the most primitive among the spiroplasmas now known, those of the *Drosophila* sex-ratio spiroplasmas (*6*) might be considered the most highly evolved. These organisms, apparently lacking a gut phase, may be wholly dependent on transovarial transmission for their survival, although transmission by mouth has been reported in one species (*24*).

We cannot yet compare the ecology of spiroplasmas from insects with those of other arthropods such as ticks. Although two tick genera have been reported to harbor spiroplasmas (*20*), the maintenance cycles of the organisms have not been elucidated. Knowledge of the host-parasite relationships of tick spiroplasmas might provide evidence concerning the existence of alternative vertebrate hosts.

Thus it is clear that a diversity of spiroplasma-insect relationships exist. However, the contamination of plant surfaces by feces or regurgitated gut contents from insects infected with spiroplasmas is an important means of spreading infections in nature. This conclusion is supported by the observation that a spiroplasma found in the bettle, *Melolontha melolontha* in Europe (*25*) is serologically identical to spiroplasmas of group III (*26*). This group of strains includes strain 23-6 of Davis (*27*) and BNR1 of Clark (*12*), both from the tulip tree (*Liriodendron tulipifera*) and OBMG of Clark (*12*) from the southern magnolia (*Magnolia grandiflora*). The name *Spiroplasma floricola* has been proposed for these strains (*28*). Although many spiroplasmas may not be pathogenic to their usual insect hosts, their exceptional stability on various surfaces (*29*) and wide experimental host ranges suggest that some may have application for biological control.

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15. Identification of Hymenoptera was made by S. W. T. Batra and D. L. Vincent, Diptera by F. C. Thompson, all Hemiptera except *R. annulicornis* by T. J. Henry, and *R. annulicornis* by R. Froeschner. *Leptinotarsus decemlineata* were supplied by W. W. Cantelo and G. Cantwell of the USDA Vegetables Laboratory in Beltsville, Md.
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30. All new hosts reported except *R. annulicornis* and *L. decemlineata* were discovered while I was assigned to the Bioenvironmental Bee Laboratory, Beltsville, Md. I thank Mrs. R. B. Henegar for technical assistance.

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Differential Breakdown of Phylogenetically Diverse Ribosomal RNA's Inserted via Liposomes into Mammalian Cells

Abstract. *Liposomes were used to deliver ribosomal RNA's from three different organisms into cultivated mouse plasmacytoma cells. Ribosomal RNA from* Escherichia coli *was degraded intracellularly within 1 hour, whereas mouse and yeast ribosomal RNA's were degraded more slowly. This indicates that cells can discriminate between different ribosomal RNA's.*

Although the regulation of protein synthesis is controlled mainly at the level of messenger RNA (mRNA) synthesis, regulation may also occur at the posttranscriptional level, by modulation either of the initiation of translation (*1*) or of the stability of RNA (*2*). Any method that would allow direct delivery of mRNA into cells and permit a study of the differential processing of RNA molecules in an unmodified environment would facilitate the investigation of posttranscriptional regulatory mechanisms.

In recent years lipid vesicles (liposomes) have been used to deliver biologically active macromolecules into a variety of eukaryotic cells in vitro (*3*). The liposomes protect the sequestered molecules from external agents and can deliver macromolecules to a large number of cells. In particular, liposome-sequestered RNA is protected from the action of ribonuclease (*4*). Translation of globin mRNA (*5*), infectivity of polio RNA (*6*), and association of myosin mRNA with ribosomes (*7*) have been demonstrated in foreign cells into which the appropriate RNA was injected via liposomes. We have shown that a substantial amount of liposome-entrapped RNA can be delivered into cells and that most of the cell-associated RNA is internalized (*8*). We now describe experiments indicating that liposome-delivered ribosomal RNA's (rRNA's) of different phylogenetic origin are degraded at different rates. Because rRNA in vivo is usually bound to proteins, these experiments were not intended as a means of studying the degradation of rRNA in vivo, but rather were performed to assess whether this technology could be used to investigate the putative differential processing of RNA molecules.

