A mammalian tRNA^{His}-containing antigen is recognized by the polymyositis-specific antibody anti-Jo-1

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SUMMARY

The mammalian cell antigen reactive with the autoantibody anti-Jo-1 has been shown to contain tRNA^{His}. The RNA sequence of this human and mouse cell tRNA was determined in a search for unusual features that might be related to antigenicity. The 5' terminal nucleotide is unique among other sequenced tRNAs in that it is a methylated guanine. The presence of the hypermodified base queuine, which occurs in the wobble position of the anticodon of tRNA^{His} from several species, was not detected in the tRNA^{His} immunoprecipitated by anti-Jo-1 from either human HeLa or mouse Friend erytholeukemia cell extracts. The binding of protein(s) appears to confer antigenicity on tRNA^{His} since either proteinase K treatment or phenol extraction resulted in the loss of immunoprecipitability. However, we have not succeeded in identifying an antigenic protein, and we find that the antigenic complex is not resolved from purified tRNA^{His} by Sephacryl S-200 column chromatography. Immunofluorescence studies indicate that the antigenic form of tRNA^{His} is located preferentially in the mammalian cell cytoplasm. The results presented here are discussed in light of an earlier report (1) on the nature of the Jo-1 antigen.

INTRODUCTION

The production of antibodies directed against cellular macromolecules is a feature of autoimmune diseases (2). Patients clinically diagnosed as having connective tissue disease synthesize predominantly one or more antibodies which recognize DNA, ribosomes or other cellular antigens described as Sm, RNP, La and Ro. Biochemical analyses of the last four antigens have shown that they are located on distinct classes of small ribonucleoprotein (RNP) particles that function in various aspects of eukaryotic cell RNA metabolism (3-6).

In a screen of patients with a variety of autoimmune and other diseases (7), we found that sera from patients having the polymyositis-specific antibody anti-Jo-1 first described by Nishikai and Reichlin (1) precipitated RNAs of the size of tRNAs from mammalian cell extracts. This report

establishes that the anti-Jo-1 antibody reacts with what is probably a ribonucleoprotein particle containing $tRNA^{His}$. The primary structure of the Jo-1 $tRNA^{His}$ from both human and mouse cells indeed has several unusual features which could be related to antigenicity. However, the exact nature of the antigenic determinant, despite various approaches, has not been established.

MATERIALS AND METHODS

Cells, Maintenance and Labeling

HeLa cells were derived from standard laboratory stocks. Friend erythroleukemia cells were originally obtained from Dr. A. Sartorelli, Yale University. Vero cells were from Dr. W.P. Summers, Yale University. All cell lines were maintained at 37°C, 5% CO_2 at approximately 2 x 10^5 cells per ml in RPMI 1640 medium, supplemented with 5% heat-inactivated bobby calf serum (GIBCO Laboratories), 60 µg/ml penicillin and 100 µg/ml streptomycin. HeLa and Friend cells were radioactively labeled at 2 x 10^5 cells/ml in phosphate-free minimal essential medium (MEM, GIBCO Laboratories) for approximately 16 hrs in the presence of 10-20 µCi/ml ^{32}P-orthophosphate.

Preparation of ^{32}P-labeled *E. coli* was according to Brownlee (8). *Saccharomyces cerevisiae* strain trpl-1 *a* RH 218 was obtained from Dr. H. Hottinger, Yale University, and grown at 30°C to mid-log phase in YEPD medium (9). Yeast cells were radioactively labeled in low phosphate YEPD medium according to Knapp *et al.* (9). Spheroplasts were generated as follows. Mid-log phase yeast cells were washed twice with 0.8 M sorbitol, 0.1 M sodium phosphate, pH 7.5, and resuspended at 1 x 10^8 cells/ml in the sorbitol buffer plus 25 mM 2-mercaptoethanol. Then zymolyase 60K (Miles Laboratories) was added to a concentration of 0.1 mg/10^8 cells and the yeast suspension incubated at 30°C for 1 hr. The resulting spheroplasts were centrifuged at 1000xg for 2 min and disrupted by the addition of 50 mM disodium ethylenediaminetetraacetic acid (Na_2EDTA), 10 mM Tris(hydroxymethyl)aminomethane (Tris)-HCl, pH 7.5.

Sera

Sera from patients with polymyositis came from the collection of M. Reichlin. Other sera were obtained from Dr. J. Hardin, Yale University. Monoclonal anti-ribosome antibodies were the gift of Dr. E. Lerner, Yale University. Before use, sera were precipitated three times with 40% saturated ammonium sulfate, pH 7.5, dialyzed against 17.5 mM sodium

phosphate, pH 7.0, at 4°C and cleared of precipitate (10). The preparations were made 0.15 M in NaCl, 0.03 M in Tris-HCl, pH 7.4, and stored in aliquots at approximately 10 A_{280} units/ml.

RNA Fractionation and Sequence Analysis

Immune complexes were precipitated using Pansorbin (Calbiochem) and the extracted RNAs were fractionated in one dimension on 10% polyacrylamide (20:1 acrylamide:N,N'-bisacrylamide) gels in 8.3 M urea, 100 mM Tris-borate, pH 8.3, 2 mM Na_2EDTA as described by Rosa et al. (11).

Two dimensional RNA fractionation was essentially according to Zuniga and Steitz (12). The first of the two dimensions was 9% acrylamide, 0.3% N,N'-bisacrylamide, 7 M urea in 0.09 M Tris borate, pH 8.3. RNA samples were dissolved in 5 M urea, 0.05% XCFF and loaded onto a 0.5 cm wide slot in the gel (14 x 40 x 1.5 cm). The upper and lower reservoir buffers were 0.045 M and 0.09 M Tris borate, respectively. Electrophoresis was at 4°C and 500 v until the XCFF had migrated 25 cm. The RNAs were detected by autoradiography and the appropriate region sliced out of the gel and positioned at the bottom of a 30 x 40 cm gel plate. The gel for the second dimension (20% acrylamide, 0.45% N,N'-bisacrylamide, 0.09 M Tris borate, pH 8.3) was then formed around the gel slice; the reservoir buffer was 0.09 M Tris borate. Electrophoresis was carried out with migration of the RNAs in the upward direction at 4°C for 40 hrs at 800 v. RNAs were extracted from gel slices by the crush and soak method (13).

