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The *Drosophila* trithorax group gene *tonalli* (*tna*) interacts genetically with the Brahma remodeling complex and encodes an SP-RING finger protein

Luis Gutiérrez[1], Mario Zurita[1], James A. Kennison[2] and Martha Vázquez[1,*]

[1]Departamento de Fisiología Molecular y Genética del Desarrollo, Instituto de Biotecnología, UNAM, Cuernavaca, Morelos 62250, México
[2]Laboratory of Molecular Genetics, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD 20892, USA
*Author for correspondence (e-mail: mvazquez@ibt.unam.mx)

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SUMMARY

The trithorax group genes are required for positive regulation of homeotic gene function. The trithorax group gene *brahma* encodes a SWI2/SNF2 family ATPase that is a catalytic subunit of the Brm chromatin-remodeling complex. We identified the *tonalli* (*tna*) gene in *Drosophila* by genetic interactions with *brahma*. *tna* mutations suppress *Polycomb* phenotypes and *tna* is required for the proper expressions of the *Antennapedia*, *Ultrabithorax* and *Sex combs reduced* homeotic genes. The *tna* gene encodes at least two proteins, a large isoform (TnaA) and a short isoform (TnaB). The TnaA protein has an SP-RING Zn finger, conserved in proteins from organisms ranging from yeast to human and thought to be involved in the sumoylation of protein substrates. Besides the SP-RING finger, the TnaA protein also has extended homology with other eukaryotic proteins, including human proteins. We show that *tna* mutations also interact with mutations in additional subunits of the Brm complex, with mutations in subunits of the Mediator complex, and with mutations of the SWI2/SNF2 family ATPase gene *kismet*. We propose that Tna is involved in postranslational modification of transcription complexes.

Key words: Homeotic gene regulation, *brahma*, Trithorax group, Sumoylation, Chromatin remodeling, SWI/SNF, *taranis*, *tonalli*, *Drosophila melanogaster*

INTRODUCTION

The trithorax and Polycomb group genes encode positive and negative factors required for the maintenance of homeotic gene expression (Francis and Kingston, 2001; Gellon and McGinnis, 1998; Kennison, 1995; Simon and Tamkun, 2002). Kennison and Tamkun (Kennison and Tamkun, 1988) first identified *brahma* (*brm)* as a trithorax group gene required for the maintenance of homeotic gene expression, but *brm* also regulates the expression or function of *engrailed* (Brizuela et al., 1994), *hedgehog* (Felsenfeld and Kennison, 1995), *wingless* (Collins and Treisman, 2000), and *E2F* (Staehling-Hampton et al., 1999). The Brm protein (Tamkun et al., 1992) is a SWI2/SNF2 family ATPase (Eisen et al., 1995). Brm is a subunit of a large protein complex that is a member of the SWI/SNF family of chromatin remodeling complexes (Papoulas et al., 1998). Several different mouse and human SWI/SNF complexes related to the Brm complex have been isolated and mutations of some subunits have revealed their roles in a variety of processes, including cell proliferation, differentiation, viral infection, and cancer (reviewed by Klochendler-Yeivin et al., 2002). In vitro studies show that SWI/SNF complexes can alter both nucleosome position and nucleosome conformation (reviewed by Flaus and Owen-Hughes, 2001; Vignali et al., 2000). The yeast SWI/SNF complex is recruited to nucleosomes proximal to the promoter by transcriptional activators. This recruitment leads to localized nucleosome disruption. Retention of SWI/SNF complexes on the promoter requires either the continued binding of the transcriptional activator or the presence of acetylated histones (Cosma et al., 1999; Hassan et al., 2001). These changes facilitate the transcriptional activation or repression by gene-specific DNA-binding proteins. It is likely that the effects of SWI/SNF complexes will have important effects on inter-nucleosomal interactions that could have consequences for higher-order chromatin structure (Francis and Kingston, 2001).

Several trithorax groups genes in *Drosophila* encode proteins involved in chromatin remodeling, including *moira* (Brizuela and Kennison, 1997; Crosby et al., 1999), *snr1* (Dingwall et al., 1995; Rozenblatt-Rosen et al., 1998), *osa* (Collins et al., 1999; Collins and Treisman, 2000; Treisman et al., 1997; Vázquez et al., 1999), and *kismet* (Daubresse et al., 1999; Therrien et al., 2000). The Brm, Mor, and Snr1 proteins are probably part of a core complex that is required for chromatin remodeling activity, whereas other subunits probably regulate and/or target this activity (Collins et al., 1999; Kal et al., 2000; Papoulas et al., 1998).

In addition to chromatin remodeling complexes, the initiation of transcription in eukaryotes also requires the

function of several other large protein complexes that may act to either relieve repression or allow transcriptional activators to interact with RNA polymerase and other basal transcription factors. Among these other protein complexes, the Mediator and TATA-binding protein (TBP)-associated factors (TAF)s function as coactivators by relaying transcriptional activation signals from DNA-bound activators to the basal transcription machinery. The Mediator complex is found from yeast to human and functions as an interface between activators and RNA polymerase II to transduce regulatory information from enhancers to promoters. There is also some in vitro evidence to suggest that some specific Mediator subcomplexes act as transcriptional corepressors (Balciunas et al., 1999; Song and Carlson, 1998; Sun et al., 1998). In flies, the Mediator complex has been purified and its interactions with different promoters, sequence-specific transcription factors and basal transcription machinery has been characterized to some extent (Park et al., 2001). In addition, many subunits have been identified in the *Drosophila* genomic DNA sequence by their similarity to yeast or human Mediator subunits (Boube et al., 2000; Rachez and Freedman, 2001). The TRAP230 and TRAP240 subunits of the Mediator complex are encoded by the trithorax group genes: *kohtalo* (*kto*) (Treisman, 2001) and *skuld* (*skd*) [described as *blind spot* (*bli*) (Treisman, 2001) and *poils aux pattes* (*pap*) (Boube et al., 2000) (J. W. Southworth and J. A. Kennison, unpublished results)]. *kto* and *skd* were first identified in the same genetic screen for regulators of homeotic genes as *brm* (Kennison and Tamkun, 1988).

In order to identify additional proteins that are required for the proper regulation of homeotic gene expression, we have screened for mutations that show genetic interactions with *brm* mutations in regulation of the *Antennapedia* (*Antp*) P2 promoter. We have previously described the isolation of mutations in the trithorax group gene *osa* in these screens (Vázquez et al., 1999). Here we report the isolation of mutations in two other genes, *taranis* (*tara*) and *tonalli* (*tna*). *tara* has been recently characterized as a new trithorax group gene required for homeotic gene expression (Calgaro et al., 2002; Fauvarque et al., 2001). In this work we show that *tna* is a novel trithorax group gene that is required to regulate the expression of the *Sex combs reduced* (*Scr*) and *Antp* homeotic genes. We also show that *tna* function is required at several developmental stages. The molecular characterization of two Tna protein isoforms reveals that *tna* could function in postranslational modification of chromatin-modifiers and/or transcriptional activator proteins.

MATERIALS AND METHODS

Fly strains

Flies were raised at 25°C on a yeast-sucrose-agar medium with either Nipagin or propionic acid or on a cornmeal-molasses-yeast-agar medium with Tegosept. Unless otherwise noted, all mutations and chromosome aberrations are described by Lindsley and Zimm (Lindsley and Zimm, 1992). tna^1, $tara^2$, and $tara^{20}$ are EMS-induced mutations recovered on the basis of the wings-out phenotype when transheterozygous to brm^2 (Vázquez et al., 1999). $tara^{03881}$ is a P-element insertion allele. tna^2 [*P{PZ}l(3)rI075*r^{I075}], tna^3 [*P{lacW}l(3)s*0583/02] and tna^4 [*P{lacW}l(3)rI075*^{L6731}] are P-element insertion alleles, that are lethal in combination with tna^1. The EP(3)0374 is a tna^+ line kindly provided by P. Rorth (Rorth et al., 1998). We will refer to *In(3R)Scr*^{Msc} simply as Scr^{Msc}.

Mutant phenotypes

The 'held-out wings' phenotype was scored if flies had both wings extended (Fig. 1A). For Pc^3, Pc^4, and Scr^{Msc}, the penetrance of the homeotic transformation was measured by the presence of ectopic sex comb teeth on the second and third legs of adult males. The expressivity of the homeotic transformation was determined by counting the number of ectopic sex comb teeth on the second and third legs and comparing it to control first legs, which have an average of 10.8 sex comb teeth per leg (Kennison and Russell, 1987). Wing extension, transformation of haltere to wing (Fig. 1B), and reductions in the numbers of sex comb teeth on the male first legs (Fig. 1C) were used to evaluate *Antp*, *Ultrabithorax* (*Ubx*) and *Scr* expressions, respectively.

