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Molecular analysis of *tra-2*, a sex determining gene in *C.elegans*

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We have cloned the *Caenorhabditis elegans* sex determining gene, *tra-2*, by transposon tagging. The *tra-2* region is delineated by mapping Southern blot differences associated with 11 *tra-2* mutations, mutant rescue, and analysis of *tra-2* RNAs. The *tra-2* gene encodes three transcripts. One transcript, a 1.8 kb RNA, is not detected in animals lacking a germ line, and therefore may be germline specific. Comparison of the two sexes shows that adult hermaphrodites have ~15-fold more *tra-2* RNA than adult males. In addition, adult hermaphrodites contain 5 kb and 1.8 kb *tra-2* RNAs whereas adult males possess 5 kb and 1.9 kb RNAs. A 1.9 kb *tra-2* RNA is also found during hermaphrodite larval development, prior to sexual differentiation of the *XX* germ line. Surprisingly, analysis of *tra-2* expression in selected sex determination mutants reveals that the sex specificity of *tra-2* RNAs is not dictated by the pathway of sex determination that has been established by genetic experiments. This result can be interpreted in two ways. Either the sex specificity of the *tra-2* RNAs is irrelevant to regulation of the sexual phenotype, which seems unlikely, or there is additional complexity within the hierarchy of sex determining genes, such as feedback regulation, which ensures that the *tra-2* product corresponds to phenotypic sex.

Key words: sex determination/*C.elegans*/*tra-2*/transposon tagging

Introduction

The sex determining genes in *Caenorhabditis elegans* have been subjected to intense genetic analysis, but their molecular analysis has just begun (for reviews, see Hodgkin, 1990; Villeneuve and Meyer, 1990). We have focused on the *tra-2* gene to analyze the molecular mechanism of sex determination in *C.elegans*. *XX* animals are normally hermaphrodite, and *XO* are male. Hermaphrodites are essentially somatic females that make sperm and oocytes. Loss-of-function (*lf*) *tra-2* mutations transform *XX* animals into non-mating pseudomales, but do not affect *XO* development (Klass *et al.*, 1976; Hodgkin and Brenner, 1977). Therefore, *tra-2* is required for hermaphrodite development. Gain-of-function (*gf*) *tra-2* mutations lead to inappropriate feminization of *XX*, and in some cases *XO*, animals (Doniach, 1986; Schedl and Kimble, 1988; P.G.Okkema, unpublished). Since *tra-2(lf)* and *tra-2(gf)* mutations result in opposite sexual transformations, this gene acts as a genetic switch to regulate the decision between hermaphrodite and male development. From epistasis experiments, it has been proposed that *tra-2* acts as a negative regulator of the male specifying *fem* genes (Hodgkin, 1986) (Figure 1). The temperature sensitive period (TSP) of *tra-2* spans larval development (Klass *et al.*, 1976) and no maternal effects have been detected (Hodgkin and Brenner, 1977). As a first step in investigating the molecular role of *tra-2*, we have cloned the gene and examined the sex, stage and tissue specificity of *tra-2* RNAs.

Results

Identification and cloning of tra-2

To clone *tra-2*, we isolated spontaneous *tra-2(lf)* mutants in strains active for Tc1 transposition as suppressors of *tra-2(q122gf)* (see Materials and methods). A novel Tc1 present in the DNA of one suppressor, *tra-2(q122q150)*, was cloned (see Materials and methods). Then, using a probe of unique DNA flanking the Tc1 insertion, pJK7, we isolated clones spanning the *tra-2* region from a *C.elegans* genomic library.

To provide evidence that *tra-2* is contained within the cloned region, we examined DNAs from *tra-2* mutants by Southern blot and attempted rescue of *tra-2(lf)* by genetic transformation. We found alterations in four more *tra-2(lf)* mutants (*q276, q270, q149, sc146*) and six *tra-2(gf)* mutants (*e2020, q101, q103, q122, q244, e2046*) (Figure 2A; data not shown). DNA changes in *tra-2(lf)* alleles are scattered across a 10 kb region, whereas those in *tra-2(gf)* alleles are clustered within 1 kb. For mutant rescue, we constructed plasmid pJK274, which was deemed to cover *tra-2* from the extents of DNA alterations in *tra-2* mutants (Figure 2A) and *tra-2* RNAs (see below). pJK274, which includes the region

