RNA surfaces as functional mimetics of proteins

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Accumulating evidence suggests that RNA molecules can form surfaces that mimic those of proteins. Reactivity of autoantibodies with RNA surfaces may be due to cross-reactivity between a protein epitope and the RNA. The structural mimicry detected by an autoantibody may reflect functional mimicry.

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Chemistry & Biology July 1996, **3**: 505–513

Conformational RNA epitopes (Fig. 1) were discovered because autoantibodies in the sera of patients with systemic autoimmune diseases react directly with discrete structural elements in the U1 RNA of the small nuclear ribonucleoproteins (snRNPs), transfer RNA (tRNA) and ribosomal RNA (rRNA) [1–3]. Such immunoreactivity with specific regions of RNA molecules was unexpected, since nucleic acids are poorly immunogenic and the net negative charge on the surfaces of antibodies generally disfavors interactions with the negatively charged phosphate backbones of RNA and DNA. Historically, the reactivity of RNA with antibodies has been viewed as a result of accidental crossreactivity of RNAs with other cellular immunogens, or from the presentation of RNA fragments to the immune system following a breakdown in discrimination of self from nonself. But recent data indicate that conformational RNA epitopes may result from crossreactivity between RNAs and proteins that is not merely due to coincidence [4–7]. On the contrary, such RNA epitopes may indicate that functional mimicry between proteins and RNAs is an important and general biological phenomenon.

Several of the characteristics of conformational RNA epitopes indicate that they may represent meaningful structural entities or even biologically interesting sites of molecular interaction. For example, autoimmune RNA epitopes are uniquely restricted to certain RNA species. The RNA epitopes identified to date are summarized in Figure 1. Reactive epitopes have been found to reside on the U1 small nuclear RNA (snRNA), but not on U2, U3 or any of the many other U-rich snRNAs [1]. Certain autoimmune patient sera react specifically with initiator methionine tRNA ($tRNAi^{Met}$) [1,8] or alanine tRNA [9], yet other sera appear to react with all 4S tRNAs [1,10,11]. Autoantibodies that react with the hY5 RNA are present in sera of Sjogren's syndrome patients of the autoimmune Ro specificity, but reactivity with hY1 through hY4 was not detected [12]. In addition, a conformational epitope defined on 28S rRNA resides uniquely at a region between nucleotides 1944 and 2002, but reactivity with 18S rRNA has not been reported [3]. Thus, it is clear that autoantibodies recognize and bind with high specificity to unique and restricted regions of cellular RNA molecules in a manner much like their binding to autoepitopes on proteins.

Are the epitopes on RNA molecules dependent on the conformation of the RNA? This idea was initially proposed for RNA epitopes by Wilusz and Keene [1]. It was later suggested that stable conformational RNA epitopes probably were not involved in interaction of the anticodon region of alanine tRNA with autoantibodies in myositis sera [9]. However, conformational autoimmune RNA epitopes have been unequivocally demonstrated to exist within U1 snRNA [2], and several other conformational RNA epitopes reactive with autoimmune sera have been described (Fig. 1).

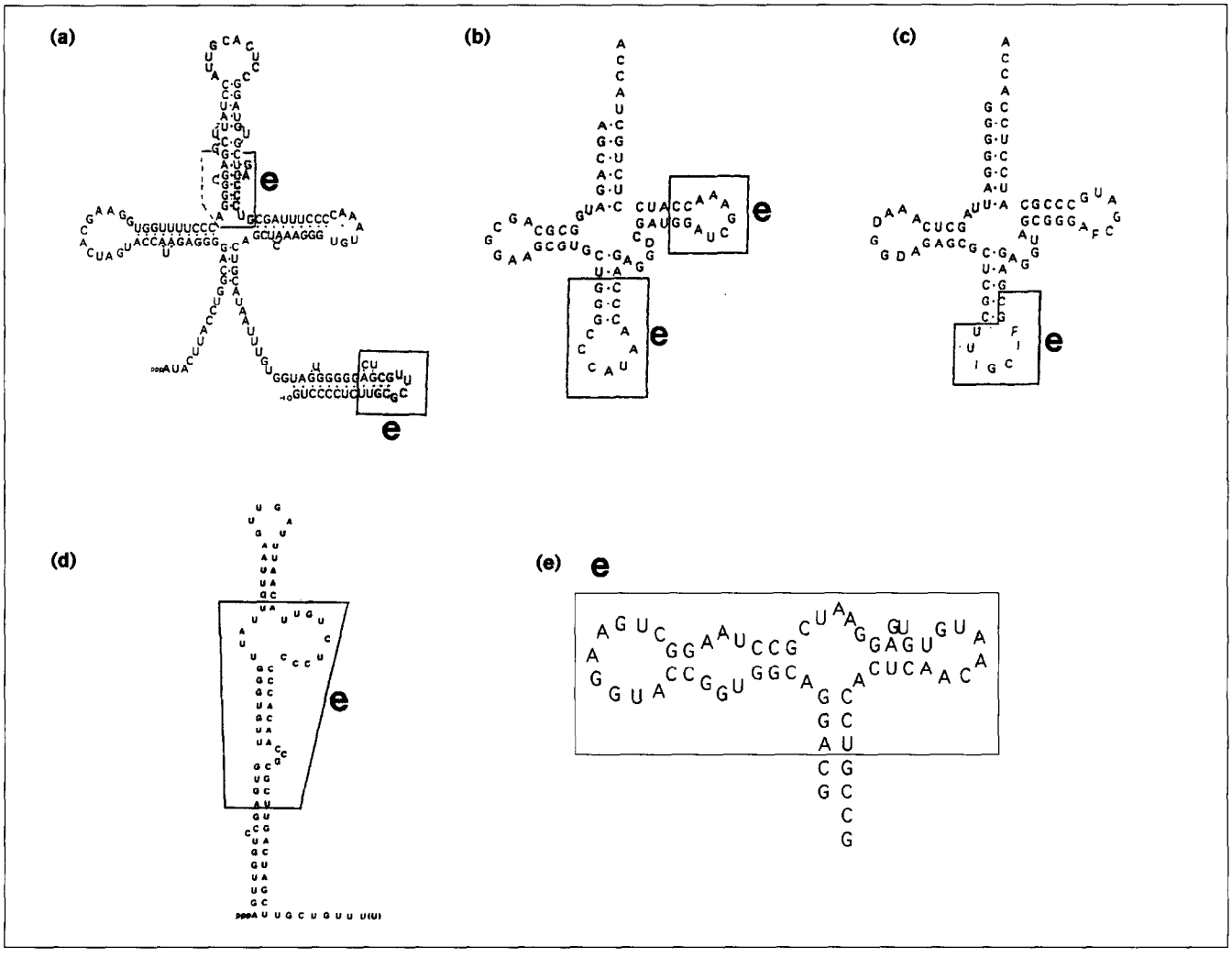
Why do anti-RNA antibodies arise?

