5'-Terminal Caps of snRNAs Are Accessible for Reaction with 2,2,7-
Trimethylguanosine-specific Antibody in Intact snRNPs*

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Immune precipitation assays with antibodies specific for 2,2,7-trimethylguanosine (m2,7G) have been used to study the accessibility of the 5'-terminal m2,7G-containing caps of eucaryotic small nuclear RNAs (snRNAs) either as naked RNAs or in intact small nuclear ribonucleoprotein (snRNPs). The antibody selectively precipitates snRNA species U1a, U1b, U2, U4, and U5 from total deproteinized RNA isolated from Ehrlich ascites cells. Binding by the antibody occurs via the m2,7G moiety of the snRNAs' caps, since complex formation with the antibody can be completely abolished by excess nucleoside m2,7G. The specificity of the antibody is further demonstrated by the complete absence of reaction with deproteinized snRNA species U6, the 5' terminus of which does not contain m2,7G. Most importantly, the cap structures of the snRNAs U1a, U1b, U2, U4, and U5 are also accessible for anti-m2,7G IgGs when intact snRNPs are reacted with the antibody. In this case, snRNA species U6 is coprecipitated, suggesting that there are intermolecular interactions between this and other snRNA species. Our data demonstrate that the 5'-terminal regions of the above snRNAs are not protected by the snRNPs. The finding of special interest for snRNA species U1, and is discussed in terms of a model which proposes that the 5'-terminal region of U1 participates in the proper alignment of splice junctions in eucaryotic pre-mRNAs (Lerner, M. R., Boyle, J. A., Mount, S. M., Wolin, S. L., and Steitz, J. A. (1980) Nature (Lond.) 283, 220-224).

Nuclei of cells from a wide variety of eucaryotic organisms contain a group of metabolically stable RNAs known as snRNAs.1 There are six major species designated U1 to U6. Of these, U3 and its structural variants are located only in the nucleolus (1), while the others are predominantly found in the nucleoplasm. With the exception of U6 RNA (2, 3), the snRNA species U1 through U5 are notable for possessing a 5'-capped end with the highly methylated base m2,7G at its 5' terminus (4).

snRNAs have frequently been detected in high molecular weight ribonucleoprotein particles, and models of hnRNP particle structure have proposed them to be integral structural components (5). However, it has only recently become clear that the snRNAs of nucleoplasmic origin exist in separate ribonucleoprotein particles (snRNPs) and are associated with a set of proteins which are related neither to histones nor to the proteins packaging hnRNAs (6). The snRNPs display sedimentation coefficients of about 10 S (7).

While the exact function of the various snRNPs in the nucleus is still unknown, a number of findings point to a crucial role of some of them in the maturation of hnRNA. (i) snRNPs are most abundant in metabolically active cells and coexist with hnRNPs isolated from the respective cell nuclei (7, 8). (ii) The coexistence of at least snRNP species U1 and U2 with hnRNPs in situ is corroborated by the finding that in intact cells the respective RNA chains could be cross-linked by a psoralen derivative (9, 10). (iii) The components of the snRNPs have been strongly conserved during evolution. Antibodies with either Sm- or RNP-specificity from patients with lupus erythematosus precipitate snRNPs from cells of a wide variety of species including man, chicken, and an insect (6). Since these antisera are directed against the protein part of snRNPs (6), this indicates a strong similarity of the snRNPs proteins in the respective species. A strong conservation through evolution is also evident for at least some snRNAs. Thus, the sequences of the snRNA species U1 from man, rat, and chicken differ at only a few positions (11), the 5' termini being completely identical. (iv) It has been noticed that the 5'-terminal sequence of U1 shows extensive complementarity to the consensus sequences at the exon-intron junctions of eucaryotic pre-mRNAs (7, 12). These findings led to the proposal that, by basepairing with an intron's consensus 5' and 3' ends, U1 might ensure the proper alignment for splicing of the respective pre-mRNAs' exon boundaries. The above model requires that the 5' terminus of U1 RNA is accessible for pre-mRNA recognition and is not buried inside the snRNP structure. The presence of the highly methylated nucleoside m2,7G in the snRNAs' cap structures provides an excellent basis to test this model by the use of m2,7G-specific antibodies. Antibodies of high specificity for m2,7G have been obtained by immunizing rabbits with an m2,7G-HSA conjugate. The isolation and characterization of the antibody is described elsewhere (17). Here, we report on the reaction of the antibody with deproteinized nucleoplasmic snRNAs, as well as with snRNPs isolated from Ehrlich ascites cells. The accessibility of the caps in the snRNPs for anti m2,7G antibodies shows that the snRNP proteins do not protect the 5'-terminal regions of the respective snRNAs.