Lethality of individuals carrying homozygous or heteroallelic combinations of *tna* alleles was determined by counting the Tb^+ progeny from crosses between *tna* alleles balanced with *TM6B, Hu e Tb*.

Isolation of DNA from the *tna* genomic region

We identified three P-element insertion strains (tna^2, tna^3 and tna^4) that failed to complement tna^1 for viability. The insertion sites of these three P elements were mapped in contig Dm3049 (Adams et al., 2000) located in the 67F1-68A1 region. To isolate genomic DNA from the *tna* locus we carried out a standard plasmid rescue of genomic DNA adjacent to the P element from the tna^2 and tna^3 strains (Sullivan et al., 2000). Both isolates were [^{32}P]dCTP-labeled and used as probes for Southern analyses of P1 clones from the 67F1-68A1 region. After standard restriction mapping and Southern hybridization of the positive P1 clones, we carried out further restriction mapping and Southern analysis of approximately 32 kb of the chromosomal region surrounding the tna^2 and tna^3 insertion sites in the DS04626 P1 clone. Several fragments of this P1 clone were used as probes to analyze the transcripts from the *tna* genomic region and to screen cDNA libraries.

Nucleic acids analyses

To identify cDNAs representing the *tna* transcripts, we screened a cDNA library in the Uni-ZAP XR vector from 2- to 14-hour Canton-S embryos (Stratagene). Three positive clones were recovered and in vivo excised to isolate the phagemids containing the cloned insert. The largest cDNA clone (ZAP1 in Results, Fig. 3A) was sequenced to confirm its identity.

Several expressed sequenced tags (ESTs) were identified by identity searches carried out using the BLASTN and BLASTX programs (Altschul et al., 1997) as provided by the NCBI and BDGP databases. The cDNA clone LD16921 (from 0-22 h embryos) was reported with the nucleotide sequence from the 5′ and 3′ ends. With primers from these 5′ and 3′ sequences we amplified an RT-PCR fragment named PCR1 (see Fig. 3A). This fragment joins the most 5′ untranslated exon to the Tna coding exons. PCR1 was amplified with the Expand High Fidelity polymerase (Roche) according to manufacturer's instructions with poly(A)$^+$ RNA from 0-3-hour embryos, using as 5′ and 3′ 24mers primers with the sequences 5′CTGTCGCTTCTTCTTCTTCAC3′ and 5′TGCCTCCGTAACCATTTCCTGCTC3′, respectively.

Southern and northern analyses were done as previously described (Vázquez et al., 1999). Five micrograms of poly(A)$^+$ RNA from the indicated developmental stages were fractionated on a 1% agarose Mops/formaldehyde gel and transferred to a Hybond™ N$^+$ nylon membrane (Amersham). RNA blots were probed with purified DNA fragments labeled with [^{32}P]dCTP by the random primer method (Prime-It II kit from Stratagene) and washed under conditions of high stringency (0.1× SSC, 0.1% sodium dodecyl sulfate, at 65°C).

We searched for *tna*-related proteins in the human genome using

the http://www.ensembl.org/Homo_sapiens/ and Online Mendelian Inheritance in Man™ (McKusick, 2000) databases.

To identify the molecular lesion in the *tna*^{1} mutant allele, we purified genomic DNA from individuals with the genotypes *tna*^{1} *red e/Df(3L)vin2* or *tna*$^{+}$ *red e/Df(3L)vin2*. *Df(3L)vin2* is a chromosomal deletion that lacks the entire *tna* gene. The *tna* coding region was PCR amplified with Expand High Fidelity polymerase (Roche) using as 5′ and 3′ primers oligonucleotides with the sequences 5′ATGAACCAGCAGGCGGGCTCCTCAAGGGCG3′ and 5′CTAGTCGAATAACGTGGCCAGCAAGTCGT3′, respectively. These primers amplify a 4.4 kb fragment with the entire *tna* open reading frame. One fragment from *tna*^{1} and *tna*$^{+}$ (the wild-type chromosome in which the *tna*^{1} mutation was induced) was sequenced in both strands and the sequences were compared. To verify the identity of the *tna*^{1} mutation, a 578 bp fragment that includes the exon 5 genomic DNA (Fig. 4A) was amplified from five *tna*^{1}*/Df(3L)vin2* individuals using a 5′ oligonucleotide with sequence from the end of exon 4 and a 3′ oligonucleotide from the beginning of exon 6 as amplification primers. The sequences of these 5′ and 3′ primers are 5′GCTATGGTGGAGTCGGAGGAG3′ and 5′ATTCGTCGGAGACGGTGACGGTATG3′, respectively. All five independently amplified 578 bp fragments contained the substitution of a cytosine for a thymidine that changes the glutamine codon at position 566 to a stop codon (Fig. 4C).

Germline clones

Germline mosaics were generated using the dominant female-sterile technique (Chou et al., 1993). *tna*^{1}, *tna*^{2} and *tna*^{3} heterozygous females were mated to *w; P[w*$^{+}$*, ovo*^{D1}*]*^{2X48}*/TM3, Sb* males and the progeny irradiated during the first larval instar (24-48 hours after egg laying) with 1000 rads of X-rays. Female offspring of the genotypes *+/w; tna*^{1} *red e/P[w*$^{+}$*, ovo*^{D1}*]*^{2X48}*, +/w; tna*^{2} */P[w*$^{+}$*, ovo*^{D1}*]*^{2X48} or *+/w; tna*^{3} */P[w*$^{+}$*, ovo*^{D1}*]*^{2X48} were crossed to males heterozygous for a *tna*$^{-}$ deficiency (*y w; Df(3L)lxd6/TM6B, Hu e Tb Dr*). Individuals produced by a female bearing a germ-line clone were dissected, mounted and examined under the light microscope.

RESULTS

tonalli and *taranis* enhance *brahma* mutant phenotypes

Flies heterozygous for some combinations of mutations in trithorax group genes have a held-out wings phenotype (Fig. 1A) that results from reduced expression of the *Antp* P2 promoter (Vázquez et al., 1999). On the basis of this phenotype we isolated several dominant enhancers of *brm*. Two of the new mutations are alleles of the trithorax group gene *taranis* (*tara*) (Fauvarque et al., 2001). These mutations, *tara*^{2} and *tara*^{20}, show genetic interactions with multiple alleles of *brm*. In addition, we isolated one mutation in a novel gene that we named *tonalli* (*tna*). *tonalli* is ‘fate’ in náhuatl, an indigenous mexican language. We mapped *tna*^{1} to polytene chromosome bands 67F3-4. Analyzing the available collection of P-element insertion lines from the BDGP we identified three P-element insertion strains [*P{PZ}l(3)rI075r*^{I075}, *P{lacW}l(3)*s0583/02, and *P{lacW}l(3)rI075*^{L6731}] that failed to complement *tna*^{1}. We will refer to these P-insertion mutations as *tna*^{2}, *tna*^{3} and *tna*^{4}, respectively.

tna is a trithorax group gene

The *Antp* gene has two alternative promoters, P1 and P2. The *Antp*^{Ns} allele derepresses the *Antp* P2 promoter in the eye-antennal disc and expresses wild-type *Antp* transcripts from the *Antp* promoter (Jorgensen and Garber, 1987; Talbert and Garber, 1994).

