756–760 *Nucleic Acids Research, 1998, Vol. 26, No. 3*

A 7-methylguanosine cap commits U3 and U8 small nuclear RNAs to the nucleolar localization pathway

Marty R. Jacobson[+] and Thoru Pederson*

Cell Biology Group, Worcester Foundation for Biomedical Research, Shrewsbury, MA 01545, USA and Department of Biochemistry and Molecular Biology, University of Massachusetts Medical School, Worcester, MA 01655, USA

Received October 17, 1997; Revised and Accepted December 3, 1997

ABSTRACT

U3 and U8 small nucleolar RNAs (snRNAs) participate in pre-rRNA processing. Like the U1, U2, U4 and U5 major spliceosomal snRNAs, U3 and U8 RNAs are transcribed by RNA polymerase II and their initial 7-methylguanosine (m^7G) 5′ cap structures subsequently become converted to 2,2,7-trimethylguanosine. However, unlike the polymerase II transcribed spliceosomal snRNAs, which are exported to the cytoplasm for cap hypermethylation, U3 and U8 RNAs undergo cap hypermethylation within the nucleus. Human U3 and U8 RNAs with various cap structures were generated by *in vitro* transcription, fluorescently labeled and microinjected into nuclei of normal rat kidney (NRK) epithelial cells. When U3 and U8 RNAs containing a m^7G cap were microinjected they became extensively localized in nucleoli. U3 and U8 RNAs containing alternative cap structures did not localize in nucleoli nor did U3 or U8 RNAs containing triphosphate 5′-termini. The nucleolar localization of m^7G-capped U3 RNA was competed by co-microinjection into the nucleus of a 100-fold molar excess of dinucleotide m^7GpppG but not by a 100-fold excess of ApppG dinucleotide. Although it was obviously not possible to assess formation of di- and trimethylguanosine caps on the microinjected U3 and U8 RNAs in these single cell experiments, these results indicate that the initial presence of a m^7G cap on U3 and U8 RNAs, most likely together with internal sequence elements, commits these transcripts to the nucleolar localization pathway and point to diverse roles of the m^7G cap in the intracellular traffic of various RNAs transcribed by RNA polymerase II.

INTRODUCTION

The spatial segregation of individual species of RNA to their correct destinations in the cell constitutes a key element in eukaryotic gene expression that has only recently begun to be understood. Certain messenger RNAs have been shown to have non-uniform distributions within the cytoplasm and in several cases this has been linked to sequence elements in the mRNA 3′-untranslated region (1–4). In the nucleus pre-mRNAs are thought to be tethered in place by virtue of physical associations among elements of the transcriptional, polyadenylation and splicing machinery (5–13), followed by rapid nuclear export once processing is completed. In the case of the small nucleolar RNA (snRNA) species RNase MRP RNA we have shown that a discrete sequence element near the 5′-end is necessary and sufficient for localization in the nucleolus (14). We have also identified specific nucleotide sequences involved in intranuclear localization of the RNA subunit of RNase P (15). It is not known whether various intracellular RNA localization events are based on direct affinity between distinct RNA sequence elements and fixed intracellular sites or, alternatively, a prior binding of key proteins to specific RNA sequences with the resulting ribonucleoprotein structure constituting the high affinity ‘ligand’ for particular loci in the cell (see for example 14–16).

U3 and U8 snRNAs are members of a family of RNAs that are defined by their nucleolar localization and association with the nucleolar protein fibrillarin. Both U3 and U8 RNAs are essential for pre-rRNA processing (17–22). Like the spliceosomal snRNAs U1, U2, U4 and U5, the snRNAs U3 and U8 are transcribed by RNA polymerase II with typical 7-methylguanosine (m^7G) 5′ cap structures, which subsequently become hypermethylated to 2,2,7-trimethylguanosine (23–25). Here we report that nucleolar localization of U3 and U8 RNAs in mammalian cells is dependent on the specific nature of their 5′ cap structure and that excess dinucleotide m^7GpppG specifically competes nucleolar localization of m^7G-capped U3 RNA.