The existence of sequence-specific conformational autoimmune epitopes on cellular RNAs has led to several hypotheses to explain their origins. Perhaps the most plausible of these is that the RNA is carried along during a response to a protein. Patients who make autoantibodies to the RNA components of RNP particles also generally make autoantibodies to at least one of the protein components of these particles [13–15], leading to the suggestion that the immune system may initially respond to a protein, then broaden its response to include the proteins and nucleic acids that are tightly associated with the autoantigen.

The suggestion is reasonable, although not proven, because of the manner in which proteinaceous autoantigens are believed to be presented to the immune system. Pre-B cells, the precursors of antibody-producing cells, express a membrane-bound form of antibody (the B-cell receptor) on their surface. Particles recognized by the B-cell receptor are internalized and processed into fragments, and the peptide fragments may then be presented on the surface of the pre-B cell, as a complex with class II molecules of the major histocompatibility complex (MHC). If an autoreactive helper T cell is present that recognizes a peptide derived from the RNP complex, the pre-B cell will receive help in the form of cytokines such as IL-4, and will differentiate into a mature, antibody-producing B cell.

It is highly unlikely that fragments of RNA molecules can be presented on class II MHC molecules in this way, and therefore 'naked' RNA (or DNA) molecules would not be expected to generate an immune response. However, if the RNA is bound tightly to a protein autoantigen, a B-cell

Figure 1



Cellular RNAs reactive with autoimmune sera. Each RNA depicted could be immunoprecipitated with sera from autoimmune patients. The predicted epitope sequences are indicated for: **(a)** U1snRNA [1,2,5,17]; **(b)** initiator methionine transfer RNA [1,8]; **(c)** alanine transfer RNA [9]; **(d)** hY5-Ro RNA [12]; and **(e)** a portion of human 28S ribosomal RNA [3]. Epitopes (e) in stem II and stem–loop IV of U1snRNA; the anticodon and T regions of transfer RNA(s); the sarcin-sensitive site of 28S ribosomal RNA [24,25]; and the stem of hY5 RNA are highlighted.

receptor that binds to the RNA may allow the internalization of the whole complex, resulting in presentation of peptide fragments of the autoantigen to the autoreactive helper T cell and T-cell help for the RNA-binding B cell.

Cross-reactivity between RNA and protein

Many other explanations for the generation of antibodies that bind to RNA have been suggested, however. In particular, several mechanisms for accidental cross-reactivity between RNA surfaces and protein surfaces have been proposed. According to the idiotype/anti-idiotype hypothesis, for example, one would expect antibodies to function as antigens themselves; anti-antibodies of this kind (anti-idiotypes) would have a shape complementary to the original antibody, and might therefore mimic the shape of the original antigen closely enough to bind to an RNA target of the original protein antigen. Extensive searches for the idiotypic RNA antibodies in sera from autoimmune patients have proved negative (J.D.K., unpublished data; [16]). In addition, the RNA epitopes in U1 snRNA do not appear to reside in regions of contact between RNA-binding proteins and the RNAs [5,12,17]. Furthermore, many autoantigenic RNA-binding proteins are attached to RNAs for which no anti-RNA antibodies have been reported.

Interestingly, unique RNA molecules can be selected *in vitro* from combinatorial RNA libraries to bind to antibody

binding sites [4–7]. Could autoimmune RNA epitopes represent a similar coincidental binding of cellular RNAs to antibodies? One observation which argues against this idea is that only a restricted set of cellular RNAs contain RNA epitopes. If RNA epitopes occur at random one would expect the distribution of epitopes to be more uniform among cellular RNAs. For example, one would expect to find epitopes on U RNAs other than U1 snRNA. One would also expect to find many epitopes in rRNA — since it is 20 times the size of U1 snRNA (which contains at least 3 such epitopes), there should be in the region of 60 RNA aptamer-type epitopes in ribosomal RNA, but in fact only 1 (or possibly 2) has been reported [3]. Thus, coincidence is unlikely to explain the whole phenomenon of autoimmune RNA reactivity. Nonetheless, it remains possible that certain RNA epitopes present on cellular RNAs coincidentally resemble structural features of protein antigens. The hypotheses outlined here are not necessarily mutually exclusive, of course.