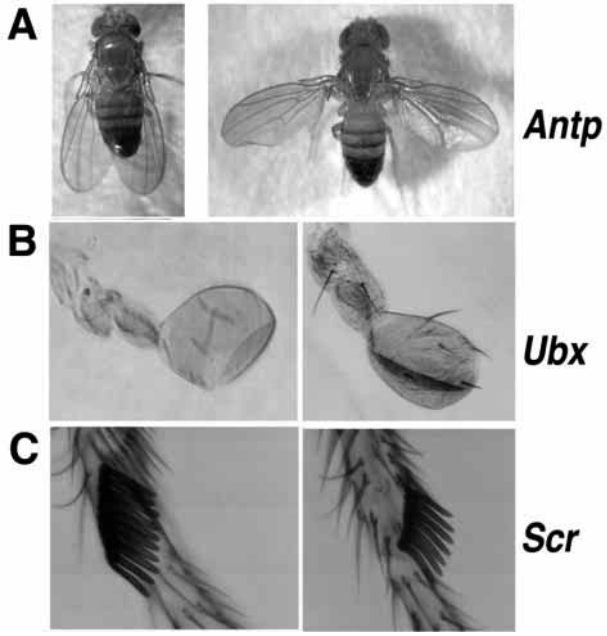


Fig. 1. *tna* mutant phenotypes mimic homeotic loss-of-function phenotypes. In all panels wild type is on the left and the mutant is on the right (A) The held-out wings phenotype of a *tna*^{1}/*brm*^{2} double heterozygote indicative of loss of *Antp* P2 function. (B) The partial transformation of haltere to wing in a *tna*^{1}/*tna*^{4} mutant fly indicative of loss of *Ubx* function. (C) The reduction in the number of sex comb teeth on the first leg of a *tna*^{2}/*tna*^{4} mutant male indicative of loss of *Scr* function.

Derepression of the *Scr* gene causes the appearance of extra sex combs on the second and third legs of males. This derepression can be caused by gain-of-function alleles of *Scr*, such as *Scr*^{Msc} (reviewed by Southworth and Kennison, 2002), or by loss-of-function mutations in Polycomb group genes, such as *Pc*^{3} or *Pc*^{4}.

Several trithorax group genes (including *brm*, *mor*, *osa*, *kis*, *skd* and *kto*) were first identified as suppressors of the extra sex combs phenotype caused by derepression of *Scr* or as suppressors of the antenna to leg transformation caused by derepression of *Antp* in the *Nasobemia* (*Ns*) allele of *Antp* and (Kennison and Tamkun, 1988). Since we identified the *tna* gene on the basis of genetic interactions with *brm*, we first tested whether *tna* mutations could also suppress these two homeotic derepression phenotypes. We found that all *tna* mutations strongly suppress the extra sex combs phenotype caused by *Pc*^{3}, *Pc*^{4} or *Scr*^{Msc} (Table 1), but only weakly suppress the antenna to leg transformation caused by the *Antp*^{Ns} mutation (Table 2).

We also analyzed other *Antp* alleles affecting the expression from the P2 promoter. We have shown through this approach, that the P2 promoter expression is sensitive to *brm* and *osa* dosages (Vázquez et al., 1999). For example, *brm* and *osa* alleles enhance the held-out wings phenotypes caused by mutations affecting the *Antp cis* region located between the breakpoints of *In(3R)Antp*^{B} and *In(3R)Antp*^{R} aberrations. We tested for genetic interactions with all of the chromosome

Table 1. Effects of *tna* mutations on *Scr* homeotic derepression phenotypes induced by *Polycomb* and *Scr* mutations

Genotype	Transformed flies/total* (%)	Expressivity† (%)
$+/Pc^4$	37/40 (93)	14
tna^1/Pc^4	8/20 (40)	4
$+/Pc^3$	20/20 (100)	52
tna^1/Pc^3	16/20 (80)	6
tna^2/Pc^3	20/20 (100)	28
tna^3/Pc^3	20/20 (100)	30
tna^4/Pc^3	19/20 (100)	17
$tna^{-‡}/Pc^3$	10/20 (50)	2
$+/Scr^{Msc}$	20/20 (100)	23
tna^1/Scr^{Msc}	18/20 (90)	8
tna^2/Scr^{Msc}	19/20 (95)	7
tna^3/Scr^{Msc}	16/17 (94)	9
tna^4/Scr^{Msc}	19/20 (95)	6
tna^-/Scr^{Msc}	0/20 (0)	0

*Numbers of male individuals showing sex comb teeth in the second and/or third leg.

†Expressivity was determined by counting the number of ectopic sex comb teeth on the second and third legs and comparing to control first legs with an average of 10.8 sex comb teeth each (100%).

‡*tna*$^-$ is *Df(3L)vin2*, a chromosomal deficiency for 67F2 to 68D6 that deletes the entire *tna* gene.

Table 2. Genetic interactions of *tna*^{1} with *Antp* chromosomal aberrations that alter *Antp* P2 promoter function

Genotype	$Antp^{Ns}$*	$Antp^{73b}$*	Flies with held-out wings/Total
+/+	236/249 (95)	253/253 (100)	–
$tna^1/+$	172/205 (84)	258/258 (100)	–
$tna^2/+$	41/47 (87)	53/53 (100)	–
$tna^-/+$†	13/75 (17)	46/46 (100)	–
$In(3R)Antp^B/+$	–	–	5/62 (8)
$tna^1/In(3R)Antp^B$	–	–	55/73 (75)
$In(3R)Antp^R/+$	–	–	0/117 (0)
$tna^1/In(3R)Antp^R$	–	–	84/130 (65)

*Numbers of individuals showing antenna to leg transformation divided by the total numbers of flies examined. The percentages are given after in parentheses.

†*tna*$^-$ refers to *Df(3L)vin2*, a large deletion that removes the entire *tna* gene.

Table 3. Genetic interactions of *tna* and *tara* with some trithorax group genes

Genotype	Numbers of flies with held-out wings/Total	% Penetrance
$+/tna^1$	19/115	17
$+/tna^3$	0/120	0
$+/tara^2$	0/129	0
$+/tara^{20}$	0/151	0
$+/tara^{03881}$	9/184	5
$brm^2/+$	9/498	2
brm^2/tna^1	43/43	100
brm^2/tna^3	0/100	0
$brm^2/tara^2$	7/31	23
$brm^2/tna^3\ tara^2$	12/20	60
$brm^2/tara^{20}$	3/37	8
$brm^2/tna^3\ tara^{20}$	40/53	75
$brm^2/tara^{03881}$	1/165	<1
$brm^2/tna^3\ tara^{03881}$	21/71	30
$osa^1/+$	6/208	3
osa^1/brm^2	100/100	100
osa^1/tna^1	35/35	100
osa^1/tna^3	0/69	0
$osa^1/tara^2$	41/57	72
$osa^1/tara^{20}$	36/48	75
$osa^1/tara^{03881}$	33/105	31
$osa^1/tna^3\ tara^{03881}$	22/59	37
$+/tna^3\ tara^{03881}$	0/137	0
$tna^1/tara^2$	36/36	100
$tna^1/tara^{20}$	27/31	87
$tna^1/tara^{03881}$	106/123	80
mor^1/tna^1	82/139	59
mor^2/tna^1	97/123	79
$snr1^{0319}/tna^1$	41/120	34
kis^{13416}/tna^1	67/108	62
kis^1/tna^1	75/137	55
skd^2/tna^1	74/103	72
skd^{L7062}/tna^1	135/175	77
skd^{rk760}/tna^1	107/219	49
kto^1/tna^1	41/120	34
$Trap80^{s2956}/tna^1$	41/152	27

For mor^1, mor^2, $snr1^{0319}$, kis^{13416}, kis^1, skd^2, skd^{L7062}, skd^{rk760}, kto^1 or $Trap80^{s2956}$ data from the controls (the same genotypes as in the table but lacking the tna^1 mutation) were not included, since no flies with the held-out phenotype were observed. At least 100 flies were examined for each control genotype.

aberrations with breakpoints between the *Antp* P1 and P2 promoters that were previously used to test for interactions with *brm* and *osa* mutations (Vázquez et al., 1999). We found that tna^1 (but not the P element *tna* alleles) enhances the held-out wings phenotype when in combination with $In(3R)Antp^B$ and $In(3R)Antp^R$ lines (Table 2). We did not observe interactions with any of the other aberrations (data not shown). Thus, we conclude that as with *brm* and *osa*, there is a *tna*-sensitive region mapping between the 5′ breakpoints of $In(3R)Antp^B$ and $In(3R)Antp^R$.