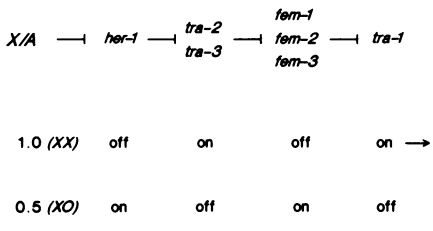

Fig. 1. Regulatory pathway of sex determination in the soma of *C.elegans* (modified from Hodgkin, 1990). The *X:A* ratio affects the activities of the sex determination pathway via the *sdc* genes and *xol-1*, which have been omitted from this diagram for simplicity. *her-1* must be active in *XO* animals in achieve male development by negatively regulating *tra-2* and *tra-3*. *tra-2* and *tra-3* negatively regulate the *fem* genes, which in turn negatively regulate *tra-1*. In somatic tissues, the state of *tra-1* dictates the sexual phenotype. In germline tissues, the situation is more complex: several germline specific sex determining genes are involved and the state of the *fem* genes, not *tra-1*, dictates the sexual phenotype. However, the position of *tra-2*, between *her-1* and the *fem* genes is the same in both germ line and soma.

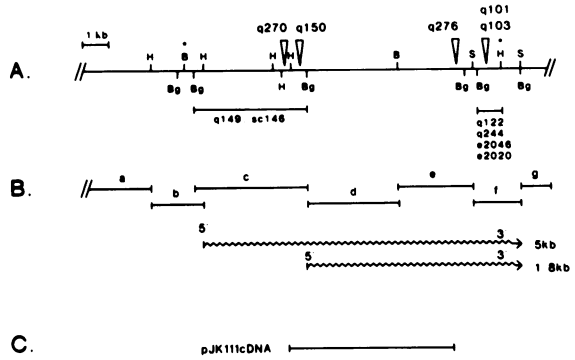

Fig. 2. The *tra-2* region and probes used in this paper. (**A**) Partial restriction map of the *tra-2* region, showing approximate positions of *tra-2* mutations. Triangles above line indicate sites of 1.6 kb (presumably Tc1) insertions: *tra-2(q270)*, *tra-2(q150)*, *tra-2(q276)*, *tra-2(q101)* and *tra-2(q103)*; bracketed lines below line indicate sites of two uncharacterized DNA alterations, *tra-2(q149)* and *tra-2(sc146)*, and four small deletions, *tra-2(q122)*, *tra-2(q244)*, *tra-2(e2046)* and *tra-2(e2020)*. H, *Hin*dIII; Bg, *Bgl*II; B, *Bam*HI; S, *Sal*I. The restriction map is oriented so that *unc-4* is located to the left and *dpy-10* is located to the right. This orientation is based upon an intragenic recombination event that regenerated *tra-2(q122)* from a *tra-2(q122q150)* double mutant (data not shown). (**B**) Genomic probes used to map the extent of the *tra-2* transcription unit. The direction and approximate extent of the 5 kb and the 1.8 kb RNAs is shown below the subclones; this agrees with partial sequence of cDNAs and genomic DNA (P.Kuwabara and J.Kimble, unpublished). The 1.9 kb RNA is detected by the same probes that detect the 1.8 kb RNA. (**C**) cDNA pJK111 was used to probe Northern blots shown in Figures 3–7.

between the *Bam* and *Hin*dIII sites marked by asterisks in Figure 2, was co-injected into *tra-2(e1425) unc-4(e120)/mnC1* animals with a plasmid carrying *rol-6(d)* DNA. Transformed animals carrying exogenous *rol-6* DNA have a distinct roller phenotype (C.Mello, V.Ambros, J.Kramer and D.Stinchcomb, personal communication). Four lines were obtained that produced feminized, but not self-fertile, *unc-4 XX* animals. Several transformants had a female soma, with hermaphrodite somatic gonad and vulva, but had a male germ line; one transformant was fertile in one gonadal arm and produced intersexual progeny. This nearly complete rescue of *tra-2(lf)* indicates that the region contains *tra-2*.

tra-2 RNAs in wild-type hermaphrodites

To identify *tra-2* RNAs, we hybridized strand specific probes spanning the DNA alterations associated with *tra-2* mutants to Northern blots of poly(A)$^+$ RNA prepared from mixed-stage N2 hermaphrodites (data not shown). Four probes (Figure 2B, probes c, d, e and f) hybridized to a 5 kb RNA, and three of these (probes d, e and f) also hybridized to a 1.8 kb RNA. Probes flanking the region (probes a, b and g) do not hybridize to either the 5 kb or 1.8 kb RNA (data not shown). Using probes to the 5 kb and 1.8 kb RNAs, novel-sized RNAs were detected in several transposon-induced and rearranged *tra-2* alleles (data not shown). Therefore, both the 5 kb and the 1.8 kb RNAs are products of *tra-2*.

