Antibodies to Homologous RNA in the Rabbit Following Stimulation By Exogenous RNA[1] (34922)

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(Introduced by R. Hiramoto)

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Antigenic cross reactivity of ribosomes from various sources has been demonstrated in both patients and animal systems.

Antiribosomal antibodies occurring in patients with systemic lupus erythematosus (SLE) have been shown to react with both human and various mammalian ribosomes (1–3). An antigenic determinant of this system has recently been shown to be ribosomal RNA (3).

Common antigenic determinants on heterologous ribosomes have been demonstrated by immunizing rabbits against ribosomes from one source and subsequently demonstrating reactivity of the immune serum with ribosomes from various other sources including the rabbit (4, 5). That the antigen involved in this cross reactivity is RNA has been demonstrated by reaction of antiribosomal antisera with RNA from various sources (6–9).

Bigley *et al.* (5) demonstrated that rabbits immunized against yeast RNA produce antibodies which react with ribosomes from various sources including rabbit and further that this reaction could be inhibited completely or partially by mononucleotides and nucleosides, suggesting strongly that the antigenic determinant of this reaction is on rRNA.

The purpose of this investigation was to further study the antigenic properties of ribosomes.

Materials and Methods. Whole ribosomes were isolated essentially by the procedure described by Wettstein *et al.* (10) from human liver, rabbit liver, and mouse myeloma tumors MOPC31C and MOPC70E (11). Ribosomal protein was separated from rRNA using urea and LiCl (12). Ribosomal RNA (rRNA) was isolated from rabbit liver and mouse myeloma tumor MOPC70E using 4-amino salicylate and phenolic solvents essentially as described by Kirby (13). Yeast RNA was used as obtained from Mann Research Laboratories, Inc. suspended in 0.01 *M* phosphate buffered saline, pH 7.4.

Immunologic Procedures:

Immunizations. Three groups of New Zealand white male rabbits containing two animals each were immunized using suspended antigen and Freund's adjuvant for initial subcutaneous doses followed by an intravenous dose without adjuvant which was repeated until precipitin lines were detectable. Antigens used were human liver ribosomes (HLR), yeast RNA and mouse myeloma (MOPC31C) ribosomal protein. The doses were initially 5 mg/week; however, intravenous doses were increased until precipitin lines were detectable.

Double diffusion was carried out in plastic petri dishes containing about 10 ml of agar (5–6.0 mm thick) which was made up with 1% agar (Difco) in Veronal buffered 0.2% saline, pH 8.6, with 7% glycine and 0.1% sodium azide.

Results. Table I summarizes the cross reactivity of ribosomes and rRNA from various sources tested against the first two groups of antisera.

The anti-yeast RNA serum produced precipitin lines in agar by double diffusion against yeast RNA and human liver

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TABLE I. Cross Reactivity of Ribosomes and rRNA from Various Sources Tested Against Antiserum to Yeast RNA and Human Liver Ribosomes.[a]

Antisera	Antigens tested				
	Yeast RNA	HLR	RLR	RLrRNA	MOPC70E rRNA
Anti-yeast RNA	+	+	+	+	—
Anti-HLR	—	+	+	+	+

[a] HLR = human liver ribosomes; RLR = rabbit liver ribosomes; and RLrRNA = rabbit liver ribosomal RNA.

ribosomes (HLR) (Fig. 1). This antiserum was also reactive against purified rabbit liver rRNA from another New Zealand white rabbit.

Antiserum produced against human liver ribosomes (HLR) reacted with both human and rabbit ribosomes (Fig. 2). That this cross reactivity was due to common antigenic determinants of the rRNA was demonstrated by the reaction of this antiserum with rabbit liver rRNA as well as mouse myeloma tumor rRNA (from MOPC70E). This antiserum was nonreactive when tested against ribosomal protein from mouse myeloma tumor and rabbit liver.

