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Repression by the 3′ UTR of *fem-3*, a sex-determining gene, relies on a ubiquitous *mog*-dependent control in *Caenorhabditis elegans*

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The *fem-3* sex-determining gene is repressed post-transcriptionally via a regulatory element in its 3′ untranslated region (UTR) to achieve the switch from spermatogenesis to oogenesis in the *Caenorhabditis elegans* hermaphrodite germ line. In this paper, we investigate the *fem-3* 3′ UTR control in somatic tissues using transgenic reporter assays, and we also identify six genes essential for this control. First, we find that a reporter transgene bearing a wild-type *fem-3* 3′ UTR is repressed in somatic tissues, whereas one bearing a mutant *fem-3* 3′ UTR is derepressed. Moreover, control by mutant 3′ UTRs is temperature sensitive as predicted from the temperature sensitivity of the *fem-3* gain-of-function *(gf)* mutations. Secondly, we find a *fem-3* 3′ UTR RNA-binding activity in somatic tissues, in addition to the previously reported germ-line-specific binding by FBF. Thirdly, we find that each of six genes, *mog-1–mog-6*, is required for repression by the *fem-3* 3′ UTR. Therefore, the *mog* genes not only affect the sperm/oocyte switch in the germ line, but also function in somatic tissues. We suggest that the *mog* genes may encode components of a ubiquitous machinery that is used for *fem-3* 3′ UTR-mediated repression and the sperm/oocyte switch.

Keywords: 3′ UTR/*fem-3*/*mog*/post-transcriptional control/RNA

Introduction

Sequence elements in the 3′ untranslated region (UTR) often regulate the stability, translation or localization of mRNAs (reviewed in Singer, 1993; Beelman and Parker, 1995; Curtis *et al*., 1995; Wickens *et al*., 1996, 1997). Although regulatory proteins have been identified that bind specifically to such *cis* elements (Legagneux *et al*., 1992; Murata and Wharton, 1995; Dubnau and Struhl, 1996; Smibert *et al*., 1996; Wang *et al*., 1996; Deshler *et al*., 1997; Kelley *et al*., 1997; Ostareck *et al*., 1997; Webster *et al*., 1997; Zhang *et al*., 1997), key questions remain unanswered. For example, by what mechanism does the RNA-binding protein regulate RNA activity? And how are regulatory processes modified during growth and development? To begin to address these questions, we have taken a molecular genetic approach to identify and characterize factors required *in trans* for the 3′ UTR-mediated regulation of *fem-3*, a sex-determining gene in *Caenorhabditis elegans*. This genetic strategy is designed to identify both regulators of the post-transcriptional machinery and components of the machinery itself.

Caenorhabditis elegans can develop either as a male or self-fertile hermaphrodite (female that briefly makes sperm), depending on its ratio of X chromosomes to sets of autosomes, the X/A ratio (reviewed in Kuwabara and Kimble, 1992; Meyer, 1997). The *fem-3* gene is required for specification of male fates: XX and X0 animals, which would normally develop as hermaphrodites and males respectively, are both transformed into females in the absence of *fem-3* activity (Figure 1A, top; Hodgkin, 1986; Barton *et al*., 1987). In contrast, hermaphrodites carrying dominant *fem-3* gain-of-function (*gf*) alleles are masculinized in the germ line, producing excess sperm and no oocytes (Figure 1A, bottom; Barton *et al*., 1987). This is the Mog phenotype (masculinization of the germ line). Molecular characterization of *fem-3(gf)* alleles revealed a point mutation element, or PME, in the *fem-3* 3′ UTR (Figure 1B; Ahringer and Kimble, 1991).

The PME appears to regulate *fem-3* activity post-transcriptionally. First, overexpression of an RNA consisting of the wild-type *fem-3* 3′ UTR masculinizes the hermaphrodite germ line, but overexpression of a mutant *fem-3* 3′ UTR with a defective PME does not have this effect (Ahringer and Kimble, 1991). A simple explanation is that the excess *fem-3* 3′ UTR titrates a post-transcriptional repressor and thereby deregulates endogenous *fem-3* RNA. Secondly, a cytoplasmic RNA-binding protein, called FBF (for *fem-3* binding factor), is required for the switch from spermatogenesis to oogenesis (Zhang *et al*., 1997). FBF binds RNAs that contain a wild-type PME, but does not bind RNAs with a mutant PME. We hypothesize that *fem-3* acts early in germ-line development to direct spermatogenesis and subsequently is repressed by a 3′ UTR-mediated control to permit oogenesis.

Genes required *in trans* for the 3′ UTR-mediated repression of *fem-3* are expected to have a loss-of-function (*lf*) Mog phenotype similar to that of the *fem-3(gf)* alleles. Mutations in six genes, *mog-1–mog-6*, satisfy this criterion (Graham and Kimble, 1993; Graham *et al*., 1993); in addition, animals lacking FBF activity as a result of RNA-mediated interference have a Mog phenotype (Zhang *et al*., 1997). Epistasis analyses have placed all six *mog* genes and FBF upstream of the *fem* genes, consistent with their participation in *fem-3* repression (Graham and Kimble, 1993; Graham *et al*., 1993; Zhang *et al*., 1997).

In this paper, we demonstrate that the *fem-3* 3′ UTR confers PME-dependent repression of a reporter gene *in vivo*. Because the reporter assay was established in

somatic tissues, we conclude that the machinery required for the post-transcriptional repression of *fem-3* is present in somatic tissues as well as in the germ line. In support of this conclusion, we report a PME-dependent RNA-binding activity in somatic tissues that is possibly distinct from FBF. Finally, we show that each of six genes, *mog-1–mog-6*, is required for 3′ UTR-mediated repression.

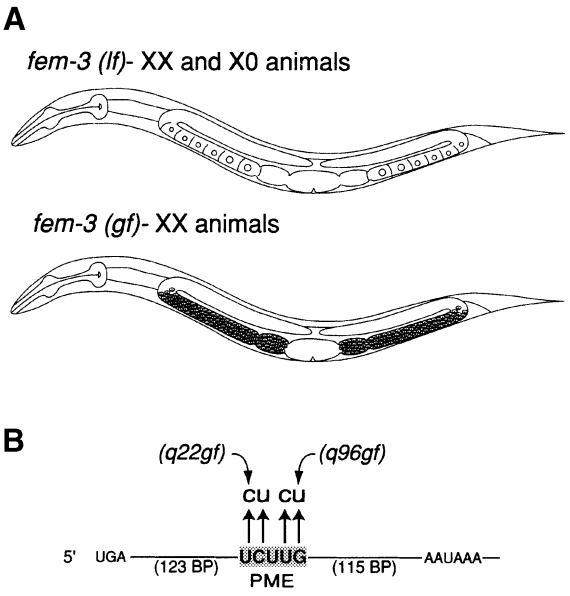

Fig. 1. The *fem-3* sex-determining gene specifies male fates in both somatic and germ line tissues. (**A**) *fem-3* mutant phenotypes. Above, both XX and X0 adults carrying a *fem-3* loss-of-function (*lf*) mutation develop as females (Hodgkin, 1986). Below, XX adults carrying a *fem-3* gain-of-function (*gf*) mutation have a masculinized germ line and therefore make only sperm (Barton *et al*., 1987). Anterior is left, dorsal is up. (**B**) The *fem-3(gf)* mutations carry point mutations within a five base nucleotide (nt) sequence of the *fem-3* 3′ UTR. PME sequence shown within the 3′ UTR (thin line). Number of nucleotides found between stop codon and PME and between PME and AAUAAA are indicated in parentheses. *fem-3(gf)* nt changes are indicated by arrows (Ahringer, 1991; Ahringer and Kimble, 1991). Phenotypically, the weakest and strongest *fem-3* (*gf*) point mutations are *q22gf* and *q96gf* (Barton *et al*., 1987).

