Transvection in the Drosophila *Abd-B* Domain: Extensive Upstream Sequences Are Involved in Anchoring Distant *cis*-Regulatory Regions to the Promoter

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ABSTRACT

The *Abd-B* gene, one of the three homeotic genes in the Drosophila bithorax complex (BX-C), is required for the proper identity of the fifth through the eighth abdominal segments (corresponding to parasegments 10–14) of the fruitfly. The morphological difference between these four segments is due to the differential expression of *Abd-B*, which is achieved by the action of the parasegment-specific *cis*-regulatory regions *infra-abdominal-5* (*iab-5*), *-6*, *-7* and *-8*. The dominant gain-of-function mutation *Frontab-dominal-7* (*Fab-7*) removes a boundary separating two of these *cis*-regulatory regions, *iab-6* and *iab-7*. As a consequence of the *Fab-7* deletion, the parasegment 12- (PS12-) specific *iab-7* is ectopically activated in PS11. This results in the transformation of the sixth abdominal segment (A6) into the seventh (A7) in *Fab-7* flies. Here we report that point mutations of the *Abd-B* gene in *trans* suppress the *Fab-7* phenotype in a pairing-dependent manner and thus represent a type of transvection. We show that the observed suppression is the result of *trans*-regulation of the defective *Abd-B* gene by the ectopically activated *iab-7*. Unlike previously demonstrated cases of *trans*-regulation in the *Abd-B* locus, *trans*-suppression of *Fab-7* is sensitive to heterozygosity for chromosomal rearrangements that disturb homologous pairing at the nearby *Ubx* locus. However, in contrast to *Ubx*, the transvection we observed in the *Abd-B* locus is insensitive to the allelic status of *zeste*. Analysis of different deletion alleles of *Abd-B* that enhance *trans*-regulation suggests that an extensive upstream region, different from the sequences required for transcription initiation, mediates interactions between the *iab cis*-regulatory regions and the proximal *Abd-B* promoter. Moreover, we find that the amount of DNA deleted in the upstream region is roughly proportional to the strength of *trans*-interaction, suggesting that this region consists of numerous discrete elements that cooperate in tethering the *iab* regulatory domains to *Abd-B*. Possible implications of the tethering complex for the regulation of *Abd-B* are discussed. In addition, we present evidence that the tenacity of *trans*-interactions in the *Abd-B* gene may vary, depending upon the tissue and stage of development.

IN eukaryotes, gene activity can be controlled by extensive regulatory regions that are located many kilobases away from the promoter. In the most widely accepted model for such long-distance interactions, the intervening sequences between the regulatory regions and the promoter are thought to "loop out" (Pirrotta 1991). Although the looping model can account for long-distance interactions between regulatory elements and promoters, it also poses a problem. Most regulatory elements are rather promiscuous in their interactions and are capable of controlling the activity of many different promoters, irrespective of their origin. Thus, the looping model raises the question of how enhancers are able to distinguish their target promoter from the promoters of other nearby genes.

One model system for studying the factors governing such long-distance regulatory interactions is provided by the phenomenon of transvection in Drosophila. Transvection, first described by Lewis (1954) in the *Ultrabithorax* (*Ubx*) locus, refers to a partial interallelic complementation that depends upon the pairing of homologous chromosomes. In the best-documented cases, such as in the *yellow* (Geyer *et al.* 1990) and the *Ubx* loci (Martinez-Laborda *et al.* 1992), transvection appears to involve *trans*-regulation, that is, a regulatory element on one of the homologues controls the promoter activity of the corresponding gene on the other, paired homologue. This unusual *trans*-interaction can be exploited to learn more about the mechanisms responsible for specifying conventional interactions between regulatory elements and promoters in *cis*.

The *Ubx* gene is a part of the homeotic bithorax complex (BX-C). Although BX-C contains only two other homeotic genes, *abdominal-A* (*abd-A*) and *Abdominal-B* (*Abd-B*) (Sánchez-Herrero *et al.* 1985; Tiong *et al.* 1985), these three genes assign proper identity to the third thoracic (T3) and all of the abdominal (A1–A9) segments, corresponding to parasegments (PS) 5 to

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PS14. This is achieved through the control exerted by PS-specific *cis*-regulatory elements on the individual genes of the complex. Thus, *abx/bx* and *pbx/bxd* elements regulate *Ubx* expression in PS5 and PS6 (Beachy *et al.* 1985; Hogness *et al.* 1985; White and Wilcox 1985), and *iab-2*, *-3* and *-4* elements regulate *abd-A* in PS7, PS8 and PS9, respectively (Karch *et al.* 1990; Macias *et al.* 1990). *iab-5* - *iab-8* elements regulate the *Abd-B* class A transcription unit that corresponds to the *Abd-B m* function (Casanova *et al.* 1986) in PS10 through PS13 (Celniker *et al.* 1990; Boulet *et al.* 1991; Sánchez-Herrero 1991). Specific regulatory elements that regulate the longer transcription units (class B, C and γ; Zavortnik and Sakonju 1989), corresponding to the *Abd-B r* subfunction (Casanova *et al.* 1986), have not yet been identified, but they are expected to specify the expression pattern of the class B, C and γ RNA species in PS14 and PS15.

Recently, an unusual type of *trans*-regulation has been described for the *Abd-B* class A transcription unit and its regulatory regions (Hendrickson and Sakonju 1995; Hopmann *et al.* 1995). In contrast to the classical transvection of Lewis (1955), this *trans*-regulation is extremely resistant to disruption of homologous pairing. In this article, we provide evidence that *trans*-interaction in the *Abd-B* gene may vary in its resistance to disruption of homologous pairing in different tissues. With the help of a dominant gain-of-function mutation, *Fab-7*, we show that transvection of a more regular type, closely resembling the classical case in the *Ubx* locus, can also be demonstrated in *Abd-B* in the adult stage. Detailed analysis of this transvection suggests the existence of a multicomponent tethering mechanism that may ensure *cis*-autonomy of the *Abd-B* domain within the BX-C. This mechanism involves an extensive region upstream of the *Abd-B* gene, which appears to be distinct from the sequences required for transcription initiation.

MATERIALS AND METHODS

General procedures: Fly stocks were maintained on standard yeast-cornmeal medium. Crosses were performed at 25° *en masse*, unless otherwise noted. Unless described in this article, all genetic variants used are described in the following references: *iab-7^{MX2}*, *Abd-B^{D14}*, *Abd-B^{D16}*, *Df(3R)C4* (Karch *et al.* 1985); *Abd-B^{RD18}*, *Df(3R)U110* (Hopmann *et al.* 1995); *Abd-B^{S1}*, *Abd-B^{S4}* (Tiong *et al.* 1985); *Abd-B^{R41}*, *Fab-7*, *In(3R)Fab-7iab-7^{R7}*, *Df(3R)R59* (Gyurkovics *et al.* 1990); *iab-7^{164}* (Celniker *et al.* 1990); *iab-7^{Sz}* (Galloni *et al.* 1993); *Mcp^{B116}* (Karch *et al.* 1994); *TM3 Sb P(ry^{+})Δ2-3* (Reuter *et al.* 1993); *UC21-10,1-d* (McCall *et al.* 1994); *Cbx^{1}*, *Df(3R)P9*, *Dp(3;3)P5*, *In(3LR)TM1*, *In(3LR)TM3 Sb Ser*, *In(3LR)TM6B Tb*, *In(3LR)TM6C Sb*, *Mc*, *Tp(3;1) bxd^{l11}*, *Ubx^{1}*, *z^{1}*, *z^{a}*, *z^{pp}*, *z^{v77h}* (Lindsley and Zimm 1992). *In(3R) Fab-7iab-7^{R5}* was isolated as an X-ray-induced revertant of *Fab-7*, and its breakpoint within the BX-C was cloned and determined by Southern analysis. Cytological analysis of polytene chromosomes was performed as described by Ashburner (1989). Adult abdominal cuticles were mounted as described by Duncan (1982), and Southern blot analyses were done as described by Bender *et al.* (1983).

Immunohistochemical staining of embryos: Embryos collected from 17-hr egglays were dechorionated, fixed and devitellinized according to the procedure of Mitchison and Sedat (1983), modified as described in Karch *et al.* (1990). We modified this procedure further as follows. The time of fixation was reduced to 12 min. Devitellinized embryos were washed and rehydrated in 1× PBT (1× PBT = 1× phosphate-buffered saline + 0.1% Triton X-100 + 0.1 bovine serum albumin, 1× phosphate-buffered saline = 137 mm NaCl, 2.7 mm KCl, 10.1 mm Na_2HPO_4, 1.8 mm KH_2PO_4; pH = 7.5). Embryos were incubated with primary antibody (monoclonal mouse-anti-ABD-B 1A2E9; Celniker *et al.* 1990) diluted in 1× PBT 1:1 on a rotating wheel overnight at 4°C. Antibody was removed, and embryos were washed six times with PBT for about 20 min on rotating wheel. Horseradish peroxidase-conjugated rabbit-anti-mouse secondary antibody (DAKO), diluted in prechilled (4°) PBT 1:200, was added and incubated with the embryos on a rotating wheel for 3 1/2 hr at 4°. These conditions reduced background staining without significant loss of specific staining. Embryos were then washed again as described above. After removing PBT, embryos were briefly rinsed twice in staining buffer (0.1 m citric acid, 0.05 m NH_4-acetate, pH = 5.7 adjusted with NH_4OH) and stained in the mixture of 980 μl buffer, 20 μl DAB solution (25 mg/ml stock solution, final cc 0.5 mg/ml) and 2 μl H_2O_2 (30% stock solution, final cc 0.06%). The reaction was stopped by dilution with PBT and embryos were rinsed six times with PBT. Embryos were stored in PBT with 0.05% NaN_3 and mounted in 9:1 glycerol:10 × PBT. For the dissection of CNS, we used tungsten needles.

Chromosomes generated by recombination: *Mcp^{B116}iab-7^{Sz} Abd-B^{D16}:* The synthesis of this chromosome was done in three consecutive steps. First, we generated the chromosome *Mcp^{B116} iab-7^{Sz}* by recombination. Out of ~29,000 male progeny of the cross between *Mcp^{B116}/iab-7^{Sz}* virgins and *Oregon-R* males, we identified two flies with darkly pigmented A4 [due to the dominant mutation, *Miscadastral pigmentation* (*Mcp*)] and an additional rudimentary seventh tergite (*iab-7^{-}*). In the second step, *Microcephalus* (*Mc*) (a dominant mutation resulting in a strong reduction of the head capsule) was recombined onto the *Mcp^{B116} iab-7^{Sz}* chromosome. *Mcp^{B116}iab-7^{Sz}/Fab-7Mc* virgins were mated with *Oregon-R* males. Among ~35,000 F_1 males, we found three recombinants showing *Mcp*, *iab-7* and *Mc*, but no *Fab-7* phenotype. Finally, we isolated recombinants between the chromosomes *Mcp^{B116} iab-7^{Sz} Mc* and *Abd-B^{D16}*. (*Mcp* and *Mc* served as flanking markers to detect recombination events.) *Dp(3;1)bxd^{l11}/+; Abd-B^{D16}/Mcp^{B116} iab-7^{Sz} Mc* virgins were allowed to mate with *Oregon-R* males and their male progeny (~90,000) were scored for the presence of *Mcp* and the absence of *Mc* phenotype. Of the 39 such males found, eight were sterile and 23 did not carry *Abd-B^{D16}* [based on complementation with *Df(3R)P9*]. The remaining eight lines carrying the *Abd-B^{D16}* were tested for their ability to suppress the *Fab-7* phenotype. Although all of them showed suppression, they fell into two discrete categories: five of them had an A6 tergite larger than the thin A7 (similar to the phenotype of *Fab-7/Abd-B^{D16}*), whereas in the remaining three these tergites were equally large both in A6 and in A7. We concluded that the first group carried *Mcp^{B116}* only, and the second group had both *Mcp^{B116}* and *iab-7^{Sz}*. We confirmed the presence of both lesions by Southern blot analysis. The same protocol was followed in the generation of recombinant chromosomes that contained *Abd-B^{D14}* instead of *Abd-B^{D16}*.

Fab-7 Abd-B^{D16}: Two apparently contradictory observations prompted us to study transvection in the *Abd-B* domain. First, we found that (when the homologues are paired) an *Abd-B* point mutation *trans* to the *Fab-7* mutation suppresses the *Fab-7* gain-of-function phenotype so that the A6 tergite in males is larger than the A7 tergite (*trans*-suppression, described in detail in the results section). Second, we noticed in an earlier study (Gyurkovics *et al.* 1990) that when the

Fab-7 mutation is present both in *cis* and in *trans* to an *Abd-B* mutation (*Fab-7*/*Fab-7 Abd-B^{R41}*) the A6 and A7 tergites are equally thin. We presumed that this phenotypic difference between the two types of mutant combinations was not due to some unusual properties of *Abd-B^{R41}* (which is an *Abd-B m$^-$* mutation, Gyurkovics *et al.* 1990), but to the presence of the *Fab-7* mutation in *cis* to *Abd-B^{R41}*. To test if this is the case, we attempted to isolate recombinants carrying both *Fab-7* and *Abd-B^{D16}* (an *Abd-B mr* null mutation; Boulet *et al.* 1991) on the same chromosome, based on the assumption that *Fab-7*/*Fab-7Abd-B^{D16}* flies should have a phenotype similar to *Fab-7*/*Fab-7 Abd-B^{R41}*. For this purpose, *ry^{506}Fab-7*/*Abd-B^{D16}* females were crossed to *ry^{506}Fab-7*/*ry^{506}Fab-7* males, and the male progeny were scored for the presence of equally thin A6 and A7 tergites. Out of ~35,000 males, three such flies were found. After establishing stocks of these lines, the presence of the *Fab-7* deletion was confirmed by Southern blot analysis and the presence of *Abd-B^{D16}* by genetic complementation test in all three putative recombinants. Having established the usefulness of this method, we constructed double mutant combinations of *Fab-7* with *Abd-B^{D14}* and *UC21-10,1-d* in the same way.

R5^{L}R7^R: For the construction of this chromosome, we took advantage of the fact that the desired product of recombination between the two inversions *In(3R)iab-7^{R7}* and *In(3R)iab-7^{R5}* should contain a deletion in the region 87C1-2;87D1-4, including the *karmoisin* (*kar*) locus at 87C8 (Gausz *et al.* 1979). *Tp(3;1)bxd^{111}*/+ ; *iab-7^{R5}*/*iab-7^{R7}* females were crossed to *cu kar*/*cu kar* males, and the progeny were scored for *kar* phenotype. Of 180 flies scored, we recovered seven *kar* individuals. Stocks of these putative recombinants were established and checked cytologically. All of them contained the inversion *In(3R)*87C1-2;89E3-4 and the deficiency *Df(3R)*87C1-2;87D1-4.