We next examined *tra-2* RNAs prepared from developmentally staged hermaphrodites (Figure 3). Whereas the 5 kb RNA is observed throughout development, RNAs of ~1.8–1.9 kb are detected at different stages of development. A 1.85 kb RNA is found in embryos; a 1.9 kb RNA is seen in L1, L2 and L3 animals; and a 1.8 kb RNA

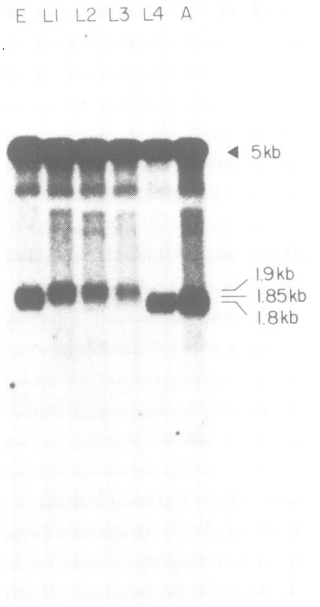

Fig. 3. *tra-2* expression during hermaphrodite development. Oligo(dT)–cellulose selected RNA (~2 μg per lane) was prepared from synchronized animals, electrophoresed through a 1.5% formaldehyde/agarose gel, and blotted to nitrocellulose. The filter was hybridized to a ^{32}P-labeled RNA probe made from *tra-2* cDNA pJK111 (Figure 2C). E, embryos; L1–L4, first to fourth larval stages; A, adults. The approximate size of each RNA is marked. A weakly hybridizing band that migrates above the large rRNA (~3 kb) is also detected. The appearance of this band is variable between RNA preparations; we believe it results from partially degraded *tra-2* RNA that is excluded from the rRNA band.

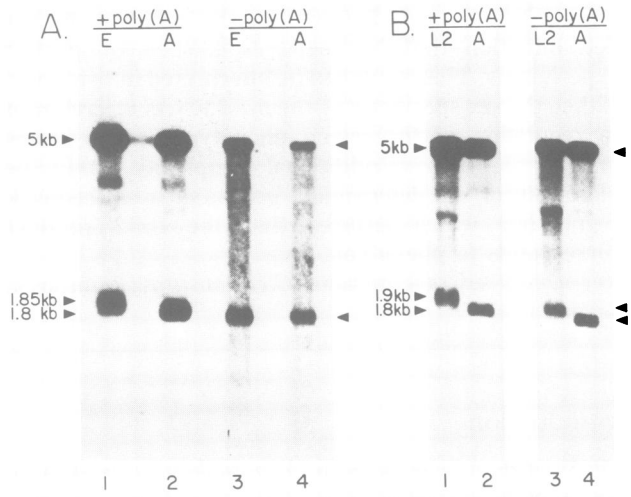

Fig. 4. Examination of *tra-2* transcripts after removal of poly(A) tails. Poly(A) tails were removed from oligo(dT)–cellulose selected RNAs by hybridization to oligo(dT) and digestion with RNase H (see Materials and methods; Vournakis *et al.*, 1975). About 2 μg RNA, either untreated [+poly(A)] or treated [−poly(A)], was electrophoresed, blotted and hybridized to pJK111 as described in the legend to Figure 3. Bands seen before poly(A) removal are marked by arrowheads on the left; bands detected after poly(A) removal are marked by arrowheads on the right. (**A**) Lanes 1 and 3, embryo RNA; lanes 2 and 4, adult RNA. (**B**) Lanes 1 and 3, L2 RNA; lanes 2 and 4, adult RNA.

is present in L4 animals and adults. Analysis of more tightly synchronized populations of animals indicates that early to mid-L4 animals contain the 1.9 kb RNA and not the 1.8 kb

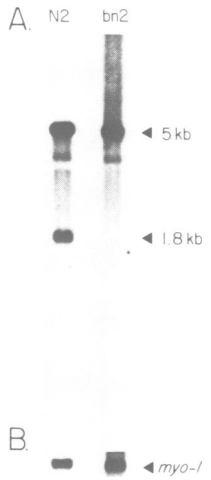

Fig. 5. Tissue specificity of *tra-2* expression. (**A**) Oligo(dT)-selected RNA was electrophoresed, blotted and hybridized to pJK111 as described in the legend to Figure 3. N2, wild-type adult hermaphrodites, which have ~2500 germ nuclei per animal; *bn2*, adult *glp-4(bn2)* animals grown at non-permissive temperature, which have ~7–16 germ nuclei per animal. (**B**) Blot in (A) rehybridized with a ^{32}P-labeled RNA probe specific for *myo-1*, a myosin expressed specifically in the pharynx, to normalize the somatic equivalents of RNA loaded.

Table I. Relative amounts of 5 kb *tra-2* RNA[a]

	tra-2[a]	*myo-1*[a]	*tra-2/myo-1*
A. Tissue differences			
wild-type[b]	162	175	0.93
glp-4(bn2)[c]	57	353	0.17
B. Sex differences			
XX hermaphrodite[d]	410[f]	276	1.49
XO male[e]	26	250	0.10

[a]Values (in arbitrary units) determined by scanning laser densitometry of Northern blots with an average of two to three scans through different regions of each band.
[b]From blot in Figure 5A.
[c]From blot in Figure 5B.
[d]From blot in Figure 6A.
[e]From blot in Figure 6B.
[f]This value is probably an underestimate, because the signal is beyond the linear range of the film.

