Reprogramming of the Transcriptional Machinery in *Xenopus* Oocytes by Injection of Mouse Poly(A)⁺ RNA

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We have devised an assay for species-specific transcription factors for the mouse rRNA gene by exploiting the ability of *Xenopus* oocytes to transcribe injected DNA and translate mRNAs. When mouse rRNA genes are microinjected into *Xenopus* oocytes, they are not transcribed. We show here that transcription of mouse rRNA genes is supported when mouse mRNAs are injected before the transcription template is injected, indicating that the necessary transcription factors are translated in the oocyte and are available to transcribe an appropriate template. The use of this assay in cloning genes for transcription factors is discussed.

Unlike RNA polymerase II and III genes, rRNA genes are transcribed by a highly species-specific transcriptional apparatus (7). This property of RNA polymerase I transcription has been exploited to identify components of the RNA polymerase I transcriptional apparatus that are involved in the species specificity of transcription (11, 16, 18). In these experiments, fractionated human transcription extracts, for example, are used to complement a mouse extract to support human rRNA transcription. The human species specificity factor, SL1, has been studied the most extensively (1, 2, 11, 12). Human SL1 does not bind to DNA without a second protein, UBF (12). SL1 has therefore not been amenable to purification by sequence-specific DNA affinity chromatography (9, 21) and has not been purified to homogeneity. As an alternate approach we describe an assay for the mRNAs encoding species-specific transcription factors that provides the means to clone the genes for these proteins. This assay has general potential for cloning other transcription factors that may be difficult to purify either because they do not bind to DNA or because they bind to DNA only in the presence of another factor.

Experimental strategy. The strategy used to reprogram the transcriptional machinery is illustrated in Fig. 1. When a mouse rRNA transcription template is injected into *Xenopus* oocytes, it is not transcribed. We reasoned that the necessary mouse transcription factor(s) could be supplied by injection of mouse mRNA. We therefore injected mouse poly(A)⁺ RNA into *Xenopus* oocytes and allowed the oocytes to translate the injected mRNAs for 24 to 48 h. The mouse transcription template was then injected into the *Xenopus* oocyte nucleus, and the oocytes were further incubated overnight to allow the transcripts from the injected mouse template to reach their steady-state levels. The oocytes were harvested the next day, the nucleic acid was purified, and the 5' ends of mouse rRNA transcripts were detected by S1 nuclease protection.

Mouse RNA was prepared from log-phase mouse myeloma cells by using the guanidinium isothiocyanate-CsCl method (13). After denaturation, the RNA was diluted to 100 µg/ml in binding buffer and applied to an oligo(dT)-cellulose column. The poly(A)⁺ fraction, which contains the bulk of the mouse 45S precursor, was collected, ethanol precip-

itated, and saved for use in the injection experiments. The poly(A)⁺ fraction was eluted from the oligo(dT)-cellulose column and concentrated by ethanol precipitation. Both poly(A)⁻ and poly(A)⁺ fractions were suspended in water for injection, and their concentrations were determined by A₂₆₀. Since the transcription assay detects small quantities of 45S rRNA, it was important to determine the relative concentrations of 45S precursor in the poly(A)⁺ and poly(A)⁻ fractions. To assay 45S precursor, an RNA probe identical to the S1 probe described below was synthesized and used in an RNase protection assay (15). Mouse RNA assayed by RNase protection yielded two major bands, while the S1 assay showed a single major band. The poly(A)⁺ fraction contained less than 1/20 as much mouse 45S precursor as did an equal mass of poly(A)⁻ RNA (Fig. 2). Thus, although the mouse 45S rRNA contains adenine tracts, we did not find it concentrated into the poly(A)⁺ fraction.

