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Antibodies in human serum that precipitate ribonuclease P

(systemic lupus erythematosus/ribonucleoprotein/tRNA processing)

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ABSTRACT **Sera from certain patients with systemic lupus erythematosus (SLE) and related rheumatic diseases contain antibodies that selectively deplete extracts of HeLa cells of RNase P activity. Most of the sera that recognize RNase P, an endoribonuclease with an essential RNA subunit, also contain antibodies against another small ribonucleoprotein known as the Th antigen. A species of RNA about 400 nucleotides in length is the only RNA species found in common in all immunoprecipitates prepared with anti-RNase P antibodies. The discovery of antibodies against RNase P defines a major class of antibodies produced by patients with autoimmune disease.**

Sera from patients with systemic lupus erythematosus (SLE) and related rheumatic diseases contain antibodies to various small nuclear and cytoplasmic ribonucleoprotein complexes designated snRNPs and scRNPs, respectively (1). These antibodies have proved to be powerful tools for defining the structure and function of a variety of macromolecules. For example, anti-U1 RNP, anti-U2 RNP, and anti-Sm snRNP antibodies were crucial to the elucidation of the role played by the U snRNPs in the processing of pre-mRNA (2).

RNase P, a ribonucleoprotein, is an enzyme essential for the biosynthesis of the 5′ termini of tRNAs (3). Under certain conditions *in vitro* the RNA component of the enzyme from *Escherichia coli* and other prokaryotes exhibits catalytic activity (4). Studies of RNase P from eukaryotic cells have proceeded slowly because the enzymatic activity is labile (5, 6) and the enzyme is much less abundant than other RNPs. We previously showed that RNase P from HeLa cells has essential RNA and protein components, but we were unable to identify unequivocally any single species of RNA associated with the enzymatic activity. We now report that a subset of patients with SLE and related rheumatic diseases produces antibodies against RNase P. These antibodies occur in nearly 25% of patients and thus constitute a major autoantibody class. By using these anti-RNase P antibodies, we have identified a major species of RNA that copurifies with RNase P and that is selectively immunoprecipitated with the enzymatic activity from RNase P preparations.

MATERIALS AND METHODS

Sera. Sera were obtained from patients followed in the rheumatology clinic at the Yale University School of Medicine. Sixty sera were selected for study because of their high titers of anti-nuclear antibodies detected by immunofluorescence.

Preparation of RNase P. RNase P was prepared from HeLa cells (purchased from the Massachusetts Institute of Technology Cell Culture Center and grown and prepared under sterile conditions) by an extension of methods previously described (7). All buffers were supplemented with 1 mM dithiothreitol/0.2 mM phenylmethylsulfonyl fluoride. An S20 extract was prepared from whole cells and loaded onto a column of DEAE-Sepharose, and the fractions that contained the peak of enzymatic activity were concentrated by using Amicon Centriflo concentrating filters. This material was then loaded onto a glycerol gradient (15–25% glycerol; 100 mM KCl/50 mM Tris·HCl, pH 8.0/10 mM $MgCl_2$) and centrifuged in an SW41 rotor at 35,000 rpm for 23 hr at 4°C. Fractions were collected through a needle at the bottom of each tube. The peak fractions of enzymatic activity were concentrated and centrifuged on a second glycerol gradient. The peak of RNase P activity was then concentrated by using Amicon Centricon filters and the enzyme was stored at 0°C. Whole cells are used as starting material rather than nuclei because the yield of activity is much higher.

Monoclonal Antibody. Y12, a mouse monoclonal antibody that recognizes and precipitates Sm snRNPs, was a generous gift from Douglas L. Black (Yale University, New Haven, CT).

Immunoprecipitation of RNase P Activity with Antibody Coupled to Sepharose Beads. Sera were incubated with protein A-Sepharose (PAS) and immunoprecipitations were carried out by using a procedure slightly modified from that described by Matter *et al.* (8). After the antibodies were coupled to PAS, the complex was washed three times with NET-2 buffer (50 mM Tris·HCl/150 mM NaCl/0.05% Nonidet P-40, pH 7.4) and once with RNase P reaction buffer, RM (30 mM Tris·HCl/100 mM NH_4Cl/5 mM $MgCl_2$/0.1 mM EDTA/0.1 mM 2-mercaptoethanol, pH 8). RNase P in RM buffer was then added and the samples were incubated with mixing for 1–2 hr at 4°C. The mixture was then centrifuged, and the supernatant was removed and assayed for RNase P activity by using standard conditions.

