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The sex-determining gene *tra* of *Drosophila*: molecular cloning and transformation studies

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In *Drosophila*, the primary signal for sex determination is given by the ratio of X chromosomes to sets of autosomes (X:A). The primary signal is read by a key gene (*Sxl*) and transmitted down to the differentiation genes by the subordinate control genes *tra, tra-2, ix* and *dsx*. Mutations in *tra* transform chromosomal females (X/X; *tra/tra*) into sterile males (pseudomales). We have cloned the *tra* region by microdissection and chromosomal walking. We identified the gene using deficiency breakpoints, DNA aberrations in three different alleles of *tra* and by P-mediated transformation. A 3.8-kb fragment perfectly rescued the mutant phenotype of X/X; *tra/tra* flies, showing that it contained all the necessary information to restore female-specific functions in the mutant flies. We present evidence that most of the function of *tra* can be provided by a subsegment of 2 kb that is differentially transcribed or processed in males and females.

Key words: sex determination/P-mediated transformation/intersexual phenotype/sex-specific RNA

Introduction

Sex determination was one of the first developmental processes studied in *Drosophila* (Bridges, 1921, 1925; Morgan, 1926; Dobzhansky and Schultz, 1934). As shown by Bridges (1921, 1925), the ratio of X chromosomes to sets of autosomes (X:A) forms the primary signal that initiates either the male or the female pathway of development in all cells of the fly. A ratio of 1.0 leads to female development, a ratio of 0.5 to male development. The Y chromosome plays no role in sex determination, but is required for male fertility (Bridges, 1921; Kennison, 1981).

Over the years, the isolation and characterization of mutations interfering with sex determination have shown that several distinct genes, *Sex lethal* (*Sxl*), *transformer* (*tra*), *transformer-2* (*tra-2*), *intersex* (*ix*), *doublesex* (*dsx*), are involved in the process of sexual differentiation. Their differential activity initiates and maintains the sexual pathway in somatic cells (for review, see Baker and Belote, 1983; Cline, 1985; Nöthiger and Steinmann-Zwicky, 1985). In response to the primary signal of the X:A ratio, the key gene *Sxl* becomes irreversibly set around the blastoderm stage (Sanchez and Nöthiger, 1983; Cline, 1984). Epistatic relationships between mutations in the different genes imply that the state of *Sxl* regulates the subordinate control genes *tra*, *tra-2* and *ix*. These in turn control *dsx* whose products transmit the sex-determining signal down to the differentiation genes (Baker and Ridge, 1980; Steinmann-Zwicky and Nöthiger, 1985). How this regulation is effected is not known. The genes may act directly on *dsx* at the transcriptional or the post-transcriptional level or indirectly, through as yet unknown intermediate genes.

The genetic data indicate that *dsx* is a bifunctional locus, active in males to suppress female-specific development, and in females to suppress male-specific development. The role of *tra*, *tra-2* and *ix* is to direct *dsx* to the female mode of operation and to maintain it in this state. Recessive mutations in *tra* or *tra-2*, corresponding to a loss of function, transform chromosomal (XX) females into pseudomales that are morphologically, physiologically and behaviorally male, but sterile; the mutations have no effect on the somatic cells of chromosomal (XY) males (Sturtevant, 1945; Watanabe, 1975). This shows that the somatic functions of *tra* and *tra-2* are only essential to promote female sexual differentiation, and suggests that these genes may be differentially active in the two sexes. Developmental and genetic analyses demonstrated that the products of *tra* and *tra-2* are required throughout development to maintain the cells in the female pathway (Baker and Ridge, 1980; Wieschaus and Nöthiger, 1982; Epper and Nöthiger, 1982; Epper and Bryant, 1983; Ehrensperger, 1983; Belote *et al.*, 1985). These experiments demonstrate that sex differentiation is under the permanent control of the sex-determining genes which specify and maintain alternative determined states in a way analogous to the specification of alternative segmental identities by homoeotic genes such as *bithorax* or *Antennapedia*.

In this paper we report the cloning of the *transformer* gene (*tra*) and the use of P-mediated transformation to identify a small DNA fragment (3.8 kb) that contains the functional *tra* gene.

Results

Cytological mapping of tra

Baker and Ridge (1980) mapped the *tra* locus cytologically to region 73A on the left arm of the third chromosome. To obtain a more precise localization, we generated a series of deficiencies in this region by X-irradiation, using the fact that the eye color mutation *scarlet* (*st*) is closely linked to *tra*. We isolated deficiencies for *st* (see Materials and methods) and tested them in *trans* over the mutation tra^1 (Sturtevant, 1945) to distinguish between those that also included the *tra* locus and those that did not. The cytological extents of the deficiencies were then determined on salivary gland chromosomes. The results, summarized in the schematic map in Figure 1, indicate that *tra* should be found between the proximal breakpoint of Df E5, a deficiency that does not include *tra*, and the proximal breakpoint of Df E52 that is tra^-. Since these two breakpoints are both around 73A9,10 and are cytologically indistinguishable, these results localize *tra* very precisely in the interval 73A9–10.

