Eukaryotic Small Ribonucleoproteins

ANTI-La HUMAN AUTOANTIBODIES REACT WITH U1 RNA-PROTEIN COMPLEXES*

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Anti-La sera from patients with autoimmune disorders precipitate a set of nuclear and cytoplasmic small RNA-protein complexes. Up to now, it has been thought that the La antigen is associated only with RNAs transcribed by RNA polymerase III, including precursors of tRNA and 5 S ribosomal RNA. Here we report that anti-La sera also react with ribonucleoprotein particles containing small nuclear RNA U1, which is transcribed by RNA polymerase II. Anti-La sera from 12 out of 12 patients tested were found to precipitate U1 RNA-protein complexes from HeLa cell nuclear extracts, under conditions where noneimmune sera do not. Ribonucleoprotein particles containing a second small nuclear RNA, U2, do not react appreciably with anti-La sera although they are present in HeLa cell nuclei at the same concentration as U1 RNA. Anti-La sera also react with U1 RNA-protein complexes in mouse and frog cells, but not in Drosophila or Chironomus, two organisms which lack the La antigen. Hybridization of cloned U1 DNA with anti-La-reactive RNA from HeLa cell nuclear extracts reveals mature U1 RNA, whereas anti-La-reactive cytoplasmic RNA contains a series of hybridizing bands that represent molecules 1–7 nucleotides longer than U1 and which may include precursors of nuclear U1 RNA (Madore, S. J., Wiesen, E. D., and Pederson, T. (1984) J. Cell Biol. 95, 471a). This paper presents data with two additional anti-La sera that represent molecules 1–7 nucleotides longer than U1 and which may include precursors of nuclear U1 RNA (Madore, S. J., Wiesen, E. D., and Pederson, T. (1984) J. Cell Biol. 95, 471a). This paper presents data with two additional anti-La sera.

Several different classes of eukaryotic small RNP particles react with antibodies from the sera of patients with autoimmune diseases such as systemic lupus erythematosus, mixed connective tissue disease, scleroderma, and Sjogren's syndrome (1–6). One class of RNP particles is recognized by sera known as anti-La (also termed anti-SS/B) and contains RNAs 80 to 130 nucleotides long (2). Most of these are RNA polymerase III transcripts (3), including precursors of tRNA and 5 S ribosomal RNA (7). On this basis, it has been suggested that the La antigen is associated only with RNA polymerase III transcripts (3, 7). Here we show that some of the RNP complexes containing small nuclear RNA U1, which is transcribed by RNA polymerase II, also specifically react with anti-La sera. This raises the possibility of common La protein binding sites in U1 RNA and RNA polymerase III transcripts.

EXPERIMENTAL PROCEDURES

Drosophila (Kc0), HeLa, and mouse erythroblast cells were cultured as described (4, 8, 9). Mammalian cells were homogenized in reticulocyte standard buffer (0.01 m NaCl, 1.5 mM MgCl2, and 0.01 mM Tris-HCl, pH 7.2) and nuclear extracts were prepared by brief sonication in 8.5 buffer as previously described (4). Xenopus laevis ovary tissue (kindly provided by Dr. Douglas Melton, Harvard University) was homogenized directly in 8.5 buffer. Drosophila nuclear extracts were prepared as described by Wiesen and Pederson (4). The procedures used for antibody selection of RNP's, purification of RNA, electrophoresis, and hybridization with cloned DNA probes have all been described (4, 10–12). Anti-La, Sm, and RNP patient sera were screened in the clinic by immunodiffusion against standard reference antigens and were further defined, as IgG (13), by their distinct patterns of reactivity with HeLa cell small nuclear and cytoplasmic RNAs, as previously described (14–11). Throughout "Results," the term "sera" should be understood to mean purified IgG.