Immunoprecipitation of Ribonucleoprotein from ^{32}P-Labeled Cell Extracts. HeLa cells (2×10^5 cells per ml) were incubated for 12 hr in phosphate-depleted RPMI 1640 medium (GIBCO) that contained $^{32}PO_4$ (10 μCi/ml; 1 Ci = 37 GBq). After the cells were harvested, a crude extract was prepared as described, except that the cells were washed once and sonicated in RSB buffer (9). Ten times concentrated RM buffer was added to give a final concentration of 1× RM buffer in the cell extract (see above). The extract was then added to antibodies previously coupled to PAS (prepared as described above) and incubated with mixing for 1 hr at 4°C. The mixture was centrifuged and the pellet was washed three times with NET-2 buffer. The pellet was resuspended in NET-2 buffer and the RNA bound to the pellet was extracted, precipitated, and analyzed by electrophoresis in a 5% polyacrylamide/7 M urea gel.

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Abbreviations: SLE, systemic lupus erythematosus; PAS, protein A-Sepharose; snRNP and scRNP, small nuclear and small cytoplasmic ribonucleoprotein, respectively.

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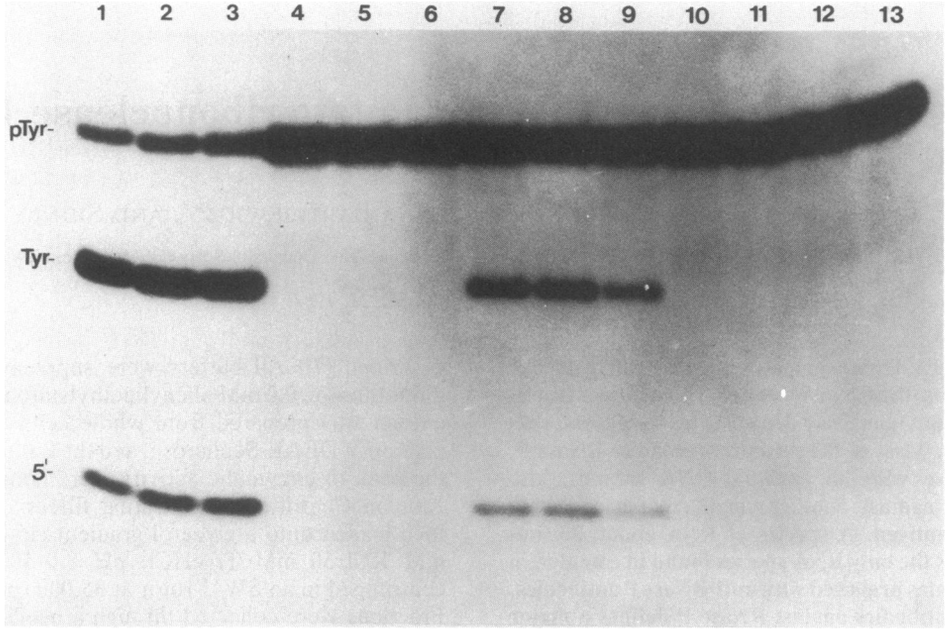

FIG. 1. Depletion of RNase P activity from extracts of HeLa cells using antibodies found in the sera of patients with SLE and related rheumatic diseases. See text for antibody coupling to PAS beads. The precursor to $tRNA^{Tyr}$, transcribed *in vitro* (C. Guerrier-Takada and S.A., unpublished data), was used as substrate in the RNase P assay. The position of the precursor is indicated by pTyr, and the cleavage products are indicated by Tyr (the segment that contains the sequence of the mature tRNA) and 5′ (the segment that contains the extra nucleotides cleaved from pTyr at the 5′ end of the mature tRNA). Lane 1, mock immunoprecipitation of RNase P (no antibodies are coupled to the PAS). Lane 2, normal human serum coupled to PAS added. Lane 3, anti-Ro scRNP serum added. Lanes 4–6, three different sera, each containing anti-Th snRNP antibodies. In lanes 1–6, 3 μl of supernatant was assayed for RNase P activity. Lanes 7–12, duplicates of the reactions shown in lanes 1–6 but only 1 μl of supernatant was assayed in each case. Lane 13, precursor alone with no RNase P added.

