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Encapsidation of heterologous RNAs by bacteriophage MS2 coat protein

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ABSTRACT

The RNA bacteriophages of *E.coli* specifically encapsidate a single copy of the viral genome in a protein shell composed mainly of 180 molecules of coat protein. Coat protein is also a translational repressor and shuts off viral replicase synthesis by interaction with a RNA stem-loop containing the replicase initiation codon. We wondered whether the translational operator also serves as the viral *pac* site, the signal which mediates the exclusive encapsidation of viral RNA by its interaction with coat protein. To test this idea we measured the ability of *lacZ* RNA fused to the translational operator to be incorporated into virus-like particles formed from coat protein expressed from a plasmid. The results indicate that the operator-*lacZ* RNA is indeed encapsidated and that nucleotide substitutions in the translational operator which reduce the tightness of the coat protein-operator interaction also reduce or abolish encapsidation of the hybrid RNA. When coat protein is expressed in excess compared to the operator-*lacZ* RNA, host RNAs are packaged as well. However, elevation of the level of operator-*lacZ* RNA relative to coat protein results in its selective encapsidation at the expense of cellular RNAs. Our results are consistent with the proposition that this single protein-RNA interaction accounts both for translational repression and viral genome encapsidation.

INTRODUCTION

The virus particle of the RNA bacteriophage MS2 is composed of 180 coat protein monomers, one molecule of the maturase protein, and one molecule of genomic RNA (1). Proper assembly of the virus requires the selective encapsidation of a single copy of the viral plus strand. Minus strands and host nucleic acids are excluded. In addition to its structural role, coat protein has a genetic regulatory function and interacts with a specific stem-loop structure in the viral RNA to translationally repress replicase synthesis (2). It is frequently assumed that this protein-RNA interaction also represents the nucleation event in virus assembly and accounts for the selectivity of RNA packaging. In this view, the translational operator is also the viral packaging signal. Other sequences might not be necessary for packaging specificity. Indeed, coat protein complexed with a synthetic 21 nucleotide RNA containing the translational operator can be incorporated into capsids *in vitro*, but the presence of the operator sequence on a heterologous RNA provides little selective advantage over nonoperator-containing RNA in *in vitro* packaging reactions (3). This leaves open the possibility that other components may be necessary.

We sought to determine whether the 21 nucleotide operator would confer MS2-specific packagability to a heterologous RNA *in vivo*. To do so we took advantage of a two-plasmid expression system in which coat protein expressed from one plasmid translationally represses synthesis of a replicase-β-galactosidase fusion protein expressed from a second plasmid (4). We reasoned that if the translational operator is also a *pac* site, the hybrid replicase-*lacZ* mRNA would be packaged into virus-like particles.

MATERIALS AND METHODS

Plasmid constructions

A two-plasmid system for analysis of coat protein mediated translational repression has been described elsewhere (4). In this system coat protein is expressed from the plasmid called pCT1 (Col-E1 type replication origin) and represses translation of hybrid replicase-*lacZ* mRNAs produced from one of the pRZ series of plasmids (P15A replication origin). Since the pCT1 and pRZ plasmids are members of different incompatibility groups and because they confer resistance to different antibiotics, they may be stably maintained together in the same bacterial host. Members of the pRZT series of plasmids were derived from the pRZ plasmids by the insertion of a BstUI-SalI fragment (containing the $rrnBT_1T_2$ transcriptional terminator) between a DraI site just downstream of *lacZ* and the SalI site. The result is the loss from the pRZ-type plasmids of *lac* operon sequences downstream of lacZ and the introduction of a strong transcriptional terminator. Plasmids pRZ5T, pRZ6T, and pRZ7T differ from one another by nucleotide substitutions in the translational operator (see Figure 1). Plasmid pZT is a translational operator deletion mutant. The pLRZT plasmids resulted from replacement of the *lac* promoter-containing SspI-EcoRI fragment of the pRZT

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plasmids with a similar fragment containing the bacteriophage lambda P_L promoter from pNS3 (5). The general structures of the plasmids used in this study are shown in Figure 1 and the details of their construction are available upon request.