RESULTS

Lanes 5–7 of Fig. 1 show the RNA patterns obtained by reacting nuclear extracts of [3H]uridine-labeled HeLa cells with anti-La sera from three different patients. In each case, the antibody reacts with a species that co-migrates with U1 RNA (see markers in lane 1). The virtually complete absence of U2 RNA from lanes 5–7 is noteworthy. The nuclear RNAs running ahead of U1 in lanes 5–7 are familiar La RNAs, including 5 S ribosomal RNA in transit to the cytoplasm (7) and other relatively stable species preferentially labeled under these conditions (see also Ref. 3). In contrast to the results for nuclear RNA (lanes 5–7), no U1 is detected by anti-La in the cytoplasmic fraction from an equivalent number of cells under these long term labeling conditions (lane 4). Neither cytoplasmic nor nuclear U1 is detected with control IgG from a healthy individual (lanes 2 and 3). Protein-free U1 RNA does not react with anti-La (not shown).

Fig. 2 presents data with two additional anti-La sera (lanes 2 and 3), along with a repeat analysis (lane 4) with the anti-La serum used in lane 5 of Fig. 1. It can be seen that an appreciable amount of U1 RNA consistently reacts. Similar
different anti-La sera. Nuclear and cytoplasmic extracts were prepared from HeLa cells that had been labeled for 22 h with [3H]uridine and were reacted with IgG from anti-La or nonimmune sera. Antigenic material was recovered with protein A-Sepharose and the RNA was purified by phenol extraction and displayed by electrophoresis in 10% polyacrylamide-7 M urea gels followed by fluorography. Lane 1, total small nuclear RNA; lane 2, cytoplasm reacted with nonimmune IgG; lane 3, nuclear extract reacted with nonimmune IgG; lane 4, cytoplasm reacted with anti-La; lanes 5–7, nuclear extracts reacted with anti-La from three different patients.

results were obtained with 7 additional anti-La sera (data not shown). It is important to note however that only a portion of the total nuclear U1 RNP reacts with anti-La under the conditions used. This is shown in Fig. 2B, lanes 2 and 3, which are RNAs from the protein A-Sepharose nonbound fractions.

To verify that the RNA species being analyzed was indeed U1, anti-La-reactive nuclear RNA was hybridized with a cloned U1 DNA probe (14) as shown in Fig. 3. With each of three different anti-La sera (lanes 2–4), RNA with the electrophoretic mobility of U1 RNA hybridizes with the U1 DNA probe, under conditions in which no hybridization is detected in the RNA reacting with nonimmune IgG (lane 1).

Sucrose gradient fractionation of nuclear extracts followed by reaction with anti-La serum revealed that most of the RNA reacting with nonimmune IgG is bound fractions were phenol-extracted, precipitated with ethanol, with anti-La sera. After passage over protein A-Sepharose, the non-bound fractions were RNAs from the protein A-Sepharose nonbound fractions.

that contain total U1 RNA, as revealed by precipitation of parallel gradient fractions with Sm antibody (Fig. 4, lanes 1–6; see also Ref. 15). This indicates that the antigenicity of U1 RNP for anti-La sera is not due to an association of this subset of U1 with large nuclear structures containing La antigen (e.g. pre-mRNP), and suggests that the protein(s) responsible for the observed reaction with anti-La sera are directly complexed with U1 RNA.