Isolation of rearrangements that eliminate *trans*-suppression: We induced chromosomal rearrangements on the *Fab-7* chromosome by irradiating *Fab-7*/*Fab-7* homozygous males with X rays (4000 rads; 1000 rads/min, 0.5-mm Al filter) and crossing them to *Abd-B^{D16}*/*TM6B Tb* virgins. After 6 days the parents were discarded. Among the male progeny (about 3000) we identified 12 flies in which *trans*-suppression was weaker or no longer visible. We then tested these new rearrangements over the *Cbx^{1}Ubx^1* chromosomes. All but one showed significant reduction of the weak dominant wing phenotype generated by the *Cbx^1* mutation through the misexpression of *Ubx$^+$* on the homologous chromosome. Cytological examination of the exceptional case revealed a breakpoint at the BX-C, 89E1,2. By complementation analysis, we showed that the breakpoint inactivated the *iab-4 cis*-regulatory region.

We isolated transvection-disrupting rearrangements on the *Fab-7 Abd-B^{D16}* chromosome in a similar way. Irradiated *Fab-7 Abd-B^{D16}*/*TM6C Sb* males were crossed to *Cbx^{1}Ubx^1*/*TM1* females. Out of ~1500 F_1 flies we isolated five individuals showing significantly reduced or no *Cbx* phenotype. Cytological examination of the two mutations that completely eliminated the *Cbx* phenotype, *TSR-11A Fab-7 Abd-B^{D16}* and *TSR-59A Fab-7 Abd-B^{D16}*, showed the presence of the rearrangement *T(3;4)*89A-B;102A in the first, and *Df(3R)*88D;89D + *Tp(3;2)*89E;98C;39A in the second mutation.

Isolation of new deletions in the Abd-B gene by *P*-element remobilization: In order to generate deletions that remove sequences around the promoter of the *Abd-B* class A transcription unit, the *P*-element insertion *UC21-10,1-d*, localized 253 bp upstream of the proximal *Abd-B* promoter (McCall *et al.* 1994), was mobilized by introducing the transposase source *P(ry$^+$) Δ2-3*. First, the *Fab-7* mutation was recombined onto the chromosome carrying the insert. [In contrast to the original chromosome, these recombinants survive as adult homozygotes, suggesting that the reported lethality (McCall *et al.* 1994) should be due to second-site mutations located proximal to the *Fab-7* mutation.] *Fab-7UC21-10,1-d*/+ flies show a moderate *Fab-7* phenotype that is strongly reduced if these chromosomes are over an *Abd-B* null mutation, suggesting that the *Fab-7* phenotype originates mainly from an interaction with the paired wild-type *Abd-B* gene in *trans*. However, the remaining weak *Fab-7* phenotype indicates that *UC21-10,1-d* is a leaky *Abd-B* mutation. Imprecise excisions of the insert that result in the deletion of the promoter region of the *Abd-B* class A transcription unit should eliminate the remaining *Fab-7* phenotype in flies heterozygous with the null mutation *Abd-B^{D16}*. (The excision of the *P* element can be detected by the loss of the *ry$^+$* marker gene carried by the *UC21-10,1-d* insertion.) We therefore isolated nine *ry$^-$* mutations with no remaining *Fab-7* phenotype from the progeny (~20,000) of the cross between *Fab-7 UC21-10,1-d*/*TM3 Sb P(ry$^+$)Δ2-3* males and *Dp(3;1)bxd^{111}*/*Dp(3;1)bxd^{111}; ry^{506}Abd-B^{D16}*/*ry^{506} Abd-B^{D16}* virgins. In contrast, all nine putative 5′ deletions showed some degree of transformation of A6 toward A7 (*Fab-7* phenotype) in *trans* to wild-type chromosomes. This phenotype allowed us to separate the chromosomes carrying the new derivatives of the insertional mutation from the *ry^{506} Abd-B^{D16}* chromosome and to establish stocks. Two of them, *Abd-B^{PSz1}* and *Abd-B^{PSz2}*, showed significant complementation over *iab-7^{Sz}*, *i.e.*, the size of A7 was reduced compared to *Abd-B^{D16}*/*iab-7^{Sz}*. Southern analysis detected a deletion of 10.8 kb between map positions +155.6–156.8 and +165.6–166.6 (3.2 kb downstream and 7.6 kb upstream of the site of the original insertion) in *Abd-B^{PSz1}*, and a 5.5-kb deletion between map positions +155.6–156.8 and +160.2–161.3 (3.2 kb downstream and 2.3 kb upstream of the site of the original insertion) in *Abd-B^{PSz2}*.

RESULTS

In adult wild-type males the program specifying the development of the A7 abdominal segment does not produce a visible tergite or sternite (Figure 1a). This developmental program depends on the normal functioning of the *iab-7 cis*-regulatory domain that is responsible for generating the appropriate level of *Abd-B* RNA/protein expression in PS12/A7. When *iab-7* is deleted on both homologues, *Abd-B* expression in PS12/A7 comes under the control of the *iab-6 cis*-regulatory domain, resulting in the transformation of PS12/A7 into a copy of PS11/A6 (Galloni *et al.* 1993). Because of the haplo-insufficiency of the *Abd-B* homeotic gene, disruptions in the normal developmental program of PS12/A7 are evident in animals heterozygous for an *iab-7* mutation, such as *iab-7^{Sz}*, which deletes the *iab-7* region (Galloni *et al.* 1993). In this case, A7 assumes an identity in between that of the normal A6 and A7. The mixed identity of A7 is manifested as a rudimentary tergite-like structure posterior to A6 with a characteristic shape and size (Karch *et al.* 1985). This phenotypic transformation is shown in Figure 1b and summarized diagrammatically in Figure 2. A similar effect on A7 development is also observed in animals heterozygous for an *Abd-B* mutation; however, in this case the disruptions in development are not restricted to A7, and the morphology of the adjacent segments A6 and A5 are altered as well.

A phenotype exactly the opposite of that produced by *iab-7* mutations is observed for the gain-of-function mutation *Fab-7*; PS11/A6 is transformed into PS12/A7. This is due to the ectopic activation of *iab-7* in PS11/

A6. As a consequence, the number of visible segments in *Fab-7* homozygous males is reduced from six to five (Gyurkovics *et al.* 1990). Although disruptions in A6 development are also found in *Fab-7*/+ males, the extent of the transformation is reduced compared to that observed in homozygous mutant animals. Instead of the PS12/A7 identity seen in homozygotes, PS11/A6 assumes a mixed PS11/A6-PS12/A7 identity in animals heterozygous for the *Fab-7* mutation. In fact, the mixed identity phenotype of PS11/A6 observed in *Fab-7*/+ males closely resembles the mixed identity phenotype of PS12/A7 observed in $iab\text{-}7^{-}$/+ (or $Abd\text{-}B^{-}$/+) male flies (see Figure 1, c and b).

The incomplete transformation of PS11/A6 in *Fab-7*/+ males could be attributed to a haplo-insufficiency for *iab-7*. In *Fab-7*/+ males, the *iab-7 cis*-regulatory domain on the *Fab-7*-containing homologue is active in PS11/A6, whereas the *iab-7 cis*-regulatory domain on the wild-type homologue is not. If regulation *only* occurs in *cis*, *Abd-B* expression in PS11/A6 should be driven by interactions between the *iab-7 cis*-regulatory domain and the *Abd-B* gene only on the *Fab-7* homologue. Such interactions should not occur on the wild-type homologue in PS11/A6 because *iab-7* is inactive. This interpretation is supported by the phenotype of *Fab-7*/$iab\text{-}7^{Sz}$ mutant animals. In this genotype, only a single copy of the *iab-7 cis*-regulatory domain should be available to drive *Abd-B* expression, not only in A6 and but also in A7. In males carrying this mutant combination, both PS11/A6 and PS12/A7 would be expected to assume an identity in between that of PS11/A6 and PS12/A7. This is the case. Moreover, as illustrated in Figure 1d and Figure 2, segments A6 and A7 in the *Fab-7*/$iab\text{-}7^{Sz}$ males closely resemble the A7 segment found in $iab\text{-}7^{-}$/+ (Figure 1b) males.

Taken together, these results would seem to suggest that, if the rest of the *Abd-B* domain remains unchanged, the phenotype/identity of PS12/A7 (or PS11/A6 in the *Fab-7* mutant) directly reflects the number of active *iab-7 cis*-regulatory domains.

***Fab-7* is suppressed by *Abd-B* point mutations in *trans*:** We next examined the effects of combining *Fab-7* with an *Abd-B* point mutation, $Abd\text{-}B^{D16}$. If our hypothesis that the identity of PS11/A6 and PS12/A7 depends upon the number of *iab-7* domains that are active in these two parasegments is correct, then *Fab-7*/$Abd\text{-}B^{D16}$ males would be expected to have the same intermediate phenotype in A6 as was observed in *Fab-7*/$iab\text{-}7^{Sz}$ males. However, as illustrated in Figure 1e and Figure 2, this is not the case. Although A7 has the same phenotype in the two mutant combinations, the phenotype of segment A6 in *Fab-7*/$Abd\text{-}B^{D16}$ males differs from that in *Fab-7*/$iab\text{-}7^{Sz}$ males. The A6 tergite in the *Fab-7*/$Abd\text{-}B^{D16}$ genotype is enlarged relative to the A7 tergite, and more closely resembles the wild-type A6 segment. This unexpected finding suggests that, unlike $iab\text{-}7^{Sz}$, the $Abd\text{-}B^{D16}$ mutation partially suppresses the gain-of-function phenotype of *Fab-7* in A6. This partial suppression is not due to some unusual properties of the $Abd\text{-}B^{D16}$; precisely the same A6 phenotype was observed when other *Abd-B* point mutations ($Abd\text{-}B^{S1}$, $Abd\text{-}B^{S4}$; Tiong *et al.* 1985) were combined with *Fab-7* (data not shown).

These observations could mean that the activity of *Abd-B* is "more haplo-insufficient" in A6 than in A7. If this were true, we would expect to observe a similar suppression of the *Fab-7* phenotype in A6 by the deletion *Df(3R)P9*, which removes the entire BX-C, including the *Abd-B* gene. However, as was observed for the *Fab-7*/$iab\text{-}7^{Sz}$ combination, segments A6 and A7 in *Fab-7*/*Df(3R)P9* males show the same intermediate A6-A7 phenotype (see Figure 1f). Similarly, the combination of *Fab-7* with a deletion, *Df(3R)R59*, which removes both *iab-6* and *iab-7* but not the *Abd-B* gene, has the same phenotype as *Fab-7*/$iab\text{-}7^{Sz}$ (data not shown), indicating that the functioning of *iab-6* in *trans* is irrelevant for the phenotype of *Fab-7* in A6.

Chromosomal rearrangements eliminate the effect of the *Abd-B* point mutations: The suppression of the *Fab-7* gain-of-function phenotype by *Abd-B* point mutations in *trans* implies that our original assumption, namely, that regulatory interactions only occur in *cis*, is incorrect. Instead, it suggests that some type of *trans*-regulatory interaction or "transvection" must also occur. In particular, a *trans*-regulatory interaction could potentially explain why the *Fab-7* gain-of-function phenotype of *Fab-7*/$Abd\text{-}B^{+}$ animals is stronger than that of *Fab-7*/$Abd\text{-}B^{-}$ animals; in the former case, the *Abd-B* genes in *cis* and in *trans* would produce functional products, whereas in the latter case, functional products would only be produced by the *Abd-B* in *cis*.

Transvection is classically tested by generating chromosomal rearrangements that disturb homologous pairing. For this purpose, we first screened for X-ray-induced mutations on the *Fab-7* chromosome, which reduced or eliminated the suppression of the *Fab-7* gain-of-function phenotype by $Abd\text{-}B^{D16}$, that is, for *Fab-7**/$Abd\text{-}B^{D16}$ males in which the A6 tergite more closely resembled the A7 tergite. [We used $Abd\text{-}B^{D16}$ as a representative allele in this and subsequent experiments because it is a molecularly characterized mutation that does not code for a detectable protein (Boulet *et al.* 1991).] Out of ~3000 mutagenized chromosomes, we recovered 12 independent *Fab-7**/$Abd\text{-}B^{D16}$ males in which the A6 tergite was as thin or nearly as thin as the A7 tergite. For further analysis, we established stocks of these 12 *Fab-7** chromosomes.

Chromosomal rearrangements that interfere with the postulated transvection between *Fab-7* and *Abd-B* might also be expected to disrupt pairing-dependent interactions elsewhere in BX-C. A well-known example of a pairing-dependent regulatory interaction in BX-C is the wing transformation induced by the $Cbx^{1}Ubx^{1}$ chromosome when it is paired with a wild-type copy of BX-C (Lewis 1955, 1985). The 12 *Fab-7** chromosomes were

