Microinjection of antisense *c-mos* oligonucleotides prevents meiosis II in the maturing mouse egg

(meiosis/protooncogene/oocyte/maternal RNA)

STEPHEN J. O'KEEFE*, HEINER WOLFES*, ANN A. KIESSLING†, AND GEOFFREY M. COOPER*

*Dana-Farber Cancer Institute and Department of Pathology, Harvard Medical School, Boston, MA 02115; and †Laboratory of Human Reproduction and Reproductive Biology and Department of Obstetrics, Gynecology and Reproductive Biology, Harvard Medical School, Boston, MA 02115

Communicated by Howard M. Temin, June 27, 1989 (received for review February 10, 1989)

ABSTRACT Injection of antisense oligonucleotides was used to investigate the function of *c-mos* in murine oocytes. Oocytes injected with antisense *c-mos* oligonucleotides completed the first meiotic division but failed to initiate meiosis II. Instead, loss of *c-mos* function led to chromosome decondensation, reformation of a nucleus after meiosis I, and cleavage to two cells. Therefore, *c-mos* is required for meiosis II during murine oocyte maturation.

The ability of activated oncogenes to induce neoplastic transformation suggests that their normal cellular progenitors, the protooncogenes, may also play important roles in regulating cell growth and differentiation. While some protooncogenes are expressed in many cell types, others display more restricted patterns of expression, suggesting that they play a role in particular developmental schemes. *c-mos* is unique among the protooncogenes in that its major sites of expression appear limited to male and female germ cells of the mouse and of several other species (1–8), suggesting a specific function for *c-mos* in meiotic cell types.

The murine oocyte, which is arrested at the diplotene stage of meiotic prophase, accumulates large amounts of *c-mos* RNA during its growth (2, 3). The *c-mos* transcripts in these oocytes lack detectable poly(A) tails but become polyadenylated after resumption of meiosis (9). Such posttranscriptional polyadenylation is indicative of recruitment of maternal mRNAs for translation in both the mouse and lower organisms (10–13). Consistent with the fate of other maternal mRNAs in the mouse (14), *c-mos* RNA is degraded by the two-cell stage (2, 15, 16). These results imply that *c-mos* is a maternal mRNA that is translated and may function during meiosis.

In the present study, we have used injection of antisense oligonucleotides to investigate the function of *c-mos* in the murine oocyte. Meiosis in the murine oocyte is easily followed *in vitro*. After the reinitiation of meiosis, germinal vesicle (nuclear) breakdown (GVBD) occurs within 4 hr. The first polar body is extruded 6–8 hr later, indicating the completion of meiosis I. Upon reaching metaphase II, the now mature oocyte (egg) will once again arrest awaiting fertilization. After sperm penetration, the egg completes meiosis II, extrudes the second polar body, and cleaves to two cells within 24 hr. The results of our experiments indicate that the loss of *c-mos* prevents meiosis II and leads to chromosome decondensation, nuclear membrane formation, and cleavage to two cells.

MATERIALS AND METHODS

Synthesis and Purification of Oligonucleotides. Oligonucleotides were synthesized on an Applied Biosystems 380A DNA synthesizer by using the phosphoramidite method.

Oligonucleotides purified with an Applied Biosystems cartridge frequently exhibited nonspecific toxicity; therefore, oligonucleotides were purified by HPLC on a μ Bondapak C₁₈ column (Waters). The oligonucleotides were eluted from the column with a 20–30% acetonitrile/H₂O gradient, detritylated, and repurified by HPLC on a second μ Bondapak C₁₈ column with a 0–35% acetonitrile/H₂O gradient.