Results

The fem-3 3′ UTR represses a lacZ reporter gene in vivo

To ask whether the *fem-3* 3′ UTR is sufficient for PME-mediated regulation, we developed a transgenic reporter assay by fusing a *fem-3* 3′ UTR to a *lacZ* reporter gene. *lacZ::fem-3(+)* contains a wild-type *fem-3* 3′ UTR, whereas *lacZ::fem-3(q96 gf)* contains a mutant *fem-3* 3′ UTR with a single base change in the PME, and *lacZ::fem-3(del8)* contains a mutant *fem-3* 3′ UTR with an eight-nucleotide (nt) deletion, removing the PME (Figure 2A). Since transgenes do not express well in the germ

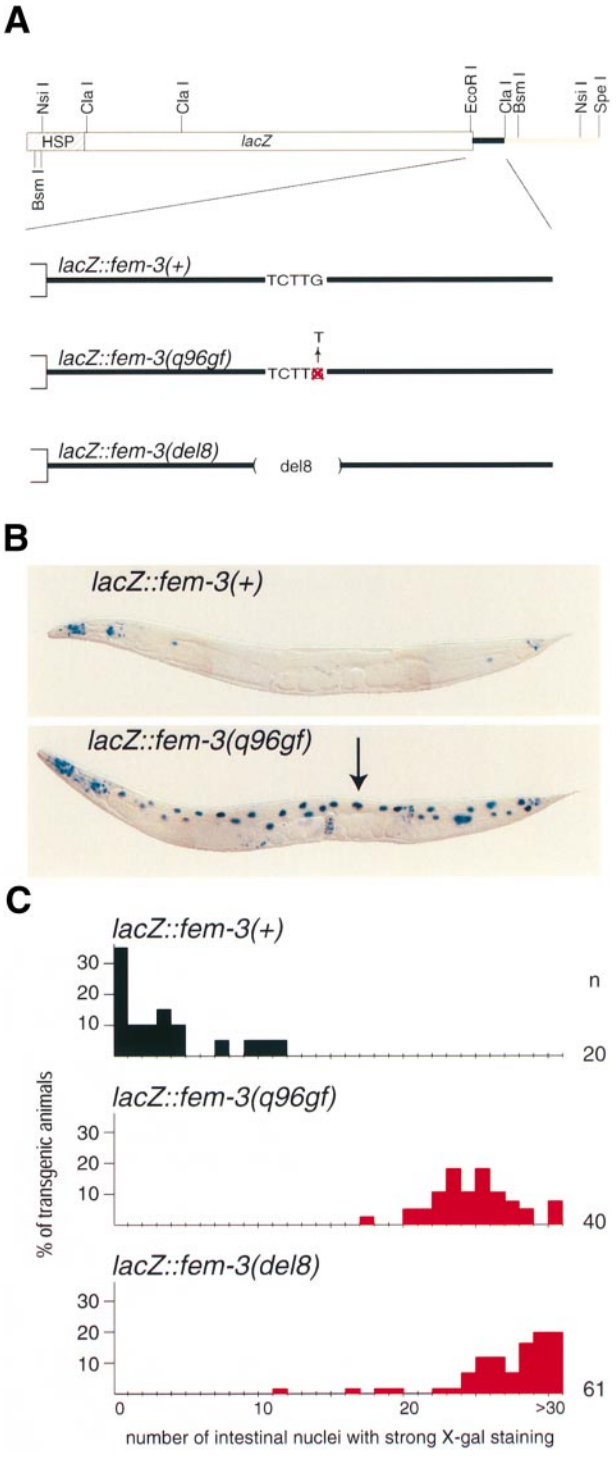

Fig. 2. Regulation of reporter expression by the *fem-3* 3′ UTR. (**A**) The *lacZ* reporter construct consists of a heat shock promoter fused to a 5′ UTR containing a synthetic intron (HSP, striped rectangle), *lacZ* coding sequence fused to an SV40 nuclear localization signal (*lacZ*, white rectangle), a wild-type or mutant *fem-3* 3′ UTR (thick black line) plus *fem-3* 3′ flanking genomic sequence (light grey line). Relevant restriction sites used to determine integrity of transgenic lines and copy number of integrated transgenes are indicated (see Materials and methods). The 3′ UTR portion of each construct is enlarged and the PME sequence indicated. The point mutation of *lacZ::fem-3(q96 gf)* contains a C to T change (middle construct). *lacZ::fem-3(del8)* contains an 8 nt deletion (bottom construct). (**B**) Nomarski micrographs showing the typical amount of X-gal staining found after heat shock of integrated transgenic lines: *qIs43* [*lacZ::fem-3(+)*] and *qIs15* [*lacZ::fem-3(q96 gf)*] (see Table I). Anterior is left, dorsal is up. Arrow points to an intestinal nucleus. (**C**) Graph of results of a single experiment with *qIs43* [*lacZ::fem-3(+)*], *qIs15* [*lacZ::fem-3(q96 gf)*] and *qIs44* [*lacZ::fem-3(del8)*]. *n*, number of animals examined. Similar results were also found with at least two independently isolated transgenic lines carrying extrachromosomal arrays (see Table I).

Table I. Transgenes used

Transgene name	Transgene type	Type of array	Transgene expression[a]	No. of transgenes per chromosome[b]
qEx212	*lacZ::fem-3(+)*	extrachromosomal	+/−	–
qEx213	*lacZ::fem-3(+)*	extrachromosomal	+/−	–
qIs43	*lacZ::fem-3(+)*	integrated	+/−	4
qEx131	*lacZ::fem-3(q96gf)*	extrachromosomal	++++	–
qEx208	*lacZ::fem-3(q96gf)*	extrachromosomal	++++	–
qIs15	*lacZ::fem-3(q96gf)*	integrated	++++	3
qEx387	*lacZ::fem-3(del8)*	extrachromosomal	++++	–
qEx388	*lacZ::fem-3(del8)*	extrachromosomal	++++	–
qEx389	*lacZ::fem-3(del8)*	extrachromosomal	++++	–
qIs44	*lacZ::fem-3(del8)*	integrated	++++	4

[a]Transgene expression levels: +/− (0–20%); + (20–40%); ++ (40–60%); +++ (60–80%); and ++++ (80–100%) of animals had >20 intestinal nuclei with strong X-gal staining. See Materials and methods for definition of strong X-gal staining.
[b]Copy number was determined by Southern analysis of integrated lines using a radioactive probe that detected both the integrated transgene and endogenous *fem-3* (see Materials and methods).

line of *C.elegans* and we did not know *a priori* which somatic tissues might contain *fem-3* repressor activity, each *lacZ::fem-3* construct was fused to the *C.elegans hsp16* heat shock promoter. This promoter drives expression in various somatic tissues following heat shock (Stringham *et al*., 1992). Multiple lines containing extrachromosomal arrays of each *lacZ::fem-3* transgene were generated and Southern blots were performed to confirm that the array carried full-length transgenes. An integrated line was also generated for each *lacZ::fem-3* transgene and the copy number was determined (Table I; Materials and methods). β-galactosidase (β-gal) activity in intestinal nuclei of adults was assayed using X-gal staining following heat shock. The intestine was assayed since it is easy to score.

Animals carrying *lacZ::fem-3(+)* expressed a low level of β-gal whereas animals carrying *lacZ::fem-3(q96 gf)* or *lacZ::fem-3(del8)* expressed a higher level of β-gal (Table I; Figure 2B and C). Among the integrated lines, no *lacZ::fem-3(+)* animals exhibited strong X-gal staining in ⩾20 intestinal nuclei, in contrast to 92% of *lacZ::fem-3(q96 gf)* and 94% of *lacZ::fem-3(del8)* animals (Figure 2B and C). We conclude that the 3′ UTR is sufficient to repress a heterologous reporter gene *in vivo* and that a wild-type PME is required for repression.

The fem-3(gf) 3′ UTR confers temperature-sensitive repression on a reporter in vivo

The *fem-3(gf)* alleles are temperature sensitive: at 15°C most *fem-3(gf)* mutants make both sperm and oocytes, whereas at 25°C they make only sperm (Barton *et al*., 1987). Therefore, the *fem-3(gf)* 3′ UTR mediates repression at 15°C, but not at 25°C. To explore further the *fem-3* 3′ UTR control, we asked whether reporter expression could be rendered temperature sensitive by a mutant *fem-3(gf)* 3′ UTR. To this end, the coding region for green fluorescent protein (GFP) was fused either to a *fem-3(q22 gf)* or *fem-3(q96 gf)* mutant 3′ UTR or, as a control, to the *fem-3(+)* wild-type 3′ UTR (Figure 3A); then each reporter was placed under the control of the *lag-2* promoter, and multiple transgenic lines carrying extrachromosomal arrays were generated for each construct. The *lag-2* promoter drives expression in the distal tip cells (DTC) of the somatic gonad (Henderson *et al*., 1994).