RNA (data not shown). The Northern blot in Figure 3 was probed with a strand specific probe made from a *tra-2* cDNA. Therefore, all four RNAs are transcribed from the same DNA strand, and they share common sequences.

The size similarity of the 1.8, 1.85 and the 1.9 kb *tra-2* RNAs suggested that they may differ simply by the amount of polyadenylation. To test this possibility, RNAs were compared before and after removal of poly(A) (Figure 4). Although the embryonic 1.85 kb RNA does not co-migrate with the adult 1.8 kb RNA before removal of the poly(A) (Figure 4A, lanes 1 and 2), these RNAs co-migrate after removal of the poly(A) (Figure 4A, lanes 3 and 4). Therefore, the 1.8 kb and 1.85 kb RNAs differ only in the length of their poly(A) tails. In contrast, the 1.9 kb RNA is not a more highly polyadenylated version of the 1.8 kb RNA. Before poly(A) removal, the L2 1.9 kb RNA forms a diffuse band that migrates more slowly than the adult 1.8 kb RNA (Figure 4B, lanes 1 and 2). After poly(A) removal, the L2 RNA still migrates more slowly than the adult RNA (Figure 4B, lanes 3 and 4). Therefore, the 1.9 kb RNA must contain sequences not present in the 1.8 kb RNA. However, the 1.8 kb and 1.9 kb RNAs are transcribed from roughly the same region (data not shown), suggesting that they are similar. We conclude that *tra-2* produces at least three transcripts in *XX* animals: a 5 kb RNA throughout development; a 1.8 kb RNA in embryos, L4s and adults; and a 1.9 kb RNA in larvae.

Tissue specificity of tra-2 expression

In *C.elegans* hermaphrodites, regulation of the sexual phenotype differs in germ line and soma (see Hodgkin, 1990 for review). We therefore compared *tra-2* RNAs prepared from wild-type hermaphrodites, which have ~2500 germ cells, with those isolated from mutant adults with few germ cells. Adult *glp-4(bn2)* animals grown at restrictive temperature (25°C) have ~7–16 germline nuclei that remain mitotic (Austin and Kimble, 1989); adult *glp-1(q224)* animals grown at restrictive temperature (25°C) have 4–8 germline nuclei that have all entered meiosis and differentiated as sperm (Austin and Kimble, 1987). We found that the 1.8 kb *tra-2* RNA is not detectable in either *glp-4(bn2)* (Figure 5A) or in *glp-1(q224)* (data not shown). The simplest interpretation of this result is that the 1.8 kb RNA is expressed only in the hermaphrodite germ line. We also found that the 5 kb RNA is about five times less abundant in mutant than in wild-type (Figure 5B; Table IA). Therefore, the 5 kb RNA appears to be expressed in both germ line and soma.

tra-2 expression in wild-type males

To investigate sex specific differences in *tra-2* RNAs, we first compared RNAs prepared from adult hermaphrodites and males (Figure 6). Whereas the *tra-2* RNAs in adult hermaphrodites are 5 kb and 1.8 kb (see above), those in adult males are 5 kb and 1.9 kb (Figure 6A and C). The 1.9 kb RNA detected in males may be the same as that in larval hermaphrodites: the RNAs co-migrate (data not shown) and each forms a diffuse band. The larval 1.9 kb RNA in hermaphrodites occurs when its germline is either not sexually determined or is preparing for spermatogenesis. Therefore, this 1.9 kb RNA may be indicative of male development even though it is present in hermaphrodites. In addition, males accumulate at least 15-fold less of the 5 kb *tra-2* RNA than do hermaphrodites (Figure 6A and B; Table IB).

RNAs from developmentally staged animals of a strain that is 50% male and 50% hermaphrodite were also examined. The developmental profile was virtually identical to that seen for hermaphrodites alone (Figure 3; data not shown). Therefore, no novel-sized male specific RNA is detected during development.

tra-2 expression in sex determination mutants

To test whether the sex specific differences in adult *tra-2* RNAs are regulated by the sex determination cascade (Figure 1), we examined *tra-2* RNAs in mutants whose phenotypic sex is not appropriate to their *X/A* ratio. *XX* animals homozygous for *tra-1(e1099)* develop as males rather than hermaphrodites (Hodgkin and Brenner, 1977); *XO* animals homozygous for *fem-3(e1996)* develop as females rather than males (Hodgkin, 1986). By genetic tests,

