

Methylphosphate cap structure increases the stability of 7SK, B2 and U6 small RNAs in *Xenopus* oocytes

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ABSTRACT

We studied the role of the methylphosphate cap structure in the stability and nucleocytoplasmic transport by microinjecting U6, 7SK and B2 RNAs into the *Xenopus* oocytes. In every case, the methylphosphate capped RNAs were 3 to 9 times more stable than the uncapped RNAs. When a methylphosphate cap structure was placed on human H1 RNA which is normally not capped, its stability was improved 2–7 fold. These data show that the methylphosphate cap enhances the stability of 7SK, B2, H1 and U6 RNAs. The methylphosphate-capped 7SK RNA was transported into the nucleus from cytoplasm, but remained in the nucleus when injected into the nucleus; in this respect, 7SK RNA exhibited properties previously shown for U6 RNA. Both U6 and 7SK RNAs with ppp on their 5' ends were transported from cytoplasm to the nucleus suggesting that the methylphosphate cap structure is not required for transport of these RNAs across the nuclear membrane.

INTRODUCTION

Eukaryotic messenger RNAs, and many small RNAs have their 5' ends blocked by cap structures (reviewed in 1–4). The cap structures contain m⁷G, m^{2,7}G or m^{2,2,7}G (TMG) and these structures play important roles in RNA and protein biosynthesis. The m⁷G cap structure in mRNAs has been shown to be important in splicing of pre-mRNAs, stability of mRNAs, and in the translation of mRNAs (1,2,5). The TMG cap structure in small RNAs also improves the stability of small RNAs (6,7) and in the case of U1 and U2 RNAs, has an essential role in transport across the nuclear membrane in *Xenopus* oocytes (8,9). The requirement of TMG cap for import into the nucleus is not universal because in mammalian cells, the TMG cap is not required for the import of U2 RNA (10). In addition, import of U5 RNA into the nucleus of *Xenopus* oocytes is not dependent on the TMG cap (10). These data suggest that small RNAs contain multiple signals for nuclear import and TMG cap is one of the signals. In eukaryotic cells, U6 (11), 7SK (12,13), rodent B2 (12), and plant U3 (14) small RNAs are known to contain the methyl-pppG/A (methylphosphate) cap structure. All known

methylphosphate-capped RNAs are transcribed by RNA polymerase III (4), in contrast to TMG-capped RNAs which are transcribed by RNA polymerase II (3).

The methylphosphate-capped RNAs are implicated in diverse cellular functions. U6 snRNA, in which the methylphosphate cap structure was initially identified (11), participates in the splicing of nuclear pre-mRNAs (15,16). Unlike TMG-capped U1 and U2 RNAs which are rapidly transported to the cytoplasm after synthesis, U6 RNA does not leave the nucleus of frog oocytes (7). In the frog oocytes, the methylphosphate cap in the U6 RNA is not required for the transport from the cytoplasm to the nucleus (10). 7SK RNA is an abundant small RNA containing methylphosphate cap on its 5' end (12,13); the function of this 330 nt long RNA is not known. The 180 nucleotide-long B2 RNA of unknown function is homologous to the B2 short interspersed repeats and appears to be present only in rodent cells (17). B2 RNA is found both in polyadenylated (poly(A) size ranging from 200 to 400 nucleotides) and nonpolyadenylated forms. Both polyadenylated and nonpolyadenylated B2 RNAs contain the methylphosphate cap structure at their 5' ends (12). B2 RNA can be found in the nucleus and cytoplasm of the mouse cells, most of the RNA being in the cytoplasm (18). U3 snRNA is involved in the processing of pre-ribosomal RNA and contains methylphosphate cap structure in plants and TMG cap structure in animal cells (14). In plants, the methylphosphate-capped U3 RNA was found associated with pre-rRNPs, whereas the TMG cap-containing U3 RNA was unable to associate with pre-rRNPs (19).

In this report, we describe the stability and transport of capped and uncapped U6, 7SK and B2 RNAs in the frog oocytes. We demonstrate that the absence of the methylphosphate cap in these three RNAs leads to decreased stability ranging from 3 to 9 fold. The methylphosphate cap structure did not affect the transport of any of these RNAs across the nuclear membrane.