Isolation and characterization of virus-like particles

Bacterial cultures were grown in 500ml of 2×YT medium (6) at 37 degrees. At the desired density (usually $A_{600}=2$) cells were harvested by centrifugation, and the pellet was stored at −20 degrees. Cells were thawed and resuspended in 10 ml of 10mM Tris, pH 7.5, 0.5 mM EDTA and lysozyme was added to a concentration of 0.05 mg/ml. After incubation at room temperature for 20 min. the cells were chilled and sonicated. Cellular debris was removed by centrifugation (20 min, 12,000 rpm) and solid ammonium sulfate was added to the supernatant fraction to 50% of saturation. The precipitated proteins were collected by centrifugation, redissolved in 10 mM Tris, pH 7.5, 100mM NaCl, 0.10 mM $MgSO_4$, 0.01mM EDTA, and applied to a 2.5×45 cm column of Sepharose CL-4B equilibrated in the same buffer. Virus-like particles elute from the column in the same position as authentic virus (4). Fractions containing coat protein were pooled and an equal volume of 20% polyethylene glycol 8000, 1 M NaCl was added to precipitate the particles. After centrifugation at 8,000 rpm for 10 min. the pellet was dissolved in 4.0 ml of 50 mM Tris, pH 7.5, 100 mM NaCl, 5mM EDTA and 2.20 g of CsCl was added and dissolved. This was centrifuged in a Beckman SW55Ti rotor at 40,000 rpm and 4 degrees for 24 hrs. Fractions were collected from the gradient and their densities were determined by refractive index. An equal volume of 20% polyethylene glycol, 1M NaCl was added to each fraction, the precipitate was collected by centrifugation, and redissolved in 0.1 ml of 10mM EDTA. Part of each fraction was subjected to electrophoresis on an SDS/polyacrylamide gel (7) and western blotting (8). Coat protein was visualized using anti-MS2 serum and ^{125}I-protein A. The remaining material from each fraction was extracted twice with phenol/chloroform and nucleic acids were precipitated from the aqueous phase with ethanol. The resulting RNA was fractionated by electrophoresis on agarose gels containing formaldehyde and either stained with methylene blue or blotted to nylon membranes. Alternatively the RNA was denatured in formaldehyde and applied directly to a nylon membrane using a slot-blot apparatus (9). ^{32}P-labeled probes were prepared by the oligo-labeling method (10) using isolated restriction fragments. The *lac*-Z specific probe was obtained by labeling a 294 bp *Hae* II fragment excised from near the 5′ end of the *lac*-Z gene. A probe for 16S rRNA was produced by labeling a 568 bp *Hind* III fragment representing sequences in the 5′-half of the mature 16S rRNA. Pre-16S rRNA was detected using a ^{32}P-labeled *Dra* I fragment conntaining sequences upstream of the mature 16S-encoding sequences in the *rrn*E operon.

RESULTS

The expression system

Translational repression of replicase synthesis in RNA phages is accomplished when coat protein binds a specific stem-loop structure in the viral RNA that contains the replicase translation initiation site. It is frequently assumed that this translational operator also represents the viral *pac* site and is responsible for the specific packaging of viral RNA. If this 21 nucleotide sequence is the major determinant of packaging specificity, we reasoned that it might confer MS2-specific packagability to heterologous RNAs. We tested this idea by asking whether hybrid *lac*Z mRNAs containing the putative *pac* site could be selectively incorporated into capsids *in vivo*. In a previous report we described a two plasmid expression system for the genetic analysis of the translational repressor activity of MS2 coat protein (4). In that system coat protein expressed from one plasmid (pCT1) represses translation of a hybrid replicase-*lac*Z mRNA transcribed from a second plasmid (e.g. pRZ5). Synthesis of β-galactosidase is efficiently repressed when the operator is derived from the wild-type MS2 sequence. Mutation of the operator results in a constititutive phenotype. It was also shown that coat protein expressed from pCT1 assembles into capsids *in vivo*. We took advantage of the existence of these plasmids to determine whether RNAs containing an efficient operator are also substrates for packaging into virus-like particles.

The plasmids used in the present study were modified versions of those previously reported (4). The pRZ family of plasmids contains most of the *lac* operon and should produce transcripts with lengths in excess of 6000 nucleotides. This substantially exceeds the length of MS2 RNA at 3569 nucleotides (11). We worried that our replicase-*lac*Z transcripts might be too long for efficient packaging. Thus, derivatives of the pRZ plasmids were constructed that contain the strong *rho*-independent transcription teminators, T_1T_2, from the *E.coli rrn*B operon inserted just downstream of the *lac*Z translation termination codon (see Methods and Figure 1). This results in transcripts with a predicted length of about 3300 nucleotides. The plasmid called pRZ5T contains the wild-type operator sequence. The related plasmids, pRZ6T and pRZ7T differ from pRZ5T by mutation of important residues of the translational operator sequence (see Figure 1B). These mutations reduce K_a for binding by coat protein *in vitro* by about 100-fold in the case of pRZ6T and by more than 1000-fold in the case of pRZ7T (12). A fourth plasmid, pZT completely deletes the operator. If the operator functions as a *pac* site, pRZ5T transcripts might be expected to be efficiently incorporated into the capsids, while the mutants should be packaged less efficiently.

