Transcription and Export of RNase MRP RNA in *Xenopus laevis* Oocytes

Sunjoo Jeong*

Department of Molecular Biology, College of Natural Sciences, Dankook University, Seoul 140-714, Korea

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RNase MRP is a ribonucleoprotein complex with a site-specific endonuclease activity. Its original substrate for cleavage is the small mitochondrial RNA near the mitochondrial DNA replication origin, thus it was proposed to generate the primer for mtDNA replication. Recently, it has been shown to have another substrate in the nucleus, such as pre-5.8S ribosomal RNA in nucleolus. The gene for the RNA component of RNase MRP (MRP RNA) was found to be encoded by the nucleus genome, suggesting an interesting intracellular trafficking of MRP RNA to both mitochondria and nucleolus after transcription in nucleus. In this study, genomic DNA encoding MRP RNA was microinjected into the nucleus of *Xenopus* oocytes, to analyze promoter regions involved in the transcription. It showed that the proximal sequence element and TATA box are important for basal level transcription; octamer motif and Sp1 binding sites are for elevated level transcription. Most of *Xenopus* MRP RNA was exported out to the cytoplasm following transcription in the nucleus. Utilizing various hybrid constructs, export of MRP RNA was found to be regulated by the promoter and the 5′ half of the coding region of the gene. Interestingly, the transcription in nucleus seems to be coupled to the export of MRP RNA to cytoplasm. Intracellular transport of injected MRP RNA can be easily visualized by whole-mount *in situ* hybridization following microinjection; it also shows possible intra-nuclear sites for transcription and export of MRP RNA.

RNase MRP (Mitochondrial RNA Processing) was originally identified as the endoribonuclease activity that cleaves mitochondrial RNA (mtRNA) in a site-specific manner (Chang and Clayton, 1987a; Chang and Clayton, 1989). Its proposed role in mitochondria was generating RNA primer for mitochondrial DNA (mtDNA) replication, by processing small mtRNA near the replication origin of mtDNA [Chang and Clayton, 1987b; see Jeong-Yu and Clayton (1996) for review]. Since some experiments showed, however, that the majority of it can be found in nucleolus (Kiss et al., 1992), its function in nucleus has been a subject of intense studies (Clayton, 1994). It seems now in agreement that RNase MRP is also involved in ribosomal RNA (rRNA) processing in nucleolus, especially in pre-5.8S rRNA processing (Schmitt and Clayton, 1993; Chu et al., 1994; Lygerou et al., 1996). Whatever the locations and the roles of RNase MRP *in vivo*, it has shown clear and accurate processing activity *in vitro* (Karwan et al., 1991).

Structure of RNase MRP is found to be a member of ribonucleoprotein (RNP) complex (MRP RNP) composed of one small RNA (MRP RNA, also known as 7-2 nucleolar RNA) and several unidentified proteins. One of the protein components is Th antigen, which is an antigenic target for autoantibody (Th antibody) found in autoimmune disease patients, such as systemic lupus erythematosus (Reddy et al., 1983). Interestingly, Th antibody could co-immunoprecipitate RNase P RNP, an endoribonuclease that processes precursor tRNA, as well as MRP RNP (Gold et al., 1989). Therefore, RNase MRP and RNase P RNPs are likely to be structurally related to each other. Genes for the MRP RNA was cloned from various organisms (Topper and Clayton, 1990a; Bennett et al., 1992; Schmitt and Clayton, 1992), which were found to be encoded by the nucleus genome (Chang and Clayton, 1989). The pathway for assembly of MRP RNA and its cognate proteins is not known, however, it suggests an interesting trafficking of MRP RNA in the cell, leading to dual localization to mitochondria and nucleolus after transcription in nucleoplasm. Considering assembly pathways of other RNPs, it is possible that MRP RNA is assembled with its cognate proteins in cytoplasm, transported as RNP and directed by a specific protein component to its destined organelle. However, the exact mechanism of assembly and transport is not understood at this point.

In previous studies, intracellular locations of MRP

* Tel: 82-2-709-2819, Fax: 82-2-793-0176

RNA was carefully examined by biochemical and cell biological methods (Li et al., 1994; Davis et al., 1995). *Xenopus* oocyte system was employed in this study to analyze its fate following microinjection of MRP RNA gene, because it is well suited to study nucleocytoplasmic transport of protein and RNA (Zasloff, 1983). Especially, the nucleus of oocyte (germinal vesicle, GV) easily dissociates away from the cytoplasm by simple manual dissection. Moreover, during development of oocytes, mitochondrial and ribosomal DNAs are enormously amplified to produce 10^5-10^6 excess of mtDNA and rDNA compared to somatic cells, leading to cytologically distinct amplified mitochondria and nucleolus in later stages of oocytes (Brown and Dawid, 1968; Chase and Dawid, 1972; Dumont, 1972).