MATERIALS AND METHODS

Human U3 RNA was transcribed with T7 RNA polymerase from *Hinc*II-linearized pHU3.1, the detailed construction of which has been previously described (15). Human U8 RNA was transcribed with T7 RNA polymerase from *Xba*I-linearized pSPU8, provided by Joan Steitz (Howard Hughes Medical Institute, Yale University School of Medicine). The transcription conditions and fluorescent labeling of RNA were as described previously (14,15,26,27). Transcription reactions were carried out in the presence or absence of 1 mM $m^7G(5′)ppp(5′)G$, $G(5′)ppp(5′)G$, $A(5′)ppp(5′)G$ or

*To whom correspondence should be addressed at: Worcester Foundation Campus, University of Massachusetts Medical Center, 222 Maple Avenue, Shrewsbury, MA 01545, USA. Tel: +1 508 842 8921; Fax: +1 508 842 7762; Email: thoru@sci.wfbr.edu

[+]Present address: Department of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder, CO 80309-0347, USA

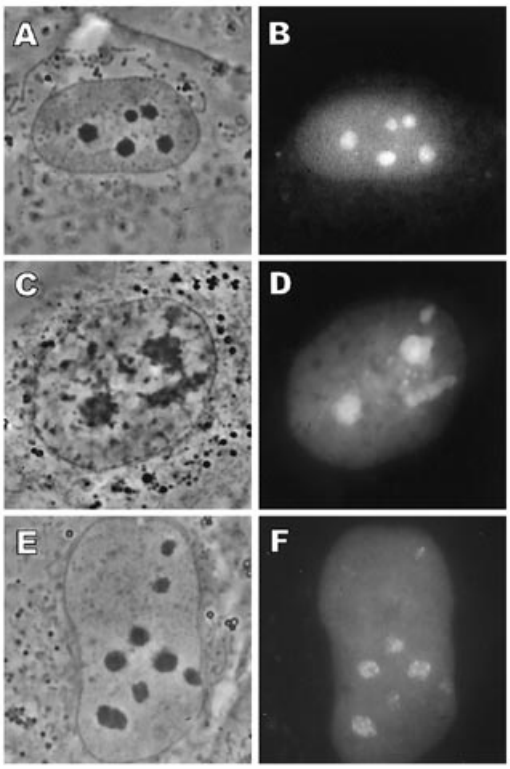

Figure 1. Nucleolar localization of m^{7}G-capped U3 RNA. Human U3 RNA was transcribed in the presence of m^{7}G(5′)ppp(5′)G and 5-(3-aminoallyl)UTP followed by rhodamine labeling, purification and microinjection into the nuclei of NRK epithelial cells (see Materials and Methods). Three representative cells are shown. (**A**, **C** and **E**) Phase contrast images ~1 min after microinjection. (**B**, **D** and **F**) Fluorescence micrographs: (B) 20 s after microinjection; (D) 30 s after microinjection; (F) 2 min after microinjection.

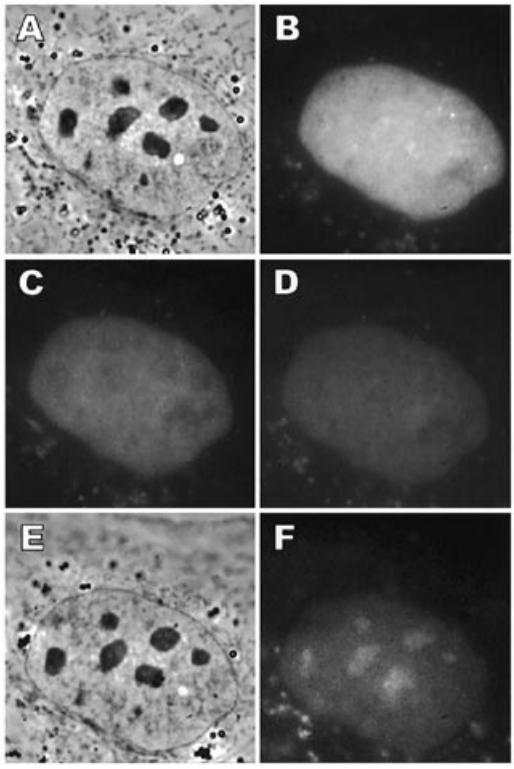

Figure 2. (5′)Appp(5′)G-capped U3 RNA. (**A**) Phase contrast image immediately after microinjection. (**B–D**) Fluorescence micrographs: (B) 1 min after microinjection; (C) 3 min after microinjection; (D) 7 min after microinjection. (**E**) Phase contrast image 20 min after microinjection. (**F**) Fluorescence micrograph 21 min after microinjection. The image in (F) was contrast enhanced using Adobe Photoshop V.4.0 software (Adobe Systems Inc., San José, CA).