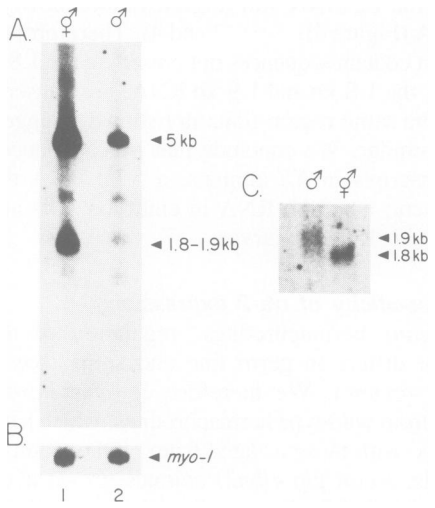

Fig. 6. *tra-2* expression in adult hermaphrodites (♂) and males (♀). Electrophoresis and blotting were done as described in the legend to Figure 3. (**A**) *tra-2* RNAs detected with cDNA pJK111. Lane 1, total RNA isolated from 1000 wild-type adult hermaphrodites; lane 2, total RNA isolated from 1000 wild-type adult males. The 5 kb and the 1.8/1.9 kb *tra-2* RNAs are indicated. Two other bands (~2.8 kb and 1.4 kb) are due to non-specific cross-hybridization to *Escherichia coli* rRNA that contaminates total RNA preparations (data not shown). (**B**) Rehybridization of blot in (A) with a probe for pharyngeal specific *myo-1* to normalize the amount of RNA loaded in each lane. The pharynges of adult hermaphrodites and males are approximately the same size. (**C**) 1.8/1.9 kb *tra-2* RNAs detected by *tra-2* cDNA pJK111. Lane 1, oligo(dT)-selected RNA from 3600 adult males; lane 2, oligo(dT)-selected RNA from 100 adult hermaphrodites.

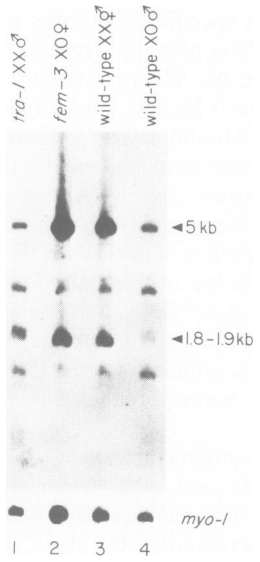

Fig. 7. *tra-2* RNA levels in adults are determined by the phenotypic sex rather than by the *X/A* ratio. Total RNA isolated from 1000 adults of the indicated genotype was electrophoresed, blotted and hybridized to pJK111 (top) and rehybridized to pharyngeal specific *myo-1* (bottom) as described in the legend to Figure 3. Lane 1, *tra-1(e1099) XX* males; lane 2, *fem-3(e1996);lon-2(e678)/O XO* females; lane 3, *lon-2(e678) XX* hermaphrodites; lane 4, *lon-2(e678) XO* males. See text for explanation. *lon-2(e678)* is an X-linked marker used to identify *XO* males (see Materials and methods). Bands of ~2.8 kb and 1.4 kb are due to non-specific cross-hybridization to *E.coli* rRNA contaminating preparations of total RNA (data not shown).

tra-1 and *fem-3* have been placed downstream from *tra-2* in the regulatory pathway for *C.elegans* sex determination (Figure 1). Therefore, *tra-1* and *fem-3* mutations should not interfere with regulation of *tra-2* by the *X/A* ratio. Surprisingly, *tra-2* RNAs in adult *tra-1 XX* males are similar to those in wild-type *XO* males (Figure 7, lanes 1 and 4). In addition, *tra-2* RNAs in adult *fem-3 XO* females are similar to those in wild-type *XX* hermaphrodites (Figure 7, lanes 2 and 3). We also examined *tra-2* RNAs in a *her-1* mutant. *XO* animals homozygous for *her-1(e1518)* develop as hermaphrodites rather than males (Hodgkin, 1980). The *her-1* gene has been placed upstream of *tra-2* in the pathway of regulation (Figure 1). As might be expected, *tra-2* RNAs in adult *her-1(e1518) XO* hermaphrodites are similar to those in wild-type *XX* hermaphrodites (data not shown). Thus, the sex specific differences in adult *tra-2* RNAs reflect the phenotypic and not the chromosomal sex of the animal.