In this paper, transcription of MRP RNA gene was studied and promoter regions responsible for high level of transcription was determined. Resulting nascent RNAs were found to be exported out to the cytoplasm, in case of *Xenopus* gene but not in mouse gene injection. Hybrid genes of these two differently transported genes were injected and found that export of MRP RNA to the cytoplasm is in some way coupled to the transcription process in the nucleus. It also suggests that the intracellular transport of MRP RNA is a dynamic process, having cytoplasmic phase as its transit to either mitochondria or nucleolus.

Materials and Methods

Microinjection

Adult female *Xenopus* laevis was purchased from *Xenopus* I, Ann Arbor, MI, USA. To obtain oocytes for microinjection, ovary was surgically removed from mature frogs and treated with collagenase (Sigma, type 1A) as previously described (Jeong-Yu and Carroll, 1992a), Oocytes were staged according to Dumont (Dumont, 1972) and incubated in OR-2 buffer (Wallace, 1973). For injection, 20 to 30 nl of DNA or RNA (corresponding to 2 to 10 ng) was targeted into the nucleus (germinal vesicle) of stage VI oocyte and incubated for various times (Jeong-Yu and Carroll, 1992b). Following incubation, nucleus and cytoplasm were manually dissected under ice-cold J-buffer (Davis et al., 1995). At least 20 oocytes were injected for each time point to reduce the variability of individual oocyte; among those, at least ten oocytes were dissected, nuclei and cytoplasm fractions were pooled and RNAs were prepared from each fraction. Among those, RNAs from two oocytes were loaded per lane of 8% polyacrylamide-8 M urea gel for Northern analysis.

DNA for microinjection

Injected plasmids were pXlMRP [genomic *X. laevis* MRP RNA gene (Bennett et al., 1992)], pMRPSP [genomic mouse MRP RNA gene (Chang and Clayton, 1989)], promoter deletion mutants of mouse genomic DNA and *Xenopus*/mouse hybrid genes. Promoter 5′-deletion mutants were generated by exonuclease III digestion of 1200 bp BamHI-EcoRI fragment of pMRPSP following PstI and BamHI digestion. TATA box linker scanning mutant was constructed as follows; 5′-deletion mutant (deletion point at −24 bp) was cleaved with HindIII and EcoRI, and treated with S1 nuclease. The insert was gel purified and ligated into 3′-deletion mutant (deletion point at −31) which had been digested with HindIII and also blunt-ended by S1 nuclease. *Xenopus*/mouse hybrid plasmids were constructed by recombinant PCR method (Jeong-Yu et al., 1996), in which PCR fragments from unrelated sequences can be specifically combined by overlapping ends of the first set of PCR products. Second PCR was done by hybridizing the first PCR products and amplifying it with outermost primers of the first two PCR reactions. PCR reactions and hybrid constructs were described (Jeong-Yu et al., 1996), except pMPXM which has the mouse promoter and *Xenopus* 5′-coding region and mouse 3′-coding region. Mouse promoter region of pMRPSP was amplified with oligo 1-2 and oligo 11, *Xenopus* 5′-coding and mouse 3′-coding regions of pXM was amplified with oligo 12 and oligo 8-2. Resulting primary PCR products were combined and second PCR was done with oligo 1-2 (5′-primer) and oligo 8-2 (3′-primer). Sequences of junction of all hybrid constructs were confirmed by dideoxy chain-termination sequencing with Sequenase (United States Biochemical).

RNA analysis, preparation of probes and whole-mount in situ hybridization

Northern blots were performed with digoxigenin-labeled probes using Genius (DIG) chemiluminescent labeling and detection kit (Boehringer Mannheim) as previously described (Bennett et al., 1992). MRP RNA was detected with either antisense MRP RNA or oligonucleotide probe. An antisense MRP RNA from *Xenopus* was made by *in vitro* transcription of XhoI-digested pXLMU with T7 RNA polymerase (Bennett et al., 1992). Mouse MRP RNA was detected with 3′-end labeled oligo 4 (Topper and Clayton, 1990b), which was prepared with digoxigenin-11-ddUTP and terminal transferase. To detect mouse and *Xenopus* MRP RNA in the same extent, 5′MRP oligonucleotide probe was designed. 5′MRP oligo (46-mer) was from 60-105 of mouse and 65-110 of *Xenopus* MRP RNA gene with 3 nucleotides mismatches to each RNA. To normalize the expression of promoter mutants, 5S RNA gene was used as a control. The hexamer-labeled HindIII fragment of *Xenopus* oocyte type 5S DNA in pHU1063 (a kind gift of Dr. Dana Carroll) was used. After exposing Northern blots to Lumi-phos chemiluminescent detection system, resulting X-ray films were scanned with the densitometer (Molecular Dynamics). To uncouple transcription and export, two methods were employed.

