*Proc. Natl. Acad. Sci. USA*
Vol. 81, pp. 1996–2000, April 1984
Biochemistry

# The Ro small cytoplasmic ribonucleoproteins: Identification of the antigenic protein and its binding site on the Ro RNAs

(small cytoplasmic RNAs/rheumatic disease/autoantibodies/RNA–protein interactions)

SANDRA L. WOLIN AND JOAN A. STEITZ

Department of Molecular Biophysics and Biochemistry, Yale University, P.O. Box 3333, New Haven, CT 06510

*Contributed by Joan A. Steitz, December 19, 1983*

ABSTRACT **Patients with systemic lupus erythematosus and other rheumatic diseases often possess autoantibodies directed against discrete classes of small ribonucleoprotein particles (RNPs). The class of particles recognized by anti-Ro antibodies contains from two to four small cytoplasmic RNAs, depending on the mammalian species examined. We find that an antigenic polypeptide of 60 kDa is the major protein residing in Ro RNPs from human HeLa cells. To determine what common feature of Ro RNA sequence or structure is recognized by the Ro protein, we carried out ribonuclease protection experiments on isolated Ro RNPs from HeLa cells. For each of the three human Ro RNAs whose sequence is known, the most highly protected portion found in immunoprecipitates corresponded to the lower section of a stem formed by base-pairing the 5′ and 3′ ends of the RNA. Within this protected helix is a highly conserved region composed of seven identical base pairs with a single bulged cytidine. We discuss possible functions for the Ro RNPs.**

Anti-Ro antibodies from patients with systemic lupus erythematosus precipitate several small cytoplasmic ribonucleoproteins (scRNPs) from mammalian cells (1). The RNA components of these particles, designated hY1–hY5 in human cells and mY1 and mY2 in mouse cells, range in size from 83 to 112 nucleotides (2–4). Sequences have been reported for three of the four unique human Ro RNAs (hY2 is a processing or degradation product of hY1). They exhibit many sequence and secondary structure homologies (3, 4). Each Ro RNA is present in about $10^5$ copies per cell, or about 1% the number of ribosomes. Because of their relatively low abundance, Ro scRNPs were not detected before the use of patient antibodies as probes.

Ro RNAs are also precipitated by another lupus antibody, anti-La, which recognizes a 50-kDa protein that binds many nascent RNA polymerase III transcripts, including adenovirus VA RNAs, Epstein–Barr virus EBER RNAs, and precursors to tRNA and 5S rRNA (1, 2, 5–8). The Ro RNAs are also synthesized by RNA polymerase III (4); reassembly experiments have demonstrated that a proportion of the Ro scRNPs actually contain the La protein in addition to protein(s) carrying the Ro determinant (2), although anti-Ro and anti-La antibodies frequently are present simultaneously in patient sera (9).

The exact number of proteins associated with Ro scRNPs has not been well defined. Proteins are required for antigenicity as the isolated RNAs are not precipitable by anti-Ro antibodies (1). In the mouse, Ro RNAs are found exclusively in anti-Ro-precipitable scRNPs, indicating that the majority of Ro RNAs are bound by the Ro protein(s) (4).

We show here that the major protein component of the Ro scRNPs is a single antigenic polypeptide of 60 kDa. Ribonuclease protection experiments indicate that this protein binds to a highly conserved feature of all Ro RNAs, a stem formed by pairing the 5′ and 3′ ends of the molecules.

## MATERIALS AND METHODS

**Cells, Extracts, and Sera.** Cells were maintained as described (4). Cell sonicates (5) were prepared for RNA analysis by labeling $2 \times 10^7$ cells with $^{32}PO_4$ (50 μCi/ml; 1 Ci = 37 GBq) in phosphate-free minimal essential medium (GIBCO) for 14–16 hr, or for protein analysis by labeling $1 \times 10^7$ cells with [$^{35}$S]methionine (10 μCi/ml) in methionine-free minimal essential medium for 20 hr.

Sera from patients with systemic lupus erythematosus or related autoimmune disorders were provided by J. Hardin (Yale University), S. Malawista (Yale University), M. Reichlin (Oklahoma Medical Research Foundation, Oklahoma), M. Akizuki (Keio University, Tokyo), and G. McCarty (Georgetown University).