Oocyte Recovery, Culture, and Injection. B6SJL (The Jackson Laboratory) or BDF1 (Charles River Breeding Laboratories) mice (22–26 days old) were injected i.p. with 5 units of pregnant mare's serum gonadotropin. Cumulus-enclosed oocytes were isolated 44–53 hr later and cultured by using a modification of the procedure of Downs *et al.* (17). Oocytes were isolated in Dulbecco's phosphate-buffered saline (DPBS) containing 5% (vol/vol) fetal calf serum and 150 μ M isobutylmethylxanthine (IBMX) and were transferred to 250- μ l microdrops of Earle's balanced salt solution (18) supplemented with minimal essential medium's essential amino acids and vitamins (M.A. Bioproducts), 5% fetal calf serum, 10 μ M EDTA, and 150 μ M IBMX (maintenance medium). Oocytes were incubated at 37°C in 7–10% CO₂ (pH 7.1–7.2).

Typically, 20–35 cumulus-enclosed oocytes were washed twice through 2.5 ml of DPBS containing 5% fetal calf serum to remove IBMX and initiate maturation. Oocytes were transferred to 350 μ l of DPBS containing 5% fetal calf serum under silicone oil, visualized by using Hoffman diffraction-interference contrast optics, and injected in the cytoplasm with 10 pl of oligonucleotide (1 μ g/ μ l) in 0.6 $\times$ DPBS containing 0.15 mM EDTA; a beveled injection pipette with an outer diameter of 2.5 μ m was used. Injection volume was controlled with a Picoinjector 100 (Medical Systems, Greenvale, NY). Injections were completed within 45 min after removal from IBMX. Injected oocytes were subsequently transferred to 200- μ l microdrops of maintenance medium without IBMX (maturation medium) under oil and cultured as above.

For several experiments, including DNA labeling and autoradiography (19) and determining the exact timing of nuclear formation, oocytes were maintained in the presence of IBMX before, during, and after injection. The following day, the surviving injected oocytes along with uninjected control oocytes were transferred to maturation medium and cultured as above.

RNA Extraction and Blot-Hybridization Analysis. Oocytes were suspended in 300 μ l of 30 mM sodium acetate containing 1 mM EDTA (pH 5.1) and extracted twice with phenol at 65°C. Yeast tRNA (50 μ g) was added as carrier, and the RNA was extracted twice with chloroform/isoamyl alcohol, 24:1 (vol/vol), and precipitated with ethanol. RNAs were electrophoresed in 1% agarose/2.2 M formaldehyde gels and analyzed by RNA (Northern) blot hybridization with a *c-mos*

probe as described (2). Filters were then rehybridized with a probe for mouse 18S ribosomal RNA (11).

Fluorescent Staining. Eggs were treated with 0.025% trypsin and 70 units/ml of hyaluronidase in DPBS containing 1 mg of bovine serum albumin (BSA) per ml until all cumulus cells were removed. Staining and fixation of the chromatin was achieved by incubation with Hoechst dye 33258 (5 μ g/ml) in DPBS containing 1.8% formaldehyde, 0.05% Triton X-100, and 2 mg of BSA per ml for 10–20 min. The eggs were washed twice with 1 ml of DPBS containing 2 mg of BSA per ml and examined with a Zeiss microscope equipped with epifluorescence.

Analysis of Protein Synthesis. Oocytes were cultured in 50 μ l of Earle's balanced salt solution containing 5% fetal calf serum and 250 μ Ci (1 Ci = 37 GBq) of [35 S]methionine (1000–1400 Ci/mmol; New England Nuclear) per ml for 15 hr. Labeled oocytes were washed three times with 1 ml of DPBS containing 250 μ g of BSA per ml, boiled for 2 min in double-strength sodium dodecyl sulfate (SDS) sample buffer (20), and electrophoresed in an SDS/11% polyacrylamide gel.

RESULTS

Injection of Antisense *c-mos* Oligonucleotides Results in Degradation of *c-mos* mRNA. In the *Xenopus* oocyte, endogenous RNase H degrades RNA-DNA hybrids formed by antisense oligonucleotides and target mRNAs (21). To determine if maternal messages in the murine oocyte could be destroyed in a similar manner, oocytes were injected with an antisense *c-mos* oligonucleotide and total RNA was isolated and analyzed by Northern blotting. A 1.4-kilobase (kb) *c-mos* transcript was observed in RNA of uninjected control oocytes, but *c-mos* RNA was not detected in the antisense *c-mos*-injected oocytes (Fig. 1). Rehybridization of the filter with a probe for mouse 18S ribosomal RNA showed the presence of oocyte RNA in both lanes (Fig. 1). Comparison of the relative signal intensities of *c-mos* and 18S rRNAs indicated that *c-mos* RNA was reduced at least 5-fold in the antisense-injected oocytes. Thus, injection of an antisense *c-mos* oligonucleotide resulted in degradation of the target RNA.