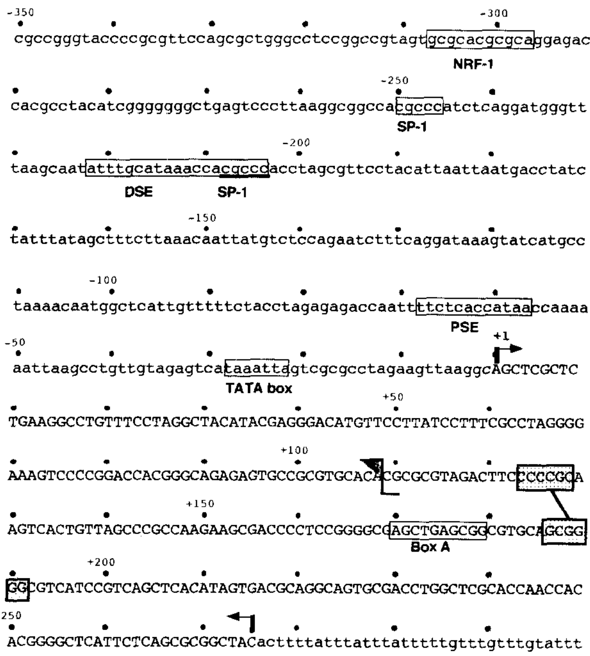

Fig. 1. Sequences of genomic mouse MRP RNA gene. Start and stop of transcription are indicated by arrows. First nucleotide of RNA is shown as +1. Putative regulatory sites are NRF-1 (nuclear respiratory factor-1), SP-1, DSE (distal sequence element), PSE (proximal sequence element), TATA box and Box A. Regions known to be involved in the pseudoknot formation (Topper and Clayton, 1990b) are indicated by dotted boxes and line between them. Hybrid junction of *Xenopus* and mouse gene is shown in +108 nucleotide.

First, to inhibit transcription by RNA polymerase II in oocytes, 500 μg/ml of α-amanitin was injected. Secondly, *Xenopus* MRP RNA was prepared after injection of pXlMR to get *in vivo* expression. Following overnight incubation, total nuclear RNA was extracted and full-length MRP RNA was gel purified and prepared for microinjection. Whole-mount *in situ* hybridization was extensively described elsewhere (Davis et al., 1995).

Results

Promoter analysis of mouse MRP RNA gene in Xenopus oocytes

Comparison of the genomic sequences of the mouse, human and *Xenopus* MRP RNA genes reveals extreme conservation of the 5′-upstream regions of promoter. Among those, several highly conserved sequence motifs closer to the transcription start site are shown in Fig. 1. Centered at −302 of mouse and human genes, there is a binding site for nuclear respiratory factor-1 (NRF-1). This sequence has been identified in the upstream of the human cytochrome c and rat cytochrome c oxidase subunit VIc genes and is believed to be involved in the coordinate regulation of nucleus-encoded mitochondrial proteins. Putative SP1 sites (CGCCC) are centered at −248 and −206. An octamer motif (ATTTGCAT) is adjacent to the proximal SP1 site; together, these two motifs comprise the Distal Sequence Element (DSE). Near the transcription start site are the Proximal Sequence Element (PSE) and TATA box, centered at −62 and −25, respectively. In addition to these upstream sequences, the Box A sequence is located downstream of the transcription start site, around +175 inside of the gene. The DSE and PSE as well as intragenic control element, Box A, are known to be involved in the transcription by RNA polymerase III (Pol III), whereas the TATA box was originally thought to be involved in transcription by RNA polymerase II (Pol II). This kind of composite regulatory sequences for transcription by Pol II and Pol III has been found in several other RNA genes, including U6 and 7SK RNAs (Das et al., 1988). To test which RNA polymerase is responsible for MRP RNA transcription, α-amanitin sensitivity test was done to show that mouse MRP RNA gene has a similar sensitivity profile as 5S rRNA gene (data not shown), suggesting that MRP RNA gene is transcribed by Pol III. These data are in agreement with others (Yuan and Reddy, 1991).

