Transvection in the *Ultrabithorax* Domain of the Bithorax Complex of *Drosophila melanogaster*

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ABSTRACT

The phenotypes of several heterozygous combinations of mutations which map within the *Ultrabithorax* gene of *Drosophila melanogaster* are modulated by the extent of somatic homologous chromosome pairing, an effect known as transvection. One can discriminate between otherwise phenotypically similar mutations via their transvection behavior. This suggested the existence of previously undetected intragenic functional units. A collection of mutations has been classified into "transvection groups" (in analogy to complementation groups) on the basis of transvection tests with *bithorax^{34e}*, *postbithorax²*, and *Contrabithorax¹ Ultrabithorax¹*. The conditions necessary for each transvection effect were determined from these transvection groups. The *bithorax^{34e}* mutation only transvects with *Ultrabithorax* mutations with a contiguous *Ultrabithorax* transcriptional unit. In contrast, *postbithorax²* transvection requires the distal part of the *bithoraxoid* region. As expected, *Ultrabithorax* mutations do not transvect with *Contrabithorax¹ Ultrabithorax¹*. However, it appears that this cross activation is not mediated solely through one of the known regulatory regions as mutations in these regions do not consistently block the response.

T HE *Ultrabithorax* domain of the bithorax complex of *Drosophila melanogaster* contains a large and complicated gene primarily required for the correct development of parasegments 5 and 6 (LEWIS 1963; SANCHEZ-HERRERO *et al.* 1985; MARTINEZ-ARIAS and LAWRENCE 1985). In an animal homozygous for an *Ultrabithorax* (*Ubx*) mutation, both parasegments are transformed into parasegment 4. There are several other types of mutant syndromes associated with lesions in the *Ultrabithorax* domain, each affecting only part of the *Ubx* type transformation (LEWIS 1955, 1981). The phenotypes of these mutations were first characterized in the adult cuticle. The *anterobithorax* and *bithorax* (*abx* and *bx*) mutations primarily transform the anterior third thoracic segment (T3a). *bithoraxoid* (*bxd*) lesions alter both the posterior third thoracic segment and the anterior first abdominal segments (T3p, A1a). *postbithorax* (*pbx*) mutations primarily affect the development of T3p. *Contrabithorax* (*Cbx*) mutations also map within the *Ultrabithorax* domain. *Cbx* mutations partially transform the second thoracic segment, producing patterns typical of the third thoracic segment.

Ultrabithorax domain mutations have been mapped both genetically and molecularly (Figure 1) (LEWIS 1955, 1978; BENDER *et al.* 1983). *Ubx* mutations lie within a 75 kb protein coding transcription unit. Also located within this region are the *Cbx¹* insertion into

the second intron, and all of the *abx* and *bx* lesions, which are in the third and final intron (BENDER *et al.* 1983; HOGNESS *et al.* 1985; PEIFER and BENDER 1986). A second transcribed region, approximately 30 kb long and immediately upstream from the *Ubx* transcription unit, contains the sites of all *bxd* and *pbx* lesions. The relation between *Ubx* mutations and the phenotypes that result from them is clear; in most cases the long transcription unit is broken or particular exons are affected, thus eliminating one or more of the *Ubx* family of proteins (WEINZIERL *et al.* 1987; PEIFER, KARCH and BENDER 1988). How the non-*Ubx* lesions cause their associated phenotypes is not yet understood. Each of these syndromes affects a different part of the animal and alters the spatial and quantitative expression of *Ubx* protein, but does not disrupt the protein coding regions of the gene (LEWIS 1963; BEACHY *et al.* 1985; WHITE and WILCOX 1985; WHITE and AKAM 1985; BENDER *et al.* 1983). Furthermore, mutations of different types can complement each other (LEWIS 1963). Clearly these non-*Ubx* mutations alter the regulation of the *Ultrabithorax* domain and many or all of them are thought to be lesions of normal regulatory regions of that gene (BEACHY *et al.* 1985; HOGNESS *et al.* 1985; PEIFER and BENDER 1986).

In *Drosophila*, interphase somatic chromosomes are normally synapsed (METZ 1916), but certain chromosome rearrangements disrupt this pairing. LEWIS (1954, 1955, 1982) discovered that the degree of
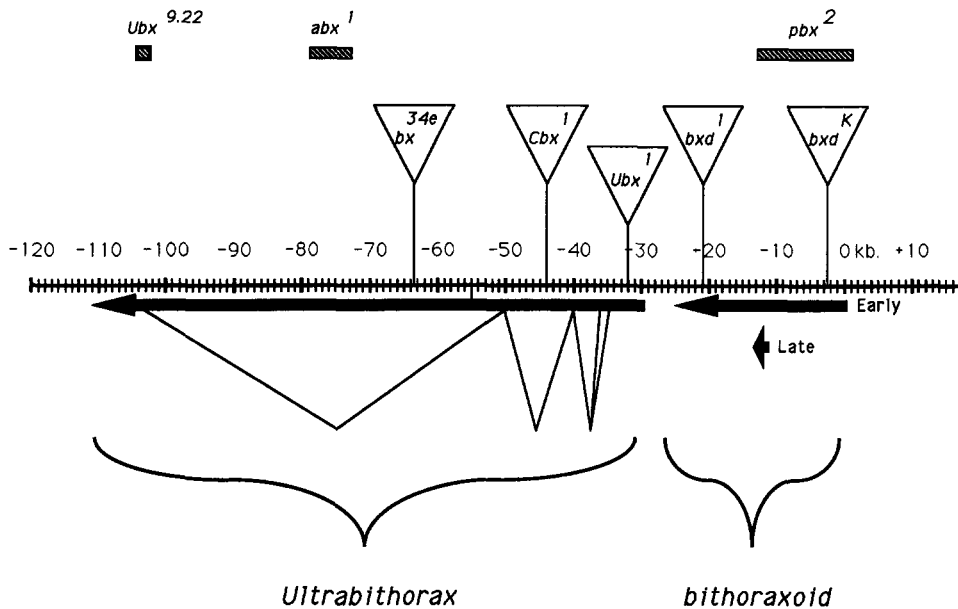
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FIGURE 1.—A schematic overview of the organization of the *Ultrabithorax* domain illustrating its two transcription units and the locations of several mutations. Hatched rectangles represent DNA deletions, inverted triangles are insertion elements, and large arrows depict transcription units. Donor and acceptor sites for *Ubx* mRNAs are also shown. The complex splicing pattern for *bxd* is not shown. Data is from BENDER *et al.* (1983) and HOGNESS *et al.* (1985).

complementation of certain *Ultrabithorax* domain genotypes is modulated by the extent of this pairing. He named pairing dependent complementation "transvection" and the transvection suppressing chromosome rearrangements "TSRs." Subsequently, the term transvection was generalized to include any situation where the paired phenotype differs from the unpaired for an otherwise similar genotype (GELBART and WU 1982).