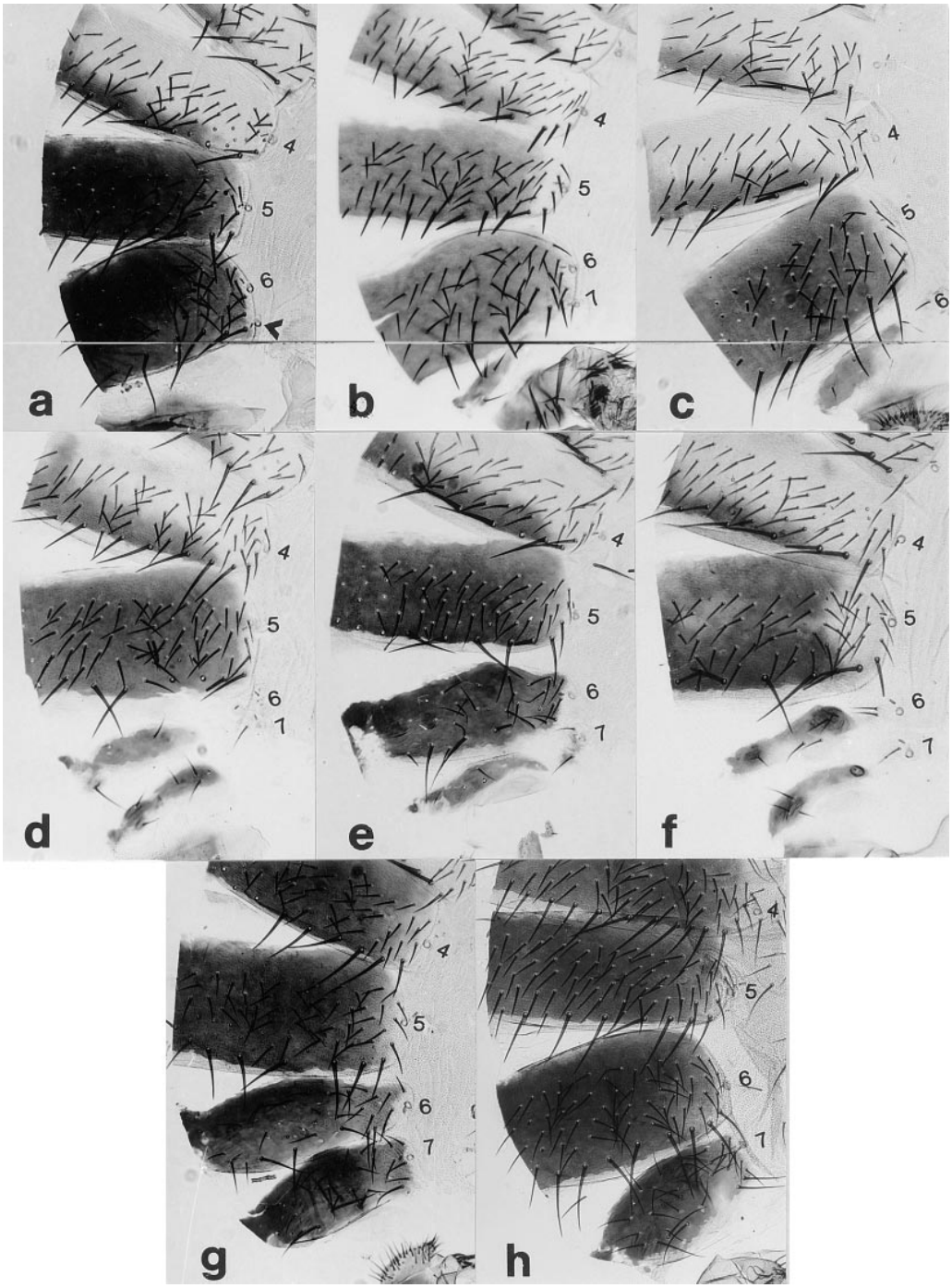

Figure 1.—Abdominal cuticles of adult males. (a) +/+. In wild type, A7 is represented only by a pair of tracheal openings (arrowhead). (b) *iab-7*^{Sz}/+. The haplo-insuffiency of the *iab-7 cis*-regulatory region results in the addition of a rudimentary, seventh tergite. (c) *Fab-7*/+. Ectopic activation of *iab-7* in A6 reduces the sixth tergite to the size of the seventh in *iab-7*^{Sz}/+. (d) *Fab-7*/*iab-7*^{Sz}. Because only a single *iab-7* is active in A6 and A7 (that of the *Fab-7* chromosome), both sixth and seventh tergites are reduced to the size of the seventh in *iab-7*^{Sz}/+. (e) *Fab-7*/*Abd-B*^{D16}. A7 is similar to that of the previous genotype, but the A6 tergite is enlarged. (f) *Fab-7*/*Df(3R)P9*. If, in addition to *iab-7*, the *trans* copy of *Abd-B* is also deleted, both A6 and A7 tergites are again similar to those of the genotype *Fab-7*/*iab-7*^{Sz}. (Additionally, due to the partial transformation of A5 into A4, lack of dark pigmentation in patches of A5 is also evident.) (g) *Fab-7*/*Mcp*^{B116}*iab-7*^{Sz}*Abd-B*^{D16}. The size of the sixth tergite is similar to that of the sixth tergite in *Fab-7*/*Abd-B*^{D16} males e, indicating that the presence or the absence of an inactive copy of *iab-7* in *trans* is indifferent for the suppression of the *Fab-7* phenotype. However, A7 is enlarged to the size of A6 in *Fab-7*/*Mcp*^{B116}*iab-7*^{Sz}*Abd-B*^{D16} *trans*-heterozygotes. As shown in h, if *Fab-7* is replaced with a wild-type chromosome in this genotype (*Mcp*^{B116}*iab-7*^{Sz}*Abd-B*^{D16}/+), A6 becomes similar to wild type, but A7 remains about the same as in *Fab-7*/*Mcp*^{B116}*iab-7*^{Sz}*Abd-B*^{D16}, indicating that the *Fab-7* deletion only ectopically activates the wild-type *iab-7* in PS11, but does not alter its ability to *trans*-regulate *Abd-B*.

TABLE 1

List of breakpoints of rearrangements that reduce *trans*-suppression in *Fab-7/Abd^{D16}* males

TSR-X^{Sz},	*Fab-7*	Cytology in the critical region	Effect on *trans*-suppression	Note
TSR-1^{Sz},	*Fab-7*	T(2;3)2R heterochromatin; 89B	+++	
TSR-2^{Sz},	*Fab-7*	heterochromatin (?); 87B	++	
TSR-3^{Sz},	*Fab-7*	T(2;3)2R heterochromatin, 87F-88A	++	
TSR-4^{Sz},	*Fab-7*	In(3R)heterochromatin; 90E-F	+++	
TSR-5^{Sz},	*Fab-7*	T(2;3)26A; 3R heterochromatin	++	
TSR-6^{Sz},	*Fab-7*	T(2;3)28E; 87B8,9	++	
TSR-7^{Sz},	*Fab-7*	T(2;3)59B-C; 81F	++	
TSR-8^{Sz},	*Fab-7*	In(2R)81F; 88B	++	
TSR-9^{Sz},	*Fab-7*	T(2;3)58F; 3R heterochromatin	++	homozygous viable
TSR-10^{Sz},	*Fab-7*	In(3LR)62B; 81F	++	homozygous viable
TSR-11^{Sz},	*Fab-7*	T(Y; 3R)87B	+	
TSR-12^{Sz},	*Fab-7*	In(3LR)79E-80B; 89E	+++	*iab-4^{-}*

Additional rearrangements outside of the critical region are present in *TSR^{Sz}−1, 3, 5, 6, 8* and *12*; only the relevant breakpoints are listed. +++, strong effect; ++, moderate effect; +, weak effect.

tested for their ability to interact with the *Cbx^{1}Ubx^{1}* chromosome, and we found that all but one significantly suppressed the wing phenotype induced by the *Cbx^{1}* mutation. Moreover, the strength of the phenotypic effects of these 11 *Fab-7** chromosomes closely paralleled that found in combination with *Abd-B^{D16}*: mutations that exerted a strong effect on the *Fab-7/Abd-B^{D16}* phenotype also strongly suppressed the wing phenotype (not shown).

As expected from the suppression of the *Cbx^{1}* phenotype, cytological examination of these 11 *Fab-7** chromosomes revealed that they all had rearrangements affecting the right arm of the third chromosome. The position of the breakpoints for the 11 *Fab-7** chromosomes that suppress the *Cbx^{1}* phenotype (*TSR-1^{Sz},Fab-7* to *TSR-11^{Sz},Fab-7*) are listed in Table 1. All 11 of them have one breakpoint between BX-C and the centromere, and a second breakpoint outside of this region. This arrangement of breakpoints follows the rules established by Lewis (1955) for chromosomal aberrations that disrupt transvection in BX-C. The one exception, *TSR-12^{Sz},Fab-7*, proved to be an *iab-4* mutation, and it has an inversion breakpoint in BX-C (see Table 1). Although this rearrangement should disrupt pairing in the *Abd-B* region of BX-C, it would not be expected to affect pairing in the *Ubx* region of BX-C.

These results argue that the suppression of *Fab-7* gain-of-function phenotype by *Abd-B* point mutations is likely to be due to a nonproductive *trans*-regulation. When a wild-type gene is present on both homologues (*Fab-7/+*), both *cis*- and *trans*-regulatory interactions can contribute to the level of ABD-B protein that is ultimately expressed in PS11/A6. Although these same *cis*- and *trans*-interactions would also occur in *Fab-7/Abd-B^{D16}* flies, functional *Abd-B* protein would only be produced by the *Abd-B* gene on the *Fab-7* chromosome. It should be noted that this model requires that the regulatory capacity of the ectopically activated *iab-7* domain in PS11/A6 is limiting; otherwise, the presence of a competing *Abd-B* point mutant in *trans* to the *Fab-7* chromosome would have no phenotypic consequences. This supposition—that the regulatory capacity of *iab-7* is limiting—seems to be correct, because the phenotype of PS11/A6, when the *Abd-B* gene in *trans* is either removed entirely (as in *Fab-7/Df(3R)P9*) or no longer available for pairing (as in *TSR,Fab-7/Abd-B^{D16}*), is indistinguishable from *Fab-7/+*.

Competition between the *iab-7 cis*-regulatory domains: In the experiments described in the previous section, nonproductive *trans*-interaction between the *iab-7* regulatory domain on the *Fab-7* homologue and the *Abd-B* point mutation on the other homologue suppressed the gain-of-function phenotype in A6. We speculated that the *trans*-interactions between *iab-7* and the mutant *Abd-B* gene were permitted in A6 because the *iab-7* regulatory domain on the homologue containing the mutant *Abd-B* gene is not active in this segment (see Galloni *et al.* 1993). In contrast, such *trans*-interactions might not be as strong in A7 because *cis*-interactions between the *Abd-B* gene and the *iab-7* regulatory domain on the same homologue would reduce the ability of the *Abd-B* promoter to interact in *trans*. This model could potentially explain the phenotypic difference between A6 and A7 in *Fab-7/Abd-B^{D16}* males (see Figure 2).

If this model is correct, *cis*-competition in segment A7 should be eliminated by deleting the *iab-7* regulatory domain on the *Abd-B^{D16}*-containing chromosome. The lack of competition from the *iab-7* domain in *cis* should result in an increase in the frequency or strength of *trans*-interactions between the *iab-7* domain on the *Fab-7* chromosome and the mutant *Abd-B* gene on the other homologue.

To test this prediction, we recombined an *iab-7* deletion mutant, *iab-7^{Sz}*, onto the *Abd-B^{D16}* chromosome. The phenotype of *+/iab-7^{Sz}Abd-B^{D16}* and *Fab-7/iab-7^{Sz}Abd-B^{D16}*

males is shown in Figure 1h and Figure 1g, respectively. Two findings are of interest.

First, the gain-of-function phenotype of *Fab-7* in A6 is still suppressed in *Fab-7*/*iab-7^{Sz}Abd-B^{D16}* males (Figure 1g), and this segment closely resembles the A6 segment found in *Fab-7*/*Abd-B^{D16}* males (Figure 1e). The similarity between these two mutant combinations would argue that the *iab-7 cis*-regulatory domain on the *Abd-B^{D16}* chromosome does not contribute to the suppression of the *Fab-7* phenotype in A6 and would be consistent with the idea that the *iab-7 cis*-regulatory domain is normally inactive in this segment (Galloni *et al.* 1993). Second, segment A7 in both +/*iab-7^{Sz}Abd-B^{D16}* (Figure 1h) and *Fab-7*/*iab-7^{Sz}Abd-B^{D16}* (Figure 1g) males is even more strongly transformed toward an A6 identity than it is in +/*Abd-B^{D16}* or in *Fab-7*/*Abd-B^{D16}* males. In fact, A6 and A7 are indistinguishable in *Fab-7*/*iab-7^{Sz}Abd-B^{D16}* males and closely resemble the appearance of A6 in *Fab-7*/*Abd-B^{D16}* and A7 in +/*iab-7^{Sz}Abd-B^{D16}*. It should be emphasized that this transformation in A7 identity cannot be attributed solely to the lack of *iab-7* and *Abd-B* function on the *iab-7^{Sz}Abd-B^{D16}* chromosome. Thus, the transformation of A7 toward A6 in *Fab-7*/*iab-7^{Sz}Abd-B^{D16}* males is much more extreme than it is when *Fab-7* is combined with deficiencies *e.g.*, *Df(3R)P9*, Figure 1f, or *Df(3R)C4*, not shown, which completely remove both *iab-7* and *Abd-B* (compare Figure 1g and f). As expected, if the *Fab-7* chromosome is replaced by its rearranged version in *Fab-7*/*iab-7^{Sz}Abd-B^{D16}*, *i.e.*, in *TSR,Fab-7*/*iab-7^{Sz}Abd-B^{D16}* (not shown), the phenotype of A6 and A7 becomes indistinguishable from that of *TSR,Fab-7*/*Abd-B^{D16}* or *Fab-7*/*Df(3R)P9* (shown in Figure 2 and Figure 1f, respectively).

These findings indicate that *trans*-regulatory interactions in segment A7 have no phenotypic consequences if an *iab-7* domain is present on the chromosome containing the mutant *Abd-B* gene. To demonstrate that this domain must not only be present but also active, we recombined *Fab-7* with the *Abd-B* point mutation *Abd-B^{D16}* and then examined the phenotype of *Fab-7*/*Fab-7Abd-B^{D16}* males. In these animals, both copies of *iab-7* will be ectopically activated in A6. If we are correct in assuming that the regulatory domain in *cis* must be active in order to reduce *trans*-regulatory interactions with the mutant *Abd-B* gene, then the phenotype of A6 and A7 in these animals should be identical and resemble the A7 segment in *Fab-7*/*Abd-B^{D16}* animals. Indeed, this is the case (see Figure 2).

Two different (but not mutually exclusive) hypotheses could explain the phenotypic difference between the segments in which one *vs.* two *iab-7* regions are active, *e.g.*, the A7 of *Fab*/*iab-7^{Sz} Abd-B^{D16}* and *Fab-7*/*Abd-B^{D16}*. The first postulates that the *iab-7* regulatory domains engage not only in *cis*- but also in *trans*-regulatory interactions (indicated by dotted arrows in Figure 2). In this case, the resulting phenotype depends upon the sum of the interactions contributed by either one or two active *iab-7* domains. In the second, *cis*-interactions between the *iab-7* regulatory domain and the *Abd-B* gene on the same homologue would, because of competition, tend to reduce or suppress *trans*-interactions between this *Abd-B* gene and the *iab-7* regulatory domain on the other homologue. When there is no competing active regulatory domain in *cis*, the *Abd-B* gene will be more readily available to engage in *trans*-interactions. It is not possible to distinguish between these two models with the genetic tools presently available; however, we favor the second model, as our analysis of the effects of promoter deletions (see below) suggests that the balance between *cis*- and *trans*-interactions can be shifted by competition.

Mutations that delete sequences near the 5′ end of the *Abd-B* gene enhance *trans*-regulation: The ability of the *iab-7* regulatory domain to interact with the *Abd-B* genes in *cis* and in *trans* is not equal. If the *cis*- and *trans*-interactions were symmetrical, the phenotype of *Fab-7*+/+*Abd-B* and *Fab-7Abd-B*/++ would be identical. However, this is clearly not the case. In contrast to the modest but detectable transformation of A6 toward A7 in *Fab-7*/*Abd-B^{D16}* flies, we see no trace of an A6 to A7 transformation in *Fab-7Abd-B^{D16}*/+ flies. Similarly, *Abd-B^{D16}* does not complement *iab-7^{Sz}* to any detectable degree in A7 (Figure 6a).