The ribotope/proteotope hypothesis

An alternative idea, that at least some antibodies to conformational RNA epitopes might arise because of crossreactivity with cellular components that is discrete, specific and non-coincidental, arose following the serendipitous finding that artificial RNAs can mimic peptide epitopes [4]. In an experiment intended to select a preferred RNA species for an RNA-binding protein from a degenerate RNA library, in which the RNA-binding protein was attached to a solid support using an antibody, we instead selected an RNA that bound directly to the peptide-binding site of the carrier antibody [4]. Because the antibody was originally generated against a 13-amino-acid peptide, it appeared that the RNA and the peptide were molecular mimics of one another (reviewed in [6]). The key evidence suggesting mimicry between these molecules was that the RNA could compete against the peptide for binding to the antibody, and *vice versa*. Thus, these data suggested that an RNA molecule might mimic the shape of a protein epitope and thereby occupy a similar or overlapping space. It was proposed that autoimmune RNA epitopes might originate by crossreactivity with cellular proteins rather than by direct presentation or by anti-idiotypic mechanisms [4–6].

Doudna *et al.* [7] also demonstrated that an RNA can mimic a peptide following *in vitro* selection against a monoclonal antibody to the insulin receptor. This *in vitro* selected RNA also was able to react with autoantibodies from patients with extreme insulin resistance and the insulin receptor was competitive with the selected RNA epitope for binding to the antibody. These findings further suggest that RNAs and proteins can recognize identical or overlapping topological surfaces on proteins (as depicted in Fig. 2) despite the fact that RNA and protein are chemically very different and are not expected to form the same shapes or to present the same chemical moieties on their surfaces.

If RNA can mimic protein *in vitro*, can it also do so *in vivo*? Can RNA epitopes bind at sites where protein molecules otherwise interact? We have suggested that there may be situations in which RNA conformers, called ribotopes, can bind to the same target as peptide epitopes (proteotopes). Perhaps signals result from the binding of a ribotope, or displacement of a proteotope by a ribotope or *vice versa*. Evidence that such mimicry is possible is accumulating from diverse sources, and it is possible that the phenomenon is widespread.

Sources of proteotopes

If ribotopes and proteotopes function in a variety of cellular processes, polyclonal sera generated by immunization with proteotope-containing proteins might help to reveal corresponding ribotopes. Which cellular proteins contain the proteotopes that might crossreact with autoimmune ribotopes? The autoantigens that provide proteotopes may be the proteins commonly observed to react with autoimmune sera [13–15] (ie. primary autoantigens), but are more likely to be secondary autoantigens which are less commonly observed. In many cases, these autoantigens may not have been identified previously. One example of a possible secondary autoantigen discussed below is elongation factor 2 (EF-2; EF-G in bacteria), which may participate in a tRNA-specific autoimmune response. As RNA-specific autoantibodies are generally less common than the protein-specific autoantibodies and may in some cases appear later in disease progression than the protein-specific autoantibodies, the idea that secondary autoantigens are the source of proteotopes is plausible. Certain crossreactive autoantigenic epitopes may be generated by unique proteolytic enzymes involved in apoptotic, as well as necrotic degradation [18]. If discrete proteotopes can be elucidated, it will be important to determine their structures; they may turn out to be presented to the immune system in an unusual way.

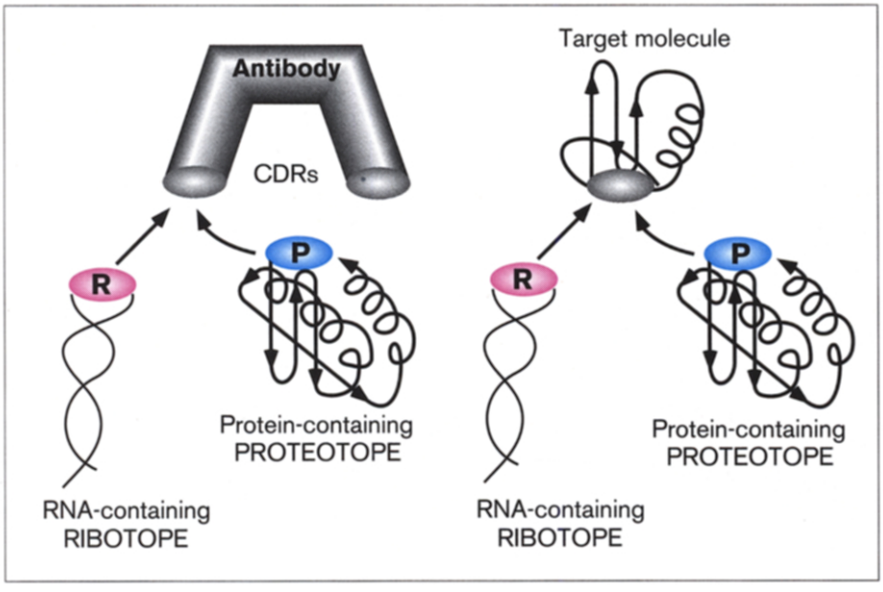
Structural mimicry involving conformational RNA epitopes

