Development 117, 119-134 (1993)
Printed in Great Britain © The Company of Biologists Limited 1993

trithorax regulates multiple homeotic genes in the bithorax and Antennapedia complexes and exerts different tissue-specific, parasegment-specific and promoter-specific effects on each

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SUMMARY

The *trithorax* (*trx*) gene is required for normal development of the body plan in *Drosophila* embryos and adults. Mutations in *trx* cause homeotic transformations throughout the body. Genetic studies suggest that *trx* encodes a positive regulatory factor required throughout development for normal expression of multiple homeotic genes of the bithorax and Antennapedia complexes (BX-C and ANT-C). To determine how *trx* influences homeotic gene expression, we examined the expression of the BX-C genes *Ultrabithorax, abdominal-A, Abdominal-B* and the ANT-C genes *Antennapedia, Sex combs reduced* and *Deformed* in *trx* embryos. We show that *trx* does indeed exert its effects by positively regulating homeotic gene expression and that its effects on expression of individual homeotic genes are complex: each of the BX-C and ANT-C genes examined exhibits different tissue-specific, parasegment-specific and promoter-specific reductions in their expression. This implies that each of these genes have different requirements for *trx* in different spatial contexts in order to achieve normal expression levels, presumably depending on the promoters involved and the other regulatory factors bound at each of their multiple tissue- and parasegment-specific *cis*-regulatory sites in different regions of the embryo. These results also imply that those components of homeotic gene expression patterns for which *trx* is dispensable, require other factors, possibly those encoded by other *trithorax*-like genes.

Key words: *Drosophila* development, Homeotic gene regulation, trans-regulatory factor, *trithorax*

INTRODUCTION

The homeotic genes of the bithorax and Antennapedia complexes (BX-C and ANT-C) encode a family of related transcription-regulatory proteins required for the determination of the segmental identities of cells throughout the body (Scott and Carroll, 1987; Akam, 1987; Ingham, 1988; Scott et al., 1989; Kaufman et al., 1990). Expression of each homeotic gene is activated at the blastoderm stage and restricted to a unique set of cells within stereotypical parasegmental and segmental boundaries along the anteroposterior axis of the embryo (Scott et al., 1983; White and Wilcox, 1984; Beachy et al., 1985; Harding et al., 1985; Kuroiwa et al., 1985; Struhl and Akam, 1985; Martinez-Arias et al., 1987; Kuziora and McGinnis, 1988a; Pultz et al., 1988; Sánchez-Herrero and Crosby, 1988; Kornfeld et al., 1989; Celniker et al., 1989; Diederich et al., 1989). Development of the normal body plan requires continuous expression of each homeotic gene throughout development. Loss of expression of a homeotic gene at any time during development causes transformation of the segmental identity of cells normally expressing that gene (Lewis, 1963; Morata and García-Bellido, 1976; Struhl, 1981, 1982; Merrill et al., 1987; Diederich et al., 1989). Conversely, ectopic homeotic gene expression can cause transformation of the segmental identities of cells normally not expressing that gene (Schneuwly et al., 1987; González-Reyes and Morata, 1990; González-Reyes et al., 1990; Mann and Hogness, 1990). Establishment and maintenance of stable heritable patterns of homeotic gene expression is therefore essential for development of the normal body plan. Initially, homeotic gene expression patterns are modulated by transiently expressed segmentation gene products (Duncan, 1986; Ingham et al., 1986; Ingham and Martinez-Arias, 1986; White and Lehmann, 1986; Riley et al., 1987; Harding and Levine, 1988; Irish et al. 1989; Ish-Horowicz et al., 1989; Tremml and Bienz, 1989a; Jack and McGinnis, 1990; Reinitz and Levine, 1990). However, expression of the segmentation genes subsides during gastrulation and additional factors are required to sustain homeotic gene expression patterns throughout the rest of development.

At least four classes of gene functions are required to maintain the patterns of homeotic gene expression initiated at the blastoderm stage. Sustained repression of each homeotic gene outside its normal expression domain is mediated by cross-regulatory interactions among the

homeotic genes themselves (Struhl, 1982; Hafen et al., 1984; Harding et al., 1985; Struhl and White, 1985; Carroll et al., 1986) and by the products of the Polycomb group genes (Lewis, 1978; Struhl, 1981; Duncan, 1982; Duncan and Lewis, 1982; Struhl, 1983; Ingham, 1984; Dura et al., 1985; Jürgens, 1985; Struhl and Akam, 1985; Breen and Duncan, 1986; Wedeen et al., 1986; Kuziora and McGinnis, 1988a; McKeon and Brock, 1991). The proteins encoded by the Polycomb group genes, *Polycomb* and *poly-homeotic*, appear to be directly involved in repression as they bind to the chromosomal sites of homeotic genes (Zink and Paro, 1989; DeCamillis et al., 1992). Maintenance of normal levels of expression of each homeotic gene within its normal domain requires the activity of the functionally related group of genes called the '*trithorax* set' (Shearn et al., 1987; Capdevila and García-Bellido, 1981; Kennison and Tamkun, 1988; Shearn, 1989; Tamkun et al., 1992) and, in some instances, autoregulatory stimulation of transcription of homeotic genes by their own protein products (Bienz and Tremml, 1988; Kuziora and McGinnis, 1988b; Chouinard and Kaufman, 1991).

Genetic evidence indicates that the genes of the *trithorax* set positively regulate homeotic gene expression and the requirement for *trithorax* (*trx*) itself has been the most thoroughly examined (Lewis, 1968; Ingham and Whittle, 1980; Duncan and Lewis, 1982; Capdevila et al., 1986; Shearn, 1989; Castelli-Gair and García-Bellido, 1990). *trx* embryos and adults exhibit homeotic transformations similar to those seen in *Sex combs reduced* (*Scr*), *Antennapedia* (*Antp*), *Ubx*, *abdominal-A* (*abd-A*) and *Abdominal-B* (*Abd-B*) mutants, suggesting that *trx* is required for the normal expression of these ANT-C and BX-C genes. Gene-dosage studies, varying the number of copies of trx^+, ANT-C and BX-C genes, further suggest that *trx* is required to maintain normal levels of homeotic gene expression (Capdevila and García-Bellido, 1981; Duncan and Lewis, 1982; Capdevila et al., 1986). However, these studies do not rule out the possibility that *trx* acts coordinately with the protein products of the homeotic genes in their regulation of other genes.

Clonal analysis (Ingham, 1981, 1985a) indicates that *trx* expression is required continuously to insure development of normal adult segment identities. Temperature-shift experiments with a t-s *trx* allele reveal that there is also a discrete early critical period (0-4 hour) during which trx^+ function is required for development of normal adult segment identities (Ingham and Whittle, 1980). This implies that lack of trx^+ during this early period cannot be compensated by the continuous presence of trx^+ thereafter. This suggests a distinct early role of *trx* in the establishment (as well as the maintenance) of stably determined states of homeotic gene expression.

The *trx* gene encodes at least three large predicted protein isoforms produced by differential splicing, the largest containing 3759 residues (Mazo et al., 1990; Breen and Harte, 1991). These proteins contain Cys-rich regions, some of which may constitute a zinc-finger-like DNA-binding domain, as well as glutamine-rich and acidic regions which might be involved in transcriptional activation (Mazo et al., 1990). The *trx* RNAs have different developmental profiles, suggesting that these proteins may function differently in regulating homeotic gene expression in embryonic and imaginal tissues (Breen and Harte, 1991).

We previously demonstrated that a null *trx* mutation causes reduced expression of *Antp*, *Ubx* and *abd-A* proteins in the embryonic ventral nerve cord (VNC) (Breen and Harte, 1991). Here, we present a detailed examination of the expression of *Dfd*, *Scr*, *Antp*, *Ubx*, *abd-A* and *Abd-B* proteins in stage 15-17 *trx* embryos. Each of these genes exhibits a complex pattern of tissue-specific, parasegment-specific and, very likely, promoter-specific requirements for *trx*, suggesting that maintenance of stable patterns of homeotic gene expression is likely to be considerably more complex than previously suspected.

MATERIALS AND METHODS

trx^{B11} embryos

Eggs were collected at 25°C for 18-24 hours from a trx^{B11} *red e* / *TM6B, Hu Tb* stock. Embryos were prepared for incubation with antibodies to homeotic proteins and for examination of cuticle phenotypes as described in Breen and Harte (1991). trx^{B11} homozygotes were unambiguously identified by their altered gut morphology (see Results) and homeotic protein expression patterns. *TM6B* homozygotes were identified by their failure to complete germ band retraction. Cuticle preparations of these embryos show that they do not complete dorsal fusion, posterior segments are still located dorsally with the telson closely apposed to the head. This phenotype was observed in several independent stocks containing the *TM6B* chromosome and another third chromosome not carrying a *trx* allele. Stage 15-17 trx^{B11} / *TM6B* embryos were identified by their normal gut morphology and wild-type patterns of homeotic protein expression. The pattern of expression of each homeotic protein was examined in 100-300 embryos. Typically, there was a greater proportion of stage 15-17 trx^{B11} and *TM6B* homozygotes than wild-type stage 15-17 embryos. This disparity arose because the trx^{B11} and *TM6B* homozygotes do not morphologically advance beyond these stages, whereas the wild-type heterozygotes continue to develop normally and hatch on schedule.

Antibody staining

Embryos were fixed and dechorionated according to the procedure of Mitchison and Sedat (1983) as modified by Goto et al. (1989). Antibody incubations were performed by the method of DiNardo et al. (1985). The anti-Antp 4C3 (Reuter and Scott, 1990), anti-Ubx FP.3.38 (White and Wilcox, 1984) and anti-Scr 6H4 (Glicksman and Brower, 1988) monoclonal antibodies were kindly provided by Danny Brower and Thom Kaufman. The anti-abd-A monoclonal antibody was produced by Dianne Mattson and Ian Duncan and kindly provided by Shige Sakonju. The anti-Dfd (Jack et al., 1988) and anti-empty spiracles (Dalton et al., 1989) polyclonal antibodies were kindly provided by Bill McGinnis. The anti-Abd-B 1A2E9 monoclonal antibody (Celniker et al., 1990) was kindly provided by Sue Celniker. Anti-eve polyclonal antibody (Frasch et al., 1987) was kindly provided by Michael Levine. After incubation with horse radish peroxidase-conjugated secondary antibodies (Cappel), staining was performed as described in Goto et al. (1989). Dehydrated embryos were cleared in toluene preparative to final mounting in Permount. Embryos were examined and photographed under Nomarski optics using a Zeiss Axioplan photomicroscope.

