The Drosophila Gene *taranis* Encodes a Novel Trithorax Group Member Potentially Linked to the Cell Cycle Regulatory Apparatus

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ABSTRACT

Genes of the Drosophila *Polycomb* and *trithorax* groups (PcG and trxG, respectively) influence gene expression by modulating chromatin structure. Segmental expression of homeotic loci (HOM) initiated in early embryogenesis is maintained by a balance of antagonistic PcG (repressor) and trxG (activator) activities. Here we identify a novel trxG family member, *taranis* (*tara*), on the basis of the following criteria: (i) *tara* loss-of-function mutations act as genetic antagonists of the PcG genes *Polycomb* and *polyhomeotic* and (ii) they enhance the phenotypic effects of mutations in the trxG genes *trithorax* (*trx*), *brahma* (*brm*), and *osa*. In addition, reduced *tara* activity can mimic homeotic loss-of-function phenotypes, as is often the case for trxG genes. *tara* encodes two closely related 96-kD protein isoforms (TARA-α/-β) derived from broadly expressed alternative promoters. Genetic and phenotypic rescue experiments indicate that the TARA-α/-β proteins are functionally redundant. The TARA proteins share evolutionarily conserved motifs with several recently characterized mammalian nuclear proteins, including the cyclin-dependent kinase regulator TRIP-Br1/p34^{SEI-1}, the related protein TRIP-Br2/Y127, and RBT1, a partner of replication protein A. These data raise the possibility that TARA-α/-β play a role in integrating chromatin structure with cell cycle regulation.

SPECIFICATION of segmental identities along the anterior-posterior (A-P) body axis in Drosophila is controlled by the spatially restricted expression of the homeotic loci (HOM) of the Antennapedia and bithorax complexes (ANT-C and BX-C; Lewis 1978; Akam 1987; McGinnis and Krumlauf 1992). The importance of precise transcriptional control throughout development is indicated by the spectacular alterations in cell fate resulting from inappropriate HOM expression, such as transformations of antenna to leg or haltere to wing, or by more discrete changes in cell identity, such as the specialized bristles of the male sex comb. The restricted A-P expression domains of the HOM genes initiated during embryogenesis by the localized transient activities of the segmentation genes (Duncan 1986; Harding and Levine 1988; Irish *et al.* 1989) are subsequently maintained by the antagonistic activities of two families of *trans*-regulator genes, the *Polycomb* and *trithorax* group loci (PcG and trxG, respectively). The PcG genes sustain a repressed state of HOM expression, while trxG genes favor an active state (Jurgens 1985; Wedeen *et al.* 1986; Dura and Ingham 1988; Kennison and Tamkun 1992; Simon *et al.* 1992). While the PcG proteins all appear to repress transcription, the trxG products maintain the expression of key developmental control genes, including the homeotic loci, acting at different levels of gene regulation (Kennison 1995; Simon 1995; Pirrotta 1997). Recently, several PcG loci whose mutations behave as enhancers of both trxG and PcG mutations have been grouped as a third family [Enhancers of trxG and PcG: the ETP group (Gildea *et al.* 2000)]. Null mutations in most PcG and trxG genes are recessive lethal, but phenotypes may be observed in heterozygous flies as a consequence of HOM misexpression. Loss-of-function alleles of many trxG genes enhance mutations in other trxG members and suppress homeotic transformations resulting from loss-of-function mutations in *bona fide* PcG loci (Capdevila and Garcia-Bellido 1981; Ingham 1983; Shearn 1989). Hence, most members of the trxG family were identified as dominant suppressors of phenotypes caused by misexpression of homeotic genes (Kennison and Tamkun 1988).

Given the structural and biochemical diversity observed for trxG genes (Francis and Kingston 2001), two operational criteria are widely used to define members of this group (Shearn 1989). First, they are functional antagonists of PcG loci. Second, they interact synergistically with other trxG genes. In addition, phenotypic manifestations of these interactions often include homeotic transformations.

Although trxG homologs have been identified in a variety of organisms (Gould 1997; Chamberlin and Thomas 2000), their mechanism of action is not clearly understood. Genetic and molecular studies have suggested that some Drosophila trxG proteins may interact physically at the level of chromatin to regulate the tran-

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scription of target genes, including the HOM loci (Collins *et al.* 1999; Vazquez *et al.* 1999; Kal *et al.* 2000). One of the best-characterized trxG proteins, BRAHMA (BRM), is a homolog of yeast SWI2/SNF2, a bromodomain-containing DNA-stimulated ATPase (Tamkun *et al.* 1992). BRM is found within a large (~2 MD) dSWI/SNF protein complex that is thought to increase target gene accessibility by using the energy of ATP hydrolysis to overcome the repressive effects of nucleosomal histones and imposing an active chromatin state (Papoulas *et al.* 1998; Kingston and Narlikar 1999). How transcriptionally active or inactive chromosomal structures can be stably transmitted through multiple cell divisions in development is largely unknown (for reviews see Lyko and Paro 1999; Francis and Kingston 2001) but represents a question of fundamental interest for better understanding cell differentiation and developmental control (Pirrotta 1998; Farkas *et al.* 2000). Recent data indicate that the acetylation status of histone H4 is one element of the epigenetic control resulting from PcG/trxG chromosomal imprints (Cavalli and Paro 1999).

Unravelling this problem will be facilitated by the identification of the full complement of trxG proteins, permitting the study of their biochemical roles in the cell. It is not clear just how large the trxG family may be nor the full extent of molecular diversity among its members (Kennison 1993). To identify functional partners of Drosophila HOM loci in directing normal development, we performed a genetic screen for mutations that act as dominant modifiers of phenotypes resulting from ectopic expression of the ANT-C gene *proboscipedia* [*pb*; *Hox-A2/-B2* homolog (Cribbs *et al.* 1995; Benassayag *et al.* 1997; Boube *et al.* 1998)]. One *P*-element-induced modifier mutation allowed us to isolate a previously unknown gene, *taranis* (*tara*), whose genetic properties identify it as a novel trxG member. Molecular analysis indicates that *tara* encodes two closely related 96-kD proteins (TARA-α/-β) comprising structural hallmarks of nuclear factors, as well as several evolutionarily conserved motifs. Recognizable mammalian counterparts include the transcriptional coactivator proteins TRIP-Br1/p34^{SEI-1}, a cyclin-dependent kinase regulator that also interacts with PHD zinc fingers and bromodomains (Sugimoto *et al.* 1999; Hsu *et al.* 2001), and RBT1, a replication protein A-binding protein (Cho *et al.* 2000). We discuss the potential implications of these findings for the epigenetic maintenance of active chromosomal states through successive cell cycles.

MATERIALS AND METHODS

Fly stocks and culture: *Drosophila melanogaster* fly stocks were maintained on standard cornmeal/yeast/agar medium at 22°. For embryo or cuticle preparations, eggs were collected on apple juice/agar plates. Strains carrying trx^{E2}/TM6C *Sb*, osa^1/TM6C *Sb*, brm^2/TM6C *Sb*, brm^2 trx^{E2}/TM6C *Sb*, Pc^{16}/TM6 *Ubx*, or the homozygous viable ph^{410} allele were obtained from J.-M. Dura. Unless otherwise noted, all mutations and chromosome aberrations are described in Lindsley and Zimm (1992). $tara^1$ is a *P*[lacW]-induced allele isolated by M.-O. Fauvarque and J.-M. Dura who named the corresponding gene *taranis* for a Celtic god (Fauvarque *et al.* 2001). *Df(3R)sbd26* and *Df(3R)sbd45* were from the Indiana University Drosophila Stock Center (IUDSC; Bloomington, IN).