MATERIALS AND METHODS

Plasmids

The mouse U6 (20), human 7SK (21) and mouse B2 (22) genes were used for generation of plasmids suitable for transcription by T7 RNA polymerase. The T7 promoter sequences and Dra

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I restriction site at the 3' ends of the genes were introduced by polymerase chain reaction. A human H1 (RNaseP RNA) gene under T7 promoter with Sma I restriction site at the 3' end of the gene was a gift from C. Guerrier-Takada, Yale University (23).

RNA synthesis *in vitro* with T7 RNA polymerase

The plasmid DNAs were linearized by cutting at the introduced restriction sites. 3–5 μ g of linearized DNAs were incubated in a 50 μ l of transcription buffer (Epicentre Technologies Corp., Madison, WI), 10 mM DTT, 0.5 mM each of ATP, CTP, UTP, 0.04 mM GTP, 50 U of T7 RNA polymerase, 50 U of RNasin, 50 μ Ci [α - 32 P]-GTP (Amersham Corp., Arlington Heights, IL) for 60 min at 37°C. Water was added to a final volume of 450 μ l, proteins were extracted with phenol-chloroform (pH 8), chloroform and RNAs were then precipitated with 3 vol of ethanol (including 20–40 μ g of glycogen as a carrier, Boehringer Mannheim Biochemicals, Indianapolis, IN). For the preparation of capped RNAs, 4 mM γ -monomethyl GTP (synthesized from GTP as described in Ref. 24) was included in the transcription reactions. Labeled RNAs were fractionated on 10% denaturing polyacrylamide gels, bands of interest were excised and RNA was extracted from the gel pieces by incubation in 0.3% SDS, 0.1 M NaCl, 50 mM sodium acetate (pH 5.3) at 37°C with shaking overnight. RNAs were again purified by extraction with phenol-chloroform, chloroform and precipitated with 3 vol of ethanol including 20–40 μ g of glycogen as carrier. RNAs were suspended in 10 μ l of sterile water and used for injection into frog oocytes.

Labeling of HeLa cell RNAs *in vivo*

For preparation of uniformly labeled RNA, HeLa cells were incubated at 37°C with [32 P]-orthophosphate for 16 hours in phosphate-free medium (25). The 4–8S RNA was prepared by centrifugation of the whole cell RNA on a sucrose density gradient and pooling the fractions corresponding to the 4–8S RNAs (26).

Oocyte injections

Approximately similar amounts of radioactivity of each of the *in vitro*-synthesized RNAs were mixed so as to obtain similar signal intensities on films during autoradiography. After a low speed centrifugation of the oocytes to facilitate accurate injection, approximately 20 nl were injected into each oocyte nucleus. For the cytoplasmic injections, 20–60 nl were injected into the vegetal half of oocytes. Total, nuclear or cytoplasmic RNA was isolated using the procedure described by Fischer et al. (10). Oocytes were dissected manually in J-buffer (70 mM NH₄Cl, 7 mM MgCl₂, 0.1 mM EDTA, 2.5 mM DTT, 20 mM Tris-HCl [pH 7.5], 10% glycerol) under a dissecting microscope and suspended in homomedium (50 mM Tris-HCl [pH 7.5], 5 mM EDTA, 1.5% SDS, 300 mM NaCl). 1.5 mg/ml of proteinase K was added and mixtures were incubated for 15 min at 37°C. RNAs were extracted with phenol-chloroform, chloroform and precipitated with 3 vol of ethanol (30 μ g/ml of tRNA was added to the nuclear RNA). Extracted RNA was analyzed on denaturing 10% polyacrylamide gels, and usually 0.5–4 oocyte equivalents of RNA were loaded on polyacrylamide gels. Immunoprecipitations using Pansorbin (Calbiochem Corp., La Jolla, CA) were carried out as described by Lerner and Steitz (27). RNAs were purified and analyzed on denaturing 10% polyacrylamide gels. Radioactivity in dried gels

was quantified using the Betascope 603 blot analyzer system (Betagen, IntelliGenetics Inc., Mountain View, CA).

For capping analysis, RNAs were digested with nuclease P1 and in some cases also with bacterial phosphatase and then subjected to electrophoresis on DEAE-cellulose paper, dried and subjected to autoradiography.

