c-mos Proto-Oncogene RNA Transcripts in Mouse Tissues: Structural Features, Developmental Regulation, and Localization in Specific Cell Types

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c-mos RNA transcripts have been previously detected in mouse gonadal tissue and in late-term embryos. Here, we show that they are also present at low levels in placenta and in adult mouse brain, kidney, mammary gland, and epididymis. Marked differences are observed in the size of the *mos* RNA transcripts detected in different tissues. All transcripts appear to end at the same 3' position, and the tissue-specific size variations appear to be due to the use of different promoters. For example, the testicular and ovarian RNA transcripts initiate ~280 and ~70 base pairs, respectively, upstream from the first initiation codon, but both end at a common site downstream from the *mos* open reading frame. The expression of *mos* is developmentally regulated in gonadal tissue. Thus, the level of *mos* transcripts in testes is low for the first 3 weeks after birth, increases at least 10-fold around day 25, and reaches adult levels by day 30. In contrast, ovaries from preweaning mice contain a higher level of *mos* mRNA compared to ovaries from adult mice. In cell fractionation experiments we show that *mos* transcripts are present in haploid germ cells. We find that these transcripts are associated with monosomes and polysomes. The peculiar pattern of *mos* expression in mouse gonadal tissue suggests a role for the *c-mos* proto-oncogene in germ cell differentiation.

The *c-mos* proto-oncogene was one of the first cellular homologs of a retroviral oncogene to be molecularly cloned (18, 28). However, the analysis of *c-mos* RNA expression and the detection of protein products in normal tissues have been difficult. Early information about putative transcription regulation sequences at the *c-mos* locus was obtained from DNA transfer experiments. It was found that a cellular DNA sequence ~1 kilobase (kb) upstream from the *mos* open reading frame has transcription termination functions and can inhibit expression of the *c-mos* gene transforming activity in NIH/3T3 cells (4, 24, 44). The presence of this *cis*-acting inhibitory sequence suggested that transcription of the *c-mos* gene may be tightly regulated and restricted to a few specific cell types (24, 32). Likewise, it emerged from similar transfection experiments that a low level (1 to 10 copies per cell) of *mos* transcripts (43, 45) and a very low level of *mos* protein (29) are sufficient to transform NIH/3T3 fibroblasts. These results prompted us to reexamine the question of *c-mos* expression in normal mouse tissues. Using a sensitive S1 nuclease assay, we have been able to detect *mos* transcripts in 19-day embryos and gonads of adult mice (32). Subsequent Northern analysis revealed a 1.7-kb transcript in testes, a 1.4-kb transcript in ovaries, and ~1.3- and 2.3-kb transcripts in male and female embryos (32).

Here we report the detection of *mos* transcripts in additional tissues. We have mapped the *mos* RNA initiation sites in both testes and ovaries and examined the pattern of expression during postnatal development of the gonads. We also show that *mos* RNA transcripts in testes are expressed in haploid germ cells.

MATERIALS AND METHODS

RNA preparations. (i) **Organs and embryos.** In general, specific organs were obtained from several adult

BALB/cNCr or C57BL/6NCr mice (34 to 39 weeks old), and the pooled material was frozen on dry ice and kept at -70°C before homogenization. Embryos free of extraembryonic membranes separated from the placenta were collected at day 18 or 19 of gestation and were sexed by microscopic examination of the gonads. The frozen tissues were homogenized with a PT 10-35 Kinematica homogenizer (Brinkman no. 2711200-5) in 5 M guanidinium isothiocyanate–2 M mercaptoethanol–0.08 M Tris hydrochloride (pH 7.4), and total RNA was prepared as described previously (11, 22, 39).

Poly(A)⁺ RNA was prepared by passing the total RNA samples once over an oligo(dT) column as described previously (22), except that washing with low-salt buffer after loading was omitted (32). This protocol yielded a ~10-fold enrichment for poly(A)⁺ RNA. The RNA concentrations of total and poly(A)⁺ RNA samples were calculated from the absorbance at 260 nm. All samples were also analyzed by gel electrophoresis to ensure that the RNA was intact and that equal amounts of total RNA were loaded from samples to be compared quantitatively for the level of *mos* transcripts.

