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Caenorhabditis elegans homologue of the human azoospermia factor *DAZ* is required for oogenesis but not for spermatogenesis

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SUMMARY

***DAZ* (*Deleted in Azoospermia*), the putative azoospermia factor gene in human, encodes a ribonucleoprotein-type RNA-binding protein required for spermatogenesis. A *Drosophila* homologue of *DAZ*, called *boule*, is also essential for spermatogenesis. A mouse homologue, *Dazla*, is implicated in both spermatogenesis and oogenesis. Here, we report the identification and characterization of *daz-1*, the single *DAZ* homologue in the nematode *Caenorhabditis elegans*. Loss of *daz-1* function caused sterility in hermaphrodites, by blocking oogenesis at the pachytene stage of meiosis I. Epistasis analysis suggested that this gene executes its function succeeding *gld-1*, which governs the early pachytene stage in the oogenic pathway. Spermatogenesis did not appear to be affected in *daz-1* hermaphrodites. Males defective in *daz-1* produced sperm fully competent in fertilization. Analysis employing sex-determination mutants indicated that the *daz-1* function was required for meiosis of female germline regardless of the sex of the soma. Transcription of *daz-1* was restricted to the germline, starting prior to the onset of meiosis and was most conspicuous in cells undergoing oogenesis. Thus, *daz-1* in *C. elegans* is an essential factor for female meiosis but, unlike other *DAZ* family members so far reported, it is dispensable for male meiosis.**

Key words: *Caenorhabditis elegans*, Germline, *DAZ*, Meiosis, Oogenesis, Pachytene

INTRODUCTION

Mechanisms underlying gametogenesis are complex and apparently divergent among metazoans. The *DAZ* (*Deleted in Azoospermia*) gene family provides one of the few lines of evidence that argue for evolutionary conservation of gametogenesis at the molecular level. DAZ family proteins carry two conserved domains, namely the ribonucleoprotein (RNP)-type RNA recognition motif (RRM) and the DAZ motif, and have been found in both vertebrates and invertebrates (Cooke et al., 1996; Eberhart et al., 1996; Reijo et al., 1995). In humans, multiple copies of *DAZ* genes cluster in a small region on the Y chromosome (Reijo et al., 1995; Saxena et al., 1996). Deletion of the *DAZ* cluster has been correlated with azoospermia and oligospermia, which makes DAZ a strong candidate for the Azoospermia factor (AZF). In addition, an autosomal *DAZ*-like gene, termed *DAZLA/DAZH*, has been found on human chromosome 3 (Reijo et al., 1996; Seboun et al., 1997). Mouse carries *Dazla*, a homologue of *DAZLA/DAZH*, on an autosome, but has no *DAZ* cluster on the sex chromosome (Cooke et al., 1996). Hence, the human autosomal gene has been proposed to be the ancestor of the *DAZ* cluster, with its transposition to the Y chromosome during the evolution of primates (Saxena et al., 1996). Knock-out of mouse *Dazla* resulted in the complete absence of gamete production in both sexes (Ruggiu et al., 1997). In contrast, a homologue of *DAZ* in *Drosophila*, named *boule*, is essential for the meiotic progression in spermatogenesis but not in oogenesis (Eberhart et al., 1996). Expression of these *DAZ* family genes appears to be confined to the germline. Human *DAZ* and *Drosophila boule* are transcribed specifically in the male germline (Eberhart et al., 1996; Reijo et al., 1995; Saxena et al., 1996), while human *DAZLA/DAZH* and its mouse, zebrafish, and *Xenopus* homologues are expressed in the germline of both sexes (Houston et al., 1998; Maegawa et al., 1999; Ruggiu et al., 1997; Seligman and Page, 1998).

The role of the *DAZ* family genes in the production of gametes is still largely unknown, partly due to the apparent phenotypic diversity caused by their defects. A human male missing the *DAZ* cluster shows a varying range of defects in spermatogenesis, from no germ cells at all to less severe spermatogenic arrest generating some mature spermatids (Reijo et al., 1995), indicating that the *DAZ* cluster is not absolutely necessary for the entry to meiosis and sperm differentiation. In the *Dazla*-defective mouse, female germ cells arrest at prophase of meiosis I, whereas male germ cells are affected at the proliferating stage (Ruggiu et al., 1997). In the *Drosophila boule* mutant, male germ cells arrest at the G_2/M transition in meiosis I, exhibiting limited postmeiotic differentiation (Eberhart et al., 1996). This has been attributed

to the lowered level of translation of *twine* mRNA encoding a Cdc25-type phosphatase (Maines and Wasserman, 1999).

Under these circumstances, it is interesting to see whether or not the *DAZ* family genes perform essentially the same molecular function in gametogenesis. Here, we report analysis of a *DAZ* family gene in the nematode *Caenorhabditis elegans* at the cellular and organismal levels. *C. elegans* is a favorable metazoan to investigate the process of gametogenesis, because of its genetic accessibility, the completed genome project facilitating reverse genetics, and the ease of observation of germline development (Schedl, 1997). We cloned the single *DAZ* family gene in *C. elegans*, *daz-1*, and isolated *daz-1* loss-of-function mutants. The *daz-1*-defective hermaphrodite turned out to be completely sterile due to arrest at meiotic prophase in oogenesis. Unexpectedly, however, *daz-1*-defective males showed no significant defect and were fully fertile. Thus, although *C. elegans daz-1* is a regulator of gametogenesis, its sex specificity is distinct from that of the human, mouse and *Drosophila* counterparts.

MATERIALS AND METHODS

General methods and strains

Maintenance and genetic manipulation of *C. elegans* were carried out as described (Brenner, 1974). Strains used were: wild-type *C. elegans* var. Bristol, strain N2 (Brenner, 1974); (LGI) *unc-13(e51, e1091), lin-11(n566), fog-3(q470), gld-1(q485), glp-4(bn2)*; (LGII) *daz-1(tj3, tj7)* (this study), *unc-4(e120), mIn1[dpy-10(e128)]* (generously provided by M. Edgley and D. L. Riddle, University of Missouri, Columbia); (LGIV) *fem-3(q20gf, e2006lf), ced-3(n717)*. Strains were maintained at 20°C, except for the temperature-sensitive mutants *fem-3(q20gf, e2006lf)* and *glp-4(bn2)*, which were maintained at 15°C and analyzed at 25°C.

Identification and cloning of *daz-1*

The *C. elegans* genome sequencing project identified a single possible homologue of *DAZ* (ORF F56D1.5; DDBJ/EMBL/GenBank accession number U39997). A corresponding cDNA clone yk62b4, which lacked part of the 5′ region, was kindly supplied by Yuji Kohara. The missing 5′-terminal sequence was cloned by RT-PCR, as described (Salles et al., 1992). Poly(A)$^+$ RNA (3 μg) prepared from N2 strain was reverse-transcribed using a *daz-1*-specific primer RACE1 (5′-CGATGGTCTCTCAGTGCGAGCTCC-3′). Produced cDNA was amplified by two rounds of PCR, first with a SL1-specific primer (5′-GGTTTAATTACCCAAGTTTGAG-3′) and a *daz-1*-specific primer RACE2 (5′-GATTGAGCAAACTGTTGATATG-GATGAGG-3′), and then with the same SL1 primer and another *daz-1*-specific primer RACE3 (5′-CCTGCTTTGATCCTGACTGCG-TAACGGAGG-3′). The isolated RT-PCR product contained additional 22 bases that were absent in yk62b4 (excluding the SL1 sequence). The sequence of the deduced full-length *daz-1* cDNA was registered to DDBJ/EMBL/GenBank nucleotide sequence databases under the accession number AB025253.

Isolation of *daz-1* deletion mutants using UV/TMP population screening

A worm library mutagenized with a combination of trimethylpsoralen and UV irradiation (Yandell et al., 1994) was screened for a deletion in the *daz-1* locus according to the protocol distributed by Moulder and Barstead (http://snmc01.omrf.uokhsc.edu/revgen/RevGen.html), essentially as we described before (Ohmachi et al., 1999). PCR primers used were as follows (the numbers given in parentheses represent the 5′ end point of each primer on the cosmid F56D1). The outer primer set: b1′, 5′-CCACAAAAATGACCTACTAC (9914), and b4′, 5′-GAACTAAAAACTTGCGGTTG-3′ (14153); the inner primer set: n1, 5′-TGGATTGACGAAGAAGACTG (10050), and n2, 5′-TCGCCTTGATTCTCTCTTTG-3′ (13471).