Cloning of the tra region

We began the molecular cloning of *tra* by microdissecting the 73A3,4–73B1,2 region from polytene chromosomes using the microcloning technique (Scalenghe *et al.*, 1981; Pirrotta *et al.*, 1983b). We obtained a total of 73 clones containing *Eco*RI frag-

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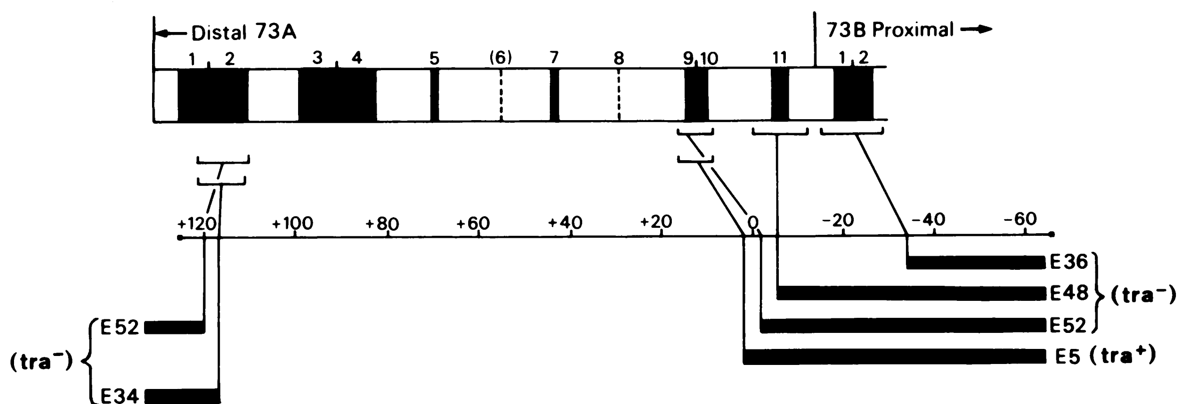

Fig. 1. Schematic drawing of the 73A–73B chromosome region. Cytological bands are drawn above, approximately aligned with the cloned DNA region, illustrated below. The extents of deficiencies E36, E48, E52, E5 and E34 are represented below with black bars indicating DNA present. Their cytological breakpoints are shown by brackets on the chromosome map above while their positions on the DNA map were deduced from genomic Southern blot hybridizations such as those in Figure 3. The deficiencies are marked as *tra*$^-$ if the *tra* gene is deleted or *tra*$^+$ if it is present.

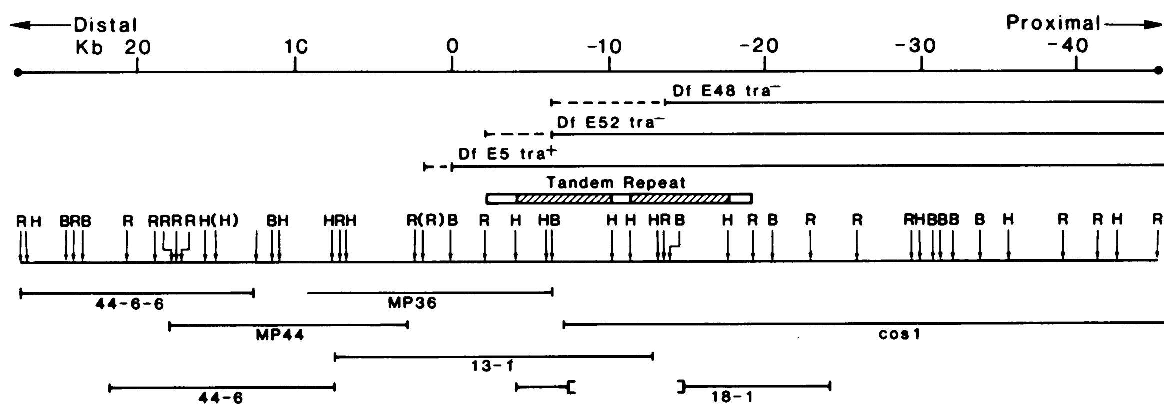

Fig. 2. Map of the region surrounding the *tra* lcous. The map is calibrated in kb and restriction enzyme sites are symbolized by R: *Eco*RI, B: *Bam*HI, H: *Hin*dIII. A tandem repeat of ~7 kb is indicated by the crosshatched bars with the white extremities representing the uncertainty in the limits. Representative phage and cosmid clones are shown below the restriction map. Clone 18-1 was isolated in a form suggestive of the deletion of one tandem repeat unit as indicated by the parenthesis. The breakpoints of three deficiencies are given with the solid line indicating DNA present and the dotted line the uncertainty in the breakpoint position.

ments from three microdissected chromosomes. By screening these clones with total nick-translated *Drosophila* DNA we eliminated those containing repetitive sequences and were left with 51 'single-copy' clones. Inserts ranging in size from 2 to 10 kb, isolated from some of these clones, were used as probes to screen both phage and cosmid libraries. Individual probes yielded groups of related phage and cosmid clones which were mapped against one another to determine overlap. We then employed chromosome walking techniques (Bender *et al.*, 1983) to connect groups of overlapping clones and to obtain a continuous cloned region. The orientation of this region was determined by *in situ* hybridization using probes from both ends.

The continuous cloned DNA region covers ~200 kb and maps to the cytological interval from 73A1,2 to 73B as shown schematically in Figure 1. Figure 2 shows a restriction map of that part of this region that is relevant to the *tra* locus. The entire region was covered by overlapping clones of which only representative examples are shown. The interval between position −17 and −3 presented some difficulties because of the presence of a tandemly repeated 7-kb sequence. As a consequence of this tandem repeat, λ clones containing this region are unstable and tend to give rise to deletion variants. For example, during growth phage 13-1 produces a shorter variant that has lost 7 kb from the insert. Clone 18-1 was isolated in a form that had already suffered a similar deletion.