tna, *tara*, *brm* and *osa* interact genetically

Since we isolated *tna* and *tara* mutations because they enhance the held-out wings phenotype of *brm*, we also looked for genetic interactions with *osa*, which is also required for *Antp* P2 function (Vázquez et al., 1999). We tested the EMS-induced alleles, tna^1, $tara^2$ and $tara^{20}$, and the P-element insertion alleles, tna^3 and $tara^{03881}$, for genetic interactions with *brm*, *osa*, and with each other (Table 3). We found that all three EMS-induced alleles interact strongly with brm^2, but that the two P-element insertion alleles do not show strong genetic interactions. The P-element insertion alleles do show genetic interactions with brm^2 in flies heterozygous for mutations in all three genes (*brm*, *tna* and *tara*). These results suggest that the P-insertion alleles are weaker than the EMS-induced alleles. We observed similar results previously with *brm* and *osa* (Vázquez et al., 1999). It is possible that the P-insertion mutations are not null alleles, but it is also possible that the EMS-induced alleles make mutant proteins that behave as dominant-negative mutations, still binding to interacting protein complexes and competing for binding of the wild-type alleles. All of the *tna* and *tara* mutations (except the P-insertion allele tna^3) show strong genetic interactions with osa^1 (Table 3). All three *tara* alleles interact strongly with tna^1, with $tara^2$ showing the strongest interactions.

tna interacts genetically with mutations in subunits of the Brm complex, the Mediator coactivator complex and with the Kismet SWI2/SNF2 family ATPase

Several members of the trithorax group proteins are subunits of chromatin remodeling or coactivator complexes. The Brm protein is the SWI2/SNF2-family ATPase subunit of the Brm chromatin remodeling complex (Tamkun et al., 1992). The trithorax group genes *mor*, *osa* and *snr1* encode other subunits of the Brm complex (Brizuela and Kennison, 1997; Collins et al., 1999; Collins and Treisman, 2000; Crosby et al., 1999; Dingwall et al., 1995; Kal et al., 2000; Papoulas et al., 1998; Rozenblatt-Rosen et al., 1998; Treisman et al., 1997; Vázquez et al., 1999). The *kismet* (*kis*) gene encodes another trithorax group SWI2/SNF2-family member and is probably the ATPase subunit of a different chromatin remodeling complex (Daubresse et al., 1999; Therrien et al., 2000). It is thought that chromatin remodeling complexes may interact physically with the basal transcription machinery, with transcriptional coactivators or corepressors, or with proteins involved in histone modification, such as acetyl-transferases and deacetylases. One of the transcriptional coactivator complexes with which chromatin remodeling complexes might interact is the Mediator complex (Rachez and Freedman, 2001). The *kohtalo* (*kto*), *skuld* (*skd*), and *Trap80* trithorax group genes encode subunits of the Mediator coactivator complex (Kennison and Tamkun, 1988; Boube et al., 2000; Treisman, 2001) (J. W. Southworth and J. A. K., unpublished results).

We tested whether *tna* mutations could genetically interact with mutations in the trithorax group genes encoding subunits of the Brm or Kis chromatin remodeling complexes or the Mediator coactivator complex to give the same held-out wings phenotype that we observed in the *brm*/+; *osa*/+ transheterozygous combinations (Vázquez et al., 1999). We also looked for genetic interactions between *tna* and several other trithorax group mutations that probably do not encode subunits of the Brm, Kis or Mediator complexes. The results are shown in Table 3. We found that tna^{1} shows strong genetic interactions with some mutations in the Brm complex (brm^{2}, osa^{1}, mor^{1} and mor^{2}), with *kis* mutations (kis^{1} and kis^{13416}), and with some mutations in the Mediator complex (skd^{2}, skd^{lL7062} and skd^{rk760}). There were no strong interactions with the $snr1^{0319}$ mutation in the Brm complex or the kto^{1} and $Trap80^{s2956}$ mutations in the Mediator complex. We also observed no strong genetic interactions with $ash2^{1}$, trx^{1}, trx^{00347}, urd^{2} or sls^{1} trithorax group mutations (data not shown).

The zygotic and maternal functions of *tna*

Transheterozygous combinations among tna^{1}, tna^{2}, tna^{3} and tna^{4} alleles result in death at the third instar larval, pupal or pharate adult stages. Heteroallelic pharate individuals (dissected from the pupal cases) present transformations typical of loss-of-function of the Antennapedia and Bithorax complex homeotic genes (Table 4). In some cases, we observed partial haltere to wing transformation that results from loss of function for the *Ultrabithorax* (*Ubx*) homeotic gene (Fig. 1B). In 100% of the male flies we observed a strong reduction in the number of bristles in the male sex comb (the sex comb teeth) (Fig. 1C). This is the phenotype observed in partial loss of function in the *Scr* homeotic gene. Thus, we found that the

Table 4. Homeotic transformations in pharate adult *tna* mutants

Genotype	Number of flies with transformed tissue/Total (%)	
	First leg* (*Scr*)	Haltere† (*Ubx*)
tna^{1}/tna^{2}	33/34 (97)	33/74 (45)
tna^{1}/tna^{3}	33/40 (83)	36/80 (45)
tna^{1}/tna^{4}	36/36 (100)	47/74 (64)
tna^{2}/tna^{3}	24/24 (100)	0/24 (0)
tna^{3}/tna^{4}	30/30 (100)	3/64 (5)
tna^{2}/tna^{4}	40/40 (100)	1/80 (1)

*Transformed tissue in the first leg measured by the reduction in numbers of sex combs teeth. A wild-type first leg has an average of 10.8 sex comb teeth, while most of the *tna* mutant males had only 5-7 sex comb teeth per leg.

†Transformed wing tissue in the haltere.

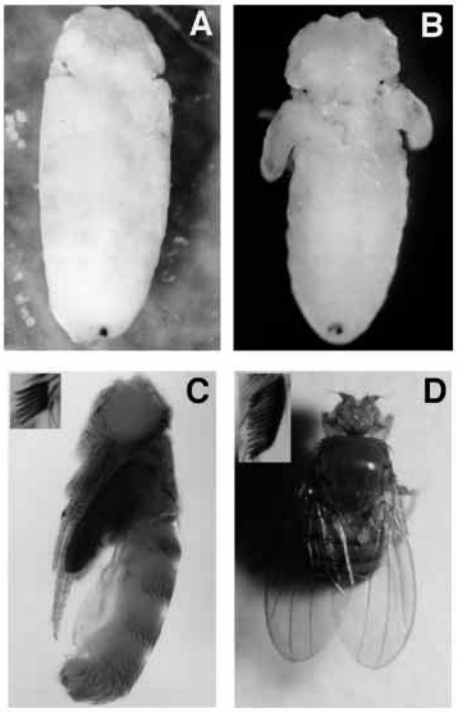


Fig. 2. Phenotypes from maternal loss of *tna* function. Prepupa (A) and young pupa (B) that lack both maternal and zygotic *tna* functions. These individuals are $tna^{1}/Df(3L)lxd6$ [tna^{-}] and very few individuals reach the pharate adult stage without paternal rescue. One of the males that reached the pharate adult stage is shown in C. The first leg has a smaller sex comb with fewer sex comb teeth (inset in C) suggesting a reduction in *Scr* function. (D) A tna^{1}/+ male that lacked maternal Tna function but was rescued by the paternally-inherited wild-type allele. The paternally rescued males have sex combs with normal numbers of sex comb teeth (inset in D).

tna zygotic function is required for proper expression of at least three homeotic genes, *Antp*, *Ubx*, and *Scr*.

At least 50% of the *tna* mutant transheterozygotes (and 85% for some heteroallelic combinations) reach the pupa stage. This late stage of lethality suggests that maternal *tna* function might be sufficient for early development. To determine if this is so we generated homozygous germ cells for the tna^{1}, tna^{2} and tna^{3} alleles. We used mitotic recombination and a transgene

carrying the dominant female-sterile mutation *ovo*^{D1} (Chou et al., 1993) to produce embryos that lacked wild-type maternal *tna* functions. The same results were obtained with all three *tna* alleles and individuals representative of this experiment are shown in Fig. 2. When both maternal and zygotic *tna* functions are lacking, most individuals die as third instar larvae. For *tna*^{1}, a few mutant individuals reach late developmental stages (Fig. 2A-C) if they lack both maternal and zygotic *tna* functions. These pharate individuals have fewer sex comb teeth in the male first legs (Fig. 2C) and show a haltere to wing transformation. In contrast, if only the zygotic function is lacking (and the maternal function is normal), the *tna* mutants die, predominantly as pupae. Individuals with no maternal *tna* can be completely rescued by a wild-type allele inherited from the father, giving rise to normal (and fertile) adults (Fig. 2D). Thus, we can conclude from these experiments that there is maternal *tna* contribution but the zygotic function is sufficient to reach late developmental stages, at least with the three alleles that we have tested.

