Cis and *Trans* Interactions Between the *iab* Regulatory Regions and *abdominal-A* and *Abdominal-B* in *Drosophila melanogaster*

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ABSTRACT

The *infra-abdominal* (*iab*) elements in the bithorax complex of *Drosophila melanogaster* regulate the transcription of the homeotic genes *abdominal-A* (*abd-A*) and *Abdominal-B* (*Abd-B*) in *cis*. Here we describe two unusual aspects of regulation by the *iab* elements, revealed by an analysis of an unexpected complementation between mutations in the *Abd-B* transcription unit and these regulatory regions. First, we find that *iab-6* and *iab-7* can regulate *Abd-B* in *trans*. This *iab trans* regulation is insensitive to chromosomal rearrangements that disrupt transvection effects at the nearby *Ubx* locus. In addition, we show that a transposed *Abd-B* transcription unit and promoter on the *Y* chromosome can be activated by *iab* elements located on the third chromosome. These results suggest that the *iab* regions can regulate their target promoter located at a distant site in the genome in a manner that is much less dependent on homologue pairing than other transvection effects. The *iab* regulatory regions may have a very strong affinity for the target promoter, allowing them to interact with each other despite the inhibitory effects of chromosomal rearrangements. Second, by generating *abd-A* mutations on rearrangement chromosomes that break in the *iab-7* region, we show that these breaks induce the *iab* elements to switch their target promoter from *Abd-B* to *abd-A*. These two unusual aspects of *iab* regulation are related by the *iab-7* breakpoint chromosomes that prevent *iab* elements from acting on *Abd-B* and allow them to act on *abd-A*. We propose that the *iab-7* breaks prevent both *iab trans* regulation and target specificity by disrupting a mechanism that targets the *iab* regions to the *Abd-B* promoter.

PROPER development of an organism requires precise regulation of gene expression. Enhancer and silencer elements provide one level of this regulation, activating and suppressing, respectively, the transcription of genes in precise temporal and spatial patterns. Enhancer/silencer elements can regulate the activity of specific target promoters from a relatively long distance in *cis* on the same chromosome (for review see ATCHISON 1988). In *Drosophila melanogaster* a genetic phenomenon called transvection suggests that enhancers and silencers can also regulate promoters in *trans* on a homologous chromosome (see JUDD 1988; WU and GOLDBERG 1989; PIRROTTA 1990; TARTOF and HENIKOFF 1991; WU 1993 for reviews). This effect was originally described for regulatory mutations for the *Ultrabithorax* (*Ubx*) gene (LEWIS 1954). In a transvection effect chromosomal rearrangements that disrupt pairing between homologous chromosomes enhance or suppress the phenotype caused by certain regulatory mutations. Thus, it has been proposed that homologue pairing allows interaction between a regulatory element on one chromosome and its target promoter on another chromosome (ZACHAR *et al.* 1985; CASTELLI-GAIR *et al.* 1990; GEYER *et al.* 1990).

The homeotic genes of the bithorax complex, *Ubx*, *abd-A* and *Abd-B*, specify segmental identities of overlapping body segments of the fly from parasegments (PS) 5 through PS 14, corresponding to the third thoracic (T3) through the ninth abdominal segment (A9) (LEWIS 1978; DUNCAN 1987). Genetic analyses have revealed two types of complementation groups, lethal and nonlethal, within the bithorax complex (Figure 1; LEWIS 1978; SÁNCHEZ-HERRERO *et al.* 1985). Lethal complementation groups identify the *Ubx*, *abd-A* and *Abd-B* transcription units that encode essential DNA-binding proteins. *Abd-B* comprises two subfunctions, *Abd-Bm* and *Abd-Br*, provided by four overlapping transcripts encoding Abd-BI and Abd-BII proteins in PS10-13 and PS14, respectively (CASANOVA *et al.* 1986; CASANOVA and WHITE 1987; DELORENZI *et al.* 1988; KUZIORA and MCGINNIS 1988; SÁNCHEZ-HERRERO and CROSBY 1988; CELNIKER *et al.* 1989; ZAVORTINK and SAKONJU 1989; DELORENZI and BIENZ 1990; BOULET *et al.* 1991). Nonlethal complementation groups, which affect subsets of *Ubx*, *abd-A* and *Abd-B* expression domains, identify regulatory elements for *Ubx*, *abd-A* and *Abd-B* transcription units. The *abx*, *bx*, *pbx* and *bxd* elements regulate *Ubx* transcription in PS 5 (T2/T3) and PS 6 (T3/A1) (BEACHY *et al.* 1985; HOGNESS *et al.* 1985; WHITE and

We dedicate this paper to E. B. Lewis, who began it all, on the occasion of his 77th birthday.

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WILCOX 1985). *iab-2, -3* and *-4* elements regulate *abd-A* in PS7 (A2), PS8 (A3) and PS9 (A4), respectively (KARCH *et al.* 1990; MACIAS *et al.* 1990). *iab-5–iab-7* elements regulate the *Abd-B* class A transcription unit that codes for the Abd-BI protein in PS10 (A5) through PS13 (A8) (CELNIKER *et al.* 1990; BOULET *et al.* 1991; SÁNCHEZ-HERRERO 1991). Although specific regulatory mutations affecting the expression of the class A *Abd-B* transcript in PS13 and the three longer transcripts (class B, C and γ) that code for the Abd-BII protein in PS14 and PS15 have not been found, they are expected to identify *iab-8* and *iab-9*, respectively.

Genetic and molecular evidence indicate that the regulatory elements in these *iab* regions act in *cis*. When mutations mapping to the transcription units are made heterozygous with mutations in regulatory elements, they partially fail to complement each other. Furthermore, expression patterns of *Ubx, abd-A* and *Abd-B* transcripts are altered in embryos mutant for these regulatory elements (BEACHY *et al.* 1985; HOGNESS *et al.* 1985; WHITE and WILCOX 1985; CELNIKER *et al.* 1990; KARCH *et al.* 1990; MACIAS *et al.* 1990; BOULET *et al.* 1991; SÁNCHEZ-HERRERO 1991), and aspects of correct expression patterns can be recapitulated by a *lacZ* reporter under the control of these regulatory elements in transformed flies (SIMON *et al.* 1990; MULLER and BIENZ 1991; QIAN *et al.* 1991; BUSTURIA and BIENZ 1993).

We describe in this paper an analysis of an unexpected complementation between mutations in the *Abd-B* transcription unit and its regulatory regions. We show that the *iab* regulatory elements can regulate the target promoter in *trans*. Unlike previously described transvection effects, this *trans* regulation by the *iab* elements, which we call ITR, is unusually resistant to disruption by chromosomal rearrangements. We also show that chromosomal rearrangements that separate the *iab* elements from their normal *Abd-B* target promoter cause *abd-A* to substitute for some *Abd-B* functions, most likely by allowing the *iab* elements that normally act on *Abd-B* to act on *abd-A* instead.

MATERIALS AND METHODS

Fly stocks: Fly stocks were maintained at 25° in a humidified incubator on standard cornmeal medium supplemented with fresh yeast. All crosses were performed under these conditions. Descriptions of the mutations used can be found in the following references: $abd\text{-}A^{M3}$ (SÁNCHEZ-HERRERO *et al.* 1985); $abd\text{-}A^{MX1}$, $abd\text{-}A^{MX2}$, $abd\text{-}A^{D24}$, $iab\text{-}7^{MX1}$, $iab\text{-}7^{MX2}$, $iab\text{-}7^{SGA62}$, $iab\text{-}4,5^{DB}$, $Abd\text{-}B^{D14}$, $Abd\text{-}B^{D16}$, *Df(3R)P9* (KARCH *et al.* 1985); $Abd\text{-}B^{M5}$ (PELAZ 1994); $Abd\text{-}B^{S4}$ (TIONG *et al.* 1985); $Abd\text{-}B^{S7}$ (TIONG *et al.* 1988); $iab\text{-}7^{S10}$ (TIONG *et al.* 1987); *Fab-7, Fab-7R7, Fab-7R9, Fab-7R41, Fab-7R59, Fab-7R73* (GYURKOVICS *et al.* 1990); $iab\text{-}7^{blt}$, $iab\text{-}7^{Sz}$ (GALLONI *et al.* 1993); *TM3 Sb, TM6 Tb*, z^1, z^{1IG3}, z^{ep}, z^a (LINDSLEY and ZIMM 1992); *zeste* alleles, 826.2s, 977.2s, 1008.1bs (WU 1984).

Mutagenesis and mapping: We induced transvection-disrupting chromosomal rearrangements on the $Abd\text{-}B^{D14}$ chromosome by irradiating $Abd\text{-}B^{D14}$ males with 4000 rad from a ^{137}Cs source and crossing them to females carrying the double mutation Cbx^1Ubx^1. Cbx^1Ubx^1 heterozygotes show a dominant phenotype of warped and held out wings because Cbx^1 acts to misexpress Ubx^+ on the homologous chromosome via a transvection effect (LEWIS 1955; CABRERA *et al.* 1985; WHITE and AKAM 1985; WHITE and WILCOX 1985; CASTELLI-GAIR *et al.* 1990). This transvection effect is disrupted by chromosomal rearrangements that break once between *Ubx* and the centromere and once outside this region. We recovered rearrangement mutations on the $Abd\text{-}B^{D14}$ chromosome that disrupt transvection between Cbx^1 and Ubx^+ by screening for $Abd\text{-}B^{D14}/Cbx^1Ubx^1$ F1 progeny with wild-type wings. The rearrangements were mapped by following segregation with autosomal markers. Some mutations were more finely mapped by cytogenetic analysis of larval polytene salivary gland chromosomes.