To investigate the importance of these elements for transcription, a series of 5′-deletion mutants were made for the promoter of mouse MRP RNA gene and injected into *Xenopus laevis* oocytes. Genomic mouse MRP RNA gene was used as starting material to generate 5′-deletion promoter mutants (see Materials and Methods). They were microinjected into the nuclei (germinal vesicle) of stage VI oocytes and allowed to express the gene for 12 h. Newly transcribed mouse MRP RNA was analyzed by Northern blotting with the mouse-specific MRP probe; 5S RNA probe was also included in the hybridization solution to visualize the endogenous frog RNA as a recovery control (Fig. 2). Frog MRP RNA was not visible in this gel, partly due to the low level of endogenous RNA compared to exogenously expressed MRP RNA and also due to the specificity of the probe used (see uninjected oocyte; lane u of Fig. 2). Newly transcribed RNA could be analyzed by injection of radiolabeled nucleotides in addition to the gene; the result of this procedure was similar to that of Northern analysis (data not shown). Fig. 2 shows that the upstream elements of mouse gene are important for the high level of transcription in this heterologous expression system. High levels of transcription were observed after injection of the gene with 840 nucleotides of upstream sequences (lane 1). Deletion of sequences between −840 to −320 resulted in slight reduction of transcription efficiency (lane 2); however, this reduction was restored by more deletion to −239 (lane 3). NRF-1 binding site and a putative Sp1 site are positioned between −320 to −239, but the mechanism of this phenomenon is not known. Deletion to −190 completely removes the octamer as well as the adjacent putative Sp1 site, causing a significant reduction in transcription (lane 4). Further deletion to −120 did not show any more reduction in transcription

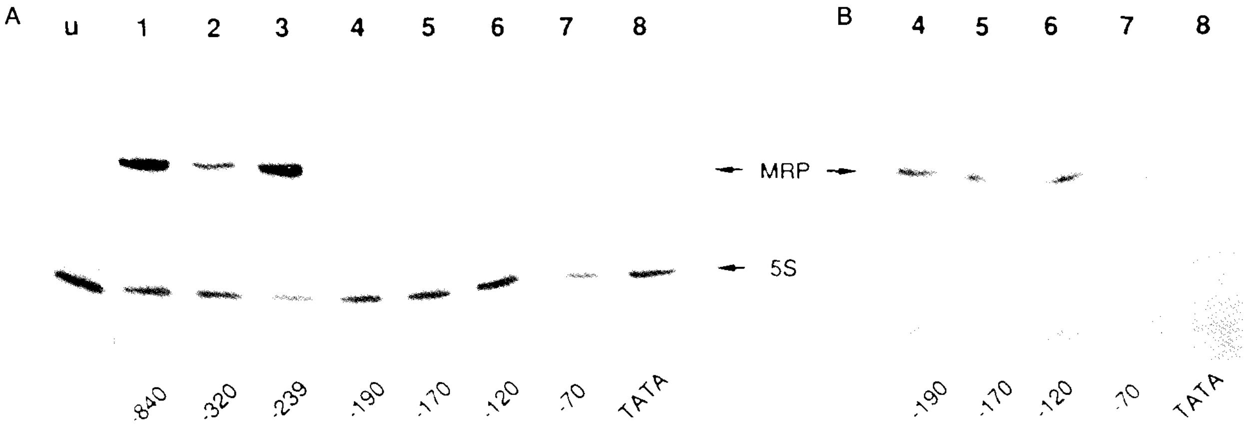

Fig. 2. Transcription of mouse MRP RNA gene in *Xenopus* oocytes. Various promoter mutants are indicated by 5′-deletion points (-840 to -70) and substitution region (TATA). Lane u represents uninjected oocyte control. Locations of MRP RNA and 5S rRNA bands are indicated. Part of panel A (lanes 4-8) was over-exposed to detect low level transcription of MRP RNA in panel B.

efficiency; however, more deletion to − 70 abolishes the basal level transcription.

Deletion of all elements upstream of − 190 reduced transcription of the mouse MRP RNA gene to the basal level, which was too low to be detected in panel A of Fig. 2. However, longer exposure of the same gel shows that basal level transcription is not affected until the PSE is deleted (lane 7 of Fig. 2B). In order to determine if the TATA box is a functional promoter element, a linker scanning mutation in the sequences of − 31 to − 25 (TCATAAA) was generated to alter it to GCGGGC. This mutation completely eliminates transcription, suggesting an importance of TATA box in the transcription of MRP RNA gene. These results generally agree with the data of transfecting these series of promoter deletion in mouse MRP RNA gene into the mouse cells (Michelotti and Clayton, unpublished data). It further shows that *Xenopus* cells might have similar proteins for transcription of mouse MRP RNA genes. More transfection experiments also showed that the intragenic control element (Box A) was not necessary for MRP RNA transcription (data not shown), even though this gene was transcribed by Pol III.

Export of MRP RNA

Heterologous mouse MRP RNA gene can be correctly transcribed by frog protein machinery as shown above. Therefore, next question would be whether heterologous mouse RNA can be correctly processed thereafter. The frog gene has been shown to be efficiently expressed and a majority of its RNA seemed to be exported out to the cytoplasm after microinjection (Bennett et al., 1992). However, mouse MRP RNA was retained in the nucleus even though its transcription was efficient (Jeong-Yu et al., 1996). To identify the cis-acting factors involved in this species-specific export, various hybrid genes of *Xenopus* and mouse MRP RNA were constructed as in Fig. 3. All of hybrid constructs were designed to have a junction in the middle of the gene (around 108) to have 5′-half of one gene (5′-flanking region and/or 5′-coding region) and 3′-half of other gene (3′-coding and 3′-flanking regions) (see Fig. 1). This junction was chosen for two reasons: one reason is not to disrupt the predicted pseudoknot formation (see Fig. 1) which seems to be important for the secondary structure of MRP RNA (Topper and Clayton, 1990b) and the other reason is for various hybrids to have similar sizes (about 275-280 nts) of RNA.

