Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 9739–9743, October 1992
Biochemistry

Autoantibodies against a serine tRNA–protein complex implicated in cotranslational selenocysteine insertion

(UGA suppressor serine tRNA that carries selenocysteine/autoimmune chronic active hepatitis)

CARMEN GELPI*, ERIK J. SONTHEIMER†, AND JOSE LUIS RODRIGUEZ-SANCHEZ*

*Department of Immunology, Sant Pau Hospital, Barcelona 08025, Spain; and †Department of Molecular Biophysics and Biochemistry, Howard Hughes Medical Institute, Yale University School of Medicine, New Haven, CT 06510

Communicated by Joan A. Steitz, June 23, 1992

ABSTRACT **We describe an autoantibody specificity present in a subgroup of patients with a severe form of autoimmune chronic active hepatitis. These antibodies precipitate a 90-nucleotide RNA from human whole cell extracts and recognize a 48-kDa polypeptide in immunoblotting assays. The RNA is a UGA suppressor serine tRNA that carries selenocysteine (tRNA$^{[Ser]Sec}$), as shown by sequence analysis. The protein does not appear to be seryl-tRNA synthetase; rather, it is an excellent candidate for a factor involved in cotranslational selenocysteine incorporation in human cells.**

Autoantibodies reactive with cellular constituents are often detected in sera from patients with autoimmune disease. Autoantibodies that immunoprecipitate tRNA have been detected primarily in sera from patients with polymyositis (1–3) and, less frequently, in patients with systemic lupus erythematosus (4) and scleroderma (5). The reactive antigens are usually tRNA-associated proteins; in only a few cases are they the tRNAs themselves (4, 6). Some of these protein antigens have been identified as aminoacyl-tRNA synthetases (3, 6–10).

Other well-characterized components of the protein synthetic machinery [for example, ribosomes (11), 5S ribonucleoprotein (12), and elongation factor 1α (13)] also act as autoantigens. One translational event, the incorporation of selenocysteine into a growing polypeptide chain, is not well-understood in eukaryotes, and to our knowledge, the factors involved in this process have not been implicated in autoimmunity. The synthesis of selenoproteins in *Escherichia coli* involves several reactions (for review, see ref. 14). Briefly, selenocysteine incorporation is cotranslational and requires an unusual suppressor aminoacyl-tRNA (selenocysteyl-tRNA^{Sec}, where Sec is selenocysteine) and a specific translation factor (the product of the *selB* gene) that substitutes for elongation factor Tu. Insertion is directed by certain UGA triplets that in other contexts act as termination codons. tRNA^{Sec} is initially charged with serine to form seryl-tRNA^{Sec} and is then converted to selenocysteyl-tRNA^{Sec} by the action of the *selA* (selenocysteine synthase) and *selD* gene products. In mammalian cells, selenocysteine incorporation also occurs at specific UGA codons, and a suppressor serine tRNA that carries selenocysteine (tRNA$^{[Ser]Sec}$) has been detected (15). However, complete elucidation of the eukaryotic pathway (and identification of the factors involved) has not yet been achieved.

Here we report that an RNA–protein complex is the target of an autoantibody specificity found in the sera of a subset of patients with autoimmune chronic active hepatitis (AI-CAH). The RNA corresponds to a previously identified human suppressor serine tRNA, and the antigenic protein may be a factor involved in the pathway of cotranslational incorporation of selenocysteine into specific polypeptide chains.

MATERIALS AND METHODS

Subjects. Sera from 35 patients with AI-CAH were collected over a period of 4 years at the Sant Pau and Valle Hebron Hospitals in Barcelona. Seven sera were selected for further study because they immunoprecipitated a characteristic RNA pattern not observed with other known autoimmune sera. All belonged to young women with hypergammaglobulinemia, anti-nuclear antibodies, and negative markers for hepatitis B and C viruses. In addition, all 7 patients were free from drug or alcohol exposure, transfusions, or metabolic abnormalities and did not respond to corticosteroid treatment.

Immunoprecipitation of Cell Extracts. Friend erythroleukemia cells and HeLa cells growing in logarithmic phase at 4×10^5 and 2×10^5 cells per ml, respectively, were labeled *in vivo* with [^{32}P]orthophosphate or [^{35}S]methionine (Amersham) as described (16). Whole cell extracts were prepared as described (17) and precleared with 0.05 vol of a 20% (wt/vol) suspension of protein A-Sepharose (Pharmacia) in NET-2 buffer (150 mM NaCl/50 mM Tris·HCl/0.05% Nonidet P-40, pH 7.5) plus bovine serum albumin (2 mg/ml); immunoprecipitations were performed as described by Lerner and Steitz (18) for ^{32}P-labeled extracts and by Matter *et al.* (19) for ^{35}S-labeled extracts. Deproteinized extracts were prepared by incubating ^{32}P-labeled extract in proteinase K (0.1 mg/ml) plus 0.2% SDS for 2 h at 37°C, followed by extraction with phenol/chloroform/isoamyl alcohol (PCA), 50:49:1 (vol/vol), and ethanol precipitation; alternatively, ^{32}P-labeled purified immunoprecipitated RNA (see below) was resuspended in the original volume of NET-2 buffer.