Location of the deletion breakpoints

The breakpoints of deficiencies in the DNA map were determined by hybridization of the clones to genomic Southern blots of the various deficiency stocks. Since all the deficiencies in question are homozygous lethal, the stocks were heterozygous for a balancer third chromosome, *TM3*. As a consequence, in the DNA of these flies, the deleted region is always represented by one copy of the sequence in the balancer chromosome. Restriction fragments containing a deletion breakpoint should give rise to a new band. We used these criteria to localize the breakpoints of a number of deficiencies (Figure 1). Three deletions, whose proximal breakpoints are closely clustered, are of particular interest. These are E48, E52 and E5 which are mapped in greater detail in Figure 2. Genomic Southern blots of these breakpoints are shown in Figure 3 where probes corresponding to different genomic intervals reveal in turn a breakpoint band in each of the deficiency mutants. Note that because of the 7-kb tandem duplication, the hybridizations in some cases reveal additional bands not contained within the probe itself.

Since the E52 and E5 breakpoints bracket the *tra* locus, we chose the *Bam*HI site midway between them as the zero point

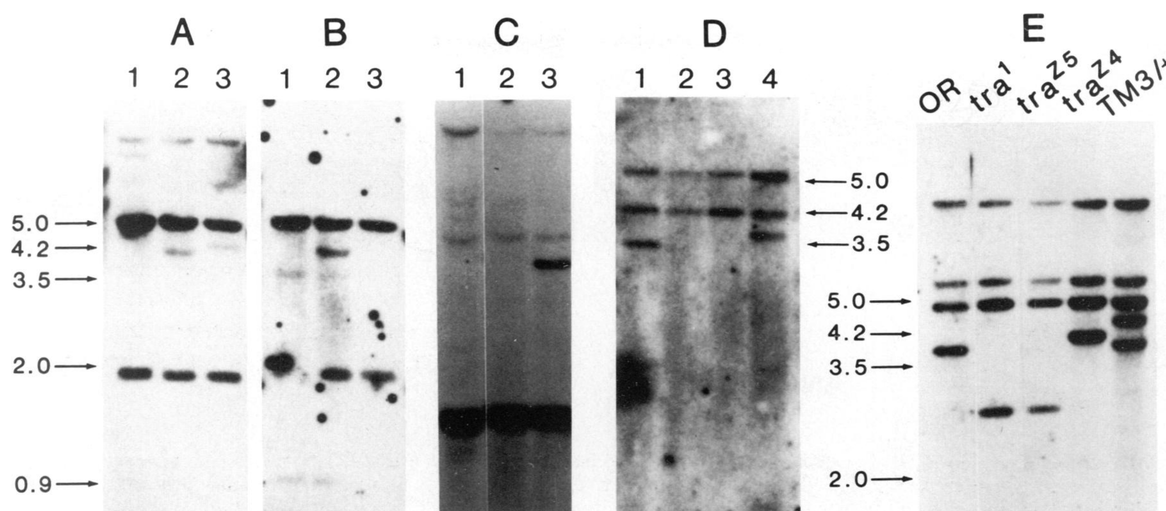

Fig. 3. Genomic Southern blots of deficiencies and *tra* mutants. In **panel A, B, C** and **D** the DNAs analysed were: **lane 1** Df E5; **lane 2** Df E48; **lane 3** Df E52; **lane 4** *TM3*/+ control. **Panel A:** the DNAs were cut with *Hin*dIII and hybridized wth clone 13-1 DNA. **Panel B:** digestion was with *Hin*dIII and hybridization with the proximal fragment of clone 13-1, from the *Bam*HI site at position −6 to the proximal end of 13-1. **Panel C:** digestion was with *Hin*dIII and hybridization with the proximal *Bam*HI fragment of clone MP36 (positions 0 to −6). **Panel D:** digestion was with *Eco*RI and hybridization with the distal part of clone MP36 from position 0 to the *Hin*dIII site at +7. All deficiency stocks were balanced with *TM3*. Note that the *TM3* chromosome lacks an *Eco*RI site at +2. As a result the *Eco*RI 3.8 fragment from *TM3* becomes 4.3 kb. In **lane 4** the *TM3*/+ DNA is shown to demonstrate the normal *Eco*RI 3.8 band. **Panel E:** genomic DNA from adult males homozygous for the different *tra* alleles indicated was digested with *Eco*RI, run on an agarose gel and the blot was hybridized with clone MP36 DNA. OR is Oregon R wild-type DNA.

of our DNA map (Figure 2). The proximal breakpoint of E48 maps then in the interval −13 to −6, that of E52 between −6 and −2 and the E5 breakpoint is between 0 and +2. These results therefore place all or part of the *tra* locus in the 8-kb interval between −6 and +2.

tra mutations

Three recessive *tra* mutations, resulting in loss of *tra* function, cause alterations in the DNA corresponding to this region. tra^1 (Sturtevant, 1945) and tra^{Z4} (M.Roost and R.Nöthiger, unpublished) are two spontaneous *tra* mutations. The tra^{Z5} mutation was isolated from a P-M/I-R dysgenic cross (E.Jacquenoud and R.Nöthiger, unpublished) but contains neither P nor I element sequences in the 73A region. In all three cases, the *Eco*RI 3.8-kb fragment spanning the zero point in Figure 2 is changed in size. Further genomic Southern blots showed that tra^1 and tra^{Z5} have a deletion of ~1 kb in the 0 to −2 interval while tra^{Z4} has a small insertion of ~200 nucleotides in the same interval. We detected no other significant differences in the restriction pattern of a 70-kb region around the origin.