RESULTS

Sera from patients with SLE and related rheumatic diseases were tested for their ability to inactivate the RNase P activity in partially purified extracts of HeLa cells. All of these sera have been characterized by their ability to precipitate known snRNPs and scRNPs. The RNase P activity was prepared from crude extracts of cells by chromatography on DEAE-Sepharose and subsequent sedimentation through glycerol gradients (see *Materials and Methods*). These preparations of RNase P, purified about 300-fold, were identical in their chromatographic behavior to preparations made from isolated nuclei. As shown in Fig. 1, antibodies from sera of selected patients were highly effective in removing RNase P activity from these extracts. In these experiments, partially purified RNase P was preincubated with (PAS) beads alone or with beads coated with antibodies from various patients or control serum. The amount of RNase P activity that is present when the enzyme is incubated in the presence of PAS alone is shown in lanes 1 and 7. When normal serum was coupled to PAS, the amount of RNase P activity was the same as that observed when no serum was added (compare lanes 1 and 7 to lanes 2 and 8). In contrast, PAS beads coated with antibodies from sera of certain patients effectively removed RNase P activity from these extracts (see lanes 4–6 and 10–12). These three sera also contain anti-Th antibodies. Although antibodies from sera of patients with SLE generally interact with protein, they precipitate ribonucleoprotein complexes and, in part, have been characterized by the RNA species they bring down (1). Thus, anti-Th antibodies were previously defined by their ability to immunoprecipitate a nucleolar RNA of 300 nucleotides from extracts of HeLa cells (10–13) by way of an undefined antigenic protein(s). Serum from another patient with SLE, which contained antibodies to the Ro scRNP (1), had no effect on RNase P activity (see lanes 3 and 9). Precipitates formed by complexes made of the PAS-coupled antibodies with the preparation of RNase P can be resuspended and some enzymatic activity can be detected in the resuspended material.

A survey of sera from 60 patients revealed that 14 had antibodies against RNase P. (Seven patients had SLE, three had scleroderma, one had primary biliary cirrhosis, and three had undefined connective tissue diseases.) The large fraction of samples of sera in which these antibodies were found suggests that RNase P may be a relatively frequent target for the autoimmune responses of SLE and related rheumatic diseases. Table 1 summarizes the antibody specificities of the various sera screened and the percentage of patients in each category who also have antibodies against RNase P. Some sera were not as effective as others in precipitating RNase P activity (see Table 1, footnote †), a result that suggests that

Table 1. Autoantibody specificities of sera

Antibody specificity*	Number of sera	Anti-RNase P activity†	% anti-RNase P
Th	9	9‡	100
Sm/U1	16	2§	12.5
Ro/La	28	3	11¶
Others	7	0	0
Total	60	14	23

*Antibody specificities were determined as described (1, 10). Non-Th sera gave negative results for the Th specificity (that is, for the presence of 7-2 RNA) even in overexposed autoradiographs.

†Assays for RNase P activity were carried out as described in the text and in the legend to Fig. 1. Assays were carried out so that controls, with no serum added, gave ≈50% cleavage of substrate and represented reactions in the linear range of the kinetics of the cleavage reaction. The inhibition of RNase P activity ranged between 70% and 100%.

‡Three of these sera also contain antibodies against Ro scRNP and one has antibodies against U1 RNP and rRNAs.

§One of these sera also has anti-Ro scRNP antibodies.

¶This percentage does not include the four sera from patients who have anti-Ro scRNP antibodies, which are listed here under the other specificities they have.

the titer of antibodies against RNase P varies among patients, as do most other autoantibodies. The only strong correlation between anti-RNase P antibodies and other known autoantibodies is with anti-Th. All sera with anti-Th also have anti-RNase P antibodies; conversely, 64% of sera with anti-RNase P antibodies also had anti-Th antibodies. An anti-Sm monoclonal antibody (see *Materials and Methods*) lacked the ability to deplete RNase P activity from extracts of HeLa cells, in agreement with our previous finding that RNase P does not bind to an anti-Sm affinity column (14).