Transgenic animals were raised at 15 or 25°C, and GFP expression was compared in animals possessing the same extrachromosomal array (Figure 3B). At 15°C, GFP expression was low in *GFP::fem-3(q22 gf)* transgenic animals, but at 25°C, GFP expression was high (Figure 3B, middle); the same was found for *GFP::fem-3(q96 gf)* transgenic animals (Figure 3B, right). However, GFP expression in *GFP::fem-3(+)*-bearing animals did not change substantially with temperature (Figure 3B, left) and it also did not change using another control transgene (*lag-2::GFP* fused to a wild-type *unc-54* 3′ UTR) (data not shown). Therefore, point mutations in the *fem-3(gf)* 3′ UTR reporter constructs render them temperature sensitive, consistent with the fact that the *fem-3(gf)* alleles are temperature sensitive. Furthermore, the temperature sensitivity of *fem-3(gf)* alleles is not germ-line specific.

A PME-dependent fem-3 RNA-binding activity

To identify an RNA-binding activity specific for a PME containing *fem-3* 3′ UTR, we used a gel retardation assay. ^{32}P-labelled RNA probes derived from the *fem-3* 3′ UTR (Figure 4A) were incubated with crude extracts prepared from wild-type or mutant adults and then analysed by electrophoresis through a non-denaturing gel.

Figure 4B shows that a PME-dependent *fem-3* RNA-binding activity is present in crude extracts of wild-type adults. Using RNAs containing either 35 or 81 nt of the wild-type *fem-3* 3′ UTR (including the PME), two complexes are formed in crude extract (Figure 4B, lane 2; and data not shown). In contrast, an RNA deleted for 8 nt spanning the PME forms no complex (Figure 4B, lane 6) and neither does an RNA with those 8 base pairs changed [chg8(81); Figure 4, legend; not shown]. To further demonstrate that these complexes are specific to a wild-type *fem-3* 3′ UTR, we compared the ability of unlabelled wild-type and mutant RNAs to compete for complex formation with a labelled wild-type RNA. A 100-fold excess of unlabelled RNA derived from the wild-type *fem-3* 3′ UTR interferes with complex formation (Figure 4B, lane 3), whereas the same amount of an RNA

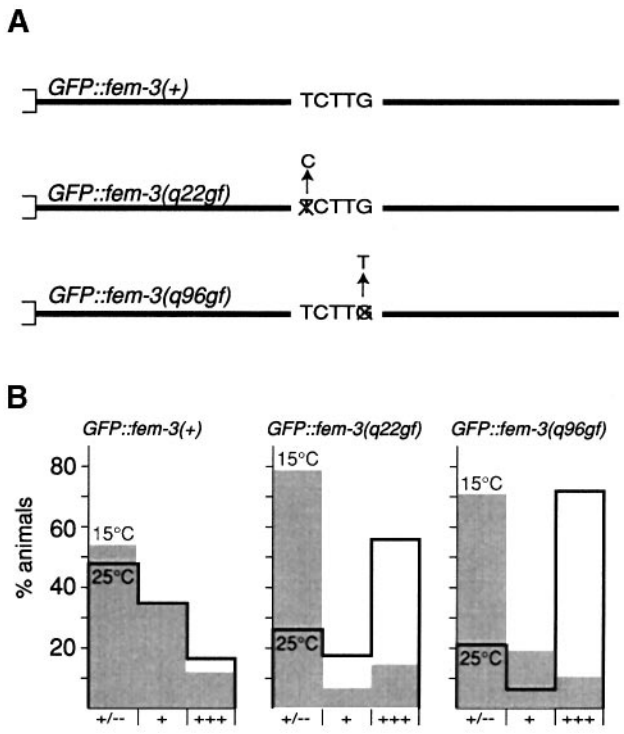

Fig. 3. Temperature-sensitive regulation of reporter expression by *fem-3 (gf)* 3′ UTRs. (**A**) The *fem-3* 3′ UTR portions of *GFP::fem-3* reporter constructs. The entire construct is described in the text and detailed in Materials and methods. *GFP::fem-3* reporters include a *lag-2* promoter, GFP-coding sequence, a *fem-3* 3′ UTR derived from *fem-3(+), fem-3(q22 gf)* or *fem-3(q96 gf),* and 766 nt of *fem-3* 3′ genomic flanking. The *fem-3(q22 gf)* and *fem-3(q96 gf)* mutations were selected for these experiments because they represent the weakest and strongest *fem-3(gf)* point mutants respectively (Barton *et al*., 1987). Only the 3′ UTR is shown and each point mutation is indicated. (**B**) GFP expression is evaluated at both 15°C (grey line) and 25°C (black line) in transgenic animals bearing extrachromosomal arrays with *GFP::fem-3(+)* (left), *GFP::fem-3(q22 gf)* (middle), and *GFP::fem-3(q96 gf)* (right). Each graph represents the combined data of four [*GFP::fem-3(+)*], five [*GFP::fem-3(q96 gf)*] or six [*GFP::fem-3(q22 gf)*] independently isolated transgenic lines. For *GFP::fem-3(+),* 89 and 91 DTCs (two per animal) were scored at 15 and 25°C, respectively; for *GFP::fem-3(q22 gf),* 89 and 100 DTCs were scored at 15 and 25°C, respectively; for *GFP::fem-3(q96 gf),* 106 and 108 DTCs were scored at 15 and 25°C, respectively. +++, bright fluorescence; +, faint fluorescence; +/–, little or no fluorescence (see Materials and methods for further explanation of scoring).

with a mutant PME [chg8(81)] sequence does not compete (Figure 4B, lane 4) and an RNA bearing a point mutation competes more poorly than wild-type (data not shown).

To determine whether this PME-dependent *fem-3* RNA-binding factor is present in somatic tissues, crude extracts were prepared from *glp-1* or *glp-4* mutants, which possess few germ cells (Austin and Kimble, 1987; Beanan and Strome, 1992). We found that complexes formed with extracts prepared from either wild-type animals that possess a full germ line or mutants that lack a germ line (*glp-1* and *glp-4*) (Figure 4C, compare lane 2 with lanes 3 and 4). The complexes are PME-specific since their formation is prevented by excess unlabelled wild-type RNA, but not by excess RNA lacking a PME (data not shown). We conclude that the somatic tissues possess a PME-dependent *fem-3* RNA-binding activity. The simplest interpretation is that at least two PME-dependent RNA-binding proteins exist: FBF, which is germ-line specific

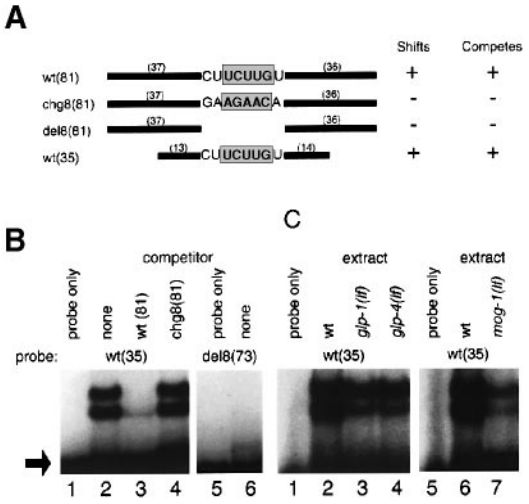

Fig. 4. Gel-shift assay using a *fem-3* 3′ UTR RNA probe. (**A**) RNA probes were derived from the *fem-3* 3′ UTR. Eight nt sequence including the PME (shaded) is shown, with number of flanking nt in parentheses. (B and C) Five fmoles of labelled RNA probe (see horizontal text above each gel) were incubated with 5 μg of worm extract. An interaction between factor(s) from extract and probe is identified as a shift in migration of the RNA through a non-denaturing gel. Arrow signifies free probe. Where indicated, 500 fmol of unlabelled competitor was included. (**B**) *fem-3* RNA-binding activity requires a PME. All extracts were prepared from wild-type adults. The type of competitor RNA used in each case is indicated in vertical text. The chg8 RNA possesses GAAGAACA instead of CTTCTTGT in the PME region. (**C**) RNA-binding activity is present in somatic tissues and *mog-1(q223)* hermaphrodites. Mutant extracts were made from animals that lacked germ lines, *glp-1(lf)* and *glp-4(lf)* (see text for details), or *mog-1(q223)* mutants. The genotype of animals used to make extract is indicated in vertical text. The weak signal in lane 7 is probably due to the limited sample size [the extract was made from 700 hand-picked *mog-1(q223)* homozygotes].

