancer–promoter interactions can be specifically facilitated by Chip and Nipped-B, two proteins identified in screens for genes that modify the ability of the *gypsy* insulator to block the *cut* wing enhancer. Chip, a chromosomally associated protein, is thought to antagonize blocking activity of *gypsy* by preventing the insulator from interfering with enhancer–promoter communication in *trans*. Mutants show pleiotropic defects in gene expression in the embryo, suggesting a general role in gene expression. Chip is homologous to mammalian LIM-domain binding proteins, and has been proposed to crosslink weakly bound transcription activators, facilitating formation of bridges between enhancers and promoters (Morcillo et al., 1997). Nipped-B is homologous to fungal adherins, proteins with a variety of roles in chromosome structure and function (Rollins et al., 1999). The molecular activity of both of these proteins remains obscure.

## 5. Long-acting switches

### 5.1. Polycomb/trithorax genes

Developmentally programmed or environmentally induced transcriptional switches can be rapidly activated, repressed, and reactivated like toggles, often a consequence of the transient appearance or activation of DNA binding regulators. In contrast, some genes such as homeotic selector genes of the HOX clusters are programmed early in development into stable active or inactive conformations (Fig. 7). Transcriptional patterns of these genes set early in embryogenesis are then propagated through development, long after the disappearance of transcription factors that set the original pattern of expression (Kennison, 1995; Pirrotta, 1998).

While a detailed understanding of these stably programmable switches is still lacking, biochemical and genetic advances have begun to provide an outline of the components and activities involved. Initial patterns of gene expression are set by conventional transcriptional regulators such as Krüppel and hunchback binding to enhancer elements. Linked maintenance elements (often termed PRE or TRE) are bound by protein complexes from the Polycomb group (PcG) or trithorax group (trxG) of proteins (Brock and van Lohuizen, 2001). Initially, it was thought that PcG proteins only act to silence associated genes, while trxG proteins are involved in gene activation, but recent work suggests that some PcG and trxG proteins may have dual roles, functioning in both silencing and activation (reviewed in Brock and van Lohuizen (2001). An epigenetic mark,

perhaps in the form of chromatin modification such as histone acetylation, permits continued association of PcG proteins and repression through subsequent rounds of DNA replication. Stable activation of a maintenance element from the *Fab-7* region of *Ubx* is associated with hyperacetylation of histone H4 (Cavalli and Paro, 1999). The number of genes regulated by PcG and trxG complexes is not known, but PcG complexes have been mapped to approximately 100 sites on polytene chromatin (Zink and Paro, 1989). Besides HOX genes in the *Bithorax* and *Antennapedia* complexes, a cluster of 3 homeobox genes located within the 140 kbp *Iroquois* complex is regulated by PcG genes, suggesting that regulation by maintenance elements may be especially well-suited for coordinate regulation of clusters of developmental genes (Netter et al., 1998). However, individual genes not known to be regulated in clusters such as *engrailed*, *hairy*, *hedgehog*, and *wingless* are also regulated by PcG and/or trxG proteins (Zuckerkindl, 1999).

### 5.2. Enzymatic activities

Two general types of chromatin remodeling systems have been implicated in this long-term regulation by PcG and trxG proteins: nucleosome remodeling complexes and histone deacetylases (HDAC). SWI/SNF remodeling complexes move histones and alter the physical nature of nucleosome structure. Three trxG proteins are found in one such SWI/SNF complex, namely *brahma* (a homolog of the yeast SWI2/SNF2 ATPase), *moira* (SWI3 homolog) and *osa* (SWI1 homolog) (Collins et al., 1999; Collins and Treisman, 2000). Additional trxG proteins are found in at least two other high molecular weight complexes whose activities have not been identified (Papoulas et al., 1998). *brahma* and *osa* are also important for reversible silencing of *wingless* target genes, indicating that this SWI/SNF complex can also have negative effects on transcription, and that these factors are not dedicated solely to long-term functions (Collins et al., 1999; Collins and Treisman, 2000).

The second type of chromatin remodeling activity involves histone deacetylation, which can produce a more compact chromatin structure and possibly alter the affinity of histone tails for specific chromatin-binding factors (Strahl and Allis, 2000). The PcG proteins Esc and E(z) are found in one such complex associated with HDAC activity (Tie et al., 2001). Other PcG proteins are found in a complex isolated from mammalian cells termed PRC1 that can block SWI2/SNF2 activity (Shao et al., 1999). Genetic interactions between PcG, trxG genes and regulators of mitotic chromatin structure have also been noted, further evidence that transcriptional control by PcG and trxG proteins is likely to involve local changes in chromatin structure (Brock and van Lohuizen, 2001; Lupo et al., 2001; Decoville et al., 2001).