Discussion

We have cloned the *C.elegans* sex determining gene, *tra-2*. Identification of *tra-2* is based on DNA alterations associated with 11 *tra-2* mutants and mutant rescue after genetic transformation. Four typical *tra-2(lf)* mutations are associated with an insertion or DNA rearrangement near the 5′ end of the gene; six *tra-2(gf)* mutations are associated with an insertion or small deletion near the 3′ end of the gene; and one unusual *tra-2(lf)* allele, *tra-2(q276)*, is associated with an insertion ~1 kb upstream from the 3′ end of *tra-2*. This novel allele, unlike the typical *tra-2(lf)* alleles, transforms *XX* animals into fertile mating males (T.Schedl, personal communication). Because this allele maps towards the 3′ end of the gene, it could produce a truncated *tra-2* protein that interferes with other components of the sex determining mechanism.

The *tra-2* gene encodes transcripts of three sizes (5 kb, 1.8 kb and 1.9 kb). The 5 kb RNA is present throughout development and is expressed in both germ line and soma. DNA alterations associated with both *tra-2(lf)* and *tra-2(gf)* mutations map to restriction fragments hybridizing to the 5 kb RNA. Therefore, this RNA appears to be important for *tra-2* function. A nearly full length cDNA from the 5 kb RNA contains a large open reading frame (P.Kuwabara and J.Kimble, unpublished), suggesting that this RNA encodes a protein. The function(s) of the 1.8 kb and 1.9 kb *tra-2* RNAs are uncertain. Several *tra-2(lf)* mutations map to a restriction fragment that does not hybridize to the smaller *tra-2* RNAs. Therefore, these RNAs may remain unaffected in these mutants. Potentially, mutations that affect all three *tra-2* RNAs could result in more severe masculinization than previously characterized alleles. *tra-2(q276)*, which masculinizes some tissues more severely than putative nulls, maps to a fragment that hybridizes to all *tra-2* RNAs; by genetic criteria, however, this unusual allele does not appear to be null (T.Schedl, personal communication).

A 1.8 kb *tra-2* RNA is first detected just before oogenesis begins and is not found in mutants lacking a germ line. We envisage two possible functions for this RNA. First, it may be a maternal RNA which directs embryonic sex determination. Because translational activation of maternal RNAs is often associated with increased polyadenylation (e.g. in the clam *Spisula*, Rosenthal and Ruderman, 1987), the

increased polyadenylation of the 1.8 kb *tra-2* RNA in embryos may indicate a similar activation. Although no maternal effect of *tra-2* has been observed genetically (Klass *et al.*, 1976; Hodgkin and Brenner, 1977; Doniach and Hodgkin, 1984; Hodgkin, 1986), the alleles used for these studies may not have affected the 1.8 kb RNA. Second, this RNA may promote the switch from spermatogenesis to oogenesis in the hermaphrodite germ line. Genetic analyses suggest that hermaphrodite spermatogenesis depends on a transient inhibition of *tra-2* activity (Doniach, 1986; Schedl and Kimble, 1988). The 1.8 kb RNA (or its product) may alleviate this inhibition by binding an inhibitor or by encoding a product resistant to the inhibition. Alternatively, it (or its product) may inhibit maternal *fem* gene products that are also in the hermaphrodite germ line (Rosenquist and Kimble, 1988).

A comparison of *tra-2* RNAs produced by adult hermaphrodites and males reveals differences in both amount and kind. Adult males accumulate less *tra-2* RNA than hermaphrodites and males express a 1.9 kb RNA, while adult hermaphrodites make a 1.8 kb RNA. Although hermaphrodites also make a 1.9 kb RNA during larval development, the germ line of this sex produces sperm before switching to oogenesis in late L4. Therefore, the 1.9 kb RNA, though expressed in both sexes, may nevertheless be male specific. We do not yet understand the significance of the sex specific differences in the *tra-2* RNAs. The *tra-2* gene is a dose sensitive locus, so the amount of *tra-2* RNAs, *per se*, may be critical to activity. For the 1.9 kb versus the 1.8 kb RNAs, an intriguing possibility is that these two RNAs may be produced by alternative splicing and generate different proteins. In *Drosophila, Sxl* and *tra* are subject to sex specific splicing, so that RNAs with large open reading frames are only made in females (Boggs *et al.*, 1987; Bell *et al.*, 1988). However, other models exist and the function of each *tra-2* RNA must await further analysis.

As summarized in Figure 1, the sex determining genes in *C.elegans* act in a cascade of negative regulation to control sexual differentiation (Hodgkin, 1990; Villeneuve and Meyer, 1990). The availability of molecular probes for genes in the pathway permits the predicted relationships among these genes to be tested at the molecular level. If the sex specific differences in *tra-2* RNAs were regulated by genes upstream of *tra-2* in the pathway (Figure 1), mutations in genes downstream of *tra-2* would not be expected to affect *tra-2* expression. However, this prediction is not met: *XX tra-1* mutant males make *tra-2* RNAs typical of an *XO* male, and *XO fem-3* mutant females make *tra-2* RNAs typical of an *XX* hermaphrodite. Given this result, it seemed plausible that the pathway might be reversed. However, *XO* hermaphrodites lacking *her-1* activity make *tra-2* RNAs typical of an *XX* hermaphrodite. Therefore, regardless of the activity of regulatory genes either upstream or downstream of *tra-2*, the *tra-2* RNAs reflect the phenotypic sex rather than the chromosomal sex. Why is this so? The simple interpretation is that the sex specific difference of *tra-2* RNAs is not regulated by the *X/A* ratio. The difference may have been regulated initially by the *X/A* ratio, but it must be maintained in the adult either by other regulatory genes or by some feedback mechanism within the cascade.

