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Interactions of *Polycomb* and *trithorax* with *cis* regulatory regions of *Ultrabithorax* during the development of *Drosophila melanogaster*

James E.Castelli-Gair and Antonio García-Bellido

Centro de Biología Molecular, C.S.I.C.-Universidad Autónoma de Madrid, 28049 Madrid, Spain

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The activity of the *Ultrabithorax* gene is continuously required during imaginal development to maintain the morphogenetic identity of the third thoracic segment of *Drosophila*. The spatial pattern of *Ultrabithorax* gene expression depends on certain *cis* regulatory regions and several *trans* regulatory genes. Amongst the latter the *Polycomb* gene is necessary to maintain *Ultrabithorax* repressed in cells where it was not initially activated and the *trithorax* gene is required for maintaining the expression of the gene where initially active. We have studied genetic interactions between several *Ultrabithorax* mutations in coding and *cis* regulatory regions in combination with *Polycomb* and *trithorax* mutations. Our results suggest that *Polycomb* and *trithorax* gene products do not interact with *Ultrabithorax* protein products but interact (directly or indirectly) with specific and discrete *cis* regulatory regions such as those where *anterobithorax* and *postbithorax* but not *bithorax* mutations map. We discuss possible mechanisms of these interactions

Key words: cis control elements/*trans* regulatory genes/*Ultrabithorax*

Introduction

The expression of the *Ultrabithorax* (*Ubx*) gene during development is controlled by several *trans* regulatory genes. Its early transcription in the blastoderm is regulated by the maternal and gap genes (White and Lehmann, 1986; Irish *et al.*, 1989) as well as by pair-rule genes (Ingham and Martínez-Arias, 1986). Once transcription is initiated the *Ubx* spatial pattern of expression evolves by the control of genes of the segment polarity group (Martínez-Arias and White, 1988) and of other homeotic selector genes (Struhl and White, 1985; White and Wilcox, 1985). Later in development the continuous activity of the *Ubx* gene is required in the metathoracic imaginal disks to maintain the proper morphogenetic identity. Clones of *Ubx* loss-of-function mutant alleles induced in any stage of development, transform the metathorax into mesothorax (Morata and García-Bellido, 1976; Kerridge and Morata, 1982). Conversely, ectopic expression of *Ubx* in the second thoracic segment produces homeotic transformations towards third thoracic segment (Lewis, 1982; Cabrera *et al.*, 1985; White and Akam, 1985; Botas *et al.*, 1988). During this maintenance period a new set of *trans* regulatory genes is required for its correct spatial expression. These genes can be grouped in two classes; the genes of the *trithorax* group (*trx*-G) (also called *Regulator of bithorax* group) coding for products necessary to maintain *Ubx* activity (Ingham and Whittle, 1980; Capdevila and García-Bellido, 1981; Shearn *et al.*, 1987) and the *Polycomb* group (*Pc*-G) coding for products necessary to repress *Ubx* expression (Lewis, 1978; Struhl, 1981; Struhl and Akam, 1985; Jürgens, 1985; Capdevila *et al.*, 1986). Mutations in the *Pc*-G genes cause ectopic expression of the *Ubx* gene in the gastrula and larval stages without affecting its early blastoderm activation (Struhl and Akam, 1985; Wedeen *et al.*, 1986). Mutations in the *trx*-G genes cause loss of *Ubx* activity in late development but its early activation is not affected (Ingham, 1983). The *Pc* and *trx* genes are the best known of these two groups of genes whose products regulate *Ubx*, as well as other homeotic selector genes.

The *Ubx* gene is composed of three transcriptional units (Figure 1) (For reviews, see Duncan, 1987; Beachy, 1990). Only one of them, the *Ultrabithorax* unit (*Ubx*-U), is transcribed throughout development (Hogness *et al.*, 1985) giving rise to the *Ultrabithorax* morphogenetic proteins (UBX) responsible for *Ubx* activity (O'Connor *et al.*, 1988; Kornfeld *et al.*, 1989). The others correspond to the early and late transcripts of the *bithoraxoid* unit (*bxd*-U). The early *bxd*-U is transcribed in blastoderm and probably does not code for protein products (Akam *et al.*, 1985; Hogness *et al.*, 1985), the late *bxd*-U is transcribed in third larval and pupal stages and could encode a peptide of 101 amino acids (Lipshitz *et al.*, 1987). In the *Ubx*-U map several mutations (Lewis, 1955, 1982; Kerridge and Morata, 1982) which have been analysed molecularly (Bender *et al.*, 1983; Peifer and Bender, 1986; Akam *et al.*, 1985; Weinzierl *et al.*, 1987). There are mutant alleles affecting all UBXs, known generically as *Ubx* alleles, which when homozygous are embryonic lethal and show the same phenotype as the deficiency of the gene (Hayes *et al.*, 1984). Other alleles correspond to mutations in non-coding regions and do not change the UBX structure. However, they affect its spatial expression in different regions of the embryo and larva (Beachy *et al.*, 1985; Ingham, 1985b; White and Wilcox, 1985; Cabrera *et al.*, 1985; Botas *et al.*, 1988) suggesting they perturb *cis* regulatory elements of *Ubx* (Beachy *et al.*, 1985; Hogness *et al.*, 1985; Ingham, 1985b; Peifer *et al.*, 1987). The phenotype of these *cis* regulatory mutant alleles in the adult is the transformation of the anterior metathorax into anterior mesothorax in the case of *abx* and *bx* alleles; the transformation of the posterior metathorax into posterior mesothorax in the case of *pbx*, and the transformation of the first abdominal segment into thorax, along with a slight pbx transformation, in the case of *bxd*.

With the aim of identifying the specific regions of the *Ubx* gene responsible for the interactions with *trans* regulatory proteins involved in *Ubx* maintenance, we studied combinations of mutations in *Pc* and *trx* with mutations affecting different regions of the *Ubx* gene.

Results

trx and Ubx interactions

Heterozygous deficiencies for the *trx* gene ($trx^-/+$) produce in 2% of individuals, partial transformations of metathoracic into mesothoracic structures mainly in the anterior compartment. These transformations are erratic in position and asymmetric in expression and are therefore designated 'bithorax-variegated' (bx-v) phenotypes (Figure 2). The number of individuals showing transformations varies depending on the number of *Ubx* wild-type genes, from 18% with one dose to <0.5% in flies with an extra copy of the Ubx^+ gene (Capdevila and García-Bellido, 1981).

