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# A clonal analysis of the requirement for the *trithorax* gene in the diversification of segments in *Drosophila*

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SUMMARY

The requirement for the *trithorax* gene during imaginal cell proliferation has been analysed by clonal analysis. Clones carrying the allelic *Regulator of bithorax* and *trithorax* mutations were induced by mitotic recombination at different developmental stages. The results indicate that the *trithorax* gene is required at least until the beginning of the third larval instar to ensure the correct differentiation of head, thoracic, and abdominal structures, and are consistent with a role for the gene in maintaining the appropriate levels of Antennapedia and bithorax complex expression.

## INTRODUCTION

Differential expression of selector genes (Garcia-Bellido, 1975, 1977) is the primary cause of segment diversification in *Drosophila*. Selector genes are defined by mutations which result in homoeotic transformations of different body segments. Bithorax complex (BX-C) mutations cause transformations of thoracic and abdominal segments (Lewis, 1978), from which it is inferred that these genes normally control the diversification of these body segments. Antennapedia complex (ANT-C) mutations, on the other hand, transform structures deriving from the head and thoracic segments (Kaufman, Lewis & Wakimoto, 1980) implying that the ANT-C controls the development of the anterior part of the fly.

In addition to those of the BX-C and ANT-C, a considerable number of other homoeotic mutations have been described in *Drosophila melanogaster*. Recent studies have provided evidence that several of these mutations define genes which function in controlling the expression of the ANT-C and BX-C. The majority of these appear to exert a negative control over the two complexes (Lewis, 1978; Struhl, 1981*a*; Duncan & Lewis, 1982; Duncan, 1982; Ingham, 1984; see also review of Ingham, 1985), whereas one, defined by the *Regulator of bithorax* (*Rg-bx*) (Capdevila & Garcia-Bellido, 1981) and *trithorax* (*trx*) (Ingham & Whittle, 1980) mutations appears formally equivalent to a positive regulator of the BX-C.

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*Key words*: *Drosophila,* clonal analysis, *trithorax*, mutations, segment diversification, homoeotic transformations.

Much interest centres around the developmental stage at which these genes act; in particular which, if any, are involved in the initiation of selector gene expression. Capdevila & Garcia-Bellido (1981) argued that *Rg-bx* defines a gene required solely for the initiation of BX-C expression. A preliminary analysis of two *trx* alleles, which fail to complement *Rg-bx*, produced results inconsistent with this interpretation (Ingham, 1981). Here I present the results of an extensive clonal analysis of both the *Rg-bx* and *trx* mutations. The data confirm that the mutations define a single gene, *trithorax*, which is required throughout the larval stage for the appropriate differentiation of adult derivatives of thoracic, abdominal and head segments.

## MATERIALS AND METHODS

### *Mutant genotypes and culture conditions*

$trx^2$, $trx^3$ are recessive lethal alleles which fail to complement $trx^1$ (see Ingham, 1981). *Rg-bx* is a recessive lethal which also fails to complement $trx^1$, $trx^2$ and $trx^3$ (Ingham, 1981). The allelism of *Rg-bx* and *trx* has been questioned by Capdevila & Garcia-Bellido (1981) but is confirmed by results of this analysis. Since the *trx* symbol conforms to the convention for *Drosophila* gene nomenclature I here refer to *Rg-bx* as *trithorax of Lewis* ($trx^L$). *Dp(3:1)kar*$^{5l}$ carries a copy of the *trx* locus on the X chromosome, which covers all *trx* alleles. Two crosses were employed to generate animals bearing *trx* mutant clones:

(1) ♀♀*y* $f^{36a}$; *mwh red* $trx^2/TM2 \times$ ♂♂ *Dp(3:1)kar*$^{5l}$ *M(1)O*$^{Sp}$; *red* $trx^3/Dp(1:3)A59$.

(2) ♀♀*y* $f^{36a}$; *mwh red* $trx^2/TM2 \times$ ♂♂ *Dp(3:1)kar*$^{5l}$ *M(1)O*$^{Sp}$; *mwh* $trx^L/Dp(1:3)A59$. *y*, $f^{36a}$, and *mwh* are cuticular markers; these, and *red* and *TM2*, are described in Lindsley & Grell (1968).

The dominant *M(1)O*$^{Sp}$ mutation causes cell autonomous slow growth. *Dp(1:3)A59* carries a wild-type copy of the *M(1)O*$^{Sp}$ locus on chromosome 3 (see Kerridge & Morata, 1982).

Clones of marked cells in $trx^1/trx^2$ flies were induced in larvae from the cross ♀♀*y*;*mwh st* $trx^1 \times$ ♂♂*y*; *Dp(1:3)sc*$^{J4}$, $y^+$ *M(3)i*$^{55}$ red $trx^2/TM1$.

Flies were reared at 25°C on a yeast/molasses/cornmeal/agar medium.

### *Induction and analysis of clones*

Eggs were collected from the appropriate crosses over 12 h periods in split glass bottles containing standard medium. The resultant larvae were subsequently irradiated using a Philips Constant Potential MG102 X-ray machine. A dose of 1000 rad was delivered in 3 min 14 s at 4 mamp, 100 kV. Larvae were irradiated at either 48 ± 6 h after egg laying (AEL) or 84 ± 6 h AEL, corresponding to the first and second larval instars. Adult progeny of the appropriate genotype were collected and preserved in 70 % alcohol.

In the case of flies irradiated during the first instar, most induced *y* $f^{36a}$ clones are expected to be sufficiently large to be detectable at low (×25) magnification. Accordingly, animals irradiated at 48 ± 6 h AEL were screened under the dissecting microscope for clones in the head, dorsal thorax and abdomen. Animals bearing clones were dissected and mounted for examination at higher magnification. Since *y* $f^{36a}$ clones in the legs are not so easily detectable, all the legs from these animals were mounted and screened at high magnification for clones. All parts of flies irradiated at later stages were similarly screened at high magnification. This screening procedure means that some clones induced during the first instar may have gone undetected, especially if they had not resulted in transformations. However, judging from their frequency amongst later stage irradiations, the number of such untransformed clones would have been small.

