plate [(5); the digested templates with 3′-terminal single-stranded regions were incubated with the Klenow fragment of DNA polymerase I of *E. coli* before transcription], and the 17-nt RNA and EGS RNAs, synthesized by T7 RNA polymerase from oligodeoxyribonucleotide templates [J. F. Milligan, D. R. Groebe, G. W. Witherell, O. C. Uhlenbeck, *Nucleic Acids Res.* **15**, 8783 (1987)], were purified as described elsewhere (7). Wild-type M1 RNA and C5 protein were prepared as described elsewhere [A. Vioque, J. Arnez, S. Altman, *J. Mol. Biol.* **202**, 835 (1988)].

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An Essential Signaling Role for the m_3G Cap in the Transport of U1 snRNP to the Nucleus

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The major small nuclear ribonucleoprotein particles (snRNPs) U1, U2, U4 + U6, and U5 have to be transported from the cytoplasm, where they are synthesized, to the nucleus, where they splice pre–messenger RNAs. Since the free core snRNP proteins in the cytoplasm do not enter the nucleus on their own, the nuclear location signal must either reside on the snRNA or be created as a result of snRNA-protein interaction. Here the involvement by the 5′-terminal cap of snRNA molecules in the nucleo-cytoplasmic transport of UsnRNPs has been studied by microinjection of synthetic U1 RNA molecules into frog oocytes; the U1 RNA bore either the normal cap (m_3G) or a chemical derivative. Antibodies in the cytoplasm against the m_3G cap inhibited the nuclear uptake of U1 snRNP. U1 RNA that was uncapped or contained an unnatural ApppG cap did not enter the nucleus, even though it carried a normal complement of protein molecules. When the ribose ring of the m_3G cap was oxidized with periodate, nuclear transport of U1 snRNPs was severely inhibited. Finally, microinjection of m_3G cap alone (but not m^7G cap) into oocytes severely inhibited the transport of U1 snRNPs to the nucleus. These data suggest that one step in the nuclear uptake of U1 snRNPs involves the m_3G cap structure.

THE TRANSPORT OF MACROMOLECULES between the cytoplasm and the nucleus appears to occur through pores in the nuclear membrane (*1–3*). Although some small proteins may diffuse passively into the nucleus, many proteins contain one or more "nuclear location signals" that direct them to the nucleus (*4, 5*), possibly by an active transport mechanism (*6, 7*). It is not yet known how larger particles such as U snRNPs are transported to the nucleus (*8*).

The proteins of the major spliceosomal snRNPs U1, U2, U4 + U6, and U5 can be divided into two classes: the proteins common to all these particles [also called Sm proteins (*9*)] and proteins that are associated specifically with U1, U2, or U5 (*10*). In *Xenopus* oocytes (*11*) and in somatic cells (*12*) the common proteins are synthesized in excess over U snRNAs and remain in the cytoplasm. Only after association of these proteins with newly transcribed U snRNA precursor molecules are the cytoplasmic snRNP proteins transported as an RNP particle to the nucleus. Microinjection studies with mutant U2 RNA genes have shown that association of the common proteins with the U2 RNA is necessary for efficient nuclear transport (*13*), whereas the presence of U2-specific proteins on the particle is not required (*14*). Since free Sm proteins do not enter the nucleus, the nuclear location signal must either reside on the snRNA or be created as a result of snRNA-protein interaction, for example, by a conformational change in the Sm proteins that exposes a nuclear location signal (*15*).

We decided to test the first possibility by seeking a determinant of nuclear transport lying on the snRNA. All U snRNA species except U6 RNA have an m_3G cap at their 5′ ends (*16*), with the sequence m_3GpppG (m_3G is 2,2,7-trimethyl guanosine); U6 RNA carries a γ-monomethyl phosphate at its 5′ end (*17*). The m_3G cap is formed in the cytoplasm by dimethylation of an m^7G cap (m^7GpppG, where m^7G is 7-methyl guanosine). It is in keeping with a role for the m_3G cap as nuclear location signal that the dimethylation does not occur until after the Sm snRNP proteins have assembled onto the pre-snRNA (*18*), that is, when the newly formed snRNP particle is ready to move to the nucleus.

We tested whether m_3G had a signaling role with a chemically synthesized m_3GpppG cap (*19*). We attached this cap at the 5′ end of U1 RNA by using it as primer for RNA polymerase SP6-directed synthesis of U1 snRNA from a suitably constructed plasmid. In this way, capped U1 snRNA and related derivatives were prepared in vitro.