Maturation of Antisense *c-mos*-Injected Oocytes Is Abnormal. Three antisense oligonucleotides, complementary to sequences near the middle and 3' terminus of the *c-mos* coding sequence have been used to examine the function of *c-mos* during meiosis. The corresponding sense oligonucleotides and one oligonucleotide complementary to the rabbit α -globin coding sequence were used as controls. In preliminary experiments, several oligonucleotide preparations caused a block to polar body extrusion after GVBD. However, this inhibition occurred with both control and antisense oligonucleotides and thus appeared to represent a nonspecific toxic effect. When new oligonucleotide preparations were

FIG. 1. *c-mos* RNA in injected oocytes. Oocytes were injected with 5–10 pl of the A-Mmos oligonucleotide (see Table 1; 4 μ g/ μ l) containing RNase H (1 unit/ μ l) and cultured for 24 hr in the presence of IBMX to maintain meiotic arrest. The oocytes were stripped of cumulus cells, and RNAs from 250 uninjected control (lanes U), and 250 A-Mmos-injected oocytes (lanes I) were isolated and analyzed by Northern blot hybridization. The filter was hybridized with a *c-mos* probe and then rehybridized with a probe for murine 18S ribosomal RNA.

purified by extensive HPLC chromatography, injected oocytes extruded polar bodies normally.

The results of a series of experiments in which the effect of antisense *c-mos* oligonucleotides on oocyte maturation was analyzed are summarized in Table 1. Typically (Table 1, experiments A), oocyte maturation was initiated immediately prior to injection. In our initial experiments, RNase H was injected with the oligonucleotide, but subsequent studies demonstrated that exogenous RNase H is unnecessary. After a 16- to 22-hr incubation (day 1), $\approx$ 70% of all oocytes—uninjected, sense injected, and antisense injected—had extruded polar bodies (Table 1, experiments A), indicating completion of the first meiotic division. Both uninjected eggs and eggs injected with control oligonucleotides appeared normally arrested at metaphase II, as evidenced by the presence of polar bodies and the absence of nuclei on day 1 (Fig. 2 A and B). The majority of control eggs ($>90\%$) remained arrested for the next 24 hr (Table 1, experiments A), although multiple abnormalities were observed after more extended culture (3–5 days after initiating maturation). In marked contrast, over half of the oocytes injected with antisense *c-mos* oligonucleotides failed to arrest at metaphase II on day 1 as evidenced by two morphological abnormalities (Table 1, experiments A). The predominant abnormality observed on day 1 was reformation of a nucleus, with a prominent nucleolus, within the body of the egg (Fig. 2 C and D). Frequently, these aberrant eggs had also extruded a large polar body that contained a nucleus (Fig. 2D). The second abnormality, most frequently observed on day 2 was cleavage of injected eggs to two cells (Fig. 2E).

The various antisense *c-mos* oligonucleotides produced the observed phenotypes with similar efficiencies (55–71% of the injected eggs were abnormal on day 2), which differed significantly from the controls ($P < 0.001$, χ^2 analysis). The day 1 results are most striking in that only one egg containing a nucleus was ever detected in the controls, while significant numbers of antisense *c-mos*-injected eggs were already expressing an abnormal phenotype. Although the number of eggs that contained a nucleus or cleaved to two cells in the control groups did increase during the next 24 hr, this abnormal development was 10- to 20-fold lower than in the antisense *c-mos*-injected eggs, and the majority of the control eggs ($>90\%$) were still arrested at metaphase II 44–48 hr after the initiation of maturation (Table 1, experiments A). In addition, the effect of antisense *c-mos* oligonucleotides is not restricted to a particular strain of mice. BDF1 oocytes (Table 1, experiments B) developed the same abnormalities at a similar frequency as B6SJL oocytes.