Alleles of the *tara* gene: The *P*[lacW] insertion mutagenesis was carried out with an X chromosome insertion line (P20) obtained from IUDSC (Bier *et al.* 1989) and isogenized for chromosomes 2 and 3 shortly before initiating the screen. New autosomal insertions were generated by mobilizing the X-linked P20 element with the Δ2-3 *P* transposase source (Robertson *et al.* 1988) and selecting male progeny of dysgenic males carrying a transposed transgene copy. These males were crossed with females harboring a *pb* gain-of-function transgene, HSPB:4d, in search of insertions modifying the effects of ectopic PB (Boube *et al.* 1997). The $tara^{L4}$ insertion led to deletions of wing veins L4 and L5 specifically in combination with HSPB. In addition to the $tara^{L4}$ allele and the independently isolated lethal *P*[lacW] insertion $tara^1$ (Fauvarque *et al.* 2001), analysis of the recently completed Drosophila genome sequence (Adams *et al.* 2000) allowed us to identify 10 other *P* insertions within the *tara* locus. The latter contain insertions of an "Enhancer Pirate" (*EP*) element containing a yeast heterologous UAS promoter that allows modular misexpression (Rorth 1996). Their positions within the *tara* genomic sequence (GenBank accession no. AF227213) are the following: 1686 (*EP3427*), 5447 (*EP1145*), 11526 (*EP3178*), 11573 (*EP3095*), 17707 (*EP1189*), 18081 (*EP508*), 18450 (*EP3463*), 18466 (*EP3294*), 18476 (*EP3530*), and 30901 (*EP693*). The *EP3427* and *EP3463* lines contain an appropriately oriented UAS promoter inserted within the *tara* locus that affords overexpression of a determined TARA isoform in combination with a tissue-specific driver for the yeast transcription factor GAL4 (see Abdelilah-Seyfried *et al.* 2000; Fernandez-Funez *et al.* 2000). To recover excision alleles, the *P*[lacW]-L4 element was mobilized by generating dysgenic males carrying the *P*[ry^+, Δ2-3]99B *Sb* chromosome (Robertson *et al.* 1988), crossing them with w^- females, and recovering w^- Sb^+ animals. Of 120 w^- chromosomes recovered, 8 (R19, R38, R40, R47, R58, R75, R91, and R108) were lethal over *Df(3R)sbd26* and $tara^{L4}$. Lethal phases were assessed by mating heterozygous $tara^-$/+ females and males (without balancer chromosomes). The parents were allowed to mate at 22° for several days to minimize the number of unfertilized eggs in the collections. The number of unhatched eggs was recorded 48 hr after the collection period.

Genetic interactions: Interactions among various combinations of the alleles $tara^{L4}$, $tara^1$, pb^4, pb^{13}, pb^5, Pc^{16}, ph^{410}, trx^{E2}, osa^1, and brm^2 were examined after culture at 22°. Labial palp phenotypes of pb^{13}/pb^4 and pb^{13}/pb^4 $tara^{L4}$ were compared in independent blind tests by three persons. For the wings-held-out phenotype, individuals with both wings extended were scored as mutant.

Isolation of *tara* genomic and cDNA clones: The *P*[lacW] element allows cloning of adjacent genomic sequences by plasmid rescue (Bier *et al.* 1989). One isolate, pL4, obtained by *Eco*RI digestion of flies bearing *P*[lacW]-L4, was ^{32}P-labeled (Megaprime kit; Amersham Pharmacia Biotech) and used to screen a genomic library of Oregon-R DNA made in λcharon4 bacteriophage (Maniatis *et al.* 1978) using standard techniques (Sambrook *et al.* 1989). Two independent genomic λ clones were isolated and characterized by restriction mapping and Southern blot analysis. All *Eco*RI genomic fragments were subcloned into pBluescript II SK+ (Stratagene, La Jolla, CA).

A pool of purified *Eco*RI inserts was prepared, ^{32}P-labeled, and used as a probe to screen a Canton-S 8- to 12-hr embryonic cDNA library (Brown and Kafatos 1988). Thirty positive inserts in the pNB40 plasmid were purified and characterized by restriction mapping. Sequencing was performed using dideoxy chain termination with a Sequenase 2.0 kit (Amersham Pharmacia Biotech). The complete double-stranded sequences of the C12 and C16 cDNAs were determined, using the SP6 and T7 primers as well as specific internal oligonucleotides (Isoprim, Toulouse, France; sequences available upon request). The GenBank accession numbers for the sequences reported in this study are AF227211, AF227212, and AF227213. Sequence similarity searches and clustering were performed at the National Center for Biotechnology Information using BLAST (http://www.ncbi.nlm.nih.gov/BLAST/) and at the Institut de Génétique et de Biologie Moléculaire et Cellulaire (Illkirch, France) using DbClustal (http://igbmc.u-strasbg.fr:8080/DbClustal/dbclustal.html). The Drosophila protein and annotation databases were searched at the Berkeley *Drosophila* Genome Project (BDGP; http://www.fruitfly.org/).

***In situ* hybridization:** *In situ* hybridizations were carried out with digoxigenin-labeled RNA probes (Roche Molecular Biochemicals, Indianapolis) by the method of Tautz and Pfeifle (1989). To allow synthesis of antisense riboprobes by transcription from the T3 or T7 promoter, three fragments were each inserted into pBluescript II SK+ cut with the appropriate enzymes: a 2.3-kb *Xho*I/*Kpn*I genomic fragment (probe C), a 0.75-kb *Apa*I/*Eco*RI fragment from C12 cDNA (probe A), or a 1.5-kb *Eco*RI/*Kpn*I genomic fragment (probe B). Following color development, the embryos or larval tissues were mounted in PBS/50% glycerol, viewed, and photographed with a Zeiss Axiophot microscope.

***P*-element-mediated germline transformation and tests for rescuing *tara* lethality:** The pUbTARA-β construct used for rescue tests was made as follows. A 3.3-kb *Mlu*I/*Xmn*I cDNA fragment from the pNB-C16 plasmid was inserted into the pUbHB1 vector between the *Mlu*I and *Eco*RV polylinker sites to yield the pUbC16 construct. The pUbHB1 plasmid contains a polylinker placed downstream of the *ubiquitin-63E* promoter [2-kb *Sal*I/*Bgl*II fragment (Lee *et al.* 1988)] and upstream of the *hsp70* 3′-untranslated and 3′-flanking genomic regions [0.5-kb *Bam*HI fragment from pCaSpeR-hs (Thummel and Pirrotta 1992)]. The map of the pUbHB1 vector is available upon request. Finally, the pUbTARA-β plasmid was generated by insertion of a 5.9-kb *Xmn*I/*Not*I fragment from pUbC16 into pCaSpeR4 (Thummel and Pirrotta 1992) between the *Stu*I and *Not*I polylinker sites. w^{1118} embryos were co-injected with pUbTARA-β and pUChsΔ2-3 (Mullins *et al.* 1989) by standard techniques (Rubin and Spradling 1982). Emerging adults were crossed individually to w^{1118} flies, and w^+ transformant progeny were identified. Five independent lines were recovered, chromosomal linkage was determined by crosses with a multiple-balancer stock, and homozygous stocks were established. Four transformant lines carrying the *P*[UbTARA-β] construct on the second chromosome (lines 2, 3, 4, and 5) were tested for rescue of lethality in $tara^{L4}$ homozogotes, in the following way: w^{1118}; *P*[UbTARA-β]/+; $tara^{L4}$/TM3, *Sb e* males were generated and mated to w^{1118}; $tara^{L4}$/TM6B, *Hu Tb e* females. Rescue was assessed by scoring for surviving progeny with the genotype w^{1118}; *P*[UbTARA-β]/+; $tara^{L4}$/$tara^{L4}$. All rescued *tara* mutant individuals (non-*Stubble*, non-*Humeral*, e^+) were mini-*w*+.