(ii) **Testis cell fractions.** We prepared cell fractions by the procedure of Velletri et al. (41) with some modifications. Briefly, the decapsulated testes of 20 adult BALB/cNCr males were treated with 0.1% collagenase (Cooper Biomedical) at 32°C for 15 min. Seminiferous tubules were isolated by allowing them to sediment at unit gravity. The supernatant was decanted and the seminiferous tubules were cut into 1- to 2-mm pieces and incubated at 32°C for 20 min with trypsin (GIBCO Laboratories) and DNase I (0.6 mg/ml). The resulting cell suspension was decanted and filtered through a Nitex cloth filter (60 mesh; Tetko, Inc.). The remaining undissociated tubules were incubated with trypsin and DNase once again. The filtered cell suspensions from both trypsin-DNase I treatments were pooled, the cells were collected by centrifugation, and the cell integrity was monitored by trypan blue exclusion.

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FIG. 1. Probes derived from the mouse *c-mos* locus. Relevant restriction sites are shown. Wavy lines represent pBR322 sequences (restriction sites and their sequence position are given below the line). Probes E and F are labeled, respectively, at the first and second *AvaI* site in the *c-mos* open reading frame (corresponding to positions 4061 and 4093, respectively, of the *v-mos* sequence [40]).

For elutriation the cells were suspended in 20 ml of phosphate-buffered saline containing 5% calf serum and introduced into the mixing chamber of the centrifugal elutriator (Beckman Instruments, Inc.). Elutriation was done at 4°C and at a rotor speed of 2,060 rpm. Four fractions were collected at flow rates of 5, 13, 25, and 50 ml/min. The cells in each fraction were pelleted and subjected to bovine serum albumin gradient centrifugation as described previously (33). The predominant band of each of the four gradients was collected, the cells were pelleted and then suspended in 5 ml of Dulbecco modified Eagle medium, and a sample was streaked out on a glass slide, Giemsa stained, and examined by light microscopy for the cell types present.

For RNA analysis the fractionated cells were washed twice in Dulbecco modified Eagle medium. The final cell pellet was dissolved in 7 M guanidine hydrochloride–0.02 M potassium acetate (pH 7.0)–5 mM EDTA and homogenized in a Dounce homogenizer, and total RNA was prepared as described previously (8, 37).

(iii) **Polysomes.** Polysomal and nonpolysomal RNA from fresh mouse testes was prepared by centrifugation of postmitochondrial supernatants through 10 to 60% linear sucrose gradients for 2.5 h at 25,000 rpm in a Beckman SW28 rotor as described previously (14), except that the postmitochondrial supernatant was incubated for 15 min on ice in the presence of 1% deoxycholate and either 6 mM MgCl₂ or 30 mM EDTA before fractionation.

RNA analysis. S1 nuclease protection assays were carried out as described previously (3, 9). Briefly, 25 µg of total RNA or 25 µg of poly(A)⁺ RNA in aqueous solution was lyophilized and dissolved in 10 µl of hybridization buffer (9) containing the end-labeled DNA probe. After denaturation for 10 to 15 min at 90°C the samples were incubated overnight at the following temperatures: probes A, B, C, and F, 60°C; probe E, 51°C. Samples were diluted to a final

volume of 310 µl with S1 digestion buffer (9) containing 10,000 U of single-strand-specific S1 nuclease (Boehringer Mannheim Biochemicals) per ml, incubated at 37°C for 60 min, and analyzed on denaturing 5% polyacrylamide gels.

For Northern analysis, RNA samples were fractionated on agarose formaldehyde gels and transferred to nitrocellulose as described previously (21, 34, 38). Hybridization was carried out for 2 days at 42°C in 50% formamide (22). The three final washes were in 0.015 M NaCl–0.0015 M sodium citrate–0.1% sodium dodecyl sulfate at 55°C for 20 min.

