Homologs of the *Caenorhabditis elegans* Masculinizing Gene *her-1* in *C. briggsae* and the Filarial Parasite *Brugia malayi*

Adrian Streit,*,[1] Weiqing Li,* Barbara Robertson,* Jacquie Schein,† Ibrahim H. Kamal,‡,[2] Marco Marra† and William B. Wood*

*Department of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder, Colorado 80309-0347, †Genome Sequencing Center, Washington University School of Medicine, St. Louis, Missouri 63108 and ‡Filarial Genome Project Resource Center, Clark Science Center, Smith College, Northampton, Massachusetts 01063

Manuscript received February 22, 1999
Accepted for publication April 23, 1999

ABSTRACT

The masculinizing gene *her-1* in *Caenorhabditis elegans* (*Ce-her-1*) encodes a novel protein, HER-1A, which is required for male development. To identify conserved elements in *her-1* we have cloned and characterized two homologous nematode genes: one by synteny from the closely related free-living species *C. briggsae* (*Cb-her-1*) and the other, starting with a fortuitously identified expressed sequence tag, from the distantly related parasite *Brugia malayi* (*Bm-her-1*). The overall sequence identities of the predicted gene products with *Ce*-HER-1A are only 57% for *Cb*-HER-1, which is considerably lower than has been found for most homologous *briggsae* genes, and 35% for *Bm*-HER-1. However, conserved residues are found throughout both proteins, and like *Ce*-HER-1A, both have putative N-terminal signal sequences. *Ce-her-1* produces a larger masculinizing transcript (her-1a) and a smaller transcript of unknown function (her-1b); both are present essentially only in males. By contrast, *Cb-her-1* appears to produce only one transcript, corresponding to her-1a; it is enriched in males but present also in hermaphrodites. Injection of dsRNA transcribed from *Cb-her-1* into *C. briggsae* hermaphrodites (RNA interference) caused XO animals to develop into partially fertile hermaphrodites. Introducing a *Cb-her-1* construct as a transgene under control of the *C. elegans unc-54* myosin heavy chain promoter caused strong masculinization of both *C. briggsae* and *C. elegans* hermaphrodites. Introduction of a similar *Bm-her-1* construct into *C. elegans* caused only very weak, if any, masculinization. We conclude that in spite of considerable divergence the *Cb* gene is likely to be a functional ortholog of *Ce-her-1*, while the function of the distantly related *Bm* gene remains uncertain.

SEX determination is an almost universal feature of animal development. However, in contrast to the genes that control other basic developmental processes like pattern formation, which often are highly conserved among taxa as divergent as mammals, flies, and nematodes (Manak and Scott 1994), the sex-determining mechanisms that have been investigated by molecular genetics exhibit little similarity (Hodgkin 1992; Ryner and Swain 1995; Marin and Baker 1998). Even within groups of animals that employ homologous sex-determining systems, the genes involved are often more divergent than those encoding components of other regulatory pathways, for reasons that are not understood (O'Neil and Belote 1992; Tucker and Lundrigan 1993; Whitfield *et al.* 1993; de Bono and Hodgkin 1996; Kuwabara 1996).

In the nematode *C. elegans*, determination of the two sexes, hermaphrodites and males, has been extensively studied (for reviews, see Cline and Meyer 1996; Meyer 1997). The primary signal is the ratio of X chromosomes to autosomes in the embryo. Normally, XX embryos develop into hermaphrodites and XO embryos into males. Hermaphrodites are somatically female, but produce and store sperm during the fourth larval stage before switching to oogenesis as adults. Their self-progeny are all hermaphrodites except for males that arise by spontaneous X nondisjunction at a frequency of ~0.2%. Hermaphrodites mated with males produce 50% male cross-progeny.

In sex determination, the primary signal acts as a switch to regulate a cascade of interacting genes that control X-chromosome dosage compensation as well as sex determination (Figure 1). The masculinizing gene *her-1*, required for male development, is the first in the sex-determining branch of the pathway. *her-1* loss-of-function (*lf*) mutations have no effect on XX animals but cause XO animals to develop as normal-appearing fertile hermaphrodites (Hodgkin 1980). The *her-1* locus encodes two transcripts of 1.2 and 0.8 kb, both regulated sex specifically at the level of transcription (Trent *et al.* 1991; Perry *et al.* 1993; Li *et al.* 1999). The smaller transcript has no known function; the larger is necessary

Corresponding author: W. B. Wood, Department of MCD Biology, Porter Biosciences Room 058, University of Colorado, Boulder, CO 80309-0347. E-mail: wood@stripe.colorado.edu

[1]*Present address:* Institute for Zoology, University of Zurich, Winterthurerstr. 190, 8057 Zurich, Switzerland.

[2]*Present address:* Department of Biochemistry, Ain Shams University, Abassiah, Cairo 11566, Egypt.

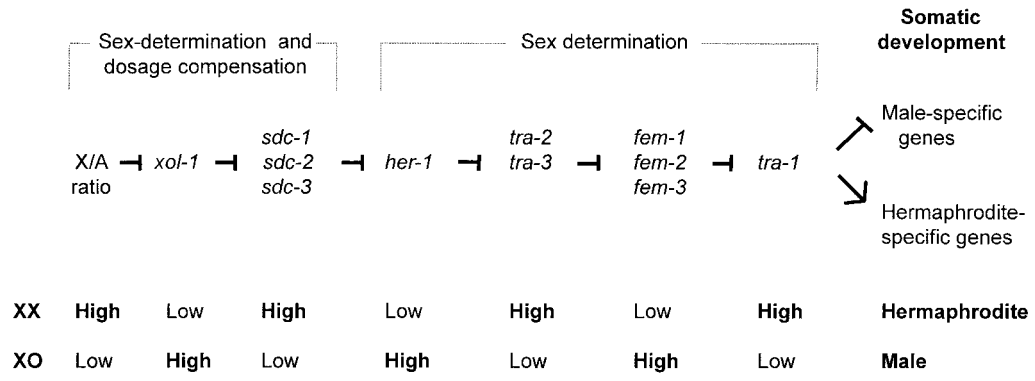

Figure 1.—Position of *her-1* in the signaling pathway controlling somatic sex determination in *C. elegans*. Blunt-ended arrows (⊣) indicate negative regulation. Indicated below the pathway is the predicted state of each gene's function in wild-type XX and XO animals. Three of these genes, *tra-2*, *fem-2*, and *tra-1*, have previously been shown to have homologs in *C. briggsae*. Adapted from Perry *et al.* (1993).

and sufficient for all known functions of *her-1* and encodes a predicted protein of 175 amino acids (HER-1A), which has no significant similarity to other proteins in the current data bases (except *Bm*-HER-1; see below). Analysis of genetic mosaics showed that *her-1* acts cell nonautonomously (Hunter and Wood 1992), and HER-1A has a predicted N-terminal secretion signal that is required for function (Perry *et al.* 1993). These findings support the view that HER-1A is secreted as the inhibitory ligand for a receptor encoded by *tra-2* (Hunter and Wood 1992; Kuwabara *et al.* 1992).

Mutations that impair *her-1* function are distributed throughout the gene, providing little insight into the relative functional importance of different domains (Perry *et al.* 1994). In an attempt to identify candidates for functionally important domains based on evolutionary conservation, we have undertaken a comparison of the *her-1* genes from *C. elegans* (*Ce-her-1*) and two other nematodes: the closely related free-living species *C. briggsae* (Fitch *et al.* 1995) and the distantly related parasitic species *Brugia malayi* (Blaxter *et al.* 1998). We have designated these homologs as *Cb-her-1* and *Bm-her-1*, respectively. In *C. briggsae*, as in *C. elegans*, the two sexes are hermaphrodites and males, and three of the sex-determining genes downstream of *her-1* (Figure 1) are similar, although poorly conserved, between the two species (*tra-2*, Kuwabara 1996; *fem-2*, Hansen and Pilgrim 1998; and *tra-1*, de Bono and Hodgkin 1996). *B. malayi* is a filarial parasite responsible for the Southeast Asian form of the lymphatic disorder elephantiasis (Blaxter and Bird 1997). The two sexes are females and males, but little is known about the sex-determining mechanism. We show here that although the *her-1* genes in the two comparison species have diverged substantially from *Ce-her-1*, residues known to be functionally important in *C. elegans* have been well conserved. The *Cb-her-1* gene exhibits masculinizing activity in both *C. elegans* and *C. briggsae* and, therefore, is likely to be a functional ortholog. Although we could not demonstrate a clear masculinizing activity for *Bm-her-1* in these species, the conservation of key residues in its predicted protein product suggests that it may share biochemical functions with *Ce-her-1*.