We reasoned that it might be possible to strengthen the interaction of *iab-7* with the *trans* copy of *Abd-B* by weakening its interactions with the *Abd-B* gene in *cis*. Because the regulatory interactions of *iab-7* with the *Abd-B* gene are likely to be mediated, at least in part, by sequences in the vicinity of the *Abd-B* promoter, we thought that it might be possible to weaken the *cis*-interactions by substituting *Abd-B* point mutants with an *Abd-B* 5′ deletion mutant, *Abd-B^{D14}* (*m*$^{-}$; Karch *et al.* 1985), which lacks sequences around the proximal promoter (Boulet *et al.* 1991). As anticipated, we found that *Abd-B^{D14}* weakly complements the *iab-7* loss-of-function mutant *iab-7^{Sz}* in A7 (Figure 6b). Moreover, unlike in *Fab-7Abd-B^{D16}*/+, we were able to detect a weak transformation of A6 into A7 in *Fab-7Abd-B^{D14}*/+ adults. This phenotype of weak *trans*-interaction is best seen in the sixth sternites of females (see Figure 3a).

To rule out the possibility that this weak transformation is due to a "leakiness" of the *Abd-B^{D14}* mutation, we examined the phenotype of *Fab-7Abd-B^{D14}*/*Df(3R)P9* hemizygotes and *Fab-7Abd-B^{D14}*/*Fab-7Abd-B^{D14}* homozygotes. In neither case did we detect transformation of A6 into A7 (not shown). In fact, it appears that the weak transformation observed in *Fab-7Abd-B^{D14}*/+ animals requires that the wild-type copy of the *Abd-B* gene in *trans* can pair. Thus, transformation is not detectable when pairing is efficiently disrupted (Figure 3b). Conversely, *trans*-interaction can be detected in both *Fab-7Abd-B^{D14}*/*iab-7^{Sz}* and *Fab-7Abd-B^{D14}*/*Df(3R)R59* flies (not shown), where the *trans* copy of the *iab-7* regulatory domain is deleted but the *Abd-B* gene is intact.

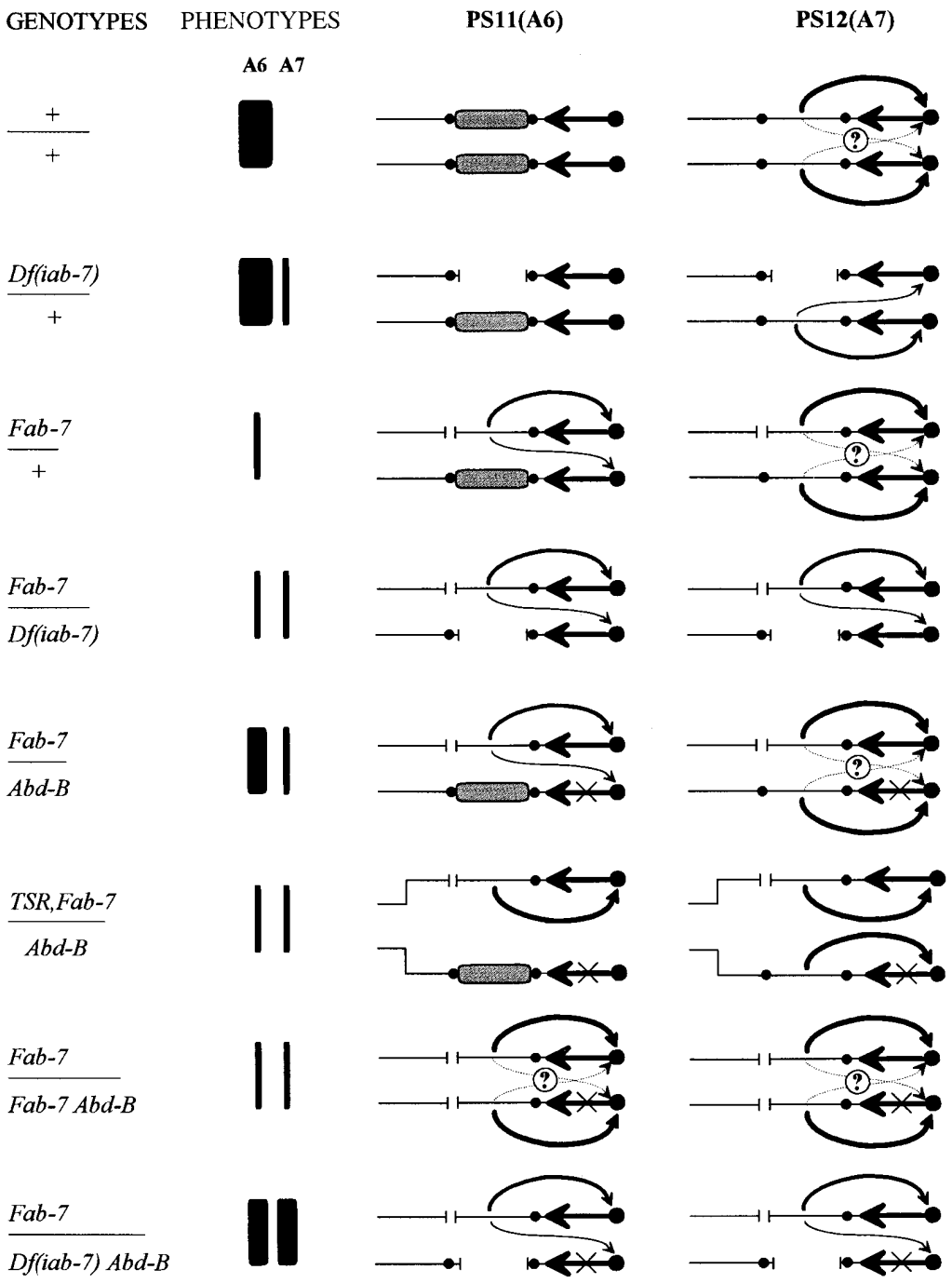

Figure 2.—Diagrammatic recapitulation of the most relevant results (presented in part in Figure 1) and their interpretation. Continuous line represents the active form of *iab-6* and *iab-7*; shaded rectangle, the inactive (closed) form of *iab-7*; small black dots, the *Fab-7* and the putative *Fab-8* boundaries; vertical lines, the endpoints of *Fab-7* and *iab-7*^{Sz} deletions; filled circle, the proximal *Abd-B* promoter; bold arrow, the *Abd-B* class A transcription unit; and X, the presence of a point mutation. The regulatory interaction between *Abd-B* and *iab-7* is represented by curved arrows. Dotted arrows and question marks symbolize the possibility of mutual *trans*-regulation when *iab-7 cis*-regulatory regions are intact and active on both homologues. The width of black oblongs in column 2 roughly corresponds to the size of the tergites of the different genotypes.

Other lines of evidence also suggest that the *Abd-B*^{D14} promoter deletion mutant is a weaker competitor for interactions with *iab-7*, either in *cis* or in *trans*, than a structurally wild-type *Abd-B* gene. First, the *trans*-suppression of the *Fab-7* gain-of-function phenotype in segment A6 of *Abd-B*^{D14} / *Fab-7* males is significantly weaker (but still detectable) than in the combination of *Fab-7* with any of the *Abd-B* point mutants (compare Figure 4, b and a). Second, the haplo-insufficient phenotype of *Abd-B*^{D14} / + in A7 is clearly weaker than that of *Abd-B* point mutations (compare Figure 4, b and a), which suggests a hyperactivation of the wild-type *Abd-B* gene in *trans*. These differences are pairing dependent, because the phenotypes of *Abd-B*^{D14} / *TSR,Fab-7* (Figure 4c) and *Abd-B*^{D16} / *TSR,Fab-7* flies are indistinguishable (not shown).

Isolation of new *Abd-B* mutations which enhance *trans*-regulation by *iab-7*: The observation that the small *Abd-B*^{D14} deletion enhances *iab-7 trans*-regulation points to sequences in the vicinity of the proximal *Abd-B* promoter as potential targets for *cis*- (and *trans*-) regulatory interactions. However, the finding that *Abd-B*^{D14} still suppresses *Fab-7* in *trans* to some extent (compare Figure 4, b and c) suggests that additional sequences may also be relevant for *trans*-regulation.

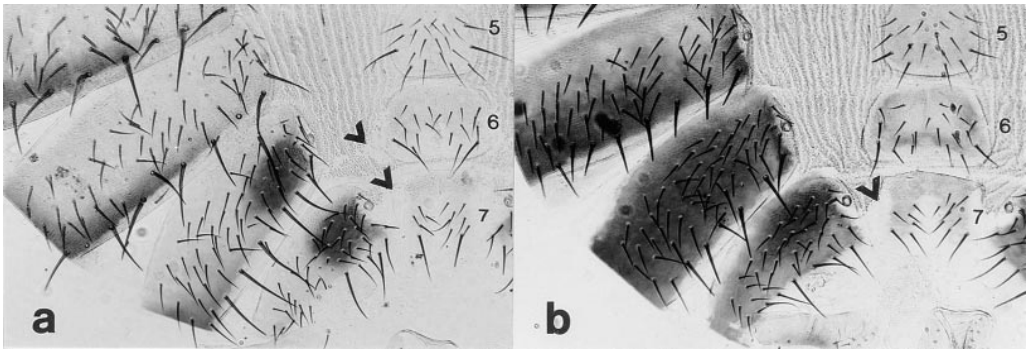

Figure 3.—Adult cuticles of (a) *Fab-7Abd-B^{D14}/+*, (b) *TSR-10^{Sz},Fab-7Abd-B^{D14}/In(3LR)TM3, Sb Ser* females. When pairing of the homologues is normal a, A6 is weakly transformed toward A7 as evidenced by the presence of characteristics that are normally found on the wild-type A7, such as hairs pointing toward the middle of the sixth sternite and the absence of trichomes around the sternite (indicated by arrowheads). When pairing is efficiently disrupted (by the rearrangements *TSR-10^{Sz}* and *In(3RLR)TM3*), this mild *Fab-7* phenotype is no longer detectable b. In addition, the haplo-insufficient phenotype in A7 is clearly stronger (the size of the seventh sternite is enlarged) than in a, indicating that the hyperactivation of the *Abd-B* gene is also eliminated in the absence of somatic pairing.

To further characterize sequences from the *Abd-B* gene that contribute to these regulatory interactions we took advantage of a hypomorphic *Abd-B* mutation, *UC21-10,1-d*, which has a *P*-transposon at position +159 on the molecular map (McCall *et al.* 1994) just upstream of the transcription start site of the *Abd-B* class A transcript. By itself, this insertional mutation, like the promoter deletion in *Abd-B^{D14}*, weakly enhances *iab-7 trans*-regulation. Thus, *Fab-7UC21-10,1-d* in *trans* to a wild-type *Abd-B* gene transforms A6 toward A7. That this transformation of A6 to A7 is not due simply to the residual *Abd-B* activity of the *UC21-10,1-d* allele, but rather arises from *trans*-activation of the wild-type *Abd-B* gene, is suggested by the finding that the A6-A7 transformation is strongly reduced when *Fab-7UC21-10,1-d* is combined with the point mutant *Abd-B^{D16}* (lethality covered by *Dp(3;1)bxd^{111}*).

To identify sequences that contribute to *cis*- (and *trans*-) regulatory interactions, we mobilized the *UC21-10,1-d* transposon on the *Fab-7UC21-10,1-d* chromosome and then selected for excision events that eliminated the remaining *Abd-B m* activity (see materials and methods). We then tested these new *Abd-B* alleles for their ability to enhance *trans*-regulation of a wild-type *Abd-B*. Out of nine new *Abd-B* alleles, we identified two mutations (*Abd-B^{PSz1}* and *Abd-B^{PSz2}*) that enhanced the *Fab-7* gain-of-function phenotype in A6 when *trans* to a wild-type copy of BX-C more strongly than *Abd-B^{D14}*. Genomic Southern blotting revealed that *Abd-B^{PSz1}* has a 10.8-kb deletion between map position +155.6–156.8 and +165.6–166.6, and *Abd-B^{PSz2}* carries a smaller deletion of about 5.5-kb from +155.6–156.8 to +160.2–161.3 (Figure 5).

Other 5′ deficiencies also enhance *trans*-regulation: When we compared the transformation of A6 into A7 in *Fab-7 Abd-B^{-}/+* flies, it appeared that the strength of the *trans*-activation of the wild-type *Abd-B* gene is greatest in the largest promoter deletion, that is, *Fab-7Abd-B^{PSz1}/+ > Fab-7Abd-B^{PSz2}/+ > Fab-7Abd-B^{D14}/+ > Fab-7Abd-B^{D16}/+*. Interestingly, precisely the same relationship is observed for the complementation of the *iab-7* deletion, *iab-7^{Sz}*, in A7. Thus, the strongest complementation is observed in *Fab-7Abd-B^{PSz1}/iab-7^{Sz}* flies, which is followed by *Fab-7Abd-B^{PSz2}/iab-7^{Sz}*, *Fab-7Abd-B^{D14}/iab-7^{Sz}*, and finally, *Fab-7Abd-B^{D16}/iab-7^{Sz}*. The similarity between these two assays suggested that we could measure the ability of any *Abd-B* mutation to promote *trans*-regulation by examining its complementation of *iab-7^{Sz}* in A7. Using this assay, we tested the *trans*-regulating ability of three previously isolated deficiencies, *Df(3R)C4* (Karch *et al.* 1985), *Df(3R)U110* and *Abd-B^{RD18}* (Hopmann *et al.* 1995) that remove the distal part of BX-C. As expected, *Df(3R)C4*, which removes *iab-7* and part of *iab-6* in addition to *Abd-B*, does not show any complementation. However, both *Df(3R)U110* and *Abd-B^{RD18}*, which leave *iab-7* intact, complement *iab-7^{Sz}* to a high degree (Figure 6, d and c, respectively). As expected, these two deletions do not suppress the *Fab-7* phenotype in *trans*, and the phenotype of A6 in *Fab-7/Df(3R)U110* and *Fab-7/Abd-B^{RD18}* (not shown) is similar to *Fab-7/+* (see Figure 1c).

The approximate limits of the complementing deletions are shown in Figure 5. These deletions can be ordered in increasing strength of *iab-7^{Sz}* complementation as follows: *Abd-B^{D14} < Abd-B^{PSz2} < Abd-B^{RD18} ≤ Abd-B^{PSz1}*. From this data it would appear that *trans*-regulation is strongest in the case of deletions that remove the largest region of *Abd-B* DNA on the 5′ side of the *Abd-B* gene (compare Figure 6, b and c). It is also clear that deficiencies that strongly enhance *trans*-regulation extend well beyond the region thought to correspond to the proximal *Abd-B* promoter (Busturia and Bienz 1993).