For primer extension experiments, a 55-base-pair (bp) *AluI*–*AvaI* restriction fragment 5' end labeled (22, 23) at the first *AvaI* site in the *c-mos* open reading frame (corresponding to position 4061 of the *v-mos* sequence [40]) was purified from a nondenaturing 5% polyacrylamide gel. This primer was hybridized in 10 µl of S1 hybridization buffer (9) to the RNA sample as described above for the S1 assay at 42°C for 3.5 days. The mixture was diluted with 300 µl of 0.3 M sodium acetate (pH 5), precipitated by adding 700 µl of ethanol, washed, and dried. Dry samples were carefully dissolved in 50 µl of a buffer containing 50 mM Tris hydrochloride (pH 8.3), 20 mM KCl, 10 mM MgCl₂, 5 mM dithiothreitol, 1 mM each of the 4 deoxynucleotide triphosphates, and 600 U of RNasin (Promega Biotec) per ml. After incubation with 30 U of reverse transcriptase (Life Sciences, Inc.) at 43°C for 60 min, the samples were extracted once with a 1:1 mixture of phenol and chloroform, precipitated with ethanol, and analyzed on a denaturing 5% polyacrylamide gel. Radioautography of dried polyacrylamide gels and nitrocellulose filters was carried out at –70°C with XAR-5 X-ray film (Eastman Kodak Co.) and an intensifying screen.

DNA probes. All *mos*-specific probes were derived from appropriate subclones of *c-mos* sequences in pBR322 (Fig. 1). 3' end labeling was carried out with the Klenow fragment

FIG. 2. *c-mos* transcripts in mouse tissues. Samples of 25 μ g were used throughout. (A) S1 analysis with probe A 3' end labeled at the *AhaII* site. Lanes: 1, yeast tRNA; 2, total ovary RNA; 3, male embryo poly(A)⁺ RNA; 4, female embryo poly(A)⁺ RNA; 5, epididymis poly(A)⁺ RNA; 6, total epididymis RNA; 7, total testis RNA. The X-ray film was exposed for 3 days. (B) S1 analysis with probe B 3' end labeled at the *AhaII* site. Lanes: 1, yeast tRNA; 2, kidney poly(A)⁺ RNA; 3, brain poly(A)⁺ RNA; 4, total testis RNA; 5, placenta poly(A)⁺ RNA; 6, total mammary gland RNA. The X-ray film was exposed for 14 days. (C) S1 analysis with probe C 5' end labeled at the *HindIII* site. Lanes: 1, epididymis poly(A)⁺ RNA; 2, total ovary RNA; 3, male embryo poly(A)⁺ RNA; 4, female embryo poly(A)⁺ RNA; 5, total testis RNA; 6, yeast tRNA. The X-ray film was exposed for 21 days. (D) Northern analysis with probe D labeled by nick translation. Lanes: 1 through 3, total RNA from, respectively, testes, ovaries, and epididymis; 4 through 6, poly(A)⁺ RNA from, respectively, epididymis and male and female embryos. The X-ray film was exposed for 19 days.

of *Escherichia coli* DNA polymerase I (Boehringer) in the presence of the appropriate α -³²P-labeled deoxynucleotides (3,200 Ci/mmol; New England Nuclear Corp.) required to fill in the respective restriction site as described previously (22), except that labeling was done in the presence of 240 mM NaCl. 5' end labeling was carried out with polynucleotide kinase (Boehringer) (22, 23) and [γ -³²P]ATP (7,000 Ci/mmol; New England Nuclear). The *AvaI-HindIII* fragment for probe D was gel purified and nick translated as described previously (36). An *abl* oncogene-specific probe, the 1.5-kb *BglII* fragment of pAB1 (12), was subcloned into the *BglII* sites of pHCT9 (Boehringer) (15). This subclone was used to obtain the gel-purified, 1.5-kb *BglII* fragment, which was labeled by nick translation (36). For the actin-specific probe a gel-purified, 1-kb *PstI* fragment from a rat β -actin cDNA clone was used (6).