To isolate *abd-A* mutations on an *iab-7* breakpoint chromosome, $iab\text{-}7^{MX2}/TM3\ Sb$ male flies, aged 1–7 days, were irradiated with 4000 rad using a ^{137}Cs source. They were immediately mated en masse to *TM3 Sb/TM6 Tb* 1–7-day-old virgin females. After 7 days the parents were removed. Individual $iab\text{-}7^{MX2}*/TM6$ Tb F1 males were allowed to mate with $abd\text{-}A^{D24}/TM6\ Tb$ females. $iab\text{-}7^{MX2}*/TM6\ Tb$ F2 progeny from vials containing no Tb^+ pupae were crossed separately to $abd\text{-}A^{MX1}$, $abd\text{-}A^{MX2}$, $abd\text{-}A^{M3}$ and $abd\text{-}A^{D24}$ to confirm that they carried an *abd-A* mutation. From 9735 individual matings we recovered two *abd-A* mutations, $abd\text{-}A^{JX1}$ and $abd\text{-}A^{JX2}$.

$abd\text{-}A^{JX1}$ and $abd\text{-}A^{JX2}$ are null for *abd-A* function based on the following criteria. Cuticle preparations of $abd\text{-}A^{JX1}/abd\text{-}A^{D24}$ and $abd\text{-}A^{JX2}/abd\text{-}A^{D24}$ embryos show transformation of ventral denticle belts toward A1, typical of *abd-A* mutants (SÁNCHEZ-HERRERO *et al.* 1985). $abd\text{-}A^{JX1}$ homozygotes also show transformations typical of *abd-A* mutants, but $abd\text{-}A^{JX2}$ homozygotes show a transformation of denticle belts toward T3. $abd\text{-}A^{JX2}/Ubx$ embryos show a transformation of A1 toward T3 and die before hatching, but $abd\text{-}A^{JX2}/bx^{34e}$ animals live to adulthood and show no obvious transformation. We suspect that the $abd\text{-}A^{JX2}$ deletion includes *pbx* and *bxd* but not *bx*. The deletion may include part of the *Ubx* promoter, causing an enhanced transvection from bx^+ that suppresses the bx^{34e} phenotype (MARTÍNEZ-LABORDA *et al.* 1992). Analysis of $abd\text{-}A^{JX1}$ or $abd\text{-}A^{JX2}$ chromosomes reveals no chromosomal abnormalities other than the parental $iab\text{-}7^{MX2}$ inversions. Southern blot analysis using fragments of genomic clones L2235, L2255, L2265, L2279, R8004 and R8019 (KARCH *et al.* 1985) reveals that $abd\text{-}A^{JX1}$ contains a deletion of 4–6 kb including the *abd-A* homebox and that $abd\text{-}A^{JX2}$ is associated with a deletion with the right endpoint between *abd-A* exons 2 and 4. Neither mutation contains lesions in the *iab* regions to the right of the *abd-A* transcriptional unit. Neither mutant expresses *abd-A* transcripts in embryos as assayed by whole mount *in situ* hybridization.

In the screen for *abd-A* alleles, we fortuitously isolated one insertional transposition mutation, *Tp(3;Y)JX3*. *Tp(3;Y)JX3* fully complements *abd-A* mutations and is genetically identical to the parental $iab\text{-}7^{MX2}$ allele, except that it segregates with the *Y* chromosome. The two components of *Tp(3;Y)JX3*, *Dp(3;Y)JX3* and *Df(3)JX3*, segregate independently, although the *Df(3)JX3* is not viable without the *Dp(3;Y)JX3*. Analysis of *Tp(3;Y)JX3* of larval polytene chromosomes reveals a transposition of 65A-64A/89E-92A, spanning the right inversion breakpoint in $iab\text{-}7^{MX2}$, to the *Y* chromosome and the corresponding deletion on the third chromosome.

Cuticle preparations: Adult cuticles were prepared using a modification of the technique described in DUNCAN (1982). The abdomen was split middorsally with a razor blade, teased away from the rest of the fly and incubated in 10% KOH for

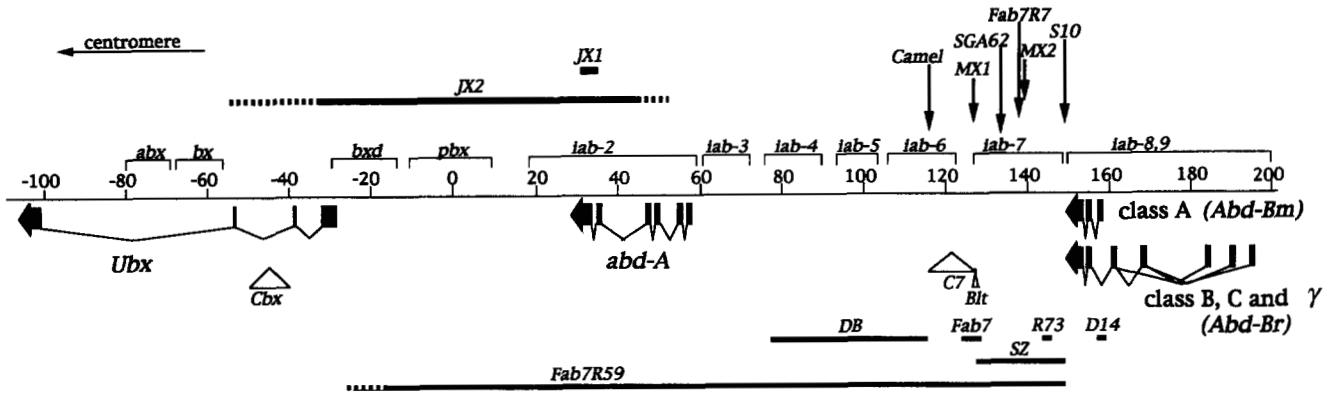


FIGURE 1.—Map of the bithorax complex. The *Ubx, abd-A* and *Abd-B* transcripts are shown. Arrows indicate the positions of breakpoint mutations; —, the regions covered by deletion mutations; △, the positions of insertion mutations; ---, uncertainty in the locations of the endpoints.

30 min at room temperature. The abdominal cuticles were then splayed out on a slide, covered with 10% KOH and flattened with a coverslip overnight. The cuticles were then washed off the slides with water and mounted in CMCP10 (Masters Chemical Company, Inc., Elk Grove, IL) mountant. The cuticle pigmentation remained a natural color for months in this mountant. For larval cuticle preparations we followed the protocol described in LAMKA *et al.* (1992).

***In situ* hybridizations and antibody stainings:** We synthesized DNA probes for *in situ* hybridizations using digoxigenin conjugated dUTP (Boehringer Mannheim) and asymmetric PCR to predominantly amplify the antisense strand. The class A and common probes were constructed as described in BOULET *et al.* (1991). The *D14*-specific probe included nucleotides +95 through +191 of the *Abd-B* sequence (ZAVORTINK and SAKONJU 1989). The *abd-A* probe included sequences from +3494 to +4043 (APPEL 1993). We detected the probes with alkaline phosphatase-conjugated sheep anti-digoxigenin antibody from Boehringer Mannheim (1:2000). Monoclonal mouse anti-invected was provided by J. KASSIS and D.-H. HUANG and used at a 1:4 dilution. We detected the anti-invected antibody with biotinylated anti-mouse (used at 1:2000) (Vector Labs) followed by streptavidin-horseradish peroxidase (HRP) (used at 5 μg/ml) (Chemicon). We used the rat anti-Abd-B antibody described in BOULET *et al.* (1991) at 1:200 to 1:400. All antibodies were preadsorbed to CanS embryos at 1:20 for 1 hr at room temperature and stored at 4° (except for the invected antibody that is preadsorbed at 1:4).

We performed *in situ* hybridizations as described (TAUTZ and PFEIFLE 1989). To double label the embryos with anti-invected antibody and a DNA probe, we modified this protocol after the hybridization washes by blocking the embryos with phosphate-buffered saline (PBS) + 0.1% Triton X-100 + 0.1% bovine serum albumin (BSA) (PBSBT) for 1 hr at room temperature and incubating the embryos with anti-invected antibody overnight at 4°, washing 12 times for 10 min with PBSBT, incubating with biotinylated anti-mouse and sheep anti-digoxigenin in PBSBT for 1 hr at room temperature, washing again and incubating with fresh streptavidin-HRP in PBSBT for 30 min at room temperature. The final wash was without BSA. HRP was detected by reacting the embryos with 0.03% hydrogen peroxide and 0.5 mg/ml diaminobenzidine. The HRP reaction was stopped with PBS plus 0.1% Tween-20, and the alkaline phosphatase reaction was performed as described (TAUTZ and PFEIFLE 1989). When possible, we identified specific genotypes by using a *TM3* balancer chromosome containing a *ftz-lacZ* construct (from S. SMOLIK-UTLAUT). We stained the embryos using *X*-gal following the protocol in RUSSELL *et al.* (1992). If we could not follow the chromosomes using this marker, we determined the ratios of the different staining patterns to confirm that the mutant expression pattern appeared in the expected ratios.