Two independent *daz-1* deletion mutants (*tj3* and *tj7*) were isolated. To eliminate possible additional mutations, backcrosses were performed five times for each strain. Sequence analysis indicated that the *tj3* allele carried a 2073 bp deletion corresponding to nucleotides 11115 to 13187 of the cosmid F56D1, whereas *tj7* carried a 1609 bp deletion corresponding to nucleotides 10484 to 12092 of the cosmid.

RNA interference experiment

Antisense RNA molecules for *daz-1* were prepared by in vitro transcription of the *daz-1* cDNA fragment (nucleotides (nt) 27-1450 in Fig. 1) cloned on the pBluescript SK(–) vector, as described (Guo and Kemphues, 1995). They were microinjected into the distal arm of the gonad in wild-type young adult hermaphrodites. F_1 hermaphrodites from these injected worms were examined for phenotypes. The RNA-injected hermaphrodites were also crossed with N2 males, and F_1 male progeny were examined. For the RNAi experiment to interfere with *gld-1*, the cDNA clone yk30e10 (provided by Y. Kohara) was used as the template to prepare antisense and sense *gld-1* RNA (Fire et al., 1998). Worms were soaked in the concentrated *gld-1* RNA solution containing both sense and antisense strands for 24 hours before phenotypic analysis (Tabara et al., 1998).

Mating test of *daz-1* male

Males with the genotype either *daz-1(tj3) unc-4(e120) II* or *unc-4(e120)II* as a positive control were test-mated to an indicator Dpy strain, *mIn1[dpy-10(e128)]*. On each mating plate, one male and four *mIn1* hermaphrodites were allowed to mate.

Observation of sperm

Both hermaphrodite and male adult worms were dissected at the position of spermatheca in 5 ml of sperm medium (50 mM Hepes (pH 7.0), 50 mM NaCl, 25 mM KCl, 1 mM $MgSO_4$, 5 mM $CaCl_2$, 1 mg/ml BSA) (Nelson and Ward, 1980). Monensin was added to 0.5 mM to the resultant sperm suspension and activated sperm were observed under Nomarski microscopy.

DAPI staining and immunofluorescence

DAPI staining was performed as described (Kadyk and Kimble, 1998). Worms were fixed in –20°C methanol and stained with 0.1 μg/ml DAPI in M9. Immunostaining was performed essentially as described (Crittenden et al., 1994). Dissected gonads were fixed with 1% paraformaldehyde in PBS and permeabilized in TBS/0.5% BSA supplemented with 0.1% Triton X-100. The sample was subsequently incubated with anti-phosphohistone H3 antibody (06-570, Upstate Biotechnology), diluted 1/300 in TBS/0.5% BSA, at 4°C overnight. The Cy3-labeled goat anti-mouse IgG antibody (AP132C, CHEMICON) was used as secondary antibody. DAPI was added to the final concentration of 2 μg/ml, and the sample was mounted for microscopy. Microscopic images taken by a cooled CCD camera (Hamamatsu Photonics) attached to a Zeiss Axiophot microscope were stored digitally using either the NIH Image or the Fish Imaging Software (Hamamatsu Photonics) program.

Dead cell staining with SYTO12

Apoptotic germ cells were stained with SYTO12 (Molecular Probes, Eugene, OR), as described (Gumienny et al., 1999). Adult worms were incubated in 33 μM SYTO12 in M9 for 4.5 hours at room temperature. Worms were then fed on seeded plates for an hour and observed microscopically with a FITC filter.

Northern analysis

Total RNA was isolated using TRIZOL reagent (Gibco BRL) from a culture of L4 to adult worms grown at 25°C, for the wild-type (N2),

glp-4(bn2), fem-3(e2006lf) and *fem-3(q20gf)* strains. Each poly(A)$^+$-selected RNA preparation was subjected to formaldehyde agarose gel electrophoresis and then transferred to Hybond N+ nylon membrane. The membrane was probed by ^{32}P-labeled *daz-1*-specific DNA prepared with the Amersham Multiprime DNA Labeling kit using a *daz-1* cDNA fragment (nt 27-1450 in Fig. 1) as the template.

In situ hybridization

Whole-mount in situ hybridization was performed according to the procedure of Tabara et al. (1996), with modifications as described by Kohara at http://watson.genes.nig.ac.jp:8080/db/method/. For in situ hybridization of dissected gonads, the procedure of Jones et al. (1996) was employed. DIG-labeled sense (control) and antisense DNA probes were synthesized using the T7 primer from cDNA yk62b4 cloned in pKS(+) and pSK(+) vectors, respectively.

RESULTS

Identification of a *DAZ* homologue in *C. elegans*

C. elegans has a single predicted gene that apparently belongs to the *DAZ* family, namely F56D1.5 on chromosome II (mapping position −1.94) (The *C. elegans* Sequencing Consortium, 1998). We call it *daz-1* hereafter. We assembled a full-length *daz-1* cDNA as described in Materials and methods. The putative initiation codon was located 27 nucleotides downstream of the SL1 *trans*-spliced leader sequence (Krause and Hirsh, 1987). The full-length *daz-1* cDNA was about 2.0 kb in length, consisting of the 5′-UTR of 48 nt (including SL1), the coding region of 1500 nt (including the termination codon), and the 3′-UTR of 461 nt (Fig. 1A).

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GGTTCTTGGAATCAAAGCGAACGGGTTATGATGTCGCCGCCTCTACGGTATCAAAAAGACCAACAAAATCAGCAACATCAGCAAAATCAATCACAGCAAGCTGCTCATCAGATGGTCTCT   120
                          M  M  S  P  P  L  R  Y  Q  K  D  Q  Q  N  Q  Q  H  Q  Q  N  Q  S  Q  Q  A  A  H  Q  M  V  S     31

TTCATGCCACAAACACCGACTTCTCTTCCATCAACTCCGATACAACTTTATCCAACCGGAGCAGCCGCACTAATACCAGCACCACCTACATACGAGCTGATTCCAAACCGTATTTTCGTT   240
 F  M  P  Q  T  P  T  S  L  P  S  T  P  I  Q  L  Y  P  T  G  A  A  A  L  I  P  A  P  P  T  Y  E  L  I  P  N  R  I  F  V     71

GGTGGCTTCCCAACTTCGACCACAGAAACTGAGTTGCGTGAACATTTCGAGAAATTCTTCGCTGTGAAGGACGTCAAAATGGTTAAATCACTTGATGGTCAGTCAAAGGGATACGGATTT   360
 G  G  F  P  T  S  T  T  E  T  E  L  R  E  H  F  E  K  F  F  A  V  K  D  V  K  M  V  K  S  L  D  G  Q  S  K  G  Y  G  F     111

ATCACCTTCGAAACCGAAGATCAAGCAGAGGAAATTCGTAAACTGACTCCAAAGCAGTTGGAATTCCGTAGCAGGAAGCTCAACCTAGGTCCAGCGATCCGAAAAATAAACTCCAATAGT   480
 I  T  F  E  T  E  D  Q  A  E  E  I  R  K  L  T  P  K  Q  L  E  F  R  S  R  K  L  N  L  G  P  A  I  R  K  I  N  S  N  S     151

TTTCAACCAAGCTACGCAATCGCCACTCCAAGTCAGCTCGTTGCGGCATCTCCAGGACCATTCAGCTACGCCATTCCGGCATCTCCAAGTCCATATAGCGGATACTCCTATCCTGCTTCG   600
 F  Q  P  S  Y  A  I  A  T  P  S  Q  L  V  A  A  S  P  G  P  F  S  Y  A  I  P  A  S  P  S  P  Y  S  G  Y  S  Y  P  A  S     191

CCTCAGATGTTCGTCTATCCTCCGTTACGCAGTCAGGATCAAAGCAGGCAGCAATCTGAGCAACAAACTACTCCGCAGAATAGTCCTACTAATCTGCAACATCAACAATCGCCACAAGTC   720
 P  Q  M  F  V  Y  P  P  L  R  S  Q  D  Q  S  R  Q  Q  S  E  Q  Q  T  T  P  Q  N  S  P  T  N  L  Q  H  Q  Q  S  P  Q  V     231

TTCTTCGGTGGAGATCAAGATCCAATAAGATCTTATGCGAGTGCAGTTGCAGGAGTTGAAAAGTCCGAAGTTTCCCCTGAAAAGCATGAATCAGTTTCTCCACAACCACTTCTTCCAAAT   840
 F  F  G  G  D  Q  D  P  I  R  S  Y  A  S  A  V  A  G  V  E  K  S  E  V  S  P  E  K  H  E  S  V  S  P  Q  P  L  L  P  N     271