RESULTS

Detection and S1 nuclease mapping of *mos* transcripts in various mouse tissues. We examined preparations of poly(A)-enriched RNA for the expression of *c-mos* transcripts in normal mouse tissues by using a sensitive S1 nuclease protection assay. In addition to embryos, testes, and ovaries (32), we found evidence for *mos* transcripts in brain, kidney, mammary gland, placenta, and epididymis (Fig. 2A and B). We also observed some slight protection of the probe with RNA from heart and lung tissue (data not shown), but not from liver, skin, intestine, and spleen. The number of *mos* transcripts was estimated to be 10 to 100 copies per cell in the gonads and 1 to 10 copies per cell in embryos by comparing the level of *mos* transcripts in these tissues to *mos* RNA in *v-mos*-transformed NIH/3T3 cells (43, 45; data

not shown). The structure of all RNA transcripts described here and previously (32) appears to be identical downstream from the *mos* open reading frame. S1 probes were consistently protected for 480 bp (Fig. 2A and B) with the endpoint of protection 130 bp downstream from the *mos* termination codon and ~20 bp downstream from a consensus polyadenylation signal (40). In contrast, the *mos* RNA transcripts examined thus far differ at their 5' end in a tissue-specific fashion (Fig. 2C). For example a ~1.6-kb fragment of probe C is protected by RNA from epididymis and embryos (Fig. 2C, lanes 1, 3, and 4). With embryonic RNA, smaller fragments ranging from 700 to 1,200 bp in length are also protected. These fragments probably derive from the class of ~1.3-kb transcripts detected in embryonic RNA by Northern analysis (Fig. 2D) (32). Note that every transcript protecting the probe for less than 1,050 bp will lack N-terminal sequences of the *mos* open reading frame. Ovarian RNA and testicular RNA protected fragments of ~1.1 and ~1.3 kb, respectively (Fig. 2C, lanes 2 and 5).

By Northern analysis, we were able to detect a 6-kb transcript in epididymis and embryonic RNA (Fig. 2D, lanes 4, 5, and 6), and an additional very faint band at 1.4 kb was observed in three independent preparations of epididymis RNA (Fig. 2D, lane 4). Whereas the S1 analyses of RNA from brain, kidney, placenta, and mammary gland were positive (Fig. 2B), Northern analyses did not reveal *mos* transcripts, perhaps due to either low levels of *mos* mRNA or size heterogeneity of the transcripts.

Mapping of transcriptional start sites on the *c-mos* gene. A primer extension analysis of testicular and ovarian *mos* transcripts was performed in parallel with an S1 nuclease protection assay. The primers to be extended by reverse transcriptase and the S1 probe were end labeled at the same *Ava*I site (Fig. 1) to allow a direct comparison of the results obtained by the two methods. Primer extension analysis of testicular RNA with this primer revealed equal amounts of four fragments 407, 396, 362, and 353 bp in length (Fig. 3B, lane 1, full arrows). These fragments are the same size as the fragments obtained by S1 nuclease mapping (Fig. 3A, lane 3). With primer extension of ovarian RNA we observe two minor and two major fragments (Fig. 3B, lane 2, asterisk and open arrows, respectively), which correspond in relative quantity and size to the fragments protected in the S1 assay (Fig. 3A, lane 2). Smaller bands observed in the primer extension experiments of testicular or ovarian RNA, for example, the 270-bp band in lane 1 (Fig. 3B), have no corresponding counterpart in the S1 assay and are probably due to premature reverse transcriptase stops. By comparing the size of protected fragments by using various probes, we can estimate the transcriptional start sites within ± 5 bp (data not shown). From our analyses it is evident that the major testicular and ovarian transcription initiation sites are clustered ~280 and ~70 bp upstream from the *mos* open reading frame, respectively (Fig. 3C). The sequences in the promoter regions are not G+C rich and do not contain obvious TATA boxes (Fig. 4). We could not identify regions of homology in sequences upstream from the individual start sites (Fig. 4).