The identification of antibodies that bind RNase P suggests that they might serve as a tool for the identification of the components of this RNA–protein complex. Moreover, the association of these antibodies with anti-Th antibodies suggests that the RNA component of RNase P may be related to the so-called 7-2 RNA (≈300 nucleotides long), which is immunoprecipitated by anti-Th sera (see Fig. 2, lanes 5–7, and refs. 10–13). However, when partially purified preparations of RNase P were immunoprecipitated with sera that contain anti-Th antibodies, the 7-2 RNA was not evident in the resulting precipitate. Instead, as seen in Fig. 3, the major components were RNAs of approximately 400 and 200 nucleotides in length, designated H1 and H2, respectively. We observed less H1 than H2 in the precipitates when using

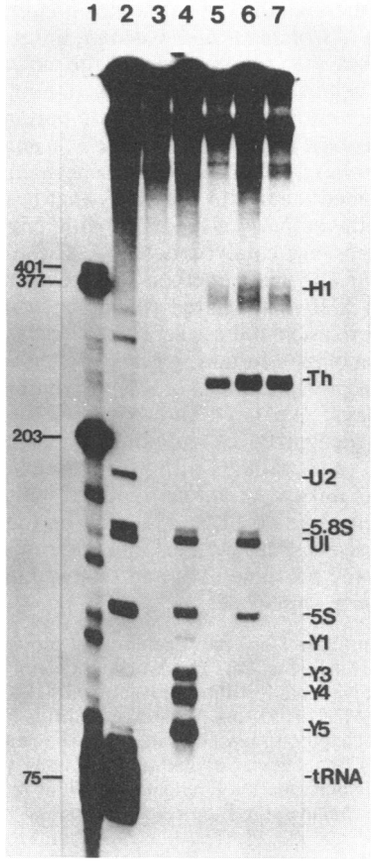

FIG. 2. Immunoprecipitation of RNAs from extracts of whole HeLa cells. Extracts were prepared and precipitated and the precipitates were analyzed. Lane 1, molecular size standards with the number of nucleotides in each shown on the left. Lane 2, total RNA from HeLa cells. Lane 3, RNAs precipitated with normal human serum. Lane 4, RNAs precipitated with serum that contains anti-Ro scRNP, anti-rRNA, and anti-U1 snRNP antibodies. Lanes 5–7, RNAs precipitated with sera that contain antibodies against Th and RNase P snRNPs. The serum used in lane 6 also has anti-U1 RNP and anti-rRNA antibodies. The positions of Th RNA, H1 (the putative RNA component of RNase P from HeLa cells), and other cellular RNAs are marked on the right. The Y RNAs are precipitated by anti-Ro serum.

older preparations of enzyme and when using sera that had lower titers of anti-RNase P antibodies. In separate experiments we have shown that H1 and H2 RNAs are greatly enriched in those fractions that contain RNase P activity during the purification of this enzyme from HeLa cells. The relative proportion of H1 and H2 RNAs changes from <1% of the total RNA in an S20 fraction to >75% in our purest fractions. In contrast, 7-2 RNA does not copurify with RNase P, as is evident especially in Fig. 3*B*. The absence of 7-2 RNA is not due to a technical artifact as this species is diminished in relative amount at every step in the RNase P purification procedure.

With a modification of buffers that stabilize RNase P activity (see *Materials and Methods*), we were able to immunoprecipitate from extracts of whole cells labeled with ^{32}P an RNA that has the same mobility as H1 RNA (see Fig. 2, lanes 5–7; the material migrating more slowly than H1 RNA in lanes 2–7 disappears after treatment of the extracts with DNase). It is apparent that there are at least two distinct RNAs that migrate at the position of H1 RNA. H1 RNA may have heterogenous ends, it may have two conformations, or there may be two distinct species of RNA in precipitates from crude extracts of HeLa cells that migrate at the position of H1 RNA. We have found, however, that H1 RNA migrates faster than an RNA of 400 nucleotides on a 5% denaturing polyacrylamide gel and slower than this standard on an 8% denaturing polyacrylamide gel and that H1 RNA migrates as a diffuse band unless it is boiled before loading on the gel. The structural properties of H1 RNA that are responsible for these different electrophoretic mobilities remain to be determined. In separate experiments, one of us (M.B., unpublished data) has recently shown that H1 RNA prepared by immunoprecipitation from whole cell extracts and isolated from a 5% denaturing gel has the same sequence as H1 RNA, the major RNA species that copurifies with RNase P activity.

