Role of the *C. elegans* U2 snRNP protein MOG-2 in sex determination, meiosis, and splice site selection

Simone Zanetti, Marco Meola, Arlette Bochud, Alessandro Puoti *

Department of Biology, University of Fribourg, Switzerland

In *Caenorhabditis elegans*, germ cells develop as spermatids in the larva and as oocytes in the adult. Such fundamentally different gametes are produced through a fine-tuned balance between feminizing and masculinizing genes. For example, the switch to oogenesis requires repression of the *fem-3* mRNA through the *mog* genes. Here we report on the cloning and characterization of the sex determination gene *mog-2*. MOG-2 is the worm homolog of spliceosomal protein U2A′. We found that MOG-2 is expressed in most nuclei of somatic and germ cells. In addition to its role in sex determination, *mog-2* is required for meiosis. Moreover, MOG-2 binds to U2B″/RNP-3 in the absence of RNA. We also show that MOG-2 associates with the U2 snRNA in the absence of RNP-3. Therefore, we propose that MOG-2 is a bona fide component of the U2 snRNP. Albeit not being required for general pre-mRNA splicing, MOG-2 increases the splicing efficiency to a cryptic splice site that is located at the 5′ end of the exon.

Introduction

How does a germ cell decide between mitotic proliferation and entry into meiosis? What are the factors that are necessary to differentiate a germ cell as a sperm or an oocyte? Such fundamental questions are addressed in the protandric nematode *Caenorhabditis elegans*. In this species, X0 males produce sperm for their whole adult life, while XX hermaphrodites make sperm only during a short period of their larval development and then switch to oogenesis (Hirsh et al., 1976). Genes involved in germline sex determination have been identified through genetic screens for animals that were defective in gamete production. For example, *fem-3 loss-of-function (lf)* alleles lead to feminized hermaphrodites that omit spermatogenesis (Hodgkin, 1986). In contrast, *fem-3 gain-of-function (gf)* mutant hermaphrodites continue spermatogenesis throughout their entire lives (Barton et al., 1987). In the latter, post-transcriptional repression of the *fem-3* mRNA is abolished through mutations in the 3′untranslated region (3′UTR; Ahringer and Kimble, 1991). The *fem-3* mRNA is repressed through a number of regulatory proteins, among which the FBF and the MOG proteins are the most prominent examples (Graham and Kimble, 1993; Graham et al., 1993; Zhang et al., 1997). The Puf proteins FBF-1 and FBF-2 bind directly to a regulatory region of the *fem-3* 3′UTR (Zhang et al., 1997). Although all *mog* genes cloned to date code for proteins that are homologous to splicing factors, splicing defects have

not been found in the respective mutants (Belfiore et al., 2004; Kasturi et al., 2010; Puoti and Kimble, 1999, 2000).

RNA splicing is a key event in the processing of the primary transcript to mRNA. Splicing takes place in the spliceosome, a large dynamic nuclear complex consisting of five snRNAs, snRNP proteins, and non-snRNP proteins (Moore et al., 1993). U1, U2, U4/U6 and U5 snRNAs play crucial roles in RNA–RNA interactions with the pre-mRNA (Konarska and Sharp, 1987). While U1 snRNP binds to the 5′ splice site of the intron, the U2 snRNP associates with the branchpoint (Black et al., 1985). In humans, U1 snRNA binds to the snRNP protein U1A, while U2 snRNA interacts with the snRNP protein U2B″ (Scherly et al., 1990b). In vertebrates, specific binding of U2B″ to U2 snRNA requires yet another RNA-binding protein, U2A′ (Mattaj et al., 1986; Scherly et al., 1990b). Splicing is essentially identical in *C. elegans* and in vertebrates, but several differences need to be mentioned. First, as in other free-living nematodes, *C. elegans* introns are much shorter than in vertebrates, with a median length between 48 and 52 nucleotides (Blumenthal and Steward, 1997). Second, although *C. elegans* introns almost exclusively start with a GU and end with an AG dinucleotide, no branchpoint consensus and no obvious polypyrimidine tract have been identified (Blumenthal and Steward, 1997). Finally, in contrast to human U2B″, the worm homolog RNP-3 binds specifically to U2 snRNA without requiring the *C. elegans* ortholog of U2A′, also known as SAP-1 (Spliceosomal-Associated Protein 1; Saldi et al., 2007).

In this study, we report the cloning and the initial characterization of the *mog-2* gene. *mog-2* encodes SAP-1, the worm homolog of U2A′ (Caspary and Seraphin, 1998; MacMorris et al., 2003; Polycarpou-Schwarz et al., 1996). We show that MOG-2/SAP-1 functions in splicing and that it is synthetically required for meiosis. Furthermore, we show that MOG-2

* Corresponding author at: Department of Biology, Chemin du Musée 10, 1700 Fribourg, Switzerland. Fax: +41 26 300 9741.
E-mail address: alessandro.puoti@unifr.ch (A. Puoti).

binds to RNP-3 and to U2 snRNA in vivo. Finally, we identify a motif in MOG-2 that is crucial for RNP-3 binding and possibly for U2 snRNA binding and sex determination.

Materials and Methods

Strains

All strains were maintained by standard procedures at 20 °C unless specified. Deletion alleles *sap-1(ok1221)/mIn1[dpy-10(e128)mIs14]II* and *rnp-3(ok1424)IV* are from the CGC. *gld-2(q497);mog-2(ok1221)* double mutants are from strain *gld-2(q497)/hT2[qIs48](I;III);mog-2 (ok1221)II* and *mog-2(q75) gld-3(q730)* double mutant came from *lin-31(n301) mog-2(q75) gld-3(q730)/mIn1[dpy-10(e128)mIs14]II*. PremRNA processing and decay were analyzed in *smg-1(r861)I, mog-2 (q75)II, mog-2(ok1221)II, smg-1(r861)I;mog-2(q75)II*, and *mog-2(q75) II;rnp-3(ok1424)/nT1g[qIs51](IV;V)* animals. *mIn1, hT2* and *nT1g* are semi-dominant fluorescent markers. Other genotypes used in 3′ splice site recognition: *dpy-10(e128)II, smg-1(r861)I;dpy-10(e128)II, mog-2 (q75)dpy-10(e128)II, unc-4(e120)dpy-10(e128)II, smg-1(r861)I;mog-2(q75)dpy-10(e128)II, dpy-10(e128)II;fem-3(q95)IV. mog-6(q465) dpy-10(e128)II* was obtained from *mog-6(q465) dpy-10(e128)II/mIn1* and *mog-5(q449)dpy-10(e128)II* is from *mog-5(q449) dpy-10(e128)/ unc-85(e1414)II*.

Cloning of mog-2 and accession number

mog-2 has been mapped on chromosome *II*, between *lin-31* and *unc-85* (Graham et al., 1993). We further mapped *mog-2* by analyzing recombinants between *mog-2* and two single nucleotide polymorphisms in cosmids R03H10 (position 9489) and ZK430 (position 13198). By sequencing candidate genes, we found that ORF H20J04.8 contained a 5-nucleotide deletion, which was not present in wild type animals. The deletion is located at the end of the first exon and removes a *Hinf*I restriction site. The accession number HM852593 has been assigned to *mog-2* cDNA sequence data.

Phenotype analyses

Fertile hermaphrodites were allowed to lay eggs at the appropriate temperature for 4 h. Developmental stages were determined by the size of the germ line. Plates were scored for dead eggs 30 h after being laid and sterile adults were observed 24 h after the vulva had formed. For sperm counting, worms were grown at 15 °C until L4 and then switched to 25 °C. To avoid larval arrest at 25 °C, we used homozygous siblings of *mog-2/mIn1* heterozygotes. Sperms were identified by their nuclear morphology upon staining with DAPI (4,6-diamino-2-phenylindole).

RNA interference

Sense and antisense RNA corresponding to the entire coding region of *mog-2* were generated using T3 and T7 RNA polymerases (Stratagene). RNA interference was performed by injecting dsRNA (0.5 μg/μl) into either young N2 or *gld-3(q730)/mIn1* adults. Worms were shifted from 20 °C to 25 °C 12 h post injection. *mog-2(q75)* and *mog-2(q75);rnp-3(ok1424)* hermaphrodites were grown at 20 °C on HT115 bacteria producing *gld-1, nos-3* or *rnp-2* double-stranded RNA. L3-L4 larvae were then switched to 25 °C. Adults were scored for germline defects by DAPI staining.

MOG-2 antibodies and immunostaining

Polyclonal antibodies were produced against Glutathione-S-Transferase (GST)-tagged MOG-2. For Western blotting, approximately 100 μg of protein extracted from adult worms were loaded. Equal loading was verified by staining with Ponceau S or with α-Tubulin antibodies (1/2000, Sigma). Blots were incubated in Blotto/Tween with anti-MOG-2 antibodies (1/800). Secondary HRP IgG conjugates (Sigma) were used at a dilution of 1/25,000.

For immunostaining, anti-MOG-2 (1/100), anti-GLD-1 (1/100), anti-UAF-1 (1/75) and anti-GLP-1(1/8) antibodies were diluted in PBT, 5% BSA. Worms were fixed with 1.5% formaldehyde (Sigma) in 100 mM phosphate buffer at pH 7.5 followed by cold methanol for 5′ at RT. FITC or Cy-3-conjugated secondary antibodies were used at a dilution of 1/1000 (Jackson Immunoresearch). Immunostaining with anti-PGL-1 (1/500) or anti-MOG-2 (1/100) was performed on entire larvae as described (Bettinger et al., 1996). All incubations were performed overnight at 4 °C, followed by several washes. Stained worms were mounted with Vectashield containing 2 μg/ml DAPI and observed under fluorescence.