T1 and RNase A digests of in vivo ^{32}P-labeled RNAs were fingerprinted as described by Barrell (14) using either thin layer homochromatography on Cel PEI 300 (Brinkmann Instruments, Inc.) or electrophoresis in 7% formic acid on DEAE paper for the second dimension. Oligonucleotides were subsequently eluted and analyzed by digestion of T1 spots with RNase A and digestion of RNase A oligonucleotides with T1 RNase, followed by electrophoretic separation on DEAE paper at pH 3.5. To identify modified nucleotides, oligonucleotides were digested by either P1 nuclease or T2 nuclease (14) and subjected to two dimensional chromatography, using the system designed by Nishimura (15) or Silberklang et al. (16). Unlabeled RNA was immunoprecipitated and labeled at the 3' terminus using T4 RNA ligase and 5'-[^{32}P]-cytidine-5'3'-bisphosphate (17). Enzymatic sequencing was as described by Donis-Keller et al. (18) and chemical sequencing was as described by Peattie (19). Analysis of the 3' terminal nucleotide was by electrophoresis on Whatman No. 3MM paper of a complete alkaline hydrolysate of 3' ^{32}P-end-labeled RNA.

Proteinase K Experiment

1 ml of HeLa sonicate was divided into 6 aliquots and 360 µg proteinase K (Merck) added to 3 of these. 30 µl samples were removed at the indicated times from each aliquot and made 3.3 mM in phenylmethylsulphonyl fluoride (PMSF) before immunoprecipitation with anti-Sm, anti-tRNA-1 (7) or anti-Jo-1.

Immunofluorescence Studies

Indirect immunofluorescence was performed using either patient sera followed by FITC-conjugated goat anti-human IgG (Miles) or mouse monoclonal anti-ribosome antibodies (20) plus FITC-conjugated rabbit anti-mouse IgG (the last three the gift of Dr. E. Lerner). Prepared HEp-2 cell substrate was purchased from Immunoconcepts, Inc. (Sacramento). The indirect immunofluorescence procedure was exactly as described (6).

Characterization of the Jo-1 Antigen

For molecular sizing of the Jo-1 antigen, 0.5 ml HeLa cell sonicate prepared from 5 x 10^7 cells labeled with 5 mCi ^{32}P-orthophosphate was applied to a 1 x 110 cm Sephacryl S-200 (Pharmacia) column in 0.1 M KCl, 10 mM Tris-HCl, pH 7.5, 10 mM 2-mercaptoethanol. 1 ml fractions were collected at a flow rate of 5 ml/hr and analyzed for the presence of immunoprecipitable Jo-1 RNA. The column was calibrated using Blue dextran 2000, ovalbumin (Stokes' radius 30.5), chymotrypsinogen (Stokes' radius 20.9) and HeLa cell $tRNA^{His}$ purified by immunoprecipitation.

RESULTS

RNA Components of the Jo-1 Antigen

Figure 1 shows a one dimensional gel fractionation of ^{32}P-labeled RNAs that are included in an anti-Jo-1 immunoprecipitate generated from a HeLa cell sonicate. Because RNase fingerprint analyses of the Jo-1 RNAs showed that the two major bands contained mixtures of molecules (data not shown), we utilized the two-dimensional gel system of Zuniga and Steitz (12). There, one dominant and homogeneous RNA species was consistently seen (Figure 2), whereas the relative amounts of other RNAs were more variable. [Note that neither dimension of this system corresponds to that used in Fig. 1.] Furthermore, the major RNA molecule precipitated using sera from five different patients determined by immunodiffusion to produce anti-Jo-1 antibodies yielded identical T1 RNase fingerprint patterns (Figure 3A).

The Jo-1 RNA is $tRNA^{His}$

To establish the identity of the Jo-1 RNA, an analysis of the major RNA immunoprecipitated from HeLa cell sonicates was undertaken using a variety of

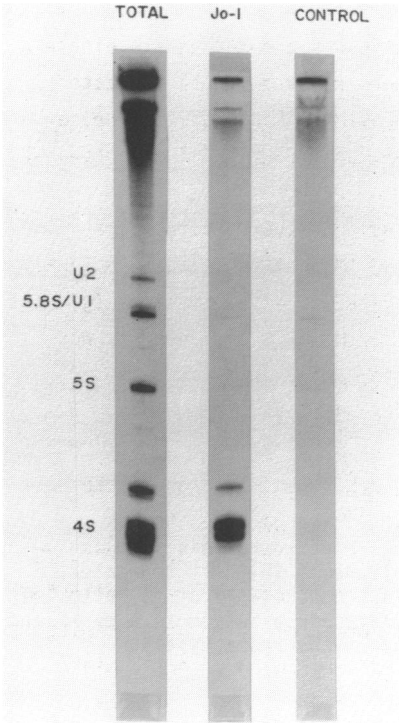


Figure 1 One dimensional gel fractionation of Jo-1 RNA. HeLa cells were labeled and RNAs were isolated from the total cell extract and from immunoprecipitates using anti-Jo-1 or normal control serum as described in Materials and Methods.

