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The *C. elegans* sex determination gene *laf-1* encodes a putative DEAD-box RNA helicase

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ABSTRACT

The *Caenorhabditis elegans* gene *laf-1* is critical for both embryonic development and sex determination. *Laf-1* is thought to promote male cell fates by negatively regulating expression of *tra-2* in both hermaphrodites and males. We cloned *laf-1* and established that it encodes a putative DEAD-box RNA helicase related to *Saccharomyces cerevisiae* Ded1p and *Drosophila* Vasa. Three sequenced *laf-1* mutations are missense alleles affecting a small region of the protein in or near helicase motif III. We demonstrate that the phenotypes resulting from *laf-1* mutations are due to loss or reduction of *laf-1* function, and that both *laf-1* and a related helicase *vbh-1* function in germline sex determination. *Laf-1* mRNA is expressed in both males and hermaphrodites and in both the germline and soma of hermaphrodites. It is expressed at all developmental stages and is most abundant in embryos. LAF-1 is predominantly, if not exclusively, cytoplasmic and colocalizes with PGL-1 in P granules of germline precursor cells. Previous results suggest that *laf-1* functions to negatively regulate expression of the sex determination protein TRA-2, and we find that the abundance of TRA-2 is modestly elevated in *laf-1*/+ females. We discuss potential functions of LAF-1 as a helicase and its roles in sex determination.

Introduction

Caenorhabditis elegans sex determination is a highly regulated process during which animals with two sets of autosomes and two X chromosomes (AA XX) develop as self-fertile hermaphrodites, while those with one X chromosome (AA XO) develop as males (reviewed in Zarkower, 2006; Ellis and Schedl, 2007). The core sex determination pathway in both somatic and germline cells involves a series of negative regulations among proteins that ultimately specify male or female cell fates (Fig. 1A). Stage-specific regulation of *tra-2* and *fem-3* in the hermaphrodite germline facilitates production of sperm in the L4 larval stage and oocytes in the adult. Translation of *tra-2* mRNA is repressed during the L2 and L3 larval stages, thereby allowing spermatogenesis. Translation of *fem-3* mRNA is repressed in adults, thereby causing a switch to oogenesis (reviewed in Puoti et al., 2001; Ellis and Schedl, 2007).

Mutations of *laf-1* affect sex determination, and genetic analysis suggests that LAF-1 promotes male cell fates. *Laf-1* mutations were isolated as dominant suppressors of *fem-3* gain-of-function (gf) alleles (Goodwin et al., 1997). The germlines of *fem-3*(*gf*) XX animals are masculinized, containing excess sperm and no oocytes (Barton et al., 1987). Such animals are sterile, but heterozygosity of *laf-1* mutations suppresses the sterility. *Laf-1*/+; *fem-3*(*gf*) animals produce both sperm and oocytes and are self-fertile (Goodwin et al., 1997). In an otherwise wild-type background, 10–30% of *laf-1*/+ XX heterozygotes are feminized, producing oocytes but no sperm (Goodwin et al., 1997). A similar percentage of XO animals are partially feminized in both the germline and soma. These data suggest that wild-type LAF-1 promotes male fates in all tissues.

Previous data suggest that the function of LAF-1 involves repression of *tra-2* expression (Goodwin et al., 1997). TRA-2 promotes female fates, and regulation of its expression is critical for normal sexual development in all tissues. Translation of *tra-2* mRNA is repressed in the hermaphrodite germline to allow spermatogenesis (Doniach 1986; Goodwin et al., 1993). This repression requires two 28-nucleotide elements termed TGEs (*tra-2* and *GLI* elements) located in the *tra-2* 3′ untranslated region (3′UTR) (Goodwin et al., 1993; Jan et al., 1997). Disruption or deletion of these elements, such as in *tra-2*(*gf*) mutants, causes excess *tra-2* activity and feminizes the hermaphrodite germline (Doniach, 1986; Schedl and Kimble, 1988). The STAR protein GLD-1 binds to the TGEs and mediates *tra-2* repression (Jan et al., 1999) (Fig. 1B). Regulation of *tra-2* also requires FOG-2, an F-box protein that physically interacts with GLD-1 (Clifford et al., 2000). Expression of *fog-2* and *gld-1* is restricted to the germline (Jones et al., 1996; Clifford et al., 2000), but *tra-2* translation is also regulated in the soma (Goodwin et al., 1997). It is unknown what factors mediate repression of *tra-2* in the soma, but LAF-1 is a good candidate (Goodwin et al., 1997).