We then tested whether antibodies to the m_3G cap structure (anti-m_3G) were able to inhibit the nuclear transport of U1 snRNPs. Increasing amounts of a monoclonal anti-m_3G (*20*) were coinjected with m_3G-capped human U1 snRNA (*21*) into the cytoplasm of *Xenopus laevis* oocytes. The injected radioactive U1 snRNA was transported efficiently to the nucleus, whereas the anti-m_3G severely inhibited this transport (at the highest antibody concentration, the rate of nuclear uptake was reduced by about 95%) (Fig. 1A). Coinjection of a monoclonal antibody (MAb) to a non-snRNP protein had no effect (Fig. 1B). The binding of U1 RNA to the Sm snRNP proteins was not impaired by the presence of anti-m_3G, as indicated by the precipitability of U1 RNA with a monoclonal anti-Sm (Fig. 1C). Although the bulky anti-m_3G molecule may sterically hinder the passage of U1 snRNP through the nuclear pores, such hindrance is unlikely, as immunoglobulin G (IgG) molecules bound to large karyophilic proteins are known to be cotransported to the nucleus (*22, 23*).

When we injected uncapped U1 RNA (that is, U1 RNA with pppG at its 5′ terminus) into the cytoplasm of oocytes, the injected RNA molecules remained stable for more than 30 hours, but no nuclear transport was observed (Fig. 2A, exp. 2) despite the fact that the U1 RNA was assembled efficiently into U1 RNP particles containing all their Sm proteins (Fig. 2C, exp. 2).

Anti-m_3G did not react with the cytoplasmic U1 RNAs or RNPs (Fig. 2B, exp. 2), showing that no cap structures had been added to the injected U1 RNA molecules in the cytoplasm. It was possible that uncapped U1 snRNPs might have entered the nucleus and eluded detection because of rapid degradation of "naked" RNA inside the nucleus. However, we could not detect any RNA molecules or radioactivity in the nucleus at 1, 2, 3, 6, or 12 hours after the oocytes were injected and the U1 RNA concentration in the cytoplasm remained roughly constant during a 30-hour period (*24*).

We tested 5′-terminal caps other than m_3G, but none of these led to the transport of U1 RNPs to the nucleus. U1 RNA

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molecules with the cap ApppG were efficiently assembled with Sm proteins into U1 RNP particles (Fig. 2C, exp. 3), but these remained entirely within the oocyte cytoplasm (Fig. 2A, exp. 3). When U1 RNAs capped with GpppG or m^7GpppG were injected into the oocyte cytoplasm, nuclear transport of the U1 snRNPs did occur, with an efficiency comparable to that attained with m_3G-capped U1 RNA (Fig. 2, A and C, exps. 4 and 5).

The effectiveness of m^7GpppG actually provides further support to the hypothesis that the m_3G cap is essential for nuclear transport as G and m^7G caps have been shown to be hypermethylated in the cytoplasm to give m_3G caps (*18*). We confirmed the relation of the m_3G and m^7G caps by showing that U1 RNPs formed from G- and m^7G-capped RNAs can be precipitated with a rabbit anti-m_3G IgG (Fig. 2B, exps. 4 and 5). The antibody used specifically reacts with cap structures containing m_3G, but not with those containing m^7G or G (*20, 25, 26*) (Fig. 3B).

To study whether the chemical integrity of the entire m_3G cap is important, the ribose ring of the 5′-terminal m_3G of m_3G-capped U1 RNA was oxidized in vitro with sodium periodate and the aldehyde groups generated were reduced to alcohol with borane *t*-butylamine complex (*27*). U1 RNA treated in this way can still be precipitated with anti-m_3G (Fig. 2B, exp. 6). The oxidation of U1 RNA at the m_3G moiety was quantitative, as was shown by removing the oxidized m_3G residue by alkaline treatment with lysine (*28*); the U1 RNA had lost completely its reactivity with anti-m_3G antibody (*24*). When treated U1 RNA was injected into the oocyte cytoplasm, the rate of nuclear transport was only about 10% of the value seen with an intact m_3G cap (Fig. 2A, exp. 6). However, stable association of U1 RNA with the Sm proteins was not disturbed, as shown by precipitation with anti-Sm (Fig. 2C, exp. 6). These data suggest that the structure and conformation of the m_3G cap moiety are important for efficient nuclear transport of U snRNPs.