m^{7}G(5′)ppp(5′)A (all obtained from New England Biolabs Inc., Beverly, MA); the concentrations of the four ribonucleoside triphosphates in the transcription reactions were each 1 mM. RNAs were either column purified or gel purified prior to microinjection into the nucleus of NRK fibroblasts (14,15,26). All microinjection experiments were carried out with sub-confluent cultures of growing NRK cells set up in special chambers in which the temperature and CO_2 level were precisely maintained during the period of observation (26). For competition experiments on U3 RNA nucleolar localization dinucleotide m^{7}GpppG or dinucleotide ApppG was mixed at a 100-fold molar excess with m^{7}G-capped rhodamine-labeled U3 RNA prior to nucleus microinjection. Microinjection of excess cap dinucleotides had no apparent effect on cell viability, as determined by phase contrast microscopy at various times (up to 1 h) after microinjection.

RESULTS

When human U3 RNA transcribed with a m^{7}G cap was microinjected into the nucleus a substantial fraction underwent very rapid nucleolar localization (Fig. 1). At the earliest post-microinjection time point it is feasible to record (~20–30 s) the majority of U3 RNA already displayed extensive nucleolar localization (Fig. 1B and D). In general each nucleolus within a given nucleus displayed approximately similar levels of fluorescent U3 RNA, although occasionally there was some nucleolus-to-nucleolus variation within a particular nucleus (as is evident in the nuclei shown in Fig. 1D and F).

Because the first transcribed nucleotide from the T7 promoter–U3 gene construct is G, the m^{7}G(5′)ppp(5′)G cap can be incorporated in both orientations during transcription by T7 RNA polymerase (28). We therefore employed the cap analog A(5′)ppp(5′)G, which can only be incorporated with the A as the U3 RNA ultimate 5′ nucleotide. In contrast to m^{7}G(5′)ppp(5′)G-capped U3 RNA (Fig. 1), U3 RNA containing the A(5′)ppp(5′)G cap did not display appreciable nuclear localization over a period of 9 min (Fig. 2B–D). However, a small degree of nucleolar localization was observed at 21 min after microinjection when the image was deliberately contrast enhanced (Fig. 2F). We also used the cap analog m^{7}G(5′)ppp(5′)A, which can only be incorporated into U3 RNA by T7 RNA polymerase in the orientation 5′-A(5′)ppp(5′)G^{7}m…3′. As shown in Figure 3, U3 RNA containing this cap displayed a low level of nucleolar localization that was evident 11 min after microinjection (Fig. 3C). The fact that a small amount of this U3 RNA carrying a 7-methylG as the cap internal nucleotide displayed some nucleolar localization probably reflects the contribution, in a minor fraction of the RNA molecules, of the 7-methylG even in this (perhaps sterically hindered) position. As shown in Figure 4, U3 RNA with no 5′ cap (i.e. containing a 5′ triphosphate end) displayed no appreciable nucleolar localization (Fig. 4B and C).

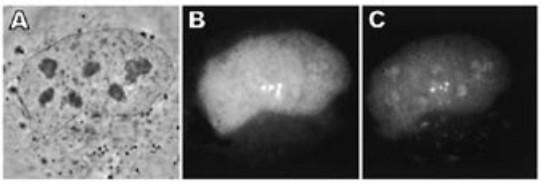

Figure 3. A(5′)ppp(5′)G^{m7}-capped U3 RNA. (**A**) Phase contrast image. (**B** and **C**) Fluorescence micrographs: (B) 5 min after microinjection; (C) 11 min after microinjection. The small, bright fluorescent spots in (B) and (C) are at or near the microinjection site. They were not coincident with nucleoli nor were they observed consistently. (This also applies to the small, bright spots in Fig. 4B and C and Fig. 6B and C.)

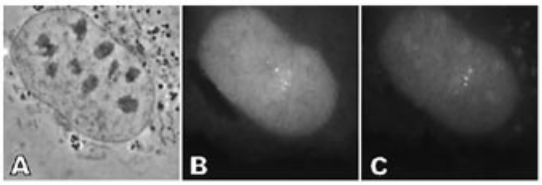

Figure 4. U3 RNA containing a triphosphate 5′-end. (**A**) Phase contrast image. (**B** and **C**) Fluorescence micrographs: (B) 9 min after microinjection; (C) 14 min after microinjection.