We also investigated the possibility that biologically significant amounts of *c-mos* protein might be translated prior to oligonucleotide-mediated destruction of *c-mos* RNA. Oocytes were injected with antisense *c-mos* oligonucleotides 24 hr prior to initiating GVBD to insure that *c-mos* RNA was degraded prior to initiation of meiosis. These oocytes (Table 1, experiments C) express the same phenotypes as oocytes injected immediately after the removal of IBMX (Table 1, experiments A).

To determine the timing of nuclear reformation relative to polar body extrusion and completion of meiosis I, eggs were examined hourly beginning 8 hr after initiating maturation. Nuclei reformed within 2–3 hr of polar body extrusion, indicating that injected oocytes failed to initiate meiosis II. Since most oocyte cleavage occurred within 24 hr of reformation of nuclei, we sought to determine if two cells arose from eggs that contained a nucleus on day 1. Antisense *c-mos*-injected oocytes that contained a nucleus on day 1 were removed and cultured separately. On day 2, some of these injected eggs had undergone cleavage, indicating that eggs with nuclei on day 1 can subsequently form two cells. Finally, tritiated thymidine labeling and autoradiography

Table 1. Morphology of oligonucleotide-injected oocytes

Oligo-nucleotide	Day 1 oocytes			Day 2 oocytes		
	PB	Nuclei	2 cells	Nuciei	2 cells	Total
B6SJL experiments A						
Uninjected	302/432	0/302	0/302	1/307	1/307	2/307
A- α glob	51/74	0/51	0/51	2/52	1/52	3/52
S-3' mos	46/62	0/46	0/46	1/56	1/56	2/56
S-Mmos	48/70	1/48	0/48	3/50	1/50	4/50
S-M2mos	11/18	0/11	0/11	1/12	0/12	1/12
A-3' mos	47/75	5/47	0/47	18/53	11/53	29/53
A-Mmos	152/221	57/152	9/152	87/187	45/187	132/187
A-M2mos	80/101	47/80	2/80	26/91	37/91	63/91
BDF1 experiments B						
Uninjected	22/44	0/22	0/22	0/24	1/24	1/24
S-Mmos	17/46	0/17	0/17	1/21	0/21	1/21
A-Mmos	18/43	12/18	0/18	8/32	11/32	19/32
B6SJL experiments C*						
Uninjected	35/44	0/35	0/35	0/35	0/35	0/35
A-M2mos	25/41	12/25	4/25	12/29	8/29	20/29

Cumulus-enclosed oocytes were injected with 10 pl of the indicated oligonucleotide (1 μ g/ μ l). Typically, 50–75% of the injected oocytes survived. The sequences of the oligonucleotides are (5'–3'): A-Mmos, CAGGCCGTTAACAAC; A-M2mos, GCTTTGTGAGTGGAG; A-3'mos, ATACTGCACGTACTG; S-Mmos, GTTGTAAACGGCCTG; S-M2mos, CTCCACTCACAAAGC; S-3'mos, CAGTACGTGCAGTAT; and A- α glob, GGACAGGAGCTTGAA. A- α glob is complementary to position 295–309 relative to the adenosine in the initiating ATG of the rabbit α -globin coding sequences (22), and the corresponding pairs of antisense and sense *mos* oligonucleotides are complementary to positions 544–558, 565–579, and 841–855, respectively (23). Results of multiple independent experiments with the same oligonucleotide (20–35 oocytes per experiment) are combined. (Experiments A) Oocytes from B6SJL mice were injected immediately after initiating maturation. After 16–22 hr in culture (day 1), the oocytes were denuded and scored for polar body (PB) extrusion (presented as the fraction of surviving oocytes), for reformation of a nucleus (presented as the fraction of eggs that extruded a polar body), and for cleavage to two cells (also presented as the fraction of eggs). The eggs and oocytes were reexamined after 40–44 hr in culture (day 2) and again scored for nuclear formation and cleavage to two cells. Oocytes that had extruded a polar body between day 1 and day 2 were included as eggs on day 2. Total is the fraction of eggs that had either reformed a nucleus or cleaved to two cells after 40–44 hr in culture. Experiments with and without addition of RNase H are combined, since this enzyme did not alter the results (data not shown). (Experiments B) Oocytes from BDF1 mice were injected, cultured, and scored as described in part A. (Experiments C) Oocytes from B6SJL mice were injected and cultured for 24 hr in the presence of IBMX to maintain meiotic arrest. The oocytes were then transferred to medium without IBMX to initiate maturation and were cultured as above. The scoring on days 1 and 2 refers to the time after the initiation of maturation.