MATERIALS AND METHODS

Antibodies—Antibodies of high specificity for m2,7G were obtained by immunizing rabbits with m2,7G-human serum albumin conjugates. Preparation of the immunogen, immunization procedures, and characterization of the antibody by radioimmunounassays is described elsewhere (17). Anti-m2,7G IgGs were obtained from the rabbit sera by ammonium sulfate precipitation followed by chromatography on Sephadex G150. 150 µg of anti-m2,7G IgGs bound about 0.1 nmol of m2,7G.

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1 The abbreviations used are: snRNA, small nuclear RNA; snRNP, small nuclear ribonucleoprotein; m2,7G, 2,2,7-trimethylguanosine; IgG, immunoglobulin G; HSA, human serum albumin; hnRNA, heterogeneous nuclear RNA; hnRNP, heterogeneous nuclear RNP; Sm, nuclear antigen.

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m$^\text{2,7-G}$ nucleoside. IgGs with anti-Sm specificity were similarly prepared from sera of two patients with systemic lupus erythematosus.

Cell Growth and Labeling Conditions—Ehrlich ascites cells were grown in suspension culture at 37 °C in a medium consisting of Eagle’s minimum essential medium (Flow Laboratory) supplemented with 5% newborn calf serum, 50 µg/ml of penicillin, and 100 µg/ml of streptomycin. For preparation of $^{32}$P-labeled RNA, 150 ml of cells (starting density, $5 \times 10^8$ cells/ml) in phosphate-free minimum essential medium were labeled with $[^3P]$orthophosphate (Amersham/Buchler, Braunschweig) at 8 mCi/liter for 14 h.

The methods for the preparation of cellular supernatants were those previously described (13). Ehrlich ascites cells were harvested by centrifugation at 150 × g for 10 min, washed once in 50 ml of buffer A (40 mm Tris-HCl, pH 7.4; 150 mm NaCl) and collected again by centrifugation. $10^6$ cells were resuspended in 3 ml of buffer A and sonicated 3 times for 40 s at setting 3 of a Branson sonifier. Cell debris and chromatin were pelleted by centrifugation at 6000 × g for 10 min.

The clear supernatant was immediately used for immune precipitation assays and as a source for preparation of total RNA.

Immune Precipitation Assays—Antibody was precipitated with Pansorbin (Calbiochem) as described (14). In a typical experiment, 150 µg of IgG were incubated with either the supernatant from 5 × $10^6$ cells or with total RNA extracted from an equivalent amount of the cell sonicate at 6 °C for 15 min. After adding 100 µl of Pansorbin (10% suspension), the mixtures were incubated for an additional 30 min at 0 °C. The immune complexes were pelleted by centrifugation for 15 s in an Eppendorf centrifuge and washed five times with NET buffer (50 mm Tris-HCl, pH 7.4, 150 mm NaCl, 0.05% Non-ident P-40) (14).

RNAS prepared by phenol extraction were fractionated on 10% polyacrylamide gels containing 7 M urea, 45 mM Tris-borate, pH 8.3, and 1 mM EDTA.

RESULTS AND DISCUSSION

Reactivity of anti-m$^\text{2,7-G}$ IgG with total deproteinized RNA from Ehrlich ascites cells was investigated by immune precipitation assays. Antibody-RNA complexes were precipitated with Staphylococcus aureus cells (Pansorbin) and immune precipitated RNAs were identified by polyacrylamide gel electrophoresis. Anti-m$^\text{2,7-G}$ antibodies selectively react with snRNA species U1a, U1b, U2, U4, and U5 (Fig. 1, lane 2), while IgGs from preimmune serum do not react with any of these RNAs (Fig. 1, lane 3). Coprecipitation of an RNA which migrates in the position usually found for the cytoplasmic 7 S RNA (1) was sometimes observed with antibodies of any specificity and is presumably due to unspecific RNA-protein interactions. The amount of this coprecipitated RNA varies considerably from experiment to experiment (compare Figs. 1 and 2).