MATERIALS AND METHODS

Nematode strains and culture: All *C. elegans* strains were derivatives of the wild-type Bristol strain N2. The *C. elegans* alleles used were *him-8(e1489)* IV (Hodgkin *et al.* 1979) and *her-1(y101hv1)* V (Trent *et al.* 1991; Perry *et al.* 1994). The strain PA43, of genotype *him-8(e1489); her-1(y101hv1)*, produces ~37% XO animals as a result of the *him-8* mutation, but they develop into fertile hermaphrodites rather than males as a result of the *her-1* null allele *y101hv1*.

The *C. briggsae* wild-type strain AF16 (Fitch *et al.* 1995) was obtained from the *Caenorhabditis* Genetics Center. The *C. briggsae* strain BW1850 was produced by backcrossing a *m*ale-*i*ncidence-*h*igh [*mih-3(s1290)*] strain, kindly provided by S. Bird and D. Baillie, twice to AF16. In BW1850 partial self-sterility of the hermaphrodites was overcome by mating to males of the same genotype, producing a population with ~50% males. All strains were cultured by techniques standard for *C. elegans* (Brenner 1974; Sulston and Hodgkin 1988).

DNA libraries: The libraries used were kindly supplied to us by the following investigators: *C. elegans* embryonic cDNA from P. Okkema and A. Fire (Carnegie Institution of Washington, Baltimore), *C. briggsae* genomic from T. Snutch and D. Baillie (Simon Fraser University, Burnaby, BC, Canada), *B. malayi* genomic DNA library-97 from U. Wagner (University of Giessen, Germany), and *B. malayi* adult male cDNA library SAW94NL-BmAM from N. Ling and S. A. Williams (Filarial Genome Project Resource Center, Smith College, Northampton, MA). A *C. briggsae* high-density fosmid grid was purchased from Genome Systems Inc. (St. Louis).

PCR primers: Sequences of primers referred to in the text are as follows:

zk287-41: AACCGTTGCCACCTGCCGCC;
zk287-42: TATGGAAAACAACGAATGCG;
zk287-43: CTGAATAATACGCAACGGCG;
zk287-44: GACAGATGAGTTGAAGGCG;
ACb1: TTTGGTCATAAAAATGAATGC;
ACb2: ACCATCTCAAAACCAGATCG;
BRU1: ATGGGACATTCTCTGATTCTAGC;
BRU2: TTATTTGGCATTCAATCTGATGC;
BRU3: GACCAATGTATACTTTCCCCGGC;
BRU4: GTTAATTATTTAATTTCGGGACC;
SL1: GGTTTAATTACCCAAGTTTGAG;
BCB1F: TAGAATCATCACTCTTCTCACCAT
BCB4R: ACGCTTCTGGAGATACGTCGTGTT;
BCB3R: CCAAAGACGGTGCAGCACACAGAA;
CB5′UTRF: GCTCTTCTCGCTAGCAGATCCGTCACACTTCTCT;
CBCE3′R: AGGCTAATGAGCCCAGATTCAGTGGATTGGACGCTTCTGGAGA;

BRU9F: CGATCAGTGCTAGCAAAGGAATATAATTATTAAG GGAACA;
BRU10R: AGGCTAATGAGCCCAGATTTATTTAATTTCGG GACCAATGTATACT.

Molecular techniques: Standard methods were as described by Sambrook *et al.* (1989). Other molecular methods are described below.

PCR protocols: Reverse transcriptase PCR (RT-PCR) was used to obtain partial *C. briggsae* cDNAs following the method of Innis *et al.* (1990). Reverse transcription was carried out with AMV Reverse Transcriptase (Promega Madison, WI), random hexamer primers (GIBCO/BRL, Gaithersburg, MD), and from 1.4 to 10 μg of *C. briggsae* RNA. The program for PCR was 1 min 95°; then 40 cycles of 1 min 95°, 1 min 55°, 1 min 72°; then 10 min 72°, 10 min 99°.

Nested PCR was used to isolate desired cDNA sequences directly from phage libraries. For the first round of PCR, 5 μl of a phage library were used as a template in a 100-μl reaction. The PCR program was 5 min 94°; then 30 cycles of 1 min 94°, 1 min 54°–58° (depending on the primers used), 1.5 min 72°; then 10 min 72°, 10 min 99°. For the second round of PCR, 1 μl of a 1:100 dilution from the above amplification product was used as the template. The PCR program used was the same as for the first round except that the initial denaturation step was shortened to 1 min. The resulting PCR product was either sequenced, labeled directly, or cloned blunt into the *Hinc*II site of pT7/T3α18 (GIBCO/BRL).

DNA sequencing: All samples were sequenced by the DNA sequencing facility in MCD Biology, Boulder, the DNA Sequencing Facility, Iowa State University, or the Genome Sequencing Center in St. Louis. Samples obtained by PCR were sequenced using the same primers as those used for amplification. The accession no. for the *B. malayi* genomic sequence (see below) is AF125985. All other sequences are available at the world wide web sites of the respective sequencing projects:

C. elegans: www.sanger.ac.uk/Projects/C_elegans/
C. briggsae: genome.wustl.edu/gsc/Projects/briggsae.shtml
B. malayi: helios.bto.ed.ac.uk/mbx/fgn/filgen1.html

Cloning *Cb-her-1* by synteny: A partial cDNA (ZK287.4-V) from exons 2–6 of the predicted protease inhibitor gene ZK287.4 was obtained by nested PCR using the *C. elegans* embryonic cDNA library as template, primers zk287-41 and zk287-43 in the first round, and primers zk287-42 and zk287-44 in the second round. Sequencing of the resulting partial cDNA sequence confirmed the splicing shown in ACeDB, as predicted by Genefinder (Sulston *et al.* 1992; see also *C. elegans* web site above).

Using ZK287.4-V to probe the *C. briggsae* genomic library at low stringency (Yochem and Greenwald 1989), a clone (C.b.λ1) consisting of two *Eco*RI fragments was isolated. Both were subcloned into pT3/T7α-18 to produce clones ZK287.4A2 (8-kb insert) and ZK287.4C4 (5.2-kb insert) that, when partially sequenced, were found to contain elements that were 70–80% identical at the nucleotide level to predicted exons of *C. elegans* ZK287.4.

A segment of ZK287.4A2 amplified with primers ACb1 and ACb2 was used to probe a *C. briggsae* high-density fosmid grid. Using the fingerprints of the cognate fosmids from the grid as a starting point, a contig was constructed by interactive consultation of a database of fingerprinted *C. briggsae* fosmids (M. Marra, J. Schein and T. Kucaba, unpublished results). A minimal tiling set of *C. briggsae* fosmids for this contig (G39O07 and G33P21) was sequenced by the Genome Center in St. Louis.

The genomic interval surrounding the *C. elegans her-1* gene was compared to the sequence obtained from the *C. briggsae* fosmids (see Figure 2) using the dot-plot program DOTTER with default parameter settings (Sonnhammer and Durbin 1996). The finished sequence was submitted to BLAST (Altschul *et al.* 1997), and the exons of *Cb-her-1* were roughly identified by similarity to the *C. elegans her-1* exons at the protein level.

Isolation of the genomic *Bm-her-1* region: Based on sequence information from the *B. malayi* cDNA sequencing project (see results), a partial cDNA (B.m.her-12E) was amplified from the adult male-derived *B. malayi* cDNA library SAW96MLW-*Bm*AM by nested PCR using primers BRU1 and BRU4 in the first round and primers BRU2 and BRU3 in the second round. Using this partial cDNA as a probe, the *B. malayi* genomic library-97 was screened and two overlapping phage clones were isolated (B.m.λ2 and B.m.λ3). A 2.2-kb *Eco*RI fragment common to both clones that hybridized to the cDNA probe was subcloned and sequenced by primer walking (DNA Sequencing Facility, Iowa State University). The cDNA sequence is contained entirely within this fragment.

RNA isolation and analysis: RNA used for RT-PCR and RNA blot analyses was isolated directly from mixed-stage populations of the *C. briggsae* strains AF16 and BW1850 and from embryos isolated by hypochlorite treatment of these populations. Isolation and analysis followed the procedure of Trent *et al.* (1991) with the following modifications. The lysis step of the isolation was simplified as follows: the guanidinium isothiocyanate solution was added to the frozen pellet and vortexed constantly as the pellet thawed. For RNA blots, the gel and running buffers were modified as recommended by Tsang *et al.* (1993), and gels were blotted onto Hybond N membranes (Amersham, Buckinghamshire, UK). Blots were probed with a partial *C. briggsae her-1* cDNA obtained by RT-PCR (see below) and, to provide a loading control, with a *C. briggsae* clone (CbIC#9) containing the ribosomal protein gene *rpl-29* obtained from D. Evans and T. Blumenthal (University of Colorado Health Sciences Center, Denver). Signals were detected and quantitated using a PhosphoImager (Storm 860, Molecular Dynamics, Sunnyvale, CA).