Transport of *tra-2* mRNA from the nucleus to the cytoplasm is regulated. Most *C. elegans* mRNAs exit the nucleus via an NXF-1-mediated pathway, but *tra-2* mRNA export occurs and is regulated by a leptomycin B-sensitive pathway that likely involves CRM-1 (Segal et

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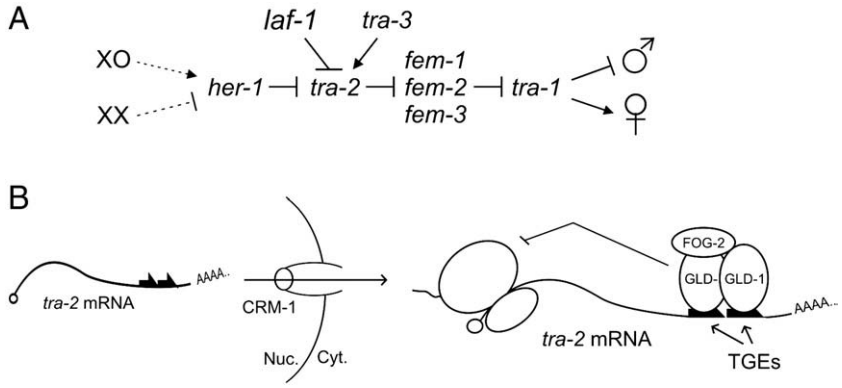

Fig. 1. *C. elegans* sex determination. (A) Simplified sex determination pathway. (B) Models for *tra-2* regulation. Export of the *tra-2* mRNA is regulated so that it exits the nucleus via a CRM-1-mediated pathway (Segal et al., 2001; Kuersten et al., 2004). GLD-1 binds to sequences in the 3′ UTR of the *tra-2* mRNA (Jan et al., 1999) and acts in conjunction with FOG-2 (Clifford et al., 2000) to repress *tra-2* translation.

al., 2001; Kuersten et al., 2004) (Fig. 1B). Alteration of *tra-2* mRNA export such that it exits via the NXF-1-mediated pathway leads to increased levels of TRA-2 protein (Kuersten et al., 2004), suggesting that normal export is needed to establish the translational regulation of *tra-2* mRNA described above.

Three lines of evidence suggest that *laf-1* regulates *tra-2*. First, the phenotypes of *laf-1*/+ animals are similar to those of strong *tra-2*(*gf*) mutants. Both of these genotypes cause XX animals to develop as females and partially feminize the XO soma and germline (Doniach, 1986; Schedl and Kimble, 1988; Goodwin et al., 1997). Additionally, both *tra-2*(*gf*) mutations and *laf-1*/+ heterozygosity suppress the germline masculinization of *fem-3*(*gf*) mutants (Barton et al., 1987; Goodwin et al., 1997). Second, tests of epistasis placed *laf-1* upstream of *tra-2*. *Tra-2*(*null*); *laf-1*/+ double mutants are pseudomales indistinguishable from *tra-2*(*null*) single mutants (Goodwin et al., 1997). Third, reporter transgenes carrying the *tra-2* 3′UTR are misregulated in *laf-1*/+ mutants (Goodwin et al., 1997). Transgenes carrying the wild-type *tra-2* 3′UTR are repressed in wild type but are derepressed in *laf-1*/+ mutants. Transgenes that lack the TGEs fail to be repressed in wild type and exhibit no additional derepression in *laf-1*/+ heterozygotes, indicating that *laf-1* acts through the TGEs (Goodwin et al., 1997). These data suggest that *laf-1* acts upstream of *tra-2* and inhibits its expression. Feminization of *laf-1*/+ heterozygotes may thus result from overexpression of TRA-2.

In addition to its role in sex determination, *laf-1* is essential for embryogenesis. *Laf-1* heterozygotes are feminized, but *laf-1* homozygotes die as embryos or early larvae (Goodwin et al., 1997). The embryonic lethality of *laf-1* homozygotes is probably not due to misregulation of *tra-2*, as such lethality is not associated with either increased or decreased *tra-2* activity. Therefore, *laf-1* likely regulates other mRNAs in the embryo or serves another function required for normal development.