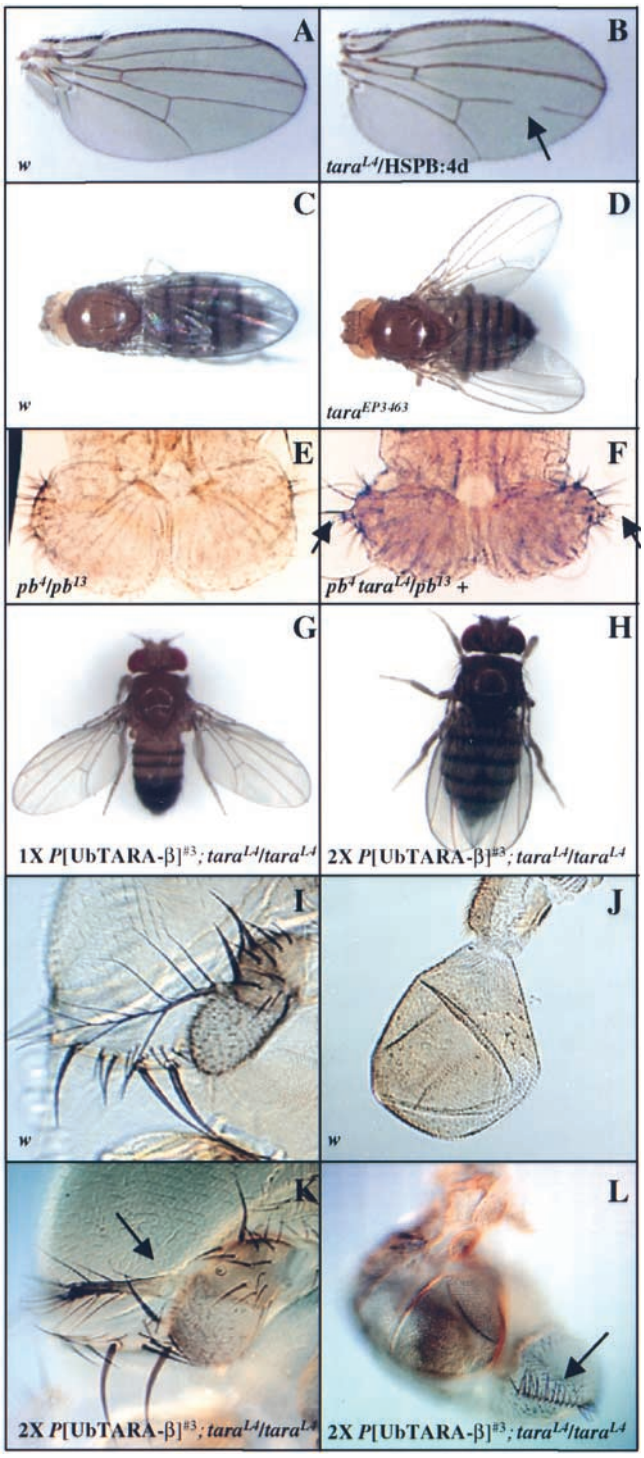
RESULTS

***tara* is an essential locus that modulates *pb* homeotic selector activity:** The homeotic locus *pb* is a genetic selector required for adult mouthparts (Pultz *et al.* 1988). Basal ectopic expression of PB protein from a *Hsp70-pb* (HSPB) transgene, including *pb* transcriptional regulatory sequences, leads to discrete dose-sensitive adult phenotypes in the wing, the eye, and the prothoracic leg (Cribbs *et al.* 1995; Boube *et al.* 1997). The HSPB minigene thus facilitates genetic screens for dominant modifiers (*cf.* Botas *et al.* 1982; Duncan 1982) that we sought by insertional mutagenesis with a marked *P* element [*P*[lacW]; Bier *et al.* (1989)]. Among ~5000 independent males tested carrying mutagenized autosomes, one new *P*[lacW] insertion was associated with partial truncation of the distal part of the longitudinal wing vein L4 in heterozygous combination with the HSPB:4d transgenic line (penetrance of ~60%; Figure 1B). This phenotype depends on both HSPB and the *P*[lacW] insert since it is detected in heterozygous combination with several HSPB lines, but not for HSPB nor for insertion heterozygotes alone (not shown). The chromosome harboring the interacting insertion, *P*[lacW]-L4, situated on chromosome 3R at 89B13-16, is homozygous lethal. This recessive lethality of the *P*[lacW]-L4 line and its interaction with ectopic PB were reverted by excision of the marked *P* element (see below), identifying the interacting locus as an essential gene. This locus, likewise isolated in an independent screen, was *tara* (Fauvarque *et al.* 2001).

The initial insertion allele $tara^{L4}$ was examined in homozygotes or in hemizygous combination with the chromosomal deficiencies *Df(3R)sbd26* and *Df(3R)sbd45* covering the 89B13-16 region. Homozygous or hemizygous animals died as late embryos or as first instar larvae without obvious cuticular pattern defects in all examined allelic combinations (Table 1). $tara^{L4}$ thus behaves genetically as a strong or complete loss-of-function (lof) mutation. To recover additional alleles by imprecise excision, the *P*[lacW]-L4 insertion was mobilized utilizing the Δ2-3 *P*-transposase source (Robertson *et al.* 1988). Among 120 recovered $white^-$ revertants, 110 were fully viable without apparent adult phenotypes (presumptive precise excisions), 8 were homozygous lethal (see materials and methods), and 2 ($tara^{R19}$ and $tara^{R51}$) led to adult escapers with prominent wing phenotypes including balloon and/or held-out wings along with L5 vein truncation (see Table 1 and Figure 1D). The new lethal alleles failed to complement the parental allele, although the lethal phases ranged from first instar larva to pupa (Table 1 and data not shown). No excision alleles were recovered showing fully penetrant embryonic lethality either as homozygotes or as hemizygotes.

To examine whether the observed dose-sensitive interaction of $tara^{L4}$ with HSPB in the wing reflects a relationship in the normal lieu of *pb* action, the adult mouthparts, we tested for a dominant interaction between $tara^{L4}$ and different *pb* alleles. The allelic combination pb^4/pb^{13} leads to reduced labial pseudotracheae, but without an overt homeotic transformation (Cribbs *et*

al. 1992). In contrast, pb^4 $tara^{L4}/pb^{13}$ + flies showed a weak but reliable distal labium-to-antenna transformation (Figure 1, compare E and F), indicating that reduced *tara* activity diminishes normal *pb* function. Similarly, the allelic combination pb^4/pb^5 transforms distal labium to antennal arista, and distal claws typical of a leg (a more severe loss of function) are not observed in a $tara^+$ context. By contrast, claws were observed in nearly half of $tara^{L4}$ heterozygotes (not shown). We infer from these interactions that *tara* acts to positively regulate *pb* selector activity.



***tara* suppresses PcG mutations:** An independent genetic screen for suppressors of the *Polycomb* group gene *polyhomeotic* (*ph*) led to the isolation of another recessive lethal *P*[lacW] insertion allele in *taranis* (Fauvarque *et al.* 2001). This allele, $tara^1$, was identified on the basis of its dominant suppression of the extra-sex-combs phenotype due to the hypomorphic ph^{410} allele (Dura *et al.* 1985). $tara^1$ also suppresses the dominant extra-sex-combs phenotype caused by the amorphic *Polycomb* allele Pc^{16} (Kennison and Tamkun 1988). Complementation tests established that $tara^{L4}$ and $tara^1$ are allelic. The former showed a stronger interaction with Pc^{16}, ph^{410} (Table 2), or HSPB:4d (data not shown). Our molecular analysis indicated that the $tara^{L4}$ and $tara^1$ insertions are situated ~0.28 kb apart within an intron shared by two nested transcription units (see below and Figure 2).

***tara* enhances mutations of trxG loci:** Interaction of *tara* loss-of-function alleles with *pb* and PcG mutations suggested that *tara* might encode a new trxG member. Apart from suppression of PcG phenotypes, synergistic interaction with mutations of established trxG genes is an additional criterion for classifying a gene as a *trithorax* group member (Shearn 1989). Representatives include *trithorax* (*trx*), *brahma* (*brm*), and *osa*. The latter two strongly interact to give a fully penetrant wings-held-out phenotype that is thought to result from a failure to properly activate the *Antp* P2 promoter (Vazquez *et al.* 1999). This phenotype strongly resembles that seen in *tara* hypomorphs (Figure 1D).

To test whether *tara* behaves like a member of the trxG family, combinations of the $tara^{L4}$ or $tara^1$ allele with

Figure 1.—Adult phenotypes associated with *tara* mutants. (A and C) Wild-type wing blade and posture, respectively, in w^{1118} adults. (B) The partial deletion of the L4 wing vein (arrow) in a w^{1118}; HSPB:4d +/+ $tara^{L4}$ fly. This phenotype is specific to the combination. (D) Held-out wings in a female homozygous for the semiviable allele $tara^{EP3463}$. (E) The labial palps of this pb^4/pb^{13} fly are nearly normal (with a slight reduction of the pseudotracheal rows). (F) Reduced *tara* function leads to a discrete, limited transformation of the distal labium to antennal arista (arrows) in a pb^4 $tara^{L4}/pb^{13}$ + adult. This phenotype is typical of reduced *pb* function. (G and H) Embryonic lethality of $tara^{L4}$ homozygotes was rescued in flies harboring one (G) or two (H) copies of the transgene *P*[UbTARA-β] (line 3; see materials and methods), regardless of gender. Note the wings-held-out phenotype in G, similar to the *tara* hypomorph in D. This defect is rectified on increasing TARA-β, as seen in H. (I and J) Wild-type antenna and haltere, respectively, in w^{1118} adults. (K and L) Apart from the wings-held-out phenotype mentioned above, other developmental defects were observed in $tara^{L4}$ homozygotes harboring a single copy of the *P*[UbTARA-β]#3 transgene. The thickened antennal arista shown in K resembles a weak antenna-to-leg transformation (arrow), while the haltere defect (L) is interpreted as a nascent transformation to wing margin, as indicated by the row of bristles (arrow).

TABLE 1

Developmental phenotypes of *tara* alleles

Genotype	Lethal phase[a]	Adult phenotype[b]
$tara^{L4}/tara^{L4}$	L1	NA
$tara^{L4}/Df(3R)sbd26$	L1	NA
$tara^{1}/tara^{1}$	L1	NA
$tara^{R19}/tara^{R19}$	Viable	Balloon wings (1%)
$tara^{R47}/tara^{R47}$	L3	NA
$tara^{R51}/tara^{R51}$	Subviable	Balloon and held-out wings (4%)
$tara^{R108}/tara^{R108}$	L2	NA
$tara^{EP3427}/tara^{EP3427}$	Viable	Held-out wings (5%)
$tara^{EP3463}/tara^{EP3463}$	Subviable	Held-out wings (100%), L5 vein truncation (15%) and ectopic ventral abdominal bristles (5%)
$tara^{EP508}/tara^{EP508}$	Viable	Wild type

NA, not applicable.