We note that unlike *Abd-B* "point mutations," the

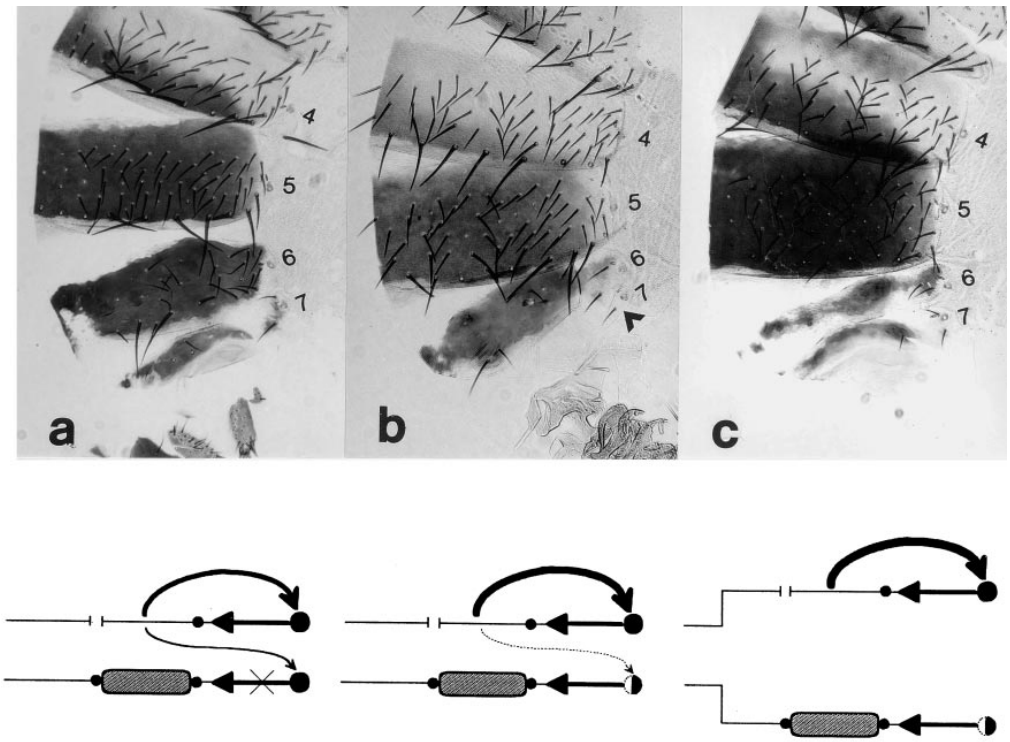

Figure 4.—Abdominal cuticles of adult males of the genotypes (a) *Fab-7*/*Abd-B*^{D16}, (b) *Fab-7*/*Abd-B*^{D14} and (c) *TSR-1*^{Sz},*Fab-7*/*Abd-B*^{D14}. Suppression of the *Fab-7* phenotype by the deletion mutant *Abd-B*^{D14} is significantly weaker (but still detectable) than in the combination with the point mutation *Abd-B*^{D16} (compare the size of the sixth tergites in a, b and c). Due to the hyperactivation of the wild-type *Abd-B* gene, the haplo-insufficient phenotype of A7 is suppressed in b (A7 tergite is missing and only a single hair at the tracheal opening, indicated by arrowhead, shows that it is not completely wild type). When pairing is efficiently disrupted c, both hyperactivation and *trans*-suppression are eliminated, and A6 and A7 tergites are equally thin. The thickness of arrows in the drawings under each of the abdomens indicates the relative strength of *cis*- and *trans*-regulation.

Abd-B^{PSz2}, *Abd-B*^{RD18} or *Abd-B*^{PSz1} deletions do not show any sign of an A7 to A6 transformation when they are *trans* to a wild-type BX-C. This difference presumably reflects the ability of these large promoter deletions to enhance *trans*-regulatory interactions with the wild-type *Abd-B* gene. In fact, in the case of these larger deletions, the hyperactivation of the wild-type *Abd-B* gene in *trans* is even stronger than that observed in the case of the smaller promoter deletion, *Abd-B*^{D14}, because a weak A7 to A6 transformation (the presence of at least a single bristle in tergite A7; Figure 4b) is observed in *Abd-B*^{D14}/+ males. However, these promoter deletions do show the expected haplo-insufficient phenotype when they are combined with a chromosome like *TSR, Fab-7* that disrupts pairing. In this case, their phenotype is indistinguishable from that of *TSR,Fab-7*/*Abd-B*^{D14} (see Figure 4c).

Df(3R)U110 is an unusual case. It is the largest of the *Abd-B* deficiencies that still retains *iab-7*, and it extends from near the 3′ end of *Abd-B* into the 90A region. As expected from the large size of this deficiency, it exhibits by far the strongest enhancement of *trans*-regulation when in *trans* to *iab-7*^{Sz}. However, unlike any of the other deficiencies, the extent of complementation by the *trans* copy of *iab-7* varies from hemitergite to hemitergite and sometimes appears to be clonal even within a hemitergite (Figure 6d). *Df(3R)U110* also differs from the other *Abd-B* deficiencies in that it fails to complement the loss-of-function *iab-7* phenotype of *Df(3R)R59*, a deficiency that extends proximally from the 3′ end of the *Abd-B* gene to the *bxd* region. The only sign of some complementation in *Df(3R)U110*/*Df(3R)R59* males is the presence of black pigmentation in tergite A6 and A7, a phenotype corresponding to the result of the pairing-insensitive, long-range interaction described by Hopmann *et al.* (1995) and Hendrickson and Sakonju (1995). All of the other *Abd-B* deletion mutations complement the *R59* deficiency in A7 to a degree roughly similar to that observed for *iab-7*^{Sz} (data not shown). The lack of this complementation between *Df(3R)U110* and *Df(3R)R59* does not seem to be due to the fact that these two deficiencies overlap in the region just 3′ to the *Abd-B* gene. Like *Df(3R)U110*, the deletion in *Abd-B*^{RD18} overlaps *Df(3R)R59* in this 3′ region, yet *Abd-B*^{RD18} shows the same degree of complementation of *Df(3R) R59* as that observed for the *Abd-B*^{PSz1}, which does not overlap.

The most likely explanation for the unusual behavior of *Df(3R)U110* is illustrated in Figure 7. In the case of nonoverlapping deficiency combinations such as

Abd-B^{RD18}/*iab-7*^{Sz} (Figure 7a), the *iab-7* and *Abd-B* DNA segments on the two homologues loop out, but remain in relatively close proximity. This also holds for the overlapping deficiency combination *Abd-B*^{RD18}/*Df(3R)R59* (see Figure 7b). In contrast, the remaining *iab-7* and *Abd-B* DNA segments in the *Df(3R)U110*/*Df(3R)R59* are located on opposite sides of the large unpaired deficiency loops (see Figure 7c), and this distance (which to a first approximation corresponds to the size of the smaller deficiency) may prevent effective *trans*-regulation. In the case of the *Df(3R)U110*/*iab-7*^{Sz}, the *iab-7* deficiency is quite small, and the remaining *iab-7* and *Abd-B* DNA segments would still be in relatively close proximity. (The hypothetical chromosome structure of *Df(3R)U110*/*iab-7*^{Sz} is essentially the same as that of *Abd-B*^{RD18}/*iab-7*^{Sz}, shown in Figure 7a.) Thus, the difference between the phenotype of *Df(3R)U110*/*iab-7*^{Sz} and that of *Df(3R)U110*/*Df(3R)R59* would suggest that proximity has a strong influence in the restoration of A7 identity by *trans*-regulation.

Transvection in the *Abd-B* domain is not *zeste*-dependent: Because most of the previously documented cases of transvection are dependent on the presence of a wild-type copy of the X-linked gene *zeste* (Kaufman *et al.* 1973; Micol and García-Bellido 1988), we tested whether *zeste* is also required for *trans*-regulatory interactions involving the *Abd-B* gene. Surprisingly, we found that mutations in the *zeste* locus (z^a, z^{op6}, z^1, z^{v77h}) had no apparent affect on any of the *trans*-regulatory interactions in the *Abd-B* domain described above. Hendrickson and Sakonju (1995) and Hopmann *et al.* (1995) have also found that *zeste* function is not required for the long-range pairing-insensitive *trans*-interaction that they have uncovered in the *Abd-B* domain.

Is transvection tissue or stage specific? We wondered whether *iab-7* is able to *trans*-regulate the *Abd-B* gene in tissues besides those giving rise to the adult cuticle. To investigate this question, we used immunostaining to examine the *Abd-B* expression in embryos of different genetic backgrounds.

The various control embryos gave the expected patterns of *Abd-B* protein expression described by others (Celniker *et al.* 1989, 1990; DeLorenzi and Bienz 1990). In wild type, ABD-B protein increases in a stepwise fashion between PS10 and PS14 (Celniker *et al.* 1989; Sánchez-Herrero 1991; see also Figure 8a). In *Fab-7* homozygous embryos, the wild-type pattern of ABD-B protein is observed in all parasegments except for PS11, where *Abd-B* is expressed in a PS12-like pattern (not shown; see Galloni *et al.* 1993). As anticipated,

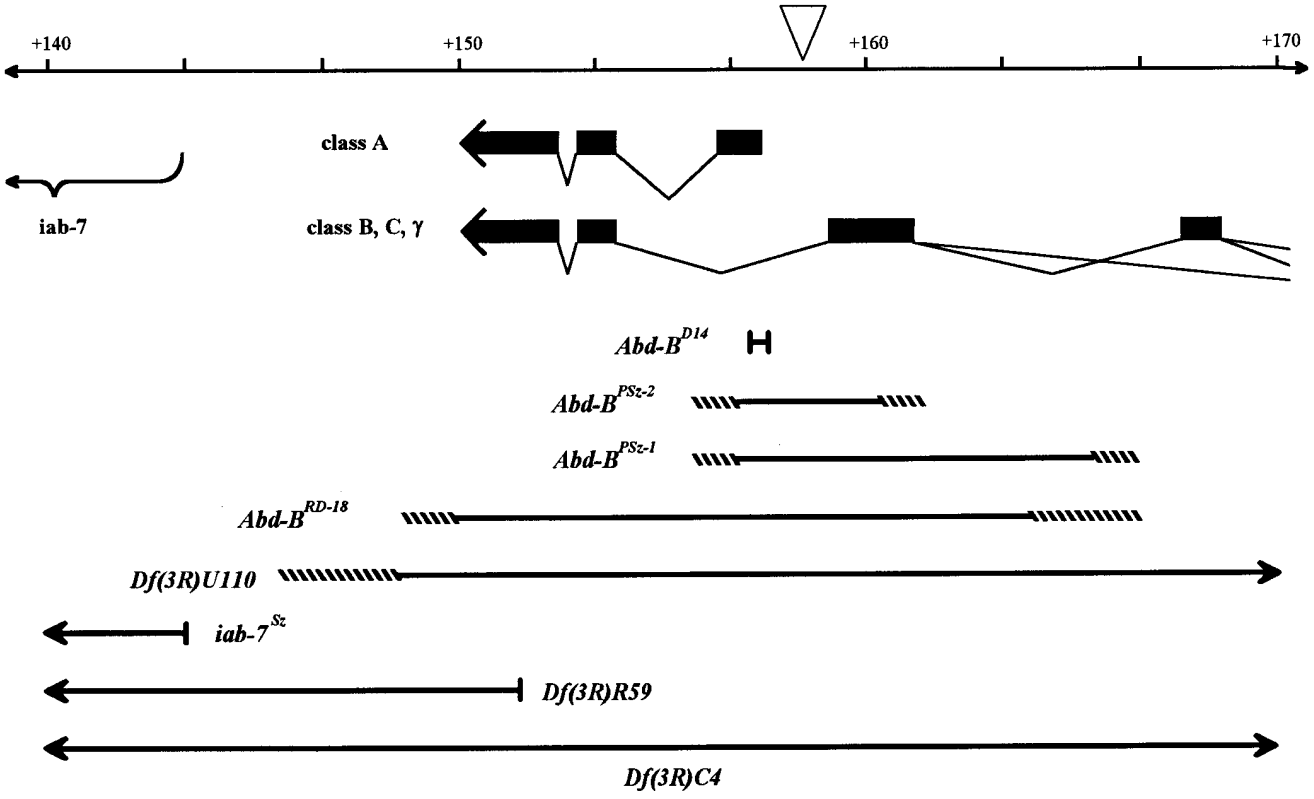

Figure 5.—Structure of *Abd-B* deletions used in complementation tests with the deletions *iab-7*^{Sz} and *Df(3R)R59*, shown on the molecular map. Continuous bold line indicates the regions covered by the deletions (Karch *et al.* 1985; Gyurkovics *et al.* 1990; Galloni *et al.* 1993; Hopmann *et al.* 1995); dashed line, uncertainty in the locations of the endpoints; triangle, the position of the insertion *UC21-10, l-d* (McCall *et al.* 1994). Transcriptional unit of class A and part of class B, C and γ are indicated under the molecular map (Zavortnik and Sakonju 1989).