CAAAATGTGTTGAACACTCAGTATTCCCAAGGTCAGCAGCAGTGGAATTCCAATGTGCAGCAGCAGCAGCAACAACAAATGGACTCGAACAACGGAGGTCCTTACTATAATGAAAATTAT   960
 Q  N  V  L  N  T  Q  Y  S  Q  G  Q  Q  Q  W  N  S  N  V  Q  Q  Q  Q  Q  Q  Q  M  D  S  N  N  G  G  P  Y  Y  N  E  N  Y     311

TCTCAAGGCTACACCCGTCCTCATCCATATCAACAGTTTGCTCAATCTGGGGTGTACATGAACAGCCAAGGAATGTACCATTCGAGTTATTCCTACATGACTCCTCCACTGGCACCAGGT   1080
 S  Q  G  Y  T  R  P  H  P  Y  Q  Q  F  A  Q  S  G  V  Y  M  N  S  Q  G  M  Y  H  S  S  Y  S  Y  M  T  P  P  L  A  P  G     351

CAATATCCACAAATGATGTCCGCTCCCTATTGGCAACAGCAACAACAGCACCATCAAAGTCATGCATACGCTGGCTATAATCCCGCCTACAACAACTGGGTTGGACCATCTGGAGATATG   1200
 Q  Y  P  Q  M  M  S  A  P  Y  W  Q  Q  Q  Q  Q  H  H  Q  S  H  A  Y  A  G  Y  N  P  A  Y  N  N  W  V  G  P  S  G  D  M     391

AATCAGTTCAAACAAAATCCGTCAAACTACTTCTATCAAAATTATCCGGGAAATTTTTCACAACAACACACGATGGGAAACAATGAAAATACATTCTCCCTTCCCCTCCAAGCTCCACGC   1320
 N  Q  F  K  Q  N  P  S  N  Y  F  Y  Q  N  Y  P  G  N  F  S  Q  Q  H  T  M  G  N  N  E  N  T  F  S  L  P  L  Q  A  P  R     431

CAAGGCAAGAAGAGCCGGAAACCATCGGAATGTCAGGATAAGAAGACAAAAAGCCCAATCAAAGGAGCTCGCACTGAGAGACCATCGTCATCAGCTTCGACTCCAGACGCGAAATATCAG   1440
 Q  G  K  K  S  R  K  P  S  E  C  Q  D  K  K  T  K  S  P  I  K  G  A  R  T  E  R  P  S  S  S  A  S  T  P  D  A  K  Y  Q     471

AAGAACCACAGATATCCAGTTCATCTTTCTCCACTTTCAGCATCCCTCCAATCACTAGCCATTTCCTCGCCCACTAAGAATAATTAAATACGAATTCTACATTTTTGTCATTAGTATTTT   1560
 K  N  H  R  Y  P  V  H  L  S  P  L  S  A  S  L  Q  S  L  A  I  S  S  P  T  K  N  N                                        499

CTGTCCTTTAGGTAGTTTTCTATTCCATTGCTCTGGTGCCTACCAAATAAGAATTAAAACACGTATTTTGTATCCTATACGCAACTGTATATTTGATTCAAATACTAGAATTAGCTAGTT   1680
TCTTTCTCTTTTTCCTATCCAAATTATCTTCCTTACGAATATTGTTCCATTCTATAATGTGCTTCTGAAGCCATCGATCTTTTCATATCTTCTAGTAAGATTGAATTTAGATCCCACGTT   1800
CCCCATTCCCATAGTATTCAAATGTTTTTGTATGCCCCCAATTTAGAATAAAATGAAATCACAATAATGACACCAGTAGCCCCTCTCTCGCATTACATGGTAATTTTGAATCACCCAAAT   1920
TGTCCACTTTAACACCTAAAAAATCCGAAGTAGAATTTTGTGTGTGATTTTAATAAATCTTTTCGCAGA                                                        1989
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B

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RRM                    ..  ....     .     .    ..  .     . ......  .                        ...   ... .
DAZH      32  LPEGKIMPNTVFVGGIDVRMDETEIRSFFARYGSVKEVKIITDRTGVSKGYGFVSFVNDVDVQKI...VESQINFHGKKLKLGPAIRKQ   117
DAZ       32  LPEGKIVPNTVFVGGIDARMDETEICSCFCRYGSVKEVKIITNRTGVSKGYGFVSFVNDVDVQKI...VCSQIIFHGKKLKLGPAIRKQ   117
Dazla     32  LPEGKIMPNTVFVGGIDVRMDETEIRSFFARYGSVKEVKIITDRTGVSKGYGFVSFVNDVDVQKI...VESQINFHGKKLKLGPAIRKQ   117
Boule     25  PKYGTLIPNRIFVGGISGDTTEADLTRVFSAYGTVKSTKIIVDRAGVSKGYGFVTFETEQEAQRL.QADGECVVLRDRKLNIAPAIKKQ   112
DAZ-1     59  PPTYELIPNRIFVGGFPTSTTETEIREHFEKFFAVKDVKMVKSLDGQSKGYGFITFETEDQAEEIRKLTPKQLEFRSRKLNLGPAIRKI   147

DAZ motif  .. .  .    .                    .   .   .
DAZH      171  YPNSPVQVITGYQLP.........VYNYQMPPQWPVGEQ   200
DAZ       315  YPNSAVQVTTGYQFH.........VYNYQMPPQSPVGEQ   344
Dazla     171  YPSSPVQVITGYQLP.........VYNYQMPPQWPAGEQ   200
Boule     159  YPPSAMQYQPFYQYY.........SVPMNVPTIWPQNYQ   188
DAZ-1     196  YPASP.QMFV.YPPLRSQDQSRQQSEQQTTPQNSPTNLQ   232
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Fig. 1. The nucleotide sequence of *daz-1* cDNA and its deduced gene product. (A) The *daz-1* cDNA sequence, with the deduced amino acid sequence given in the single letter code underneath. The RNA recognition motif (RRM) is doubly underlined and the DAZ motif is underlined. The nucleotide sequence between the last nucleotide of the SL1 *trans*-spliced leader and the first nucleotide of the poly(A) tail, both of which are marked with a dot, is shown. (B) Comparison of the primary structure of *C. elegans* DAZ-1 with those of other *DAZ* family proteins. The RRM of DAZ-1 shows the highest similarity to that of *Drosophila* Boule. The DAZ motif in DAZ-1 appears to be slightly diverged from those of others.

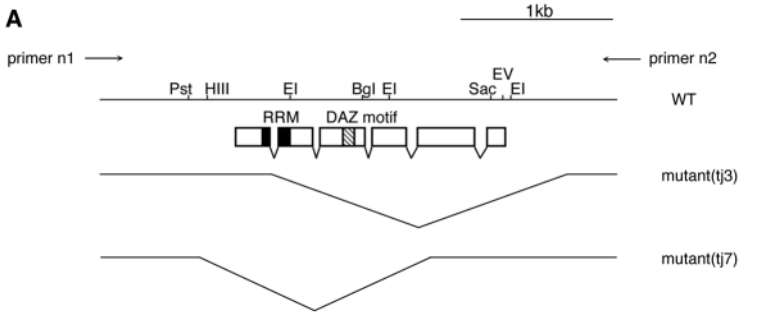

Fig. 2. Genomic structure of the *daz-1* gene and its deletion alleles.
(A) Schematic illustration of the genomic *daz-1* locus. The six exons are shown by boxes. Exon 1 carries part of the RRM including the RNP2 motif, while exon 2 carries the rest of the RRM including the RNP1 motif (black boxes). The hatched box represents the DAZ motif. Two deletion alleles of *daz-1* (*tj3* and *tj7*) are illustrated under the gene structure. Two PCR primers used for the deletion screen are shown on the top, flanking a restriction map of the *daz-1* locus. Restriction enzymes are abbreviated as follows: Bgl, *Bgl*II; EI, *Eco*RI; EV, *Eco*RV; HIII, *Hin*dIII; Pst, *Pst*I; and Sac, *Sac*I.
(B) PCR analysis of the genomic DNA of the wild-type and the *daz-1*-deletion mutants (*tj3* and *tj7*) using the primers shown in panel A.