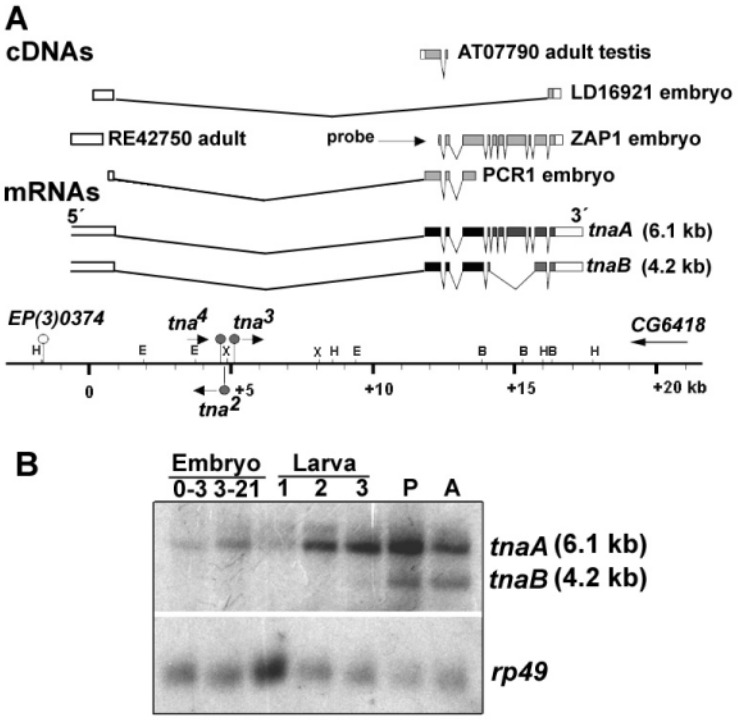


Fig. 3. Molecular organization and developmental expression of the *tna locus*. (A) The *tna* genomic region is represented at the bottom of the panel. The *tna*$^{-}$ P-element insertion sites are indicated by gray circles. The arrows by the insertions represent the orientation of the respective P-element with respect to the *tna* transcription direction. The *tna*$^{+}$ EP0374 insertion site is shown as a white circle. The restriction sites are: B, *Bam*HI; X, *Xba*I; E, *Eco*RI; H, *Hin*dIII. CG6418 is an RNA helicase transcribed towards the 3′ end of the *tna* locus. The transcripts (mRNAs) are depicted in the middle of the panel. The BDGP, release 2-predicted transcripts containing the translated exons (black rectangles for shared, grey rectangles for the non-shared exons between *tnaA* and *tnaB*) are shown. We have added the 5′ untranslated exon and the 3′ poly(A)$^{+}$ regions (white rectangles) deduced from our analysis of the locus. The 5′ UTR exon is open on the left to indicate that the *tna* transcription initiation start site has not been determined. The indicated sizes of both transcripts are in agreement with the northern analysis shown in B. The upper part A (cDNAs) shows representative cDNAs isolated from the *tna* locus. AT07790 is one of several ESTs identified in adult testis. RE42750 is an EST from adult heads. The ZAP1 embryo cDNA clone was isolated from the UNI-ZAP library from 0-12-hour embryos (see Material and Methods) and was the probe for the northern blot shown in B. The PCR1 embryo cDNA clone was RT-PCR amplified with 5′ and 3′ primers sequences from the reported LD16921 embryonic clone (see Material and Methods). (B) RNA poly(A)$^{+}$ was prepared from 0-3-hour and 3-21-hour embryos (0-3 and 3-21), first, second and third instar larvae (L1, L2, L3), pupae (P) and adults (A) . Samples were blotted and run under standard conditions. The blot was probed with the ZAP1 cDNA (A). The blot was washed and rehybridized using a probe for *rp49* as a loading control. The sizes of the detected bands are indicated.

Molecular analyses of *tna*

The closest *tna*$^{+}$ insertion line, EP(3)0374 (Fig. 3A, open circle), has an EP element 6kb upstream of the *tna*$^{-}$ P-element insertion sites (Fig. 3A, full circles). Thus, at least part of the gene could be between the *tna*$^{-}$ P and *tna*$^{+}$ EP element insertion sites.

To isolate genomic DNA from the *tna* region we selected P1 clone DS04626, which includes the genomic DNA flanking the sites of the *tna*^{2}, *tna*^{3} and *tna*^{4} P-element insertions. We did a chromosomal walk from DS04626 and tested several genomic probes to identify putative *tna* transcripts (data not shown). The *tna*$^{-}$ insertion sites are within the first large intron of an annotated gene, CG7958. We will present the evidence below that CG7958 is *tna*, but we will first describe our efforts to characterize the structure and limits of the transcription unit (Fig. 3A). There is another gene (CG6418, which may encode an RNA-binding protein) about 1.8 kb downstream of the last exon of *tna*. The 3′ end of the *tna* transcription unit should be within this 1.8 kb region. Although there is an annotated gene (CG12523) about 60 kb upstream of the predicted 5′ exon of *tna*, the first predicted gene (CG6449) for which there is EST evidence is about 160 kb upstream of *tna*. Although the *tna* promoter should be somewhere within this large genomic region, we have not yet identified the transcriptional start site. There is one P-element insertion available within this large region, EP(3)0374, which is about 1.6 kb upstream of the predicted *tna* 5′ exon. This P-element insertion complements the *tna* mutations, i.e., it is *tna*$^{+}$.

Our northern analyses identified two transcripts (6.1 and 4.2 kb in size) within the *tna* region, which derive by alternative splicing (see below). To characterize the structure of these transcripts, we isolated cDNA clones from an embryonic library. ZAP1, which is the longest, is shown in Fig. 3A. We also characterized cDNA clones from the BDGP. The BDGP clone LD16921, which was isolated from a 0- to 24-hour mixed stage embryonic library, was particularly useful and is also shown in Fig. 3A. We were able to amplify several RT-PCR fragments using, as a 5′ primer, an oligonucleotide with the 5′LD16921 sequence and as 3′ primers oligonucleotides with the sequence of diverse translated *tna* exons (see Materials and Methods). One of these fragments, PCR1, is a cDNA made from poly(A)$^{+}$ RNA purified from 3-24-hour embryos. To corroborate its identity it was cloned and sequenced (Fig. 3A). There are at least two alternative untranslated 5′ exons. The 5′ exon of the embryonic LD16921 cDNA clone (and the adult cDNA

clones RE42750 and RE27454) differs from the 5′ exon found in several testis ESTs (AT07790, Fig. 3A). There is also alternative splicing within the translated exons (described in detail below).

The *tonalli* transcripts are differentially expressed during development

We performed northern blot analyses with RNA samples purified from different developmental stages using the ZAP1 cDNA clone (Fig. 3A) as a probe. This clone was isolated from a λZAP embryonic library and overlaps all of the *tna* translated exons. We found two signals (6.1 and 4.2 kb) (Fig. 3B) that correspond to major *tna* transcripts. The 6.1 kb transcript was present at all stages, but its expression increased at the second larval instar and reached its maximum in the pupal stage. The 4.2 kb transcript was first detected in third instar larvae, but it was most abundant in the pupal and adult stages.

One of the Tna protein isoforms belongs to an SP-RING Zn-finger domain family

The northern and sequence analyses of *tna* predict at least two alternative transcripts (CT41698 and CT23982 from BDGP, release 2) (Fig. 3A, mRNAs) encoding products of 1109 and 610 residues (Fig. 4A). The long form of the protein (TnaA) is translated from 10 coding exons and may have three different amino termini (CG7958-RA, -RB and -RC, BDGP, release 3). The mRNA for the short form (TnaB) lacks exons 5-8 and part of exon 9. Both proteins have similar amino termini, which have two Gln-rich regions, but they do not share the same carboxyl termini; the alternative splicing of the short form generates a frameshift that changes the open reading frame after the alternative splice (Fig. 4A). This frameshift generates a stop codon in the middle of exon 9.

Exon 7 is present only in TnaA and encodes a possible bipartite nuclear location signal and an SP-RING (Siz/PIAS-RING) (Hochstrasser, 2001) putative zinc finger (Fig. 4, see below).

Blast analyses of the TnaA protein sequence allowed us to identify four regions (Fig. 4A). Region I and IV (residues 1-494, and residues 799-1109, respectively) do not show homology to any other reported protein in any organism. Region I contains two blocks of glutamine residues.