RESULTS

Capped U6, 7SK, B2 and H1 RNAs are more stable than their uncapped counterparts

To study the role of the methylphosphate cap structure in the RNA stability, we chose U6, 7SK and B2 RNAs that naturally have methylphosphate cap on their 5' ends and also H1 RNA

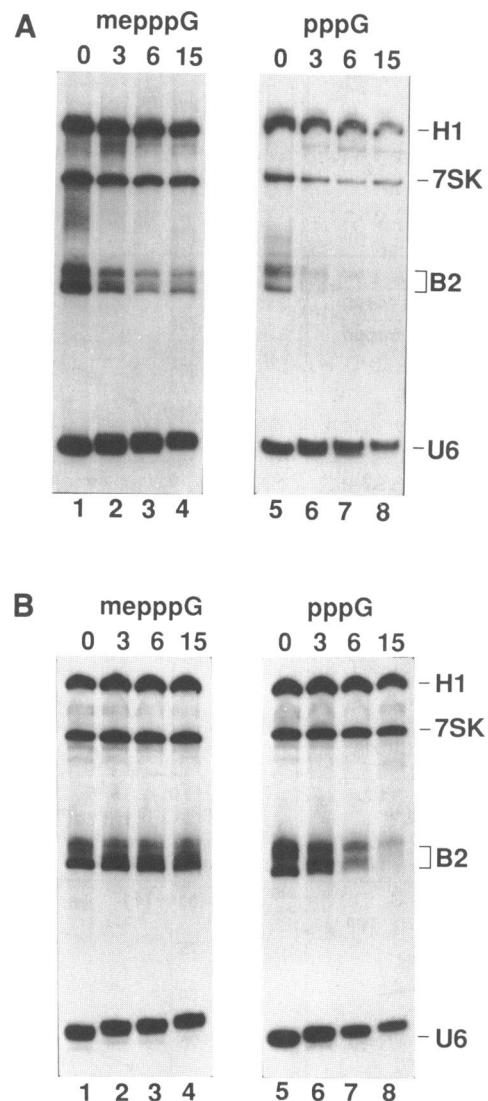


Figure 1. Determination of the stability of four small RNAs with and without the methylphosphate cap structure. Frog oocytes were injected with ~20 nl of a mixture containing capped (mepppG) or uncapped (pppG) U6, 7SK, B2 and H1 RNAs into the nucleus (A) or cytoplasm (B). Total RNAs from groups of five oocytes were isolated after 0, 3, 6 and 15 hrs of incubation, purified, fractionated on denaturing 10% polyacrylamide gels, dried and subjected to autoradiography. The period of incubation (in hours) of frog oocytes with injected RNAs is shown on top of each lane.

that normally has a triphosphate on its 5' end. RNAs containing methyl-pppG or pppG were synthesized *in vitro* and incorporation of methyl-pppG into RNAs was monitored by digestion of the synthesized RNAs with appropriate nucleases and subjecting the products to chromatography; analysis of the data showed that over 90% of the RNA molecules contained the methylphosphate on their 5' ends (data not shown). A mixture of four capped RNAs or uncapped RNAs was injected into the *Xenopus* oocyte nuclei. After incubation of the injected oocytes for various time periods, total RNA was isolated, purified, fractionated on a denaturing polyacrylamide gel and subjected to autoradiography