We next investigated the signaling potential of the m_3G cap in the transport of a truncated U1 RNA molecule that had lost the ability to stably interact with the Sm proteins. For this purpose we made a deletion mutant of U1 RNA, which contained only the 5′-terminal 115 nucleotides of normal U1 RNA (the normal length of U1 RNA is 165 nucleotides). This truncated RNA, here termed ^{trunc}U1 RNA, was synthesized by transcription from a plasmid that had been cleaved with Taq I restriction endonuclease. The ^{trunc}U1 RNA lacks the domain that is needed for stable binding of the Sm proteins (*13*), but it includes domains that have been implicated in the binding of U1-specific proteins (*29*).

After injection into the cytoplasm of oocytes, ^{trunc}U1 RNA behaved as expected in terms of RNA-protein interactions: it could not be precipitated with a monoclonal anti-Sm (Fig. 3C), but it could be precipitated by an antibody that reacts with the 70-kD protein, which belongs exclusively to U1 snRNP (Fig. 3D, exps. 1 and 2). ^{trunc}U1 RNA was also precipitated by antibodies to the U1-specific protein A, though to a lesser extent (*24*). These results were obtained irrespective of whether the U1 RNA was capped with m_3G or with m^7G (compare experiments 1 and 2 in Fig. 3, C and D). The efficiency of the nuclear uptake of m_3G-capped ^{trunc}U1 RNA was low (about 10% of the values observed with full-length U1 RNA) (*30*), but in view of the fact that the Sm proteins cannot associate stably with ^{trunc}U1 RNA, it is remarkable that the m_3G-capped ^{trunc}U1 RNA was transported to the nucleus at all (Fig. 3A, exp. 1). The ^{trunc}U1 RNA accumulating in the nucleus could be precipitated with anti-m_3G IgG, which indicates that the RNA still possessed its m_3G cap (Fig. 3B, exp. 1). In contrast, m^7G-capped ^{trunc}U1 RNA remained in the cytoplasm of the oocyte and was not transported to the nucleus (Fig. 3, A and B, exp. 2). The experiments with (i) ^{trunc}U1 RNA indicate that the nuclear uptake of the m_3G-capped ^{trunc}U1 RNA cannot be explained by simple diffusion through the nuclear pores, and (ii) the U1-specific 70-kD and A proteins, which associate with ^{trunc}U1 RNA in the cytoplasm, might have cotransported the U1 RNA into the nucleus by a nuclear location signal contained on these proteins. If the nuclear transport of m_3G-capped ^{trunc}U1 RNA proceeded by either of these two mechanisms, then it would have occurred irrespective of the nature of the 5′-terminal cap. Thus, these data may indicate