In order to ascertain which regions of the *Ubx* gene contribute to these dose effects we have studied heterozygous combinations for both deficiency $Df(3R)red^{P52}$, lacking the *trx* gene (trx^-) and different *Ubx* mutant alleles affecting different elements in *Ubx*. These mutations correspond to lack of UBX or perturbations of *cis* regulatory regions (*abx, bx, bxd, pbx*). For this study we have used *Ubx* mutant alleles in which the molecular nature and location of the mutation in *Ubx* is known (Figure 1), helping us to define the extent and nature of the particular element involved in the interaction. We shall first analyse pseudopoint (not visible cytologically) mutations in the *Ubx* gene and subsequently chromosome rearrangements with a breakpoint in the gene.

In combinations of trx^- with *Ubx* pseudopoint mutations that only affect UBX (not the *cis* regulatory regions), the percentage of bx-v transformations remains as in wild-type flies (Table I). This result applies to all kinds of pseudopoint mutations affecting the *Ubx* coding region [deletions of the homeobox in $Ubx^{9.22}$ (Bender *et al.*, 1983; Akam *et al.*, 1985; Weinzierl *et al.*, 1987), Ubx^{MX18} and Ubx^{MX15} (Vernós, 1989); a point mutation introducing a stop codon in Ubx^{195} (Weinzierl *et al.*, 1987); and an insertion of a transposable element, that probably abolishes UBX, in Ubx^1 (Bender *et al.*, 1983; Hogness *et al.*, 1985)].

Interactions with mutations that only affect *Ubx cis* regulatory elements yield different results. In the intronic region of the *Ubx*-U map *abx* and *bx* alleles. Combinations with any *abx* allele increase the percentage of bx-v transformations to similar levels to those observed with a complete deficiency of *Ubx*. In contrast, heterozygotes carrying *bx* alleles do not show a stronger bx-v phenotype, confirming previous results (Capdevila and García-Bellido, 1981), with the exception of two *bx* alleles: bx^{F31} and $bx^{34e-prv}$. The bx^{F31} allele is caused by the insertion of an I transposable element in the 5′ end of the region affected by *abx* deletions (Peifer and Bender, 1986) (see Figure 1). The $bx^{34e-prv}$ allele is a partial revertant of bx^{34e} in which the gypsy element has disappeared causing a 9.5 kb deletion of *Ubx* DNA overlapping one of the *abx* deletions (Peifer and Bender, 1986).

Interactions with different *bxd* alleles do not affect the frequency of *trx* transformations. Interactions with *pbx* deletions increase it, but now a large fraction of the trans-

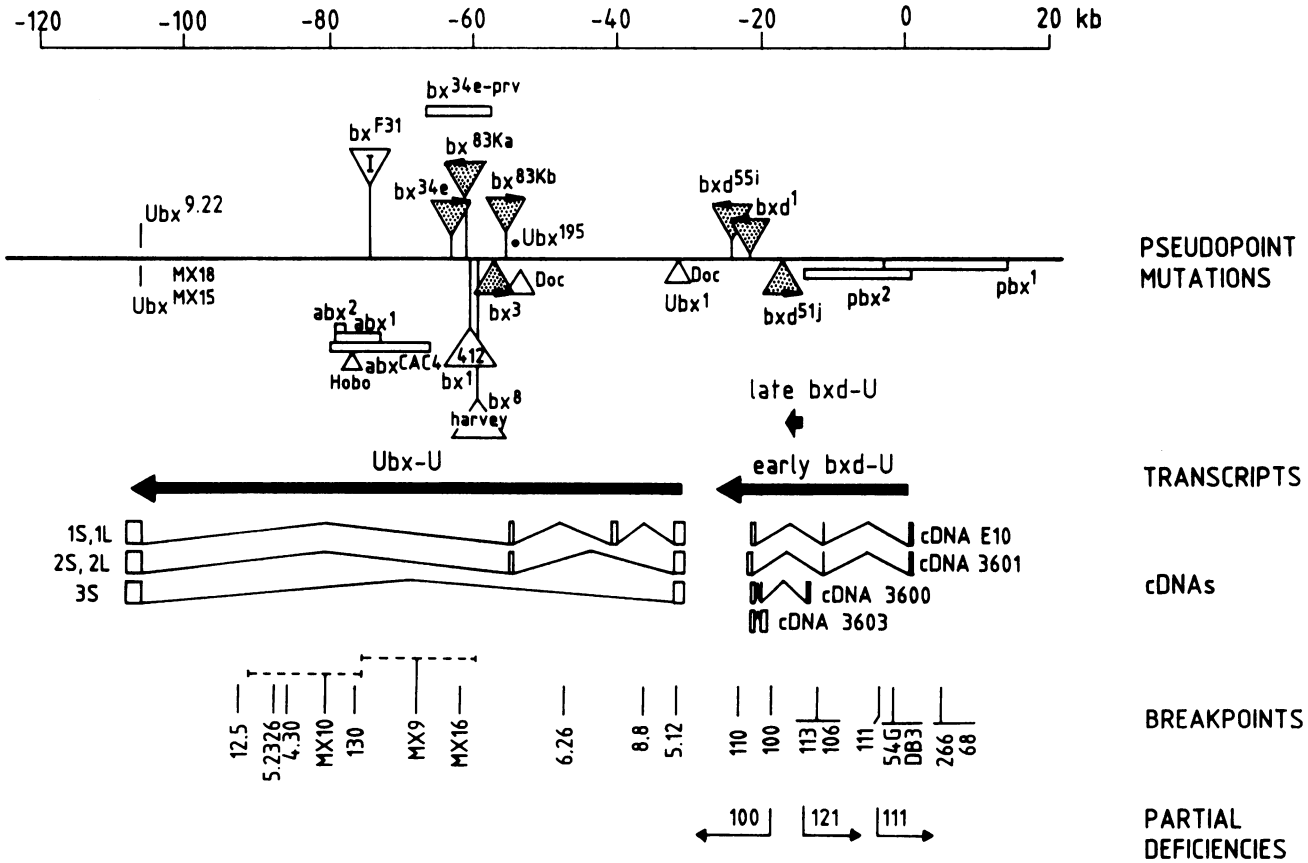

Fig. 1. Molecular map of the *Ubx* gene showing the mutations used in this work. The three transcription units of the *Ubx* gene are shown as thick arrows. The *Ubx*-U gives rise by differential splicing to the different proteins responsible for all morphogenetic *Ubx* functions. *Ubx* pseudopoint mutations abolish all UBX functions, while *abx, bx, pbx* and *bxd* mutations affect *cis* regulatory elements necessary for *Ubx* spatial expression. Rectangles indicate DNA deletions and triangles DNA insertions of transposable elements indicated by their name. Dotted triangles correspond to gypsy insertions with an arrow indicating the direction of their transcription. (Data from Bender *et al.*, 1983, 1985; Peifer and Bender, 1986; Lipshitz *et al.*, 1987; O'Connor *et al.*, 1988; Weinzierl *et al.*, 1987).