Immunoprecipitated ^{32}P-labeled RNAs were PCA-extracted, ethanol precipitated, electrophoresed on 10% polyacrylamide denaturing gels in 0.1 M boric acid/0.1 M Tris base/2 mM EDTA (1× TBE), and subjected to autoradiography. Alternatively, RNAs immunoprecipitated from unlabeled HeLa cell extracts were prepared as described (17), fractionated by electrophoresis on a 10% polyacrylamide denaturing gel in 1× TBE, and visualized by staining with ethidium bromide. [Immunodepleted supernatants were saved for use in tRNA aminoacylation assays (see below).] Individual bands were excised, and RNAs were eluted by incubating the gel slice in 0.4 ml of 10 mM Tris·HCl, pH 7.4/1 mM EDTA/0.5% SDS/1 unit of RNasin (Promega) overnight at 4°C. Eluted RNAs were PCA-extracted and ethanol-precipitated.

RNA Sequencing. Purified RNAs were dephosphorylated with calf intestinal phosphatase (Boehringer Mannheim) ac-

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: AI-CAH, autoimmune chronic active hepatitis; tRNA$^{[Ser]Sec}$, UGA suppressor serine tRNA that carries selenocysteine.

cording to the manufacturer's protocol and labeled at the 3′ end with [5′-^{32}P]pCp (New England Nuclear) and T4 RNA ligase (Pharmacia) according to the method of England and Uhlenbeck (20); alternatively, dephosphorylated RNAs were labeled at the 5′ end with [γ-^{32}P]ATP and T4 polynucleotide kinase (Pharmacia) as described (21). End-labeled RNAs were repurified on a 10% polyacrylamide denaturing gel, eluted as described above, and sequenced by digestion with base-specific RNases (T1, U2, Phy M, *Bacillus cereus*, and CL3) (Pharmacia) according to the manufacturer's protocol; sequencing reaction products were fractionated on a 20% polyacrylamide denaturing gel and subjected to autoradiography. The 3′-terminal nucleotide was identified by TLC of RNase T2-digested 3′-end-labeled RNA on Avicel plates (Analtech) (22). Purified end-labeled 5S rRNA was used as a control for sequencing.

Immunoblots. Immunoblot analysis was performed as described by Towbin *et al.* (23), with modifications. Briefly, unlabeled HeLa cell extracts (17) were subjected to gel electrophoresis and the proteins were transferred to nitrocellulose with methanol in the absence of SDS. The membrane was blocked with 3% (wt/vol) nonfat dry milk in 20 mM potassium phosphate, pH 7.4/130 mM NaCl (PBS) for 4 h and then incubated overnight with serum (1:100 dilution) in the same buffer. The nitrocellulose strips were washed sequentially with PBS, PBS/0.1% Tween 20, and again with PBS and then incubated for 30 min with ^{125}I-labeled rabbit anti-human IgG (Amersham; 0.1 μCi/ml; 1 Ci = 37 GBq). After four washes with PBS, the strips were air-dried and subjected to autoradiography.

Affinity Purification of Antibodies. Affinity-purified antibodies were prepared as described by Olmsted (24) with minor modifications (25) and used without dilution in immunoblot or immunoprecipitation assays (see above).

Aminoacylation. Aminoacylation assays were performed as described (10). The reaction mixture contained either 0.1 μg of purified immunoprecipitated RNA (see above) or 2 A_{260} units of whole calf liver tRNA (Boehringer Mannheim), except for the threonine reaction mixture, which contained 1.5 A_{260} units of whole calf liver tRNA. Antisera were tested over a range of dilutions (1:80 to 1:10). Radiolabeled amino acids were purchased from Amersham; asparagine and glutamine were labeled with ^{14}C, cysteine and methionine were labeled with ^{35}S, and the other amino acids were labeled with ^{3}H. ATP (5 mM) and Mg^{2+} (10 mM) were included in each reaction mixture. HeLa cell extract (see above) was used as the enzyme source. Control reactions used immunodepleted extracts (see above); the extent of immunodepletion was assessed by immunoblots and immunoprecipitations.