Many of the regulatory mutations of the *Ultrabithorax* domain participate in transvecting genotypes. Transvection may result when regulatory regions, which normally act over long distances in *cis*, occasionally "miss" and cross-regulate the closely paired homologous allele (LEWIS 1954). It is common to classify mutations into complementation groups. In this paper this approach is extended by using transvection tests, rather than complementation tests, to define the groups. Transvection is assayed for bx^{34e}, pbx^2, and Cbx^1 Ubx^1 *vs.* a large number of other *Ultrabithorax* domain mutations. Since most of the mutations utilized have been mapped at the molecular level, such groups indicate which parts of the *Ultrabithorax* domain are required for the different transvection effects tested.

MATERIALS AND METHODS

Crosses were performed at 25° in shell vials. J. CASANOVA who also isolated bxd^{MX1}, constructed the chromosome $Ubx^{9.22}$ *C1* by recombination. I. VERNOS and E. MARTIN characterized bxd^{MX1}. All other stocks are described in LINDSLEY and GRELL 1968; LEWIS 1978; KERRIDGE and MORATA 1982; BENDER *et al.* 1983; AKAM *et al.* 1985; BENDER *et al.* 1985; KARCH *et al.* 1985; SANCHEZ-HERRERO *et al.* 1985; LINDSLEY and ZIMM 1987; WEINZIERL *et al.* 1987; or CASANOVA, SANCHEZ-HERRERO and MORATA 1988. Chromosome rearrangements and new *Ubx* alleles were induced with X-rays (100 kV, 15 mA, 2-mm aluminum filter), typi-

cally with a dose of 3000 R. Cytology was determined from conventional lactic acid/orcein polytene chromosome squashes (LEFEVRE 1976).

The phenotypes in tests of bx^{34e}, pbx^2 and Cbx^1 Ubx^1 transvection were assayed directly under the dissecting microscope. Numerical values were assigned for each phenotype.

For the *pbx* transformation of posterior haltere to posterior wing: 0 = wild type; 1 = slight enlargement of posterior haltere; 2 = as 1, but including posterior marginal wing bristles; 3 = as 2, but including some wing blade; 4 = sufficient wing blade so that the appendage is longer than it is wide; 5 = complete transformation.

For the bx^{34e} transformation producing notum in T3a: 0 = no transformation; 1 = cuticle visible, but no bristles; 2 = <5 bristles per side; 3 = <25 bristles per side; 4 = <50 bristles per side; 5 = maximal transformation (about 60 bristles per side).

For the transformation of wing to haltere associated with Cbx^1 Ubx^1 transvection (which is somewhat variable even within a given cross): 0 = wild type wing; 1 = intermediate phenotype (differs from 0 in having at least a reduction of the alula, may also have spread and/or slightly warped wings, but never so extreme as Cbx^1 $Ubx^1/+$); 2 = as Cbx^1 $Ubx^1/+$ (extreme alula reduction, wings strongly warped).

For the purpose of these experiments, designed to map regions involved in transvection, these measurements were thought to be sufficiently precise. It is possible that a very weak complementation of bx^{34e} or pbx^2 would have been scored as a 5 (rather than 4).

The definition of the transvection effect used here is general and can be applied to all transvection effects so far described. It is easiest to present this definition symbolically. Let **a** and **b** represent two combinations of alleles for a chromosome and let (**a/b**) be the heterozygous genotype. A genotype can be in one of two states, written //(**a/b**) and Λ(**a/b**), and indicating when the two chromosomes are completely paired and when they are completely unpaired. A particular phenotype of a given genotype is written *P*(**a/b**). *T*(**a,b**) is the result of the transvection test for **a** and **b**.

T(**a,b**) is positive when *P*(//(**a/b**)) ≠ *P*(Λ(**a/b**)).

T(**a,b**) is negative when all of the following are true:

$$P(//(\mathbf{a/b})) = P(\Lambda(\mathbf{a/b}));$$

//(a/b) and ∧(a/b) can both be demonstrated;
$P(a/b) \neq$ one or more of $P(a/+)$, $P(+/b)$, $P(\wedge(a/+))$, or $P(\wedge(+/b))$.

Otherwise, **T(a,b)** is undefined.

Applying the preceding tests in the real world requires some approximations. In practice $P(//(a/b))$ is replaced by $P(a/b)$, while $P(\wedge(a/b))$ is replaced by $P(R(a)/b)$, where $R( )$ is the symbol for a TSR (LEWIS 1954). It must be emphasized that this substitution greatly complicates the interpretations of observed phenotypes since //(a/b) and ∧(a/b) represent completely nonoverlapping states whereas (a/b) and (R(a)/b) correspond only approximately to these basis states. In particular, if **b** contains a breakpoint mutation, then the associated rearrangement will generally have some TSR properties. In this case, (a/b) can be anything from wholly //(a/b) to wholly ∧(a/b), depending on the strength of the TSR. Nevertheless, the transvection test will still be determinate as long as (a/b) contains a detectable level of the state //(a/b).

The following genetic test can often be used to infer the existence of //(a/b): if there exists a point or pseudopoint mutation **b'** such that $T(b/b')$ is positive, and **a** is also a point or pseudopoint mutation, then //(a/b) exists and is present to some extent in (a/b). This test does not say that all of the DNA in (a/b) is paired, only that it is paired somewhere within the gene. In particular, if **b** has a breakpoint which lies between **b'** and **a**, then (a/b) may not be paired in the vicinity of **a**. Such crosses are still useful because their phenotype tells us if the region which is paired is sufficient for transvection of (a/b). In the present work **a** is pbx^2, bx^{34e}, or $Cbx^1 Ubx^1$. Since these are all pseudopoint mutations, if $T(a/b)$ is positive with one of the three, then //(a/b) exists for all three **a**. If the other conditions (existence of the unpaired state and transvecting phenotype detectable) are satisfied, then $T(a/b)$ is negative when $P(a/b) = P(R(a)/b)$. Typically, ∧(a/b) can be guaranteed by choosing a strong TSR for (R(a)/b). In the present work, the only case where $T(a, b)$ is invariably undefined results from a failure to detect a phenotype. This occurs for $a = bx^{34e}$ and $b = bxd$ or pbx, where $P(a/b) = P(a/+) = P(+/b) = P(a/R(+)) = P(R(+)/b)$.

One unwieldy, but unavoidable, consequence of a complete definition of the transvection test is that $T(a,b)$ has three possible values. These will be written as "transvection positive, negative, or undefined." Although awkward, this nomenclature avoids even worse problems which arise when the negation of "transvect" is not necessarily equivalent to "does not transvect." By using this construction, it should always be clear that "not transvection positive" is the same as "transvection negative or undefined."

RESULTS

A brief description of the three different transvection effects analyzed are presented here as an aid to the reader. The first effect is complementation of pbx^2. When heterozygous with pseudopoint or point mutations of *Ubx* (for example, Ubx^1), the pbx^2 phenotype is almost completely complemented. However, in the presence of a TSR, the same genotype produces a strong transformation of T3p to T2p (LEWIS 1982). The second effect is complementation of a part of the bx^{34e} phenotype. Flies which are bx^{34e}/Ubx^1 do not have a T3a to T2a notal transformation; with the addition of a TSR, the notum is strongly transformed (LEWIS 1954). The last effect is the transformation produced

by the double mutant chromosome $Cbx^1 Ubx^1$, which induces a weak *Cbx* transformation of wing to haltere only when paired with a normal chromosome (LEWIS 1955). The numerical scales used to describe these phenotypes are described in MATERIALS AND METHODS.

