*Proc. Natl. Acad. Sci. USA*
Vol. 84, pp. 4509–4513, July 1987
Developmental Biology

# Expression of c-*mos* RNA in germ cells of male and female mice

(oncogene/oogenesis/spermatogenesis)

DEBRA S. GOLDMAN*†, ANN A. KIESSLING‡, CLARKE F. MILLETTE§, AND GEOFFREY M. COOPER*¶‖

*Dana–Farber Cancer Institute and Departments of †Microbiology and Molecular Genetics, ‡Obstetrics and Gynecology, §Anatomy and Cellular Biology, and ¶Pathology, Harvard Medical School, Boston, MA 02115

*Communicated by Elizabeth D. Hay, March 23, 1987*

**ABSTRACT** **We have investigated the cell types in mouse testis and ovary in which the c-*mos* protooncogene is normally transcribed. Blot hybridization analysis of electrophoretically fractionated RNAs from testes of mice with defects in germ-cell development and from prepubertal and adult mice indicated that c-*mos* was transcribed during male germ-cell development. Analysis of purified populations of spermatogenic cell types detected c-*mos* RNA in the earliest haploid postmeiotic germ cell, the round spermatid, indicating that c-*mos* was expressed transiently during spermatogenesis. c-*mos* RNA was detected by blot hybridization in the ovaries of prepubertal mice and decreased in relative concentration following gonadotropin-stimulated proliferation of granulosa cells. These results suggested that c-*mos* was transcribed in oocytes and were confirmed by detection of high levels of c-*mos* RNA in isolated grown oocytes. Thus, c-*mos* is expressed in both male and female germ cells, suggesting possible roles for this protooncogene in meiosis, germ-cell development, fertilization, and early embryogenesis.**

Cellular oncogenes have been identified by three approaches: (*i*) as homologs of retroviral oncogenes, (*ii*) as genes that induce transformation upon transfection of cultured cells, and (*iii*) as genes that are frequently altered in neoplasms by DNA rearrangement or amplification (see refs. 1–3 for reviews). Together, these approaches have identified 40–50 cellular genes that, as activated oncogenes, can induce at least some aspects of neoplastic transformation. The formation of activated oncogenes from their normal cellular homologs (termed protooncogenes) can occur as a consequence of changes in the regulation of gene expression, point mutations resulting in single amino acid substitutions, or DNA rearrangements resulting in the synthesis of recombinant fusion proteins from which portions of the normal amino acid sequence have been deleted.

A physiologic role for normal cellular progenitors of four oncogenes, *sis, erbB, fms* and *erbA*, is indicated by their identification as the genes encoding platelet-derived growth factor, epidermal growth factor receptor, macrophage colony-stimulating factor receptor, and thyroid hormone receptor, respectively (4–9). However, normal functions of other protooncogenes remain obscure. While some protooncogenes are expressed in many types of proliferating cells, others display more restricted patterns of expression, suggesting that they may function in specific developmental pathways.

An example of developmental regulation of a protooncogene is provided by the expression of high levels of a unique c-*abl* transcript during postmeiotic differentiation of male germ cells (10). A possible role for another protooncogene, c-*mos*, in reproductive processes is suggested by its specific transcription in testes and ovaries of adult mice (11). The testis and ovary contain specialized, hormonally responsive somatic cells, Sertoli and Leydig cells in the male and granulosa cells in the female, as well as germ cells (12, 13). In the male, the germ cells are comprised of a constantly renewing stem-cell population and the full array of spermatogenic cells undergoing meiosis and differentiation culminating in the mature spermatozoa (12). In contrast, ovaries contain no stem-cell population but contain oocytes that are uniformly arrested in the diplotene stage of meiotic prophase (13–15). A subpopulation of oocytes undergo growth and accumulate macromolecular stores in preparation for ovulation and fertilization (13–15). To begin to define the possible normal function of c-*mos*, we have investigated the cell types in testis and ovary in which this protooncogene is expressed. We report that c-*mos* is transcribed in both male and female germ cells and that accumulation of c-*mos* RNA is confined to a discrete phase of postmeiotic male germ-cell development.

## MATERIALS AND METHODS

**Mouse Strains and Cell Preparations.** WBB6F1 $W/W^v$, WCB6F1 $Sl/Sl^d$, C57BL/6 *Tfm*, B6A$^w$ *Sxr*, and matched control mice were from The Jackson Laboratory. BALB/c, CD-1, Swiss Webster, and BDF1 (BCD2$F_1$/J) mice were purchased from The Jackson Laboratory, Taconic Farms (Germantown, NY), and Charles River Breeding Laboratories (Kingston, NY).