RESULTS

AI-CAH Sera Immunoprecipitate a Small RNA. Sera collected from 7 of the 35 AI-CAH patients studied contain antibodies that immunoprecipitate RNA species migrating more slowly than the bulk of tRNA in a denaturing 10% polyacrylamide gel (Fig. 1, lanes 3–9). Although band X appears as a triplet in Fig. 1, we usually observed a tight doublet, with the upper band predominating. In addition, serum ACN precipitated large amounts of high molecular weight nucleic acids, serum RGG precipitated mY1 and mY2 Ro RNAs, and sera DPP and DOD precipitated 5S and 5.8S rRNAs as well as high molecular weight nucleic acids (Fig. 1, lanes 3–9). With the exception of the anti-Mas serum (Fig. 1, lane 17) from a patient with polymyositis and alcoholic rhabdomyolysis (29, 30), RNA X was not immunoprecipitated by any of the control sera, nor was it seen using sera from patients with other hepatic diseases, nonhepatic autoimmune diseases, or from healthy donors.

Since most small RNAs precipitated by autoimmune sera are associated with an antigenic protein, we tested the ability of the AI-CAH sera to immunoprecipitate deproteinized RNA. ARA and JCA sera precipitated band X about 60% as efficiently from proteinase K-treated extracts; the other five sera showed no detectable precipitation. Similar results were obtained when gel-purified RNA X was used as antigen (data not shown). We conclude that in most cases the RNA is indirectly immunoprecipitated as part of a protein–RNA complex, but some sera contain antibodies that directly target the RNA.

AI-CAH Sera Recognize a 48-kDa Protein. To identify the protein antigen, the seven AI-CAH patient sera were used to probe Western blots of HeLa cell extract; six of the seven sera recognized a protein of ≈48 kDa (Fig. 2, lanes 1–7). Anti-Mas control serum (29, 30) recognized a protein of the same size (lane 10), and serum ACN, which inefficiently precipitates the RNA (Fig. 1, lane 7), did not detect a 48-kDa species (Fig. 2, lane 3). Although other proteins were recog-

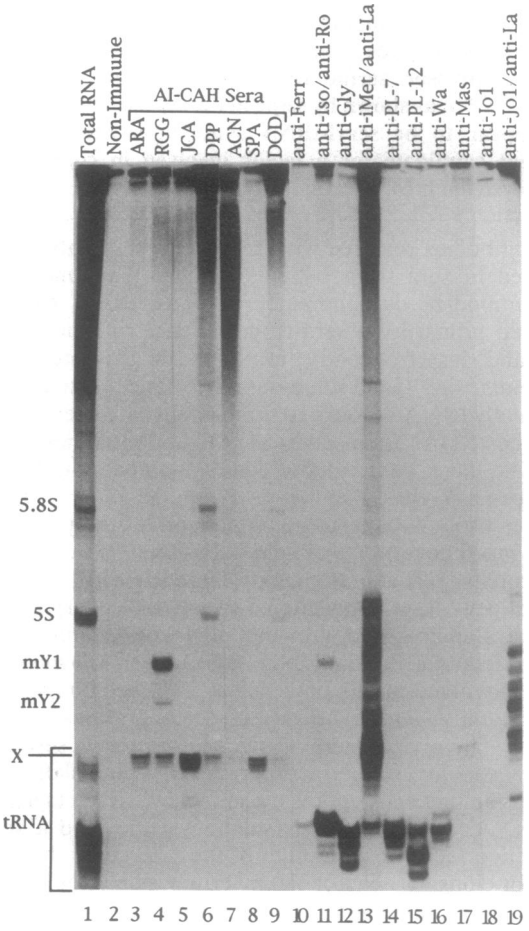

FIG. 1. Immunoprecipitation of small RNAs by sera from patients with AI-CAH. ^{32}P-labeled Friend erythroleukemia cell sonicates were immunoprecipitated and gel-fractionated. Serum specificities are shown at the top of each lane. Known RNAs are indicated on the left; X denotes the RNA(s) precipitated by AI-CAH sera (lanes 3–9) and anti-Mas serum (lane 17). Lane 1 shows total RNA extracted from the Friend erythroleukemia cell sonicate prior to immunoprecipitation, and lane 2 shows RNAs precipitated by serum from a healthy nonautoimmune donor. Other specificities are as follows: anti-Ro (lane 11) (26) and anti-La (lanes 13 and 19) (27); anti-Ferr (lane 10), an anti-elongation factor 1α (13); anti-Iso (lane 11), an anti-isoleucyl-tRNA synthetase (10); anti-Gly (lane 12), an anti-glycyl-tRNA synthetase (10); anti-iMet (lane 13), an anti-initiator methionine (28); anti-PL-7 (lane 14), an anti-threonyl-tRNA synthetase (8, 9); anti-PL-12 (lane 15), an anti-alanyl-tRNA synthetase (6); anti-Wa (lane 16), unknown specificity (5); anti-Mas (lane 17), unknown specificity from a patient with polymyositis and alcoholic rhabdomyolysis (29, 30); anti-Jo1 (lanes 18 and 19), an anti-histidyl-tRNA synthetase (3).