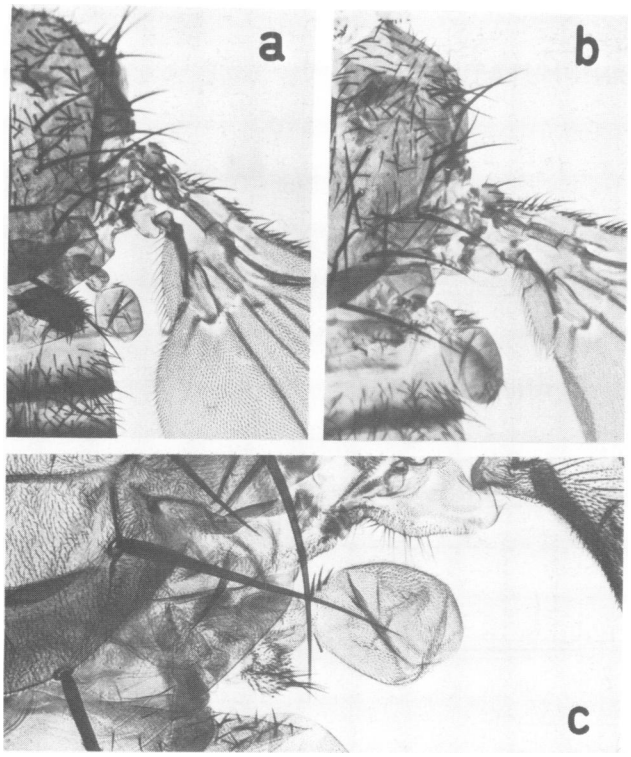

Fig. 2. *trithorax* mutant transformations. (**a**) Partial transformation of the metanotum (which is devoid of chaetae) into mesonotum (with chaetae). (**b**) Partial transformation of the anterior haltere into anterior wing showing the presence of structures of wing costa. (**c**) Partial transformations of the posterior haltere into posterior wing (allula) and anterior structures (costa). The genotype of all flies is $trx^{-}/+$; *abx bx pbx*/+.

formations are to posterior mesothoracic structures rather than to anterior ones. This effect with pbx^{1} passed unnoticed (Capdevila and García-Bellido, 1981) possibly because most transformations are small and difficult to classify as occurring in the posterior compartment.

We have also studied interactions of trx^{-} with *Ubx* mutations associated with breakpoints in the gene or carrying partial deficiencies of it (Table I). Breakpoints in the *bxd*-U do not directly affect the *Ubx* protein coding region but separate possible *cis* regulatory regions from the *Ubx* promoter. In combinations with these *bxd*-U breakpoints, no significant increase of the bx-v transformations was observed. All breakpoints in the *Ubx*-U certainly affect the UBXs but they can also perturb *Ubx cis* regulatory elements. And yet, of these mutations, only breakpoints in the *Ubx*-U that map near the region affected by *abx* deletions (Figure 3) increase the frequency of the bx-v transformations. Those mapping both 5′ or 3′ to it do not modify these frequencies.

Partial deficiencies can also increase bx-v transformations. Thus $Dfbxd^{100}$ (a complete deletion of the *Ubx*-U) increases anterior transformations (bx-v) in the metathorax, while $Dfbxd^{121}$ and $Dfbxd^{111}$ [which delete the most 5′ regions of the *bxd*-U including the *pbx* region (Figure 1)] cause an increase in transformations in the posterior metathorax. These results suggest that *trx* products maintain *Ubx* activity interacting with the *Ubx* gene in functions or structures associated with specific *cis* regulatory regions (*abx* and *pbx*), but not the *Ubx* protein products.

Pc and Ubx interactions in the mesothorax

The *Pc* gene has been interpreted by its genetic behavior as coding for a repressor of *Ubx* activity (Lewis, 1978; Capdevila and García-Bellido, 1981). This has been

Table I. Increase in the penetrance of trx^{-} heterozygous phenotype in combination with heterozygous *Ubx* alleles

	$trx^{-}/+$, '*bx*'/+		$trx^{-}/+$, +/+			$trx^{-}/+$, '*bx*'/+		$trx^{-}/+$, +/+	
	n	%	*n*	%		*n*	%	*n*	%
Control					Breakpoints				
+	314	1.2			$Ubx^{5.12}$	151	1.3	112	0
DfP9	94	12.7	109	0	$Ubx^{8.8}$	114	0	60	0
					$Ubx^{6.26}$	108	0.9	73	1.3
Pseudopoint					Ubx^{MX16}	175	5.7	152	1.3
					Ubx^{MX9}	226	7.0	132	0.7
Ubx^{1}	143	0	81	0	Ubx^{130}	195	21.0	144	2.7
Ubx^{195}	109	0	107	0	Ubx^{MX10}TM1	244	40.9	251	1.5
$Ubx^{9.22}$	122	4.1	105	0	$Ubx^{4.30}$	124	17.7	134	0.7
Ubx^{MX18}	206	0.4	121	0.8	$Ubx^{5.2326}$	148	0	129	0
Ubx^{Mx15}	123	2.4	256	0.7	$Ubx^{12.5}$	112	2.6	107	0.9
abx^{1}	152	17.7	109	0.9	$bx^{1}Tpbxd^{110}$	217	0	96	1.0
abx^{2}	112	21.4	110	1.8	$Tpbxd^{100}$	77	0	66	1.5
abx^{CAC4}	330	24.8	94	1.0	bxd^{113}	154	0.6	129	0
					$bx^{3}bxd^{106}$	92	0	77	0
bx^{F31}	332	19.5	80	3.7	$Tpbxd^{111}$	101	6.9	185	0.5
bx^{1}	130	0.7	44	0	bxd^{54G}	152	1.3	134	0
bx^{34e}	92	4.3	ND		bxd^{DB3}	122	1.6	47	2.1
bx^{3}	161	2.4	91	1.0	bxd^{266}	114	0	ND	
bx^{83ka}	182	1.0	157	0.6					
bx^{83kb}	196	0	194	0.5	Deficiencies				
bx^{8}	123	1.6	120	4.1					
$bx^{34e\text{-}prv}$	166	15.0	96	0	$Dfbxd^{100}$	193	9.8	198	0
					$Dfbxd^{111}$	121	16.5	224	0.4
bxd^{55i}	112	0	ND		$bx^{34e}Dfbxd^{121}$	117	16.2	89	1.1
bxd^{1}	144	1.3	113	0	*DfP9*	94	12.7	109	0
bxd^{51j}	182	1.0	50	0					
pbx^{1}	356	11.8	281	0.7					
pbx^{2}	430	7	253	0.8					

The number of individuals studied for each experiment (*n*), and the penetrance of the phenotype as a percentage of individuals with a transformation (%) is represented. As an internal control the value of the trx^{-}/Balancer siblings is presented. *DfP9* lacks the *Ubx* gene. ND, not determined.