We next asked whether this 5′ cap structure-dependent nucleolar localization of U3 RNA was the case also for U8 snRNA, another nucleolar RNA that is involved in pre-rRNA processing (20,22) and, like U3, binds the nucleolar protein fibrillarin (29). As shown in Figure 5B–D, U8 RNA containing a m^{7}G(5′)ppp(5′)G cap displayed nucleolar localization, although this took place somewhat more slowly than in the case of U3 RNA. The cell shown in Figure 5A was binucleate and the two nuclei were closely juxtaposed. Interestingly, a small amount of the microinjected U8 RNA moved into the other nucleus and there, too, displayed nucleolar localization (Fig. 5B and C). U8 RNA containing the non-methylated cap G(5′)ppp(5′)G did not become localized in nucleoli (Fig. 6) nor did non-capped U8 RNA containing a triphosphate 5′-end (Fig. 7). We also attempted to investigate the intranuclear behavior of U3 and U8 RNA containing a 5′ trimethylguanosine cap structure, but the m^{2,2,7}G(5′)ppp(5′)G preparation we employed was not appreciably incorporated during transcription of U3 or U8 RNA by T7 RNA polymerase (M.R.Jacobson and T.Pederson, unpublished results).

To further investigate the m^{7}G cap dependence of U3 RNA nucleolar localization competition experiments were carried out in which an excess of the cap dinucleotide m^{7}G(5′)ppp(5′)G or the cap analog dinucleotide A(5′)ppp(5′)G was co-microinjected into the nucleus together with fluorescent m^{7}G-capped U3 RNA. As shown in Figure 8, the presence of a 100-fold molar excess of m^{7}G(5′)ppp(5′)G prevented nucleolar localization of fluorescent U3 RNA (Fig. 8B), whereas the presence of a 100-fold molar excess of A(5′)ppp(5′)G did not (Fig. 8D).

DISCUSSION

Ever since they were discovered (30,31) the m^{2,2,7}G cap structures on the 5′-ends of snRNAs have remained enigmatic as regards function. We previously speculated (32) that the trimethylguanosine

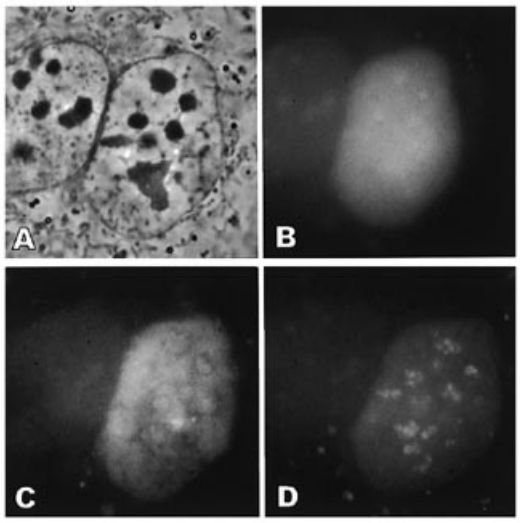

Figure 5. Nucleolar localization of m^{7}G(5′)ppp(5′)G-capped U8 RNA. (**A**) Phase contrast image. (**B–D**) Fluorescence micrographs: (B) 4 min after microinjection; (C) 9 min after microinjection; (D) 25 min after microinjection.

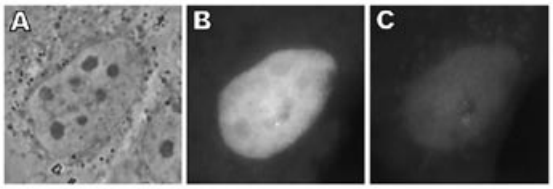

Figure 6. G(5′)ppp(5′)G-capped U8 RNA. (**A**) Phase contrast image. (**B** and **C**) Fluorescence micrographs: (B) 2 min after microinjection; (C) 6 min after microinjection.

caps might serve to keep the cytoplasmic precursors of U1, U2, U4 and U5 RNAs from associating with the translational machinery, with which they might otherwise become engaged if bearing m^{7}G caps like most mRNAs. However, the subsequent findings that synthetic mRNAs containing m^{2,2,7}G caps are capable of translation (33) and that *trans*-spliced mRNAs contain m^{2,2,7}G caps (34,35) makes it very unlikely that the m^{2,2,7}G caps on U1, U2, U4 and U5 pre-snRNAs are designed to keep them from interacting with the translational apparatus. Studies of spliceosomal snRNP biosynthesis in *Xenopus* oocytes have revealed a bipartite signal for nuclear import of U1 and U2 snRNPs, consisting of the Sm domain and its associated proteins and the 5′-m^{2,2,7}G cap (36–38). However, in the case of U4 and U5 snRNPs the m^{2,2,7}G cap is of reduced importance for nuclear import into the nucleus of *Xenopus* oocytes (36,39). Moreover, the m^{2,2,7}G cap is not required for nuclear import of U1 or U2 snRNPs in mammalian cells (40,41). That the m^{2,2,7}G cap is not invariably involved in nuclear–cytoplasmic traffic of snRNAs has been further reinforced by studies of U3 RNA biosynthesis, which have revealed that in both *Xenopus* oocytes and mammalian cells this RNA does not leave the nucleus during maturation, including cap hypermethylation (42,43).