RESULTS

Unexpected complementation at the *Abd-B* locus: The mutant phenotype caused by rearrangement mutations with a break in the *iab-7* region (*iab-7^{break}*) depends on the type of *Abd-B* mutations on the homologous chromosome. When an *iab-7^{break}* allele is heterozygous with a deletion of the bithorax complex *DfP9*, abdominal segments A5–A7 transform toward A4. This transformation is evident in adult male flies by the loss of male-specific pigment in A5 and A6 tergites (dorsal plates) and the presence of A7 segment, which is normally suppressed in males (Figure 2B). It is also indicated by an increase in trichome density in A6 and A7 tergites and the presence of sternite (ventral plate) bristles in A6 and A7. In contrast, *iab-7^{break}* heterozygous with *Abd-B* point or pseudopoint mutations (henceforth referred to as *Abd-B^{-}*) that do not affect the *iab* regulatory regions cause a less severe mutant phenotype. A5 segment appears normal, and A6 and A7 tergites show male-specific pigmentation and reduced trichome density, whereas their sternites resemble that of A5 (Figure 2C). We shall call this mixed identity assumed by A6 and A7 the "A5/A6 identity". Thus, contrary to expectations, *iab-7^{break}/Abd-B^{-}* flies show a less severe transformation of the posterior abdominal segments than do *iab-7^{break}/DfP9* flies.

We eliminated two trivial explanations for the unexpected A5/A6 identity in *iab-7^{break}/Abd-B^{-}* flies. First, this phenotype is not due to uncharacterized mutations outside the bithorax complex, because several *iab-7^{break}* mutations heterozygous with several *Abd-B^{-}* mutations (listed in Table 1) show the nearly

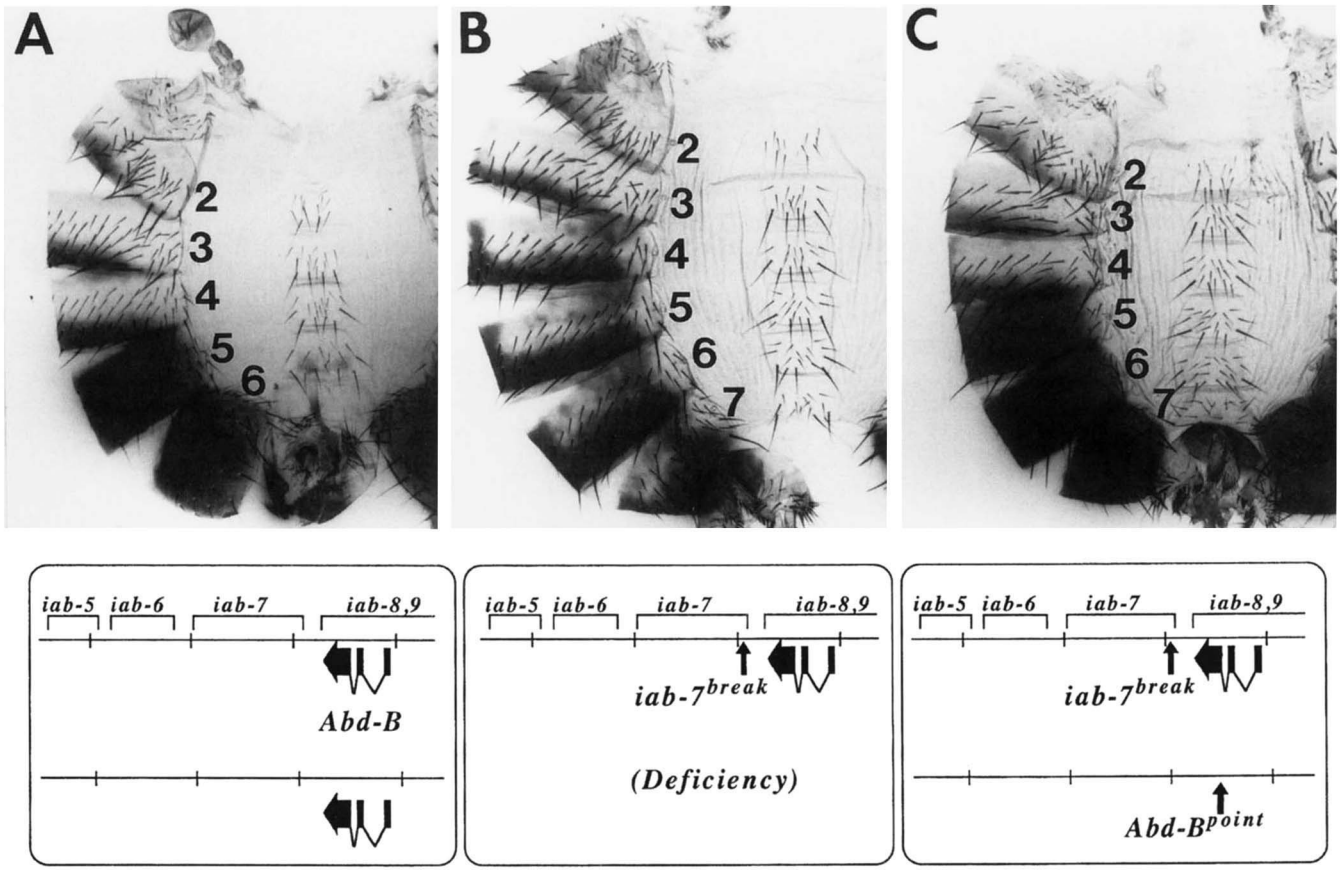


FIGURE 2.—Unexpected complementation at the *Abd-B* locus. Adult male phenotypes of wild type (A), *iab-7*^{MX2}/*DfP9* (B) and *iab-7*^{MX2}/*Abd-B*^{D16} (C). Each abdominal cuticle shows the ventral sternites on the right and half of the dorsal tergites on the left. Abdominal segments are labeled. Wild-type male flies show a characteristic dark pigmentation pattern (male-specific pigmentation) on the dorsal part of the sclerotized cuticle (tergite) in abdominal segments A5 and A6 and suppress the formation of A7 cuticle. Small hairs called trichomes cover the A5 tergite but are very sparse on the A6 tergite, and there are bristles on the A5 sternite (ventral cuticle plate) but not on the A6 sternite. *iab-7*^{MX2}/*DfP9* males show derepression of A7 tergite and sternite formation, the loss of dark male-specific pigment and an increase in trichome density on A5, A6 and A7 tergites, and presence of bristles on A6 and A7 sternites. *iab-7*^{MX2}/*Abd-B*^{D16} males show male-specific pigment on A5, A6 and A7. In addition, the A6 tergite has patches devoid of trichomes, the A7 tergite nearly completely lacks trichomes, and the A6 and A7 sternites are larger, indicating a greater amount of posterior identity in A6 and A7 than in A5.

identical pigmentation phenotype. Second, the A5/A6 identity is not due to the possible hypomorphic nature of the *Abd-B*$^-$ alleles used. All of the *Abd-B*$^-$ alleles we have tested behave genetically null for the *Abd-Bm* function (see Table 1 and MATERIALS AND METHODS for references). Furthermore, in two cases where associated DNA lesions have been determined, one mutant allele (*Abd-B*^{D14}) has a 411-bp deletion of the *Abd-B* class A promoter and transcription start site, obliterating detectable levels of *Abd-B* class A transcription, and another (*Abd-B*^{M5}) carries a point mutation that causes premature termination of the *Abd-B* open reading frame (ORF) in the first exon (see Table 1 for references).

We also tested the possibility that other essential functions of the bithorax complex still present on the *Abd-B*$^-$ chromosome, but missing on the *DfP9* chromosome, contribute to the A5/A6 identity in *iab-7*^{break}/*Abd-B*$^-$ heterozygotes. We examined *iab-7*^{break} mutations heterozygous with a chromosome carrying the triple mutant chromosome *Ubx*^{MX12} *abd-A*^{M1} *Abd-B*^{M8}, which behaves as *Ubx*$^-$ *abd-A*$^-$ *Abd-Bm*$^-$*r*$^-$ (CASANOVA *et al.* 1987). These heterozygous flies show the same phenotype as *iab-7*^{break}/*Abd-B*$^-$ flies (data not shown). Therefore, neither *Ubx*, *abd-A* nor *Abd-Br* on the *Abd-B*$^-$ chromosome contribute to the less severe phenotype in *iab-7*^{break}/*Abd-B*$^-$ flies. Because the only remaining significant difference between the *Abd-B*$^-$ chromosome and the *DfP9* chromosome is the presence or absence, respectively, of the *iab* regions, we hypothesized that the A5/A6 identity in *iab-7*^{break}/*Abd-B*$^-$ heterozygotes is due to the activity of the *iab* regions located on the *Abd-B*$^-$ chromosome. We further hypothesized that the intact *iab-5*–*iab-7* regions on the *Abd-B*$^-$ chromosome activate, in *trans*, the *Abd-B* promoter on the *iab-7*^{break} chromosome, normally quiescent in A5 through A7. We shall

TABLE 1

Characterization of *iab*-7 and *Abd-B* alleles

Mutation	Phenotype	Protein	RNA	Cytology	Molecular lesion
iab-7^{break} mutants					
iab-7^{MX1}	*iab-5,-6,-7*			T(Y;2;3)	126–129
iab-7^{MX2}	*iab-5,-6,-7*	13, 14, 15[a]	13, 14, 15[b,c]	In(89E,89A,64A)	139.5–142
iab-7^{SGA62}	*iab-5,-6,-7*			In(89C-89E)	133–139.5
iab-7^{S10}	*iab-5,-6,-7*			Tp(89c-89E;Y)	154–158
Fab-7R7	*iab-5,-6,-7*			In(87D;89E)	141.5–143
Abd-B^{-} mutants					
*Abd-B^{D14}**	*Abd-Bm-*	14, 15[a,c,d]	14, 15[c]	Normal	Deletes −66 to +345 of class A transcript[e]
Abd-B^{M5}	*Abd-Bm-*	14, 15[f]		Normal	Stop codon in first class A exon[g]
*Fab-7R9**	*Abd-Bm-*			Normal	0.5-kb insert at 157–158
Fab-7R41	*Abd-Bm-*			Normal	0.5-kb insert at 154–156
Abd-B^{D16}	*Abd-Bm-r-*	None[c]		Normal	
Abd-B^{S4}	*Abd-Bm-r-*				
Abd-B^{S7}	*Abd-Bm-r-*				

Characterization of *iab-7^{break}* and *Abd-B^{-}* alleles used in this study. The names of alleles according to the nomenclature established by DUNCAN (1987) are shown with their hemizygous phenotype. * denotes two alleles that exhibit a slightly weaker mutant phenotype (a lower density of trichomes in A6 and A7 tergites) than other *Abd-B^{-}* alleles when transheterozygous with *iab-7^{break}* alleles. However, because *Abd-B^{D14}* and *Fab-7R9* behave similarly to other *Abd-Bm^{-}* alleles when hemizygous, we suspect that this weaker mutant phenotype is due not to these alleles' hypomorphic nature, but rather to an ectopic activation of *abd-A* (see DISCUSSION for a possible explanation). The Protein column indicates in which embryonic parasegments Abd-B protein is detected. The RNA column indicates in which embryonic parasegments *Abd-B* transcript is detected. We have indicated references for molecular data.

