Mutant Alleles of the Drosophila *trithorax* Gene Produce Common and Unusual Homeotic and Other Developmental Phenotypes

Thomas R. Breen

Department of Zoology, Southern Illinois University, Carbondale, Illinois 62901-6501

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ABSTRACT

trithorax (*trx*) encodes chromosome-binding proteins required throughout embryogenesis and imaginal development for tissue- and cell-specific levels of transcription of many genes including homeotic genes of the ANT-C and BX-C. *trx* encodes two protein isoforms that contain conserved motifs including a C-terminal SET domain, central PHD fingers, an N-terminal DNA-binding homology, and two short motifs also found in the TRX human homologue, ALL1. As a first step to characterizing specific developmental functions of TRX, I examined phenotypes of 420 combinations of 21 *trx* alleles. Among these are 8 hypomorphic alleles that are sufficient for embryogenesis but provide different levels of *trx* function at homeotic genes in imaginal cells. One allele alters the N terminus of TRX, which severely impairs larval and imaginal growth. Hypomorphic alleles that alter different regions of TRX equivalently reduce function at affected genes, suggesting TRX interacts with common factors at different target genes. All hypomorphic alleles examined complement one another, suggesting cooperative TRX function at target genes. Comparative effects of hypomorphic genotypes support previous findings that TRX has tissue-specific interactions with other factors at each target gene. Some hypomorphic genotypes also produce phenotypes that suggest TRX may be a component of signal transduction pathways that provide tissue- and cell-specific levels of target gene transcription.

THE Drosophila *trithorax* gene (*trx*) encodes a large protein (TRX) that is required throughout development to maintain tissue- and cell-specific levels of homeotic and other gene transcription (Capdevila and García-Bellido 1981; Ingham 1981; Duncan and Lewis 1982; Cabrera *et al.* 1985; Ingham 1985a,b; Capdevila *et al.* 1986; Mazo *et al.* 1990; Breen and Harte 1991, 1993; Sedkov *et al.* 1994). In *trx* mutants, transcription of homeotic genes of the Antennapedia complex (ANT-C) and bithorax complex (BX-C) is reduced or absent in a specific subset of cells within a gene's normal expression domain. Besides homeotic genes, the transcription of *engrailed* (Breen *et al.* 1995), *fork head* (Kuzin *et al.* 1994), and *polyhomeotic* (Fauvarque *et al.* 1995) is also TRX dependent. TRX associates with at least 76 sites on salivary gland polytene chromosomes, suggesting many additional target genes (Kuzin *et al.* 1994; Chinwalla *et al.* 1995; Paro and Harte 1996). *trx* transcripts are found in all cells during embryogenesis and are similarly widely distributed in imaginal discs (Mozer and Dawid 1989; Kuzin *et al.* 1994; Sedkov *et al.* 1994; Stassen *et al.* 1995). Characterized TRX target genes, such as homeotic genes, encode transcriptional regulatory factors that specify cell fates. It is not known if this is a common feature of TRX target genes.

There are at least five differentially spliced *trx* mRNAs that encode two protein isoforms, TRXI of 3358 amino acids and TRXII of 3726 amino acids. The protein isoforms differ by 368 N-terminal amino acids that are encoded in an alternatively used exon (Mazo *et al.* 1990; Breen and Harte 1991; Sedkov *et al.* 1994; Stassen *et al.* 1995). The 10- and 12-kb mRNAs that encode only TRXI are maternally supplied to oocytes and are present at decreasing levels through embryogenesis (Mozer and Dawid 1989; Breen and Harte 1991; Sedkov *et al.* 1994). A 14-kb mRNA that encodes TRXII, and could potentially translate TRXI, too, is expressed from early embryogenesis through pupation (Mozer and Dawid 1989; Breen and Harte 1991; Sedkov *et al.* 1994). Only the larger mRNA encoding TRXII is expressed during imaginal cell proliferation. Western blot analysis showed that TRXI is the most prevalent isoform during early embryogenesis, while TRXII is the predominant isoform during the final third of embryogenesis (Kuzin *et al.* 1994). It has not been reported whether TRXII is the only isoform present during larval growth and imaginal proliferation.

Both TRX isoforms have a C-terminal SET domain found in other proteins known or suspected to modulate chromatin structure. These proteins include SU(VAR)3-9, which modulates heterochromatin-mediated repression (Tschiersch *et al.* 1994), and E(Z), a Polycomb group (PcG) protein required for transcriptional repression of homeotic genes (Jones and Gelbart 1990, 1993). E(Z) is required for binding of TRX and

Author e-mail: tbreen@zoology.siu.edu

other proteins to specific chromosomal sites where they may interact with other chromatin factors to alter target gene transcription (Rastelli *et al.* 1993; Kuzin *et al.* 1994; Platero *et al.* 1996). The SET domain of HRX (aka MLL, ALL1, HTRX), the human homolog of TRX, interacts with human myotubularin, a dual-specificity phosphatase, and Sbf1 (Cui *et al.* 1998). Apparently, Sbf1 protects the SET domain from dephosphorylation by myotubularin. This protection delays cell maturation and differentiation, which are promoted after SET domain dephosphorylation effected by myotubularin (Cui *et al.* 1998; De Vivo *et al.* 1998). The TRX SET domain associates with SNR1, a homolog of the yeast SWI/SNF protein SNF5 that participates in chromatin remodeling to facilitate transcription (Rozenblatt-Rosen *et al.* 1998). By this interaction, the SET domain of TRX may abet the recruitment of Drosophila SWI/SNF complexes to some target genes to help modulate transcription by altering chromatin.

The TRX isoforms also contain four centrally located PHD-finger motifs (Mazo *et al.* 1990; Aasland *et al.* 1995; Stassen *et al.* 1995) found in other proteins that appear to interact with chromatin (Lonie *et al.* 1994; Adamson and Shearn 1996; Tripoulas *et al.* 1996). Aasland *et al.* (1995) speculate that PHD fingers may mediate protein-protein interactions between regulatory factors or they may recognize specifically modified histones. N-terminal to the PHD finger motifs, TRX isoforms have a C4 zinc-finger motif similar to the DNA-binding domain (DBD) of nuclear receptors (Stassen *et al.* 1995), though it is not known whether TRX can bind DNA. TRX shares three additional conserved motifs with ALL1 and the closely related ALR (Djabali *et al.* 1992; Gu *et al.* 1992; Tkachuk *et al.* 1992; Domer *et al.* 1993; Rowley 1993; Prasad *et al.* 1997). One motif, of unknown significance, precedes the C-terminal SET domain. Two other short motifs, located on either side of the DBD homology (Stassen *et al.* 1995), promote nuclear localization and may be necessary for chromosomal binding (Yano *et al.* 1997).

TRX and other members of the trithorax group (trxG) of homeotic gene positive regulators behave genetically as antagonists of PcG transcriptional silencing (Capdevila and García-Bellido 1981; Ingham 1983; Capdevila *et al.* 1986; Sato and Denell 1987; Kennison and Tamkun 1988; Shearn 1989). TRX and another trxG protein, ASH1, colocalize with some PcG proteins at many sites along salivary gland polytene chromosomes (Chinwalla *et al.* 1995; Tripoulas *et al.* 1996). Genetic and cytological data suggest that TRX and PC assemble relatively near each other at Polycomb response elements (PREs) near the genes *Ultrabithorax* (*Ubx*) and *Sex combs reduced* (*Scr*; Chan *et al.* 1994; Chang *et al.* 1995; Chinwalla *et al.* 1995; Gindhart and Kaufman 1995). More recently, it was shown that TRX colocalizes with PC at the major PREs in the BX-C (Orlando *et al.* 1998). These observations suggest that the genetic behavior of some trxG and PcG genes may reflect direct functional interactions between their proteins. However, the significance of TRX and PC colocalization remains unknown. The genetic evidence cited above suggests some trxG and PcG proteins colocalize at PREs in other tissues to exert their regulatory effects. The mechanism by which TRX stimulates transcription and preempts PcG silencing is unknown. However, PcG silencing appears to occur by default if a target gene is not transcriptionally active at the time PcG silencing is implemented during germband elongation (Pirrotta *et al.* 1995; Poux *et al.* 1996; Pirrotta 1997, 1998).

The genetic, cytological, biochemical, and structural analyses of TRX suggest it may interact with a variety of proteins at its target genes to stimulate transcription through chromatin modulation. TRX is widely distributed during embryogenesis and imaginal development, as are its PcG antagonists. Either may exert its effect on a target gene depending on other factors that determine the target gene's transcriptional status during germband elongation. Within a homeotic gene's expression domain, TRX is used to different extents to stimulate that gene's transcription. Depending on the target gene, TRX may be generally required to boost its transcription in specific cells or tissues. For some target genes, TRX is essential for transcription in specific cells or tissues. The differential use of TRX at its target genes strongly suggests that tissue- or cell-specific combinations of regulatory factors interact with unique combinations of TRX functional groups to elicit appropriate use of TRX in a cell. As a first step to identifying distinct functions of TRX, I performed a detailed analysis of phenotypes associated with *inter se* combinations of 21 *trx* alleles.

Eclosing and pharate adults were examined for phenotypes associated with reduced expression of the *trx* homeotic target genes *Scr* of the ANT-C and *Ubx*, *abdominal-A* (*abd-A*), and *Abdominal-B* (*Abd-B*) of the BX-C. I hoped to identify allele-specific phenotypes that may reveal different functional domains of TRX. I characterized eight hypomorphic alleles, seven of which primarily affect imaginal development. Results demonstrate that TRX is used in tissue-specific contexts at the target genes examined. Some *trx* genotypes appear to have almost no *Scr* function in T1 leg discs, no *Ubx* function in T3 leg discs, and greatly reduced function of the other genes examined in their respective imaginal tissues. Some *trx* genotypes exhibited additional phenotypes, some of which are also seen in trx^{-} somatic clones (Ingham 1981, 1985a). These latter phenotypes are similar to ones produced by mutations in elements of signal transduction pathways. I suggest that the differential effects of *trx* mutations on different tissues and cells may be due in part to the differential regulation of TRX by cell-signaling mechanisms. I present a model of TRX regulation consistent with its signal transduction and homeotic mutant phenotypes.

MATERIALS AND METHODS

Fly crosses: Crosses were set in vials containing USB/Amersham Fly Diet. About five male and five virgin female flies were placed in each vial. Each cross consisted of at least four vials. Fly crosses were maintained at 22° except for those involving trx^{1}, which were maintained at 25°, the temperature at which trx^{1} has its highest penetrance and expressivity (Ingham and Whittle 1980). Adults were discarded from vials after 10 to 12 days. Fly stocks used in the crosses were trx^{M17} *red e*/*TM6B*, trx^{Z32} *red e*/*TM6B*, *st* trx^{1}/*st* trx^{1}, trx^{E3}/*TM6B*, trx^{Z16} *red e*/*TM6B*, trx^{Z11} *red e*/*TM6B*, trx^{M18} *red e*/*TM6B*, *cu* trx^{JY16} *red e*/*TM6B*, *mwh* $trx^{6.1}$ *red e*/*TM6B*, trx^{D} *sr e*/*TM6B*, trx^{B11} *red e*/*TM6B*, trx^{A7} *red e*/*TM6B*, trx^{M14} *red e*/*TM6B*, trx^{Z15} *red e*/*TM6B*, *T(Y;3)JY25, cu* trx^{JY25} *red e*/*red cv-c sbd*^{2}, *mwh* $trx^{7.1}$ *red e*/*TM6B*, trx^{Z44} *red e*/*TM6B*, *st* trx^{3} *red*/*TM6B*, *cu* trx^{JY21} *red e*/*TM6B*, *Df(3R)redP52*/*TM1*, and *Df(3R)redP6*/*TM1*. *TM1* is *In(3LR)TM1, Me ri sbd*^{l}. *TM6B* is *In(3LR)TM6B, Hu e Tb ca*. See references in Table 1 for origins of *trx* mutant chromosomes.

Lethal phase: Vials were inspected for dead embryos, small or sickly larvae, and dead pupae as a component of lethal phase determination. A lethal phase in this report indicates the latest phase in which animals of an indicated genotype are seen. For each *trx* mutant genotype, the lethal phase is embryonic if no second instar larvae were detected, larval if no pupae were detected, and pupal if no adults were detected. Except in crosses involving trx^{JY25}, *Df(3R)redP52*, and *Df(3R)redP6*, transheterozygotes beyond the first larval instar are identifiable as Tb^{+} animals and as Hu^{+} adults because they are the only offspring that do not carry *TM6B*. Also, except in crosses involving trx^{1}, trx^{E3}, and trx^{D}, *trx* transheterozygotes and hemizygotes are *red* homozygotes or hemizygotes, respectively, and identifiable by their red Malpighian tubules after the first larval instar and *red* mutant eye color as adults. Crosses that involve the intersection of the two exceptional categories can produce heteroallelic larvae and pupae that cannot be distinguished by being either Tb^{+} or *red* mutants. Their lethal phases were determined by other criteria. Crosses between *st* trx^{1} flies and flies carrying trx^{JY25}, *Df(3R)redP52*, and *Df(3R)redP6* produced mutant adults distinguished by their phenotypes. Crosses between trx^{E3}/*TM6B* flies and flies carrying trx^{JY25}, *Df(3R)redP52*, and *Df(3R)redP6* produced disproportionate numbers of dead pupae as did other crosses using trx^{E3}/*TM6B* flies in which the dead pupae were scored as mutant transheterozygotes because they were Tb^{+}. Crosses between trx^{D}/*TM6B* flies and flies carrying trx^{JY25}, *Df(3R)redP52*, and *Df(3R)redP6* produced disproportionate numbers of dead embryos as did other crosses using trx^{D}/*TM6B* flies in which transheterozygotes were determined to die as embryos because they did not produce Tb^{+} second instar larvae.

Alleles examined: I examined phenotypes produced by *inter se* combinations of 21 of 67 available *trx* alleles (Table 1). The 21 alleles used in this study were chosen because (1) they have previously described phenotypic effects, (2) they correlate with described molecular lesions, (3) they were used in previous developmental studies, and (4) they have comparatively high penetrance of haploinsufficient phenotypes, or a combination of these characteristics.

The eight hypomorphic alleles described in this study are trx^{M17}, trx^{Z32}, trx^{1}, trx^{E3}, trx^{Z16}, trx^{Z11}, trx^{M18}, and trx^{JY16}. Mortin *et al.* (1992) reported that trx^{M17} and trx^{Z32} are hemizygous viable at 22°. Homozygous trx^{1} adults from homozygous trx^{1} mothers show an array of transformation phenotypes associated with reduced homeotic gene expression in imaginal tissues. The penetrance of transformation phenotypes increases with increasing temperature to 25°. trx^{1} is associated with an ~9-kb insert in the region encoding the first intron of *trx* (Figure 1). trx^{1} probably has no qualitative effect on *trx* proteins. trx^{E3} is associated with an in-frame deletion that removes 271 amino acids from TRXI and TRXII (Figure 1). The deleted residues are located on the C-terminal side of the central cysteine-rich domain that contains PHD fingers. trx^{E3} is homozygous embryonic lethal, but is a pupal lethal allele in combination with null alleles. trx^{Z16} and trx^{Z11} correlate with point mutations in the central cysteine-rich and terminal SET domains, respectively. They are pupal lethal alleles in combination with null alleles. trx^{M18} has low penetrance of haploinsufficient phenotypes and is a strong hypomorphic, pupal lethal allele in combination with null alleles. trx^{JY16} is associated with a chromosomal break in the region encoding exon 3 (Figure 1). The resulting fusion gene can express unaltered TRXI and perhaps a form of TRXII with novel N-terminal residues, assuming a fusion initiation codon is used. It is a larval lethal allele in combination with null alleles.