RESULTS

We examined the expression of *Ubx*, *Antp*, *abd-A*, *Abd-B*,

Scr and *Dfd* proteins in homozygous trx^{B11} embryos and their heterozygous siblings, the latter always exhibiting a wild-type expression pattern of these proteins. We also examined trx^{B11} / $Df(3R)red^{P52}$ hemizygotes, which showed the same patterns of homeotic protein expression and mutant morphology seen in trx^{B11} homozygotes (see below). The red^{P52} deletion removes *trx* and ten neighboring loci (Mortin *et al.*, 1992). The trx^{B11} *red e* chromosome complements lethal alleles in each of the loci removed by the red^{P52} deletion except *trx*. Thus the altered levels and patterns of homeotic gene expression that we observed are almost certainly due to loss of *trx* function, especially in light of the correlation that we observe between decreased homeotic gene expression and mutant cuticular phenotype of *trx* embryos (see Discussion).

Some of the effects of *trx* mutations on the expression of *Ubx* (Mazo et al., 1990; Breen and Harte, 1991) and *Antp* and *abd-A* proteins (Breen and Harte, 1991) in the embryonic VNC have been described previously. This report presents a detailed examination of the effects of loss of *trx* function on the expression of six homeotic proteins in most embryonic tissues.

Homozygous trx^{B11} embryos probably lack any significant *trithorax* function

The trx^{B11} mutation is very likely a null allele since it is a small frame-shifting deletion in the *trx* open reading frame of common exon 3 (Mazo et al., 1990; Breen and Harte, 1991), which would produce a truncated protein consisting of the N-terminal 17% of the predicted large protein isoform (Mazo et al., 1990). The cuticular phenotype of homozygous trx^{B11} embryos is the same as that of embryos containing overlapping deletions of the *trx* locus (Duncan and Lewis, 1982; Breen and Harte, 1991). However, these phenotypes may not be those associated with complete elimination of *trx*-encoded products, since maternally synthesized *trx* RNAs are present in the egg (Mozer, 1989; Breen and Harte, 1991) and *trx* mutations and deletions have been shown to exert a maternal effect on penetrance and expressivity of adult phenotypes (Garcia-Bellido, 1977; Garcia-Bellido and Capdevilla, 1978; Ingham and Whittle, 1980). However, *trx* embryos derived from trx^{-} germ line clones, and therefore entirely lacking *trx* function, are phenotypically indistingushable from genotypically identical embryos derived from heterozygous parents (Ingham, 1983; Breen and Harte, 1991). This suggests that maternally contributed trx^{+} products do not contribute significantly to the development of normal segment identities of larval epidermal cells, but are required, at least quantitatively, in adult progenitors. Nevertheless, the possibility exists that maternally contributed trx^{+} products may affect development of embryonic tissues other than the cuticle, or that the alleles used to make germ line clones caused only partial loss of *trx* function. Therefore, we cannot rule out the possibility that the presence of maternally synthesized *trx* products in the embryos that we examined might result in higher levels and less severely altered patterns of homeotic gene expression than would be seen in embryos from which all maternally synthesized products were eliminated.

Homozygous trx^{B11} embryos have a stereotypically abnormal midgut morphology which correlates with altered homeotic protein expression

During stage 15, three constrictions (first, second and third in anterior to posterior order) divide the midgut into four regions of comparable size (Campos-Ortega and Hartenstein, 1985). In trx^{B11} homozygotes and trx^{B11} / $Df(3R)red^{P52}$ hemizygotes, only the second midgut constriction forms in its normal position. The first and third constrictions form posterior to their wild-type positions. Consequently, the region between the proventriculus and the first constriction is larger than normal and the region between the first and second constrictions is smaller than normal. The posterior shift of the third constriction is less pronounced, but it is evident that the region between the second and third constrictions is larger than normal, and the region between the third constriction and the hindgut is smaller than normal. These relationships are best illustrated by comparing the stage 16 embryos in Fig. 6A and 6B. As we describe below, the posterior shift of the first and third midgut constrictions correlates with loss of *Antp* expression and reduction of *abd-A* expression, respectively, in the visceral mesoderm (VM). We speculate in the discussion on the possible role of altered homeotic gene expression in the VM in causing the ectopic formation of the first and third midgut constrictions.

The altered patterns of homeotic protein expression that we describe below are always seen in embryos with the stereotypical altered midgut morphology that we ascribe to *trx* homozygotes. Their sibs either showed both wild-type midgut morphology and homeotic protein expression patterns (*trx* heterozygotes) or the arrested germ band retraction phenotype (*TM6B* homozygotes). This correlation provides convincing evidence beyond counting embryos that those embryos with both mutant midgut morphology and altered homeotic protein expression must be trx^{B11} homozygotes.

Ultrabithorax expression

In *trx* embryos, *Ubx* expression is reduced in all tissues throughout its normal domain except in the VM, where *Ubx* autoregulation occurs (Bienz and Tremml, 1988). This reduction is greatest in PS6 tissues, where *Ubx* expression is normally at its highest level (Fig. 1A,B).

The amount of *Ubx* protein expressed in mutant VM appears similar to wild type, but its expression domain is expanded. In wild-type VM, cells expressing *Ubx* occupy the anterior surface of the second constriction, the highest level of *Ubx* expression being in the posterior of its domain, which extends to the point where the second midgut constriction forms. Lower levels of *Ubx* are normally found in the anterior of its domain, which extends to slightly less than halfway between the points where the first and second midgut constrictions form (Figs 1C, 7A,C). This domain corresponds to PS7 in the VM (Bienz et al., 1988; Tremml and Bienz, 1989b). In *trx* embryos, the highest level of *Ubx* is still found where the second midgut constriction forms, and lower levels extend anteriorly the same distance as in wild type (Figs 1D, 7B,D). However, the *Ubx* expression domain is expanded posteriorly into PS8, approximately

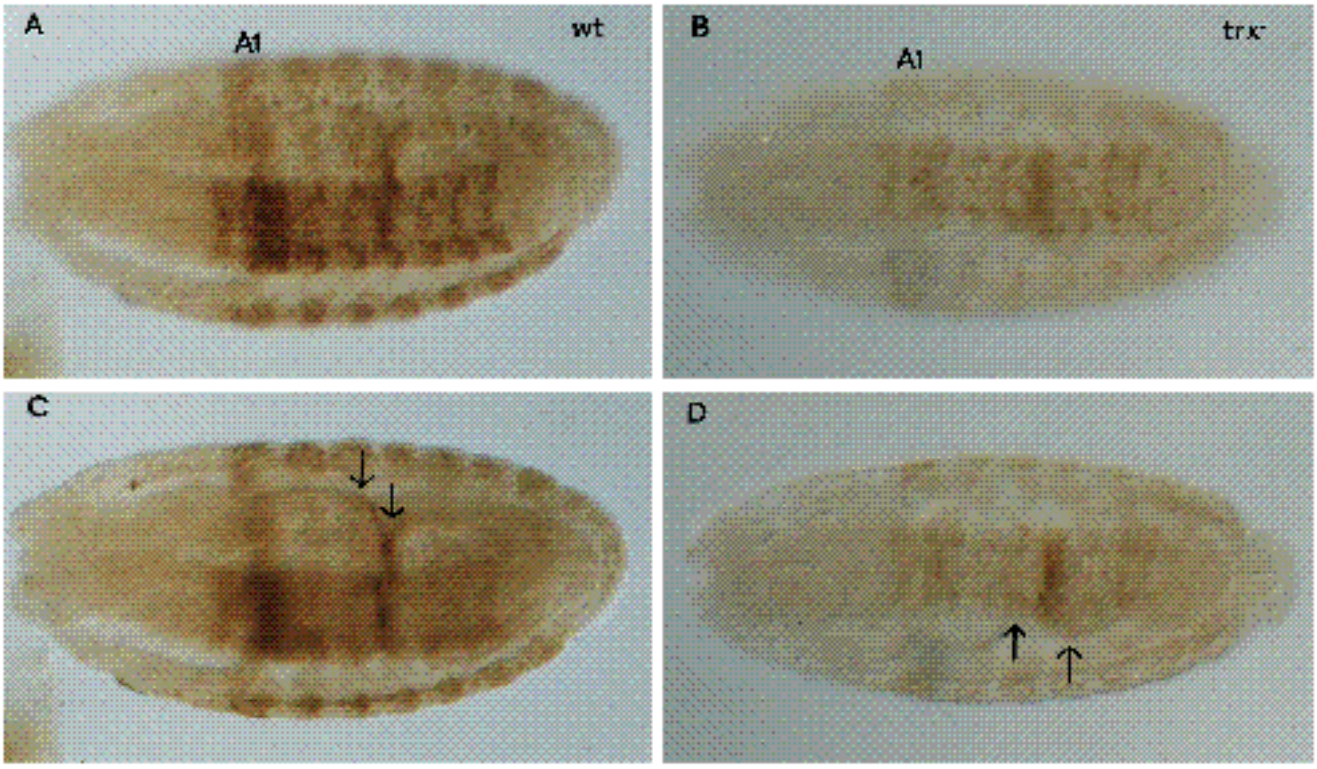

Fig. 1. The pattern of *Ubx* protein expression in late stage 15 to early stage 16 embryos. Anterior is to the left in Figs 1-8. A and C show the same wild-type embryo. B and D show the same homozygous trx^{B11} embryo. A and B compare wild-type and mutant *Ubx* expression patterns in the ventral nerve cord (VNC) and ectodermal derivatives. A1 indicates the position of the first abdominal segment. In both embryos, the first abdominal neuromere is located slightly posterior to the position of the epidermal first abdominal segment. C and D compare *Ubx* protein expression in the visceral mesoderm of a wild type and a mutant. In C, wild-type *Ubx* protein expression extends from anterior to the position of the second midgut constriction (anterior arrow) to within the second constriction (posterior arrow). In D, *Ubx* protein expression in the VM of a trx^{B11} mutant extends from the position of the first midgut constriction (anterior arrow) to about half the distance between the second and third midgut constrictions (posterior arrow).

halfway between the second and third midgut constrictions (Figs 1D, 7B,D). This point also corresponds to the anterior boundary of *abd-A* expression in *trx* embryos (see below), suggesting that ectopic posterior expression of *Ubx* is due to derepression in the absence of *abd-A* protein (Bienz and Tremml, 1988). The more posterior position of the first midgut constriction in *trx* embryos corresponds to the anterior boundary of *Ubx* VM expression (Figs 1D, 7B,D). This could be coincidental, since the first constriction normally only requires *Antp* expression to develop (Tremml and Bienz, 1989b), or it could indicate an abnormal involvement of *Ubx* in the formation of this constriction in the absence of normal *Antp* expression in the VM.