The RNA epitopes depicted in Figure 1 were discovered over the past decade using sera from patients with autoimmune diseases. It is evident that these epitopes occur in regions of the RNAs that involve higher order structures and that overlap both paired and unpaired RNA bases.

tRNA epitopes

Human autoantibodies generally reactive with 4S RNA [10,11] and mouse anti-4S antibodies [19] have been known for some time. However, no specific sequences or structures within a given tRNA were found to be immunoreactive until $tRNAi^{Met}$ (Fig. 1) was shown to be precipitable using sera from patients with Sjogren's syndrome [1,8]. These studies compared the $tRNAi^{Met}$ sequences of other species with those of human and suggested that epitopes reside in

Figure 2



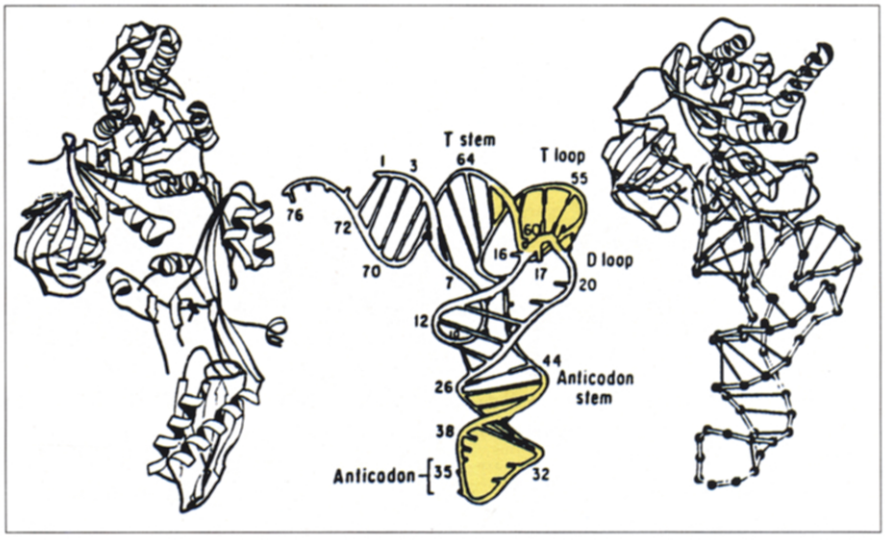
The ribotope/proteotope model of functional mimicry. The antibody with its complementarity determining regions (CDRs) is shown at left, and the *in vivo* target molecule is shown at right, with a similar surface feature to which both the RNA ribotope (R) and the protein proteotope (P) are able to bind [4–6]. The molecular shapes of R and P mimic one another, allowing binding to the same target site. Consequently either molecule may elicit a conformational change or functional effect on the target molecule. The antibody CDRs are formed by antigenic presentation of P which results in crossreactivity with R. According to this hypothesis, autoantibodies against the RNA epitopes shown in Fig. 1 were elicited during an autoimmune response to autoantigens containing proteotopes, and these sera contain antibodies crossreactive with the corresponding ribotopes. It is likely that only a very small subset of functional mimicry of this kind would be detected by autoantibodies.

the anti-codon stem–loop and in the T-stem–loop regions of the RNA based upon enzyme protection studies and the presence of phylogenetically conserved sequences. Interestingly, both of these regions, which are highly conserved among most species of tRNA, appear (when modeled using yeast $tRNA^{Phe}$) to have similar structures to that of *Escherichia coli* EF-G [20]. This finding is consistent with the ribotope/proteotope hypothesis and suggests that human EF-2, or a closely related protein (e.g. bacterial EF-G itself [21,22]), may be an immunizing antigen, giving rise to tRNA-specific autoantibodies in patient sera (Fig. 3, highlighted). According to the ribotope hypothesis (Fig. 2), one would predict that polyclonal sera reactive with autoepitopes on EF-2 might also react with tRNA. It will be important to determine whether the antigen must be processed in a manner similar to digestion by apoptotic proteases, thought to produce unique antigenic epitopes in the autoimmune response. Likewise, autoimmune patient sera reactive with the tRNA epitopes might be reactive with EF-2 protein. Therefore, RNA epitopes on the anticodon stem–loop of tRNA may represent a topological similarity between bacterial and human species, both of which represent correspondingly strong homology between bacterial EF-G and mammalian EF-2 proteins. A similar analogy can be made with the RNA epitope predicted by Sri-Widada *et al.* [8] in the T stem-loop of $tRNAi^{Met}$ (Fig. 1), which is highly homologous among species.

