Published in final edited form as:

Science. 1981 January 23; 211(4480): 396–398.

Mouse Oocytes Transcribe Injected *Xenopus* 5S RNA Gene

Ralph L. Brinster, Howard Y. Chen, and Myrna E. Trumbauer

Laboratory of Reproductive Physiology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia 19104

Abstract

Transcripts produced after injection of the *Xenopus* 5S RNA gene into oocyte germinal vesicles of mice migrate electrophoretically with the 5S RNA marker, an indication that the gene is transcribed and processed with considerable accuracy. Approximately two 5S RNA molecules are transcribed per gene per hour. This system may be useful in studying DNA processing and gene regulation by the mammalian ovum and might be modified to allow permanent incorporation of specific genes into mice.

The *Xenopus* oocyte has been used extensively to study the biological activity of macromolecules introduced by microinjection (1–3). The oocyte translates numerous types of injected messenger RNA (mRNA) (2) and transcribes several types of DNA with great fidelity (4–5). This system has been extraordinarily useful in studying the processing of injected mRNA and DNA by a normal living cell (5). Globin mRNA microinjected into the mouse oocyte or into a one-cell fertilized ovum is translated to globin protein (6). The translation characteristics of the mouse oocyte are different from those of the *Xenopus* oocyte, and therefore injection of the mouse ovum provides a valuable additional technique for studying mRNA processing in the intact cell. We now report that the mouse oocyte is also capable of transcribing foreign genes introduced by microinjection and can thus be used to study DNA processing by the mammalian egg cell.

Growing mouse oocytes (50 to 60 μ m in diameter) were dissected from the ovaries of 14-day-old hybrid C57 $\times$ SJL females. The oocytes were collected in Brinster's medium for ovum culture (BMOC) (7) modified by the addition of bovine serum albumin (4 mg/ml), glucose (1 mg/ml), Eagle's essential and nonessential amino acids, and 10 percent fetal calf serum (BMOC-2-M). Mature oocytes (just prior to ovulation) were dissected from the ovaries of 6- to 8-week-old mice. The medium used for collection and maintenance was BMOC-2 (7). Fertilized ova were flushed from the oviduct with BMOC-2 on day 1 of pregnancy. The gene used in these experiments was for somatic 5S RNA from *Xenopus borealis*; two repeating units were cloned in plasmid pBR 322. The characteristics of the cloned sequence and gene (pXbs 1) have been described (8). The concentration of the DNA was 1 mg/ml in a dilute salt solution (0.015M NaCl, 0.5 mM EDTA, and 5 mM tris at pH 7.8). The injection procedure was similar to that previously employed for injecting cells into blastocysts (9) and mRNA into one-cell fertilized ova (6). Growing oocytes were placed on a depression slide in BMOC-2 containing cytochalasin B (5 to 10 μ g/ml) and held with a blunt pipette (6). The tip of the injector pipette was filled with the DNA solution and inserted into the oocyte nucleus (germinal vesicle). The volume injected was determined by measuring

the germinal vesicle before and after injection. The average increase in volume was 2 picoliters. Injections into the germinal vesicle of mature oocytes and the pronucleus (male) of fertilized ova were accomplished in the same manner. The survival rate was approximately 75 percent for growing oocytes, 25 percent for mature oocytes, and 50 percent for fertilized ova. The reason for the difference in survival is not known but may be related to the speed with which the punctured membranes can be repaired. After injection, the oocytes or ova were incubated in the appropriate medium (see above) containing [³H]guanosine (4 μ Ci/ μ l; specific activity, 28 Ci/mmol; Amersham/Searle) for 24 hours. After the oocytes were labeled, they were washed three times in medium and placed in a tube (6 by 60 mm) containing 20 μ g of 4S RNA from *Escherichia coli* as carrier and 20 μ g of proteinase K in 10 μ l of 50 mM tris (pH 7.5), 10 mM EDTA, and 0.5 percent sodium dodecyl sulfate (10). The tubes were stored at -70°C. Immediately before electrophoresis, the tubes containing the oocytes and proteinase K were incubated for 2 hours at 37°C to liberate RNA. (Extraction with a mixture of phenol and chloroform did not improve RNA separation.) Nonradioactive 5S RNA (25 μ g) from *E. coli* (Boehringer Mannheim) was added to each tube as a marker, and the samples were subjected to electrophoresis on 8 percent acryl-amide slab gels containing 0.2 percent sodium dodecyl sulfate by the tris-acetate system (pH 7.2) (11).