The reprogramming experiment was done according to the following protocol. Mouse poly(A)⁺ or poly(A)⁻ RNA (100 ng) was injected into the cytoplasm of each of 20 *Xenopus* oocytes. The oocytes were incubated at 20°C for 48 h in Barth buffer supplemented with gentamicin. On the third day after injection of the mouse RNA, 1 ng of transcription template containing the mouse rRNA promoter was injected into each oocyte nucleus. After overnight incubation, the healthy oocytes were collected and homogenized in 200 mM NaCl-10 mM EDTA-1% sodium dodecyl sulfate and digested with proteinase K. The nucleic acids were purified by phenol extraction and ethanol precipitation.

Mouse rRNA transcripts were assayed by an S1 nuclease assay because we obtained consistently lower background using the S1 assay on oocyte injections. A uniformly labeled probe was prepared by extending a primer annealed to a single-stranded DNA containing sequences of the mouse rRNA gene from -170 to +150. After the primer was extended in the presence of radioactive nucleotides, the DNA was cleaved with *Sal*I at -170 and the probe was purified on a denaturing acrylamide gel. The radioactive probe was annealed to the RNA from the injected oocytes in 0.3 M NaCl-20 mM Tris hydrochloride (pH 7.5)-5 mM EDTA at 65°C for 2 h. Samples were then diluted 10-fold with 0.3 M NaCl-50 mM sodium acetate (pH 4.5)-1 mM ZnSO₄-5% glycerol and treated with 30 or 45 U of S1 nuclease for 30 min at 37°C. The reaction was stopped by the addition of 1/5 volume of 1.5 M sodium acetate-50 mM

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FIG. 1. Strategy for detection of transcription factor expression. The time course of the injection experiment is depicted. Mouse poly(A)⁺ RNA was isolated as described in the text. The mouse transcription template contained promoter sequences from -170 to +3300.

EDTA, and the nucleic acids were precipitated by the addition of 2.5 volumes of ethanol. The precipitate was suspended in formamide and electrophoresed on an 8% acrylamide-8 M urea denaturing gel buffered by Tris-borate. The dried gel was exposed to preflashed Kodak XAR5 film at -80°C with screens.

Injected mouse mRNA supplies essential mouse transcription factors. The results from one reprogramming experiment are shown in Fig. 3. When only the mouse rRNA transcription template was injected, no mouse rRNA transcript could be detected (lanes 3 and 4), indicating that the *Xenopus* transcription machinery was unable to transcribe the mouse

template. In contrast, *Xenopus* transcription templates are readily transcribed when these injection conditions are used (for example, see reference 4). However, when mouse poly(A)⁺ RNA was injected into the oocyte cytoplasm and then mouse rRNA transcription template was injected into the nucleus (lanes 5 and 6), the mouse rRNA transcripts were detected. To exclude the possibility that the mouse transcript detected in lanes 5 and 6 was 45S precursor

FIG. 2. Assay of mouse 45S rRNA precursor in poly(A)⁺ and poly(A)⁻ fractions. Total mouse RNA was chromatographed on oligo (dT)-cellulose as described in the text. Mouse 45S precursor was measured by an RNase protection assay that resulted in a protected ribonucleotide of 150 bases (larger band indicated by arrow) and a second, smaller band that also resulted from protection of the intact precursor. For poly(A)⁺ and poly(A)⁻ RNA, 1 µg each was assayed.

FIG. 3. Reprogramming of transcription by mRNAs encoding mouse transcription factors. Each of 20 oocytes was injected with 100 ng of mouse RNA or buffer as indicated and incubated at 19°C for 48 h. Mouse transcription template (1 ng) was then injected into each oocyte nucleus. The oocytes were incubated overnight at 19°C and harvested the next day. Mouse rRNA transcripts were detected by an S1 nuclease assay in which a uniformly labeled probe was used and five oocytes were assayed. Because of variations in the sensitivity of the assay due to incomplete digestion of the S1 probe, each sample was assayed with 30 U (lanes 1, 3, 5, and 7) and 45 U (lanes 2, 4, 6, and 8) of S1 nuclease. Lanes: 1 and 2, injection of mouse poly(A)⁻ RNA followed by injection of mouse transcription template; 3 and 4, injection of buffer followed by injection of mouse transcription template; 5 and 6, injection of mouse poly(A)⁺ RNA followed by injection of mouse transcription template; 7 and 8, 100 ng of the poly(A)⁻ RNA preparation used in this injection experiment assayed for mouse 45S precursor.