The nine null alleles examined are $trx^{6.1}$, trx^{D}, trx^{B11}, trx^{A7}, trx^{M14}, trx^{Z15}, trx^{JY25}, $trx^{7.1}$, and trx^{Z44}. They are homozygous and hemizygous embryonic lethals. Tripoulas *et al.* (1994) reported that $trx^{6.1}$ and $trx^{7.1}$ enhance homeotic transformation phenotypes in double heterozygous combination with *ash1*. trx^{D} is the *Rg-bx* of Lewis (1968) used in many developmental studies. trx^{B11} is associated with an 833-bp deletion. It could encode truncated proteins consisting of 8.6% of the N terminus of TRXI and 17.7% of the N terminus of TRXII (Figure 1). It has been used as a null allele in several studies. trx^{A7} and trx^{M14} have low penetrances of haploinsufficient phenotypes for null alleles, whereas trx^{Z15} and trx^{Z44} have relatively high penetrances of haploinsufficient phenotypes. trx^{JY25} is associated with a Y;3 translocation, but cytological examination showed the breakpoint is distant from *trx* (not shown).

trx^{3} and trx^{JY21} have relatively high penetrance of haploinsufficient phenotypes (Table 1). Ingham (1985a) reported that trx^{3} may have some antimorphic characteristics. This study shows trx^{JY21} also may be slightly antimorphic.

Df(3R)redP52 and *Df(3R)redP6* are cytologically visible deletions of the region encoding *trx*. *Df(3R)redP52* completely removes *trx* and at least 10 other surrounding complementation groups. The centromere proximal break of *Df(3R)redP6* maps between the second and third *trx* exons (Figure 1). *Df(3R)redP6* removes at least 5 more distal complementation groups. It was examined because a remaining *trx* fusion gene could express TRX. However, *Df(3R)redP6* is a *trx* amorph.

Quantified *trx* mutant phenotypes: Reduced *Scr* expression in first thoracic segment (T1) leg discs can lead to transformation of ventral T1 segmental structures into homologous ventral second thoracic (T2) segmental structures (Lewis *et al.* 1980; Struhl 1982). *trx* mutant males were scored for 10 *Scr*-related transformations, and females for 8. A large anterior preapical bristle, a large posterior apical bristle, or both can develop distally on a T1 tibia. One animal can have 1 to 4 such transformations. Anterior bristle transformations occur more often than posterior bristle transformations. Genotypes that produce successively more T1 transformations increasingly produce posterior bristle transformations. Males can have reduced numbers of sex comb teeth on the basitarsus of one or both T1 legs. Proximal transformations include development of T1 sternopleural and mesosternal bristles (Figure 2, B, E, and I). One or two of both of these structures can appear in a *trx* mutant.

Reduced *Ubx* expression in third thoracic segment (T3) haltere and leg discs can lead to transformation of T3 segmental structures into homologous T2 segmental structures (Lewis 1963, 1978). *trx* mutant males and females were scored for four dorsal and eight ventral *Ubx*-related transformations. Dorsally, haltere disc transformations include development of wing tissue in place of normal haltere (Figure 2, C and G) and mesonotal tissue in place of metanotum (Figure 2B). One

TABLE 1

***trithorax* alleles**

Allele	Hemizygous phenotype[a]	Heterozygous penetrance[b]	Molecular lesion[c]	References[d]
trx^{M17}	ts, viable at 22°	0.000 (1255)	Unknown	1
trx^{Z32}	ts, viable at 22°	0.000 (799)	Unknown	1
trx^{1}	Viable, ts ↑ P&E	0.008 (1698)	~9-kb insert in region encoding first intron	2, 3
trx^{E3}	Pupal lethal	0.016 (1135)	Causes a 271-aa in-frame deletion from aa 2130	3–7
trx^{Z16}	Pupal lethal	0.000 (1017)	Causes R to W at aa 1753 in Cys-rich domain	1, 8
trx^{Z11}	Pupal lethal	0.013 (668)	Causes G to S at aa 3601 in SET domain	1, 8
trx^{M18}	Pupal lethal	0.007 (534)	Unknown	1, 23
trx^{JY16}	Larval lethal	0.199 (1139)	Breakpoint within region encoding aa's 172–276	3
trx$^{6.1}$	Embryonic lethal	0.082 (514)	Unknown	9
trx^{D}	Embryonic lethal	0.067 (390)	Unknown	10–16
trx^{B11}	Embryonic lethal	0.045 (222)	Causes truncated protein after aa 659	1, 3, 6, 7, 17
trx^{A7}	Embryonic lethal	0.019 (368)	Unknown	1, 23
trx^{M14}	Embryonic lethal	0.015 (334)	Unknown	1, 23
trx^{Z15}	Embryonic lethal	0.102 (422)	Unknown	1
trx^{JY25}	Embryonic lethal	0.155 (161)	Unknown, a T(Y;3) not in *trx*	This study
trx$^{7.1}$	Embryonic lethal	0.097 (527)	Unknown	9
trx^{Z44}	Embryonic lethal	0.052 (192)	Unknown	1, 23
trx^{3}	Embryonic lethal	0.208 (525)	Unknown	15, 18, 19
trx^{JY21}	Embryonic lethal	0.136 (309)	Unknown	This study
Df(3R)redP52	Embryonic lethal	0.105 (500)	Deletes *trx*, removes 88A4 to 88B4-5	1, 3, 12, 18, 20, 21
Df(3R)redP6	Embroynic lethal	0.331 (136)	Breakpoint in second intron, removes 88B1 to 88B3-C2	1, 3, 22

The top-to-bottom organization of the alleles reflects their relatively increasing contribution to the penetrance and expressivity of the homeotic transformations examined in this study (see Table 2). The exception to this organization is that *trx*^{3} and *trx*^{JY21} cause a slightly more transformed phenotype than the two deficiencies that are listed at the bottom for convenient reference.

[a] Phenotypes are for animals heterozygous for the *trx* mutant chromosome and a *Df(3R)redP52* chromosome. ts, temperature sensitive; ts ↑ P&E, increasing penetrance and expressivity with increasing temperature.

[b] Numbers on the left are the frequency of appearance of at least one transformation phenotype in adults heterozygous for the *trx* mutant chromosome and *TM1* or *TM6B* balancers. Numbers of adults examined are in parentheses.

[c] See Figure 1 for more detailed descriptions.

[d] Numbers refer to the following list: 1, Mortin *et al.* (1992); 2, Ingham and Whittle (1980); 3, Breen and Harte (1991); 4, Kennison and Tamkun (1988); 5, Mozer and Dawid (1989); 6, Mazo *et al.* (1990); 7, Sedkov *et al.* (1994); 8, Stassen *et al.* (1995); 9, Tripoulas *et al.* (1994); 10, Lewis (1968); 11, García-Bellido and Capdevila (1978); 12, Capdevila and García-Bellido (1981); 13, Duncan and Lewis (1982); 14, Botas *et al.* (1982); 15, Ingham (1985a); 16, Capdevila *et al.* (1986); 17, Kuzin *et al.* (1994); 18, Ingham (1981); 19, Ingham (1983); 20, Lewis (1981); 21, Parkhurst *et al.* (1988); 22, Gans *et al.* (1980); 23, D. B. Bailey and P. J. Harte (unpublished results).

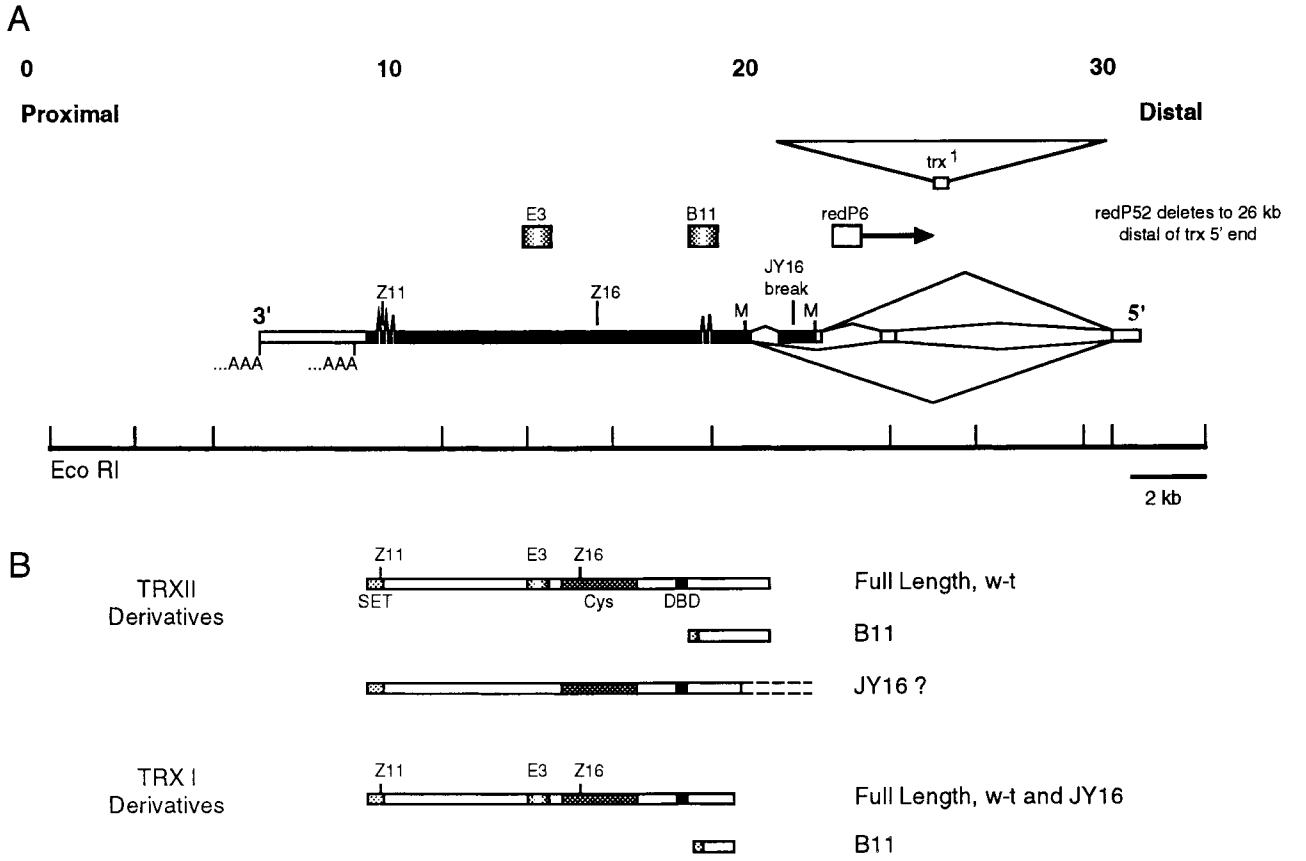

Figure 1.—Characterized mutations within the *trx* transcription unit. (A) The map depicts 32 kb containing the *trx* transcribed region. It is derived from previously published reports (Mozer and Dawid 1989; Mazo *et al.* 1990; Breen and Harte 1991; Sedkov *et al.* 1994; Stassen *et al.* 1995). The thin line with vertical tick marks represents an *Eco*RI map of the region. Above it are nine rectangles that indicate regions encoding exons. Unfilled areas depict 5′- and 3′-untranslated sequences; filled areas show translated sequences. Connectors between the exons represent splicing alternatives. The M's on the 5′ sides of the third and fourth exons label positions of likely initiation codons. The TRXII isoform of 3726 amino acids is translated from mRNAs that contain the third exon. The TRXI isoform of 3358 amino acids initiates within the fourth exon. The tick marks labeled ". . . AAA" show the positions of alternatively used polyadenylation signals. The positions of the point mutations associated with the trx^{Z11} and trx^{Z16} alleles are indicated above the exon rectangles, as is the approximate location of a rearrangement breakpoint associated with the trx^{JY16} allele. The gradient shaded boxes show the approximate sizes and locations of deletions associated with the trx^{E3} and trx^{B11} alleles. The proximal breakpoint of *Df(3R)redP6* is located within the region represented by the open box labeled redP6. The rightward arrow indicates that *Df(3R)redP6* deletes distally beyond the extent of the map. The inverted triangle depicts the approximate position of the 9-kb insert associated with the trx^{1} allele. The open box labeled trx^{1} indicates the region of uncertainty within which the insert is located; the base of the triangle represents the size of the insert. (B) TRXII and TRXI mutant isoforms are depicted. The C termini of these protein representations are to the left, consistent with the orientation of the transcription unit in A. The trx^{Z11} allele is associated with a missense mutation causing a G- to S-substitution at amino acid 3601 in the conserved SET domain (lightly shaded region). The trx^{Z16} allele is associated with a missense mutation causing an R- to W-substitution at amino acid 1753 in the conserved Cys-rich, PHD finger domain (medium gray region). The black region labeled DBD shows the region with similarity to DNA-binding domains of steroid receptors. The trx^{E3} allele is associated with an in-frame deletion that leads to the removal of 271 amino acids from both isoforms. The region removed is indicated by the gradient shaded box. The trx^{B11} allele is associated with an 833-bp deletion that encodes truncated isoforms with 83 novel, C-terminal residues (shaded boxes at left of B11 isoforms). The trx^{JY16} allele is associated with a rearrangement (possibly a small inversion) breakpoint that occurs in the region encoding the third exon. The resulting fusion gene must be transcribed as determined in this analysis. Fusion mRNAs encode normal TRXI. A fusion form of TRXII is also possible that would have the N-terminal *ca.* 172–276 amino acids replaced by residues encoded by the fusion partner.

or both halteres can be affected in a *trx* mutant, and the metanotal transformation can be unilateral or bilateral. Ventrally, transformed T3 legs can have a large anterior preapical bristle, a large posterior apical bristle, or both, on a distal tibia (Figure 2E). One animal can have one to four transformed T3 leg bristles. Proximal T3 ventral transformations include development of sternopleural and mesosternal bristles (Figure 2, A, C, and E). One or two of both of these can appear in a *trx* mutant. Dorsally and ventrally, transformations that affect only anterior compartment structures are more frequent than those that also include posterior compartment structures, and genotypes that produce successively more T3 transformations increasingly develop larger transformed regions that extend into posterior compartments.