(Zhang *et al*., 1997), and a distinct activity, which occurs in somatic tissues.

The mog genes are required for fem-3 3′ UTR-mediated repression

Six *mog* genes encode candidate negative regulators of *fem-3* (Graham and Kimble, 1993; Graham *et al*., 1993). To determine whether *mog-1–mog-6* are required for the 3′ UTR-mediated repression of *fem-3*, we examined expression of *lacZ::fem-3(+)* in *mog* mutants. For these experiments, we used *qIs43*, an integrated line of *lacZ::fem-3(+)* (Figure 2 and Table I), and scored β-gal levels by X-gal staining in the self-progeny of parents homozygous for *lacZ::fem-3(+)* and heterozygous for the mutant (m) of interest. These progeny included non-Mog hermaphrodites of genotype *lacZ::fem-3(+); +/+* or *lacZ::fem-3(+); m/+* and Mog animals of genotype *lacZ::fem-3(+); m/m* (*m* = *mog-1*, *-4*, *-5*, *-6*) (Figure 5A). *mog-2(lf)* and *mog-3(lf)* were assayed one generation later and only *lacZ::fem-3(+)*; *m/m* and *lacZ::fem-3(+)*; *+/+* were compared (see Materials and methods).

Non-Mog hermaphrodites carrying *lacZ::fem-3(+)* produced a low level of β-gal, whereas their *lacZ::fem-3(+)*; *m/m* Mog siblings produced a higher level of β-gal (Figure 5B and C; Table II). Only 2% of non-Mog *lacZ::fem-3(+)* animals exhibited strong X-gal staining in >20 intestinal nuclei. In contrast, 93% (*mog-1*), 42% (*mog-2*), 62% (*mog-3*), 95% (*mog-4*), 86% (*mog-5*) and 64% (*mog-6*) of *lacZ::fem-3(+)*; *mog-x* (*x* = 1–6) mutants exhibited

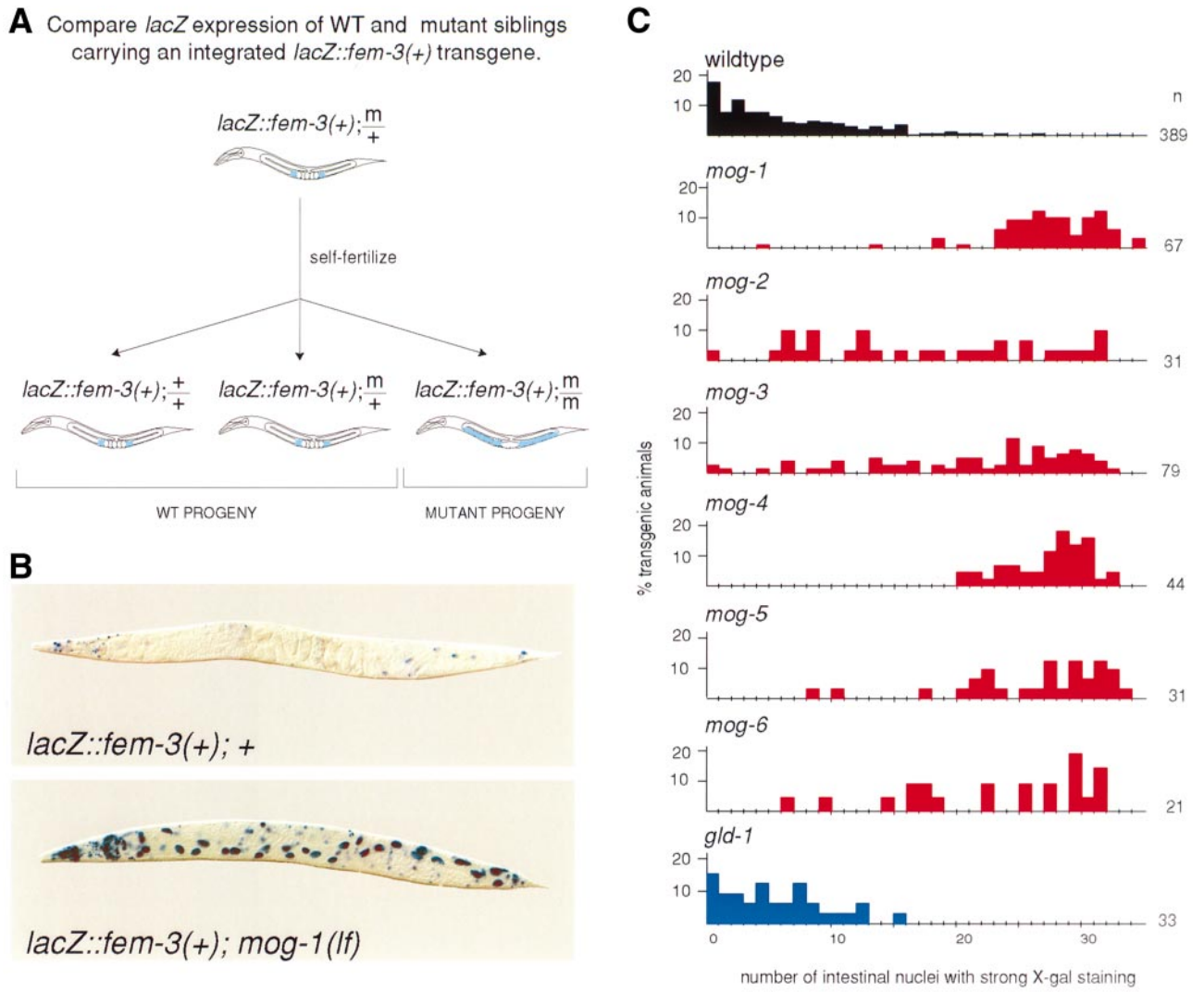

Fig. 5. Expression of *lacZ::fem-3(+)* in *mog* mutants. (**A**) The basic strategy used to examine *qIs43* [*lacZ::fem-3(+)*] in various mutant backgrounds is outlined here (see Materials and methods for details and exceptions). Animals homozygous for *lacZ::fem-3(+)* and heterozygous for the mutant (m) of interest were self-fertilized. X-gal staining following heat shock of mutant [*lacZ::fem-3(+); m/m*] and phenotypically wild-type [*lacZ::fem-3(+); +/+* and *lacZ::fem-3(+); m/+*] siblings were compared. (**B**) Nomarski micrographs representing the typical amount of heat-induced X-gal staining seen with *lacZ::fem-3(+)* in a wild-type background (top) or *mog-1* mutant background (bottom). Anterior is left, dorsal is up. (**C**) Data represented in graph form. Derepression is seen as a shift to the right in the *mog* graphs compared with the wild-type graph. The *y*-axis represents the percentage of animals, the *x*-axis represents the number of intestinal nuclei with strong X-gal staining per animal. The mutant background is labelled above each graph on the left. *n* represents the number of animals examined. Each graph represents the combined results of at least two independent experiments. Control graph (black) represents a combination of all the wild-type animals examined in parallel with the *mog* and *gld-1* mutant siblings.

strong X-gal staining in >20 intestinal nuclei. The weaker derepression of *lacZ::fem-3(+)* in *mog-2(lf)* and *mog-3(lf)* mutants is consistent with the fact that the *mog-2(lf)* and *mog-3(lf)* alleles used in this assay are temperature sensitive and not likely to be null (Graham *et al*., 1993). The *mog-1* allele, however, is a molecular null (A.Puoti and J.Kimble, unpublished data), consistent with the stronger *lacZ::fem-3(+)* derepression observed in *mog-1* mutants.