These results support the conclusion derived from the deficiency breakpoints that *tra* is located near the origin in our map. This hypothesis was put to the test directly by reintroducing DNA from this region into the genome of *tra* flies by P-mediated germ line transformation (Spradling and Rubin, 1982; Rubin and Spradling, 1982).

P-mediated transformation

Although we tested several DNA fragments in the interval between the breakpoints of Df E48 and Df E34, only three are relevant to this discussion. These are the *Eco*RI 3.8-kb fragment (R) spanning the origin, the 7.5-kb *Bam*HI fragment from position 0 to the distal border of clone MP36 (BH) and the 6-kb *Bam*HI fragment from position 0 to −6 (BL) (Figure 4). These fragments were cloned in the P-vector pUChsneo (Steller and Pirrotta, 1985) which carries the bacterial neomycin resistance gene and renders larvae resistant to G418.

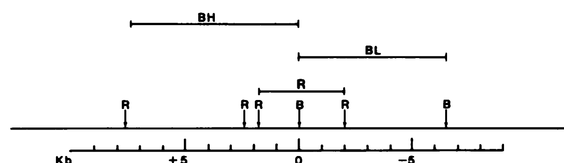

Fig. 4. Transposons used for germ line transformation. The segments indicated in the map were cloned in the pUChsneo vector for P-mediated transformation (Steller and Pirrotta, 1985) and injected into tra^+/tra^+ embryos.

Each construct was injected into embryos and the surviving adults were mated with suitably marked *tra*/*TM3* flies (see details in Materials and methods). In each case the progeny was selected on food containing G418 and crossed again to suitable *tra* partners to test for rescue of the *tra* phenotype in X/X; *tra*/*tra* flies. Since, for convenience, the injected flies were tra^+, transposons inserted in the third chromosome were linked to a tra^+ allele and the corresponding flies could not be easily tested for transposon-mediated *tra* activity. We therefore discarded lines in which G418 resistance was linked to the third chromosome.

Eight lines transformed with the R transposon were retained. In these lines, the tra^+ allele segregated with the X or with the second chromosome and *in situ* hybridization confirmed the presence of the transposon on the corresponding chromosome. In each case, one copy of the R transposon gave perfect rescue of X/X; *tra*/*tra* flies so that they developed into morphologically normal and fertile females. Full rescue was obtained regardless of the *tra* allele used: tra^1, tra^{Z4} or even tra^1/DfE48, showing that a fully functional *tra* gene is contained within the 3.8-kb *Eco*RI fragment.

None of the five G418-resistant lines established with the BH transposon gave any visible sign of rescue of the *tra* phenotype. In contrast, all seven lines established with the BL transposon

