

Lupus Antiribosomal P Antisera Contain Antibodies to a Small Fragment of 28S rRNA Located in the Proposed Ribosomal GTPase Center

By Jia-Li Chu,* Nathan Brot,† Herbert Weissbach,‡ and Keith Elkon*

From the *The Hospital for Special Surgery, Cornell University Medical Center, New York, New York 10021; and †The Roche Institute of Molecular Biology, Roche Research Center, Nutley, New Jersey 07110

Summary

The ribosomal P proteins are necessary for GTPase activity during protein synthesis. In addition to antibodies to the P proteins, sera from lupus patients contain anti-rRNA activity. To determine whether lupus antiribosomal sera recognize the region of 28S rRNA recently proposed to form part of the ribosomal GTPase center, an rRNA fragment corresponding to nucleotides (nt) 1922-2020 was transcribed in vitro and tested for antigenicity. 18 of 24 (75%) lupus sera containing anti-P antibodies, but only 2 of 24 (8%) lupus sera without anti-P, immunoprecipitated this rRNA fragment ($p < 0.001$). The binding was specific, since no significant differences were observed between anti-P positive and negative lupus sera in binding to the RNA fragment transcribed in the antisense orientation or to a control region of rRNA. The majority of sera tested protected a rRNA fragment of approximately 68 nucleotides. To evaluate the fine specificity of the anti-28S antibodies, deletions and site-directed mutations were made in the RNA fragment. The anti-28S antisera required nt 1944-1955 for recognition and were remarkably sensitive to destabilizing as well as nondestabilizing mutations in the stems of the RNA fragments. Detection of anti-protein and anti-RNA antibodies directed against a functionally related domain on the ribosome, together with the remarkable specificity of anti-28S antibodies, strongly suggests a direct role for this region of the ribosome in initiating and/or maintaining antiribosomal autoantibody production.

Antibodies reactive against single (1) and double (2, 3) stranded RNA have been detected in the sera of patients with systemic lupus erythematosus (SLE). However, these autoantibodies did not show specificity for the type or sequence of RNA (1-3). Previous studies have also suggested the possible existence of antibodies against ribosomal (r)¹ RNA although the specificity of these antibodies were not characterized (4-7). In our study of antiribosomal antibodies, we detected an RNase-sensitive antigen by counterimmunoelectrophoresis (8). Further efforts to characterize the antigen were, however, unsuccessful due to high background binding of all sera to rRNA (our unpublished observations). Recently, Uchiumi et al. (9) identified a SLE serum that contained antibodies against the ribosomal phosphoproteins P0, P1, and P2 as well as 28S rRNA. This anti-rRNA antibody was sequence specific since it bound to a 59 nucleotide fragment of 28S rRNA but did not bind to other regions of 28S, 18S

rRNA or tRNA (9). Sequence analysis of the RNA segment protected by the antibody revealed that the epitope was located between nucleotides 1944-2002 of 28S RNA (9). Since the P proteins (10-12) and 28S rRNA (9) may form part of a functional GTPase domain on the large ribosomal subunit, we investigated the relationship between anti-P and anti-28S RNA antibodies.

Materials and Methods

Polymerase Chain Reaction Amplification of Segments of 28S DNA. Two regions of 28S DNA, approximately 100 bp in length, were selected for study. The region 1922 to 2020 (28S-Ag) included the 59 base pair fragment (1944-2002) of 28S DNA encoding the RNA precipitated by the reference serum (9). Nucleotides 4430 to 4528 (28S-C) was selected as a control test antigen on the basis of similar size and similar GC content to 28S-Ag (50.5% for 28S-Ag and 48.5% for 28S-C). The following primer pairs were used to amplify the segments of 28S DNA: 5'-GCGAATTCAGGIGTTGGT-TGATATAGAC-3' and 5'-GCTCTAGATTTTCAGGGCTAGTTG-ATTCG-3' for 28S-Ag; 5'-GTGAAGCAGAATTCGCCAAGC-3'

¹ Abbreviations used in this paper: AS, antisense; r, ribosomal.

and 5'-GCTCTAGATCAGTAGGGTAAAACCTACC-3' for 28S-C. 1 μ g of genomic DNA isolated from HeLa cells was amplified through 30 cycles by the PCR using Taq polymerase (13). Following amplification, the primers were removed by ultrafiltration through Ultrafree membranes (Millipore Corp., Bedford, MA). The DNA fragments were restricted with Eco RI and Xba I and subcloned into the plasmid pGem 3 (Promega, Madison, WI). All of the DNA sequences were verified by dideoxy sequencing.

Transcription. Plasmids containing either the 28S-Ag or 28S-C DNA were linearized by Eco RI or Xba I. Transcription was performed by the method of Melton et al. (14) using either T7 or SP6 polymerase and [α - 32 P]CTP. Since pGem3 contains SP6 and T7 promoters in opposite orientations, sense and antisense RNA were synthesized from the pGem3-28S-Ag construct (Fig. 1). Following transcription, the plasmids were digested with RNase-free DNase and the RNA isolated by phenol/chloroform extraction and ethanol precipitation.

Sera and Immunoprecipitation. Sera from patients with SLE containing anti-P antibodies have been described in detail elsewhere (8, 15, 16). Anti-P levels were quantified by ELISA using a recombinant human P2 fusion protein (rHuP2) as antigen (17). Sera from patients with other multisystem autoimmune diseases (Sjogren's syndrome, polymyositis, and scleroderma) as well as normal healthy controls have also been described (18).