Density gradient analysis of capsids

The association of RNA with coat protein should increase its buoyant density. Purified MS2 phage particles form a narrow band at 1.45g/cc^3 when sedimented to their buoyant density in CsCl density gradients (see Figure 2A). This compares favorably with the previously reported value of 1.46 g/cc^3 (13). Virus-like particles produced in cells containing pCT1 and the various pRZ derivatives were partially purified by gel-exclusion chromatography on Sepharose CL-4B (4), concentrated by PEG precipitation, and applied to CsCl gradients (see Methods). Gradient fractions were analyzed for the presence of coat protein by SDS-polyacrylamide gel electrophoresis (7) and western blotting (8). Anti-MS2 serum and ^{125}I-protein A were used to visualize the blotted coat protein. The resulting autoradiagrams were scanned with a densitometer for quantitation and the data were plotted as shown in Figure 2A. The distribution of coat protein from strains containing pCT1 and either pRZ5T, pRZ6T, pRZ7T or pZT were determined. The patterns obtained from each were virtually identical and for the sake of simplicity only one of them (pCT1/pRZ5T) is shown. Coat protein forms a broad peak centered at about 1.40 g/cc^3. The higher density compared to that of naked coat protein (about 1.30 g/cc^3) indicates a likely association with RNA, but the breadth of the peak suggests

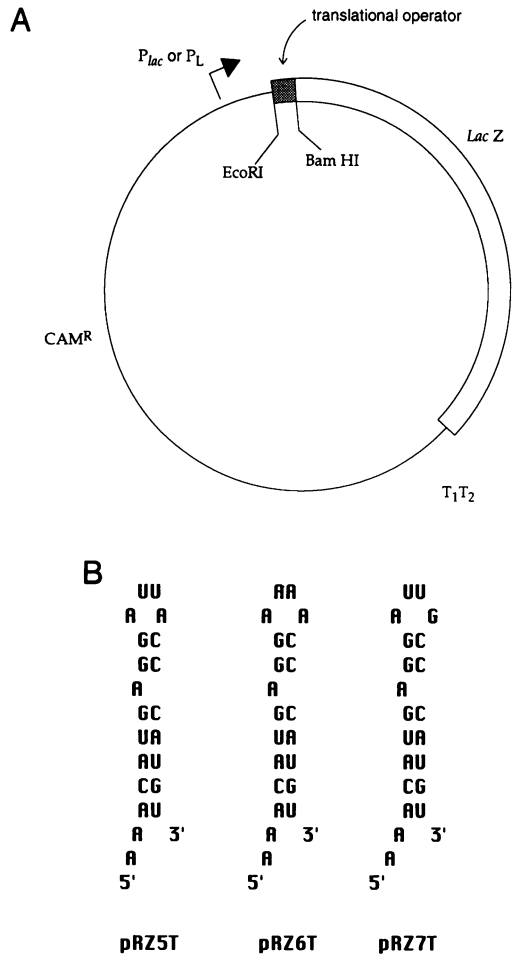

Figure 1. A. The general structure of the pRZ-type plasmids used in this study. Each plasmid of this type is about 10 kb and is characterized by the presence of the wild-type or one of the mutant translational operator sequences shown in B. As described in the text some derivatives use the *lac* promoter and others use the P_L promoter. **B.** Nucleotide sequences and predicted secondary structures of the translational operators.