*Microinjection 24 hr prior to initiating GVBD.

demonstrated that at least some of the injected eggs that reformed nuclei underwent DNA synthesis. Thus, oocytes injected with antisense *c-mos* oligonucleotides under several conditions do not initiate meiosis II. Loss of *c-mos* function, instead, leads to the reformation of a nucleus after the completion of meiosis I and can result in DNA synthesis and subsequent cleavage to two cells.

Chromatin Decondensation in Antisense *c-mos*-Injected Oocytes. To verify that control oocytes were undergoing normal meiosis leading to metaphase II arrest *in vitro* and to examine the distribution of DNA in the antisense *c-mos*-injected oocytes, we carried out DNA labeling studies with the fluorescent dye Hoechst 33258 (Fig. 3). Uninjected and control injected eggs had condensed chromosomes arranged on a metaphase plate, confirming their metaphase II arrest. Additionally, the polar bodies contained condensed chromosomes dispersed throughout the cytoplasm. In contrast, antisense *c-mos*-injected eggs containing a nucleus showed diffuse staining of the nucleus with a single prominent unstained nucleolus typical of a normal germinal vesicle. Similar diffuse nuclear staining was observed in the two cells and in polar bodies of both eggs and the two cells. Thus, the nuclei in either one or two cells contained decondensed chromatin rather than condensed meiotic chromosomes.

Protein Synthesis Is Unaltered in Antisense *c-mos*-Injected Oocytes. Since transient inhibition of protein synthesis in metaphase I or II oocytes can also induce chromosome decondensation, nuclear formation, and cleavage to two cells (19, 24), we examined protein synthesis in antisense *c-mos*-injected oocytes (Fig. 4). The overall pattern of protein synthesis was very similar in both control and antisense

c-mos-injected oocytes, indicating that the abnormal development of the antisense *c-mos*-injected oocytes did not result from general protein synthesis inhibition.

DISCUSSION

To directly address the biological role of *c-mos*, we have injected murine oocytes with antisense oligonucleotides. When injected oocytes underwent meiosis *in vitro*, two abnormalities were observed: (i) decondensation of metaphase chromosomes and reformation of a nucleus within the body of the egg 2–3 hr after extrusion of the polar body and (ii) cleavage to two cells. When examined at the same times, control eggs were arrested at metaphase II and contained condensed chromosomes arranged on a metaphase plate. Thus, the loss of *c-mos* function in the maturing murine oocyte prevents meiosis II and leads to chromosome decondensation, nuclear membrane formation, and oocyte cleavage.

Interestingly, similar phenotypes result from transient inhibition of protein synthesis during meiotic maturation (19, 25). Metaphase II-arrested eggs, incubated in the presence of puromycin for 6 hr, are parthenogenetically activated and cleave to form two cells after resumption of protein synthesis. Oocytes treated with puromycin for 6 hr at metaphase I extrude the first polar body normally, but the meiotic chromosomes decondense, and a nucleus forms between 18 and 21 hr after initiating maturation. These oocytes also become parthenogenetically activated as long as they are maintained in interphase for 10–15 hr after protein synthesis is allowed to resume. This phenotype most closely resembles that which we observe in the antisense *c-mos*-injected oocytes since neither group completes meiosis II.