A 10% (v/v) suspension of Sepharose-protein A (Pharmacia Fine Chemicals, Piscataway, NJ) was incubated with 5 μ l of test serum for 30 min at room temperature. The beads were centrifuged, washed with buffer A (20 mM Tris, pH 7.6, 100 mM KCl, and 5 mM MgCl₂) and then incubated with the *in vitro* transcribed, 32 P-labeled 28S rRNA fragments for 45 min at 4°C. 10 μ g of yeast tRNA (Sigma Chemical Co., St. Louis, MO) was added to the reaction to minimize degradation of the 32 P-labeled RNA. The beads were washed three times with buffer A and then counted in liquid scintillation counter. Values greater than 3 SDs above the mean of 10 normal controls were considered positive. In some cases, the immunoprecipitated RNA was extracted from the protein A beads and analyzed on a 10% polyacrylamide gel containing 7 M urea, 90 mM Tris, 90 mM borate and 1 mM EDTA, pH 8.6 (7 M urea PAGE).

RNA Protection. To determine the size of 28S RNA protected by anti-28S antibodies, IgG fractions were incubated with 32 P-labeled RNA synthesized by *in vitro* transcription in the presence of 2 μ g of tRNA for 20 min at 30°C (9). 2 U of RNase T1 (Pharmacia Fine Chemicals) were added to the reaction and incubation continued for an additional 15–30 min. Immunoprecipitation was performed as described above and the RNA fragments detected by 7 M urea PAGE and autoradiography. The sizes of the major fragments protected by the antibodies were determined by comparison with the mobility of RNA fragments of known size.

Results

Anti-P Sera Immunoprecipitate 28S-Ag rRNA. The amount of radiolabeled 28S-Ag rRNA precipitated by SLE sera positive (+) or negative (-) for anti-P antibodies is shown in Fig. 2, panel Ag. 18 of 24 (75%) anti-P positive sera precipitated the 28S-Ag rRNA compared to only 2 of 24 (8%) anti-P negative SLE controls (Yates $\chi^2 = 19.3$, $p < 0.001$). None of the normal controls (Fig. 2, Ag) and only 1/15 (7%) sera obtained from patients with other autoimmune diseases precipitated the 28S-Ag rRNA (not shown).

To determine whether lupus sera containing anti-P anti-

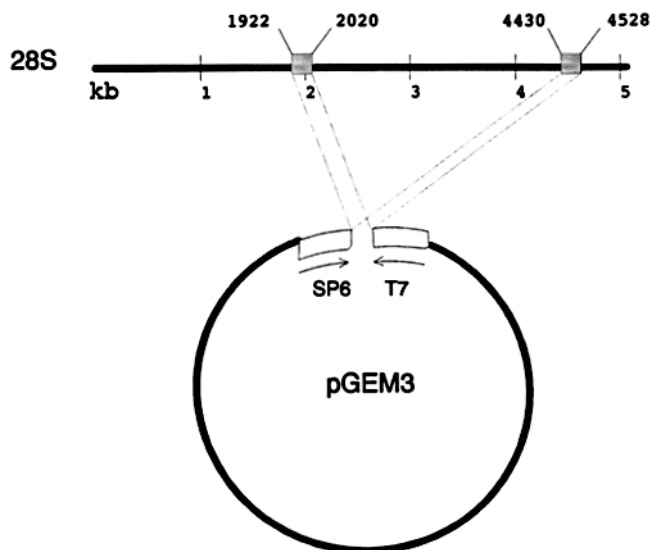


Figure 1. Schematic diagram of the construction of plasmids for transcription. Following amplification of bp 1922–2020 (28S-Ag) and 4430–4528 (28S-C) of 28S DNA by PCR, the DNA fragments were subcloned into pGem3 and transcribed by SP6 or T7 polymerase. The transcribed RNAs were used as test antigens for immunoprecipitation.

bodies bound specifically to the 28S-Ag fragment, the complementary strand of 28S-Ag DNA was transcribed to produce the 28S antisense (AS) rRNA. In addition, a control region of 28S DNA was transcribed to produce the rRNA fragment, 28S-C (Fig. 1). These DNA fragments were transcribed with similar efficiency to 28S-Ag and were of the ap-

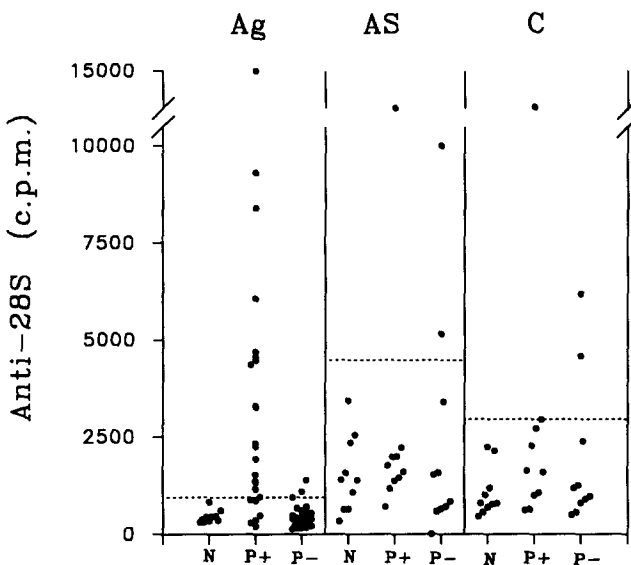


Figure 2. Immunoprecipitation of 28S-Ag (Ag), antisense (AS), and control (C) fragments of 28S-rRNA. The RNA fragments were transcribed *in vitro* in the presence of [α - 32 P]CTP and incubated with IgG-bound to Sepharose protein A beads. The results are expressed as the amount of radiolabeled RNA precipitated. The horizontal dashed lines represent the mean + 3 SD of radiolabeled RNA precipitated by the normal control sera.