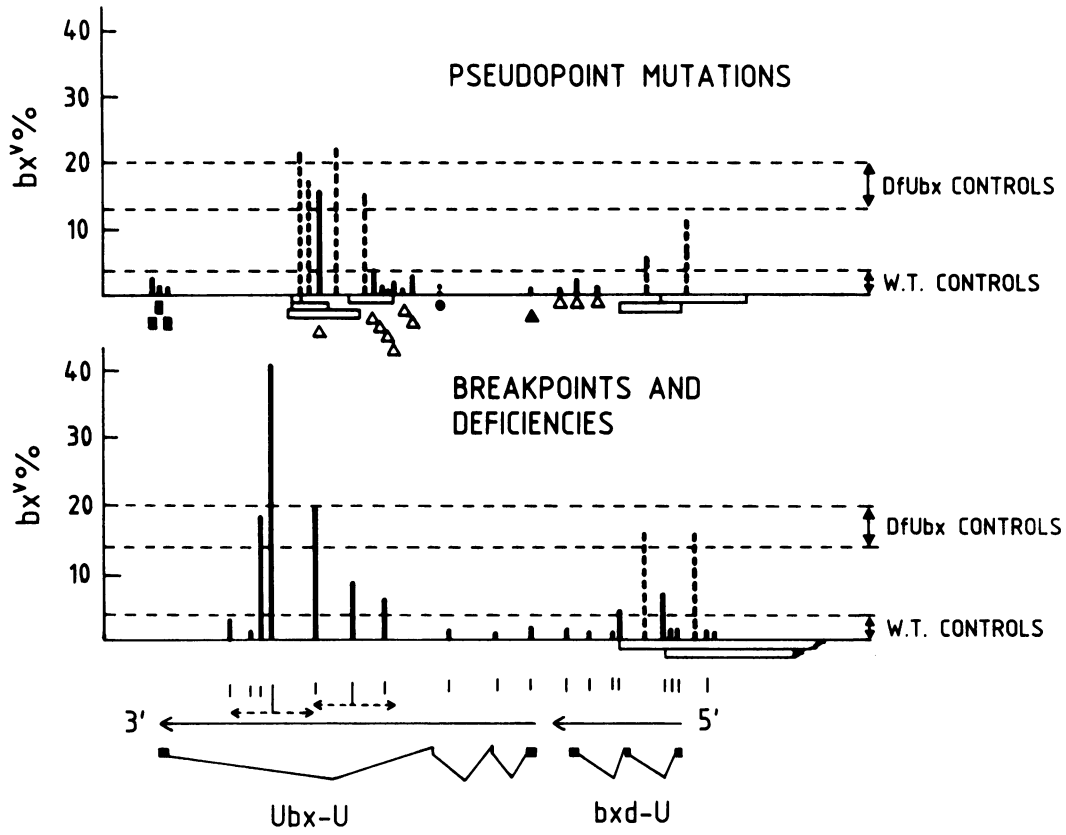

Fig. 3. Penetrance of *trx*$^-$ transformations in heterozygotes with *Ubx* mutant alleles. The number of flies showing transformations of haltere to wing (bx-v) is represented in percentages by a bar (discontinuous for deletions and deficiencies) along the map position of the mutation considered. Symbols for mutations are as in Figure 1. In both graphs the intervals represent the limits of control values obtained with one or two *Ubx*$^+$ doses. Observe the significative increases in mutants affecting the *abx* and *pbx* regions (in this representation bx-v includes anterior and posterior compartment transformations).

confirmed by the observation that in Pc^3 homozygous embryos *Ubx* is expressed in regions where normally it is absent (Beachy *et al.*, 1985; Wedeen *et al.*, 1986). The Pc^3 mutation when heterozygous produces ectopic expression of *Ubx* in the wing disc causing partial transformations to metathorax. As in the *trx* interactions this effect is proportional to the number of *Ubx*$^+$ genes present in the organism (Duncan and Lewis, 1982; Capdevila *et al.*, 1986; Botas *et al.*, 1988). Pc^3 heterozygotes show strong transformations of wing towards haltere with three *Ubx*$^+$ genes, less so with two, and are virtually wild-type in individuals carrying only one *Ubx*$^+$ gene.

As we did in the previous section for *trx* here we study the interactions of Pc^3 and different *Ubx* mutant alleles in heterozygotes (Table II). Mutations that abolish UBX function normalize the wing towards haltere transformation caused by *Pc* mutants. This result was expected since the ectopic expression of a mutant UBX lacks the morphogenetic activity responsible for the visible homoeotic transformation. Pseudopoint mutations in the *Ubx*-U *cis* regulatory regions that do not affect UBX have no effect, with some exceptions, on the ectopic UBX expression. Again *abx* alleles provide one such exception. Breakpoints in the *bxd*-U do not affect the mutant transformation either, with the exception of $Tpbxd^{100}$ which increases it.

Since there are no large phenotypical differences between $Pc^3/+$ and wild-type wings we have studied the same *Ubx* combinations in flies heterozygous for both Pc^3 and

Table II. *Pc*-G mutant mesothoracic phenotypes in combination with several *Ubx* mutant alleles

	$Pc^3/+$	$Pc^3Scm^{XF24}/++$
Control		
+/+	B	D
Pseudopoint		
$Ubx^1/+$	A	B
$Ubx^{9.22}/+$	A	B
$abx^1/+$	A	B
$abx^2/+$	A	B
$abx^{CAC4}/+$	A	B
$bx^{34e}/+$	B	D
$bx^{83Ka}/+$	A	C
$bx^1/+$	B	D
$bx^8/+$	ND	D
$bx^3/+$	B	D
$bx^{83Kb}/+$	A	D
$bxd^1/+$	B	E
$bxd^{51j}/+$	B	D
$pbx^1/+$	B	D
$pbx^2/+$	A	D
Breakpoints		
$bx^1Tp(3)bxd^{110}/+$	A	D
$Tp(3)bxd^{100}/+$	C	E
$In(3)bxd^{113}/+$	A	C
$bx^3In(3)bxd^{106}/+$	B	D
$T(1,3)bxd^{111}/+$	A	D
$bxd^{54G}/+$	B	C
$T(2,3)bxd^{DB3}/+$	B	D
Deficiencies		
$Df(3)bxd^{100}/+$	A	B
$Df(3)bxd^{111}/+$	A	B
$Df(3)bxd^{121}/+$	A	B
$Df(3)Ubx^{109}/+$	A	B

Phenotypes are classified from A (wild-type) to E (maximal wing to haltere transformation found). ND, not determined.