**Immunoprecipitation of Proteins and RNA.** A procedure based on that of Matter *et al.* (10) was used. Protein A Sepharose CL-4B (Pharmacia) preswollen in NET-2 (150 mM sodium chloride/ 10 mM Tris·HCl, pH 7.5/0.05% Nonidet P-40) was incubated with 2 μl of crude serum for 1 hr at room temperature, and then washed 3 times with NET-2. The antibody-bound beads were incubated for 15 min at 4°C with an aliquot of labeled cell sonicate corresponding to 5 ml of cells. After three washes with NET-2, the bound material was extracted either with $NaDodSO_4$ gel sample buffer (for proteins) (11) or treatment with phenol/$NaDodSO_4$ (phenol/chloroform/isoamyl alcohol, 50:50:1/0.1% $NaDodSO_4$) (for RNA) as described by Lerner and Steitz (12).

RNAs were fractionated on 15% polyacrylamide (acrylamide/bisacrylamide, 27:1) gels in 7 M urea/45 mM Tris borate, pH 8.3/1.25 mM EDTA. Bands were extracted by the crush and soak method (13). T1 and pancreatic ribonuclease fingerprints of eluted RNAs were prepared (14) using thin-layer homochromatography on PEI 300 (Brinkmann) for the second dimension (12), and the resulting oligonucleotides were subjected to secondary analysis (14).

Proteins were analyzed by electrophoresis on 10% polyacrylamide/$NaDodSO_4$ gels (11). Gels were soaked for 30 min in 0.5 M sodium salicylate, dried, and autoradiographed.

**Ribonuclease Protection Experiments.** $^{32}$P-labeled immune complexes bound on protein A-Sepharose beads (see above) were resuspended in 500 μl of NET-2 containing 5 mM $MgCl_2$ and 40 μg of carrier yeast RNA. This mixture was digested for 15 min at 25°C with pancreatic ribonuclease at concentrations ranging from 10 μg/ml to 1 mg/ml. The beads were then washed 4 times with NET-2 and extracted with phenol/$NaDodSO_4$. The nuclease-resistant RNA fragments were precipitated with ethanol in the presence of 20 μg of carrier RNA, and electrophoresed as described above. As

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: RNP, ribonucleoprotein; scRNP, small cytoplasmic ribonucleoprotein.

controls, immunoprecipitates were extracted with phenol/NaDodSO$_4$, precipitated with ethanol, resuspended in the buffer described above and mixed with antibody bound on protein A-Sepharose beads prior to digestion with pancreatic ribonuclease. The digested mixture was extracted with phenol/NaDodSO$_4$ and processed as described above.

**Protein Blots.** Cytoplasmic extracts of HeLa cells (15) (provided by E. Gottlieb, Yale University) were separated by electrophoresis on 10% polyacrylamide/NaDodSO$_4$ gels and the protein was transferred to nitrocellulose sheets (16, 17). Nitrocellulose sheets were blocked overnight in 1% gelatin in phosphate-buffered saline (50 mM $KH_2PO_4$, pH 7.4/130 mM NaCl). The sheets were washed 3 times (5 min each) in phosphate-buffered saline containing 0.05% Triton X-100 (buffer T), then incubated with antisera in buffer T for 2 hr at room temperature. After 3 washes in buffer T, the sheets were probed with $^{125}$I-labeled protein A in buffer T, washed as described above, and autoradiographed.

## RESULTS

**Anti-Ro Sera Precipitate a Single Major Antigenic Protein of 60 kDa from Mammalian Cell Extracts.** To examine the protein components of Ro scRNPs we labeled human HeLa cells with [$^{35}$S]methionine and incubated samples of a whole-cell extract with anti-Ro sera. The gel in Fig. 1*A* shows that autoantibodies from four different Ro patients (lanes 3–6) and a Ro,La patient (lane 7) precipitate a common major polypeptide of ≈60 kDa, although additional bands unique to individual sera are sometimes seen. Anti-Ro sera from seven different patients all immunoprecipitate this protein. When a mixture of $^3$H amino acids was used to label cells, no additional polypeptides were seen (data not shown). The 60-kDa protein appears conserved across mammalian species: a protein that comigrates with the HeLa cell protein is precipitated from mouse Friend erythroleukemia cells (data not shown) by anti-Ro antibodies.

To determine whether the 60-kDa polypeptide seen in immunoprecipitates corresponds to the antigenic moiety of Ro scRNPs, immunoblots were prepared using a cytoplasmic extract of HeLa cells (15). Fig. 1*B* compares the proteins recognized by a serum containing anti-La antibodies (lane 6), a serum containing mostly anti-Ro antibodies but also low-titer anti-La antibodies (lane 4), and a normal (nonimmune) serum (lane 8). The serum containing high-titer anti-Ro antibodies reacts with a 60-kDa protein as well as the 50-kDa La protein (lane 4) (8, 18); normal serum (lane 8) and the serum containing only anti-La antibodies do not detect the 60-kDa protein. These results indicate that the 60-kDa protein that dominates anti-Ro immunoprecipitates is antigenic, and that the presence of bound RNA is not absolutely required for Ro antigenicity. All seven anti-Ro sera blot a 60-kDa protein, although in certain cases it is necessary to use an anti-Ro immunoprecipitate as a concentrated source of antigenic protein. All seven sera also show anti-La specificity (as seen in Lane 4) in immunoblots, although these secondary autoantibodies are not usually evident from examining immunoprecipitated RNAs.