appropriate size when analyzed on a denaturing polyacrylamide gel (not shown). 10 normal sera and 10 randomly selected sera from the anti-P (+) and anti-P (-) groups were tested for binding to these RNAs. As shown in Fig. 2, panels AS and C, only a minority of lupus sera bound to these rRNA fragments and there were no significant differences between anti-P (+) and anti-P (-) sera. Preincubation of IgG fractions obtained from anti-28S-Ag sera with 5 μ g of *Escherichia coli* rRNA either had no effect, or increased the amount

of 28S-Ag rRNA precipitated (presumably by preventing nonspecific adsorption or degradation of the labeled RNA).

Relationship between Anti-P and Anti-28S-Ag Antibodies. To ensure that quantitative differences in anti-28S-Ag levels could be detected within the range studied, a standard curve was constructed with serial two-fold dilutions of the highest titer anti-28S-Ag serum. A sigmoid curve was obtained indicating that the anti-28S Ag levels could be quantified by the immunoprecipitation assay (not shown). We then compared anti-28S-Ag and anti-P levels in the same 24 SLE anti-P (+) sera. There was no correlation between the levels of these two antibodies ($r = 0.15, p > 0.5$).

Patients with certain neuropsychiatric forms of SLE show changes in serum anti-P levels which parallel disease activity (16, 19). To determine whether anti-P and anti-28S-Ag levels fluctuated in parallel, the levels of these two antibodies were compared in sera obtained from three patients with lupus psychosis. As shown in Fig. 3, only one of the three sera (C) demonstrated parallel changes in anti-P and anti-28S-Ag levels. Since the antibody profile shown in A was compatible with an idio/anti-idio mechanism, a mixing experiment was performed. Equal volumes of serum from the first and fourth bleeds were mixed and tested for anti-P and anti-28S activity. No decrease of either antibody activity, other than that accounted for by dilution, was observed.

Nucleotide Sequence Comparisons. It was previously shown that the epitope on human 28S rRNA was identical to nucleotides 1767-1825 of mouse 28S rRNA (9). To further evaluate the degree of conservation of the epitope, the 59 bp sequence of human 28S rRNA 1944-2002, as well as approximately 175 bp flanking this sequence were compared with the DNA sequences of the genes encoding yeast (*Saccharomyces cerevisiae*) 25S rRNA (20) and *E. coli* 23S rRNA (21). Comparisons were made by the method of Wilbur and Lipman (22) using the DNASTAR program Align. As shown in Fig. 4, there was a striking similarity between the human and yeast rRNA in the region of epitope (97% similarity and no gaps). For comparison, the flanking sequences showed 53-77% similarity with 7 gaps (not shown). Although the similarity between

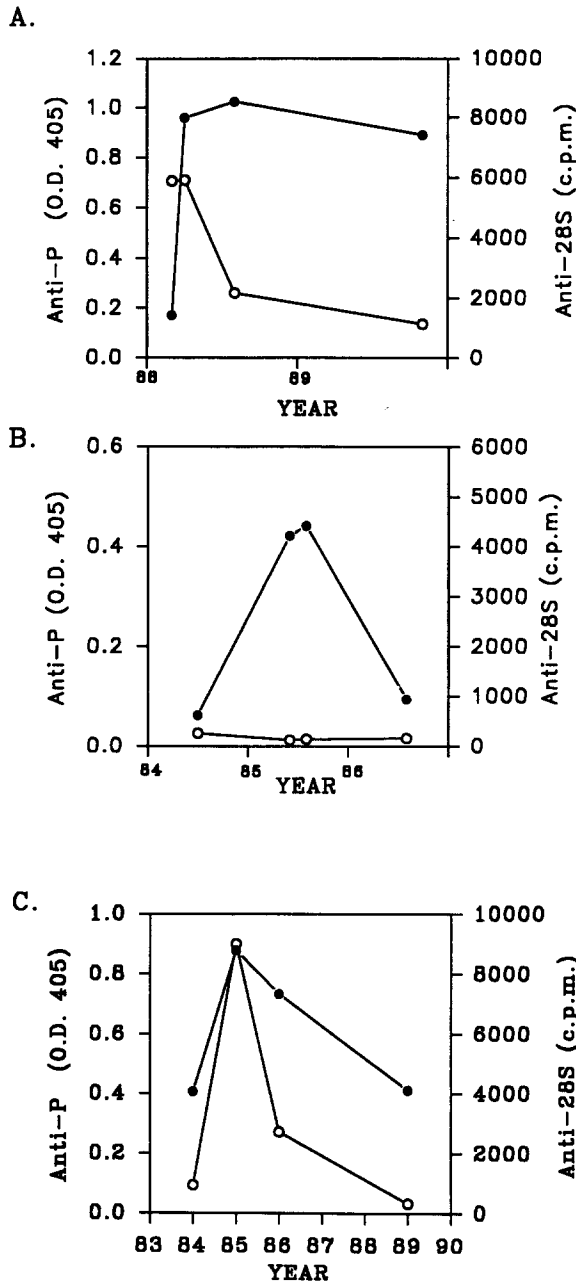


Figure 3. Longitudinal studies of anti-28S and anti-P levels in patients with lupus psychosis. Stored serum samples were tested for anti-28S-Ag (O) by immunoprecipitation and for anti-P (●) by ELISA using a recombinant human P2 fusion protein (17). A, B, and C represent studies performed on three different SLE patients.