[a] Lethal phase is the farthest developmental stage reached by the *tara* mutants.

[b] The percentage of adult flies (>200 males and females were examined) with the indicated phenotypes shown in parentheses.

strong or null alleles of *trx*, *brm*, or *osa* were examined specifically for wing phenotypes. Double heterozygotes for $tara^{L4}$ with trx^{E2}, brm^{2}, or osa^{1} showed a wings-held-out phenotype not observed in single heterozygotes (Table 2). The penetrance of these interactions ranged from 2 to 36%, with the strongest expressivity observed in $tara^{L4}$ +/+ osa^{1} heterozygotes. Weakly penetrant wing phenotypes were observed in + $tara^{L4}/trx^{E2}$ + or in brm^{2} trx^{E2}/+ + double heterozygotes (2 and 5%, respectively). By contrast, on examining triple heterozygotes for *tara*, *brm*, and *trx*, 45% of flies of + + $tara^{L4}/brm^{2}$ trx^{E2} + flies displayed held-out wings. Similar synergistic effects were seen with the $tara^{1}$ allele (see Table 2), ruling out that the genetic interaction between *tara* and trxG loci is due to a second-site mutation on the $tara^{L4}$ chromosome. Taken together, these results indicate that *tara* behaves genetically as expected for a member of the trxG family.

TABLE 2

Interaction of *tara* with PcG and trxG loci

Genotype	% of male legs with three or more ectopic sex-comb teeth on T2 legs/T3 legs
$ph^{410}/+$	38/24 (n = 120)
$ph^{410}/tara^{L4}$	2/0 (n = 176)
$ph^{410}/tara^{1}$	4/2 (n = 152)
$Pc^{16}/+$	69/15 (n = 106)
$Pc^{16}/tara^{L4}$	8/2 (n = 116)
$Pc^{16}/tara^{1}$	14/4 (n = 132)
Genotype	**% of flies with held-out wings[a]**
$tara^{L4}/+$	0 (n = 123)
$tara^{1}/+$	0 (n = 135)
$osa^{1}/+$	0 (n = 153)
$+ osa^{1}/tara^{L4}$ +	36 (n = 185)
$+ osa^{1}/tara^{1}$ +	28 (n = 115)
$brm^{2}/+$	2 (n = 160)
$brm^{2}+/+tara^{L4}$	12 (n = 220)
$brm^{2}+/+tara^{1}$	10 (n = 215)
$trx^{E2}/+$	0 (n = 88)
$trx^{E2}+/+tara^{L4}$	2 (n = 129)
$trx^{E2}+/+tara^{1}$	1 (n = 85)
brm^{2} $trx^{E2}/++$	5 (n = 52)
brm^{2} $trx^{E2}+/++tara^{L4}$	45 (n = 232)
brm^{2} $trx^{E2}+/++tara^{1}$	38 (n = 152)

For each tested genotype the number of scored flies is indicated in parentheses.

[a] Flies with at least one held-out wing were counted as mutant.

***tara* encodes two classes of mRNA:** The origin of $tara^{L4}$ as a *P*-element insertion allowed us to pursue the molecular analysis of the *tara* gene. A 1.5-kb fragment of genomic DNA flanking the *P*[lacW]-L4 element was cloned by plasmid rescue (Wilson *et al.* 1989) and then used to screen an Oregon-R genomic DNA library in the λcharon4 bacteriophage (see materials and methods). Among the recombinant phages recovered, two overlapping clones (λC5 and λC13) spanned 30 kb of genomic sequence (Figure 2A). Subcloned fragments covering this genomic region were used as probes in Northern blot analysis to identify transcribed regions. Two embryonic polyadenylated RNA species of ~2 and ~4.5 kb were detected (not shown). The ~2-kb mRNA, detected only with a probe from one end of the cloned region, corresponds to a novel gene encoding a putative novel member of the AAA family of ATPases that we named *belphegor* (*bor*; see Figure 2A; GenBank accession no. AF227209). In contrast, the ~4.5-kb mRNA was detected with probes separated by 15 kb (probes B and C) and flanking the $tara^{1}$ and $tara^{L4}$ insertions (Figure 2A). These results strongly suggest that both *P*[lacW] insertions reside within an intron of a transcription unit giving rise to the ~4.5-kb mRNA species. Consistent

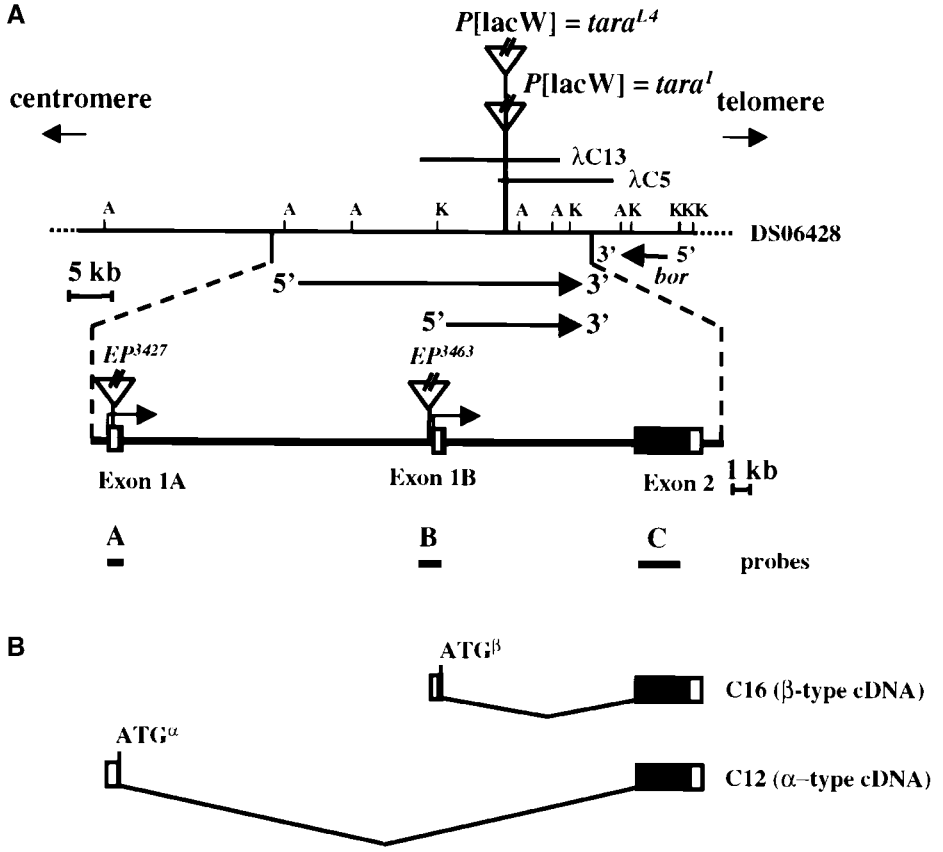


FIGURE 2.—Molecular analysis of the *tara* locus. (A) Physical map of the *tara* region on chromosome 3R, cytological interval 89B13-16 (centromere, left; telomere, right). The restriction map of the P1 bacteriophage DS06428 (A, *Apa*I; K, *Kpn*I) corresponds to nucleotides 85321–158160 of GenBank accession no. AE003712 containing the *tara* locus. The positions of the two λ-bacteriophage used to initiate the structural characterization of the locus (λC5 and λC13) are shown above the restriction map. The positions of the neighboring *P*[lacW] insertion mutations $tara^{LA}$ and $tara^{1}$ are indicated above the genomic map by inverted triangles. The 5′-to-3′ orientation and the length of the two alternative transcription units that define the *tara* locus are shown below the DNA line, as well as the approximate location (not to scale) and orientation of the neighboring gene *bor*. *tara* exons are indicated as solid boxes for coding sequences and open boxes for untranslated mRNA sequences, respectively. The putative transcription initiation sites (arrows) have been inferred from the presence of an additional 5′ G in the longest α- or β-class cDNAs. The positions of the *P* insertions *EP3427* and *EP3463* allowing overexpression of the TARA isoform α or β, respectively, are indicated by inverted triangles. The approximate positions and sizes of DNA fragments in A–C, employed to generate probes for Northern blots and whole-mount *in situ* hybridizations, are marked beneath the map. (B) Structure of representative cDNAs derived from the *tara* region. Shown are *tara* coding sequences (solid) and positions of the alternative initiator ATGs, as deduced from the apparent full-length cDNAs C12 (α-class) and C16 (β-class).

with this, a much weaker *in situ* hybridization signal was observed in $tara^{LA}$ homozygous embryos compared to wild type (not shown). Apart from indicating that the ~4.5-kb mRNA corresponds to *tara*, these data also suggested that $tara^{LA}$ is not a molecular null allele.