As a preliminary step to determine whether any of the *mog* genes is required for the PME-dependent *fem-3* RNA-binding activity detected by gel shift, we assayed binding activity in *mog-1(q223)* mutants, a molecular null (A.Puoti and J.Kimble, unpublished data). Complex formation is indistinguishable between extracts prepared from wild-type animals (Figure 4C, lane 6) or *mog-1* nulls (Figure 4C, lane 7). In addition, complex formation in *mog-1*, as in wild-type, is specific since it is disrupted by co-incubation with unlabelled wild-type *fem-3* 3′ UTR RNA competitor [wt(81)], but not *fem-3* RNA with a mutant PME [chg8(81)] (data not shown). This result is consistent with the finding that *mog-1* fails to interact with the *fem-3* 3′ UTR in a yeast 3-hybrid assay (A.Puoti and J.Kimble, unpublished data).

We conclude that each of the six *mog* genes is required for 3′ UTR-mediated repression of *fem-3* but that *mog-1*, at least, is not required for PME-dependent *fem-3* RNA-binding activity. Whereas germ-line function is implied by the germ-line phenotype of *mog* mutants, this reporter assay provides *in vivo* evidence that the *mog* genes also function in the soma. Molecular evidence is also consistent with such a somatic function: *mog-1* RNA is detected in

Table II.

Line	Transgene type[a]	Temperature (°C)	Animals with strong X-gal staining (%) in[b]:			Levels[c]	Number
			<10	10–20	>20 intestinal nuclei		
1	*lacZ::fem-3(+)*[d]	33	81	17	2	+/−	556
2	*lacZ::fem-3(+); mog-1*	33	1	6	93	++++	67
3	*lacZ::fem-3(+); mog-2*	33	29	29	42	++	31
4	*lacZ::fem-3(+); mog-3*	33	15	23	62	+++	79
5	*lacZ::fem-3(+); mog-4*	33	0	5	95	++++	44
6	*lacZ::fem-3(+); mog-5*	33	7	7	86	++++	31
7	*lacZ::fem-3(+); mog-6*	33	9	27	64	+++	22
8	*lacZ::fem-3(+); gld-1(q93)*	33	88	12	0	+/−	33
9	*lacZ::fem-3(+); fog-1*	33	89	11	0	+/−	100
10	*lacZ::fem-3(+); glp-4*	33	73	22	5	+/−	37
11	*lacZ::fem-3(+); unc-17*	33	98	2	0	+/−	48
12	*lacZ::fem-3(+); tra-2*	33	96	4	0	+/−	48
13	*lacZ::fem-3(q96gf)*	30	81	17	2	+/−	47
14	*lacZ::fem-3(q96gf); mog-1*	30	86	14	0	+/−	50
15	*lacZ::fem-3(del8); dpy-19*	30	82	4	14	+/−	22
16	*lacZ::fem-3(+); dpy-19 mog-1*	30	95	5	0	+/−	39
17	*lacZ::tra-2(+)*	33	100	0	0	+/−	219
18	*lacZ::tra-2(+); mog-1*	33	97	3	0	+/−	111

[a]*qIs43* [*lacZ::fem-3(+)*], *qIs15* [*lacZ::fem-3(q96)*] and *qIs44* [*lacZ::fem-3(del8)*] integrated transgenes were used in these experiments (see Table I).
[b]See Materials and methods for definition of strong X-gal staining.
[c]Transgene expression levels: +/− (0–20%); + (20–40%); ++ (40–60%); +++ (60–80%); and ++++ (80–100%) of animals had >20 intestinal nuclei with strong X-gal staining.
[d]Results represent the combined data from control experiments done in parallel with rows 2–12.

the soma and *mog-1::GFP* promoter fusion is expressed in somatic tissues, including the intestine (A.Puoti and J.Kimble, unpublished data). Expression in the intestine is consistent with the 3′ UTR-mediated repression of *lacZ* in that tissue.

Other genes with mog-1-like mutant phenotypes do not derepress lacZ::fem-3(+)

To determine whether derepression of *lacZ::fem-3(+)* is specific to the *mog* genes, we examined the expression of *lacZ::fem-3(+) (qIs43*) in other mutant backgrounds that share some of the phenotypic effects of *mog* mutants. The *mog-1* mutant phenotype is characterized by a masculinized hermaphrodite germ line, reduced germ-line proliferation and a slow growth defect (Graham and Kimble, 1993; A.Puoti and J.Kimble, unpublished data). To test whether derepression might be linked to one of these phenotypes instead of the *mog* mutant genes themselves, we examined *lacZ::fem-3(+)* in an *unc-17(lf)* background, which is a mutant with a slow growth defect (Rand and Russell, 1984), and in mutants with defective germ lines: *fog-1(lf)* feminizes the germ line (Barton and Kimble, 1990), *glp-4(lf)* reduces germ-line proliferation (Beanan and Strome, 1992), *gld-1(q93)* masculinizes the germ line (Mog phenotype) (Francis *et al*., 1995), and *tra-2(b202 ts)* transforms XX hermaphrodites into pseudomales (Hodgkin and Brenner, 1977). Furthermore, *tra-2*, like the *mog* genes and FBF, is positioned upstream of *fem-3* in a negative regulatory pathway (Hodgkin, 1986). To perform these experiments, we followed the protocol described in Figure 5A and in Material and methods.

Unlike our finding with the *mog* mutants, *lacZ::fem-3(+)* remained repressed in these other mutant backgrounds. Thus, 0% (*gld-1*), 0% (*fog-1*), 5% (*glp-4*), 0% (*tra-2*) and 0% (*unc-17*) of mutants carrying *lacZ::fem-3(+)* expressed a high level of β-gal in >20 intestinal nuclei when placed in the mutant background indicated in parentheses. These numbers are comparable with the 2% of *lacZ::fem-3(+)* wild-type control animals that produced a high level of β-gal in >20 intestinal nuclei (Table II, compare line 1 with lines 8–12; for *gld-1*, Figure 5C).

We conclude that the derepression of *lacZ::fem-3(+)* observed in the *mog* mutant background does not occur due to the lack of a sperm/oocyte switch, the reduced germ-line proliferation or slow growth of *mog* mutants. Instead, we propose that the *mog* genes themselves are required for *fem-3* 3′ UTR-mediated repression. In addition, because *lacZ::fem-3(+)* remains repressed in pseudomales [XX *tra-2(lf)* animals], we suggest that repressor activity is present in both hermaphrodites and males. Consistent with this result, *lacZ::fem-3(+)* expresses β-gal at a low level in X0 males (data not shown) and factor(s) from crude extracts prepared from X0 males also contain detectable *fem-3* RNA-binding activity (Ahringer, 1991).

mog-1-mediated repression requires a PME-containing fem-3 3′ UTR

To determine whether the *mog* genes derepress any mRNA regardless of sequence, we next assayed a different reporter transgene, *lacZ::tra-2(+)*, in a *mog-1* mutant background. *lacZ::tra-2(+)* contains the *lacZ* gene fused to a *tra-2* 3′ UTR and is driven by a heat shock promoter (Goodwin *et al*., 1997). The *tra-2* 3′ UTR contains a negative-acting regulatory element distinct from the *fem-3* 3′ UTR