DISCUSSION

This study demonstrates that certain sera from patients with rheumatic diseases recognize RNase P and selectively immunoprecipitate an RNA 400 nucleotides in length, designated H1 RNA. This RNA is only immunoprecipitated by sera that inhibit RNase P activity. Moreover, this RNA is the major RNA species that copurifies with RNase P activity and, since that enzymatic activity can be inactivated by treatment with other RNases (16), it is likely that H1 RNA is the essential RNA component of HeLa cell nuclear RNase P. Rigorous proof of this last statement will come from a demonstration of catalytic activity of a transcript of H1 RNA from the DNA of the corresponding, cloned gene.

The ability of antibodies to immunoprecipitate Th and H1 RNA from extracts of whole cells, as well as the finding that some sera that have anti-RNase P antibodies do not have anti-Th antibodies, led us to conclude that antibodies against RNase P constitute a major class of antibodies produced by patients with autoimmune disease. Our results indicate that the sera we employed are polyclonal. Sera having more than one antibody specificity have been previously described for many other autoantibody systems (17). Our findings that most of the sera that contain anti-RNase P antibodies have anti-Th antibodies and that every sample of serum that contains anti-Th antibodies also contains anti-RNase P antibodies suggest that the antibodies to Th and RNase P be classified as a linked set (18).

We previously reported that there were at least three major species of RNA smaller than 200 nucleotides in preparations of RNase P partially purified from the nuclei of HeLa cells. We also indicated that the enzymatic activity was very unstable and that these species of RNA could be breakdown products of a larger molecule. We have now been able to