Seven different hybrid DNAs were injected into the nuclei of *Xenopus* oocytes, allowed to express for 12-18 h and their transcripts were analyzed by Northern blot hybridization (Fig. 4A). Nuclei were manually dissected out from the cytoplasm and MRP RNA from these two compartments were visualized by the 5′-probe which could detect mouse and *Xenopus* RNAs with similar sensitivity. Injection of *Xenopus* gene (X) resulted in the transport of expressed RNA to the cytoplasm, whereas mouse gene (M) failed to produce exported MRP RNA to the cytoplasm as previously shown. Hybrid gene with 5′-flanking region and 5′-coding sequences of *Xenopus* (XM) showed similar level of export to the cytoplasm as *Xenopus* gene (X) did. In contrast, hybrid gene with 5′ of mouse genes (MX) was incapable of exporting MRP RNA to the cytoplasm just as mouse gene was (M). Smaller fragments were observed in cytoplasmic fractions (C lane in Fig. 4A), which might come from more degradation of RNA in cytoplasm than in nucleus. These data suggest that there must be signal(s) for export of MRP RNA in the 5′-region of *Xenopus* gene. The 5′-region includes 5′-flanking upstream promoter region and 5′-half of coding sequences; thus, it would be critical to divide these two regions to test whether promoter and/or coding region is important for export. More hybrid constructs (XpM, MpX and MpXM) were generated to have a promoter from one DNA and the 5′-coding region from the other. Injection of these DNA showed that the export of these hybrid

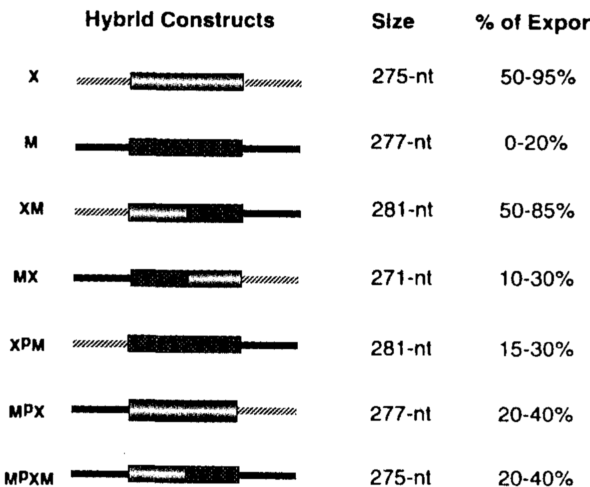

Fig. 3. Structure and size of various mouse/*Xenopus* hybrid MRP RNA genes. Percentages of export following injection of hybrid genes are also shown. Dark lines, non-coding regions of mouse gene; light lines for those of *Xenopus* gene. Dark box, coding region of mouse gene; light box, coding region of *Xenopus* gene.

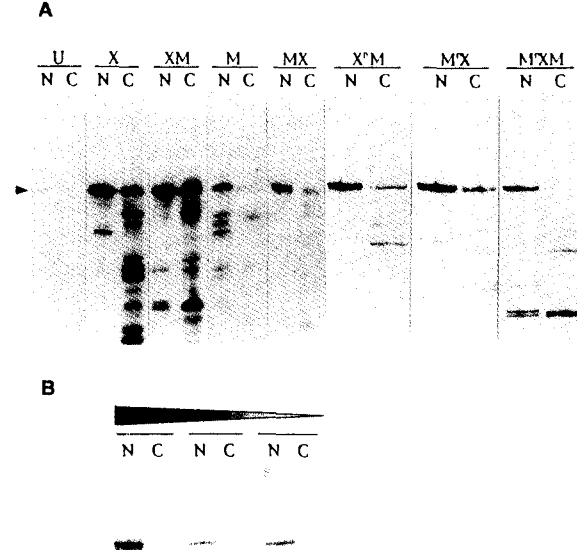

Fig. 4. Transcription and export of various mouse/*Xenopus* hybrid genes. A, Various hybrid genes were injected and incubated 12-18 h in oocytes. Northern blot analysis was performed to visualize MRP RNA. It is indicated by small arrow on the left side of the gel. Degradation products are also shown as smaller fragments. Refer to Fig. 2 for the structures of hybrid constructs. U, uninjected; X, *Xenopus;* M, mouse; N, nuclear fraction; C, cytoplasmic fraction. B, Varying amounts of mouse gene were injected. Following incubation for 12-18 h, nuclear (N) and cytoplasmic (C) fractions were separated and MRP RNA was visualized. Amount of injection is following: from left to right, 20 ng, 1 ng and 0.1 ng DNA per nucleus.