Testicular cell populations were obtained as described (16, 17). In brief, testes were removed, decapsulated, and immediately placed into cold Krebs–Ringer enriched bicarbonate buffer (16). Suspensions of single germ cells were prepared by sequential enzymatic digestions of the testis parenchyma with collagenase (0.5 mg/ml) to yield seminiferous tubules, followed by trypsin (0.5 mg/ml) (16, 17). Isolated cell types were then obtained by unit-gravity sedimentation in 2–4% (wt/vol) bovine serum albumin gradients in the Krebs–Ringer buffer at 4°C. Testes of adult CD-1 mice (70–120 days old) were used to obtain purified populations of late pachytene spermatocytes (average purity 91%; contaminants predominantly Sertoli and Leydig cells), round spermatids (average purity 95%; contaminants predominantly residual bodies), and residual bodies (average purity 91%; contaminants predominantly condensing spermatids). Populations of late pachytene spermatocytes with no spermatid contamination were obtained by restrictive pooling of cells after unit-gravity sedimentation. Testes of prepubertal CD-1 male mice (16–17 days old) were used to obtain early pachytene spermatocytes (87% pure; contaminants predominantly leptotene/zygotene spermatocytes) and leptotene/zygotene spermatocytes (97% pure; contaminants predominantly preleptotene spermatocytes) (18). Cell identifications were conducted using phase-contrast and differential interference microscopy according

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

‖To whom reprint requests should be addressed at: Dana–Farber Cancer Institute, 44 Binney Street, Boston, MA 02115.

to well-established criteria (16, 18). All cell populations immediately after isolation were >95% viable as determined by the exclusion of trypan blue.

Ovaries were collected from prepubertal female mice (20–27 days old) of strain CD-1 (Charles River) or BDF1 (The Jackson Laboratory). Pregnant-mare serum gonadotropin (5 units; Sigma) was administered i.p. to stimulate follicle development, and ovaries or oocytes were recovered 36 hr later. Germinal-vesicle-stage oocytes were recovered by teasing apart stimulated ovaries in Dulbecco's phosphate-buffered saline containing bovine serum albumin (4 mg/ml) plus $N^6$,$O^2$-dibutyryl cyclic AMP (100 μg/ml; Sigma) and mechanically pipetting the zona-enclosed oocytes to free them of granulosa cells.

**RNA Extraction and Blot Hybridization Analysis.** RNA was extracted in guanidinium thiocyanate and precipitated in LiCl as described (19). Total cellular RNA was electrophoresed in a 1% agarose/2.2 M formaldehyde gel (20) with ethidium bromide to allow visualization of 28S and 18S ribosomal RNAs. The gel was rinsed in water, and the RNAs were transferred to nitrocellulose (Schleicher & Schuell) in 20× standard saline citrate (SSC; 1× SSC is 0.15 M NaCl/0.015 M sodium citrate, pH 7.0) and baked for 2 hr at 80°C in a vacuum oven. Prehybridization was performed overnight at 42°C in 50% (vol/vol) formamide/5× SSC/5× Denhardt's solution (1× is 0.02% Ficoll/0.02% polyvinylpyrrolidone/0.02% bovine serum albumin)/50 mM sodium phosphate, pH 6.8/0.2% $NaDodSO_4$ containing sonicated, single-stranded salmon sperm DNA at 100 μg/ml. Hybridization was performed overnight at 42°C in 50% formamide/5× SSC/1.25× Denhardt's solution/50 mM sodium phosphate, pH 6.8/0.2% $NaDodSO_4$/10% dextran sulfate containing sonicated, single-stranded salmon sperm DNA (100 μg/ml) and denatured, $^{32}$P-labeled DNA probe (1–3 × $10^6$ cpm/ml). Filters were washed twice in 2× SSC/0.5% $NaDodSO_4$ for 1–2 hr at 65°C and autoradiographed at −70°C with an intensifier screen. Exposure times were 4–14 days for the c-*mos* probe, 3–4 days for the v-*abl* probe, and 1–2 days for the actin probe.

**Nick-Translated Probes.** c-*mos* probe was prepared from an electrophoretically purified fragment derived from pMS1 (21) and subcloned into a bacterial expression vector (unpublished) that contained all of the c-*mos* predicted coding sequence (nucleotides 94–1154) (22). v-*abl* probe was prepared from the electrophoretically purified *Eco*RI insert of pK2 (23). β-Actin probe was prepared from a mouse β-actin plasmid (24). DNAs were labeled by nick-translation (20) with [α-$^{32}$P]dGTP and [α-$^{32}$P]dCTP (>600 Ci/mmol, New England Nuclear; 1 Ci = 37 GBq). Unincorporated nucleotides were removed by use of a Sephadex G-50 column (20).