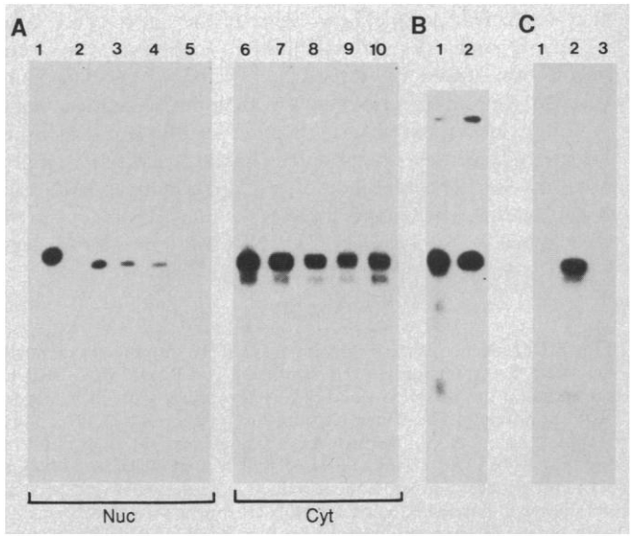

Fig. 1. (**A**) Inhibition of the nuclear uptake of m_3G-capped U1 RNA by anti-m_3G. The m_3G-capped ^{32}P-labeled U1 snRNA (25 fmol at 2×10^6 cpm/pmol), prepared in vitro by transcription with SP6 RNA polymerase (*34*), was injected into the cytoplasm of fully grown *Xenopus* oocytes in triplicate, along with 0, 80, 800, 1125, and 1500 fmol of monoclonal anti-m_3G IgG H-20 in a total volume of 25 nl of phosphate-buffered saline (PBS) (lanes 1 to 5 and 6 to 10, respectively). Oocytes were incubated for 18 hours in Barth's solution (*35*) and then transferred to J-buffer (*36*). The nuclei of the triplicate cells were isolated manually, and their RNA was pooled and separated by electrophoresis (lanes 1 to 5) alongside the corresponding RNA from the cytoplasms (lanes 6 to 10) in 5% polyacrylamide–7 M urea gels as described (*14*). ^{32}P-labeled RNA was detected by autoradiography. The results of the cytoplasmic injection experiments shown here were reproduced in three independent experiments with oocytes from different frogs. The higher relative mobility of U1 RNA in lane 1 as compared to the others is a gel artifact. (**B**) Absence of inhibition of the nuclear uptake of m_3G-capped U1 RNA by the control MAb C383. U1 RNA (25 fmol) and MAb C383 (1.5 pmol) (*37*) (dissolved in PBS buffer,which was 20 mM KH_2PO_4 and 300 mM NaCl, adjusted to pH 8.0) were injected into the cytoplasm of oocytes in triplicate, and analysis of RNA migration into the nuclei was carried out essentially as described in (A). Lane 1 shows RNA from the cytoplasms of three oocytes and lane 2 shows the RNA from their nuclei. (**C**) Controls to show that Sm snRNP proteins assemble onto U1 RNA even in the presence of anti-m_3G. The m_3G-capped U1 RNA (25 fmol) was injected into the oocyte cytoplasm, together with the monoclonal anti-m_3G H-20 [1.5 pmol, the quantity that caused maximal inhibition of transport of U1 RNA into the nucleic; (A), lane 5] and was incubated for 18 hours at 18°C. [^{32}P]U1 RNA was then precipitated from ten manually isolated nuclei (lane 1) or the corresponding cytoplasms (lane 2) with monoclonal anti-Sm Y12 (*38*) that had been covalently bound to CNBr-activated Sepharose as described (*20*). Immunoprecipitation was carried out in the presence of PBS (pH 8.0), and Sepharose-bound immunocomplexes were washed five times with IPP_{150} buffer (*9*) before extraction of [^{32}P]U1 RNA from immunocomplexes and analysis by polyacrylamide gel electrophoresis. MAb Y12 reacts primarily with Sm proteins D and B/B′ (*39*), but not with naked RNA. The immunoprecipitation of radioactive U1 RNA therefore reflects association of the RNA injected into the cell with oocyte proteins. Lane 3 shows a control experiment in which a MAb, unrelated to snRNP proteins, was used for immunoprecipitation of U1 snRNPs from oocyte cytoplasms.

that the m_3G cap can function as a weak nuclear location signal on its own, yet we cannot exclude the possibility that, despite the failure of anti-Sm to precipitate ^{trunc}U1 RNA (Fig. 3C, exp. 1), the Sm proteins could still associate loosely with ^{trunc}U1 RNA and thus, together with the m_3G cap, mediate its transport to the nucleus. However, any interaction between the Sm proteins and the ^{trunc}U1 RNA is too weak to bring about the trimethylation of the m^7G-capped ^{trunc}U1 RNA (Fig. 3B, exp. 2).

Nuclear uptake of certain karyophilic proteins is mediated, at least in part, by the interaction between one or more nuclear location signals in these molecules and receptors located in the nuclear envelope, nucleus, or cytoplasm (*31, 32*). To investigate the interaction of m_3G-capped U snRNPs with a possible cognate receptor, we injected m^7G-capped U1 RNA (full-length) together with increasing amounts of free m_3GpppG cap into the oocyte cytoplasm. The nuclear uptake of U1 RNA was efficiently blocked by the free m_3GpppG cap (Fig. 4A, lanes 1 to 4). The presence of the m_3GpppG molecules did not inhibit trimethylation of the m^7G cap of the injected U1 RNA (immunoprecipitability with anti-m_3G, Fig. 4B) or assembly of U1 RNA into U1 snRNP particles (immunoprecipitability with anti-Sm). The inhibition by m_3GpppG is thus selective: m_3GpppG inhibits the nuclear uptake of m_3G-capped U1 RNPs but does not inhibit the cytoplasmic cap methyl transferase enzyme. Only m_3GpppG inhibits the nuclear uptake of U1 RNP, since coinjection of free m^7GpppG with the U1 RNA did not inhibit the nuclear transport of U1 RNP (Fig. 4C; free m^7G derivatives are not permethylated by the enzyme). Finally, we were able to show that the m_3G moiety of the m_3GpppG cap in isolation must be structurally intact to exert its inhibitory effect; when the m_3G purine ring system was destroyed by alkaline hydrolysis before injection into oocytes, the inhibition of nuclear uptake of U1 snRNP was completely abolished (Fig. 4D). This behavior correlates well with the requirement for an intact m_3G cap at the 5′ terminus of U1 RNA for the efficient transport of this RNA into the nucleus (Fig. 2, exp. 6). These data are consistent with competitive inhibition by free m_3GpppG of a protein that recognizes the m_3G cap of U1 RNP in a process leading to the passage of U1 RNP through the nuclear pores.