contaminating the poly(A)⁺ RNA, the following controls were done. Mouse poly(A)⁻ RNA was injected into oocyte cytoplasm prior to template injection using the same protocol as for poly(A)⁺ RNA. When the poly(A)⁻ injected oocytes were assayed for mouse rRNA transcript, none was detected (lanes 1 and 2). However, the 45S precursor was easily detected when the same preparation of mouse poly(A)⁻ RNA was assayed for 45S precursor prior to injection (lanes 7 and 8). These results show that mouse rRNA precursor is degraded in the cytoplasm as expected for an uncapped RNA (5, 6). Further, the amount of 45S precursor detected after poly(A)⁺ injection cannot be accounted for by mouse 45S precursor contaminating the poly(A)⁺ RNA; this fraction contains only 5% as much precursor as the poly(A)⁻ fraction, yet the levels of 45S precursor synthesized in the reprogrammed oocyte were similar to those in uninjected poly(A)⁻ RNA. Clearly then, the mouse rRNA transcripts must have resulted from transcription of the injected template. We conclude that the mouse poly(A)⁺ RNA supplied mRNAs encoding mouse rRNA transcription factors and that translation of these factors reprogrammed the transcriptional apparatus of the oocytes.

When the mouse poly(A)⁺ RNA used in this experiment was fractionated on a sucrose gradient and each fraction was injected, no mouse rRNA transcripts were detected. However, when the fractions of poly(A)⁺ RNA were combined and injected, mouse rRNA transcripts could again be detected (data not shown). These data suggest that multiple mRNA fractions, and presumably multiple mouse transcription factors, are required to transcribe the mouse template. Our results also indicate that *Xenopus* species and mouse show species specificity of rRNA transcription. The ability of mouse extracts to transcribe *Xenopus* genes in vitro (3) may be due to greater permissiveness of the in vitro system. Although *Xenopus* and human UBF proteins give identical footprints on the human rRNA promoter, *Xenopus* UBF in conjunction with human SL1 is unable to initiate transcription on the human template (2). We assume that in our experiments, the endogenous *Xenopus* rRNA transcription factors in the injected oocytes must have competed with the translated mouse factors for binding to the mouse templates. If so, the mouse transcription factors may have been present at higher concentrations than the transcription results suggest.

The sensitivity of the assay was important for the success of this experiment. While transcription of *Xenopus* templates can easily be detected by using end-labeled S1 probes and overnight exposures, we used uniformly labeled probes synthesized by using high-specific-activity radiolabeled nucleotides in the reprogramming assay. From a comparison of the two transcription assays, we estimate the difference in transcription to be about 20-fold. For injected *Xenopus* templates, approximately 1% of the injected genes are transcribed (10), which would suggest that less than 0.05% of the injected mouse templates are transcribed.

An assay for expression of proteins translated from injected RNAs is a starting point for cloning the genes for the transcription factors. When oocyte injection has been used to clone genes, either RNAs are directly expressed from cDNA library pools (20) or pools of cDNAs from these libraries are used to hybrid select the corresponding RNAs (for example, see reference 19). These RNAs are then injected, and the injected oocytes are assayed for the gene product. However, since these approaches are inherently limited in cases when more than one protein is required for

activity, an alternative approach will be necessary. Rather than using a positive assay that requires translation of an RNA derived from a functionally complete cDNA, an assay utilizing inhibition of the pool of mouse mRNAs can be devised. Pools of clones expressing the antisense strand of the cDNAs could be used to inhibit the activity of the total poly(A)⁺ pool (8, 14), or single-stranded cDNAs could be used to target complementary RNAs for destruction by RNase H (17, 22). Two, or even more, factors could be independently cloned using such a strategy.

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