In order to produce a transvection group, in analogy to a complementation group, it is essential to consider the exact definition of the transvection test (see MATERIALS AND METHODS). In particular, it is essential to remember that the result of a transvection test can be positive, negative, or undefined. A secondary genetic test is frequently invoked in this work to demonstrate that two chromosomes are capable of pairing, and thus to distinguish between transvection negative and transvection undefined results. This test is based on the assumption that TSRs disrupt pairing over large contiguous chromosomal regions. That this might be true was suggested by the observation that TSRs follow the same rules for both the $Cbx^1 Ubx^1/+$ and the bx^{34e}/Ubx transvection effects (LEWIS 1954, 1985). One might expect then, that TSRs not having a breakpoint within the *Ultrabithorax* domain, would act uniformly throughout it. To determine whether TSRs behave as hypothesized, it was necessary to produce $R(Ubx)$ chromosomes. Irradiated $Ubx^1/TM6B$ males were crossed with $pbx^2/TM1$ virgin females. Rearrangements were detected by an enlargement of the haltere in the $pbx^2/R(Ubx^1)$ progeny. The putative $R(Ubx)$ chromosomes were then tested over bx^{34e}. A total of 62 such chromosomes was produced; in every case but one, the strength of a TSR was about the same in both systems (data not shown). The exceptional chromosome decreases synapsis dependent complementation of pbx^2, but not of bx^{34e}. This is probably not a TSR effect since the associated lesion, $Df(3R)88F;89B$, does not visibly disrupt polytene chromosome pairing at the *Ultrabithorax* locus. In some other cases the observed phenotypes were extremely variable, but in all such cases, both the *pbx* and the *bx* phenotypes were affected. Since pbx^2 and bx^{34e} are 70 kb apart, it appears that TSRs with breakpoints outside of the *Ultrabithorax* domain act uniformly within it.

In order to determine the members of each transvection group, each mutation in a large collection of *Ultrabithorax* domain lesions was tested for the three transvection effects studied here. The TSR used in most transvection tests was $T(2;3)53B,81$ while the very similar rearrangement $T(2;3)57B,81F$ was used in three cases. These two TSRs almost completely disrupt homologous pairing. The phenotypic, cytogenetic, and molecular characterizations of these crosses are presented in the tables. A summary of these crosses with commentary is presented below.

The extent of complementation of bx^{34e} with various

TABLE 1

Breakpoint mutations

Name	Location	Cytology	pbx^2 Phenotype pbx^2	$R(pbx^2)$	Results	$Cbx^1\,Ubx^1$ Phenotype No Dp	Dp	Results
bxd^{266}	+6.5 (3)	$T(2;3)40;89E$	0	0	U	0	·	U
bxd^{54G}	−0.5 (9)	$T(2;3)59C;89E + In(3R)88C-D;92$	5	5	N	1	0	P
bxd^{DB3}	−0.5 (9)	$T(2;3)48;89E$	5	5	N	2	1	P
bxd^{DB6}	−0.5 (9)	$T(2;3)22A;43A-C;80D;84;89E;92F$	5	5	U	·	·	·
bxd^{MX1}	−2.0 (6)	$IN(3R)89E;90EF$	5	5	N	1	1	P
bxd^{111}	−2.5 (2)	$Tp(3;1)4D;89E;90B2$	5	5	N	1	1	P
bxd^{657}	−6.0 (7)	$T(1;3)X;81?;89E$	5	5	U	·	0	U
bxd^{106} $[bx^3]$	−12.5 (5)	$In(3LR)72D11-E1;89E$	5	5	N	·	1	P
Uab^1	−14.0 (2)	Normal $[In(3R)89E,89E]$	3–5	4–5	N/P	·	2	P
bxd^{C5A}	−17.5 (5)	$In(3R)89B;89E$	2–3	*4–5	P	·	1	P
bxd^{100}	−17.5 (5)	$Tp(3;3)66;89B5;89E$	1–4	5	P	·	0	N
bxd^{110}	−23.0 (7)	$Tp(3;3)89E;91D;92A$	2–5	*5	P	·	0	N
bxd^{183}	−23.0 (7)	$In(3R)89C;89E$	1–3	*3–5	P	·	1	P
$Ubx^{5.12}$	−30.0	$In(3R)89E;90A$	2–3	4–5	P	·	0	N
$Ubx^{8.8}$	−37.0	Normal $[In(3R)89E,89E]$	1–2	4–5	P	·	0	N
$Ubx^{5.22}$	−45.0	$Tp(3;4?)89A-89E1.2;?$	2–4	5	P	·	0	N
$Ubx^{6.26}$	−47.0	$In(3LR)75C;89E + T(2;3)59E;75C$	5	5	U	·	0	U
Ubx^{882}	−75.0	$In(3R)87;89E1.2$	3–5	5	P	·	0	N
$Ubx^{4.30}$	−85.0	$T(2;3)34;89E1.2$	5	5	U	·	0	U
$Ubx^{5.2326}$	−87.0	Normal [?]	2–4	4–5	P	·	0	N
$Ubx^{12.5}$	−92.0	$In(3R)88B;89E1.2$	2–4	4–5	P	·	0	N

Mutations in brackets are in *cis* with the preceding allele. Sizes/locations are in kilobases, the size of the affected restriction fragment in parenthesis. Cytology in brackets derives from genetic or molecular data. The TSR in $R(pbx^2)$ is $T(2;3)53B,81$ or, if marked with an "*," $T(2;3)57B,81F$. Dp is $DpP10$, an insertion of one wild-type copy of the *Ultrabithorax* domain into the left arm of the second chromosome. For an explanation of the numerical phenotype scores in the rightmost six columns, see MATERIALS AND METHODS. The results column indicates whether the genotype is transvection positive (P), negative (N), undefined (U), or no information (·). Molecular data from KERRIDGE and MORATA (1982), BENDER et al. (1983, 1985), KARCH et al. (1985), SANCHEZ-HERRERO et al. (1985), LINDSLEY and ZIMM (1987), WEINZIERL et al. (1987), ROWE and AKAM (1988). Cytology from these references or from personal observations.

alleles of *Ubx* in the absence of other chromosome rearrangements has been extensively documented elsewhere (KERRIDGE and MORATA 1982; LEWIS 1954). This information and some of my own experiments are summarized here because combining the results allows the determination of the bx^{34e} transvection group. These phenotypes are not listed in the tables, although the molecular characterizations of the mutations are. Point and pseudopoint *Ubx* alleles (Ubx^1, $Ubx^{9.22}$, Ubx^{195}) are transvection positive with bx^{34e} (notum scores of 0–2), but no breakpoint allele is (notum score of 5). It has been previously reported that $Ubx^{5.2326}$ complements bx^{34e} to a level intermediate between pseudopoint and breakpoint mutations and is cytologically normal, yet is a breakpoint mutation (KERRIDGE and MORATA 1982; AKAM et al. 1985). Unfortunately, on reexamination, the notum transformation score was 5. Hence, it is impossible to determine if the originally reported phenotype was due to transvection with bx^{34e}.