References: [a] CELNIKER *et al.* (1990), [b] SANCHEZ-HERRERO and AKAM (1989), [c] BOULET *et al.* (1991), [d] SÁNCHEZ-HERRERO (1991), [e] ZAVORTINK and SAKONJU (1989), [f] DELORENZI and BIENZ (1990), [g] PELAZ (1994).

refer to this postulated *trans* activity of the *iab* regions as *iab trans* regulation or ITR.

Molecular evidence for ITR: To test if the *Abd-B* promoter is activated in *trans* in ITR, we examined *Abd-B* expression patterns using *in situ* hybridization in embryos. Although embryonic cells that express *Abd-B* do not necessarily become imaginal precursor cells of adult tergites and sternites, *Abd-B* expression patterns in the embryo have been previously shown to correlate remarkably well with adult mutant phenotypes (CELNIKER *et al.* 1990; BOULET *et al.* 1991; SÁNCHEZ-HERRERO 1991). In wild-type embryos *Abd-B* class A transcripts are expressed in PS10-13, and class B, C and γ transcripts are expressed in PS14 and PS15 (Figure 3A) (KUZIORA and MCGINNIS 1988; SÁNCHEZ-HERRERO and CROSBY 1988; CELNIKER *et al.* 1989; ZAVORTINK and SAKONJU 1989). In *iab-7^{MX2}* (*iab-7^{break}*) homozygous embryos *Abd-B* class A transcript is not detected in PS10-12 (Figure 3B), and in *Abd-B^{D14}* (*Abd-B^{-}*) homozygous embryos, it is no longer expressed in PS10-13 (Figure 3C). In contrast, we observe that *iab-7^{MX2}/Abd-B^{D14}* embryos express *Abd-B* transcripts in PS11–PS15 in a graded manner similar to the wild-type embryos (Figure 3D). These transcripts are translated, because immunostaining detects Abd-B protein in a similarly graded manner (A. BOULET, unpublished results).

To determine whether *Abd-B* on the *iab-7^{MX2}* chromosome is activated, we hybridized *iab-7^{MX2}/Abd-B^{D14}* embryos with a class A-specific probe containing only the sequences deleted in the *Abd-B^{D14}* mutation. In *iab-7^{MX2}/Abd-B^{D14}* embryos we detect this sequence in PS 11, 12 and 13 (Figure 3E), whereas we detect this sequence only in PS 13 in *iab-7^{MX2}* homozygotes, and we do not detect this sequence in *Abd-B^{D14}* homozygotes (data not shown). Therefore, when *iab-7^{MX2}* is transheterozygous with *Abd-B^{D14}*, the *Abd-B* class A promoter on the *iab-7^{MX2}* breakpoint chromosome is activated in PS 11 and 12.

A notable difference between the expression patterns of *iab-7^{MX2}/Abd-B^{D14}* embryos and the wild-type embryos is that *Abd-B* transcripts are not expressed in PS 10 in the mutant embryos. Our failure to detect hybridization signal in PS 10 could be due either to absence of *Abd-B* expression or to insensitivity of the technique. We suspect, however, that the absence of hybridization signal is real, because, as will be shown below, genetic experiments indicate that *Abd-B* contributes to A5/A6 identity only in A6 and A7 (corresponding to PS11 and PS12) and not in A5 of *iab-7^{MX2}/Abd-B^{D14}* heterozygotes.

Genetic evidence that *Abd-B* is activated in *trans*: To test whether *trans* activation of the *Abd-B* promoter can confer A5/A6 identity in *iab-7^{break}/Abd-B^{-}* adult flies, we used an X-ray-induced insertional transposition derived from the *iab-7^{MX2}* inversion chromosome. This transposition, *Tp(3;Y)JX3*, moves the *Abd-B* transcription unit and promoter to the *Y* chromosome [*Dp(3;Y)JX3*], leaving the *iab* regions on the third

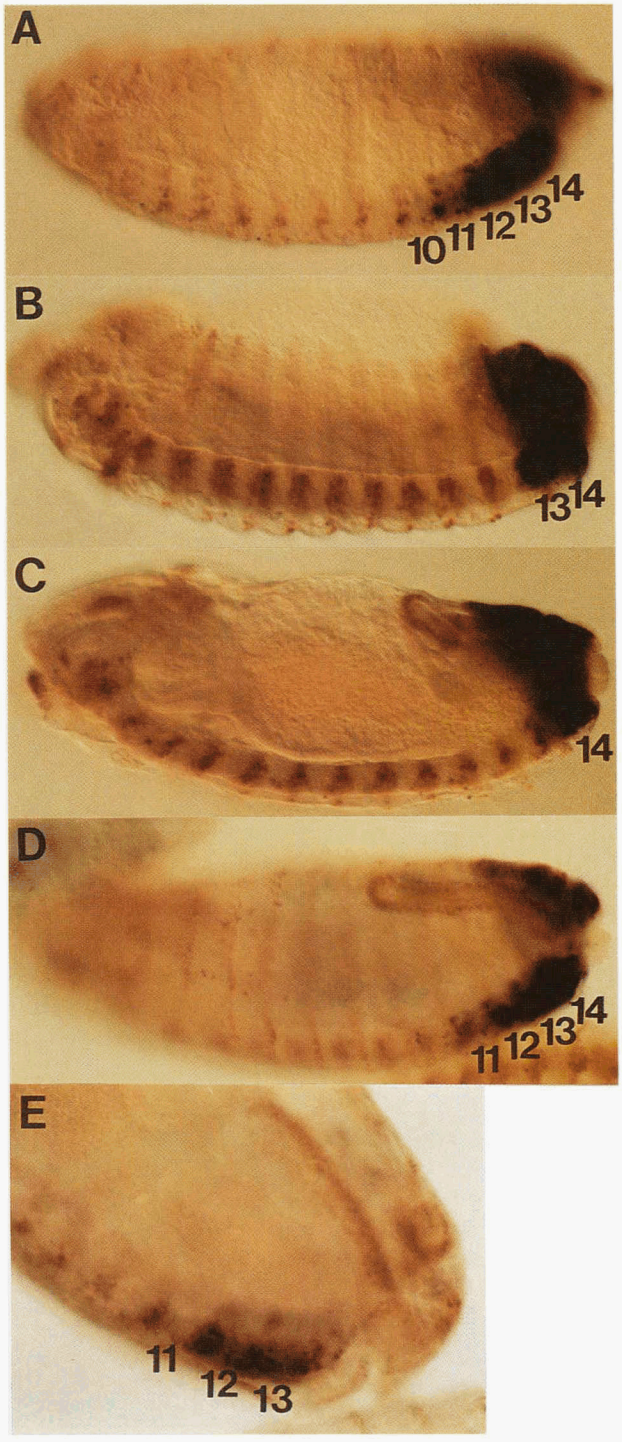


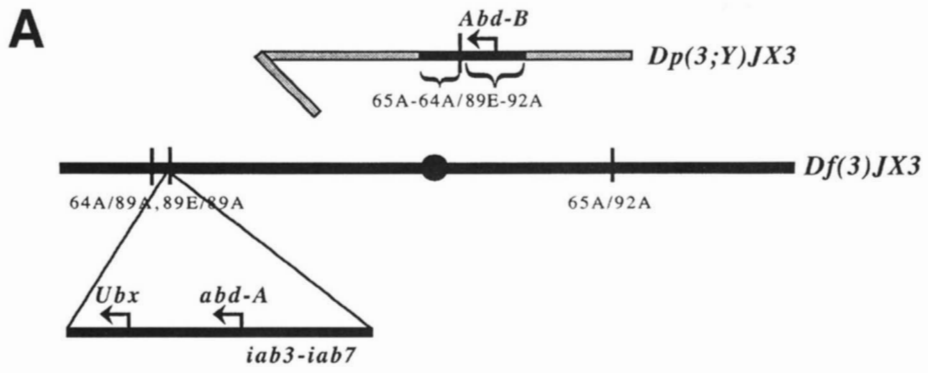
FIGURE 3.—Expression of *Abd-B* in embryos with homozygous and transheterozygous combinations of *iab-7*^{MX2} and *Abd-B*^{D14} mutations. (A) Wild-type embryos, (B) *iab-7*^{MX2} homozygotes, (C) *Abd-B*^{D14} homozygotes and (D and E) *iab-7*^{MX2}/*Abd-B*^{D14} transheterozygotes. Anterior is to the left, and dorsal is up. Embryos in A–D were hybridized to a probe antisense to *Abd-Bm* and *r* sequences (blue); the embryo in E was hybridized to a probe antisense to the sequences missing in the *Abd-B*^{D14} deletion (blue). All embryos were also stained for invected protein (brown). Invected protein is detected at the anterior boundary of each parasegment. Parasegments are labeled.