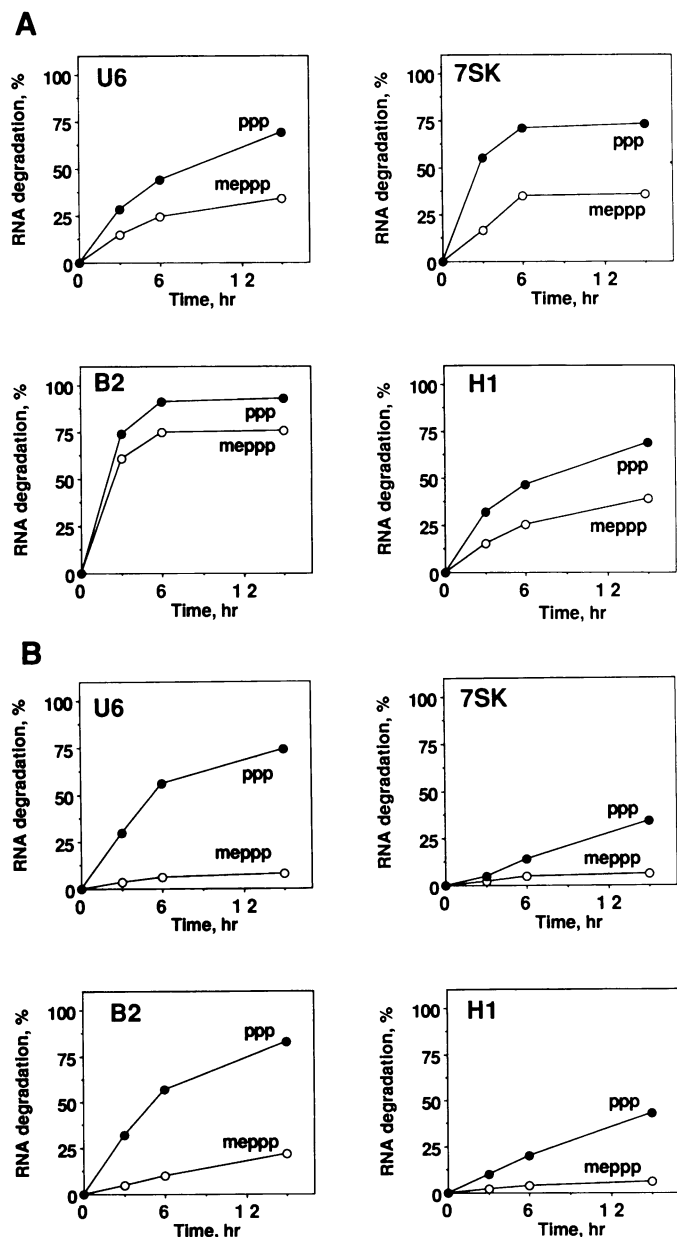


Figure 2. Quantitation of RNAs remaining after incubation of frog oocytes for different time periods. The radioactivity in different RNA bands shown in Fig. 1 was quantified using a Betagen counter and the activity at 0 time is taken as 100. The percentage of radioactivity degraded after incubation for different time periods is shown in the form of graphs. The values used are averages from three independent experiments. Fig. 2A and 2B are related to Fig. 1A and 1B, respectively.

(Fig. 1A). All the RNAs containing methylphosphate cap structure were more stable compared to the corresponding uncapped RNAs (Fig. 1A, compare lanes 1–4 with lanes 5–8, respectively). Since scanning autoradiograms after exposure for any one time period may not provide actual quantitation, we used Betascope to obtain actual counts in each band and the fraction of RNA degraded at different time periods is shown in Fig. 2A. In the case of U6, 7SK, H1 and B2 RNAs, there was less degradation of capped RNAs when compared to their uncapped counterparts, up to 15 hrs, the longest time period tested. Of all the RNAs tested, B2 RNA was the most unstable with more than 60% of the injected RNA degraded in 3 hrs (Fig. 2A).

The RNAs were also injected into the cytoplasmic compartment of the frog oocytes and the stability of these RNAs was monitored (Fig. 1B). The capped RNAs were very stable when injected into the cytoplasm with less than 10% of the radioactivity degraded even after 15 hrs. Only in the case of capped B2 RNA, there was marginal (15–20%) RNA degradation. All four RNAs were less stable in the absence of the cap structure (Fig. 1B and Fig. 2B). Here again, the B2 RNA was the least stable, with over 80% of the RNA degraded after 15 hrs after injection. Uncapped U6 RNA was also rapidly degraded when injected into the cytoplasm with 55% of the RNA degraded in 6 hrs and 75% degraded in 15 hrs. 7SK and H1 RNAs without the cap structure were relatively more stable with less than 20% of the RNA degraded in 6 hrs (Fig. 2B). All four RNAs were more stable in the cytoplasm than when the corresponding RNAs were injected into the nucleus. The increase in stability was most pronounced in the case of U6 RNA injected into the cytoplasm; in contrast to about 8% loss of capped U6 RNA after 15 hrs, there was 75% loss in the case of uncapped RNA. This is a 9-fold improvement in the stability of U6 RNA. In the case of other RNAs, the stability was improved by 3-fold to 7-fold (Fig. 2B). The stability of H1 RNA, which normally has a triphosphate on its 5' end, was also increased when a methylphosphate cap was introduced on its 5'-end (Figs. 1 and 2). These data show that the protective function of methylphosphate cap is not restricted to RNAs that usually contain the methylphosphate cap structure. There were no RNAs of intermediate size at any time point (Fig. 1A, 1B), indicating that RNAs are rapidly degraded to completion once they are targeted for degradation. The observed increase in the stability of U6, 7SK and B2 RNAs supports the