Previous studies have defined elements within U3 RNA that are required for binding of specific proteins (44–46) and for pre-rRNA processing (17,18). Our results demonstrate that the

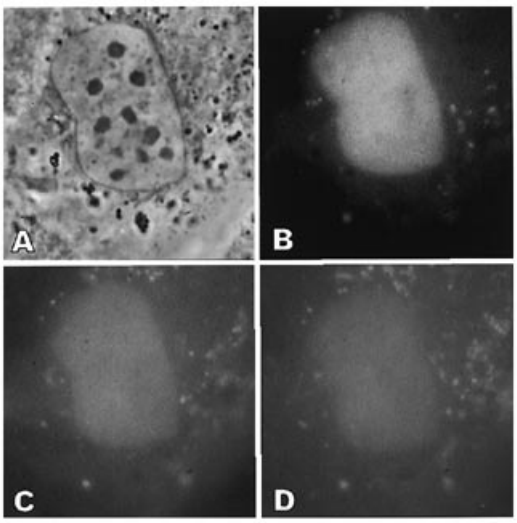

Figure 7. U8 RNA containing a triphosphate 5′-end. (**A**) Phase contrast image. (**B**–**D**) Fluorescence micrographs: (B) 1 min after microinjection; (C) 9 min after microinjection; (D) 15 min after microinjection. The images in (C) and (D) were contrast enhanced (each to the same quantitative extent) to confirm the absence of any nucleolar localization.

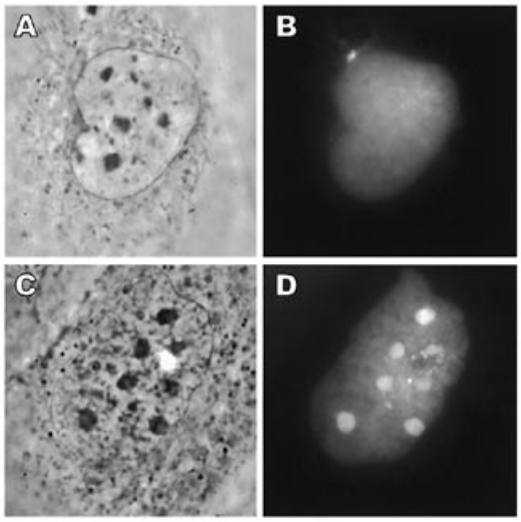

Figure 8. Nuclear microinjection of fluorescent m^{7}G(5′)ppp(5′)G-capped U3 RNA with a 100-fold molar excess of dinucleotide m^{7}G(5′)ppp(5′)G (**A** and **B**) or dinucleotide A(5′)ppp(5′)G (**C** and **D**). (A and C) Phase contrast images 1 min after microinjection. (B and D) Fluorescence micrographs 30 s after microinjection.

initial presence of a m^{7}G cap on U3 RNA is a determinant of subsequent nucleolar localization and we show that the same is true for U8 RNA. Our finding that the initial presence of a m^{7}G cap is required for subsequent nucleolar localization of both U3 and U8 RNAs is compatible with the fact that these two RNAs share several other properties, including their association with fibrillarin (29), their roles in pre-rRNA processing (17–22) and their maturation within the nucleus without a detectable cytoplasmic phase (42,43,47). One difference betwen U3 and U8 RNAs as regards the present study is that the amount of fluorescent U8 RNA that becomes localized in nucleoli appears to be less than that observed when an approximately equimolar amount of U3 RNA is microinjected (Fig. 1 versus Fig. 5 and data not shown). This may reflect a relative difference in the number of nucleolar binding sites for U3 and U8 RNA. Endogenous U8 RNA is ~20% as abundant as U3 RNA (29). Moreover, U3 and U8 RNAs function at temporally distinct steps in pre-rRNA processing (17–22) and it is possible that this is reflected in the relative affinities of the two RNAs for their respective nucleolar binding sites. Indeed, the spatial localization of U8 RNA within the nucleolus as observed by *in situ* hybridization has been reported to differ from that of U3 RNA (48).