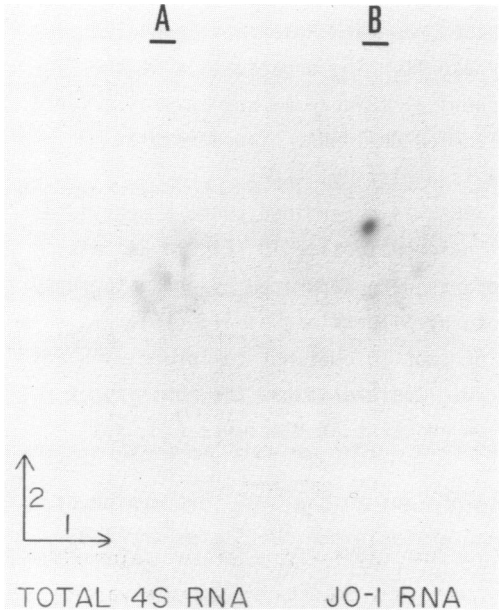


Figure 2 Two dimensional gel fractionation of Jo-1 RNA. Total 4S RNA (A) and RNAs extrated from an anti-Jo-1 immunoprecipitate (B) were fractionated on the same gels in two dimensions, as described in Materials and Methods.

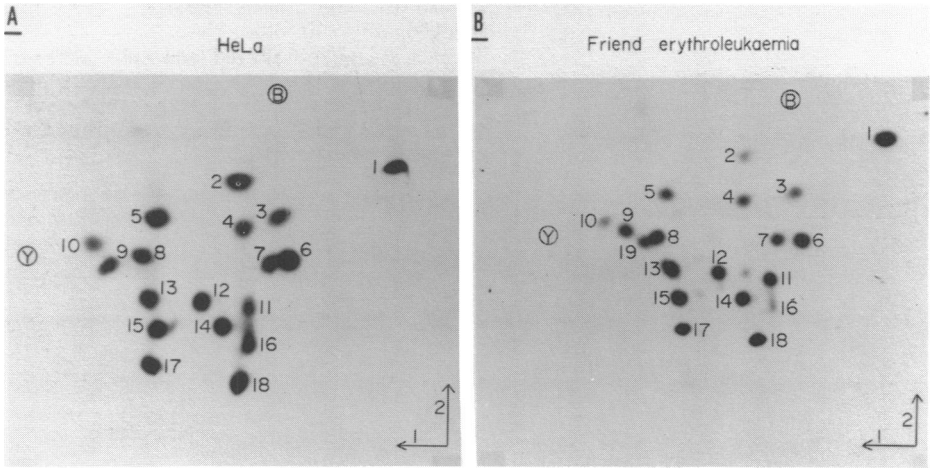


Figure 3 RNase T1 fingerprints of Jo-1 RNA.
Jo-1 RNA was prepared by two dimensional gel electrophoresis from immunoprecipitates of HeLa (A) or Friend erytholeukemia (B) cell sonicates. The RNase T1 fingerprints were obtained by electrophoresis from right to left and by homochromatography on PEI thin-layer plates from bottom to top. B and Y indicate the positions of the blue and yellow dyes, respectively.

RNA sequencing techniques. The RNase T1 oligonucleotides (Figure 3A) were examined further by digestion with RNases A, T2 or P1. An RNase A fingerprint (not shown) was also obtained and each RNase A oligonucleotide analyzed by secondary cleavage with RNase T1. The organization of the T1 oligonucleotides was established by chemical (Figure 4) and enzymatic (data not shown) sequencing of 3' ^{32}P-end-labeled Jo-1 RNA. These results (Figure 5) revealed that the Jo-1 RNA is a histidyl tRNA.

The 5' terminal nucleotide of Jo-1 RNA was determined to be a methylated guanylic acid residue as follows. T1 oligonucleotide 10 (Figure 3A) was digested with nuclease P1 and the products were separated by chromatography in the two-dimensional system described by Silberklang et al. (16). As shown in Figure 6, the P1 cleavage product of spot 10 does not comigrate with any of the mononucleotides pA, pG, pC or pU. Instead it has the mobility of either pm^{1}G or pm^{2}G in both this system and that of Nishimura (15; not shown).

Unusual base modifications are also present in the UΨCG loop region of Jo-1 tRNA^{His}. By chemical RNA sequence analysis the UΨCG stem and loop region was determined to contain the sequence GGUΨCGXAUCCG (Figure 4).

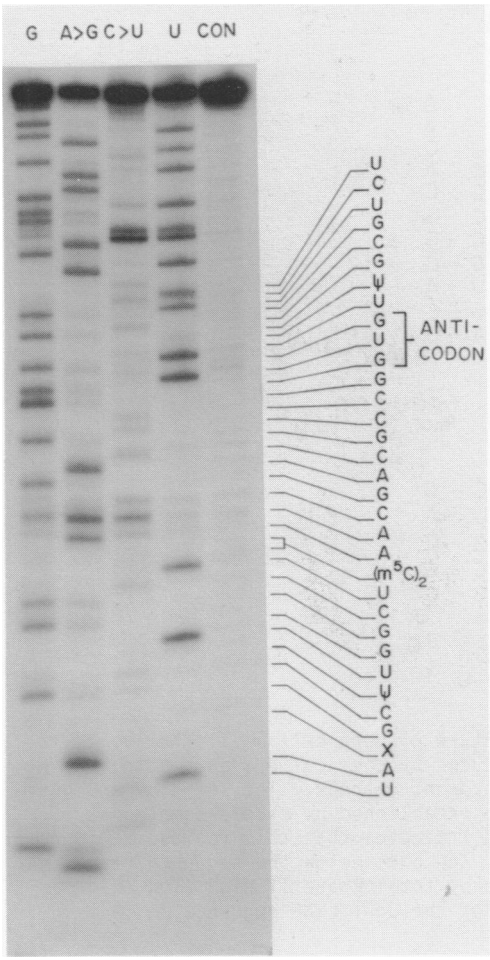


Figure 4 Chemical RNA sequencing of 3' ^{32}p-end-labeled Jo-1 RNA. Products are displayed on a 8% polyacrylamide gel run in 8.3 M urea after base-specific chemical modification and cleavage according to Peattie (19). Jo-1 RNA was obtained as described in Materials and Methods. X represents a modified nucleotide.