Reduced *abd-A* expression in dorsal histoblasts can lead to abdominal tergite transformations (Sánchez-Herrero *et al.*

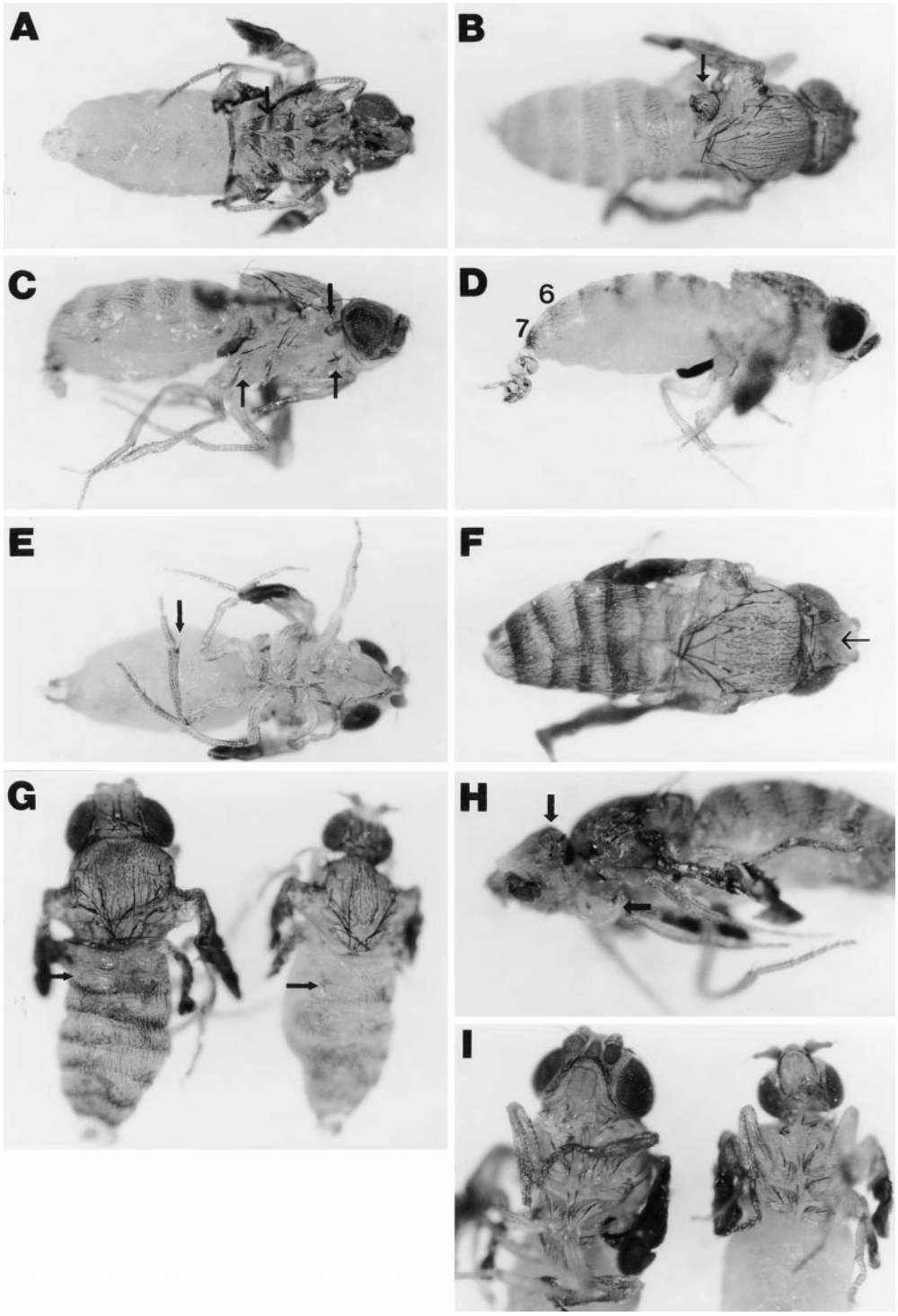

Figure 2.—*trithorax* mutant phenotypes. All flies shown have combinations of *trx* alleles that are lethal by the end of pupal development. Because these flies did not eclose, their wings and halteres partially transformed to wings are not expanded. Flies in A–E are trx^{1Y21}/trx^{Z16}, the fly in F is trx^{Z16}/trx^{E3}, flies in G and I are trx^{3}/trx^{Z11}, and the fly in H is $trx^{6.1}/trx^{M17}$. (A) The arrow indicates ventral T3 with mesosternal bristles normally found only in T2. Mesosternal bristles are also visible in ventral T1. Above the arrow is a haltere partially transformed to a wing. Anterior sternites fail to fuse at the ventral midline. (B) The arrow indicates a transformation of dorsal T3 to mesonotum typical of T2. The A3 tergite is not fused at the dorsal midline. (C) The lower arrows show sternopleural bristles in T1 and T3. These are normally found only in T2. The upper arrow indicates wing tissue that developed in association with a T1 spiracle. The A6 spiracle has unusual material protruding from it. (D) The labels 6 and 7 indicate sixth and seventh tergites of this male. Males normally have fully pigmented fifth and sixth tergites and no seventh tergite. This male has his external genitalia transformed to a leg complete with terminal claws not visible in this focal plane. (E) The arrow shows a T3 leg with a large anterior preapical bristle normally found only on T2 legs. (F) The arrow indicates the presence of a right ocellus and ocellar bristle and the absence of left and center ocelli and a left ocellar bristle. The dorsal anal plate of this female is incomplete. The posterior border of the A1 tergite has *Uab*-like large bristles and dark pigmentation. (G) Arrows show A2 tergites with patches of small bristles normally found only in A1 tergites. Similar patches are also found in the A3 tergites of these flies. The left fly has *Uab*-like dark pigmentation and large bristles at the posterior of its A1 tergite. The right fly has a small head and anterior thorax compared to the one on the left. (H) Top arrow indicates a mirror image duplication of the right eye. The lower arrow shows several abnormal bristles on the lateral labellum. (I) Ventral view of same flies as G. The right fly has complete complements of T1 sternopleural bristles. Almost all of its ventral T1 is transformed to ventral T2. The maxillary palps of the right fly did not develop. Its antennae have *dpp*-like abnormal outgrowths and reduced aristae.

1985). *trx* mutant males and females were scored for five *abd-A*-related transformations. Tergites of abdominal segments two through seven (A2–A7) can develop patches with small bristles and lightly pigmented cuticle normally found in A1 tergites (Figure 2G). A *trx* mutant can have one to six transformed tergites. However, transformations of A6 to A1 tergite were so rare that they were not scored. Transformed patches that encompass only anterior tergite are more frequent than

patches that extend into posterior tergite. Genotypes that produce successively more tergite transformations have successively larger transformed patches that extend into the posterior.

Abd-B is required for normal development of adult posterior segments including A5–A7 (Karch *et al.* 1985; Sánchez-Herrero *et al.* 1985; Tiong *et al.* 1985). *trx* mutant males were scored for two *Abd-B*-related transformations, and females for one. Reduced *Abd-B* expression in dorsal histoblasts can cause males to develop A7 tergites (Figure 2D) that are normally suppressed by *Abd-B* and females to develop enlarged A7 tergites that are similar to more anterior tergites. Males normally have dark pigmentation in A5 and A6 tergites. Reduced *Abd-B* expression can cause loss of this pigmentation (Figure 2D), indicating transformation of underlying cuticle-secreting cells into more anterior identities. Again, transformations that encompass only anterior tergite tissue are more common than those that also include posterior tissue, and genotypes that produce successively more tergite transformations have successively larger transformed patches that include posterior tergite structures.

RESULTS

Penetrance and expressivity of *trx* homeotic phenotypes: Reduced *trx* function leads to reduced expression of homeotic genes during embryogenesis (Mazo *et al.* 1990; Breen and Harte 1991, 1993; Sedkov *et al.* 1994) and imaginal development (Cabrera *et al.* 1985; Ingham 1985b). For each *trx* mutant genotype that produced pharate and eclosing adults, I measured the penetrance and expressivity of that part of the *trx* mutant phenotype that correlates with reduced expression of homeotic genes in imaginal tissue.

Each fly of each genotype was scored for transformations associated with reduced expression of *Scr*, *Ubx*, *abd-A*, and *Abd-B*. *trx* mutant expressivity was measured as the total number of transformed structures, and among those described in materials and methods and in Figure 3, that a fly developed. As a partial example, the number of large T1 leg preapical and apical bristles, diminished sex combs, T1 sternopleural bristle groups, and T1 mesosternal bristles a *trx* mutant male can develop ranges from 0 to 10 and is a measure of *trx* function at *Scr* in T1 leg discs. Males were scored for 29 possible transformed structures, and females for 26. The penetrance of each *trx* mutant transformation is the frequency with which it appears in flies of a particular genotype. Therefore, each genotype produces a combined penetrance and expressivity (P&E) that is expressed as the average number of transformed structures it produces per fly. P&E measurements are used to make quantitative comparisons among the genotypes (Table 2). Male and female transformation averages were combined to obtain a single average for each genotype taking into account the male:female ratio. For each genotype, male:female distributions were within expected values (not shown). P&E values for informative hypomorphic heteroallelic combinations are graphically depicted in Figures 3–6 to illustrate their different qualitative effects.

Below, I describe phenotypes for heteroallelic combinations of eight hypomorphic mutations, nine amorphic mutations, two possible antimorphic mutations, and two deletions. Flies of most combinations that include at least one hypomorphic allele can survive from pupal stages through adulthood. Amorphic combinations are embryonic lethal. The hypomorphic trx^1 allele appears to encode normal TRX that is produced in reduced amounts. Phenotypes of flies with heteroallelic genotypes that contain trx^1 reveal that TRX is used differently among the homeotic genes examined, and it is used differently at *Ubx* in haltere discs compared to T3 leg discs. The relative sensitivities to reduced *trx* function are summarized: *Abd-B* > *Scr* > *Ubx* in haltere discs > *abd-A* > *Ubx* in T3 leg discs. The seven other hypomorphic alleles complement trx^1 and, to some extent, each other. This indicates that the hypomorphic alleles encode impaired proteins, and TRX cooperates at target genes. Hypomorphic mutant proteins are sufficient for embryogenesis. Compared to trx^1, the other hypomorphic mutations have reduced function at *Ubx* in T3 leg discs but greater function at *abd-A*. One hypomorphic mutation, trx^{JY16}, complements loss-of-*trx* function in haltere discs. trx^{E3} has a marked effect on *Scr* and produces head and growth defects. These observations show that TRX has unique interactions at different target genes. trx^{M17}, trx^{Z11}, and trx^{E3} produce unusual phenotypes reminiscent of those produced by hypomorphic signal transduction mutations.

Effects of trx^1: The ~9-kb insert associated with trx^1 is located outside coding sequences. It is likely that the mutant gene produces normal TRXI and TRXII but at reduced levels due to either impaired RNA processing, such as splicing of the first intron, or impaired translation of an mRNA with an unspliced first intron. Consequently, the effect of trx^1 is probably quantitative, and it supplies insufficient TRX to accumulate properly at affected target genes. This is consistent with the findings of Chinwalla *et al.* (1995), who showed that fewer TRX-binding sites on polytene chromosomes are occupied by TRX in trx^1/*Df(3R)redP52* mutants compared with trx^1/trx^1 mutants.

trx^1 has measurably different effects on *Abd-B*, *Scr*, *abd-A*, *Ubx* in haltere discs and *Ubx* in T3 leg discs compared with effects produced by genotypes with high P&E values (Figures 3 and 4). *Abd-B* is most sensitive to reduced levels of TRX in trx^1 hemizygotes followed by *Scr*, *Ubx* in haltere discs and *abd-A*, and *Ubx* in T3 leg discs. This suggests concentration-dependent differences in the ability to assemble or maintain sufficient TRX at different PREs and in different tissues.

The seven other *trx* hypomorphic alleles complement trx^1. Transheterozygotes of these alleles with trx^1 have significantly lower P&E values than trx^1 homozygotes and hemizygotes (Table 2). P&E values of trx^1/hypo-

TABLE 2

Heteroallelic penetrance and expressivity

♂ / ♀	trx^{M17}	trx^{Z32}	trx^{1}	trx^{E3}	trx^{Z16}	trx^{Z11}	trx^{M18}	trx^{JY16}
trx^{M17}	Larva	0 (75)	0.30 (45)	0.35 (37)	0.55 (11)	1.18 (49)	0.18 (22)	3.83 (33)[b]
trx^{Z32}	0 (48)	Larva	2.07 (95)	0.95 (155)	1.36 (36)	4.19 (21)[b]	2.95 (12)[d]	2.68 (30)
trx^{1}	0.01 (89)	2.11 (75)	7.16 (105)	4.82 (125)	3.20 (38)	2.56 (72)	1.90 (50)	3.51 (105)
trx^{E3}	0.12 (65)	0.80 (85)	4.40 (112)	Embryo	5.89 (11)[c]	7.90 (79)[b]	6.75 (40)[b]	Pupa
trx^{Z16}	0.25 (44)	1.20 (66)	2.29 (159)	3.08 (36)[a]	Larva	9.41 (26)[d]	9.50 (4)[e]	14.07 (27)[e]
trx^{Z11}	0.27 (107)[a]	2.63 (94)[b]	2.75 (125)	4.21 (51)[a]	7.36 (28)[d]	14.69 (31)[e]	13.30 (10)[e]	15.13 (16)[c]
trx^{M18}	0.42 (12)	3.91 (22)[c]	3.55 (80)	5.78 (20)[d]	10.58 (4)[c]	16.39 (14)[e]	Larva	17.50 (32)[e]
trx^{JY16}	1.86 (29)	2.47 (56)[a]	3.40 (55)	Pupa	11.82 (25)[d]	13.32 (34)[e]	16.40 (22)[e]	Larva
$trx^{6.1}$	2.37 (56)	5.62 (8)[c]	1.36 (157)	Pupa	18.87 (8)[e]	19.50 (2)[e]	22.20 (10)[e]	Larva
trx^{D}	0.50 (6)	6.12 (8)[b]	12.36 (41)	Pupa	17.00 (2)[e]	Pupa	Larva	Larva
trx^{B11}	2.30 (10)[a]	4.19 (7)[a]	8.86 (50)	Pupa	15.29 (7)[e]	Pupa	14.00 (1)[e]	Larva
trx^{A7}	1.00 (16)	5.34 (31)[e]	8.54 (103)[a]	13.00 (1)[e]	21.00 (3)[e]	Larva	Larva	Larva
trx^{M14}	1.54 (45)[b]	5.29 (12)[e]	9.14 (34)[a]	Pupa	18.10 (10)[e]	17.00 (2)[e]	Embryo	Larva
trx^{Z15}	3.16 (64)	7.19 (39)[e]	13.23 (75)	Pupa	15.75 (4)[e]	Pupa	Embryo	Larva
trx^{JY25}	4.37 (8)[a]	3.29 (7)[d]	11.35 (77)	Pupa	17.74 (8)[e]	Pupa	21.00 (2)[e]	Larva
$trx^{7.1}$	2.06 (31)[a]	5.56 (34)[d]	12.08 (112)[a]	Pupa	20.67 (9)[e]	23.00 (3)[e]	21.00 (2)[e]	Larva
trx^{Z44}	3.40 (5)[e]	5.73 (45)[e]	10.24 (34)[d]	Pupa	17.00 (1)[e]	Pupa	Larva	Larva
trx^{3}	2.93 (14)[a]	6.33 (6)[c]	12.27 (59)	Pupa	19.50 (2)[e]	21.70 (5)[e]	25.00 (1)[e]	Larva
trx^{JY21}	3.31 (78)[a]	7.79 (32)[e]	17.41 (30)[a]	Pupa	22.45 (9)[e]	22.33 (3)[e]	23.29 (11)[e]	Larva
redP52	1.25 (9)[a]	5.96 (14)	13.20 (117)	11.33 (3)[d]	18.15 (10)[e]	21.74 (7)[d]	26.00 (1)[e]	Larva
redP6	3.82 (41)[b]	5.86 (7)	12.26 (28)	Pupa	20.14 (14)[e]	22.60 (3)[e]	Embryo	Larva

♂ / ♀	$trx^{6.1}$	trx^{D}	trx^{B11}	trx^{A7}	trx^{M14}	trx^{Z15}	$trx^{7.1}$	trx^{Z44}	trx^{3}	trx^{JY21}	*redP52*	*redP6*
trx^{M17}	6.75 (31)[b]	3.60 (20)[b]	3.60 (60)[d]	2.88 (8)[b]	4.00 (7)[e]	5.67 (10)[e]	5.60 (5)[b]	5.82 (26)[d]	7.60 (10)[c]	6.39 (46)[b]	5.35 (52)	4.88 (11)[b]
trx^{Z32}	5.90 (67)[a]	5.42 (12)[a]	4.08 (52)[b]	4.93 (7)[e]	4.72 (28)[c]	6.70 (5)[c]	7.58 (13)[e]	6.15 (17)[e]	8.95 (13)[a]	7.27 (23)[e]	6.19 (14)	7.01 (18)[a]
trx^{1}	1.51 (83)	3.46 (30)	6.74 (75)	2.05 (57)	4.60 (20)	6.90 (209)	9.25 (76)	Larva	6.51 (62)	5.86 (195)	7.01 (45)	8.69 (23)
trx^{E3}	Pupa	Pupa	Pupa	Pupa	Pupa	Pupa	Pupa	Pupa	Pupa	Pupa	Pupa	Pupa
trx^{Z16}	17.97 (11)[e]	16.50 (14)[c]	16.64 (22)[e]	18.17 (6)[d]	17.97 (6)[e]	18.43 (50)[e]	17.61 (22)[e]	17.25 (4)[e]	21.33 (12)[e]	20.22 (18)[e]	20.50 (6)[e]	16.67 (6)[e]
trx^{Z11}	20.80 (5)[e]	18.70 (10)[e]	20.00 (6)[e]	Larva	18.67 (3)[e]	19.00 (1)[e]	22.20 (5)[e]	21.00 (1)[e]	23.40 (5)[e]	22.49 (11)[e]	23.00 (10)[e]	18.83 (6)[e]
trx^{M18}	20.50 (4)[e]	Larva	20.00 (1)[e]	Larva	Embryo	Embryo	Larva	Larva	Larva	23.84 (6)[e]	Larva	Embryo
trx^{JY16}	Larva	Larva	Larva	Larva	Larva	Larva	Larva	Larva	Larva	Larva	Larva	Larva

Column headings represent maternally contributed alleles. Row headings (far left column) indicate paternally contributed alleles. Numbers are the average number of tranformations seen in adults of the genotypes determined by reading the column/row headings. Numbers in parentheses show the number of flies examined of the given genotypes. Males have a maximum of 29 transformations, females 26. The maximum average when there is a 1 male:1 female ratio is 27.5. An entry of embryo indicates that no larvae of the given genotype were seen. An entry of larva indicates that the genotype was lethal by the larval third instar stage. An entry of pupa indicates that the genotype was lethal during the pupal stage. Amorphic and antimorphic genotypes not included are embryonic lethal.