Total RNA from *Escherichia coli* A 155 (U^-) was labeled with ^3H or ^{32}P (*4*). Mouse rRNA labeled with ^{32}P was prepared from P3XAg6593 cells (derived from the myeloma MOPC21) grown for 24 hours in Eagle's minimal essential medium containing one-tenth the normal amount of inorganic phosphate and supplemented with [^{32}P]phosphate (10 to 20 μCi/ml). Cells were lysed in a buffer containing 10 m*M* sodium acetate (*p*H 5.1), 100 m*M* NaCl, 1 m*M* EDTA, and 0.5 percent sodium dodecyl sulfate and extracted with a phenol-chloroform mixture (1:1). The total nucleic acid was precipitated with ethanol and repeatedly suspended in 3*M* sodium acetate (*p*H 6.0) to solubilize 4*S* to 5*S* RNA and

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DNA. Polyadenylated (poly A) RNA was eliminated by oligodeoxythymidylate cellulose chromatography (*9*). Yeast rRNA labeled with ^{32}P was extracted from *Saccharomyces cerevisiae* grown in low-phosphate medium in the presence of [^{32}P]phosphate (10 to 20 μCi/ml) (*10*). The 4*S* to 5*S* RNA, DNA, and poly A RNA were eliminated as described for mouse RNA. The specific radioactivities of the rRNA preparations were never less than 10^5 count/min per microgram. The RNA's carrying different labels (at least 100 μg or 10^7 count/min) were sequestered simultaneously in liposomes (13 μmole of lipids) prepared by the reverse-phase evaporation technique (*11*) and containing phosphatidylcholine and phosphatidylserine at a molar ratio of 1:2. Liposomes were purified by chromatography on a column of Sepharose 2B (1 by 50 cm) with phosphate-buffered saline as the elution buffer. The liposomes, which routinely sequestered 20 to 30 percent of the RNA, eluted with the void volume.

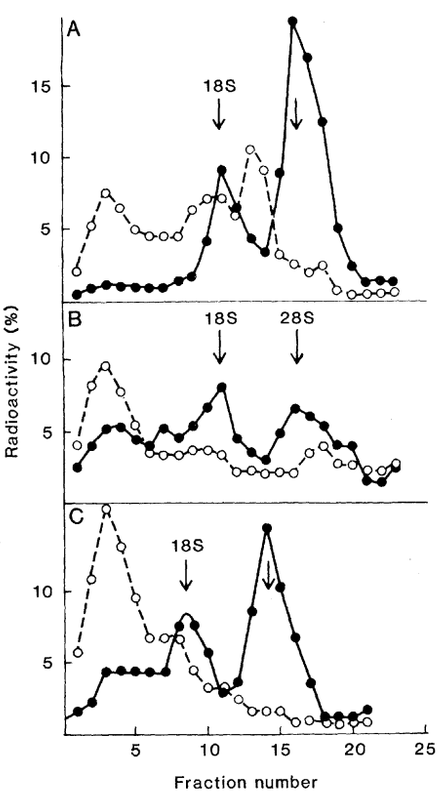

Fig. 1. Differential breakdown of RNA from *E. coli* and from mouse, delivered by liposomes to mouse plasmacytoma cells; (○) ^3H-labeled *E. coli* RNA; (●) ^{32}P-labeled mouse rRNA. (A) RNA extracted from liposomes: ^3H, 15,000 count/min; ^{32}P, 2500 count/min. (B) RNA extracted from cells incubated with liposomes in the absence of actinomycin D: ^3H, 1300 count/min; ^{32}P, 1500 count/min. (C) RNA extracted from cells incubated with liposomes in the presence of actinomycin D: ^3H, 2700 count/min; ^{32}P, 4200 count/min. The radioactivity of each fraction is expressed as a percentage of total radioactivity of each isotope.

To assess whether mouse myeloma P3XAg6593 cells can discriminate between "naked" homologous and a heterologous (*E. coli*) rRNA, liposomes containing the two RNA's carrying different labels were incubated with these cells in serum-free RPMI 1640 medium for 60 minutes (Fig. 1). In one experiment, actinomycin D added at a concentration of 5 μg/ml inhibited more than 95 percent of the endogenous RNA synthesis. After the liposome-cell interaction, the cells were washed four times with RPMI 1640—a procedure that completely frees cells from adhering liposomes (*8*). RNA was extracted from the cell pellets and from the liposomes in the supernatant of the first wash and analyzed on sucrose gradients (*12*). The ^3H-labeled RNA from *E. coli* and the ^{32}P-labeled RNA from mouse were extracted from the liposomes that had not interacted with the cells; their sedimentation profiles were characterized by 16*S* and 23*S* and by 18*S* and 28*S* peaks, respectively (Fig. 1A) and were undistinguishable from the sedimentation profiles of the original RNA's (not shown). We concluded that RNA was not degraded during the process of sequestration.