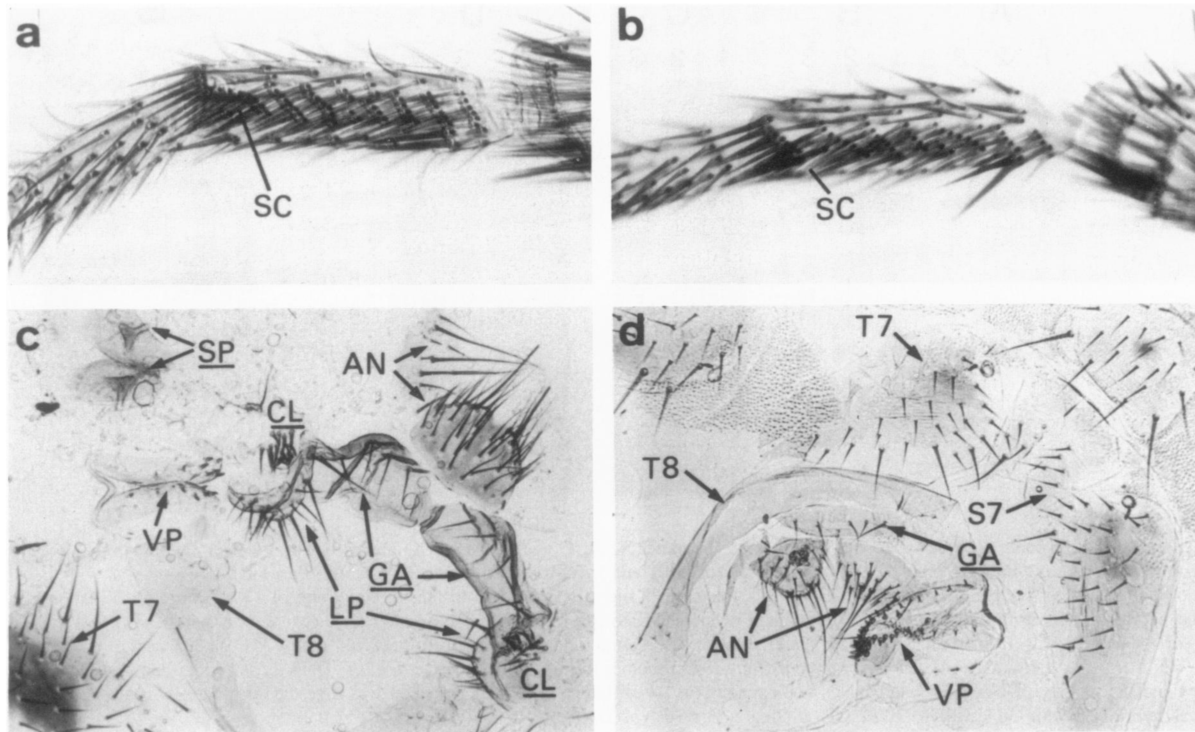

Fig. 5. Sexual phenotypes of foreleg basitarsi **(a,b)** and terminalia **(c,d)** in X/X; *tra/tra* animals carrying one or two doses of a BL transposon. **(a)** one dose of BL2, **(b)** two doses of BL2, **(c)** one dose of BL15, **(d)** two doses of BL15. BL2 and BL15 are two lines in which the BL transposon had integrated at different positions on the second chromosome, resulting in different degrees of rescue (see text). Note the rotated position of the sex comb (SC) in **(a)** compared with SC in **(b)** where this row of bristles is almost female in appearance. **(c)** and **(d)** show the terminalia (genitalia and analia) and tergites (T7) and sternites (S7) of abdominal segment 7. The animal in (c) is strongly intersexual with reduced and abnormal male and female genitalia. Male genitalia (underlined symbols): CL, clasper; GA, genital arch; LP, lateral plate; SP, sperm pump; female genitalia: T7, T8, tergites 7 and 8; VP, vaginal plate. The anal plates (AN) are intersexual. In contrast, the animal in (d) is practically a normal female, with a small remnant of a male genital arch (GA). Some animals with two doses of BL15 have no traces of male structures left and can be fertile females. The sex comb in **(a)** and the genitalia in **(c)** are typically intersexual like those of *dsx* mutants.

gave at least partial rescue. The degree of rescue was characteristic of each line, ranging from weak in the BL3 line to nearly complete in the BL15 line. In line BL3, females homozygous for tra^{Z4} but carrying one copy of the BL transposon have rudimentary male genitalia, in most cases rotated to various degrees. In the basitarsus of the foreleg, the row of bristles that forms the sex comb in males is only partially rotated to the male position, and the bristles are intermediate in shape and thickness. Figure 5a and b shows such intermediate sex combs in the case of line BL2. In lines with intermediate degrees of rescue both female and male genital structures are present to various extents depending on the line (Figure 5c and d). These structures, however, are morphologically abnormal and rudimentary to different degrees. This phenotype is very reminiscent of that displayed by flies that are mutated at the *dsx* locus (Hildreth, 1965; Baker and Ridge, 1980).

Two doses of the BL transposon increase the degree of rescue (Figure 5). In the best cases (lines BL11 and BL15), the rescued animals are almost indistinguishable from normal females. Some are even fertile and produce viable progeny although at a lower rate than normal.

The difference in degree of rescue obtained with the R as opposed to the BL transposon might be due to the additional 1.8 kb of DNA in R acting as a buffer, protecting the gene from position effects, or to specific sequences present in the R but absent in the BL transposon, or to the integrity of a coding region extending across the *Bam*HI site at the zero point. To distinguish between these possibilities, we modified the R transposon by cleaving it at the *Bam*HI site, filling in the *Bam* cohesive ends with the Klenow fragment of DNA polymerase and religating the blunt ends thus produced. The resulting transposon, called R[B], is identical to the R transposon except for a four-nucleotide insertion at the *Bam* site which would be unlikely to cause changes in transcription but which would introduce a frameshift in a coding sequence. Of three independent lines transformed with this transposon, one showed complete rescue of the *tra* phenotype while two displayed the partial rescue typical of the majority of the BL lines. This result indicates that the integrity of the region surrounding the *Bam* site is required for the high level of rescue displayed by the R transposon.

Transcription of the tra region

The genetic data predict that the *tra* function should be expressed in females but not in males. Preliminary analyses of the RNA species transcribed from the *tra* region and surroundings suggest a remarkable transcriptional complexity (Figure 6). RNA species of five size classes (mol. wts 4.0, 1.9, 1.6, 1.3 and 0.9 kb) are detected in adult females.

The 4-kb RNA is transcribed from right to left in the map shown in Figure 6b and clearly initiates outside of the region that contains a functional *tra* gene as defined by the R transposon.