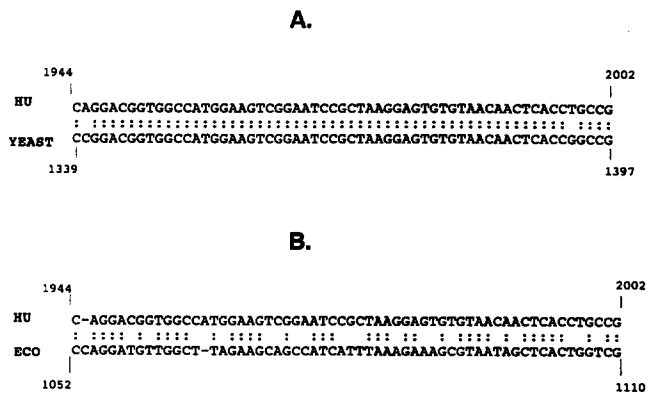


Figure 4. Nucleotide sequence similarities between the 28S-Ag epitope (nucleotides 1944-2002) of human (Hu) 28S (42) and the corresponding regions of Yeast 25S (A) and *E. coli* (Eco) 23S (B) ribosomal RNAs (19, 20). Nucleotide identity is shown by a colon.

the human 28S and *E. coli* 23S rRNA was much lower in the region of the epitope (63%, Fig. 4), the flanking regions showed only 53–56% similarity with large gaps to maximize homology. To determine whether the 28S-Ag epitope contained sequence homology to nonribosomal nucleotide sequences, a homology search was performed with the sequences contained in GenBank^R, release 64. No significant sequence similarities to foreign bacteria or viral RNAs were observed.

RNA Protection. To determine whether the boundaries of the 28S Ag were the same for all anti-28S sera, an antibody RNase protection experiment was performed. Seven sera which exhibited minimal nonspecific binding to the control RNAs, 28S-C and 28S-AS, were tested. As shown in a representative experiment (Fig. 5), the smallest fragment protected was approximately 68 nt (lanes 3–6). Increasing the amount of RNase T1 to 20 U resulted in a loss of the 105 nt fragment and accumulation of the 68 nt fragment (not shown). One of the seven sera (from patient, Pa), protected two fragments of approximately 50 and 46 nt (lane 2).

Fine Specificities of Anti-28S Antibodies. To further evaluate the fine specificities of the anti-28S Ag antibodies, changes in the sequence and conformation of the 28S RNA Ag were made (Table 1). These changes are described in the legend to Fig. 6 and their predicted effects on the secondary structure of RNA (23) are shown in Fig. 6, A–E. The deletion mutant, A, was created by restricting the 28S Ag DNA at the unique Nco I site (bp 1955). Mutants B through E, were created by oligonucleotide synthesis and were ligated into the Eco RI and Sma I sites of pGEM 3. All of the mutants were verified by dideoxy nucleotide sequencing and the sizes of the labeled RNA transcripts evaluated by 7 M urea PAGE (not shown).

To determine whether anti-28S sera would bind to shorter fragments of the 28S Ag RNA, immunoprecipitation of mutant A was performed with the 7 anti-28S sera described above. As shown in Table 1, deletion of the twelve most 5' nucleotides resulted in almost a 90% decrease in binding when compared to the reference 28S Ag. When the same sera were tested for binding to mutant B, which lacked only the 3 most 3'

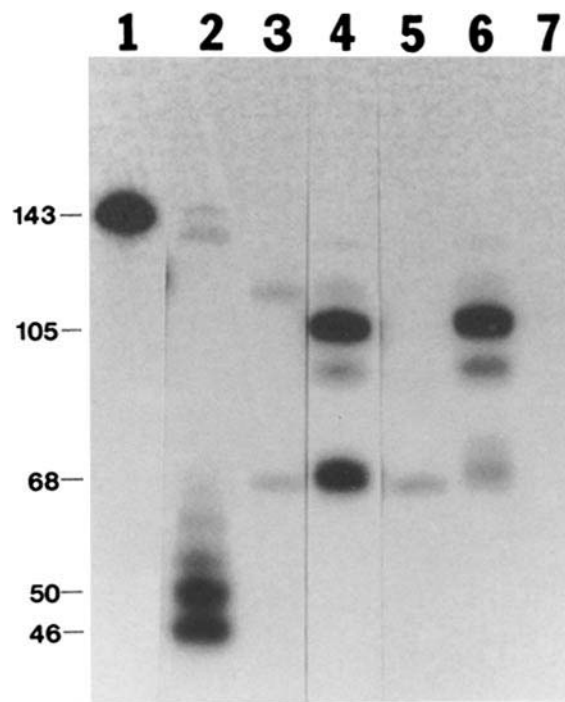


Figure 5. RNA protection by anti-28S IgG. ³²P-labeled 28S Ag rRNA was incubated with IgG obtained from five patients with anti-28S antibodies (lanes 2–6) or a normal control (lane 7). 2 U of RNase T1 was added to the reaction and the protected RNA isolated by protein A immunoprecipitation and phenol/chloroform extraction. The protected fragments were analyzed on a 7 M urea 10% polyacrylamide gel. Lane 1 contains the 28S Ag without exposure to IgG or RNase T1. The estimated size (nt) of the RNA fragments are shown on the left.