In the RNA extracted from the cells after interaction with liposomes, the ^{32}P-labeled mouse RNA gave a sedimentation pattern characterized by large 18*S* and 28*S* peaks, whereas the ^3H-labeled *E. coli* RNA had lost its characteristic 16*S* and 23*S* peaks and appeared almost totally degraded (Fig. 1B). When liposome-cell interaction was carried out in the presence of actinomycin D, *E. coli* RNA was totally degraded, with only a small shoulder at 16*S* being evident (Fig. 1C). Conversely, the presence of 18*S* and 28*S* peaks of ^{32}P radioactivity shows that homologous rRNA was not degraded. We conclude that mouse rRNA was preferentially protected from degradation within the cells. Since actinomycin D did not affect the results, it is unlikely that both RNA's were degraded and that the ^{32}P-labeled, homologous rRNA in the cells resulted from a preferential reutilization of the ^{32}P. To eliminate the possibility that residual RNA synthesis (not inhibited by actinomycin D) was responsible for the appearance of ^{32}P in mouse rRNA, *E. coli* RNA preparations labeled with ^{32}P or ^3H were sequestered within the same liposomes and delivered to myeloma cells in the presence of actinomycin D. Both labels were found in populations of RNA molecules that had been completely degraded (not shown).

We investigated the time course of degradation of homologous naked rRNA delivered into P3XAg6593 cells. Mouse ^{32}P-labeled rRNA was sequestered within liposomes together with ^3H-labeled *E. coli* RNA as a positive control for the degradation and reutilization of the label. After 60 minutes of liposome-cell interaction in the absence of actinomycin D, cells were suspended in RPMI 1640 medium at 37°C (time 0). RNA extracted from equal samples of the culture at time 0 and at 3 and 19 hours was analyzed on sucrose gradients. At time 0 (Fig. 2A), *E. coli* RNA, but not mouse rRNA, was degraded, indicating that no liposomes containing intact rRNA were adhering to

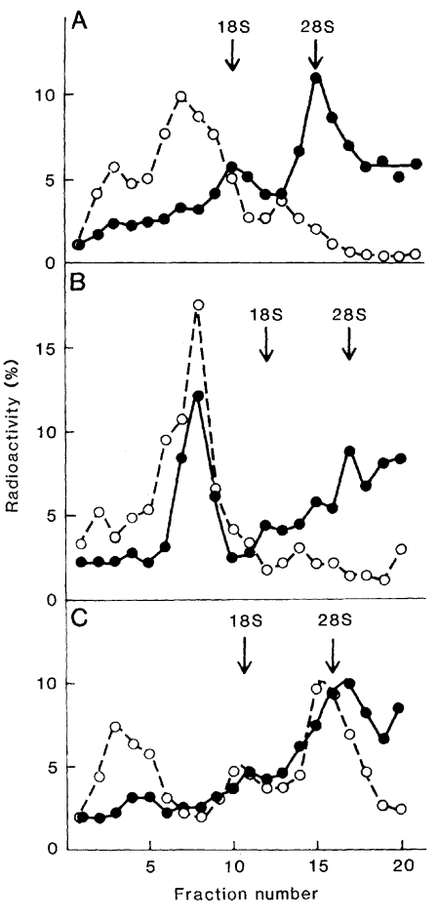

Fig. 2. Kinetics of breakdown of RNA extracted from cells incubated with liposomes in the absence of actinomycin D: (○) ^3H-labeled *E. coli* RNA; (●) ^{32}P-labeled mouse rRNA. (A) RNA extracted from cells immediately after incubation with liposomes: ^3H, 1000 count/min; ^{32}P, 3400 count/min. (B) RNA extracted from cells after 3 hours in culture: ^3H, 200 count/min; ^{32}P, 1800 count/min. (C) RNA extracted from cells after 19 hours in culture: ^3H, 500 count/min; ^{32}P, 2200 count/min. The radioactivity of each fraction is expressed as a percentage of total radioactivity of each isotope.

the cells. The presence of a peak of ^{3}H activity in the 8*S* to 12*S* region may be partly due to incorporation of the label from *E. coli* RNA into mammalian mRNA. After 3 hours (Fig. 2B), little ^{32}P activity was found in 18*S* and 28*S* regions, indicating that homologous rRNA had largely been degraded by this time. Activity resulting from both ^{3}H and ^{32}P in the 8*S* to 12*S* region may indicate their incorporation into host mRNA. After 19 hours (Fig. 2C), all radioactivity showed a sedimentation profile typical of bulk mammalian RNA. We conclude that naked homologous rRNA is mostly degraded in 3 hours and that its radioactivity can be utilized by host cells.