It is important to note that anti-m$^\text{2,7-G}$ antibodies do not react at all with snRNA-species U6 (Fig. 1, lane 2) although this snRNA is present in the RNA preparation used for the precipitation assays (Fig. 1, lane 1). This satisfactorily corroborates the high specificity of the antibody, since U6 snRNA from mouse cells does not display an m$^\text{2,7-G}$-containing cap structure at its 5' terminus (2, 6). The contention that binding of the other snRNAs by the antibody does indeed occur via the m$^\text{2,7-G}$ moiety of their caps is demonstrated by the complete abolition of snRNA-antibody complex formation in the presence of excess nucleoside m$^\text{2,7-G}$ (Fig. 1, lane 4). As a control, other nucleosides such as adenosine or guanosine which are not recognized by the antibody do not inhibit at all (data not shown).

Most importantly, anti-m$^\text{2,7-G}$ not only reacts with deproteinized snRNA molecules but also selectively precipitates snRNPs from sonicated Ehrlich ascites cells (Fig. 2, lane 3). The antibody predominantly reacts with snRNP-particles containing the snRNAs U1a, U1b, U2, U4, and U5 (Fig. 2, lane 3). In addition, snRNPs-species U6 is also precipitated to some extent. This is surprising, since m$^\text{2,7-G}$ is absent from U6 RNA and the antibody was shown not to react with the free RNA molecule (see above and, for direct comparison, lane 2 in Fig. 2). The most likely explanation for this finding is that coprecipitation of U6 snRNP is due to noncovalent interactions between this and other snRNP species. Whether such intermolecular interactions between individual snRNPs reflect a genuine state inside the cell nucleus, or whether they are simply in vitro artifacts due to the isolation procedure cannot be decided at the moment.

The antibody binding of snRNPs is specific for anti-m$^\text{2,7-G}$ since IgGs from preimmune serum precipitate only negligible amounts of the various snRNPs (Fig. 2, lane 4).

As a second control, the supernatant of sonicated Ehrlich ascites cells was also reacted with a serum from a lupus erythematosus patient characterized as anti-Sm. Anti-Sm antibodies recognize antigenic determinants solely on the protein moiety of the snRNPs and do not react at all with naked
snRNA Caps Are Accessible for Anti-m$^{2,7}G$ Antibody in snRNPs

The accessibility of the snRNA caps for the m$^{2,7}G$-specific antibody further suggests that the extreme 5'-terminal regions of the snRNAs are not buried inside the RNP structure. This is of special importance for snRNP species U1 and is consistent with the notion that the 5'-terminal region might be utilized for intermolecular RNA-RNA interactions to provide accurate processing of RNA transcripts (7, 12).

While our data show that the snRNA caps are accessible for anti-m$^{2,7}G$ antibodies in isolated snRNPs, it remains to be clarified whether this is also true when the snRNPs are present in the intact cell. Cellular fractionation experiments have localized several of the nucleoplasmic snRNPs in association with unique nuclear substructures. The most abundant snRNP species have been found associated with hnRNPs (7, 8). Recent data indicate that snRNPs may be part of the nuclear matrix as well (16). Experiments are in progress to study the accessibility of the snRNAs' 5' termini in these substructures. Elucidation of these questions should also provide valuable information as to the role of the snRNA caps themselves. It is hard to believe that the caps merely function to protect the snRNAs against potential nuclease attack, since they are already packaged with specific proteins into RNP particles. However, it is tempting to assume the existence of a m$^{2,7}G$ cap binding protein which might be present in one or both of the above mentioned nuclear substructures. This putative protein could direct the snRNPs to their site of action.

In addition to its potential application for studies on the functional role of the 5'-terminal regions of snRNAs, the anti-m$^{2,7}G$ antibody turns out to be a powerful tool for isolating snRNPs as well. Preliminary results indicate that snRNP species U1, U2, U4, and U5 may be quantitatively recovered from nuclear extracts by affinity chromatography with m$^{2,7}G$-specific IgGs.

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Our results demonstrate that the 5'-terminal caps of snRNAs U1a, U1b, U2, U4, and U5 are not protected by the snRNP proteins. Considering that the antigen binding sites of an IgG molecule are located in a cavity at the top of the bulky Fab arms (15), the accessibility of the snRNA caps for the m$^{2,7}G$-specific antibody further suggests that the extreme 5'-terminal regions of the snRNAs are not buried inside the RNP structure. This is of special importance for snRNP species U1 and is consistent with the notion that the 5'-terminal region might be utilized for intermolecular RNA-RNA interactions to provide accurate processing of RNA transcripts (7, 12).
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