The phenotypes of the clones in each segment are described in Results. The detailed morphology of adult structures have been described by various authors. This study follows the descriptions of Struhl (1981*b*) for the proboscis; Morata & Lawrence (1979) for the eye antenna; Roseland & Schneiderman (1979) for the abdomen; Schupbach, Wieschaus & Nothiger (1978)

for the genitalia; sources of descriptions of thoracic structures are as previously cited (Ingham, 1981).

## RESULTS

The mutant alleles $trx^2$, $trx^3$ and $trx^L$ are all homozygous lethal; they fail to complement the homoeotic phenotype of $trx^1$, as well as each others lethality. Genetic analysis suggests that all three alleles abolish most, if not all, of the wild-type function of the *trx* locus (Ingham, 1981); in addition, there is some evidence that $trx^3$ may have an antimorphic component (unpublished observations).

Clones trans-heterozygous for pairwise combinations of these alleles allow an investigation of the requirement for *trx* wild-type activity in cells of different segments and at different developmental stages. It has previously been shown that cells trans-heterozygous for $trx^2$ and $trx^3$ are viable in clones in the adult thoracic cuticle (Ingham, 1981). Here the viability and differentiative potential of cells lacking *trx* activity has been investigated in the adult derivatives of head, thoracic and abdominal segments. To rule out allele-specific effects, two independent series of clones were induced, genotypically either $y\,f^{36a}\,M(1)O^+;trx^2/trx^3$ or $y\,f^{36a}\,M(1)O^+;trx^2/trx^L$. The Minute technique (Morata & Ripoll, 1975) was employed, in order to generate large clones.

In all regions of the animal, clones of these two genotypes are phenotypically indistinguishable. Thus in the following description the two series of clones have been pooled and will be referred to simply as $trx^-$ clones. The characteristics of clones in each adult structure are described below:

### (1) *The eye antennal segment*

A total of 79 antennal clones and 49 clones in the eye or head capsule induced during the first and second instars (see Table 1) were examined; the majority of clones exhibit the following phenotype.

Clones in the antenna result in the transformation of distal structures (arista and third segment) to homologous leg structures (the tarsal segments) bearing characteristic structures such as bracted bristles, claws and pulvilli (Fig. 1B). In contrast, the proximal regions of the antenna develop normally in the absence of $trx^+$.

Clones in the eye were detected since they result in an outgrowth of bristle-bearing cuticle (see Fig. 1C). This cuticle also bears trichomes; the identity of the bristles is not clear, but they are of a size comparable to those found on the notum. Clones in other regions of the head are integrated into the head capsule but cause disruption of the normal pattern. In particular, clones on the top of the head capsule result in the absence of the ocelli and of the orbital bristles (Fig. 1A).

### (2) *The proboscis*

27 clones induced in the first instar were examined. Of these, sixteen are located in the anterior compartment whilst eleven are of posterior provenance (see Table 1).

Table 1. trx⁻ *clones in the head*

| Genotype of clone | Stage irradiated | No. of heads scored | Number and location of clones | | | |
|---|---|---|---|---|---|---|
| | | | Proboscis | | Antenna | Eye/head capsule |
| | | | A | P | | |
| $trx^2/trx^3$ | 1st instar | 193 | 9 | 9 | 25 | 17 |
| | 2nd instar | 41 | 4 | 2 | 18 | 15 |
| $trx^2/trx^L$ | 1st instar | 176 | 7 | 2 | 45 | 32 |