## RESULTS

**Expression of c-*mos* in Testes of Mutant Mice.** Our first approach to identify the cell type expressing c-*mos* in the testis took advantage of the availability of mutant mice with defects in germ-cell development. Expression of c-*mos* was analyzed in testes of four strains of mice, each of which lacked mature germ cells but retained their complement of somatic cells, including Leydig and Sertoli cells. The testes of male $W/W^v$ and $Sl/Sl^d$ mice are devoid of germ cells as a result of failure of the primordial germ cells to migrate to and/or proliferate in the gonadal ridge during embryogenesis (25). X/X sex-reversed (*Sxr*) mice are phenotypic males in which germ cells do not survive in the testes past 10 days after birth (25). Male mice carrying the X chromosome-linked testicular-feminization (*Tfm*) gene develop small testes but no male accessory organs as a consequence of a lack of androgen receptors (25). Their testes contain spermatogonia in the mitotic stages of stem-cell proliferation, but spermatogenesis does not progress past meiotic prophase (25).

Total cellular RNA was extracted from testes of mutant and control adult mice (>35 days old) and analyzed by blot hybridization with c-*mos* probe. c-*mos* RNA was not detected in the testicular RNA of $Sl/Sl^d$, $W/W^v$, *Sxr*, and *Tfm* mice (Fig. 1, lanes 2, 4, 6, and 8), whereas a 1.7-kilobase (kb) c-*mos* transcript was detected in RNA from testes of controls, including wild-type littermates of $Sl/Sl^d$ mice, $W/+$ heterozygotes, BALB/c mice, and wild-type (*Ta*/Y) littermates of *Tfm* mice (lanes 1, 3, 5, and 7). We confirmed the correlation of c-*mos* transcription with germ-cell development by hybridization, on the same filters, with a v-*abl* probe, which detected a testis-specific transcript of 4.7 kb in postmeiotic germ cells, in addition to the 6.2- and 8.0-kb transcripts that are present in a variety of somatic cell types (10). The 4.7-kb c-*abl* transcript was detected in the testicular RNA of normal control mice but not of $Sl/Sl^d$, $W/W^v$, *Sxr*, or *Tfm* mice (Fig. 1). As a further control, the filters were incubated with a β-actin probe. The 2.1-kb actin RNAs, present in both somatic and germ cells, were detected in the RNA of testes of both mutant and control mice, whereas an additional 1.5-kb transcript specific for postmeiotic germ cells (26) was detected only in testicular RNA of the controls (Fig. 1). Expression of c-*mos* in the testis is thus correlated with normal germ-cell differentiation. Transcription of c-*mos* may therefore either be germ-cell-specific or occur in testicular somatic cells only in the presence of normal germ-cell development.

**Temporal Expression of c-*mos*.** We next examined c-*mos* expression in testes of 6-, 20-, and 35-day-old mice. At these ages there are both qualitative and quantitative differences in the somatic and germ-cell types present in the testis (17). At 6 days of age, more than 80% of the cells within the seminiferous epithelium are the somatic Sertoli cells, and the

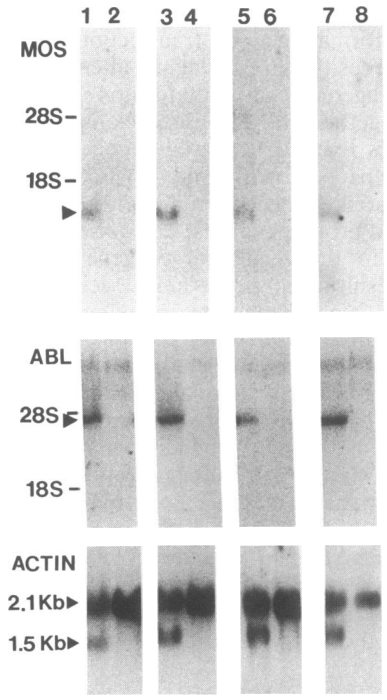


FIG. 1. Blot hybridization analysis of RNA isolated from testes of mutant mice. Total testicular RNA was electrophoresed in formaldehyde/agarose gels and hybridized with c-*mos*, v-*abl*, and actin probes (allowing for decay of the previous signal between hybridizations). Lanes: 1, wild-type littermate of $Sl/Sl^d$; 2, $Sl/Sl^d$; 3, $W/+$; 4, $W/W^v$; 5, BALB/c; 6, *Sxr*; 7, *Ta*/Y; 8, *Tfm*. Each lane contained 16 μg of RNA, except for lanes 5 and 6, which contained 20 μg. Positions of 28S and 18S ribosomal RNAs are indicated. The 1.7-kb c-*mos*, 4.7-kb c-*abl*, and actin transcripts are indicated by arrowheads.

germ cells are represented solely by the mitotically proliferating spermatogonia. By 20 days of age, the germ cells are more prominently represented, comprising about 70% of the cells within the seminiferous epithelium. At this age, the germ-cell population consists of spermatogonia and spermatocytes in a continuum of developmental stages of meiotic prophase: ≈50% of the spermatocytes are in the pachytene stage of late meiotic prophase. By 35 days of age, postmeiotic round and condensing spermatids comprise about 70% of the cells within the seminiferous epithelium and represent the major germ-cell types within the testis.