Region III (647-798) includes the SP-RING finger (residues 718-760), which is present in several proteins from organisms ranging from yeast to human (Fig. 4B). One family of SP-RING finger proteins are the PIAS [protein inhibitor of activated STAT (signal transducer and activator of transcription)] family. One of the PIAS proteins, Miz1 (ARIP3/PIASXα) (Wu et al., 1997) has also been identified as a cofactor of homeotic gene function in mice. In the *Drosophila* genome, the only other SP-RING finger proteins are ZimpA and ZimpB (zinc finger-containing, Miz1, PIAS3-like) (Mohr and Boswell, 1999). The Zimp proteins belong to the PIAS family and are encoded by the *Su(var)2-10* locus (Hari et al., 2001). Region III also includes the putative bipartite nuclear location signal (residues 668-686, Fig. 4C).

Although there are many proteins with similarities to Regions II (residues 495-646) or III (residues 647-798), there are only a few proteins that have similarity to both. These include proteins from the mouse (EST B6863016), *Xenopus laevis* (EST BJ075201), *Gallus gallus* (EST AJ396794), *Caenorhabditis elegans* (predicted protein NM_069604), *Arabidopsis thaliana* (AB011483), and human (KIAA1224 and KIAA1886). The two human proteins (retinoic acid-induced KIAA1224, EMBL AB033050, and KIAA1886, GenBank source AL136572) are 60% identical to TnaA in a region spanning almost 300 residues (from TnaA residues 495 to 798) (Fig. 4A,C). We searched the OMIM database (McKusick, 2000) but did not find any associated diseases attributed to mutations in the KIAA1224 (10q23.2) and KIAA1886 (7p15.1) genes to date. This family of proteins differs from the PIAS family in having Region II. We believe that the 300 amino acid domain spanning both Regions II and III identifies a new signature that we have named the XSPRING (eXtended SP-RING finger) domain (Fig. 4A,C).

The TnaB form shares regions I and II with TnaA, but has a unique carboxyl terminus. It does not show any additional homology to other known or predicted proteins.

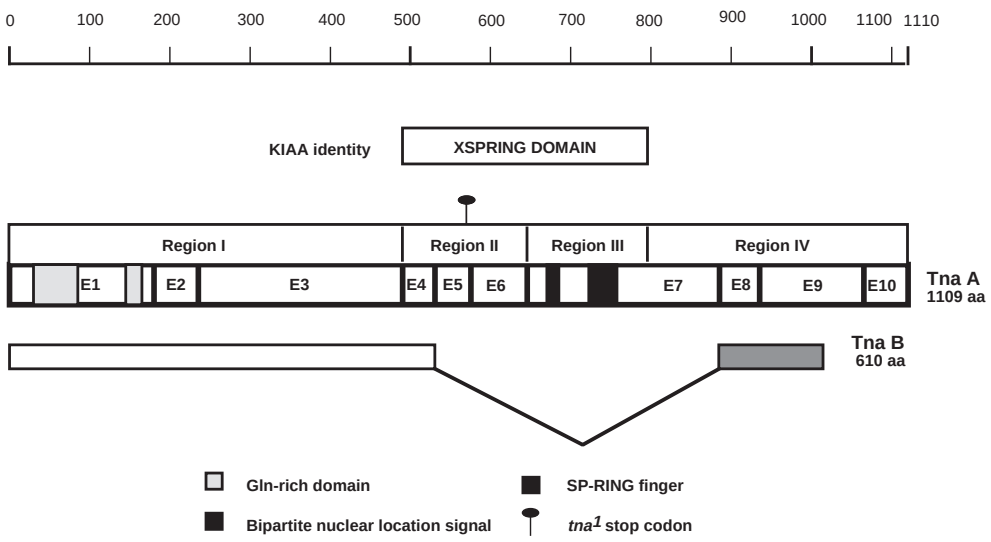
The *tna*^{1} allele carries a mutation that affects only the TnaA protein product

The *tna* locus produces at least two different proteins, TnaA and TnaB. We are interested in characterizing the functions of each one of these forms and in dissecting more accurately whether the *tna* mutant phenotypes are caused by the failure of one or both Tna proteins. Individuals with the EMS-induced *tna*^{1} allele have different phenotypes from those resulting from the P-element insertion alleles (*tna*^{2}, *tna*^{3} and *tna*^{4}). *tna*^{1} is the allele that interacts strongest with several trithorax group mutations to reduce *Antp* P2 function and cause a held-out wings phenotype (Fig. 1A, Tables 2 and 3). The *tna*^{1} allele is also the allele that shows the strongest loss-of-function *Ubx* phenotype (Fig. 1B, Table 4) when heterozygous with the P-element insertion alleles or the deletions. Thus, we characterized molecularly the nature of the mutation in *tna*^{1} (see Materials and Methods). We purified DNA from *tna*^{1}*/Df(3L)vin2* individuals that survive until third instar larvae, PCR amplified, and sequenced the *tna*^{1} genomic region. We found only one change within the entire open reading frame of the *tna*^{1} mutant chromosome, a transition (C to T) that changes glutamine 566 (Fig. 4C) to a stop codon. This change would generate a truncated product at the end of exon 5 (Fig. 4A) that will resemble the amino-terminal region of the TnaB protein without its carboxyl terminus. These data suggest that *tna*^{1} should affect only TnaA, with TnaB still functional. The truncation of the TnaA protein may be responsible for the phenotypes we observe with the *tna*^{1} allele. The fact that this truncated form resembles the amino terminus of the wild-type TnaB, together with the *tna*^{1} genetic data, leads us to suggest that TnaB cannot substitute for TnaA. As TnaB mRNA appears for the first time late in development, the role of TnaB could be to negatively modulate the TnaA function.

DISCUSSION

To study the mechanism of action of the Brm complex on different homeotic genes, we have characterized genes that interact with Brm in regulating the expression of the *Antp* P2 promoter in the imaginal wing disc. Reduced expression of the Antp P2 promoter in the imaginal wing disc causes flies to