X is a modified nucleotide, as evinced by the gap in the sequencing ladder; in most tRNAs the nucleotide at the position of X is m^1A (21). However, the expected RNase T1 oligonucleotide m^1AAUCCG was not observed in the T1 fingerprint of Jo-1 RNA (Figure 3). Instead, two T1 oligonucleotides (11 and 16), each appearing in about 0.5 molar yield, were found to be derived from this region. Since only oligonucleotide 16 was observed in fingerprints where the second dimension was run in 7% formic acid on DEAE paper instead of by homochromatography, a labile modified nucleotide(s) appeared to be involved. Indeed, RNase A cleavage products of oligonucleotide 16 from either type of fingerprint included, in addition to 2 Cp and 1 Gp, an

pmG G CCG UG AUCG UAΨAG DG G DDAG UACUCUG CG ΨUG UG m^1G CCG CAG CAAm^{5}Cm^{5}CUCG G UΨCG X[A]UCCG AG UCACG G CACCA$_{OH}$

10 2 6 5 12 15 5 2 8 17 3 9 5 2 6 7 18 2 13 11/16 4 14 2 1

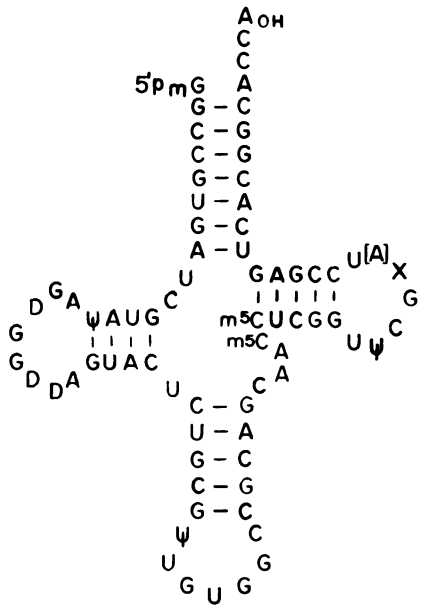


Figure 5 Primary and secondary structure of HeLa cell Jo-1 tRNA^{His}.
The nucleotide sequence (A) was deduced by chemical and enzymatic RNA sequencing methods together with fingerprint methods. The identity and position of most modified nucleotides were established by standard methods; others are discussed in the text. [A] indicates that this residue could be identified only by chemical cleavage. The bars below the sequence represent the numbered oligonucleotides obtained by total RNase T1 digestion (Figure 3). (B) shows the cloverleaf folding of the Jo-1 tRNA sequence.

oligomer migrating between ApApCp and ApApUp (data not shown). Analyses of the RNase T2 cleavage products of oligonucleotide 16 from paper fingerprints by both the method of Silberklang et al. (16) and that of Nishimura (15) consistently revealed 2Cp, Up, Gp, little or no Ap, and an additional streaky spot which did not exhibit the mobility expected for m^1Ap in either system. (not shown). Therefore, the Jo-1 tRNA^{His} appears to contain unusual modifications certainly at the first and perhaps at both the first and second positions within the sequence X[A]U.

Although the modified base queuine is present in the anticodon of some species of tRNA^{His} (22), the Jo-1 tRNA^{His} from the two mammalian tissue culture cell lines we examined did not appear to contain queuine. Queuosine-3'-monophosphate was not detected by two-dimensional

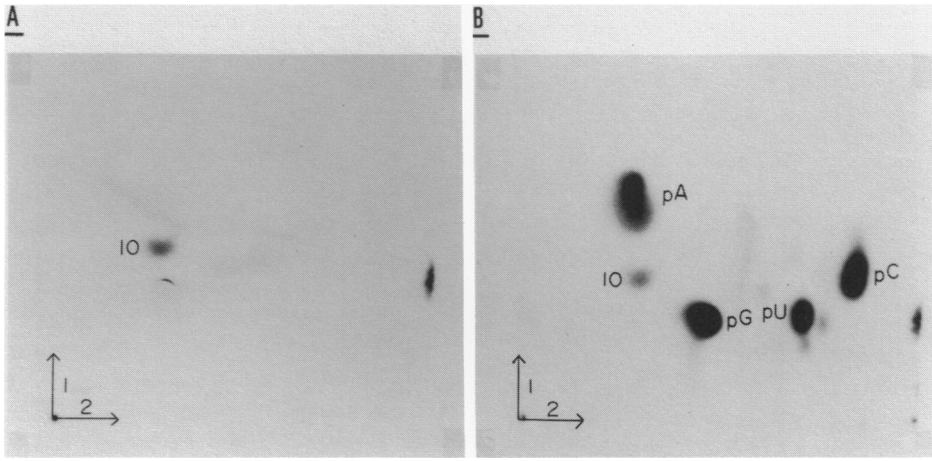


Figure 6 Analysis of the 5' terminal nucleotide of Jo-1 $tRNA^{His}$.
RNase T1 oligonucleotide 10 of Jo-1 RNA (Figure 3A) was cleaved by nuclease P1 in the absence (A) or presence (B) of marker RNA and chromatographed in the two dimensions described by Silberklang et al. (16).

chromatographic analyses (15) either of a total RNase T2 digest of Jo-1 $tRNA^{His}$ or of an RNase T1 secondary digest of the pancreatic RNase products of Jo-1 $tRNA^{His}$ (data not shown).