C1 is a hybrid *abd-A/Ubx* gene produced by a large deletion of parts of the *Ubx* and *abd-A* domains; it is functionally bx^+ and bxd^- (CASANOVA, SANCHEZ-HERRERO and MORATA 1988; ROWE and AKAM 1988). The double mutant chromosome $Ubx^{9.22}\,C1$ is func-

tionally Ubx^- due to the *Ubx* mutation, yet a large region surrounding the site of the bx^{34e} lesion is intact. Flies of the genotype $Ubx^{9.22}\,C1/bx^{34e}$ (±TSR) have a notum score of 5. The *abx* alleles are transvection positive with both bx^{34e} and Ubx^1 (PEIFER and BENDER 1986). Therefore, the phenotypes of bx^{34e}/abx genotypes are not examined here because the identity of the mutation which complements in the heterozygote can only be determined from genotypes such as $abx\,Ubx^1/bx^{34e}$.

The phenotypes of flies carrying pbx^2 heterozygous with many different mutations, both in the presence and absence of a TSR, are shown in the tables. pbx^2 is transvection positive with *Ubx* point, pseudopoint, and most breakpoint alleles. The $Tp(3;3)bxd^{100}$ breakpoint marks the distal limit for transvection positive rearrangements. Pairing dependent complementation is mediated by the *bxd* (distal) part of the transposition since $Df(3R)bxd^{100}$ has the same phenotype (Tables 1 and 3). This clearly demonstrates that no *Ubx* transcription unit DNA is necessary in *trans* to pbx^2 for pairing dependent complementation to occur. The *bxd* breakpoints distal to bxd^{100} were not transvection positive with pbx^2. As expected, neither bxd^{121} nor *C1*, two deficiencies spanning pbx^2, was transvection posi-

TABLE 2

Insertion and point mutations

| Name | Location | Mutation | pbx^2 | | | $Cbx^1 Ubx^1$ | | |
| | | | Phenotype | | | Phenotype | | |
			pbx^2	$R(pbx^2)$	Results	No Dp	Dp	Results
bxd^K	−2.5	Gypsy insertion	2–5	2–5	N	2	.	P
bxd^{51j}	−17.5	Gypsy insertion	0–2	2–3	P	1	1	P
bxd^1	−21.0	Gypsy insertion	0	0–2	P	2	1	P
Ubx^1	−32.0	Doc insertion	1–2	4–5	P	.	0	N
Cbx^1	−44.0	17kb [= pbx^1 DNA] insert	.	.	.	.	.	.
Ubx^{195}	−50.0	Nonsense mutation	1–2	3–5	P	.	0	N
Ubx^{M4}	?	Point mutation?	0	1	P	1	.	P
bx^3	−57.0	Gypsy insertion	.	.	.	2	.	P
bx^{34e}	−63.5	Gypsy insertion	.	.	.	2	.	P

For details, see legend to Table 1.

tive with it. Of the pseudopoint mutations in the *bxd* region, *pbx¹* and *bxdᴷ* were not transvection positive, while *bxd¹* and *bxd⁵¹ʲ* were. Transvection of *pbx²* with *bxd¹* was more evident in the combination *bx³ bxd¹*, which has a stronger haltere transformation. The phenotypes of *bxdᴷ* genotypes were extremely variable, even between one side of the fly and another (see below). *Uab¹* also has a somewhat variable *pbx* phenotype; if this mutant transvects with *pbx²*, the effect is too slight to be reliably detected in this background. *bxd¹¹⁰* was transvection positive, but the penetrance of the complemented phenotype was low.

The *Cbx¹ Ubx¹/+* transvection effect differs from the two cases described above in that when the chromosomes are synapsed, the extra function is produced by the chromosome located in *trans* to the mutations (LEWIS 1955). Thus the transvection group for *Cbx¹ Ubx¹* contains mutations which block the response to the double mutant. Crosses were made to a series of mutations similar to that described above for *pbx²*, with results shown in the tables. The resulting gain of function phenotype, which is not present with any of the tested chromosomes until they are made heterozygous with *Cbx¹ Ubx¹*, is assumed to result from transvection. However, this was only demonstrated directly for +, *bx³*, *bxd⁵¹ʲ* and *pbx²* (data not shown). One copy of *DpP10*, an insertional duplication of the *Ultrabithorax* domain located in the second chromosome, was used to rescue lethal or poorly viable genotypes. The phenotypes recorded here are generally in agreement with those reported in BABU, SELVAKUMAR, and BHOSEKAR (1987) and MICOL and GARCIABELLIDO (1988).

Of the point and pseudopoint mutations, only lethal *Ubx* alleles eliminated the *Cbx¹ Ubx¹* transformation. A homozygous viable allele, *Ubxᴹ⁴*, greatly reduced the transformation. None of *abx¹*, *abx²*, *bx³⁴ᵉ*, or *bx³* strongly suppressed the *Cbx¹ Ubx¹* effect. Heterozygotes of *Cbx¹ Ubx¹* with *abx¹ bx² pbx¹* did not differ

significantly in the wing transformation from heterozygotes with *abx¹*, *bx³*, or *pbx¹*. A synergistic interaction of *bx* and *bxd* was observed: *DpP10/+*; *Cbx¹ Ubx¹/ Test* had a much weaker wing transformation when *Test* was *bx³ bxd¹* than it was when *Test* was *bxd¹*, *bx³*, or *abx¹ bx³ pbx¹*.

All *Ubx* breakpoint alleles tested were not transvection positive with *Cbx¹ Ubx¹*. This was also true for *C1*, despite its residual *Ubx⁺* activity. The behavior of small lesions in the *bxd* region was complicated; *bxd⁵¹ʲ* definitely decreased the transformation while *bxdᴷ*, *pbx¹*, *bxd¹*, and *pbx²* did not. The *bxd* breakpoint mutations generally suppressed the phenotype, but not completely. The copy of *Ubx⁺* in *DpP10* pairs rarely, if at all, with the third chromosome, since *Cbx* transformations were not observed in either *DpP10/+;Cbx¹ Ubx¹/DfP10* or *DpP10/+;Cbx¹ Ubx¹/Ubxˣ* genotypes. However, *DpP10* apparently can affect the phenotype of certain genotypes in a nonpairing dependent manner. For every *bxd* allele which was tested both with and without the duplication, the latter had a significantly stronger wing transformation. This is less evident in the tables since the phenotypic classes used were quite broad. Additionally, both *DpP115* (an insertion of the entire bithorax complex into the heterochromatin of the *X* chromosome) and *DpP10* eliminated the weak wing transformation seen with the *TM1* balancer chromosome.