The results presented so far could have a trivial explanation, viz. the presence of antibodies against U1 RNP in the polyclonal anti-La sera. This possibility was examined by determining if anti-La sera react with U1 RNP in Drosophila cells. While human autoantibodies specific for mammalian U1 RNP also react with Drosophila U1 RNP (4, 16), Drosophila does not contain RNPs that react with La antibodies (16). Hence, if anti-La sera were contaminated by antibody against mammalian U1 RNP, they should precipitate Drosophila U1. However, no reaction whatsoever of Drosophila U1 with three different anti-La sera were detected (Fig. 5, lanes 7–12). Lanes 3 and 4 of Fig. 5 are controls to confirm that Drosophila U1
The reaction of U1 RNP with anti-La sera was confirmed in vertebrate cells other than human (HeLa). Murine cells contain two species of U1 RNA, termed U1a and U1b, and both of these react with anti-La serum. This can be seen in lane 2 of Fig. 6, which is the U1 DNA hybridization of RNA selected by anti-La from nuclear extracts of mouse erythroleukemia cells. Lane 3 confirms that both U1a and U1b also react with U1 RNP-specific U1 RNP serum (3). As lanes 2 and 3 represent equivalent amounts of nuclear extract, these results again demonstrate that only a fraction of the U1 RNA detected by anti-RNP reacts with anti-La, which agrees with the previous conclusion in HeLa cells (Fig. 4). An analysis similar to that in lanes 1–3 is shown for an extract of Xenopus oocytes in lanes 4–6. Again, a substantial fraction of the U1 RNP reacting with anti-RNP (lane 6) also reacts with anti-La (lane 5).

Previous studies have suggested that U1 RNA biosynthesis involves a set of cytoplasmic precursor molecules that are 1–12 nucleotides longer than mature U1 RNA (12, 17–20). It was therefore of interest to determine whether these cytoplasmic U1 RNA precursors also react with anti-La sera. Lanes 1 and 2 of Fig. 7 represent equivalent amounts of nuclear and cytoplasmic fractions from HeLa cells. After reaction with anti-La sera, RNAs were hybridized in gel blots with cloned U1 DNA as in Fig. 3. In contrast to the nuclear La-reactive material which is entirely mature U1 RNA (lane 1), the anti-La-reactive RNAs from the cytoplasmic fraction contain a series of closely spaced bands estimated to be molecules ranging from 1 to 7 nucleotides longer than mature U1 (lane 2). These species correspond in electrophoretic mobility to the HeLa cytoplasmic U1 RNA molecules that behave as biosynthetic precursors to mature U1 RNA, which we have recently identified employing RNP and Sm antibodies and pulse-chase experiments (12). We emphasize that the "cytoplasmic" assignment of these apparent precursors of U1 RNA is strictly operational, with the usual caveats invoked in all cell fractionation studies. However we do find similar amounts of these molecules in fractions prepared by homogenization under isotonic conditions as when a hypotonic buffer is used (12). In addition, these molecules are found in the cytoplasm when HeLa cells are fractionated by a nonaqueous procedure (19, 20).

Because the U1 DNA hybridization of anti-La-selected HeLa cytoplasmic RNA (Fig. 7, lane 2) revealed the presence

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**Fig. 5.** Reaction of *Drosophila* small nuclear RNAs with autoantibodies. Nuclear extracts were prepared from *Drosophila* Kc0 cells that had been labeled for 22 h with [3H]uridine. Lane 1, total HeLa cell small nuclear RNA markers; lanes 2–7, antibody-selected *Drosophila* nuclear RNAs; lane 2, nonimmune IgG; lane 3, anti-Sm; lane 4, anti-RNP; lanes 5–7, three different anti-La sera. The lower band in lane 4 is a U1 RNA fragment (see Fig. 2 in Ref. 4).

**Fig. 6.** Reaction of mouse and Xenopus U1 RNA with anti-La sera. Extracts from mouse erythroleukemia cell nuclei or *X. laevis* ovary were reacted with antibodies and the selected RNA was analyzed by U1 DNA hybridization as in Figs. 3 and 4. Lanes 1–3, mouse; lane 1, nonimmune IgG; lane 2, anti-La; lane 3, anti-RNP; lanes 4–6, Xenopus; lane 4, nonimmune IgG; lane 5, anti-La; lane 6, anti-RNP.