Our findings show that the m_3G cap is essential for the nuclear transport of U snRNPs. The low efficiency of nuclear uptake of the m_3G-capped ^{trunc}U1 RNA indicates that other structural features, such as the snRNP proteins, also make a contribution. As evidenced by the work of Mattaj and co-workers (*14, 18*), the most likely candidates for this helper function are the Sm proteins, which on association with snRNA are believed to signal or assist the transport of the U snRNP to the nucleus. However, the core snRNP structure is not a sufficient nuclear location signal, as nuclear

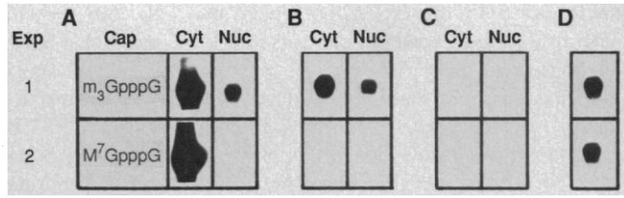

Fig. 3. Nuclear migration of U1 RNA that is m_3G-capped, but truncated. ^{trunc}U1 RNA was prepared by cutting the plasmid pHU1 with Taq I restriction endonuclease and then transcribing it in vitro with SP6 RNA polymerase in the presence of primer containing the cap m_3GpppG (exp. 1) or m^7GpppG (exp. 2) as described (*24*). ^{32}P-labeled ^{trunc}U1 RNA (25 fmol) was injected into the cytoplasm of oocytes in triplicate. After the microinjected oocytes had been incubated for 6 hours at 18°C, the migration of ^{trunc}U1 RNA into nuclei was determined by direct RNA analysis (**A**), by immunoprecipitation with rabbit anti-m_3G IgG (R1131) (**B**), and with the anti-Sm MAb Y12 (**C**) as described in Fig. 2. (**D**) Immunoprecipitation of ^{trunc}U1 RNA from oocyte cytoplasms with MAb H386, which reacts selectively with the U1-specific 70-kD protein (*42*). The results shown here reproduced in more than ten microinjection experiments (A) and three immunoprecipitation experiments (B).