MOG-2 interaction assays and screen

mog-2, rnp-3 and *rnp-2* full length cDNAs were either introduced into pBTM116 (Bartel and Fields, 1995) to create LexA protein fusions, or into pACTII to make GAL4 activation domain fusion proteins. The plasmid form of a random-primed cDNA library (λACT-RB2) was screened for MOG-2 binding proteins using a MOG-2::LexA fusion construct on minimal medium lacking leucine, tryptophan, and histidine and supplemented with 2.5 mM of 3-aminotriazole. Two-hybrid assays were performed in strain L40. All constructs were sequenced and tested for the presence of the fusion protein by Western blotting using either anti-LexA or anti-GAL4 activation domain antibodies (Upstate Biotechnology).

For in-vitro protein–protein binding assays, full-length *rnp-3* cDNA was cloned into pCITE (Novagen). [^{35}S]Met-labeled RNP-3 protein was produced using TNT-coupled reticulocyte lysate (Promega). *mog-2 (+)* and *mog-2(q75)* cDNAs were introduced into pGEX. Binding assays were performed as described (Belfiore et al., 2004).

For immunoprecipitations, mixed-stage worms were lysed in a mortar in liquid nitrogen, collected with homogenization buffer (10 mM KCl, 1.5 mM MgCl$_2$, 1 mM DTT, 10 mM Tris–HCl pH 8.0, 50 mM sucrose, 0.05% Nonidet P-40) and treated with RNasin (0.025 U/μl). Protein G Dynabeads (Invitrogen) were coupled with anti-MOG-2 antibodies and incubated 1 h at 4 °C with worm extract in IP Buffer (100 mM KCl, 0.1 mM DTT, 20 mM Tris–HCl pH 8.0, 0.1% Nonidet P-40, 0.2 mM EDTA). Beads were washed with 1× PBS and eluted as indicated in the manufacturer's instructions with 1× NuPAGE LDS sample buffer. Mock and IP were treated with RNase-free DNase (0.05 U/μl), with Proteinase K, and finally incubated 5′ at 95 °C. Phenol/chloroform precipitates were analyzed by Northern blotting with a U2 snRNA full-length probe.

Northern analysis and RT-PCR

Poly(A)$^+$ RNA and total RNA were extracted and analyzed as described (Puoti and Kimble, 1999). RT-PCR was performed on total RNA from adult worms (Puoti and Kimble, 1999). For RT-PCR, 1 μg of DNase-treated total RNA and 100 ng of random primers were processed as described (Belfiore et al., 2004). As a positive control we used genomic DNA and random-primed single-stranded cDNAs from wild type animals. Sequences of primers are available on request. After 21 cycles of PCR, *dpy-10(e128)* cDNAs were separated on a 2% agarose gel and probed with a template that equally recognizes both spliced and unspliced variants. Quantification was performed with an Amersham Biosciences Phosphoimager.

Results and discussion

The MOG-2 protein and the Mog-2 mutant phenotype

The *mog-2(q75)* allele was isolated in a screen for recessive sterile mutants (Graham et al., 1993). *mog-2* has been mapped on chromosome

II, between *lin-31* and *unc-85* (Graham et al., 1993). We have found that *mog-2* corresponds to ORF H20J04.8, which codes for Spliceosomal-Associated Protein SAP-1 (Fig. 1A; MacMorris et al., 2007; Saldi et al., 2007). MOG-2 contains 3 Leucine-rich repeats (LRR; Bairoch, 1991), while its yeast homolog harbors six LRRs (Caspary and Seraphin, 1998). LRRs are implicated in protein-protein interactions (Kobe and Deisenhofer, 1993). The *mog-2(q75)* allele has a 5-nucleotide deletion at the end of the first exon of ORF H20J04.8. The use of a cryptic splice acceptor site generates a 24-nucleotide in-frame deletion. The predicted mutant MOG-2(q75) protein therefore lacks 8 amino acids (Fig. 1B). Interestingly, the deleted amino acids belong to a most conserved motif (Fig. 1C). We found that the mutant MOG-2(q75) protein is produced at low levels (Fig. 3B,C) and therefore asked whether *mog-2(q75)* behaved like a genetic null allele. We thus compared the phenotypes of *mog-2(q75)* with a deletion allele that removes the entire first exon. This allele, *sap-1(ok1221)*, is likely null and was named *mog-2(ok1221)* for simplicity in the rest of this study (Fig. 1A). We found that the phenotypes caused by *mog-2(q75)* or *mog-2 (ok1221)* are very similar, indicating that both alleles are null. Furthermore, *mog-2(q75)/mog-2(ok1221)* trans-heterozygotes are indistinguishable from the respective homozygotes (data not shown). While mostly fertile at 15 °C, more than 20% of *mog-2* hermaphrodites developed as somatic females that made excess of sperm and no oocytes at 20 °C (the Mog phenotype, masculinization of the germ line; Fig. 1E,H). Similarly, RNA interference against *mog-2* led to Mog animals (51%, n = 149; Fig. 1D). At 25 °C, most germ lines are fully masculinized with only 1% of

fertile progeny producing sperm and oocytes (Fig. 1E,F,G). In masculinized *mog-2(q75)* animals we counted 180 ± 88 (n = 20) sperm per gonadal arm, while *mog-2(ok1221)* had 227 ± 111 (n = 20) sperm. Regarding the extent of masculinization, both *mog-2* alleles are therefore comparable. In addition, we observed that the number of germ cells depends on the developmental stage when animals are shifted to restrictive temperature. If shifted from 15 °C to 25 °C, late-L4 larvae developed as Mog adults containing 300 or more sperm (*q75*: 276 ± 90, n = 20 and *ok1221*: 333 ± 92, n = 20; Graham et al., 1993). In contrast, when mid-L3 to mid-L4 larvae were shifted to 25 °C, they developed into adults with smaller germ lines containing 137 ± 46 (n = 14) and 155 ± 42 (n = 14) sperm for *mog-2(q75)* and *mog-2(ok1221)*, respectively (Fig. 1F). In addition to germline defects, both *mog-2* alleles cause slow growth and maternal effect lethality (Fig. 1H and Graham et al., 1993). At 25 °C, 100% of the progeny of homozygous *mog-2* mutants died as embryos, without showing signs of morphogenesis (Fig. 1H). When larvae were shifted at 25 °C before they had reached mid-L3, they typically arrested somatic and germline development (data not shown). The only difference we observed between the two *mog-2* alleles was their growth kinetics. Both *mog-2* alleles grew significantly slower than wild type siblings at 15 °C and 20 °C. Furthermore, *mog-2(q75)* larvae required more time to develop into adults than *mog-2(ok1221)* suggesting that the former produce a defective protein that interferes with growth (Fig. 1H). Finally, we show that *mog-2* is required maternally for early embryonic development, and zygotically for germline and somatic development (Fig. 1H).
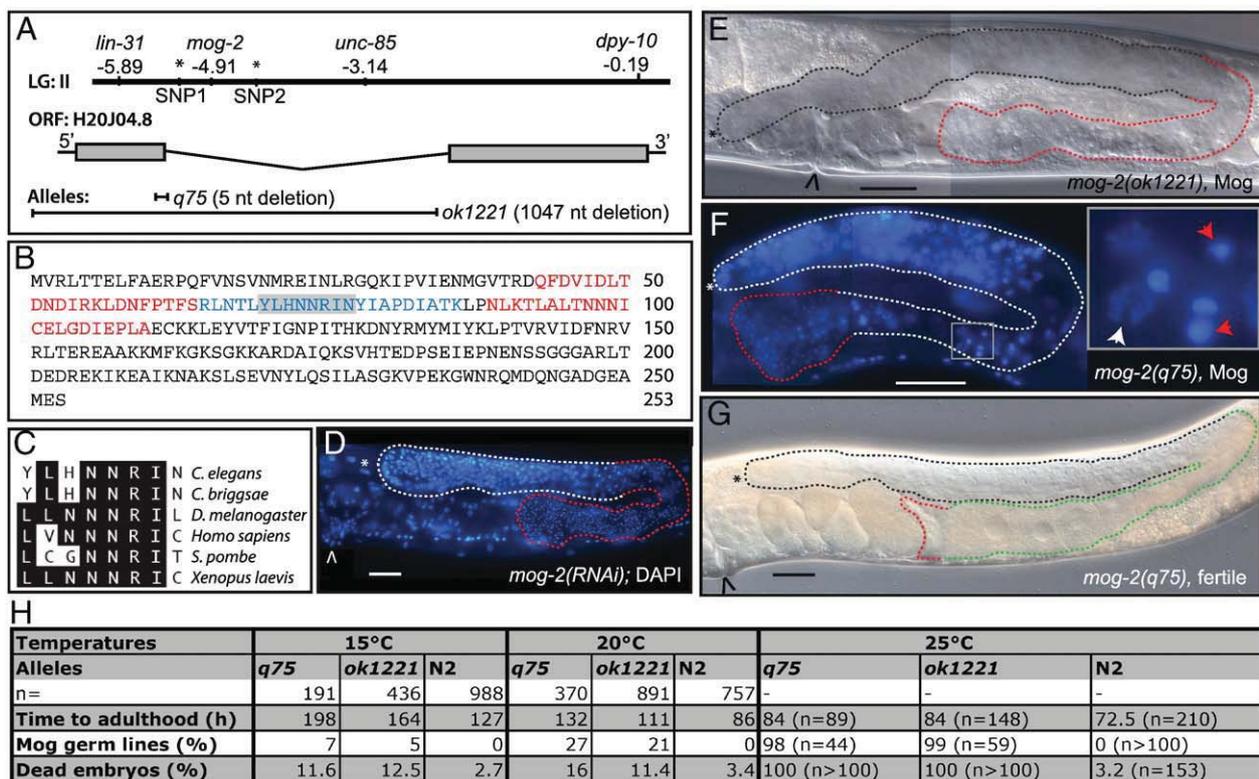