Antennapedia expression

Compared to *Ubx*, expression of *Antp* generally appears to be only slightly reduced in the VNC and in ectodermal and somatic mesodermal derivatives in *trx* embryos (Fig. 2B); however, its expression is almost completely absent in mutant VM (Fig. 2D).

Close examination reveals a reduction in *Antp* expression in all abdominal neuromeres, particularly in A8 and A9 neuromeres, where it is expressed in only a few nuclei. Its expression is reduced slightly in the anterior compartment of the first thoracic (T1) neuromere and in the T2 and T3 neuromeres. It is somewhat more reduced in the thoracic ectodermal derivatives, particularly in T3.

The *Antp* gene has two separate promoters, P1 and P2, whose transcripts are predicted to encode the same proteins (Bermingham and Scott, 1988; Stroeher et al., 1988). The P1 and P2 transcripts are expressed in largely overlapping domains (Bermingham et al., 1990), but there are some differences in their levels and patterns of expression, the P2 domain being more extensive than that of P1. The pattern of *Antp* expression that we see in *trx* embryos is similar to the pattern of P1 RNA expression described by Bermingham et al. (1990), suggesting that *trx* may have a greater effect on P2 than P1 transcription, which we address further in the discussion.

Normally, the highest level of *Antp* expression in the VM is at the position where the first midgut constriction forms. Its domain extends anteriorly and posteriorly from this constriction (Figs 2C, 7A,C) and corresponds to PS5-6 in the VM (Tremml and Bienz, 1989b). In *trx* embryos, *Antp* expression is limited to one or two cells at both ends of the normal expression domain in each of the four rows of cells which invest the midgut (Figs 2D, 7D, 8B). A cluster of about five cells that are closely apposed to the VM near the center of the normal *Antp* domain also still express *Antp* protein (not shown). These cells, which we believe are part

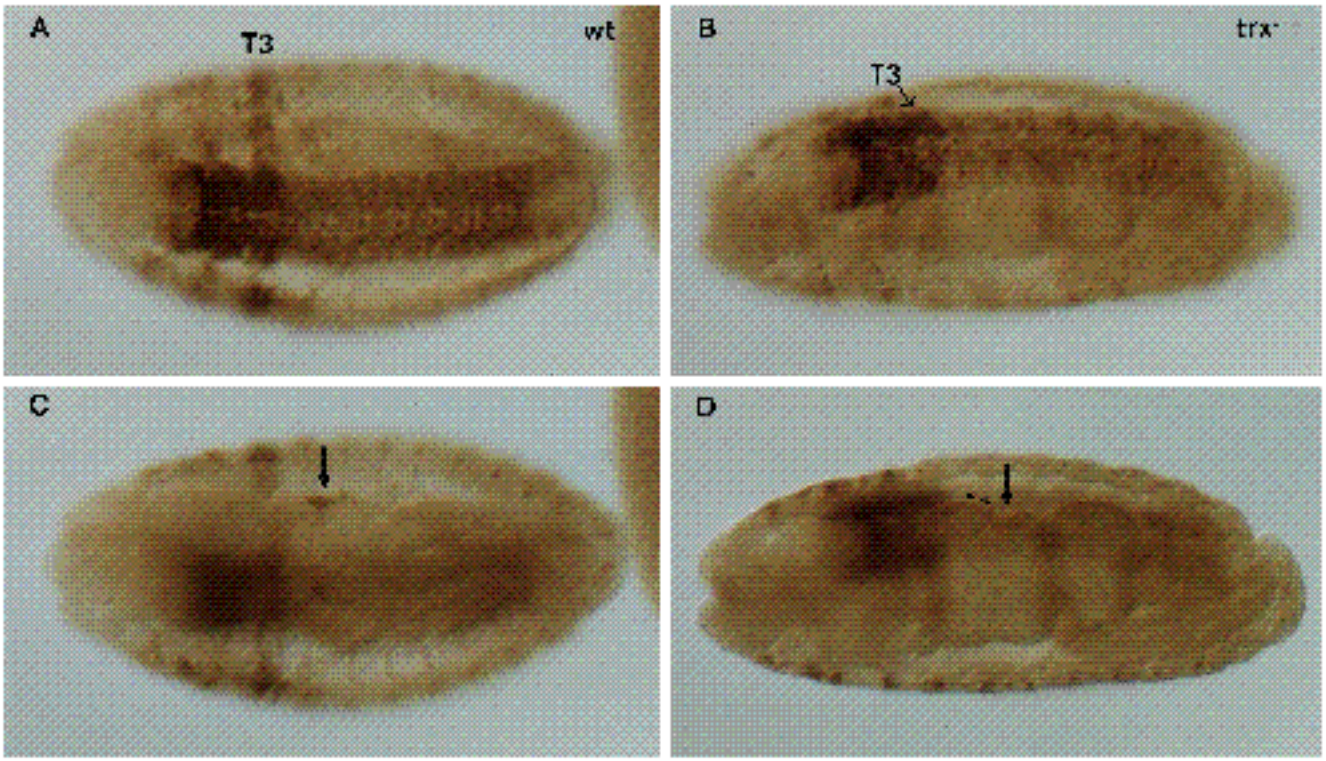

Fig. 2. The pattern of *Antp* protein expression in late stage 15 to early stage 16 embryos. A and C show the same wild-type embryo, B and D show the same trx^{B11} homozygote. A and B compare VNC and epidermal expression, C and D compare VM expression. The T3 neuromere in A directly underlies the T3 epidermal segment. The T3 neuromere in B (arrow) is shifted slightly posterior to the epidermal T3 segment. In C, *Antp* protein is expressed in VM cells within and flanking the forming first midgut constriction (arrow). In D, there is very little *Antp* protein expression in trx^{B11} VM cells. It is expressed in only two cells at each end of the normal *Antp* domain (double head arrow) in each of the four rows of VM cells. The first midgut constriction (heavy arrow) forms immediately posterior to those cells expressing *Antp*.

of the fat body, also express *Antp* in wild-type embryos, but are difficult to detect above the background of normal *Antp* expression in the VM. As we consider in the discussion, this pattern could also reflect a greater effect of *trx* on P2 than P1 transcription.

In *trx* embryos, the first constriction develops precisely posterior to the VM cells which still express *Antp* protein (Figs 2D, 8B). These cells lie immediately adjacent to cells that express *Ubx* protein, probably at the PS6-7 junction (Tremml and Bienz, 1989b), so that the constriction actually forms at a point where VM cells are now expressing *Ubx*.

abdominal-A expression

trx embryos have moderately reduced levels of *abd-A* expression in the VNC (Fig. 3B) and in ectodermal and somatic mesodermal tissues (Fig. 3D). Its expression in the VM is also reduced. In wild-type embryos, *abd-A* is expressed from the second midgut constriction to the hindgut (Fig. 3C). This domain corresponds to PS8-12 in the VM (Tremml and Bienz, 1989b). In *trx* embryos, *abd-A* expression is absent from the most anterior part of its domain, probably no more than PS8, and is expressed at reduced levels throughout the remainder of its normal domain (Fig. 3D). Loss of *abd-A* expression in the anterior of its domain coincides with ectopic expression of *Ubx* in those cells, probably due to derepression caused by the loss of *abd-A*. The more posterior formation of the third midgut constriction in *trx* embryos (Fig. 3D) also coincides with the anterior contraction of the *abd-A* expression domain, the significance of which we consider in the Discussion.

abd-A expression is completely abolished in the heart and supporting alary muscles in *trx* embryos (Fig. 3F). It is normally expressed at high levels in the pericardial cells and alary muscles of A5-A7 (Fig. 3E and Karch et al., 1990). The loss of *abd-A* expression in this tissue constitutes a more severe loss than that seen in *trx* mutant epidermal and somatic mesodermal cells, where *abd-A* protein is normally expressed at a level comparable to that in the pericardial cells.

The different extents to which *abd-A* protein expression is altered in ectodermal and somatic mesodermal derivatives, VM and heart structures in *trx* embryos demonstrate that there are three qualitatively different requirements for *trx* function to obtain the normal pattern of *abd-A* expression.

Abdominal-B expression

In wild-type embryos, *Abd-B* protein is expressed at high levels in PS13-15 tissues and at increasingly lower levels from PS12 to PS10 (Fig. 4A,C,E, and Celniker et al., 1990; DeLorenzi and Bienz, 1990; Boulet et al., 1991). The *Abd-*

