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NOTES

mRNAs That Mature through *trans*-Splicing in *Caenorhabditis elegans* Have a Trimethylguanosine Cap at Their 5′ Termini

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Approximately 10% of the mRNAs in the nematode *Caenorhabditis elegans* mature through a *trans*-splicing mechanism that involves the transfer of a 22-nucleotide spliced leader to the 5′ end of the pre-mRNA. The spliced leader RNA exists as a small nuclear ribonucleoprotein particle and has the trimethylguanosine cap that is characteristic of eucaryotic small nuclear RNAs. We found that the trimethylguanosine cap present on the spliced leader RNA was transferred to the pre-mRNA during the *trans*-splicing reaction. Thereafter, the trimethylguanosine cap was maintained on the mature mRNA. This is the first example of eucaryotic cellular mRNAs possessing a trimethylguanosine cap structure.

Eucaryotic mRNAs possess modified 5′ termini of the form $m^7G(5')ppp(5')N$ (monomethylguanosine). This modification, known as a cap, is important in binding factors involved in the initiation of translation (for reviews, see references 19 and 20). The cap influences splicing of pre-mRNA and contributes to the stability of mRNA (4, 5, 10, 11, 16, 17).

Small nuclear RNAs (snRNAs) are an important component of the splicing machinery in eucaryotes (for reviews, see references 7 and 21). These RNAs are complexed with proteins into small nuclear ribonucleoprotein particles (for reviews, see references 14 and 15). snRNAs have a trimethylguanosine cap [$m_3^{2,2,7}G(5')ppp(5')N$] at their 5′ ends (for a review, see reference 18) and are not translated.

Approximately 10% of the mRNAs in the nematode *Caenorhabditis elegans* mature through an unusual mechanism in which the 5′ 22 nucleotides of the mRNA are acquired through a *trans*-splicing reaction (1, 12). This reaction involves a unique small nuclear ribonucleoprotein particle containing spliced leader (SL) RNA (2, 22, 23). SL RNA provides the 5′ exon (the SL) in the *trans*-splicing reaction and is thought to act as its own U1 snRNA during the *trans*-splicing reaction (2). SL RNA also possesses the trimethylguanosine cap that is characteristic of eucaryotic snRNAs (22, 23).

Because of the importance of caps in mRNA maturation and translation, we examined whether the trimethylguanosine cap on the SL RNA was transferred to and retained on *trans*-spliced mRNAs in *C. elegans*. RNA purified from a mixed population of *C. elegans* containing all developmental stages was immunoprecipitated by using the monoclonal antibody recognizing trimethylguanosine (as described by Van Doren and Hirsh [23]). RNAs immunoprecipitated by the monoclonal antibody and RNAs remaining in the supernatant were analyzed by RNase protection (as described by Van Doren and Hirsh [23]), using three antisense RNA probes: antisense SL RNA, antisense actin 1 RNA, and antisense actin 4 RNA. SL RNA has been shown to be immunoprecipitable by this monoclonal antibody (22, 23) and served as a positive control (Fig. 1A, lanes 6 and 7). Protection of the antisense SL RNA probe yielded a fragment of approximately 90 bases. The actin 1 gene is one of four *C. elegans* actin genes. Actin 1 mRNA acquired the SL through *trans*-splicing and was immunoprecipitated by the monoclonal antibody, showing that it has a trimethylguanosine cap (Fig. 1B, lanes 6 and 7). Actin 1 mRNA will protect 220 bases of the antisense actin 1 RNA probe. The actin 4 gene is the only member of the actin gene family that does not undergo the *trans*-splicing reaction. The fragment of the antisense RNA probe protected by actin 4 mRNA was 83 bases. In contrast to actin 1 mRNA, actin 4 mRNA was not immunoprecipitated by the monoclonal antibody (Fig. 1C, lanes 5 and 6). This result shows that the cap structure of actin 4 mRNA is something other than trimethylguanosine. These data demonstrate that actin 1 mRNA has a trimethylguanosine cap and that the 5′ terminus of actin 4 mRNA is different from that of actin 1 mRNA. Experiments using a different RNA preparation and assaying two other *trans*-spliced mRNAs that code for ribosomal proteins show that these mRNAs also have trimethylguanosine caps (data not shown). Within the limits of detection, all RNA for the three *trans*-spliced mRNAs studied was immunoprecipitable by the monoclonal antibody. Therefore, we conclude that mRNAs that mature through *trans*-splicing possess trimethylguanosine caps.