[a] Between 100 and 75% of the scored adults eclosed.

[b] Between 75 and 50% of the scored adults eclosed.

[c] Between 50 and 25% of the scored adults eclosed.

[d] Between 25% and 0 of the scored adults eclosed.

[e] None of the scored adults eclosed. If no superscript, 100% eclosed.

morph transheterozygotes form an increasing series that suggests progressively decreasing function among the hypomorphic alleles: $trx^{M17} > trx^{Z32} > trx^{Z16} > trx^{Z11} > trx^{M18} = trx^{JY16} > trx^{E3}$. As hypomorphic complementation decreases in this series, *Abd-B* is most sensitive to declining *trx* function, followed by *Scr* and, to a lesser extent, *Ubx* and *abd-A* (Figure 5). This agrees with the relative sensitivities of the target genes seen in trx^{1} hemizygotes. It is also consistent with previous reports of trx^{-} haploinsufficient phenotypic frequencies (Capdevila and García-Bellido 1981; Capdevila *et al.* 1986; Castelli-Gair and García-Bellido 1990; T. R. Breen, unpublished results) that show that transformations associated with decreased *Abd-B* and *Scr* functions are most frequent, followed by transformations associated with decreased *Ubx* and *abd-A* functions. Slight differences in target gene sensitivities seen among trx^{1}/hypomorph transheterozygotes can be attributed to allele-specific effects seen in hypomorph hemizygotes (Figure 3).

The hypomorphic alleles must encode proteins that cooperate with normal TRX supplied by trx^{1} in imaginal cells. Hemizygous P&E values for trx^{Z16}, trx^{Z11}, trx^{M18}, and the larval lethality of trx^{JY16} (Table 2) show that they are strong hypomorphs, yet these alleles provide substantial imaginal *trx* function in combination with trx^{1} (Table 2) except at *Abd-B* (Figure 5). Thus, the defective proteins encoded in the hypomorphic alleles must be present in sufficient quantity, together with low levels of normal TRX, to form and maintain functional TRX structures at many PREs other than those associated with *Abd-B*.

García-Bellido and Capdevila (1978), Ingham and Whittle (1980), and Capdevila and García-Bellido (1981) recognized that reduced *trx* function during imaginal cell determination and proliferation produces patches of tissue with cell identity transformations that occur most frequently in anterior compartments of segments. In this study, all heteroallelic combinations with trx^{1} similarly produce patchy transformations that occur most frequently in anterior compartments (not shown; see materials and methods). All other hypomorphic genotypes also produce transformed patches, but a greater percentage of them extend into posterior compartments than are seen in trx^{1} genotypes. Furthermore, genotypes that produce higher P&E values concomitantly produce larger transformed patches, sometimes encompassing most of a segment (Figure 2).

Ingham and Whittle (1980) and Ingham (1985a) suggested that transformed patches are clones derived from progenitor cells that have lost the ability to transmit functional TRX structures to their descendants. The preponderance of anterior compartment transformations produced by trx^{1} genotypes suggests that production of heritable or functional TRX assemblies in anterior cells is more susceptible to reduced TRX concentration than in posterior cells. Strong hypomorphic hemizygotes can form some functional and heritable TRX structures (Figure 3), but they are more often lost during early imaginal cell proliferation and increasingly in posterior cells. This is seen in the increased penetrance of flies with three and four transformed leg bristles that include posterior apical bristles (Figure 3). These flies also show increased penetrance of posterior haltere to wing transformations and posterior tergite transformations (Figure 2).

Effects of other hypomorphic alleles: trx^{M17}, trx^{Z32}, trx^{Z16}, trx^{Z11}, and trx^{M18} in heteroallelic combination with amorphic or antimorphic alleles have proportionally less function at *Ubx* in T3 leg discs than at *Ubx* in haltere discs and at *Scr*, *abd-A*, and *Abd-B* in their tissues compared with equivalent trx^{1} and trx^{E3} heteroallelic combinations (Figures 3 and 4). They have a relatively small effect on *abd-A* expression compared with that caused by trx^{1} genotypes. These effects are also evident in flies with heteroallelic combinations of hypomorphic alleles as exemplified in Figure 6. trx^{JY16} shows a slightly more exaggerated example of this profile in heteroallelic combinations with trx^{M17}, trx^{Z32}, trx^{Z16}, trx^{Z11}, or trx^{M18}, as demonstrated in Figure 6.

trx^{M17}, trx^{Z32}, trx^{E3} trx^{Z16}, trx^{Z11}, trx^{M18}, and trx^{JY16} complement each other to varying extents (Table 2 and compare Figures 3 and 4 with Figure 6), but almost all combinations supply sufficient function at *abd-A* for normal tergite development. The complementation supplied by these defective proteins shows that they can cooperate at PREs. Differing levels of complementation among the hypomorphic genotypes reflect that the mutant proteins have different abilities to assemble at PREs, associate with each other, or interact with other factors to influence target gene transcription.

Molecular lesions associated with trx^{Z16}, trx^{Z11}, and trx^{JY16} affect different regions of TRX (Figure 1), yet they have similar proportional effects on *Scr*, *Ubx*, *abd-A*, and *Abd-B* in imaginal cells. It is likely that mutations associated with these and some of the other hypomorphic alleles disrupt separate functions that have the same consequence on development. This is supported by the ability of trx^{M17}, trx^{Z32}, trx^{E3}, and trx^{Z16} to complement trx^{Z11} (Figure 6), suggesting that one mutant protein supplies the missing function of the other and vice versa. Other cases of heteroallelic complementation can be gleaned from Table 2. trx^{M18}/trx^{Z11} and trx^{JY16}/trx^{Z11} are examples of genotypes for which it is difficult to determine if there is complementation. For point mutations, lack of complementation may indicate both alleles affect the same functional domain. Other combinations are more difficult to interpret.

trx^{JY16} homozygotes and hemizygotes die as small, lethargic third instar larvae. They do not have obvious segmental transformations, but their tracheae are convoluted and may be disjointed (not shown) as can occur when decapentaplegic protein (DPP) or epidermal growth factor (EGF) tracheal signaling is disrupted (Vincent *et al.* 1997; Wappner *et al.* 1997). Homozygotes or hemizygotes of the other hypomorphic alleles

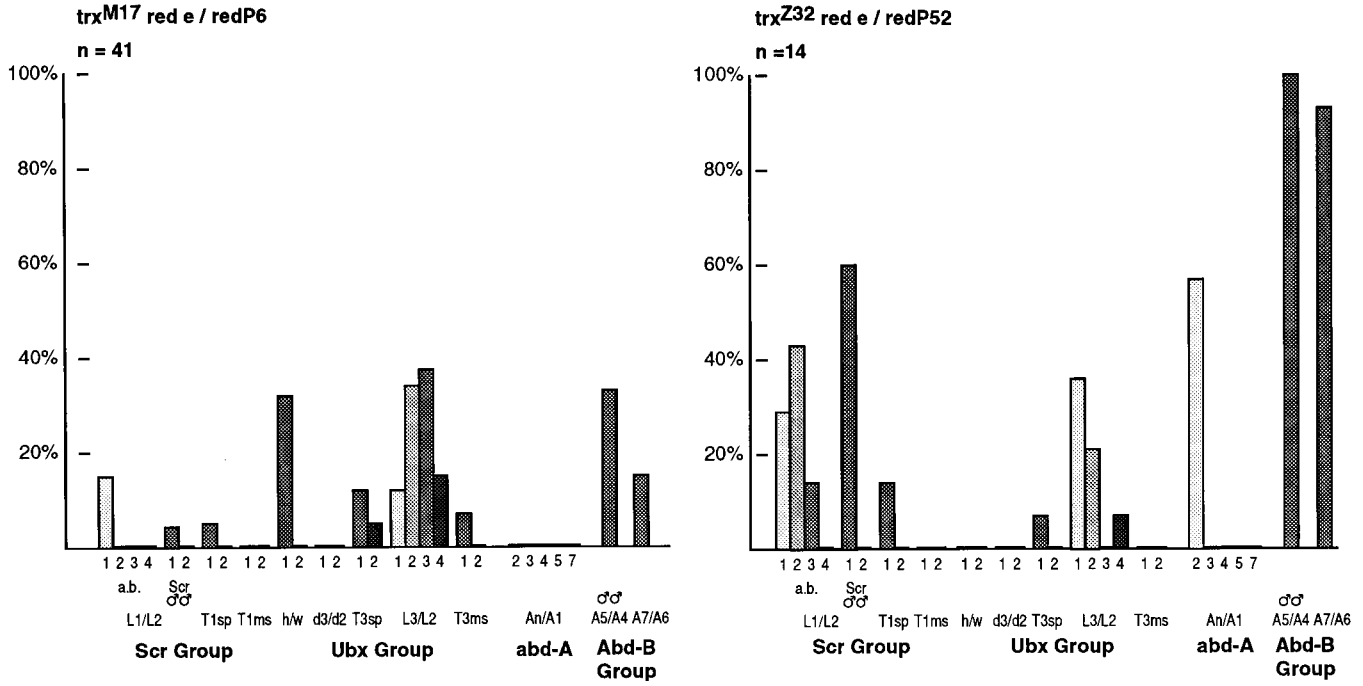

Figure 3.—Mutant penetrance and expressivity of hemizygous hypomorphs. Vertical bars show the percentage of flies with the indicated number of transformed structures associated with reduced expression of *Scr*, *Ubx*, *abd-A*, or *Abd-B*. The number of transformed structures for a category measures its expressivity. The percentage of flies with transformed structures in a category measures its penetrance. Transformation penetrance and expressivity infer a level of *trx* function affecting homeotic gene expression specifying cell fate identity. *Scr* group transformations. Decreased *Scr* function causes development of T1 structures with T2 identity. T1 to T2 leg transformations (L1/L2) include development of an anterior preapical bristle, a posterior apical bristle, or both (a.b.) on the distal tibia of one or two T1 legs and reduced numbers of sex comb teeth in males only (Scr ♂♂) on one or two legs. Other ventral transformations of T1 to T2 include development of one or two sets of T1 sternopleural bristles (T1sp) and one or two T1 mesosternal bristles (T1ms). *Ubx* group transformations. Decreased *Ubx* function leads to development of T3 structures with T2 identity. Dorsal T3 to T2 transformations include development of one or two halteres to wing (h/w) and one or two hemimetanota to hemimesonota (d3/d2). Ventral T3 to T2 transformations include development of one or two sets of T3 sternopleural bristles (T3sp), an anterior preapical bristle, a posterior apical bristle, or both on the distal tibia of one or two T3 legs (L3/L2), and one or two T3 mesosternal bristles (T3ms). *abd-A* group transformations. Decreased *abd-A* function results in development of A2–A7 structures with A1 identity. Dorsal transformations are evidenced by development of small hairs typical of an A1 tergite in more posterior tergites (An/A1, n = 2–7). So few A6 tergites were transformed, the category was excluded. Vertical bars show the percentage of each transformed tergite. Transformation of more posterior tergites correlates with increased expressivity. *Abd-B* group transformations. Decreased *Abd-B* function causes development of posterior abdominal segments with more anterior abdominal identities. Transformation phenotypes include anterior abdominal pigmentation in A5 and A6 tergites in males (A5/A4 ♂♂) and enlargement of A7 tergites (A7/A6). These phenotypes were scored for penetrance only. The genotype and number of flies examined are indicated at the top left of each chart. The chromosome indicated to the left of the slash was maternally inherited. The trx^{M17} allele supplies significant function at the target genes examined except *Ubx* in ventral structures. Other transformations can be attributed to haploinsufficiency. The trx^{Z32} allele is a weak hypomorph at the four loci examined. The trx^{1} allele is a strong hypomorph at the loci examined. It has a noticeably greater effect on *abd-A* expression than the other hypomorphs. This effect is also seen when trx^{1} is paternally inherited. The trx^{E3} allele is a moderate hypomorph at the loci examined. Few trx^{E3} hemizygotes develop to the pharate stage. Of those that do, most have unchitinized cuticles and fail to evert head structures. The trx^{Z16} and trx^{Z11} alleles are strong hypomorphs at the loci examined. trx^{Z16} has some residual function at *Scr* and *Ubx* compared to trx^{Z11}.

develop through the third larval instar with no obvious defects. Thus, all eight hypomorphic alleles supply sufficient *trx* function for normal embryogenesis with one dose of maternally supplied, wild-type *trx*, and only trx^{JY16} is insufficient for normal larval development. It is unknown how imaginal cell proliferation is affected in trx^{JY16} homozygotes and hemizygotes. As stated above, trx^{JY16} in combination with trx^{M17}, trx^{Z32}, trx^{Z16}, trx^{Z11}, or trx^{M18} disproportionally complements *Ubx* in haltere discs compared with equivalent heteroallelic combinations of the other hypomorphs, as typified in Figure 6. This reveals a different use of TRX to regulate *Ubx* in haltere *vs.* T3 leg discs.

trx^{E3} hemizygotes occasionally develop to pharate adults. More often they die as pupae with some head and thorax chitinization but little in the abdomen, and their heads often fail to evert. In hemizygotes, trx^{E3} has a proportionally greater effect on *Scr* than *Ubx* and *abd-A* compared with other hypomorphic alleles (Figure 3). Its effect on *Scr* is more evident in transformed distal