The c-*mos* transcript was detected in total cellular RNA of 35-day-old but not 20- or 6-day-old mouse testes (Fig. 2). Similarly, the 4.7-kb c-*abl* transcript and the 1.5-kb actin transcript were expressed in 35-day-old but not in 6- or 20-day-old testes (Fig. 2). Thus, c-*mos* expression is temporally correlated with the development of postmeiotic germ cells.

**Separation of Germ Cells.** We sought to identify the specific spermatogenic cell types expressing c-*mos* by purifying RNA from populations of germ cells enriched for specific stages of development. We began by purifying spermatocytes from 16- to 17-day-old mice to determine if c-*mos* RNA could be detected in these purified cell populations in spite of the absence of detectable c-*mos* in total testicular RNA of prepubertal mice. However, c-*mos* RNA was not detected in either leptotene/zygotene or pachytene spermatocytes, which represent progressive stages in meiotic prophase (Fig. 3, lanes 1 and 2). As expected, neither the 4.7-kb c-*abl* nor the 1.5-kb actin postmeiotic transcript was detected in these RNAs (Fig. 3, lanes 1 and 2). Thus, c-*mos* RNA was not detectable in prepubertal spermatocytes.

We next separated cells from adult testes. The seminiferous-tubule fraction, which consists of Sertoli cells and all germ-cell types, contained c-*mos* RNA as well as the 4.7-kb c-*abl* and 1.5-kb actin transcripts (Fig. 3, lane 7). Three germ-cell fractions were then purified from the seminiferous tubules: pachytene spermatocytes (late meiotic prophase), round spermatids (the earliest postmeiotic cell type), and residual bodies. Residual bodies are the cytoplasmic fraction normally lost from condensing spermatids during the late stages of spermatid condensation. They are obtained in the cell separation as a result of fragmentation of condensing spermatids. Neither c-*mos* RNA, the 4.7-kb c-*abl* transcript, nor the 1.5-kb actin transcript was detected in pachytene spermatocytes (Fig. 3, lane 4). In contrast, RNA of postmeiotic round spermatids contained the 1.7-kb c-*mos* transcript as well as the 4.7-kb c-*abl* and 1.5-kb actin transcripts (Fig. 3, lane 5). Thus c-*mos* RNA, like testis-specific c-*abl* and actin transcripts, is first detected in postmeiotic germ cells.

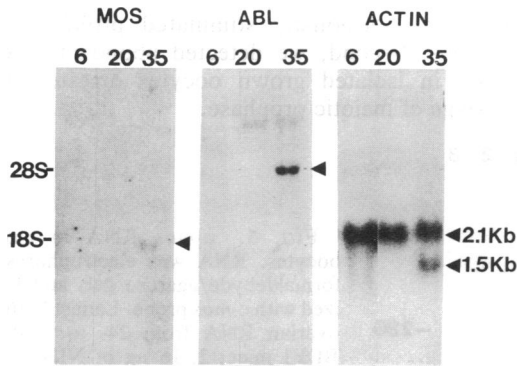


FIG. 2. Temporal expression of c-*mos* RNA in testes. RNA (20 μg) isolated from testes of 6-, 20-, and 35-day-old Swiss Webster mice was electrophoresed in formaldehyde/agarose gels and hybridized with c-*mos*, v-*abl*, and actin probes. The 1.7-kb c-*mos*, 4.7-kb c-*abl*, and actin transcripts are indicated by arrowheads. Positions of 28S and 18S ribosomal RNAs are indicated at left.