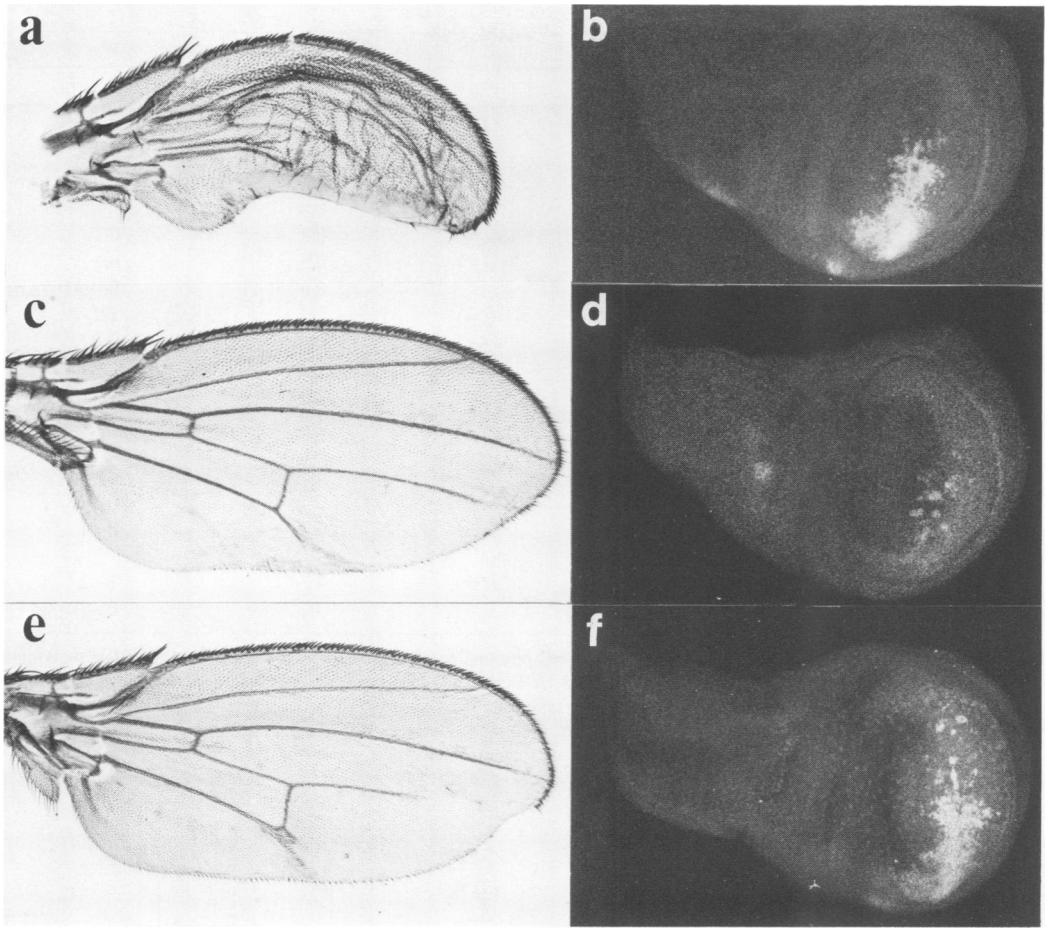

Fig. 4. Adult phenotypes and UBX expression of Pc^3 Scm^{XF24} heterozygotes in different *Ubx* mutant backgrounds. (**a–b**) Pc^3 $Scm^{XF24}/++$ wing and wing disc. The transformation to haltere corresponds to the region expressing UBX as detected by FP3.38 with immunostaining. (**c–d**) Pc^3 $Scm^{XF24}/++$; $abx^1/+$. Both adult wing transformation and UBX expression in the wing disc are reduced (compare with a–b) showing that abx^+ is necessary for *Ubx* ectopic expression in a *Pc*-G mutant background. (**e–f**) Pc^3 $Scm^{XF24}/++$; $Ubx^{9.22}/+$ wing and wing disc. The adult wing transformation to haltere is reduced as in (c), although the signal in discs is as in (b), indicating that there is no impairment of *Ubx* transcription in $Ubx^{9.22}$ mutants. ($Ubx^{9.22}$ is a homeobox deletion; it codes for UBX detectable by FP3.38 antibody but without morphogenetic activity.)

Sex comb on midleg (Scm^{XF24}), another member of the *Pc*-G. These individuals show a more extreme mutant transformation in the wing (Jürgens, 1985; Botas, 1985) correlating with an increase in UBX expression as detected by immunofluorescence with FP3.38 (see Figure 4a and b) and any reduction in the phenotype would be more readily classifiable. The results obtained in these combinations (Table II) are consistent with those obtained with Pc^3 heterozygotes. Thus, mutations affecting UBX normalize the wing phenotype. However, in cases where the *Ubx* mutation gives rise to an inactive UBX detectable by FP3.38 antibody (e.g. $Ubx^{9.22}$), it is possible to see that the mutant protein product is still being expressed (Figure 4e and f). Again, as in Pc^3 heterozygotes the only *cis* regulatory mutations reducing the wing phenotype are those affecting the *abx* region. This is true both for the adult transformation and for the pattern of protein expression (see Figure 4c and d).

Pc and Ubx interactions in the metathorax

In this section we analyse the effects of *Pc* insufficiency on the expression of *Ubx* in the metathorax, in order to ascertain if *Pc* insufficiency is also differentially perceived by the same *cis* regulatory regions in cells where *Ubx* expression occurs normally in development.