RNAs were intermediate levels, suggesting that the high level of export with *Xenopus* 5′-region was by combined effect of both upstream promoter region and 5′-coding region of about 110 nucleotides. More work needs to be done to identify the factors involved in this phenomenon.

One of the possibilities for the low export level of mouse RNA is simply the low transcription level of mouse gene in *Xenopus* oocytes. To test this question, various amounts of mouse DNA were injected and their levels of export were examined in Fig. 4B. Since it is generally believed that the amount of injected DNA is proportional to the level of transcription in *Xenopus* oocytes, 10-fold more mouse DNA (20 ng of DNA per nucleus) was injected and compared to the usual concentration of 1-2 ng for each nucleus was injected. Even though high concentration of mouse gene produced high level of transcripts as *Xenopus* DNA did, almost all of mouse RNA was retained in the nucleus (see lanes 1 and 2 of Fig. 4B). Similarly, when the amount to *Xenopus* DNA was reduced down to 0.1 ng per nucleus, the level of export was not changed (data not shown). These results suggest that the level of transcription is independent of the level of export, therefore the defect of mouse MRP RNA export is genuinely related to the 5′-upstream region and 5′-coding sequences, not to the promoter strength of MRP RNA gene.

Relationship of transcription and export of MRP RNA

To study the transport of MRP RNA in previous experiments, the gene for MRP RNA was injected into oocytes to transcribe nascent RNA *in vivo*, rather than *in vitro* transcription followed by microinjection. The reason for this is that it would be natural to have every biological transcriptional and post-processes transcriptional in the same cell not to miss any coupled processes. However, as mentioned above, export seems to be regulated by the 5′-upstream promoter region, suggesting two possibilities. One is the importance of upstream sequence itself which might be bound to protein(s) directing MRP RNA to the nuclear pore complex to direct it to the cytoplasm. The other more plausible possibility is that transcription machinery is in some way related to the export proteins, therefore transcription and export are coupled processes. To test these possibilities, two approaches were used to uncouple transcription from export and to see if the separation has any effects on the high export level of *Xenopus* MRP RNA. First approach is to stop transcription with the RNA polymerase inhibitor and examine the transport of previously transcribed RNA. The logic for this experiment is the following. First, *Xenopus* gene is injected as usual, let it be transcribed by endogenous RNA polymerase for 1 h and then stop further transcription by re-injecting high level of α-amanitin into the same cell to inhibit Pol III activity. Oocytes were further incubated for various time, to test whether transcription-inhibited RNA can be exported as usual. Fig. 5A showed that injection of DNA resulted in the much higher level of exogenous MRP RNA (compare injection - to injection +) during 1 h incubation. The α-amanitin injected oocytes have similar level of MRP RNA compared to α-amanitin - lanes, suggesting an effective

inhibition by the drug. The distributions of MRP RNA in nucleus (N lanes) and cytoplasm (C lanes) were not changed during 3 h of incubation after second injection (4 h after initial DNA injection). Further incubation up to 18 h did not increase the level of export in α-amanitin injected oocytes. In contrast, when water was injected instead of α-amanitin as a second injection, export of *Xenopus* MRP RNA was as efficent as previously seen (data not shown). Therefore, α-amanitin inhibition of transcription also reduce the otherwise efficient export of *Xenopus* MRP RNA.

Second method for uncoupling is injecting previously transcribed RNA, instead of injecting the gene for RNA. To do it, two sequential injection was done: first, DNA for *Xenopus* MRP RNA was injected and incubated for 24 h to get high expression of RNA *in vivo*. During the first injection, it was expected that some biological modifications on newly transcribed MRP RNA occur. Then, nuclear RNA was purified from injected oocytes; during purification, it is expected that any RNA binding