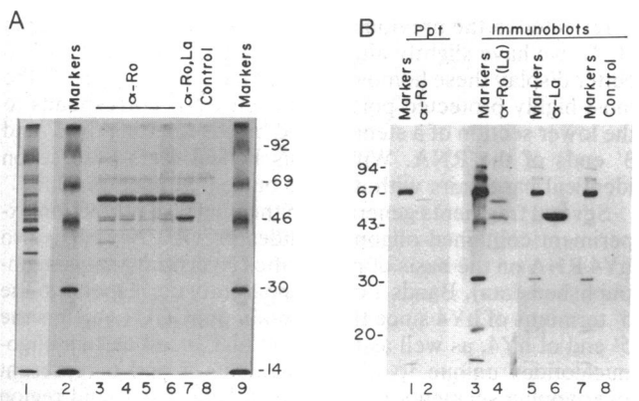


FIG. 1. Proteins reactive with anti-Ro autoantibodies. (*A*) $^{35}$S-labeled HeLa cell proteins contained in immunoprecipitates were analyzed as described in *Materials and Methods*. Lanes: 1, a light exposure of total cell proteins; 2 and 9, $^{14}$C-methylated molecular size marker proteins (in kDa); 3–6, proteins immunoprecipitated by anti-Ro sera from four different patients; 7, proteins immunoprecipitated by a patient serum characterized as containing both anti-Ro and anti-La antibodies; 8, proteins immunoprecipitated by normal (nonimmune) human serum. (*B*) Immunoblot comparison of proteins identified by serum from a patient with anti-Ro antibodies (lane 4), serum from a patient with anti-La antibodies (lane 6), and a normal (nonimmune) human serum (lane 8). Lanes 1, 3, 5, and 7 contain $^{125}$I-labeled molecular size marker proteins (in kDa). Lane 2, a $^{35}$S-labeled anti-Ro immunoprecipitate from a HeLa whole-cell sonicate. The anti-Ro serum used in lane 4 is the same as that used in *A*, lane 6.