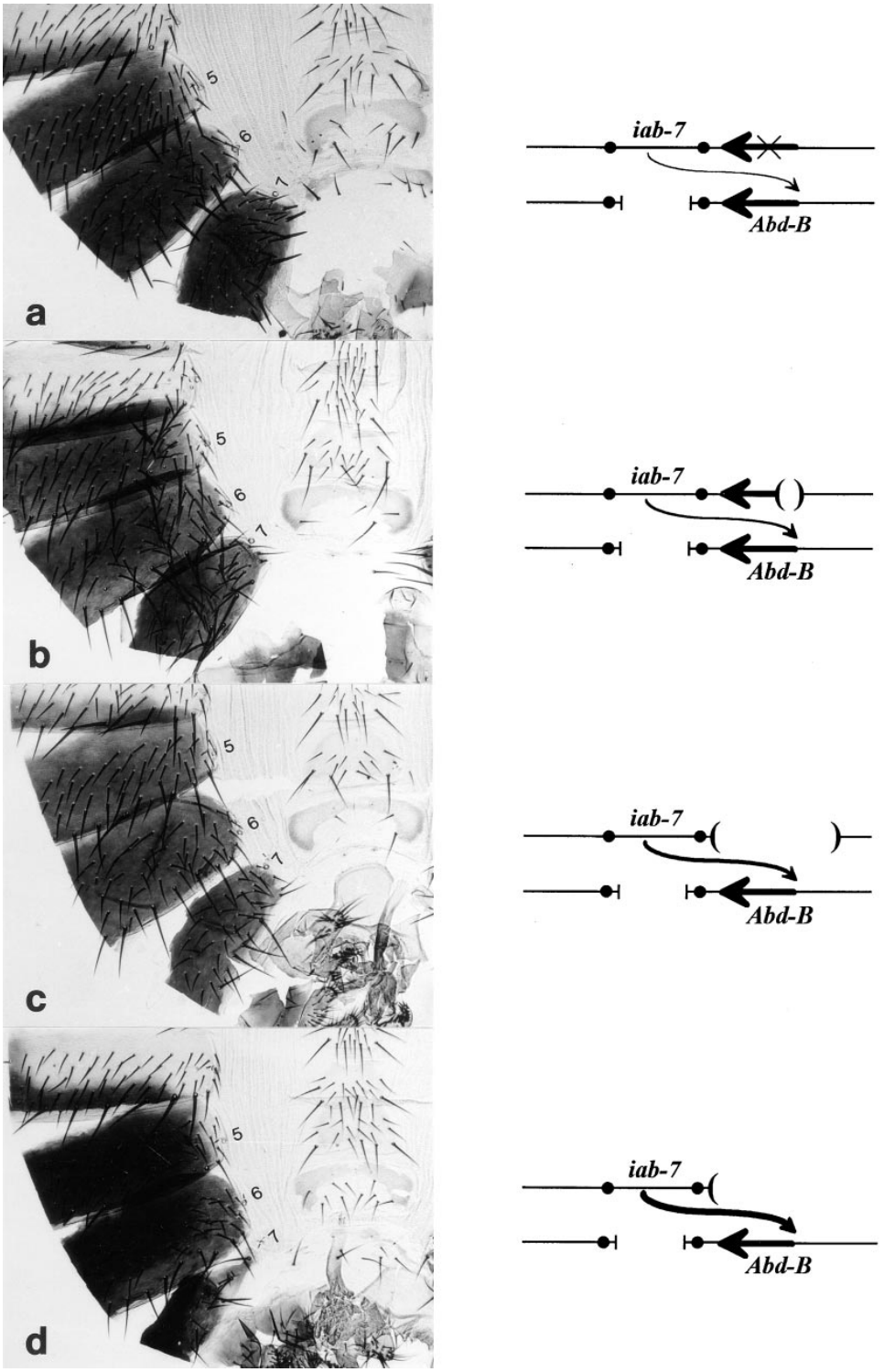

Figure 6.—Abdominal cuticles of adult males of the genotype: (a) *Sb Abd-B*^{D16}/*iab-7*^{Sz}, (b) *Abd-B*^{D14}/*iab-7*^{Sz}, (c) *Abd-B*^{RD18}/*iab-7*^{Sz} and (d) *Df(3R)U110*/*iab-7*^{Sz}. The point mutation *Abd-B*^{D16} does not complement *iab-7*^{Sz} to any detectable degree, as A7 appears to be completely transformed into A6. However, the size of the A7 tergite and sternite is progressively reduced in b–d as compared to a, indicating that deletions that remove increasingly larger regions upstream of the *Abd-B* class A transcription unit complement the *iab-7*^{Sz} mutation to increasingly higher degrees. In the right column, the diagrammatic interpretation of the phenotype for each genotype is presented. The thickness of curved arrows corresponds to the increasing strength of *trans*-interaction (*cis*-interaction is not shown).

no protein can be detected when the *Fab-7* embryos are also homozygous for the *Abd-B* point mutation, *Abd-B*^{D16} (not shown; Boulet *et al.* 1991). In the case of *Fab-7Abd-B*^{D14} homozygotes, protein is observed in the parasegment PS14, but not in PS10–PS13 (Figure 8b). This is expected because the *Abd-B*^{D14} deletion removes the transcription start site of the class A *Abd-B* transcripts, which are specifically expressed in PS10–PS13. Finally, there is no ABD-B protein in PS10–PS12 of embryos homozygous for the deletion *Df(3R)R59*, whereas the normal pattern of protein staining is observed in PS13 (where the expression of *Abd-B* is under the control of the *iab-8 cis*-regulatory domain) and in PS14 (Figure 8c).

We next examined the *Abd-B* expression pattern in *Fab-7Abd-B*^{D16}/*Df(3R)R59* and *Fab-7Abd-B*^{D14}/*Df(3R)R59*

embryos. On the basis of regulatory interactions inferred from the phenotype of the adult cuticle, we anticipated that the *Abd-B* gene on the *R59* homologue would be *trans*-activated in the embryos carrying the $Abd\text{-}B^{D14}$ deletion mutant, but not in the embryos carrying the $Abd\text{-}B^{D16}$ point mutation. For $Fab\text{-}7Abd\text{-}B^{D14}$ / *Df(3R)R59* embryos, we found the same levels of ABD-B protein in the CNS of PS11 and PS12 (see Figure 8d). This is the result expected if the *iab-7* regulatory domain on the *Fab-7* chromosome is able to *trans*-regulate the *Abd-B* gene on the *R59* homologue in PS11 and PS12. Thus, for this mutant combination, *trans*-regulation is the same in the embryonic CNS as it is in the adult. Surprisingly, although we were unable to detect *trans*-regulation of the *Abd-B* gene on the *R59* homologue in adult $Fab\text{-}7Abd\text{-}B^{D16}$ / *Df(3R)R59* animals, *trans*-regulation could be detected in the embryonic CNS. As in $Fab\text{-}7Abd\text{-}B^{D14}$ / *Df(3R)R59* embryos, we found the same levels of ABD-B protein in the CNS in PS11 and PS12 of $Fab\text{-}7Abd\text{-}B^{D16}$ / *Df(3R)R59* embryos (not shown).

Although we found clear evidence for *trans*-regulatory interactions between the $Fab\text{-}7Abd\text{-}B^{-}$ and *R59* homologues in the embryonic CNS, such interactions were not evident in other embryonic tissues. Thus, in animals of the same two genotypes, we were unable to detect ABD-B protein in either the ectoderm or mesoderm of PS11 and PS12. This was true even at the beginning of germ-band retraction, when *Abd-B* expression is most readily evident in wild-type embryos in these parasegments (not shown).

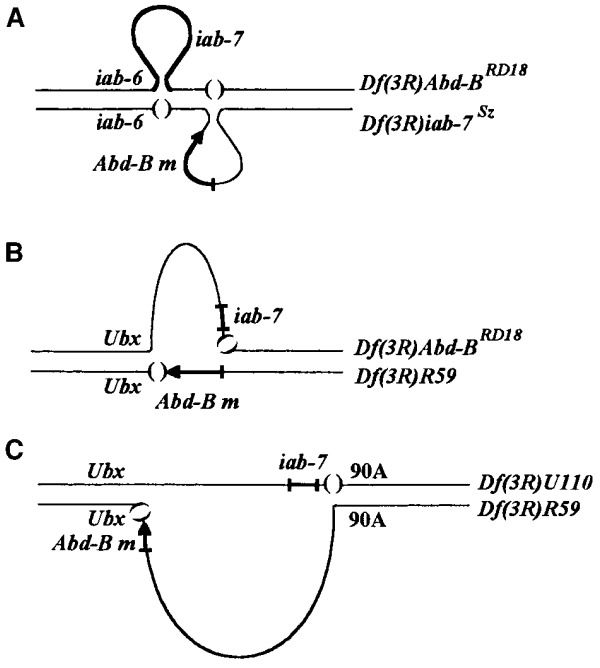

Figure 7.—The position of the *iab-7* regulatory region and the *Abd-B* class A transcription unit (marked with bold line and arrow, respectively) in different pair-wise combinations of deletions. Continuous line represents DNA; parentheses indicate the breakpoints of deletions. (A) nonoverlapping; (B, C) overlapping combinations of deletions. Note that in B the regions marked with bold are relatively close to each other, and in C they are localized at the far ends of the unpaired region.

In some experiments, we saw faint *Abd-B* specific staining of CNS in PS10 of both $Fab\text{-}7Abd\text{-}B^{D14}$ / *Df(3R)R59* and $Fab\text{-}7Abd\text{-}B^{D16}$ / *Df(3R)R59* embryos. This observation suggested that *iab-5* and *iab-6* may also be able to *trans*-regulate the *Abd-B* gene on the *R59* homologue. To test this possibility, we stained $Mcp^{B116}iab\text{-}7^{Sz}Abd\text{-}B^{D16}$ / *Df(3R)R59* embryos. The Mcp^{B116} mutation is a deletion that removes a putative boundary between *iab-4* and *iab-5*, ectopically activating the PS10-specific *iab-5* in PS9 (Karch *et al.* 1994). In animals carrying a wild-type *Abd-B* gene in *cis*, the ectopic activation of *iab-5* results in the appearance of ABD-B protein in PS9 of the embryonic CNS at a level normally found in PS10 (Sanchez-Herrero 1991). As *iab-7* is also deleted on this Mcp^{B116} chromosome, *Abd-B* expression in both PS11 and PS12 can only be driven by the *iab-6* regulatory domain. Since the *Abd-B* gene in *cis* to Mcp^{B116} and $iab\text{-}7^{Sz}$ does not code for a detectable protein, ABD-B protein can only be expressed in these embryos by *trans*-regulation of the *Abd-B* gene on the *Df(3R)R59* chromosome in PS9–PS12. As can be seen in Figure 8e, we can detect weak ABD-B expression in PS11–PS12, and an even weaker, but unmistakable expression is seen in PS9–PS10. This pattern of protein expression argues that the *iab-5* and *iab-6 cis*-regulatory domains on $Mcp^{B116}iab\text{-}7^{Sz}Abd\text{-}B^{D16}$ chromosome are capable of *trans*-regulation much like the *iab-7* regulatory domain.

Long distance *trans*-regulatory interactions in the embryonic CNS: The results described in the previous section indicate that *trans*-regulatory interactions in the *Abd-B* domain may be significantly stronger in the embryonic CNS than in the adult epidermis. If this were true, it might be possible to detect *trans*-regulation in the CNS of embryos carrying chromosomal rearrangements that substantially perturb pairing in BX-C and eliminate transvection in the cells giving rise to the adult epidermis. To investigate this possibility we examined *Abd-B* expression in the CNS of embryos heterozygous for either $Fab\text{-}7Abd\text{-}B^{D14}$ or $Fab\text{-}7Abd\text{-}B^{D16}$ and chromosomes that have rearrangements that disrupt *Abd-B* and *Ubx* *trans*-regulation in the adult. These rearrangements include *TSR-11A*, *TSR-59A*, $iab\text{-}7^{MX2}$ (In(3LR)64A; 89A;89E; Karch *et al.* 1985), $iab\text{-}7^{164}$ (In(3LR)65;89E superimposed on In(3R)1000 with breaks in 81C and 90C; Celniker *et al.* 1990).

Even though these rearrangements suppress *trans*-regulatory interactions in the cells giving rise to the adult epidermis, they do not prevent *trans*-regulatory interactions in the embryonic CNS, and we observe equal levels of ABD-B protein in PS11 and PS12 (as would be expected if the *iab*-7 regulatory domain on the $Fab\text{-}7Abd\text{-}B^{-}$ chromosome is driving the expression of the *Abd-B* gene on the rearranged chromosome). The one exceptional case is the doubly rearranged

iab-7^{164} chromosome. In both *Fab-7Abd-B*^{D16} / *iab-7*^{164} and *Fab-7Abd-B*^{D14} / *iab-7*^{164} embryos, the *Abd-B* antibody staining is weak and irregular (Figure 8h), suggesting that the configuration of the rearrangement in this particular chromosome interferes with *trans*-regulation even in the CNS.

Long-distance regulatory interactions in the embryonic CNS are suppressed by the uninterrupted pairing of homologues: Our ability to detect long-distance *trans*-regulatory interactions in the CNS prompted us to investigate the parameters governing this phenomenon further. The chromosomal rearrangement *In(3R) Fab-7iab-7*^{R5} was isolated as a revertant of *Fab-7*. It has a distal breakpoint at position +152 on the molecular map in the BX-C, very close to the 3′ end of the *Abd-B m* transcription unit. From its position, it seems likely that the break in BX-C may leave both the *iab-7* regulatory domain and the *Abd-B* gene functionally intact. The proximal breakpoint is in the region 87C1-2,3.

Because *iab-7* as well as *iab-5* and *iab-6* are separated by a large distance from the *Abd-B* gene in this inversion-bearing chromosome, these regulatory domains should be unable to contact the *Abd-B* gene, and the gene should not be expressed in PS10–PS12 in the CNS. This is the case in embryos homozygous for the *In(3R)Fab-7iab-7*^{R5} inversion; we only see *Abd-B* staining in PS13–PS14 (not shown). Surprisingly, however, a different result is obtained when *In(3R)Fab-7iab-7*^{R5} is combined with the deficiency chromosome *Df(3R)P9* that removes the entire BX-C. In this combination, we detect a low but uniform level of *Abd-B* antibody staining in the CNS of PS11 and PS12 (Figure 8f).

From these results, it would appear that *iab-7* is able to regulate a distantly located *Abd-B* gene on the same chromosome when the inversion is in *trans* to a BX-C deficiency, but not when it is homozygous. In the former case, two factors might facilitate this regulatory interaction. First, the loop formed by homologous pairing between the deficiency and the inversion chromosomes would bring the BX-C sequences on either side of the inversion breakpoints in close proximity. Second, there would be unpaired regions around the breakpoints of the inversion that at one end might expose the *iab-7* regulatory domain and at the other end might expose the *Abd-B* gene and 5′ flanking regions. In the latter case, the *iab* regulatory domains and the *Abd-B* gene would not be brought in close proximity when the two inversion chromosomes pair. Instead, these sequences would be separated by the length of the inversion. Additionally, the pairing of the BX-C regions across the inversion breakpoint would be uninterrupted.

We wondered whether unpaired DNA segments could by themselves facilitate long-distance interactions. To investigate this possibility, we combined *In(3R)Fab-7iab-7*^{R5} with another inversion, *In(3R)Fab-7iab-7*^{R7}, which has its breakpoints in 87D1,2 and 89E3,4 (Gyurkovics *et al.* 1990). Because these two inversions have similar but not precisely identical breakpoints, they will be paired over much of their length without forming the loop that is typically generated when an inversion chromosome pairs with a structurally wild-type homologue. However, at the proximal inversion breakpoint of one chromosome, there will be a locally unpaired DNA segment that is homologous to an unpaired DNA segment at the distal breakpoint of the other chromosome (see Figure 9). Thus, at the distal end of *R5* and the proximal end of *R7*, there will a large unpaired DNA segment containing the 87C interval. Similarly, at the proximal end of *R5* and at the distal end of *R7*, there will be a short ~10-kb unpaired DNA segment from BX-C that is located just downstream of the *Abd-B* transcription unit (between map position +141.5–143 and +152) (see Figure 9). If these unpaired DNA segments are able to facilitate long-distance regulatory interactions, *Abd-B* expression should not be restricted to PS13 and PS14 but instead should also be observed in PS11 and PS12. The results we obtained are consistent with this model. As illustrated in Figure 8g, *Abd-B* expression can be detected in PS11 and PS12 in the CNS of the *R5* / *R7* embryos, but not in embryos homozygous for either the *R5* or the *R7* inversion. The expression pattern in the *R5* / *R7 trans*-heterozygous embryos is somewhat irregular, suggesting that *trans*-regulation occurs in some cells but not in others. (We suspect that the *Abd-B* expression in these embryos may be due to the activity of *iab-7* on the *R5* chromosome alone. This possibility is suggested by the observation that there is no detectable ABD-B protein in PS10–PS12 of *In(3R)Fab-7iab-7*^{R7} / *Df(3R)P9* or *In(3R)Fab-7iab-7*^{R7} / *Df(3R)R59* embryos. This observation would argue that *iab-7* on the *R7* chromosome is inactivated by the breakpoint in the BX-C.)