Our molecular analysis of *tra-2* raises several questions about the role of *tra-2* in regulating the sexual phenotype. What is the true null phenotype? Mutations that appear to be null based on genetic criteria appear to affect only one *tra-2* RNA, whereas a mutation that is not null based on genetic criteria appears to affect all three *tra-2* RNAs. What are the functions of the two smaller *tra-2* RNAs? What is the significance of the sex specific difference in amount and type of *tra-2* RNA made? How do lesions at the 3′ end of the gene affect the regulation of *tra-2*? Is there feedback regulation within the regulatory cascade? Although the *tra-2* locus appeared to be rather simple genetically (Hodgkin and Brenner, 1977; J.Hodgkin, personal communication), molecular analysis of this locus has revealed unexpected levels of complexity.

Materials and methods

Strains

C.elegans strain Bristol (N2) has ~30 Tc1 elements that do not transpose (Emmons *et al.*, 1983; Emmons and Yesner, 1984; Eide and Anderson, 1985b). Each of two other *C.elegans* strains, TR403 and Bergerac (EM1002), has ~300 Tc1s that actively transpose (Moerman and Waterston, 1984; Eide and Anderson, 1985a; Rosenquist and Kimble, 1988). The markers, *unc-4(e120)* and *dpy-10(e128)*, and balancer for chromosome II, *mnC1*, are described elsewhere (Hodgkin *et al.*, 1988). Most *tra-2* alleles have been described [*tra-2(q122)* and *tra-2(q244)*, Schedl and Kimble (1988); *tra-2(e2046)* and *tra-2(e2020)*, Doniach (1986); *tra-2(q101)* and *tra-2(q103)*, Rosenquist and Kimble (1988) and Schedl and Kimble (1988)]. *tra-2(sc146)* was provided by R.Edgar. For Figure 7, *tra-1(e1099) XX* males were picked from strain *tra-1(e1099);eDp6*; also *fem-3(e1996)*; *lon-2(e678)*/O *XO* females were picked from the F1 of the following cross: *fem-3(e1996)*; *lon-2(e678)* females × *fem-3(e1996)*/+ males. Finally *XX* hermaphrodite and *XO* males were picked from *lon-2(e678)* adults.

Isolation of spontaneous tra-2 mutants

Two strains, JK750 and JK742, were constructed to place *tra-2(q122)* into a Tc1 active background. To make JK750 [*tra-2(q122) unc-4(e120)/mnC1* in a hybrid TR403/EM1002 background], a primarily TR403 animal carrying the *tra-2(q122) unc-4(e120)* Bristol chromosome was crossed to a primarily Bergerac animal carrying cross-over suppressor *mnC1*. To make JK742 [*tra-2(q122) unc-4(e120)/mnC1* in a Bergerac background], a primarily Bergerac animal carrying a *tra-2(q122) unc-4(e120)* Bristol chromosome was crossed to the primarily Bergerac animal with *mnC1*. To select for spontaneous *tra-2(lf)* mutants, $\sim 5 \times 10^5$ animals of the male/female mating strains (JK750 or JK742) were transferred from plates to liquid culture and grown for two generations at room temperature (~22°C). Embryos present in this culture were isolated using hypochlorite (Sulston and Hodgkin, 1988). Because *tra-2(q122)* is incompletely penetrant when grown in liquid culture, many embryos were isolated. These embryos were predominantly *XX* females, but some spontaneous *XO* males were present (Hodgkin *et al.*, 1979). To find self-fertile animals, L4 *XX* animals were separated to screen the following day for self-fertility. Two suppressors, *tra-2(q122q150)* and *tra-2(q122q149)*, were isolated using JK750, and three, *tra-2(q122q146)*, *tra-2(q122q147)* and *tra-2(q122q148)*, were isolated using JK742. Each suppressed stock segregated Unc pseudomales and no females, suggesting that a *tra-2(lf)* allele had been induced *in cis* to *tra-2(q122)*. Each suppressor was confirmed to be *tra-2(lf)* by complementation testing.