A



B

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                718                                                760                 779
TnaA            SLKCPITKSRIRLPARGHECKHVQCFDLEAYLMINSERGSWRCPECSKSAITDTLEIDQYIWAIL
KIAA1224        SLKCPITFRRIQLPARGHDCKHVQCFDLESYLQLNCERGTWRCPVCNKTALLEGLEYDQYMWGIL
KIAA1886        SLKCPITFRRIQLPARGHDCRHIQCFDLESYLQLNCERGTWRCPVCNKTALLEGLEVDQYMLGIL
Su(var)         SLNCPLGKMKMLLPCRASTCSHLQCFDASLYLQMNERKPTWNCPVCDKPAIYDNLVIDGYFQEVL
Miz1            SLMCPLGKMRLTIPCRAVTCTHLQCFDAALYLQMNEKKPTWICPVCDKKAAYESLILDGLFMEIL
PIAS1           SLLCPLGKMRLTIPCRALTCSHLQCFDATLYIQMNEKKPTWVCPVCDKKAPYEHLIIDGLFMEIL
KCh             SLMCPLGKMRLTVPCRALTCAHLQSFDAALYLQMNEKKPTWTCPVCDKKAPYESLIIDGLFMEIL
PIAS3           SLMCPLGKMRLTVPCRALTCAHLQSFDAALYLQMNEKKPTWTCPVCDKKAPYESLIIDGLFMEIL
PIASy           SLICPLVKMRLSVPCRAETCAHLQCFDAVFYLQMNEKKPTWMCPVCDKPAPYDQLIIDGLLSKIL
CEW10D5         SLLDPLCKTRMTTPSRCQDCTHLQCFDLLSYLMMNEKKPTWQCPVCSSNCPYDRLIVDDYFLDML
VICIA           SLNCPISFTRIKTPVKGRSCKHFQCFDFDNFIKINSKRPSWRCPHCNQNVSYTEIRLDRNMIEIL
SEP-INT         SLKCPLSFSRISLPVRSVFCKHIQCFDASAFLEMNKQTPSWMCPVCASHIQFSDLIIDGFMQHIL
NFI1/SIZ2       SLQCPISCTRMKYPAKTDQCKHIQCFDALWFLHSQSQVPTWQCPICQHPIKFDQLKISEFVDNII
SIZ1            SLQCPISYTRMKYPSKSINCKHLQCFDALWFLHSQLQIPTWQCPVCQIDIALENLAISEFVDDIL
                SL.cPl.k.R...P.r...CkHlQCFDa...l......ptW.CPvC.k.a.y..l.id.....iL
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C

```
                                                                    495                 518
                                  451                                NY HSPVPGNPTPP TP  S  PY
Tonalli                            SHQGQFYPGSGQSAGMQQAGGMCPAGPGAVATTGNPYQNQGFQQNYQHSPVPGNPTPPLTPACSV-PY
KIAA1224  APNPPRPLTSPNYPGQRMPSQPSSGQYPPPTVNMGQYYKPEQFNGQNNTFSGSSYSNYSQGNVNRPPRPVPVANYPHSPVPGNPTPPMTPGSSIPPY
KIAA1886                                                                                    SV-PY
                                                                566                 595
           SP         PD KP               E RLTFPVRDG  L PFRL HNL VSNHVF L   V  TL  R DLELQ KC H  DRQMNTNW
Tonalli   VSPN--------PDIKPPMDNSE----------EMRLTFPVRDGIILAPFRLLHNLSVSNHVFHLKQNVYNTLMCRNDLELQLKCFHQDDRQMNTNW
KIAA1224  LSPSQDVKPPFPPDIKPNMSALPPPPANHND--ELRLTFPVRDGVVLEPFRLEHNLAVSNHVFHLRPTVHQTLMWRSDLELQFKCYHHEDRQMNTNW
KIAA1886  MSPNQEVKSPFLPDLKPNLNSLHSSPSGSGPCDELRLTFPVRDGVVLEPFRLQHNLAVSNHVFQLRDSVYKTLIMRPDLELQFKCYHHEDRQMNTNW

          P  V VS NATPL IER   N T    L LK VCQPGRNT Q T   CCCSHLFVLQLVHRPSVR VLQ L K  LLP EH   KIKRN S   692
Tonalli   PHTVTVSANATPLNIERSEKNSTALRPLYLKAVCQPGRNTLQLTASSCCCSHLFVLQLVHRPSVRQVLQTLHKRNLLPLEHSVQKIKRNLSQPEANA
KIAA1224  PASVQVSVNATPLTIERG-DNKTSHKPLHLKHVCQPGRNTIQITVTACCCSHLFVLQLVHRPSVRSVLQGLLKKRLLPAEHCITKIKRNFSSVAASA
KIAA1886  PASVQVSVNATPLTIERG-DNKTSHKPLYLKHVCQPGRNTIQITVTACCCSHLFVLQLVHRPSVRSVLQGLLKKRLLPAEHCITKIKRNFS---SGT

                                  ****************************************                  789
                        Q   K SLKCPIT  RI LPARGH C H QCFDLE YLM N ERG WRCP C K A    LE DQY   IL     S   E
Tonalli   GPDATPQQQQQQGGGQQCAKISLKCPITKSRIRLPARGHECKHVQCFDLEAYLMINSERGSWRCPECSKSAITDTLEIDQYIWAILNTLGNSDVDEV
KIAA1224  G---NTTLNGEDGVEQTAIKVSLKCPITFRRIQLPARGHDCKHVQCFDLESYLQLNCERGTWRCPVCNKTALLEGLEVDQYMWGILNAIQHSEFEEV
KIAA1886  IP-GTPGPNGEDGVEQTAIKVSLKCPITFRRIQLPARGHDCRHIQCFDLESYLQLNCERGTWRCPVCNKTALLEGLEVDQYMLGILIYIQNSDYEEI

           ID    W              P                                                    D      G884
Tonalli    IIDSSANWRALQHNGGMPNAPPPSNVPSNPSGGSGSNSGNGSVNPTLPVIKQELCDDI-AKVMSPGSTQLPTWDSAQAMSPYNMHDMNSIASGNMM
KIAA1224   TIDPTCSWRPVPIKSDLHIKDDPDGIPSKRFKTMSPSQMIMPNVMEMIAALGPGPSPY-PLPPPPGGT-------------NSNDYSS--QGNNY
KIAA1886   TIDPTCSWKPVPVKPDMHIKEEPDGPALKRCRTVSPAHVLMPSVMEMIAALGPGAAPFAPLQPPSVP--------------APSDYPG--QGSSF

           G G                    G                  EK          M          H                      972
Tonalli    -GNGGNTNQHGNRSSYDGFSGNHSDGSGGLPGGDGGVNSLDQLNAMEKSLSDQ-----MPHTPHTPGAASHPMTP--GGPPSVSSSHMEPISGGTP
KIAA1224   QGHGNFDFPHGNPGGTSMNDFMH--GPPQLSHPPDMPNNMAA---LEKPLSH-PMQETMPHAGSSDQP--HPSIQQGLHVPHPSSQSGPPLHHSGA
KIAA1886   LGPGTFPESFPPTTPSTPTLAEFTPGPPPISYQSDIPSSLLT---SEKSTACLPSQ--MAPAGHLDPT--HNPGTPGLHTSNLGAPPGPQLHHSNP
                                                                                                  1066
                                   PS    G                                                        L
Tonalli    NANGSGSATNGSGNNN-SSTGHNSPQTPGT-PSRMGGGMGGSGSADSQQQQQEQLLNSLMSSQTQLKFSESDLSAELQSFDAAAAAINDTAHDLNL
KIAA1224   PPPPPSQPPRQPPQAAPSSHPHSDLTFN---PSSALEGQAGAQGASDMPEPSI----------------------------------------NL
KIAA1886   PPAS-RQSLGQASLGPTGELAFSPATGVMGPPSMSGAGEA--------PEPAL----------------------------------------DL

           L     P E LSYL P PDL    P       N  DDLL   1109
Tonalli    LQDVD-PMEILSYLDPQPDLNTPPSSGSSNNNASDDLLATLFD
KIAA1224   LPELTNPDELLSYLDP-PDL---PS------NSNDDLLSLFENN
KIAA1886   LPELTNPDELLSYLGP-PDL---PT------NNNDDLLSLFENN
```

Fig. 4. The Tonalli proteins. (A) The two alternatively spliced forms predicted by the BDGP, release 2, are shown. In the upper part is a scale that indicates the aminoacid residues. In the TnaA protein the exons are indicated as E. E1 to E4 are exons shared between the TnaA and the TnaB forms. The glutamine rich domains are indicated by lightly shaded boxes. The bipartite nuclear location signal is indicated by the hatched box. The SP-RING finger is indicated by a black box. The TnaB carboxyl termini is indicated by the grey box and is different from the one in TnaA. The XSPRING domain, which is present in the human KIAA proteins and in proteins in other organisms, is indicated by the box above the proteins. (B) Multiple alignment of the SP-RING finger region in different proteins. KIAA1224 and KIAA1886 human proteins (accession numbers in Results); Su(var), *D. melanogaster* Su(var)2-10/ZimpA/B (gb/AAD29287.1); Miz1, (Msx-interacting-zinc finger) from mouse (gb/AAB96678.1); PIAS1 from mouse (gb/AAC36702.1); KCh, K^+ channel-associated protein from rat (gb/AAC40114.1); PIAS3 from mouse (dbj/BAA78533.1); PIASy from human (gb/AAC36703.1); CEW10D5 predicted protein from *C. elegans* (pir/T26331); VICIA, *Vicia faba* transcription factor (pir/T12184); SER-INT, a *Schizosaccharomyces pombe* homologue (pir/T37748) of *Saccharomyces cerevisiae* Siz proteins; NFI1/SIZ2, CDC12 and septin-interacting protein in *S. cerevisiae* (gb/AAA86121.1); SIZ1, septin-interacting protein from *S. cerevisiae* (pir/S69691). The bottom line is the identical (in uppercase letters) and most common (lowercase) residues in all sequences. (C) Multiple alignment of *Drosophila* TnaA, human KIAA1224, and human KIAA1886 XSPRING domains (495-798). The glutamine 566 that changes for a stop codon in tna^1 is indicated by the residue number. The bipartite nuclear location signal residues are underlined. The SP-RING finger residues are indicated with asterisks. Consensus sequence of the same amino acid present in the three proteins is indicated.

extend their wings out from the body (a held-out wings phenotype). While flies heterozygous for a null *brm* allele usually hold their wings properly, they often extend their wings when also heterozygous for *brm*-interacting mutations. We have previously used this genetic screen to isolate mutations in the *osa* gene (Vázquez et al., 1999), which encodes a subunit of the Brm chromatin remodeling complex. Here we report the isolation of mutations in two additional *brm*-interacting genes, *tara* and *tna*. *tara* is pleiotropic and has been identified in several other genetic screens (e.g. Fernandez-Funez et al., 2000). Of particular importance to our own results was the recent identification of *tara* as a dominant suppressor of the extra-sex-combs phenotype displayed by loss-of-function mutations in the Polycomb group gene *polyhomeotic* (Fauvarque et al., 2001) and its modification of phenotypes associated with ectopic expression of the homeotic gene *proboscipedia* (*pb*) (Calgaro et al., 2002). The published results, as well as the results presented here, suggest that *tara* and *tna* are both members of the trithorax group of homeotic gene regulators.

tara encodes twin proteins, Tara-α and Tara-β, which have a cyclinA-binding motif (also present in the cell cycle regulatory transcription factors E2F1-3), a SERTA domain [which is the largest conserved region among TRIP-Br (transcriptional regulator interacting with the PHD-bromodomain) proteins] and a PHD-bromo interaction domain (Calgaro et al., 2002). Trip-Br1/$p34^{SEI-1}$ is a Tara-related protein in mice that is a cyclin-dependent kinase regulator (Sugimoto et al., 1999) and a transcriptional regulator. Trip-Br1/$p34^{SEI-1}$ can interact with PHD and/or bromodomains (Hsu et al., 2001). It has been proposed that this family of proteins could link the cell cycle with chromatin remodeling (Sugimoto et al., 1999).