nucleotides, 5/7 sera showed similar recognition of the 28S Ag and mutant B whereas 2 sera immunoprecipitated only 2% (serum Pa) and 43% as much RNA compared to the 28S Ag. Further studies on the effect of site directed mutagenesis were therefore performed with the 5 sera that bound to mutant B RNA. The 8 nucleotide substitutions of mutant C were designed to destabilize the secondary structure

Table 1. Recognition of 28S RNA Ag Mutants by Anti-28S rRNA Sera

Mutant	Change*	Length	Predicted* change in secondary structure	% Immunoprecipitated†
		nt		
A	Δ1944–1955	47	Yes	11 (3–25)
B	Δ2000–2002	56	No	75 (2–125)
C	8 substitutions	56	Yes	9 (2–14)
D	2 substitutions	56	Yes	10 (3–16)
E	4 substitutions	56	No	22 (8–39)

* The positions of the changes and their predicted effect on the secondary structure of 28S RNA are shown in Fig. 6.

† The values for each mutant were calculated from (cpm mutant RNA precipitated/cpm 28S Ag RNA precipitated) × 100. The mean (and range) of 7 test sera are shown for mutants A and B. Only 5 sera were studied for mutants C–E since 2 sera demonstrated low binding to mutant B.

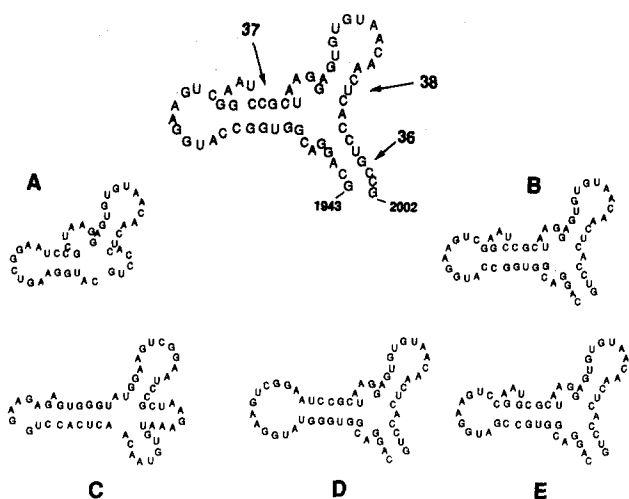


Figure 6. Deletions and site-directed mutations of the 28S Ag RNA. (Top) predicted secondary structure of human 28S rRNA nt 1943-2002. (Bottom) predicted secondary structures of the 28S Ag RNA mutants. Mutation A was produced by the deletion of nt 1944-1955. Mutants B-E were produced by oligonucleotide synthesis and all contained a deletion of the three 3' terminal nucleotides (nt 2,000-2,002) of 28S Ag. Mutants C-E contained additional site-directed changes at the nucleotide numbers indicated: mutant C, 1944 (C→G), 1946 (G→A), 1949 (C→G), 1950 (G→A), 1955 (C→G), 1956 (C→T), 1979 (G→A) and 1981 (G→A); mutant D, 1955 (C→G) and 1956 (C→T); mutant E, 1954 (G→C), 1956 (C→G), 1966 (G→C) and 1971 (C→G). The secondary structures of the RNA fragments were predicted by the FOLD program (23). The helices are labeled 36-38 according to the secondary structure proposed for human 28S RNA (37).

of 28S RNA by preventing base pairing of all 3 stems, 36-38 (Fig. 6 C). As shown in Table 1, antibody binding to this RNA fragment was minimal. To determine the effect of destabilization of a single stem, only 2 nucleotide substitutions were introduced into stem 37 of mutant D (Fig. 6 D). Despite the minimal sequence changes and predicted alteration in secondary structure, limited antibody recognition of this fragment was observed (Table 1). Since all previous alterations in the RNA sequence (except B) were also predicted to have effects on the secondary structure of 28S RNA, reciprocal changes in two G-C pairs were made in stem 37 of the 28S Ag so as not to disrupt base pairing (Fig. 6 E). Antibody recognition of this structure was also poor (Table 1). Although mutants were constructed with substitutions in the loops of the 28S Ag, recombinant plasmids could not be isolated from *E. coli* for reasons which are not understood.

Finally, to determine whether anti-28S antibodies could distinguish between ribo- and deoxyribo nucleic acids with the same predicted secondary structure, mutant B oligonucleotides (sense and anti-sense strands) were end labeled with [γ - 32 P]-ATP and T4 polynucleotide kinase. Immunoprecipitation was performed with the same 5 sera that bound to mutant B RNA. No significant differences in binding to sense and antisense mutant B oligonucleotides were observed. Similarly, there was no correlation between the levels of binding to the sense mutant B oligonucleotide and 28S Ag RNA.

Discussion

The major finding in this study is that the majority of lupus anti-P sera, but <10% of anti-P negative SLE sera or sera from patients with other autoimmune disorders, contained antibodies that bound to a small fragment of 28S RNA (28S-Ag). This binding was specific for 28S-Ag since anti-P sera did not show increased binding to RNA transcribed from the same 28S DNA fragment in the opposite direction antisense (28S-AS) or to a control RNA fragment (28S-C) of similar size and GC composition. Furthermore, the binding of anti-28S-Ag antibodies to the 28S-Ag could not be inhibited by yeast tRNA or *E. coli* rRNA. The higher background binding of all sera to the control segments of human 28S rRNA may explain our previous failure to detect sequence specific anti-28S antibodies using the intact 28S RNA molecule. Although the studies performed here do not exclude the possibility that anti-P or other lupus antisera bind to additional epitopes on 28S RNA, detailed analysis of a single high titer anti-P and anti-28S serum revealed that the only RNA epitope that could be identified on either 28 or 18S rRNA was located between nucleotides 1944-2002 of 28S rRNA (9).