To recover *tara* cDNAs, relevant λC5 and λC13 genomic fragments were used to probe a cDNA library from 8- to 12-hr embryos (see MATERIALS AND METHODS). Among 30 positive clones recovered, insert sizes ranged from 0.9 to 4.3 kb. Restriction mapping and partial sequence analysis indicated that all of the cDNA inserts had similar 3′ extremities, ending in an A-rich region with oligoadenylated tails. However, two classes of 5′ extremities (α and β) were detected among the longest cDNAs. All but one cDNA insert shared a common 5′ region of the β-class and differed only in length. The longest of these β-type inserts, C16, contained a sequence of 4000 bp (Figure 3). The complete sequence of the single α-type clone (C12) was 4316 bp, again in reasonable agreement with a fully polyadenylated mRNA of ~4500 nucleotides (nt) detected by Northern blots. C12 was identical to C16 for most of its length, sharing a long open reading frame of 2724 bp (see Figures 2B and 3). However, C12 and C16 diverged toward their 5′ ends, containing distinct 5′ mRNA untranslated regions of 767 and 499 bp, respectively (see Figure 3). The C12 cDNA thus appeared to represent an alternatively spliced mRNA, potentially derived from an independent transcriptional promoter.

Comparison of the complete C12 and C16 cDNA sequences with newly available BDGP genomic sequences (P1 bacteriophage DS06428) allowed us to establish the following three points: (i) Both cDNAs are likely to be full length, since each contains a G nucleotide at its 5′ end lacking in the genomic sequence that might correspond to a 7-methyl-G cap added post-transcriptionally (HIRZMANN *et al.* 1993); (ii) the sequences shared by C12 and C16 correspond to a single large 3′ exon of 3.5 kb (annotated as CG6889 by the complete *D. melanogaster* genome database), ending with a putative polyadenylation signal (ATTAAA; PROUDFOOT and BROWNLEE 1976) in the immediate vicinity of the A-rich 3′-terminal region found in all cDNA clones (not shown); and (iii) this 3′ exon is linked to alternative 5′ exons of 790 and 511 bp situated 30 and 13.5 kb distant and corresponding to C12 and C16 cDNAs, respectively (see Figure 2B). The most direct interpretation of these data is that the α- and β-type cDNA inserts correspond to alternatively

spliced 5′ exons (termed 1A and 1B, respectively) initiated from distinct transcriptional start sites (see Figure 2, A and B).

The exons 1A and 1B each include an in-frame initiator ATG in a favorable context compared to the Drosophila consensus start site (Cavener 1987; see Figure 3). The expected 4.5- and 4.2-kb polyadenylated mRNA species thus encode two distinct proteins (hereafter referred to as TARA-α and -β isoforms) with predicted molecular weights of 96,274 and 95,730 D, respectively. These isoforms differ only in their amino (N)-terminal sequences, sharing 908 residues of 916 (α) or 912 (β; Figure 3).

Genetic evidence for partially independent *tara* promoters: In addition to $tara^{LA}$ and $tara^{1}$ (see above), analysis of the recently completed Drosophila genomic sequence (Adams *et al.* 2000) allowed us to identify 10 other *P* insertions distributed throughout the *tara* locus (see materials and methods), including four (*EP3294*, *EP3427*, *EP3463*, and *EP3530*) that are located within or near either one of the two alternative 5′ exons. The 7 examined insertions (*EP508*, *EP693*, *EP1189*, *EP3294*, *EP3427*, *EP3463*, and *EP3530*) are all homozygous viable or subviable (see Table 1 for representative *EP* alleles). When those insertions were tested in combination with themselves, with each other, with $tara^{LA}$ or the $tara^{-}$ deficiency *Df(3R)sbd26*, various adult phenotypes were observed including held-out wings, defects of wing veins L2 and/or L5, and abnormally located bristles on the ventral abdomen (Table 1, Figure 1D, and data not shown). Interestingly, two insertions located within exon 1A and close to exon 1B (*EP3427* and *EP3463*, respectively; see Figure 2A) complement each other. This observation suggests that transcription of the two alternative mRNAs may depend on distinct promoter sequences and give rise to functionally redundant TARA-α/-β isoforms.

***tara* is broadly expressed throughout development:** The spatial expression of the *tara* gene during various developmental stages was examined by whole-mount tissue *in situ* hybridization. Three antisense RNA probes were prepared, permitting detection of overall gene expression and discrimination between the α- and β-type mRNA species (for details, see Figure 2A and materials and methods).

tara mRNAs are detected at all embryonic stages with a common probe derived from exon 2 (probe C). Weak but reproducible signal is obtained in blastoderm embryos (not shown), presumably reflecting maternally contributed mRNA. By the onset of gastrulation (stage 6), stronger staining is evident in invaginating cells in the cephalic furrow, the prospective mesoderm, and posterior midgut (Figure 4C). This heightened local accumulation is transient and broadens to encompass the entire germ band at full extension. By stage 13, and until stage 17, *tara* mRNAs are present at uniformly low levels, except for the ventral nerve cord, brain, and visceral mesoderm (Figure 4, F and I). As in embryos, generalized *tara* expression is detected in late third instar larval tissues including the imaginal discs, the brain, and ventral nerve cord (not shown).

To reveal mRNA species specific for the TARA-α or -β isoform, probes were derived from the 5′ part of the C12 cDNA insert corresponding to exon 1A (probe A) and from a genomic fragment overlapping the exon 1B (probe B), respectively (see Figure 2A). The spatial and temporal expression patterns observed with the α-specific probe closely resemble that obtained with the common probe (above). In contrast, although a low-level uniform staining is detected, the β-specific probe revealed mRNA accumulation in the developing gut throughout embryogenesis. As a whole, these data indicate that *tara* is broadly expressed throughout development. However, the difference in accumulation between α and β mRNAs again suggests that their expression depends on distinct regulatory sequences.

Ubiquitous expression of the TARA-β isoform is sufficient for viability: To address the relative contributions of the α- and β-transcription units to *tara* activity, we performed phenotypic rescue experiments using a transgene coding for only the β isoform. A β-coding cDNA fragment was subcloned into the *P*-transformation vector pCaSpeR4 (Thummel and Pirrotta 1992), downstream of a ubiquitously active promoter from the *ubiquitin-63E* gene (Lee *et al.* 1988; see materials and methods for details). Four independent transformant lines carrying the resulting *P*[UbTARA-β] construct were tested for their capacity to rescue the embryonic/larval lethality of $tara^{LA}$ homozygote animals. In all four cases *P*[UbTARA-β]/+; $tara^{LA}/tara^{LA}$ animals were fully viable (Table 3), indicating that TARA-β protein possesses *tara* genetic function. However, these rescued flies showed poor fertility and a fully penetrant wings-held-out phenotype (Figure 1G). Furthermore, a small number of these incompletely rescued adults showed clear homeotic phenotypes, including partial arista-to-tarsa and haltere-to-wing transformations (Figure 1, compare K and L with I and J, respectively), supporting our previous conclusion that *tara* is a *bona fide* trxG gene since the homeotic selectors are sensitive to trxG function. Two simple models (not mutually exclusive) might readily explain the incomplete rescue observed. The level of transgene expression might be inadequate for complete rescue of some specific *tara* functions. Alternatively, the α isoform might be required in dorsal mesothoracic cells. To distinguish between these two possibilities, we asked whether the rescue could be improved by increasing the dose for the same isoform. Many animals carrying a second transgene copy (*P*[UbTARA-β]/*P*[UbTARA-β]; $tara^{LA}/tara^{LA}$) now showed normal wing posture (Figure 1H) and increased fertility allowing stable stocks (see Table 3). These data, as well as overexpression experiments (not shown), therefore suggest (i) that *tara* function is dose sensitive in specific tissues and (ii) that

α-type cDNA (C12)

```
GAATCCGAATTGAACCGAGCCAAGGCGAGTTGGCTTTGTTTGTTGGTCGGGCCCACTAAAATAGTTCAGGAAATGATGAAAACGTGGAGCTGGGGCGAAG  100
CAGAGAGGGCGGAAGGCATGGTACATTGAAAGCATTGTGGGTCTGAGTTTTGGTATTGGGATTGGAGCCATGGCTTTCGCCCGATTGTGTCCCAGCCTGG  200
TGGCTTTCCACAGAGCAACTGAAAATACGAAAATTCGAGGCGGAAAAGCTGGCAACAACAACGCGCGCATGTGGCAGAGTTGCCAAAGCGCAGAGAGTTT  300
TCCAGGCGCGGCGACGGCAACCCTGGCCACCCGGCTGTCGAGTGTGTTGGCCAGGCCGAAAGCATTCAGTACGAACTCTGCACTTCGCACAACAAGACGC  400
CAAGCGACCAGCGACGACGACGCCAAAGGCGCTCGGCCAAAGCCACAGAGCCCGAGCAAAAACTACGTAAATTTTCCACAGCGATTTTCCCCATTTTTCC  500
CAGTTGAACAACAGCGCCAAAAACAAAAACATCGGACCAGCGGTCTGCATGTGTGTTTGTGCCAGAGCGTGTGTGTTTTGTGTGTGTTATATAGTGAATC  600
GTTCTTGGCCCGACGATCGACTTCCCCATCACCCTCAAACCCGCAAACATGTGGAGCTGGGGCTAAGGAACACCACGGAACCCAAAACAACCAGCGACTC  700
AGAAAAAGGCGCCAAACTTAAATTATTGCCAGCCGAGATTACCTCCTCAAGACTTCGCAGGAAACCATGAAAATGTGGACGGAATCCAAT              790
                                                                M  K  M  W  T  E  S  N                    aa9
```