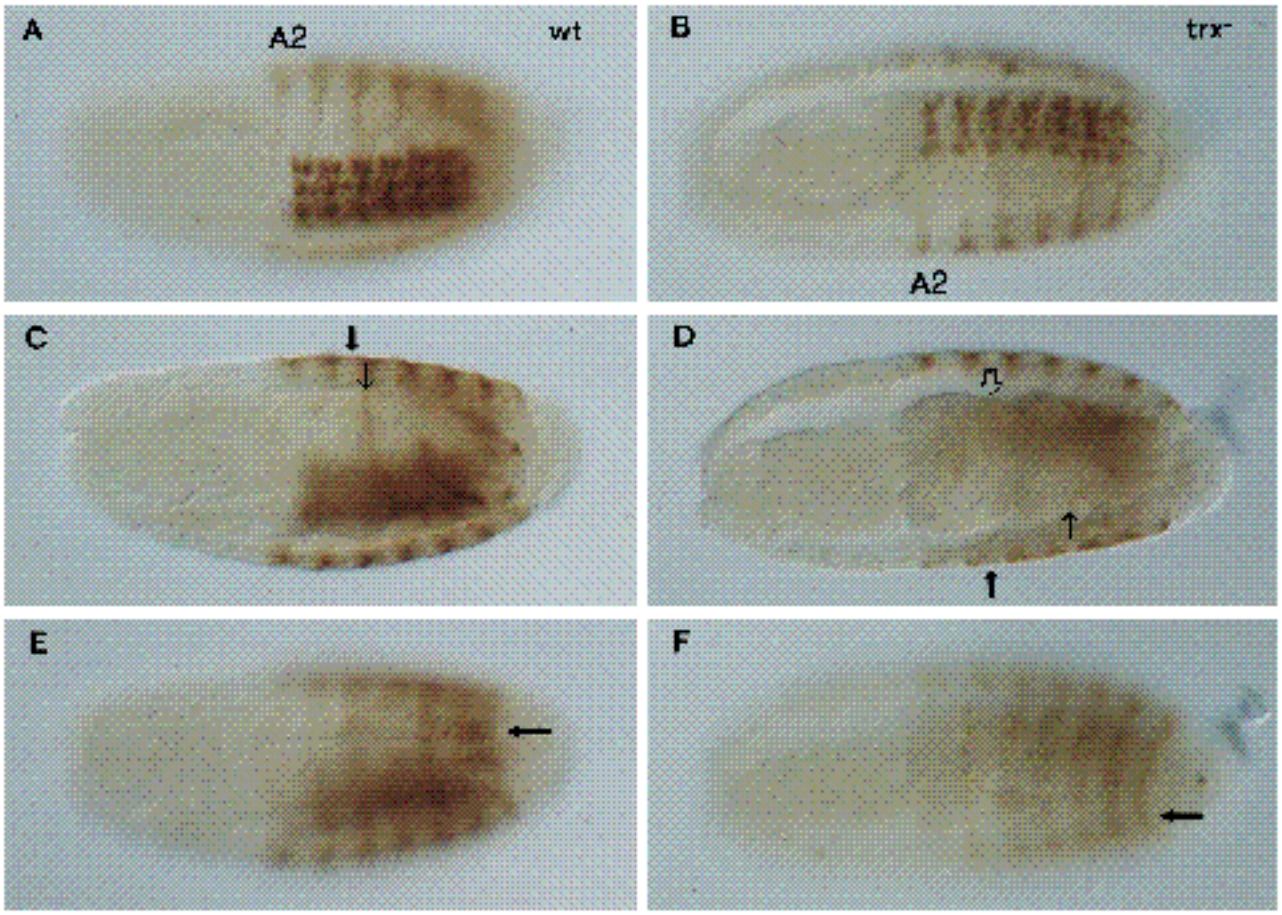

Fig. 3. The pattern of *abd-A* protein expression in late stage 15 to early stage 16 embryos. A, C and E show the same wild-type embryo. B, D and F show the same homozygous trx^{B11} embryo. A and B compare *abd-A* protein expression in the VNC. A2 indicates the position of the second abdominal segment. In B, there is reduced expression of *abd-A* protein in the VNC as evidenced by its decreased levels in the central regions of each neuromere, which shows as gaps in the expression pattern compared to the more contiguous appearance of the wild-type pattern. C and D compare expression in the epidermis and VM. In C, the dorsal epidermal expression of *abd-A* protein is in contiguous nuclei with its lowest level in the middle of each segment (heavy arrow). In D, there are gaps in the dorsal epidermal expression of *abd-A* protein in the middle of each segment (heavy arrow). In C, the normal extent of *abd-A* protein expression in VM cells is from within the second midgut constriction (light arrow) to the junction of the midgut and hindgut. In D, the mutant anterior limit of *abd-A* protein expression is between the second and third midgut constrictions (light arrow). The open arrow indicates the posterior shift in the position of the developing first midgut constriction. E and F compare *abd-A* protein expression in the pericardial cells of the heart. In E, *abd-A* protein is expressed in the pericardial cells underlying segments A5-A7 (heavy arrow). In F, trx^{B11} embryos completely lack *abd-A* protein in the pericardial cells (heavy arrow) and supporting tissues.

B protein isoform expressed in PS10-13 is encoded by the mRNA transcribed from P4, one of the four *Abd-B* promoters. This protein, ABD-BI (Celniker et al., 1990), is the agent of the *Abd-B* m function described by Casanova et al. (1986). The *Abd-B* protein isoform expressed in PS14-15 and the hindgut VM is encoded by alternatively spliced mRNAs transcribed from the more distal P1, P2 and P3 promoters. This protein, ABD-BII (Celniker et al., 1990), is responsible for *Abd-B* r function (Casanova et al., 1986).

In *trx* embryos, *Abd-B* expression is greatly reduced in PS10-12 in the VNC (Fig. 4B), and is completely absent in ectodermal and somatic mesodermal tissues of these parasegments (Fig. 4D,F). Its expression in PS13-15 and in the hindgut VM appears comparable to wild type. Normally, *Abd-B* expression is also detected in the posterior midgut VM, but this fades during development and cannot be detected in the stage 15-17 embryos that we examined (DeLorenzi and Bienz, 1990). Because ABD-BI is the only isoform normally expressed in PS10-12 and PS13, these observations imply that the *Abd-B* gene has a parasegment-specific requirement for *trx* to achieve normal expression from the P4 promoter in PS10-12 but not in PS13. This pattern of *Abd-B* expression is very similar to that seen in *iab-7* mutants, which also express no ABD-BI in PS10-12, but apparently exhibit normal expression of ABD-BII from the more distal promoters in PS14-15 (Celniker et al., 1990; Boulet et al., 1991). As we consider in the discussion, phenotypic defects in PS14-15 in *trx* embryos suggest that despite the apparently normal levels of total *Abd-B* protein in PS14-15, there is some deficit of Abd-B r function there.

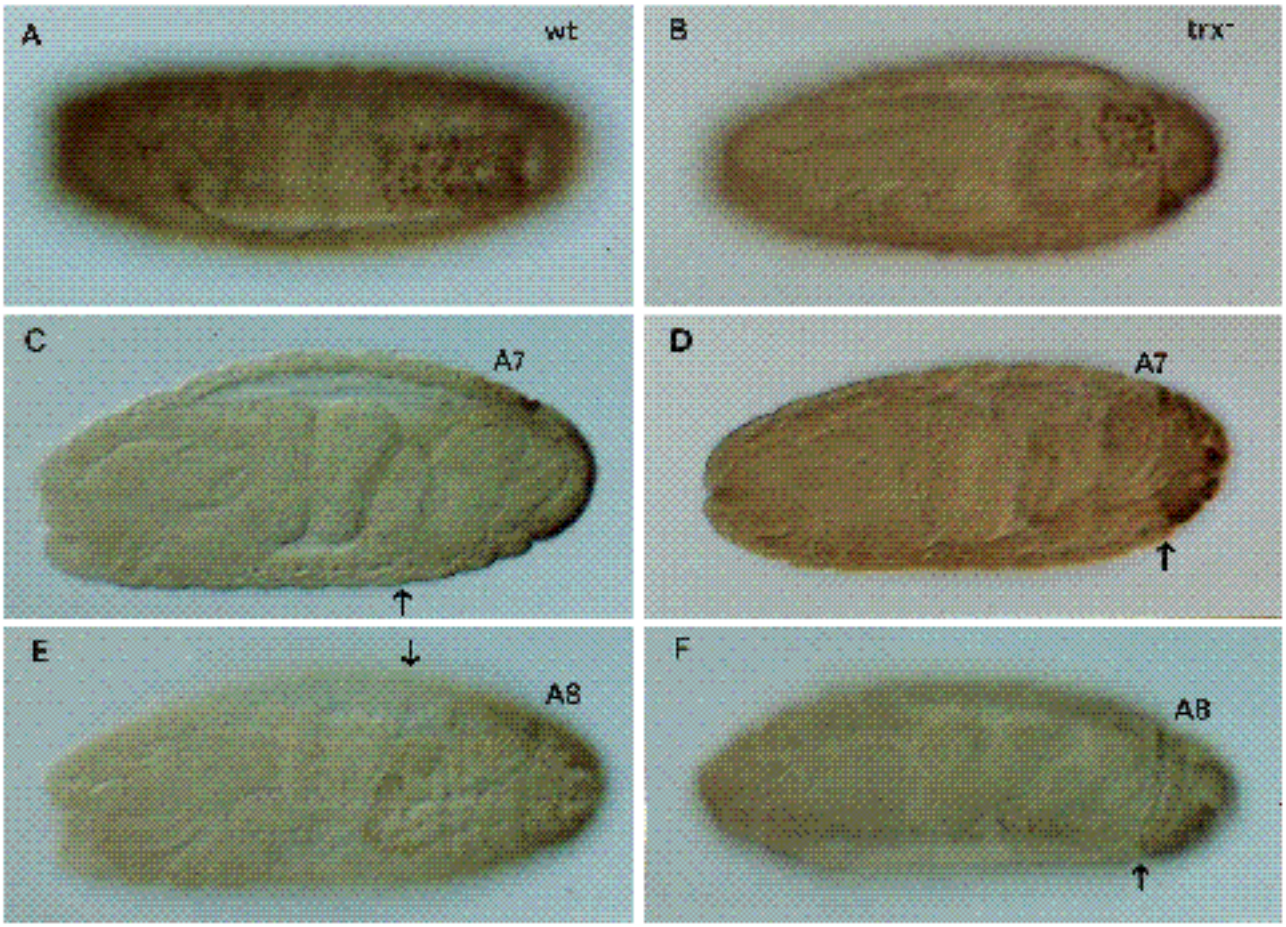

Fig. 4. The pattern of *Abd-B* protein expression in middle to late stage 16 embryos. A, C and E show wild-type embryos; B, D and F show homozygous trx^{B11} embryos. A and B compare *Abd-B* protein expression in the VNC. In A, the most anterior expression of *Abd-B* protein is largely within the A5 neuromere, PS10. In B, *Abd-B* protein is expressed in only a few cells in the A5-A7 neuromeres. It is expressed at near normal levels in the A8 and A9 neuromeres. C and D compare *Abd-B* protein expression in oblique optical sections of the epidermis. In C, low levels of expression are visible in the posterior of A4 which is the anterior of PS10 (arrow). The level increases in more posterior parasegments. In D, the most anterior *Abd-B* protein expression is in the posterior of A7 which is the anterior of PS13 (arrow). *Abd-B* protein is not expressed in most of the epidermal derivative of PS15, the anal plates, in either wild-type or mutant embryos. E and F compare epidermal expression, focussing on surface cells. Again, in wild-type embryos (E), *Abd-B* protein expression is seen in increasing amounts from PS10-15 (arrow). In trx^{B11} mutants (F), it is expressed at apparently wild-type levels in only PS13-15 (arrow). *Abd-B* protein expression is seen in the underlying hindgut VM in both E and F, showing that expression in this tissue is unaffected in mutants.