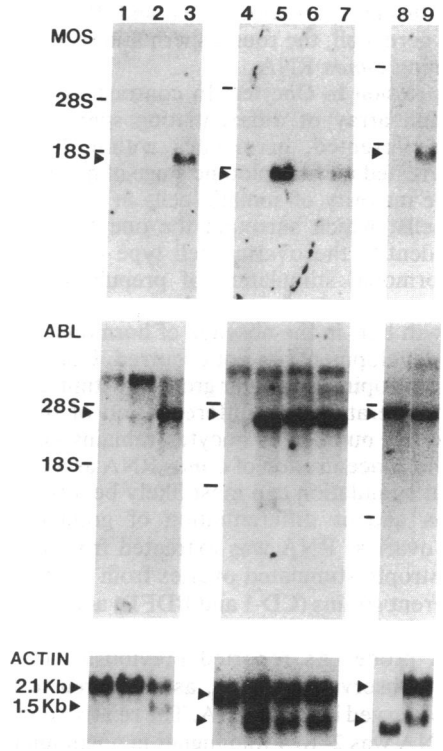


FIG. 3. Analysis of separated spermatogenic cell populations. RNAs of the indicated cell populations were analyzed by blot hybridization with c-*mos*, v-*abl*, and actin probes. Lanes: 1, leptotene/zygotene spermatocytes from 16-day-old mice; 2, pachytene spermatocytes from 16-day-old mice; 3, total testis of adult mice; 4, pachytene spermatocytes from adult mice; 5, round spermatids from adult mice; 6, total testis of adult mice; 7, seminiferous tubules from adult mice; 8, residual bodies from adult mice; 9, total testis of adult mice. Each lane contained 16 μg of RNA, except for lane 8, which contained 12 μg. Positions of 28S and 18S ribosomal RNAs are indicated with dashes. The 1.7-kb c-*mos*, 4.7-kb c-*abl*, and actin transcripts are marked with arrowheads.

The c-*mos* transcript, however, appears to differ from those of c-*abl* and actin in later stages of spermatogenesis. c-*mos* RNA was not detected in residual bodies (Fig. 3, lane 8), whereas the 4.7-kb c-*abl* and 1.5-kb actin transcripts were present in residual-body RNA at levels comparable to those in whole testis and round spermatid RNAs (Fig. 3, lane 8). Note that the testis-specific actin transcript in residual-body RNA migrated slightly faster than that in total testis RNA. Thus, while c-*abl* and actin RNAs appear to persist until formation of mature spermatozoa, c-*mos* RNA is present only transiently during postmeiotic germ-cell development.

Further evidence for this difference in the cell specificity of c-*mos* and c-*abl* is the 2- to 5-fold enrichment of c-*mos* transcript in round-spermatid RNA as compared to total testis RNA (Fig. 3, compare lanes 5 and 6). This is consistent with the fact that round spermatids comprise about 30% of the germ cells within the testis (17). However, the 4.7-kb c-*abl* and 1.5-kb actin transcripts were not significantly enriched in round spermatid as compared to total testis RNA, indicating that other cell types (i.e., condensing spermatids and residual bodies) contribute to the c-*abl* and actin RNA content of the testis. Since the round and condensing spermatid populations together comprise about 70% of the germ cells within the testis (17), the persistence of c-*abl* and actin transcripts throughout postmeiotic germ-cell development accounts for their lack of enrichment in round-spermatid RNA. Thus we conclude that c-*mos* is expressed

transiently during spermatogenesis and that the earliest postmeiotic germ cell, the round spermatid, is the major cell type containing c-*mos* RNA.

**c-*mos* Expression in Oocytes.** In contrast to the testis, in which the full array of differentiating spermatogenic cell types are represented, germ cells within the ovary are uniformly arrested in the diplotene stage of meiotic prophase (13–15). The majority of somatic cells in the ovary are the granulosa cells, which surround the oocyte. Our first approach to identify the ovarian cell type expressing c-*mos* involved hormonal stimulation of prepubertal mice. The ovaries of prepubertal mice contain oocytes at all stages of oocyte growth but, in the absence of hormonal stimulation, full follicle development has not occurred. Upon administration of gonadotropins, follicular growth is stimulated, involving rapid proliferation and differentiation of the granulosa cells. Since the number of oocytes remains constant, any change in the concentration of c-*mos* RNA as a consequence of hormonal stimulation can most likely be attributed to the proliferation and/or differentiation of granulosa cells in stimulated ovaries. RNA was extracted from unstimulated and gonadotropin-stimulated ovaries from prepubertal mice of two different strains (CD-1 and BDF1), and equal amounts of total ovarian RNAs were analyzed by blot hybridization with c-*mos* probe. As reported previously (11), a 1.4-kb transcript is observed in ovary, as compared to the 1.7-kb transcript observed in testis RNA. The relative concentration of c-*mos* RNA was 2- to 4-fold higher in unstimulated than in stimulated ovaries (Fig. 4, compare lanes 2 and 3 and lanes 4 and 5). Since the effect of hormonal stimulation is to increase substantially the number of granulosa cells while the number of oocytes remains constant, the relative contribution of RNA from the oocyte is greater in unstimulated ovaries than in stimulated ovaries. Therefore the decrease in relative concentration of c-*mos* RNA in stimulated ovaries implicates the oocyte as the likely source of c-*mos* transcription. The RNAs of unstimulated and stimulated ovaries were also hybridized with v-*abl* and actin probes. As previously reported (10, 26), the 4.7-kb c-*abl* and 1.5-kb actin transcripts were not present in ovarian RNAs (Fig. 4, lanes 2–5), in contrast to round-spermatid RNA (lane 1). However, similar amounts of somatic actin transcripts were detected in all RNA preparations.