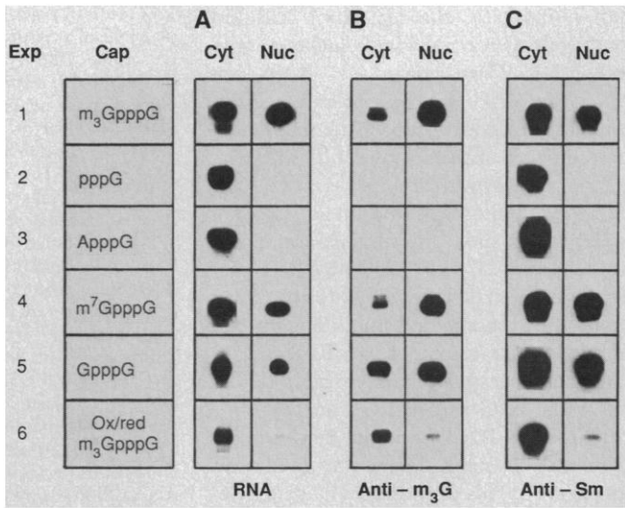

Fig. 2. Dependence of the migration of U1 RNA into the oocyte nucleus on an intact 5′-terminal m_3G cap structure. U1 RNAs were prepared by transcription in vitro of plasmid pHU1 (linearized with Bam HI) by SP6 RNA polymerase. The primer included m_3GpppG (exp. 1), no cap (exp. 2), ApppG (exp. 3), m^7GpppG (exp. 4), or GpppG (exp. 5). In experiment 6 we used U1 RNA in which the m_3GpppG had been modified by oxidation with sodium periodate and subsequent reduction of the ribose aldehyde groups with borane *t*-butylamine complex (*40*). Treatment of m_3G-capped U1 RNA with borane *t*-butylamine complex without previous periodate cleavage did not impede nuclear transport of the U1 RNA. Incorporation of the 5′-terminal cap structures into U1 RNA was confirmed by immunoprecipitation with anti-m_3G MAb H-20 or by electrophoresis in 5% polyacrylamide gels with denaturing buffer, or both. In addition, cap analysis, in particular the incorporation of $A_{ppp}G$ cap into U1 RNA molecules, was carried out by two-dimensional chromatography on polyethylenimine plates after digestion of ^{32}P-labeled U1 RNA molecules with ribonuclease T_2 (*41*). Injection of [^{32}P]U1 RNA into the cytoplasm of *Xenopus* oocytes and incubation of microinjected oocytes were as described in Fig. 1A. [^{32}P]RNA was detected by autoradiography; autoradiograms shown in (A) to (C) are from independent experiments. The results shown in these three panels were reproduced in three to five independent repetitions with oocytes from different frogs. In addition, experiments 3 and 6 were repeated with two independent U1 RNA preparations. (**A**) U1 RNA migration in oocytes. In each experiment, three microinjected oocytes were separated manually into nucleus and cytoplasm, and the [^{32}P]U1 RNAs extracted from the nuclei (left column) and cytoplasms (right column) were analyzed on denaturing gels containing 5% polyacrylamide. (**B**) Immunoprecipitation of differently capped U1 RNAs by anti-m_3G IgG. U1 RNA with the desired cap was microinjected into the cytoplasms of *Xenopus* oocytes. After 18-hour incubation, nuclei and cytoplasms were separated manually (10 oocytes were used for each experiment). Their [^{32}P]U1 RNA was extracted, pooled, and immunoprecipitated with rabbit anti-m_3G IgG (R1131) that had been conjugated to protein A–Sepharose beads essentially as described (*20*). R1131 anti-m_3G IgG reacts exclusively with m_3G cap structures but does not cross-react at all with m^7G-capped RNAs [exp. 4 and (*20*)]. The bands shown are from nuclei (left column) and cytoplasms (right column). (**C**) Immunoprecipitation of differently capped U1 RNAs by anti-Sm IgG. After microinjection and 18-hour incubation, as described in (B), immunoprecipitation of [^{32}P]U1 RNAs by anti-Sm MAb Y12 was carried out on the nuclear and cytoplasmic extracts directly. Extraction of [^{32}P]U1 RNAs from immunoprecipitates and gel electrophoretic analysis were carried out as described above. Extracts from five oocytes were used for each experiment: left column, nuclei and right column, cytoplasm.

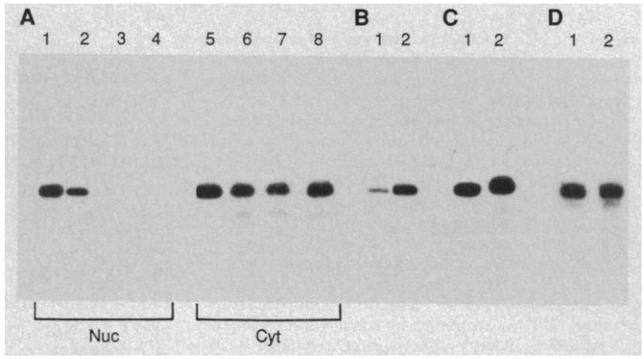

Fig. 4. (**A**) Inhibition of nuclear transport of U1 RNA by free m_3GpppG cap. The m^7G-capped U1 RNA (25 fmol), dissolved in water, was injected with 0, 3.75, 37.5, and 190 pmol free m_3GpppG into the cytoplasm of *Xenopus* oocytes in triplicate (lanes 1 to 4 and 5 to 8). After incubation of the oocytes for 18 hours at 18°C, the RNA was extracted from nuclei (lanes 1 to 4) and cytoplasm (lanes 5 to 8), separated by electrophoresis as described in Fig. 1A and detected by autoradiography. (**B**) Immunoprecipitation of U1 RNA by anti-m_3G antibodies microinjected into oocytes along with m_3GpppG cap; m^7G-capped (25 fmol) U1 RNA and free m_3GpppG (37.5 pmol) were injected into the cytoplasm of oocytes (conditions equivalent to those of Fig. 4A, lanes 3 and 7) and incubated for 18 hours at 18°C before dissection into nuclear and cytoplasmic fractions. The extracted RNA was immunoprecipitated with anti-m_3G rabbit IgG (R1131) as described in Fig. 1B. Lanes 1 and 2 show autoradiograms of gel-fractionated [^{32}P]U1 RNA extracted from immunoprecipitates obtained from nuclear and cytoplasmic fractions, respectively. (**C**) Failure of free m^7GpppG to inhibit nuclear uptake of U1 RNA. The m^7G-capped U1 RNA (25 fmol) and free m^7GpppG (475 pmol) were coinjected into the oocyte cytoplasm. Incubation and RNA analysis were carried out as described above (Fig. 1A). Lane 1 shows an autoradiogram of gel-fractionated [^{32}P]U1 RNA extracted from nuclei and lane 2 the corresponding cytoplasmic RNA. (**D**) Abolition of the inhibitory effect of the free m_3GpppG molecule by alkaline hydrolysis. The m^7G-capped U1 RNA (25 fmol) was injected into oocytes along with m_3GpppG (37.5 pmol) that had been treated with 100 mM NH_4HCO_3, pH 11.0. Incubation and RNA analysis were carried out as described before (Fig. 1A). Lane 1 shows the nuclear and lane 2 the cytoplasmic fraction of the U1 RNA. ^{32}P-labeled U1 RNA was detected by autoradiography. The alkaline hydrolysis of the purine ring of m_3GpppG cap was carried out as follows. A solution containing 0.5 mM m_3GpppG and 100 mM NH_4HCO_3, pH 11.0, was incubated overnight at room temperature. The solution was vacuum-dried and resuspended in 2 μl of water containing 2 μM m^7G-capped U1 RNA. A portion (25 nl) of this mixture was then injected into the oocyte cytoplasm. As a control, free m_3GpppG was treated with 100 mM NH_4HCO_3 at pH 7.5 under the same conditions as described above. In this case, the m_3GpppG molecule retains its purine ring and its ability to inhibit the nuclear transport of U1 RNA (*43*).