The specificity of the antitrimethylguanosine monoclonal antibody for trimethylguanosine caps is critical to the experiments described in this work. Therefore, the extent of antibody cross-reactivity to monomethylguanosine caps was assayed. RNA purified from COS-1 cells was immunoprecipitated by using the monoclonal antibody and analyzed for U1 snRNA, an RNA known to have trimethylguanosine at its 5′ terminus (18), and simian virus 40 T antigen, an mRNA known to have a monomethylguanosine cap (9). U1 snRNA was immunoprecipitated by the monoclonal antibody. No detectable level of simian virus 40 T-antigen mRNA was

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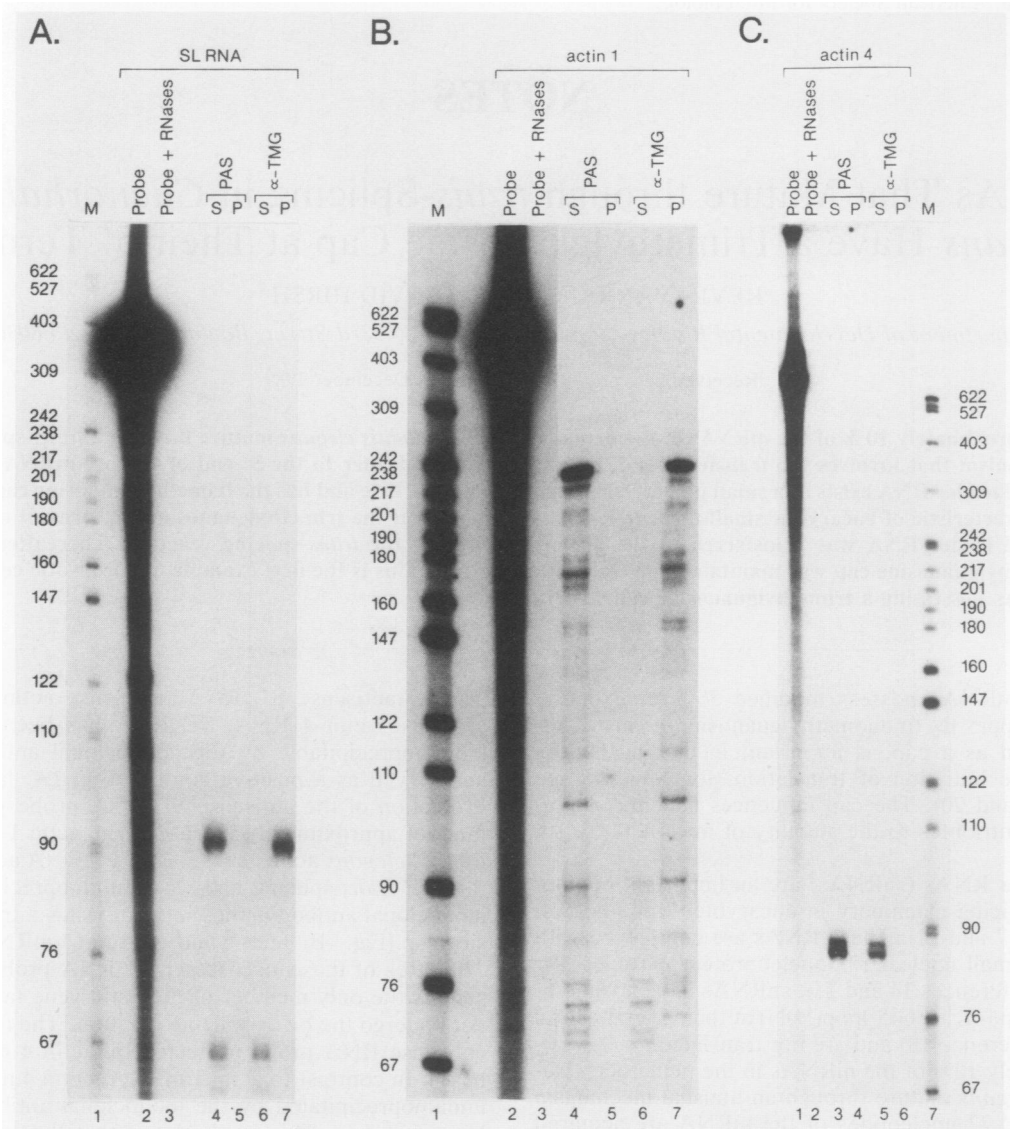