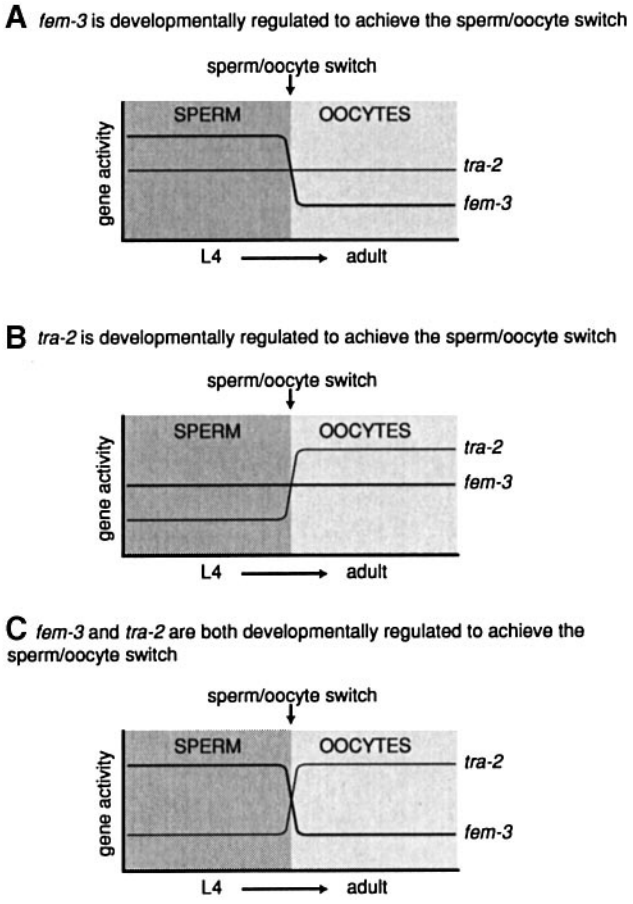

Fig. 6. Models for genetic regulation of the sperm/oocyte switch. All three models result in a high *fem-3*/*tra-2* ratio early in germ-line development to make sperm and a low *fem-3*/*tra-2* ratio later to make oocytes. (**A**) In the first model, the *fem-3* repressor (e.g. FBF and/or the *mog* genes) is developmentally regulated: its activity is first low, resulting in a high relative level of *fem-3* (thick line) and spermatogenesis, and then is increased to repress *fem-3* and switch to oogenesis. In this model, *tra-2* activity (thin line) remains constant. (**B**) In the second model, the *tra-2* repressor is developmentally regulated such that its initial activity is high, resulting in a low relative level of *tra-2* (thin line) so spermatogenesis can occur, and then is decreased to derepress *tra-2* and switch to oogenesis. In this model, *fem-3* activity (thick line) is kept to a constant low level. (**C**) In the third model, both *fem-3* and *tra-2* are developmentally regulated.

(Goodwin *et al*., 1993). We found that *lacZ::tra-2(+)* remained repressed in a *mog-1* mutant background (Table II, compare line 17 with line 18).

To test whether the PME itself mediates *mog* repression, we asked whether *lacZ::fem-3(q96 gf)* or *lacZ::fem-3(del8)* can become further derepressed in a *mog-1* mutant background. If, on the one hand, the *q96* and *del8* lesions do indeed abolish PME function, *lacZ::fem-3(q96 gf)* and *lacZ::fem-3(del8)* should not become derepressed further in a *mog-1* mutant background if *mog-1* functions through the *fem-3* PME. On the other hand, if *mog-1* functions through a *cis*-element independent of the PME, additional derepression of *lacZ::fem-3(q96 gf)* and *lacZ::fem-3(del8)* should occur. Heat shock was carried out at 30°C since *lacZ::fem-3(q96 gf)* and *lacZ::fem-3(del8)* already express a high level of β-gal when heat shocked at 33°C. The *hsp-16* promoter is less active at 30 than 33°C (Jones *et al*., 1989).

Neither *lacZ::fem-3(q96 gf)* nor *lacZ::fem-3(del8)* become derepressed further when placed in a *mog-1* mutant background (Table II, compare lines 13 and 14, and lines 15 and 16). Two per cent of *lacZ::fem-3(q96 gf)* animals express β-gal in ⩾20 intestinal nuclei, which is not substantially different from 0% of *lacZ::fem-3(q96 gf)*; *mog-1(lf)* animals. Furthermore, 14% of *lacZ::fem-3(del8)*; *dpy-19(lf)* animals express β-gal in 20 intestinal nuclei in comparison with 0% of *lacZ::fem-3(del8)*; *mog-1(lf) dpy-19(lf)* animals. If *lacZ::fem-3(del8)*; *mog-1(lf) dpy-19(lf)* had been derepressed, a percentage >14% would have been expected. Therefore, in both experiments, the *mog* mutant animals are not derepressed when compared with their wild-type counterparts.

We conclude that the *mog* genes act, either directly or indirectly, through the *fem-3* 3′ UTR. Moreover, *mog-1*, and perhaps the other *mog* genes, appear to function through the PME itself. These results suggest that the *mog* genes do not regulate all mRNAs but may regulate only those with a specific regulatory element.

Discussion

Three major conclusions can be drawn from the results reported here: (i) the *fem-3* 3′ UTR is sufficient to confer repression of a reporter gene in a PME-dependent manner; (ii) the *trans*-acting factors required for *fem-3* 3′ UTR repression are ubiquitous (present in both soma and germ line, males and hermaphrodites); and (iii) repression by the *fem-3* 3′ UTR requires the *mog* genes, *mog-1–mog-6*.

A ubiquitous mechanism of post-transcriptional control

The regulatory machinery that confers *fem-3* 3′ UTR regulation appears to be ubiquitous. Its presence in the germ-line tissue is supported by the germ-line phenotypes of *fem-3(gf)* and *mog* mutants (Barton *et al*., 1987; Graham and Kimble, 1993; Graham *et al*., 1993), and by the germ-line specificity of FBF, a PME-dependent *fem-3* RNA-binding protein required for the sperm/oocyte switch (Zhang *et al*., 1997). Its presence in somatic tissues is established by the fact that two distinct reporter transgenes show PME-dependent repression by the *fem-3* 3′ UTR in the soma. First, a *lacZ::fem-3(+)* reporter is repressed in intestinal cells and secondly, a *GFP::fem-3(+)* reporter transgene is repressed in the distal tip cell of the somatic gonad. Although other somatic tissues were not scored quantitatively, the *lacZ::fem-3(+)* reporter appears to be repressed in other somatic tissues as well. In addition, a PME-dependent RNA-binding activity is present in extracts prepared from mutants lacking a germ line. Therefore, a specific RNA-binding activity, which is possibly distinct from FBF, is present in somatic tissues.

In addition to its presence in both germ line and somatic tissues, the *fem-3* 3′ UTR regulatory machinery is found in both XX hermaphrodites and X0 males, as assayed using the *lacZ::fem-3(+)* reporter transgene. Consistent with this finding, an extract prepared from males contains *fem-3* 3′ UTR-binding activity (Ahringer, 1991). Furthermore, whereas *tra-1(gf)* X0 single mutants develop as females, the somatic tissues of *tra-1(gf); fem-3(gf)* X0 double mutants are partially masculinized (Schedl *et al*., 1989). Therefore, repressor activity appears to be ubiquitous.

The ubiquitous nature of the *fem-3* 3′ UTR-mediated repressor activity suggests that this control mechanism is not likely to play a major role in somatic sex determination: neither *mog* nor *fem-3(gf)* mutants exhibit obvious sexual transformations of XX somatic tissues (Barton *et al*., 1987; Graham and Kimble, 1993; Graham *et al*., 1993), and X0 wild-type animals develop as males despite the presence of *fem-3* 3′ UTR repressor activity. It is possible that somatic sex is regulated primarily by the X:A ratio controlling the major sex-determination pathway (reviewed in Meyer, 1997) and not by 3′ UTR-mediated repression of *fem-3*. The absence of somatic masculinization of *fem-3(gf)* or *mog* mutant XX animals may result from controls that restrict *fem-3* RNA to the hermaphrodite germ line. Indeed, *fem-3* RNA appears to be expressed primarily in germ-line tissues during post-embryonic development (Rosenquist and Kimble, 1988).

fem-3 3′ UTR regulation, the mog genes and the sperm/oocyte switch

How might a ubiquitous regulatory mechanism achieve a fine-tuned fate decision such as the sperm/oocyte switch? We envision three possible models (Figure 6), all of which bring in the sex-determining gene, *tra-2*, because the sperm/oocyte decision depends on a balance between *tra-2* and *fem-3* activities (Barton *et al*., 1987). The *tra-2* gene represses the *fem* genes (Hodgkin, 1986), and TRA-2 protein physically interacts with FEM-3 protein (A.Mehra, L.Heck, P.E.Kuwabara and A.M.Spence, personal communication). Of great importance to this paper, an increase in *tra-2* activity relative to that of *fem-3* promotes female development, whereas the converse, an increase in *fem-3* activity relative to *tra-2*, leads to male development.

In the first model, the regulatory machinery that represses *fem-3* (e.g. FBF/*mog* genes) is regulated developmentally. A temporary decrease in *fem-3* repressor activity would result in a temporary increase in *fem-3* activity and hence the transient production of sperm (Figure 6A). This model requires the tissue-specific regulation of a ubiquitous machinery. In the second model, *fem-3* is continually kept at a low level and, at that low level, is efficiently repressed by TRA-2. By this scenario, *tra-2* repression (Doniach, 1986; Schedl *et al*., 1989; Goodwin *et al*., 1993) is high early in development, releasing enough *fem-3* for spermatogenesis (Figure 6B). A third model invokes developmental regulation of both *fem-3* and *tra-2* repressors to achieve the switch (Figure 6C). To distinguish among these models, the developmental regulation of the *fem-3* and *tra-2* regulators must be understood.