rRNA epitopes

Among the most readily apparent immunoreactive RNAs in autoimmune sera is rRNA [1,3,10,13–15,23], which often appears as a large smear at the top of acrylamide gels following immunoprecipitation experiments. Autoantibodies reactive with ribosomal RNA, including the 5.8S rRNA [1], have been known for many years [23] and appear to be quite common among serum samples and across disease types. Although more than one RNA epitope are probably present within 28S rRNA, only one conformational epitope (Fig. 1) of apparent biological importance has so far been identified [3]. This region of 28S rRNA resides within the GTPase center of translation elongation and corresponds to residues 1944–2020 in humans. Interestingly, this region is nearly identical among metazoans and yeast and is highly conserved in prokaryotes. Within this region of bacterial 23S RNA, which is homologous to the 28S human ribosomal RNA epitope, resistance to the antibiotic thiostrepton has been shown to result following 2'-O methylation of a critical A residue [24]. Several studies have shown that this portion of rRNA in all organisms is involved in elongation and GTP hydrolysis when ribosomes translocate from the A to the P site of the translation apparatus. By analogy with this conformational RNA epitope at 1944–2020 of human 28S rRNA, the homologous region of bacterial 23S RNA binds to EF-G, the bacterial elongation factor analogous to EF-2 [25]. As noted above, this region of 28S ribosomal RNA interacts with the ternary complex consisting of elongation factors 1α, tRNA and GTP and EF-2 in the eukaryotic ribosome to form part of the GTPase catalytic center. This is the same RNP complex and site of action of RNA epitopes in the anticodon arm and in the T arm of tRNA (Figs 1,3). Supportive data include the fact that autoantibodies specific for the 28S RNA epitope at 1944–2020 are able to inhibit both GTP hydrolysis and the binding of elongation factors to the 80S ribosomal complex [3]. Therefore, it is striking that three different conformational RNA epitopes reactive with autoantibodies appear to reside

Figure 3



Ribbon diagrams of elongation factor G (left) [21,22], $tRNA^{Phe}$ (middle) and the EF-Tu ternary complex (right) [20], depicting three-dimensional structural similarities believed to represent functional mimetics [20]. Sequences in the T stem–loop and in the anticodon stem–loop have been implicated as conformational RNA epitopes in certain tRNAs (see Fig. 1). Note that the $tRNA^{Phe}$ represented in the middle structure is perpendicular to the page and has a slightly different orientation from those of the ternary complex and EF-G, which are slightly turned. The highlighted regions represent ribotopes proposed to result from crossreactivity with proteotopes on EF-G (EF-2).

within the ribonucleoprotein complex and to interact directly with components of the GTPase center which induces the translocation step within the translational apparatus.

U1 snRNA epitopes

The discovery of epitopes on U1 snRNA (Fig. 1) resulted from attempts to immunoprecipitate deproteinized radiolabeled RNA from HeLa cells using sera from patients with systemic lupus erythematosus (SLE) [1]. These sera were known to immunoprecipitate proteins bound to U1 snRNP particles [13–15] and some sera were reactive also with proteins on other snRNP particles. Following deproteinization and immunoprecipitation, only U1 RNA was able to bind to antibodies in these sera. This study found that ~30 % of the patients with SLE contained U1 snRNA-reactive autoantibodies, but their prevalence may be higher when more sensitive methods are used. In none of these experiments were other uridylate-rich small nuclear RNAs, such as U2 through U7, found in the precipitated extracts. Thus, the restricted nature of the U1 snRNA epitopes is striking.

Attempts to identify the exact epitopes on the U1 snRNA involved the creation of complex pools of partially degraded *in vitro* transcripts followed by immunoprecipitation and RNA sequencing [2]. The optimal RNA epitope on U1 snRNA appeared to involve ~35 nucleotides encompassed within stem–loop II, and an intact stem and loop were found to be necessary for antibody reactivity. This demonstration of a conformational RNA epitope confirmed the previous supposition [1] that autoantibodies react with higher order conformers of RNA. Although this study [2] used an iterative procedure involving steps of synthesis, immunoprecipitation, sequence identification and resynthesis of deleted variants (outlined in [26]), iterative methods were not necessary to isolate the RNA epitopes, as the efficiency of antibody precipitation is high. These and other studies have indicated that the binding specificity and affinity of autoantibodies to RNA epitopes is comparable to that of proteinaceous antigens.

Efforts to pinpoint the numbers and locations of RNA epitopes on U1 snRNA have involved systematic deletion ([2,17]; C.S. Lutz and J.D.K., unpublished data), as well as *in vitro* selection using degenerate pools of synthetic RNA transcripts [5,27]. These studies identified at least three RNA epitopes located in stem–loop II and one located in stem–loop IV of U1 snRNA (Fig. 1). In addition to deletion analysis, RNAase protection and *in vitro* selection from combinatorial libraries, a variety of RNA competition experiments were carried out to confirm the sequence requirements for antibody binding to these RNA epitopes [5]. These studies were the first to indicate the restricted nature of the autoimmune response to U1 snRNA and demonstrated that multiple epitopes with various potential conformations can reside on a single cellular RNA.

Although candidate proteotopes for the U1 snRNA epitopes have not been identified, autoantibodies specific for U1 snRNA should be useful in this search. When the U1 snRNP docks with the spliceosome, protein–protein and RNA–RNA interactions are known to take place. Contacts between U1 snRNA and spliceosomal proteins or between U1 snRNP proteins and the pre-mRNA have not been reported, but it seems reasonable to suggest that such interactions occur. If the RNA epitopes on U1 snRNA mimic protein epitopes, perhaps the proteins in the splicing complex have proteotopes that bind to the same target site as the U1 snRNA ribotopes. If so, then the U1 snRNA-specific autoantibodies should serve as a useful tool to reveal

the proteotope(s), and may even be mimics of the target site on the spliceosome.

hY5 RNA epitope

Examination of sera from patients with Sjogren's syndrome and lupus that have the autoimmune Ro specificity [13–15] found that one of the human Y RNAs is immunoreactive [12]. The lack of autoantibody binding to the hY1–hY4 RNAs confirmed the restricted nature of the response, but examination of additional sera might yet reveal broader reactivity. hY5 RNA is highly structured (Fig. 1) and the epitope has been localized to a large region in the middle of the molecule encompassing a large internal loop. The structural epitopes may be confined to a smaller portion of hY5, and it is possible that more than one epitope resides in this blocked region.