The *E. coli* ribosomal proteins, L10, L7/12 (L7 is the acetylated form of L12), are the prokaryotic homologues of the eukaryotic phosphoproteins P0, P1, and P2. Although the exact mechanism is not known, the presence of the L7/L12 protein is required for the interaction of elongation factors with the ribosome and for subsequent GTP hydrolysis (reviewed in reference 24). A role for 23S rRNA in GTPase activity was also suggested by inhibition of GTP hydrolysis by the antibiotic, thiostrepton (25). The binding site for thiostrepton has been localized to nucleotides 1055-1081 on *E. coli* 23S rRNA (26). Thus, both the L7/L12 protein and a specific region of 23S may constitute the rRNA functional domain responsible for elongation factor binding and GTP hydrolysis on the *E. coli* ribosome. The epitope on 28S RNA recognized by SLE antisera is highly conserved in eukaryotic organisms (97% sequence identity with yeast rRNA) and shares 62% sequence identity with nucleotides 1051-1110 of *E. coli* 23S rRNA (Fig. 6). Since both anti-P antibodies (12, 27) and the prototype anti-28S-Ag antibody (9) inhibited elongation factor binding and GTPase activity, it seems likely that the P proteins and nucleotides 1944-2002 of 28S rRNA constitute a similar functional domain to that observed on *E. coli* ribosomes. The properties of the 28S-Ag epitope viz. sequence conservation and participation in an active functional domain are common to most other epitopes mapped on SLE autoantigens (28).

Case reports of antibodies reactive against individual tRNAs (29, 30) and U1 RNA (31) have been described. It was suggested that these anti-RNA autoantibodies arise through an idiotype/antiidiotype mechanism since the patients' sera also contained antibodies reactive against the proteins to which the RNAs bind (29, 31). An idiotype/anti-idiotype mechanism is unlikely to explain the presence of anti-28S-Ag antibodies in SLE anti-P sera for the following reasons. The purified P protein homologues from *E. coli* (32) and from

yeast (33) do not bind directly to rRNA. We also failed to detect binding of isolated P1/P2 to the 28S rRNA Ag by a nitrocellulose filter binding assay or by RNase protection (our unpublished observations). Longitudinal analysis of antibody levels in individual patients with lupus psychosis and elevations in anti-P levels, showed reciprocal fluctuations of anti-28S-Ag antibodies in only one out of three patients studied. Mixing experiments in sera obtained from this patient did not suggest an idiotype/antiidiotype mechanism. Considerable evidence supports the idea that autoantibodies in SLE are antigen-driven (reviewed in reference 34). Since, as discussed above, the P proteins and the 28S-Ag rRNA appear to constitute an active functional site, we favor the idea that SLE patients produce autoantibodies against the proposed GTPase domain on the 60S subunit (9). Parallel fluctuations in the levels of anti-P and anti-28S autoantibodies in only one of three patients analyzed longitudinally may be explained by different patterns of the immune response to protein versus nucleic acid antigens. Rather than an idiotype/antiidiotype pattern, the antibody profile shown in Fig. 3, panel A, could reflect an initial response to 28S RNA followed by autoantibody production against the P proteins. Longitudinal studies of large numbers of patients will be required to determine the relationship between anti-P and anti-28S antibodies.

Although considerable information is now available regarding autoantibody recognition of protein antigens (reviewed in reference 28), little is known about antibody recognition of specific RNA epitopes. Detailed analysis of a single anti-U1 RNA antibody (31), revealed that the smallest epitope recognized was a 40 nt fragment located within the second stem loop structure. A very recent study of anti-U1 RNA

activity (35) reported that specific anti-U1 RNA antibodies were common in sera with anti-RNP antibodies. These anti-U1 RNA antisera showed heterogeneity in epitope recognition, binding to any one or more stem loops of U1 RNA. Investigation of an anti-tRNA^{ala} antibody (36) revealed that the antibody bound to 7-9 nt fragment located in the anticodon loop. In the present study, we have shown that the dominant epitope recognized by the majority of anti-28S sera is similar, or identical, to that identified by the prototype anti-28S serum (9). One serum, however, protected a smaller fragment of 28S RNA and lost total reactivity when three of the 3' nucleotides were removed from the RNA fragment. All sera tested, failed to bind to an RNA fragment from which the 12 most 5' nucleotides had been deleted indicating that this region is essential for antibody recognition. The proposed secondary structure of the 28S Ag shown in Fig. 6 is virtually identical to the secondary structure proposed for the same region of intact human 28S rRNA (37) and the corresponding regions of mouse (38), *Zenopus* (39), yeast (40), and *E. coli* (41) rRNA. Site-directed mutations of the 28S fragment revealed that antibody recognition was remarkably sensitive to sequence and conformational changes. Substitution of only 4 nt without a predicted change in secondary structure, resulted in a striking decrease in antibody binding.

Although the factors that initiate autoantibody production remain unknown, the demonstration of autoantibodies reactive against a very limited number of ribosomal proteins (8), identification of a single immunodominant epitope on the P proteins (15) and localization of anti-28S-Ag antibodies to a specific, functionally related domain on 28S rRNA, provide important clues to the origin of antiribosomal antibodies in SLE.