Fig. 1. The MOG-2 protein and the Mog-2 phenotype. (A) The *mog-2* locus was mapped between two SNPs on chromosome II. In *mog-2(q75)* mutants, open reading frame H20J04.8 contains a 5-nucleotide deletion. A larger deletion (*ok1221*) is available through the knockout consortium. (B) Amino acid sequence of MOG-2; the residues highlighted in gray are absent from the mutant MOG-2(q75) protein. The three Leucine-rich repeats are shown by alternating red and blue colors. (C) Deletion *q75* removes residues that belong to a LRR and that are highly conserved from yeast to mammals. (D) Injection of double-stranded *mog-2* RNA yields Mog progeny. (E) At 25 °C, *mog-2* germ lines produce only sperm (outlined in red). (F) *mog-2* L3 larvae shifted to 25 °C develop into adults with small masculinized germ lines (Nuclear DAPI staining). Magnification of the pachytene region boxed. Arrowheads point towards meiotic nuclei in oocytes (white) or sperm (red). (G) *mog-2* adults kept at 15 °C make both sperm (outlined in red) and oocytes (outlined in green; DIC image). Bar, 10 μm. The asterisk indicates the distal end of the germ line, and the open arrowheads point towards the vulva. (H) Temperature-dependent phenotypes of *mog-2* alleles *q75* and *ok1221*. L4 larvae were shifted from 15 °C to the temperature indicated and scored for the Mog phenotype. Depending on the developmental stage when shifted to 25 °C, sperm counts are variable among Mog animals, ranging from more than 300 to less than 100 (panels D, E, and F). Tested animals are the progeny of homozygous mothers kept at permissive temperature. n, number of animals checked.

mog-2 is required for the switch from mitosis to meiosis

In hermaphrodites, the switch from mitotic proliferation to meiosis is promoted through two redundant branches that function downstream of GLP-1/Notch. These two branches include GLD-1 and GLD-2. While GLP-1 is necessary for germline proliferation, GLD-1 and GLD-2 function redundantly in meiosis (Austin and Kimble, 1987; Kadyk and Kimble, 1998). Remarkably, most genes that are necessary for meiosis also function in germline sex determination (Belfiore et al., 2004; Eckmann et al., 2004; Hansen et al., 2004a; Kasturi et al., 2010; Kerins et al., 2010). The adult *C. elegans* hermaphrodite germ line features different regions with distinct stages of germ cell development. In the distal part, nuclei are mitotic and proliferate. In the transition zone, nuclei start entering into meiosis, stay in pachytene and finally differentiate as spermatids or oocytes (for review see Schedl, 1997). In previous studies, we have found that *mog-1, mog-3, mog-4, mog-5,* and *mog-6* are synthetically required with *gld-2* or *gld-3* for the switch from mitosis to meiosis (Belfiore et al., 2004; Kasturi et al., 2010). *mog;gld* double mutant germ lines contain mitotic nuclei in both their distal and proximal ends, while germ nuclei in the central region are meiotic with signs of gametogenesis (Pepper et al., 2003). To test if *mog-2* functions in parallel with *gld-2*, we constructed a *gld-2;mog-2* double mutant. We found that its germ line contains proliferating nuclei in both distal and proximal parts (Fig. 2A,B). Proximal proliferation was confirmed by staining for the mitotic marker phosphohistone H3 (Fig. 2B,C). *gld-3;mog-2* double mutants arrested as L1 larvae and could not be used to analyze germline phenotypes. The arrested L1 larvae expressed PGL-1 only in the germline primordium (Fig. 2D,E), indicating that, in contrast to *mep-1* mutants, arrested *gld-3;mog-2* larvae make no ectopic germ cells (Unhavaithaya et al., 2002). To circumvent larval lethality we treated *gld-3(q730)* animals with *mog-2(RNAi)*: 33% (n = 29) of *gld-3(q730); mog-2(RNAi)* mutants produced animals showing proximal proliferation, indicating that *mog-2* and *gld-3* are synthetically required for the switch from mitosis to meiosis (not shown). Ectopic mitosis was also observed by following the expression of germ line markers GLP-1

and GLD-1. GLP-1 is necessary for proliferation and is found only in the distal part of the germ line (Fig. 2F,G; Crittenden et al., 1994). In *gld-2 mog-2* animals, GLP-1 expands proximally, indicating ectopic proliferation (Fig. 2H). The central region however is meiotic, as shown by the expression of the meiosis marker GLD-1 (Francis et al., 1995; Hansen et al., 2004b). In wild type hermaphrodite germ lines, GLD-1 is present throughout the transition zone and the pachytene region, but rapidly disappears from the proximal portion (Fig. 2I; Jones et al., 1996). In *gld-2;mog-2* germ lines, GLD-1 staining is similar to wild type, indicating that oocyte precursors are made but do not differentiate (Fig. 2J). Our data show that in the absence of *gld-2* or *gld-3*, *mog-2* mutant germ lines are mitotic in both distal and proximal ends. In the central region, germ line nuclei transiently initiate meiosis. Proximal proliferation was found in *mog-2(q75);gld-1(RNAi)* animals, but not in *mog-2(q75);nos-3(RNAi)* (not shown). Therefore, *mog-2* functions synthetically with *gld-2* or *gld-3*, while it does not enhance the tumorous phenotype of *gld-1*. Taken together, *mog-2* functions in the same branch as *gld-1, nos-3*, and the other *mog* genes (Belfiore et al., 2004; Kasturi et al., 2010). The switch to meiosis is impaired in favor of mitosis if both branches are inactive (Hansen et al., 2004a). Therefore, *mog-2* and *gld-2* or *gld-3* are synthetically required for the decision between mitosis and meiosis.

The nuclear protein MOG-2 is expressed throughout germline development

As predicted by its maternal effect lethality and its role in oogenesis, *mog-2* mRNA is most abundant in embryos and at later larval stages, when spermatogenesis switches to oogenesis (Fig. 3A). While the *mog-2* transcript is absent in *mog-2(ok1221)* animals, it is present at low levels in *mog-2(q75)* (Fig. 3B). The presence of *mog-2 (q75)* was confirmed by RT-PCR and sequencing of the cDNA. Furthermore, *mog-2(q75)* mutants produced low levels of the mutated MOG-2 protein that lacks 8 amino acids, as predicted by the cDNA sequence (Fig. 3C). No MOG-2 protein was found in *mog-2 (ok1221)* mutants. Although the MOG-2(q75) protein is produced, the
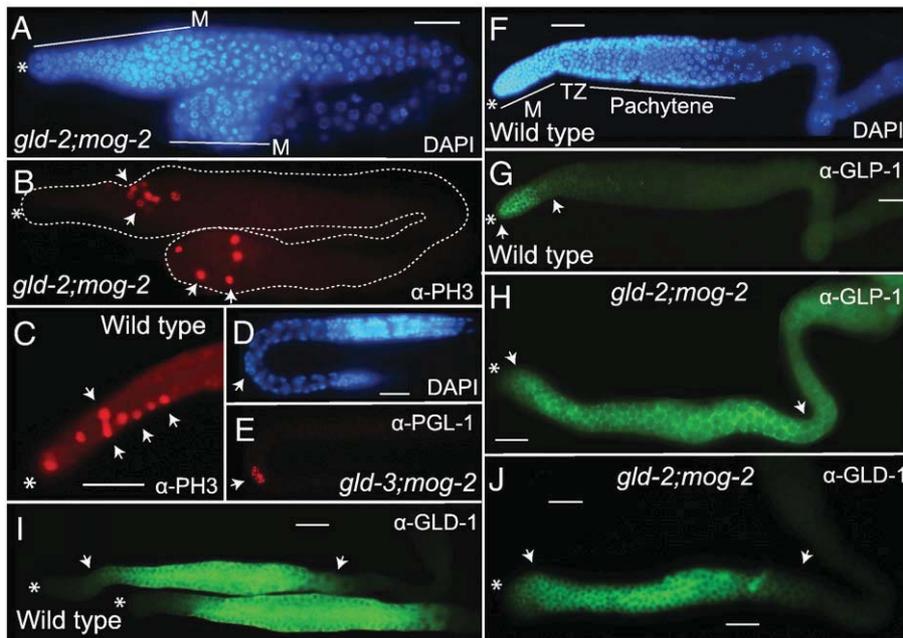