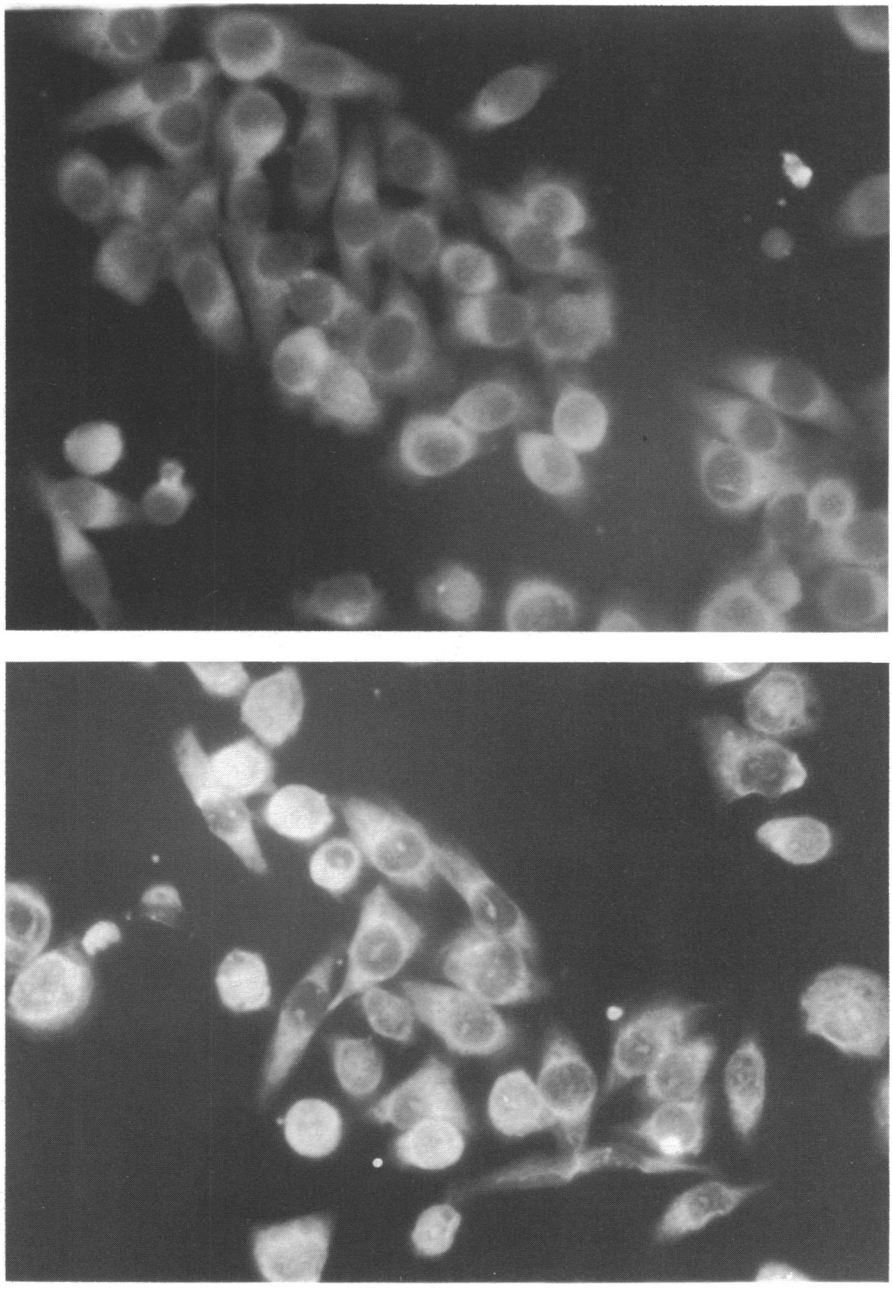
Species Conservation of the Jo-1 Antigen

To ascertain whether the Jo-1 antigen is conserved across species, immunoprecipitates were prepared from sonicates of ^{32}P-labeled E. coli, yeast, Drosophila, and Friend erythroleukemia (mouse) cells. The human antibodies did not precipitate any detectable RNAs from either E. coli or yeast cell extracts (data not shown). The Jo-1 antigen in Drosophila Kc cells appears weakly cross-reactive (23), but attempts to identify the RNAs were unsuccessful.

Immunoprecipitates generated from Friend erythroleukemia cell sonicates contained a Jo-1 RNA whose RNase T1 fingerprint (Figure 3B) is nearly identical to that of HeLa Jo-1 RNA (Figure 3A). Interestingly, the Friend cell Jo-1 RNA contains both 5'p^mG and 5'pG.

Cellular Location of the Jo-1 Antigen

Indirect immunofluorescence studies were undertaken using both Vero (monkey) cells and HEp (human) cells (Figure 7) to localize the Jo-1 RNP within the mammalian cell. The pattern obtained with anti-Jo-1 serum (A) is shown in comparison to that with anti-ribosome (B), anti-Sm (C) and normal