The discovery that *bxdᴷ* was transvection negative with *pbx²* prompted the series of crosses shown in Table 5. The variability of *bxdᴷ* is a property of that allele independent of pairing. It produces patchy transformations and a similar distribution of mild to extreme phenotypes with *Ubx¹*, *Df(3R)Ubx¹⁰⁹*, *Tpbxd¹⁰⁰*, *pbx¹*, and *pbx²*. Conceivably, *bxdᴷ* completely blocks *pbx²* transvection, but this variable *cis* regulatory defect masks the effect. Accordingly, the *bxdᴷ* chromosome was X-rayed and two new *Ubx* alleles recovered. These mutations are functionally *Ubx⁻* and

TABLE 3

Deletion mutations

| Name | Proximal | Distal | pbx^2 Phenotype | | | $Cbx^1 Ubx^1$ Phenotype | | |
			pbx^2	$R(pbx^2)$	Results	No Dp	Dp	Results
$DfUbx^{109}$	«—Ubx	+89.5 (7)	5	5	U	.	.	.
$DfP10$	«—Ubx	+35.5 (1)	.	.	.	.	0^a	U
bxd^{111}	-2.5 (2)	Ubx—»	5	5	N	1	1	P
pbx^1	-3.0	+14.0	5	5	N	2	.	P
pbx^2	-13.0 (2)	+1.0 (4)	5	.	N	2	.	P
bxd^{121} [bx^{34e}]	-15.0	+62.5 (2.5)	5	5	U	.	0	U
bxd^{100}	«—Ubx	-17.5 (5)	1-4	5	P	.	0	N
$C1$	-51.0	+48.0	5	5	U	.	0	U
abx^1	-79.0	-73.0	.	.	.	2	.	P
abx^2	-79.0	-77.5	.	.	.	2	.	P
$Ubx^{9.22}$	-104.5	-103.0	1-2	3-5	P	.	0	N

For details, see legend to Table 1.

^{a} $TpP10/Cbx^1 Ubx^1$.

are similar to other Ubx breakpoint alleles when heterozygous with bx^{34e} and Ubx^{M4} (data not shown). Ubx^{S205-2} is associated with the rearrangement $In(3R)87D,89E$. Ubx^{S205-1} has one breakpoint at 89E and the other in heterochromatin, it segregates with the Y and is presumably $T(Y;3)89E$. Ubx^{S205-2} has the sort of rearrangement which typically has only weak TSR effects (see above and Table 1). The double mutant $Ubx^{S205-2} bxd^K$ still partially complements pbx^2. The transformation of the noncomplemented portions of the haltere frequently is patchy, suggesting that bxd^K only blocks that fraction of the pairing dependent complementation which corresponds to its own variable loss of function phenotype. bxd^K is a *gypsy* mutation and is suppressed in a $su(Hw)^2$ homozygous background (BENDER *et al.* 1983). In this background, $Ubx^{S205-2} bxd^K/pbx^2$ complement to a greater degree and the patchy transformation typical of bxd^K is not observed. This suggests that it is the action of the *gypsy*, rather than the lesion associated with its insertion, which is responsible for the phenotype of bxd^K.

DISCUSSION

The transvection groups for pbx^2 and $Cbx^1 Ubx^1$ can be read directly from Tables 1 through 4. Mutations with an "N" in the results column are members of the respective transvection group, those with a "P" are not members, and those with a "U" cannot be assigned. The transvection group for pbx^2 is composed of bxd^{54G}, bxd^{DB3}, bxd^{MX1}, bxd^{111}, bxd^{106}, bxd^K, and pbx^1. The transvection group for $Cbx^1 Ubx^1$ includes bxd^{100}, bxd^{110}, and as expected, all Ubx lethal alleles as well. For bxd^{100} it is more likely that this classification results from the transposition of the Ubx transcription unit to another chromosome than from the position of the breakpoint within bxd. The inclusion of the bxd^{110} lesion in this transvection group is also somewhat problematical. Since the pairing dependent complementation of bxd^{110} with pbx^2 is not very penetrant, it is possible that the corresponding feeble response to $Cbx^1 Ubx^1$ was simply not detected.

The members of the transvection group for bx^{34e}, from KERRIDGE and MORATA (1982) and Table 1, are $Ubx^{5.12}$, $Ubx^{8.8}$, $Ubx^{5.22}$, Ubx^{882}, $Ubx^{5.2326}$, and $Ubx^{12.5}$. The pseudopoint mutations Ubx^1, $Ubx^{9.22}$, and Ubx^{195} are not members. Surprisingly, the double mutant $Ubx^{9.22} C1$ is transvection undefined for bx^{34e}. It is not possible from the current data to determine what role, if any, the bxd region plays in this transvection effect. (Such an analysis requires $bxd^{breakpoint} Ubx^{pseudopoint}$ chromosomes.) At least the pbx^1 DNA is not required since the double mutant $Ubx^1 pbx^1$ is transvection positive with bx^{34e} (D. MATHOG, unpublished observations).

Before discussing these transvection groups further, it is important to consider the synapsis disrupting effects of breakpoints within the *Ultrabithorax* domain. In particular, to what extent do TSR effects determine membership in a transvection group? In this paper 61 TSRs outside of the *Ultrabithorax* domain are shown to disrupt pairing throughout the gene. Only one mutation had differential effects on bx^{34e} and pbx^2 transvection, but it was not associated with a classical TSR (LEWIS 1954). Nevertheless, one would expect that TSR effects could be either symmetric or asymmetric around a single breakpoint within the *Ultrabithorax* domain. Apparently, small paracentric chromosomal inversions have symmetric TSR effects since bxd^{C5A} and bxd^{183}, when paired with pbx^2 and $Cbx^1 Ubx^1$, have approximately proportional phenotypes (data not shown). Other classes of chromosome rearrangements, such as translocations between the second and third chromosomes and pericentric inversions, may very well have asymmetric effects on pairing. If we assume that disruption of pairing distal to

breakpoints is entirely responsible for membership in the pbx^2 transvection group, then DNA absolutely required to complement pbx^2 lies distal to the bxd^{MX1} breakpoint and one cannot determine whether or not sequences from there to the bxd^{106} breakpoint are required as well. If, on the other hand, the bxd^{106} chromosome can pair distal to its breakpoint, then DNA from around that breakpoint and distal to it is required for pbx^2 transvection. The distal and proximal ends of the bx^{34e} transvection group are defined by small inversions which demonstrably pair distal to their breakpoints and should pair proximally as well (based on their similarity to bxd rearrangements which are transvection positive with $Cbx^1 Ubx^1$). This constitutes a strong argument that TSR effects do not determine membership in this group.

Position effects near breakpoints, distinct from any longer range TSR effects, may also exist. Aside from the data at hand, there is no indication whether this local disruption of pairing extends for bases or megabases. No consistent estimate of the maximum range of this putative effect can be derived from the current data. For example, the distance separating the lesions in the transvection negative pair $Ubx^{12.5}$ and bx^{34e} is about 30 kb, whereas the distance for the transvection positive pair bxd^{C5A} and pbx^2 is between 4 and 17 kb. There is no evidence of phenotypic gradients as breakpoints approach bx^{34e} and pbx^2. Instead, one finds a contiguous block of transvection negative mutations and phenotypes outside of this block correlate very well with the expected TSR properties of the rearrangements (Table 1). If the layout of the transvection groups results from a local pairing disrupting effect, such an effect must act in predefined chromosomal regions, perhaps corresponding to DNA loops or other structures. Such an explanation has its own difficulties: what sort of chromosomal structure would correspond to all of one transcription unit, but only part of another? In any case, simple proximity to a breakpoint does not define membership in a transvection group. For example, $Ubx^{5.12}$ is closer to pbx^2 than it is to bx^{34e}, yet it is transvection positive with the former.