Functional mimicry

As discussed above and depicted in Figure 3, further evidence of structural mimicry between RNA and protein has been provided by the costructure of the ternary complex of yeast $tRNA^{Phe}$ bound to elongation factor Tu (EF-Tu) from *Thermus aquaticus* [20]. The costructure included the GTP nucleotide analog, GDPNP, which was bound to EF-Tu in the crystallized complex. One of the striking findings of this study is that the ternary complex appears to resemble the crystal structure of the EF-G from *Thermus thermophilus* (analogous to eukaryotic EF-2) whose structure with bound GDP was solved previously [21,22]. One prediction of the ribotope/proteotope hypothesis is that RNA epitopes on tRNA would each have a structurally similar protein(s) with which it may crossreact (Fig. 2). The findings of Nissen *et al.* [20] are consistent with this hypothesis because RNA epitopes have been found in the anticodon arm and in the T arm of tRNA [1,8,9], which were proposed to have structural mimics on EF-G. Thus, it is possible that autoantibodies reactive with these conformational RNA epitopes were originally generated to portions of EF-2 (or EF-G) and subsequently crossreacted with tRNA. One caveat of the structural mimicry model of Nissen *et al.* [20] is that the known structure of EF-G was determined in the GDP-bound state, which might differ slightly from that of the GTP-bound state. Although there are critical functional differences between these nucleotide-bound states of EF-G, the substitution of GDP for GTP may not result in major differences in the regions of structural similarity to tRNA in the ternary complex.

Structural mimicry (Fig. 3) between the ternary complex and EF-G [20] suggests that they bind to a common target site on the ribosome. Both EF-G and the ternary complex are involved at the active site of GTP hydrolysis and translocation during translation (Fig. 4). This represents a key regulatory step in the dynamic transition of amino acid addition from the A to the P site of the assembled ribosome. Therefore, it is presumed that these similarities evolved because of the need for functional mimicry at overlapping sites on the ribosome. The autoantibodies may be revealing a structural aspect of the mimicry.

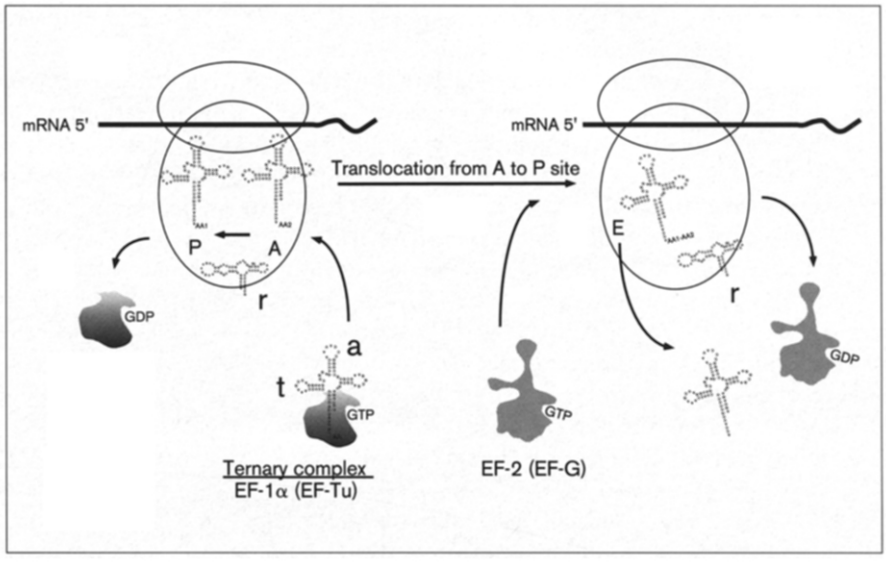
Interestingly, the presence of the 28S RNA epitope (Fig. 1) at the GTPase center of the ribosome further suggests a targeted RNA autoimmune response involving the translocation apparatus (Fig. 4). However, a candidate proteotope which corresponds to the 28S rRNA epitope has yet to be identified, but is predicted to be another proteinaceous surface that interacts at the GTPase center and involves the ternary complex and other ribosomal components. The ribotope/proteotope hypothesis would predict that the proteotope corresponding to this epitope of 28S rRNA might itself be capable of interaction with EF-2 given that the 28S rRNA epitope is reported to interact with this protein. Thus, the autoantibody to the 28S ribotope may itself represent a mimic of the EF-2 target site for interaction of both the RNA and the protein mimetic epitopes. This prediction is speculative, but autoantibodies to 28S rRNA should serve as useful reagents to dissect interactions within this dynamic region of the ribosome and to identify the putative proteotope(s). As noted above, autoantibodies to the 28S rRNA epitope can inhibit the GTPase and elongation factor binding. It will be interesting to determine whether the RNA epitopes revealed on U1 snRNA and on hY5-Ro RNA (Fig. 1) are indicators of dynamic *trans*-acting functions originating in these small ribonucleoproteins and competing with proteins for functions at target sites on larger RNP complexes such as the spliceosome.