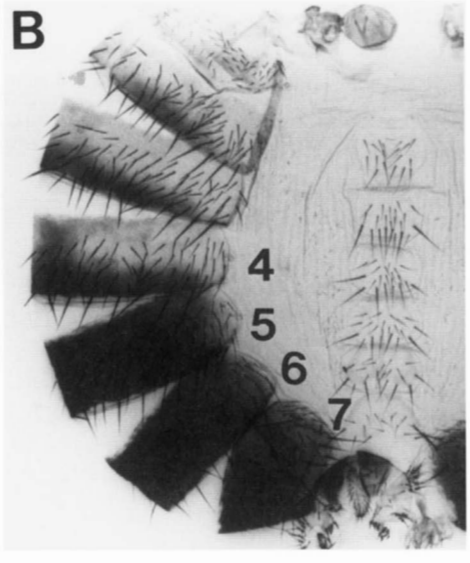
chromosome [*Df(3)JX3*] (Figure 4A) (MATERIALS AND METHODS).

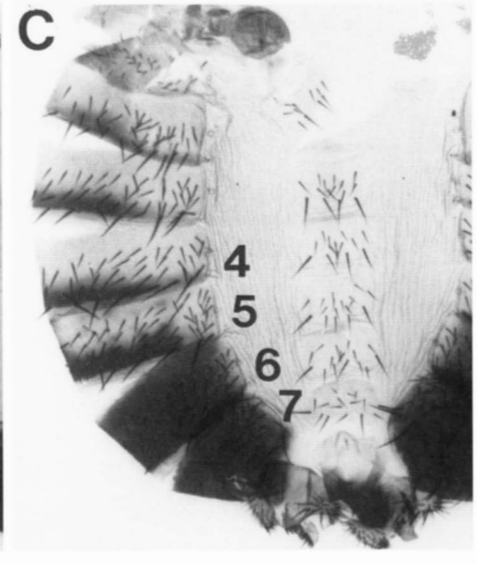
To ascertain that the transposition event did not alter the state of *Abd-B*, we constructed *Tp(3;Y)JX3/DfP9* flies. These control flies show the same mutant phenotype (data not shown) as the parental *iab-7*^{MX2}/*DfP9* flies (Figure 2B), indicating that the original *iab-7* break mutation still exists on this chromosome and that the transposition did not cause ectopic activation of *Abd-B*. We then constructed another control genotype, *Tp(3;Y)JX3/Abd-B*$^{-}$, that is equivalent to *iab-7*^{MX2}/*Abd-B*$^{-}$ heterozygotes, except that the *Abd-B* transcription unit is transposed to the *Y* chromosome (Figure 4B). These flies show a phenotype identical to that of *iab-7*^{MX2}/*Abd-B*$^{-}$ heterozygotes (Figures 2C and 4B). This result indicates that all the genetic factors that contribute to the unexpected A5/A6 identity in *iab-7*^{MX2}/*Abd-B*$^{-}$ flies are still present in *Tp(3;Y)JX3/Abd-B*$^{-}$ flies.

To test whether the transposed copy of *Abd-B* confers A5/A6 identity, we constructed the genotype *Dp(3;Y)JX3; Abd-B*$^{-}$/*DfP9*. In this genotype the *Abd-B* transcription unit and promoter from the original *iab-7*^{MX2} chromosome is on the *Y* chromosome, and the rest of the bithorax complex from the original *iab-7*^{MX2} chromosome is missing. Another copy of the bithorax complex, including the *iab* regions, is supplied on the *Abd-B*$^{-}$ chromosome. These flies are fully viable and show male-specific pigment in A6 and A7 (Figure 4C). In comparison, the genotype *Abd-B*$^{-}$/*DfP9* in female siblings is lethal. In addition, it has been demonstrated previously that escapers of *Abd-B*$^{-}$/*DfP9* flies or *Abd-B*$^{-}$ clones show no male-specific pigment in the abdominal segments (SÁNCHEZ-HERRERO *et al.* 1985; TIONG *et al.* 1985, 1988). Together, these results indicate that *Abd-B* on the *Y* chromosome, transposed from the *iab-7*^{MX2} chromosome, provides essential functions for viability and confers male-specific pigmentation in A6 and A7. In support, we have confirmed, using *in situ* hybridization, that *Abd-B* is expressed in *Tp(3;Y)JX3/Abd-B*$^{-}$ mutant embryos in a pattern identical to that of *iab-7*^{MX2}/*Abd-B*$^{-}$ embryos (data not shown). Because *Abd-B* activity has not been altered by the transposition event (see above), the result obtained with *Dp(3;Y)JX3* should also reflect the activity of *Abd-B* on the parental chromosome *iab-7*^{MX2}. We conclude that *trans*-activation of *Abd-B* promoter on the *iab-7*^{MX2} chromosome explains ITR observed in A6 and A7 of *iab-7*^{MX2}/*Abd-B*$^{-}$ flies. At the end of RESULTS, we will present our examination of the factors that confer male specific pigmentation in A5.

Genetic evidence that *iab-6* and *iab-7* activate *Abd-B* in *trans*: The phenotype of *Dp(3;Y)JX3; Abd-B*$^{-}$/*DfP9* flies suggests that the *iab* regions, located on the *Abd-B*$^{-}$ chromosome, can activate an *Abd-B* promoter in *trans*. To test more directly whether *iab* elements are responsible for the *trans* activation of the *Abd-B* promoter that confers







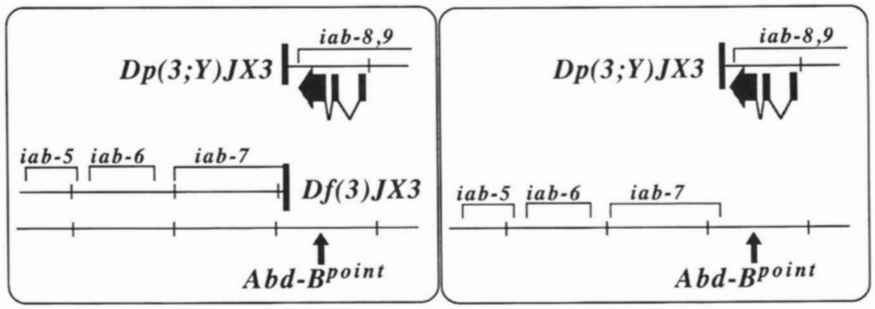


FIGURE 4.—Phenotypes of adult males carrying the transposition of a segment from the *iab-7*^{MX2} chromosome, including *Abd-B*, to the *Y* chromosome, *Dp(3;Y)JX3*. (A) Map showing gross structure of *Tp(3;Y)JX3* mutation induced on the *iab-7*^{MX2} chromosome. Adult male phenotype of *Tp(3;Y)JX3/ Abd-B*^{D16} (B) and *Dp(3;Y)JX3; Abd-B*^{D16}*/ DfP9* (C) shown above maps depicting each genotype. Note that *Tp(3;Y)JX3/ Abd-B*$^{-}$ male looks like parental *iab-7*^{MX2}*/ Abd-B*$^{-}$ male (Figure 2C). *Dp(3;Y)JX3; Abd-B*^{D16}*/ DfP9* male, in contrast, shows pigmentation in A6 and A7 but not in A5, indicating that the transposed *Abd-B* on the *Y* chromosome makes pigment in A6 and A7, whereas factors on the *Df(3)JX3* chromosome make pigment in A5.

A5/A6 identity, we constructed the genotype *iab-7*^{break}*/ Fab-7R59* (Figure 5) and compared the phenotype of these flies to the phenotype of *iab-7*^{break}*/ Abd-B*$^{-}$ flies (Figure 2C). The *Fab-7R59* mutation deletes all sequences left from the 3′ end of the *Abd-B* transcript, including all of the *iab* regions, through *bxd* but retains a functional *Abd-B* transcription unit (Figure 1) (GYURKOVICS *et al.* 1990). *iab-7*^{break}*/ Fab-7R59* flies do not show male-specific pigmentation in A5–A7 (Figure 5) (see DISCUSSION for an explanation of residual pigmentation), observed with *iab-7*^{MX2}*/ Abd-B*^{D14} flies (Figure 2C). We conclude that the *iab* elements on the *Abd-B*$^{-}$ chromosome, which are missing from the *Fab-7R59* chromosome, contribute to A5/A6 identity in *iab-7*^{break}*/ Abd-B*$^{-}$ flies.