Comparison of the cDNA sequence with the genomic sequence revealed that *daz-1* has six exons (Fig. 2A). The predicted exons for F56D1.5 based solely on the genomic sequence was slightly incorrect.

The deduced *daz-1* gene product consists of 499 amino acid residues. It has a single RNA-recognition motif (RRM) of the ribonucleoprotein (RNP) type (Burd and Dreyfuss, 1994; Nagai et al., 1995), which includes the RNP1 octamer and the RNP2 hexamer motifs (Fig. 1). The RRM of the *C. elegans* DAZ-1 is most similar to that of *Drosophila* Boule (41% identity, 49% similarity), and slightly less similar to the vertebrate DAZ family proteins (e.g., 35% identity to human DAZ) (Fig. 1B). The C-terminal 12 amino acids of the RRM domain, which have been thought to determine the RNA-binding specificity (Nagai et al., 1995), are highly conserved among all DAZ family members, suggesting that DAZ proteins may bind to a specific RNA sequence.

Besides the RRM, the DAZ family proteins carry another conserved domain of unknown function in their C-terminal region. This domain, called the DAZ motif, consists of approximately 24 amino acid residues rich in glutamine, proline and tyrosine (Reijo et al., 1995). Each DAZ family protein contains one DAZ motif, except for human DAZ, which has seven tandem repeats of the motif. *C. elegans* DAZ-1 has a single apparent DAZ motif, which is divergent from ones in mammals (Fig. 1B). The entire DAZ-1 polypeptide except the RRM domain was rich in glutamine (65/410), proline (48/410), and tyrosine (29/410), as is often the case with RRM proteins, including DAZ family members.

Isolation of *daz-1* deletion alleles

To see the function of the *daz-1* gene product in *C. elegans*, we isolated two *daz-1* deletion mutants. One allele, termed *tj3*, apparently carried a 2.1 kb-long deletion, whereas the other, termed *tj7*, carried a 1.6 kb-long deletion (Fig. 2B). Sequence analysis confirmed that the *daz-1(tj3)* allele lacked exons 2 through 6, hence missing most of the ORF including the DAZ motif and part of the RRM, and that *daz-1(tj7)* lacked the first four exons, missing both the DAZ motif and the RRM (Fig. 2A). Thus, these alleles appeared unlikely to produce functional DAZ-1 protein. The *trans*-heterozygotes *daz-1(tj3)/ccDf5*, where *ccDf5* represents a chromosomal deletion that encompasses the *daz-1* locus, were phenotypically indistinguishable from either the *daz-1(tj3)* or the *daz-1(tj7)* homozygotes (data not shown; see below). This finding supported the contention that these *daz-1* alleles were null rather than reduction-of-function. Because *daz-1(tj3)* and *daz-1(tj7)* were phenotypically indistinguishable in the initial characterization, we used *daz-1(tj3)* mainly in subsequent analyses.

Loss of *daz-1* function causes sterility in hermaphrodites but not in males

Hermaphrodites homozygous for *daz-1(tj3)*, born of heterozygous mothers, grew to adulthood without showing any phenotypic abnormality in somatic tissues. However, *daz-1(tj3)* adult hermaphrodites were found to be sterile with complete penetrance (Table 1). In wild-type adult hermaphrodites, each gonad exhibited distal-to-proximal polarity, in which distal germ cells proliferated whereas proximal cells entered meiosis and underwent oogenesis (Fig. 3A). Sperm were produced just before reaching adulthood and were stored in the spermatheca located proximal to the gonadal arm. In contrast, the gonad of *daz-1* adult hermaphrodites contained no oocytes, but was filled mostly with small nuclei. None of the cells therein displayed morphological characteristics of the developing or mature oocytes, such as granular cytoplasm and enlarged nuclei. Apparently normal sperm were observed in the spermatheca of *daz-1* hermaphrodites, although the mutant spermatheca was typically elongated compared with the wild-type (Fig. 3B). Spermatids dissected from *daz-1* hermaphrodites were morphologically normal and could be properly activated by monensin in vitro (Fig. 3G,H), suggesting that the sperm produced by *daz-1* hermaphrodites were not defective.

Males homozygous for *daz-1(tj3)* appeared to have no obvious defect in either formation of sperm or their activation (Fig. 3I,J). We attempted crosses between *daz-1* males and *mIn1[dpy-10]* hermaphrodites, the latter of which have the Dpy phenotype. The mutant male could give rise to non-Dpy

Table 1. Fertility of the *daz-1* mutant

Genotype	Sex	Number of fertile adults
unc-4	hermaphrodite	30/30
	male	26/26
unc-4 daz-1	hermaphrodite	0/30
	male	23/23

Hermaphrodites were tested for self fertility. Male animals were test-mated to a hermaphrodite indicator strain.

progeny as efficiently as the wild-type male (Table 1), indicating that *daz-1* sperm were functionally normal. These results lead to the conclusion that *daz-1* is dispensable for spermatogenesis, which makes a striking contrast to the other *DAZ* family members previously analyzed.

To confirm that the observed phenotype of the *daz-1* mutant was caused by the deletion of *daz-1* but not any other mutation, we performed an RNA interference (RNAi) experiment (Fire et al., 1998; Tabara et al., 1998). The phenotype of *daz-1(RNAi)* hermaphrodites and that of the *daz-1* mutant were indistinguishable from each other, supporting strongly that the deletion of the *daz-1* gene was indeed responsible for the mutant phenotype (data not shown).

Germ nuclei show pachytene arrest during oogenesis in the *daz-1* mutant

To determine which stage of oogenesis is defective in *daz-1* hermaphrodites, they were stained with DAPI, and morphology of their germ nuclei was compared with that of the wild-type. In wild-type worms, transition from mitosis to meiosis occurs at the middle of the distal arm and pachytene nuclei with distinct thread-like chromosomes become visible beyond this transition zone (Schedl, 1997; Fig. 3C). Prophase of meiosis I continues as nuclei travel to the proximal arm, where late stage oocytes show highly condensed chromosomes of the diakinesis stage (Fig. 3C). DAPI-stained sperm nuclei were seen as small dots in the spermatheca in wild-type hermaphrodites (Fig. 3C).

The *daz-1* hermaphrodites had mitotic and transition zone nuclei at the distal end of the gonad, as in the wild-type, but the rest of the mutant gonad was filled with pachytene nuclei. Occasionally smaller nuclei were seen among pachytene nuclei, which appeared similar to mitotic germ nuclei in size (Fig. 3D). Nuclei at the diakinesis stage could never be observed in the mutant gonad. DAPI-stained sperm in the mutant spermatheca showed no obvious aberrance, confirming the results of the Nomarski microscopy. The number of sperm per gonad was about 130-150 in both the wild-type and the *daz-1* mutant. DAPI-stained sperm in *daz-1* males revealed no obvious defect, either (Fig. 3E,F).