Sex combs reduced expression

The expression of *Scr* protein in *trx* embryos is slightly reduced in the labial ganglion (Fig. 5B), the floor of the atrium anterior to the salivary duct (Fig. 5D) and the anterior margin of dorsal T1 epidermis (Fig. 5F). Its expression in anterior midgut VM nuclei appears unaffected (Fig. 5B). In wild-type embryos, *Scr* expression is required in the anterior midgut VM for normal development of the gastric caeca (Reuter and Scott, 1990). These develop normally in *trx* embryos (not shown), consistent with our observation that *Scr* expression in the VM is unaffected.

The reduction in *Scr* expression in ectodermal and somatic mesodermal structures is not dramatic, suggesting a minor role for *trx* in the regulation of *Scr* expression in these tissues. However, there does appear to be a difference between its effect on *Scr* expression in VM and in ectodermal and somatic mesodermal tissues, suggesting that the *Scr* gene also has different requirements for *trx* in different tissues.

Deformed expression

Loss of *trx* function affects *Dfd* expression in a manner similar to *Scr* expression. There is a slight reduction in *Dfd* protein expression in the mandibular/maxillary ganglion (Fig. 6B), and in ectodermal and somatic mesodermal tissues (Fig. 6D). In wild-type embryos, *Dfd* is not expressed in VM, hence there is no comparison for a tissue-specific requirement for its expression.

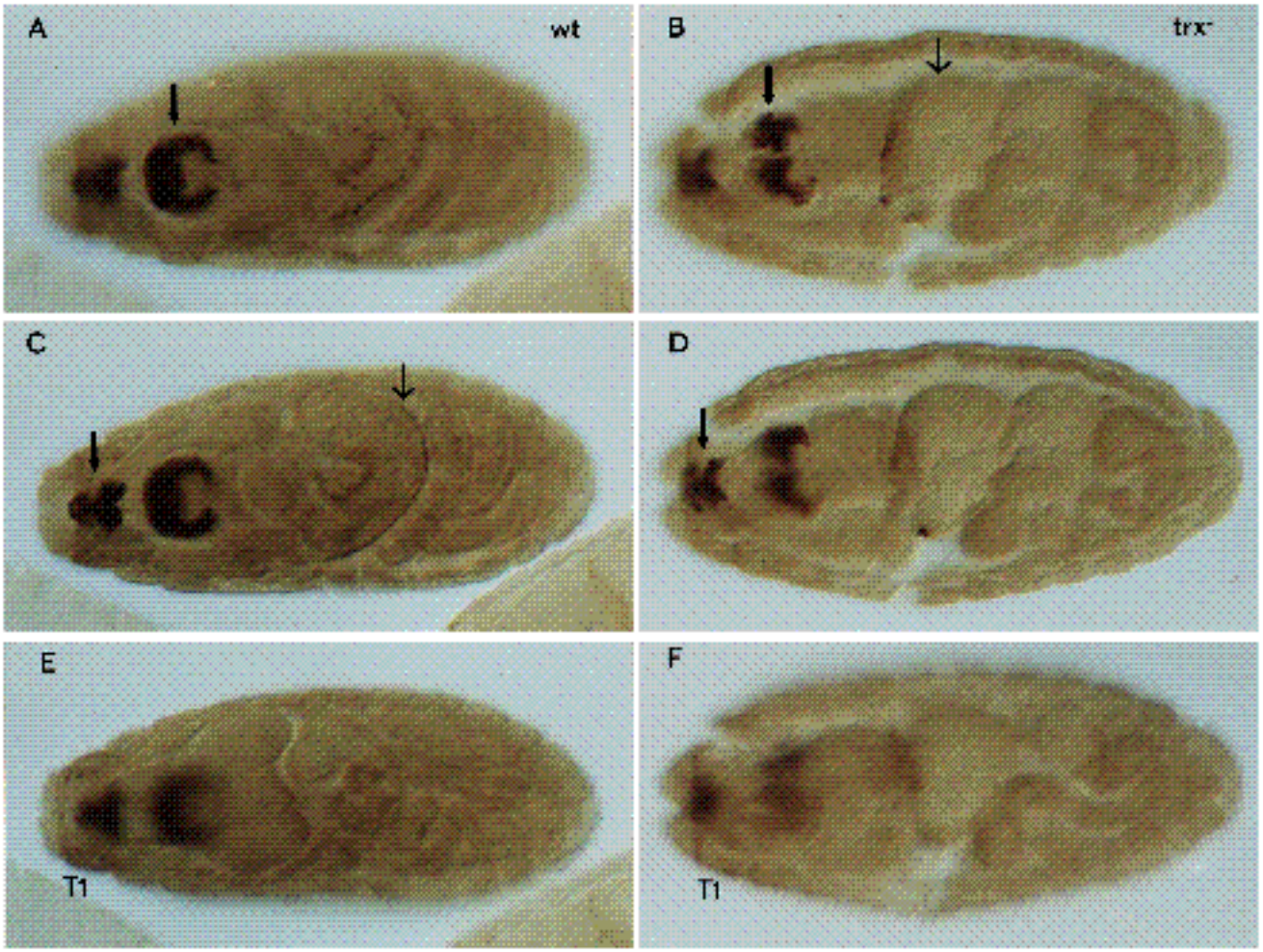

Fig. 5. The pattern of*Scr* protein expression in early to middle stage 17 embryos. A, C and E show the same wild-type embryo; B, D and F show the same homozygous trx^{B11} embryo which has artifactual breaks in the T1 and A1 epidermis. A and B compare expression in the labial ganglion (heavy arrows in A and B). In B, *Scr* expression is patchy and does not extend to the anterior of the ganglion. C and D compare expression in the floor of the atrium (heavy arrows in C and D), just anterior to the salivary duct. In C, *Scr* protein is expressed in the midgut VM from the junction of the proventriculus and the anterior midgut to about half the distance between the proventriculus and the position of the first midgut constriction (light arrow). In B, *Scr* protein expression can be detected in the same domain as wild type (light arrow). E and F compare *Scr* protein expression in dorsal T1 epidermis. In E, it is expressed in two rows of cells at the anterior margin of T1. In F it is expressed in only one row of cells.

Further correlation of *trithorax* midgut phenotype and homeotic protein expression

We provide further evidence that altered homeotic protein expression and mutant midgut morphology are correlated by simultaneously observing the expression patterns of more than one homeotic protein in embryos from the trx^{B11} */TM6B* stock. Comparison of Fig. 7A and C to Fig. 7B and D clearly demonstrate that the loss of most of the *Antp* expression in the VM around the first midgut constriction is associated with normal *Scr* expression in the anterior midgut VM and an expansion of the domain of *Ubx* expression in the VM posterior to the position of the second midgut constriction. These expression patterns are seen together in embryos that have a posteriorly shifted first midgut constriction (Fig. 7D). The near loss of *Antp* expression in the VM of embryos with a posteriorly shifted first midgut constriction is also associated with the loss of expression of *Abd-B* in PS10-12 epidermis (Fig. 8B).

trithorax is not required for normal expression of all homeodomain proteins

In *trx* embryos, *even-skipped* and *empty spiracles* proteins are expressed as in wild type (not shown). Furthermore, the cuticular phenotype of *trx* embryos implies that the major requirement for *trx* expression is for the normal expression of only the homeotic genes that we examined. Furthermore, other than the midgut, the internal structures of *trx* embryos are not noticeably affected. It is possible that other genes may have a requirement for *trx* expression similar to *Dfd*, but these would currently be equally difficult to ascertain due to a lack of an observable phenotypic effect.

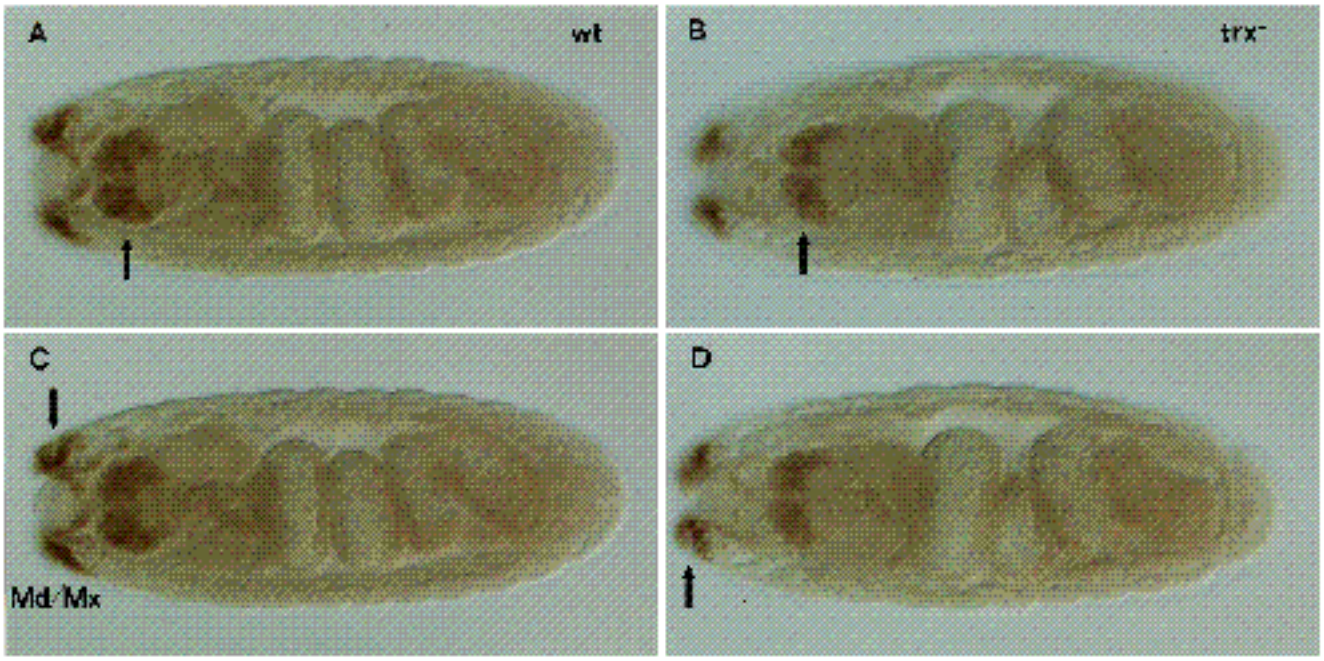

Fig. 6. The pattern of *Dfd* protein expression is shown in stage 16 wild-type (A and C) and homozygous trx^{B11} (B and D) embryos. A and B show *Dfd* protein expression in the mandibular/maxillary ganglion (arrows in A and B). In B, its expression is patchy and is not uniform to the anterior of the ganglion. C and D show *Dfd* expression in the mandibular/maxillary epidermis and derivatives (heavy arrows in C and D). In D, fewer epidermal cells express *Dfd* protein. In B and D, notice the shift in positions of the first and third midgut constrictions characteristic of trx^{B11} mutant embryos (see text).