A = anterior compartment
P = posterior compartment

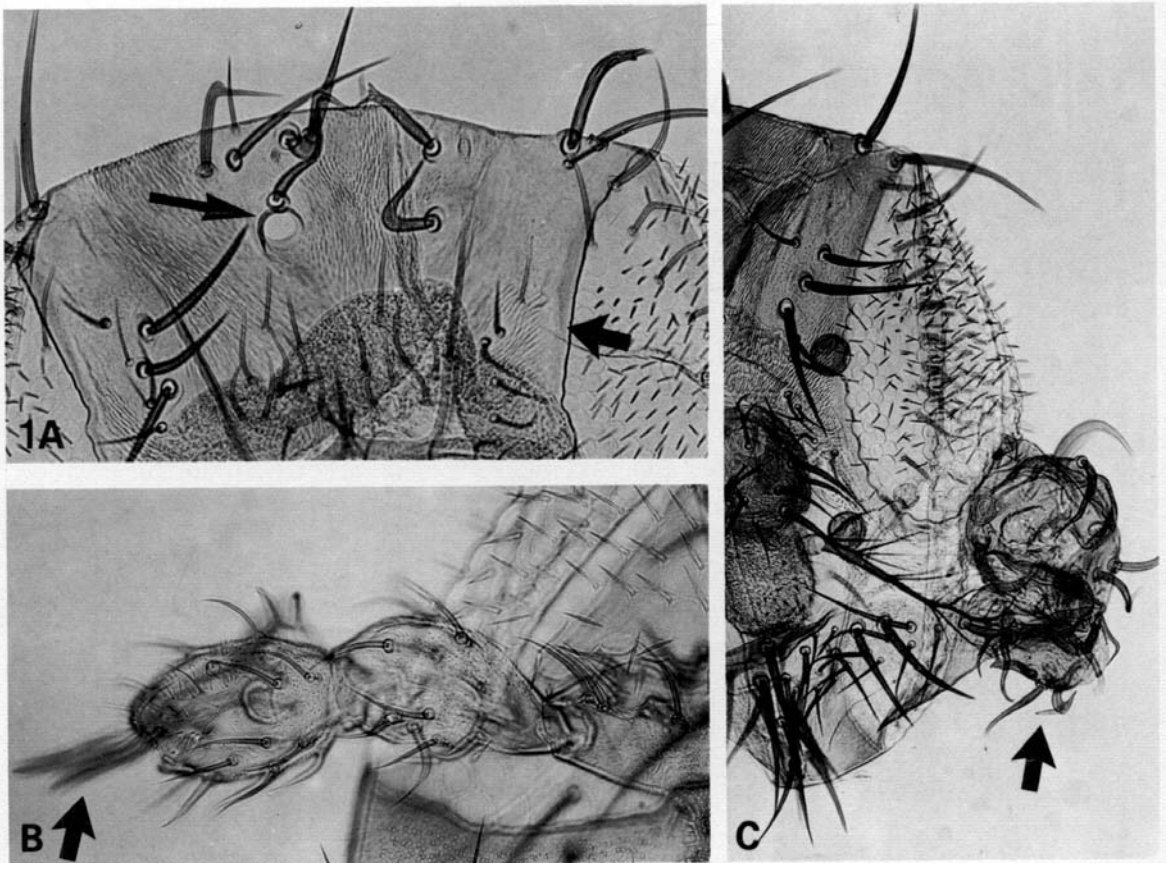


Fig. 1. *trx*⁻ clones in the head. (A) A clone in the dorsal region of the head capsule. The abnormalities caused by the mutation are apparent in a comparison between the right side of the head (differentiated by the clone) and the left side, which is wild type. The major effects are the absence of the lateral and medial ocelli (the wild-type ocellum is indicated by the thin arrow) and the failure to differentiate the orbital bristles in the region indicated (thick arrows). (B) A clone in the antenna resulting in the transformation of the arista to tarsal segments. Note the claw (arrowed). (C) A clone (arrowed) transforming part of the eye to cuticle bearing large bristles resembling those of the notum.

All the anterior clones show evidence of transformation of the palps to leg tissue. In most cases this is indicated by the presence of an apical bristle (characteristic of the second leg); some clones also differentiate claws and pulvilli (Fig. 2A). In one exceptional case, the clone bears both bracted bristles and a structure resembling an arista (Fig. 2B). Posterior clones in the prementum often cause unusual bristle arrangements. These cannot be identified as being characteristic of any particular tissue (Fig. 2C); however they are similar to those caused by clones of lethal *engrailed* alleles (see fig. 1 in Lawrence & Struhl, 1982). In the posterior region of the palps, clones differentiate large bristles, similar to apical bristles. Though apical bristles are characteristic of the anterior leg compartment, similar bristles are also found in $trx^-$ clones in the posterior leg compartment (see below).

(3) *The ventral thorax*

A total of 217 clones induced in the three thoracic legs during the first larval instar were examined. The number and distribution of clones is presented in Table 2.

A further 35 clones induced during the second instar in the first and third thoracic legs were found to have a similar phenotype. As previously reported

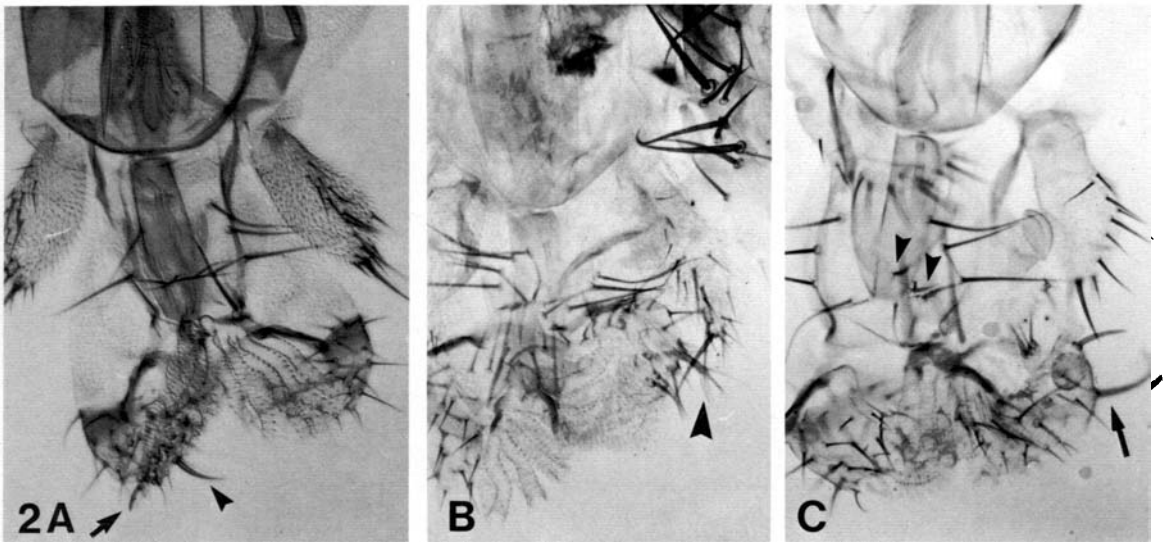


Fig. 2. $trx^-$ clones in the proboscis. (A) Transformation of the palps of the anterior compartment to leg tissue. The arrowhead indicates a large bristle not associated with a bract; the arrow indicates a well-formed claw. Just to the right of the claw but hardly visible in this photograph is a pulvillus. (B) In this exceptional case the clone has differentiated bristles and an arista (arrowhead), typical of the antenna. (C) A clone in the posterior compartment of the proboscis. The arrowheads indicate several bristles in the prementum belonging to the clone; three of these are in an ectopic location, resembling those differentiated by *en* lethal clones (Lawrence & Struhl, 1982). Note also the large bristles (arrowed) belonging to the clone in the palps.

(Ingham, 1981) clones lacking $trx^+$ activity in the prothoracic and metathoracic legs exhibit changes in pattern consistent with a transformation towards mesothoracic leg. These changes include the presence of stenopleural bristles in the presumptive propleural (Fig. 3A) and metapleural regions and of apical bristles on the distal tibia (Fig. 3B). The pattern differentiated by these clones is not however strictly that of a normal mesothoracic leg; furthermore, clones in the mesothoracic leg also differentiate abnormally. In the tarsal segments of all three legs, clones bear larger than normal bristles at the distal tip of each tarsal segment (Fig. 3B), whilst those including the distal anterior tibia frequently bear a duplicated apical bristle (Fig. 3B) [(see also fig. 1a, Ingham, 1981)].

Clones in the posterior tibia also bear a larger than normal bristle at the distal tip, adjacent to the (anterior) pre-apical bristle. In the posterior femur of all three legs, clones differentiate some large bractless bristles in an arrangement not typical of any leg (Fig. 3C,D and E). Similar ectopic bristles are also produced by clones of *en* lethal alleles in the posterior femur (Lawrence & Struhl, 1982). In other respects, posterior clones in the first and third legs exhibit a clear transformation to second leg; this is indicated in the first leg by the reduction in size of the typical proximal distal row of large femoral bristles. In the posterior third leg, clones bear typically mesothoracic bristles in the coxa (*e.g.* the $BH^-$ bristle), trochanter and femur. In addition, the transverse rows of bristles unique to the posterior metathoracic tibia and tarsal segments are removed.