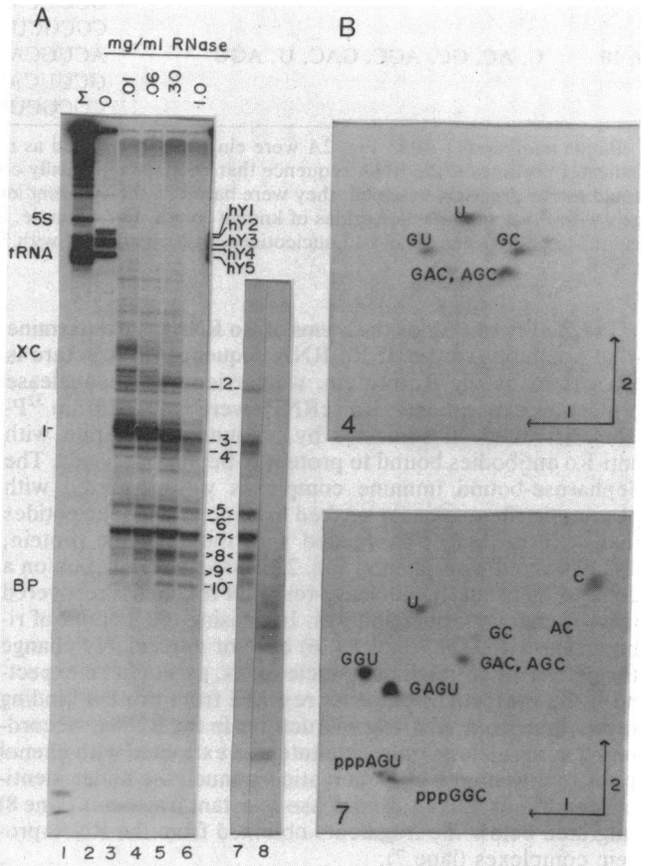


FIG. 2. RNA fragments protected by Ro protein from pancreatic ribonuclease digestion. (*A*) Anti-Ro immunoprecipitates isolated from equal portions of $^{32}$P-labeled HeLa whole-cell extracts were treated with increasing amounts of pancreatic ribonuclease (0.01, 0.06, 0.30, or 1.0 mg/ml), and the nuclease-resistant bound fragments were analyzed (lanes 3–6). A profile of total cellular RNAs (lane 1) and undigested Ro RNAs (lane 2) representing 1/5th of the amount of sample loaded in lanes 3–6 are also shown. Bands numbered 1–10 were eluted and analyzed. XC and BP indicate the positions of the xylene cyanole FF and bromophenol blue marker dyes. In a separate experiment, we compared nuclease-resistant fragments obtained from immunoprecipitates (lane 7) with fragments obtained from immunoprecipitates that were extracted with phenol (lane 8) prior to digestion with pancreatic ribonuclease (1.0 mg/ml). (*B*) Pancreatic ribonuclease fingerprints of nuclease-resistant bands 4 and 7 were prepared, and the resulting oligonucleotides were analyzed (12, 14). Electrophoresis was from right to left and homochromatography was from bottom to top.

Table 1. Oligonucleotide composition and identity of nuclease-resistant fragments isolated from anti-Ro immunoprecipitates

| Gel band | Oligonucleotides | Sequence | RNA |
|---|---|---|---|
| 1 | pppGGC, U, GU, GGU, AGU, GAU, (AG, G)U, C | 5′ end | hY4 |
| 2 | C, AC, AAC, GC, AGC, GAC, U, GU | UGUCUCCCCCCACAACCGCGCUUGACUAGCUUGCUGUUU(U)$_{OH}$ | hY5 |
| 3 | pppGGC, C, GC, U, GU, AGU, GGU, GAGU, GAAGGU | **pppGGCUGGUCCGAAGGUAGUGAGUU**p | hY1 |
| | | **pppGGCUGGUCCGAGUGCAGUGGUGUUU**p | hY3 |
| | C, AC, GC, AGC, GAC | UUCUCCACUCCCACU**GCUUCACUUGACUAGC**CUU(U)$_{OH}$ | hY3 |
| 4 | C, GC, GAC, AGC, U, GU | CGCGCUU**GACUAGC**UUGCUGUUU(U)$_{OH}$ | hY5 |
| 5 | GC, GAC, GGC, U, AAAU | ? | hY4 |
| | pppGGC, C, U, GGU, GAAGGU | **pppGGCUGGUCCGAAGGU**p | hY1 |
| 6 | GAC, GGC U, AAAU | ? | hY4 |
| 7 | pppGGC, pppAGU, C, U, GGU, GAGU | **pppGGCUGGUCCGAGU**p | hY3 |
| | | **pppAGUUGGUCCGAGU**p | hY5 |
| | C, AC, GC, AGC, GAC, U | GCUUCACUU**GACUAGC**CUU(U)$_{OH}$ | hY3 |
| | | CGCGCUU**GACUAGC**UUGCUp | hY5 |
| 8 | pppGGC, C, U, GAU, GGU | 5′ end | hY4 |
| | C, AC, GC, AGC, GAC, U, AGU | ACUGCACUU**GACUAGU**CUU(U)$_{OH}$ | hY1 |
| | | GCUUCACUU**GACUAGC**CUU(U)$_{OH}$ | hY3 |
| | | CGCGCUU**GACUAGC**UUGCUp | hY5 |
| 9, 10 | C, AC, GC, AGC, GAC, U, AGU | ACUGCACUU**GACUAGU**CUU(U)$_{OH}$ | hY1 |
| | | GCUUCACUU**GACUAGC**CUU(U)$_{OH}$ | hY3 |
| | | CGCGCUU**GACUAGC**UUGCUp | hY5 |

Bands numbered 1–10 in Fig. 2*A* were eluted, fingerprinted as in Fig. 2*B*, and the oligonucleotides were analyzed as described. Boldface indicates portions of the RNA sequence that were unequivocally contained within the protected fragment. When the endpoints of a fragment could not be precisely assigned, they were based on the apparent length of the fragment, determined by comparing its migration on denaturing gels with those of oligonucleotides of known length. For example, in band 7, the size of the fragment corresponding to the 3′ end of hY3 was judged to be between 10 and 15 nucleotides, as it comigrated with 5′-terminal fragments that are 13 nucleotides long but contain triphosphate ends.