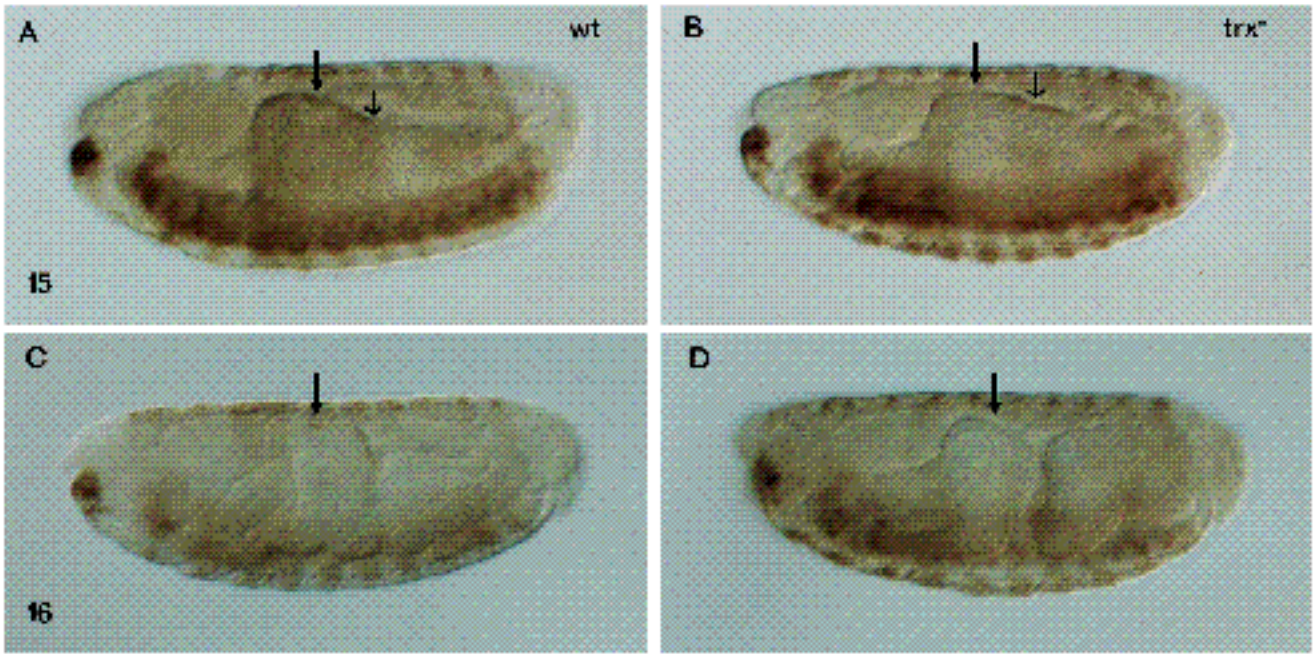

Fig. 7. Composite of *Scr, Antp* and *Ubx* protein expressions in the midgut VM of stage 15-16 embryos. A and C show wild-type embryos, B and D show homozygous trx^{B11} embryos. A and B compare stage 15 patterns, C and D compare early to late stage 16 patterns. In wild-type embryos, *Antp* protein is expressed where the first midgut constriction will form (compare heavy arrows in A and C). *Scr* protein is expressed from the junction of the proventriculus to about two cells anterior to the anterior border of the *Antp* domain. *Ubx* protein is expressed from the posterior border of the *Antp* domain to the position where the second midgut constriction forms (small arrow in A). In homozygous trx^{B11} embryos *Antp* protein is mostly not expressed in the VM (heavy arrow in B and D). *Scr* protein appears to be expressed in its normal domain, though the posterior of that domain is out of focus in B. *Ubx* protein is expressed from its normal anterior limit to almost half the distance between the second and third midgut constrictions. The small arrow in B indicates the position of the forming second constriction with the mutant *Ubx* domain extending beyond it.

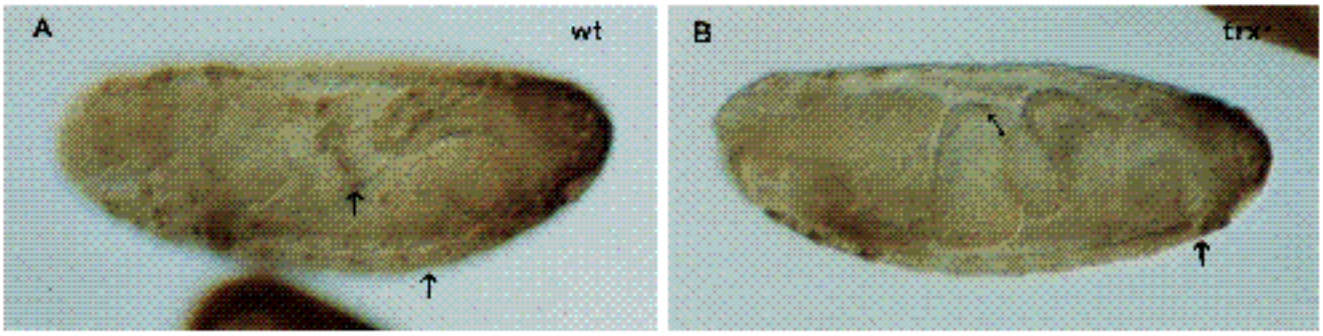

Fig. 8. Correlation of *Antp* and *Abd-B* mutant expression. *Antp* and *Abd-B* protein expressions are shown in early stage 17 wild-type (A) and homozygous trx^{B11} (B) embryos. In A, normal *Antp* protein expression is seen in the first midgut constriction (anterior arrow), and normal *Abd-B* protein expression is seen in the epidermis from PS10 (posterior arrow) through PS15. In B, The trx^{B11} mutant pattern of *Antp* protein expression is seen in the VM (double head arrow) and the mutant pattern of *Abd-B* protein expression is seen in PS13-15 (posterior arrow).

DISCUSSION

Correlation of homeotic protein expression and cuticular phenotype in *trithorax* embryos

The embryonic cuticle of *trx* mutants displays segmental identity transformations resembling those associated with mutations in a number of BX-C and ANT-C homeotic genes (Fig. 9).

Dfd mutants fail to develop mandibular and maxillary structures (Wakimoto and Kaufman, 1981; Regulski et al., 1987), which reduces the size of the mandibular/maxillary segment, located anterior to the large ventral setal belt (VSB) of the first thoracic segment (T1). In *trx* embryos, this segment and its structures appear normal, indicating that the slight decrease in *Dfd* protein expression in these mutants has no detectable developmental consequence.

In *Scr* mutants, the anterior of the cephalopharyngeal apparatus (CA) is reduced, and the anterior T1 VSB is composed of small denticles instead of large heavily pigmented ones (Wakimoto and Kaufman, 1981; Sato et al., 1985). In addition, the small posterior T1 VSB is reduced in *Scr* mutants. In *trx* embryos, the CA and the posterior T1 VSB appear unaffected, but there are fewer large denticles in the anterior T1 VSB. These phenotypes reflect the minor reduction in *Scr* protein expression observed in labial segment derivatives and the slightly more noticeable reduction in anterior T1 cells.

Antp mutants develop T1 structures in T2 (Wakimoto and Kaufman, 1981). This phenotype is not due to a derepression of *Scr* in T2, but has been attributed to the derepression of one or more head-specific genes there (Struhl, 1983). In the cuticle of *trx* embryos, T2 appears nearly wild type, despite a noticeable reduction in *Antp* expression in the epidermis. While the minimum level of *Antp* required for normal development is not known, *Antp* mutations that selectively disrupt P1 transcripts cause more severe T2 to T1 transformations than those that appear to affect only P2 transcripts (Wakimoto and Kaufman, 1981; Abbot and Kaufman, 1986; Sato and Dennell, 1987). This implies that the protein translated from P1 transcripts plays a more important qualitative or quantitative role in determining T2 development. Qualitative differences in their effects could arise from the P1 and P2 transcripts being expressed in different patterns in T2, each determining different aspects of T2 development. However, since the proteins encoded by P1 and P2 transcripts are predicted to be the same (Bermingham and Scott, 1988; Stroeher et al., 1988) and are expressed in largely overlapping patterns in the epidermis, it seems more likely that the more severe phenotype of P1-specific mutations arise from differences in their normal levels of expression, P1 being expressed at a higher level. The spatial patterns of P1 and P2 transcription have been examined in wild-type embryos (Bermingham et al., 1990), but the results do not allow us to confirm this possibility. Nevertheless, we would argue that *trx* embryos have a greater reduction in P2 than P1 expression, since this could decrease the level of *Antp* expression without severe phenotypic consequences, as observed. Furthermore, the pattern of residual *Antp* protein expression in *trx* embryos suggests that *trx* may have a greater effect on P2 than P1 transcription (see below).