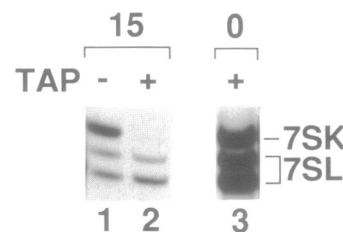


Figure 3. Stability of pyrophosphatase-treated 7SK and 7SL RNAs labeled *in vivo*. Human 7SK and 7SL RNAs treated with tobacco acid pyrophosphatase (TAP +) or untreated RNAs (TAP -) were injected into the nuclei of frog oocytes. After 15 hours of incubation, total RNA was isolated, fractionated on denaturing 10% polyacrylamide gels and subjected to autoradiography. The period of incubation (in hours) of frog oocytes with injected RNAs is shown on top of each lane.

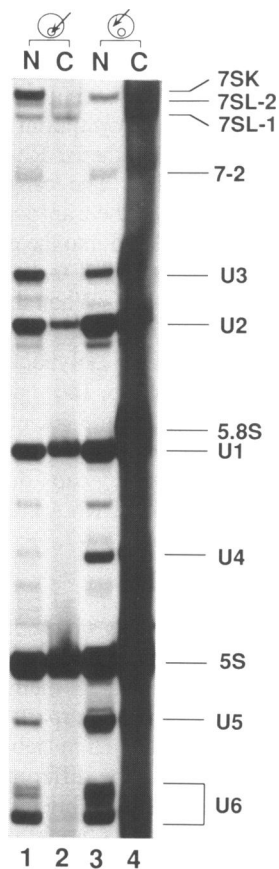


Figure 4. Transport of snRNAs across the nuclear membrane in frog oocytes. HeLa 4–8S RNAs labeled *in vivo* with [32 P]-phosphate were injected into the nucleus or cytoplasm of *Xenopus* oocytes. After 15 hours of incubation, nuclei (N) and cytoplasm (C) from groups of five oocytes were separated and RNA from each fraction purified, fractionated on a 40 cm long denaturing 10% polyacrylamide gel, dried and subjected to autoradiography.

hypothesis that methylphosphate cap structure protects these RNAs from degradation by 5' exonucleases.

To confirm this observation further, the methylphosphate cap structure was removed from the 5' end of the human 7SK and 7SL RNAs labeled *in vivo* with [32 P]-phosphate. 7SK and 7SL RNAs were treated with tobacco acid pyrophosphatase to remove the methylphosphate cap structure. The completion of pyrophosphatase reaction was monitored by direct analysis of the 5' ends (data not shown). 7SK and 7SL RNAs were co-injected into the nuclei of frog oocytes and after 15 hours of incubation, total RNA was isolated and fractionated on a polyacrylamide gel and subjected to autoradiography. Figure 3 shows that 7SK RNA with pG as its 5' end was less stable (lane 2) than 7SK RNA with the methylphosphate cap structure (lane 1). However, the stability of 7SL RNA was not affected by this treatment (compare Fig. 3, lane 1 with lane 2) demonstrating that the stability of 7SL RNA is the same with pppG or pG as its 5' end. The stability of U6 RNA from HeLa cells also decreased by treatment with pyrophosphatase (data not shown). Thus, similar results were obtained with the *in vitro* and *in vivo* synthesized RNAs showing that methylphosphate cap structure plays an important role in improving the stability of U6, 7SK and B2 RNAs.