Fig. 2. *mog-2* functions in meiosis. (A,B) Proximal proliferation in *gld-2; mog-2* germ lines. Mitotic nuclei are present in both distal and proximal parts. Anti-phospho-histone H3 (PH3) staining of mitotic nuclei (arrows). (C) Wild type adult germ lines are mitotic only at the distal end (arrows). (D,E) *mog-2; gld-3* double mutants stained with DAPI and anti-PGL-1. The germline primordium is indicated (arrow). (F,G) In wild type germ lines, mitosis marker GLP-1 is restricted to the distal part (between the two arrows). (H) A *gld-2; mog-2* germ lines express GLP-1 well beyond the distal part. (I,J) Meiosis marker GLD-1 is expressed from the transition zone to the end of the pachytene region in both wild type and *gld-2; mog-2* mutants (between the two arrows). In the mutant, nuclei exit mitosis then re-enter mitosis at the proximal end. M, mitotic region; TZ, transition zone. Asterisks indicate the distal end of the germ line; Bar, 10 μm.
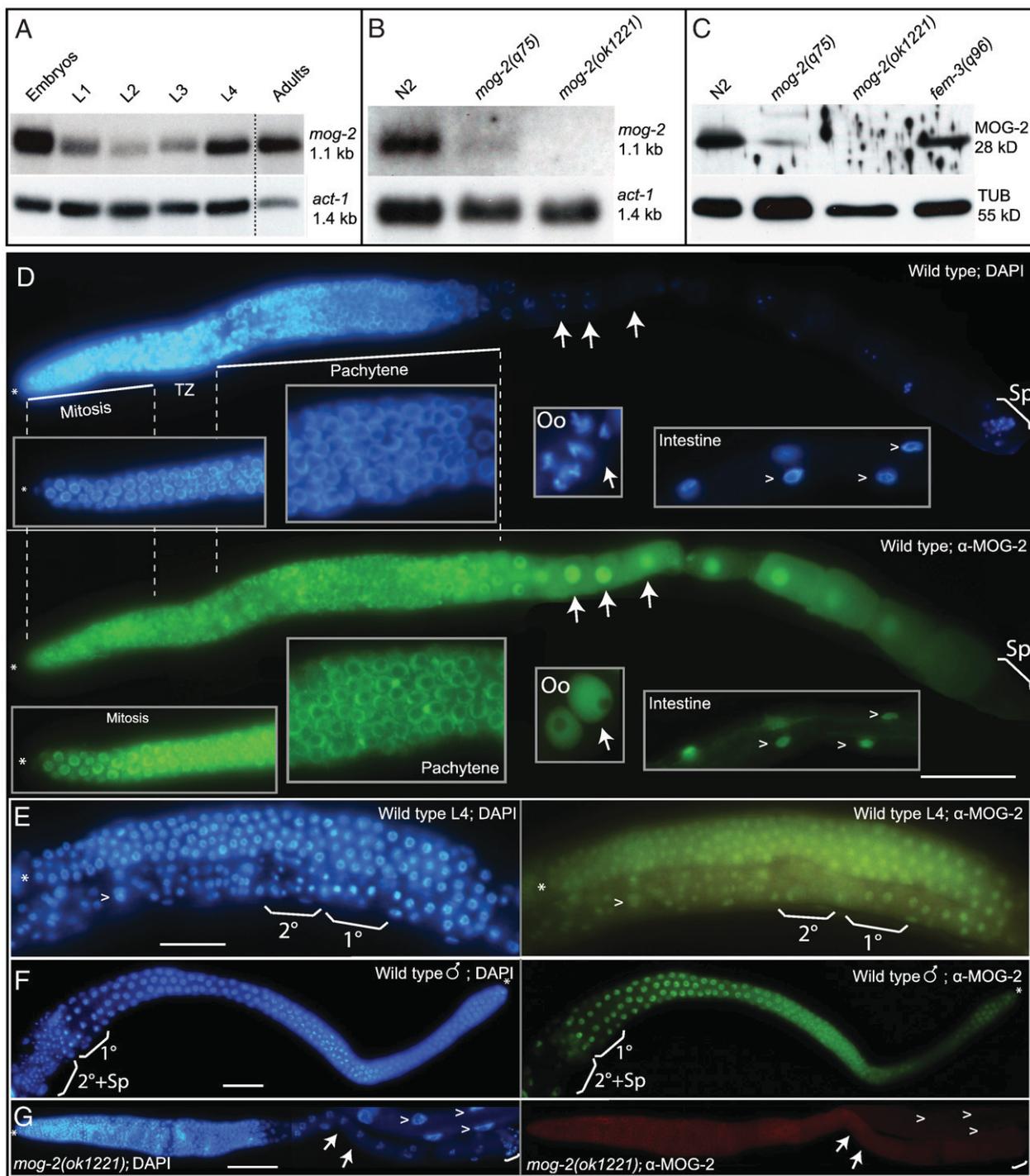
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Fig. 3. Expression of *mog-2*. (A) *mog-2* is transcribed into a single 1.1 kb mRNA, which was found in embryos, larval stages (L1 to L4) and adults. *act-1* was used as a loading control. The dotted line indicates that encompassing lanes have been removed. (B) Northern analysis of total RNA. Low levels of *mog-2* are present in masculinized *mog-2(q75)* mutants. No *mog-2* transcript was detected in *ok1221*. (C) The MOG protein is produced in adult wild type, masculinized *fem-3(gf)* and *q75* mutants, but not in *ok1221*. Tubulin was used as a loading control. (D) Immunocytochemistry on dissected adult hermaphrodite germ lines. MOG-2 was detected in mitotic nuclei, in the transition zone (TZ) and in the pachytene region. MOG-2 was found in oocytes (Oo; arrows), but not in sperm (Sp). Intestinal nuclei are indicated by open arrowheads. Nuclei were stained with DAPI. (E) MOG-2 immunostaining on a whole L4 hermaphrodite larva. One gonad arm is shown. MOG-2 was found in all germ nuclei, including primary spermatocytes (1°) but not in secondary spermatocytes (2°). (F) Dissected gonad of a wild type male stained for MOG-2. (G) No MOG-2 staining was detected in *mog-2(ok1221)* germ lines and soma. Bar, 10 μm. Asterisks indicate the distal end of the germ line.

corresponding mutant behaves as a genetic null allele (Fig. 1H). MOG-2 is expressed in many somatic and germline nuclei. Here we focus on its distribution in the germ line. MOG-2 was detected in all germline nuclei, including those in mitosis (M), in meiosis (TZ, pachytene) and in oocytes (Fig. 3D). However, MOG-2 was not found in spermatids. In L4 larvae, which transiently produce sperm, we

found MOG-2 in mitotic and meiotic nuclei, except in secondary spermatocytes (Fig. 3E). Similarly, male germ lines also expressed MOG-2 in all nuclei except in secondary spermatocytes and mature sperm (Fig. 3F). No signal was detected in mutant *mog-2(ok1221)* germ lines (Fig. 3G). *mog-2(q75)* did not stain for MOG-2, possibly because the protein levels are too low to be detected (Fig. 3C, not

shown). Taken together, our results show that MOG-2 is a nuclear protein that is present in most embryonic and somatic cells. In the germ line, MOG-2 was found in all nuclei, except in secondary spermatocytes and spermatids. Previous reports show that *fem-3* is post-transcriptionally repressed, and that the *mog* genes are necessary for this repression (Ahringer and Kimble, 1991; Gallegos et al., 1998). The distribution of MOG-2 in the hermaphrodite germ line suggests that MOG-2 is present in the whole oogenic lineage, but also to some extent in nuclei that are committed to spermatogenesis. Our results suggest that MOG-2 is present, possibly to repress *fem-3,* until germ cells have completed their second meiotic division.

MOG-2 is a component of U2 snRNP

U2A′, the vertebrate homolog of MOG-2, is a component of the U2 snRNP (Mattaj et al., 1986; Scherly et al., 1990a; Sillekens et al., 1989). The U2 snRNP functions in spliceosome assembly by interacting with the branchpoint located near the 3′ splice site (Moore et al., 1993; Padgett et al., 1984). In addition to U2A′, the vertebrate U2 snRNP includes the U2 snRNA, the RRM protein U2B″, and other proteins (Jurica and Moore, 2003). In mammals and trypanosomes, U2A′ binds to U2B″ in the absence of the U2 snRNA. Nevertheless, U2A′ is required for efficient binding of U2B″ to the U2 snRNA (Boelens et al., 1991; Caspary and Seraphin, 1998; Polycarpou-Schwarz et al., 1996; Preusser et al., 2009; Scherly et al., 1990b; Simpson et al., 1991). The *C. elegans* genome encodes two homologous proteins, RNP-2 and RNP-3, which correspond to U1A and U2B″ respectively. While RNP-2/U1A binds to the U1 snRNA, RNP-3/U2B″ interacts with U2. Nevertheless, in the absence of RNP-3, RNP-2 can bind to U2, indicating that both proteins play redundant roles in U2 snRNA binding (Saldi et al., 2007). In *Drosophila,* U1A and U2B″ are represented by one single protein, SNF which binds to both U1 and U2 snRNA, (Albrecht and Salz, 1993; Polycarpou-Schwarz et al., 1996). However, dU2A′, the *Drosophila* homolog of U2A′, significantly enhances the binding specificity of SNF to the U2 snRNA (Nagengast and Salz, 2001). Furthermore, *Drosophila* U2A′ interacts with SNF, associates with the U2 snRNP, and is essential for viability (Nagengast and Salz, 2001). Similarly, yeast Lea1p/U2A′ and Yib9p/U2B″ are essential for growth and viability at 37 °C, but have no independent function (Caspary and Seraphin, 1998). The situation is different in *C. elegans,* where U2A′ is neither required for U2B″ binding to the U2 snRNA, nor for viability (Saldi et al., 2007).