FIG. 2. Phenotypes of antisense *c-mos*-injected oocytes. Oocytes were injected and cultured as described in Table 1, experiments A, and were photographed at 20 hr (A–D) or 44 hr (E) after the initiation of maturation with a $\times 40$ Hoffman diffraction interference contrast objective. (A) Uninjected control egg. (B) A-aglob oligonucleotide-injected egg. (C) A-3' mos oligonucleotide injected egg containing a nucleus. (D) A-Mmos oligonucleotide-injected egg containing a nucleus and an enlarged polar body. (E) A-Mmos-injected egg containing a nucleus (Left) and two cells resulting from the cleavage of an A-Mmos-injected egg (Right).

In contrast to our results with the murine oocyte, a similar study (5) has demonstrated that loss of *c-mos* function in the *Xenopus* oocyte prevented GVBD. This is not necessarily unexpected since the utilization of maternal RNA is quite different in mammalian and amphibian development (26). In particular, new protein synthesis is a prerequisite for GVBD in the *Xenopus* oocyte (27); whereas, new protein synthesis is not required until sometime near metaphase I in the murine oocyte (28). Therefore, although the phenotypes produced by antisense *c-mos* injections are different in the two systems, each occurs at a stage during oocyte maturation that requires translation of maternal mRNA. Moreover, the results of Sagata *et al.* (5) do not rule out an additional role for *c-mos* during meiosis II in the *Xenopus* oocyte.

The present results suggest several plausible mechanisms by which the *c-mos* protooncogene, which encodes a serine/threonine protein kinase, might function. A number of proteins are specifically modified by phosphorylation during oocyte maturation, and protein phosphorylation is thought to play a regulatory role in meiosis (26). Certainly, the effects of protein synthesis inhibitors indicate that newly synthesized proteins are required for meiosis II. Our results imply that *c-mos* may be one of these proteins, with a direct role in

FIG. 3. DNA fluorescent staining of control and antisense *c-mos*-injected eggs. Uninjected control eggs and A-M2mos-injected (see Table 1) eggs were removed from culture at the indicated times, stained with Hoechst dye 33258, and visualized by using a Zeiss microscope equipped with epifluorescence. (Top) $\times 25$ phase-contrast objective. (Middle) $\times 25$ epifluorescence objective. (Bottom) $\times 100$ epifluorescence objective. The nuclei photographed at $\times 100$ are not the same as those photographed at $\times 25$ but are typical of the nuclei observed. h, Hours.

initiating meiosis II. Alternatively, *c-mos* may act indirectly by modifying mRNA utilization and protein synthesis.

One possible target of the *c-mos* gene product is maturation-promoting factor (MPF), which was originally described

FIG. 4. Protein synthesis in injected oocytes. Oocytes were labeled with [35 S]methionine for 15 hr beginning 4 hr after the initiation of maturation. Total trichloroacetic acid-precipitable radioactivity incorporated by injected eggs was 80% of that incorporated by control eggs. The lysates from 10 oocytes were electrophoresed in each lane. Molecular weight marker proteins are indicated in kilodaltons. Lanes: 1, uninjected control oocytes; 2, A-aglob oligonucleotide-injected oocytes; 3, A-M2mos oligonucleotide-injected oocytes.

as an activity found in unfertilized frog eggs that could induce GVBD in the immature oocyte (29, 30). A latent form of MPF, present in immature oocytes of both *Xenopus* (31) and mouse (32), is apparently activated by phosphorylation prior to GVBD (27, 33). The major difference between the two species is that protein synthesis is required for the initial activation of MPF in *Xenopus* (27) but not murine oocytes (32). *In vitro*, *Xenopus* MPF induces nuclear membrane breakdown and chromosome condensation (34). *In vivo*, MPF activity cycles during meiosis in both the mouse and *Xenopus*, increasing to high levels before GVBD, reaching a peak near metaphase I, declining to almost undetectable levels after the metaphase-anaphase transition, and peaking once again at metaphase II (31, 32). In *Xenopus*, the loss of MPF activity at fertilization is extremely rapid and coincides with the resumption of meiosis (31). Thus, MPF appears to be responsible for breakdown of the nuclear membrane and chromosome condensation, the initial steps in GVBD, and maintenance of chromosome condensation and arrest at metaphase II. Consequently, loss of MPF activity after puromycin treatment or antisense *c-mos* oligonucleotide injection could account for the phenotypes generated in both *Xenopus* and mouse oocytes.