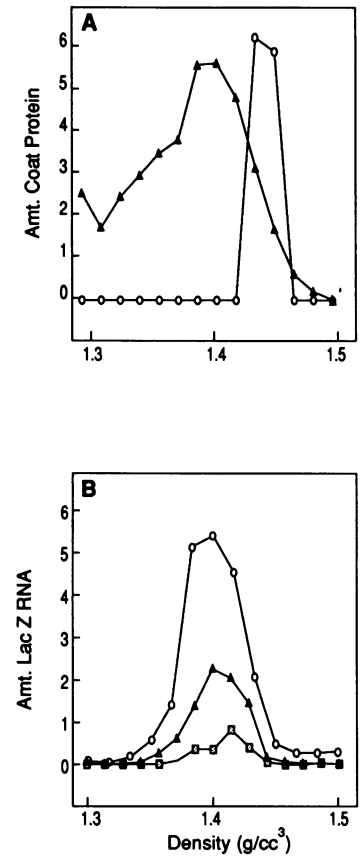

Figure 2. Buoyant density centrifugation of MS2 virus and virus-like particles in CsCl gradients. Panel A shows the coat protein profiles of viral (circles) and pCT1-expressed coat protein (triangles) prepared as described in Methods. Panel B shows the distribution of *lacZ*-specific RNAs in identical gradients containing extracts from cells bearing pCT1 and either pRZ5 (circles), pRZ6 (triangles), or pRZ7 (squares). The amounts of coat protein and *lacZ* RNA are expressed in arbitrary units.

substantial heterogeneity of RNA content. These results are reminiscent of those obtained with the defective particles produced by MS2 mutants that fail to synthesize maturase (13). Those particles also became broadly distributed in CsCl gradients with densities lower than observed with intact virus. In that case it was shown that the heterogeneous behavior resulted from the RNase sensitivity of maturase-defective virus particles. Since the particles we have produced also contain no maturase, they may be similarly susceptible to RNase. On the other hand, heterogeneity may result from the packaging of heterogenous RNAs or RNAs already degraded to varying degrees.

Packaging of lacZ-specific RNA

To assess the RNA content of these particles nucleic acids were extracted from gradient fractions with phenol-chloroform, concentrated by ethanol precipitation, denatured with formaldehyde, and applied to nylon filters using a slot-blot apparatus (9). The filter was incubated in a hybridization reaction using a *LacZ*-specific probe representing sequences near the 5′ end of the *LacZ* gene, and the resulting autoradiograph was scanned with a densitometer. The *LacZ* RNA CsCl gradient profile for the strain containing pRZ5T peaks in the same position as the profile for coat protein, suggesting that the RNA is in fact associated with capsids (Figure 2B). *LacZ* RNA from pRZ6T was packaged less efficiently. We estimate a 2.5-fold reduction in packaging of this species. *LacZ* RNAs from pRZ7T and from pRZT, the operator deletion mutant, were incorporated into virus-like particles about 10-fold less efficiently than wild-type. The failure of pRZ6T, pRZ7T and pZT RNAs to be packaged was not the result of reduced expression since cells containing these plasmids produce nearly identical amounts of *LacZ*-specific RNA when analyzed by Northern blots of total cellular RNA using *LacZ* probe (results not shown). The RNAs present in total RNA and in CsCl gradient purified virus-like particles were also subjected to Northern-blot analysis (14) using the same *Lac* gene-specific probe (see Figure 3). Note, however, that *Lac*-specific RNAs are mostly degraded to species smaller than the 3000 nucleotides expected of full-length transcripts. A species of about 500 nucleotides predominates. This is consistent with previous observations that demonstrated the rapid degradation of *Lac* mRNA and the accumulation of a 500 nucleotide species (15). We do not know whether these RNAs are degraded before or after their uptake into virus-like particles.

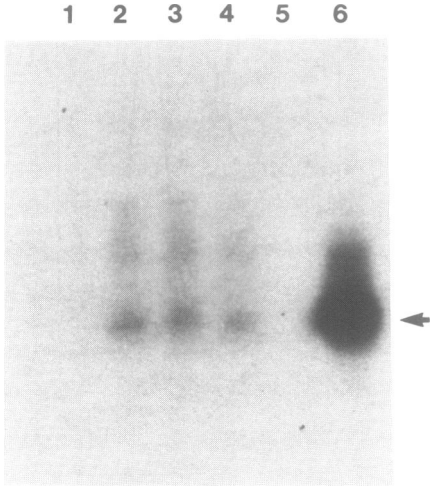

Figure 3. Northern-blot analysis of *lacZ*-specific RNA in total RNA from *E.coli* containing pCT1 (lane 1), pCT1 and pRZ5 (lane 2), pCT1 and pRZ5T (lane 3), or pCT1 and pZT (lane 4), and capsid RNA from *E.coli* bearing pCT1 (lane 5), or pCT1 and pRZ5T (lane 6). The arrow indicates a molecular size of about 490 nucleotides.