Virtually all RNA polymerase II transcripts have 5′-m^{7}G caps and yet most do not localize in the nucleolus, so clearly the m^{7}G cap is not in and of itself a nucleolar localization signal. This same conclusion follows from our finding that several other RNAs containing m^{7}G caps (e.g. U2 RNA, pre-mRNAs and spliced mRNA) do not localize in nucleoli when microinjected into the nucleus (49; M.R.Jacobson and T.Pederson, unpublished results). The presence of nucleolar localization signals in U3 and U8 RNAs in addition to the 5′ cap itself is also indicated by the fact that other RNA polymerase II transcripts, i.e. pre-mRNAs and spliceosomal snRNA precursors, are exported from the nucleus via a m^{7}G cap-dependent mechanism (50,51). It is likely that U3 (and probably U8) RNA cap hypermethylation temporally precedes nucleolar localization, since m^{7}G-capped U3 RNA undergoes cap hypermethylation in the nucleoplasmic but not the nucleolar fractions of both *Xenopus* oocyte germinal vesicles (47) and HeLa cell nuclei (M.R.Jacobson and T.Pederson, unpublished results). A specific internal region of U3 and U8 RNAs, box D, has been implicated in cap hypermethylation of these RNAs in *Xenopus* oocytes (47) and it is possible that this internal region also plays a role in nucleolar localization. This is further suggested by the observation that the nucleolar function of the intron-encoded U20 snRNA in directing site-specific 2′-*O*-ribose methylation of pre-rRNA is dependent upon a box C/D structure that forms a 5′,3′-terminal stem (52).

In a previous study on the role of U8 RNA in pre-rRNA processing in *Xenopus* oocytes it was found that ApppG-capped U8 RNA was able to rescue pre-rRNA processing in oocytes depleted of endogenous U8 RNA, suggesting that a methylated guanosine 5′ cap is not required for nucleolar localization of U8 RNA in this system (21). We consider it likely that this reflects a difference between the *Xenopus* oocyte and the mammalian cell we have used in the present investigation. As mentioned earlier, the role of the 5′ cap structure in snRNA intracellular traffic has already been found to differ significantly between *Xenopus* oocytes and mammalian cells in numerous previous studies (36–41).

Considering the present results in a broader context, it is apparent that the various RNAs imported into the nucleolus display a diversity of 5′-end structures. In addition to the trimethlyG-capped U3, U8 and U14 snRNAs, several nucleolar RNAs have triphosphate 5′-termini, e.g. 5S rRNA, RNase MRP RNA and RNase P RNA (53–55). U3 RNA in higher plants is transcribed by RNA polymerase III and contains the 5′ γ-monomethyl phosphate ester cap structure (56) first discovered on U6 snRNA (57), which is also transcribed by RNA polymerase III (58,59). A very large number of snRNAs are processed from introns of pre-mRNAs (60–63) and are therefore presumably imported into the nucleolus with monophosphate 5′-termini. Finally, plant viroid RNAs, which are closed circular single-stranded RNAs (64), are localized in

nucleoli (65), thus providing an example of nucleolar localization of RNAs that have no 5′-termini. It is therefore clear that there are a considerable number of different signals and mechanisms for nucleolar localization of RNAs, as opposed to a single canonical targeting element, and that these diverse nucleolar localization mechanisms operate on RNA molecules with several different types of 5′-ends.

ACKNOWLEDGEMENTS

This investigation was supported by NIH grant GM-21595-21 to T.P. We thank Joan Steitz (Howard Hughes Medical Institute, Yale University School of Medicine) for providing the human U8 plasmid. We gratefully acknowledge the expert assistance of Roxanne Labrecque and Susan Kilroy in the transcription and fluorescent labeling of RNAs and we thank Qian Huang for help with the U8 RNA experiments.