We searched for binding partners of MOG-2 by yeast two-hybrid screening. We found that MOG-2 strongly interacts with RNP-3/U2B″ but not with RNP-2/U1A. (Fig. 4A). Interestingly, the MOG-2(q75) deletion abrogates binding to RNP-3 (Fig. 4A). In vitro, a MOG-2::GST fusion protein was found to interact with RNP-3 independently of RNA. Again, this interaction requires the eight amino acids that are deleted in MOG-2(q75) (Fig. 4B). No RNP-3 was retained on beads loaded with plain GST. Our binding data indicate that MOG-2 belongs to the U2 snRNP and confirm that RNP-2 and RNP-3 are the *C. elegans* homologs of U1A and U2B″, respectively. Furthermore, we provide evidence that the interaction between RNP-3/U2B″ and MOG-2/U2A′ is conserved in *C. elegans* and that although RNP-2 can substitute RNP-3 in U2 RNA binding, only RNP-3 is able to interact with MOG-2. We therefore tested interactions with other proteins that function in *C. elegans* sex determination but found that MOG-2 binds neither to FBF-1 nor to MOG-3 (Fig. 4A). Remarkably, MOG-2 does not associate with MEP-1, which otherwise interacts with all other MOG proteins cloned to date (Fig. 4A; Belfiore et al., 2002, 2004; Kasturi et al., 2010).

mog-2 mutants are characterized by slow growth, embryonic lethality and sterility (Graham et al., 1993; Saldi et al., 2007). Removal of *rnp-3* resulted in slow growth and reduced viability, while *mog-2 (ok1221)*;*rnp-3(ok1424)* double mutants are more severely affected (Saldi et al., 2007). Similarly, we found that at permissive temperature, *mog-2(q75)*;*rnp-3(ok1424)* double mutants arrested larval development at L2, without showing signs of oogenesis or spermato-genesis. In arrested larvae, germline and somatic nuclei were enlarged, indicating cell degeneration as a possible cause of arrested development (Fig. 4C). Furthermore, in such double mutants, the severity of the maternal-lethal phenotype was increased at 20 °C, with 31% (n = 322) of *mog-2(q75)*;*rnp-3(ok1424)* animals dying as embryos, compared to 16% for *mog-2(q75)* single mutants. The phenotype was even stronger in *mog-2(q75)*;*rnp-3(ok1424)*;*rnp-2 (RNAi)* animals in which 45% (n = 170) of the progeny arrested as embryos at 20 °C. Therefore, the functions of *mog-2* and *rnp-3* overlap only partially.

In vitro binding studies and crystal structure analysis suggest that human U2A′ binds to the U2 snRNA (Boelens et al., 1991; Price et al., 1998). To test the role of MOG-2 in the spliceosome, we analyzed its ability to associate with the U2 snRNP. Native protein extracts were prepared from either wild type or mutant *rnp-3(ok1424 null)*, *mog-2 (q75),* and *mog-2(ok1221 null)* nematodes. The extracts were incubated with beads coupled to anti-MOG-2 antibodies and the bound RNAs were analyzed by Northern blotting. U2 snRNA was detected in the total lysate of all extracts, and immunoprecipitated with anti-MOG-2 antibodies thereby indicating that it binds to MOG-2 (Fig. 4D). In the absence of RNP-3, MOG-2 still bound to the U2, but to a lesser extent (Fig. 4D). Reduced U2 snRNA binding by MOG-2(q75) can be explained as follows: on one hand the mutant MOG-2(q75) protein is produced in smaller amounts than wild type MOG-2 (Fig. 3C). In addition, U2 snRNA is present at reduced levels in a U2B″/RNP-3 mutant in yeast (Caspary and Seraphin, 1998) and in *C. elegans* (data not shown). No U2 snRNA was detected in *mog-2(q75)* mutant extracts, indicating that the deleted motif in MOG-2 is necessary for U2 snRNA binding (Fig. 4D). However, alternative possibilities need to be considered: first, we found that MOG-2(q75) does not interact with RNP-3 in the yeast two-hybrid system, indicating that both proteins could be necessary for robust U2 snRNA binding. Second, MOG-2 levels are significantly lower in the *mog-2(q75)* mutants as compared to wild type (Fig. 3C). As expected, U2 snRNA was not co-immunoprecipitated in the absence of MOG-2 in a *mog-2(ok1221)* null mutant (Fig. 4D). We propose that in the absence of RNP-3, MOG-2 can still associate with the U2 snRNA. Similar results have been obtained with *Drosophila* U2A′, which interacts with U2 snRNP in the absence of SNF (Nagengast and Salz, 2001). Similarly, the trypanosome U2A′ homolog U2-40 K has been shown to be a component of the U2 snRNP, but the requirement for U2B″ has not been tested (Nagengast and Salz, 2001; Preusser et al., 2009). Our data show that in *C. elegans,* MOG-2/U2A′ is able to bind to the U2 snRNA in vivo and that this interaction does not require RNP-3/U2B″. MOG-2 might therefore interact with the U2 snRNA either directly, or through other proteins such as RNP-2/U1A, which functions redundantly with U2B″ in *C. elegans* (Saldi et al., 2007). However, our data from the yeast two-hybrid system tend to exclude RNP-2 in this interaction. The fact that *C. elegans* MOG-2 is able to associate with the U2 snRNP in the absence of RNP-3 supports an earlier finding that *Drosophila* SNF is not essential for U2A′ binding to U2 snRNP. Therefore, U2B″-independent association of U2A′ to the U2 snRNP is conserved at least between *C. elegans* and *Drosophila* (Nagengast and Salz, 2001).

We found that MOG-2 and UAF-1 co-localize in the nuclei of germ cell precursors, oocytes, but not in sperm. Surprisingly, both proteins do not localize to nuclear speckles, as expected for splicing factors, but are evenly distributed throughout the nucleoplasm (Fig. 4E; Zamore and Green, 1991). Neither MOG-2 nor UAF-1 are found in the nucleolus. Albeit not a proof, the co-localization of MOG-2 with a well-characterized splicing factor such as UAF-1 indicates that the former could function in pre-mRNA splicing.

mog-2 is involved in pre-mRNA splicing

MOG-1, MOG-4, and MOG-5 are the worm homologs of well-characterized yeast splicing factors (Puoti and Kimble, 1999, 2000).
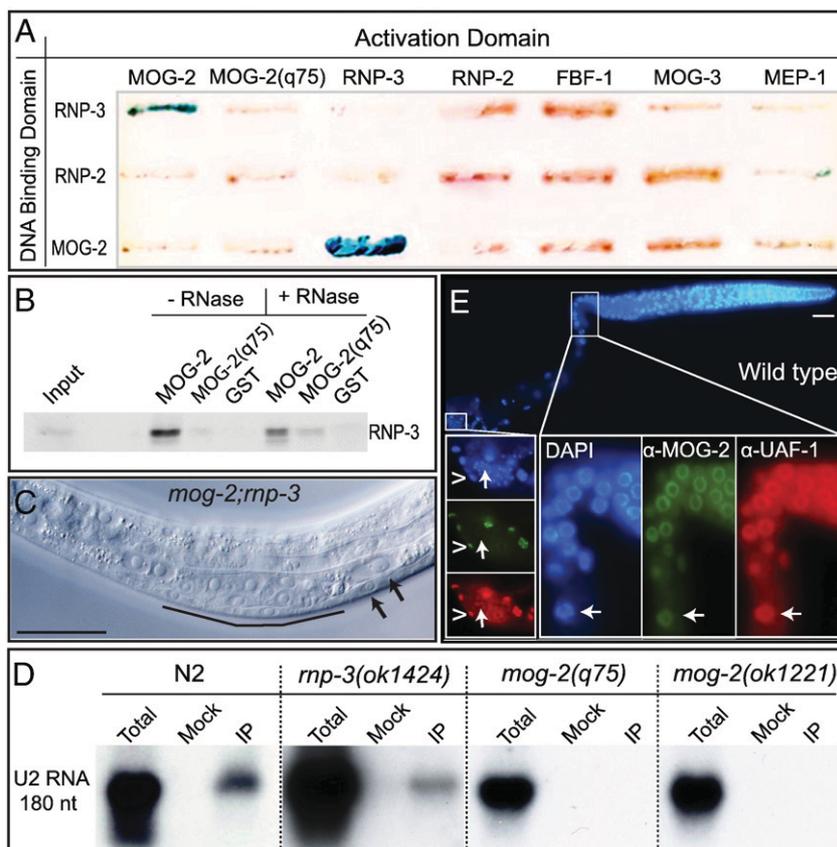
Fig. 4. MOG-2 binds to U2 snRNP components. (A) Interaction of MOG-2 with RNP-3 in the yeast two-hybrid system. Only MOG-2 and RNP-3 bind to each other (blue color). The *q75* deletion abolishes MOG-2 binding to RNP-3 (lane 2). (B) Radiolabeled, in-vitro translated RNP-3 is retained on beads coupled to MOG-2::GST. The *q75* deletion dramatically reduces RNP-3 binding in vitro. Beads coupled to plain GST do not bind RNP-3. The input corresponds to 1/10 of radiolabeled RNP-3 used for pulldowns. (C) *mog-2(q75);rnp-3(ok1424)* double mutants arrest development at larval stage 2. Arrows indicate condensed nuclei, the germ line is underlined. (D) MOG-2 binds the U2 snRNA in vivo. 10% of native total protein extracts were loaded as a positive control and analyzed by Northern blotting. The U2 RNA was immunoprecipitated by anti-MOG-2 antibodies (IP). The mock control corresponds to similar conditions, but in the absence of the antibody. No U2 RNA was detected in extracts originating from either *mog-2(q75)* or *mog-2(ok1221)* mutants. (E) MOG-2 colocalizes with splicing factor UAF-1 in the nuclei of developing oocytes (horizontal arrows) and germ cell precursors, as well as in somatic nuclei of the spermatheca (open arrowhead). Sperm nuclei stain for UAF-1, but not for MOG-2 (vertical arrow). UAF-1 is the *C. elegans* homolog of U2AF65 (Zorio et al., 1997). Bar, 10 μm.

