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A HUMAN AUTO-IMMUNE ANTIBODY SPECIFICALLY RECOGNIZING INITIATOR METHONINE tRNA FROM YEAST AND HIGHER EUCARYOTES

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Analysis of sera from 168 patients with autoimmune disorders revealed that one patient with Sjôgren's syndrome produced antibodies against deproteinized initiator methionine tRNA in addition to those against La protein. This anti-$tRNA_i^{met}$ recognizes also $tRNA_i^{met}$ from yeast but not from Phaseolus vulgaris chloroplasts (bean) or E. coli. It appears therefore that the epitope could be located in the TF loop in which an A residue in position 60 and the AUCG sequence are the only common features in yeast and human $tRNA_i^{met}$. © 1986 Academic Press, Inc.

Patients suffering from autoimmune disorders, such as mixed connective tissue disease (MCTD), systemic lupus erythematosus (SLE) and rheumatoid arthritis produce antibodies that are directed against a variety of nuclear and cytoplasmic components (for reviews, see references 1 and 2). Many of these antibodies are directed against protein(s) that reside in discrete small ribonucleoprotein particles generally containing a single small RNA and one or more proteins. In this respect, anti-RNP, anti-Sm, anti-La and anti-Ro antibodies are well known. They provided important clues for the diagnosis of autoimmune diseases as well as a basis for a molecular approach of their pathology and pathogenesis. Last but not least, they provided invaluable tools for probing both the structure and function of some of these ribonucleoproteins.

Other antibodies recognizing DNA (1), rRNAs (3) and tRNAs (3-6) have been described. In this last case, the antibody can be directed either against the cognate tRNA synthetase - as an example, the Jo-1 antibody is

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known to immunoprecipitate $tRNA^{His}$ in association with histidyl-tRNA synthetase (7) - or against a subset of tRNAs (3,4).

In a search for antibodies of novel specificities, we have screened a series of 168 sera from patients with autoimmune disorders. Among them, one serum from a patient with Sjôgren'syndrome was found to precipitate deproteinized initiator methionine tRNA from Hela and yeast but not from Phaseolus vulgaris chloroplasts (bean) or E. coli.

MATERIALS AND METHODS

Cell growth, labeling and cellular extract

HeLa cells were grown in spinner culture to a density of approximately 5×10^5 cells/ml in the presence of 0.05 mM phosphate and 5 µCi/ml of carrier-free $H_3{}^{32}PO_4$ for 36 hrs. After harvesting and washing under isotonic conditions, cells were suspended in one volume of buffer A (10mM Tris-HCl, pH 7.4, 10 mM NaCl, 1.5 mM $MgCl_2$) and sonicated. The cellular extract was then layered onto a 30% sucrose (w/v in buffer A) cushion and centrifuged at 3,000 x g for 30 min.

Immunoprecipitation techniques

5 µl of patient serum were added to 1 mg of protein A-Sepharose (Pharmacia) preswollen in 100 µl of 50 mM Tris-HCl pH 8.0, 100 mM NaCl, 0.1% Nonidet P40 and allowed to react for 2 hrs at 4°C with gentle stirring. After 6 washes, each with 400 µl of buffer B (10 mM Tris-HCl pH 7.4, 100 mM NaCl, 1.5 mM $MgCl_2$) a volume of cellular extract corresponding to about 4×10^6 cpm of TCA precipitable radioactivity was added to antibody-bound protein A-Sepharose and incubated with gentle stirring, for at least 4 h at 4°C. After 4 washes with 10 mM Tris-HCl, pH 7.2, 500 mM LiCl, 0.5% Nonidet P40 and one more without LiCl, bound RNA was extracted with phenol and separated by electrophoresis on a 12.5% polyacrylamide:urea gel in Tris-borate-EDTA buffer. Immunoprecipitations from deproteinized RNAs were conducted similarly either from in vivo ^{32}P labeled RNA or pure RNA species labeled in vitro at their 3' end (see below).

RNA analysis

Deproteinized HeLa RNA was prepared by overnight incubation of cellular extracts with proteinase K (200 µg/ml in 0.1% SDS) at room temperature. Proteinase K was removed by phenol extraction and ethanol precipitation of the RNA. RNA to be identified was selectively immunoprecipitated with Ma serum, extracted from Sepharose beads by phenol and labeled at its 3' end using cytidine (3',5' ^{32}P) bisphosphate and RNA ligase. Chemical sequencing was according to the method of Peattie (8). E. coli $tRNA_i^{met}$ was from Boehringer and $tRNA_i^{met}$ from yeast as well as Phaseolus vulgaris chloroplasts were generous gifts from Dr. G. Keith.