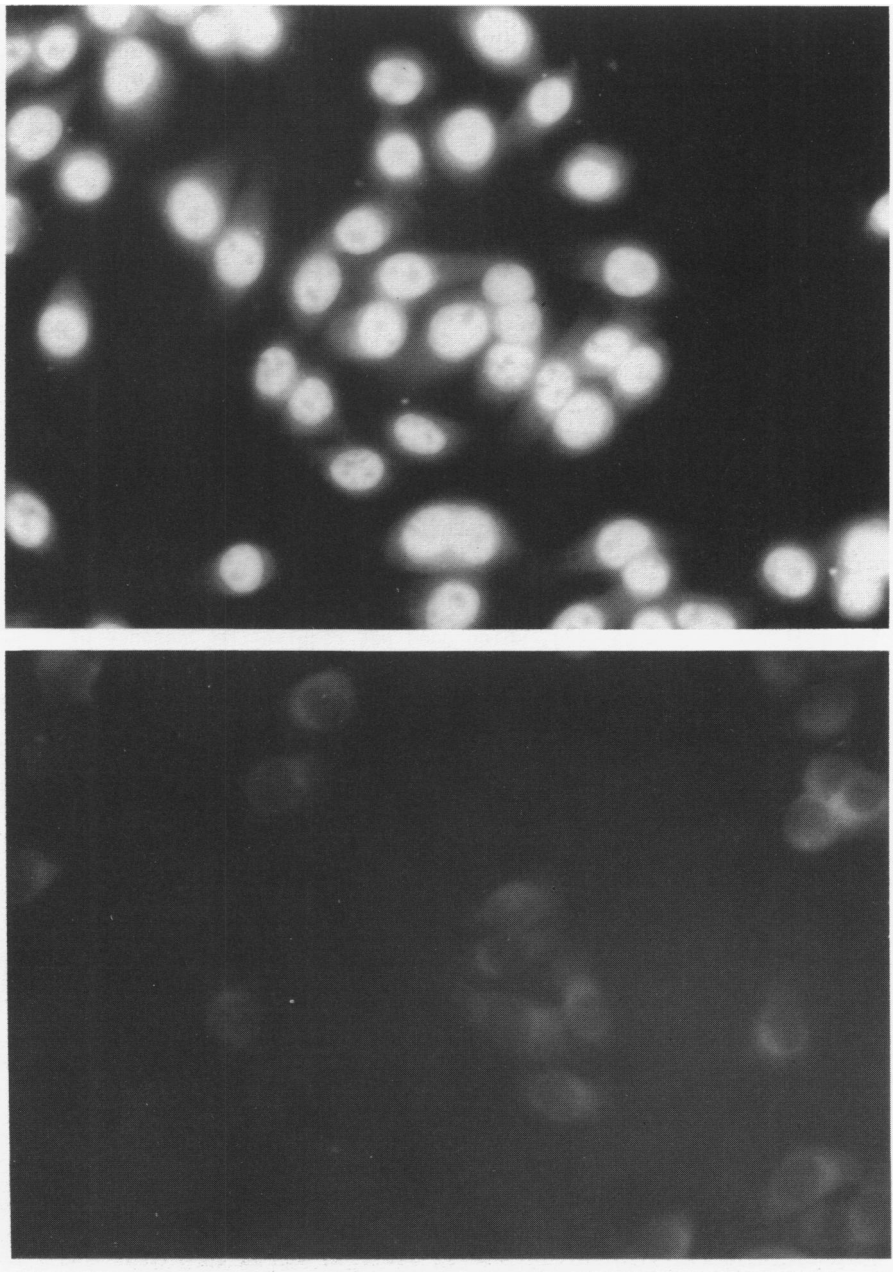


Figure 7 Cellular location of the Jo-1 antigen.
HEp-2 cells (Immunoconcepts, Inc.) were stained with (A) anti-Jo-1, (B) anti-ribosome monoclonal, (C) anti-Sm, or (D) normal control antibodies.

human (D) serum. The fluorescence observed with this and several other anti-Jo-1 sera is similar to the cytoplasmic staining seen with the anti-ribosome serum (20), except that it lacks nucleolar fluorescence. The Jo-1 antigen therefore appears to be located preferentially within the cell cytoplasm. This contrasts with the nuclear localization reported previously (1).

Properties of the Jo-1 Antigen

The following experiments suggested that the Jo-1 RNA most likely exists in the form of a ribonucleoprotein particle in mammalian cell extracts. First, the tRNA was not immunoprecipitable after phenol extraction (not shown), although control experiments showed that it was quantitatively recovered in an undegraded form in the aqueous phase.

Second, Jo-1 antigenicity is sensitive to proteinase action. HeLa cell sonicates were incubated at 4°C in the presence of proteinase K, aliquots were removed after various times, and further proteinase K activity was inhibited by the addition of phenylmethylsulfonyl fluoride (PMSF). Figure 8B reveals that the Jo-1 RNA rapidly lost all of its ability to be immunoprecipitated upon the addition of proteinase K. As a control, we showed that a tRNA antigen (7), which does not require the presence of protein, retained antigenicity throughout the time course of the experiment (Figure 8A); after an initial loss (which may be due to proteinase-released nuclease), the level of precipitable RNA remained constant. Conversely, most of the U RNAs associated with the Sm antigen, which has long been known to be a protein [see (3) for references], lost immunoprecipitability after the addition of proteinase K (not shown).

Attempts were first made to characterize the protein(s) associated with Jo-1 RNA by analyzing anti-Jo-1 immunoprecipitates from ^{35}S-methionine labeled cells. However, in a number of trials involving both 3 hour and 14 hour (1 generation) labeling times and different percentage gels (that would detect proteins from 8,000 to 150,000 mw), no protein band specifically present in anti-Jo-1 immunoprecipitates was ever observed. Likewise, labeling with a mixture of 14 ^{3}H-amino acids yielded no positive results.

Numerous attempts were made to detect a Jo-1 polypeptide using protein blot methodology that has been successful with anti-(U1)RNP, anti-Sm, and anti-La autoantibodies (24, 25). These again failed to identify a polypeptide reactive with any of several anti-Jo-1 sera, both crude and preselected for the IgG component.

Thus, determination of the size of the Jo-1 antigen was undertaken using chromatography of ^{32}P-labeled HeLa cell sonicates on molecular sieving