The mog genes and the regulatory machinery controlling the fem-3 3′ UTR

Previous work showed that the *mog* genes are critical for the sperm/oocyte switch and that they possibly act genetically upstream of *fem-1*, *fem-2*, *fem-3*, *fog-1* and *fog-3*, five genes required for specification of the sperm fate (Graham and Kimble, 1993; Graham *et al*., 1993; Ellis and Kimble, 1995). In this paper, we show that the *mog* genes are essential for repression by the *fem-3* 3′ UTR. The *mog* genes are therefore excellent candidates for encoding components of the regulatory machinery responsible for *fem-3* 3′ UTR repression. The only other component of the *fem-3* 3′ UTR regulatory machinery identified to date is FBF. FBF is a PME-dependent *fem-3* RNA-binding activity that functions as a *fem-3* repressor in the germ line and is essential for the sperm/oocyte switch (Zhang *et al*., 1997). The identity of the PME-dependent *fem-3* RNA-binding activity in somatic tissues is not known. Either this somatic binding activity is distinct from the currently known FBF or previous experiments were not sufficiently sensitive to detect FBF in somatic tissues.

The functions of FBF and the *mog* genes are similar in several ways. On the one hand, both are required for *fem-3* 3′ UTR regulation during the sperm/oocyte switch and for robust germ-line proliferation (Graham and Kimble, 1993; Graham *et al*., 1993; Zhang *et al*., 1997; A.Puoti and J.Kimble, unpublished data; this paper). On the other hand, FBF does not appear to be involved with other *mog* functions: FBF protein and function seems to be limited to the germ line as assayed by immunocytochemistry and RNA-mediated interference (RNAi) (Zhang *et al*., 1997), whereas the *mog* genes function in the soma as well as the germ line (this paper). Since RNAi is particularly powerful for knocking out maternal gene functions, the lack of an embryonic lethal phenotype in *fbf(RNAi)* animals is striking given the strong maternal embryonic lethality of *mog* mutations (see below). We suggest that the *mog* genes function in both FBF-dependent and FBF-independent RNA regulatory events.

The molecular relationship between the *mog* genes and FBF for regulation of the *fem-3* 3′ UTR is not clear. The *mog-1* gene encodes a DEAH-helicase (A.Puoti and J.Kimble, unpublished data), which possibly functions in RNA regulation. It is not yet known whether *mog-1* functions directly or indirectly with FBF to achieve the post-transcriptional repression of *fem-3*. Indeed, we cannot rule out the possibility that the *mog* gene products and FBF may act independently to regulate *fem-3* RNA.

The mog genes and regulatory functions distinct from sex determination

The *mog* genes possibly regulate other RNAs and other processes in addition to *fem-3* and the sperm/oocyte switch. For example, all *mog* genes are required maternally for embryogenesis (Graham and Kimble, 1993; Graham *et al*., 1993). Furthermore, *mog-2*–*mog-6* mutants have a variety of partially penetrant somatic defects (Graham *et al*., 1993), and *mog-1* is required for robust germ-line proliferation and a wild-type growth rate (A.Puoti and J.Kimble, unpublished data). Therefore, the *mog* genes function broadly in both germ line and somatic tissues and in both embryonic and post-embryonic processes.

The identity of the other RNAs regulated by the *mog* genes is not known. Such RNAs may or may not contain a PME regulatory element. For example, one might speculate that the *mog* genes are designed to act with FBF and other RNA binding proteins that recognize a PME-like element. Alternatively, the *mog* genes may encode a complex that is brought to specific RNAs by a variety of different RNA-binding proteins with distinct specificities. A precedent for this latter form of regulation is found among sequence-specific transcriptional regulators, which provide promoter specificity for co-regulators that regulate transcription more generally (e.g. the SWI/SNF complex, Pazin and Kadonaga, 1997; or *groucho*, Parkhurst, 1998).

Materials and methods

Strains

In addition to wild-type animals, Bristol N2 (Brenner, 1974), the following mutant strains were used: *mog-1(q223)/dpy-19(e1259 ts) unc-69(e587) III* [JK885], *mog-1(q151)/dpy-19(e1259 ts) unc-69(e587) III* [JK1167], *mog-2(q75 ts) III; him-8(e1489) IV* [JK1960], *mog-3(q74 ts) III* [JK784], *mog-4(q233)/mnC1[dpy-10(e128) unc-52(e444)] II* [JK907], *mog-5(q449)/unc-85(e1414) dpy-10(e128) II* [JK1330], *mog-6(q465)/sqt-1(e1350) II; him-8(e1489) IV* [JK1500], *gld-1(q93)/dpy-5(e61) unc-13(e51) I* [JK648], *unc-17(e113) IV* [CB113], *him-5(e1490) V* [CB1490], *dpy-19(e1259 ts) III* [CB1259], *dpy-19(e1259 ts) mog-1(q223) III/eT1(III;V)* [JK1380], *tra-2(b202 ts) II* [DH202], *fog-1(q253 ts) I* [JK560]. Strains with extrachromosomal and integrated arrays are: *+/+; qEx212* [JK1939], *+/+; qEx213* [JK1940], *+/+; qIs43* [JK2421], *+/+; qEx131* [JK1935], *+/+; qEx208* [JK1948], *qIs15* [JK1950], *+/+; qEx389* [JK2546], *+/+; qEx387* [JK2547], *+/+; qEx388* [JK2548], *qIs44* [JK2567]. *qIs15*, *qIs43* and *qIs44* are integrated arrays of *qEx208*, *qEx213* and *qEx389*, respectively. Table I lists the constructs used to make these arrays.

Reporter constructs

lacZ::fem-3 constructs were made by inserting a 1028 bp *Eco*RV–*Hin*dIII fragment from pJK164 [*fem-3(+)*] or pJK172 [*fem-3(q96)*] into pPD50.14 digested with *Stu*I and *Spe*I. The 1028 bp *Eco*RV–*Hin*dIII fragment includes 262 nt of the *fem-3* 3′ UTR and 766 nt of 3′ flanking genomic sequence. pPD50.14 (provided by Andrew Fire) contains the *C.elegans* heat shock promoter 16.41-2 (Stringham *et al*., 1992), a synthetic 5′ UTR containing an intron, *lacZ*-coding region (targeted to the nucleus by the SV40 NLS) and the *unc-54* 3′ UTR. The *unc-54* 3′ UTR is replaced by the *fem-3* genomic sequence. The 3′ UTR of *lacZ::fem-3(del8)* transgene was made by ligation PCR (Ho *et al*., 1989) using the *lacZ::fem-3(+)* construct as template. The 3′ UTR of *lacZ::fem-3(del8)* was confirmed by sequencing. *lacZ::tra-2(+)* was constructed as described previously and contains the entire *tra-2* 3′ UTR (Goodwin *et al*., 1997). *GFP::fem-3(q22 gf)* and *GFP::fem-3(q96 gf)* were made as follows: the 1028 bp *Eco*RV–*Hin*dIII fragment (described above) from pJK164 [*fem-3(+)*]. pJK165 [*fem-3(q22)*] or pJK172 [*fem-3(q96)*] was cloned into pOCUS (Novagen). This fragment was then cut from pOCUS using *Eco*RI and *Spe*I and cloned into the *Eco*RI to *Spe*I sites downstream of GFP in pPD95.81 (provided by Andrew Fire), a *C.elegans* transformation vector that encodes GFP. In addition, a 3.0 kb *Bam*HI fragment containing the *lag-2* promoter (D.Gao and J.Kimble, unpublished data) was cloned into pPD95.81 digested with *Bam*HI.