(4) *The dorsal thorax*

(i) *The humerus and notum*

A total of 23 humeral and 35 notal clones were examined. None of these show any evidence of transformation or other abnormalities.

(ii) *The wing*

144 clones induced in the wing during the first and second instars were examined.

In the posterior compartment (54 cases) most clones exhibit a consistent abnormal phenotype. Large socketed bristles are formed along the posterior margin of the wing blade (Fig. 4B). In the wild-type wing, bristles are present along the anterior margin but not the posterior margin. The bristles formed by clones in the proximal region of the posterior margin resemble those of the costal

Table 2. $trx^-$ *clones induced in the ventral thorax during the first instar*

| Genotype of clone | No. legs scored | Number and location of clones | | | | | |
|---|---|---|---|---|---|---|---|
| | | Leg 1 | | Leg 2 | | Leg 3 | |
| | | A | P | A | P | A | P |
| $trx^2/trx^3$ | 316 | 37 | 12 | 33 | 13 | 33 | 10 |
| $trx^2/trx^L$ | 198 | 25 | 6 | 18 | 8 | 24 | 9 |

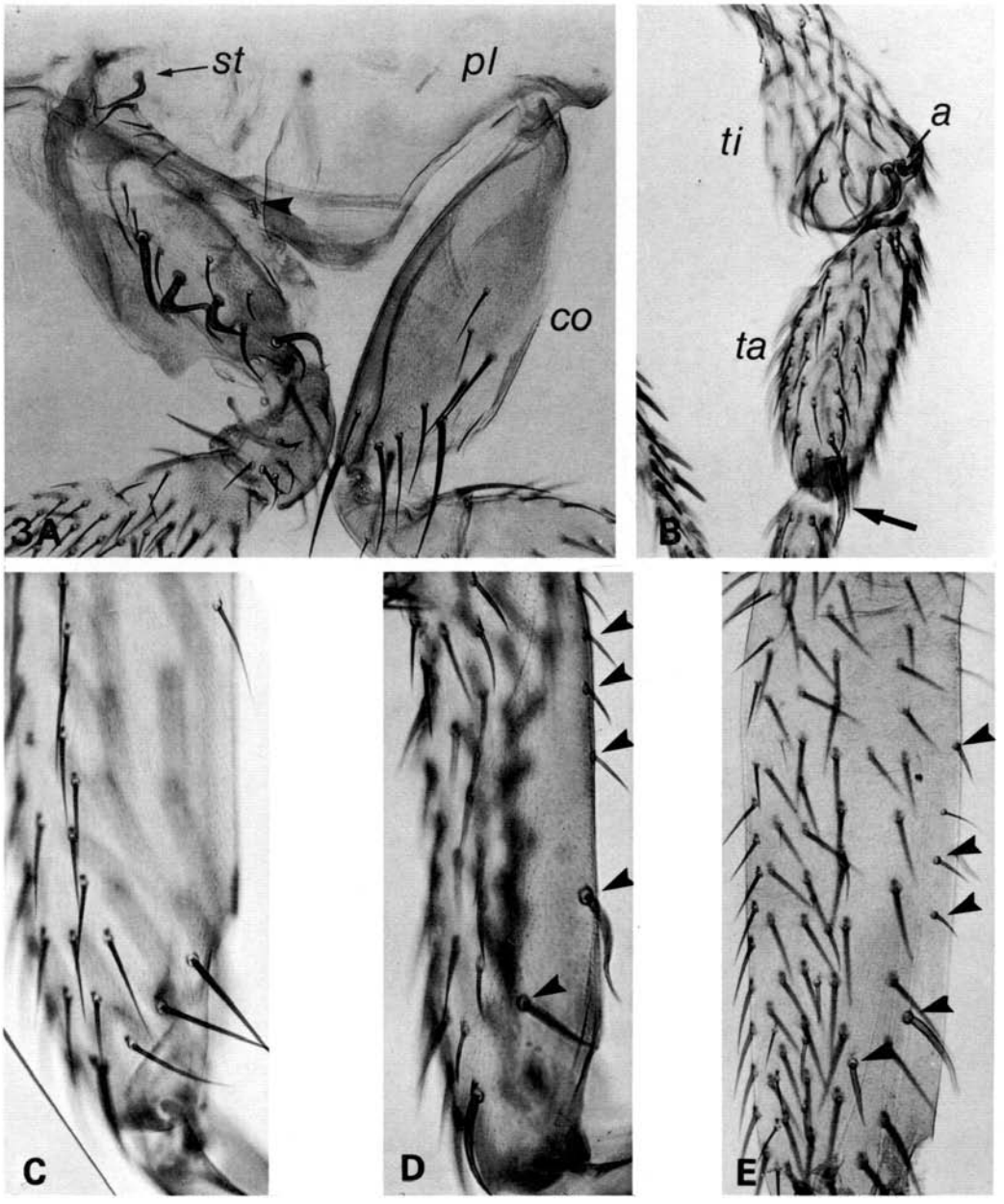


Fig. 3. *trx*$^{-}$ clones in the legs. (A) Clone in the anterior prothoracic leg. Note the presence of sternopleural bristles (st), characteristic of the mesothoracic leg in the propleura; compare with the wild-type propleura (*pl*) of the complementary leg (right). Note also the additional large bristles and ectopic location of the group of sensilla (arrowed) in the coxa (co). (B) Anterior clone in the distal metathoracic leg. This has differentiated two apical bristles (*a*) in the tibia (*ti*) and a bristle of similar morphology at the distal tip of the basitarsus (*ta*) (arrowed) (D) and (E) show clones in the posterior compartments of the mesothoracic and metathoracic femura; a wild-type mesothoracic femur is shown for comparison in (C). Note the differentiation of bractless ectopic bristles by both clones (arrowed).