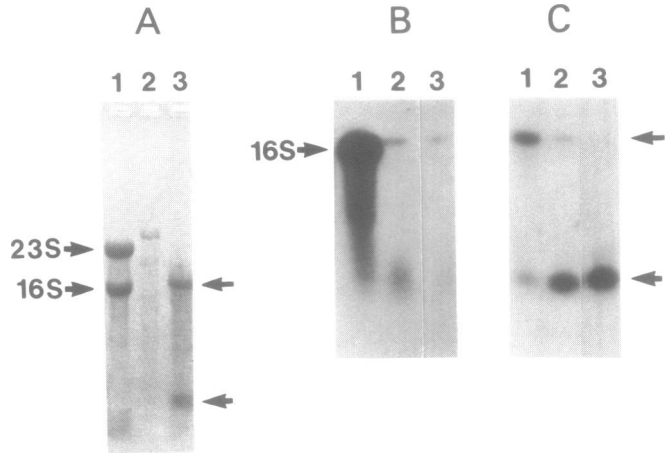

Figure 4. A. A methylene blue-stained agarose gel of total *E.coli* RNA (lane 1), MS2 RNA (lane 2), and RNA extracted from virus-like particles from a strain containing pRZ5 and pCT1. B. Northern-blot analysis of total *E.coli* RNA (lane 1), Capsid RNA from a strain containing pCT1 and pRZ5 (lane 2), and capsid RNA from a strain containing pCT1 (lane 3). The probe is derived from sequences encoding mature 16S rRNA. C. The same samples as in B probed with pre-16S rRNA probe. The unlabeled arrows indicate positions corresponding to approximate molecular sizes of 1800 and 200 nucleotides.

Packaging of host RNAs

In spite of the failures of pRZ6T, pRZ7T and pZT *Lac*Z RNAs to be efficiently packaged, the density profiles of capsids isolated from all three strains are similar, suggesting they contain similar amounts of RNA. This raised the possibility that cellular RNAs were being incorporated. To analyze their total RNA content nucleic acids were extracted from CsCl gradient purified virus-like particles and fractionated by electrophoresis in agarose gels containing formaldehyde (16). RNA was visualized by staining the gel with methylene blue. Figure 4A indicates that although the capsids contain a heterogenous population of RNAs, there are two especially prominant species corresponding to approximate sizes of 1800 and 200 nucleotides. These species are clearly enriched in virus-like particles since they are not visible in total RNA extracted directly from cells (Figure 4A, lane 1).

What is the identity of these RNAs? In a computer search of known *E.coli* sequences, Romaniuk, *et al.* previously noted the presence of sequences very similar to the MS2 translational operator within some ribosomal RNA precursors (12). The ribosomal RNAs of *E.coli* are synthesized from seven operons (see reference 17 for a review). They are produced initially as large precursor transcripts which contain sequences for all the rRNAs. The precursors are processed by a series of endonucleolytic cleavage events which release the mature rRNA species and the intervening spacer RNAs. Transcripts from rrnE and rrnH contain a potential operator-like structure near their 5′ ends, upstream of the 16S rRNA sequence. A third candidate lies within the spacer separating 16S and 23S sequences in rrnB. None of the potential operator-like structures is found in mature rRNAs. These observations raised the possibility that specific rRNA precursors might be capable of encapsidation by MS2 coat protein. To test this possibility various packaged RNAs isolated from CsCl gradient fractions were subjected to Northern-blot analysis using probes from two different portions of the *rrn*E operon. Figure 4B shows the pattern obtained when the blot is hybridized to a ^{32}P-lableled restriction fragment representing a portion of the mature 16S rRNA sequence. This results in hybridization to the 16S rRNA present in total RNA (lane 1 of Figure 4B). In the RNAs extracted from virus-like particles the probe predominantly detects a RNA species slightly larger than 16S rRNA corresponding to the 1800 nucleotide RNA detected by methylene blue staining. When this same blot is stripped of the 16S rRNA probe and rehybridized with a probe representing the 5′ precursor region upstream of the mature 16S sequence, a RNA of about 200 nucleotides is observed in addition to the 1800 nucleotide species (see Figure 4C). The predicted size of the 5′ fragment cleaved from the precursor RNA is 173 nucleotides, roughly consistent with the size of the fragment we obtain. Hybridization is observed even under conditions of relatively high stringency (i.e. washing in 15mM NaCl, 0.75mM sodium citrate at 65 degrees). These results, therefore, suggest that under the conditions of this experiment plasmid-produced coat protein encapsidates host RNAs, including species derived from rRNA precursors.