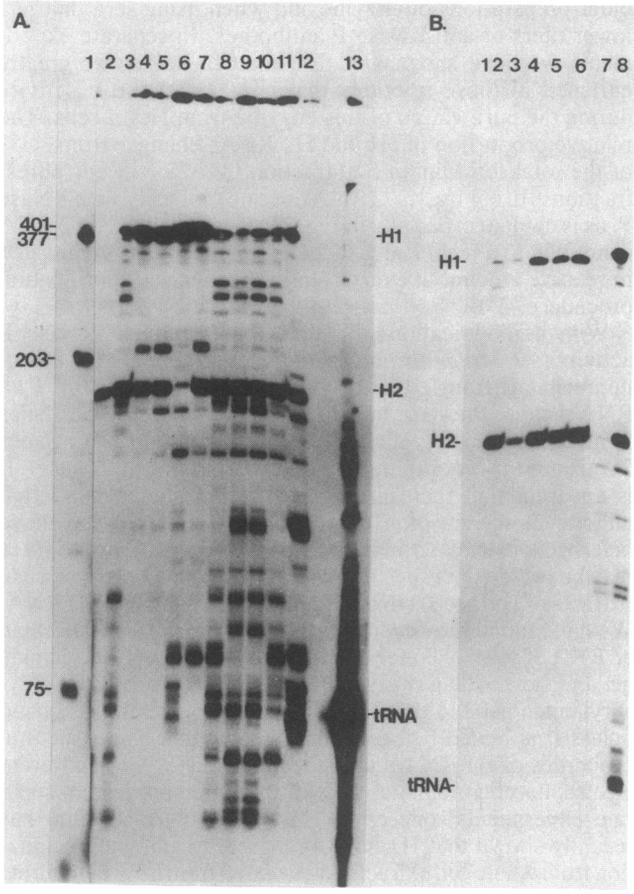

FIG. 3. RNAs immunoprecipitated from RNase P partially purified from HeLa cells. After immunoprecipitation of partially purified RNase P with antibodies coupled to PAS, the beads were washed three times with NET-2 buffer and then resuspended in NET-2 buffer. The bound RNAs were then extracted and precipitated with oyster glycogen as carrier. The RNAs were then labeled at their 3′ terminus with [^{32}P]pCp as described (15). The labeled RNAs were analyzed on an 8% polyacrylamide/7 M urea gel. The positions of H1 RNA, H2 RNA, and tRNAs are shown. (*A*) RNAs that were immunoprecipitated from a fresh preparation of RNase P. Lane 1, molecular size markers with the number of nucleotides in each shown on the left. Lanes 2–11, RNAs that were immunoprecipitated when RNase P from HeLa cells was incubated with anti-RNase P antibodies coupled to PAS beads. Lanes 3–7, RNAs that were immunoprecipitated when antibodies that inhibit RNase P activity by close to 100% were used. Lanes 2 and 8–11, RNAs that were immunoprecipitated when antibodies that inhibit RNase P activity by ≈70% were used. The enzyme that was used to immunoprecipitate the RNAs shown in lanes 4–7 was ≈1 day old, whereas the enzyme that was used to immunoprecipitate the RNAs shown in lanes 2, 3, and 8–11 was 10 days old. Lane 12, total RNA extracted from the same RNase P that was used for the experiments shown in lanes 2, 3, and 8–11. Lane 13, total RNA extracted from an S20 extract of HeLa cells. (*B*) RNAs resulting from an immunoprecipitation experiment that was done by using the same preparation of RNase P that was used in the experiments shown in *A*, except that it was 3 weeks old at the time of use. The autoradiograph shown in *B* was exposed for ≈20 min, whereas the autoradiograph shown in *A* was exposed for ≈2 hr. An extended exposure of the autoradiograph shown in *B* reveals the presence of the minor species of RNA visible in *A*. Lane 1, RNAs immunoprecipitated with normal serum. Lanes 2–6, RNAs immunoprecipitated with sera that contain anti-RNase P antibodies. The serum used in lane 3 inhibited enzymatic activity less strongly than the sera used in lanes 2 and 4–6. Lane 7, RNAs immunoprecipitated with serum that does not contain anti-RNase P antibodies but does contain antibodies against Sm RNP and U1 RNP. Lane 8, total RNA extracted from the partially purified RNase P.

stabilize the enzymatic activity and have found that H1 and H2 RNAs are by far the dominant RNA species in fresh preparations of enzyme. However, we have found that H1 RNA is rather unstable and that, as the preparation ages, H1 RNA disappears. Nucleotide sequence analysis indicates that the two species of RNA that migrate at the position of H2 RNA (on a 5% polyacrylamide/7 M urea gel, the two RNAs are resolved) are the result of a single break in H1 RNA and that H1 RNA is different from any mammalian RNA of previously known sequence. Although the evidence is uncertain, it is possible that H1 RNA is the previously identified 8-2 RNA, which is immunoprecipitated by two sera with anti-Th antibodies (12, 13). However, the latter RNA is part of a 10S particle, in contrast to RNase P, which occurs in a 15S particle. The 8-2 RNA has also been reported to be cytoplasmic, whereas RNase P is a nuclear enzyme.

It seems most likely that antibodies from the sera of patients with autoimmune diseases bind RNase P through interaction with an epitope constituted at least in part of protein. Only in two cases have autoantibodies been noted to recognize nucleotides directly (19, 20). In preliminary studies utilizing immunoblots (21–23), we have observed that sera containing anti-RNase P antibodies recognize in common a 38-kDa protein in cell extracts enriched for RNase P activity.

We have shown that sera from certain patients with SLE and related rheumatic diseases contain antibodies that are highly effective in binding RNase P from HeLa cells. [We have also found that anti-Th antibodies precipitate RNase P from *E. coli* and *Halobacterium volcanii*, though with a much lower efficiency than they precipitate the enzymatic activity from HeLa cells (H.A.G. and S.A., unpublished experiments).] These antibodies provide an important tool for the investigation of the structure of RNase P, a snRNP involved in the biosynthesis of tRNA. More specifically, they will facilitate detailed studies of the RNA that is thought to be essential for the activity of the eukaryotic enzyme and that may, itself, possess catalytic activity. These observations also provide an enzymatic method for categorizing sera from patients with SLE and related rheumatic diseases. In the past, characterization of these sera has been based upon their ability to precipitate certain species of RNA or upon the appearance of precipitin lines of identity in double immunodiffusion assays. We have shown that RNase P and Th snRNPs are recognized by autoantibodies that appear together in the sera of patients with SLE and related rheumatic diseases. This linkage of autoantibodies, which was previously noted in cases of SLE (17), may be indicative of an intracellular association, perhaps in a large macromolecular complex like the prosome (24), and related functions of the Th and RNase P snRNPs.

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