Cloning tra-2 and isolation of tra-2 cDNAs

To remove extraneous Tc1s, *tra-2(q122q150)* was back-crossed to Bristol six times. Tc1s linked to *tra-2* were removed by isolating recombinants first between *tra-2(q122q150)* and *unc-4(e120)* and then between *tra-2(q122q150)* and *dpy-10(e128)*. JK794, *dpy-10(128) tra-2(q122q150)/mnC1*, carries this backcrossed and recombined chromosome in a Bristol background. To clone the novel 7 kb Tc1-containing *Bgl*II restriction fragment associated with *q150*, genomic JK794 DNA was digested with *Bgl*II and fractionated by electrophoresis through 0.55% low melting temperature agarose (Sigma). Fragments of 6–9 kb were isolated from the gel by standard techniques and cloned into the *Bam*HI site of Charon 27 (Rimm *et al.*, 1980). A recombinant carrying the 7 kb Tc1 containing *Bgl*II fragment (JK#L13) was isolated using a ^{32}P-labeled Tc1 probe. pJK7 was made by cloning a random *Eco*RI–*Eco*RV fragment flanking the Tc1 from the insert DNA of JK#L13 into plasmid vector pIBI76 (International

Biotechnologies, Inc.). Clones spanning wild-type *tra-2* were isolated from a *C.elegans* genomic DNA library in EMBL3 (provided by C.Cummins and P.Anderson) using a ^{32}P-labeled pJK7 probe. Subclones of the region (probes a–h, Figure 3) were constructed in pIBI76 from these clones. A *C.elegans* cDNA library (kindly provided by J.Ahringer) was screened with pJK67 (probe e, Figure 2). pJK67 hybridizes to all identified *tra-2* RNAs. One *tra-2* cDNA with a 2.2 kb insert (pJK111) was used as the probe for examining *tra-2* RNAs here.

Manipulation of nucleic acids

Standard methods for manipulating nucleic acids were used (Maniatis *et al.*, 1982). Isolation of *C.elegans* DNA and RNA followed standard protocols (Emmons and Yesner, 1984; Rosenquist and Kimble, 1988). DNA blots were hybridized for ~16 h in 5 × SSC, 5 × Denhardt's, 0.5% SDS, 1.5 mM sodium pyrophosphate, 100 μg/ml herring testes DNA at 68°C using ~2 × 10^6 c.p.m./ml ^{32}P-labeled RNA probe. Blots were washed in 0.2 × SSC, 0.25% SDS at 65°C. RNA blots were hybridized for ~16 h at 66°C in 50% formamide, 5 × SSC, 5 × Denhardt's, 50 mM sodium phosphate, pH 6.5, 1.5 mM sodium pyrophosphate, 100 μg/ml herring sperm DNA (Melton *et al.*, 1984) using ~2 × 10^6 c.p.m./ml ^{32}P-labeled RNA probe. Blots were washed in 0.2 × SSC, 0.25% SDS at 65°C.

^{32}P-labeled RNA probes were made using SP6 or T7 RNA polymerase using reaction conditions similar to those described (Melton *et al.*, 1984). Tc1 probes were synthesized from pJC163 (provided by J.Collins and P.Anderson). Probes diagrammed in Figure 2 are as follows: a = pJK64, b = pJK63, c = pJK69, d = pJK68, e = pJK67, f = pJK66 and g = pJK65. The pharyngeal specific *myo-1* (Miller *et al.*, 1986) probe was pJK191.

Synchronizing animals

Developmentally staged animals were grown from embryos isolated by hypochlorite treatment of gravid hermaphrodites (Sulston and Hodgkin, 1988). For more tightly synchronized animals, embryos isolated as above were allowed to develop for ~6 h at room temperature (~22°C), followed by a second hypochlorite treatment. L3 and L4 animals were staged by scoring vulval development (Sulston and Horvitz, 1977).

Digestion of poly(A) tails

Poly(A) tails were removed as described by Vournakis *et al.* (1975). Approximately 2 μg poly(A)$^+$ RNA in 10 μl of 1 mM EDTA, 2 mM dithiothreitol were incubated in a boiling water bath for 90 s, briefly cooled on ice, and warmed to room temperature. Twenty units RNasin (Promega) and 1 μg oligo(dT) (Pharmacia) were added, and the mixture incubated at room temperature for 10 min. Ten μl of 100 mM KCl was added and the mixture was incubated at room temperature for 10 min. Twenty μl of 56 mM $MgCl_2$, 40 mM Tris, pH 8.0, 1 mM EDTA, 2 mM dithiothreitol and 2 U RNase H (Pharmacia) were added and the mixture was incubated at 37°C for 40 min. The mixure was then extracted with phenol/$CHCl_3$ and resuspended for loading on a gel.

Quantitation of band intensity on Northern blots

Quantitation of transcript bands on autoradiographs was done using a Zeineh model SL-504-XL scanning laser densitometer. Each lane was scanned in two to three locations and the mean peak area calculated.

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