In *Ubx* mutants, T3 and A1 VSBs look like a normal T2 VSB, comprising two to three rows of small denticles (Lewis, 1978). In addition, the A2-A7 VSBs do not contain the posterior two rows of smaller denticles found in wild-type embryos (Sánchez-Herrero et al., 1985; Casanova et al., 1987). In *trx* embryos, the T3 VSB contains only small T2 type denticles, and the A1 VSB is slightly reduced, with fewer large denticles than wild type. The posterior rows of small denticles in the A2-A7 VSBs are reduced such that they contain fewer denticles and do not form two complete rows. These morphological alterations are consistent with the decrease in *Ubx* expression seen in PS5-12 epidermis in *trx* embryos.

abd-A mutants do not develop the four anterior rows of denticles in the A2-A7 VSBs (Sánchez-Herrero et al., 1985; Casanova et al., 1987). In *trx* embryos, these rows of denticles are considerably reduced. The *trx* mutant VSBs contain fewer than four anterior rows of denticles and those rows that do develop contain fewer denticles than the corresponding wild-type VSBs. This phenotype is consistent with the moderate reduction in *abd-A* expression observed in the A2-A7 epidermis in *trx* embryos.

In wild-type embryos, the denticles in the most anterior

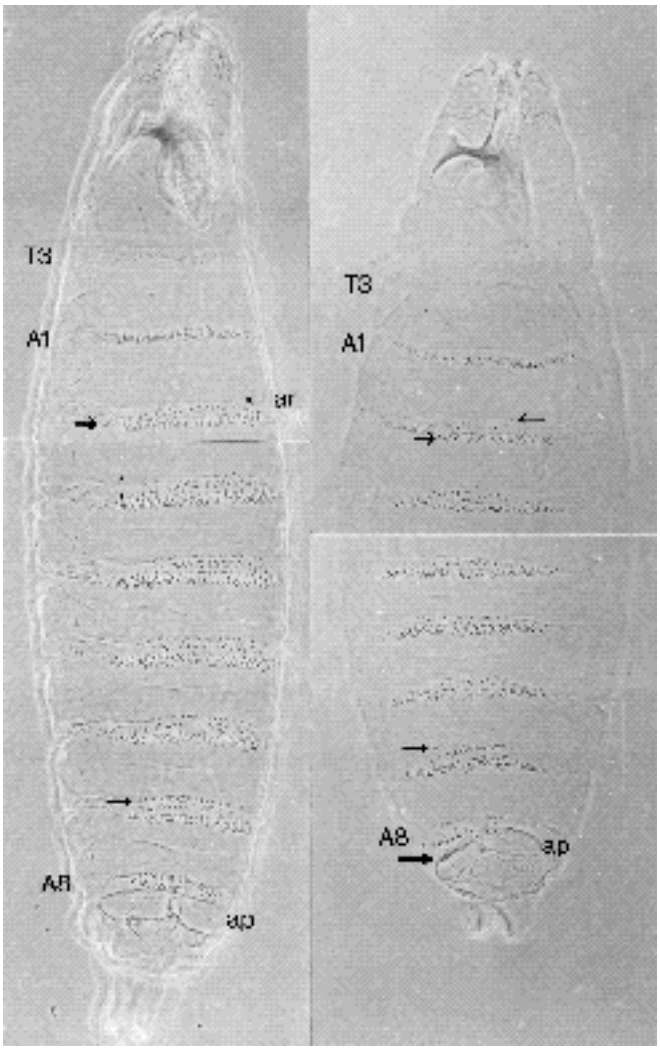

Fig. 9. trx^{B11} mutant cuticle. At the left is a late embryonic cuticle from our Oregon-R wild-type stock; at the right is an embryonic cuticle from a trx^{B11} homozygote. T3, A1 and A8 indicate the positions of the VSBs of the third thoracic, first abdominal and eighth abdominal segments which are located at the anterior margin the segment. The heavy arrow to the left of the A2 VSB in the wild type and trx^{B11} mutant indicates the posterior two to three rows of smaller denticles in this and each VSB to A7. In the trx^{B11} cuticle, there are fewer of these denticles in the A2-A7 VSBs. The two arrows to the left of the wild-type A3 VSB indicate the four most anterior rows of denticles, which fail to develop in A2-A7 in *abd-A* mutants. trx^{B11} mutants have many fewer of these denticles in the A2-A7 VSBs. The small arrow to the right of the wild-type and trx^{B11} mutant A2 VSB indicates the anterior row (ar) of fine denticles. In the wild-type cuticle, the denticles of the anterior row are increasingly larger in more posterior VSBs to A7 (arrow at left in A7). In trx^{B11} mutants, the denticles of the anterior row remain small in more posterior VSBs to A7 (arrow at left in A7). The A4-A7 VSBs of trx^{B11} mutants do not extend as far laterally as in wild type. The trx^{B11} mutant A8 VSB is only slightly reduced compared to wild type and there is no naked cuticle between the A8 VSB and the anal pads (ap) as in wild type. The large arrow at the left anterior margin of the anal pad in the trx^{B11} mutant indicates a sclerotic plate. The remainder of the PS14-derived telson appears nearly normal in the trx^{B11} mutant.

row in the A2-A7 VSBs are larger in more posterior VSBs. This size increase is pronounced between the A3 and A5 VSBs. In *Abd-B* mutants, the anterior row in each of the posterior VSBs contains small denticles like those normally found in A2 and A3 VSBs (Sánchez-Herrero et al., 1985; Casanova et al., 1986, 1987). Also, the four anterior rows of denticles in each VSB do not extend as far laterally in posterior VSBs as in wild type. In *Abd-B* mutants, the A8 VSB resembles one normally found in A4 or A5 and there is a region of naked cuticle between the A8 VSB and the anal pads, which is not present in wild-type embryos (Casanova et al., 1986). Additionally, *Abd-B* mutants develop sclerotic plates resembling mouth hooks just anterior to the anal pads, in posterior A8 (anterior PS14). Casanova et al. (1986) demonstrated that the *Abd-B* locus has two separable functions. *Abd-B* m function is required for the normal development of the A5-A8 VSBs, and its function in PS13 prevents the formation of naked cuticle between the A8 VSB and the anal pads. They also showed that sclerotic plate development is prevented by *Abd-B* r function in PS14. The *Abd-B* m and r functions are implemented by two different protein isoforms encoded by the *Abd-B* locus (DeLorenzi et al., 1988; Kuziora and McGinnis, 1988a; Celniker et al., 1989; Zavortink and Sakonju, 1989). The m function protein, ABD-BI (Celniker et al., 1990), is normally expressed in PS10-13, and the r function protein, ABD-BII, is normally expressed in PS14 and 15 (Celniker et al., 1990; DeLorenzi and Bienz, 1990; Boulet et al., 1991).

In *trx* embryos (Fig. 9), the denticles in the most anterior row of the A5-A7 VSBs are as small as those in more anterior VSBs and the smaller anterior rows of denticles in each of these VSBs (caused by decreased *abd-A*) do not extend as far laterally as in wild type. These phenotypes correlate with the loss of ABD-BI expression seen in PS10-12 in *trx* embryos. The A8 VSB, though somewhat reduced, has the same general shape as found in wild type (Duncan and Lewis, 1982; Breen and Harte, 1991). Also, as in wild type, *trx* embryos have no naked cuticle between the A8 VSB and the anal pads. This near normal development of posterior A7 and anterior A8 in *trx* embryos reflects the near normal levels of ABD-BI that they express in PS13.

Although total *Abd-B* protein appears to be expressed at normal levels in PS14 in *trx* embryos (Celniker et al., 1990; DeLorenzi and Bienz, 1990; Boulet et al., 1991), ABD-BII cannot be present at normal levels since sclerotic plates develop there (Fig. 9), just anterior to the anal pads in posterior A8, as they do in r-specific *Abd-B* mutants (Casanova et al., 1986). We cannot unambiguously confirm this since (1) the *Abd-B* antibody that we used (Celniker et al., 1990) cannot distinguish ABD-BI and ABD-BII and (2) although only ABD-BII is normally expressed in PS14-15, ABD-BI is derepressed in PS14 when ABD-BII expression is eliminated by mutation (Celniker et al., 1990; Boulet et al., 1991). Therefore, it is possible that the *Abd-B* expression that we observe in PS14 is a combination of some derepressed ABD-BI and a reduced level of ABD-BII, insufficient to suppress sclerotic plate development in PS14. However, since the other transformations associated with a loss of *Abd-B* r function in A9 (Casanova et al., 1986) are not seen in *trx* embryos, ABD-BII expression in PS14 cannot

be substantially reduced. Therefore, the *Abd-B* protein detected in PS14 in *trx* embryos could also reflect imperceptibly reduced levels of ABD-BII with little or no derepressed ABD-BI and an extreme sensitivity of sclerotic plate development to any reduction of ABD-BII. To determine the correct explanation, we are presently examining expression from each of *Abd-B* promoters individually in *trx* embryos by RNA in situ hybridization using promoter-specific probes.

Altered patterns of homeotic gene expression in the visceral mesoderm are associated with stereotypically displaced midgut constrictions in *trithorax* embryos

Formation of the second midgut constriction requires both *Ubx* and *abd-A* expression (Tremml and Bienz, 1989b). In *trx* embryos, the second midgut constriction develops in its normal location, where *Ubx* expression is still at its highest. However, since the anterior boundary of *abd-A* expression is shifted posteriorly from PS8 to PS9 in *trx* embryos, VM cells expressing *abd-A* are now removed from the site where the constriction forms by as much as a parasegment. Since *abd-A* expression is absolutely required for the formation of this constriction, it is possible that we are unable to detect very low levels of *abd-A* protein there, which are sufficient to promote formation of the constriction, but inadequate to repress *Ubx*. It is also possible that *abd-A* need not be expressed in the VM cells where the second constriction forms, but only in nearby cells, which then induce the formation of the constriction in adjacent VM cells.

The slight posterior shift in the location of the third midgut constriction correlates with the posterior shift of the anterior boundary of *abd-A* expression. *abd-A* is the only homeotic gene required for the development of this constriction (Tremml and Bienz, 1989b). It is possible that this constriction forms in response to a critical level of *abd-A* expression in the VM, which is only attained at a more posterior position in *trx* embryos. It is also possible that *Ubx* protein normally inhibits the formation of the third midgut constriction and its ectopic posterior VM expression in *trx* embryos displaces posteriorly the site at which this constriction forms.