Gene structures of *Cb-her-1* and *Bm-her-1*: To determine the precise splice sites of the *C. briggsae* exons, a partial cDNA of *Cb-her-1* was obtained by RT-PCR. The primers for the PCR step, designed on the basis of the genomic *C. briggsae* sequence, were BCB1F and BCB4R. Samples from six 100-μl PCR reactions were gel purified, pooled, and sequenced. The initiator AUG was inferred from inspection of the genomic sequence. Splice sites for the *Bm-her-1* exons were determined by comparison of the genomic and cDNA sequences obtained as described above. The 5′ end of the *Bm-her-1* transcript was defined as the site of SL1 *trans*-splicing (see results).

Plasmid constructs for functional studies: Plasmids containing the *C. briggsae* or *B. malayi* cDNAs driven by the *Ce-unc-54* promoter and followed by the *Ce-her-1* 3′UTR were made by three-part ligation using the vector pPD30.38 (which contains the *unc-54* promoter; Fire *et al.* 1990). The *C. elegans her-1* 3′UTR fragment was 0.8 kb in length, starting immediately after the termination codon. The *C. briggsae* cDNA was generated by RT-PCR using the primers CB5′UTRF (located 54 bp upstream of the putative initiator AUG) and CBCE3′R. The *B. malayi* cDNA was produced using the *B. malayi* library as template, the PCR program described above for RT-PCR, and the primers BRU9F (located 46 bp upstream of the inferred initiator AUG) and BRU10R. Candidate clones were checked by comparison to genomic sequence to eliminate those with PCR-introduced errors in protein-coding sequences. The clones used were designated pU54P/CbcDNA/Ce3′ for the *C. briggsae* cDNA clone and pU54P/BmcDNA/Ce3′ for the *B. malayi* clone. For control experiments, plasmid pU54P/CbFS/Ce3′, containing a frameshift mutation in the

C. briggsae cDNA, was made from pU54P/CbcDNA/Ce3′ by filling in a unique *Xba*I site in exon 2. These constructs were chosen for comparison of protein functions because preliminary experiments with *Ce-her-1* cDNA in such a transgenic construct had demonstrated dominant masculinization in both *C. elegans* and *C. briggsae* XX animals, presumably as the result of ectopic expression from the transgene (M. D. Perry and W. B. Wood, unpublished results).

The plasmid from which RNA was transcribed *in vitro* for RNA interference (RNAi) experiments (see below), designated pT7/T3CbcDNA, was made by cloning a 0.4-kb blunt-ended *Ssp*I-*Fsp*I fragment of the partial *C. briggsae her-1* cDNA obtained by RT-PCR into the *Sma*I site of the vector pT7/T3α-18 (GIBCO/BRL).

Transgenic worms: To observe effects of overexpression from cloned *her-1* genes, DNA of the experimental plasmid was coinjected with the pRF4 plasmid carrying the *rol-6(su1006dm)* marker, each at a concentration of 100 μg/ml (Mello *et al.* 1991), to yield transgenic lines carrying extrachromosomal arrays.

RNAi: The likely phenotype resulting from loss of function of the *her-1* gene in *C. briggsae* was investigated by injection of double-stranded RNA transcribed *in vitro* from a *Cb-her-1* cDNA plasmid into mated adult hermaphrodites, whose F_1 XO progeny were then scored for feminization. This technique has been shown to be a potent, specific method of silencing many genes in *C. elegans* (Fire *et al.* 1998; Montgomery and Fire 1998). Control experiments in *C. elegans* verified that *her-1(RNAi)* caused a phenotype similar to that resulting from a known *her-1* null mutation.

DNA sequence analysis: Searches for regions of sequence similarity in the 5′ flanking region and intron 2 of *Ce-her-1* and *Cb-her-1* were carried out using a program for analyzing pairwise alignments of long sequences (Schwartz *et al.* 1991). Searches for smaller conserved sequence motifs were carried out using a pattern discovery program (Hertz *et al.* 1990; Hertz and Stormo 1999).

RESULTS

Cloning of *Cb-her-1* by synteny: Several attempts to clone *Cb-her-1* by low stringency hybridization to a *Ce-her-1* probe were unsuccessful, suggesting that like the sex-determining genes *tra-2* (Kuwabara 1996) and *tra-1* (de Bono and Hodgkin 1996), the *her-1* gene might be poorly conserved between these species. Therefore, we attempted to identify *Cb-her-1* by taking advantage of synteny, which previously allowed cloning of *tra-2* (Kuwabara and Shah 1994).

From a *C. briggsae* genomic library we isolated a clone including predicted exons that were 70–80% identical, at the nucleotide level, to the *C. elegans* predicted protease inhibitor gene ZK287.4, which is located 11.7 kb upstream of the *her-1* cap site. We used this *C. briggsae* clone to identify a contig of overlapping fosmids, two of which (G39O07 and G33P21, totaling 65 kb) together included most of the contig.

Sequencing of these fosmids indicated synteny in a region of >45 kb spanning the *her-1* locus (Figure 2). Clear sequence similarities were evident at the nucleotide level between seven of eight predicted genes as well as several intergenic regions, but little if any similarity could be seen at the position of *Ce-her-1*. Moreover, a shift of the diagonal in the similarity plot (Figure 2) suggested that the region between the two genes that flank *her-1* is ~10 kb larger in *C. briggsae* than in *C. elegans*. Nevertheless, at this position in the *C. briggsae* sequence a BLAST search (Altschul *et al.* 1997) identified a predicted gene product that appeared to be a HER-1A homolog, with 57% identity and 76.7% similarity in the amino-acid sequence (Figure 3). We subsequently refer to this gene as *Cb-her-1*.

***Cb-her-1* produces a single transcript:** Sequencing of a partial *Cb-her-1* cDNA showed that the intron positions in the *C. briggsae* transcript are the same as in *C. elegans* (Figure 3A). Compared to most *C. elegans* introns, *Cb-her-1* introns 2 and 3 are unusually large (4076 and 1912 bp, respectively). *Ce-her-1* intron 2 is also large (3437 bp) and contains a second promoter (P2), which drives production of the smaller (0.8-kb) her-1b transcript (Trent *et al.* 1991; Perry *et al.* 1993; Li *et al.* 1999). When probed with the *Cb-her-1* partial cDNA, blots of RNA from mixed-stage populations of the *C. briggsae* wild type (AF16; <1% males) and *mih-3(s1290)* (strain BW1850, ~50% males) showed a single band of ~1.1 kb, which was enriched in the *mih-3* sample but present in both (Figure 4). When quantitated by PhosphoImager the difference in band intensity between the two samples indicated that this transcript is about threefold more abundant in males than in hermaphrodites. Similar results were obtained in a comparison of embryonic RNAs from these two strains (data not shown). We detected no band corresponding to the *C. elegans* smaller transcript her-1b in these experiments.

In *C. elegans*, her-1b, but not the larger transcript her-1a, is trans-spliced to the leader sequence SL1 (Perry *et al.* 1993). Using seminested PCR to assay for trans-splicing of the *Cb-her-1* mRNA with an SL1 primer and primers from exon 4 and then exon 3, we could detect no reproducible amplified product. This result is consistent with absence in *C. briggsae* of a smaller SL1-spliced transcript equivalent to *Ce*-her-1b, and it suggests that the *Cb-her-1* 1.1-kb transcript, like *Ce*-her-1a, is not trans-spliced to SL1.

The HER-1 amino acid sequence is partially conserved: *Cb-her-1* encodes a predicted protein of 174 amino acids (*Cb*-HER-1), compared to the predicted 175-residue *Ce*-HER-1A protein (Figure 3B). Whereas most homologous proteins in the closely related species *C. briggsae* and *C. elegans* exhibit high levels of identity (11 of 13 proteins compared ranged from 83 to 100% identity; de Bono and Hodgkin 1996), *Cb*-HER-1 is only 57% identical and 77% similar to *Ce*-HER-1A.