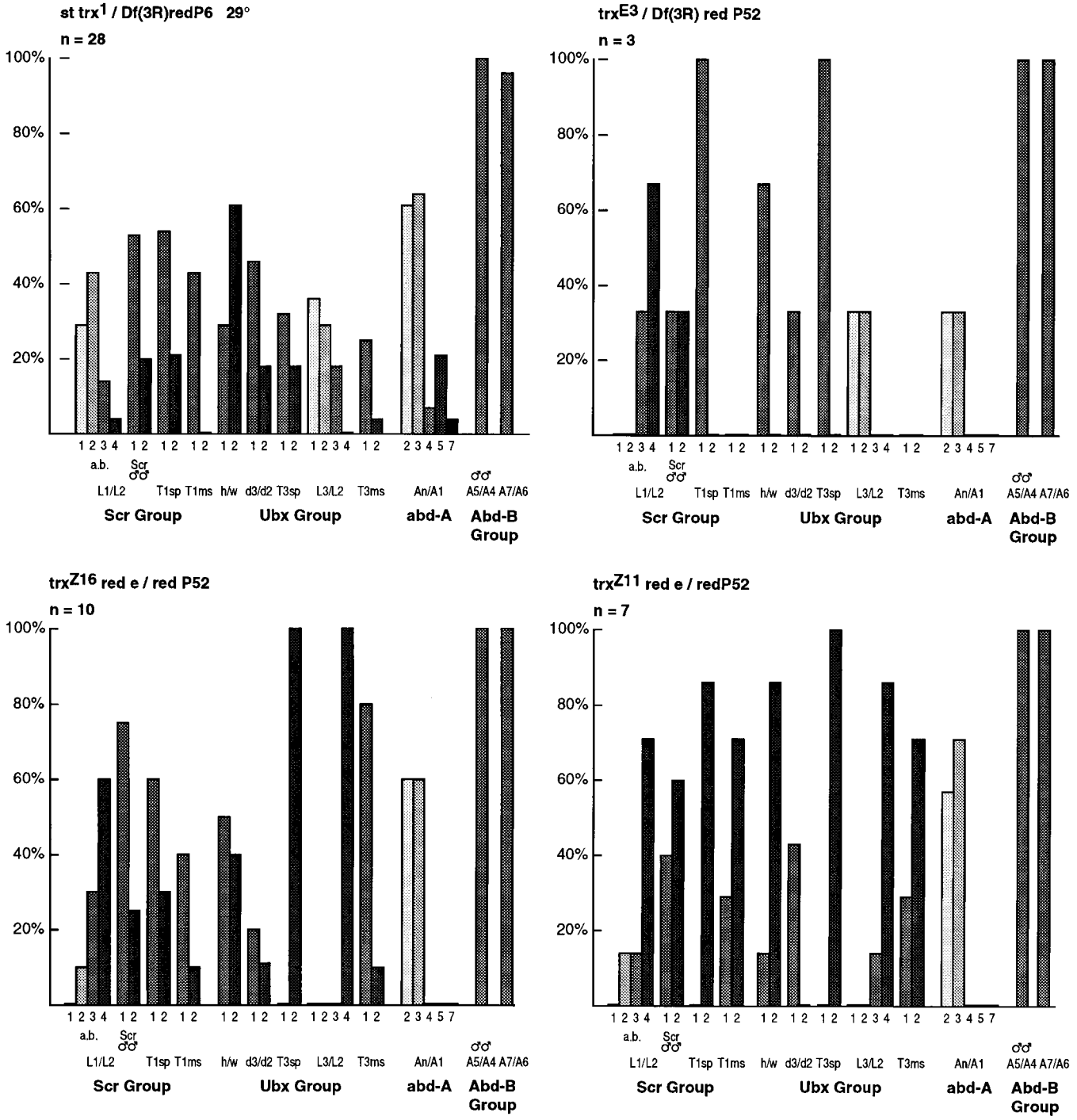

Figure 3.—*Continued.*

T1 leg structures (preapical and apical bristles and sex combs) than in proximal structures (sternopleural and mesosternal bristles). trx^{E3} also proportionally provides greater complementation at *Ubx* than at *Scr* in heteroallelic combination with other hypomorphic alleles, as seen in Figure 6. These observations are consistent with those of Sedkov *et al.* (1994) who noted that trx^{E3} reduces ANT-C, but not BX-C, expression during embryogenesis. Pharate adult trx^{E3} hemizygotes do have reduced BX-C expression, but much of it may be attributed to haploinsufficiency. This seems likely because trx^{E3} substantially complements reduced *Ubx*, *abd-A*, and *Abd-B* function caused by other hypomorphic alleles (Figures 5 and 6). The ability of two doses of trx^{E3} to complement BX-C function more fully cannot be observed because trx^{E3} homozygotes die as embryos. This lethality is probably not caused solely by trx^{E3} because trx^{E3} hemizygotes develop to pharate adults. As noted

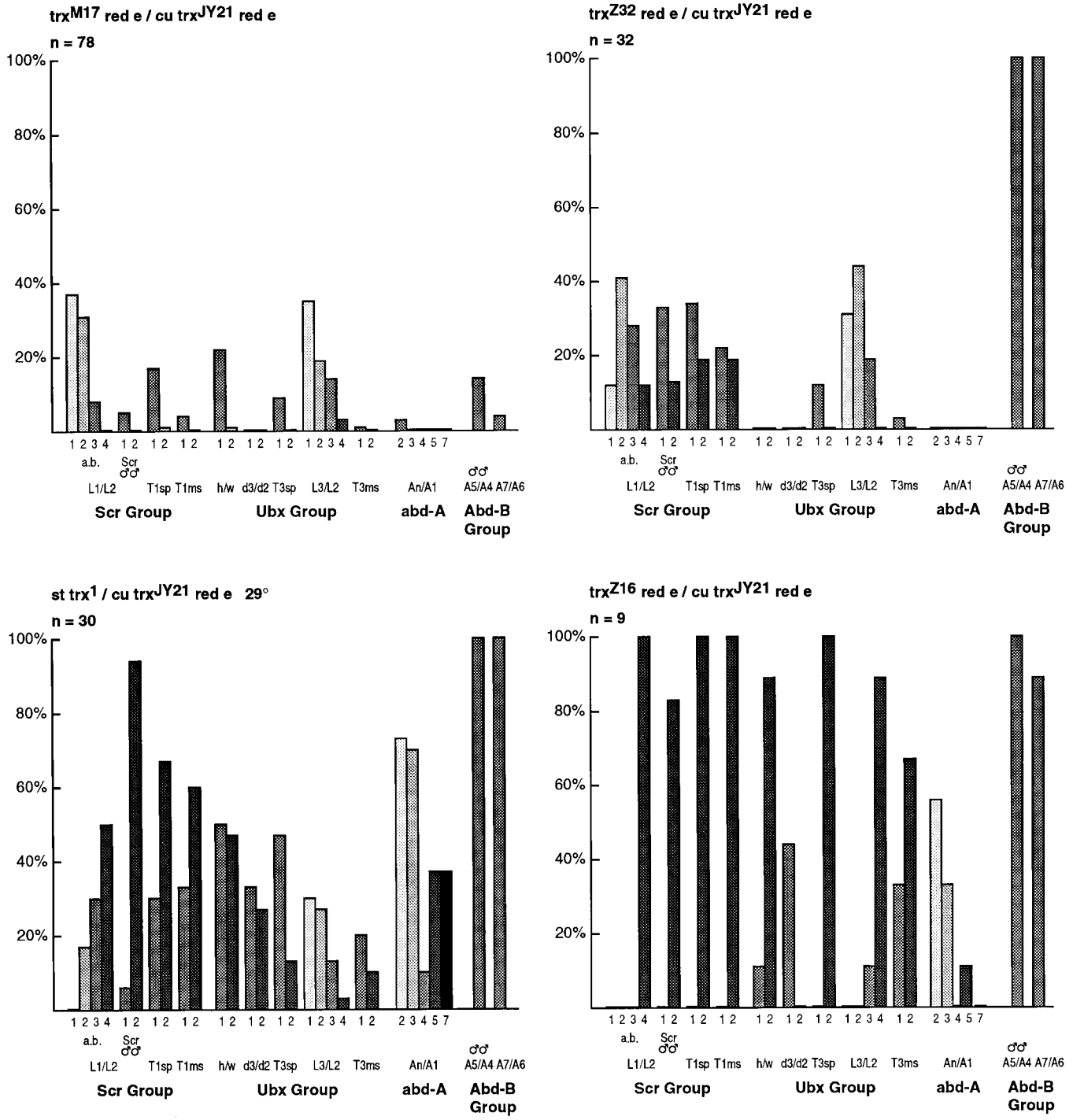

Figure 4.—*trx*^{JY21} may be a weak antimorph. Bar graphs are as in Figure 3. Compare hypomorph/*trx*^{JY21} profiles to those of the same hypomorphic hemizygotes in Figure 3. Penetrance and expressivity are higher in heteroallelic combinations with *trx*^{JY21}. *trx*^{E3}/*trx*^{JY21} is pupal lethal and not available for comparison. Note that *trx*^{JY21} impairs the already decreased function of *trx*^{1} at *abd-A*. *trx*^{M18} is a stronger hypomorph than *trx*^{Z11}. It may be a near amorph in imaginal cells at the loci examined.

below, *trx*^{E3} genotypes frequently have anterior dorsal eye-disc defects including missing ocelli, ocellar bristles, and postvertical bristles. This phenotype is not associated with ANT-C function (Merrill *et al.* 1987, 1989; Pultz *et al.* 1988), but it may be related to deficient epidermal growth factor receptor (EGFR; Clifford and Schupbach 1989; Finkelstein *et al.* 1990; Gabay *et al.* 1996) or *hedgehog* (*hh*) protein (HH) signaling (Royet and Finkelstein 1996) in eye imaginal discs.

Effects of amorphic and antimorphic alleles: *trx*$^{6.1}$, *trx*^{D}, *trx*^{B11}, *trx*^{A7}, *trx*^{M14}, *trx*^{Z15}, *trx*^{JY25}, *trx*$^{7.1}$, and *trx*^{Z44} homozygotes and hemizygotes die as embryos (Table 2). *Inter se*

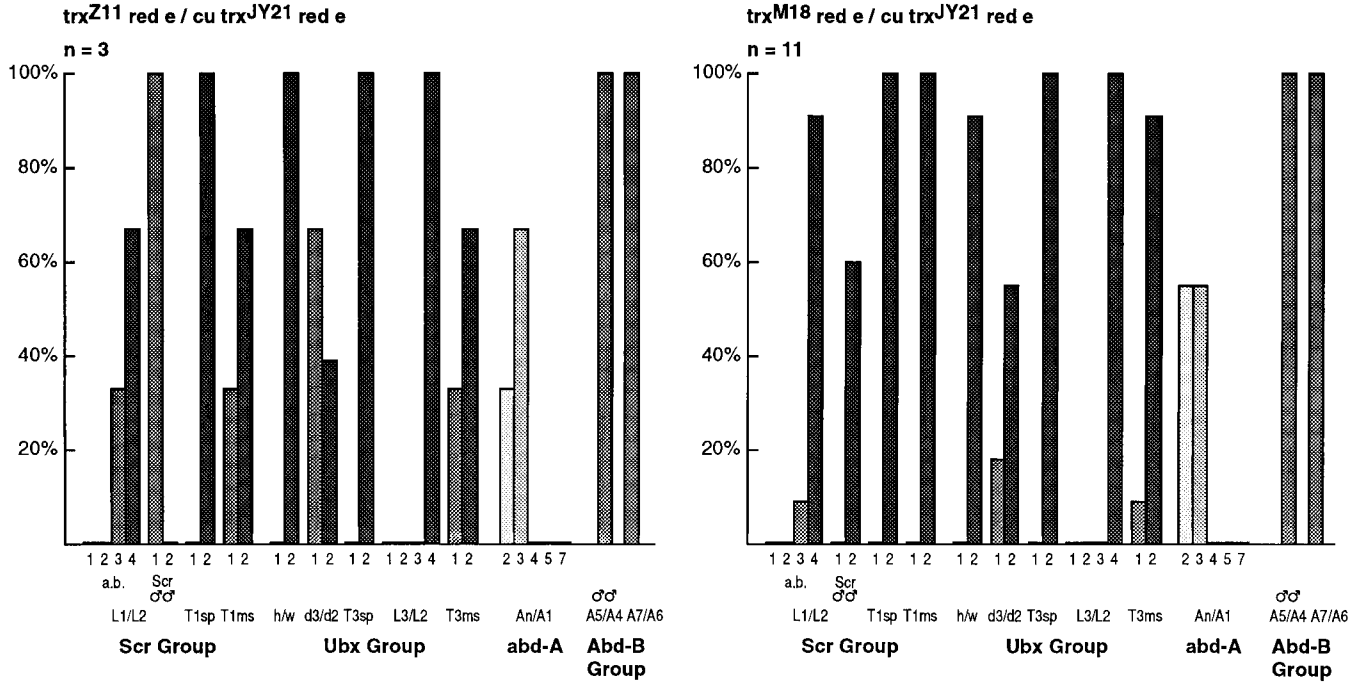

Figure 4.—*Continued.*

combinations of these alleles are also embryonic lethal. $trx^{6.1}$, trx^{B11}, trx^{A7}, and trx^{M14} variably complement trx^{1} (Table 2), but otherwise behave as amorphic alleles. Thus, these alleles may encode defective proteins that supply some imaginal function with wild-type TRX, though the complementation of trx^{B11}, trx^{A7}, and trx^{M14} is marginal enough that it may be attributed to background effects (Capdevila and García-Bellido 1981; T. R. Breen, unpublished results).

$trx^{6.1}$ provides greater complementation of trx^{1} than do hypomorphic alleles other than weak trx^{M17} (Table 2). Thus, $trx^{6.1}$ mutant proteins probably form functional TRX structures with normal TRX supplied zygotically and maternally by trx^{1} or zygotically by trx^{1} and maternally by one dose of trx^{+} from $trx^{6.1}$/*TM6B* mothers. Proteins encoded in $trx^{6.1}$ do not complement functions affected by the hypomorphic alleles other than trx^{1}. $trx^{6.1}$ may encode a truncated protein that easily interacts with intact TRX but cannot compensate for a defective function.

trx^{D} and trx^{A7} weakly complement trx^{M17} (Table 2). They proportionally rescue trx^{M17} function (not shown). Thus, defective proteins encoded in trx^{D} and trx^{A7} may weakly interact with deficient trx^{M17} proteins, but they are unable to rescue mutations that remove successively greater *trx* function.

trx^{JY21}/hypomorph genotypes typically have higher P&E values (Table 2) than equivalent amorphic genotypes, suggesting trx^{JY21} is slightly antimorphic. Furthermore, trx^{JY21} genotypes often produce phenotypes not used for P&E analysis (Figure 2; see below) that are not seen in hypomorphic hemizygotes. trx^{JY21} genotypes also produce larger transformed patches, sometimes encompassing entire structures (Figure 2, A–D), than equivalent amorphic genotypes. Indeed, trx^{M18}/trx^{JY21} pharate adults have nearly completely transformed ventral T1 and T3 structures, suggesting little or no *trx* function at *Scr* in T1 leg discs or at *Ubx* in T3 leg discs. In combination with trx^{1}, trx^{JY21} disproportionally reduces *Scr* expression compared with what is seen in trx^{1} hemizygotes (compare Figures 3 and 4). trx^{JY21} similarly reduces the function of strong hypomorphic alleles at *Scr* and *Ubx*, but not at *abd-A*.

In a few combinations with hypomorphic alleles, trx^{3} produces higher P&E values than equivalent amorphic genotypes; otherwise it behaves as an amorphic allele. Like trx^{JY21}, trx^{3} genotypes often produce phenotypes not seen in hypomorphic hemizygotes, including male genitalia to leg, humerus to wing, and others mentioned below. By this criterion, trx^{3} may be slightly antimorphic.

Additional *trx* mutant phenotypes: Some *trx* mutant genotypes produce unusual phenotypes similar to those reported by Ingham and Whittle (1980) or that develop in amorphic *trx* mutant clones (Ingham 1981, 1985a).