Developmental regulation of *mos* expression. Total RNA was prepared from testes of mice at various times after birth. By Northern analysis the 1.7-kb *mos* RNA transcripts were detected beginning at day 25 (Fig. 5A, lanes 3, 4, and 5). The more sensitive S1 nuclease assay reveals that very low levels of *mos* expression are detectable in testes as early as 1 day after birth and through day 23. The levels for days 17 and 20 are shown (Fig. 5B, lanes 2 and 3). By day 30 the level of

mos RNA is equivalent to the level found in adult mouse testis (Fig. 5B). As with *mos* transcripts, the 4.7-kb testis-specific *c-abl* transcript (26) shows a similar pattern of developmental regulation in the testis (Fig. 5A, lanes 4 and 5), whereas the more common 5.3- and 6.5-kb *c-abl* transcripts do not (Fig. 5A, lanes 1 through 5). The differential appearance of the *c-abl* transcripts compared to the *c-mos* transcripts was not observed in a subsequent experiment.

In contrast to the pattern of *mos* expression in testes the levels in ovaries are highest in 5- to 20-day-old animals (Fig. 6), and a three- to fivefold decrease occurs during the next 10 days. Thereafter, the level of *mos* transcripts stays constant for at least 7 weeks but is further decreased in older animals (34 to 39 weeks) (Fig. 6). We have also observed lower levels of *mos* RNA at days 1 and 10 (Fig. 6, lanes 1 and 3) in several experiments. It is possible that this is a feature of *mos* expression in postnatal ovarian development. Alternatively,

FIG. 3. Combined primer extension and S1 analysis of gonadal *c-mos* transcripts. (A) S1 analysis with probe E 5' end labeled at the *Ava*I site. Lanes: 1, 25 μ g of total NIH/3T3 cell RNA; 2 and 3, 25 μ g of total RNA from, respectively, ovaries and testes. (B) Primer extension analysis. The primer to be extended was a 55-bp *Alu*I-*Ava*I restriction fragment 5' end labeled at the same *Ava*I site as probe E. Lanes: 1 and 2, 25 μ g of poly(A)⁺ RNA from, respectively, testes and ovaries; 3, 25 μ g of total NIH/3T3 cell RNA. The X-ray film was exposed for 21 days. The results shown in A and B are from separate gels. In similar experiments we observed that the bands marked by arrows or asterisks comigrated when analyzed in parallel on the same gel. (C) Schematic representation of the two clusters of start sites. Testicular and major and minor ovarian start sites are indicated by, respectively, full arrows, open arrows, and asterisks. The number of the first nucleotide of each transcript (determined with an accuracy of ± 5 bp) is given counting back from the first ATG codon in the open reading frame.

FIG. 4. Sequence of the promoter regions of gonadal *c-mos* transcripts. Testicular and major and minor ovarian start sites are indicated by, respectively, full arrows, open arrows, and asterisks. The sequence was taken from Wood et al. (44).

it may be due to variation in the amount of contaminating uterine tissue isolated with the ovary from these very young mice, hence lowering the concentration of ovarian RNA.

Expression of *c-mos* in specific testicular cell types. We used the method of centrifugal elutriation (41) followed by bovine serum albumin gradient centrifugation (33) to separate postmeiotic germ cells from other cell types present in the adult testes. We obtained four fractions. Fraction 1 contained residual bodies and elongated spermatids, fractions 2 and 3 contained elongated and round spermatids, and fraction 4 contained round spermatids and multinucleated round spermatids. With the *mos*-specific probe we detected little difference in the level of the 1.7-kb transcript in total RNA of

the four fractions (Fig. 7). In agreement with previously published results (31), we detected the testis-specific *c-abl* transcript (26) in postmeiotic fractions enriched for elongated and round spermatids (Fig. 7, lanes 3 and 4). The level of this *c-abl* transcript was dramatically decreased in fractions 1 and 4 (Fig. 7, lanes 2 and 5). We also detected the larger, more common 6.5- and 5.3-kb *c-abl* transcripts (2, 26) and consistently observed higher levels of the 6.5-kb transcript in fraction 4 (Fig. 7, lane 5). The testis-specific actin transcript (42) was detected in fractions 1 through 3 (Fig. 7, lanes 2 through 4). This is consistent with the presence of this transcript in round spermatids and residual bodies as reported by Waters et al. (42). The level of this transcript like the testis-specific *c-abl* transcript was reduced in the cells in fraction 4 (Fig. 7, lane 5). From these analyses we conclude that testicular *mos* transcripts are present in haploid germ cells, but we cannot rule out *mos* expression in premeiotic germ cells or testicular somatic cells.