FIG. 1. Immunoprecipitation of trimethylguanosine-capped RNA. *C. elegans* RNA was isolated from a mixed population of nematodes containing all developmental stages as described previously (1). Trimethylguanosine-capped RNA was immunoprecipitated from purified total RNA by using an antitrimethylguanosine monoclonal antibody. RNA was analyzed by protecting ^{32}P-labeled antisense probes against degradation by RNases A and T_1. (A) Lanes: 1, ^{32}P-labeled products of an *Msp*I digest of pBR322, used as relative molecular mass standards; 2 and 3, antisense probes for SL RNA before and after, respectively, treatment with the RNases; 4 to 7, results of hybridization of the antisense SL RNA probe to RNA in the supernatant (S) or precipitate (P) after immunoprecipitation with either protein A-Sepharose (PAS; lanes 4 and 5) or the antitrimethylguanosine monoclonal antibody (α-TMG) complexed to protein A-Sepharose (lanes 6 and 7). (B) RNA immunoprecipitated by the antitrimethylguanosine monoclonal antibody and analyzed for actin 1 mRNA. Lanes: 1, relative molecular mass standards; 2 and 3, antisense RNA probe before and after, respectively, treatment with RNases A and T_1; 4 to 7, same order as lanes 4 to 7 in panel A. (C) RNA analyzed for actin 4 mRNA after incubation with the antitrimethylguanosine monoclonal antibody. Lanes: 1 and 2, antisense RNA probes before and after, respectively, treatment with RNases A and T_1; 3 to 6, same order as lanes 4 to 7 in panel A; 7, relative molecular mass standards.

immunoprecipitated by the monoclonal antibody (data not shown). These results indicate that the monoclonal antibody has little cross-reactivity to monomethylguanosine-capped mRNA.

Previously, we showed that binding of trimethylguanosine-capped RNAs to the monoclonal antibody could be inhibited by including high concentrations of the cap analog m^7GpppG in the immunoprecipitations (23). Thus, although the results described above showed that the monoclonal antibody had low cross-reactivity with monomethylguanosine, it did have cross-reactivity. We used the cross-reactivity to the monomethylguanosine cap analog to show that U2 snRNA and SL RNA had the same affinity for the antibody in the presence of increasing concentrations of cap analog (23). The major inhibition of binding to the monoclonal antibody occurs between 100 and 500 nM cap analog. The majority of U2 snRNA and SL RNA is immunoprecipitated by the monoclonal antibody at 100 nM cap analog, whereas most U2 snRNA and SL RNA is found in the supernatant at 500 nM cap analog (23). In the study de-