In Pc^3 interactions with *Ubx* mutant combinations we have first to ascertain possible effects of *Pc* insufficiency on general increase of *Ubx* transcription that would rescue, partially or totally, all weak *Ubx* metathoracic transformations due to partial loss-of-function mutations (Capdevila *et al.*, 1986). This is not the case in heterozygous combinations of Pc^3 with Ubx^1 where only the transformations of *abx*, *bxd* and *pbx* alleles are rescued (Table III). In addition, we have studied Pc^3 combinations with mutations affecting *Ubx cis* regulatory regions over *DfUbx*, in such a way that the observed effects result from the *Pc* interactions with the gene carrying the *Ubx* mutant allele, the only *Ubx* gene present in the genome. In these conditions (Table III, Figure 5) the rescue of the metathoracic mutant phenotype is not proportional to the extent of the transformation (which reflects the degree of loss-of-function of the specific alleles), but depends on the particular type of allele. Thus, there is rescue with all *abx* and *bxd* alleles, while the phenotypes of *pbx* and many *bx* alleles remain as in Pc^+ controls.

The fact that a Pc^3 background is capable of rescuing the pbx phenotype of pbx^1/Ubx^1 but not that of $pbx^1/DfUbx$ (Figure 6) may be due to 'transvection' of the Ubx^1 with its homologue (Lewis, 1954, 1982; Kerridge and Morata,

Table III. Reduction of metathoracic mutant phenotypes in a Pc^3 heterozygous background

	Df(3R)UbxP9			*Ubx*^{1}		
	+/+	Pc^3/+		+/+	Pc^3/+	
abx^1	2−4/3−4	0−3/1−3	(−)	0−2/1−2	0/1	(−)
abx^2	2−3/3−4	0−1/1−3	(−)	0−2/1−2	0/1	(−)
abx^{CAC4}	4/4	1−3/2−4	(−)	0−2/3	0/2−3	(−)
bx^{F31}	1/2	1/2	(=)	0/1	0/1	(=)
bx^{34e}	3/2	3/2	(=)	0/1	0/1	(=)
$bx^{34e\text{-}prv}$	0−2/0−3	ND		0−2/0−1	0/0−1	(−)
bx^{83Ka}	4/4	4/4	(=)	1/2	1/2	(=)
bx^1	0−1/0−1	0−(1)/0	(−)	0/0	0/0	(=)
bx^8	4/4	4/4	(=)	2/3	2/2	(−)
bx^3	4/4	4/4	(=)	1/3	1/3	(=)
bx^{83Kb}	4/4	4/4	(=)	1/3	1/3	(=)
bxd^{55i}	1	0−1	(−)	1	0−1	(−)
bxd^1	2	0−1	(−)	2	0−1	(−)
bxd^{51j}	2	0−1	(−)	2	0−1	(−)
pbx^1	4	4	(=)	4	1−2	(−)
pbx^2	4	4	(=)	1	0−1	(−)

Heterozygotes between *DfUbx* (*Df(3)P9*), and *Ubx*^{1} over different *abx, bx, pbx* and *bxd* mutant alleles were analysed in a Pc^3/+ compared with a wild-type background. The expressivity of the phenotypes is represented by figures (0, wild-type; 4, complete transformation of anterior metanotum to anterior mesonotum) as described in Materials and methods. For the anterior metathorax proximal and distal values are given separately (notum/haltere), while for the posterior metathorax only distal values are presented due to the difficulty of classifying transformations in the postnotum. Symbols in brackets indicate whether the Pc^3 mutation rescues mutant phenotype (−), or leaves it unaffected (=). ND, not determined.

1982) opening the question of a possible role of *Pc* in the mechanism of transvection. Transvection in *Ubx* reflects partial complementation between *cis* regulatory elements present in a chromosome unable to produce UBX (*Ubx*^{1} in this case), with another homologue capable of producing UBX, but lacking the *cis* regulatory element (*pbx* in this case). Therefore in combinations with *DfUbx*, *pbx* would be unable to complement any *cis* regulatory function from the homologue. We have analysed if breakpoints proximal to *Ubx* or *zeste* mutant alleles that perturb transvection (Lewis, 1954; Babu and Bhat, 1981; Micol and García-Bellido, 1988) prevent the rescuing caused by Pc^3 alleles. As seen in Figure 6 the rescue of the pbx transformation by *Pc* insufficiency is abolished under these conditions. In addition, we have also studied the phenotype of Pc^3 heterozygous individuals with no *pbx* regulatory regions, but with two wild-type *Ubx*-U. As shown in Figure 6e, there is no rescue of the pbx transformation by *Pc* insufficiency. This indicates that in the specific control of *Ubx* expression in the posterior metathorax. *Pc* interaction is mediated through *pbx cis* regulatory elements.

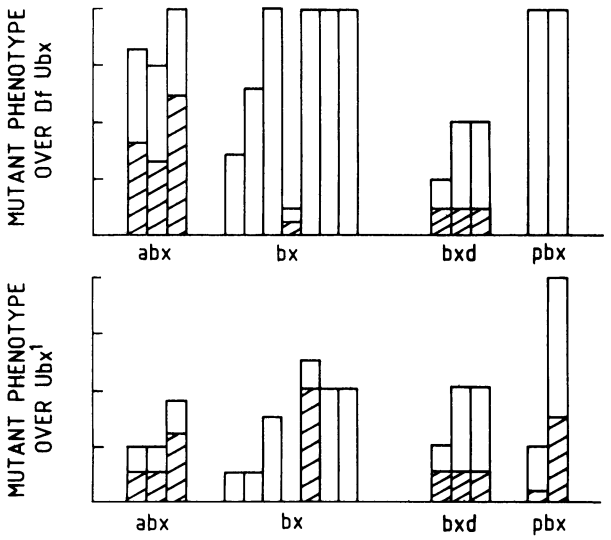

Fig. 5. Rescue of different *Ubx* mutant metathoracic phenotypes in a Pc^3 heterozygous background. The phenotype of recessive alleles in hemizygous individuals (upper graph) and with *Ubx*^{1} (bottom graph) represented in percentages of maximal transformations by a white bar. The same combinations may show a reduced phenotype (represented by a hatched bar) in a Pc^3 heterozygous background. Where hatching is not shown the phenotypes are unaffected. The alleles are represented in the order of their DNA perturbations (see Figure 1).