Individuals with low dosages of *tara* (Calgaro et al., 2002) or *tna* (this work) have a held-out wings phenotype. As we have isolated *tara* mutations because they interact genetically with *brm* mutations, the simplest hypothesis is that Tara proteins physically interact with Brm proteins through the Brm bromodomain.

One Tna protein isoform is related to the PIAS family

Analysis of *tna* ESTs shows that there are at least two different 5′ ends (represented by RE42750 and AT07790), suggesting that the *tna* gene may have alternative promoters. The *tara* gene also appears to have two promoters (Calgaro et al., 2002). In addition to the possibility of two promoters, alternative splicing within the *tna* open reading frame gives rise to at least two different protein isoforms, TnaA and TnaB.

The TnaA isoform has an SP-RING (Siz/PIAS RING) finger (Saurin et al., 1996), which is present in the PIAS [protein inhibitor of activated STAT (signal transducer and activator of transcription)] family of proteins. PIAS proteins are co-regulators of many gene-specific transcription factors. For example, PIAS proteins co-repress STAT factors (which act as signal transducers of cytokine receptors) to transcriptionally activate specific target genes (Chung et al., 1997; Liu et al., 1998). PIAS proteins also coactivate steroid receptor-dependent transcription (Kotaja et al., 2000; Tan et al., 2000). The PIAS protein Miz1/ARIP3/PIASXα possesses intrinsic transcriptional-activating function (Kotaja et al., 2000), interacts with the homeobox protein Msx2 to enhance its affinity for DNA (Wu et al., 1997) and is an androgen receptor (AR)-interacting protein (ARIP). The *Drosophila zimp* (zinc finger-containing, Miz1, PIAS3-like) gene encodes proteins with similarity to the Miz1/PIAS3 protein (Mohr and Boswell, 1999). The *zimp* gene is also known as *Su(var)2-10* (Hari et al., 2001). In addition to the SP-RING zinc finger domain, the Su(var)2-10 proteins have a putative DNA-binding domain (the SAP domain) that is found in diverse nuclear proteins. The Su(var)2-10 proteins regulate chromosome structure and chromosome condensation, and function in interphase nuclei (Hari et al., 2001). Recently a SUMO-protein ligase (E3) activity has been found in several SP-RING finger proteins (Johnson and Gupta, 2001; Sachdev et al., 2001) (reviewed by Hochstrasser, 2001).

tna and a role for sumoylation in regulating homeotic gene expression

SUMO (small ubiquitin-related modifier) is a ubiquitin-like protein (UBL) that is covalently attached to other proteins in a manner analogous to that of ubiquitin (reviewed by Muller et al., 2001). Conjugation of SUMO-1 to all protein targets requires the E1-activating heterodimer Aos1/Uba2 and the single E2-conjugating Ubc9 enzyme. The target specificity is conferred by the SUMO E3 ligases. There are at least two types of SUMO E3 ligases that are structurally unrelated. The first type is represented by the PIAS family of SP-RING finger proteins. The second type is represented by RanBP2, a nuclear pore complex protein. TnaA has an SP-RING finger within the larger XSPRING domain (Fig. 4B). The XSPRING domain is present in a new group of human, mouse and *Arabidopsis*

proteins and may be the signature for a new subgroup of SUMO E3 ligases within the PIAS family.

Although the role of sumoylation is not clear, it has been suggested that sumoylation could be an address tag for protein targeting. Most of the identified substrates of sumoylation are nuclear proteins, and the sumoylated forms are often found in specific subnuclear protein complexes. Preferential accumulation sites for sumoylated proteins are the PML nuclear bodies. PML, a protein found in PML nuclear bodies, is a RING-finger protein. Another core component of PML nuclear bodies is Sp100, a protein that interacts with HP1 and HMG1/2 families and a major cellular substrate for sumoylation. In vitro, sumoylated Sp100 has a higher affinity for the HP1 protein (Seeler et al., 2001). Relocalization of proteins to nuclear bodies after sumoylation can modulate transcriptional activity (Fogal et al., 2000; Ishov et al., 1999; Lehembre et al., 2001; Li et al., 2000; Schmidt and Muller, 2002). It has been suggested that nuclear bodies might stimulate SUMO conjugation, and that proteins transiently associated with nuclear bodies include SUMO targets (Muller et al., 2001). Thus, sumoylation can modulate the interaction of transcription factors with transcriptional corregulators. In *Drosophila*, the transcriptional repressor Tramtrack 69 protein (Ttk69), which inhibits neuronal differentiation, has been identified as a SUMO substrate (Lehembre et al., 2000). The Dorsal protein also undergoes sumoylation, which facilitates its nuclear import (Bhaskar et al., 2000).

The SUMO ligation target consensus sequence is ΨKxE (where Ψ is an aliphatic residue) surrounding the substrate lysine(s) that is sumoylated. Although this consensus sequence is short, all of the proteins encoded by the trithorax groups genes that interact genetically with *tna* (including TnaA itself) (Table 3) have one or more blocks of this consensus sequence (L. G. and M. V., unpublished results). However, some trithorax group genes that do not interact with *tna*, such as *trithorax* (*trx*), also encode proteins with the 'sumoylation consensus'. Sumoylation of the HDAC4 deacetylase is catalysed by the RanBP2 SUMO E3 ligase. While HDAC4 has several 'sumoylation consensus' sequences, only one functions in vitro and in vivo (Kirsh et al., 2002). The possibility that subunits of the Brm and/or Kismet complexes might be targets for sumoylation opens the window for a new level of regulation of the activity of chromatin remodeling complexes. This level of regulation could involve the modification of their subnuclear localization within the nucleus, although mutation of the SUMO acceptor site in HDAC4 did not change its subcellular distribution (Kirsh et al., 2002). Alternatively, is that sumoylation could target the homeotic function itself or its cofactors.

Another possible role for sumoylation is as an antagonist of ubiquitylation. Ubiquitylation is a key regulator of transcription (reviewed by Conaway et al., 2002) and it has been suggested that sumoylation could be an inhibitor of ubiquitylation. The RING (reviewed by Jackson et al., 2000) and PHD (Lu et al., 2002) fingers have been described in proteins that have E3 ubiquitin ligase activities. In that sense it is intriguing that Trip-Br1 (the *tara* homolog in mice) (Hsu et al., 2001) was identified because it binds the PHD-bromodomain of Krip1/TIF1β which also has an RBCC (RING finger-B boxes-coiled coil) RING finger (Saurin et al., 1996). Krip1/TIF1β has a dual role because it has been described as a corepressor of a subset of Krüppel-type zinc finger proteins (Witzgall et al., 1994) and as a hormone-dependent coactivator that interacts with several nuclear hormone receptors (Chang et al., 1998; Le Douarin et al., 1996). Mutations in a ubiquitin-conjugating enzyme (UbcD1) have been shown to affect homeotic gene silencing (Fauvarque et al., 2001). Since *tna* mutations affect homeotic gene activation, antagonism between the ubiquitylation and sumoylation post-translational modifications may play a key role in homeotic gene regulation. Antagonism of ubiquitylation and targeting nuclear sublocalization are not mutually exclusive roles for sumoylation, and it is possible that both will be found to have roles in regulating the functions of chromatin remodeling and/or transcriptional co-activator complexes.

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