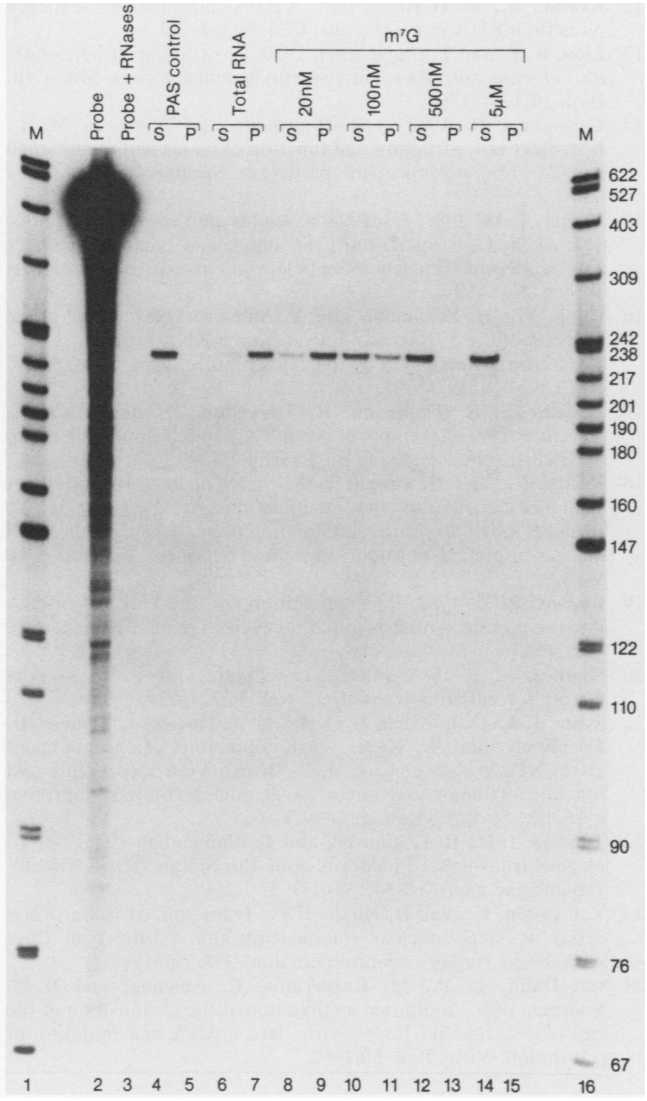

FIG. 2. Monomethylguanosine as a competitor in the immunoprecipitations by the antitrimethyl guanosine monoclonal antibody. Shown is an autoradiogram of RNA immunoprecipitated by the antitrimethylguanosine monoclonal antibody analyzed for actin 1 mRNA by RNase protection. Lanes: 1 and 16, ^{32}P-labeled products of an *Msp*I digest of pBR322, used as relative molecular mass standards; 2 and 3, ^{32}P-labeled antisense probe before and after, respectively, treatment by RNases A and T_1; 4 to 15, pairs of RNA from the supernatants (S) and precipitates (P), using protein A-Sepharose (PAS) alone (lanes 4 and 5) or the antitrimethylguanosine monoclonal antibody complexed to protein A-Sepharose in the presence of the indicated amount of monomethylguanosine as a competitive inhibitor. Total RNA (lanes 6 and 7) indicates that no competitive inhibitor was added to the immunoprecipitation.

scribed here, the RNA from the cap analog competition experiments was analyzed for actin 1 mRNA (Fig. 2). Inhibition of actin 1 mRNA binding to the monoclonal antibody occurred at the same concentration of cap analog as did inhibition of U2 snRNA and SL RNA. RNA was found in both the precipitate and the supernatant at 100 nM cap analog (Fig. 2, lanes 10 and 11), whereas almost all of the RNA was in the supernatant at 500 nM cap analog (Fig. 2, lanes 12 and 13). These results indicate that the 5′ terminus of actin 1 mRNA has the same affinity for the antitrimethylguanosine monoclonal antibody as do U2 snRNA and SL RNA. We conclude that the termini of these RNAs have the same cap structure, trimethylguanosine.

The data presented here show that *trans*-spliced mRNAs are immunoprecipitated by an antitrimethylguanosine monoclonal antibody. Previously, it had been reported that *trans*-spliced mRNAs do not have the trimethylguanosine cap present on the SL RNA (22). However, these data and data described in the accompanying report (13) show that *trans*-spliced mRNAs do possess trimethylguanosine caps.

The observation of eucaryotic cellular mRNAs that possess trimethylguanosine caps is unprecedented. Eucaryotic mRNAs typically have a monomethylguanosine cap structure. Infection by the togaviruses Semliki Forest virus and Sindbis virus results in a low percentage of viral mRNAs synthesized that have hypermethylated versions of monomethylguanosine caps (8, 24). The function of these RNAs is unknown. However, the proportion of hypermethylated guanosine-capped RNAs found in polysomal RNA is lower than in nonpolysomal RNA, suggesting that these RNAs are translated less efficiently (24).

Recently, the difference in translational efficiency between monomethyl- and trimethylguanosine-capped RNAs was investigated in a rabbit reticulocyte in vitro translation system. Darzynkiewicz et al. (3) demonstrated that substitution of the monomethylguanosine cap with trimethylguanosine decreases translational efficiency fourfold. These results are intriguing with respect to the presence of trimethylguanosine caps on *trans*-spliced mRNAs in *C. elegans*. The RNAs analyzed in this study were isolated from mixed populations of nematodes containing all developmental stages. The nematode must synthesize actin and the ribosomal proteins to grow and survive during the various stages of development. Virtually all of the actin 1 and ribosomal protein mRNAs have been shown to have trimethylguanosine caps, suggesting that they must be functional for translation in this nematode. We cannot exclude the possibility that a small population of mRNA exists beyond our limits of detection that does not have trimethylguanosine caps and is used for protein synthesis, but this seems unlikely, particularly in view of the results in the accompanying report (13) showing that trimethylguanosine-capped mRNAs are present on polysomes. It is possible that trimethylguanosine caps do not inhibit translation in *C. elegans* even though they decrease the efficiency of translation in a rabbit reticulocyte in vitro translation system. An additional cap-binding protein(s) may be responsible for interacting specifically with trimethylguanosine-capped mRNAs, or the cap-binding proteins in *C. elegans* may recognize both monomethyl- and trimethylguanosine caps. Another possibility is that the SL increases translational efficiency so that having a trimethylguanosine cap on the mRNA is not inhibitory. No cap structures have been determined for *C. elegans* mRNAs that do not participate in *trans*-splicing, such as actin 4 mRNA. It is possible that mRNAs without the SL do not have caps. If this is the case, then having a trimethylguanosine cap might stabilize the mRNA and be an improvement over not having a cap. If the stability of the mRNA is increased to a greater extent than the loss in translational efficiency, the overall effect would be to increase the amount of protein product derived from *trans*-spliced mRNAs. These explanations are not mutually exclusive. Functional tests to distinguish between these possible explanations await either an in vitro translation system for *C. elegans* or an in vivo transient assay system.

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