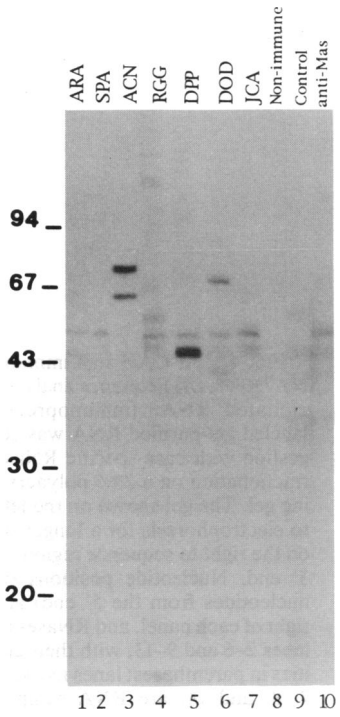

FIG. 2. Immunoblots of HeLa cell sonicate with AI-CAH sera. Unlabeled HeLa cell sonicate was fractionated on a 10% polyacrylamide/SDS gel, blotted onto nitrocellulose, and probed with antisera as indicated. The positions of molecular mass markers (in kilodaltons) are shown on the left. Lanes 1–7 are AI-CAH sera that immunoprecipitate RNA X, and control serum (lane 9) is an AI-CAH serum that does not precipitate small RNA. Nonimmune (lane 8) is serum from a healthy nonautoimmune donor; anti-Mas (lane 10) is a polymyositis/alcoholic rhabdomyolysis serum that precipitates a small RNA (29, 30).

nized, the 48-kDa band was the only one common to all the high-titer sera.

To determine whether the 48-kDa protein was associated with the immunoprecipitated RNA, antibodies eluted from the 48-kDa band of a Western blot were assayed by immunoprecipitation of a ^{32}P-labeled HeLa cell extract. These affinity-purified antibodies clearly precipitated the same RNA (Fig. 3, lane 9), and when used to reprobe a Western blot, they detected only the 48-kDa protein (data not shown). Control experiments using eluates from an unrelated region of the nitrocellulose did not precipitate band X (Fig. 3, lane 10) and did not react with any proteins in an immunoblot (data not shown).

The Immunoprecipitated RNA Is tRNA$^{[Ser]Sec}$. Sequence analysis was used to identify RNA X present in the AI-CAH immunoprecipitates. Sera DPP and ARA were incubated with a HeLa cell extract, and the precipitated RNA was gel-purified. After 3′- or 5′-end-labeling, the RNA was repurified by gel electrophoresis, giving a tight doublet with a major upper band and a minor lower band (data not shown). The major RNA species was then sequenced in its entirety by digestion with base-specific RNases using both 5′- and 3′-end-labeled samples.

Fig. 4 shows an example of sequencing with 3′-end-labeled RNA. A few residues (e.g., bases 36, 39, 42, 74, and 78) yielded no enzymatic cleavage products, indicating nucleotide modifications or sequestration within a structured region. The remainder of the bases, however, were unambiguously identified. The 3′-terminal 30 nucleotides of the minor band were also sequenced; in those positions that could be resolved, no differences were seen between it and the major species. The 3′-terminal base was identified as an adenine residue in both cases by TLC of RNase T2-digested 3′-end-labeled samples (data not shown). Similar results were obtained by sequencing the 3′-terminal 38 bases of the comigrating RNA precipitated by anti-Mas serum.

Comparison of the 90-nucleotide full-length sequence with all human sequences in the GenBank data base revealed that RNA X is a UGA suppressor serine tRNA (31) [also referred to as tRNA$^{[Ser]Sec}$ (32)]. Fig. 4 (lanes 1–7) shows that the anticodon sequence at nucleotides 36–38 is NCA (N is an unknown nucleotide), indicating the presence of a modified base in the wobble position as in bovine tRNA$^{[Ser]Sec}$ (33, 34). The corresponding sequence in the cloned human gene is TCA (31).

To confirm the identity of the tRNA as a serine acceptor, we showed that the RNA immunoprecipitated by sera ARA and DPP was aminoacylated efficiently with [^{3}H]serine but not [^{3}H]histidine (data not shown). Control reactions with unfractionated calf liver tRNA showed incorporation of both amino acids (data not shown).