β-type cDNA (C16)

```
GATTTGTGATTTTAGCACGGCGCTGCGAGGTCCACGTCGCTCATTTGCTCCGTGCCCGCTCGACAGCTCCAATTCGAAAACGACGTAAACGCCAGCCGTT  100
CGCCAAGCGCGCGTAATTCAAAGTTATCAACTCGAAACACTGTTTCCCGGAAAAAGTGCACACCGTTAAATGTGAAATATTCAATCAAGTCAACTGGAGA  200
ATATAAAAAAAATATTAAAAAAAATTAAAGTGAACTGCATTATACACAGATTGATCAGTTTAAGTAGTGCCAGCCATGTCGTCCAAGTTCTGTGATGCCGC  300
TTTGGGTCGCTCCTACGCCTGTTCGTCCCGCTTTCCTTCGGGACACCTGCTGGCCACCCTCTGATCCGCCCACGCCGCCCAGTGACTGACTGAAAGGGAT  400
CGTACCGCCCTGAACAAAAACTCCAACGCGTTACCTTTTTTTTTCGTTTCCCATCTATTTGGCTATAAACGTTGTGAAACTGGGAACGGCCCAGCCGAAAA  500
                                                                                                        _
                ▼
TGTGCACTGAGGTGAATTCCGCGATGGGTCTTCAAGCAACAGCCGCCACCAAGCGGAAGCATGAGCTGACCTTCGACAGCAAGGATGCCAACACCACCTA  600
M  C  T  E  V  N  S  A  M  G  L  Q  A  T  A  A  T  K  R  K  H  E  L  T  F  D  S  K  D  A  N  T  T  Y  aa34
CACGGGCAACTGTGCCCCGCCCCCCGTCAAGGCCAACAAGTGGGCCATCAGCAACAACAACTACCTGGAGTCGCTCGAGGAGCAGCAGCAGCAACAGCAA  700
  T  G  N  C  A  P  P  P  V  K  A  N  K  W  A  I  S  N  N  N  Y  L  E  S  L  E  E  Q  Q  Q  Q  Q  Q  aa67
TCGCCATCGGAGCCAGCTGTTGAGTCCAATAACAACCACATTGTTTTAGAAGCATCAATGGATGCGTTGAAGCCAACGGAGCCAAGTATTAGCAATGGTC  800
 S  P  S  E  P  A  V  E  S  N  N  N  H  I  V  L  E  A  S  M  D  A  L  K  P  T  E  P  S  I  S  N  G  aa100
ATGAAGTTACTACCGCCGTCGCAGCAATGAAAAGTCAAGCGGAGGTGCCACTCCCGCCAACAGCGAGTGCGGCGATACCGGAGGACAGTATCGCCAGGTT  900
H  E  V  T  T  A  V  A  A  M  K  S  Q  A  E  V  P  L  P  P  T  A  S  A  A  I  P  E  D  S  I  A  R  L  aa134
AGAGGTGGTCACCTCAGCCGTTCCTTGTGAGCCCTGGACGAGCAATGGGCCCACTACTCCTTCCGCGGTGGCAGGACCCGCAGCATCCGCGGAACCTGTG 1000
 E  V  V  T  S  A  V  P  C  E  P  W  T  S  N  G  P  T  T  P  S  A  V  A  G  P  A  A  S  A  E  P  V  aa167
GATTGCATCTCCAAGCTGCAGGCCGTCGCGGTGCCCAGTGATCCTTGGGGTAGCATTGCCACCCGCTCCACTCTGGCAACGACTTTGCTCAGTGCCGATG 1100
 D  C  I  S  K  L  Q  A  V  A  V  P  S  D  P  W  G  S  I  A  T  R  S  T  L  A  T  T  L  L  S  A  D  aa200
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 Q  Q  Q  Q  Q  H  P  Q  Q  Q  R  S  Y  A  P  G  Y  G  S  R  Q  P  N  Y  Y  S  E  P  F  P  Q  Q  N  aa300
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 E  N  G  K  S  Y  L  D  L  G  C  S  S  G  A  S  G  N  A  G  G  S  P  I  S  P  P  P  S  S  A  P  V  aa400
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E  L  E  H  P  A  H  I  M  V  G  S                                                                    aa912
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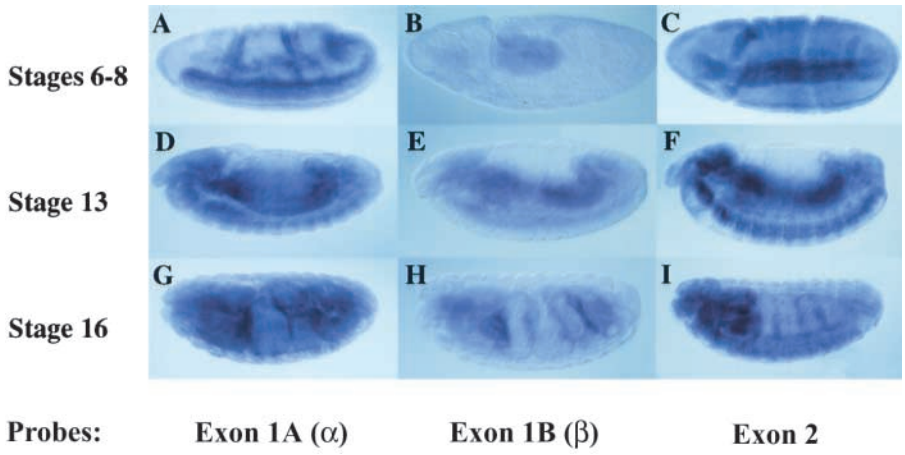

FIGURE 4.—Spatial distribution of *tara* mRNAs during embryonic development. Whole-mount preparations of wild-type (w^{1118}) embryos at indicated stages were hybridized with antisense *tara* riboprobes corresponding to exon 1A (α) (A, D, and G), exon 1B (β) (B, E, and H), or exon 2 (C, F, and I). Anterior is to the left; dorsal is uppermost. α- and β-type mRNAs are broadly expressed throughout embryogenesis. In stage 6 embryos note the higher mRNA accumulation in invaginating tissues, in particular the ventral mesodermal cells. By stage 13, also note the higher accumulation of β-type mRNA species throughout the gut.

the two TARA protein isoforms perform largely or fully redundant functions.

The TARA proteins belong to a novel evolutionarily conserved family apparently restricted to higher eukaryotes: The common primary sequence of TARA-α/-β proteins contains several regions with a strong amino acid (aa) bias. These include a long acidic stretch (residues 200–217 in TARA-β) as well as several runs of alanine and glutamine residues (see Figure 3). The carboxy(C)-terminal third of the predicted TARA-α/-β proteins is markedly rich in alanine, serine, and threonine residues (58% over 264 amino acids). Finally, they share a potential nuclear localization signal (ATKRKH, positions 16–21 of TARA-β) near the N terminus. These are all frequently encountered structural features of nuclear regulatory components.

Apart from the preceding general traits, initial analyses of the TARA twin isoforms showed no motif diagnostic of established biochemical or functional properties. However, more detailed sequence database searches revealed several mammalian proteins structurally related to TARA-α/-β (Figure 5). Based on DbClustal analysis (THOMPSON *et al.* 2000) the highest identity score (E value of 0.098) was with human Y127 (NAGASE *et al.* 1995), a hypothetical 34-kD protein. In turn, searches for protein homology with Y127 as the query sequence revealed at least three human paralogs and presumptive mouse orthologs of the four Y127 family members (not shown). TARA-α/-β and the four mammalian Y127 family proteins (hereafter referred to as TRIP-Br family members; see below) show four distinct regions of clear similarity (see Figure 5A). The four evolutionarily conserved regions comprise (i) an N-terminal basic cyclin A-binding motif homologous to that of the cell cycle regulatory transcription factors E2F1–3 (KREK *et al.* 1994; ADAMS *et al.* 1996); (ii) a novel motif that we designate SERTA (for *SE*I-1, *R*BT1, and *TA*RA), corresponding to the largest conserved region among TRIP-Br proteins; (iii) a PHD-bromo interaction domain (HSU *et al.* 2001); and (iv) a conserved C-terminal (C-ter) motif of unknown biochemical function. Each of the five proteins contains all four motifs, organized in the same order in the linear sequence (see Figure 5B). These considerations led us to conclude that TARA and mouse/human TRIP-Br proteins are members of a novel evolutionarily conserved family. The TARA-related human proteins include (a) TRIP-Br1/p34^{SEI-1}, recently identified both as a cyclin-dependent kinase regulator (SUGIMOTO *et al.* 1999) and as a transcriptional regulator interacting with PHD and bromodomains (HSU *et al.* 2001), two motifs widely found in chromosomal proteins (AASLAND *et al.* 1995; WINSTON and ALLIS 1999); (b) RBT1, a potent transcriptional coactivator that interacts with the second subunit of replication protein A (CHO *et al.* 2000); and (c) HEPP, a novel protein expressed preferentially in hematopoietic progenitors and mature blood cells (ABDULLAH *et al.* 2001). As shown in Figure 5A, a careful analysis of protein databases revealed a SERTA region conserved in predicted human (GenBank accession no. CAB81635) and Drosophila proteins [Drosophila Genome Annotation Database (GadFly) identifier CG2865 (ADAMS *et al.* 2000)]. No conservation with TRIP-Br proteins was detected outside of the SERTA motif. In particular, no C-ter motif was detected in CG2865, nor in any other predicted Drosophila protein. Interestingly, no SERTA-