Growing oocytes serving as controls and growing oocytes injected with 5S DNA from *X. borealis* both show considerable radioactive 18S and 28S ribosomal RNA at the top of the gel (Fig. 1). There is a band of labeled RNA migrating with the 4S carrier and a radioactive band migrating with the dye front. The latter band has not been identified, but all of the bands disappear with ribonuclease treatment of the lysed oocyte. The only difference between separations in the control and injected oocyte samples is a clearly visible radioactive band migrating with the 5S marker RNA in the DNA-injected oocyte sample. We interpret this finding to indicate that 5S RNA is synthesized from the *Xenopus* 5S DNA gene present in the plasmid. There is no evidence of plasmid DNA transcription. Synthesis of 5S RNA from 5S DNA also occurs when pXbs 1 is injected into the germinal vesicle of the mature oocyte. The plasmid alone does not stimulate transcription from endogenous 5S genes, since no new bands appear when pBR 322 without the 5S gene is injected (Fig. 2). However, if the plasmid containing a 150-nucleotide adenovirus-associated RNA gene is injected simultaneously with pXbs 1, a new RNA band appears above the 5S band (Fig. 2). This band is in the location expected for an RNA transcribed from a 150-nucleotide gene. The band is less dense than the 5S band, perhaps because pAd123 has one cloned viral gene sequence and pXbs 1 has two repeating units of the cloned 5S gene.

The location of the 5S band, which migrates with the 5S marker, suggests that the gene is transcribed with some accuracy. If small pieces of plasmid DNA were transcribed concomitantly with the gene, one would not expect to see exact comigration. In fact, transcription of 5S genes by *E. coli* or *Xenopus* polymerases in vitro does not produce significant quantities of 5S transcripts (4). Most of the transcripts obtained in vitro are incorrect. The location of the adenovirus RNA on the gel also suggests accurate transcription by the oocyte. The accuracy of transcription in the *Xenopus* and mouse oocyte systems is an important characteristic of the technique.

We have not yet detected 5S RNA synthesis after injection of the plasmid into one-cell fertilized ova. Since the volume of plasmid solution injected (2 picoliters) is the same in the one-cell fertilized ovum as in the growing oocyte, the absence of detectable 5S RNA (or any other new RNA bands) must result from the conditions that are responsible for the low levels of RNA synthesis in one-cell fertilized ova and cleaving embryos (12). It is not known why detectable 5S RNA synthesis is absent in control oocytes and fertilized ova, even though 18S and 28S RNA are synthesized in considerable amounts. However, the synthesis of 5S RNA does not always parallel synthesis of 18S and 28S RNA, and their rates of production vary independently in the *Xenopus* oocyte and early embryo (4, 13). These relationships have not been determined for mouse oocytes and fertilized ova.

The size of the growing oocyte (50 to 60 μm) and the number of oocytes that can be injected in each experiment (50 to 100) make it difficult to quantify precisely the response of the cell to the injected DNA, but some estimates are possible. The radioactivity present in the 5S RNA band of injected oocytes is approximately 1 percent of that for 18S plus 28S synthesis. There was no indication that the synthesis of the *Xenopus* 5S RNA depressed the synthesis of 18S and 28S RNA in the injected oocytes in comparison to controls. The incorporation into 18S plus 28S RNA was 501 ± 60 disintegrations per minute for controls and 523 ± 38 for injected ova per hour per ovum. The 4S band and the bands that migrate just ahead of and behind the 4S RNA marker are slightly less dense in some samples from injected ova than in controls. The difference in radioactivity is not statistically significant but could represent the appearance of competition of the injected 5S gene for some component of the polymerase III transcription machinery that handles both 5S and 4S genes. On the basis of the radioactivity in the 5S band, the specific activity of guanosine, and the amount of DNA injected, it is estimated that at least two 5S RNA molecules are synthesized each hour from each *Xenopus* gene in an injected oocyte. This is similar to the transcription rate for 5S DNA injected into the germinal vesicle of the *Xenopus* oocyte (5, 14).

The significant increase in 5S RNA synthesis by the mouse oocyte after injection of the plasmid containing the *Xenopus* 5S gene and the very precise migration of the radioactive material with the nonradioactive 5S RNA marker provide evidence that the gene has been accurately transcribed by the oocyte nucleus.