Each of the three transvection effects may be modeled using the functions *send, receive* and *respond*. Ultimately, the *respond* function for all three effects is the synthesis of *Ubx* protein(s). Conversely, *receive* may be different for all three of the transvection effects. pbx^2 and bx^{34e} are defective in their respective *send* functions, but are complemented when a working *send* is paired with them. Cbx^1 has a neomorphic *send* which acts strongly in *cis* and weakly in *trans* on *receive*. The resulting *Cbx* transformation can be blocked either in *cis* or in *trans* by an *Ubx* mutation (*respond*).

The DNA corresponding to the pbx^2 *send* unit is either similar in size to the pbx^2 deficiency or it is located within the distal few kilobases of that deficiency (see above). Either interpretation includes the 5' end of the *bxd* early transcription unit (HOGNESS *et al.* 1985, LIPSHITZ, PEATTIE and HOGNESS 1987). One argument favoring the more extensive *send* unit is that chromosomes which are transvection positive with pbx^2, and thus have a functioning *send* unit, all contain a piece of wild type DNA extending both proximally and distally beyond the pbx^2 deficiency.

The DNA corresponding to the bx^{34e} *send* unit is more extensive. Mutations which do not *send* to bx^{34e} map throughout the *Ubx* transcription unit. However, no suitable breakpoint mutations fall within the final 10 kb of the gene, so it has not been possible to determine if this region is also required.

Cbx^1 *receive* is difficult to map within the *Ubx* transcription unit since *respond* requires the production of *Ubx* protein. Nevertheless, it is possible to exclude the *abx, bx* and *pbx* regulatory regions as mediating *receive* either singly or in combination (Tables 2 and 3). $Cbx^1 Ubx^1$ transvection is often partially suppressed by *bxd* mutations, but the location of the breakpoint or insertion and the magnitude of the resulting suppression are poorly correlated (Tables 1 and 2). Therefore, either bxd^+ is not *receive*, or it is not the only *receive*, for Cbx^1 *send*.

The two deficiencies $C1$ and bxd^{121}, which are roughly similar in size and position, have unexpected phenotypes. $Cbx^1 Ubx^1$ is transvection positive with both *pbx* alleles (which more or less cover the *bxd* DNA deleted in bxd^{121}), with Uab^1 (which has a breakpoint and phenotype close to that of bxd^{121}), and with bxd^{106} (which is also a *bx bxd* double mutant). Therefore, one must posit some new effect to explain why bxd^{121} is transvection undefined with $Cbx^1 Ubx^1$. Presumably such an effect could also occur with $C1$, which is a deletion of comparable scale and location (Table 3). This new effect might also explain why $Cbx^1 Ubx^1/ C1$ and $bx^{34e}/Ubx^{9.22} C1$ are not transvection positive. One explanation might have been that deficiencies of this size have anomalously large effects on pairing. This is apparently not the case for bxd^{121} since it is transvection positive with the distally located lesion iab-$4,5^{DB}$ (KARCH *et al.* 1985; E. B. LEWIS, personal communication). Whether $C1$ also exerts a polar effect on transvection is not yet known.

Two effects are noted with respect to $Cbx^1 Ubx^1$ transvection. The first is a synergistic interaction between bx^3 and bxd^1 such that the double mutant responds to $Cbx^1 Ubx^1$ more weakly than does either mutation alone. BABU, SELVAKUMAR and BHOSEKAR (1987) also observed this synergism in their studies of the interactions between multiple *gypsy* mutations in the same gene. In the second effect, which has not previously been reported, $DpP10$ reduces the wing to haltere transformation of $Cbx^1 Ubx^1/bxd$ genotypes,

TABLE 4

Combinations of mutations and miscellaneous

Name	pbx^2			$Cbx^1\ Ubx^1$		
	Phenotype			Phenotype		
	pbx^2	$R(pbx^2)$	Results	No Dp	Dp	Results
$abx^1\ bx^3\ pbx^1$	.	.	.	2	2	P
$bx^3\ bxd^1$	0–2	3–4	P	.	1	P
$Ubx^1\ pbx^1$	5	5	N	.	.	.
$Cbx^1\ Ubx^1$	.	.	.	.	0	N
$bx^3\ Cbx^1\ Ubx^1\ bxd^1\ pbx^1$	5	5	U	.	.	.
+	.	.	.	2	2	P
$TM1\ [In(3L)63C;72E + In(3LR)69E;91C + In(3R)89B;97D]$	0	0	U	1	0	P

For details, see legend to Table 1.

TABLE 5

Phenotypes of bxd^K

Genotype (a/b)	a/b	$a/R(b)$	$\dfrac{Su(Hw)^2/a}{su(Hw)^2/b}$
bxd^K/pbx^2	2–5	2–5	0
bxd^K/pbx^1	2–5	.	.
$bxd^K/Tpbxd^{100}$	2–5	.	.
bxd^K/Ubx^1	2–5	2–5	.
$bxd^K/Df(3R)Ubx^{109}$	2–5	.	0
$bxd^K\ In(3R)Ubx^{S205-2}/pbx^2$	3–5	.	2–3
$bxd^K\ T(3;Y)Ubx^{S205-1}/pbx^2$	5	.	5

For details, see legend to Table 1.

for all alleles of *bxd* tested. The suppression is not evident in either $Cbx^1\ Ubx^1/+$ or $Cbx^1\ Ubx^+/+$ (LEWIS 1955; CASANOVA, SANCHEZ-HERRERO and MORATA 1985), which perhaps explains why it has not been previously detected. The suppression is apparently not due to a maternally acting modifier since it is evident in reciprocal crosses of bxd^{51j} with $DpP10/+;Cbx^1\ Ubx^1/+$. Neither can it be due to a second chromosome dominant modifier linked to *DpP10* since another Ubx^+ duplication. $Dp(3;1)P115$, has the same effect. Lastly, it does not appear that pairing of *DpP10* with the *Ultrabithorax* domain is involved: $TpP10/Cbx^1\ Ubx^1$ flies have normal wings and no synapsis of the transposed region was seen in a small number of polytene squashes (D. MATHOG, unpublished results). These results suggest either that the duplication competes for a required factor or that the duplication responds to the misexpression of *Ubx* proteins in the wing by producing some other product (also *Ubx*?) which partially suppresses that misexpression.

The abstract concepts of *send*, *receive* and *respond* are useful for discussing transvection effects. However, the final goal is to discover the molecular mechanisms underlying these abstract terms. A simple class of transvection models has been suggested by ZACHAR, CHAPMAN and BINGHAM (1985), BIGGIN *et al.* (1988), and by others. In this class of models, regulatory regions move passively to the promoter upon which

they act. Left unanswered is how, given the distances involved and the ability to act on the homologous promoter on a paired chromosome, a regulatory region acts specifically on its target rather than on an equidistant, but incorrect, promoter. The *abx/bx* region, for example, would have to move at least 30 to 40 kb (BENDER *et al.* 1983). How does it avoid the neighboring promoters in *lethal left of bithorax, bxd,* and *abd-A* (SANCHEZ-HERRERO *et al.* 1985)? Furthermore, this simple mechanism does not predict the observed asymmetry in pbx^2 and bx^{34e} transvection: the former is complemented by a relatively small region of DNA which does not extend to the *Ubx* promoter whereas the latter requires an enormous unbroken piece of DNA which probably does include that promoter.