The requirement for protein synthesis to maintain chromosome condensation also suggests the possibility that *c-mos* might act at the level of translational regulation. When the murine oocyte undergoes maturation, the pattern of protein synthesis is altered, reflecting translation of stored maternal RNAs (26, 28). One early event during *Xenopus* oocyte maturation is phosphorylation of the ribosomal protein S6 (27), which has been implicated in mRNA recruitment in several cell lines after the addition of external stimuli (27, 35) and may be one method for regulating the use of maternal messages. When *Xenopus* oocytes mature, an endogenous S6 kinase is activated by serine phosphorylation prior to GVBD (36). Interestingly, several oncogenes, including tyrosine kinases, induce maturation in the *Xenopus* oocyte and stimulate S6 kinase (37–39). Thus, the change in the pattern of protein synthesis during maturation may be the result of a phosphorylation cascade culminating in activation of S6 kinase. Since protein synthesis is vital for meiosis II in the murine oocyte and for GVBD in the *Xenopus* oocyte, a role for *c-mos* in regulating translation would account for the phenotypes observed in both species. Although inhibition of *c-mos* did not affect the gross pattern of protein synthesis in the maturing oocyte, more subtle changes reflecting altered translational regulation would not have been detected in the present experiments.

In summary, the *c-mos* protooncogene functions as a maternal message needed for normal meiotic maturation of the murine oocyte. Loss of *c-mos* leads to chromosome decondensation and formation of a nucleus after the completion of meiosis I, phenotypes that would also be produced by the loss of MPF activity. Thus, the *c-mos* protein may interact directly with MPF or other components of the MPF pathway, or it may act on MPF indirectly by regulating protein synthesis. Experiments are now underway to identify the targets of *c-mos* in the oocyte.

We thank Kathy Jackson for expert technical assistance, Dr. J. Robl for assistance and advice concerning microinjection, Dr. R. Bachvarova for the 18S RNA probe, and Dr. A. Nussbaum for expeditious synthesis and purification of oligonucleotides. This work was supported by National Institutes of Health Grants CA21082, CA28946, HD21890, and HD21988 and by fellowships to S.J.O. (CA08224) and H.W. (DFG Wo 371/1-1).