**The Ro Protein Binds the Stems of Ro RNAs.** To determine what common feature of Ro RNA sequence or structure is recognized by the Ro protein, we carried out ribonuclease protection experiments. Ro scRNPs were isolated from $^{32}$P-labeled HeLa cell sonicates by immunoprecipitation with anti-Ro antibodies bound to protein A-Sepharose beads. The Sepharose-bound immune complexes were digested with pancreatic ribonuclease, washed to remove oligonucleotides that were no longer associated with the antigenic protein, and extracted with phenol. Fig. 2*A* shows fractionation on a denaturing gel of the nuclease-resistant fragments recovered from such immunoprecipitates. Increasing the amount of ribonuclease 100-fold (lanes 3–6) did not appreciably change the pattern of selected oligonucleotides, as would be expected if the protected fragments resulted from protein binding rather than from secondary structures in the RNAs. Accordingly, when an immunoprecipitate was extracted with phenol prior to digestion with pancreatic ribonuclease under identical conditions, all of the nuclease-resistant fragments (lane 8) migrated below the fragments obtained from the RNA–protein complexes (lane 7).

To determine their identity, the gel bands labeled 1–10 (lanes 3–6) were subjected to pancreatic ribonuclease fingerprint analysis. Although some bands contained a mixture of protected fragments, secondary analyses of the oligonucleotides allowed their unambiguous identification (Table 1). All fragments derived from hY1, hY3, or hY5 (the three Ro RNAs whose sequence is known) corresponded to homologous regions of these RNAs that had been predicted to base-pair to form a long stem (3, 4). Strikingly, RNA sequences from both strands of this stem were seen among the smallest protected fragments. For instance, in the fingerprint of band 7 (Fig. 2*B*), some of the spots are more intense than others, indicating a nonequimolar mixture of two or more fragments comigrating on the gel. In fact, the presence of four fragments can be deduced (Table 1). Two of these correspond to the 5′ ends of hY3 and hY5, since pppGGC and pppAGU (the 5′ ends of hY3 and hY5, respectively) appear as well as oligonucleotides derived from the first 13 nucleotides of each of these two RNAs. The spots of lowered intensity can be unambiguously assigned to regions of hY3 and hY5 near their 3′ ends.

The regions of the sequenced Ro RNAs that were retained in immunoprecipitates after pancreatic ribonuclease digestion are diagrammed in Fig. 3. Because we noticed that the protected regions exhibit similarities that were not completely reflected in the previously proposed secondary structures (3, 4), we have slightly altered the stems of hY1 and hY5 to better display these homologies. For hY1, hY3, and hY5, the most highly protected portion of each RNA corresponds to the lower section of a stem formed by base-pairing the 5′ and 3′ ends of the RNA. Within this helical region are seven identical base pairs with a single bulged cytidine residue.

Several fragments generated in the nuclease protection experiment contained oligonucleotides previously assigned to hY4 RNA on the basis of pancreatic fingerprint analysis (unpublished data). Bands 1 and 8 apparently correspond to the 5′ terminus of hY4 since they contain pppGGC (which is the 5′ end of hY4, as well as of hY1 and hY3) and certain oligonucleotides unique to hY4. Fragments 5 and 6 represent overlapping segments that do not include the 5′-end region (Table 1). From the oligonucleotide composition of these fragments, we were able to derive hypothetical sequences for the 5′ and 3′ ends of hY4 RNA. These sequences can be base-paired to form a stem similar to that found in hY1, hY3, and hY5 RNA; the conserved seven-base-pair region differs only in that the first A·U base pair is replaced by a G·U base pair.

## DISCUSSION

We have shown that the major protein component of the Ro RNPs is an antigenic polypeptide of 60 kDa. This size agrees well with the conclusions of Venables *et al.* (21) but not with those of Francoeur and Mathews (18). The Ro protein binds to a highly conserved region of the Ro RNAs, the lower part

of a stem formed by base-pairing the 5′ and 3′ ends of the molecule.

**Antigenic Proteins Contained in Ro scRNPs.** Although the 60-kDa protein is clearly the major protein component of Ro scRNPs, other proteins may also be associated with these particles. A small percentage of the Ro RNPs contain the 50-kDa La protein, a polypeptide that binds at least initially to virtually every known RNA polymerase III transcript. [A protein that comigrates with the La protein is visible as a faint band in $^{35}$S-labeled immunoprecipitates on long exposures (Fig. 1*A*, lanes 3-6).] Similarly, if other proteins were associated either transiently or permanently with only one of the less abundant Ro scRNPs, such as that containing hY1 (4), they might not be detectable by immunoprecipitation of total cellular Ro particles.