To extend our observations and to determine whether an evolutionarily distant eukaryotic rRNA is protected from rapid degradation in mouse myeloma cells, we used liposomes to deliver ^{32}P-labeled rRNA from yeast into P3XAg6593 cells in the presence of actinomycin D (5 μg/ml); ^{3}H-labeled *E. coli* RNA was also delivered to provide a reference to the previous experiments. RNA was extracted from target cells immediately after liposome-cell incubation (time 0) and after 3 hours of culture in RPMI 1640 in the presence of actinomycin D. At time 0, the yeast rRNA appeared intact. In fact, peaks of ^{32}P activity at 16*S* and 25*S* were present, whereas most of the *E. coli* RNA was degraded (Fig. 3A). In the RNA preparation obtained after 3 hours of culture, the radioactivity due to *E. coli* RNA was greatly reduced and the RNA was totally degraded, while most of the yeast rRNA was not degraded. During the 3 hours of incubation (when endogenous RNA synthesis was effectively blocked by actinomycin D), the total acid-precipitable ^{32}P activity associated with the cells also decreased (by a factor of 3). We have not investigated the mechanism by which this loss of radioactivity occurred.

These experiments show a differential rate of breakdown of eukaryotic and prokaryotic rRNA's delivered into mouse cells by liposomes. Whether the RNA is delivered into the cells by fusion or endocytosis, is not certain; in the latter case most of the RNA would be delivered to the lysosomes (*3*). However, evidence that most of the RNA was inserted into the cytoplasm includes the following: (i) cytochalasin B, an inhibitor of endocytosis, reduces the amount of RNA delivered into the cells by cholesterol-free liposomes by no more than 30 percent (*4*) and (ii) liposome-delivered mRNA can be faithfully translated (*5, 6*). The breakdown of RNA was assessed by sucrose gradient centrifugation, a technique that allows the use of relatively large amounts of RNA to maximize the amount of radioactivity analyzed.

Mouse myeloma cells appeared to degrade liposome-delivered *E. coli* rRNA faster than they degraded eukaryotic rRNA. The more rapid degradation of *E. coli* rRNA may be due to its different pattern of methylation, or cytoplasmic proteins may preferentially bind eukaryotic rRNA.

The rate of degradation of both RNA's under the conditions used is higher than that observed in vivo. In fact, mammalian rRNA has a half-life of about 100 hours or more (*13*), whereas *E. coli* and yeast rRNA's have half-lives of about 20 hours (*14*). This more rapid degradation was very likely the result of the use of naked RNA. The RNA preparations extracted from cells after 3 hours of incubation (Figs. 2B and 3B) contained a large peak of radioactivity due to *E. coli* RNA in the 4*S* to 5*S* region. This is probably attributable to degraded rRNA as well as to transfer RNA (tRNA), which was present in the *E. coli* RNA preparations used. The persistence of tRNA in the cells is consistent with data (*15*) indicating that prokaryotic tRNA can remain biologically active when delivered into cells by cultivation of cells and tRNA together.

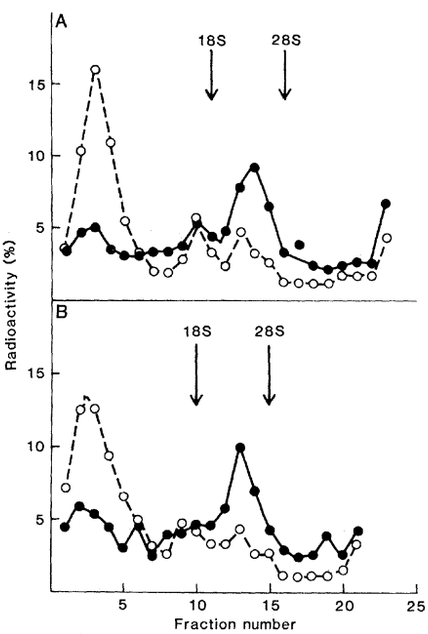

Fig. 3. Kinetics of breakdown of RNA extracted from cells incubated with liposomes in the presence of actinomycin D; (○) ^{3}H-labeled *E. coli* RNA; (●) ^{32}P-labeled yeast rRNA. (A) RNA extracted from cells immediately after incubation with liposomes: ^{3}H, 2600 count/min; ^{32}P, 7700 count/min. (B) RNA extracted from cells after 3 hours in culture: ^{3}H, 1000 count/min; ^{32}P, 2800 count/min. The radioactivity of each fraction is expressed as a percentage of total radioactivity of each isotope.

These data indicate that liposome-mediated RNA delivery into cells constitutes an efficient model system that might be used to study mRNA discrimination by different cell types. The advantage of this model system lies in the ease with which liposomes can be prepared and in the protection from external agents that they provide to the sequestered molecules. Moreover, in contrast to microinjection techniques (*16*), liposomes can deliver their content to a very large number of cells.

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