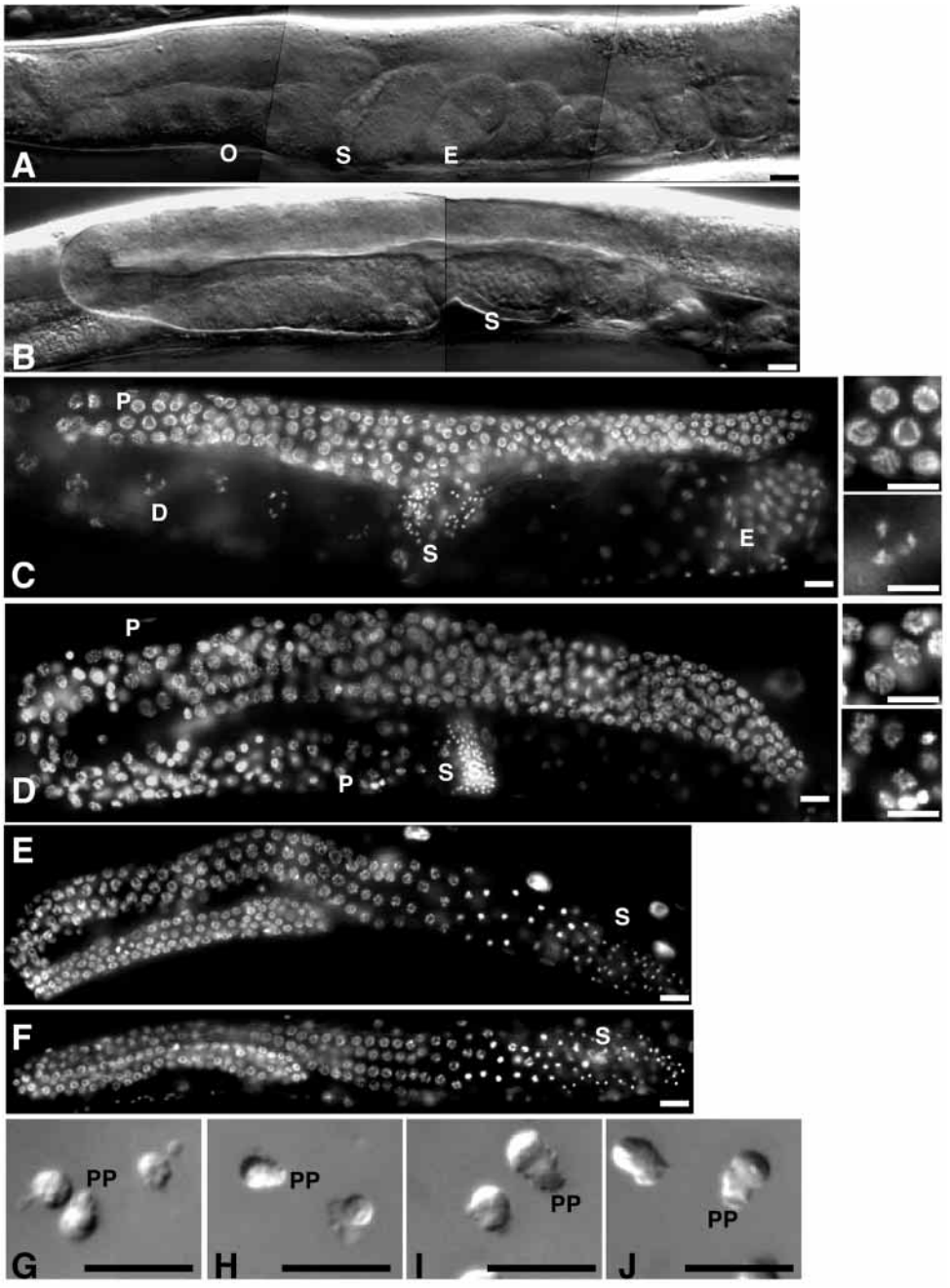

Fig. 3. Gametogenesis in the *daz-1* deletion mutant. (A,B) Images of the gonad of adult hermaphrodites by Nomarski microscopy. A wild-type hermaphrodite (A) and a *daz-1* hermaphrodite (B) were examined. O, oocytes; S, sperm; E, fertilized eggs. (C-F) Images of the gonad stained by DAPI. A wild-type hermaphrodite (C), a *daz-1* hermaphrodite (D), a wild-type male (E), and a *daz-1* male (F) were examined. Pachytene nuclei seen in both wild-type and *daz-1* hermaphrodites are enlarged in the upper insets of panels C and D, respectively. Diakinesis nuclei in the wild-type are enlarged in the lower inset of panel C. Typical smaller nuclei seen in *daz-1* are evident in the lower inset of panel D. P, pachytene nuclei; D, diakinesis nuclei. (G-J) Morphology of activated sperm. Sperm from a wild-type hermaphrodite (G), a *daz-1* hermaphrodite (H), a wild-type male (I), and a *daz-1* male (J) were examined by Nomarski microscopy. PP, pseudopod. Scale bar: 10 μm throughout.

To examine further the status of arrested germ cells in the *daz-1* mutant, we asked whether dying germ cells could be visualized by a vital dye SYTO12 in *daz-1* adult hermaphrodites. In wild-type animals, a small population of female germ cells undergoes programmed cell death at the late pachytene stage, which can be seen typically in the distal arm of the gonad (Gumienny et. al, 1999; Fig. 4A). In *daz-1* animals, the proximal arm of the gonad revealed many cell corpses (Fig. 4B). Cell corpses seen in the mutant appeared to be produced by the apoptotic pathway, because *daz-1* animals exhibited no corpses if they are defective in *ced-3*, a gene essential for apoptosis (Ellis and Horvitz, 1986) (Fig. 4C). These results imply that the arrested *daz-1* germ cells reached the cell-death sensitive, late pachytene stage in oogenesis, although we cannot completely exclude the possibility that an unusual cell death pathway might be activated by the abnormal meiotic arrest. A possible reason for the increase of cell corpses in *daz-1* will be that arrested germ cells stay longer in the cell-death-sensitive period, raising the probability that they die. Alternatively, germ cells that are unable to exit pachytene in a certain period of time may be destined to cell death, in both the mutant and the wild-type.

gld-1 is epistatic to *daz-1* at the pachytene stage

We suspected that the smaller nuclei seen in the proximal arm of the mutant gonad might be mitotic, as is the case with the *gld-1* mutant (Francis et al., 1995a). To test this possibility, we stained the *daz-1* hermaphrodites with anti-phosphohistone H3 antibody, which specifically stains condensed mitotic chromosomes at M-phase (Ajiro et al., 1996). We stained the *gld-1(null)* hermaphrodites for comparison, in which germ nuclei reach the meiotic pachytene phase and then reinitiate mitosis, giving rise to a 'tumorous' germline (Francis et al., 1995a). The results indicated that the mitotic nuclei were scattered up to the proximal arm in *gld-1(null)* hermaphrodites, whereas they were restricted to the distal region of the gonad in *daz-1* hermaphrodites and the wild-type (Fig. 5A,C). Thus, the majority of the nuclei seen in the proximal region of the *daz-1* mutant gonad are not mitotic and are likely to be arrested at an early phase of meiosis.

We set out to examine the epistasis between *daz-1* and *gld-1* using RNAi. The wild-type adult hermaphrodites treated with the *gld-1* RNA solution exhibited the tumorous germline phenotype within 48 hours (Fig. 5B). The *gld-1* dsRNA treatment of homozygous *daz-1(tj3)* adult hermaphrodites gave rise to similar tumorous germline, and its immunostaining by anti-phosphohistone H3 revealed a pattern clearly distinct from that of the untreated *daz-1* germline (Fig. 5D). Analysis using the *daz-1(tj3); gld-1(null)* double mutant gave essentially the same results (data not shown). Thus, the defect in *gld-1* is epistatic to *daz-1*, suggesting that *daz-1* function is not necessary for *gld-1* tumor formation. If one assumes that *daz-1* and *gld-1* act in a pathway of dependent events (Hartwell, 1974), then *daz-1* normally functions later than *gld-1* in the course of oogenesis. This hypothesis is consistent with the observation of cell death in the gonad of *daz-1* hermaphrodites described above: germ cells defective in *daz-1* can apparently proceeded to the cell-death sensitive stage (Fig. 4B), whereas ones defective in *gld-1* do not reach this stage (Gumienny et al., 1999) (see Discussion for additional arguments).

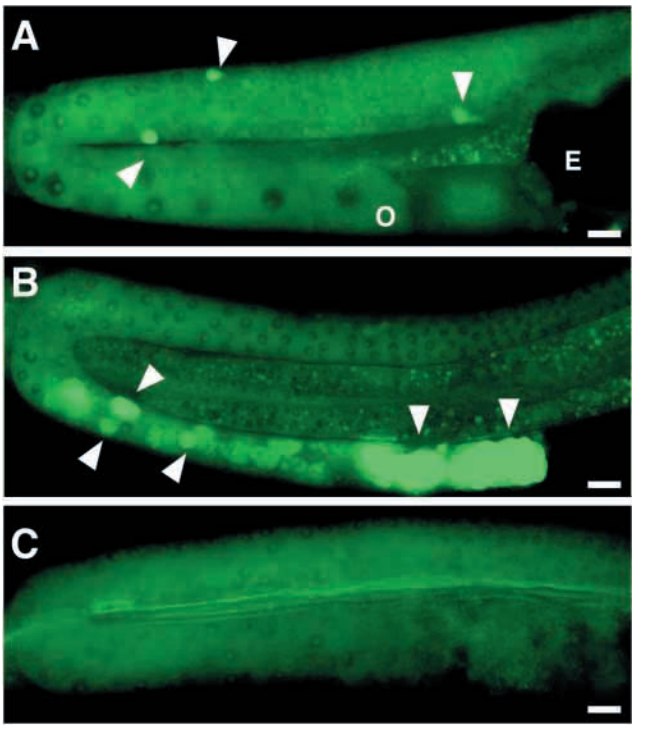

Fig. 4. SYTO12 staining of adult hermaphrodite gonads. Apoptotic germ cells were stained with SYTO12 (green). Stained corpses are indicated with arrowheads. In the wild-type, several cell corpses were seen in the distal arm of the gonad (A). In *daz-1* animals, many corpses were seen in the proximal arm (B). In *daz-1; ced-3*, no corpses were seen, suggesting that corpses in the *daz-1* gonads were produced by the *ced-3*-dependent cell death pathway. Scale bar: 50 μm.