Anti-tRNA$^{[Ser]Sec}$ Sera Do Not Inhibit Aminoacylation *in Vitro*. All of the sera that immunoprecipitate tRNA$^{[Ser]Sec}$ were tested for their ability to inhibit the aminoacylation of all 20 amino acids by using crude HeLa cell extract as enzyme source. None of the AI-CAH sera significantly (>30%) inhibited any synthetase activity, including seryl-tRNA synthetase (Table 1 and data not shown). Conversely, five autoimmune anti-aminoacyl-tRNA synthetase sera [anti-Jo1 (anti-histidyl-tRNA synthetase), anti-PL-7 (anti-threonyl-tRNA synthetase), anti-PL-12 (anti-alanyl-tRNA synthetase), anti-glycyl-tRNA synthetase, and anti-isoleucyl-tRNA synthetase] (6–10) showed reproducible >80% inhibition of the expected specific aminoacyl-tRNA synthetase activity relative to two normal human control sera (Table 1 and data not shown). Furthermore, extracts immunodepleted of detectable 48-kDa antigenic protein showed as much seryl-tRNA synthetase activity as nondepleted or mock-depleted extracts (data not shown). Although it is possible that the sera recognize seryl-tRNA synthetase epitopes not required for enzymatic activity *in vitro* or that there is a distinct seryl-tRNA synthetase that charges only tRNA$^{[Ser]Sec}$, we conclude that the 48-kDa protein is not likely to be human seryl-tRNA synthetase.

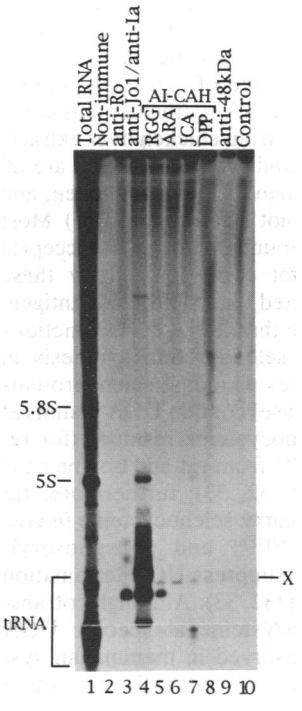

FIG. 3. Immunoprecipitation of RNA X by affinity-purified anti-48-kDa protein antibodies. Whole sera (lanes 2–8), anti-48-kDa protein antibodies eluted from an immunoblot (lane 9; see Fig. 2), and a control eluate from an unrelated region of the immunoblot (lane 10) were used to immunoprecipitate a ^{32}P-labeled HeLa cell sonicate, and the RNAs were analyzed. The mobilities of known RNAs are given on the left, and RNA X precipitated by AI-CAH sera is indicated on the right. Total RNA (lane 1) is RNA from the HeLa cell sonicate prior to immunoprecipitation. Specificities of sera used in lanes 2–8 are as described in Fig. 1.