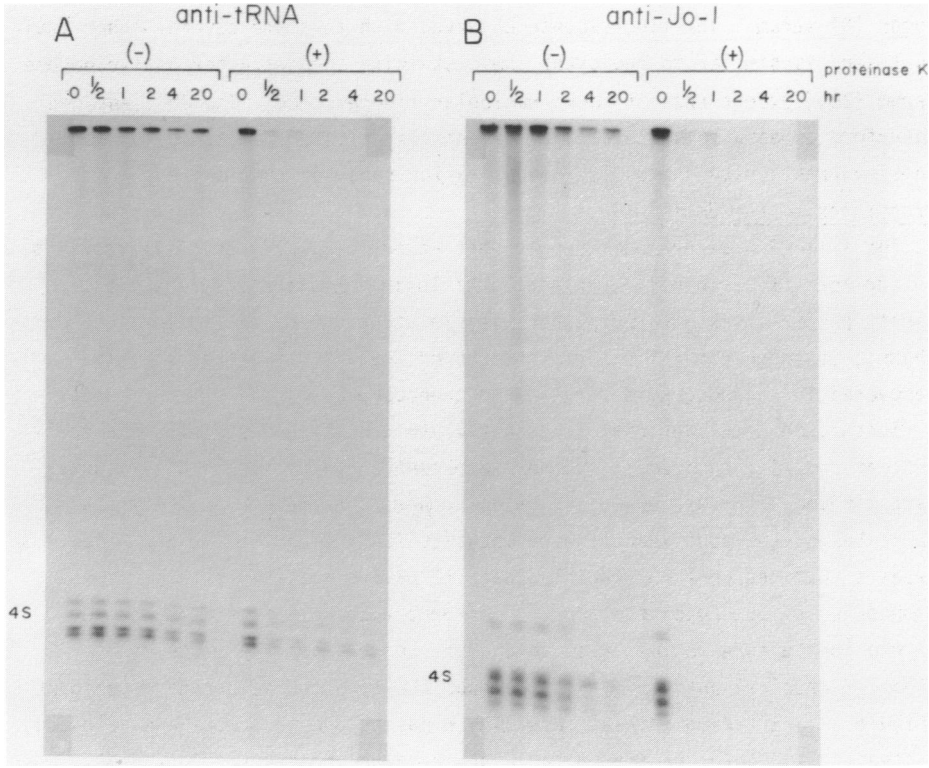


Figure 8 Analysis of immunoprecipitates from a ^{32}P-labeled HeLa cell sonicate following proteinase K treatment.
^{32}P-labeled HeLa cell sonicates were incubated in the presence (+) or absence (-) of proteinase K for increasing times (from 0 to 20 hr) before immunoprecipitation by (A) anti-tRNA-1 (7) or (B) anti-Jo-1 antibodies.

columns. Analysis of the fractions eluting from a Sephacryl S-200 column (Figure 9) revealed that the peak of Jo-1 antigenicity, as indicated by the level of immunoprecipitable $tRNA^{His}$, coincided with the elution of tRNAs. Subsequent chromatography of purified HeLa $tRNA^{His}$ through the same column (data not shown) confirmed that $tRNA^{His}$ elutes with the bulk of the tRNAs. This result establishes that the Stokes' radius of the Jo-1 antigen, at least to the extent of the resolving power of Sephacryl S-200, is not significantly different from that of the Jo-1 RNA. The possibility that proteolysis of a protein moiety was occurring during chromatography seems unlikely since larger $tRNA^{His}$-containing immunoprecipitable complexes were never detected in several reruns of the same experiment.

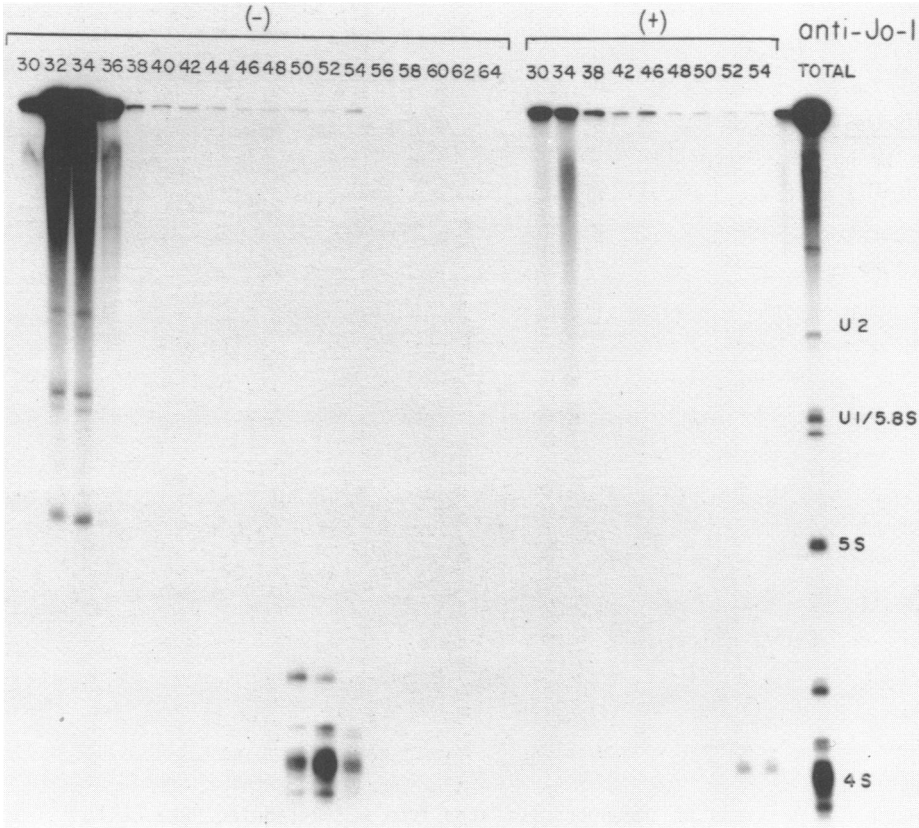


Figure 9 Molecular size of the Jo-1 antigen.
Chromatography of a HeLa cell sonicate through a Sephacryl S-200 column and subsequent analysis of the total and anti-Jo-1 precipitable RNAs were as described in Materials and Methods. The column fractions are identified by the numbers on top of the gel slots. The first radioactive material emerged in fraction 30, the void volume determined by Blue dextran 2000 elution. Ovalbumin (Stokes' radius 30.5) and chymotrypsinogen (Stokes' radius 20.9) eluted from the column in fractions 43 and 51, respectively. Purified Jo-1 $tRNA^{His}$ eluted in tubes 52 and 53 and free 5S rRNA eluted in tubes 44 to 46.

Although the possibility exists that the Jo-1 antigen might be histidyl-$tRNA^{His}$, it seems unlikely. The aminoacyl bond is labile at pH 7.5 (26); yet the Jo-1 antigen can still be immunoprecipitated after 20 hrs at this pH (Figure 8B). Furthermore, the phenol-extracted Jo-1 RNA did not acquire antigenicity upon incubation in a reaction mixture containing

histidine and a HeLa cell fraction containing aminoacyl tRNA synthetases (data not shown).