To test if *iab-6* and *iab-7* elements can each activate an *Abd-B* promoter in *trans*, we used various *iab* mutants in which only one of these elements is inactivated, leaving the other still functional. We compared the deletion mutants *iab-4,5*^{DB}, *iab-7*^{Sz} and *Fab-7R73* and the insertion mutants *iab-7*^{blt} and *iab-6*^{C7} (Figure 1) as hemizygotes *vs.* as heterozygotes with *Abd-B*$^{-}$ mutations. In all cases the phenotype of segments A6 and A7 showed more posterior segment identity when the mutants were heterozygous with *Abd-B*$^{-}$ mutations (data not shown). For instance, *iab-4,5*^{DB}*/ DfP9* flies show a loss of male-specific pigment in A5 and A6 and a small, pigmented seventh tergite. In contrast, *iab-4,5*^{DB}*/ Abd-B*$^{-}$ flies show a loss of male specific pigment in A5 but not in A6, and

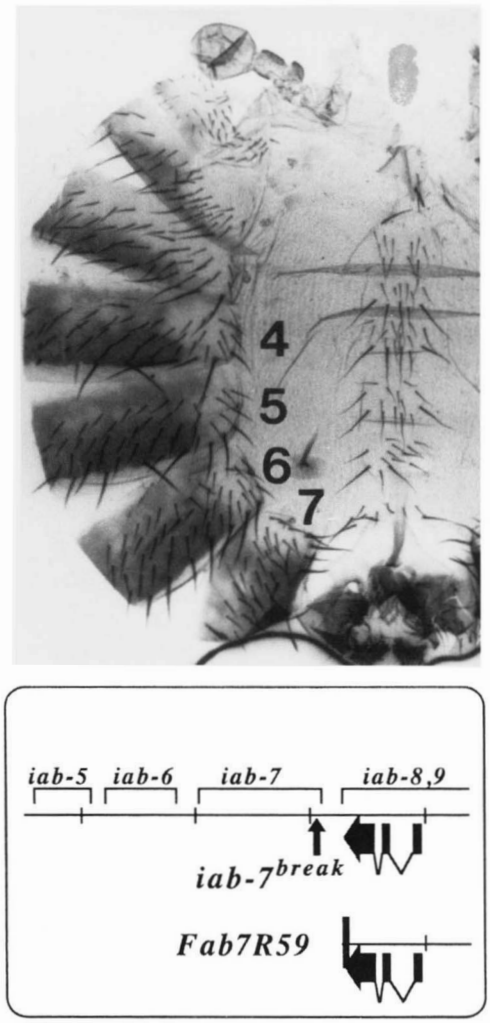


FIGURE 5.—*Fab-7R59* mutation, lacking the *iab* regions, fails to complement $iab\text{-}7^{MX2}$ mutation. Adult male phenotype of $iab\text{-}7^{MX2}/Fab\text{-}7R59$ is shown above a map depicting the genotype. Note that the loss of male-specific pigmentation on A5–A7 is similar to that in $iab\text{-}7^{MX2}/DfP9$ (Figure 2B), implicating the *iab* elements in the transregulation of *Abd-B* to produce male-specific pigment.

the seventh tergite is much smaller. $iab\text{-}7^{SZ}/DfP9$ flies have a seventh tergite that looks like A6, whereas $iab\text{-}7^{SZ}/Abd\text{-}B^{-}$ flies have a much smaller seventh tergite, indicative of an incomplete transformation to A6. These results indicate that the function of each of the regions deleted or disrupted on the *iab* mutant chromosomes can be partially complemented by the *trans* regulation of *Abd-B* by the wild-type *iab* element located on the $Abd\text{-}B^{-}$ chromosome. We conclude that both *iab-6* and *iab-7* elements can contribute to ITR.

Direct comparison of ITR with transvection at *Ubx*: *Trans* activation of *Abd-B* by its regulatory regions suggests that ITR may be similar to previously described transvection effects. One of the hallmarks of transvection effects is that they are sensitive to chromosomal rearrangements that apparently disrupt pairing between homologous chromosomes. For example, LEWIS (1954) has found that transvection effects are disrupted by chromosomal rearrangements that break once between *Ubx* and the centromere and once outside this region. To compare ITR directly with the transvection effect at *Ubx*, we induced chromosomal rearrangements on the $Abd\text{-}B^{D14}$ chromosome that disrupt the dominant Cbx^{1} transvection effect at the *Ubx* locus (see MATERIALS AND METHODS). Because these rearrangements will break between *Ubx* and the centromere, they would be predicted to disrupt a transvection effect at *Abd-B* because *Abd-B* is distal to *Ubx* with respect to the centromere (see Figure 1).

We recovered 23 rearrangements, called *R(D14)*, that disrupt transvection at the *Ubx* locus. To ascertain that our newly isolated transvection-disrupting mutations conform to the expected type of rearrangements, we mapped these mutations. All of the mutations map to the third chromosome, and 13 of them map to another chromosome as well. We examined larval salivary gland polytene chromosomes from six of the mutations and found that each mutation has one break between the centromere and the bithorax complex at map position 89E and at least one break outside this region (Table 2) and conforms to the type of rearrangements found by LEWIS (1954) for Cbx^{1} transvection-disrupting mutations.

To test if these rearrangements disrupt ITR at *Abd-B*, we crossed flies carrying *R(D14)* chromosomes to flies carrying $iab\text{-}7^{MX2}$. The phenotypes of the F1 $iab\text{-}7^{MX2}/R(D14)$ flies (data not shown) are identical to the phenotypes of $iab\text{-}7^{MX2}/Abd\text{-}B^{D14}$ flies (Figure 2C). To eliminate the possibility that the identical phenotypes result from fortouitously pairing of new rearrangements on the $Abd\text{-}B^{D14}$ chromosome with the rearranged $iab\text{-}7^{MX2}$ chromosome, we tested *R(D14)* rearrangements heterozygous with the nonrearranged *Fab-7R59* chromosome. $R(D14)/Fab\text{-}7R59$ flies also show an identical phenotype to that of the parental $Abd\text{-}B^{D14}/Fab\text{-}7R59$ flies, which show pigmentation in A6 and A7, like the phenotype of $Dp(JX3);\ Abd\text{-}B^{-}/DfP9$ flies (Figure 4C). These results indicate that ITR is not sensitive to the same chromosomal rearrangements that disrupt the Cbx^{1} transvection effect at the nearby *Ubx* locus. By this criterion ITR at the *Abd-B* locus is distinct from transvection effect at the *Ubx* locus.

As a further test of similarity of ITR to transvection, we examined $iab\text{-}7^{MX2}/Abd\text{-}B^{D14}$ heterozygotes in *zeste* backgrounds. *zeste* is a gene that has been shown to disrupt transvection effects at *Ubx* and other loci (GELBART and WU 1982). We tested several classes of *zeste* alleles (listed in the MATERIALS AND METHODS). None of these *zeste* alleles altered the phenotype of $iab\text{-}7^{MX2}/Abd\text{-}B^{D14}$ flies. These results also indicate that ITR is not

a transvection effect of the type that has been described for the *Ubx* locus.

Contribution of *abd-A* on the *iab-7^{break}* chromosome to pigmentation in A5: We have shown that ITR is responsible for A5/A6 identity in A6 and A7 of *iab-7^{break}/Abd-B^{-}* flies. Still unexplained is the male-specific pigmentation in A5 in *iab-7^{break}/Abd-B^{-}* flies. The comparison of *Dp(3;Y)JX3; Abd-B^{-}/DfP9* flies (Figure 4C) with *Dp(3;Y)JX3; Abd-B^{-}/Df(3)JX3* flies (Figure 4B) suggests that the *Df(3)JX3* chromosome contains some factor, not present on the *DfP9* chromosome, that contributes to male-specific pigmentation in A5. A candidate for this factor is the *abd-A* gene. Some genetic data indicate that *abd-A* may be able to perform some of the same functions as *Abd-B*; for instance, both *Abd-B^{-}/iab-5* and *abd-A^{-}/iab-5* heterozygotes show a partial transformation of A5 toward A4 (KARCH *et al.* 1985; DUNCAN 1987). We have already discussed that because *iab-7^{break}/Ubx^{-} abd-A^{-} Abd-B^{-}* flies show a phenotype identical to *iab-7^{break}/Abd-B^{-}* flies, *abd-A* on the *Abd-B^{-}* chromosome does not contribute to the A5 male-specific pigmentation in these heterozygotes. We hypothesized therefore that the *abd-A* gene on the *iab-7^{break}* chromosome contributes to male-specific pigmentation in A5 of *iab-7^{break}/Abd-B^{-}* flies. To test this hypothesis, we induced *abd-A* mutations on the *iab-7^{MX2}* chromosome. We irradiated *iab-7^{MX2}* males and screened for lethality over *abd-A^{D24}*. We recovered two mutations, *abd-A^{JX1}* and *abd-A^{JX2}*. Both of these mutations are null for *abd-A* based on genetic and molecular evidence (see MATERIALS AND METHODS), and neither contain mutations in the *iab* regions (see Figure 1). We crossed flies carrying the double mutant chromosome *abd-A^{JX1} iab-7^{MX2}* or *abd-A^{JX2} iab-7^{MX2}* to flies carrying *Abd-B^{-}* chromosome to assess the effect of the new *abd-A* mutations. We find that *abd-A^{JX1} iab-7^{MX2}/Abd-B^{-}* or *abd-A^{JX2} iab-7^{MX2}/Abd-B^{-}* heterozygotes show loss of dark pigment from anterior and lateral parts of the A5 tergite (Figure 6), compared with the parental *iab-7^{MX2}/Abd-B^{-}* flies (Figure 2C). This pigmentation pattern is similar for all the heterozygous flies with either the *abd-A^{JX1} iab-7^{MX2}* or the *abd-A^{JX2} iab-7^{MX2}* chromosome and shows 100% penetrance. We conclude that *abd-A* on the *iab-7^{MX2}* chromosome contributes, at least partially, to the specification of male-specific pigmentation in A5 of *iab-7^{MX2}/Abd-B^{-}* flies. We suggest that the *iab-5* element on the *iab-7^{MX2}* chromosome regulates *abd-A* to produce this pigmentation.