These findings indicate that unpaired chromosomal DNA segments can facilitate long-distance interactions. One plausible hypothesis is that the two large unpaired 87C chromosomal DNA segments may be able to interact with each other (a form of "homologous pairing"), and it is this interaction that brings the inverted BX-C regulatory regions in close proximity to the *Abd-B* gene (see Figure 9). This hypothesis is supported by our finding that in ~50% of salivary gland polytene chromosomes, the *In(3R)Fab-7iab-7*^{R5} and *In(3R)Fab-7iab-7*^{R7} homologues form a "ring" or "circle," apparently held together by pairing between the two 87C loops (not shown).

A prediction of this hypothesis is that long-distance regulatory interactions should only be observed when there are unpaired 87C chromosomal DNA segments at both the proximal and the distal breakpoints. To test this prediction, we recombined *R5* and *R7* to generate a chromosome, *R5*^{L}*R7*^{R}, which contains the proximal breakpoint of *R5* and the distal one of *R7*. In the *R5*^{L}*R7*^{R} chromosome, the 87C region is deleted, and a ~10-kb BX-C segment 3′ to the *Abd-B* gene is present at both breakpoints in an inverted orientation. When the

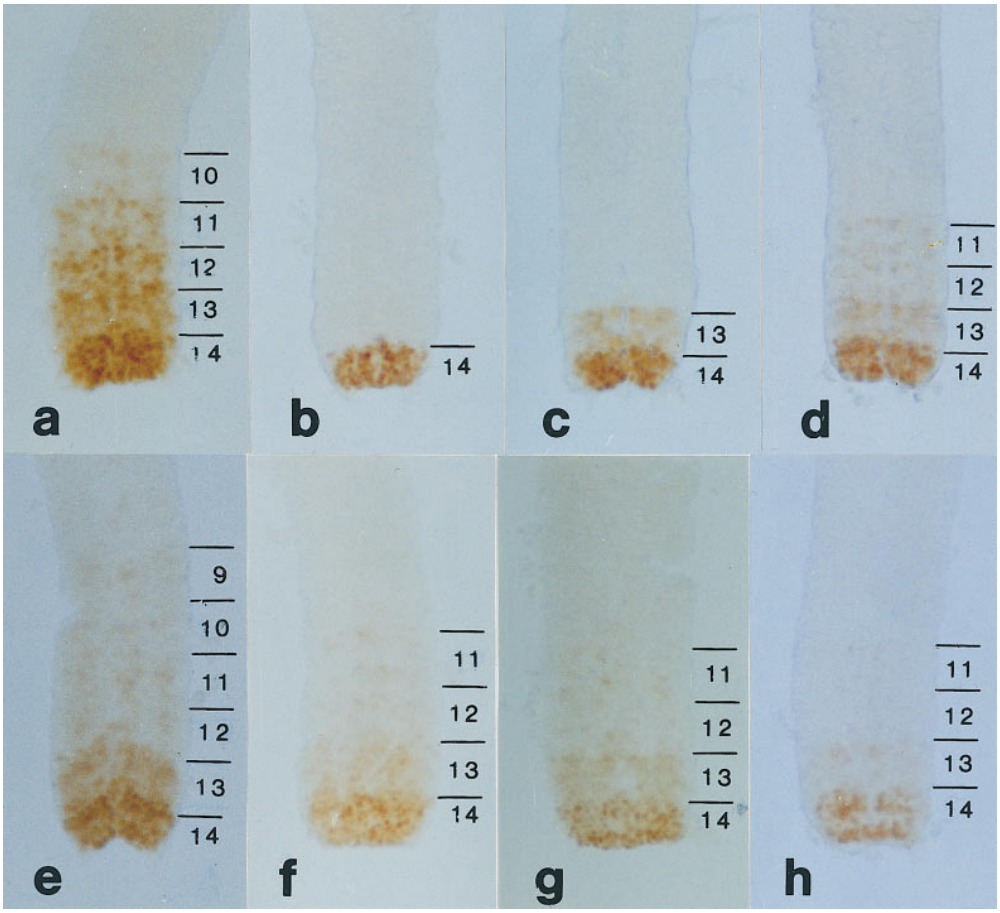

Figure 8.—Immuno-histochemical staining of ABD-B proteins in the ventral trunk region of the CNS dissected from Drosophila embryos. (a) wild type (+/+). Staining gradually increases from PS10 to PS14. (b) *Fab-7Abd-B^{D14}/Fab-7Abd-B^{D14}*. Staining is missing from PS10 to PS13. The intensity of staining in PS14 is similar to that in wild type. (c) *Df(3R)R59/Df(3R)R59*. Staining is missing from PS10 to PS12. (d) *Fab-7Abd-B^{D14}/Df(3R)R59*. A very low level of protein is detected in PS10 and stronger staining of equal intensity can be seen in PS11 and PS12, indicating *trans*-regulation of the wild-type *Abd-B*. (e) *Mcp^{B116}iab-7StAbD-B^{D16}/Df(3R)R59*. Weak staining of equal intensity is detected in both PS9 and PS10, and stronger staining is apparent in both PS11 and PS12. (f) *In(3R)Fab-7iab7^{R5}/Df(3R)P9*. Weak staining is observed in PS11 and PS12, indicating that, although the paracentric inversion *In(3R)Fab-7iab7^{R5}* separates the *cis*-regulatory region *iab-7* from the *Abd-B* promoter, they can interact via formation of a loop in hemizygous condition. (g) *In(3R)Fab-7iab-7^{R5}/In(3R)Fab-7iab-7^{R7}*. Weak staining with irregular pattern is detected in both PS11 and PS12, indicating *trans*-regulation in some cells. (h) *In(3LR)iab-7^{164}/Fab-7Abd-B^{D16}*. The doubly rearranged chromosome *In(3LR)iab-7^{164}* efficiently disrupts pairing of homologous chromosomes, and staining in PS11 and PS12 is significantly decreased as compared to d. Additionally, the pattern of the remaining staining is irregular, indicating that *trans*-regulation occurs only in some of the cells.

R5^{L}R7^{R} chromosome is combined with the *R5* chromosome, there is only a single, unpaired 87C DNA segment located at the distal *R5* breakpoint (see Figure 9). As anticipated, *R5^{L}R7^{R}/R5 trans*-heterozygotes differ from *R5/R7 trans*-heterozygotes in that they do not form a "circle" in salivary gland polytene chromosomes (data not shown). However, even though this "circle" cannot be detected, the *Abd-B* expression pattern in PS11 and PS12 of *R5^{L}R7^{R}/R5* animals is indistinguishable from that observed in *R5/R7* animals (data not shown). This is also true for the combination of *R5^{L}R7^{R}/R7*, which has only a single, unpaired 87C DNA segment, in this case at the proximal *R7* breakpoint.

Hence, contrary to our expectations, the regulation of *Abd-B* by a distant *iab-7* regulatory domain does not appear to require interactions between unpaired 87C chromosomal DNA segments at the proximal breakpoint of one homologue and the distal breakpoint of the other. On the other hand, there is still a requirement for an unpaired DNA sequence because no long-distance regulation is observed when *R5^{L}R7^{R}* chromosome is homozygous (see Figure 9). We suspect that this unpaired sequence is most likely the short 10-kb BX-C DNA segment immediately downstream of the *Abd-B* gene. As mentioned above, in the *R5/R7* combination there is an unpaired copy of this DNA sequence at the proximal breakpoint of one homologue that could potentially interact with a copy of the same sequence at the distal breakpoint of the other homologue (see Figure 9). In the *R5^{L}R7^{R}* chromosome, there are two

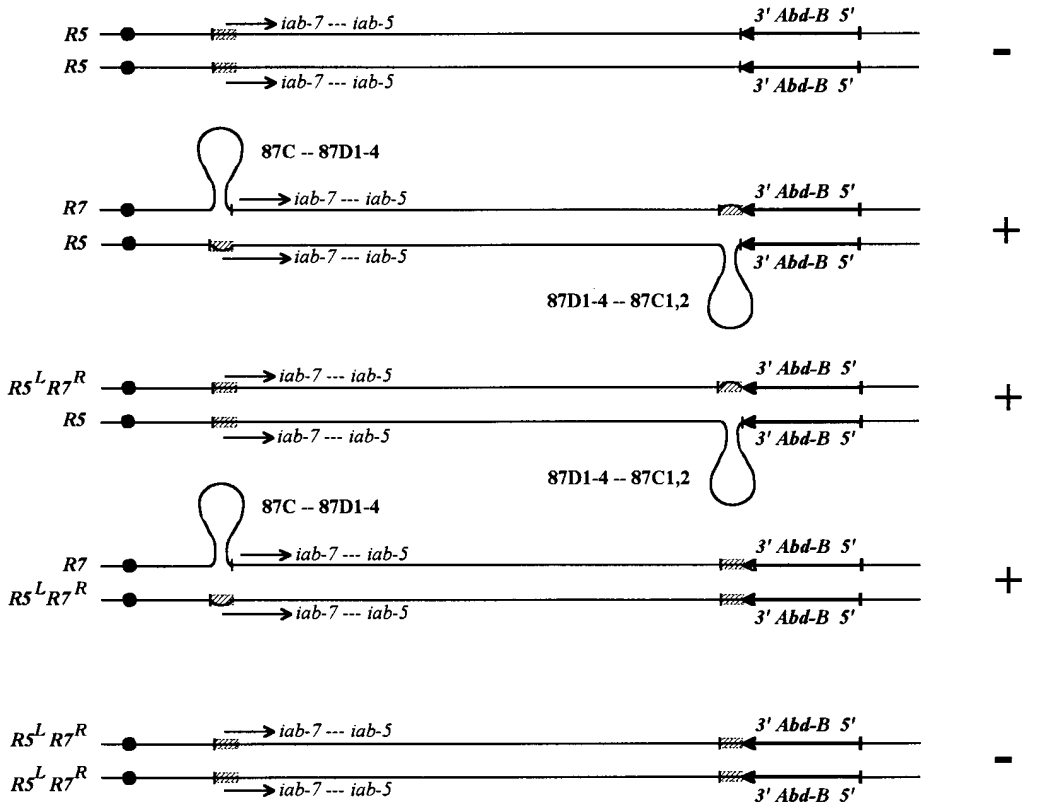

Figure 9.—The structure of chromosome pairs carrying different combinations of the inversions *In(3R) Fab-7iab-7*^{R5}, *In(3R)Fab-7iab-7*^{R7} and *In(3R)Fab-7R5*^{L}*R7*^{R}. Some of these combinations exhibit *trans*-regulation (marked by +) in the embryonic CNS, and others do not (−). Continuous lines represent chromosomes, short vertical lines the breakpoints of the inversions, and bold arrows the *Abd-B* class A transcription unit. Thin arrows show the position and the orientation of *cis*-regulatory regions of *Abd-B*. Regions within dashed areas are supposed to play a crucial role in transvection. Note that in *trans*-regulating combinations (+) these regions are unpaired at least at one of the breakpoints.

copies of this sequence, one at each breakpoint. Consequently, when *R5*^{L}*R7*^{R} is combined with either *R5* or *R7* there are three copies of the sequence (see Figure 9). One of these is always unpaired and could potentially interact with the paired copies at the opposite breakpoint. (In this hypothesis, the presence of a single, unpaired 87C region at one end of the otherwise paired combinations of *R5*^{L}*R7*^{R}/*R7* or *R5*^{L}*R7*^{R}/*R5* may only facilitate the looping out of BX-C material and would not play a major role in long-distance regulation.)

DISCUSSION

Classical transvection in the *Abd-B* domain: Recently, an unusual type of transvection has been demonstrated at the *Abd-B* domain in the adult stage (Hendrickson and Sakonju 1995; Hopmann *et al.* 1995). This weak *trans*-interaction between the regulatory regions of the *Abd-B* domain and the *Abd-B* gene itself is nearly insensitive to rearrangements that disturb homologous pairing. However, this is not the only type of *trans*-regulation in this region of BX-C. In the studies reported here, we have detected a more regular type of transvection in the adult cuticle by taking advantage of the dominant gain-of-function mutation *Fab-7*.

Transvection is usually seen as a partial complementation between a mutation in the *cis*-regulator and another one in the coding region of the same gene in *trans* (Bender *et al.* 1983; St. Johnston *et al.* 1990; Leiserson *et al.* 1994). However, in the adult animal we have been unable to see any degree of complementation in such mutant combination, that is, between the *cis*-regulatory region mutant *iab-7*^{Sz} and the *Abd-B* point mutation *Abd-B*^{D16}. We attribute this apparent lack of complementation to the strong haplo-insufficiency of *Abd-B*. Thus, although *trans*-regulation may increase the level of ABD-B protein in PS12, this effect is insufficient to produce a detectable change in the phenotype of A7. For this reason we have assayed the consequence of *trans*-regulation indirectly, by the ability of *Abd-B* point mutations in *trans* to suppress the *Fab-7* gain-of-function phenotype. However, it must be emphasized that we have used *Fab-7* in these experiments only as a convenient tool for detecting *iab-7 trans*-regulation in A6; precisely the same interaction can be shown in A7 using the combination of a wild type and the double mutant chromosome *iab-7*^{Sz}*Abd-B*^{D16}. The transvection detected in this indirect way in the *Abd-B* domain closely resembles that found in the *Ubx* domain (Lewis 1954, 1955; Micol and García-Bellido 1988), and it is disrupted by chromosomal rearrangements that also affect the *Cbx* phenotype of *Cbx*^{1}*Ubx*^{1}/++ (Lewis 1985; Mathog 1990). Additionally, both transvecting systems respond to deletions or insertions near the respective promoter regions (Martinez-Laborda *et al.* 1992). In fact, the only significant difference between transvection at the two loci is that, although in most cases of *Ubx* transvection the product of the *zeste* gene appears to play an important role (Kaufman *et al.* 1973; Micol and García-Bellido 1988), it does not seem to affect trans-

vection in the *Abd-B* locus (see also Hopmann *et al.* 1995).

What is the relationship between this classical *Abd-B* transvection and the pairing-insensitive, long-range *trans*-interaction (ITR) described for *Abd-B* by Hendrickson and Sakonju (1995) and Hopmann *et al.* (1995)? We suspect that phenotypes used to detect *trans*-interactions in each case require quite different levels of *Abd-B* expression. ITR is detected as the deposition of black pigment in tergites A6 and A7 ("A5/A6 identity"). This change in pigmentation is likely to require only limited amounts of ABD-B protein, which could be produced by a relatively weak *trans*-interaction. In contrast, we assay *trans*-regulation by determining how closely a segment approaches the appearance of the wild-type A7, a segmental phenotype likely to require a considerably higher amount of ABD-B, and consequently, a *trans*-interaction that is not only stronger but much more dependent on proximity. In addition to this quantitative difference, the two transvecting systems also appear to differ qualitatively. In general, ITR is relatively insensitive to chromosomal rearrangements. In those cases in which the rearrangements do cause a significant impairment of *trans*-interactions, the resulting phenotype is variegated (and presumably clonal) (Hopmann *et al.* 1995). In contrast, suppression of the *Fab-7* phenotype by *Abd-B* point mutations responds to interference with pairing in a different way: rearrangements that weakly interfere with pairing of the BX-C region cause a moderate reduction in the size of tergite A6, whereas rearrangements that cause a severe disruption of pairing result in a strong reduction of the A6 tergite but without any apparent "variegation" (Table 1 and not shown). Therefore, it seems likely that the classical transvection reported here involves a mechanism different from those that mediate the long-range *trans*-interactions described by Hendrickson and Sakonju (1995) and Hopmann *et al.* (1995).