However, no defects in general splicing were found in the corresponding *C. elegans* mutants (Belfiore et al., 2004; Kasturi et al., 2010; Puoti and Kimble, 1999). The identity of MOG-2 clearly indicates a role in RNA splicing. We therefore analyzed by Northern blotting several candidates involved in sex determination, including *fbf*, *nos-3* and the three *fem* genes, but they all appeared to be correctly spliced (data not shown). We therefore focused on RNAs that are alternatively spliced. As a first attempt, we tested the splicing of *unc-52*, which is alternatively processed in mutants of the RRM domain protein MEC-8 (Lundquist et al., 1996). We detected alternatively spliced products in which either exons 15 or 16 were joined to exon 19. However, we did not observe differences between wild type and *mog-2* mutants, indicating that MOG-2 does not alter *unc-52* alternative splicing (data not shown). Most introns in *C. elegans* are as small as 50 nucleotides and could remain unnoticed in larger transcripts (Blumenthal and Thomas, 1988), therefore we amplified fragments of selected cDNAs using primers designed to span small introns. In *C. elegans*, the 5′ donor splice site consensus is AG/*GU*RA*RU* and the 3′ splice acceptor site is *UUUC***AG**/R (intronic nucleotides are in italics, the cleavage site is represented by the slash, R stands for purine; Blumenthal and Steward, 1997). The nucleotides shown in bold are conserved in *C. elegans* and other species. Using specific oligonucleotides that span 122 introns in 32 different genes, we analyzed discrete regions encompassing small introns that would have escaped observation upon Northern blot analysis. Most introns analyzed were correctly spliced in both *mog-2* mutant alleles, as well as in *mog-2; rnp-3* double mutants. These include the *mrg-1* and *drsh-1* RNAs, which are

predicted to produce alternatively spliced variants (Stein et al., 2001). To ensure that incompletely-spliced RNAs are not degraded by nonsense-mediated decay, we included *smg-1* mutants in our analysis. However, the processing of such introns was similar in wild type, *smg-1,* and *smg-1;mog-2* mutants (data not shown). Similarly, the first intron of the *fem-3* transcript was correctly processed in all mutant backgrounds analyzed, indicating that absence of *mog-2* does not generate a gain-of-function isoform of FEM-3 (Fig. 5A, top row). Therefore, if *mog-2* was involved in splicing, it could control the splicing of another mRNA, which regulates *fem-3*. *nos-3* is implicated in the sperm-oocyte switch and could therefore represent a target of *mog-2* (Wang et al., 2002). We analyzed its third intron and found that *mog-2* does not affect the processing of this particular intron (Fig. 5A). Although a subpopulation of *nos-3* mRNAs retained the third intron, this splicing defect was caused by the absence of *smg-1* (Fig. 5A second row, compare lanes 2 with 5 and 6). A similar observation was made for the first intron of the *daz-1* RNA, which is also required for germline sex determination (Karashima et al., 2000). We found, however, that a significant proportion of a 50-nucleotide intron of *ama-1* was not excised in *smg-1;mog-2* and *mog-2;rnp-3* double mutants (Bird and Riddle, 1989). Such spliced variant was found neither in *smg-1,* nor in wild type animals (Fig. 5A 3rd row). Similarly, the *mog-1* transcript retained its second intron in the absence of *mog-2* (Fig. 5A 4th row). Failure in removing this intron of 43 nucleotides resulted in a truncated MOG-1 protein that lacks most of its sequence, including conserved motifs that are essential for MOG-1 function. It should be noted that normally processed transcripts were
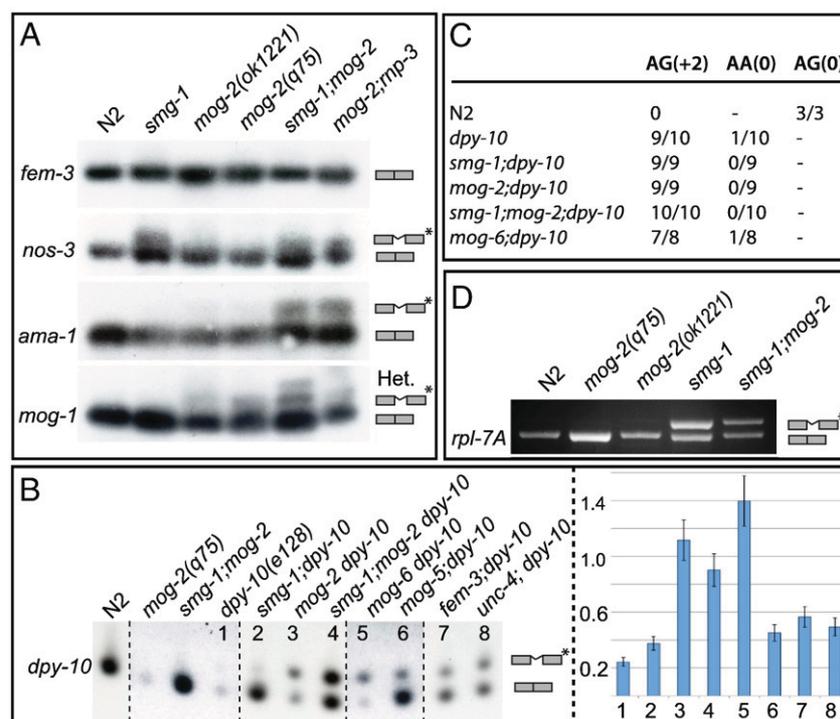
Fig. 5. MOG-2 functions in splicing. (A) Selected regions of different mRNAs have been analyzed by RT-PCR and Southern blotting. Exons are shown as boxes and introns as lines joining them. Abnormal spliced products are shown for *ama-1*, *mog-1*, and *nos-3* (*asterisks*). Het.: heterodimer of spliced and unspliced cDNAs. All products were sequenced. (B) cDNAs from wild type (3′ splice site: AG) or *dpy-10(e128)* (3′ splice site: AA) were tested for splicing efficiency of the corresponding intron in mutant backgrounds as indicated. The unspliced product is shown by the asterisk. The bar graph indicates the calculated ratio of unspliced versus spliced *dpy-10(e128)* cDNAs of three independent experiments. (C) Usage of 3′ splice sites: spliced *dpy-10* mRNAs were sequenced as cDNAs. In most cases, a cryptic AG(+2) splice site was used instead of the mutated AA(0) splice site. This selection occurred independently of the *mog-2* mutation. Numbers of cDNA clones sequenced are indicated. (D) The presence of the unspliced variant of *rpl-7A* is dependent on the absence of *smg-1* rather than *mog-2*.

found in all cases, in addition to the spliced variant. Therefore, proteins other than MOG-2 and RNP-3 must function in the recognition of such introns. Our data show that *mog-1* splicing is slightly reduced in the absence of *mog-2*. Therefore, it is conceivable that the phenotype observed in *mog-2* mutants is caused, at least in part, by the reduction of *mog-1* function. What makes the two introns of *ama-1* and *mog-1* different from the other 120 introns that have been analyzed in our survey? Both the 5′ intron splice donor and the 3′ intron splice acceptor signals are not significantly divergent from those found in most *C. elegans* introns. Also the sizes of both introns are within those commonly found in *C. elegans* (Blumenthal and Steward, 1997). Therefore, *mog-2* must play a role in recognizing other signals within the intron or exon.

mog-2 is required for the recognition of a weak splice site

The U2 snRNP recognizes the branchpoint and possibly interacts with the 3′ splice site (Moore et al., 1993). However, among the transcripts analyzed, we did not find divergences among 3′ splice acceptor sites. We therefore tested the *dpy-10(e128)* allele, which modifies the invariant AG of the 3′ splice site in the second intron (AG to AA; Aroian et al., 1993). In *dpy-10(e128)* mutants, three products were found: the unspliced variant, low amounts of *dpy-10* mRNA that has been spliced to the mutated AA site and *dpy-10* transcripts that have been spliced to a cryptic AG site that is located two nucleotides downstream of the mutated AA. The presence of a fraction of spliced *dpy-10(e128)* mRNA indicates that the AG dinucleotide at the end of intron 2 is not essential for splicing, because a small fraction of *dpy-10(e128)* mRNA was spliced to the mutated AA site (Aroian et al., 1993). We asked if the selection of the cryptic splice site was dependent on *mog* activity. To do so, we compared RT-PCR products between several mutants, and calculated

the ratio between the unspliced and spliced variants. In three independent experiments, we found that in the absence of *mog-2*, the unspliced variant was two times more abundant, compared to *smg-1; dpy-10* (Fig. 5B, compare lanes 1–4). An even more pronounced effect was observed in *mog-6 dpy-10* animals. However, the ratio between the unspliced and spliced cDNAs was close to that found in single *dpy-10(e128)* mutants when tested with either *mog-5, fem-3* or *unc-4* (Fig. 5B, compare lane 1 with lanes 5–8). When *dpy-10(e128)* was spliced, the splicing occurred in most cases at a cryptic slice site [AG(+2)] located 2 nucleotides downstream of the regular splice site instead of the mutated [AA(0)] position (Aroian et al., 1993; Fig. 5C). Therefore, MOG-2 and MOG-6 lead to the activation of a nearby cryptic splicing acceptor. Since normal splicing still occurs in a fraction of the *dpy-10(e128)* mRNA, it is likely that other proteins or splice signals are required. These remain to be uncovered.