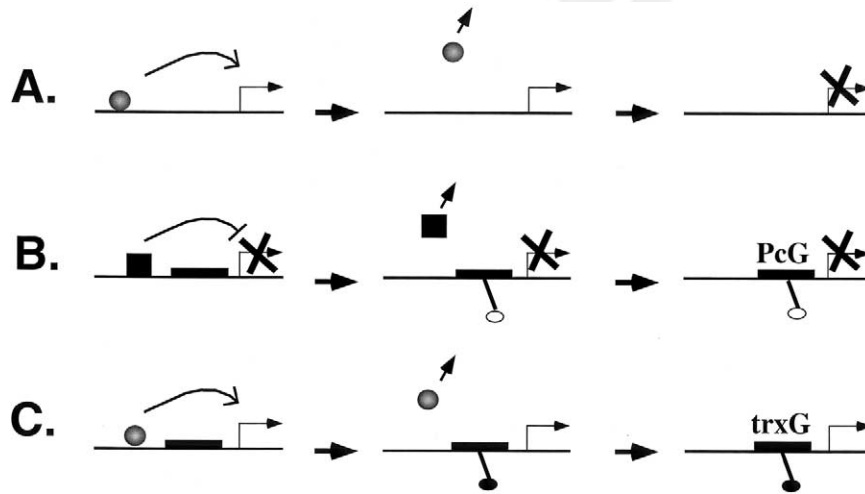


Fig. 7. Comparison of reversible vs. stable transcriptional switch elements. **A.** Sequence-specific transcription factors activate as long as they are associated with a gene. As soon as they are removed, transcription ceases, as is seen with heat shock promoters. **B.** A stable transcriptional switch containing a PRE "maintenance element" (black rectangle) is programmed early in development by transiently acting factors, in this case a transcriptional repressor (black square). The gene is repressed and epigenetically marked (ball and stick). Polycomb group (PcG) proteins stably associate with the gene and continue to silence transcription after the initial transiently acting factors are no longer present. An example of this type of regulation is seen in the early repression of the *Ubx* promoter by the hunchback protein, with subsequent repression mediated by PcG proteins (reviewed in Bienz and Müller (1995)). **C.** trithorax group (trxG) proteins, including SWI/SNF proteins, play a similar role in perpetuating an active state in response to activation of a gene early in development (Bienz and Müller, 1995).

### 5.3. Establishment

One surprising aspect of PcG and trxG regulation is that the SWI2/SNF2 and histone deacetylation functions are reversible in many circumstances, yet the signal set on a maintenance element early in embryogenesis persists through many mitotic cycles. One explanation is that the molecular mark, an epigenetic modification such as histone acetylation, may be separable from the actual protein complex responsible for repression activity. In this scenario, a PcG complex containing Esc and E(z) acts early to modify the chromatin, causing a PRC1-like complex to stably associate and repress gene activity (Tie et al., 2001; Ng et al., 2000). Consistent with this model, the continued presence of the maintenance element is essential for stable repression (Busturia et al., 1997). Clonal analysis of PcG mutants reveals that the repression activity mediated by PcG complexes appears to be readily reversible, in contrast to the mark, which is longer-lasting. When individual PcG genes were removed from cells using mitotic recombination, gene expression from the repressed locus was rapidly reactivated. If only a relatively short time was allowed to elapse, repression was restored by reintroduction of the protein, but after 72 h., restoration of repression was not possible, possibly due to loss of the epigenetic mark (Beuchle et al., 2001). The mark induced by active transcription of a transgene regulated by the *Fab-7*-associated maintenance element appears to be longer-lived, enduring even through meiosis in some cases (Cavalli and Paro, 1999).

### 5.4. Targeting

Establishment of a long-lived transcriptional state by PcG and trxG proteins is a multi-step process, including early transient promoter activation or repression, epigenetic marking, and recruiting of PcG or trxG proteins. The actual interaction of PcG proteins with a gene appears to be mediated by sequence-specific DNA binding proteins, including the YY1 homolog pleiohomeotic, the GAGA binding factor (Trithorax-like), and zeste, proteins that have been shown to interact with the *iab-7* PRE and other elements (Brown et al., 1998; Fritsch et al., 1999; Mishra et al., 2001). Interaction of the trxG protein-containing brahma complex with the DNA might involve interactions with the SWI1-like component, *osa*, which contains an ARID-type DNA binding domain, but this protein is not required for all *brahma* functions (Collins and Treisman, 2000). At least on some promoters, *brahma* may be recruited by *zeste* (Kal et al., 2000).

### 5.5. Future studies of transcriptional switch design

Transcriptional switch design has been studied for over 40 years using genetic and biochemical approaches. On a promoter-by-promoter basis, we have been successful in identifying the *cis* elements and the protein factors that interact with promoters. However, we lack an understanding of the general principles of enhancer architecture and transcription factor function that can provide a global understanding of transcriptional switch design. Recent genomic sequencing projects have

yielded rich insights into the protein coding capacity of organisms, but the transcriptional regulatory information embedded in higher eukaryotic genomes is still elusive. A major challenge in understanding the transcriptional wiring of a genome is identifying the function of individual switch elements, and predicting which genes are regulated by these elements.

New approaches promise to provide significant new insights in this area; first, genome-wide phylogenetic comparisons will highlight evolutionarily conserved intergenic blocks that may represent transcriptional regulatory information; such comparisons have already been used productively on individual genes (Loots et al., 2000). While protein binding sites are often conserved in regulatory regions, interpretation of results will have to take into account the flexibility of enhancer design, as seen with evolution of the *eve* stripe 2 enhancer. Secondly, new computational tools are being developed to search for and recognize patterns of binding sites present in putative regulatory regions. These new sources of information will be very powerful when combined with genome-wide expression information and comprehensive identification of in vivo transcription factor binding sites, as has been recently described for the yeast Gal4 activator (Ren et al., 2000). A fundamental understanding of transcriptional switch design will greatly aid in interpreting the regulatory information present in genomes and allow a better understanding of the function of biological systems. In addition, with recent progress in genetic manipulation of insects, this information will facilitate the design of transcriptional switches suitable for controlled expression of transgenes in species of economic and medical importance.

## Acknowledgements

I would like to thank the members of the Arnosti laboratory, past and present, who have contributed to our research on transcriptional repressors in the *Drosophila* embryo, members of the MSU Gene Expression in Development and Disease Focus Group for useful discussions, and helpful comments from the reviewers. Work in our laboratory is supported by a grant from the National Institutes of Health.

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