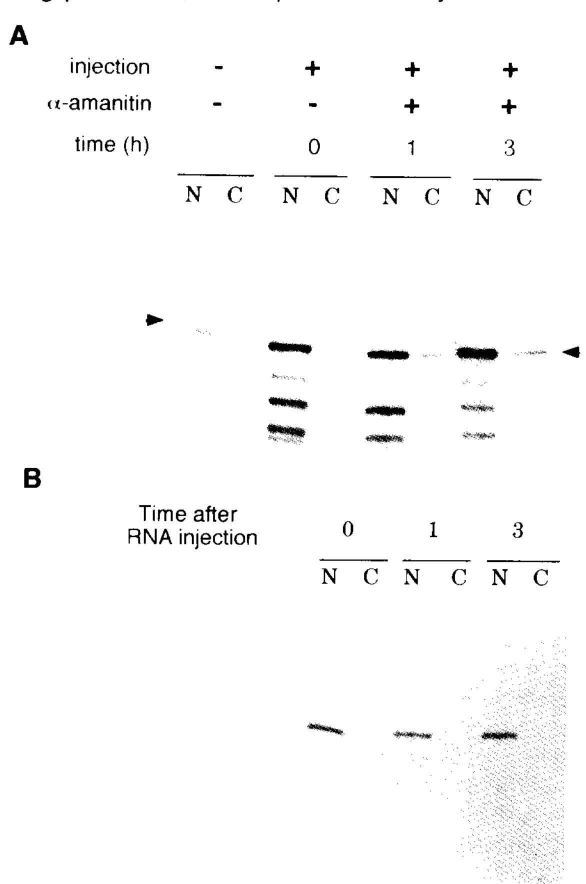

Fig. 5. Uncoupling of export from transcription. A, After injection of *Xenopus* gene, α-amanitin was injected to stop further transcription and newly transcribed MRP RNA was followed in nuclear (N) and cytoplasmic (C) fractions of oocytes. Location of MRP RNA is indicated by the arrow. Injection of DNA is indicated (injection) in top line, α-amanitin injection (α-amanitin) in second line and the time after α-amanitin injection is indicated in third line. B, Injection of previously transcribed *Xenopus* MRP RNA into the nucleus. Incubation time after RNA injection is indicated.

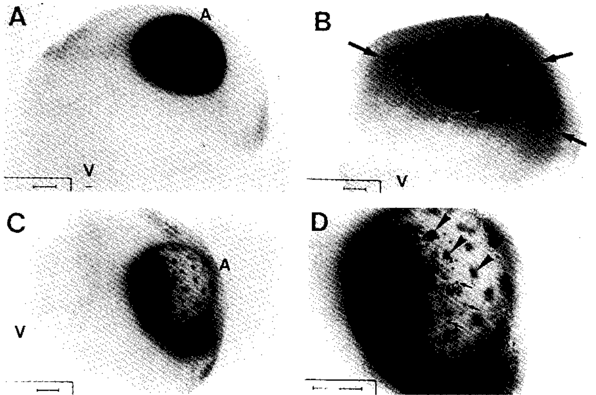

Fig. 6. Whole-mount *in situ* hybridization of injected oocytes. A, Mouse MRP RNA gene injection. Oocytes were incubated for 24 h after injection. Animal pole (A) and vegetal pole (V) of stage VI oocyte are indicated, therefore nucleus is in animal pole as stained by MRP RNA probe and yolky cytoplasm is in vegetal hemisphere. Clear retention of injected mouse RNA in nucleus is shown. B, *Xenopus* MRP RNA gene injection. Boundary of nucleus is indicated by small arows and diffused signal of *Xenopus* RNA to the cytoplasm around nucleus is indicated by large arrows. C, α-amanitin injected oocyte after *Xenopus* gene injection. In contrast to panel B, MRP RNA signal is refined to nucleus. D, Higher magnification of panel C. Locations of multiple, amplified nucleoli are indicated by small arrows. Clear accumulation of injected RNA is visulized as large plaque in the periphery of the nucleus (Arrowheads). Scale bars=10 μm.

proteins are expected to be removed, but any modifications of RNA to be maintained. Purified RNA from DNA injection was re-injected into the nucleus of second set of oocytes and its transport pattern was examined in Fig. 5B. *Xenopus* MRP RNA injected into the nucleus was retained in the nucleus (see N lanes of Fig. 5B), unlike DNA injection (see X injection in Fig. 4A). These experiments suggest that uncoupling of transcription either by transcription inhibition or by physical separation leads to accumulation of normally exporting MRP RNA in the nucleus.

Intracellular locations of injected RNA can be easily visualized by whole-mount *in situ* hybridization (Fig. 6). It clearly shows the exclusive localization of injected mouse RNA in the nucleus (Fig. 6A), compared to nuclear and cytoplasmic localization of injected *Xenopus* RNA (Fig. 6B). In this micrograph, endogenous *Xenopus* MRP RNA is not detectable due to its low level (data not shown). In Fig. 5A, injection of α-amanitin inhibits export of RNA as well as its transcription, as determined by cell fractionation and Northern blotting. The same set of oocytes used in Fig. 5A was also prepared to visualize RNA by *in situ* hybridization in Fig. 6C. It shows the retention of injected exogenous *Xenopus* MRP RNA in nucleus, in contrast to α-amanitin non-treated cells in Fig. 6B. More interestingly, it shows clear accumulation of newly-transcribed RNA in the periphery of nucleus, presumably near the nuclear pore complex (large arrowheads in Fig. 6D). Exact identity of these plaques were not examined, but it might represent an intermediate stage of transport blocked during its exit to cytoplasm. Some of MRP RNA is also localized to the multiple nucleoli (indicated by small arrows), as expected. Therefore, these *in situ*

analyses provide clear visual demonstration of nucleocytoplasmic transport of MRP RNA. Especially due to its large size, intranuclear localization of RNA can also be clearly discerned as in Fig. 6.