RESULTS AND DISCUSSION

We have screened a series of 168 sera containing antibodies against extractable nuclear antigen (ENA) as detected by Ouchterlony's method. Examples of the most representative sera specificities are shown in Figure 1 where immuno-precipitated RNA species were analysed in urea/acrylamide gels. Some sera contain only one type of auto-immune specificity: anti-RNP precipitates only U1 snRNA (lane a), monoclonal anti-Sm precipitates all U snRNAs (lane l), anti-La precipitates a large spectrum of RNAs (lane e)

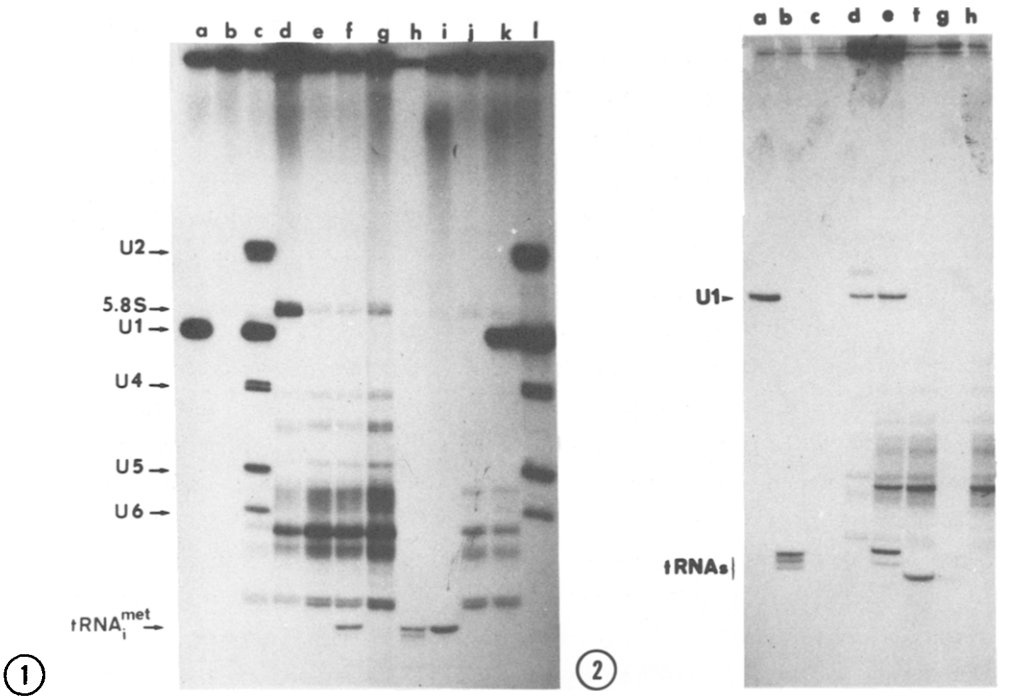

Figure 1: Immunoprecipitation of small RNAs from in vivo ^{32}P labeled HeLa cell extracts. Sera used represent a selection among the 168 samples we have screened. All lanes are described in the text except lane b in which a normal serum was used.
Figure 2: A selection of sera exhibiting anti-4S RNA specificities (lanes b, e, f, g). Ma serum is in lane f and normal serum in lane c. Sera with anti-RNP, anti-Ro and anti-La specificities were used repectively in lanes a, d, h.

associated with the unique La protein as monitored by immuno-blots (not shown) and anti-Ro (lane j) gives the typical pattern of small cytoplasmic RNAs which are present in the cell in low abundance (9). As might be expected, some sera contained several specificities. As a matter of fact, serum in lane c precipitated the typical U snRNAs as well as Ro RNAs and therefore contains anti-Sm and anti-Ro antibodies. Similarly, serum in lane k contained RNP and Ro specificities and serum in lane d had anti-rRNA (presence of 5.8 S RNA) as well as anti-La antibodies.

The Ma serum (lane f) precipitated La RNAs as well as a single species which appears to migrate in the size range of tRNAs. Thus, Ma serum appears to contain, besides anti-La, another antibody with novel specificity. This was confirmed by showing that this additional RNA species could be immunoprecipitated with Ma serum (lane h) from a cellular extract previously depleted by an anti-La serum (lane g). Unlike RNAs immunoprecipitated with anti-RNP, anti-Sm, anti-Ro and anti-La antibodies, this rapidly migrating component remained immunoprecipitable after deproteinisation (lane i) clearly demonstrating that its antigenic determinant must solely reside in the RNA.

Among the 168 sera tested, 8 comprising Ma serum were found to have antibodies precipitating RNA molecules of tRNA size. However, as seen in Figure 2 in which precipitated RNAs by 4 of the 8 sera tested are shown, all turned out to be different in size (lanes b, e) from $tRNA_i^{met}$ or at least showed an additional tRNA band (lane g). Furthermore, the band migrating at the $tRNA_i^{met}$ position in lane g was still immunoprecipitable after the extract was depleted with Ma serum. This last serum appears therefore to be unique so far in our collection.

This unknown RNA band could be easily purified by selective immunoprecipitation from unlabeled total cellular RNA with Ma serum together with protein A-Sepharose as it is the only one which can be precipitated in deproteinized form (Figure 1, lane i). After elution from Sepharose beads, this single RNA species was labeled at its 3' end with (^{32}P)pCp, purified by gel electrophoresis and chemically sequenced according to Peattie (8). Although the presence of modified bases only allowed determination of a partial sequence, sufficient diagnostic features were found for its unambiguous identification as initiator methionine tRNA (not shown).