Despite its low degree of overall sequence similarity to *Ce*-HER-1A, the *Cb*-HER-1 sequence has several conserved features. First, both proteins include predicted secretion signal sequences at the N terminus (von Heijne 1986), with identical predicted cleavage sites. Second, the positions of the 14 cysteine residues are identical in the two proteins. Third, the two sequences

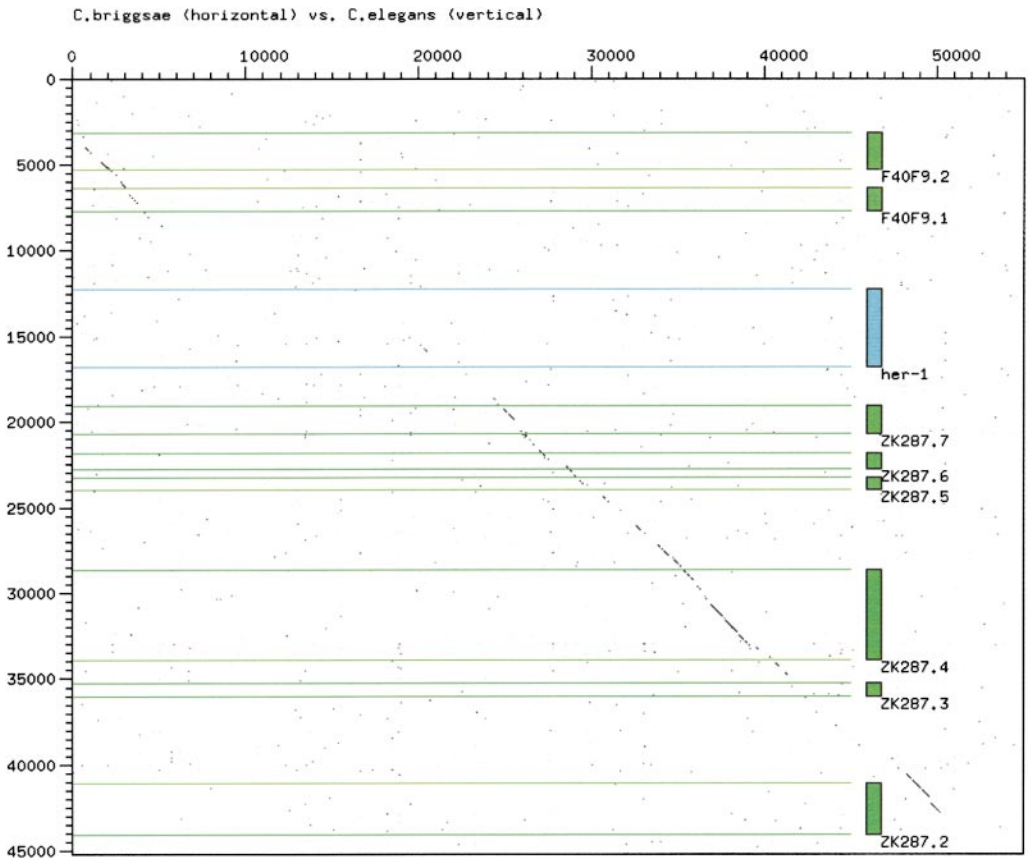

Figure 2.—Synteny is conserved in the *her-1* regions of *C. elegans* and *C. briggsae*. Genomic organization of the *C. elegans* and *C. briggsae her-1* regions were compared and displayed using the dot-matrix comparison program Dotter (Sonnhammer and Durbin 1996), with default parameter settings. The *C. elegans* sequence is represented on the vertical axis and the *C. briggsae* sequence on the horizontal axis. Axes are labeled in base pairs. Extensive DNA sequence similarity between the two species is visible as a diagonal line. Also shown are the approximate locations and names of genes predicted by the *C. elegans* Genome Sequencing Consortium. Shaded boxes indicate the approximate extent of predicted genes, including both introns and exons. Lines extending from the colored boxes to the left axis of the plot indicate the sequence coordinates of the predicted genes. See text for further explanation.

are identical at each of the 13 positions (including seven of the cysteines) at which amino acid substitutions resulting from known missense mutations in *C. elegans* cause loss of *her-1* function (Perry *et al.* 1994).

***C. briggsae her-1* has masculinizing activity:** Consistent with the masculinizing role of the *Ce*-HER-1 protein, transgenic extrachromosomal arrays including *Ce-her-1* cDNA driven by a *Ce-unc-54* (myosin heavy-chain) promoter can masculinize *C. elegans* XX animals (Perry *et al.* 1993) and in preliminary experiments were also seen to masculinize *C. briggsae* (M. D. Perry and W. B. Wood, unpublished results). To test whether *Cb-her-1* has masculinizing activity, we made transgenic *C. elegans* and *C. briggsae* hermaphrodites carrying arrays of a construct that included the *Cb-her-1* cDNA sequence driven by the *Ce-unc-54* promoter and terminated by sequence from the *Ce-her-1* 3′UTR (see materials and methods). We coinjected the chimeric construct with a *C. elegans rol-6(dm)* marker plasmid (Mello *et al.* 1991). In *C. elegans* we observed 59 masculinized F_1 progeny, all assumed to be transgenic since this phenotype is never seen among progeny of uninjected animals (Table 1 and Figure 5c). In support of this assumption, 57 of these animals were strongly masculinized and exhibited the Roller (Rol) phenotype. The remaining 2 were nonRol intersexes (note that only Rol transgenic progeny are listed in the body of Table 1). In *C. briggsae* we observed 371 F_1 progeny, again assumed to be transgenic, exhibiting a range of masculinization from intersexes with partially masculinized tails and protruding vulvas (not shown) to apparent males (~80% of the total; male gonad, no vulva) with imperfect tails (Figure 5d). All Rol animals were masculinized. However, only ~20% (43) of the masculinized animals displayed the Rol phenotype, possibly because, relative to the *Cb-her-1* construct, the *Ce-rol-6* promoter is less effective or the phenotype resulting from the *Ce-rol-6(dm)* mutation has a lower penetrance in *C. briggsae* than in *C. elegans*. In a control experiment using a derivative of the *Cb-her-1* construct with a frameshift mutation in exon 2 (*Cb-her-1FS*), we obtained 39 transgenic (Rol) F_1 animals; all of these, as well as their non-Rol siblings, were hermaphrodites with no apparent masculinization. These results suggest that the *Cb-her-1* gene has masculinizing activity when expressed ectopically from a transgenic extrachromosomal array in either *C. elegans* or *C. briggsae*.

A possible alternative explanation might be that the observed masculinization results from the *Ce-her-1* 3′-UTR sequences present in transcripts from the *Cb-her-1* construct, and that these sequences are absent in the *Cb-her-1FS* transgenic animals as a result of transcript degradation by the *smg* system (Pulak and Anderson

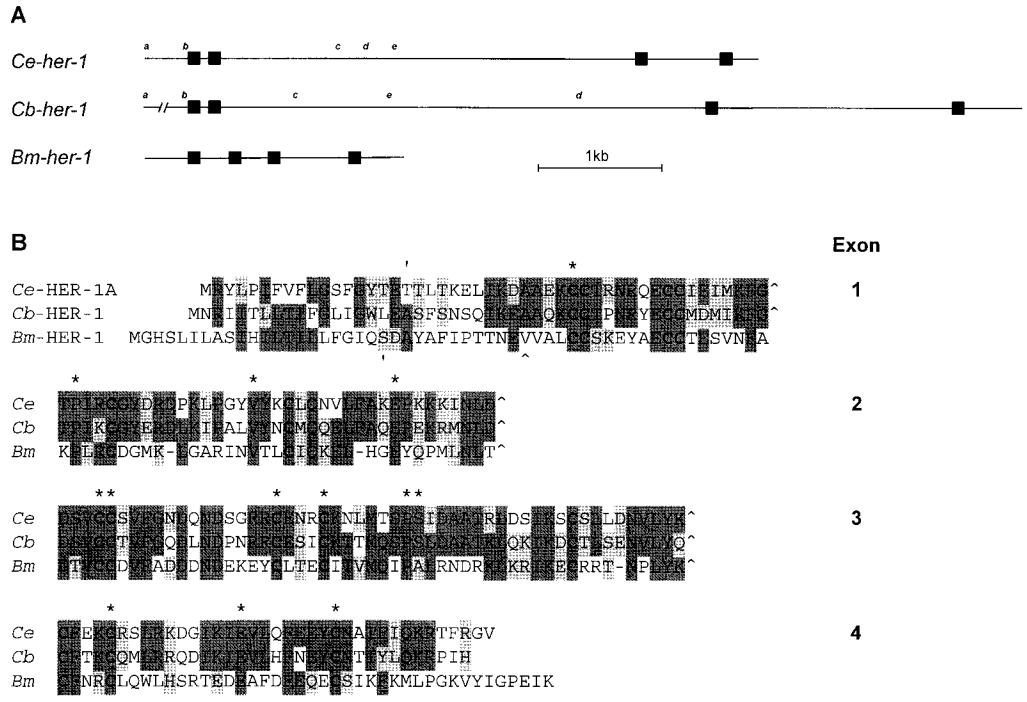

Figure 3.—(A) Structure of *her-1* genes from *C. elegans*, *C. briggsae*, and *B. malayi*. Lengths (in base pairs) of introns 1, 2, and 3, respectively, are the following: in *C. elegans*, 49, 3437, and 509; in *C. briggsae*, 48, 4076, and 1912; and in *B. malayi*, 224, 198, and 538. Italicized letters indicate approximate positions of common motifs or regions of similarity in the *Ce* and *Cb* genes that could include regulatory elements (see text). (B) Predicted amino acid sequences, shown using standard one-letter amino acid abbreviations, of HER-1 proteins encoded by the three homologs. Exon boundaries are indicated by carets (^). Sequences encoded by each of the four exons, as spliced in the two-Caenorhabditis species, are shown in a separate group of lines. In *B. malayi* the location of intron 1 is different, as shown by the caret in the first line. Predicted cleavage sites for secretion signal sequences are indicated by apostrophes in the first line, above for the two Caenorhabditis species and below for *B. malayi*. The cysteines (C) are printed in bold face. Identical residues are indicated by dark shading; residues similar to those in *C. elegans* are indicated by light shading. Asterisks indicate the amino acids altered by missense mutations in *C. elegans* that cause *her-1* loss-of-function phenotypes.