Discussion

In this report, the promoter of mouse MRP RNA was analyzed to determine regulatory elements necessary for basal and elevated levels of transcription. TATA box sequences seem to be a necessary element for basal level transcription, even though MRP RNA is transcribed by RNA Pol III (Yuan and Reddy, 1991). Moreover, the usual Pol III-type intragenic regulatory element Box A is not required. This result is consistent with the presence of Pol II and Pol III composite promoters (Das et al., 1988) and common subunits for previously separately regarded transcription machineries. For the MRP RNA gene, PSE seems to be necessary for basal level transcription, and DSE including Sp1 and octamer binding site is needed for elevated transcription.

During the transcription study, an interesting observation was made when the behaviors of mouse and *Xenopus* MRP RNA were compared. Even though the level of transcription was not that different for either species of RNA in *Xenopus*, transport of RNA was vastly different. A majority of *Xenopus* MRP RNA was transported out to the cytoplasm, but, most of mouse RNA was retained in the nucleus. Various hybrid RNAs were examined for their transportability in *Xenopus* oocytes, suggesting an importance of 5′-upstream region and/or 5′-half of coding regions for transport to cytoplasm. This opens interesting possibilities for the mechanism of export after transcription in the nucleus. First possibility is that the sequences in promoter and/or 5′-coding region are bound by protein(s) to direct exportable genes to the nucleus pore complex for transport. In this case, transcription process itself may or may not be coupled for export. Second possibility is that protein factors bound to the promoter sequences are interacting with transcription machinery via protein-protein interactions to make a transcription-export complex. If this is the case, export is in some way coupled to the transcription; export can occur where transcription occur in the nucleus, therefore the location of MRP RNA transcription is closer to the nuclear pore complexes where export occur. *In situ* analysis suggest that the transcription in nucleus are fairly well refined in the specific regions of the nucleus, making it a plausible hypothesis. Third possibility could be the maturation of 5′-portion of RNA, including cap structure and secondary structure of RNA is regulated by factors bound to 5′-regions of gene and these structural features direct RNA to export as other U-series RNA does (Hamm and Mattaj, 1990). One of the candidate protein for MRP RNA is 40-kDa Th autoantigen which binds in 5′-region of RNA. However, recent experiment deleting the binding site for Th antigen did not show any effect on the export of MRP RNA, thus making it unlikely (Jeong-Yu et al., 1996). At this point, no experiments were performed to distinguish these possibilities, but the experiments using agents that could uncouple transport from transcription suggest that export is related to the transcription in time and space. More work needs to be done to understand its mechanism.

The cellular location of MRP RNA has been a great deal of debate, because RNase MRP, the ribonucleoprotein complex of MRP RNA, has been originally identified for the mitochondrial RNA processing activity, but later studies suggested that MRP RNA is as same as nucleolar 7-2 RNA (Yuan et al., 1989). These results can be interpreted by assuming the dual localization and activities for RNase MRP. Biochemical fractionation showed that the majority of MRP RNA is in the nucleus (Kiss et al., 1992), but a more detailed analysis of cellular location of RNA with *in situ* hybridization (Li et al., 1994) suggests the dual localization in nucleus and mitochondria. Using *Xenopus* oocytes of various stages, a small portion of MRP RNA was found to be localized in the perinuclear mitochondrial network, especially where the mitochondrial biogenesis is active, but not in other mitochondria in the cell (Davis et al., 1995). This suggests that RNase MRP can be localized only to the specific mitochondria where DNA replication is active. However, in most somatic cells where mitochondria are not actively replicated, major location of RNase MRP seems to be in nucleus, especially in nucleolus (Reimer et al., 1988). In fact, recent genetic and biochemical studies suggested the role of RNase MRP in the processing of 5.8S rRNA in nucleolus (Schmitt and Clayton, 1993; Lygerou et al., 1996).

Whatever the final location of RNase MRP, its intracellular transport seems to have a cytoplasmic phase as an intermediate stages after synthesis in the nucleus. Microinjection as well as *in situ* hybridization experiments in this study showed that the large percentage of *Xenopus* RNA can be exported out to the cytoplasm. Whole-mount *in situ* hybridization allows quick and easy localization of injected RNA, especially intra-nuclear sites for transcription and/or export which could provide interesting clues to the nucleocytoplasmic transport of RNA.

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