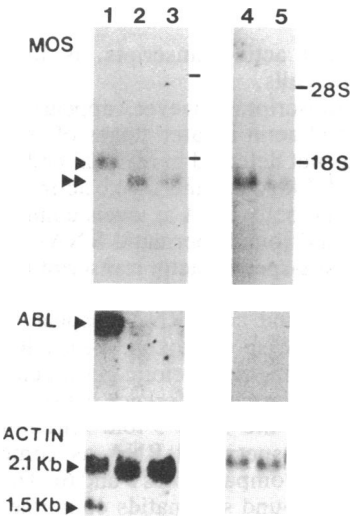


FIG. 4. c-*mos* expression in ovaries of prepubertal mice. RNA (16 μg per lane) was analyzed by blot hybridization with c-*mos*, v-*abl*, and actin probes. Hormonally stimulated ovaries were collected 36 hr after injection of 20- to 23-day-old mice with gonadotropin. Lanes: 1, round spermatid; 2, ovaries of 24- to 27-day-old unstimulated BDF1 mice; 3, ovaries of hormonally stimulated BDF1 mice; 4, ovaries of 20- to 23-day-old unstimulated CD-1 mice; 5, ovaries of hormonally stimulated CD-1 mice. Positions of 28S and 18S ribosomal RNAs are indicated with dashes. The 1.7-kb c-*mos*, 4.7-kb c-*abl*, and actin transcripts are indicated with arrowheads and the 1.4-kb c-*mos* transcript is indicated with a double arrowhead.

To determine directly whether c-*mos* is transcribed in oocytes, we purified oocytes from the ovaries of 20- to 23-day-old hormonally stimulated mice. At this age, the ovary contains ≈6000 oocytes, of which 10% are growing oocytes and 1–2% are large oocytes (13–15). These large oocytes contain about 200 times more RNA (≈0.45 ng of RNA per oocyte) than typical somatic cells (14, 15). Ovaries of 70 mice were dissected and oocytes, ≈80% of which were large germinal-vesicle-stage oocytes, were harvested. Oocytes were collected and mechanically denuded of granulosa cells in the presence of dibutyryl cyclic AMP to prevent spontaneous maturation (27). RNA was extracted from about 1800 oocytes in the presence of NIH 3T3 cell RNA as carrier, and RNA from 900 oocytes (≈0.4 μg) was analyzed by blot hybridization with the c-*mos* probe. The 1.4-kb c-*mos* transcript was detected in the oocyte RNA (Fig. 5, lane 3) but not in NIH 3T3 cell carrier RNA (lane 2). The intensity of c-*mos* hybridization to ≈0.4 μg of oocyte RNA was greater than to 16 μg of RNA from unstimulated ovary (Fig. 5, lane 1). Oocytes thus contain a high level of c-*mos* RNA that is sufficient to account for the c-*mos* RNA detected in the ovary. The concentration of c-*mos* in purified round-spermatid RNA is similar to that in total ovary RNA (Fig. 4, lanes 1 and 2). Thus, the concentration of c-*mos* RNA in oocytes is ≈100-fold higher than in round spermatids.

## DISCUSSION

Our results demonstrate that c-*mos* is transcribed in both male and female germ cells. Identification of the round spermatid, a haploid postmeiotic germ cell, as the major source of c-*mos* RNA in the testis arises from three approaches. First, c-*mos* expression was not detected in the testes of mice with defects in germ-cell development. Second, taking advantage of the temporal appearance of spermatogenic cell types, we detected c-*mos* RNA only in mice in which spermatogenesis had proceeded past meiotic prophase. Finally, we purified discrete populations of germ cells and detected c-*mos* RNA only in the round spermatid population. Two approaches were taken to analyze c-*mos* expression in the ovary. First, we observed a reduction in the relative concentration of c-*mos* RNA in the ovaries of prepubertal mice following administration of gonadotropin. This is attributable to dilution of oocyte RNA in the ovary as a consequence of hormonally stimulated proliferation of granulosa cells. Second, we detected abundant levels of c-*mos* RNA in isolated grown oocytes arrested at the diplotene stage of meiotic prophase.