***Cis*-regulators appear to be tethered to the *Abd-B* promoter:** We have found that the *Abd-B* genes in *cis* and *trans* compete for interaction with a single *iab-7* regulatory domain. This competition depends upon an extensive region at the 5′ end of the *Abd-B* class A transcription unit. Deletion of this region greatly enhances activation of the *Abd-B* gene in *trans* by the *iab-7* regulatory domain on the deletion-bearing chromosome. The strength of this activation seems to be correlated with the size of the deletion. For example, although the $Abd\text{-}B^{D14}$ mutation, which deletes just the 5′ start site for the class A transcription unit, enhances *trans*-interactions of the *iab-7* in *cis*, its effect is rather modest compared to deficiencies that remove more extensive regions upstream of the *Abd-B* class A transcription unit (see Figure 6). Additionally, unlike these larger deletions, $Abd\text{-}B^{D14}$ suppresses the *Fab-7* phenotype in A6 to some extent in *trans* (compare Figure 4, b and c). These findings indicate that $Abd\text{-}B^{D14}$ is still capable of competing with the wild-type *Abd-B* gene and suggest that important determinants of competition are located farther upstream. Although we cannot determine the maximum extent of the region that mediates competition from the present data, our deletion analysis suggests that at least 7.6 kb of DNA may be involved.

Why do these 5′ deletions enhance *trans*-activation by the *iab-7* regulatory domain (*cis* to the deletions)? One possibility is that they disrupt pairing between the *Abd-B* genes on each homologue, allowing the 5′ region upstream of the *Abd-B* promoter on the wild-type homologue to loop out. This looping out would then facilitate *trans*-regulation. Although we cannot exclude this model, we consider it unlikely. In particular, one expectation of the pairing disruption model is that *trans*-regulation should be dependent upon the overall size of loop and the relative position of the promoter within the loop. By this hypothesis, $Abd\text{-}B^{RD18}$ should be more efficient in *trans*-regulation than $Abd\text{-}B^{PSz\text{-}1}$. First, the $Abd\text{-}B^{RD18}$ deletion is larger than $Abd\text{-}B^{PSz\text{-}1}$. Second, the promoter is located in the middle of the loop in $Abd\text{-}B^{RD18}$, and it is at one end of the loop in $Abd\text{-}B^{PSz\text{-}1}$. However, this prediction is not correct: $iab\text{-}7^{Sz}$ is complemented by $Abd\text{-}B^{PSz\text{-}1}$ at least as well (if not better) as $Abd\text{-}B^{RD18}$.

An alternative hypothesis is that the 5′ flanking region of the *Abd-B* promoter contains "tethering elements" that normally mediate *cis*-interactions between the *Abd-B* gene and the *iab-7* regulatory domain or the other *iab* regulatory domains. When these upstream tethering elements are deleted, *cis*-interactions are weakened or eliminated and *trans*-interactions with tethering elements upstream of the *Abd-B* gene on the other homologue become stronger. A similar tethering mechanism has been proposed to anchor the upstream enhancers of the *white* gene to the *white* basal promoter (Qian *et al.* l992). In *white*, the tethering region is thought to be no more than ~95 bp in length. By contrast, the tethering region for the *Abd-B* gene appears to be larger than 7.6 kb. Moreover, because the strength of *trans*-regulation increases roughly in proportion to the size of the upstream deletion, it would appear that there may be multiple "tethering elements" distributed through this 5′ flanking region.

Why does the *Abd-B* gene have such a large tethering region? We suspect that several factors may be important. First, the regulatory domains that generate the parasegment specific patterns of *Abd-B* expression are located at a considerable distance from the promoter. Second, the regulation of *Abd-B* is exceedingly complex, and in each parasegment elements from a different regulatory domain must interact with and control the activity of the *Abd-B* promoter. Third, because of the arrangement and parasegment specificity of the *iab* regulatory domains, at least three of the *iab* regulatory domains (*iab-5*, *iab-6* and *iab-7*) must contact the promoter across an intervening DNA segment that contains boundary elements (like *Fab-7*) and one or more *Pc-G-*

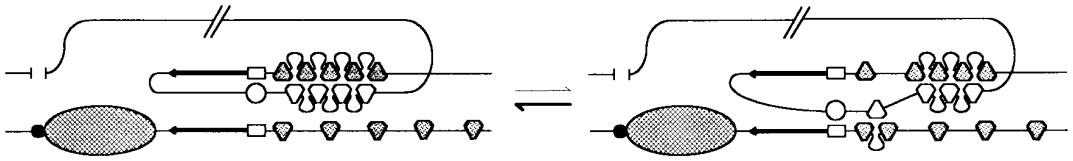

Figure 10.—Schematic model of *trans*-regulation in the A6 of *Fab-7*/+ heterozygotes. Continuous line represents DNA; open rectangle, promoter; open circle, enhancer; shaded oval, inactive form of *iab-7*; black dot, the *Fab-7* boundary; vertical line, the end points of the $Fab\text{-}7^{1}$ deletion; bold arrow, the *Abd-B* class A transcription unit; filled and open triangles, protein complexes bound to DNA near the promoter and the enhancer sequences, respectively. An anchoring complex is supposed to be formed between the enhancer and the promoter-proximal region through cooperative interactions among bound protein complexes. We assume a dynamic equilibrium between the more stable *cis*-complex (formed earlier in time) and the less stable *trans*-complex. This is supported by the nonclonal appearance of phenotypes resulting from *trans*-regulation.

silenced regulatory domains. Moreover, these boundary elements are of sufficient strength to prevent the regulatory domains from contacting heterologous promoters (Galloni *et al.* l993).

A fourth function of this extensive tethering region may be to ensure *cis*-preference/*cis*-autonomy (Martinez-Laborda *et al.* 1992). Our data show that interactions between an *iab-7* domain and an *Abd-B* gene on the same homologue are, under normal circumstances, strongly preferred to *trans*-interaction. However, as has been pointed out by Martinez-Laborda and his coworkers (1992), it is difficult to see a priori how the distant *iab* regulatory domains are able to distinguish a promoter in *cis* from the same promoter in *trans*, particularly when the homologues are the tightly paired. One possibility is that the tethering element array in the 5′ flanking region of the *Abd-B* gene helps ensure *cis*-preference by making multiple contacts with corresponding elements in the regulatory domains. Once these interactions are established in *cis*, it would be difficult to rebuild the same set of contacts with the promoter on the other homologue. Even under conditions favorable for *trans*-regulation, such as when there is only a single active *iab-7* regulatory domain (see Figure 10), our results would suggest that there may only be a partial conversion of the *cis*-complex into a *trans*-complex. A similar mechanism could account for the apparent *cis*-autonomy of *Ubx* (Martinez-Laborda *et al.* 1992). However, although the presence of an extensive tethering region in the *Abd-B* 5′ flanking region may help explain how *cis*-preference is maintained, it does not explain why the *cis*-complex is established in the first place. It is conceivable that the *cis*-complex is "inherited" from the early stages of embryogenesis, when the BX-C does not appear to be paired (see under next heading). Alternatively, *cis*-interactions may be established (or reestablished) at a point in the cell cycle, *e.g.*, early G1, that is prior to the time when the homologues become tightly paired.

The proposed tethering mechanism may also be utilized in selecting the appropriate *cis*-regulators by the genes in BX-C. For example, *iab-6* does not seem to contribute significantly to the expression of *Abd-B* in PS12 when the *iab-7* regulatory domain is intact (Celniker *et al.* 1990). However, when *iab-7* is deleted, as in $iab\text{-}7^{Sz}$, the *iab-6* domain takes over, and it controls the expression of *Abd-B* in both PS11 and PS12 (Galloni *et al.* 1993). On the other hand, the deletion of *iab-7* has no apparent effect on the regulation of *Abd-B* in PS13 by *iab-8*. These findings suggest that there is a sequential displacement of one *iab* regulatory domain by the next in progressively more posterior parasegments. That is, in PS11 the *Abd-B* promoter is complexed with *iab-6*. In PS12, *iab-7* displaces *iab-6* from the promoter complex, while in PS13, *iab-7* is displaced from the promoter complex by *iab-8*. This sequential switching could be explained by a difference in the affinity of the *iab* regulatory domains for the *Abd-B* tethering region. For example, *iab-7* may be able to interact with a larger number of upstream tethering elements and thus form a more stable complex with the *Abd-B* promoter than *iab-6*. This model would explain why *trans*-regulation of *Abd-B* by *iab-7* is not detectably influenced by the presence or absence of *iab-6* (and *iab-5*) in *trans*. It would also account for the ability of an ectopically activated *iab-7* regulatory domain in a *Fab-7* chromosome to regulate not only the *cis* but also the *trans Abd-B* gene in PS11. In the latter case, it must be able to overcome *cis*-preference, displacing the *iab-6* domain on the *trans* homologue from the *Abd-B* promoter.

Our model implies that interacting partners of the tethering elements at the 5′ end of the gene should be present in each of the *cis*-regulatory regions. Although this may be yet another reason for the large size of regulatory regions in the BX-C, we do not have direct evidence for their existence. Likewise, we do not know the protein components/*trans*-acting genes involved in the formation of the proposed complex. Based on the expected loss-of-function phenotype of these genes, they are likely to belong to the *trithorax* group. Perhaps with the help of the highly sensitive $Fab\text{-}7/Abd\text{-}B^{point}$ phenotype it will be possible to identify those genes that are specifically involved in anchoring *cis*-regulators to the *Abd-B* gene.

Transvection is different in different tissues: We were unable to detect *trans*-interactions between the *iab-7* (or

iab-5-iab-6) regulatory region and the *Abd-B* gene in either the ectoderm or the developing mesoderm during early to mid-embryogenesis. Although we might not be able to detect small amounts of ABD-B protein in the more anterior parasegments where *Abd-B* expression is low even in wild type, the complete lack of staining in PS12 of extended germ-band embryos suggests that there is essentially no *trans*-regulation at this stage of development. A plausible explanation is that BX-C is not sufficiently paired for *trans*-regulation during early embryogenesis. A similar conclusion has been reached by Martinez-Laborda and coworkers (1992). These authors found that the early *ppx* function of *Ubx*, which is required before 10 hr of development, is not restored by interallelic complementation, even under conditions most favorable for *trans*-regulation (Martinez-Laborda *et al.* 1992). In this context it is interesting to note that Hiraoka and coworkers (1993) found that pairing of the chromosome segment harboring the histone genes is already evident after 2–3 hr of embryogenesis. The apparent lack of transvection in BX-C, even at the extended germ-band stage (at about 7 hr of development), suggests that tight pairing of BX-C might occur even later than in the histone gene cluster.

We did, however, find evidence for *trans*-regulation at slightly later stages of development in the central nerve cord (see also Hendrickson and Sakonju 1995). Moreover, it appears that the *trans*-interactions observed in the embryonic CNS are considerably more tenacious than they are in the adult abdomen (with the caveat that different assays are used to measure *trans*-regulation in each case). First, *trans*-regulation in the embryonic CNS can even be detected when the competing *Abd-B* gene in *cis* has a wild-type upstream tethering region like that of the point mutant $Abd\text{-}B^{D16}$. Second, chromosomal rearrangements that would strongly interfere with *trans*-regulation in the adult have little or no effect in the embryonic CNS.

In fact, in the embryonic nervous system *iab* regulatory domains are able to interact with the *Abd-B* gene over quite large distances. This long-distance interaction is best illustrated by the expression of ABD-B protein in PS11 and PS12 in three inversion combinations, *In(3R)R5/In(3R)R7*, $In(3R)R5/In(3R)R5^{L}R7^{R}$ and $In(3R)R7/In(3R)R5^{L}R7^{R}$. In these inversion combinations, the *iab* regulatory regions on each homologue are separated from the corresponding *Abd-B* genes by a chromosomal DNA segment extending from 87C-D to 89E. These *trans*-regulating combinations of inversions identify a relatively short sequence (~10 kb) just downstream of the *Abd-B* gene which, when locally unpaired, appears to be able to mediate long-distance interaction (Figure 9). Interestingly, these sequences roughly correspond to the region (termed "transvection mediating region" by Hopmann *et al.* 1995) that was implicated in the weak but extremely tenacious *trans*-interaction of *Abd-B* in the adult (Hopmann *et al.* 1995; Hendrickson and Sakonju 1995). However, in contrast to their findings, we have found that this region can support long-distance interaction in the embryonic CNS even if its unpaired copy is contiguous to the regulated gene *Abd-B* (as in $In(3R)R5/In(3R)R5^{L}R7^{R}$; see Figure 9), and not to the *cis*-regulatory regions.

What is responsible for mediating the long-distance interactions in the CNS? One plausible hypothesis is that these interactions are facilitated by Polycomb Response Elements (PREs). PREs are short DNA segments that can initiate the assembly of silencing complexes composed of the *Polycomb*-group (*Pc-G*) proteins (Simon *et al.* 1993). The silencing activity of PREs in *mini-white* transgene assays typically depends upon cooperative interactions between two copies of the PRE (Kassis *et al.* 1991; Hagstrom *et al.* 1997). These cooperative interactions are normally observed when the PRE:*mini-white* transgene is homozygous; however, cooperative interactions can also be detected when the transgenes are inserted into the genome at sites distant from each other (Kassis 1994; Hagstrom *et al.* 1997; M. Müller, personal communication). These observations raise the possibility that PREs may play some role in mediating the long-distance transvection that we have observed. In this respect, it is interesting to note that a PRE has been found (M. Müller, K. Hagstrom and P. Schedl, unpublished results) in the region we have identified as essential for long-distance interactions in the embryonic CNS. Because this PRE appears to be in the *iab-8* regulatory domain (our unpublished data), its silencing activity need not interfere with the activation of *Abd-B* anterior to PS13 by *iab-7* and other, more proximal *cis*-regulatory regions. The looping out of the DNA segment containing this PRE could enhance its ability to pair with its distant partner, thus bringing the regulatory regions in proximity to the regulated gene. Alternatively, looping out may facilitate the integration of this PRE into a *Polycomb* compartment (Messmer *et al.* 1992) in the nucleus, which would provide the required proximity for the regulatory region(s) and the regulated gene.

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