Distribution of testicular *mos* RNA transcripts in polysomal and nonpolysomal RNA fractions. The presence of *mos* transcripts in the testicular germ cell fraction led us to ask whether the RNA was present on polysomes. Postmitochondrial supernatants of fresh testicular homogenates were fractionated on 10 to 60% linear sucrose gradients in the absence or presence of EDTA (Fig. 8). The fractions were pooled as indicated in Fig. 8, and the level of *mos* RNA in each pool was determined by Northern analysis. *mos* RNA transcripts are present on monosomes and polysomes and are dissociated from the latter in the presence of EDTA (Fig. 8). Only a small percentage of *mos* transcripts is detected in the 4S to 18S fraction (data not shown).

DISCUSSION

c-mos RNA transcripts are expressed in a novel fashion in normal mouse tissues. Thus, we find tissue-specific variations in the size, number, and level of *mos* transcripts expressed. Low levels of RNA were found to be expressed in brain, kidney, placenta, and mammary gland and, to a lesser extent, in heart and lung, but these transcripts are only detectable by S1 analysis and are either too low in amount or too heterogeneous in size to be detected by Northern analysis. Previous studies have shown that extremely low levels of *v-mos* RNA (43, 45) and *v-mos* protein (29) expression are sufficient to transform NIH/3T3 cells. In light of these results it is not surprising that the level of *mos* expression is very low in tissues such as brain, kidney, placenta, and mammary gland. These results indicate that the *c-mos* gene might have a function in somatic cells of adult animals.

Higher levels of *mos* RNA are detected in term embryos, epididymes, testes, and ovaries (32). We have found that *mos* transcripts were identical for a major portion of the open reading frame and appear by S1 analysis to terminate at a common site 130 bp 3' of the *mos* termination codon. We cannot exclude the possibility that this is a processing site,

FIG. 5. Analysis of changes in the level of *c-mos* and *c-abl* transcripts during the postnatal development of mouse testes. (A) Northern analysis. Each lane contains 25 μ g of total RNA prepared from testes collected at the indicated times after birth. The blot was first hybridized to the *mos*-specific probe D and exposed to X-ray film for 21 days and then hybridized to the *abl*-specific probe and exposed for 5 days. Both probes were labeled by nick translation. (B) S1 analysis with probe F 3' end labeled at the *Ava*I site. Lanes: 1, 25 μ g of yeast tRNA; 2 through 6, 25 μ g of total RNA of the RNA preparations as used in panel A, lanes 1 through 5, respectively; 7, 25 μ g of total RNA from testes of adult mice (34 to 39 weeks old).

FIG. 6. Northern analysis of changes in the level of *c-mos* transcripts during the postnatal development of mouse ovaries. Each lane contains 25 μ g of total RNA prepared from ovaries collected at the indicated times after birth. Probe D labeled by nick translation was used for hybridization. The X-ray film was exposed for 4 days.

but circumstantial evidence strongly suggests that this site represents the 3' end of all *mos* transcripts. First, this site is 20 bp downstream from a consensus polyadenylation signal (40). Second, this polyadenylation signal is conserved in its position between chicken (Schmidt and Vande Woude, manuscript in preparation), mouse (40), and human *mos* genes (Propst and Vande Woude, manuscript in preparation). Finally, size constraints of the ovarian and testicular transcripts for which internal structure and the transcription initiation sites have been determined (Fig. 3) are consistent with this being the polyadenylation site. These results indicate that the *mos* proto-oncogene expressed in gonadal tissue contains a single coding exon and, in contrast to all other proto-oncogenes, does not appear to be processed posttranscriptionally.