1993). This explanation seems highly unlikely because (1) a *Bm-her-1* transgenic construct (discussed below), carrying the same *Ce-her-1* 3′-UTR sequence, has only very weak, if any, masculinizing activity (Table 1), and (2) it was previously shown that the smaller her-1b transcript with an identical 3′-UTR sequence, when expressed in XX animals under control of either a heat-shock or a myosin heavy-chain (*unc-54*) promoter, had no detectable masculinizing activity (Perry *et al.* 1993).

As a further test for the normal activity of the *her-1* gene in *C. briggsae*, we used RNA-mediated interference (RNAi) to determine its probable loss-of-function phenotype (Fire *et al.* 1998). As an assay, we compared the percentage of male F_1 progeny produced by mated *C. briggsae mih-3(s1290)* (strain BW1850) hermaphrodites before and after injection with dsRNA made from *Cb-her-1* cDNA. Before injection these animals produced ~44% male progeny, whereas after injection they produced only ~8% male progeny (Table 2), as well as some presumably XO intersexual animals (Figure 5f). For comparison, this experiment was also carried out with *C. briggsae* wild type (AF16), using the same dsRNA, and with *C. elegans* wild type (N2), using dsRNA transcribed from *Ce-her-1* cDNA. As shown in Table 2, similar results were obtained in all three cases.

To ascertain the karyotypes of the F_1 hermaphrodites, we plated some of them individually and allowed them to produce F_2 self-progeny for analysis (Table 2). All the F_1 hermaphrodites from AF16 before injection produced normal-sized F_2 broods of hermaphrodites only. Most of the F_1 hermaphrodites from AF16 after injection also produced normal F_2 broods of hermaphrodites, but 12% of these F_1 hermaphrodites produced either completely nonviable F_2 broods or broods with very small numbers of viable animals, predominantly males. We observed similar effects from dsRNA made from *Ce-her-1* injected into mated *C. elegans* wild type. These results suggest that *her-1(RNAi)* in both *C. briggsae* and *C. elegans* results in transformation of XO animals into marginally fertile hermaphrodites, which is also the phenotype resulting from strong *her-1(lf)* alleles in *C. elegans* XO animals (Hodgkin 1980). We conclude that *Cb-her-1* and *Ce-her-1* are likely to be functional orthologs, required for male development in both species.

Searches for conserved *her-1* non-coding sequences: In the hope of identifying potential regulatory elements, we first searched visually for conserved nucleotide sequences in upstream and intron 2 regions identified previously as important for *Ce-her-1* regulation (Perry *et al.* 1993; Li *et al.* 1999). The region of the *Ce-her-1* P1 promoter (*b* in Figure 3A), as defined by loss-of-function mutations (Perry *et al.* 1994), contains two short sequence motifs separated by five nucleotides, which are also found as a single sequence with no separation upstream of *Cb-her-1* (Table 3). In intron 2, which contains the *Ce-her-1* P2 promoter, an 8-bp sequence is found as

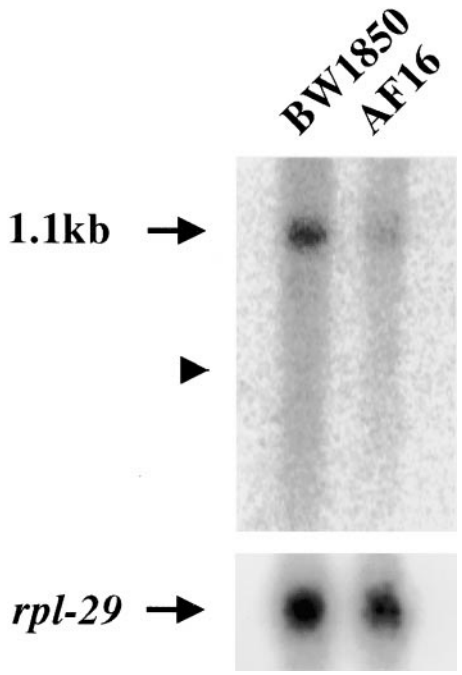

Figure 4.—*C. briggsae her-1* mRNA is enriched in males but also present in hermaphrodites. Blots of electrophoretically separated RNAs from mixed-stage populations of BW1850 (~50% males) and AF16 wild type (<1% males) were probed with a partial cDNA for *Cb-her-1* (upper panel) and with a ribosomal protein gene (*rpl-29*) to provide a loading control (lower panel; see materials and methods). Quantitation of all four bands by PhosphoImager and correction for the difference in loading indicated an approximately twofold higher level of *Cb-her-1* RNA in BW1850 relative to AF16, indicating an approximately threefold higher level of this RNA in males. Positions relative to the more slowly migrating ribosomal RNA bands (not shown) indicated the size of the *her-1* band to be ~1.1 kb, close to that of the larger *Ce-her-1* transcript her-1a. Arrowhead shows the position expected for a smaller transcript of 0.7 kb, corresponding to *Ce*-her-1b, which was not detected.

TABLE 1

Effects of *Cb-her-1* and *Bm-her-1* ectopic expression in *C. briggsae* and *C. elegans*

Injected strain	Injected transgene	Transgenic roller progeny produced		
		Total	Male[a]	Intersex[b]
C. elegans N2	*Cb-her-1*	57	57	0[c]
C. briggsae AF16	*Cb-her-1*	43[d]	37	6
C. briggsae AF16	*Cb-her-1FS*[e]	39	0	0
C. elegans N2	*Bm-her-1*	513	0	4
C. elegans PA43	*Bm-her-1*	48	0	0
C. briggsae AF16	*Bm-her-1*	79	1	0

[a] Male refers to animals that appeared under the dissecting microscope to have a male-like tail and no obvious hermaphroditic characteristics.

[b] Intersex refers to animals with both a male-like tail and a hermaphrodite vulva, based on scoring with a dissecting microscope.

[c] We observed two non-Roller intersex progeny, which must have been transgenic animals because this phenotype is never seen among progeny of uninjected animals.

[d] We observed an additional 328 non-Roller male-like progeny, of which 70 were intersexes. These must also have been transgenic animals because these phenotypes are never seen among progeny of uninjected animals.

[e] A derivative of the *Cb-her-1* construct that contains a frameshift mutation in exon 2.

an inverted repeat at the upstream end of a 400-bp sequence suspected from *in vivo* titration experiments to contain negative regulatory elements (Li *et al.* 1999). In *Cb-her-1*, only the right half of the palindrome is present in a more distal region of the intron (Table 3; *d* in Figure 3A).

Additional searches of upstream and intron 2 sequences were carried out using computer algorithms with finer resolution than Dotter (Figure 2). Using an algorithm for analyzing pairwise alignments of long sequences (Schwartz *et al.* 1991; with assistance from W. Miller, Pennsylvania State University), we identified three additional regions with significant similarity (63–65%) indicated in Figure 3A as *a* (70 nucleotides), *c* (105 nucleotides), and *e* (142 nucleotides; also located in the 400-bp *C. elegans* intron 2 sequence mentioned above). Using a pattern discovery program (Hertz *et al.* 1990; Hertz and Stormo 1999; with assistance from G. Stormo, University of Colorado, Boulder), we identified smaller sequence motifs common to the two genes, including two occurrences of CCGCCC in intron 2 of *Ce-her-1* and three in intron 2 of *Cb-her-1*, as well as a few other closely approximate matches in both sequences. Based on the previous mutational analysis, the P1 motif *b* could be a positive regulatory element common to the two *her-1* genes. The remaining similarities represent potential regulatory sites, but at present there is no evidence that any of them have functional significance.