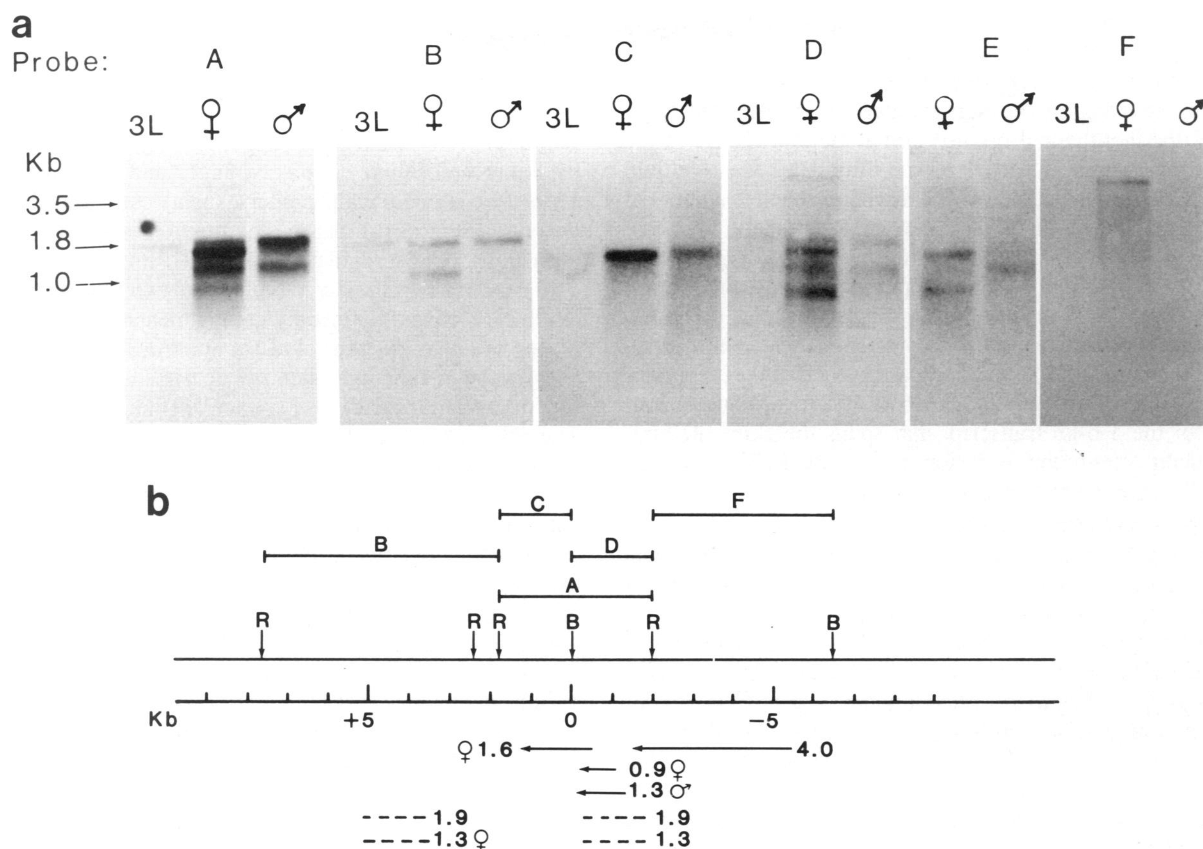

Fig. 6. (a) Northern blot hybridization. Poly(A)$^+$ RNA (5−10 μg) from the third instar larvae, adult females or adult males was electrophoresed on agarose gels, transferred to nylon filters and hybridized to nick-translated probes A−F, representing the intervals shown in (b). Probe E was single-stranded from the same interval as probe D (0 to −2) and complementary to RNA transcribed from right to left in the map. **Panels A, C** and **E** represent a single filter, stripped of the preceding probe and rehybridized in succession. **Panels B, D** and **F** represent a second filter similarly reused. (b) Summary of probes used and transcripts detected. The extent of probes A−F is indicated above the map. Below are shown the transcripts deduced from the Northern blot experiments. The dotted lines represent RNA species of 1.9 and 1.3 kb detected with probes B and D but not with probe C. The precise start and end points of the transcripts have not been determined. ♀ and ♂ indicate transcripts wholly or partly female- and male-specific, respectively.

It is therefore unlikely to be involved in *tra* function. The RNA of 0.9 kb appears to be female specific while that of 1.6 kb is at least several times more abundant in females than in males. The 0.9-kb species is contained in the 0 to −2 interval while the 1.6-kb species straddles the *Bam* site that defines the zero point of the map. Hybridization with a single-stranded probe E (0 to −2 interval) shows that these two RNAs are transcribed from the same strand, from right to left in the map shown in Figure 6b.

RNAs of 1.9 and 1.3 kb are seen with both probes D and B, but not with probe C. It is possible that the transcripts contain introns spanning the entire 0 to +2 interval. RNAs of these two sizes are seen in both males and females using probe A (the *Eco*RI 3.8-kb fragment) and probe D (the 0 to −2 interval) but the 1.3-kb species seen in the male must be a different transcript from that seen in the female since it is not detected with probe B. This is confirmed by hybridization with the single-stranded probe E which reveals the male but not the female 1.3-kb species.

In summary, these results suggest that six different RNA species are encoded by the region surrounding the *tra* locus. Three of these, of 1.6, 1.3 and 0.9 kb, are predominantly or exclusively female and one of 1.3 kb is male specific. These transcripts are also represented in third instar larvae but at a much lower level than in adults.

Discussion

The tra functional unit

The chromosomal walk ranges from 73A3,4 to 73B1,2. It covers ~200 kb and includes both the *scarlet* locus at the distal end and the *Dash* locus (Hoffman *et al.*, 1984; Telford *et al.*, 1985) at the proximal end. The deficiencies place the *tra* locus in the middle of this region, at 73A9,10, in an interval of <8 kb, a position that is supported by the mapping of DNA aberrations in the tra^1, tra^{Z4} and tra^{Z5} mutants.

Conclusive proof of the presence of the *tra* gene in this interval comes from the germ line transformation experiments. These show that the entire functional *tra* gene is contained in a 3.8-kb *Eco*RI fragment mapping from +1.8 to −2. Furthermore, most, if not all of the gene must reside in the proximal 2 kb of this fragment since the BL transposon containing the DNA of the 0 to −6 interval supplies partial and, in some lines, nearly complete *tra* function at least when present in two copies.