Generation of transgenic animals

Transgenic animals were generated as described previously (Mello and Fire, 1995). Each injection mix contained either 1–2 ng/μl of the *lacZ* reporter with 50 ng/μl pRF4 or 5 ng/μl of the GFP reporter with 100 ng/μl pRF4. pRF4 contains the dominant roller marker, encoded by the *rol-6(su1006)* allele. Extrachromosomal arrays were integrated into the genome by 4000 Rads of gamma irradiation. The integrity of the *lacZ* transgenes in each line (both extrachromosomal and integrated arrays) was examined by Southern analysis: genomic DNA from each line was digested with *Bsm*I (except *qIs15* and *qIs42*), *Nsi*I and *Cla*I (Figure 2A). Only transgenic lines that contained the appropriate sized band following hybridization with a probe that recognizes *lacZ*-coding sequences were used for further analysis (Table I). The importance of Southern analyses of transgenic lines became evident when truncated transgenes with no [*lacZ::fem-3(+)*] or low [*lacZ::fem-3(q96 gf)* or *lacZ::fem-3(del8)*] levels of X-gal staining were found. In addition, Southern analysis of integrated lines was used to determine the copy number of each transgene per chromosome: genomic DNA prepared from each integrated line was digested with *Nsi*I and probed with an *Eco*RI–*Spe*I fragment from the *fem-3* 3′ UTR of *lacZ::fem-3(+)* (Figure 2A). This probe detects both transgene and endogenous *fem-3*. Only lines containing a comparable number of intact transgenes were used (Table I).

Scoring reporter expression

lacZ. Adults carrying *lacZ::fem-3* reporter transgenes were heat shocked at 30 or 33°C for 2 h and then allowed to recover at 25°C for another 2 h. X-gal staining was performed at 37°C overnight as described previously (Edgar, 1995). Following X-gal staining, animals were mounted onto slides and examined by DIC optics. If an intestinal nucleus was filled entirely with blue precipitate or partially with a dark blue precipitate, the staining was scored as 'strong'. The total number of intestinal nuclei with strong X-gal staining was counted for each animal and plotted on a graph. The level of transgene expression was also converted into a symbol: +/– for 0–20%, + for 20–40%, ++ for 40–60%, +++ for 60–80% and ++++ for 80–100% of animals with >20 intestinal nuclei with strong X-gal staining.

GFP. Worms carrying *GFP::fem-3* reporter transgenes were grown either at 15 or 25°C and also examined as adults. Because the GFP lines were not assayed for copy number or transgene integrity, we did not compare GFP expression between lines, but instead compared GFP expression at different temperatures within the same line. A combination of DIC optics to find the distal tip cell (DTC) and fluorescein filter sets to detect fluorescence was used to score GFP expression. The intensity of GFP fluorescence was evaluated as follows: +/– if GFP was either not seen or detected only faintly at 63× after locating the distal tip cell with DIC optics; + if GFP was difficult to see but could be detected without first locating the DTC by DIC optics; +++ if GFP was very bright and easily scored. Data were combined from four *GFP::fem-3(+)*, five *GFP::fem-3(q96 gf)* or six *GFP::fem-3(q22 gf)* transgenic lines carrying extrachromosomal arrays. Lines were scored during the first four to six generations.

Transgene expression in mutant backgrounds

To assess expression of *lacZ::fem-3(+)* in most mutant (m) backgrounds, *lacZ::fem-3(+);m/+* hermaphrodites were placed singly onto Petri plates and allowed to self-fertilize [at 20°C for *mog-1*, *-4*, *-5* and *-6*, *gld-1* and *unc-17* or 25°C for *tra-2(lf ts)*]. Their self-progeny include phenotypically wild-type hermaphrodites of genotype *lacZ::fem-3(+); +/+* or *lacZ::fem-3(+); m/+* or mutants of genotype, *lacZ::fem-3(+)3′ UTR; m/m* (see Figure 5A). 1% of *gld-1(q93)/+* animals are Mog (Francis *et al*., 1995); therefore a small percentage of these mutant animals was actually *lacZ::fem-3(+); gld-1(q93)/+*. Wild-type and mutant siblings were heat shocked and stained in parallel. Recovery after heat shock was at 20°C for *mog-1*, *-4*, *-5* and *-6*, *gld-1* and *unc-17* and at 25°C for *tra-2*. *lacZ* expression was determined by counting the number of intestinal nuclei with strong X-gal staining as described above. Each experiment was carried out at least twice.

The procedure to assess expression of *lacZ::fem-3(+)* in *mog-2*, *mog-3* and *glp-4* mutant backgrounds was carried out in the following way. Strains were made at 15°C and X-gal staining of *lacZ::fem-3(+); +/+* and *lacZ::fem-3(+) 3′ UTR; m/m* adults was compared following growth at 25°C from the second larval stage (L2). Assays were performed one generation later; therefore, β-gal levels of fertile and sterile 'cousins' were compared following heat shock and recovery.

To assess expression of *lacZ::fem-3(del8)* and *lacZ::fem-3(q96 gf)* in a *mog-1* mutant background, the same procedure was followed except that recovery of *lacZ::fem-3(q96 gf); mog-1(lf)*, *lacZ::fem-3(q96 gf); mog-1(lf)/+* and *lacZ::fem-3(q96 gf); +/+* was performed at 25°C. In addition, heat shock of *lacZ::fem-3(del8); dpy-19 mog-1* (Table II, line 15) and *lacZ::fem-3(del8); dpy-19* (Table II, line 16) were not performed in parallel. Instead each was performed in parallel with the same control: *lacZ::fem-3(del8); +/+*. We justified comparing these two lines since *lacZ::fem-3(del8); +/+* in each case produced similar amounts of X-gal staining.

DNA constructs for gel shift analysis

The vector pBSKS II+ (Stratagene) was used for all clones. chg8 and del8 mutations were generated by ligation PCR using the wild-type *fem-3* 3′ UTR as template (technique described in Ho *et al*., 1989). The inserts for wt(230), del8(222), sgf(230) and chg8(230) are a *Cla*I–*Eco*RV fragment containing the entire *fem-3* 3′ UTR. wt(81), wt(35), sgf(35) chg8(81) and del8(73) were constructed by PCR using the above constructs as templates. All constructs were sequenced.

Crude extracts for gel retardation assays

Adults [wild-type N2, *glp-1(q224 ts)*, *glp-4(bn2 ts)* and *mog-1(q223)* animals] raised at 25°C were washed twice in M9 buffer and frozen in liquid nitrogen. Unlike *glp-1(q224 ts)* and *glp-4(bn2 ts)*, *mog-1(q223)* is not temperature sensitive; therefore, extract was made from hand-picked homozygotes. An equal volume of extract buffer (10 mM HEPES pH 7.6, 10 mM KCl, 1.5 mM MgCl, 1.0 mM EDTA, 5% glycerol, 1.0 mM DTT, 10 mM benzamidine, 10 μg/ml PMSF, 10 μg/ml leupeptin) was mixed with the worm pellet and then dounced by hand until only empty carcasses remained (30–50×). The homogenate was spun for 15 min at 4°C to remove debris, and aliquots were frozen in liquid nitrogen.

Protein concentrations were generally 2–10 mg/ml as determined by Bradford assay (Pierce).

RNA probes

[^{32}P]UTP labelled RNA, synthesized *in vitro* using T3 or T7 RNA polymerase was purified from a denaturing polyacrylamide gel. A trace amount of [^{32}P]UTP was added to reactions synthesizing unlabelled RNA for quantification. After synthesis, unlabelled RNA was phenol/chloroform extracted, spun through Sephadex G-50 column to remove unincorporated nucleotides and counted by the Cerenkov method to measure [^{32}P]UTP incorporation. All RNAs were precipitated with glycogen and resuspended in DEPC-treated water.

Gel shift reactions and electrophoresis

The standard reaction contained 10 µl of 10 mM HEPES pH 7.6, 40 mM KCl, 1 mM DTT, 5 mM MgCl, 5% glycerol, 100 µg/ml yeast tRNA, 2000 U RNAsin (Promega) and 5 µg of extract and 5 fmol of labelled RNA (Pikielny and Rosbash, 1986). Where indicated, 500 fmol of unlabelled competitor was used. Generally, a mix of all components except RNA was made and then added to a mixture of probe and competitor RNAs. After incubation at room temperature for 10 min, 2 µl of either 10 mg/ml or 25 mg/ml heparin was added and the entire reaction loaded on a 4% acrylamide, 1.3% bis gel. The running buffer and the gel contained 0.5× TBE and 5 mM MgCl.

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