Our results suggest, but do not rigorously prove, that each Ro RNA is contained in a separate antigenic complex. The identification of a binding site for the Ro protein on each of the four unique human Ro RNAs argues that each RNA molecule is bound by at least one molecule of the antigenic protein. Ro scRNPs sediment at ≈7 S in sucrose gradients (unpublished data), consistent with a total molecular size of about 93 kDa for each Ro RNP (60 kDa for one protein molecule and 33 kDa for one RNA). This is somewhat lower than the 100- to 150-kDa size determined by gel filtration (22).

The reaction of anti-Ro antibodies with the 60-kDa Ro protein in immunoblots is quite weak, in comparison to that seen with other classes of autoimmune antisera (8, 17, 18, 21, 23–25). This might be due to the low abundance of this antigen in cell extracts relative to other autoantigens, or to an intrinsic lability of the Ro antigenic determinant on exposure to $NaDodSO_4$ and transfer to nitrocellulose. Alternatively, bound Ro RNA, which is eliminated by $NaDodSO_4$ gel electrophoresis, might enhance Ro antigenicity.

One curious observation made in our immunoblot studies is that most anti-Ro sera contain detectable levels of anti-La antibodies. This is true even of sera that have been designated "monospecific" anti-Ro based on immunodiffusion (T. Mimori, personal communication) as well as on immunoprecipitation of $^{32}$P- or $^{35}$S-labeled cell extracts. The frequent presence of anti-Ro antibodies in patients with anti-La antibodies, but not the converse, has been noted (9). The fact that even our most "monospecific" anti-Ro sera recognize a 50-kDa protein on immunoblots suggests that these sera do contain low levels of anti-La antibodies. Certain other specificities have been noted to co-occur in patients with systemic lupus erythematosus, including anti-(U1)RNP and anti-Sm (26), and antibodies to histones H1 and H2b (27). As in the Ro–La case, the two autoantigens coexist in specific protein–nucleic acid complexes (i.e., small RNPs or nucleosomes), suggesting that the immune system may target the particle as a whole. Alternatively, the two antigenic proteins might be related at the sequence or structural level.

**RNA Binding Site of the Ro Protein.** The assignment of the Ro protein binding site to the base of a stem formed by the 5′ and 3′ ends of Ro RNAs agrees well with data on the inclusion of shortened forms of these RNAs in anti-Ro precipitable particles. Earlier, we observed that hY2 and hY3* RNAs, which are slightly truncated forms of hY1 and hY3 RNAs, respectively, are immunoprecipitable by anti-Ro antibodies (4). (hY2 terminates between nucleotides 103 and 107 of the hY1 sequence, while hY3* terminates between nucleotides 92 and 96 of the hY3 sequence.) Conversely, a severely shortened form of hY3 called hY3**, which terminates with a $U_{OH}$ between nucleotides 59 and 61, is not immunoprecipitable (4) presumably because it totally lacks the 3′ portion of the stem to which Ro protein binds.

The Ro protein binding site contains a single bulged nucleotide within a helix. This observation adds weight to the idea that bulged helices may be a general structural feature of ribonucleic acid–protein binding sites (28). Uhlenbeck and coworkers (29, 30), studying the interaction of R17 coat protein