Discussion

trx interacts with abx and pbx cis regulatory regions

Loss-of-function *trx* alleles cause *Ubx* loss-of-function phenotypes (Ingham and Whittle, 1980), due to the loss of UBX expression in metathoracic cells (Cabrera *et al.*, 1985). The frequency of these phenotypes is increased in heterozygotes with *Ubx* deficiencies or certain mutations in the *Ubx* gene (Capdevila and García-Bellido, 1981; this work). The detailed analysis of these different mutations has shown that the increase in *trx* transformations is due to perturbations in *cis* regulatory regions not involved in UBX coding, in particular the *abx* region and the *pbx* region. These effects consist in homeotic transformations of metathorax to mesothorax in the anterior compartment in the case of *abx* and in the posterior one in the case of *pbx* alleles. Although in many of these cases the alleles correspond to DNA deletions it is not a singularity of this kind of mutation, as the insertion of an I transposable element and breakpoints in the *abx* region produce the same effect as *abx* deletions. Interestingly, this effect is found in heterozygotes with chromosome rearrangements with breakpoints within the *abx* region, but not outside of it (either proximal or distal

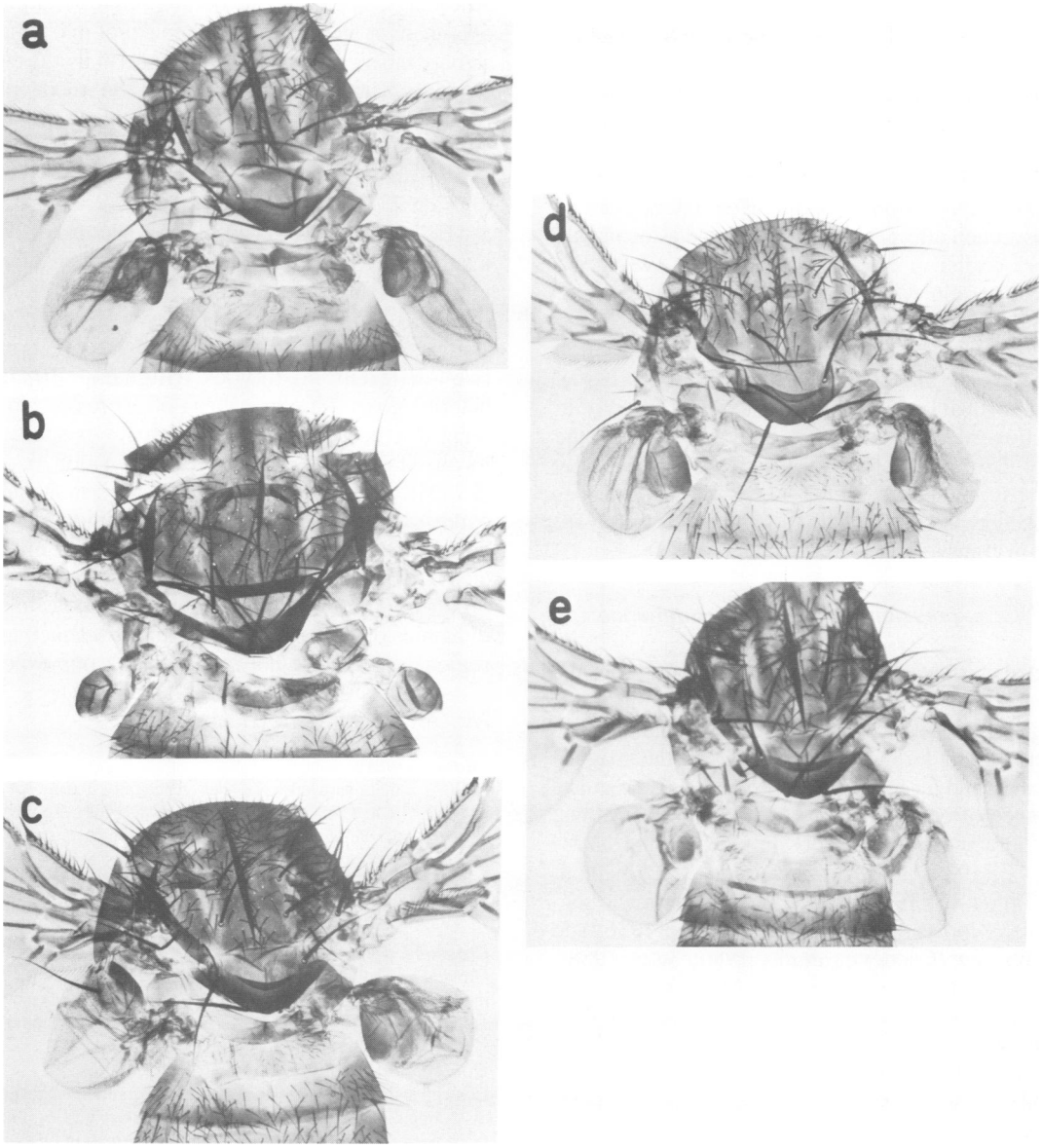

Fig. 6. Rescue of *pbx* phenotypes in different mutant backgrounds. (**a**) Ubx^1/pbx^1. The posterior compartment of the metathorax is transformed into posterior wing. (**b**) $Pc^3/+;Ubx^1/pbx^1$. The posterior haltere phenotype is rescued by the presence of Pc^3 heterozygous. (**c**) $Pc^3/+;Ubx^1/R(pbx^1)$. The same genotype as in (b) but the *pbx* allele is associated to a rearrangement (R: T(2;3)bw^{VDe3}) perturbing transvection. In these conditions *Pc* insufficiency does not reduce the pbx phenotype. (**d**) $z^{a69.3}$; $Pc^3/+$; Ubx^1/pbx^1. The same genotype as in (b) but with a *z* homozygous allele perturbing transvection. In this case *Pc* insufficiency cannot reduce the pbx phenotype. (**e**) $Pc^3/+;pbx^1/pbx^1$. In homozygotes for *pbx* deletions Pc^3 insufficiency cannot rescue the pbx phenotype, even in the presence of two functional *Ubx*-Us.

to the *Ubx*-U promoter). These findings suggest that these regions contain specific *cis* regulatory elements involved in the maintenance of *Ubx* expression. It would be with these elements with which the *trx* gene products directly or indirectly interact.

Pc interacts with abx and pbx cis regulatory regions

Pc is a member of a group of genes (*Pc*-G) that according to genetic results repress *Ubx* during development. We have observed that in the mesothoracic wing imaginal disc the derepression of *Ubx* caused by *Pc* insufficiency is abolished by heterozygous *abx* mutant alleles, but not by *bx* or mutant alleles affecting the UBX protein. In the metathorax, *Pc* insufficiency reduces *abx* mutant transformations but not *bx* ones and can also reduce *pbx* transformations if the mutant combination still has at least a *pbx cis* regulatory region. This is the case in the reduction of the pbx transformation of *bxd* alleles which are not null for *pbx* function (Lewis, 1955, 1985), and for Ubx^1/pbx heterozygotes where the *pbx cis* regulatory region of Ubx^1 chromosome is not damaged by the mutation (Micol *et al.*, 1990). The interpretation that the rescuing effect of *Pc* insufficiency upon the pbx transformation is due to the remaining *pbx* wild-type function is reinforced by the observation that the pbx phenotype in *pbx* homozygotes or heterozygotes over *DfUbx* is not rescued by Pc^3 insufficiency (Figure 6e). The finding that *bx* and *abx* mutations behave differently in interaction with *Pc* as well as with *trx*, suggests that *abx* is affecting a *cis* regulatory element necessary for *Ubx* gene maintenance not affected by *bx* mutations. This conclusion agrees with E.B. Lewis's suggestion (1982) that *abx* and *bx* mutations affect different *Ubx* gene functions.