REFERENCES

1 Kislauskis,E. and Singer,R.H. (1992) *Curr. Opin. Cell Biol.*, **4**, 975–978.
2 Wilhelm,J.E. and Vale,R.D. (1993) *J. Cell Biol.*, **123**, 269–274.
3 St Johnston,D. (1995) *Cell*, **81**, 161–170.
4 Hesketh,J.E. (1996) *Exp. Cell Res.*, **225**, 219–236.
5 Osheim,Y.N., Miller,O.J. and Beyer,A.L. (1985) *Cell*, **43**, 143–151.
6 Beyer,A.L. and Osheim,Y.N. (1988) *Genes Dev.*, **2**, 754–765.
7 Strouboulis,J. and Wolffe,A.P. (1991) *J. Cell Sci.*, **109**, 1991–2000.
8 Spector,D.L. (1993) *Annu. Rev. Cell Biol.*, **9**, 265–313.
9 Spector,D.L. (1996) *Exp. Cell Res.*, **229**, 189–197.
10 Du,L. and Warren,S.L. (1997) *J. Cell Biol.*, **136**, 5–18.
11 McCracken,S., Fong,N., Yankulov,K., Ballantyne,S., Pan,G., Greenblatt,J., Patterson,S.D., Wickens,M. and Bentley,D.L. (1997) *Nature*, **385**, 357–361.
12 Fay,F.S., Taneja,K.L., Shenoy,S., Lifshitz,L. and Singer,R.H. (1997) *Exp. Cell Res.*, **231**, 27–37.
13 Zeng,C., Kim,E., Warren,S.L. and Berget,S.M. (1997) *EMBO J.*, **16**, 1401–1412.
14 Jacobson,M.R., Cao,L.-G., Wang,Y.-L. and Pederson,T. (1995) *J. Cell Biol.*, **131**, 1649–1658.
15 Jacobson,M.R., Cao,L.-G., Taneja,K., Singer,R.H., Wang,Y.-L. and Pederson,T. (1997) *J. Cell Sci.*, **110**, 829–837.
16 Ross,A.F., Oleynikov,Y., Kislauskis,E.H., Taneja,K.L. and Singer,R.H. (1997) *Mol. Cell. Biol.*, **17**, 2158–2165.
17 Kass,S., Tyc,K., Steitz,J.A. and Sollner-Webb,B. (1990) *Cell*, **60**, 897–908.
18 Savino,R. and Gerbi,S.A. (1990) *EMBO J.*, **9**, 2299–2308.
19 Hughes,J.M.X. and Ares,M. (1991) *EMBO J.*, **10**, 4231–4239.
20 Peculis,B.A. and Steitz,J.A. (1993) *Cell*, **73**, 1233–1245.
21 Peculis,B. and Steitz,J.A. (1994) *Genes Dev.*, **8**, 2241–2255.
22 Peculis,B.A. (1997) *Mol. Cell. Biol.*, **17**, 3702–3713.
23 Reddy,R., Ro-Choi,T.S., Henning,D., Shibata,H., Choi,Y.C. and Busch,H. (1972) *J. Biol. Chem.*, **247**, 7245–7250.
24 Reddy,R. and Busch,H. (1988) In Birnstiel,M.L. (ed.), *Structure and Function of Major and Minor Small Nuclear Ribonucleoprotein Particles*. Springer-Verlag, Berlin, Germany, pp. 1–37.
25 Zieve,G.W. and Sauterer,R.A. (1990) *CRC Crit. Rev. Biochem. Mol. Biol.*, **25**, 1–46.
26 Jacobson,M.R. and Pederson,T. (1997) In Richter,J.D. (ed.), *Analysis of mRNA Formation and Function. Methods in Molecular Genetics*. Academic Press, New York, NY, pp. 341–359.
27 Jacobson,M.R., Pederson,T. and Wang,Y.-L. (1998) In Celis,J. (ed.), *Cell Biology: A Laboratory Handbook*. Academic Press, San Diego, CA, Vol. IV, pp. 5–10.
28 Pasquinelli,A.E., Dahlberg,J.E. and Lund,E. (1995) *RNA*, **1**, 957–967.
29 Tyc,K. and Steitz,J.A. (1989) *EMBO J.*, **8**, 3113–3119.
30 Reddy,R., Ro-Choi,T.S., Henning,D. and Busch,H. (1974) *J. Biol. Chem.*, **249**, 6486–6494.
31 Shibata,S., Ro-Choi,T.S., Reddy,R., Choi,Y.C., Henning,D. and Busch,H. (1975) *J. Biol. Chem.*, **250**, 3909–3920.
32 Patton,J.R., Patterson,R.J. and Pederson,T. (1987) *Mol. Cell. Biol.*, **7**, 4030–4037.