This paper describes the molecular analysis of *laf-1* and further characterization of its role in sex determination. We demonstrate that *laf-1* encodes a putative DEAD-box RNA helicase related to *S. cerevisiae* Ded1p and *Drosophila* Vasa. We investigated *laf-1* mRNA expression and the sub-cellular localization of LAF-1 protein. Finally, we tested whether TRA-2 expression is increased in *laf-1*/+ mutants.

Materials and methods

Worm strains

The following strains were used in this work: N2 Bristol (wild type), CB4856 (Hawaiian isolate), *glp-4*(*bn2ts*) *I*, *tra-2*(*e2020gf*) *II*, *tra-2*(*q122gf*) *II*, *tra-2*(*e1095*) *II*; *unc-24*(*e138*) *fem-3*(*e1996*)/+ *IV*, *unc-119*(*ed3*) *III*, *glp-1*(*q231ts*) *III*, *laf-1*(*q267*) *unc-32*/*C1 III*, *dpy-1* (*e1*) *laf-1*(*q267*)/*qC1 III*, *laf-1*(*q267*)/+*III*, *dpy-1*(*e1*) *laf-1*(*q217*)/*qC1 III*, *laf-1*(*q217*)/+*III*, *laf-1*(*q80*)/*qC1 III*, *fem-2*(*e2105*)/*unc-45*(*r450*) *dpy-1*(*e1*) *III*, *fem-1*(*e1965*)/*unc-5*(*e53*) *mor-2*(*e1125*) *IV*, and *fem-3* (*e1996*)/*unc-24*(*e138*) *dpy-20*(*e1282*) *IV*.

Fine mapping laf-1

dpy-1 laf-1(*q267*)/*qC1* or *laf-1*(*q267*) *unc-32*/*qC1* females were mated with males of the Hawaiian isolate CB4856, and F1 hermaphrodites were allowed to self-fertilize. Dpy non-Laf or Unc non-Laf F2 animals were isolated, and single nucleotide polymorphisms (snpA-snpF, see Fig. 2A) in the *laf-1* region were assayed using single worm PCR followed by restriction enzyme digest or sequencing. From mothers of genotype *dpy-1 laf-1*(*q267*)/+(*Hawaiian*), 113/461 Dpy non-Laf recombinants were homozygous for the N2 Bristol allele of snpA. In 9/65 of these animals, the crossover occurred to the right of snpC and in 1/39 it occurred to the right of snpD, placing *laf-1* to the right of snpD. From mothers of genotype *laf-1*(*q267*) *unc-32*/+(*Hawaiian*), 96/1048 Unc non-Laf recombinants were homozygous for the N2 Bristol allele of snpB. In 4/61 of these animals, the crossover occurred to the left of snpF and in 2/96 it occurred to the left of snpE, placing *laf-1* to the left of snpE. SnpA-snpE are WormBase alleles snp_Y71H2B[2], pkP3093, snp_y71H2AM [4], hw41389, and hw41420, respectively. SnpF was identified by sequencing and is an A at nucleotide III: 2,793,914 in the Hawaiian isolate compared to a G in N2 Bristol.

Sequencing laf-1 mutations

Genomic DNA from *laf-1*/*qC1* heterozygotes was PCR amplified in ~2 kb sections using Elongase enzyme (Invitrogen) and cloned into a vector for sequencing. Base changes (relative to the sequence of wild-type *C. elegans*) seen in multiple clones were confirmed by sequencing PCR products from individual *laf-1*/*qC1* heterozygotes or homozygous *laf-1* dead eggs. Wild-type animals were also tested to verify the WormBase sequence.

Phylogenetic analysis of LAF-1 and related proteins

Proteins related to LAF-1 were identified by BLAST search through the UniProt website (http://www.pir.uniprot.org/), using the full LAF-1 protein sequence as the query. An alignment of the most closely related proteins from selected species was made with the ClustalX program (http://bips.u-strasbg.fr/fr/Documentation/ClustalX/) using a Gonnet 250 protein weight matrix. A phylogenetic tree based on the alignment was constructed by the Neighbor-Joining method using the Phylip software package (http://evolution.genetics.washington.edu/phylip.html). *S. cerevisiae* eIF4A, a more distantly related DEAD-box protein, was included in the analysis and assigned