We thank T. Uchiumi, Niigata University School of Medicine, for helpful discussions.

This work was supported, in part, by Grant AR-38915 and a Research Career Development Award (to K. B. Elkon) from the National Institutes of Health, Bethesda, MD, and the New York Chapter of the Arthritis Foundation.

Address correspondence to Keith Elkon, The Hospital for Special Surgery, 535 East 70th St., New York, NY 10021.

Received for publication 8 May 1991.

References

1. Eilat, D., A.D. Steinberg, and A.N. Schechter. 1978. The reaction of SLE antibodies with native, single-stranded RNA: radioassay and binding specificities. *J. Immunol.* 120:550.
2. Schur, P.H., and M. Monroe. 1969. Antibodies to ribonucleic acid in systemic lupus erythematosus. *Proc. Natl. Acad. Sci. USA.* 63:1108.
3. Attias, M.R., R.A. Sylvester, and N. Talal. 1973. Filter radioimmunoassay for antibodies to Reovirus RNA in systemic lupus erythematosus. *Arthritis Rheum.* 16:719.
4. Lamon, E.W., and J.C. Bennett. 1970. Antibodies to ribosomal ribonucleic acid (rRNA) in patients with systemic lupus erythematosus (SLE). *Immunology.* 19:439.
5. Sturgill, B.C., and R.R. Carpenter. 1965. Precipitating antibodies to ribosomes in the serum of patients with systemic lupus erythematosus. *Arthritis Rheum.* 8:213.
6. Schur, P.H., L.A. Moroz, and H.G. Kunkel. 1967. Precipitating antibodies to ribosomes in the serum of patients with systemic lupus erythematosus. *Immunochemistry.* 4:447.
7. Francoeur, A.-M., C.L. Peebles, K.J. Heckman, J.C. Lee, and E.M. Tan. 1985. Identification of ribosomal protein antigens.