DISCUSSION

We have isolated and characterized an antigenic form of mammalian $tRNA^{His}$. It is specifically immunoprecipitated by the most frequently occurring antibody system, anti-Jo-1, found in the autoimmune disease polymyositis (1). The identities of other RNA species present in anti-Jo-1 immunoprecipitates have not been established due to their variable occurrence in terms of quantity and composition (Figures 1 and 2). It remains to be established whether other autoantibody systems associated with polymyositis (1) precipitate other specific tRNAs or tRNA-protein complexes.

Properties of Jo-1 $tRNA^{His}$

To identify the tRNA-sized RNA component of the Jo-1 antigen we elucidated its nucleotide sequence. The GUG anticodon and sequence homology to sheep liver $tRNA^{His}$ (27) and *Drosophila* $tRNA^{His}$ (28) establish that the Jo-1 RNA is $tRNA^{His}$.

There are several discrepancies between the human and mouse Jo-1 $tRNA^{His}$ sequence we determined (Figure 5) and that of sheep liver $tRNA^{His}$ (27). They are as follows. 1) Despite considerable scrutiny, we did not detect substantial levels of modification of the human or mouse sequences in the acceptor stem to yield either the m^2G or m^5C present in the sheep $tRNA^{His}$. 2) Likewise, queuine was not detected in the first position of the anticodon. 3) However, we did observe modifications not seen in the sheep sequence at both the extreme 5' end of the molecule (Figure 6) and in the UΨCG loop. 4) We clearly identified the sequence AAm^5C in the extra loop region of the Jo-1 $tRNA^{His}$ but did not see any C cleavage in the chemical sequencing reaction in this region (Figure 4). Since oligonucleotide 18 migrates slower than a 7-mer (spot 12) (Figure 3), we conclude that this region probably contains two m^5Cs. 5) In agreement with the sheep liver $tRNA^{His}$ sequence (27) but not with the sequence deduced from a mouse histidine tRNA gene (29), we find that nucleotide 16 in the Jo-1 $tRNA^{His}$ is a dihydrouridine. These differences may be due to species variation or to the fact that we examined cultured cells; some involve unusual features of $tRNA^{His}$ and are considered in more detail below because of their possible involvement in Jo-1 antigenicity.

First, Jo-1 $tRNA^{His}$ contains an unpaired guanine at its 5' terminus. The same configuration had previously been reported for histidine tRNAs isolated

from E. coli (30), yeast mitochondria (31), Drosophila melanogaster (28), and sheep liver (27). Curiously, this nucleotide does not appear in the genomic sequences for either the human or mouse mitochondrial $tRNA^{His}$ genes (32, 33) or in $tRNA^{His}$ genes from mouse (29), D. melanogaster or S. Pombe (34). Thus it appears that the additional G nucleotide is added post-transcriptionally; the mechanism of this modification reaction has recently been analyzed by Cooley et al. (34).

Second, the 5' terminal guanine is fully methylated in HeLa cell Jo-1 $tRNA^{His}$; both methylated and unmethylated 5' terminal guanines appear in Friend erythroleukemia Jo-1 $tRNA^{His}$. This particular modification has not been previously noted in histidine tRNAs; however, the added 5' pG of Drosophila and yeast $tRNA^{His}$ transcribed and processed in vitro does appear to be further modified (34), but not simply by methylation as observed here.

Third, as mentioned in Results, either queuine or guanine can occur in the first position of the anticodon of $tRNA^{His}$ from a number of both prokaryotic and eukaryotic species (22). The level of this modification, which also occurs in tRNAs specific for asparagine, aspartic acid and tyrosine (22), appears to correlate with cell age, nutritional state and the presence of oncogenic properties (35). Undermodified tRNAs containing guanine instead of queuine are specifically detected in a variety of tumor cells (35). Since HeLa and Friend erythroleukemia are both transformed cell lines, it is perhaps not surprising that anti Jo-1-immunoprecipitated $tRNA^{His}$ does not contain queuine. Indeed, we do not know whether any queuine-containing tRNAs are present in our HeLa and Friend erythroleukemia cell sonicates.

The Jo-1 antigenic moiety could therefore be a protein involved in any of the above unusual modifications of $tRNA^{His}$. It seems least likely to be the queuosine modifying enzyme (35) bound to the undermodified tRNA since the other tRNAs that normally contain queuosine (22) should also have been major components of immunoprecipitates. Alternatively, the Jo-1 antigen could be the histidyl tRNA synthetase, but then we would have expected the complex to elute considerably earlier from the molecular sizing column (Figure 9). Finally, we cannot eliminate the remote possibility that the $tRNA^{His}$ itself might possess a phenol-labile, peptide-containing modified base that is specifically recognized by anti-Jo-1 sera.

Comparison with Previous Studies of the Jo-1 Antigen

The biochemical nature of the Jo-1 antigen deduced here differs from that reported previously by Nishikai and Reichlin (1). Those authors (1)

concluded that the Jo-1 antigen was a protein of 150,000 daltons. The difference between our results and those of Nishikai and Reichlin (1) are not due to the use of different sera since in both cases the Jo-1 sera were from the same collection. The divergent properties ascribed to the Jo-1 antigen might be reconciled in the following way. If the antigenic moiety is a small protein which is capable of associating with both another protein and with $tRNA^{His}$ the two antigenic activity peaks observed to elute from DE-52 columns (1) could be explained. The peak eluting at 0.1 M NaCl and shown to be a protein of 150,000 daltons (1) would contain the protein-protein complex while the antigenic activity eluting at 0.4 M NaCl would contain $tRNA^{His}$. The assay we have used here to analyse the ^{32}P-labeled components of immunoprecipitates would not detect a protein-protein complex. It is difficult to reconcile our current cytoplasmic localization of the Jo-1 antigen with the nuclear immunofluorescence reported for spleen cells (1). More extensive studies will therefore be required to define unambiguously the nature of the Jo-1 antigen.

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