Increasing the level of operator containing lacZ RNA

It has been estimated that the MS2 infected cell produces at least 10,000 copies of the viral genome (1). Given the relatively low level of β-galactosidase expression from pRZ5 (4) we suspected that coat protein is synthesized in substantial excess relative to the hybrid replicase-lacZ mRNA. This could account for the packaging of cellular RNAs, and caused us to wonder whether higher level expression of replicase-*lacZ* mRNA relative to coat protein might allow it to be packaged more selectively at the expense of cellular RNAs. To accomplish this, several additional plasmids were constructed that place replicase-*lacZ* and various operator mutants under control of the strong P_L promoter from phage lambda (see Figure 1). These plasmids are analogues of those already described above and are called pLRZ5T (wild-type operator), pLRZ7T (operator point mutant), and pLRZT (operator deletion). Analysis of the *lacZ*-specific RNAs produced in cells containing these plasmids showed that they are produced

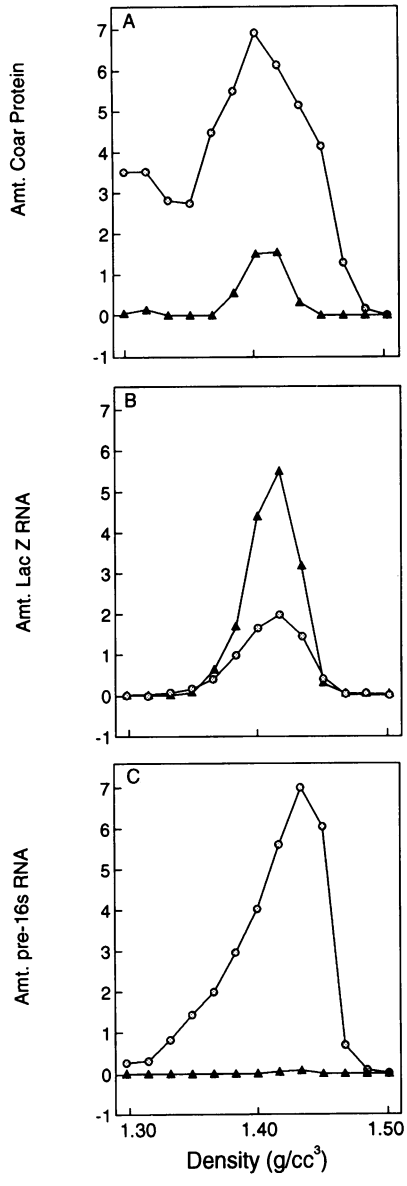

Figure 5. The effects of increasing the ratio of replicase-*lacZ* RNA to coat protein. **A.** CsCl gradient profiles of coat proteins produced from pCT1 at high constitutive levels (open circles) and under partially repressed conditions (closed triangles). **B.** The distribution and amounts of *lacZ*-specific RNA in the same samples as in A. **C.** The distribution of pre-16S rRNA sequences in the same samples as in A and B. In all cases the amounts of coat protein and *lacZ* RNA are expressed in arbitrary units.

at least 10 to 20-fold more abundantly than from plasmids like pRZ5T.

We also constructed a derivative of CSH41 F^- (we call it GGP1) by mating with strain XL1-blue (18). This resulted in the transfer of a F-factor which contains a gene for the $lacI^q$ superrepressor, and permitted us to control the level of coat protein synthesis in GGP1(pCT1) cells by adjustments in the concentration of isopropyl-β-D-thiogalactoside (IPTG), an inducer of the *lac* operon. Thus, we were able to independently alter the amount of coat protein relative to the hybrid replicase-β-galactosidase mRNA.