- J. Immunol.* 135:2378.
8. Elkon, K.B., A.P. Parnassa, and C.L. Foster. 1985. Lupus autoantibodies target the ribosomal P proteins. *J. Exp. Med.* 162:459.
 9. Uchiumi, T., R.R. Traut, K.B. Elkon, and R. Kominami. 1991. A human autoantibody specific for a unique conserved region of 28S ribosomal RNA inhibits the interaction of elongation factors 2 with ribosomes. *J. Biol. Chem.* 266:2054.
 10. Van Agthoven, A.J., J.A. Maasen, and J. Moller. 1977. Structure and function of an acidic protein from 60S ribosomes and its involvement in elongation factor-2 dependent GTP hydrolysis. *Biochem. Biophys. Res. Comm.* 77:989.
 11. Sanchez-Madrid, F., R. Reyes, P. Conde, and J.P.G. Ballesta. 1979. Acidic ribosomal proteins from eukaryotic cells. Effect on ribosomal functions. *Eur. J. Biochem.* 98:409.
 12. Uchiumi, T., R.R. Traut, and R. Kominami. 1990. Monoclonal antibodies against acidic phosphoproteins P0, P1, and P2 of eukaryotic ribosomes as functional probes. *J. Biol. Chem.* 265:89.
 13. Saiki, R.K., D.H. Gelfand, S. Stoffel, S.J. Schare, R. Higuchi, G.T. Hoen, K.B. Mullis, and H.A. Erlich. 1988. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science (Wash. DC)*. 239:487.
 14. Melton, D.A., P.A. Krieg, M.R. Rebagliati, T. Maniatis, K. Zinn, and M.R. Green. 1984. Efficient *in vitro* synthesis of biologically active RNA and RNA hybridization probes from plasmids containing a bacteriophage SP6 promoter. *Nucleic Acids Res.* 12:7035.
 15. Elkon, K.B., S. Skelly, A.P. Parnassa, W. Moller, H. Weissbach, and N. Brot. 1986. The identification and synthesis of a ribosomal protein antigenic determinant in systemic lupus erythematosus. *Proc. Natl. Acad. Sci. USA.* 83:7419.
 16. Bonfa, E., S.J. Golombek, L.D. Kaufmann, S. Skelly, H. Weissbach, N. Brot, and K.B. Elkon. 1987. Association between lupus psychosis and antiribosomal P protein antibodies: measurement of antibody using a synthetic peptide antigen. *N. Engl. J. Med.* 317:265.
 17. Magsaam, J., A.E. Gharavi, A.P. Parnassa, H. Weissbach, N. Brot, and K.B. Elkon. 1989. Quantitation of lupus antiribosome P antibodies using a recombinant P2 fusion protein and determination of the predicted amino acid sequence of the autoantigen in patients' mononuclear cells. *Clin. Exp. Immunol.* 76:165.
 18. Bonfa, E., A.P. Parnassa, D.D. Rhoads, D.J. Roufa, I.G. Wool, and K.B. Elkon. 1989. Antiribosomal S10 antibodies in humans and MRL/lpr mice with systemic lupus erythematosus. *Arthritis and Rheum.* 32:1252.
 19. Schneebaum, A.B., J.D. Singleton, S.G. West, J.K. Blodgett, L.G. Allen, J.C. Cheronis, and B.L. Kotzlin. 1991. Association of psychiatric manifestations with antibodies to ribosomal P proteins in systemic lupus erythematosus. *Am. J. Med.* 90:54.
 20. Bayev, A.A., O.I. Georgiev, A.A. Hadjiolov, N. Nikolaev, K.G. Skryabin, and V.M. Zakharyev. 1981. The structure of the yeast ribosomal RNA genes. 3. Precise mapping of the 18S and 25S rRNA genes and structure of the adjacent regions. *Nucleic Acids Res.* 9:789.
 21. Brosius, J., T.J. Dull, and H.F. Noller. 1980. Complete nucleotide sequence of a 23S ribosomal RNA gene from *Escherichia coli*. *Proc. Natl. Acad. Sci. USA.* 77:201.
 22. Wilbur, W.J., and D.J. Lipman. 1983. Rapid similarity searches of nucleic acid and protein data banks. *Proc. Natl. Acad. Sci. USA.* 80:726.
 23. Jaeger, J.A., D.H. Turner, and M. Zuker. 1989. Improved predictions of secondary structures for RNA. *Proc. Natl. Acad. Sci. USA.* 86:7706.
 24. Weissbach, H. 1980. Soluble factors in protein synthesis. In *Ribosomes: Structure, Function, and Genetics*. G.R. Craven, J. Davies, K. Davis, L. Kahan, and M. Nomura, editors. University Park Press, Baltimore. pp. 377-412.
 25. Cundliffe, E. 1980. Antibiotics and prokaryotic ribosomes: action, interaction, resistance. In *Ribosomes: Structure, Function, and Genetics*. G. Chambliss, G.R. Craven, J. Davies, K. Davis, L. Kahan, and M. Nomura, editors. University Park Press, Baltimore. pp. 555-581.
 26. Thompson, J., F. Schmidt, and E. Cundliffe. 1982. Site of action of a ribosomal RNA methylase conferring resistance to thiostrepton. *J. Biol. Chem.* 257:7915.
 27. Stacey, D.W., S. Skelly, T. Watson, K. Elkon, H. Weissbach, and N. Brot. 1988. The inhibition of protein synthesis by IgG containing antiribosome P autoantibodies from systemic lupus erythematosus patients. *Arch. Biochem. Biophys.* 267:398.
 28. Tan, E.M. 1989. Antinuclear antibodies: diagnostic markers for autoimmune diseases and probes for cell biology. *Adv. Immunol.* 44:93.
 29. Bunn, C.C., R.M. Bernstein, and M.B. Mathews. 1986. Autoantibodies against alanyl-tRNA synthetase and tRNA coexist and are associated with myositis. *J. Exp. Med.* 163:1281.
 30. Wilusz, J., and J.D. Kreene. 1986. Autoantibodies specific for U1 RNA and initiator methionine tRNA. *J. Biol. Chem.* 261:5467.
 31. Deutcher, S.L., and J.D. Keene. 1988. A sequence-specific conformational epitope on U1 RNA is recognized by a unique autoantibody. *Proc. Natl. Acad. Sci. USA.* 85:3299.
 32. Dijk, J., R.A. Garret, and R. Muller. 1979. Studies on the binding of the ribosomal protein complex L7/12-L10 and protein L11 to the 5' one third of 23S RNA: a functional center of the 50S subunit. *Nucleic Acids Res.* 6:2717.
 33. El-Baradi T.A.L., V.C.H.F. de Regt, S.W.C. Einerhand, J. Teixido, R.J. Planta, J.P.G. Ballesta, and H.A. Rave. 1987. Ribosomal proteins EL 11 from *Escherichia coli* and L15 from *Saccharomyces cerevisiae*, bind to the same site in both yeast 26S and mouse 28S rRNA. *J. Mol. Biol.* 195:909.
 34. Gharavi, A.E., J.L. Chu, and K.B. Elkon. 1988. Autoantibodies in systemic lupus erythematosus are not due to random polyclonal B cell activation. *Arthritis Rheum.* 31:1337.
 35. Van Venrooij, W.J., R. Hoet, J. Castrop, I.W. Mattaj, and L.B. van de Putte. 1990. Anti-(U1) small nuclear RNA antibodies in anti-small nuclear ribonucleoprotein sera from patients with connective tissue diseases. *J. Clin. Invest.* 86:2154.
 36. Bunn, C.C., and M.B. Mathews. 1987. Autoreactive epitope defined as the anticodon region of alanine transfer RNA. *Science (Wash. DC)*. 238:1116.
 37. Gorski, J.L., I.L. Gonzalez, and R.D. Schmickel. 1987. The secondary structure of human 28S rRNA: the structure and evolution of mosaic rRNA gene. *J. Mol. Evol.* 24:236.
 38. Michot, B., J.P. Bachelierie, and F. Raynal. 1983. Structure of mouse rRNA precursors. Complete sequence and potential folding of the spacer regions between 18S and 28S rRNA. *Nucleic Acids Res.* 12:4259.
 39. Clark, C.G., B.W. Tague, V.C. Ware, and S.A. Gerbi. 1984. *Xenopus laevis* 28S ribosomal RNA: a secondary structure model and its evolutionary and functional implications. *Nu-*

- cleic Acids Res.* 12:6197.
40. Veldman, G.M., J. Klootwijk, V.C.H.F. de Regt, R.J. Planta, C. Branlant, A. Krol, and J.P. Ebel. 1981. The primary and secondary structure of yeast 26 rRNA. *Nucleic Acids Res.* 9:6935.
41. Noller, H.F. 1984. Structure of ribosomal RNA. *Annu. Rev. Biochem.* 53:119.
42. Gonzalez, I.L., J.L. Gorski, T.J. Campen, J.M. Erickson, J.E. Sylvester, and R.D. Schmickel. 1985. Variation among human 28S ribosomal RNA genes. *Proc. Natl. Acad. Sci. USA.* 82:7666.