Transvection and Pc function

Transvection has been defined as allelic complementation depending on pairing (Lewis, 1954). This complementation is usually detected between alleles affecting different *cis* regulatory elements of a gene or a *cis* regulatory element in one homologue and the protein products in the other (For reviews see Judd, 1988; Wu and Goldberg, 1989). Recently, several cases of suppression of *zeste−white* interactions (a classical transvection effect) have been reported as being due to *Pc*-G genes (Adler *et al.*, 1989; Wu *et al.*, 1989). We have seen that certain *Ubx* transvection interactions (e.g. Ubx^{1}/*pbx*) are also sensitive to the level of *Pc* products and similar results were obtained with other *Ubx* transvection interactions in *Cbx* alleles (Castelli-Gair *et al.*, 1990). However it is probable that *Pc* is not involved directly in the transvection mechanism, as it does not modify the phenotype of *Ubx*/*bx* combinations which are classically affected in transvection experiments (Lewis, 1954), and does modify the phenotype of *abx*/*DfUbx* combinations which are not affected in transvection experiments.

Control of Ubx expression during the maintenance period

The *Ubx* gene is activated early in embryogenesis. After the initial activation in particular segmental domains it is maintained in its spatial realm of expression. Maintenance in embryogenesis and during cell proliferation of the imaginal discs requires *Pc* and *trx* products. These genes also regulate the maintenance of other selector genes' expression; thus, in the metathoracic cells *Pc* is possibly repressing *abd-A* (Wedeen *et al.*, 1986) and *trx* maintaining *Ubx* (Ingham, 1985b; Cabrera *et al.*, 1985), while in the prothoracic segment *Pc* is also repressing *Ubx* (Wedeen *et al.*, 1986) and *trx* maintaining *Scr* (Capdevila *et al.*, 1986; Sato, 1988). Molecular analysis has shown that the spatial expression of *trx* and *Pc* is ubiquitous (Mozer and Dawid, 1989; R.Paro personal communication). We have shown that both *Pc* and *trx* modulate *Ubx* phenotypes in both meso and metathorax. In this modulation they distinguish the same *cis* regulatory regions (*abx* and *pbx*) and they do not appear to interact with the *bx* region, nor UBX products. The fact that *Pc* and *trx* elements do not interact with UBX in the epidermis is in accordance with other results showing that UBX products are not involved in their positive autoregulation in the ectoderm, although they are in the visceral mesoderm (Bienz *et al.*, 1988; Bienz and Treml, 1988; Beachy *et al.*, 1988).

From our experiments we cannot conclude whether or not *trx* and *Pc* products are interacting with *abx* and *pbx* regions directly by binding to the *cis* regulatory region, or interacting with other *trans* regulatory elements involved in the process of controlling transcription from the *Ubx* promoter. Gene dose titration analysis has shown that *Pc* and *trx* act as antagonists in *Ubx* control (Capdevila and García-Bellido, 1981). The fact that the *Pc* protein binds in polytene chromosomes to the *Ubx* region (Zink and Paro, 1989) and that the DNA sequence of the *trx* gene has domains with DNA binding properties (Mazo *et al.*, 1990), suggests that, during maintenance of *Ubx* expression, the *Ubx cis* regulatory regions interact with *Pc*/*trx* gene products and the *Ubx* promoter later.

The question of how those common *trans* regulatory elements specifically maintain the correct gene activity in different segments could be answered assuming that they operate over an ongoing mechanism of maintenance of *Ubx* activity inherited from early blastoderm by the *cis* regulatory elements. Thus, *abx* would be in an 'on' mode in the anterior metathorax interacting with *trx* to maintain *Ubx* activity, while in more anterior segments *abx* would be in 'off' mode and *Pc* maintain its repression over *Ubx* through subsequent divisions.

This model does not imply that *Pc* or *trx* heterozygous insufficiencies act irreversibly on the state of *Ubx* activity in proliferating cells. In fact, although *trx* transformations appear as erratic and compact spots of wing tissue, mosaic analysis has shown that clones of marker cells can cross the two different histotypes (Ingham, 1985a). In *Pc* heterozygotes the *Ubx* pattern of expression in cells of the wing disc caused by *Pc* insufficiency is spotty, regionally localized and the adult transformation is not clonal (J.L.Micol, unpublished). Thus the maintenance of *Ubx* expression in the haltere and its repression in the wing discs is reversible and must be exposed to the effects of other elements in addition to the availability of *Pc* and *trx* gene products. Those could be regional variations in proliferation dynamics or interactions with other *trans*-acting genes with regional specificity not monitored in our experiments.

Materials and methods

Mutant stocks

All *Ubx* mutations used have been genetically and molecularly analysed in Lewis (1978, 1982); Kerridge and Morata (1982); Vernós (1989); Bender *et al.* (1985); Peifer and Bender (1986); Weinzierl *et al.* (1987).

Pc^{3} is described in Duncan and Lewis (1982); Scm^{XF24} in Jürgens (1985) and Wu *et al.* (1989). *Df(3R) red*^{P52} is described in Capdevila and García-Bellido (1981).

Crosses

Crosses and cultures were carried out at 25°C. Since *trx* mutations have maternal effects (Ingham and Whittle, 1980; Capdevila and García-Bellido, 1981), *Df red*^{P52} was carried in all crosses by the mother.

Observation of phenotypes

Control and experimental genotypes were simultaneously studied under dissecting microscope and classified in arbitrary classes [in the mesothorax from A (wild-type) to E (maximum transformation observed), and in the metathorax from 0 (wild-type) to 4 (maximum transformation)]. For the anterior metathorax, proximal (notum) and distal (haltere) values are given separately, while for posterior metathorax only distal values are presented due to the difficulty of classifying transformations in the postnotum. Variable expressivity is represented by ranges between extreme values.

Antibody staining

Immunofluorescent staining of third instar larval imaginal discs was done with the FP3.38 anti-UBX monoclonal antibody as described in White and Wilcox (1984).

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