- Propst, F. & Vande Woude, G. F. (1985) *Nature (London)* **315**, 516–518.
- Goldman, D. S., Kiessling, A. A., Millette, C. F. & Cooper, G. M. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 4509–4513.
- Mutter, G. L. & Wolgemuth, D. J. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 5301–5305.
- Propst, F., Rosenberg, M. P., Iyer, A., Kaul, K. & Vande Woude, G. F. (1987) *Mol. Cell. Biol.* **7**, 1629–1637.
- Sagata, N., Oskarsson, M., Copeland, T., Brumbaugh, J. & Vande Woude, G. F. (1988) *Nature (London)* **335**, 519–525.
- Schmidt, M., Oskarsson, M. K., Dunn, J. K., Blair, D. G., Hughes, S. & Vande Woude, G. F. (1988) *Mol. Cell. Biol.* **8**, 923–929.
- Paules, R. S., Propst, F., Dunn, K. J., Blair, D. G., Kaul, K., Palmer, A. E. & Vande Woude, G. F. (1988) *Oncogene* **3**, 59–68.
- Herzog, N. K., Singh, B., Elder, J., Lipkin, I., Trauger, R. J., Millette, C. F., Goldman, D. S., Wolfes, H., Cooper, G. M. & Arlinghaus, R. B. (1988) *Oncogene* **3**, 225–229.
- Goldman, D. S., Kiessling, A. A. & Cooper, G. M. (1988) *Oncogene* **3**, 159–162.
- Huarte, J., Belin, D., Vassalli, A., Strickland, S. & Vassalli, J. D. (1987) *Genes Dev.* **1**, 1201–1211.
- Paynton, B. V., Rempel, R. & Bachvarova, R. (1988) *Dev. Biol.* **129**, 304–314.
- Rosenthal, E. T. & Ruderman, J. V. (1987) *Dev. Biol.* **121**, 237–246.
- Standart, N., Minshull, J., Pines, J. & Hunt, T. (1987) *Dev. Biol.* **124**, 248–258.
- Bachvarova, R., DeLeon, V., Johnson, A., Kaplan, G. & Paynton, B. V. (1985) *Dev. Biol.* **108**, 325–331.
- Keshet, E., Rosenberg, M. P., Mercer, J. A., Propst, F., Vande Woude, G. F., Jenkins, N. A. & Copeland, N. G. (1988) *Oncogene* **2**, 235–240.
- Mutter, G. L., Grills, G. S. & Wolgemuth, D. J. (1988) *EMBO J.* **7**, 683–689.
- Downs, S. M., Schroeder, A. C. & Eppig, J. J. (1986) *Gamete Res.* **12**, 305–316.
- Jackson, K. V. & Kiessling, A. A. (1989) *Fertil. Steril.* **51**, 675–681.
- Clarke, H. J. & Masui, Y. (1983) *Dev. Biol.* **97**, 291–301.
- Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685.
- Dash, P., Lotan, I., Knapp, M., Kandel, E. R. & Golet, P. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 7896–7900.
- Cheng, J., Raid, L. & Hardison, R. C. (1986) *J. Biol. Chem.* **261**, 839–848.
- Seth, A. & Vande Woude, G. F. (1985) *J. Virol.* **56**, 144–152.
- Siracusa, G., Whittingham, D. G., Molinaro, M. & Vivarelli, E. (1978) *J. Embryol. Exp. Morphol.* **43**, 157–166.
- Clarke, H. J., Rossant, J. & Masui, Y. (1988) *Development* **104**, 97–103.
- Davidson, E. H. (1986) *Gene Activity in Early Development* (Academic, Orlando, FL).
- Maller, J. L. (1985) in *Oogenesis*, ed. Browder, L. W. (Plenum, New York), Vol. 1, pp. 289–311.
- Bachvarova, R. (1985) in *Oogenesis*, ed. Browder, L. W. (Plenum, New York), Vol. 1, pp. 453–524.
- Masui, Y. & Markert, C. I. (1971) *J. Exp. Zool.* **177**, 129–146.
- Smith, L. D. & Ecker, R. E. (1971) *Dev. Biol.* **25**, 233–247.
- Gerhart, J., Wu, M. & Kirschner, M. (1984) *J. Cell Biol.* **98**, 1247–1255.
- Hashimoto, N. & Kishimoto, T. (1988) *Dev. Biol.* **126**, 242–252.
- Cyert, M. S. & Kirschner, M. W. (1988) *Cell* **53**, 185–195.
- Dunphy, W. G., Brizuela, L., Beach, D. & Newport, J. (1988) *Cell* **54**, 423–431.
- Blenis, J. & Erikson, R. L. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 7621–7625.
- Erikson, E., Stefanovic, D., Blenis, J., Erikson, R. L. & Maller, J. L. (1987) *Mol. Cell. Biol.* **7**, 3147–3155.
- Maller, J. L., Foulkes, J. G., Erikson, E. & Baltimore, D. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 272–276.
- Maller, J. L., Pike, L. J., Freidenberg, G. R., Cordera, R., Stith, B. J., Olefsky, J. M. & Krebs, E. G. (1986) *Nature (London)* **320**, 459–461.
- Spivack, J. G., Erikson, R. L. & Maller, J. L. (1984) *Mol. Cell. Biol.* **4**, 1631–1634.