A *her-1* homolog in the parasitic nematode *B. malayi*: The cDNA sequence that originally identified a potential *Bm-her-1* homolog was found as an EST (GenBank accession no. AA068389) in the course of the Filarial Genome Project (by I. H. Kamal and S. A. Williams). A complete cDNA was isolated and sequenced (by I. H. Kamal, R. M. Ramzy, D. Guiliano, and S. A. Williams; GenBank accession no. AF004290). We amplified and sequenced a partial cDNA clone from an adult male cDNA library, and then isolated and sequenced clones from a *B. malayi* genomic library to confirm the cDNA sequence and determine the gene structure, shown in Figure 3A and discussed below. The cDNA encodes a predicted protein of 183 amino acids (Figure 3B), which is only 35% identical and 42% similar to the sequence of *Ce*-HER-1A between residues 38 and 169. As observed with *C. briggsae*, however, despite the limited overall sequence similarity, the predicted Brugia sequence exhibits several conserved features. First, it includes a pre-

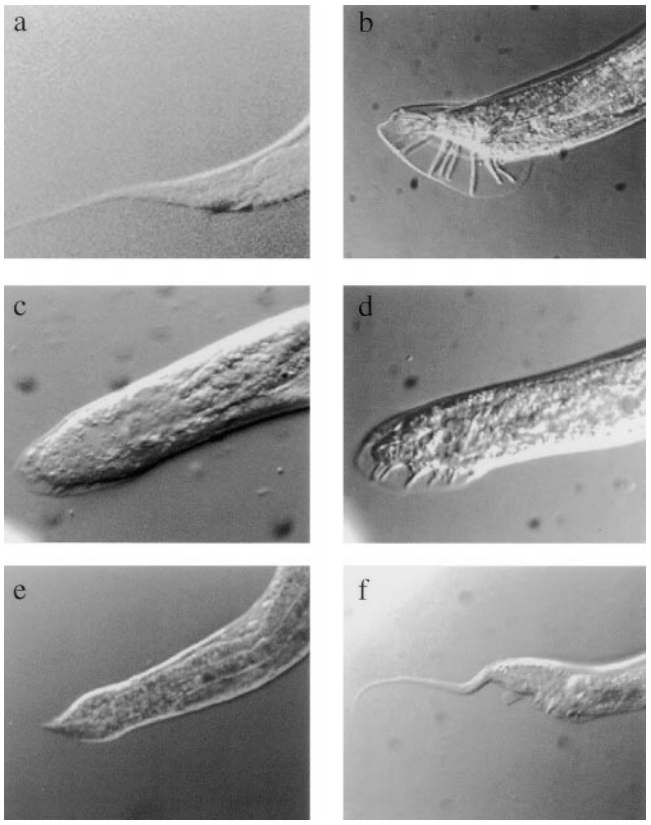

Figure 5.—Effects on tail morphology caused by *Cb-her-1* and *Bm-her-1* transgenes in XX animals and *Cb-her-1* dsRNA in an XO animal. (a and b) Hermaphrodite and male tails, respectively, of wild-type *C. elegans*. Tail structures of the two sexes in *C. briggsae*, not shown, are virtually identical to those in *C. elegans* except for a fusion of rays 3 and 4 in the *briggsae* male. (c and d) Masculinized tails of *C. elegans* and *C. briggsae* XX animals, respectively, carrying transgenic arrays of *Cb-her-1*. (e) Masculinized tail of a *C. elegans* XX animal carrying a transgenic array of *Bm-her-1*. (f) F_1 intersexual offspring, presumably XO, of a *C. briggsae mih-3* hermaphrodite injected with *Cb-her-1* dsRNA. See text for further explanation.

dicted secretion signal sequence at the N terminus, with a predicted cleavage site two residues upstream of the cleavage sites in the Caenorhabditis genes. Second, it includes 14 cysteine residues, which can be aligned with those of the Caenorhabditis proteins by the introduction of three single amino acid gaps. Third, with this alignment, the Brugia sequence is identical to that of *Ce*-HER-1A at 12 of the 13 positions (including seven of the cysteines) at which amino acid substitutions resulting from missense mutations in *C. elegans* cause loss of *her-1* function (Perry *et al.* 1994). (At the 13th position, the Brugia sequence has alanine in place of the serine in Caenorhabditis, whereas the *C. elegans* missense mutant has a less similar residue, phenylalanine, at this position.) Based on these conserved features, we will refer subsequently to this gene as *Bm-her-1*.

Like *Ce-her-1* and *Cb-her-1*, *Bm-her-1* includes four exons (Figure 3A). Intron positions are partially conserved: intron 1 is somewhat farther 5′, but introns 2 and 3 are located at precisely the same positions relative to the coding sequence as in the two Caenorhabditis genes when the cysteine codons are aligned as in Figure 3B. In contrast to the Caenorhabditis genes, all the introns in *Bm-her-1* are quite small.

The first four nucleotides of the *Bm-her-1* cDNA clone are identical to the last four nucleotides of SL1, which is highly conserved among all nematodes (Blaxter and Liu 1996). Using PCR with an SL1 primer and nested primers in exons 4 and 3, we were able to reproducibly amplify one major fragment of the expected size from the *B. malayi* cDNA library. Sequencing of this product confirmed the boundaries of exons 1 and 2, including the SL1 splice site. These results indicate that the *Bm-her-1* mRNA (in contrast to *Ce*-her-1a and *Cb*-her-1) is trans-spliced to SL1 and suggest that *B. malayi* does not make an SL1-trans-spliced shorter transcript corresponding to *Ce*-her-1b.

To test for masculinizing activity of the *Bm-her-1* gene, we carried out transgene experiments similar to those described above for *Cb-her-1*. When a construct consisting of the *Ce-unc-54* promoter driving the Brugia cDNA with the *Ce-her-1* 3′UTR appended was injected into *C. elegans* wild-type hermaphrodites with the *rol-6* marker construct, we observed 513 transgenic Rol F_1 progeny, most of which looked like normal hermaphrodites (Table 1). However, we also found 4 progeny with abnormal tails (Figure 5e), including 1 clearly masculinized animal in which ray structures were present (not shown). Similar injections into hermaphrodites of the *C. briggsae* wild-type strain AF16 resulted in only 1 strongly masculinized animal among the 80 transgenic Rol progeny recovered. These results could be interpreted to indicate that the *Bm-her-1* gene has a very weak masculinizing activity in both *C. elegans* and *C. briggsae* XX animals. An alternative possibility is that the small effects observed could result from the *Ce-her-1* 3′UTR sequence that was present in the *Bm-her-1* constructs. Previous experiments, mentioned above in connection with Table 1, found that no masculinization of *C. elegans* XX animals resulted from ectopic expression of the smaller her-1b transcript, which carries the same 3′-UTR sequence (Perry *et al.* 1993); however, the number of animals analyzed may have been too small to detect a very weak effect.

To test whether the chimeric *Bm-her-1* cDNA construct might be capable of rescuing (masculinizing) XO animals lacking an endogenous *her-1* gene, we injected into *C. elegans* hermaphrodites of genotype *him-8(e1489); her-1(y101hv1)* (strain PA43; this strain produces ~37% XO animals, which develop as hermaphrodites because *her-1* function is lacking). Whereas injection of *Ce-her-1* constructs allows these animals to produce XO male progeny (Perry *et al.* 1993), injection of the *Bm-her-1* construct resulted in no masculinized animals among 48 transgenic progeny that could be identified by their Rol phenotype (Table 1).

TABLE 2

Phenotypes resulting from *her-1 (RNAi)* in *C. briggsae* and *C. elegans*

F_1 progeny males produced before and after injection of mated hermaphrodites			F_2 broods from single-plated F_1 hermaphrodites produced before and after injection		
Strain injected	Before / after	% F_1 males (n)[a]	Hermaphrodites only	Inviable only	Mostly males
C. briggsae mih-3[b]	Before	44 (294)	—	—	—
	After	8 (173)	—	—	—
C. briggsae AF16[c]	Before	22 (907)	88	0	0
	After	5 (1031)	236	23 (8%)	12 (4%)
C. elegans N2[d]	Before	26 (53)	—	—	—
	After	5 (39)	27	8 (21%)	3 (8%)

[a] *n*, total number of progeny counted.
[b] Hermaphrodites of the *C. briggsae* strain BW1850 *mih-3(s1290)* were mated to males of the same genotype and then injected with dsRNA of the *Cb-her-1* gene. Progeny were scored from nine animals that produced >30% males before injection, indicating that mating had been successful.
[c] Hermaphrodites of the wild-type *C. briggsae* strain AF16 were mated to BW1850 males and then injected with dsRNA of the *Cb-her-1* gene. The presence of greater than a few percent males in the progeny from before injection was taken to indicate that mating had been successful. Progeny were scored from eight injected animals.
[d] Hermaphrodites of the wild-type *C. elegans* strain N2 were mated to *him-8(e1489)* males and then injected with dsRNA of the *C. elegans her-1* gene. Progeny were scored from one animal that produced more than a few percent males before injection, indicating that mating had been successful.