Flies transheterozygous for the weakly antimorphic trx^{JY21} or trx^{3} and any of the strong hypomorphs trx^{Z11}, trx^{Z16}, or trx^{M18} occasionally develop wing tissue adjacent to their prothoracic spiracles (Figure 2C). This is consistent with decreased *Scr* expression in humeral disc anterior compartments (Ingham and Whittle 1980; Ingham 1985a; Rogers *et al.* 1997). Males of the same genotypes occasionally develop T2 legs from their genital discs (Figure 2D) that may be caused by a relative

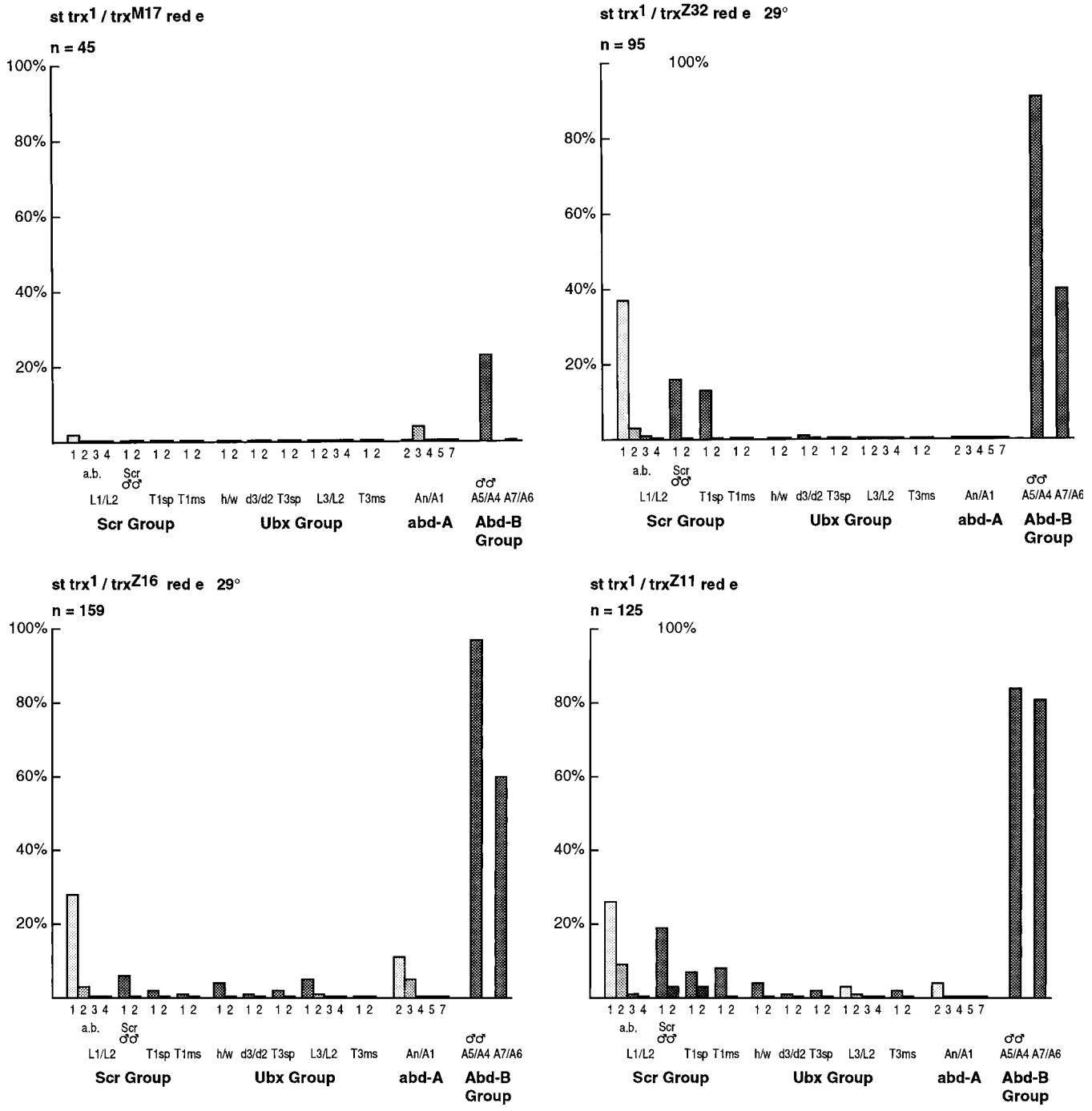

Figure 5.—*trx*^{1}/hypomorph heterozygotes. Bar graphs are as in Figure 3. Compare *trx*^{1}/hypomorph profiles to those of hemizygous hypomorphs in Figure 3. Penetrance and expressivity are lower in heteroallelic combinations with *trx*^{1}. Thus, *trx*^{1} retains significant function. The effect shown here is almost the same as when *trx*^{1} is paternally inherited. The maternal effect of *trx*^{1} is more pronounced when penetrance and expressivity of *trx*^{1}/amorph heterozygotes from *trx*^{1} homozygous mothers to amorph/*trx*^{1} heterozygotes from amorph/*trx*$^{+}$ mothers are compared (Table 2). Amorph/*trx* $^{+}$ mothers supply more *trx* function to eggs than *trx*^{1}/*trx*^{1} mothers. *trx*^{JY16} hemizygotes die as small, third instar larvae. *trx*^{JY16} is a strong imaginal hypomorph as seen when it is heterozygous with other strong hypomorphs (Figure 6 and Table 2). *trx*^{3} is an amorph when paternally inherited, but slightly antimorphic when maternally inherited (Table 2). Assuming *trx*^{1} reduces the amount of normal TRXI and TRXII made, *Abd-B* and *Scr* functions in developing T1 legs are most easily affected by reduced levels of these proteins.

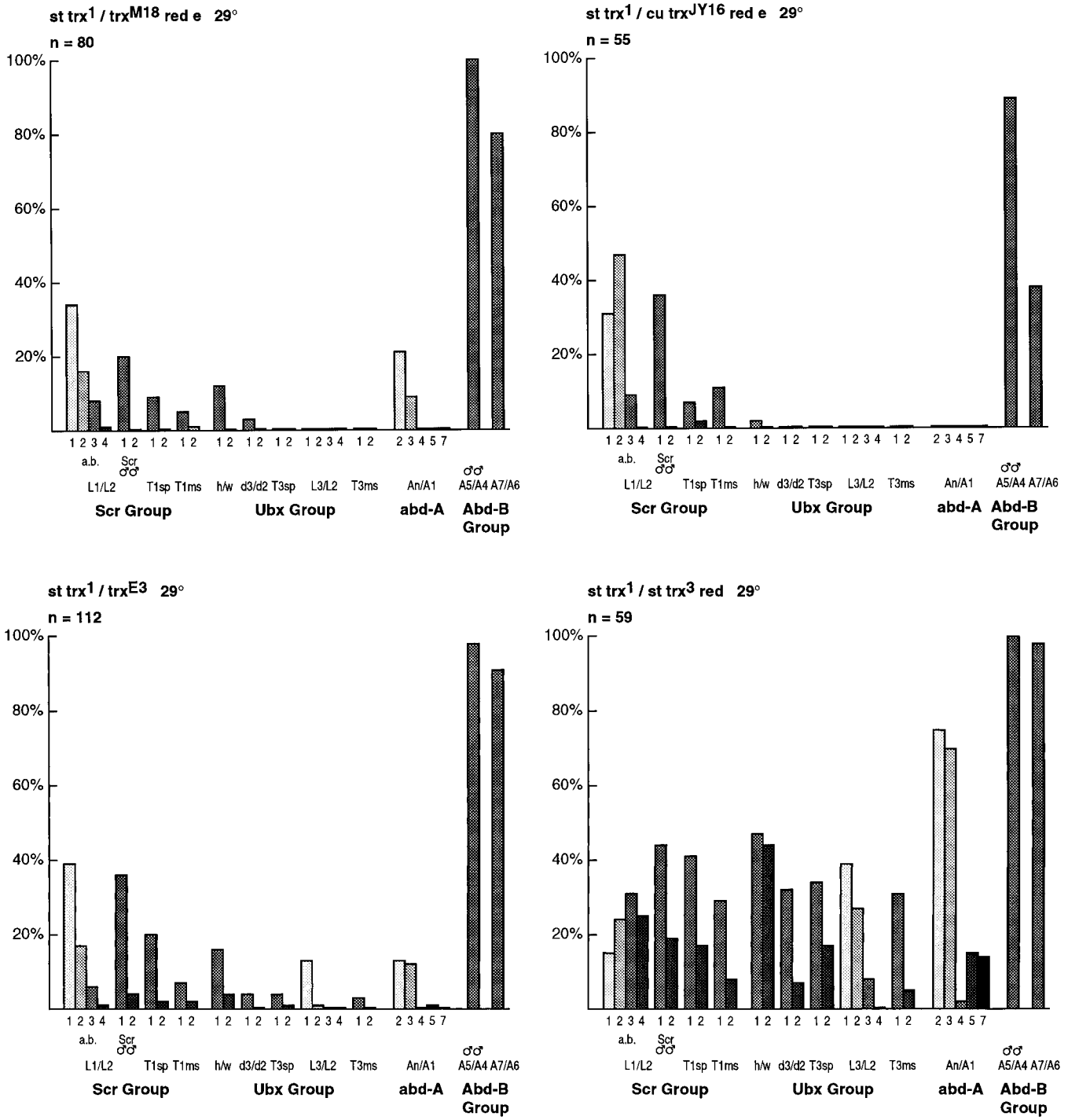

Figure 5.—*Continued.*

increase in *Antp* expression compared with *Abd-B* r expression in male genital discs (Casares *et al.* 1997).

Flies transheterozygous for trx^{E3} and one of the strong hypomorphs frequently have missing dorsal head structures including different combinations of ocelli, ocellar bristles, and postvertical bristles (Figure 2F). This phenotype is occasionally seen in amorph/trx^{Z11} pharate adults, too. The dorsal head phenotype is similar to that seen in *Egfr* (Drosophila EGF receptor) and *ocelliless* (*oc*) hypomorphs (Clifford and Schupbach 1989; Finkelstein *et al.* 1990). Many trx^{E3} hemizygotes develop as incomplete pharate adults whose heads fail to evert (and appear headless). They also have incomplete chitinization. Homeotic phenotypes could not be scored for these animals and they did not contribute to the P& E values in Table 2. Three of five trx^{3}/trx^{Z11} pharate adults developed small heads and anterior thoracic segments (Figure 2, G and I). They also had abnormal

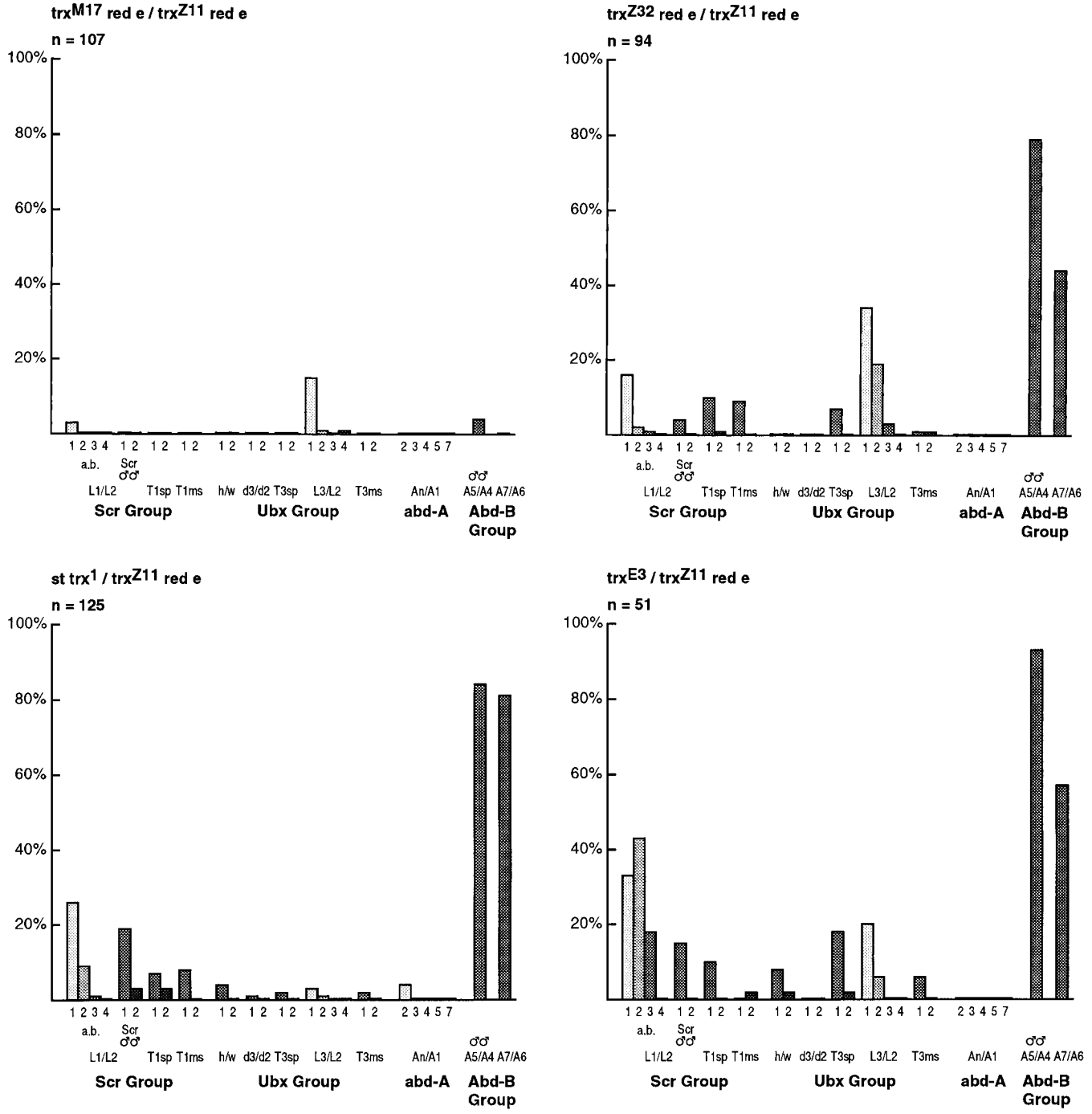

Figure 6.—Penetrance and expressivity of hypomorphs heterozygous with a strong hypomorph, trx^{Z11}. Bar graphs are as in Figure 3. Compare with profiles in Figures 3 and 4. Hypomorphs other than trx^{1} and trx^{E3} proportionally have a greater effect on *Ubx* expression in developing T3 legs than at the other loci examined in other tissues. Compare the profile for trx^{1}/trx^{Z11} to those of trx^{1}/trx^{JY21} heterozygotes and trx^{1} hemizygotes. trx^{Z11} supplies substantial *trx* function to the loci examined. Weaker hypomorphs, trx^{M17}, trx^{Z32}, trx^{1}, and trx^{E3} complement trx^{Z11} to an extent expected on the basis of their penetrance and expressivity when hemizygous and in combination with trx^{JY21}. trx^{Z16} complements trx^{Z11} more than expected on the basis of the same comparisons, suggesting it has true functional complementation of trx^{Z11} at *Scr*, *Ubx*, and *abd-A*. This effect is only seen when trx^{Z16} is maternally inherited. All hypomorph/trx^{Z11} genotypes have nearly wild-type function at *abd-A*.

antennae (Figure 2I) similar to *decapentaplegic* (*dpp*) disc III mutants (Spencer *et al.* 1982) and reduced or absent maxillary palps that may be associated with reduced *Dfd*, *proboscipedia* (*pb*), or *labial* (*lb*) expression in antennal discs (Kaufman 1978; Merrill *et al.* 1987, 1989; Pultz *et al.* 1988). A total of 6 out of 17 trx^{M18}/trx^{JY21} pharate adults also had small heads.