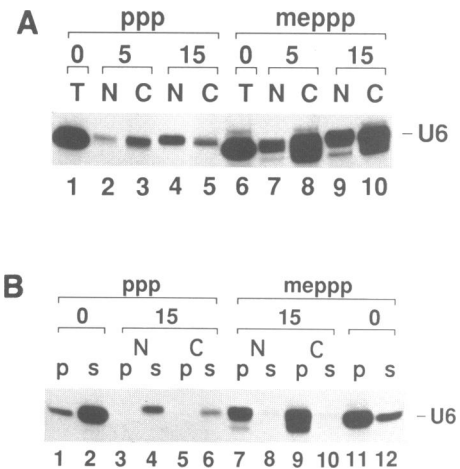


Figure 5. (A) Transport of capped and uncapped U6 RNA from the cytoplasm to the nucleus of frog oocytes. 20 nl of labeled U6 RNA with pppG or mepppG on its 5' end were injected into the cytoplasm of frog oocytes. The nuclei and cytoplasm were separated after 5 or 15 hours and purified RNAs fractionated on polyacrylamide gels and visualized by autoradiography. (B) Immunoprecipitation of the RNAs with anti-methylphosphate cap antibodies. The RNAs analyzed in Fig. 5A were immunoprecipitated with anti-methylphosphate cap antibodies. The antibody-bound RNA was recovered in the pellet (p) and the uncapped RNA was recovered from the supernatant (s). The period of incubation (in hours) of frog oocytes with injected RNAs is shown on top of each lane. T, N, C, RNA extracted from total oocytes or from nuclear or cytoplasmic fractions, respectively.

Nucleocytoplasmic transport of 7SK snRNA

In HeLa cells, U6 and 7SK RNAs are the most abundant RNAs containing the methylphosphate cap structure. Initially, HeLa cell 4–8S RNA uniformly labeled *in vivo* with [32 P]-phosphate was injected into the nucleus or cytoplasm of oocytes and the transport of small RNAs across the nuclear membrane was monitored after 15 hours of incubation. U4, U5 as well as U6 snRNAs stayed in the nucleus when injected into the nucleus (Fig. 4, lanes 1 and 2) and were transported into the nucleus when injected into the cytoplasm (Fig. 4, lane 3). These data are identical to those reported by De Robertis et al. (28). Since the purpose of this experiment was to see the transport of minor 7S-sized RNAs, the autoradiogram was overexposed to see whether any of these RNAs migrate from nucleus to cytoplasm or vice versa. Among the 7S-sized RNAs, the 7SL RNA remained in the cytoplasm when injected into the cytoplasm and did not migrate to the nucleus (Fig. 4, lanes 3 and 4) and migrated to cytoplasm when injected into the nucleus (Fig. 4, lane 2). This is consistent with the established cytoplasmic localization of 7SL RNA. 7SK RNA and 7–2 RNA behaved like U5 and U6 snRNAs in that they remained in the nucleus when injected into the nucleus and were transported into the nucleus when injected into the cytoplasm (Fig. 4). These data show that 7SK RNA behaves like U6 RNA in its nucleocytoplasmic transport and confirm the nuclear localization for 7SK RNA.

Transport of U6 and 7SK RNAs from cytoplasm to the nucleus of frog oocytes does not require the methylphosphate cap structure

Unlike U1 and U2 RNAs which are rapidly transported to the cytoplasm after synthesis, U6 RNA appears to remain in the nucleus (7); however, after microinjection into the cytoplasm of

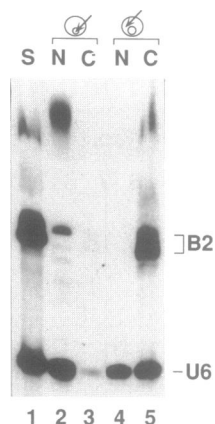


Figure 6. Migration of B2 RNA across the nuclear membrane. T7-transcribed B2 RNA with methylphosphate cap on its 5' end was injected into the nuclei or cytoplasm of frog oocytes. Lane 1, RNA extracted from total oocytes immediately after injection (S); lanes 2 and 4, RNA from nuclear (N) or lanes 3 and 5, RNA from cytoplasmic (C) fractions 10 hours after injection. The purified RNAs were fractionated on a denaturing 10% polyacrylamide gels and subjected to autoradiography.