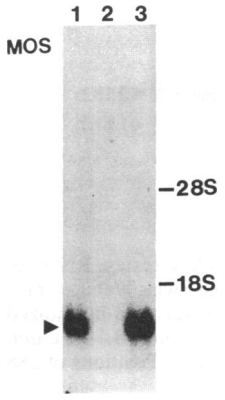


FIG. 5. c-*mos* RNA in isolated oocytes. RNA was electrophoresed in formaldehyde/agarose gels and hybridized with c-*mos* probe. Lanes: 1, 16 μg of ovarian RNA from 24- to 27-day-old BDF1 mice; 2, 16 μg of NIH 3T3 cell RNA; 3, RNA of 900 large oocytes of BDF1 mice (≈0.4 μg of RNA) extracted in the presence of 16 μg of NIH 3T3 cell RNA. The 1.4-kb c-*mos* RNA is indicated with an arrowhead. Positions of 28S and 18S ribosomal RNAs are indicated at right.

The expression of c-*mos* in both spermatogenesis and oogenesis differs from that of the testis-specific 4.7-kb c-*abl* transcript, which is expressed only in postmeiotic male germ cells (10). In addition, c-*mos* and c-*abl* RNAs are detected at different stages of spermatogenesis. Both the c-*mos* transcript and the testis-specific c-*abl* transcript are detected in postmeiotic round spermatids but not in meiotic prophase pachytene spermatocytes. However, the c-*abl* transcript (ref. 10 and Fig. 3), like the testis-specific transcripts of actin (26), tubulin (28), and protamine (29), persists throughout the further differentiation of postmeiotic spermatids and is present in residual bodies. In contrast, c-*mos* RNA was not detected in residual bodies, indicating that c-*mos* RNA is present only transiently during spermatogenesis. The differentiation of postmeiotic round and condensing spermatids to mature spermatozoa occurs over a period of about 14 days and involves extensive morphological changes (12). The observed enrichment of c-*mos* RNA in the round-spermatid fraction indicates that the c-*mos* transcript is accumulated in cells that represent early stages of this differentiation process.

A c-*mos* transcript was detected in meiotic prophase female germ cells but in postmeiotic male germ cells. However, since diplotene spermatocytes appear only transiently in spermatogenesis and are a minor testicular cell population (12), it is possible that c-*mos* RNA detected in round spermatids may in part reflect transcription that occurred in the diplotene stage of meiotic prophase. Additionally, c-*mos* RNA synthesized and accumulated in the growing diplotene oocyte may function at later stages of meiotic maturation.

Grown oocytes contained ≈100-fold higher concentrations of c-*mos* RNA than round spermatids. The total amount of c-*mos* RNA per cell is therefore ≈20,000-fold higher in grown oocytes than in round spermatids, since the oocytes contain ≈0.4 ng of RNA per cell as compared to ≈2 pg of RNA per round spermatid (14, 15, 30). The mRNAs produced in the growing oocyte constitute the mRNA pool present in the fertilized egg and function in fertilization, the first cleavage division, and possibly subsequent stages of early embryogenesis (14, 15). The accumulation of c-*mos* RNA in the growing oocyte may therefore indicate a role for c-*mos* as a maternal message in fertilization and early embryonic development. Although we have not detected c-*mos* RNA in the F9 and P19 embryonal carcinoma cell lines (unpublished observations), c-*mos* expression in early cleavage-stage embryos remains to be investigated.

The expression of c-*mos* in both oogenesis and spermatogenesis suggests potential roles for this protooncogene in meiosis, male and female germ-cell development, fertilization, and early embryogenesis. The retroviral v-*mos* oncogene encodes a protein of ≈37 kDa that is localized in the cytoplasm of infected cells and displays serine/threonine protein kinase activity (31–33). Further studies of the expression and function of the protein encoded by the c-*mos* protooncogene may help to define the biological roles suggested by its expression in germ cells.

**Note Added in Proof.** Expression of c-*mos* in haploid spermatids has been observed by Propst *et al.* (34) and developmental regulation of c-*mos* in spermatogenesis and oogenesis by Mutter and Wolgemuth (35).

We are grateful to L. A. Feig for many helpful suggestions; to F. Apter, D. Loutradis, D. John, K. Jackson, G. Monastersky, and A. Segel for assistance; and to J. Edelman for preparing the manuscript. We thank the Center for Population Research, National Institute of Child Health and Human Development, for stimulating this investigation. This research was supported by Grants CA21082 and CA18689 (G.M.C.), HD11267 and HD15269 (C.F.M.), and HD16561 (A.A.K.) from the Public Health Service.