Since alternatively spliced *daz-1* and *nos-3* mRNAs were found in both *smg-1* and *mog-2* animals (Fig. 5A; not shown), we tested *mog-2* for a role in nonsense-mediated decay. The spliced variant of *rpl-7A* is a hallmark for defective nonsense-mediated decay and is therefore detected in *smg* mutants (Mitrovich and Anderson, 2000). While an alternatively spliced variant of the *rpl-7A* was detected in *smg-1* and *smg-1; mog-2* mutants, it was observed neither in wild type, nor in *mog-2* animals (Fig. 5D). Therefore, *mog-2* does not function in RNA surveillance.

Taken together, our data suggest that *mog-2* and *mog-6* activate a cryptic splice site in the mutant *dpy-10(e128)* RNA. However, both genes are not sufficient, because a small fraction of the transcript is spliced even in their absence. Then, how do defective *mog* genes lead to a mutant phenotype? The simplest explanation is that in the absence of *mog*, the dose of correctly spliced mRNAs decreases, thereby shifting the balance in germline sex determination towards the male fate.

8

Conclusion

Post-transcriptional regulation of the *fem-3* RNA requires the action of the *mog* genes (Gallegos et al., 1998; Puoti et al., 2001). The *mog* genes code for proteins that have homologs that are either directly or indirectly involved in pre-mRNA splicing (Belfiore et al., 2004; Kasturi et al., 2010; Kerins et al., 2010; Konishi et al., 2008; Mantina et al., 2009; Puoti and Kimble, 1999). In addition to regulating sex determination, such genes also function in the decision between mitosis and entry into meiosis. In *Drosophila*, the U1A/RNP-2 and U2B″/RNP-3 homolog SNF functions in sex determination by establishing the autoregulatory feedback loop to maintain female-specific splicing of *Sxl* (Oliver et al., 1988; Steinmann-Zwicky, 1988). SNF is required for both germline and somatic sex determination, but its function in the soma is visible only when levels of *Sxl* are low. In the germ line, SNF is necessary for germ cell differentiation (Albrecht and Salz, 1993; Oliver et al., 1988). Similarly to SNF, MOG-2 could function in pre-mRNA splicing, perhaps by promoting the processing of particular transcripts. We found a few abnormally spliced mRNAs, among which *mog-1* is an interesting candidate. *mog-1* codes for the worm homolog of Prp16p (Puoti and Kimble, 1999), which not only acts as a general splicing factor, but is also required for the proofreading of spliced products in *Saccharomyces cerevisiae* (Schwer and Gross, 1998; Schwer and Guthrie, 1991). An important next step is to find additional RNA targets that lead to a Mog phenotype if alternatively spliced. High throughput sequencing techniques are likely to uncover such targets.

References

Ahringer, J., Kimble, J., 1991. Control of the sperm-oocyte switch in *Caenorhabditis elegans* hermaphrodites by the *fem-3* 3′ untranslated region. Nature 349, 346–348.

Albrecht, E.B., Salz, H.K., 1993. The *Drosophila* sex determination gene *snf* is utilized for the establishment of the female-specific splicing pattern of sex-lethal. Genetics 134, 801–807.

Aroian, R.V., Levy, A.D., Koga, M., Ohshima, Y., Kramer, J.M., Sternberg, P.W., 1993. Splicing in *Caenorhabditis elegans* does not require an AG at the 3′ splice acceptor site. Mol. Cell. Biol. 13, 626–637.

Austin, J., Kimble, J., 1987. *glp-1* is required in the germ line for regulation of the decision between mitosis and meiosis in *C. elegans*. Cell 51, 589–599.

Bairoch, A., 1991. PROSITE: a dictionary of sites and patterns in proteins. Nucleic Acids Res. 19 (Suppl.), 2241–2245.

Bartel, P.L., Fields, S., 1995. Analyzing protein–protein interactions using two-hybrid system. Methods Enzymol. 254, 241–263.

Barton, M.K., Schedl, T.B., Kimble, J., 1987. Gain-of-function mutations of *fem-3*, a sex-determination gene in *Caenorhabditis elegans*. Genetics 115, 107–119.

Belfiore, M., Mathies, L.D., Pugnale, P., Moulder, G., Barstead, R., Kimble, J., Puoti, A., 2002. The MEP-1 zinc-finger protein acts with MOG DEAH box proteins to control gene expression via the *fem-3* 3′ untranslated region in *Caenorhabditis elegans*. RNA 8, 725–739.

Belfiore, M., Pugnale, P., Saudan, Z., Puoti, A., 2004. Roles of the *C. elegans* cyclophilin-like protein MOG-6 in MEP-1 binding and germline fates. Development 131, 2935–2945.

Bettinger, J.C., Lee, K., Rougvie, A.E., 1996. Stage-specific accumulation of the terminal differentiation factor LIN-29 during *Caenorhabditis elegans* development. Development 122, 2517–2527.

Bird, D.M., Riddle, D.L., 1989. Molecular cloning and sequencing of *ama-1*, the gene encoding the largest subunit of *Caenorhabditis elegans* RNA polymerase II. Mol. Cell. Biol. 9, 4119–4130.

Black, D.L., Chabot, B., Steitz, J.A., 1985. U2 as well as U1 small nuclear ribonucleoproteins are involved in premessenger RNA splicing. Cell 42, 737–750.

Blumenthal, T., Steward, K., 1997. RNA processing and gene structure. In: Riddle, D.L., Blumenthal, T., Meyer, B.J., Priess, J.R. (Eds.), *C. Elegans* II. Cold Spring Harbor Laboratory Press, pp. 117–145.

Blumenthal, T., Thomas, J., 1988. Cis and trans mRNA splicing in *C. elegans*. Trends Genet. 4, 305–308.

Boelens, W., Scherly, D., Beijer, R.P., Jansen, E.J., Dathan, N.A., Mattaj, I.W., van Venrooij, W.J., 1991. A weak interaction between the U2A′ protein and U2 snRNA helps to stabilize their complex with the U2B″ protein. Nucleic Acids Res. 19, 455–460.

Caspary, F., Seraphin, B., 1998. The yeast U2A′/U2B complex is required for pre-spliceosome formation. EMBO J. 17, 6348–6358.

Crittenden, S.L, Troemel, E.R., Evans, T.C., Kimble, J., 1994. GLP-1 is localized to the mitotic region of the *C. elegans* germ line. Development 120, 2901–2911.

Eckmann, C.R., Crittenden, S.L., Suh, N., Kimble, J., 2004. GLD-3 and control of the mitosis/meiosis decision in the germline of *Caenorhabditis elegans*. Genetics 168, 147–160.

Francis, R., Barton, M.K., Kimble, J., Schedl, T., 1995. *gld-1*, a tumor suppressor gene required for oocyte development in *Caenorhabditis elegans*. Genetics 139, 579–606.

Gallegos, M., Ahringer, J., Crittenden, S., Kimble, J., 1998. Repression by the 3′ UTR of *fem-3*, a sex-determining gene, relies on a ubiquitous *mog*-dependent control in *Caenorhabditis elegans*. EMBO J. 17, 6337–6347.

Graham, P.L., Kimble, J., 1993. The *mog-1* gene is required for the switch from spermatogenesis to oogenesis in *Caenorhabditis elegans*. Genetics 133, 919–931.

Graham, P.L., Schedl, T., Kimble, J., 1993. More *mog* genes that influence the switch from spermatogenesis to oogenesis in the hermaphrodite germ line of *Caenorhabditis elegans*. Dev. Genet. 14, 471–484.

Hansen, D., Hubbard, E.J., Schedl, T., 2004a. Multi-pathway control of the proliferation versus meiotic development decision in the *Caenorhabditis elegans* germline. Dev. Biol. 268, 342–357.

Hansen, D., Wilson-Berry, L., Dang, T., Schedl, T., 2004b. Control of the proliferation versus meiotic development decision in the *C. elegans* germline through regulation of GLD-1 protein accumulation. Development 131, 93–104.

Hirsh, D., Oppenheim, D., Klass, M., 1976. Development of the reproductive system of *Caenorhabditis elegans*. Dev. Biol. 49, 200–219.

Hodgkin, J., 1986. Sex determination in the nematode *C. elegans*: analysis of *tra-3* suppressors and characterization of fem genes. Genetics 114, 15–52.

Jones, A.R., Francis, R., Schedl, T., 1996. GLD-1, a cytoplasmic protein essential for oocyte differentiation, shows stage- and sex-specific expression during *Caenorhabditis elegans* germline development. Dev. Biol. 180, 165–183.