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2 H. Sass and T. Pederson, unpublished data.
were prepared, reacted with anti-La, and the selected and the remainder was incubated then added to 5 mM. One-half of the cells was immediately harvested and the selected RNAs displayed by electrophoresis and fluorography. Lane 1, pulse; lane 2, 45-min chase.

of species previously implicated as possible U1 RNA precursor molecules, it was of interest to know whether these same species could be recognized by anti-La as pulse-labeled RNAs and, in addition, to determine if they decay during a chase, in keeping with the idea that they may be U1 RNA precursors. Fig. 8 shows the results of an experiment in which HeLa cells were pulse-labeled with \[^{3}H\]uridine for 45 min. One-half of the culture was then chased for 45 min with unlabeled uridine (see figure legend for details). Lane 1 is the material selected from a cytoplasmic extract by anti-La at the end of the pulse label. In agreement with previous studies (3), a large spectrum of pulse-labeled small cytoplasmic La RNAs are seen, including 5 S rRNA and tRNA precursors (7). There is also a labeled band at the position of mature U1 RNA and, in addition, several larger bands whose positions and relative intensities correspond very closely to those of the bands hybridizing with U1 DNA in Fig. 7, lane 2. These bands show a pronounced decrease in radioactivity following the chase (Fig. 8, lane 2) except for one which we suspect to be 5.8 S rRNA, as indicated. (Being a stable component of cytoplasmic ribosomes, 5.8 S rRNA would not be expected to chase. The interpretation of this labeled band as a stable cytoplasmic RNA is further suggested by its persistence when the chase was extended to 90 min (data not shown).) There is also a decrease of radioactivity in the mature U1 RNA band itself during a chase. This may reflect a progressive loss of La antigenicity during U1 RNA maturation, or a movement of La-antigenic U1 to the nucleus, and is consistent with the absence of U1 from anti-La-reactive cytoplasmic RNA when a long (22 h) \[^{3}H\]uridine label is used (Fig. 1, lane 4).

**Discussion**

The results of this investigation indicate that in cells of several vertebrate species, a fraction of small nuclear RNA U1 reacts with anti-La sera. Several controls speak to the specificity of this result. First, 12 out of 12 different anti-La sera tested all gave the same result, and these sera also precipitated the familiar pattern of other La RNAs previously established (2, 3, 7). Thus, there is no reason to believe that the collection of patient sera we have used differs from the anti-La sera previously described. Second, the presence in these polyclonal sera of antibody against a non-La component of U1 RNP is ruled out by their lack of reaction with U1 RNP in Drosophila cells (Fig. 5), which do not contain La antigen (16). Third, although U1 and U2 RNAs are present at approximately equimolar concentrations, about 10\(^{6}\) molecules/HeLa cell nucleus, only U1 reacts significantly with anti-La (Figs. 1 and 2A). We wish to stress however that, while our results show that U1 RNA consistently reacts with anti-La sera, we have not directly demonstrated that this is due to an association between U1 and the 45,000-50,000 molecular weight protein previously identified as the major La antigen (21, 22). It is possible that all anti-La sera contain multiple specificities, and that these sera react with U1 RNA by virtue of an antigen different from the major La antigen. This notwithstanding, the consistency with which anti-U1 RNA activity appears in La sera (12 out of 12 tested) is striking.

The functional meaning of the observed reaction between nuclear U1 RNA and anti-La sera is unclear at present. An obvious possibility is that some U1 RNP is complexed with another small nuclear RNP that contains La antigen. Arguing against this is the fact that RNP antibody, which is specific for U1 RNP, does not precipitate any La RNA species (2-4, 11, 23). Another possibility is that U1 RNP is precipitated by anti-La sera by virtue of its association with large RNPs that contain pre-mRNAs (9), to which certain La RNAs may also be bound (e.g. Refs. 24 and 25). Arguing against this possibility is the fact that the anti-La-reactive U1 RNA sediments in 20 S nuclear particles which are the same size as those containing total nuclear U1 RNA (Fig. 4), and much smaller than the particles that contain pre-mRNA (9, 26). In addition, anti-La sera react with apparent cytoplasmic precursors of U1 RNA, which are not expected to be associated with nuclear pre-mRNA. A third line of evidence against this possibility is that high molecular weight RNA is not seen at the top of gels containing anti-La-selected nuclear RNA from pulse-labeled cells (not shown), as would be expected if anti-La sera were reacting with large ternary complexes containing hnRNA, U1 RNA, and La antigen.