Mammalian oocytes and fertilized ova may respond to injected DNA in exactly the same way as *Xenopus* does. However, while mouse and *Xenopus* oocytes have at least a similar capability for response, there may be differences in transcription ability, as has been demonstrated for mRNA translation (6). It is possible that our technique can be extended or modified to allow incorporation of the injected gene into the chromosomes of the ovum and thereby into the mouse, an extension that would allow a wide range of studies related to differentiation and carcinogenesis.

Note added in proof: By employing the techniques described here and in collaboration with Dr. Carlo Croce of Wistar Institute, we have obtained incorporation of injected genes into DNA of the mouse.

References and Notes

1. Gurdon JB, Lane CD, Woodland HR, Marbaix G. Nature (London). 1971; 233:177. [PubMed: 4939175]
2. Gurdon, JB. Protein Synthesis in Reproductive Tissue. Diczfalusy, E., editor. Karolinska Institutet; Stockholm, Sweden: 1973.
3. Asselbergs FAM, Van Venrooij W, Bloemendal H. Eur J Biochem. 1979; 94:249. [PubMed: 436841]
4. Gurdon JB. Proc R Soc London Ser B. 1977; 198:211. [PubMed: 19752]
5. Brown DD, Gurdon JB. Proc Natl Acad Sci USA. 1977; 74:2064. [PubMed: 266727] *ibid.* **75**, 2849 (1978);Gurdon JB, Brown DD. Dev Biol. 1978; 67:346. [PubMed: 738532]
6. Brinster RL, Chen HY, Avarbock MR, Trumbauer ME. J Cell Biol. 1979; 83:32A.Brinster RL, Chen HY, Trumbauer ME, Avarbock MR. Nature (London). 1980; 283:499. [PubMed: 7352032]
7. Brinster RL. J Reprod Fertil. 1965; 10:227. [PubMed: 5836270] Rothblat, G.; Cristofalo, V., editors. Growth, Nutrition and Metabolism of Cells in Culture. Academic Press; New York: 1972.
8. Peterson RC, Doering JL, Brown DD. Cell. 1980; 20:131. [PubMed: 6248230]
9. Brinster RL. J Exp Med. 1974; 104:1049. [PubMed: 4610074]
10. Mertz JE, Gurdon JB. Proc Natl Acad Sci USA. 1977; 74:1502. [PubMed: 193103]
11. Bishop DHL, Claybrook JR, Spiegelman S. J Mol Biol. 1967; 26:373. [PubMed: 5339793]
12. Young RJ, Sweeney K, Bedford JM. J Embryol Exp Morphol. 1978; 44:133. [PubMed: 650131] Tasca RJ, Hillman N. Nature (London). 1970; 225:1022. [PubMed: 5416466] H. R. Woodland and C. F. Graham, *ibid.* **221**, 327 (1969);Crampton JM, Woodland HR. Dev Biol. 1979; 70:467. [PubMed: 478171]
13. Gurdon, JB. The Control of Gene Expression in Animal Development. Harvard Univ. Press; Cambridge, Mass: 1974.
14. Birkenmeier EH, Brown DD, Jordan E. Cell. 1978; 15:1077. [PubMed: 569551]
15. We thank D. D. Brown for providing the cloned 5S DNA and cloned adenovirus-associated RNA gene. We also thank N. Avadhani, D. Brown, C. Croce, and B. Paynton for helpful suggestions. Supported by NIH grants HD 12384 and HD 00239 (H.Y.C.) and NSF grant PCM 78-22931.

Fig. 1.

Fluorograph of polyacrylamide gel electrophoretic separation of ^3H -labeled RNA synthesized by 95 growing mouse oocytes after injection with (A) dilute salt solution and (B) dilute salt solution containing pXbs1 plasmid DNA (1 mg/ml). Each plasmid contained two repeating units of somatic 5S gene from *Xenopus borealis*. The positions of the nonradioactive marker 5S RNA and 4S RNA are indicated.

Fig. 2.

Fluorograph of polyacrylamide gel electrophoretic separation of ^3H -labeled RNA synthesized by 120 growing mouse oocytes after injection with (A) dilute salt solution, (B) dilute salt solution containing pXbs1 (0.5 mg/ml) and pAd123 (0.5 mg/ml), and (C) dilute salt solution containing pBR322 (1 mg/ml). Plasmid pAd123 has a 1.8-kilobase insert including the type 2 adenovirus-associated RNA gene (VA) and flanking regions (prepared by David Bogenhagen). The adenovirus gene is approximately 150 nucleotides (5S and 4S RNA genes are approximately 120 and 90 nucleotides, respectively). All three genes are transcribed by polymerase III. Procedures and labeling are as in Fig. 1.