Jurica, M.S., Moore, M.J., 2003. Pre-mRNA splicing: awash in a sea of proteins. Mol. Cell 12, 5–14.

Kadyk, L.C., Kimble, J., 1998. Genetic regulation of entry into meiosis in *Caenorhabditis elegans*. Development 125, 1803–1813.

Karashima, T., Sugimoto, A., Yamamoto, M., 2000. *Caenorhabditis elegans* homologue of the human azoospermia factor DAZ is required for oogenesis but not for spermatogenesis. Development 127, 1069–1079.

Kasturi, P., Zanetti, S., Passannante, M., Saudan, Z., Muller, F., Puoti, A., 2010. The *C. elegans* sex determination protein MOG-3 functions in meiosis and binds to the CSL co-repressor CIR-1. Dev. Biol. 344, 10.

Kerins, J.A., Hanazawa, M., Dorsett, M., Schedl, T., 2010. PRP-17 and the pre-mRNA splicing pathway are preferentially required for the proliferation versus meiotic development decision and germline sex determination in *Caenorhabditis elegans*. Dev. Dyn. 239, 1555–1572.

Kobe, B., Deisenhofer, J., 1993. Crystal structure of porcine ribonuclease inhibitor, a protein with leucine-rich repeats. Nature 366, 751–756.

Konarska, M.M., Sharp, P.A., 1987. Interactions between small nuclear ribonucleoprotein particles in formation of spliceosomes. Cell 49, 763–774.

Konishi, T., Uodome, N., Sugimoto, A., 2008. The *Caenorhabditis elegans* DDX-23, a homolog of yeast splicing factor PRP28, is required for the sperm-oocyte switch and differentiation of various cell types. Dev. Dyn. 237, 2367–2377.

Lundquist, E.A., Herman, R.K., Rogalski, T.M., Mullen, G.P., Moerman, D.G., Shaw, J.E., 1996. The *mec-8* gene of *C. elegans* encodes a protein with two RNA recognition motifs and regulates alternative splicing of unc-52 transcripts. Development 122, 1601–1610.

MacMorris, M., Brocker, C., Blumenthal, T., 2003. UAP56 levels affect viability and mRNA export in *Caenorhabditis* elegans. RNA 9, 847–857.

MacMorris, M., Kumar, M., Lasda, E., Larsen, A., Kraemer, B., Blumenthal, T., 2007. A novel family of *C. elegans* snRNPs contains proteins associated with trans-splicing. RNA 13, 511–520.

Mantina, P., MacDonald, L., Kulaga, A., Zhao, L., Hansen, D., 2009. A mutation in *teg-4*, which encodes a protein homologous to the SAP130 pre-mRNA splicing factor, disrupts the balance between proliferation and differentiation in the *C. elegans* germ line. Mech. Dev. 126, 417–429.

Mattaj, I.W., Habets, W.J., van Venrooij, W.J., 1986. Monospecific antibodies reveal details of U2 snRNP structure and interaction between U1 and U2 snRNPs. EMBO J. 5, 997–1002.

Mitrovich, Q.M., Anderson, P., 2000. Unproductively spliced ribosomal protein mRNAs are natural targets of mRNA surveillance in *C. elegans*. Genes Dev. 14, 2173–2184.

Moore, M.J., Query, C.C., Sharp, P.A., 1993. Splicing of precursors to mRNA by the spliceosome. In: Gesteland, R.F., Atkins, J.F. (Eds.), The RNA World. Cold Spring Harbor Laboratory Press, pp. 303–357.

Nagengast, A.A., Salz, H.K., 2001. The *Drosophila* U2 snRNP protein U2A′ has an essential function that is SNF/U2B″ independent. Nucleic Acids Res. 29, 3841–3847.

Oliver, B., Perrimon, N., Mahowald, A.P., 1988. Genetic evidence that the sans fille locus is involved in *Drosophila* sex determination. Genetics 120, 159–171.

Padgett, R.A., Konarska, M.M., Grabowski, P.J., Hardy, S.F., Sharp, P.A., 1984. Lariat RNA's as intermediates and products in the splicing of messenger RNA precursors. Science 225, 898–903.

Pepper, A.S., Killian, D.J., Hubbard, E.J., 2003. Genetic analysis of *Caenorhabditis elegans glp-1* mutants suggests receptor interaction or competition. Genetics 163, 115–132.

Polycarpou-Schwarz, M., Gunderson, S.I., Kandels-Lewis, S., Seraphin, B., Mattaj, I.W., 1996. *Drosophila* SNF/D25 combines the functions of the two snRNP proteins U1A and U2B′ that are encoded separately in human, potato, and yeast. RNA 2, 11–23.

Preusser, C., Palfi, Z., Bindereif, A., 2009. Special Sm core complex functions in assembly of the U2 small nuclear ribonucleoprotein of *Trypanosoma brucei*. Eukaryot. Cell 8, 1228–1234.

Price, S.R., Evans, P.R., Nagai, K., 1998. Crystal structure of the spliceosomal U2B″–U2A′ protein complex bound to a fragment of U2 small nuclear RNA. Nature 394, 645–650.

Puoti, A., Kimble, J., 1999. The *Caenorhabditis elegans* sex determination gene *mog-1* encodes a member of the DEAH-Box protein family. Mol. Cell. Biol. 19, 2189–2197.

Puoti, A., Kimble, J., 2000. The hermaphrodite sperm/oocyte switch requires the *Caenorhabditis elegans* homologs of PRP2 and PRP22. Proc. Natl. Acad Sci. U.S.A. 97, 3276–3281.

Puoti, A., Pugnale, P., Belfiore, M., Schlappi, A.C., Saudan, Z., 2001. RNA and sex determination in *Caenorhabditis elegans*. Post-transcriptional regulation of the sex-determining *tra-2* and *fem-3* mRNAs in the *Caenorhabditis elegans* hermaphrodite. EMBO Rep. 2, 899–904.

Saldi, T., Wilusz, C., MacMorris, M., Blumenthal, T., 2007. Functional redundancy of worm spliceosomal proteins U1A and U2B″. Proc. Natl. Acad. Sci. U.S.A. 104, 9753–9757.

Schedl, T., 1997. Developmental genetics of the germ line. In: Riddle, D.L., Blumenthal, T., Meyer, B.J., Priess, J.R. (Eds.), *C. Elegans* II. Cold Spring Harbor Laboratory Press, pp. 241–269.

Scherly, D., Boelens, W., Dathan, N.A., van Venrooij, W.J., Mattaj, I.W., 1990a. Major determinants of the specificity of interaction between small nuclear ribonucleo-proteins U1A and U2B″ and their cognate RNAs. Nature 345, 502–506.

Scherly, D., Dathan, N.A., Boelens, W., van Venrooij, W.J., Mattaj, I.W., 1990b. The U2B″ RNP motif as a site of protein–protein interaction. EMBO J. 9, 3675–3681.

Schwer, B., Gross, C.H., 1998. Prp22, a DExH-box RNA helicase, plays two distinct roles in yeast pre-mRNA splicing. EMBO J. 17, 2086–2094.

Schwer, B., Guthrie, C., 1991. PRP16 is an RNA-dependent ATPase that interacts transiently with the spliceosome. Nature 349, 494–499.

Sillekens, P.T., Beijer, R.P., Habets, W.J., van Verooij, W.J., 1989. Molecular cloning of the cDNA for the human U2 snRNA-specific A′ protein. Nucleic Acids Res. 17, 1893–1906.

Simpson, G.G., Vaux, P., Clark, G., Waugh, R., Beggs, J.D., Brown, J.W., 1991. Evolutionary conservation of the spliceosomal protein, U2B″. Nucleic Acids Res. 19, 5213–5217.

Stein, L., Sternberg, P., Durbin, R., Thierry-Mieg, J., Spieth, J., 2001. WormBase: network access to the genome and biology of *Caenorhabditis elegans*. Nucleic Acids Res. 29, 82–86.

Steinmann-Zwicky, M., 1988. Sex determination in Drosophila: the X-chromosomal gene liz is required for *Sxl* activity. EMBO J. 7, 3889–3898.

Unhavaithaya, Y., Shin, T.H., Miliaras, N., Lee, J., Oyama, T., Mello, C.C., 2002. MEP-1 and a homolog of the NURD complex component Mi-2 act together to maintain germline-soma distinctions in *C. elegans*. Cell 111, 991–1002.

Wang, L., Eckmann, C.R., Kadyk, L.C., Wickens, M., Kimble, J., 2002. A regulatory cytoplasmic poly(A) polymerase in *Caenorhabditis elegans*. Nature 419, 312–316.

Zamore, P.D., Green, M.R., 1991. Biochemical characterization of U2 snRNP auxiliary factor: an essential pre-mRNA splicing factor with a novel intranuclear distribution. EMBO J. 10, 207–214.

Zhang, B., Gallegos, M., Puoti, A., Durkin, E., Fields, S., Kimble, J., Wickens, M.P., 1997. A conserved RNA-binding protein that regulates sexual fates in the *C. elegans* hermaphrodite germ line. Nature 390, 477–484.

Zorio, D.A., Lea, K., Blumenthal, T., 1997. Cloning of *Caenorhabditis* U2AF65: an alternatively spliced RNA containing a novel exon. Mol. Cell. Biol. 17, 946–953.