Table II summarizes the reactivity of antiserum to mouse myeloma ribosomal protein with ribosomes from various sources. The antiserum reacted only with ribosomes from mouse myeloma tumors of Balb/c mice and gave no reaction with whole ribosomes from other mammalian sources. It is evident that specific antiserum to ribosomal proteins has a much more limited cross reactivity than antiserum to whole ribosomes or RNA.

Discussion. These data suggest strongly that the major antigenic determinant responsible for immunologic cross reactivity between ribosomes from various sources is on rRNA and that ribosomal proteins are not responsible for this cross reactivity in spite of their similar acrylamide gel electrophoretic patterns (14).

The reason for this apparent greater cross reactivity of RNA than of proteins between various species may be a very simple one. Using five residues as a somewhat arbitrary optimal antigenic determinant (15, 16) the probability that any particular sequence of 5 residues may appear in a random selection from either a nucleotide pool of RNA or an amino acid pool for proteins may be compared. There are 20^5 possible antigenic determinants five residues long which may occur in a given protein. The probability that any one of these possibilities will repeat itself n times is represented by the expression $(1/20^5)^n$. Similarly there are 4^5 possible antigenic determinants five residues long which may occur in a given molecule of RNA and the probability that one of these will repeat itself is given by the expression $(1/4^5)^n$. Comparison of these two expressions shows that any given antigenic determinant of RNA is $(5^5)^n$ times more likely to repeat itself n times than any given antigenic determinant of a protein. Although one might consider several exceptions to our mathematical assumptions in this model, it serves to point out that RNA is much more limited in its

TABLE II. Reactivity of Antiserum to Mouse Myeloma Ribosomal Protein with Ribosomes from Various Sources.[a]

Antiserum	Antigens tested			
	HLR	RLR	MOPC31C R	MOPC70E R
Anti-MOPC31C-rProtein	—	—	+	+

[a] MOPC31C R, MOPC70E R = whole ribosomes from these myeloma tumors; and rProtein = ribosomal protein.

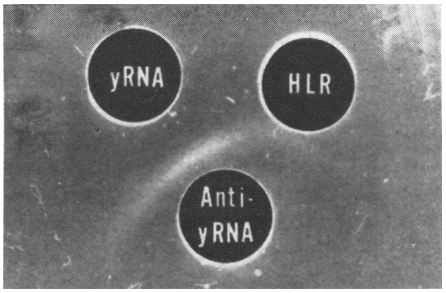

FIG. 1. Double diffusion in Agar: reaction of yeast RNA (yRNA) and human liver ribosomes (HLR) with antiserum to yeast RNA (Anti-yRNA).

possible antigenic determinants simply because it has fewer kinds of residues from which to choose.

Thus, cross reactivity between nucleotides of various sources may be the basis for "autoimmune" type antibodies being produced in such diseases as SLE. The recent report of myxovirus-like particles found in tissues of patients with SLE (17) seems to point even more to the possibility that their "autoimmune" antibody production may be in response to exogenous nucleotide stimulation.

Summary. Rabbits were immunized against yeast RNA, human ribosomes, and mouse myeloma ribosomal protein. Analysis of these antisera by double diffusion in agar against a variety of ribosomes and ribosomal RNAs demonstrated antigenic cross reactivity between ribosomes and isolated rRNA of varied mammalian species including rRNA from the rabbit, but not between ribosomal protein of varied species. Greater interspecies antigenic similarity of RNAs than of proteins appears to be based on a more limited structure of polynucleotides.

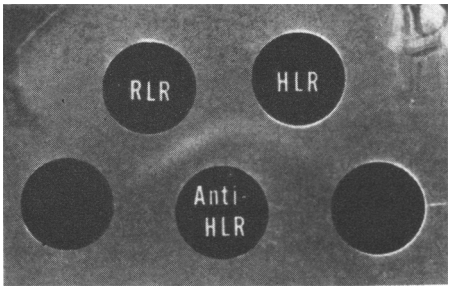

FIG. 2. Double diffusion in Agar: reaction of human liver ribosomes (HLR) and rabbit liver ribosomes (RLR) with antiserum to human liver ribosomes (Anti-HLR).

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