By S1 mapping we have been able to demonstrate that the major tissue-specific size variation in *mos* RNA transcripts occurs in the 5' portion of the RNA (Fig. 2C). For example, we have shown that RNA from epididymis (and term embryo) protected probe C to a site at least 0.5 kb upstream from the *mos* open reading frame (Fig. 2C), and by Northern analysis we can detect a 6-kb *mos* RNA transcript in this tissue (Fig. 2D). Thus, apparently no additional protein-coding sequences are spliced into the *mos* open reading frame in this 6-kb transcript, and it therefore contains the same *mos*-related open reading frame as the shorter testicular and ovarian transcripts. (We cannot exclude the possibility that other open reading frames may be present in the upstream portion of this 6-kb message.)

Our results indicate that certain *mos* RNA transcripts can lack the N-terminal portion of the open reading frame. Size considerations and the 5' S1 mapping data suggest that the protected fragments in the size range of <1,050 bp observed with embryonic RNA (Fig. 2C) are probably derived from the ~1.3-kb transcripts detected by Northern analyses (Fig. 2D) (32). We have observed similar short transcripts in human and rat tissues (Propst and Vande Woude, in preparation). These short transcripts would lack the N-terminal portion of the open reading frame and could only code for truncated proteins starting at an internal AUG (position 66 or 142) (40). These proteins would seem to be quite unusual. However, Bold and Donoghue (5) have shown that deletion of the AUG in *v-mos* corresponding to position 1 in *c-mos* does not abolish transforming activity, but the additional

FIG. 7. Northern analysis of partially purified testicular cell fractions for the level of *c-mos* transcripts. Each lane contains 25 μ g of total RNA. Lanes: 1, unfractionated testes; 2, fraction 1 containing 80% residual bodies and 20% elongated spermatids; 3, fraction 2 containing 20% residual bodies and 45% elongated and 35% round spermatids; 4, fraction 3 containing 50% elongated and 50% round spermatids; 5, fraction 4 containing 85% multinucleated round spermatids and 15% round spermatids. The faster migration of *mos*, *abl*, and *actin* transcripts as well as the ribosomal RNAs (data not shown) in lane 3 is due to an artifact and was not observed on other gels with the same samples. The blot was first hybridized to the *mos*-specific probe D and exposed to X-ray film for 14 days, hybridized to the *abl*-specific probe and exposed for 2 days, and then hybridized to the *actin*-specific probe and exposed over night. All probes were labeled by nick translation.

deletion of the following AUG corresponding to position 66 in *c-mos* does.

The size differences in *mos* RNA transcripts present in embryos, epididymes, testes, and ovaries are most likely due to tissue-specific utilization of different promoters. Primer extension analyses of testicular and ovarian RNA reveal for both tissues multiple transcription start sites (Fig. 3). The testicular start sites are clustered around -280 bp and are clearly distinct from the ovarian start sites, which are clustered around -70 bp. Thus, there are at least two promoter regions in front of the *mos* open reading frame. The fact that neither region contains TATA boxes (Fig. 4) could explain the multiple start sites (7). Likewise, these regions do not contain G+C-rich tracts as has been found for promoters of other genes (16, 17, 35). Testicular and ovarian RNA transcripts differ only in their 5' untranslated sequence and contain the same putative *mos* open reading frame. This raises the questions of how and why different promoters are used in a tissue-specific manner. Testicular *mos* transcripts are expressed in germ cells (Fig. 7). Perhaps then the ovarian *mos* transcripts are also expressed in germ cells, and the differences in the genesis of the male and female gametes have led to the selection of special features of the *mos* transcripts. For instance the male gametes are produced de novo in the mature testicle and undergo differentiation from immature diploid spermatogonia to mature haploid spermatozoa in ~5 weeks (27). The diploid daughter cells of the self-renewing spermatogonia stem cell population differentiate in several morphologically distinct steps into pachytene spermatocytes. These cells contain four complements of the genome and give rise to four haploid round spermatids by