The only universal feature revealed by comparison of the primary sequences is the occurrence of four GC base pairs (Figure 3) at identical positions in all initiator tRNAs (10). Initiator tRNAs from higher eucaryotes

Figure 3: Immunoprecipitation of 3' end labeled $tRNA_i^{met}$ from HeLa (lane a), Phaseolus vulgaris chloroplasts(lane b), yeast (lane c) and E. Coli (lane d).

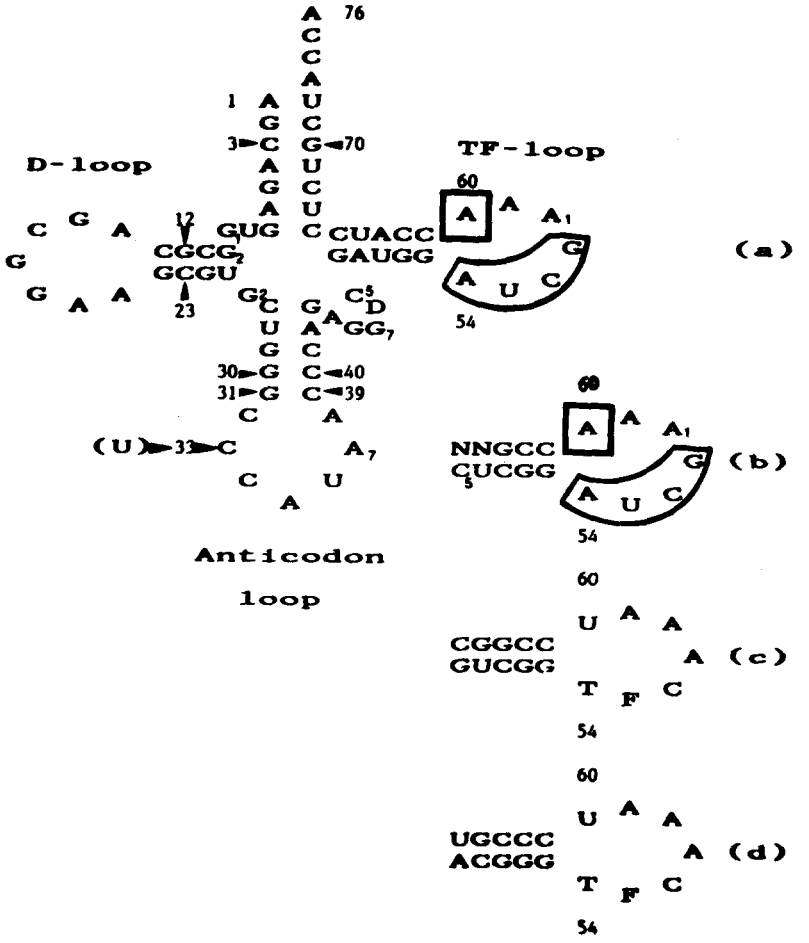

Figure 4: Sequence and secondary structure of human $tRNA_i^{met}$ (a). TF stems and loops for yeast, E. coli and Phaseolus vulgaris chloroplasts are shown respectively in b, c and d. GC base pairs common to all $tRNAs_i^{met}$ are indicated (positions 3-70, 12-23, 30-40 and 31-39). Features distinctive for initiator tRNAs from human and yeast (10) are boxed. (see ref. 11 for a compilation of tRNA sequences and modified bases).

are distinguished by three features: a) the presence of AUCG instead of TψCG (T is ribothymidine) at positions 54 to 57 in the TF loop; b) the presence of A instead of the usual pyrimidine at position 60; c) the presence of C instead of the invariant U in position 33 adjacent to the anticodon (Figure 3). In order to survey which one of these features is responsible for antigenicity, we have tested three 3' end labeled initiator tRNAs respectively from E. coli, yeast and Phaseolus vulgaris chloroplasts in comparison to that from HeLa cells. Figure 4 clearly shows that only yeast and human tRNAs are recognized by Ma serum excluding the possibility that the universal GC base pairs might be epitopes. Similarly the presence of C at position 33 of human tRNA must also be excluded since yeast tRNA is recognized and contains U at this position. It appears therefore that the

epitope recognized by this antibody could be a tertiary structure encompassing the A in position 60 and the AUCG sequence in the TF loop (boxed in Figure 3).

While this work was being completed, Wilusz and Keene (12) reported the existence of two sera with antibodies directed against $tRNA_i^{met}$. One of them was found to recognize E. coli $tRNA_i^{met}$ and is thus clearly different from that described here. Conversely, the second one does not cross-react with E. coli $tRNA_i^{met}$ and therefore could have the same specificity as Ma serum. It is noteworthy that the two antibodies reported by Wilusz and Keene (12) and that reported in this paper were found in sera which exhibited conventional anti-La specificity. Our serum was from a patient with Sjôgren's syndrome known to produce in most cases anti-La and/or anti-Ro antibodies and with a very low frequency anti-RNP (1). It therefore appears that the presence of anti-$tRNA_i^{met}$ in Sjôgren's syndrome could correspond to a distinctive form of this disease and could be a diagnostic marker.

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