33 Darzynkiewicz,E., Stepinski,J., Ekiel,I., Jin,Y., Haber,D., Sijuwade,T. and Tahara,S.M. (1988) *Nucleic Acids Res.*, **16**, 8953–8962.
34 Liou,R.-F. and Blumenthal,T. (1990) *Mol. Cell. Biol.*, **10**, 1764–1768.
35 Van Doren,K. and Hirsch,D. (1990) *Mol. Cell. Biol.*, **10**, 1769–1772.
36 Fischer,U. and Lührmann,R. (1990) *Science*, **249**, 786–790.
37 Hamm,J., Darzynkiewicz,E., Tahara,S.M. and Mattaj,I.W. (1990) *Cell*, **62**, 569–577.
38 Fischer,U., Darzynkiewicz,E., Tahara,S.M., Dathan,N.A., Lührmann,R. and Mattaj,I.W. (1991) *J. Cell Biol.*, **113**, 705–714.
39 Wersig,C., Guddat,U., Pieler,T. and Bindereif,A. (1992) *Exp. Cell Res.*, **199**, 373–377.
40 Kleinschmidt,A.M. and Pederson,T. (1990) *Proc. Natl. Acad. Sci. USA*, **87**, 1283–1287.
41 Fischer,U., Heinrich,J., van Zee,K., Fanning,E. and Lührmann,R. (1994) *J. Cell Biol.*, **125**, 971–980.
42 Terns,M.P. and Dahlberg,J.E. (1994) *Science*, **264**, 959–961.
43 Cheng,Y., Dahlberg,J.E. and Lund,E. (1995) *Science*, **267**, 1807–1810.
44 Parker,K.A. and Steitz,J.A. (1987) *Mol. Cell. Biol.*, **7**, 2899–2913.
45 Baserga,S.J., Yang,X.W. and Steitz,J.A. (1991) *EMBO J.*, **10**, 2645–2651.
46 Lübben,B., Marshallsay,C., Rottmann,N. and Lührmann,R. (1993) *Nucleic Acids Res.*, **21**, 5377–5385.
47 Terns,M.P., Grimm,C., Lund,E. and Dahlberg,J.E. (1995) *EMBO J.*, **14**, 4860–4871.
48 Matera,A.G., Tycowski,K.T., Steitz,J.A. and Ward,D.C. (1994) *Mol. Biol. Cell*, **5**, 1289–1299.
49 Wang,J., Cao,L.-G., Wang,Y.-L. and Pederson,T. (1991) *Proc. Natl. Acad. Sci. USA*, **88**, 7391–7395.
50 Izaurralde,E., Stepinski,J., Darzynkiewicz,E. and Mattaj,I.W. (1992) *J. Cell Biol.*, **118**, 1287–1295.
51 Terns,M.P., Dahlberg,J.E. and Lund,E. (1993) *Genes Dev.*, **7**, 1898–1908.
52 Cavaillé,J., Nicoloso,M. and Bachellerie,J.-P. (1996) *Nature*, **383**, 732–735.
53 Forget,B.G. and Weissman,S.M. (1967) *Science*, **158**, 1695–1699.
54 Reddy,R., Li,W.-Y., Henning,D., Choi,Y.C., Nohga,K. and Busch,H. (1981) *J. Biol. Chem.*, **256**, 8452–8457.
55 Reddy,R., Tan,E.M., Henning,D., Nohga,K. and Busch,H. (1983) *J. Biol. Chem.*, **258**, 1383–1386.
56 Shimba,S., Buckley,B., Reddy,R., Kiss,T. and Filipowicz,W. (1992) *J. Biol. Chem.*, **267**, 13772–13777.
57 Singh,R. and Reddy,R. (1989) *Proc. Natl. Acad. Sci. USA*, **86**, 8280–8283.
58 Kunkel,G.R., Maser,R.L., Calvet,J.P. and Pederson,T. (1986) *Proc. Natl. Acad. Sci. USA*, **83**, 8575–8579.
59 Reddy,R., Henning,D., Das,G., Harless,M. and Wright,D. (1987) *J. Biol. Chem.*, **262**, 75–81.
60 Maxwell,E.S. and Fournier,M.H. (1995) *Annu. Rev. Biochem.*, **35**, 897–934.
61 Bachellerie,J.-P., Michot,B., Nicoloso,M., Balakin,A., Ni,J. and Fournier,M.J. (1995) *Trends Biochem Sci.*, **20**, 261–264.
62 Maden,B.E.H. and Hughes,J.M.X. (1997) *Chromosoma*, **105**, 391–400.
63 Smith,C.M. and Steitz,J.A. (1997) *Cell*, **89**, 669–672.
64 Diener,T.O. (1979) *Viroids and Viroid Diseases*. John Wiley & Sons, New York, NY.
65 Shumacher,J., Sänger,H.L. and Riesner,D. (1983) *EMBO J.*, **2**, 1549–1555.