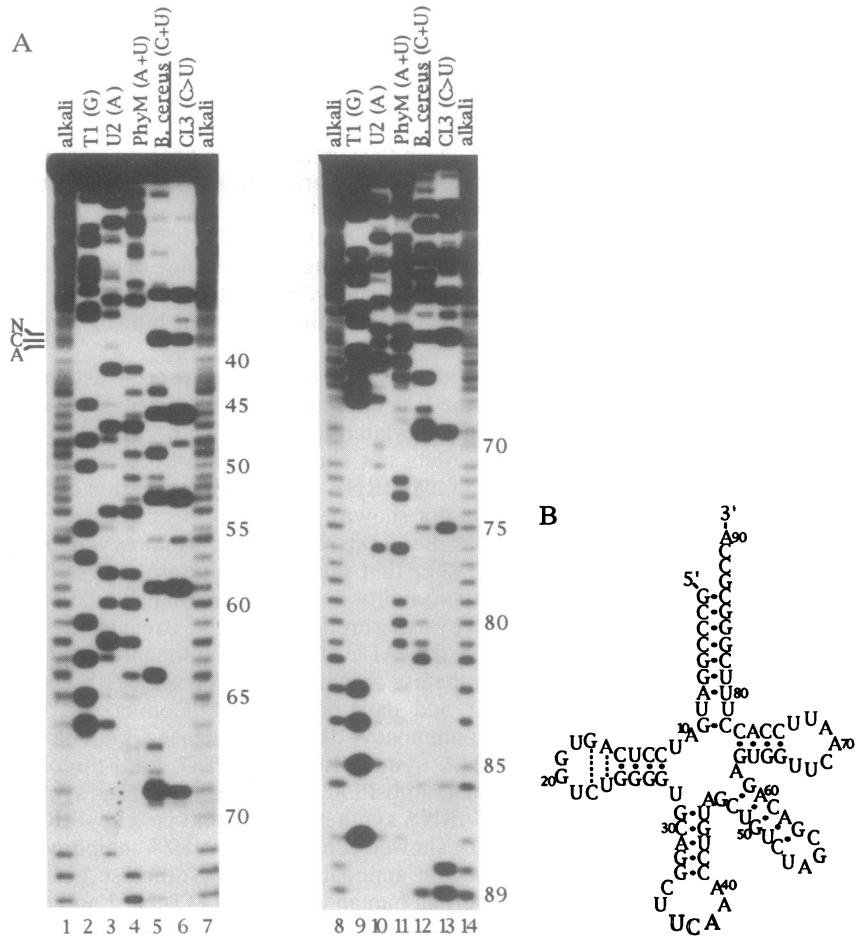

FIG. 4. AI-CAH sera immunoprecipitate tRNA$^{[Ser]Sec}$. (*A*) Sequence analysis of immunoprecipitated RNA. Immunoprecipitated 3'-end-labeled gel-purified RNA was sequenced by digestion with base-specific RNases, followed by fractionation on a 20% polyacrylamide denaturing gel. The gel shown on the left was subjected to electrophoresis for a longer time than the gel on the right to sequence regions further from the 3' end. Nucleotide positions (i.e., number of nucleotides from the 5' end) are shown to the right of each panel, and RNases are identified for lanes 2–6 and 9–13, with their cleavage specificities in parentheses; lanes marked alkali (lanes 1, 7, 8, and 14) are RNA samples subjected to nonspecific alkaline hydrolysis. The three bases of the anticodon (NCA, where N is an unidentified nucleotide) are indicated on the left. (*B*) Sequence and proposed secondary structure of tRNA$^{[Ser]Sec}$, derived from the cloned human gene (31); the 3'-terminal CCA is added post-transcriptionally. The secondary structure is as proposed by Bock *et al.* (14). The anticodon is shown in open letters.

DISCUSSION

We have identified and characterized an autoantibody specificity in patients with AI-CAH that precipitates the human UGA suppressor tRNA$^{[Ser]Sec}$. Most of these sera react with tRNA$^{[Ser]Sec}$ indirectly, as a result of its association with a 48-kDa antigenic protein. Four lines of evidence indicate that the 48-kDa protein is not seryl-tRNA synthetase. (*i*) Unlike anti-aminoacyl-tRNA synthetase sera from poly/dermatomyositis patients (6–10), the AI-CAH antisera reactive with the 48-kDa antigen do not inhibit aminoacyl-tRNA synthetase activity *in vitro*. (*ii*) Extracts depleted of detectable 48-kDa antigen show undiminished seryl-tRNA synthetase activity *in vitro*. (*iii*) Seryl-tRNA synthetases from chicken liver extracts (35), bovine liver extracts (36), and mouse cells (37) are all significantly larger (60–65 kDa) than this human antigen, and such divergence in size would not be expected. (*iv*) Most conclusively, the much more abundant major isoacceptor tRNA^{Ser} species (38–40) are not coprecipitated by these AI-CAH sera, as would be expected for a synthetase antigen.

Several lines of evidence argue that tRNA$^{[Ser]Sec}$ functions specifically in the pathway of selenoprotein synthesis in human cells. (*i*) Mammalian genes encoding selenoproteins (e.g., human glutathione peroxidase) contain UGA codons at positions corresponding to selenocysteine residues (for review, see ref. 14). (*ii*) tRNA$^{[Ser]Sec}$ from rat and bovine cells binds UGA codons *in vitro* (15, 32, 33); furthermore, rat tRNA$^{[Ser]Sec}$ has been shown to carry selenocysteine *in vivo* (15), and bovine seryl-tRNA$^{[Ser]Sec}$ and phosphoseryl-tRNA$^{[Ser]Sec}$ have been shown to suppress UGA termination codons during *in vitro* translation (32, 33). Although cotranslational incorporation of selenocysteine at specific UGA codons has not been directly observed in mammalian systems, this process has been proven to occur in *E. coli*, where it requires a suppressor tRNA^{Sec} and the protein product of the *selB* gene (for a review, see ref. 14). The SELB protein acts as a tRNA^{Sec}-specific elongation factor, performing the function executed by elongation factor Tu for all other aminoacyl-tRNAs; in addition, the products of the *selA* (selenocysteine synthase) and *selD* genes are required for the conversion of seryl-tRNA^{Sec} to selenocysteyl-tRNA^{Sec}. Thus, selenocysteine qualifies as the 21st amino acid to be directly encoded by the *E. coli* genome, and considerable circumstantial evidence indicates that selenocysteine incorporation occurs comparably in mammalian cells. It is therefore possible that the 48-kDa autoantigen is either an enzyme involved in the conversion of seryl-tRNA$^{[Ser]Sec}$ to selenocysteyl-tRNA$^{[Ser]Sec}$ or the human analog of the SELB translation factor from *E. coli*. If true, these AI-CAH sera could prove invaluable in dissecting the process of selenoprotein synthesis in mammalian systems.