The different degrees of rescue shown by the independent lines carrying the BL transposon clearly reflect a position effect that causes variable levels of expression of the gene inserted at different chromosomal sites. The involvement of a quantitative effect is also shown by the fact that two copies of the BL transposon achieve better rescue than one, in some cases resulting in fertile females. In contrast, no position dependence of rescue was ob-

served in the eight lines carrying the 3.8-kb R transposon. Its additional 1.8 kb of DNA might protect the *tra* gene from the influence of position effects, for example by shielding the promoter or by directing efficient termination of a primary transcript. However, the fact that a 4-bp insertion at the *Bam*HI site of the R transposon results in partial rescue eliminates the shielding hypothesis but shows that normal *tra* function requires additional sequences that include the *Bam* site but are absent in the BL transposon. Such sequences could be either regulatory, e.g. needed for high level transcription or for proper processing of the RNA, or they might be additional protein coding sequences. It is possible, though unlikely, that the *Bam* site should happen to form part of a regulatory sequence such as an enhancer. It is more probable that the insertion at the *Bam* site affects a coding region, e.g. that of the 1.6-kb transcript that spans the *Bam* site and, by introducing a frameshift mutation, renders the R[B] transposon functionally equivalent to the BL transposon.

Sex-specific transcripts and tra

Northern blot hybridization with the 3.8-kb *Eco*RI fragment shows at least five RNA species in the adult female. Two RNA species are likely candidates for encoding the *tra* functions required in the female: a 0.9- and a 1.6-kb species. The first of these is female specific and is contained within the 0 to -2 interval in which, according to the transformation experiments, resides the *tra* function. The second RNA, 1.6 kb long, extends across the *Bam*HI site into the region contained in the R transposon but absent in the BL transposon. Although this transcript, or one of the same size, is also found in males at a much lower level, it might encode a function required for full *tra* activity. This could explain the incomplete rescue obtained with the BL and R[B] transposons, in which part of the 1.6-kb RNA coding region is missing or contains a frameshift mutation. Whatever the role of this RNA, the principal function required for female development is most likely encoded by the 0.9-kb species since two copies of BL11 or BL15 can restore femaleness to XX; *tra/tra* flies. A possible interpretation of our transformation results might be that the 0.9-kb RNA species encodes a function essential for female development but that the 1.6-kb RNA supplies an auxiliary function that facilitates the action of the first or helps to suppress male development.

The presence of a male-specific transcript from the 0 to -2 region is unexpected and not predicted by the genetic data. It is transcribed in the same direction as the 0.9- and 1.6-kb RNAs and may represent an alternative processing product. Could this transcriptional unit still be functional in the *tra* mutants? Since this 1.3-kb RNA is only detected in the -2 to 0 interval and since the tra^1 mutation causes a 1-kb deletion in the same interval, it is very unlikely that the sequence encoding this RNA remains unaffected by the mutation. Therefore this RNA is probably not essential for male development since tra^1/tra^1 males are normal and fertile.

The fact that six RNA species hybridize to a 2-kb fragment of single-copy DNA is surprising. We cannot exclude the possibility that some of the RNA species originate from another locus that bears some homology to *tra*. Although such a locus was not detected at the stringency of our genomic blots, its transcripts might be visible in the Northern blots, particularly if they are more abundant than the *tra* transcripts.

Belote *et al.* (1985a) have also reported the cloning of the *tra* region. The results of their transcriptional analysis differ from ours in some respects but they detect a female-specific RNA of 1.1 kb that corresponds to our 0.9-kb species and to which they ascribe the *tra* mRNA function.

The intersexual phenotype of BL lines

Flies of the genotype X/X; *tra/tra* carrying a BL transposon are typical intersexes with the same kind of intermediate sexual differentiation (Figure 5) as that observed in *doublesex* (*dsx*) mutants (Hildreth, 1965; Baker and Ridge, 1980; Epper, 1981; reviewed by Baker and Belote, 1983; Nöthiger and Steinmann-Zwicky, 1985). We suppose that the intersexuality caused by the BL transposon results from ambiguous expression of the *dsx* locus which is inappropriately regulated in our BL lines.

In genetic terms the *dsx* locus harbors two functions, designated dsx^m and dsx^f and defined by complementation. Genetic models place *dsx* under the control of *tra* and *tra-2* so that the locus expresses the *m*-function when *tra* or *tra-2* is inactive, and the *f*-function when *tra* and *tra-2* are active (Baker and Ridge, 1980). The role of tra^+ (and $tra\text{-}2^+$) in normal females is apparently to inactivate the *m*-function and to activate the *f*-function. The *m*-function prevents the expression of the female-specific differentiation genes, the *f*-function that of the male-specific differentiation genes. Introducing a constitutive dsx^m allele, e.g. dsx^D, into X/X animals (genotype X/X; $dsx^D/+$) leads to the simultaneous expresion of dsx^m and dsx^f. This situation appears to produce an ambiguous signal to which the cells respond by intersexual differentiation (Baker and Ridge, 1980; Nöthiger and Leuthold, 1986). We believe that our BL lines simulate the $dsx^D/+$ situation. Qualitatively or quantitatively insufficient *tra* function in the different weakly rescued lines results in expression of both *m* and *f* functions in varying proportions, producing masculinized intersexes. Increasing *tra* activity progressively raises the level of *f* and lowers that of *m* until essentially complete rescue is obtained, as is the case with two doses of BL11 and BL15.