uptake of U1 RNPs does not occur in the absence of a 5′-terminal m_3G cap (Fig. 2, exp. 2). Our data provide evidence for the existence of a protein molecule that has to recognize the m_3G cap as a prerequisite for the nuclear transport of an snRNP particle and raise the possibility that this protein not only recognizes the m_3G cap, but also the structure of the snRNP core, before it can efficiently mediate the nuclear uptake of U snRNPs. Alternatively, there may exist two proteins that recognize two signal structures independently but mediate the nuclear transport of a U snRNP particle cooperatively. The isolation and characterization of the m_3G cap–recognizing protein should help to clarify these questions. A dual recognition by a protein of both the m_3G cap and a signal on the snRNP core before it can mediate nuclear transport of U snRNPs might be beneficial for certain organisms that possess trans-splicing mechanisms, as it would prevent the nuclear transport of cytoplasmic mRNAs that have acquired their m_3G cap by a trans-splicing event in the nucleus (*33*).

We have restricted our investigation to the nucleo-cytoplasmic transport of the U1 RNA and RNP. However, the m_3G cap may participate as part of a nuclear location signal for the other Sm protein–containing nucleoplasmic snRNPs as well. Provisionally, this idea is supported by results that we have obtained from microinjection experiments with U5 snRNA carried out as described for U1 snRNA.

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27. These reactions leave the trimethylated purine ring and the *N*-glycosidic bond intact; however, the cleavage of the ribose between C-2′ and C-3′ allows free rotation of the purine ring around the *N*-glycosidic bond and prevents the m_3G cap moiety from adopting a rigid structure.
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H (0.1 U/μl) in a buffer containing 20 mM Hepes, pH 7.9, 10 mM $MgCl_2$, 50 mM KCl, 1 mM DTE, and the DNA oligonucleotide 5'-CTCTAGAGTCGACCTGCAGCCCAAG-3' (0.1 μg/μl). After incubation of the reaction mixture for 1.5 hours at 37°C, the DNA oligonucleotide was digested with DNase I. The DNA was finally extracted and precipitated as described above and resuspended in water.
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Hypertriglyceridemia as a Result of Human Apo *CIII* Gene Expression in Transgenic Mice

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Primary and secondary hypertriglyceridemia is common in the general population, but the biochemical basis for this disease is largely unknown. With the use of transgenic technology, two lines of mice were created that express the human apolipoprotein *CIII* gene. One of these mouse lines with 100 copies of the gene was found to express large amounts of the protein and to be severely hypertriglyceridemic. The other mouse line with one to two copies of the gene expressed low amounts of the protein, but nevertheless manifested mild hypertriglyceridemia. Thus, overexpression of apolipoprotein *CIII* can be a primary cause of hypertriglyceridemia in vivo and may provide one possible etiology for this common disorder in humans.