The presence of anti-aminoacyl-tRNA synthetase antibodies appears to be a hallmark of myositis (1–3, 6–10). To our knowledge, anti-tRNA or anti-tRNA-protein antibodies had not previously been observed in AI-CAH patients. The anti-tRNA$^{[Ser]Sec}$ specificity was found only in a subset of these patients who had a worse prognosis because they failed to respond to corticosteroid immunosuppression treatment. We propose that the presence of anti-tRNA$^{[Ser]Sec}$ specificity may serve as a diagnostic marker for a subset of severe "classical" AI-CAH, in the same way that anti-Scl-70 specificity is used for scleroderma and anti-Jo1 specificity is used for poly/dermatomyositis.

We are indebted to Joan Steitz (Yale University, New Haven, CT) for materials, advice, and criticism, I. N. Targoff (Oklahoma Research Foundation, Oklahoma City) for materials and help with the charging assay and for kindly providing anti-tRNA control sera, and

Table 1. Inhibition of aminoacylation activity *in vitro*

Sample/serum	Amino acid	cpm ($\times 10^{-3}$)	% inhibition	Diagnosis
No enzyme	Ser	0.2	—	—
No antiserum	Ser	7.6	—	—
Nonimmune serum	Ser	8.7	0	—
ARA	Ser	7.9	9	AI-CAH
RGG	Ser	6.9	21	AI-CAH
ACN	Ser	7.1	19	AI-CAH
JCA	Ser	7.9	9	AI-CAH
DOD	Ser	6.4	27	AI-CAH
DPP	Ser	6.8	23	AI-CAH
SPA	Ser	6.7	23	AI-CAH
Anti-PL-7	Ser	6.6	24	Polymyositis
Anti-isoleucyl-tRNA synthetase	Ser	6.7	23	Polymyositis
Anti-Jo1	Ser	6.1	18	Polymyositis
No enzyme	Thr	0.1	—	—
No antiserum	Thr	9.2	—	—
Nonimmune serum	Thr	10.1	0	—
Anti-PL-7	Thr	0.3	97	Polymyositis
No enzyme	Ile	0.2	—	—
No antiserum	Ile	6.3	—	—
Nonimmune serum	Ile	6.0	0	—
Anti-isoleucyl-tRNA synthetase	Ile	0.7	88	Polymyositis
No enzyme	His	0.2	—	—
No antiserum	His	10.2	—	—
Nonimmune serum	His	8.4	0	—
Anti-Jo1	His	0.3	96	Polymyositis

Assays were performed as described (10). All values are for 1:10 serum dilutions; cpm is the amount of radioactivity converted to trichloroacetic acid-precipitable form. The positive control sera [anti-isoleucyl-tRNA synthetase (10), anti-PL-7 (anti-threonyl-tRNA synthetase) (8, 9), and anti-Jo1 (anti-histidyl-tRNA synthetase) (3)] are specific for their particular synthetases.

Kazio Tyc and Karen Wassarman (Yale University) for help with RNA sequencing. We thank Dr. M. Buti (Hospital de Valle Hebron, Barcelona) and Dr. J. Enriquez (Hospital de Sant Pau, Barcelona) for providing the AI-CAH patient sera. We are grateful also to John Hardin (Yale University) for providing materials and Ms. C. Newey for help with the preparation of the manuscript. This study was supported by a grant from the Fondo de Investigaciones Sanitarias de la Seguridad Social (Spain) and a grant to J. A. Steitz from the National Institutes of Health.