Molecular recognition and protein–nucleic acid mimicry

The topological nature of RNA and protein are significantly different, and one would expect the structures of their normal targets, even the antigen binding sites of antibodies, to be very different. Indeed, the usual sites or motifs for binding of RNA to proteins and proteins to proteins appear to be very different. Furthermore, the backbone of a nucleic acid is highly charged, whereas that of a protein is not charged. As a result, nucleic acids are thought to have more difficulty forming globular topologies, a property more characteristic of proteins. Despite these considerations, the finding that an RNA can mimic a peptide in binding to the complementarity-determining regions (CDRs), or ligand-binding site, of an antibody [4,7] suggests that there can be similar chemistry or similar topology among these epitopes. Peptides in the range of 10–12 amino acids may be able to achieve some local conformation, while RNA stem–loops of the order of 10 to 12 nucleotides are sometimes able to form stable tertiary structure. In space, approximately three amino acids are equivalent to a single nucleotide. Thus, in a topological sense, the ability of RNA and protein to fit against a common surface is unexpected, but the data of Tsai *et al.* [4] and Doudna *et al.* [7] suggest that it is possible.

Figure 4

Translation components that interact at the GTPase center during translocation from the A to the P and then to the E site during protein synthesis. Regions of transfer RNA (t for T stem-loop and a for anticodon stem-loop) and ribosomal 28S RNA (r) represent RNA conformational epitopes, implicated as the sites reactive with autoimmune sera [1,8,9] (see Fig. 1). Molecular mimicry may exist between the tRNA component of the prokaryotic ternary complex (consisting of GTP, tRNA and EF-Tu) and EF-G (consisting of GTP and EF-G). The eukaryotic analogs of EF-Tu and EF-G are EF-1α and EF-2, respectively. Competition between these elongation factors for binding to the ribosome mediates translocation and the hydrolysis of two molecules of GTP during translocation. The exact function of the rRNA epitope (r) is unknown, but it has been shown to have a role at the GTPase center during translocation [3,16,24,25].



Both polypeptide and nucleic acid conformations are determined by a variety of geometric and steric constraints. Hydrogen bonding, hydrophobic considerations and electrostatic forces are critical in dictating tertiary interactions of both kinds of macromolecules. However, there are distinct structural differences that affect the folding properties of nucleic acids and polypeptides. The high degree of rotational freedom about six chemical bonds in the RNA backbone is compatible with the formation of multiple conformations. It is the highly ordered structure required for base pairing that normally constrains the degrees of rotation available to RNA (and DNA), and therefore its possible conformations. In general, nucleic acids have fewer hindrances to torsional freedom of rotation about their backbone than peptides, which is compatible with greater topological flexibility. Proteins do not form complementary base pairs and have fewer conformational possibilities in the backbone, and have far less complex backbone structures than RNA, although they do have the diversity provided by the 20 or more possible amino acid sidechains. The conformational flexibility available to single-stranded nucleic acids in solution is considerable. It is likely that this potential for complex shapes and surfaces of RNA evolved to fulfill a variety of functional roles in addition to catalysis. Ribotopes may represent one such role.

Antibody CDRs are not necessarily rigid, but can themselves be flexible due to the torsional freedom inherent in certain amino-acid sidechains that commonly occur at antigen-recognition sites [28]. Thus, molecules that bind to the same CDRs are not necessarily molecular mimics of one another unless 'static' structural features are being recognized. Two molecules that recognize the same cellular target and have common topologies might also recognize the same antibody CDRs even without precise structural mimicry. Molecular recognition between topological surfaces is likely to involve dynamic and static properties of both the ligand and the target. In cases where suspected mimicry involves ligands with distinctive chemical properties, such as those of proteins and nucleic acids, competitive binding to the target is the key feature of common function and constitutes a sufficient operational definition of mimicry.

Contrary to some expectations, RNA binding to protein can be of higher affinity than protein–protein interactions. In addition, it is evident that RNA can acquire many conformational options resulting in a wide biological targeting potential [6,29,30]. *In vitro* RNA selection technologies and aptamer experiments have demonstrated that RNA has the ability to bind and inhibit enzymes and to disrupt protein–protein interactions. It is easy to imagine that RNA binding to a protein target *in vivo* could displace another protein able to bind to the same target surface as in the ribotope hypothesis. Likewise, the proteotope and the target could be part of the same molecule such that the ribotope alters the conformation of the target when displacing its mimetic proteotope.

DNA–protein mimicry

Although double-stranded DNA is more conformationally constrained than either single-stranded DNA or RNA, examples of analogous modes of recognition between DNA and proteins have emerged. For example, a recent study has demonstrated potential molecular mimicry between DNA and an enzyme, uracil-DNA glycosylase [31]. This finding

resulted from the solution of a crystallized complex between the inhibitor of glycosylase and glycosylase itself, which showed a twisted five stranded antiparallel β-sheet and two α-helices. In this structural model, the inhibitor mimics the DNA-binding groove to which the enzyme normally binds. This case, and another example of proteins that bind to the DNA-binding globular domains of proteins such as histones [32], add to the possible role of mimicry between nucleic acids and proteins as a regulatory paradigm for biological structure and function. It is relatively straightforward to consider that certain DNA-binding autoantibodies may originate by crossreactivity between the proteins that mimic DNA and DNA. Given that certain DNA-binding proteins lack sequence-specific or structure-specific recognition, it is expected that antibodies against the proteins that mimic the DNA to which they bind might themselves have little or no sequence specificity.