daz-1 phenotype depends on the sexual identity of the germline

To examine how the oogenic defect in *daz-1(tj3)* depends on the sexual fate of the germline, we performed genetic epistasis analysis using sex determination mutants. First, we tested the effect of *daz-1(tj3)* in the feminization mutant, *fem-3(lf)*. In the wild-type hermaphrodite (*XX*) germline, spermatogenesis takes place during late L4 stage, then the germline switches its sex in young adult so that it undergoes oogenesis (Ellis and Kimble, 1994). In the germline of *XX fem-3(lf)*, spermatogenesis never occurs and only oocytes are produced from the start of germ cell differentiation (Hodgkin 1986; Fig. 6A). The *XX* adults of *daz-1; fem-3(lf)* that we constructed did not produce mature oocytes, and their germ nuclei stained with DAPI showed morphology characteristic to the pachytene stage (Fig. 6B), as in the *daz-1* hermaphrodite germline. Thus, *daz-1* is required for oogenesis, whether or not spermatogenesis precedes it.

We then examined the effect of *daz-1* mutation in the masculinized germline in *fem-3(gf) XX* animals, which showed a Mog (masculinization of germline) phenotype (Barton et al., 1987). In *fem-3(gf)* hermaphrodites, switching from spermatogenesis to oogenesis does not occur, and sperm are continuously produced in the female soma. The *fem-3(gf); daz-1* animals showed masculinized germlines, producing apparently normal sperm (data not shown). They produced

sperm throughout adulthood, excluding the possibility that maternally contributed *daz-1* mRNA permits spermatogenesis in young adults but is not sufficient to allow gametogenesis to continue as animals get older.

Finally, we asked whether somatic sex may affect the oogenic defect brought by the *daz-1* mutation. *fog-3(lf)* (feminization of germline) *XO* animals are somatically male, but their germline is feminized and produces only oocytes (Ellis and Kimble 1995; Fig. 6C). The *daz-1; fog-3(lf) XO* animals that we constructed produced neither oocytes nor sperm. DAPI staining of these animals showed that the germ nuclei were arrested in the pachytene stage (Fig. 6D), suggesting that the meiotic defect in *daz-1; fog-3(lf)* animals was due to the same dysfunction as in the *daz-1* hermaphrodite or the *daz-1; fem-3(lf)* female. Taken together, the meiotic prophase arrest caused by *daz-1* loss of function is dependent on the female fate of germline, regardless of chromosome sex or sex of somatic tissues.

The transcription of *daz-1* is limited to the germline and is developmentally regulated

Northern blot analysis using poly(A)$^+$ RNA prepared from wild-type adults revealed a single band of approximately 2.0 kb in length as the *daz-1* transcript (Fig. 7), which is consistent with the size of the full-length cDNA. In the *glp-4* mutant, which produces few germ cells (Beanan and Strome, 1992), the *daz-1* transcript was not detectable (Fig. 7). In the *fem-3(lf)* mutant, in which the germline is feminized, *daz-1* transcripts were slightly more abundant than in the wild-type, whereas they were less abundant in the *fem-3(gf)* mutant, in which the germline is masculinized (Fig. 7). These results indicate that *daz-1* is expressed exclusively in the germline, more strongly during oogenesis than during spermatogenesis.

To analyze temporal and spatial distribution of *daz-1* mRNA, we performed in situ hybridization against the wild-type worms at each developmental stage (Fig. 8). In hermaphrodites, the *daz-1* mRNA was first detected in the germline of the L2 stage larva (Fig. 8B). Since germ cells enter the meiotic pathway at late L3, the onset of transcription of *daz-1* appeared to precede the initiation of meiosis. The hybridization signal increased its intensity as the developmental stage progressed (Fig. 8C-E). During the larval stages, the *daz-1* mRNA appeared more abundant at the distal region than the proximal region of the gonad. In young adult hermaphrodites, the *daz-1* transcripts were detected at a very low level in the distal mitotic region of the gonad, began to accumulate in the meiotic transition zone, and reached the maximum level in the proximal pachytene region (Fig. 8E,G). The transcript level dropped sharply in mature oocytes. No hybridization signal was seen in the spermatheca. Compared to hermaphrodites, a much weaker signal, if any, was detected in the germline of adult males (Fig. 8F). In *XX fem-3(gf)* adults, *daz-1* transcripts were detectable but restricted to the distal region of the gonad (data not shown), which may account for the low level of mRNA detected by northern blot analysis in Fig. 6. Thus, *daz-1* transcription is virtually confined to the female germline and is regulated according to the developmental stages of oogenesis.

DISCUSSION

Relationship between *daz-1* and other meiosis/gametogenesis-related genes in *C. elegans*

This study has shown that the function of *C. elegans daz-1* is required for the germ cells to traverse the pachytene stage of meiosis I. So far only a limited number of genes have been shown to be relevant to the meiotic regulation in *C. elegans.* The *glp-1* signaling pathway, involving *glp-1*, *lag-1* and *lag-2*, is essential for the inhibition of precocious entry of germ nuclei into meiosis (Austin and Kimble, 1987; Christensen et al., 1996; Henderson et al., 1994). LAG-2, a ligand secreted by the distal tip cell, binds to the receptor GLP-1 located on the germ

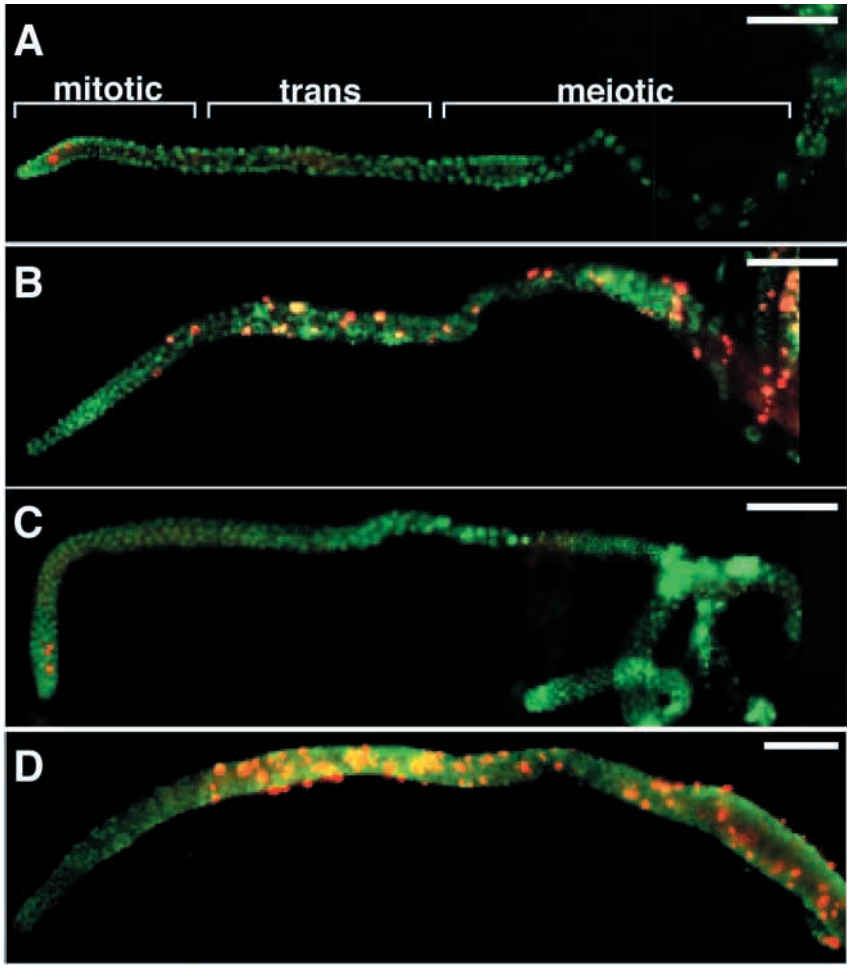

Fig. 5. Anti-phosphohistone H3 staining of gonads. Gonads were dissected from the wild-type (A), *gld-1* (B), *daz-1* (C) and *daz-1; gld-1(RNAi)* (D) animals and stained with the antibody. Staining by anti-phosphohistone H3 is represented in red, while staining by DAPI is represented in green. Scale bar: 50 μm.