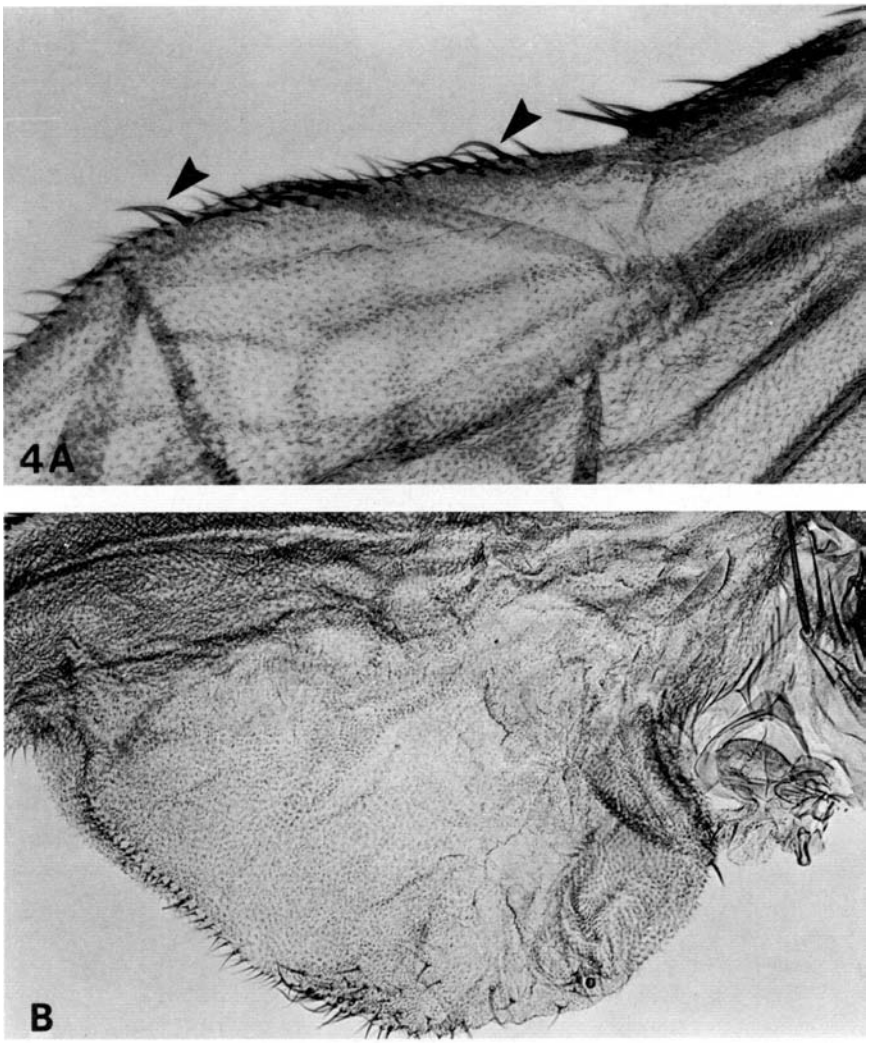


Fig. 4. *trx*$^{-}$ clones in the wing. (A) Anterior clone in which venation is disrupted and large bristles (similar to those of the wild-type costa) are present at intervals along the anterior margin of the wing (arrowed). (B) Clone in the posterior compartment. This clone has caused a considerable distortion of the normal wing morphology. In addition it has disrupted the venation and has differentiated bristles along the margin as well as within the wing blade.

region of the anterior wing. However, in clones extending distally along the margin, the bristles do not form the triple row arrangement characteristic of the homologous region of the anterior margin; rather, even in this region the bristles resemble those of the anterior costa. The clones frequently cause considerable distortion of the posterior wing, increasing its size relative to the anterior compartment (see Fig. 4B). Bristles are also formed within the wing blade itself whilst the posterior wing veins (L4 and L5) are disrupted. Most of the 90 anterior clones

also exhibit an abnormal phenotype. Venation is disrupted (Fig. 4A) as is the triple row of bristles along the anterior margin. This is replaced by an arrangement of bristles which, like those differentiated by clones along the posterior margin, resemble those of the costa. Clones extending along the length of the anterior margin exhibit a reiterated pattern of such bristles (Fig. 4A). This pattern bears a striking resemblance to the phenotype of the *Dipr* mutation (Kerridge, 1981).

(iii) *The haltere*

34 first instar clones were detected in the haltere on the basis of their being transformed to wing tissue (Fig. 5). Only anterior compartment structures (*i.e.* bristles) could be unequivocally identified; no clones were found which differentiated posterior wing elements such as the axilliary cord or alula. This is consistent with the observation that $trx^-$ clones in the posterior wing result in a transformation toward anterior compartment structures.

5. *The abdominal segments*

Clones in the tergites of abdominal segments one to seven (A1–A7) of animals irradiated during the first and second instars differentiate normal patterns (Fig. 6A). There is no evidence of transformation towards segment A1 in clones in segments posterior to A1. This is in contrast to the transformations of tergites of animals homozygous or hemizygous for the hypomorphic $trx^1$ allele (Ingham & Whittle, 1980). Transformation of abdominal clones towards mesothorax might be expected to result in the loss of the transformed clone as happens with $bxd^-$ clones induced in A1 (Morata & Garcia-Bellido, 1976; Kerridge & Morata, 1982). A comparison of the frequency of abdominal clones in control and experimental animals irradiated during the second instar (Table 3) provides no evidence for such loss. A possible explanation of this paradoxical lack of effect of removal of $trx^+$ on abdominal development will be discussed below.

In contrast to clones in segments A1 to A7, clones in the genitalia (which derives from A8, Schupbach *et al.* 1978) are transformed to tissue which bears pattern elements resembling those of the thorax. 19 clones in the genitalia induced during the first instar were examined. In each case tissue bearing *yellow forked* bristles is associated with the absence of part or all of a vaginal plate (Fig. 6B,C). Thus $trx^-$ clones cause abnormal differentiation of cells which would normally contribute to this structure. The tissue differentiated by the clones is usually disorganized; however in several cases evidence of segmentation, similar to that of the tarsus, is visible. In addition, some of the bristles formed by the clone are associated with bracts (Fig. 6C,D). Bracts are normally found in association with bristles only in the legs and a very small region of the wing. Given the ventral origin of the vaginal plate a transformation of this structure to leg, as suggested by the bracted bristles, would not be inconsistent. However it should be emphasized that many of the bristles within the clones are not associated with bracts. Occasionally other tissue, bearing some resemblance to the wing hinge/notum region is associated with these clones.