Flies transheterozygous for trx^{M17} and amorphic alleles

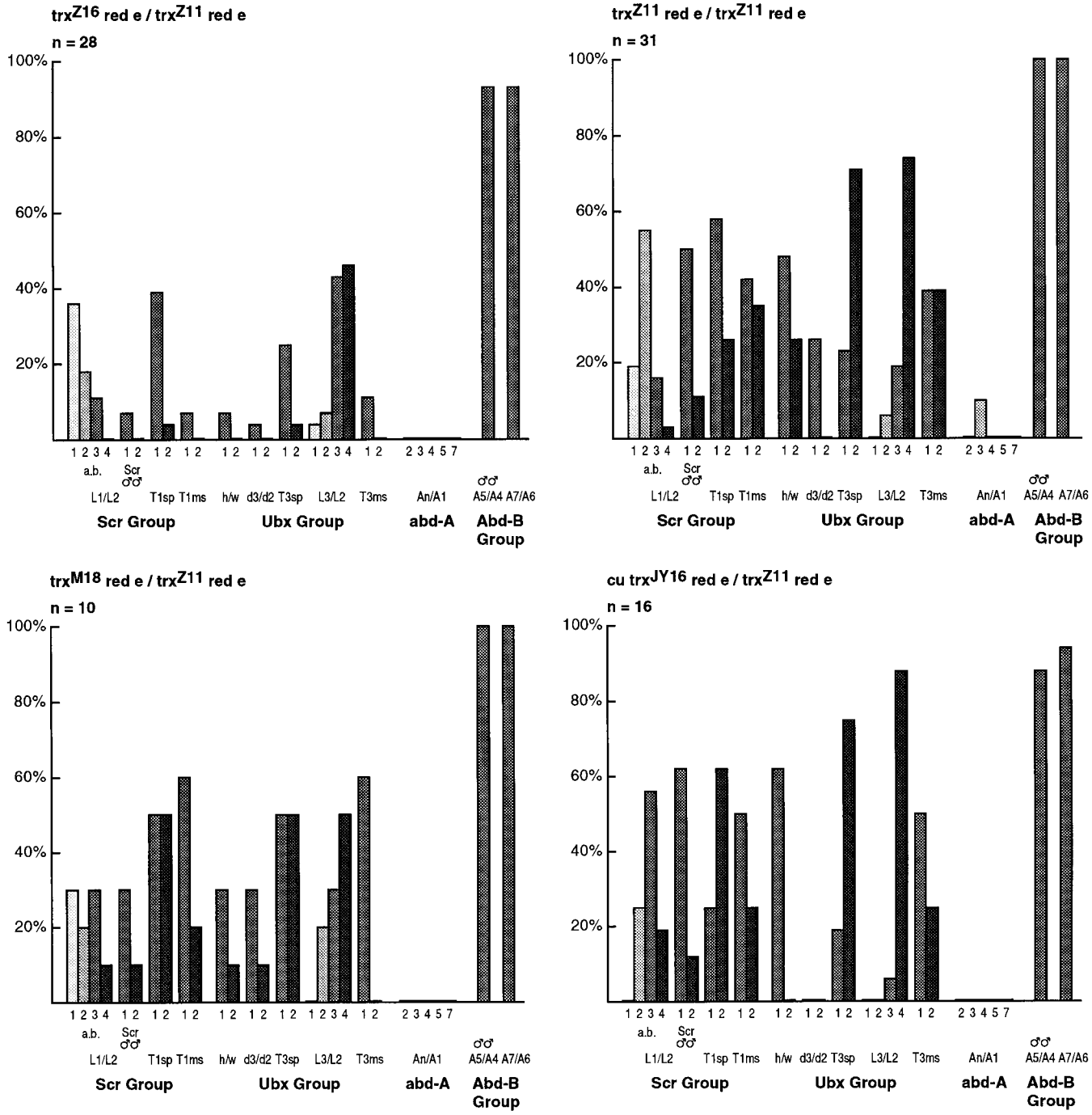

Figure 6.—*Continued.*

(Figure 2H) occasionally develop large bristles on their labial palps that may be associated with reduced *pb* and *Scr* expression in labial discs (Kaufman 1978; Pultz *et al.* 1988; Percival-Smith *et al.* 1997). They also develop mirror image eye duplications that have been seen in trx^{1} homozygotes from trx^{1} homozygous mothers (T. R. Breen, unpublished results). Rarely, trx^{M17}/amorph flies develop posterior wing abnormalities and anterior wing duplications (not shown). Similar posterior wing disruptions are seen in *en* mutant clones (Morata and Lawrence 1975; Lawrence and Struhl 1982) and in mutant clones that remove function downstream of *dpp* protein (DPP) signaling (Singer *et al.* 1997). Similar anterior wing duplications are seen also in DPP receptor mutant clones (Penton and Hoffmann 1996; Singer *et al.* 1997).

Some phenotypes described above probably arise due to overall lack of *trx* function at target genes such as is produced by combinations of trx^{JY21} with strong hypomorphs. Others may be due to altered *trx* function at

specific target genes or in specific cell types such as those associated with trx^{E3}, trx^{Z11}, trx^{M17}, and perhaps trx^{M18}.

Many *trx* mutant genotypes produce additional phenotypes. Frequently, A1 tergites develop with dark pigmentation and large bristles at their posterior borders (Figure 2, F and G), similar to *Ultra-abdominal* (*Uab*) phenotypes (Lewis 1978; Karch *et al.* 1985). Other frequent phenotypes include incomplete dorsal fusion of tergites (Figure 2, B and F), abnormal abdominal spiracles (Figure 2C), and abnormal sternites (Figure 2A). These phenotypes are associated with decreased *abd-A* and *Abd-B* function in abdominal histoblasts (Karch *et al.* 1985).

DISCUSSION

Comparative effects of trx^{1} on homeotic phenotypes: The P&E profiles of trx^{1} genotypes suggest different quantitative requirements for TRX at the four homeotic loci examined. Three factors may contribute to the different sensitivities of target genes to decreased levels of TRX: (1) Each target gene accumulates a unique amount of TRX at its PRE(s). Genes that normally accumulate less TRX may be more sensitive to decreased levels of TRX. (2) Target genes with normally equal TRX accumulation may have different threshold TRX levels below which they no longer function for proper structural determination. (3) Different tissues undergo different numbers of cell divisions, which may lead to differential loss of limiting quantities of TRXs. These three factors are further described below.

1: Polytene chromosomal analyses show that different target genes do accumulate different amounts of TRX at their PREs (Kuzin *et al.* 1994; Chinwalla *et al.* 1995). The level of accumulation of TRX proportionally decreases at all target genes in trx^{1} mutants, which shows that target genes that normally accumulate more TRX recruit limiting amounts of TRX more efficiently. Even if there is not a linear relationship between the amount of TRX accumulated at a target gene and the amount needed for function, target genes that normally accumulate less TRX should be more susceptible to reduced nuclear TRX concentration than those that normally accumulate more. Due to low resolution, polytene chromosomal analysis did not determine if *Scr* in the ANT-C and *Ubx*, *abd-A*, and *Abd-B* in the BX-C accumulate different amounts of TRXs (Chinwalla *et al.* 1995).

2: Some *trx* target genes in specific tissues may require relatively small amounts of TRX to be transcribed sufficiently for normal development, though they may normally be supplied with abundant TRX. Only very low TRX levels would elicit a mutant phenotype in such tissues. Reduced *trx* function would produce low P&E of phenotypes associated with such a relatively TRX-insensitive target gene.

3: trx^{1} mutants have reduced maternal and/or zygotic production of TRX. Thus, target genes in imaginal precursor cells of trx^{1} mutants initially accumulate less TRX than those in wild-type cells. During subsequent cell divisions, target genes are increasingly susceptible to insufficient TRX accumulation caused by continually impaired *trx* transcription or translation. Different imaginal tissues begin with different numbers of precursor cells and proliferate to different extents (Cohen 1993). Target genes expressed in imaginal tissues with more precursor cells and greater proliferation would be more likely to have TRX accumulation fall below threshold levels than those in tissues with fewer precursors and less proliferation. This scenario requires that all imaginal precursor cells initially have similarly reduced levels of TRX and all proliferating imaginal cells have the same reduced transcription or translation of *trx*.

Comparative effects of other hypomorphic alleles on homeotic phenotypes: Hypomorphic *trx* alleles encode proteins that can assemble and provide some function at target genes. They function in combination with one wild-type maternal dose of *trx* for seemingly normal embryogenesis, which is consistent with their complementation of trx^{1} during imaginal development. However, a maternal dose of trx^{+} is not sufficient to complement hypomorphic mutant function in imaginal precursors whose progeny cells produce only mutant protein.

P&E qualitative profiles produced by different hypomorphic genotypes, excluding trx^{1} and trx^{E3}, are proportionally similar. This suggests that the mutant proteins equivalently impair TRX function at the homeotic genes examined. However, instances of complementation among hypomorphic alleles suggest different mutations alter different functional domains. These observations infer, not surprisingly, that different hypomorphic mutant proteins inefficiently interact with different factors present at many, if not all, target genes.

trx^{Z16} and trx^{Z11} are associated with point mutations in the PHD finger and SET domains, respectively (Stassen *et al.* 1995). As mentioned previously, the SET domain of HRX at least mediates protein-protein interactions used in signal transduction and maturation (Cui *et al.* 1998; De Vivo *et al.* 1998). PHD fingers may also mediate protein-protein interactions (Aasland *et al.* 1995). trx^{M17}, trx^{Z32}, and trx^{M18} are point or pseudopoint mutations (Breen and Harte 1991) that impair different functional interactions as determined by complementation. Disruption of any TRX protein-protein interaction would reduce the ability of a mutant protein to function at any target gene. Results of this study show that *Ubx* transcription in T3 leg discs is most easily affected by any of several small changes in different regions of TRX. The observed different sensitivities of *trx* target genes to a variety of mutant TRX infers that each target gene employs TRX uniquely, though similar factors are present at each.

Proteins encoded in hypomorphic alleles have greater

function at *abd-A* than reduced levels of TRX in trx^{1} mutants. There does not appear to be any quantitative mechanism that could produce this difference. It is unlikely that maternally supplied, wild-type TRX could endure to complement hypomorphic mutant proteins through tergite development. It seems more likely that hypomorphic mutations do not compromise the ability of their mutant proteins to function at *abd-A* as much as they do at other homeotic target genes. This is consistent with the previous comment that TRX has unique structural interactions at each of its target genes.

In hypomorphic *trx* mutants, there is higher penetrance of A2 and A3 tergite transformations to A1 than of more posterior tergite transformations. Thus, hypomorphic mutant *trx* proteins are less efficient in stimulating *abd-A* transcription in A2 and A3 than in more posterior tergites. Reduced levels of wild-type TRX in trx^{1} mutants also have a greater effect on A2 and A3 than on more posterior tergites. In wild-type flies, *abd-A* is expressed at lower levels in PS7 and PS8 than in more posterior parasegments (Karch *et al.* 1990). Thus, moderately impaired TRX may lower *abd-A* expression slightly but still below a threshold needed to ensure proper A2 and A3 development. Reduced levels of TRX in trx^{1} mutants may lead to a greater decrease in *abd-A* transcription sufficient to affect development of more posterior tergites.

trx^{JY16} encodes wild-type TRXI and a TRXII fusion protein that has at least 172 N-terminal residues replaced by fusion partner residues (Figure 1). These proteins must be present in sufficient quantity with sufficient function for successful embryogenesis. However, they do not support larval growth. trx^{JY16} does supply significant function to complement trx^{1}, and it can form functionally impaired complexes with some of the other hypomorphic proteins. Normally, large mRNAs that encode TRXII are the only forms expressed during larval and pupal stages (Breen and Harte 1991; Sedkov *et al.* 1994); however, they also encode TRXI. It may be that TRXII supplies *trx* function for larval growth and imaginal development, though a role for TRXI cannot presently be excluded. If the larval and imaginal role for TRXII is correct, it is likely that the fusion TRXII encoded in trx^{JY16} cannot supply that function. This implies that the N terminus of TRXII is necessary for proper stimulation of target gene transcription in imaginal cells.

In combination with other strong hypomorphic alleles, trx^{JY16} partial complementation of *Ubx* function in haltere discs, but not T3 leg discs, demonstrates tissue-specific use of TRX at the same target gene. This is consistent with the observation that *trx* has tissue-specific effects on expression of *Ubx* and other homeotic target genes during embryogenesis (Breen and Harte 1993). Abnormal tracheal development (Vincent *et al.* 1997; Wappner *et al.* 1997) and lack of larval growth in trx^{JY16} mutants may indicate that TRX regulates target gene expression as an element of DPP and/or EGFR signaling pathways (see below).

The protein encoded in trx^{JY21} interferes with residual function of the other hypomorphic alleles at *Scr, Ubx*, and *Abd-B*, suggesting that it is antimorphic, but its interference with *trx* function at *Scr* is most noticeable. This is particularly evident when comparing trx^{1}/amorph to trx^{1}/trx^{JY21} P&E profiles. These results allow that trx^{JY21} protein may inhibit wild-type and hypomorphic TRX from forming into complexes or interfere with their function in complexes. Regardless, its differential effect on *Scr* expression again illustrates the differential use of TRX in the context of other factors at different target genes. In combination with strong hypomorphic alleles, trx^{JY21} may prove particularly useful in that it appears to almost completely remove *trx* function at *Scr* in T1 leg discs and *Ubx* in T3 leg discs.

Male genitalia to leg phenotype: trx^{Z11}, trx^{Z16}, and trx^{M18} in combination with trx^{JY21} often produce pharate adult males with genitalia transformed to T2 leg. This may occur because reduced *Abd-B* r expression in A9 primordia of male genital discs allows *Antp* P2 expression, which contributes significantly to leg development (Schneuwly and Gehring 1985; Abbott and Kaufman 1986), to have increased influence on the specification of these cells. Casares *et al.* (1997) showed that *Abd-B* r is expressed in male genital disc cells that derive from A9 and produce male genitalia (Freeland and Kuhn 1996). It is not known if *Antp* P2 is expressed in male genital discs, but it is expressed in A9 during embryogenesis (Bermingham *et al.* 1990). In A9 of *trx* mutant embryos, *Abd-B* r expression is reduced (Duncan and Lewis 1982; Breen and Harte 1993) and *Antp* P2 expression appears nearly wild type (T. R. Breen, unpublished results). Thus, it is possible that male genital disc precursors from A9 have relatively enhanced ANTP expression from *Antp* P2 directing T2 leg development. This situation is unique to male genital discs. Though female genital disc precursors would experience the same relative levels of *Abd-B* r and *Antp* P2, cells that express *Abd-B* r in female genital discs do not produce female genitalia (Freeland and Kuhn 1996; Casares *et al.* 1997).

Model of *trx* function: TRX is recruited to PREs of target genes (Chan *et al.* 1994; Kuzin *et al.* 1994; Chinwalla *et al.* 1995; Orlando *et al.* 1998). Once assembled, it acts with other trxG proteins to stimulate target gene transcription through chromatin remodeling as inferred by the SET domain it shares with other proteins known to alter chromatin (Jones and Gelbart 1993; Tschiersch *et al.* 1994). Pirrotta (1998) offers the interesting possibility that TRX influences the level of target gene histone acetylation. It is not clear if TRX participates in the initial transcription of its target genes. In specific cells, it is necessary for detectable levels of target gene transcription. In others, it is needed only for enhanced target gene transcription (Breen and Harte

1993; Kuzin *et al.* 1994; Sedkov *et al.* 1994; Breen *et al.* 1995). Functional TRX assemblies are inherited by progeny cells (Ingham 1981, 1985a) so that they will have levels of target gene transcription similar to their parent cells. From this information, it is conceivable that TRX assembles in a lineage-dependent manner to act as a constitutive facilitator of other transcription factors. If transcription of a target gene is not initiated in a cell, PcG proteins bound to the gene's PRE chromatin form a silencing structure that supersedes colocalized TRX (Poux *et al.* 1996). A gene's PcG protein-silencing structure is then inherited by progeny cells.

Phenotypic and gene expression analyses of two trxG genes, *ash2* (Adamson and Shearn 1996) and *mor* (Brizuela and Kennison 1997), suggest that their proteins participate in downstream functions of developmental signaling pathways. Phenotypic results of this study allow that TRX activity may be modulated downstream of cell signaling to attain cell-specific levels of target gene transcription. This role of TRX is supported by findings that propose a similar role for HRX. The interaction of a dual-specificity phosphatase inhibitor with the SET domain of HRX suggests it is activated through signal transduction and later deactivated to promote differentiation (Cui *et al.* 1998; De Vivo *et al.* 1998). In this light, I present a model in which TRX acts as a downstream mediator in multiple signal transduction pathways, including those signaled by morphogens, to elicit ligand concentration-dependent responses at target genes. Here, TRX provides a dynamic response capacity to a variety of cell-signaling events.

The model shown in Figure 7 is based on activities in PS7 of the visceral mesoderm (Immerglück *et al.* 1990; Panganiban *et al.* 1990; Reuter *et al.* 1990; Hursh *et al.* 1993; Thüringer and Bienz 1993; Staehling-Hampton and Hoffmann 1994) where *trx* is needed for normal levels of *Ubx* expression (Breen and Harte 1993). However, other ligands and their receptors may be substituted to account for the effects of *trx* mutations on other cell types. In the model, TRX is modified as a downstream substrate of signaling pathways, whose ligands may include the TGF-β homologue (Padgett *et al.* 1987), *decapentaplegic* protein (DPP), the WNT-1 homologue (Rijsewijk *et al.* 1987) *wingless* protein (WG), *hedgehog* protein (Mohler and Vani 1992; Tabata *et al.* 1992; Ingham and Hidalgo 1993; Tashiro *et al.* 1993), and ligands of the Drosophila EGFR (Livneh *et al.* 1985; Thompson *et al.* 1985; Wadsworth *et al.* 1985) such as *spitz* protein (Rutledge *et al.* 1992). Signaling intermediates may include factors such as *Mothers against dpp* (*Mad*) and *schnurri* (*shn*) proteins in the DPP pathway (Grieder *et al.* 1995; Staehling-Hampton *et al.* 1995; Newfeld *et al.* 1996, 1997). They may also include known chromosomal trxG proteins and trxG proteins that may be proven to be nonchromosomal (Kennison and Tamkun 1988; Farkas *et al.* 1994; Kennison 1995; Adamson and Shearn 1996; Tripoulas *et al.* 1996; Rozenblatt-Rosen *et al.* 1998).