1. Cooper, G. M. (1982) *Science* **217,** 801–806.
2. Land, H., Parada, L. F. & Weinberg, R. A. (1983) *Science* **222,** 771–778.
3. Bishop, J. M. (1987) *Science* **235,** 305–311.
4. Doolittle, R. F., Hunkapiller, M. W., Hood, L. E., Devare, S. G., Robbins, K. C., Aaronson, S. A. & Antoniades, H. N. (1983) *Science* **221,** 275–277.
5. Waterfield, M. D., Scrace, G. T., Whittle, N., Stroobant, P., Johnsson, A., Wasteson, Å., Westermark, B., Heldin, C.-H., Huang, J. S. & Deuel, T. F. (1983) *Nature (London)* **304,** 35–39.
6. Downward, J., Yaden, Y., Mayes, E., Scrace, G., Trotty, N., Stockwell, P., Ullrich, A., Schlessinger, J. & Waterfield, M. D. (1984) *Nature (London)* **307,** 521–527.
7. Scherr, C. J., Rettenmier, C. W., Sacca, R., Roussel, M. F., Look, A. T. & Stanley, E. R. (1985) *Cell* **41,** 665–676.
8. Sap, J., Muñoz, A., Damm, K., Goldberg, Y., Ghysdael, J., Leutz, A., Beug, H. & Vennström, B. (1986) *Nature (London)* **324,** 635–640.
9. Weinberger, C., Thompson, C. C., Ong, E. S., Lebo, R., Gruol, D. J. & Evans, R. M. (1986) *Nature (London)* **324,** 641–646.
10. Ponzetto, C. & Wolgemuth, D. J. (1985) *Mol. Cell. Biol.* **5,** 1791–1794.
11. Propst, F. & Vande Woude, G. F. (1985) *Nature (London)* **315,** 516–518.
12. Bellvé, A. R. (1979) in *Oxford Reviews of Reproductive Biology*, ed. Finn, C. A. (Oxford Univ. Press, London), pp. 159–261.
13. Peters, H. (1969) *Acta Endocrinol.* **62,** 98–116.
14. Bachvarova, R. (1985) in *Developmental Biology: A Comprehensive Synthesis*, ed. Browder, L. W. (Plenum, New York), Vol. 1, pp. 453–524.
15. Wasserman, P. M. (1983) in *Mechanism and Control of Fertilization*, ed. Hartman, J. F. (Academic, New York), pp. 1–54.
16. Romrell, L. J., Bellvé, A. R. & Fawcett, D. W. (1976) *Dev. Biol.* **49,** 119–131.
17. Bellvé, A. R., Millette, C. F., Bhatnagar, Y. M. & O'Brien, D. A. (1977) *J. Histochem. Cytochem.* **25,** 480–494.
18. Bellvé, A. R., Cavicchia, J. C., Millette, C. F., O'Brien, D. A., Bhatnagar, Y. M. & Dym, M. (1977) *J. Cell. Biol.* **73,** 68–85.
19. Cathala, G., Savouret, J.-F., Mendez, B., West, B. L., Karin, M., Martial, J. A. & Baxter, J. D. (1983) *DNA* **2,** 329–335.
20. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
21. Oskarsson, M., McClements, W. L., Blair, D. G., Maizel, J. V. & Vande Woude, G. F. (1980) *Science* **207,** 1222–1224.
22. Seth, A. & Vande Woude, G. F. (1985) *J. Virol.* **56,** 144–152.
23. Srinivasan, A., Reddy, E. P. & Aaronson, S. A. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 2077–2081.
24. Spiegelman, B. M., Frank, M. & Green, H. (1983) *J. Biol. Chem.* **258,** 10083–10089.
25. Green, M. C. (1981) in *Genetic Variants and Strains of the Laboratory Mouse*, ed. Green, M. C. (Fischer, Stuttgart, F.R.G. and New York), pp. 8–278.
26. Waters, S. H., Distel, R. J. & Hecht, N. B. (1985) *Mol. Cell. Biol.* **5,** 1649–1654.
27. Cho, W. K., Sterns, S. & Biggers, J. D. (1974) *J. Exp. Zool.* **187,** 383–386.
28. Distel, R. J., Kleene, K. C. & Hecht, N. B. (1984) *Science* **224,** 68–70.
29. Kleene, K. C., Distel, R. J. & Hecht, N. B. (1984) *Dev. Biol.* **105,** 71–79.
30. Kleene, K. C., Distel, R. J. & Hecht, N. B. (1983) *Dev. Biol.* **98,** 455–464.
31. Papkoff, J., Nigg, E. A. & Hunter, T. (1983) *Cell* **33,** 161–172.
32. Maxwell, S. A. & Arlinghaus, R. B. (1985) *Virology* **143,** 321–333.
33. Singh, B., Wittenberg, C., Reed, S. I. & Arlinghaus, R. B. (1986) *Virology* **152,** 502–506.
34. Propst, F., Rosenberg, M. T., Iyer, A., Kaul, K. & Vande Woude, G. F. (1987) *Mol. Cell. Biol.* **7,** 1629–1637.
35. Mutter, G. L. & Wolgemuth, D. J. (1987) *Proc. Natl. Acad. Sci. USA*, in press.