FIGURE 3.—Sequence of the alternative *tara* mRNAs and proteins. The nucleotide sequence and deduced amino acid sequence for a putative full-length embryonic cDNA of each class (*cf.* Figure 2B) is shown. For the unique α-class cDNA, only the alternative 5′-untranslated and flanking coding sequence is reported. The position of the alternative intron is indicated by an arrowhead. The running tallies for the nucleotides and aa sequences are shown on the right. Putative initiator ATGs are underlined, as are the four evolutionarily conserved motifs. The GenBank accession numbers for the C12 and C16 *tara* cDNA sequences are AF227211 and AF227212, respectively.

TABLE 3

Rescue of *tara*^{LA} lethality with *P*[UbTARA-β] constructs

Genotype	Viability	Fertility[a]	Adult phenotype
w^{1118}; *P*[UbTARA-β]#2, 3 or 5/+	+	+	Wild type
w^{1118}; $tara^{LA}/tara^{LA}$	−	NA	NA
w^{1118}; *P*[UbTARA-β]#2/+; $tara^{LA}/tara^{LA}$	+	+/−	Held-out wings (100%)
w^{1118}; *P*[UbTARA-β]#3/+; $tara^{LA}/tara^{LA}$	+	+/−	Held-out wings (100%)
w^{1118}; *P*[UbTARA-β]#4/+; $tara^{LA}/tara^{LA}$	+	+/−	Held-out wings (100%)
w^{1118}; *P*[UbTARA-β]#5/+; $tara^{LA}/tara^{LA}$	+	+/−	Held-out wings (100%)
w^{1118}; *P*[UbTARA-β]#3/*P*[UbTARA-β]#3; $tara^{LA}/tara^{LA}$	+	+	Held-out wings (30%)

NA, not applicable.
[a] Fertility of rescued males and females was qualitatively tested by crosses with w^{1118} flies.

containing proteins were discernible in the yeast or *Caenorhabditis elegans* genomes, suggesting that functional roles for TRIP-Br proteins may be restricted to higher eukaryotes. Taken together, these data support the notion that *tara* encodes the Drosophila homologs of the mammalian TRIP-Br proteins. The implications of this observation, in particular for the epigenetic maintenance of active chromosomal states with regard to the cell cycle, are discussed below.

DISCUSSION

We have identified a novel member of the trxG family, *taranis*, based notably on the dose-sensitive effects of reduced $tara^{+}$ function (i) in suppression of the extra-sex-combs phenotype resulting from mutations in the PcG genes *Polycomb* and *polyhomeotic* and (ii) in enhancement of a specific wing phenotype in combination with mutations of the trxG genes *trithorax*, *brahma*, and *osa*. Further, the developmental transformations in incompletely rescued *tara* mutants resembling homeotic phenotypes (Figure 1), and genetic interactions with a HOM gene (here, *proboscipedia*), correspond to often-encountered properties of trxG members.

As for most other trxG genes, *tara* is required for viability. Reduced *tara* activity during larval/pupal development leads to a wings-held-out phenotype resembling certain mutations of *Antp*. One potential function of *tara*, as for *brm* and *osa*, is thus to ensure proper transcriptional expression of *Antp* in cells of developing wing imaginal discs. In support of this possibility, a genetic interaction was detected with certain *Antp* gain-of-function alleles, which was associated with detectably reduced accumulation of ANTP protein (data not shown). However, the wing vein defects and diminished fertility observed on reducing normal function indicate that *tara* is in all likelihood also required for the expression of other target genes in addition to homeotic genes. Zygotic lethality was not accompanied by a detected cuticular phenotype for the alleles used in this study. The isolation of a null mutation of *tara* may reveal additional roles in embryonic and adult development.

Several trxG proteins function as chromatin modifiers: The PcG and trxG genes are believed to encode proteins that play out a direct functional antagonism at the level of nucleosomal and higher-order chromatin structures. Most PcG members encode chromosomal proteins belonging to common complexes that repress gene transcription, although the mechanism of this repression remains poorly understood (for a recent review, see Francis and Kingston 2001). The recent purification and analysis of one such complex, PRC1, containing PC and PH proteins, suggested that PRC1 and SWI/SNF complexes might compete for the nucleosomal template *in vivo* (Shao *et al.* 1999). However, only 4 of the 17 cloned trxG genes (BRM, MOR, SNR1, and OSA) correspond to dSWI/SNF subunits (Papoulas *et al.* 1998; Collins *et al.* 1999; Crosby *et al.* 1999; Kal *et al.* 2000). Hence, most trxG proteins are likely to function via other mechanisms. Among these, ASH1, ASH2, and possibly KIS, have been shown to belong to multiprotein chromatin-remodeling complexes distinct from the BRM complex and of unknown functions (Papoulas *et al.* 1998; Daubresse *et al.* 1999).

A major issue in the field is how the assembly and activity of these diverse chromatin-modifying complexes are regulated to control transcription of specific target genes in a mitotically stable manner (for a recent review, see Farkas *et al.* 2000). Several Drosophila trxG proteins that directly interact with regulatory DNA sequences (TRX, GAGA/TRL, and ZESTE) are thought to recruit those complexes to overcome the repressive effects of nucleosomal histones and higher-order chromatin organization (Rozovskaia *et al.* 1999; Kal *et al.* 2000). GAGA/TRL is of particular interest among the trxG proteins since, contrary to most transcription factors (see Martinez-Balbas *et al.* 1995), it remains associated with chromosomes during mitosis (Raff *et al.* 1994; O'Brien *et al.* 1995). Characterization of other trxG proteins should help to better understand how the activities of the diverse chromatin-modifying machines are regulated throughout development.

A role for TARA family proteins in linking chromatin-remodeling complexes to cell cycle regulators? The *tara*

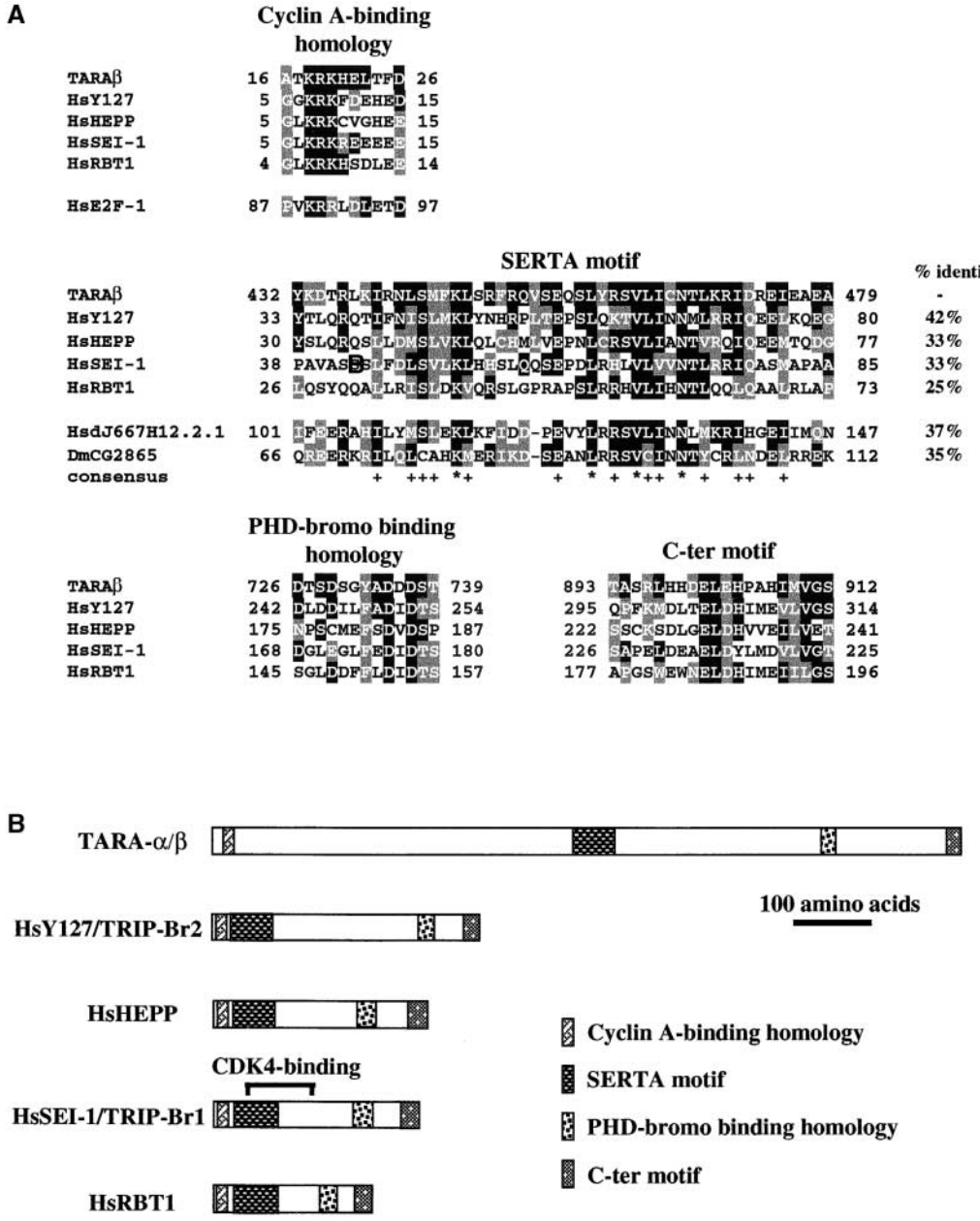