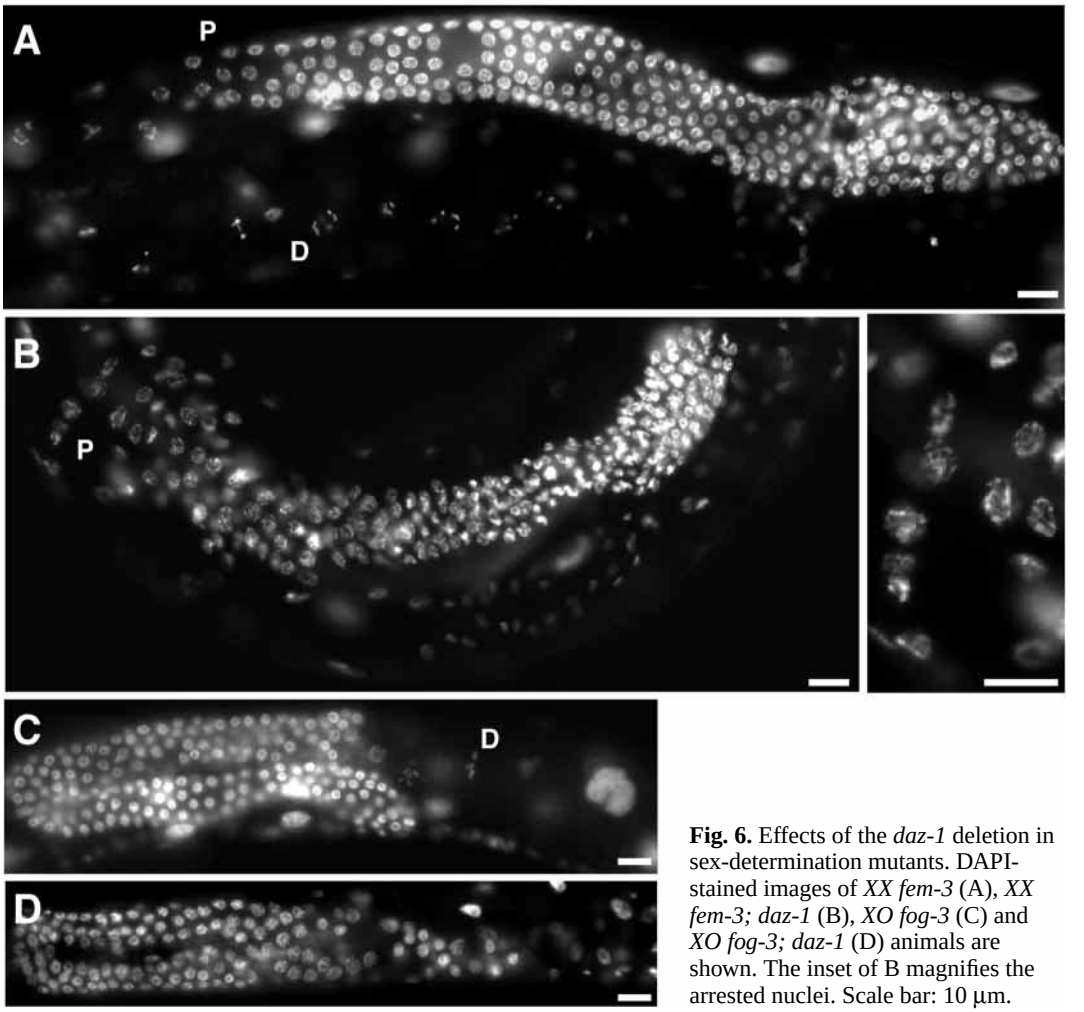

Fig. 6. Effects of the *daz-1* deletion in sex-determination mutants. DAPI-stained images of *XX fem-3* (A), *XX fem-3; daz-1* (B), *XO fog-3* (C) and *XO fog-3; daz-1* (D) animals are shown. The inset of B magnifies the arrested nuclei. Scale bar: 10 μm.

cell membrane (Henderson et al., 1994). The receptor then activates the transcription factor LAG-1, which promotes mitotic proliferation of germ nuclei (Christensen et al., 1996). When LAG-2 signaling is no longer effective, germ cells enter the meiotic pathway by virtue of the redundant function of *gld-1* and *gld-2* (Kadyk and Kimble, 1998). In addition, the RAS/MAPK signaling pathway, involving *mek-2, mpk-1/sur-1* and *let-60 ras*, is necessary for the transition from pachytene to diplotene/diakinesis (Church et al., 1995).

Among the above genes, *gld-1* appears to share several features with *daz-1*. First, loss of function of *gld-1* results in a failure of the progression of meiotic prophase in oogenesis (Francis et al., 1995a). Second, *gld-1* function is required only when the germline sex is set as female (Francis et al., 1995b). Third, *gld-1* is expressed specifically in the germline, at a much higher level in hermaphrodites than males (Jones et al., 1996). In addition, GLD-1 also is an RNA-binding protein, although its RNA recognition motif is a KH domain (Jones and Schedl, 1995) and hence is different from the RNP-type RRM in DAZ-1. Germ cells in the *gld-1(null)* hermaphrodites enter the meiotic pathway and proceed up to the pachytene stage. However, unlike the *daz-1* mutant, they do not arrest at this stage but resume aberrant mitotic growth, giving rise to a 'tumorous' germline (Francis et al., 1995a). Our epistasis analysis suggests that *daz-1* executes its function after the meiotic progression is ensured by the function of *gld-1*. This is supported by the observation that germ cells do not reach the cell-death-sensitive late pachytene stage in the absence of GLD-1, whereas they do so in the absence of DAZ-1. Strictly speaking, however, the possibility remains that apoptosis may occur in the meiotic region but elude detection in the *gld-1* mutant, because the vast majority of germline cells are mitotic in this mutant. Obviously more work is required to clarify the functional relationship between *daz-1* and *gld-1*.

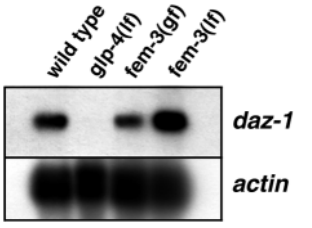

Fig. 7. Northern analysis of *daz-1* mRNA. Poly(A)+ RNA was prepared from *XX* adults that were genetically either wild-type (N2), *glp-4, fem-3(lf)* or *fem-3(gf)*. Upper panel, *daz-1* probe; lower lane, actin probe.

C. elegans mutants defective in a component of the RAS/MAP kinase pathway also show pachytene arrest but in both sexes (Church et al., 1995).The arrested nuclei in these mutants are clumped in the gonad, whereas those in the *daz-1* germline are uniformly distributed. Furthermore, the RAS/MAPK mutants show virtually no germ cell death