DISCUSSION

Conservation of genomic organization: The Spirurid parasitic nematode *B. malayi* is only distantly related to the Rhabditid species *C. elegans* and *C. briggsae*. It differs markedly in morphology as well as life cycle, and although current estimates are highly uncertain, these two orders of nematodes may have diverged as long as 400 mya (Blaxter 1998; Blaxter *et al.* 1998; M. Blaxter, personal communication). In contrast, *C. elegans* and *C. briggsae* are almost indistinguishable morphologically and are considered to be closely related. Nevertheless, genomic differences between these two species suggest that they may be as divergent as different orders of mammals, with perhaps as much as 50 million years of separation (reviewed in Fitch and Thomas 1997; Blaxter 1998). In spite of this divergence, preliminary results suggest some synteny between *C. elegans* and *B. malayi*, and between the two Caenorhabditis species there is extensive synteny (reviewed in Blaxter 1998), as there is between representatives of different mammalian orders. This feature has been exploited previously in cloning the *C. briggsae* homologs of *C. elegans* genes with too little sequence similarity for recognition by DNA hybridization, starting from closely linked genes with higher similarity (Kuwabara and Shah 1994). We have demonstrated synteny over at least 45 kb in the *her-1* region, allowing a gene that is quite distant from *her-1* (~10 kb) to be used as a starting point.

Conservation of the *her-1* locus and transcripts: The intron-exon structures of the *her-1* genes are highly conserved among the three species. Introns 2 and 3 are at the same positions in all three species; intron 1 is somewhat farther 5′ in *B. malayi* (Figure 3B). Unlike most *C. elegans* genes (Blumenthal and Steward 1997), both the *Ce-her-1* and *Cb-her-1* genes have an exceptionally long intron 2; in the latter, intron 3 is also

TABLE 3

Similarities in noncoding sequences of *Ce-her-1* and *Cb-her-1*

Sequence	Species, position
GAGTATCTAAGTCTCTTC[a]	*C. elegans*, upstream (P1 region; −186 to −168)[b]
GAGTATC.....CTCTTC	*C. briggsae*, upstream (−110 to −97)[b]
TTACTGAT|ATCAGTAA[c]	*C. elegans*, middle of intron 2
AATGTTTC|ATCAGTAA	*C. briggsae*, distal region of intron 2

[a] Underlined nucleotides are those altered in three *C. elegans her-1(lf)* mutations (Perry *et al.* 1994).
[b] Positions are indicated in base pairs upstream of the initiator ATG codon (indicated as *b* in Figure 3A).
[c] Vertical line indicates center of inverted repeat in *C. elegans* (indicated as *d* in Figure 3A). See text for further explanation.

very long (Figure 3A). In *Ce-her-1*, intron 2 contains a second promoter (P2), which drives a shorter transcript (Perry *et al.* 1993); we have shown that there is probably no such shorter transcript from *Cb-her-1*. Interestingly, in *C. elegans* the sex-determining genes *tra-1* and *tra-2* also have shorter transcripts with functions that are not well understood, and all these shorter transcripts are either absent or different in *C. briggsae* (de Bono and Hodgkin 1996; Kuwabara 1996; Kuwabara *et al.* 1998). A second difference between *Cb-her-1* and *Ce-her-1* is in the sex specificity of transcript accumulation. Whereas in *C. elegans*, males were estimated to have about 100-fold higher levels of her-1b mRNA than hermaphrodites (Trent *et al.* 1991), our preliminary analysis of *C. briggsae* expression shows that males have only ~3-fold higher levels of *her-1* transcripts than hermaphrodites (see further discussion below). Despite these differences in expression, our sequence comparisons identified several conserved regions that could represent common regulatory elements; however, there is so far no evidence that they function in sex-specific regulation.

Conservation of the HER-1 proteins: The sequences of the three HER-1 proteins compared here are considerably more divergent than those of other proteins not involved in sex determination that have been compared among *C. elegans*, *C. briggsae* (de Bono and Hodgkin 1996), and in a few cases, *B. malayi* (M. Blaxter, personal communication; see discussion of evolutionary implications below). We had hoped that this divergence would help in identification of domains important for HER-1 function. However, except for the hydrophobic N termini, which presumably serve as secretion signals, we did not find localized conserved domains. Instead, we found that the similarities extend over virtually the entire protein (Figure 3). Most striking is the perfect conservation of the 14 cysteine residues. The apparent distribution of functionally important elements is consistent with the finding that loss-of-function missense mutations are also found distributed throughout most of the *her-1* coding regions in *C. elegans* (Perry *et al.* 1994). It is noteworthy that all of the 13 amino acids affected by these mutations are conserved in *C. briggsae*, and all but one are conserved in *B. malayi*. These conserved features and the apparent ability of at least two of these proteins to masculinize *C. elegans* suggest that some biochemical functions are conserved as well. Whereas we have demonstrated directly that the *C. briggsae her-1* gene is required for normal sex determination, we can draw no conclusions about whether the *B. malayi her-1* gene also controls male development. Unfortunately, little is known about the sex determining mechanism in *B. malayi*. The finding of the *Bm-her-1* cDNA in a male-derived library is consistent with a masculinizing function. Alternatively, however, it could play a biochemical role similar to that of *Ce-her-1* but in a different signaling pathway, as may be the case for murine homologs of the *C. elegans* masculinizing gene *fem-1* (Ventura-Holman *et al.* 1998).

Evolutionary implications: Sex-determining mechanisms appear to evolve relatively rapidly (reviewed by Marin and Baker 1998). In several classes of animals, it has been observed that gene products involved in sex determination are more divergent than most other proteins compared among species (*e.g.*, O'Neil and Belote 1992; Tucker and Lundrigan 1993; Whitfield *et al.* 1993). Three more examples have been reported among Rhabditid nematodes in comparisons of the sex-determining proteins TRA-2 (Kuwabara 1996), TRA-1 (de Bono and Hodgkin 1996), and FEM-2 (Hansen and Pilgrim 1998) and their homologs between *C. elegans* and *C. briggsae*, and we have here reported on the HER-1 proteins as a fourth example. We have also obtained evidence that, although *her-1* is male determining in both Caenorhabditis species, its mechanism of sex-specific regulation may also have diverged. In *C. elegans her-1* activity is regulated at the transcript level (Trent *et al.* 1991; Perry *et al.* 1993), while in *C. briggsae*, transcript concentrations in the two sexes appear similar enough to suggest that post-transcriptional regulation may also be necessary.

Rapid divergence of sex-determining mechanisms and component proteins may be selected for as contributing to speciation. Whatever its causes, aspects of this divergence suggest a "bottom-up" model for evolution of sex determination (Wilkins 1995; Marin and Baker 1998). In general, downstream elements of sex-determining pathways appear to be more conserved; the farther up the pathways one looks the more divergent sex-determining mechanisms become. For example, the *mab-3* gene, a downstream target of the sex-determining *C. elegans* transcription factor TRA-1, appears homologous to the downstream Drosophila sex-determining gene *doublesex* (Raymond *et al.* 1998). Upstream of *mab-3*, the *C. elegans* genes *her-1*, *tra-2*, *fem-2*, and *tra-1* (Figure 1) appear to have no Drosophila homologs involved in sex determination, although they do have functional homologs in *C. briggsae* as reviewed above. *her-1* is so far the farthest upstream gene in *C. elegans* for which the *C. briggsae* homolog has been found. There is evidence that regulation of *tra-2*, at least in the soma, is similar in *C. elegans* and *C. briggsae* (Kuwabara 1996). Our results suggest that, in contrast, *her-1* may be controlled differently in the two species. It is tempting to speculate that *her-1* might be a point at which sex-determining mechanisms in *C. elegans* and *C. briggsae* have diverged. It will be interesting to determine whether homologs of genes farther upstream in the *C. elegans* pathway, such as the *sdc* genes and *xol-1*, exist and exhibit conserved functions in *C. briggsae*. The completion of the *C. elegans* genome sequencing project and the availability of a *C. briggsae* fosmid library of genomic clones should allow straightforward searches for such homologs, even if their sequence conservation is minimal.

We are grateful to J. Yochem for protocols, to members of the Wood lab for helpful discussions, to D. Baillie and collaborators for the *C. briggsae* strains and genomic library, to M. Blaxter for originally bringing the Brugia *her-1* homolog to our attention and for comments on the manuscript, to S. A. Williams and the Filarial Genome Project Resource Center as well as to U. Wagner for *B. malayi* libraries, and to G. Stormo and W. Wilson for help with genomic sequence comparisons. Some *C. elegans* strains were supplied by the Caenorhabditis Genetics Center, which is funded by the National Institutes of Health (NIH) National Center for Research Resources. A.S. received postdoctoral fellowship support from the Swiss National Science Foundation and the Ciba-Geigy Jubiläumsstiftung, and I.H.K. was funded by a WHO-UNDP-World Bank Tropical Diseases Research Program grant to the Filarial Genome Project. Research carried out in St. Louis was supported by the Washington University Genome Sequencing Center. Research carried out in Boulder was supported by NIH grant HD-11762 to W.B.W.

LITERATURE CITED

Altschul, S. F., T. L. Madden, A. A. Schaffer, J. Zhang, Z. Zhang *et al.*, 1997 Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. **25:** 3389–3402.

Blaxter, M., 1998 *Caenorhabditis elegans* is a nematode. Science **282:** 2041–2046.