A model for the regulation of the *Ultrabithorax* domain can be derived by combining some elements of the above regulator-promoter models with ideas first presented in JACK and JUDD (1979) and LEWIS (1954). This model explains many of the unexpected observations reported here and suggests a physical nature for *send* and *receive*. The normal *cis*-regulation of *Ubx* by bxd^+ is not included in this model (but see below). Furthermore, this model only covers the *initiation* of regulatory interactions—it is assumed that a system exists to maintain the state of the gene.

The proposed model assumes that regulatory regions such as those in Cbx, pbx^+, and bx^+ act by physical contact on or near the regulated promoter. Two separate actions are required to bring about this juxtaposition (Figure 2). When these regions find themselves in the immediate vicinity of their target promoter, "passive transport" physically brings the two into working contact. The thermal motions of the DNA combined with some sticking mechanism (protein-protein, for instance) probably suffice. However, such regions are located in *cis* at distances of several tens of kilobases, and therefore require prior use of "active transport" to move close enough together so that passive transport will work. This is accomplished
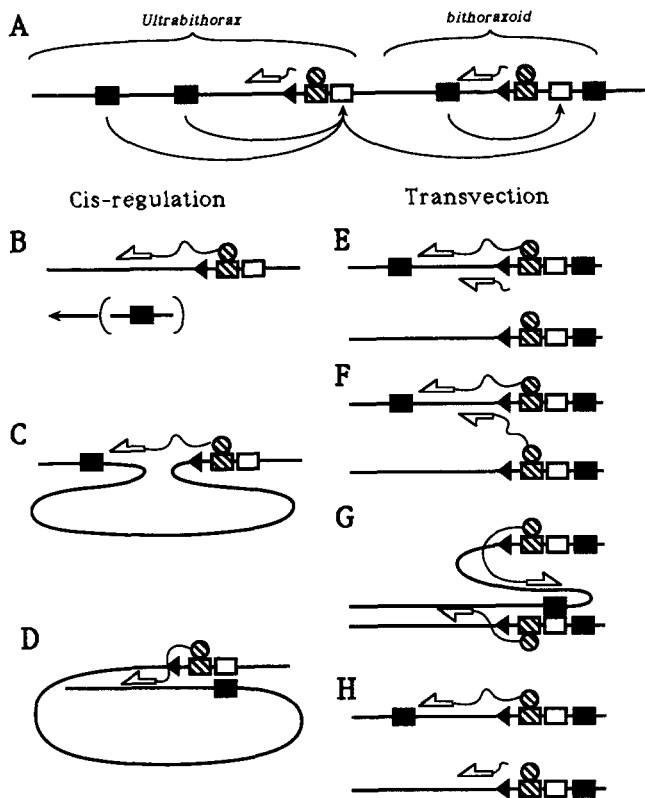
FIGURE 2.—A) A schematic illustration of the components of the *Ultrabithorax* domain which are involved in regulation. Solid boxes are regulatory *send* units, empty boxes are regulatory *receive* units, hatched boxes are "attach" sites for the 5' ends of localizing RNAs, hatched circles are "attach" mediating proteins, the open half-arrow is an RNA polymerase (shown shortly after the initiation of transcription), the thin wavy line is RNA, the thick line is DNA, and the solid arrowheads are transcription initiation sites. Regulatory interactions are indicated by the arrows. B) A localizing transcript is shown shortly after initiation. The regulatory region to be transported is far away. B→C) Active transport: the localizing transcript is attached at both ends while RNA processing and packaging makes the distance between these ends much shorter than the equivalent path along the DNA. Consequently, *send* is brought near to *receive*. C→D) Passive transport: once within range, *send* diffuses to, and interacts with, *receive*. The position of the RNA polymerase with respect to *send* in the illustration is arbitrary. E→H) Transvection between *Dfbxd*^{100} (upper chromosome) and *pbx*^{2}. The latter chromosome lacks DNA which includes the proximal *send* unit and regions up to, and possibly including, the normal *bxd* promoter. The promoter on the *pbx*^{2} chromosome is either the mutant remnant of the *bxd* promoter or a cryptic promoter which is activated in its absence. This promoter is not transcribed in *pbx*^{2} homozygotes. E) A localizing transcript on the *Dfbxd*^{100} chromosome is shown as is a second transcript which cannot "attach" to the same chromosome because the "attach" site is filled. F) The 5' end of a transcript from the *Dfbxd*^{100} chromosome is captured by the *pbx*^{2} chromosome. G) After active and passive transport, *pbx*^{2} *receive* is activated by *Dfbxd*^{100} *send*. H) The *pbx*^{2} chromosome begins producing its own transcripts. One of these becomes a localizing transcript which transports distal *pbx send* to *Ubx receive*.

by a "localizing" RNA transcript. Such transcripts are initially produced at some low rate which may be altered by subsequent regulatory interactions. The "localizing" transcripts either begin or end in the

vicinity of the target promoter. Initially, the 5' end of the "localizing" transcript "attaches" to the chromosome in the vicinity of its own promoter (which does not need to be the promoter to be regulated). The RNA polymerase "attaches" the 3' end of this "localizing" transcript. Transcription proceeds and the nascent RNA is shortened by various processes. For example, it may be packaged into RNPs (SKUGLAND *et al.* 1983), spliced (OSHEIM, MILLER and BEYER 1985), or even degraded by an exoribonuclease (see below). In this manner regulatory regions which lie in or near the localizing transcript are dragged towards the target promoter.

Transvection in this model results from an error in active transport. First, the 5' end of the localizing transcript cross attaches to the homologous chromosome. Following this misdirected active transport, interchromosomal passive transport occurs, leading to the observed transvection effects. Since the number of attachment sites for 5' ends is expected to be limited, a few localizing transcripts present on one chromosome may block the action of similar transcripts originating on the homologous allele.

The *cis* regulatory actions of *Cbx*^{1}, *pbx*$^+$, and *bx*$^+$ can all be described by the proposed model. For *Cbx*^{1} and *bx*$^+$ the localizing transcripts are either nonpolyadenylated transient transcripts or the nascent form of one of the known *Ubx* mRNA transcripts (HOGNESS *et al.* 1985; O'CONNOR *et al.* 1988; KORNFELD *et al.* 1989). It is probable that one of the regulatory regions involved in *Cbx*^{1} action is within the *Cbx*^{1} insert and that *bx*$^+$ lies in the vicinity of the *abx* lesions. The *bx*^{34e} transvection group has members proximal to *abx* and mutations with phenotypes similar to *Cbx*^{1} map in the extreme proximal (3') end of the gene (BENDER *et al.* 1983). Therefore, a region in the proximal end of *Ubx* may also be required in both cases.