Normally the first midgut constriction forms at the center of the PS5-6 domain of *Antp* VM expression (Tremml and Bienz, 1989b). In *trx* embryos, the first midgut constriction forms posterior to its normal position, directly behind the one or two VM cells that still express *Antp* protein at the posterior of PS6. Thus *Ubx* protein is now expressed at the site where this constriction forms. *Antp* is the only homeotic gene required for the formation of this constriction (Tremml and Bienz, 1989b). If formation of the first midgut constriction requires *Antp* expression in the VM itself, then the *Antp* expression in the few cells adjacent to where this constriction forms in *trx* embryos must be sufficient. It is also possible that the closely apposed tissue (fat body?), which still expresses *Antp* in *trx* embryos, is able to direct or induce the formation of the first midgut constriction. Although *Ubx* is not normally required for the formation of this first constriction (Tremml and Bienz, 1989b), its coincident expression at the site where the constriction forms raises the possibility that it may be abnormally influencing this process. We are presently examining whether the first midgut constriction can form in *trx Ubx* double mutants.

Ultrabithorax, *abdominal-A* and *Sex combs reduced* have different tissue-specific requirements for *trithorax*

trx is required for normal levels of expression of *Ubx*, *abd-A*, *Scr* and *Dfd* in somatic mesodermal and ectodermal derivatives, including neural tissues. In *trx* embryos, all four proteins are expressed within their normal domains in these tissues, but at reduced levels. *Ubx* appears to have the most severely reduced expression, particularly in PS6. *abd-A* expression is reduced in the middle of each segment from A2 to A7, but it does not appear to be reduced to the same extent as *Ubx* expression. Expression of *Scr* and *Dfd* are only slightly reduced in these tissues, indicating their minor requirement for *trx*.

Loss of *trx* expression affects *Ubx*, *abd-A* and *Scr* differently in the VM than in ectodermal and somatic mesodermal tissues. In *trx* embryos, *Ubx* is expressed at normal levels in the VM, though its domain of expression is extended posteriorly due to derepression where *abd-A* is absent. Interestingly tissue-specific *Ubx* autoregulation has been demonstrated for this tissue (Bienz and Tremml, 1988), suggesting that this process does not require *trx* for its implementation. *abd-A* expression is reduced throughout its VM domain, being completely absent from PS8, the anteriormost extent of its normal expression. This differs from its ectodermal and somatic mesodermal expression in *trx* embryos, as it is still expressed in the anterior of its domain in those tissues. *Scr* expression in the VM of *trx* embryos appears unaffected. *Dfd* is expressed only in ectodermal and somatic mesodermal tissues and does not show tissue-specific requirements for *trx* expression. *abd-A* also shows a tissue-specific requirement for *trx* in the pericardial cells of A5-A7 and their supporting alary muscles, where its expression is totally abolished. In *trx* embryos, *Antp* shows a similar loss of expression in the pericardial cells and their supporting alary muscles, where it is also expressed at high levels in wild type (not shown).

These observations indicate that the different homeotic genes require *trx* to different extents in different tissues to achieve their normal levels and patterns of expression, being absolutely required for expression in some and nearly dispensable in others. Since the homeotic genes all contain very large regulatory regions composed of many *cis*-acting elements for directing expression in different tissues and parasegmental domains, this suggests that *trx* interacts differently with unique configurations of regulatory factors present at each of these *cis* regulatory elements in different tissues. This is consistent with previous findings that homeotic genes have different regulatory requirements in the VM compared to other tissues (Bienz et al., 1988; Bienz and Tremml, 1988; Tremml and Bienz, 1989a,b; Irvine et al., 1991). It also implies that those components of homeotic gene expression patterns for which *trx* is dispensible may require other factors, possibly encoded by other trithorax-group genes.

Abdominal-B and *Antennapedia* expression patterns suggest that *trithorax* is differentially required for transcription from their multiple promoters

In *trx* embryos, *Abd-B* appears to be expressed at normal levels in PS13-15 and in the hindgut VM, but is undetectable in PS10-12 except for low levels in the VNC. This is very similar to the pattern of *Abd-B* expression in *iab-7* mutants (Celniker et al., 1990; Boulet et al.,1991). Although this anti-*Abd-B* antibody cannot distinguish ABD-BI from ABD-BII, the normal development of A8 and A9 structures in *iab-7* mutants (Duncan, 1987) implies that there is normal expression of ABD-BI in PS13 and ABD-BII in PS14-15. The PS13 *Abd-B* expression in *trx* embryos probably also reflects near normal levels of ABD-BI there, since their A8 and A9 landmarks develop almost normally. The selective loss of ABD-BI in PS10-12, but not PS13 thus represents a parsegment-specific requirement for *trx* to achieve normal expression of the P4 promoter in PS10-12, but not PS13.

iab-7 mutations disrupt regulatory regions 3′ of the *Abd-B* transcription unit (Karch et al., 1985; Duncan, 1987; Celniker et al., 1990; Boulet et al., 1991) which are required for parasegment-specific P4 transcription in PS10-12, but not PS13. This region is not required for transcription from the distal *Abd-B* promoters in PS14-15 and the hindgut VM. The overall similarity of *Abd-B* expression in *iab-7* and *trx* mutants suggests that the *trx* protein interacts, directly or indirectly, with *iab-7* regulatory sequences and mediates *iab-7* regulatory effects on P4 transcription in PS10-PS12. It may also be required to interact with *iab-5* and *iab-6* regulatory regions to direct P4 transcription in PS10 and PS11, but these effects are obscured by the more encompassing *iab-7*-like phenotype of *trx*. The inferred small reduction of ABD-BII expression in PS14 suggests that *trx* is required to a lesser extent to sustain normal levels of expression from one or more of the distal *Abd-B* promoters. Nevertheless, loss of *trx* function does have phenotypic effects in PS14, implying that its minor role in ABD-BII expression is nonetheless essential for normal PS14 development.

The pattern of *Antp* protein expression in *trx* embryos closely parallels that of P1 RNA expression in wild-type embryos, suggesting that *trx* may affect P2 expression to a greater extent than P1 expression. The features of the residual *Antp* protein expression in the VNC of *trx* embryos that resemble the wild-type P1-specific expression pattern are: (1) very low expression in A8 and A9 neuromeres, where P1 is normally not expressed, (2) low expression in the anterior compartment of the T1 neuromere (aT1), where P1 is not expressed, (3) higher levels of expression in pT1 compared to T2 and T3 neuromeres, like the P1 pattern, and (4) significantly lower levels of expression in the A1-7 neuromeres, like the P1 pattern. *Antp* expression in epidermal and subepidermal cells of *trx* embryos also resembles the wild-type P1 expression pattern. It is expressed (1) at very low levels posterior to PS5, where P1 expression is also very low, (2) at very low levels in PS3, where P1 is not expressed, and (3) at only moderate levels in PS4-5, like P1. In all the regions mentioned above, P2 RNA is expressed at substantial levels in wild type so as to visibly alter the total pattern of *Antp* RNA expression when added to the P1 pattern.

In the VM of wild-type embryos, P2 RNA is expressed in all the cells within the PS5-6 *Antp* expression domain (Tremml and Bienz, 1989b). P1 is only expressed in a cluster of cells in the center of this domain (Bermingham et al., 1990). In *trx* embryos, *Antp* protein is expressed in only one to two nuclei at either extreme of the PS5-6 domain and in a few nuclei near the center of this domain, not in the VM itself, but in a closely apposed tissue, possibly fat body. Therefore, most P2 expression is missing in the VM of *trx* embryos.

Because the P1 expression domain appears to be a subset of the P2 domain, anti-Antp antibodies can only distinguish P2-specific expression unambiguously in P2-specific regions and cannot distinguish P1-specific expression. Therefore, we cannot conclusively rule out the possibility that *trx* embryos have reduced *Antp* expression from both promoters. Furthermore, the previous study of P1 and P2 expression does not provide the resolution in the VM required to address this question. If P1 is normally expressed only in the same central cluster of cells where *Antp* protein is observed in *trx* embryos, then its expression is not severely reduced, since there *Antp* expression is comparable in *trx* and wild-type embryos. However, if P1 is normally expressed in the central region of the VM itself, then expression of both promoters is greatly reduced there. In situ hybridization with P1- and P2-specific probes will be required to resolve this.

Mechanism of action of *trithorax* proteins

How might *trx* function? About a dozen other *trx*-like genes have been identified by mutations that suppress the dominant homeotic phenotypes of *Pc* mutations (Kennison and Tamkun, 1988). One of these, *brahma* (*brm*), displays strong genetic interactions with *trx* and *Pc*, and encodes a protein with extensive homology to a family of DNA helicases (Tamkun et al., 1992), including the yeast SWI2/SNF2 protein, one of a group of global transcription activators (Peterson and Herskowitz, 1992). This, together with the recent evidence that the *Pc* protein shares a 48-residue motif with the heterochromatin-specific protein HP1 (Paro and Hogness, 1991; Eissenberg et al., 1990) has prompted the suggestion that proteins encoded by *trx*-group and *Pc*-group proteins act on chromatin (Paro, 1990). *brm* has been proposed to regulate the local topological state of DNA and thereby the stability or positioning of nucleosomes (Tamkun et al., 1992; Travers, 1992). This might counter the ‘heterochromatinizing’ action of factors like *Pc* and control accessibility of DNA to other locus-specific transcription factors that are otherwise unable to recognize their target sites in closed chromatin. The primary structure of the *trx* protein suggests that it may bind to DNA, but provides few clues to its mode of action beyond that. However, recent evidence indictes that like *trx*, some of the yeast SNF/SWI proteins are also required to different extents by the different genes that they regulate, depending on the promoters involved, the other activating factors bound and the number of binding sites for these other activators at each promoter (Laurent and Carlson, 1992). The observation that other homeobox-containing genes are expressed normally

in *trx* embryos suggests *trx* is also required for the normal expression of a limited number of genes, as suggested by its mutant phenotype, and does not act as a general regulatory factor. While no functional counterpart of *trx* has yet been found in yeast, these results lend further support to the possibility that *trx*, *brm* and other *trx*-like proteins may share a similar mode of action to these yeast proteins, as intermediary regulators that facilitate transcriptional activation by other gene-specific transcriptional regulatory proteins (Laurent and Carlson, 1992).

We would like to thank Danny Brower, Thom Kaufman, Shige Sakonju, Bill McGinnis, Sue Celniker and Mike Levine for antibodies and Mark Mortin for the trx^{B11} allele. We also thank Vijay Shankaran for excellent technical assistance. This work was supported by a National Institutes of Health Grant, GM-39255 to P. J. H.

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(Accepted 29 September 1992)