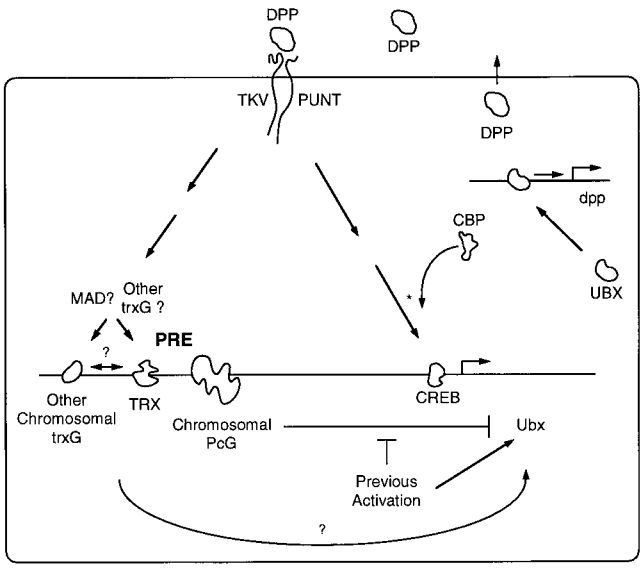

Figure 7.—Model of TRX activation through signal transduction. The model is based on experimental findings on UBX and DPP regulatory interactions in the visceral mesoderm and DPP receptor functions in imaginal discs (see discussion). UBX with other factors (Sun *et al.* 1995) initiates *dpp* transcription. DPP may signal the cell from which it came (autocrine, shown in model) or nearby cells (exocrine). DPP signals through receptor heterodimers. Each receptor heterodimer consists of one TGF-β type I subunit and one TGF-β type II subunit. In Drosophila, *thickvein* and *saxophone* proteins (TKV and SAX) are type I receptors and *punt* protein (PUNT) is a type II receptor. DPP signaling through undetermined intermediates may lead to phosphorylation (*) of CREB bound to a promoter proximal CRE (Eresh *et al.* 1997). Phosphorylated CREB recruits CBP (Chrivia *et al.* 1993) to the promoter proximal region where it may participate in histone acetylation that may form transcriptionally permissive chromatin (Bannister and Kouzarides 1996; Ogryzko *et al.* 1996). DPP signaling may additionally act through chromosomal trxG proteins including TRX to boost *Ubx* expression. Upstream elements of this signaling cascade may include the known DPP-signaling intermediate, MAD, and perhaps nonchromosomal trxG proteins. Signaling may affect any combination of chromosomal trxG proteins that would subsequently interact in an unknown way to augment *Ubx* transcription. Other known chromosomal trxG proteins include ASH1 (Tripoulas *et al.* 1996), GAGA factor (Farkas *et al.* 1994), and perhaps BRM (Dingwall *et al.* 1995), which is at least nuclear. These may work cooperatively or in parallel. They may be components with TRX of a signal response pathway, or they may function independently to prepare *Ubx* for elevated transcription. TRX transcriptional augmentation appears to operate in cells where *Ubx* was previously activated. PcG proteins silence *Ubx* transcription in cells where there is no *Ubx* transcription when PcG proteins are activated during germ band elongation. Therefore, TRX mediates DPP-signaled elevation of *Ubx* transcription in cells where *Ubx* is previously activated and its previous activation prevents PcG silencing. Other target genes in other cells may similarly use TRX to respond to DPP and other signal transduction pathways. The TRX-mediated signaling response of a cell may be quantitatively controlled by the concentration of a signaling ligand within its gradient distribution and by the number of TRXs recruited to a target gene's regulatory elements.

It is also possible that TRX is required for normal levels of expression of signaling pathway genes. Thus, *trx* mutants would develop hypomorphic signal transduction phenotypes. The expression of *dpp* in *trx* mutants does not support this possibility (T. R. Breen, unpublished results), though the expression of many other signaling element genes in *trx* mutants needs to be examined.

Below, I interpret aspects of *trx* mutant phenotypes that are consistent if TRX is modulated by signaling intermediates.

Higher frequency of anterior transformations: As noted by Ingham and Whittle (1980), trx^{1} genotypes produce adults with a higher frequency of transformed patches that include only anterior metameric structures compared to transformed patches that include more posterior structures. I observed that successively stronger hypomorphic genotypes produce successively larger transformed patches that encompass increasingly more posterior regions of affected adult segmental structures. It is important to note that these transformed posterior segmental structures are not limited to posterior compartment derivatives, but include structures derived from more posterior regions of anterior compartments. Thus, precursor cells that give rise to structures closer to the anterior margin of an adult segment are more susceptible to reduced *trx* function than precursor cells that give rise to more posterior structures, including those of posterior compartment origin.

Successively more severe *trx* hypomorphic genotypes reveal an anterior-to-posterior gradient of TRX activity. This activity gradient may be due, in whole or in part, to an anterior-to-posterior gradient of TRX in imaginal tissues, as is seen in thoracic discs (Kuzin *et al.* 1994). It is also possible, as suggested in the model presented above, that TRX activity is dependent not only on TRX levels at homeotic and other target genes, but may be modulated in response to gradients of DPP, WG, *hedgehog* protein (HH), or *spitz* protein (SPI) that are generated near segmental anterior/posterior (A/P) borders. Threshold levels of TRX may exist below which signal input is insufficient to effect or enhance target gene transcription. Morphogen concentrations diminish at increasing distances from their sources. Weak *trx* hypomorphs may produce insufficient TRX to interpret low morphogen concentrations only in anterior cells that are most distant from morphogen sources. Stronger *trx* hypomorphs may not have sufficient TRX to interpret morphogens even in cells at a morphogen source during imaginal proliferation. Consistent with either possible cause of the graded *trx* mutant phenotype is the observation that *Ubx* expression is diminished in anterior cells of haltere and T3 leg discs in moderate *trx* hypomorphs (Cabrera *et al.* 1985; Ingham 1985b). How hypomorphic *trx* mutations affect expression of other target genes in other imaginal tissues remains to be seen.

***Uab* phenotype:** Many *trx* hypomorphic genotypes produce a *Uab* phenotype, particularly the development of dark pigmentation and large bristles at the posterior of A1 tergites. The *Uab* phenotype was originally proposed to be caused by *abd-A* dominant gain-of-function alleles producing ectopic *abd-A* protein (ABD-A) in A1 tergite precursors (Lewis 1978). Most *Uab* mutations do not noticeably alter ABD-A expression patterns (Karch *et al.* 1990) including those associated with the recessive *abd-A* phenotype examined in this study. Similarly, ABD-A is not ectopically expressed in A1 tergite precursors in *trx* mutants (Breen and Harte 1993).

The posterior of a tergite is the posterior of a parasegment corresponding to the posterior of an anterior compartment (Kornberg 1981; Hama *et al.* 1990). Tergites develop from anterior dorsal histoblasts whose precursors arise in anterior abdominal compartments during embryogenesis. Struhl *et al.* (1997) showed that dark pigmentation and large bristles develop in A2–A6 tergites when anterior dorsal histoblasts receive HH signals from adjacent posterior dorsal histoblasts. This process should also occur at the A/P border of the A1 tergite, yet its posterior border is unpigmented and develops small bristles typical of the rest of the tergite.

A difference present at the A1 A/P border not present at the A/P borders of more posterior segments is the relative level of expression of UBX and ABD-A. UBX is expressed in reiterated gradients in PS7-12 with high levels at the posterior and lower levels toward the anterior of each parasegment (White and Lehmann 1986). UBX is expressed at very high levels throughout PS6. ABD-A is expressed in reiterated gradients in PS7-12 with high levels at the anterior and lower levels toward the middle of each parasegment (Karch *et al.* 1990). ABD-A gradients in PS7 and PS8 are weaker than in more posterior parasegments. Thus, there are very high levels of UBX on the anterior side of the A1 A/P border juxtaposed to moderate levels of ABD-A on the posterior side of the border. More posterior segments have lower levels of UBX juxtaposed to higher levels of ABD-A. Additionally, there is no ABD-A on the anterior side of the A1 A/P border, whereas there are low levels of ABD-A on the anterior sides of A/P borders of more posterior segments.

It is possible ABD-A contributes to HH signaling from posterior dorsal histoblasts and UBX contributes to its interpretation in anterior dorsal histoblasts. In *trx* and *Uab* mutants, relative levels of UBX and ABD-A at A1 A/P borders may establish and interpret HH signaling as at more posterior abdominal A/P borders. Weatherbee *et al.* (1998) showed UBX contributes to regulation of genes downstream of HH signaling in imaginal discs, which may be thought of as an interpretation function. Again, it will be interesting to determine if this downstream regulation by *Ubx* is parallel to signaling regulation or more directly controlled by HH signaling through TRX.

Other possible modulation of TRX activity by signal

transduction pathways: trx^{Z11} and trx^{E3} genotypes frequently cause ocelliless phenotypes similar to those caused by decreased *oc* and *Egfr* function (Clifford and Schupbach 1989; Finkelstein *et al.* 1990). EGFR may function through the *pointed* (*pnt*) transcription factor (PNT) to regulate *oc* transcription in eye imaginal discs as it does in embryogenesis (Gabay *et al.* 1996). It is noteworthy that the cytological location of *pnt* (94F) is a site of TRX localization (Chinwalla *et al.* 1995). A possible scheme is that EGFR activation of TRX boosts *pnt* expression leading to proper levels of *oc* expression. Loss of function of any of these genes would result in ocelliless phenotypes. It is also possible that TRX may mediate HH signaling for proper *oc* expression as HH also affects dorsal head development (Royet and Finkelstein 1996).

A possible target of the *oc* transcription factor (often called by its alternative name, *orthodenticle* protein, or OTD) in eye discs is *en* (Royet and Finkelstein 1995). *en* is a target of *trx* function, particularly in later developing cells (Breen *et al.* 1995). It is possible that reduced *en* function in *trx* and *oc* mutants contributes to the ocelliless phenotype. However, it has yet to be demonstrated that loss of *en* activity produces an ocelliless phenotype (Lawrence and Struhl 1982), and, unfortunately, dorsal head structures were not examined in *en*; *trx* double mutants (Breen *et al.* 1995). Regardless, it appears that *en* contributes only to ocellar development (Royet and Finkelstein 1995). Hence, reduced *en* function alone cannot account for the full range of ocelliless phenotypes seen in *trx* mutants. Perhaps TRX stimulates the transcription of multiple genes affected by a signaling pathway, such as *pnt* and *en*, creating positive impetus toward a particular level of cell fate determination.

trx^{Z11}, trx^{M18}, and trx^{E3} genotypes frequently cause growth deficiencies manifested as small or incomplete head development, small anterior thorax, and incomplete chitinization. These phenotypes suggest incomplete development of imaginal tissues that may be caused by abnormal cell death or impaired cell proliferation. DPP and EGFR pathways are necessary for normal growth and differentiation of imaginal tissues (Spencer *et al.* 1982; Clifford and Schupbach 1989; Baker and Rubin 1992; Burke and Basler 1996). Perhaps TRX mediates growth control by these signaling pathways through homeotic and uncharacterized target genes. Phenotypic analyses of trxG members *ash1* and *ash2* suggest they similarly function downstream in cell proliferation and differentiation pathways and their proteins may act in concert with TRXs (Shearn *et al.* 1987; Shearn 1989; Adamson and Shearn 1996).

trx^{Z11} genotypes produce growth deficiency phenotypes seen in *dpp* and proximal ANT-C mutants. Abnormal antennal outgrowths similar to those caused by *dpp* disc III mutations may indicate that TRX interprets DPP signals in antennal discs. trx^{Z11} mutants occasionally have reduced or missing maxillary palps seen in *Dfd*, *lb*, and *pb* hypomorphs. It is apparent that *Dfd*, *lb*, and *pb* contribute to normal growth of maxillary palps (Merrill *et al.* 1987, 1989; Pultz *et al.* 1988), but it is not known how these genes respond to developmental signals during imaginal proliferation. During embryogenesis, *trx* function is needed for elevated levels of *Dfd* expression in the anterior of its domain (Breen and Harte 1993). It is not known if *trx* is similarly required for normal *Dfd*, *lb*, and *pb* expression during imaginal proliferation. It will be interesting to determine the role of *trx* and homeotic genes in regulating imaginal proliferation as a component of developmental signaling responses.

trx^{Z11} is associated with the change of a conserved glycine to a serine in the SET domain (Stassen *et al.* 1995). Cui *et al.* (1998) showed that Sbf1 binds with the SET domain of HRX and may oppose maturation and differentiation promoted by the interaction of the SET domain with dual-specificity phosphatases such as myotubularin. It is possible that in imaginal cells the trx^{Z11} mutation prevents protection by an Sbf1 homologue, allowing premature growth repression and differentiation.

It is difficult to guess how trx^{M18} and trx^{E3} may interfere with cell proliferation and differentiation. trx^{M18} is uncharacterized and may map to the SET domain or a region of TRX that interacts with it. It may also interfere with normal phosphorylation, thus inhibiting the activation of TRX needed to promote growth. The region of TRX missing due to the trx^{E3} deletion may also be necessary for SET domain function or signal reception. In imaginal cells, trx^{E3} appears to affect ANT-C transcription primarily as it does in embryogenesis (Sedkov *et al.* 1994), yet its growth deficiencies appear to affect all imaginal tissues. It is possible that the deletion reduces the ability of the protein to assemble at ANT-C PREs or receive developmental signal input unique to the ANT-C in head and thoracic imaginal cells. At the same time, it may reduce the protein's ability to assemble into structures or receive signal input at target genes involved with cell proliferation in many tissues.

trx^{M17}/amorph genotypes occasionally cause labial palp transformations, unilateral eye duplications, posterior wing abnormalities, and anterior wing duplications. Labial palp transformations suggest that trx^{M17} proteins have reduced function at some combination of *pb* and *Scr* in labial discs (Percival-Smith *et al.* 1997). Mirror image eye duplications are reminiscent of phenotypes associated with disruption of a signal transduction pathway, yet this phenotype is not associated with a described signaling mutation. The posterior wing compartment effects are similar to those seen in *en*; *trx* double hypomorph mutants (Breen *et al.* 1995), posterior compartment *en* mutant clones (Lawrence and Struhl 1982), or clones mutant for the DPP receptor SAX (Singer *et al.* 1997). The anterior compartment duplications are similar to those produced by clones mutant for the DPP

receptor PUNT (Penton and Hoffmann 1996). trx^{M17} proteins may have reduced activity at *en*. Reduced *en* function in the wing disc may impair HH signaling that could reduce the level of DPP expressed at the A/P border (Zecca *et al.* 1995). Reduced DPP levels would produce a weaker gradient of this morphogen in the wing disc, leading to the *dpp* deficient phenotypes. These phenotypes are unique to trx^{M17}, indicating that the mutation disrupts some specific target gene interactions, but the mutation also has a low P&E effect on other homeotic genes.

Differences between the phenotypes discussed above for hypomorphic genotypes and those observed by Ingham (1985a) in trx^{-} somatic clones are probably due to differences in alleles used and timing of *trx* loss of function. In this study, the source of wild-type TRX was from a single maternal dose of trx^{+} from heterozygous mothers or reduced amounts supplied by trx^{1}. Otherwise, only mutant gene products were available throughout development. trx^{-} clones derived from progenitors that had one maternally contributed dose of wild-type *trx* mRNA and one wild-type zygotic dose until the first or second larval instar time of clone induction. Thereafter, progeny cells had no source of functional TRX except reserves of wild-type products that diminish through each round of cell division.

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