The disappearance of anti-La-reactive, pulse-labeled cytoplasmic U1 RNA during a chase (Fig. 8) raises the possibility of a transient association of La antigen with U1 RNA molecules during processing. Recent RNase T1 digestion experiments of end-labeled cytoplasmic U1 RNA suggest that these molecules are processed at their 3'-ends to generate mature U1 (17). All RNAs previously shown to be associated with La antigen have short transcribed oligo(U) tracts at their 3' termini. These RNAs include precursors of 5 S rRNA and of tRNA (7, 27, 28), adenovirus VA RNAs (29), the Epstein-Barr virus-encoded small RNAs EBER-1 and EBER-2 (30), mouse 4.5 S RNA (31), and two small RNAs transcribed \textit{in vitro} from an Alu DNA sequence 3' of the human \textit{a1-globin} gene (32). The 3'-end of VA RNA may be involved in La antigen binding (22), and we have recently found that La antigen binds strongly to poly(U) \textit{in vitro}.\(^{3}\) VA RNA ends with CUUU, which are also the 3'-terminal nucleotides of human 5 S RNA (7, 33). 5 S RNA precursors end with CUUU, CUUUU, and CUUUUU (7). The sequences CTAT and CTTTTT have also been found at the 3'-ends of several mouse tRNA genes (27). In light of all these considerations, it is therefore striking that the sequence CUUU appears at the 3'-end of human U1 RNA precursors at nucleotides 167 to 170 from the (and including the) U1 RNA cap (34). These are

\(^{3}\) S. J. Madore, E. D. Wieben, and T. Pederson, manuscript in preparation.
the very lengths of the putative U1 RNA precursors (+2 to +6 nucleotides) that appear most reactive with anti-La sera (see lane 2 of Fig. 7).

If La antigen is associated with U1 RNA precursors during processing, why then does a small portion of U1 RNA react with anti-La sera? It may be that mature U1 retains La antigen for a short time after completion of processing, as appears to be the case for 5 S rRNA (e.g., see Fig. 1A in Ref. 5) or, alternatively, reacquires it. In this respect it is interesting that the same sequence discussed above, CUUU, also appears in mature human U1 RNA at nucleotides 156–159 (CUCU in mouse U1, Ref. 35), and perhaps this serves as a secondary La antigen binding site. This idea is also compatible with our observed lack of a significant reaction between anti-La sera and HeLa U2 RNP, as human U2 RNA lacks CUUU or oligo(U) sequences at its 3′-end (36). On the other hand, we find in preliminary experiments that U6 RNA reacts with anti-La sera.1 U6 RNA contains a run of Us at its 3′-end (37).

The final steps in U1 RNA processing may take place after these molecules return to the nucleus (12, 17). The La antigen could be a karyophilic protein (either intrinsically or as a property acquired, or intensified, when it combines with polymerase). A high nuclear affinity of La antigen could also be the mechanism by which 5 S rRNA and tRNA precursors are retained in the nucleus until their processing is completed (7, 38). These and related ideas concerning RNA intracellular traffic signals can be explored in Xenopus oocytes.

Although it is transcribed by RNA polymerase II (12, 34, 39), U1 RNA maturation may have some features of polymerase III transcript processing. The presence of the sequence CUUU in the 3′ termini of VA RNA (29), EBER RNAs (30), and the precursors of 5 S rRNA, tRNAs, and U1 RNA is consistent with this possibility, particularly if this sequence represents a polymerase III transcription termination site as has been proposed (27). The reaction of U1 RNA with anti-La sera reported here provides further impetus for considering this hypothesis.

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REFERENCES
Eukaryotic small ribonucleoproteins. Anti-La human autoantibodies react with U1 RNA-protein complexes.

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