frog oocytes U6 RNA can be imported into the nucleus (29). Fischer et al. (10) observed that U6 RNA with pppG at the 5' end is very unstable in frog oocytes. These authors showed that U6 RNA with other blocked structures is transported from cytoplasm to the nucleus indicating that the type of the 5' blocking group is not important in transport into the nucleus. In our experiments, both U6 and 7SK RNAs with pppG 5' ends were stable enough to monitor their transport. U6 and 7SK RNAs with mepppG or pppG on the 5' ends were injected into the cytoplasm of frog oocytes and RNA was isolated from nuclear and cytoplasmic compartments after 5 hrs or 15 hrs of incubation. As expected, the capped U6 RNA was more stable and was transported from the cytoplasm to the nucleus (Fig. 5A, lanes 7 and 9). Uncapped U6 RNA was also transported from the cytoplasm to the nucleus at 5 hrs (Fig. 5A, lane 2), as well as after 15 hrs (Fig. 5, lane 4). Similar results were obtained when capped and uncapped 7SK RNAs were injected into the cytoplasm and transport into the nucleus was monitored (data not shown).

It is possible that capping of the uncapped U6 RNA occurred in the cytoplasm after injection and then the capped RNA was transported into the nucleus. To test this possibility, the RNAs in the nuclear and cytoplasmic compartments were analyzed for the presence of methylphosphate cap structure by immunoprecipitation with anti-methylphosphate cap antibodies. When uncapped U6 RNA was used as the antigen, very little RNA was in the pellet bound to the antibodies (Fig. 5B, lane 1) and most of the radioactivity remained in the supernatant (Fig. 5B, lane 2); in contrast, capped RNA was mostly in the pellet bound to the antibodies (Fig. 5B, compare lane 11 with lane 12). With uncapped U6 RNA, neither nuclear nor cytoplasmic RNAs were precipitable with the anti-methylphosphate cap antibodies and the radioactivity was in the supernatant (Fig. 5B, lanes 4 and 6, respectively). As expected, the capped U6 RNA was quantitatively precipitated (Fig. 5, lanes 7 and 9). The same experiment was conducted with 7SK RNA and we obtained similar results (data not shown). These data clearly demonstrate that U6 and 7SK RNAs can be transported from cytoplasm to the nucleus without any cap structure on its 5' ends.

Nucleocytoplasmic transport of B2 RNA

Studies on mouse B1 RNA, another small cytoplasmic RNA homologous to highly repeated sequences, showed that it is transported from the nucleus to the cytoplasm of *Xenopus* oocytes (30). Previous reports showed that the B2 RNA is predominantly cytoplasmic in localization (18). The capped B2 RNA was injected into the frog oocytes and the transport of this RNA was studied. The B2 RNA when injected into the cytoplasm remained in the cytoplasm (Fig. 6, lane 5) and there was no detectable radioactivity in the nucleus (lane 4). When the methylphosphate cap-containing B2 RNA was injected into the nucleus, substantial percentage of B2 RNA was polyadenylated but both polyadenylated B2 RNA and the 180 nucleotide-long non-polyadenylated B2 RNA stayed in the nucleus (Fig. 6, lane 2); there was no detectable B2 RNA in the cytoplasm (Fig. 6, lane 3). Thus, the methylphosphate cap does not facilitate nucleocytoplasmic transport of B2 RNA.

DISCUSSION

The main observation made in this investigation is that the 5' methylphosphate cap dramatically increases the stability of three small RNAs naturally containing 5' methylphosphate when compared to their uncapped counterparts. In the case of cytoplasmic U6 RNA, the difference in stability was 9-fold; in the case of 7SK and B2 RNAs, the difference in stability was 4-fold and 6-fold, respectively. In addition, the stability of H1 RNA which normally has ppp on its 5' end was increased when methylphosphate cap was introduced. These data suggest that the stability of RNAs in general may be improved by introducing a methylphosphate cap structure on their 5' ends. It would have been more appropriate to calculate half-life of these RNAs rather than fold increase in stability. Even after 15 hrs of incubation, the optimum period that the oocytes remain healthy under laboratory conditions, in most instances the percentage of RNA degraded was less than 50%. Only in the case of B2 RNA there was over 50% RNA degraded whether RNA contained cap or not (Fig. 2). This might be because the 7SK, U6 and H1 RNAs bind appropriate proteins and then are shielded from further degradation. B2 RNA undergoes degradation faster than three other RNAs tested, probably because, as a rodent-specific RNA, it is not bound to any protective *Xenopus* oocytes proteins. Therefore, it was not possible to calculate half-life of these RNAs and we calculated the fold stability from the percentage of RNA degraded.