Materials and methods

Generation of deficiencies

The original *tra* allele was isolated by Sturtevant (1945). In this paper we have generally referred to this allele as tra^1 to distinguish it explicitly from other alleles (for other genetic symbols see Lindsley and Grell, 1968). The locus of *tra* was placed by Sturtevant (1945) at position 3-45.0 on the genetic map of the third chromosome, between *st* (3-44) and *cp* (3-45.3). We therefore decided to use *st* and *cp* as visible markers to isolate deficiencies in this region. We irradiated males of the genotype $+/B^SY$; *bw/bw; e/e* with 4000 rad and crossed them to *bw; th st cp ri* p^p virgins. Loss of st^+ in a paternal sperm yields a fly with white eyes (flies homozygous for *bw* and *st* have white eyes); loss of cp^+ would produce a fly with clipped wings, but no such animals were found. Single F_1 males or females with white eyes were test-crossed to *y; TM3, Sb Ser/th st tra cp ri* p^p partners. If the irradiation had stimultaneously eliminated st^+ and tra^+, transformed X/X; *tra/tra* pseudomales appeared. At the same time, the cross established a stock with the mutant chromosome balanced over *TM3*. All chromosomes having lost *st* were homozygous lethal. For cytological analysis, flies from a balanced stock were crossed to homozygous *Ki* p^p *e* partners so that larvae carrying the deficiency over a standard chromosome could be identified and selected. The breakpoints of the deficiencies were determined with the competent help of Henrik Gyurkovics (Biocenter, Szeged, Hungary).

Preparation of flies

For microscopical examination of the cuticular structures, samples of flies were macerated in hot NaOH, washed several times in H_2O and mounted in Faure's solution under coverslips. The internal reproductive organs were studied with a dissecting microscope.

Microdissection and microcloning

The procedure followed was that described by Pirrotta *et al.* (1983b). Salivary gland chromosome squashes were made from homozygous *gt* w^a larvae. Three chromosome fragments were dissected from the 73A3,4 to 73B1,2 region. The combined DNA, cut with *Eco*RI, was ligated to the λNM 641 vector (Murray, 1983) yielding 73 independent microdissection clones.

Genomic libraries and chromosome walking

Inserts excised from selected microdissection clones were used to screen both phage and cosmid genomic clone libraries. We used two phage libraries of partial *Sau*3A fragments cloned in λEMBL 4 (Frischauf *et al.*, 1983). One was made from Oregon R DNA, the other from Canton S flies (Pirrotta *et al.*, 1983a; Mariani

et al., 1985). The cosmid libraries were constructed as described by Pirrotta *et al.* (1983b) or using the cosPneo vector (Steller and Pirrotta, 1985). For chromosome walking, suitable restriction fragments were purified from low melting temperature agarose gels (Maniatis *et al.*, 1982), labelled by nick translation and used to screen the genomic libraries.

Drosophila DNA and genomic Southern blots

DNA was extracted from up to 100 flies disrupted with a Dounce homogenizer, according to Kidd *et al.* (1983). Southern blots were made from agarose gels according to Southern (1975). The nitrocellulose filters were baked for 2 h, prehybridized in 2 × SSC, 10 × Denhardt's solution containing 100 μg/ml denatured calf thymus DNA at 65°C for 4−6 h and then hybridized under the same conditions. The filters were washed with 2 × SSC at 65°C, followed by repeated washes at 65°C with 0.2 × SSC.

Microinjection and selection of transformed flies

Restriction fragments were subcloned in the pUChsneo vector (Steller and Pirrotta, 1985) and injected into early embryos from stock *Df(1)* $w^{67c23(2)}$,*y w*. The injection solution contained the pUChsneo transposon DNA (500 μg/ml) and the phsπ helper plasmid (Steller and Pirrotta, 1986) at 100 μg/ml. Injection procedures were as previously described (Steller and Pirrotta, 1985) except that embryos were chemically dechorionated in 50% Chlorox for 3 min as suggested by A. Spradling (personal communication). The surviving G_0 flies were crossed to corresponding partners with genotype *mwh jv* tra^{Z4} p^p*/TM3, Ser Sb* p^p and the G_1 progeny were selected on instant food (Carolina Biological Supply Co.) reconstituted in water containing 800−1000 μg/ml G418. Males carried a Y chromosome marked with B^S. Resistant flies, carrying the transposon, were crossed repeatedly to tra^{Z4}*/TM3* partners to obtain G_2 or G_3 X/X progeny homozygous for *mwh jv* tra^{Z4} p^p which were then inspected for a rescuing effect of the transposon. Flies carrying the transposon on the third chromosome (bearing the endogenous tra^+ allele) were recognized because they gave only tra^{Z4}/+ and *TM3*/+ progeny when selected on G418 food. These lines were discarded without attempting to test them for ability to rescue *tra*. In some of the lines, the tra^{Z4} chromosome was replaced by tra^1/tra^1 and by tra^1*/DfE48* to test whether the transposon could also rescue these genotypes whose *tra* gene is more severely affected.

Drosophila RNA and Northern blot analysis

RNA was prepared from adult females, males or from unsexed third instar larvae using guanidine thiocyanate and CsCl purification (Chirgwin *et al.*, 1979; Glisin *et al.*, 1974). Poly(A)$^+$ RNA was isolated by two cycles of binding and elution from oligo(dT) cellulose (Collaborative Research).

For Northern blot analysis, 5−10 μg poly(A)$^+$ RNA were dissolved in 0.5 M deionized glyoxal, 50% dimethyl sulfoxide, 10 mM sodium phosphate, pH 7.0. After incubation at 50°C for 1 h the sample was fractionated on 1% agarose gels containing 10 mM phosphate buffer and transferred to nylon filters (GeneScreen plus, New England Nuclear).

The filters were prehybridized to 0.5 M phosphate buffer pH 7.2, 1% bovine serum albumin and 7% sodium dodecylsulfate at 65°C and then hybridized under the same conditions for 48 h. The filters were washed as described by Church and Gilbert (1984).

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