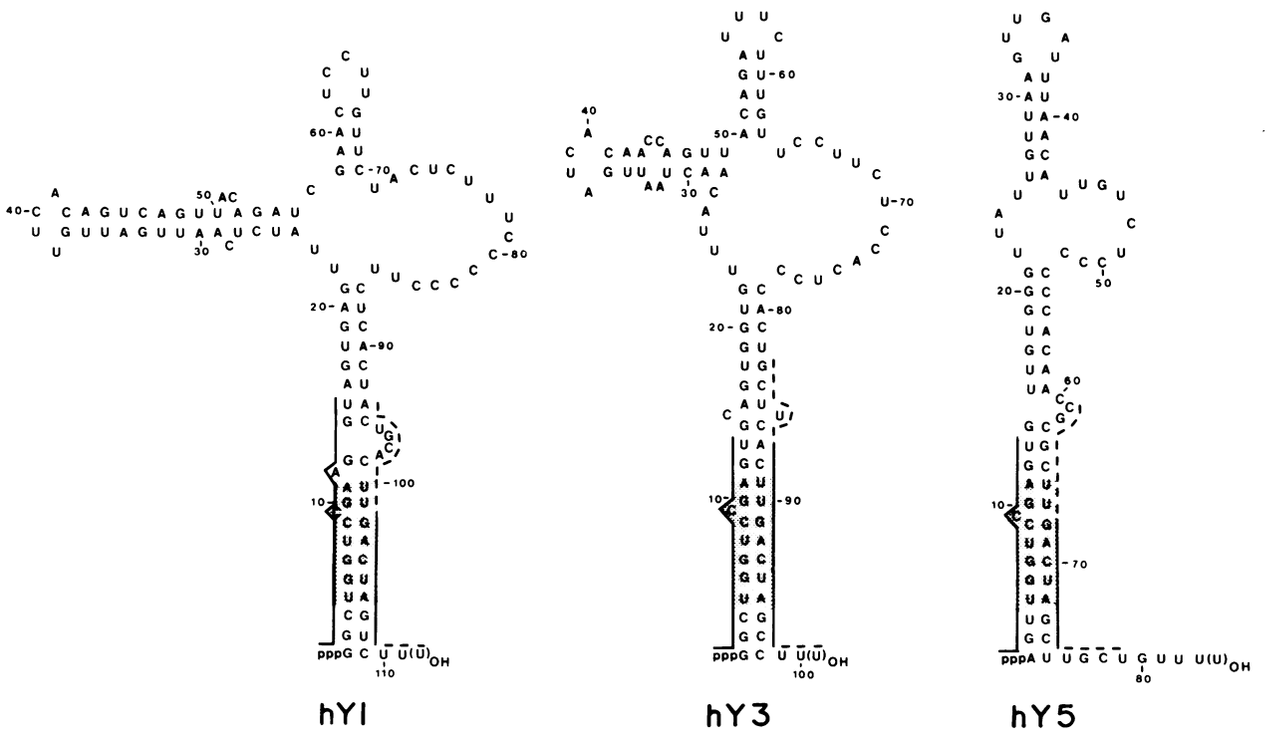


FIG. 3. Diagrammatic representation of regions of Ro RNAs protected from nuclease digestion in immunoprecipitates. Secondary structures of hY1 and hY5 (modified slightly from refs. 3 and 4) have calculated stabilization energies (−ΔG) (19, 20) that are similar to the previously proposed structures (3, 4). The secondary structure of hY3 is from ref. 4. Solid lines indicate oligonucleotides that were unambiguously identified in fingerprints of protected fragments. In some cases the endpoints of a fragment could not be precisely determined and are indicated by broken lines. The stippled area identifies a region exactly conserved between hY1, hY3, and hY5 RNAs.

with its RNA, have constructed synthetic binding sites in which the single bulged adenine was deleted or replaced by cytidine. Both of these alterations greatly decreased coat protein binding. More extensive studies of the Ro protein binding site, such as using *in vitro* mutagenesis to alter bases in this region, will be required to determine what features of the site are absolutely essential for protein recognition.

Finally, it must be noted that the ribonuclease protection experiments presented here can only identify those parts of the RNAs that remain tightly bound after digestion. There may be additional sites that contact the protein but are released by nuclease treatment.

**Cytoplasmic Roles for Ro scRNPs.** Although the function of the Ro scRNPs has not yet been determined, it seems likely that they participate in translation-related events. Three other scRNPs have been recently assigned roles as positive or negative effectors of protein synthesis in mammalian cells (31–33). Using cloned human Ro RNA genes (4) to probe RNA extracted from mouse and rat tissues, we have observed that Ro RNPs are about 10-fold more abundant, relative to total cytoplasmic RNA, in brain and heart tissue than they are in liver (unpublished data). One intriguing possibility is that Ro scRNPs function in the translation of a subset of mRNAs abundant in brain and heart. It is perhaps relevant that maternal anti-Ro antibodies are strongly associated with the occurrence of atrioventricular conduction defects in neonates (34, 35). One prediction would be that the cells of the atrioventricular node, at least at some stage in development, have high concentrations of Ro scRNPs.

We thank H. LaBranche-Chabot for help with fingerprinting; Drs. S. Mount, O. Uhlenbeck, H. Robertson, and J. Stefano for advice and criticism; and E. Gottlieb for HeLa cell extracts. We are grateful to Drs. J. Hardin, S. Malawista, M. Reichlin, M. Akizuki, T. Mimori, and G. McCarty for providing patient sera; and to Drs. J. Carey and O. Uhlenbeck for communication of results prior to publication. This work was supported by a grant from the National Institutes of Health (GM 26154) to J.A.S. and a U.S. Public Health Service training grant to S.L.W.