FIG. 8. Analysis of testicular polysomal and nonpolysomal RNA fractions for the presence of *c-mos* transcripts. The absorbance at 260 nm was determined in 1-ml fractions of sucrose gradients run in the presence of $MgCl_2$ (●) or EDTA (○). Fractions were pooled as indicated by the solid bars and analyzed for *mos* transcripts (insert). Probe D labeled by nick translation was used for hybridization. The X-ray film was exposed for 3 days.

meiotic division. The round spermatids undergo further differentiation to elongated spermatids and finally mature spermatozoa (27). In contrast the female is born with a finite, ever-decreasing number of oocytes, most of which are arrested at a specific state of differentiation, the premeiotic diplotene stage. The regulation of *mos* expression could involve male- and female-specific, *trans*-acting factors to permit the utilization of testis- and ovary-specific promoters. Alternatively, the lower temperature in the testes might influence the choice of the promoter and impose specific constraints on testicular *mos* expression. It is conceivable that the differences in female and male gametogenesis require differential stability or translational efficiency of the *mos* transcripts. This could be accomplished by varying the 5' untranslated sequences. For instance the major ovarian *mos* transcripts contain one or two AUGs in the 5' untranslated region, whereas the testicular transcripts have the two ovarian AUGs plus an additional two further upstream. In each case the reading frame opened by one of these initiation codons is terminated before the *mos* AUG. A similar con-

figuration was described for the *Saccharomyces cerevisiae* *GNC4* gene, and a strong influence of the upstream AUGs on the translational efficiency was demonstrated (25). The *mos* transcripts in testes are associated with monosomes and polysomes (Fig. 8). The presence of *mos* transcripts in the monosome fraction could result from a low translational efficiency caused by the presence of the four upstream AUGs. Thus, the rate of initiation of protein synthesis from *mos* transcripts could be diminished by the upstream AUGs. We also note the poor homology of the first *mos* initiation codon to the consensus sequence of translational start sites (20). As a result initiation might be slow compared with elongation, which could explain the abundance of *mos* transcripts in the monosome RNA fraction. Since attempts to detect the *mos* protein in testes have so far been unsuccessful, we cannot exclude that the transcripts are not translated at all. However, this seems unlikely since *c-mos* is transcribed in testes from mice, rats (Propst and Vande Woude, unpublished results), and humans (Propst and Vande Woude, in preparation) and in mouse ovaries. In addition, *mos* expression in the mouse gonads follows a concise pattern of developmental regulation.

The data presented here support the hypothesis that the *c-mos* proto-oncogene has a general function in male and female gametogenesis. Thus, the results of our cell fractionation experiments indicate that *c-mos* is expressed in haploid round spermatids in the testes. This interpretation is supported by the pattern of *mos* expression during the postnatal development of the testes, since the increase in the level of *mos* transcripts coincides with the appearance of large numbers of haploid round spermatids (1). Furthermore, we do not detect *mos* transcripts in testes of sterile mouse mutants in which differentiation of the germ cells does not proceed beyond premeiotic stages (Propst et al., manuscript in preparation). Haploid gene expression has previously been reported for other genes specifically expressed in the testes, including phosphoglycerate kinase-2 and the proto-oncogene *c-abl* (10, 13, 19, 31, 42). Our expression results confirm those previously reported for *c-abl* (31). Interestingly, in our cell fractionation experiments we obtained a fraction of round and multinucleated round spermatids (fraction 4) that expressed the 1.7-kb *mos* transcripts but not the testis-specific *c-abl* and actin transcripts (Fig. 7). The spermatids elutriated in this fraction differ from those in fractions 2 and 3 in sedimentation behavior and gene expression. Thus, they are biochemically distinct and may represent a novel round spermatid cell type.

c-mos transcripts are also found in ovaries, and again the level of expression changes during the postnatal development. Consistent with the assumption that *mos* is expressed in oocytes, the postnatal decrease in the level of the 1.4-kb *mos* transcripts could be explained by an increase in the number of somatic cells and a decrease in the number of oocytes in the developing and aging ovary (30). On the other hand, the number of oocytes decreases approximately two-fold during the first 3 weeks of life (30), a change not reflected by the level of *mos* transcription during this period of time. Thus, additional factors might contribute to the observed pattern of *mos* expression.

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