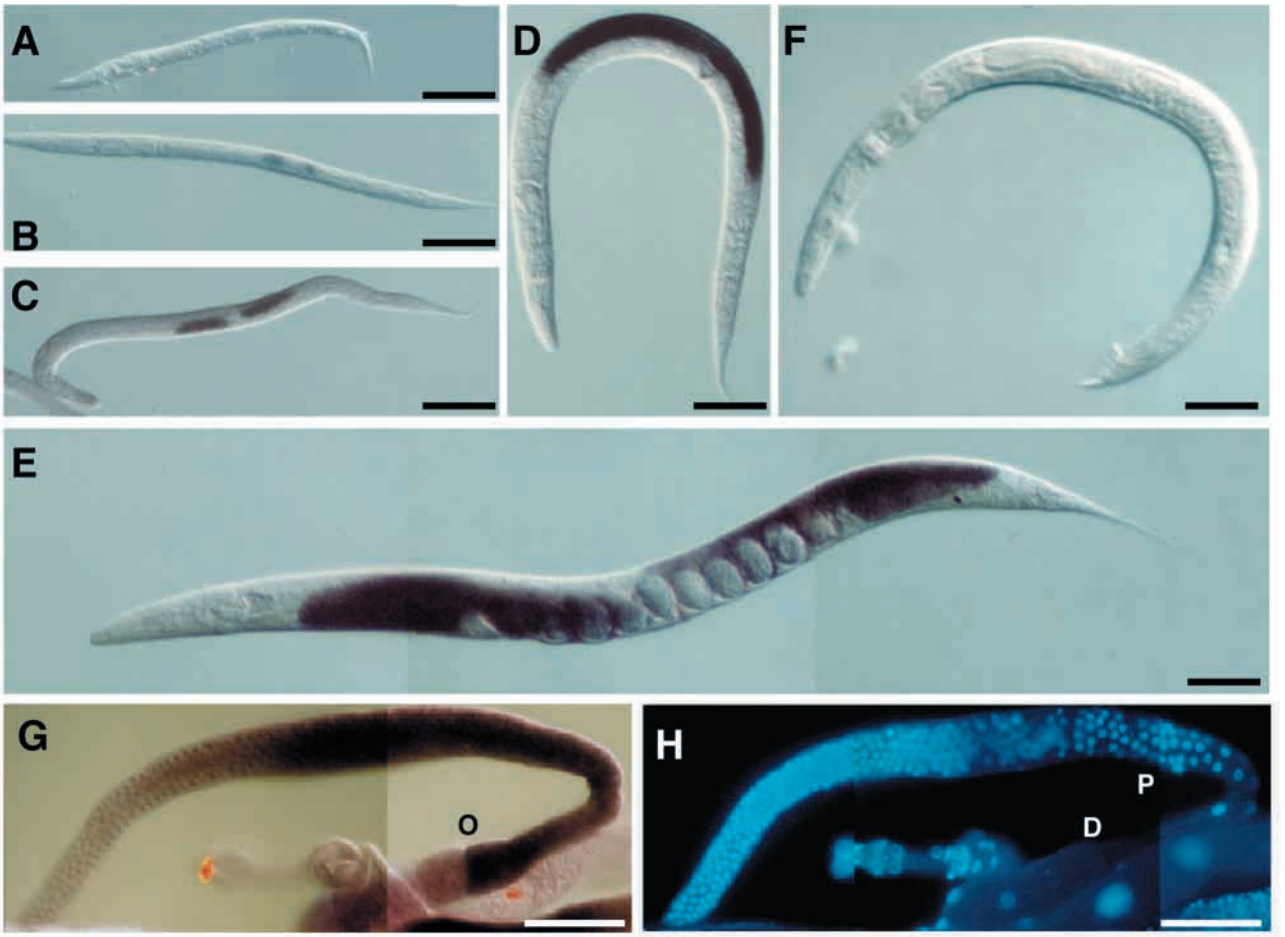

Fig. 8. In situ hybridization of *daz-1* transcripts. Whole-mount in situ hybridization was performed using the *daz-1* probe against L1 (A), L2 (B), L3 (C), L4 (D) and adult (E) hermaphrodites. An adult male (F) and a dissected adult hermaphrodite gonad (G) were also examined. (H) DAPI-stained image of (G). Scale bar: 50 μm.

(Gumienny et al., 1999). These apparent differences may indicate that *daz-1* and the RAS/MAPK cascade contribute differently to pachytene progression.

Target RNA of DAZ-1

DAZ-1 contains an RNP-type RNA-binding motif. This motif has been implicated in a variety of different functions, such as regulation of splicing, processing of the 3′-end of pre-mRNA, and transport and translation of mature mRNA (Nagai et al., 1995). Identification of RNA species that interact with DAZ-1 should await further work, but the following consideration may be beneficial for this purpose.

In *C. elegans*, RNA-protein interaction has been shown to play a pivotal role in the regulation of germline sex determination. For example, GLD-1 represses translation of *tra-2*, one of the sex-determination genes, by binding to the 3′-UTR of the *tra-2* mRNA (Jan et al., 1999). GLD-1 is likely to regulate translation of other genes also, because loss of translational repression of *tra-2* alone cannot account for all the phenotypes brought by the *gld-1* deficiency. TRA-1, a member of the GLI protein family, also binds to the 3′-UTR of the *tra-2* mRNA and promotes its export from the nucleus (Graves et al., 1999). Another example can be seen in the repression of translation of *fem-3* mRNA by FBF-1, the latter of which is a member of a new family of RNA-binding proteins including *Drosophila* Pumilio (Zhang et al., 1997).

In contrast to their role in sex determination, the role of RNA-binding proteins in the regulation of meiosis remains obscure. Although GLD-1 is essential for completion of the pachytene stage, no RNA molecule has been identified that interacts with GLD-1 to promote meiosis. It appears interesting to see whether DAZ-1 regulates translation of specific mRNA, similarly to GLD-1. Recent evidence suggests that *Drosophila* Boule is involved in the control of translation of *twine* mRNA (Maines and Wasserman, 1999), although direct interaction between the protein and the mRNA remains to be substantiated. *twine* encodes a Cdc25-type phosphatase essential for the meiotic G2/M transition (Alphey et al., 1992; Courtot et al., 1992; White-Cooper et al., 1993). Expression of fission yeast *cdc25* is significantly enhanced following the completion of premeiotic DNA synthesis, and its function is essential for both meiosis I and II (Iino et al., 1995). Thus, an attracting question is whether *C. elegans* DAZ-1 upregulates the activity of a Cdc25-type phosphatase by interacting with its mRNA during female meiosis.

In fission yeast, an RNA-binding protein Mei2 serves as a key regulator of meiosis. Mei2 carries three RRMs and is essential for both premeiotic DNA synthesis and meiosis I

(Watanabe et al., 1997; Watanabe and Yamamoto, 1994). An RNA species, called meiRNA, interacts with Mei2 and assists its translocation to the nucleus, where the protein performs a function essential for meiosis I (Yamashita et al., 1998). Although the molecular function of Mei2 is still veiled, this protein is unlikely to regulate translation in order to promote meiosis I, judging from its nuclear localization. Thus, concerning the role of DAZ-1, one may speculate the possibility as well that DAZ-1 itself performs an active function, possibly aided by RNA, rather than modifies an activity of certain RNA species.

Evolutionary twist of the *DAZ* family genes

DAZ family genes are conserved from nematode to mammals. However, no *DAZ* homologue has been found in unicellular organisms, including not only bacteria but also meiosis-proficient eukaryotic microbes like budding and fission yeast. This may imply that the emergence of the *DAZ* family gene in the course of evolution was associated with the increase of complexity in the mechanisms of meiosis in multicellular organisms.

All of the *DAZ* family genes so far investigated are expressed exclusively in the germline, but their sex-specificity is not identical. The *DAZ* family genes in *Drosophila* and *C. elegans* are required for gametogenesis in one sex: *Drosophila boule* for spermatogenesis (Eberhart et al., 1996) and *C. elegans daz-1* for oogenesis. Murine *Dazla* is required for both oogenesis and spermatogenesis, but differently (Ruggiu et al., 1997): in *Dazla*-deficient mice, oogenesis appears to be normal until it is arrested at the pachytene stage, hence mimicking the situation in *C. elegans*, whereas the male germline generates fewer germ cells, which do not proceed beyond the spermatogonial stage. It is unknown whether the human counterpart of murine *Dazla*, namely *DAZLA*/*DAZH*, is involved in gametogenesis. A deletion of the human *DAZ* cluster on Y chromosome results in a wide range of spermatogenic deficiency (Reijo et al., 1995). This variability may reflect the functional redundancy and divergence between *DAZ* and *DAZLA*/*DAZH* (Agulnik et al., 1998; Saxena et al., 1996).

How have the *DAZ* family genes acquired such versatile sex-specificity during evolution? We speculate the following. Yeast has only one mode of meiosis, i.e., diploid cells generate four spores. In contrast, higher organisms produce asymmetrical gametes. To regulate spermatogenesis and oogenesis independently according to each program, they will require two modes of meiosis, which should be distinct from each other at least partly. Thus, we assume that multicellular organisms have developed two types of mechanisms for meiotic regulation, one of which uses the *DAZ* family gene at the pachytene stage and the other not. Meanwhile, strategies for sex determination are strikingly divergent among species, with no overlapping molecular mechanisms being discovered in *C. elegans*, *Drosophila* and mammals (Marin and Baker, 1998; Parkhurst and Meneely, 1994). Taken together, during the evolution from a primitive multicellular organism, some organisms may have employed the *DAZ*-dependent meiosis for spermatogenesis and the *DAZ*-independent meiosis for oogenesis, whereas others did vice versa. Hopefully more extensive analysis of the *DAZ* family genes and more intensive analysis of their molecular function will prove or disprove the validity of this hypothesis.

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