FIGURE 5.—Constitution of a family of TARA-related proteins. (A) The sequences of the conserved portions of TARA-β are shown aligned with homologous motifs from the related human TRIP-Br proteins, human transcription factor E2F-1, as well as predicted human (Hs) and Drosophila (Dm) proteins. Numbers flanking the amino acid sequences indicate positions in the complete protein sequences. Shown on a solid or shaded background are amino acids that are identical or similar, respectively, to TARA proteins. The following amino acids were treated as similar: R/K/H; S/T; E/D/Q/N; A/G/P; and I/L/V/M/C/F/Y/W. For the Cyclin A-binding homology motif the equivalent region of E2F-1 is shown below. For the SERTA motif homologous regions from the predicted proteins HsdJ667H12.2.1 and DmCG2865 (without discernible Cyclin A-binding, PHD-bromo-binding, or C-ter motifs) are shown below with consensus positions displayed farther below. In these positions, similar or identical amino acids were found in at least six of the seven aligned sequences. Note that only four positions were identical in all nine SERTA motifs compared (marked by an asterisk). The percentage of amino acid identity with respect to the TARA-α/-β SERTA motif is indicated on the right. The GenBank accession numbers are hsY127/TRIP-Br2 (BAA09476); HsSEI-1/TRIP-Br1 (AAF08349); HsRBT1 (AAF05761); HsHEPP (AAK31075); HsdJ667H12.2.1 (CAB81635); DmCG2865 (AAF45770); and HsE2F-1 (Q01094). (B) Comparison of the motif structure of TARA-α/-β and human TRIP-Br proteins. For each alignment the overlying box refers to the corresponding motif in A. Note the equivalent order of the four conserved motifs in TARA and the mammalian TRIP-Br proteins. The minimal region of TRIP-Br1/SEI-1 required for interaction with CDK4 is indicated by brackets (aa 44–161).

gene structure suggests a possible molecular mechanism of action. Our molecular analysis of the twin TARA proteins revealed significant homologies with members of a novel family of mammalian proteins including human p34^{SEI-1}/TRIP-Br1 (Figure 5), a potent transcriptional activator reported to regulate the cyclin D1-CDK4 pair implicated in control of the G1 phase of the cell cycle (SUGIMOTO *et al.* 1999; HSU *et al.* 2001). p34^{SEI-1}/TRIP-Br1 has been reported to favor cyclin D1-CDK4 complex formation by antagonizing the inhibitory action of the tumor suppressor p16^{INK4a}. The positive effect of p34^{SEI-1}/TRIP-Br1 on the assembly and activation of cyclin D1-CDK4 complex is thought to involve a specific interaction of TRIP-Br1 with the kinase domain of CDK4 (SUGIMOTO *et al.* 1999). Hence, it may be significant that the CDK4-interacting segment of p34^{SEI-1} (amino acid residues 44–161) includes most of the SERTA motif (see Figure 5A).

Taken together, these data suggest that TARA-α/-β proteins might participate in a cell memory process that couples chromatin structure to cell cycle progression. The observed detrimental consequences of under- and

overexpression indicate a probable stoichiometric role for *tara*. Mitotic recombination experiments employing the strong hypomorphic $tara^{LA}$ allele indicate that $tara^{+}$ is required for viability and/or proliferation of imaginal cells, since clones of mutant cells were strongly reduced compared to reference wild-type clones (data not shown). Conversely, the reduction or loss of adult structures noted in overexpression experiments suggests that TARA protein quantity must be finely weighed in normal development of most examined adult external tissues (not shown). Apart from CDK4, other CDKs identified in Drosophila include CDK1/CDC2 and CDK2 (the catalytic partners of cyclins A and E, respectively), as well as several other CDKs for which no function has yet been assigned (Sauer *et al.* 1996; Follette and O'Farrell 1997). Interestingly, the wing vein defects observed for viable *tara* alleles resemble those observed with hypomorphic alleles of *cyclin E* (Duronio *et al.* 1998), raising the possibility of a shared molecular role that it will be of interest to examine.

Some hints emerge from recent results of molecular and genetic interaction screens. CDKs are thought to initiate and coordinate cell division processes by sequentially phosphorylating key protein targets (Dynlacht 1997). Three potential targets are human trxG homologs of the dSWI/SNF proteins BRM and MOIRA. Physical and functional interactions detected between components of the human SWI/SNF complex (hSWI/SNF) and Cyclin E (Shanahan *et al.* 1999), and the phosphorylation of these proteins detected during mitosis (Sif *et al.* 1998), may be relevant to a cyclin-mediated role in modulating hSWI/SNF chromatin-remodeling activity. A potential link between TARA and the cell cycle machinery is also suggested by the ability of related mammalian TRIP-Br proteins to interact *in vitro* with Cyclin A (Hsu *et al.* 2001). The Cyclin A-interacting domain of TRIP-Br1 is conserved among all TRIP-Br family members including TARA (Figure 5). Hence, one prediction is that the TRIP-Br proteins should serve as specific substrates for phosphorylation by one or several CDK complex(es).

Additional connections have been established between chromatin-remodeling complexes and E2F proteins, key transcription factors coupling the transcriptional program to cell cycle progression (Harbour and Dean 2000) and to the retinoblastoma tumor-suppressor protein pRB, itself a target of Cyclin-D1 complexes (Dyson 1998). Recent genetic interaction screens in Drosophila establish functional ties between dSWI/SNF, E2F, and pRB (Staehling-Hampton *et al.* 1999; Gildea *et al.* 2000). Interestingly, the TRIP-Br1 and TRIP-Br2 proteins interact *in vitro* with the E2F1 partner DP1 and stimulate transcriptional activity of the E2F1/DP1 heterodimer in a manner that is regulated by RB (Hsu *et al.* 2001). The ability of TRIP-Br proteins to specifically contact PHD and bromodomains raises the clear possibility that they might function as molecular integrators coupling the cell cycle machinery to transcriptional activity. Interestingly, the genetic screen for dominant suppressors of *polyhomeotic* that yielded $tara^{1}$ also allowed us to identify *toutatis*, a novel trxG gene encoding a PHD zinc-finger- and bromodomain-containing protein (Fauvarque *et al.* 2001). As BRM and TRX also contain a bromo and a PHD domain, respectively, it will be important to ascertain which pairs or groups of trxG molecules are capable of direct physical interaction.

In summary, these and other related data hint at regulated activities of trxG proteins, including TARA, as cells traverse the mitotic cycle. Available genetic data and structural conservation of identified functional domains of related TRIP-Br proteins noted above suggest that the TARA-α/-β proteins might intervene as integrators linking key cell cycle regulators to chromatin-remodeling complexes. The availability of cloned cyclins and CDKs and corresponding loss-of-function alleles should allow us to test for specific molecular relations of TARA proteins with CDK complexes and chromatin components in establishing and maintaining cellular "memory" of active transcriptional states.

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