Prejudices against the use of antibodies to precipitate DNA and RNA have discouraged investigators from testing sera for nucleic-acid-reactive antibodies. It is possible that polyclonal antibodies raised in animals against viral and cellular proteins will be found to react with specific RNA epitopes and thus reveal new ribotopes and their crossreactive proteotopes. On the other hand, this may be a feature unique to autoantibodies formed in response to distinct epitopes [18]. Given the lack of research so far in this area, it is possible that ribotopes will be discovered in many RNAs and found to be important in regulatory processes in the nucleus and cytoplasm. For example, ribotopes may be involved in RNA editing, telomerase functions, RNA discard and signal recognition. Perhaps a better understanding of the regulatory nature of ribotopes and proteotopes will provide clues about why autoimmune reactivity tends to involve nucleic acids and nucleic-acid-associated proteins.

Do ribotopes exist in viruses?

Reports of autoantibodies that react with viral RNAs [33] have received little attention and probably little investigation. On the other hand, many examples of crossreactivity between virus proteins and cell proteins have been reported ([34] and references therein). The structural similarities between translation elongation factors and tRNAs revealed by Nissen *et al.* [20] may relate to the observation that many plant and animal viruses contain aminoacylatable tRNA-like structures in their genomes ([35] and references therein). Transfer RNA-like structures present in certain plant and animal virus genomic RNAs resemble the structures of folded tRNA and may also mimic portions of EF-2 (EF-G) [21,22]. These genomic tRNA-like structures have stem–loops that mimic the anticodon arm and the T arm where RNA epitopes have been mapped (Fig. 1). Certain picornaviruses are implicated in the autoimmune inflammatory muscle disease, myositis (reviewed in [36]) and picornaviruses are known to dramatically alter translational regulation. As noted above, myositis patients contain autoantibodies reactive with aminoacyl-tRNA synthetases and with tRNA [9,13,15,36]. However, neither the mechanisms of translational blockage by viruses, nor their role in autoimmunity are well understood. It will be interesting to determine whether such viral genomes are reactive with autoantibodies. Mimicry could also be affected by the presence or absence of modified bases in tRNA, which do not seem to be present in the viral tRNA-like structures ([35] and references therein). Thus, it remains to be seen whether viruses contain *trans*-acting RNA structures, which physically displace cellular components at sites where other cell proteins normally interact.

Do ribotopes exist in messenger RNA?

Although RNA epitopes were discovered originally in small cellular RNAs, their presence in rRNA was detected, in part, because of the relative abundance of 18S and 28S rRNAs. The low abundance of individual mRNA species makes the detection of any RNA epitopes that they may contain more difficult. Thus, it is possible that RNA epitopes reactive with antibodies are as common in cellular mRNA as in small RNAs and rRNA, but this has not been explored. As the untranslated regions of mRNA often have significant secondary structure, and presumably possess regulatory functions, one might expect *trans*-active RNA structures to reside in 5'- and 3'-UTRs. It has been suggested that 3' UTRs may represent Nature's own combinatorial RNA library, allowing the RNA greater plasticity and redundancy of sequence with relatively few constraints [26]. Recent experiments suggest that *trans*-acting RNA sequences as short as 200 nucleotides can be found in the 3'-UTRs of cytoskeletal mRNAs that have the unexpected property of blocking cell division, causing differentiation and suppressing tumor formation [37]. Although the mechanisms of action of these RNA sequences and their respective target proteins have not been determined, they may fit the ribotope/proteotope model if proteins that bind the same targets also exist. Another example of a potential *trans*-acting RNA is the transcription product of the parentally imprinted mouse H19 locus [38]. This RNA lacks an open reading frame, but is the most highly abundant RNA in the developing embryo. When expressed transgenically, this RNA results in prenatal lethality, indicating its regulatory importance in development. It is reasonable to speculate that this might be a *trans*-acting RNA with an important target molecule. Two other examples of RNA transcripts that are involved in genetic imprinting, lack open reading frames and may act in *trans* at target sites to affect gene expression include the products of the XIST and IPW loci ([39] and references therein). Although there is no evidence that these or other *trans*-acting RNAs can be immunogenic and thus be ribotopes, the possibility is untested and antibodies are likely to prove highly valuable in the discovery of *trans*-acting RNA species such as these. The possibility that antisense transcripts contain ribotopes

involved in regulation of gene expression is highly speculative, but may help explain the often unpredictable functional consequences of antisense RNA expression.

The area of *trans*-acting functional RNA is undeveloped, but may be stimulated if more investigators test antibodies for reactivity with RNA molecules. It is reasonable to predict that numerous ribotopes and their corresponding proteotopes are waiting to be discovered, and antibody reactivity with RNA could represent a valuable method of exploration. If, indeed, ribotopes are acting at similar or overlapping protein-binding sites on target molecules, it is probable that many will prove to be functionally important.

Acknowledgments

I thank Daniel Kenan for critical reading of the manuscript and for stimulating discussions and Dr Stephen White for help with graphic modeling from available coordinates. This paper is dedicated to Dr Eng Tan for his many contributions to our knowledge of autoimmunity.

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