*Cell lineage analysis of the* trx$^1$ *mutation*

Animals homozygous or hemizygous for the hypomorphic allele *trx*$^1$ exhibit partial transformations of many adult body segments. In the prothoracic segment,

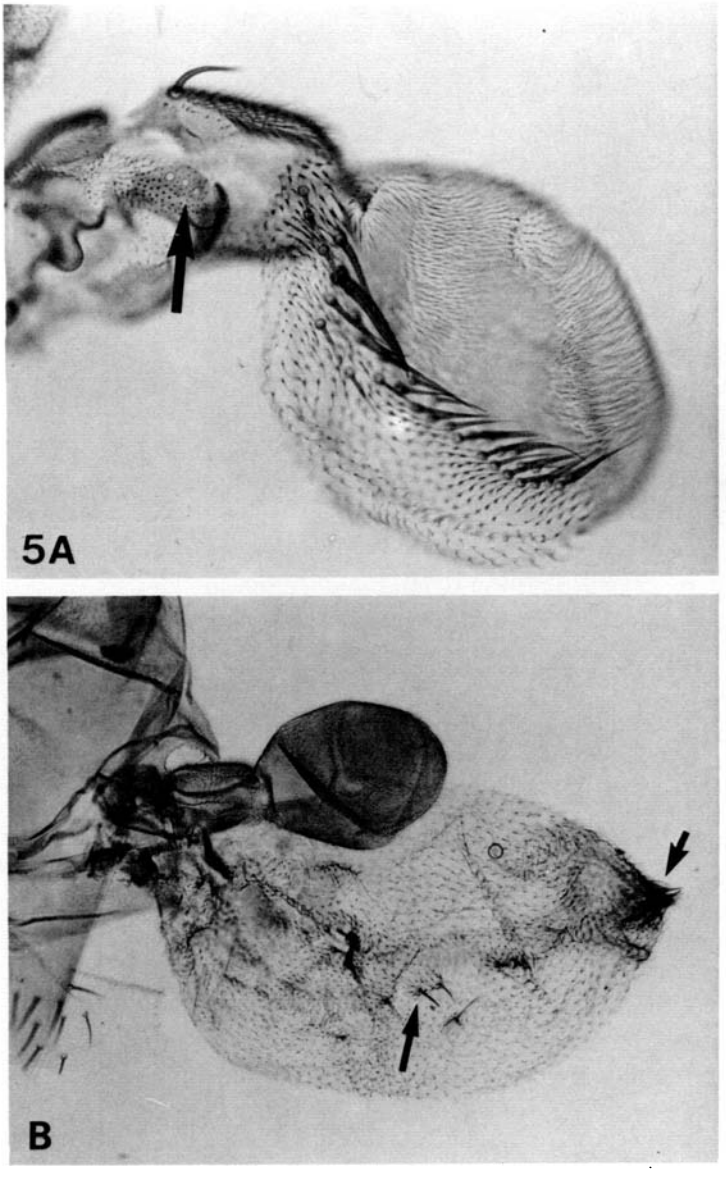


Fig. 5. *trx*$^-$ clones in the haltere. (A) Clone in the anterior compartment which is transformed to wing tissue, as indicated by the increased trichome size, the presence of marginal bristles and the morphology of companiform sensilla (arrowed). (B) Judging by its location and the haltere structures still present, this clone is in the posterior compartment. Note the transformation to wing tissue, but the presence of bristles (arrowed) typical of the anterior wing blade. Compare with *trx*$^-$ clones in the posterior wing (Fig. 4B).

Table 3. trx$^-$ *and* trx$^+$ *clones in the abdominal segments induced during the second instar*

| Genotype of clones | No. of abdomens scored | Number and location of clones | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | A1 | A2 | A3 | A4 | A5 | A6 | A7 | Genitalia* | Analia |
| *trx*$^2$/*trx*$^3$ | 41 | 5 | 7 | 8 | 7 | 12 | 9 | 8 | 4 | 4 |
| *trx*$^3$/*TM2* | 50 | 4 | 10 | 9 | 5 | 7 | 6 | 2 | 0 | 3 |

* Because of the markers used, clones in the genitalia were only identified if they resulted in a transformation.

this results in a foreleg which is a mosaic of prothoracic and mesothoracic structures (Ingham & Whittle, 1980). The early phenocritical period of this allele suggested that these transformations might arise as a result of the failure of the gene to function during early embryogenesis rather than at a later stage of development. To test this, the fate of the progeny of single cells marked at different developmental stages in mosaic forelegs was analysed.

Clones of cells marked with *yellow* and *multiple wing hairs* were induced in the first legs of *trx*$^1$/*trx*$^2$ flies during the first and second larval instars (see Materials and Methods). Clones were given a growth advantage by using the *Minute* technique (Morata & Ripoll, 1975). The numbers and locations of clones induced are shown in Table 4.

Amongst thirteen clones induced during the first instar, eight marked regions of the partially transformed legs bearing only structures characteristic of the prothoracic leg. The remaining five marked both prothoracic and mesothoracic structures, principally the sex comb and the apical and/or pre-apical bristles. The expected number of legs bearing two independently arising clones in such a sample is 0·6. Therefore most or all of these marked regions must represent the descendants of single cells.

Amongst 30 clones induced during the second instar nine include both prothoracic and mesothoracic structures. The expected number of legs bearing two independent clones in this sample is five.

## DISCUSSION

### trx *is required for the development of segments controlled by the BX-C*

The identity of all segments posterior to the mesothorax depends upon a successive increase in the expression of BX-C elements (Lewis, 1978, 1981*a*,*b*). Partial loss of function of the *trx* gene results in homoeotic transformations corresponding to a partial loss of BX-C activity. To test whether there is an absolute requirement for the *trx* gene for BX-C expression, *trx*$^-$ clones have been induced in all the segments in which the BX-C is required.