The contribution of *abd-A* to A5 identity has been postulated previously (CELNIKER *et al.* 1990), because posterior abdominal segments in *iab-7^{break}* transheterozygotes show an A5 identity rather than the expected A4 identity despite the fact that no *Abd-B* transcript or protein can be detected in *iab-7^{break}* transheterozygote embryos (CELNIKER *et al. 1990;* CROSBY *et al.* 1993). We used our new mutants to test whether *abd-A* is involved in conferring A5 identity in *iab-7* breakpoint heterozygotes as well. We tested our double mutants *abd-A^{JX1} iab-7^{MX2}* and *abd-A^{JX2} iab-7^{MX2}* as heterozygotes with *iab-7^{break}* chromosomes and find that pigment is reduced compared to *iab-7^{break}* heterozygotes (data not shown). This result indicates that *abd-A* contributes to pigment in these heterozygotes. *iab-7^{break}/DfP9* flies also show patchy pigmentation in the posterior abdominal segments (Figure 2B); this patchy pigmentation can now be explained as the result of *abd-A* activity from the *iab-7^{break}* chromosome. We suggest that *iab-5* regulates *abd-A* on *iab-7^{break}* chromosomes in these genotypes as well as in *iab-7^{break}/Abd-B^{-}* heterozygotes.

To explain the remaining pigmentation in A5 of *abd-A^{JX1} iab-7^{MX2}/Abd-B^{-}* flies (Figure 6), we compared these flies to *Dp(3;Y)JX3; Abd-B^{-}/DfP9* flies (Figure 4C) that show a complete loss of male-specific pigment in A5. A major difference between the two genotypes is the presence of a copy of the *iab* regions on the *abd-A^{JX1} iab-7^{MX2}* chromosome. Although we have presented evidence that *iab* regions on the *iab-7^{break}* chromosome are not capable of regulating *Abd-B*, they could still regulate *abd-A*. Therefore, we propose that *iab-5* on the *abd-A^{JX1} iab-7^{MX2}* or *abd-A^{JX2} iab-7^{MX2}* chromosome regulates *abd-A* on the homologous chromosome in *trans*. The *trans*-activated *abd-A* would then contribute to the male-specific pigmentation in A5 seen in *abd-A^{JX1} iab-7^{MX2}/Abd-B^{-}* and *abd-A^{JX2} iab-7^{MX2}/Abd-B^{-}* flies. We tested whether this proposed *trans iab-5* function is due to a transvection effect by crossing *abd-A^{JX1}* and *abd-A^{JX2}* to our rearrangement *R(D14)* alleles. We observe the same pigmentation patterns in *abd-A$^{JX1\ or\ JX2}$/R(D14)* as in the parental *abd-A$^{JX1\ or\ JX2}$/Abd-B^{D14}*. Thus, the proposed *trans* interaction between *iab-5* and *abd-A* is similar to ITR involving *Abd-B* and unlike transvection effects at *Ubx*.

DISCUSSION

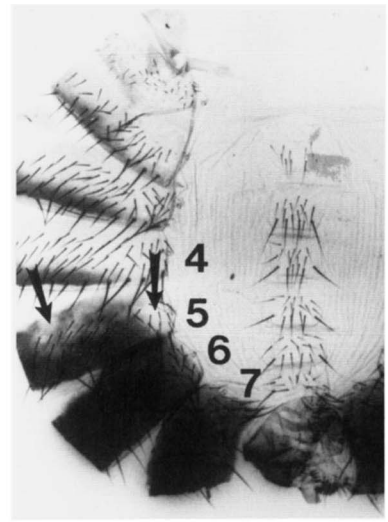
Similarities between ITR and transvection: We have demonstrated that *iab* regions, known to regulate *Abd-B* in *cis*, can also regulate *Abd-B* in *trans*. This *trans* regulation by the *iab* regions is reminiscent of previously described transvection effects and related pairing- or proximity-dependent phenomena in Drosophila. Many of these phenomena have been correlated with the altered transcription of the affected gene (BINGHAM and ZACHAR 1985; KORNHER and BRUTLAG 1986; HENIKOFF and DREESEN 1989; GEYER *et al.* 1990), leading to the hypothesis that transvection effects result when transcriptional regulatory elements on one chromosome influence the expression of their target gene on the (paired) homologous chromosome (ZACHAR *et al.* 1985). Transvection at *Ubx* has been correlated with altered expression of *Ubx* (CASTELLI-GAIR *et al.* 1990). Similarly, we have shown that the phenotype of *iab-*

TABLE 2

Induced chromosomal rearrangements

Mutant	Linkage	Cytology
R(D14)b	*3*	In(64,88A)
R(D14)c	*Y, 2, 3*	Tp(88D-94;Y), T(59A;94), T(88D;59A)
R(D14)i	*2, 3*	T(24;82)
R(D14)j	*2, 3*	T(58;89A)
R(D14)k	*3*	In(61F;82)
R(D14)o	*Y, 3*	T(89D;Y)

Locations of breakpoints for some R(D14) chromosomes that disrupt transvection at the *Ubx* locus, as described in the text.



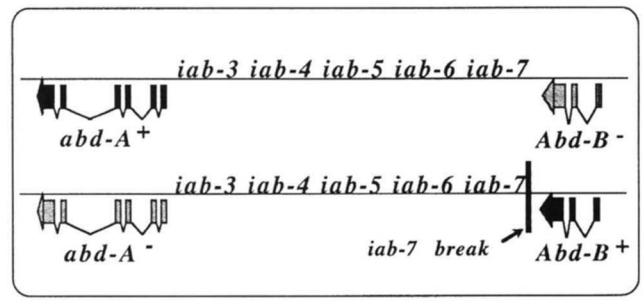


FIGURE 6.—*abd-A* on the *iab-7*^{MX2} chromosome contributes to the male-specific pigmentation in A5. Adult male phenotype of *abd-A*^{JX1} *iab-7*^{MX2}/*Abd-B*^{D16}. The arrows point to the loss of pigment in A5 compared with *iab-7*^{MX2}/*Abd-B*^{D16} or *Tp(3:Y)JX3*/*Abd-B*^{D16} flies shown in Figures 2C and 4B.

7^{break}/*Abd-B*$^{-}$ flies correlates with transcriptional activation of *Abd-B* in embryos. The *Abd-B* class A promoter is activated by *iab* elements in *trans* in qualitatively the same way as it is in *cis* in wild-type animals. However, the expression of *Abd-B* in *iab-7*^{break}/*Abd-B*$^{-}$ flies appears to be quantitatively less than in wild-type flies, because it fails to restore the wild-type phenotype completely. For transvection effects, including those at *Ubx*, the *trans* interaction appears to be weaker than the *cis* interaction as well, resulting in incomplete complementation (GEYER *et al.* 1990; MARTÍNEZ-LABORDA *et al.* 1992). Thus, ITR is similar to other transvection effects and related phenomena in that transcription of the affected gene appears to be regulated in a manner similar to but weaker than its wild-type *cis* regulation.

Differences between ITR and transvection: Transvection and related phenomena show some degree of pairing or proximity dependence, suggested by their sensitivity to chromosomal rearrangements (LEWIS 1954). In contrast, ITR is not affected by chromosomal rearrangements, including those that clearly disrupt a transvection effect at *Ubx* or a translocation of the *Abd-B* promoter and transcription unit to the *Y* chromosome. Furthermore, *iab-7*^{break} mutations do not disrupt ITR between *iab* elements on an intact homologue and the *Abd-B* promoter on the *iab-7*^{break} chromosome (as in *iab-7*^{break}/*Abd-B*$^{-}$ flies), even though these rearrangement mutations might be expected to disrupt any pairing to the right of the *iab-7* breakpoint because they break once between *Abd-B* and the centromere and once outside this region. These differences between transvection and ITR can be interpreted in one of two ways: ITR may not be pairing dependent, at least to the extent required for transvection effects at *Ubx*; alternatively, the *Abd-B* locus, but not the *Ubx* locus, has a sufficiently strong affinity to pair with its homologous copy despite the negative effects of chromosomal rearrangements.

Our data do not rule out the possibility that ITR requires pairing between homologous chromosomal segments. In all the genotypes in which we observed ITR, there were two copies of the *Abd-B* chromosomal region that could potentially pair. In *Dp(3;Y)JX3*; *Abd-B*$^{-}$/*DfP9* flies, for example, the transposed chromosomal segment from position 89E to 92A could pair with the corresponding chromosomal segment on the third chromosome (Figure 4A). If pairing does occur in this case, a functional copy of *iab* elements, which themselves do not have a homologous copy to pair, might be brought in close enough proximity to activate the *Abd-B* promoter on the homologous chromosome.

The differential sensitivity of transvection and ITR to chromosomal rearrangements may also indicate that pairing along homologous chromosome arms need not be uniform. Chromatin in the vicinity of *Abd-B* could pair much more strongly than chromatin in the vicinity of *Ubx*. Such differential pairing could occur if proteins that mediate pairing have more binding sites in the *Abd-B* region than in the *Ubx* region. For instance, Polycomb, a chromosomal protein intimately involved with regulation of the bithorax complex genes, has been found to be present at a higher density on chromatin in the *abd-A*/*Abd-B* regions than on chromatin in the *Ubx* region (ORLANDO and PARO 1993). Similarly, a chromosomal protein involved in ITR could be present

at a higher density in the *Abd-B* region, allowing this region on two chromosomes to pair more avidly.

We have shown that ITR is insensitive to chromosomal rearrangements except to those with a breakpoint within the *iab-7* region. This relative insensitivity to chromosomal rearrangements is different from transvection at the *Ubx* locus but similar to a few other reported cases of *trans*-regulatory phenomena. For instance, the *zeste-white* interaction, a proximity-dependent regulatory phenomenon, is evidently resistant to chromosome rearrangements except to those with breakpoints in the immediate vicinity of the *white* locus (SMOLIK-UTLAUT and GELBART 1987). Another example has been suggested by CASTELLI-GAIR *et al.* (1992), who found that a putative negative regulatory element in the *bxd* region of the bithorax complex can apparently exert its negative effect in *trans* independent of homologous chromosome pairing. Thus ITR may represent an emerging class of *trans*-regulatory interaction phenomena that are not readily disrupted by chromosomal rearrangements.