The application of the model to *pbx*$^+$ regulation is somewhat different because there is strong evidence that *pbx*$^+$ does not directly activate the *Ubx* promoter. This statement is based on the following observations: *pbx*$^+$ *send* maps within the DNA deleted in *pbx*^{2}; pairing in the vicinity of the *Ubx* promoter occurs in heterozygotes of *Ubx*^{1} with *pbx*^{2}, *pbx*^{1}, and many *bxd* pseudopoint and breakpoint mutations (from the *Cbx*^{1} *Ubx*^{1} data); yet only *pbx*^{2} is transvection positive with *Ubx*^{1}. The simplest explanation for these observations is that *pbx*$^+$ *send* lies within and *pbx*$^+$ *receive* lies just distal to the *pbx*^{2} deletion, and that subsequent activation of the *Ubx* promoter is mediated entirely in *cis* from *pbx*$^+$ *receive*. These observations are inconsistent with *pbx send* acting directly upon the *Ubx* promoter. Diffusible RNA models (JACK and JUDD 1969; MICOL and GARCIA-BELLIDO 1988) also fit the observations if only a small piece of the early *bxd* transcript is required and it acts near the early *bxd* promoter. However, the

subsequent *cis* activation of the *Ubx* promoter would then require a distinct mechanism. In the model proposed here the obligate *cis* interaction is not an *ad hoc* assumption but it must hold true for any upstream regulatory region whose active transport is mediated by localizing transcripts which run toward the target promoter.

The passive transport mechanism is widely accepted and will not be discussed further here. With respect to "active transport," there are two previously described biochemical activities which could serve to "attach" the 5' end of a localizing RNA. The first is a processive 5'→3' exoribonuclease (LASATER and EICHLER 1984). If this protein binds directly or indirectly near the promoter of the localizing transcript, it could then capture the 5' end of an RNA transcript before it is capped or bound by other proteins. The subsequent processive exonuclease activity, if sufficiently rapid, would greatly enhance the transport of regulator to promoter. Another possible "attach" mechanism is the formation of a hybrid between the 5' end of the RNA and the template or, more likely, a nearby region of DNA. In this second mechanism, requirements for sequence complementarity would preclude erroneous interactions with nonhomologous genes, but allow transvection. There is some precedent for this mechanism: RNA/DNA hybrids are required for Tetrahymena telomerase action (GREIDER and BLACKBURN 1989) and for initiation of replication at the origin of replication of certain plasmids in *E. coli* (ITOH and TOMIZAWA 1980).

The requirement for a process beyond passive transport follows from a number of results. For example, the transvection negative results of bx^{34e} with $Ubx^{12.5}$ and $Ubx^{5.2326}$ are not predicted by passive transport only models (because there is no obvious reason why the wild type regulatory regions on these chromosomes cannot reach the promoter on the *trans* chromosome). Furthermore, even if the *Ubx* promoter on the bx^{34e} chromosome is unpaired when heterozygous with $Ubx^{9.22}$ *C1*, it is not obvious why the bx^+ region on the double mutant cannot interact with it. How has the diffusion distance changed? It is possible to rationalize these sorts of interactions with previously undetected chromosome pairing interactions. However, the model presented here is simpler. Indeed, it is essentially the model originally proposed by LEWIS (1954), except in the current version only the transcript, not the transcript and the polymerase, changes chromosomes.

The proposed model provides a rationale for several genetic results. Ubx^1 does not transvect with *bxd* mutations or pbx^1 for the *pbx* phenotype because cross attachment of the localizing transcript from the Ubx^1 chromosome either cannot occur (pbx^1, some *bxd* alleles) and/or regulation of the *Ubx* promoter is still

blocked in *cis* even when pbx^+ *receive* is activated (*bxd* mutations). The lack of transvection of $Ubx^{9.22}$ *C1* with bx^{34e} and of Cbx^1 Ubx^1 with *C1* is caused by the absence of cross attachment at the 5' end of the respective localizing transcripts. Yet *C1* can still be bx^+ because a new localizing transcript from the *abd-A* promoter is available (ROWE and AKAM 1988).

Phenotypic gradients in the *bx* and *bxd* regions are associated with different *gypsy* insertions (BENDER et al. 1983; PEIFER and BENDER 1986). The direction of each gradient is aligned with its localizing transcript. In the present model the phenotypes could arise either from competition of some *gypsy* sequence with normal regulatory regions subsequent to active transport or from disruption of active transport by "detaching" the localizing transcript. The same mechanism can also explain how bxd^K blocks pbx^+ function either in *cis* or in *trans*. As a specific example, the "detach" mechanism can explain why bxd^1 has a *pbx* mutant phenotype whereas a phenotypic revertant (which leaves one *gypsy* LTR) does not, even though both have early *bxd* RNAs of identical (but mutant) sizes (HOGNESS et al. 1985). Explicitly, bxd^1 is inserted very near the end of several of the early *bxd* transcripts (BENDER et al. 1983; LIPSHITZ, PEATTIE and HOGNESS 1987). If one of these transcripts is acting as a localizing transcript, then termination in the first *gypsy* LTR would "detach" that transcript at least 7.3 kb short of its target, whereas active transport in the phenotypic revertant would terminate in essentially the normal location (MODOLELL, BENDER and MESELSON 1983).

There is little experimental evidence contradictory to the proposed model. To my knowledge, the transient DNA loops which are predicted by both the proposed and enhancer only models have not yet been observed in the electron microscope. A report that *bxd* transcripts are absent in parasegment 6 (AKAM et al. 1985) led to conclusions that these transcripts play no role in pbx^+ function (HOGNESS et al. 1985; LIPSHITZ, PEATTIE and HOGNESS 1987). However, more recent observations have detected a small amount of *bxd* RNA in embryos in parasegment 6 (IRISH, MARTINEZ-ARIAS and AKAM 1989). The model only concerns the *initiation* of regulated states, so the lack of *bxd* transcripts later in development does not argue against it. This initiation of a regulated state for the *bxd* region early in development would seem to require the appearance of clones of transformed cells resulting from early transvection of pbx^2—which is not evident in the observed phenotypes. Patches of transformation resulting from many *Ubx* regulatory mutations are nonclonal, suggesting that intercellular signals modulate *Ubx* expression late in development (reviewed in BOTAS, CABRERA and GARCIA-BELLIDO 1988). Since pbx^2 need not be mutant for this cell-cell regulatory function, subsequent regulation by this

mechanism could smear the borders of such clones and result in the observed phenotypes.

Lastly, it is worth commenting on the generality of active transport. It seems likely that an RNA polymerase may not be able to pass through the site where the 5' end of a localizing transcript is fixed to the chromosome. If so, such sites would normally lie outside the transcription unit which produces a gene's product. This appears to be the case for both the *Ubx* (coding) and *bxd* (noncoding) units. This is the expected result if the *Ultrabithorax* domain evolved from a two promoter gene like Antennapedia (LAUGHON *et al.* 1986; O'CONNOR *et al.* 1988). Other genes may have upstream regulatory units transcribed in the opposite direction from their structural units. Localizing transcripts which have never been coding may be "attached" downstream from their promoter. This would be efficient since the single localizing transcript extant at any given time could repeatedly scan for distant regulatory elements. It would also make the detection of such transcripts exceedingly difficult. Indeed, if "attach" is mediated by an exoribonuclease, such transcripts might only exist transiently as short oligoribonucleotides. Until recently such entities would have been totally undetectable, but with the advent of the polymerase chain reaction (SAIKI *et al.* 1985) it may now be possible to test this hypothesis.

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