As described in a previous report, (Ingham, 1981) *trx*$^-$ clones in the metathoracic (T3) segment are transformed to mesothorax (T2). This is consistent with

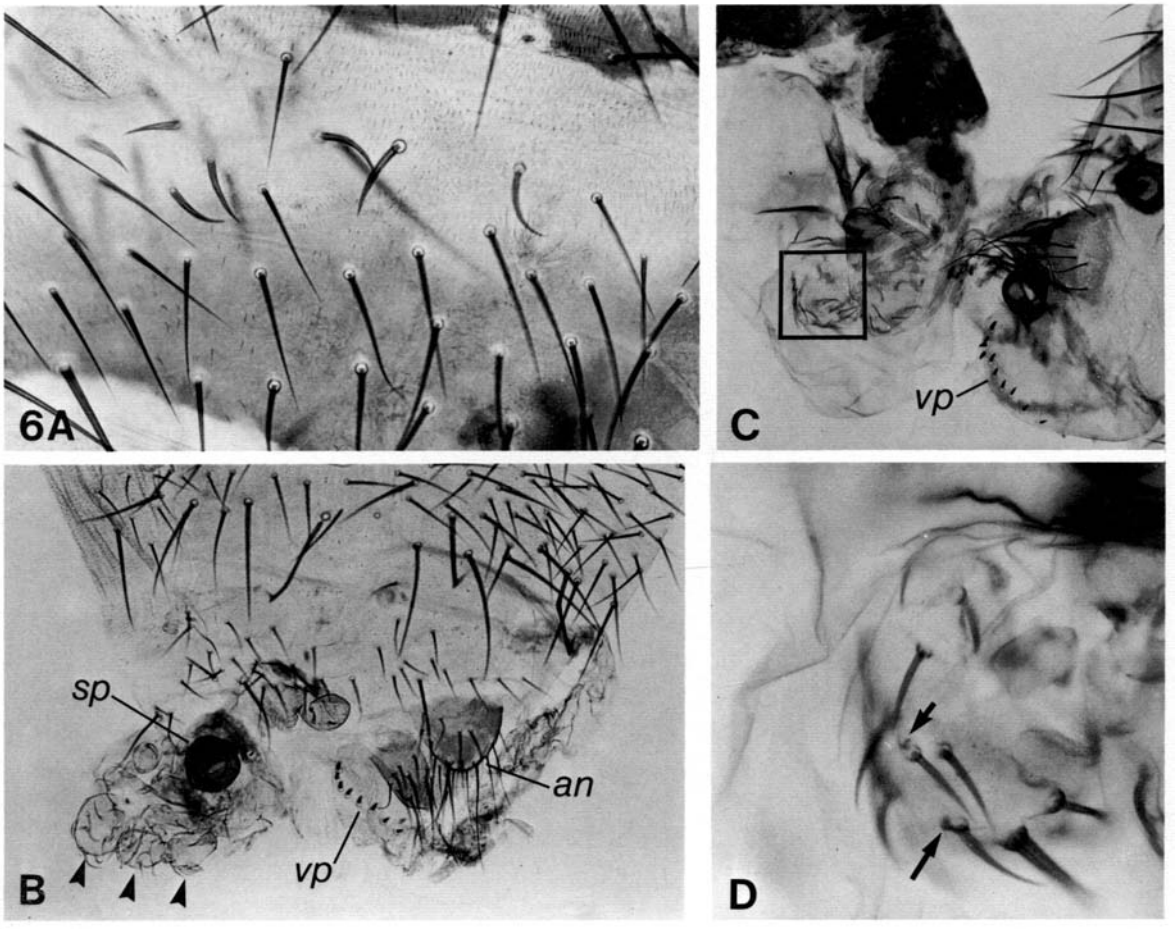


Fig. 6. *trx*$^{-}$ clones in the abdominal tergites and genitalia. (A) Clone in the tergite of a sixth abdominal segment. The morphology of such clones is essentially wild type. (B) Transformation of a vaginal plate (*vp*) to a structure resembling a leg. In particular the segmented nature of the transformed structure (arrows) resembles the tarsal segments of a thoracic leg. The round structure apparently associated with the clone is a spermatheca (*sp*) displaced during preparation of the specimen. (*an* = analia). (C) A further example of a transformation of vaginal plate to a leg-like structure. Evidence for the identity of the structure comes in this case from the association of bracts with some of the bristles differentiated by the clone. This is shown in the inset (D) of the bordered region. The inset shows the opposite surface to that visible in (C).

absence of *trx*$^{+}$ causing a loss of function of the *Ubx* gene. The BX-C is postulated to be fully expressed in the eighth abdominal segment (Lewis, 1978, 1981*a*,*b*), the segment from which the female genitalia is derived (Schupbach *et al.* 1978). Since *trx*$^{-}$ clones cause an apparent transformation of female genital structures to

Table 4. *The number and provenance of fast growing* y mwh *clones in* $trx^2/trx^1$ *flies*

| Stage irradiated | No. of legs scored | Number and location of clones | | | | |
|---|---|---|---|---|---|---|
| | | Anterior compartment | | | Posterior | Frequency |
| | | T1 | T1 + T2 | Total | | |
| First instar | 475 | 8 | 5 | 13 | 15 | 0·06 |
| Second instar | 309 | 21 | 9 | 30 | 10 | 0·13 |

Surprisingly, $trx^-$ clones in abdominal segments one to seven differentiate normally. Complete removal of the *trx* gene would be expected to have at least an equal effect to that caused by the hypomorphic $trx^1$ allele (Ingham & Whittle, 1980). A probable explanation of this lack of transformation depends upon the growth dynamics of these segments. Unlike the cells of most imaginal discs, abdominal histoblasts grow without dividing throughout the larval stages (Madhavan & Schneiderman, 1977). Hence recombinant chromosomes induced during this period do not segregate to give rise to mutant clones in the abdominal segments until the pupal stage. One possibility is that sufficient $trx^+$ product is produced prior to clone formation to support normal development even in the subsequent absence of the $trx^+$ gene. This could be tested by inducing $trx^-$ clones at the blastoderm stage.

### $trx^+$ *is required in the prothoracic and head segments*

The transformation of $trx^-$ clones in the ventral prothorax (T1) to T2 is consistent with a role for *trx* in the expression of the *Scr* gene, which promotes the prothoracic developmental pathway (Lewis, Kaufman, Denell & Tallerico, 1980*a*; Lewis, Wakimoto, Denell & Kaufman, 1980*b*).