All RNA polymerase II transcripts (U1, U2, U4, U5 and mRNAs) acquire a m⁷GpppN cap co-transcriptionally, which facilitates transport from the nucleus to the cytoplasm (31). Later in the cytoplasm, m⁷G in snRNAs is converted to TMG. The TMG cap structure was shown to increase the stability of snRNAs (6,7). Therefore, enhancing the stability of RNAs is a common feature of all known cap structures. Other RNA polymerase III transcripts, like 5S RNA, do not contain a methylphosphate cap on their 5' end; however, these RNAs are quite stable in the cell. This may reflect the fact that 5S RNA is extensively integrated into protective ribonucleoprotein structure within the ribosome. It will be interesting to test whether the stability of these uncapped RNAs will be further improved by introduction of a methylphosphate cap structure.

The second observation is related to the role of cap structures in the transport of RNAs from cytoplasm to the nucleus. TMG

cap is a part of the bipartite nuclear targeting signal of U1, U2, U4 snRNAs in the case of *Xenopus* oocytes (8,9,10,32). Data presented here show that the methylphosphate cap structure is not required for the transport of U6 and 7SK RNAs from cytoplasm to the nucleus. We have shown, for the first time, that U6 and 7SK RNAs with ppp on their 5' ends are able to migrate from the cytoplasm to the nucleus and the methylphosphate cap is not involved in this process. These data show that the methylphosphate capped U6 and 7SK RNAs have signals in the RNA sequence or they bind to some chaperone molecules which help them to move from the cytoplasm to the nucleus. Since U6 and 7SK RNAs do not leave the nucleus, the migration of these RNAs from the cytoplasm to the nucleus is not a normal phenomenon in interphase cells. It is likely that the cytoplasm to nucleus pathway observed when U6 and 7SK RNAs are injected into the cytoplasm is diffusion-driven, with nucleus containing binding sites for these two nuclear RNA species.

Studies from other groups have recently showed that snRNAs are imported into the oocyte nuclei by three distinguishable pathways (8, 9, 10, 31, 33, 34). Further studies are needed to see whether 7SK and B2 RNAs use any of these pathways or have distinct pathways for transport. 7SK RNA isolated from HeLa cells (Fig. 4), as well as T7-generated (data not shown) was transported to the nucleus when injected into the cytoplasm and stayed in the nucleus when injected into the nucleus. The nucleocytoplasmic transport of 7SK RNA was similar to U6 RNA and results presented here also confirm the nuclear localization of 7SK RNA. We also observed that after injection either into the nucleus or cytoplasm, T7-generated U6 RNA changed its length with time which affected its electrophoretic mobility (see Fig. 1B, and 5B). Terns et al. (35) studied the processing of U6 RNA injected into frog oocytes and reported similar changes in electrophoretic mobility with time. Slower migrating bands probably represent U6 RNA molecules with added U residues (36) and faster migrating bands are consistent with U6 RNA molecules that have 2', 3'-cyclic phosphate at the 3' end (35).

Thirdly, B2 RNA when injected into the nucleus, but not into the cytoplasm, became polyadenylated (Fig. 6); however, B2 RNA did not migrate across the nuclear membrane. Unlike nuclear U6 and 7SK RNAs, B2 RNA is normally cytoplasmic; however, it showed no cytoplasmic tendency in our experiments. We suggest that the transport machinery in frog oocytes does not recognize possible cytoplasmic localization signals in the B2 RNA sequence, probably because of the lack of the proteins involved in B2 RNA transport. The other possibility is that B2 RNA is transported in association with some messenger RNAs and these mRNAs may not be present in the frog oocytes. Zasloff and his associates studied the transport of B1 RNA, another rodent-specific cytoplasmic transcript of RNA polymerase III, in frog oocytes and found that B1 RNA is transported from the nucleus to the cytoplasm (30,37). Therefore, it is surprising that B2 RNA was not transported in our studies.

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