1. Bernstein, R. M., Bunn, C. C., Hughes, G. R. V., Francoeur, A. M. & Mathews, M. B. (1984) *Mol. Biol. Med.* **2**, 105–120.
2. Reichlin, M. & Arnett, F., Jr. (1983) *Arthritis Rheum.* **27**, 1150–1156.
3. Rosa, M. D., Hendrick, J. P., Jr., Lerner, M. R., Steitz, J. A. & Reichlin, M. (1983) *Nucleic Acids Res.* **11**, 853–857.
4. Epstein, P., Lidsky, M., Reddy, R., Tan, E. & Busch, H. (1982) *Biochem. Biophys. Res. Commun.* **109**, 548–555.
5. Miyachi, K. J., Takano, S., Mimori, T., Yamagata, H., Mita, S., Matsuoka, Y., Irimajiri, S., Tani, K., Akizuki, M. & Homma, M. (1991) *J. Rheumatol.* **18**, 373–378.
6. Bunn, C. C., Bernstein, R. M. & Mathews, M. B. (1986) *J. Exp. Med.* **163**, 1281–1291.
7. Mathews, M. B. & Bernstein, R. M. (1983) *Nature (London)* **304**, 177–179.
8. Mathews, M. B., Reichlin, M., Hughes, G. R. V. & Bernstein, R. M. (1984) *J. Exp. Med.* **160**, 420–434.
9. Okada, N., Mukai, R., Harada, F., Fabashima, T., Nakao, Y., Yamane, K., Ohshima, Y., Sakamoto, K., Itoh, M., Kashiwagi, H. & Hamaguchi, H. (1984) *Eur. J. Biochem.* **139**, 425–429.
10. Targoff, I. N. (1990) *J. Immunol.* **144**, 1737–1743.
11. Sturgill, B. C. & Carpenter, R. R. (1965) *Arthritis Rheum.* **8**, 213–218.
12. Hardin, J. A., Rahn, D. R., Shen, C., Lerner, M. R., Wolin, S. L. & Steitz, J. A. (1982) *J. Clin. Invest.* **70**, 141–147.
13. Targoff, I. N. & Hanas, J. (1989) *Arthritis Rheum.* **32**, Suppl., S81.
14. Bock, A., Forchhammer, K., Heider, J. & Baron, C. (1991) *Trends Biochem. Sci.* **16**, 463–467.
15. Lee, B. J., Worland, P. J., Davis, J. N., Stadtman, T. C. & Hatfield, D. L. (1989) *J. Biol. Chem.* **264**, 9724–9727.
16. Mimori, T., Hinterberger, M., Pettersson, I. & Steitz, J. A. (1984) *J. Biol. Chem.* **259**, 560–565.
17. Forman, M. S., Nakamura, M., Mimori, T., Gelpi, C. & Hardin, J. A. (1985) *Arthritis Rheum.* **28**, 1356–1361.
18. Lerner, M. R. & Steitz, J. A. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 5495–5499.
19. Matter, L., Schopfer, K., Wilhelm, J. A., Nyffenegger, T., Parisot, R. F. & DeRobertis, E. M. (1982) *Arthritis Rheum.* **25**, 1278–1283.
20. England, T. E. & Uhlenbeck, O. C. (1978) *Nature (London)* **275**, 560–561.
21. Richardson, C. C. (1971) in *Procedures in Nucleic Acids Research*, eds. Cantoni, G. L. & Davies, D. R. (Harper & Row, New York), Vol. 2, p. 815.
22. Silberklang, M., Gillum, A. & RajBhandary, U. (1979) *Methods Enzymol.* **59**, 58–109.
23. Towbin, H., Staehelin, T. & Gordon, J. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 4350–4354.
24. Olmsted, J. B. (1981) *J. Biol. Chem.* **256**, 11955–11957.
25. Gelpi, C., Alguero, A., Martinez, M. A., Vidal, S., Juarez, C. & Rodriguez-Sanchez, J. L. (1990) *Clin. Exp. Immunol.* **81**, 59–64.
26. Hendrick, J. P., Wolin, S. L., Rinke, J., Lerner, M. R. & Steitz, J. A. (1981) *Mol. Cell. Biol.* **1**, 1138–1149.
27. Stefano, J. E. (1984) *Cell* **36**, 145–154.
28. Wilusz, J. & Keene, J. D. (1986) *J. Biol. Chem.* **261**, 5467–5472.
29. Bernstein, R. M. & Mathews, M. B. (1987) *J. Rheumatol.* **14**, Suppl. 13, 83–88.
30. Love, L. A., Leff, R. L., Fraser, D. D., Targoff, I. N., Dalakas, M., Plotz, P. H. & Miller, F. W. (1991) *Medicine* **70**, 360–374.
31. O'Neill, V. A., Eden, F. C., Pratt, K. & Hatfield, D. L. (1985) *J. Biol. Chem.* **260**, 2501–2508.
32. Lee, B. J., Rajagopalan, M., Kim, Y. S., You, K. H., Jacobson, K. B. & Hatfield, D. (1990) *Mol. Cell. Biol.* **10**, 1940–1949.
33. Diamond, A., Dudock, B. & Hatfield, D. (1981) *Cell* **25**, 497–506.
34. Hatfield, D., Diamond, A. & Dudock, B. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 6215–6219.
35. Dang, C. V. (1986) *Mol. Cell. Biochem.* **71**, 107–120.
36. Walker, E. J., Treacy, B. & Jeffrey, P. D. (1983) *Biochemistry* **22**, 1934–1941.
37. Miseta, A., Woodley, C. L., Greenberg, J. R. & Slobin, L. I. (1991) *J. Biol. Chem.* **266**, 19158–19161.
38. Hong, H. J., Yoo, S. H. & Yoo, O. J. (1987) *Nucleic Acids Res.* **15**, 4987.
39. Krupp, J. L., Shu, H. H. & Martin, N. C. (1988) *Nucleic Acids Res.* **16**, 770.
40. Hong, H. J., Hoe, K. L., Ryu, C. J. & Yoo, O. J. (1992) *Nucleic Acids Res.* **20**, 1144.