HYPERTRIGLYCERIDEMIA (HTG) IS common in the general population, particularly in people with premature coronary heart disease (*1, 2*). HTG can occur on a primary genetic basis or be associated with conditions such as diabetes mellitus, obesity, pancreatitis, chronic renal failure, alcoholism, stress, or infection (*3*). Plasma triglycerides are carried principally in chylomicrons and very low density lipoprotein (VLDL). Metabolic turnover studies indicate that increased production or decreased catabolism of these lipoproteins, or both, can occur in patients with HTG (*4, 5*). In the majority of these patients, the cause of altered synthesis or breakdown of the triglyceride-rich lipoproteins is not known. In familial hyperchylomicronemia (Type I hyperlipoproteinemia), a functional defect exists in the lipoprotein lipase enzyme, which hydrolyzes the triglycerides in chylomicrons and VLDL. This is due to either defects in the lipoprotein lipase gene or defects in the gene that codes for its cofactor apolipoprotein (apo) CII (*6–9*). The frequency of these individuals in the general population is less than one in a million.

In our studies of the cis-acting DNA sequences required for tissue-specific expression of the apolipoprotein genes, we introduced the intact human apo *CIII* gene with flanking sequences into mice. The apo CIII protein is a major constituent of chylomicrons and VLDL. Apo *CIII* is 3.1 kb in length and maps within an apolipoprotein gene cluster on human chromosome 11q23, between the apo *A-I* and apo *A-IV* genes. Apo *CIII* contains four exons and encodes a mature protein of 79 amino acids (*10–13*). Two different lines of transgenic mice that expressed human apo *CIII* were established. In a line containing approximately 100 copies of the human gene, the mice were severely hypertriglyceridemic. In a second line, animals with one to two copies of the human gene manifested mild HTG. These experiments revealed that in an in vivo animal model, overexpression of apo *CIII* can lead to HTG, and suggest one possible etiology for this disorder in humans.

A recombinant bacteriophage clone containing the human apo *CIII* λ apo *A-I-8* (*10, 13*) was subjected to restriction endonuclease digestion and yielded a 6.7-kb DNA fragment between the Kpn I site 2.5 kb 5′ to apo *CIII* and the Xba I site 1.1 kb 3′ to the gene. This DNA fragment was gel-purified and used for microinjection. Transgenic mice were created as previously reported (*14*). Approximately 400 copies of human apo *CIII* were injected into the male pronucleus of fertilized eggs from superovulated (C57BL/6J × CBA/J) F1 females that had been mated to males of the same genetic background. Injected eggs were surgically transferred to oviducts of surrogate females. Of 26 mice born, 3 had integrated human apo *CIII*. Two mice were bred to (C57BL/6J × CBA/J) F1 mice, and two transgenic lines were established (2721 and 2674). The inheritance pattern of the transgene in both lines was compatible with a single autosomal integration site.

We determined the copy numbers of human apo *CIII* in these two transgenic lines with quantitative Southern (DNA) blotting

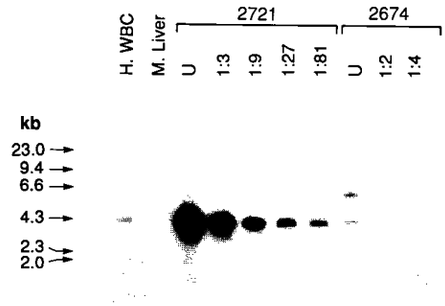

Fig. 1. Quantitative Southern blotting analysis of human apo *CIII* transgenic mouse lines 2721 and 2674. DNA was extracted from tail tissue of two lines of transgenic mice (Tg) expressing apo *CIII*, digested with Pst I, subjected to electrophoresis in a 0.7% agarose gel, transferred to a nylon membrane (Oncor), and hybridized with a 517-bp human apo *CIII* cDNA probe derived from pCIII-607 (*15*). Tg line 2721 DNA was loaded in serial dilutions of 10 μg [undiluted (U)], 3.33 μg (1:3), 1.11 μg (1:9), 0.37 μg (1:27), and 0.12 μg (1:81). Similarly, DNA from a progeny of the founder mouse Tg-2674 was loaded in dilutions of 10 μg (U), 5 μg (1:2), and 2.5 μg (1:4). The cDNA probe hybridized to a 4.2-kb band representing a Pst I fragment beginning at the Pst I site 200 bp 5′ to the start of transcription of the human apo *CIII* and terminating at the Pst I site located approximately 900 bp 3′ to the gene. A copy number of 100 to 110 for Tg line 2721 and 1.8 for Tg line 2674 was obtained by a densitometric scan of the 4.2-kb band, after normalization for the human white blood cell (H. WBC) signal. The additional band at 6 kb in the Tg line 2674 blot probably results from the loss of one of the apo *CIII* flanking Pst I sites in an end copy of the human genomic DNA insert. Control, mouse liver DNA (M. Liver).

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