The dorsal derivatives of segment T1 are the subject of some debate. Gynandromorph fate mapping suggests that the humerus is derived from dorsal T1. However, amongst $trx^1$ homozygotes or hemizygotes, prothoracically located mesonotum and wing tissue is always additional to and not in place of the humerus. One explanation of this finding depends upon the proposal that the humerus is derived wholly from the posterior compartment of this segment (Lawrence & Struhl, 1982). In this case it is possible that the ectopic mesonotum and wing tissue may be exclusively of anterior origin. $trx^-$ clones in the humerus might thus be expected to be transformed to posterior T2, whilst clones in the anterior compartment of T1 should result in additional notal tissue adjacent to the humerus. No examples of this latter type of clone were observed, whilst all clones in the humerus are morphologically normal. The explanation for this lack of effect of *trx* in dorsal T1 is unclear. It is possible that *trx* is only required in this compartment during early development. Alternatively, at least in the case of the humerus, it may reflect the delay between the time of irradiation and the subsequent segregation of the recombinant chromosomes, since, like the abdominal

histoblasts, the humeral disc cells do not begin dividing until the pupal stage (Madhavan & Schneiderman, 1977).

*trx*$^-$ clones have revealed a requirement for the gene in derivatives of the head segments unaffected by the *trx*$^1$ mutation. Absence of *trx*$^+$ in the proboscis and antenna results in a transformation of these structures to mesothoracic leg. In both cases, this transformation is incomplete in that clones form only distal leg structures (tarsal segments and tibia) whilst the proximal region of both the proboscis and antenna appear untransformed.

The genetic control of development of the head segments is less well understood than that of the thoracic and abdominal segment. Dominant *Antp* mutations result in transformation of the antenna to mesothoracic leg, which may be more or less complete. The *Antp* gene is not essential for antennal development (Denell, Hummels, Wakimoto & Kaufman, 1981; Struhl, 1981*c*). Rather, it is required in the mesothoracic segment to suppress antennal development (Struhl, 1981*c*, 1983). A likely interpretation of the dominant *Antp* phenotype therefore is that it is the consequence of inappropriate expression of *Antp*$^+$ in the antennal segment, resulting in the repression of an unspecified antennal gene or genes. The identity of these latter genes is uncertain. *spineless anstapedia* (*ss*$^a$) mutant alleles result in a partial transformation of the antenna to leg; interestingly, like *trx*$^-$ clones, the effects of these mutations are limited to the distal-most regions of the antenna. Hence the effect of absence of *trx*$^+$ in the antennae is equivalent to loss of expression of the *ss*$^a$ gene. Two genes are known to be required for the correct developmental programme of cells of the labial segment, from which the proboscis is derived. In the absence of the *Scr* gene the proboscis is transformed to maxillary palps (Lewis *et al.* 1980*a*,*b*) whilst absence of the *proboscipedia* gene, on the other hand, transforms the proboscis to prothoracic leg (see Kaufman *et al.* 1980). Since *trx*$^-$ clones in the proboscis are most likely transformed to second rather than first leg, their phenotype corresponds to the loss of neither *Scr* nor *pb* alone. However, the transformation is consistent with the combined effect of loss of *Scr*$^+$ from a proboscis which is also *pb*$^-$.

In addition to the requirement for the *ss*$^a$ and *pb* genes in adult head development, it is expected that other 'head' genes are involved in controlling the development of derivatives such as the eye and head capsule. *trx*$^-$ clones cause transformations of these structures to tissue resembling mesonotum. Thus it is clear that the *trx* gene is required during the larval stages for the normal development of much of the head. It seems likely, that by analogy to its effects on BX-C expression, *trx* is essential for the correct expression of several 'head' genes (Struhl, 1983) in imaginal cells.

### trx$^+$ *is required during embryonic and larval development*

Since animals homozygous for *trx* null alleles or deficiencies die at the end of embryogenesis, it is clear that there is an early requirement for *trx*. Capdevila & Garcia-Bellido (1981) proposed that the activity of *trx* is indeed restricted to

embryogenesis, its function being the initiation of the selective expression of the BX-C genes in different segment primordia. This has been refuted by the demonstration that the requirement for *trx* in the thoracic segments persists beyond the completion of embryogenesis (Ingham, 1981). The results of the present analysis show that there is a continuing requirement for *trx* in many adult tissues at least until the early third instar. Paradoxically, whilst removal of the $trx^+$ gene results in the transformation of adult derivatives of thoracic, some head, and at least one (but probably all) abdominal segments to mesothorax, a corresponding transformation of larval segments does not occur (Duncan & Lewis, 1982; Ingham, 1983). Thus BX-C expression is totally suppressed in the imaginal A8 segment, but only slightly reduced in its larval counterpart. This difference in requirement between larval and adult cells suggests that the *trx* gene may play a crucial role in the maintenance of expression of the BX-C and other selector genes during the proliferation of the imaginal cells.

This interpretation is supported by the analysis of cell lineage of partially transformed forelegs in $trx^1/trx^2$ flies. Cells marked during the first larval instar can still contribute to both normal prothoracic and transformed mesothoracic tissue. This suggests that the $trx^1$ mutation causes an instability in the heritability of the decision to become prothoracic, such that some cells may revert to the mesothoracic level. This is not inconsistent with the early temperature-sensitive period and maternal effect of the $trx^1$ mutation (see Ingham & Whittle, 1980). These may reflect a particularly high requirement for the *trx* gene early in embryogenesis but they do not rule out a subsequent continual function for the gene. If *trx* were autogenously regulated, either directly or via an intermediate such as a BX-C product, then a shortfall in *trx* activity early in embryogenesis could result in a reduction in activity of the gene thereafter.

### trx$^+$*is required for correct compartmental identity*

$trx^-$ clones in the posterior wing cause the differentiation of structures resembling those of the anterior wing. This phenotype is similar to that caused by mutations of the *engrailed* gene, which controls the development of the posterior compartments (Morata & Lawrence, 1975; Lawrence & Morata, 1976). Though analagous posterior to anterior transformations are not observed in other structures, it is interesting that $trx^-$ clones in the posterior compartments of the legs and proboscis exhibit some abnormalities characteristic of clones of lethal *engrailed* alleles. Taken together, these effects of loss of $trx^+$ on posterior structures suggest a role for the gene in maintaining the anterior/posterior distinction. It is possible that *trx* is required for expression of the *en* gene itself; however it should be noted that no evidence of $trx^-$ clones transgressing compartment boundaries was found.

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