A unique property of *iab-7^{break}* alleles suggests a mechanism for ITR: The resistance of ITR to chromosomal rearrangements can also be explained if the *iab* regions have a very strong affinity for the *Abd-B* promoter. This strong interaction could allow the *iab* elements to regulate *Abd-B* either in the absence of pairing between homologous chromosomal regions or during transient or weak pairing. The clue to the mechanism of this interaction may be revealed by the only type of mutations we have found that disrupt ITR: chromosomal rearrangements with breakpoints in the *iab-7* regions. These rearrangement mutations not only inactivate the *iab-7* function but also prevent ITR between the adjacent *iab* elements and *Abd-B* promoter in *cis* or *trans*, as indicated by the phenotypes of *iab-7^{break}/DfP9* or *iab-7^{break}/Fab-7R59* flies (Figures 2B and 5). Because *iab* elements in other genotypes can regulate *Abd-B* in *trans*, we might expect that structurally intact *iab* elements on *iab-7^{break}* chromosomes would be able to function. Why then don't these *iab* elements function in *cis* or *trans* when there is a break in the *iab-7* region? We consider three possible models.

In the first model *iab-7^{break}* chromosomes may impose some physical constraints for *iab* elements to interact with *Abd-B*. It is not clear, however, what these constraints may be because our data suggest that *iab* elements can interact with an *Abd-B* promoter anywhere in the genome. In the second model *iab-7* breakpoint chromosomes may inactivate *iab* regions to the left of the break and thus prevent them from regulating *Abd-B*. Nearby *iab* elements could be inactivated if the breakpoint mutations juxtapose the *iab* regions to new chromatin domains that inactivate the adjoining elements. We consider this possibility unlikely because the different *iab-7* breaks we used behave similarly with respect to abdominal phenotypes even though they are fused to different chromosomal regions.

Because neither of these explanations appears fully satisfactory, we propose a third model: *iab-7* chromosomal breaks may disrupt a mechanism that targets *iab* elements to the *Abd-B* promoter. In this model we speculate that the *iab-7* breaks interrupt the requisite mechanism that initiates at a site on the right-hand side of the *iab-7* breaks and propagates proximally toward the *iab-6* element. In molecular terms this could be initiation of transcription across the *iab* regions. Transcripts from the *iab* regions have been detected (SÁNCHEZ-HERRERO and AKAM 1989); at least one of these transcripts initiates to the right of *iab-7* breaks and extends the entire span of the *iab* regions (S. SAKONJU, unpublished results). Alternatively, this mechanism could involve a nucleation site for formation of chromosomal protein aggregates or an element similar to the locus control region of the human β-globin gene cluster (FELSENFELD 1992) that might be required for a transcriptionally active conformation of chromatin (PARO 1990). The existence of such a mechanism would also explain the behavior of the *iab-5* element on the *iab-7* breakpoint chromosomes. We have shown that, on unbroken chromosomes, *iab-5* elements preferentially interact with an *Abd-B* promoter in *cis* or in *trans*, rather than with an *abd-A* promoter. On the chromosomes with *iab-7* breaks, however, *iab-5* element is released from this mechanism that directs it to the *Abd-B* promoter, becoming free to regulate the *abd-A* promoter in *cis* or *trans*. In sum, we suggest that structural discontinuity between the *Abd-B* transcription unit and downstream *iab* regions may disrupt the mechanism required for ITR.

The role of *abd-A* in conferring posterior abdominal segment identity: CELNIKER *et al.* (1990) have proposed previously that in flies heterozygous for different *iab-7^{break}* alleles, *iab-5*, unable to regulate *Abd-B*, regulates *abd-A* instead, and *abd-A* substitutes for *Abd-B*. We have directly tested this hypothesis by inducing *abd-A* mutations on an *iab-7^{break}* chromosome. We find that these *abd-A* mutations do indeed result in loss of male-specific pigment from A5, as if under control of the *iab-5* element.

That *abd-A* can substitute for *Abd-B* seems surprising because the abd-A and Abd-B proteins share only 38 out of 61 residues in the homeodomain (SCOTT *et al.* 1989) and have no amino acid sequence similarity outside the homeodomain (DELORENZI *et al.* 1988; CELNIKER *et al.* 1989; ZAVORTINK and SAKONJU 1989; KARCH *et al.* 1990). Furthermore, experiments with heat-shock constructs show that these two proteins, when ectopically expressed, confer different larval phenotypes (LAMKA *et al.* 1992; KUZIORA 1993; SÁNCHEZ-HERRERO *et al.* 1994). In the adult, however, *abd-A* and *Abd-B* may specify the formation of some of the same morphologi-

cal features. For instance, *Abd-B* specifies the expression of dark pigment in a male-specific pattern on the tergites. *abd-A* may also be able to specify dark pigment on the tergite because in abdominal segments where *abd-A* is expressed but *Abd-B* is not expressed (A2–A4), dark pigment appears, although only in a stripe at the posterior edge of the tergite. If *abd-A* were to come under the control of *iab-5*, it may be expressed in the pattern that would allow it to confer the formation of dark pigment over the entire tergite. *abd-A* could then substitute for *Abd-B* by acting on its own downstream genes in an *Abd-B* pattern.

Implicit in this model is the assumption that *iab-5* would prefer to interact with the *Abd-B* promoter and only acts on the *abd-A* promoter when it cannot find the *Abd-B* promoter. Our data supports the validity of this assumption because *abd-A* mutations on an *iab-7*^{break} chromosome result in loss of pigment, whereas *abd-A* mutations on an intact *Abd-B*$^{-}$ chromosome do not. This interpretation may also explain other unexpected complementation patterns in *Abd-B* mutants. For instance, adult escapers of flies homozygous for the mutations *Abd-B*^{D14} and *Fab-7R9* show male-specific pigmentation on the posterior abdominal segments (CELNIKER *et al.* 1990; J. E. HENDRICKSON and S. SAKONJU, unpublished results). These mutations also exhibit weaker mutant phenotypes when heterozygous with *iab-7*^{break} mutations (see Table 1 legend). *Abd-B*^{D14} and *Fab-7R9* have been molecularly characterized and have a deletion and an insertion, respectively, within the promoter region for the class A transcript, which codes for the Abd-BI protein (ZAVORTINK and SAKONJU 1989; GYURKOVICS *et al.* 1990). One explanation for these results is that mutations in the promoter region prevent *iab-5* from interacting with *Abd-B* and thus free it to look for another suitable target, namely *abd-A*, that then substitutes for *Abd-B*.

iab-7^{break} chromosomes over *DfP9* or over *Fab-7R59* show patches of dark pigmentation in A5–A7, which we have explained as being the result of the regulation of one copy of *abd-A* by *iab-5*. These flies also show patches of dark pigmentation in A4. We have not investigated the cause of this pigmentation. It could result from very low levels of ectopic *Abd-B* expression from the *iab-7*^{break} chromosomes in this segment (CROSBY *et al.* 1993). As an alternative explanation, we hypothesize that it may be a result of the inactivation of silencer elements in the *iab* regions. The loss of posterior identity from A5 to A7 is undoubtedly due to the inability of the enhancer elements in *iab-5–iab-7* that activate *Abd-B* in those segments. Similarly, the gain of posterior identity in A4 may be due to the inability of silencer elements in *iab-5–iab-7* to repress *abd-A* or *Abd-B* in A4. Perhaps the disruption of the intact *iab* region in *iab-7*^{break} chromosomes alters the correct interactions of silencer and enhancer elements in a way that upsets the balance between the two, resulting in ectopic activation of *abd-A* in A4.

Evolutionary considerations: *Trans* regulation appears to be a general phenomenon in the bithorax complex. *Trans* regulation between the regulatory regions for *Ubx* and the *Ubx* promoter, referred to as transvection, has been known for a long time (LEWIS 1954, 1955). More recently, *trans* interactions between *iab* elements and the *abd-A* promoter have also been found (JIJAKLI and GHYSEN 1992). We have discovered the existence of ITR, a *trans* regulation of the *Abd-B* promoter by the *iab* regulatory elements. Because all of these examples of *trans* regulation are so far seen only in mutant combinations, we can only speculate on the wild-type function of *trans* regulation. We propose that *trans* regulation may be a byproduct of extremely high affinities of the regulatory elements in the bithorax complex for their promoters. These high affinities between regulatory elements and promoters could have resulted from promoter competition during the evolution of the bithorax complex, as described below.

It has been suggested that the bithorax complex and other HOM-C complexes arose through gene duplication (LEWIS 1978). Further evolution could also arise from changes in the regulation of these genes. For example, AKAM *et al.* (1988) have suggested that, in the ancestral state, *abd-A* controlled the specification of abdominal segments, whereas *Abd-B* was used exclusively in the terminalia. The interaction of *iab* elements with *abd-A* may reflect this ancestral state. *Abd-Bm* function in the abdominal segments could arise from the creation of a new promoter able to recruit *iab* functions away from *abd-A*. Such a promoter competition may result in higher and higher affinities of promoters for their regulatory elements. *Trans* regulation may result from these very high affinities between promoters and enhancers. It is intriguing to think that the cases of *trans* regulation that are more difficult to disrupt, such as that at *Abd-B*, reflect the higher affinity interactions of more recently evolved promoters.

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