Blaxter, M., and D. Bird, 1997 Parasitic nematodes, pp. 851–878 in *C. elegans II*, edited by D. L. Riddle, T. Blumenthal, B. J. Meyer and J. R. Priess. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Blaxter, M., and L. Liu, 1996 Nematode spliced leaders—ubiquity, evolution and utility. Int. J. Parasitol. **26:** 1025–1033.

Blaxter, M. L., P. De Ley, J. R. Garey, L. X. Liu, P. Scheldeman *et al.*, 1998 A molecular evolutionary framework for the phylum Nematoda. Nature **392:** 71–75.

Blumenthal, T., and K. Steward, 1997 RNA processing and gene structure, pp. 117–145 in *C. elegans II*, edited by D. L. Riddle, T. Blumenthal, B. J. Meyer and J. R. Priess. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Brenner, S., 1974 The genetics of *Caenorhabditis elegans*. Genetics **77:** 71–94.

Cline, T. W., and B. J. Meyer, 1996 Vive la difference: males vs. females in flies vs. worms. Annu. Rev. Genet. **30:** 637–702.

de Bono, M., and J. Hodgkin, 1996 Evolution of sex determination in Caenorhabditis: unusually high divergence of tra-1 and its functional consequences. Genetics **144:** 587–595.

Fire, A., S. W. Harrison and D. Dixon, 1990 A modular set of lacZ fusion vectors for studying gene expression in *Caenorhabditis elegans*. Gene **93:** 189–198.

Fire, A., S. Xu, M. K. Montgomery, S. A. Kostas, S. E. Driver *et al.*, 1998 Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. Nature **391:** 806–811.

Fitch, D. H. A., and W. K. Thomas, 1997 Evolution, pp. 815–850 in *C. elegans II*, edited by D. L. Riddle, T. Blumenthal, B. J. Meyer and J. R. Priess. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Fitch, D., B. Bugai-Gaweda and S. Emmons, 1995 18S Ribosomal RNA gene phylogeny for some Rhabditidae related to Caenorhabditis. Mol. Biol. Evol. **12:** 346–358.

Hansen, D., and D. Pilgrim, 1998 Molecular evolution of a sex determination protein: *fem-2* (pp2c) in Caenorhabditis. Genetics **149:** 1353–1362.

Hertz, G. Z., and G. D. Stormo, 1999 Identifying DNA and protein patterns with statistically significant alignments of multiple sequences. Bioinformatics (in press).

Hertz, G. Z., G. W. Hartzell and G. D. Stormo, 1990 Identification of consensus patterns in unaligned DNA sequences known to be functionally related. Comput. Appl. Biosci. **6:** 81–92.

Hodgkin, J., 1980 More sex-determination mutants of *Caenorhabditis elegans*. Genetics **96:** 649–664.

Hodgkin, J., 1992 Genetic sex determination: mechanisms and evolution. BioEssays **14:** 253–261.

Hodgkin, J., H. Horvitz and S. Brenner, 1979 Nondisjunction mutants of the nematode *Caenorhabditis elegans*. Genetics **91:** 67–94.

Hunter, C., and W. B. Wood, 1992 Evidence from mosaic analysis of the masculinizing gene *her-1* for cell interactions in *C. elegans* sex determination. Nature **355:** 551–555.

Innis, M. A., D. H. Gelfand, J. J. Sninsky and T. J. White, 1990 *PCR Protocols*. Academic Press, San Diego.

Kuwabara, P. E., 1996 Interspecies comparison reveals evolution of control regions in the Nematode sex-determining gene tra-2. Genetics **144:** 597–607.

Kuwabara, P. E., and S. Shah, 1994 Cloning by synteny: identifying *C. briggsae* homologues of *C. elegans* genes. Nucleic Acids Res. **22:** 4414–4418.

Kuwabara, P., P. Okkema and J. Kimble, 1992 *tra-2* encodes a membrane protein and may mediate cell communication in the *Caenorhabditis elegans* sex determination pathway. Mol. Biol. Cell **3:** 461–473.

Kuwabara, P. E., P. G. Okkema and J. Kimble, 1998 Germ-line regulation of the *Caenorhabditis elegans* sex-determining gene *tra-2*. Dev. Biol. **204:** 251–262.

Li, W., A. Streit, B. Robertson and W. B. Wood, 1999 Evidence for multiple promoter elements orchestrating male-specific regulation of the *her-1* gene in *C. elegans*. Genetics **152:** 237–248.

Manak, J., and M. Scott, 1994 A class act: conservation of homeodomain protein functions. Development (Suppl.)**120S**: 61–71.

Marin, I., and B. S. Baker, 1998 The evolutionary dynamics of sex determination. Science **281:** 1990–1994.

Mello, C., J. Kramer, D. Stinchcomb and V. Ambros, 1991 Efficient gene transfer in *C. elegans*: extrachromosomal maintenance and integration of transforming sequences. EMBO J. **10:** 3959–3970.

Meyer, B. J., 1997 Sex determination and X chromosome dosage compensation, pp. 209–240 in *C. elegans II*, edited by D. L. Riddle, T. Blumenthal, B. J. Meyer and J. R. Priess. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Montgomery, M. K., and A. Fire, 1998 Double-stranded RNA as a mediator in sequence-specific genetic silencing and co-suppression. Trends Genet. **14:** 255–258.

O'Neil, M. T., and J. M. Belote, 1992 Interspecific comparison of the transformer gene of Drosophila reveals an unusually high degree of evolutionary divergence. Genetics **131:** 113–128.

Perry, M., W. Li, C. Trent, B. Robertson, A. Fire *et al.*, 1993 Molecular characterization of the *her-1* gene suggests a direct role in cell signaling during *Caenorhabditis elegans* sex determination. Genes Dev. **7:** 216–228.

Perry, M. D., C. Trent, B. Robertson, C. Chamblin and W. B. Wood, 1994 Sequenced alleles of the *C. elegans* sex-determining gene *her-1* include a novel class of conditional promoter mutations. Genetics **138:** 317–327.

Pulak, R., and P. Anderson, 1993 mRNA surveillance by the *Caenorhabditis elegans smg* genes. Genes Dev. **7:** 1885–1897.

Raymond, C. S., C. E. Shamu, M. M. Shen, K. J. Seifert, B. Hirsch *et al.*, 1998 Evidence for evolutionary conservation of sex-determining genes. Nature **391:** 692–695.

Ryner, L., and A. Swain, 1995 Sex in the '90s. Cell **81:** 483–493.

Sambrook, J., E. F. Fritsch and T. Maniatis, 1989 *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Schwartz, S., W. Miller, C. M. Yang and R. C. Hardison, 1991 Software tools for analyzing pairwise alignments of long sequences. Nucleic Acids Res. **19:** 4663–4667.

Sonnhammer, E. L. L., and R. Durbin, 1996 A dot-matrix program with dynamic threshold control suited for genomic DNA and protein sequence analysis. Gene **167:** GC1–10.

Sulston, J., and J. Hodgkin, 1988 Methods, pp. 81–122 in *The Nematode Caenorhabditis elegans*, edited by W. B. Wood. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Sulston, J., Z. Du, K. Thomas, R. Wilson, L. Hillier *et al.*, 1992 The *C. elegans* genome sequencing project: a beginning. Nature **356:** 37–41.

Trent, C., B. Purnell, S. Gavinski, J. Hageman, C. Chamblin *et al.*, 1991 Sex-specific transcriptional regulation of the *C. elegans* sex-determining gene *her-1*. Mech. Dev. **34:** 43–56.

Tsang, S. S., X. Yin, C. Guzzo-Arkuran, V. S. Jones and A. J. Davison, 1993 Loss of resolution in gel electrophoresis of RNA: a prob-

lem associated with the presence of formaldehyde gradients. Biotechniques **14:** 380–381.

Tucker, P. K., and B. L. Lundrigan, 1993 Rapid evolution of the sex determining locus in Old World mice and rats. Nature **364:** 715–717.

Ventura-Holman, T., M. F. Seldin, W. Li and J. F. Maher, 1998 The murine Fem1 gene family: homologs of the *Caenorhabditis elegans* sex-determination protein FEM-1. Genomics **54:** 221–230.

von Heijne, G., 1986 A new method for predicting signal sequence cleavage sites. Nucleic Acids Res. **14:** 4683–4690.

Whitfield, L. S., R. Lovell-Badge and P. N. Goodfellow, 1993 Rapid sequence evolution of the mammalian sex-determining gene SRY. Nature **364:** 713–715.

Wilkins, A. S., 1995 Moving up the hierarchy: a hypothesis on the evolution of a genetic sex determination pathway. Bioessays **17:** 71–77.

Yochem, J., and I. Greenwald, 1989 *glp-1* and *lin-12*, genes implicated in distinct cell-cell interactions in *C. elegans*, encode similar transmembrane proteins. Cell **58:** 553–563.

Communicating editor: R. K. Herman