1. Lerner, M. R., Boyle, J. A., Hardin, J. A. & Steitz, J. A. (1981) *Science* **211,** 400–402.
2. Hendrick, J. P., Wolin, S. L., Rinke, J., Lerner, M. R. & Steitz, J. A. (1981) *Mol. Cell. Biol.* **1,** 1138–1149.
3. Kato, N., Hoshino, H. & Harada, F. (1982) *Biochem. Biophys. Res. Commun.* **108,** 363–370.
4. Wolin, S. L. & Steitz, J. A. (1983) *Cell* **32,** 735–744.
5. Lerner, M. R., Andrews, N. C., Miller, G. & Steitz, J. A. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 805–809.
6. Rosa, M. D., Gottlieb, E., Lerner, M. R. & Steitz, J. A. (1981) *Mol. Cell. Biol.* **1,** 785–786.
7. Rinke, J. & Steitz, J. A. (1982) *Cell* **29,** 149–159.
8. Steitz, J. A., Wolin, S. L., Rinke, J., Pettersson, I., Mount, S. M., Lerner, E. A., Hinterberger, M. & Gottlieb, E. (1983) *Cold Spring Harbor Symp. Quant. Biol.* **47,** 893–899.
9. Mattioli & Reichlin (1974) *Arth. Rheum.* **17,** 421–429.
10. Matter, L., Schopfer, K., Wilhelm, J. A., Nyffenegger, T., Parisot, R. F. & De Robertis, E. M. (1982) *Arth. Rheum.* **25,** 1278–1283.
11. Laemmli, U. K. (1970) *Nature (London)* **227,** 680–685.
12. Lerner, M. R. & Steitz, J. A. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 5495–5499.
13. Maxam, A. M. & Gilbert, W. (1980) *Methods Enzymol.* **65,** 499–560.
14. Barrell, B. G. (1971) in *Procedures in Nucleic Acid Research,* eds. Cantoni, G. L. & Davies, D. R. (Harper, New York), Vol. 2, pp. 751–779.
15. Weil, P. A., Segall, J. Harris, B., Ng, S.-Y. & Roeder, R. G. (1979) *J. Biol. Chem.* **254,** 6163–6173.
16. Towbin, H., Staehelin, T. & Gordon, J. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 4350–4354.
17. Mimori, T., Hinterberger, M., Pettersson, I. & Steitz, J. A. (1984) *J. Biol. Chem.* **259,** 560–565.
18. Francoeur, A. M. & Mathews, M. B. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 6772–6776.
19. Tinoco, I., Borer, P. N., Dengler, B., Levine, M. D., Uhlenbeck, O. C., Crothers, D. M. & Gralla, J. (1973) *Nature (London) New Biol.* **246,** 40–41.
20. Salser, W. (1977) *Cold Spring Harbor Symp. Quant. Biol.* **42,** 985–1002.
21. Venables, P. J. W., Smith, P. R., Maini, R. N. (1983) *Clin. Exp. Immunol.* **54,** 731–738.
22. Clark, G., Reichlin, M. & Tomasi, T. B. (1976) *J. Immunol.* **102,** 117–122.
23. White, P. J. & Hoch, S. O. (1981) *Biochem. Biophys. Res. Commun.* **102,** 365–371.
24. Takano, M., Golden, S. S., Sharp, G. C. & Agris, P. F. (1981) *Biochemistry* **20,** 5929–5936.
25. Billings, P. B., Allen, R. W., Jensen, F. C. & Hoch, S. O. (1982) *J. Immunol.* **128,** 1176–1180.
26. Mattioli, M. & Reichlin, M. (1973) *J. Immunol.* **110,** 1318–1324.
27. Hardin, J. A. & Thomas, J. O. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 7410–7414.
28. Peattie, D. A., Douthwaite, S., Garrett, R. A. & Noller, H. F. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 7331–7335.
29. Carey, J., Lowary, P. T. & Uhlenbeck, O. C. (1983) *Biochemistry* **22,** 4723–4730.
30. Uhlenbeck, O. C., Carey, J., Romaniuk, P., Lowary, P. & Beckett, D. (1983) *J. Biomol. Struct. Dyn.* **1,** 539–552.
31. Thimmappaya, B., Weinberger, C., Schneider, R. J. & Shenk, T. (1983) *Cell* **31,** 543–551.
32. Bhat, R. A. & Thimmappaya, B. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 4789–4793.
33. Walter, P. & Blobel, G. (1982) *Nature (London)* **299,** 691–698.
34. Scott, J. S., Maddison, P. J., Taylor, P. V., Esscher, E., Scott, O. & Skinner, R. P. (1983) *N. Engl. J. Med.* **309,** 209–212.
35. Weston, W. L., Harmon, C., Peebles, C., Manchester, D., Franco, H. L., Huff, J. C. & Norris, D. A. (1982) *Br. J. Dermatol.* **107,** 377–382.