In the experiment shown in Figure 5A constitutive production of coat protein in CSH41F^-(pCT1/pRZ5T) cells was compared

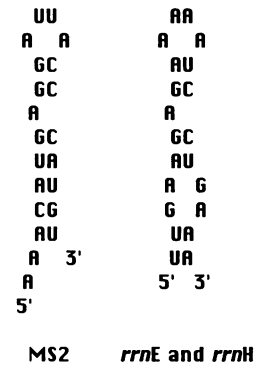

Figure 6. Similarity of the MS2 translational operator to a potential stem loop structure near the 5′ end of precurosor ribosomal RNA.

to the amounts synthesized in GGP1(pCT1/pLRZ5T) cells induced with a low level of IPTG. In the latter strain coat protein synthesis is reduced due to partial repression of the *lac* promoter while synthesis of the hybrid replicase-*lacZ* RNA is elevated due to its being under control of the strong P_L promoter. We estimate that under these conditions coat protein is reduced 12-fold in amount compared to constitutive levels. This reduction in coat protein levels resulted in a narrower distribution of coat protein in the CsCl gradient, but the peak was still centered at about 1.40 g/cc. This, combined with the increased level of replicase-*lacZ* RNA, resulted in increased specificity of packaging of *lacZ*-specific RNA. Figure 5B shows the distribution of *lacZ* RNAs in the same gradient shown in Figure 5A. Even though GGP1(pCT1/pLRZ5T) produces 12-fold less coat protein, it packages about 2-fold more replicase-*lacZ* RNA than does CSH41F^-(pCT1/pRZ5T). Thus, the average *lacZ* RNA content of the particles increases about 25-fold. The effect of these manipulations on encapsidation of host RNA is shown in Figure 5C where the distribution of pre-16S rRNA-hybidizing RNAs in the gradient is displayed. Although these results do not permit us to determine the precise ratio of coat protein to any specific RNA species, it is clear that the combined effect of elevated amounts of replicase-*lacZ* RNA and decreased quantities of coat protein is the dramatically reduced incorporation of this host RNA into capsids.

DISCUSSION

The results reported here confirm that, in addition to its role as a genetic regulatory element, the coat protein binding site on MS2 RNA can function as a *pac* signal. Its presence causes a heterologous RNA to be incorporated into virus-like particles, and mutation or deletion of the binding site dramatically reduces packaging efficiency. These results are consistent with the idea that the translational operator is also the viral *pac* site.

The distribution of the virus-like particles in CsCl gradients suggests substantial heterogeneity of nucleic acid content, and this is born out by electrophoretic analysis of RNA extracted from the particles. This heterogeneity probably reflects the uptake of a mixed population of RNAs. The particles may also be subject to RNase digestion, an additional potential cause of heterogeneity. Such RNase sensitivity has been observed with maturase-defective mutants of MS2 (13). Of course, our plasmid-produced particles also lack maturase.

Why do these capsids contain such large quantities of host RNA? The packaging of virus-specific RNA probably requires non-specific interactions in addition to the specifc binding of the 21-mer stem-loop. In fact a lysine/arginine-rich patch has been observed on the surface of coat protein facing the virus interior (19). This may provide a basis for weak interaction with random RNA sequences. It has long been known that capsid formation *in vitro* can be promoted with non-specific RNAs such as that of tobacco mosaic virus, or even with non-nucleic acid polyanions such as poyvinylsulfate (20). Given that in our system coat protein is probably expressed in excess over the hybrid replicase-*lac*Z RNA it is not surprising that these capsids contain other RNAs. Moreover, the results shown in Figure 4 suggest that the major RNA species that accumulates inside virus-like shells is a precursor form of 16S ribosomal RNA which contains 5′ sequences that are normally absent from mature 16S RNA. Interestingly, inspection of the sequences of the seven ribosomal RNA operons of *E.coli* reveals that several of them contain operator-like sequences within this 5′ region (12). The similarity of these sequences to the *bona fide* operator in shown in Figure 6. This raises the possibility that these species are preferentially packaged because of the presence of a stem-loop structure that mimics the MS2 translational operator. The abundance of ribosomal RNA transcripts in actively growing cells, where coat protein is being produced in excess over the hybrid replicase-*lac*Z RNA, could account for the selective packaging of an RNA containing a relatively poor operator. Also it is possible that some unpredicted RNA secondary structure partly interferes with the proper formation of the operator structure in *lac*Z RNA. We emphasize that we do not know whether the pre-16S rRNA and *lac*Z RNAs are the only species packaged in these experiments, although this assertion is consistent with our results. Clearly, however, the ability of these two RNAs to compete for encapsidation is dramatically altered with changing coat protein:*lac*Z RNA ratios. These results illustrate that the selectivity of protein-nucleic acid interactions depends not only on the binding affinities of competing species, but also on their relative concentrations.

We do not know how to explain why operator-containing *lac* RNAs were not packaged *in vitro* (3) with the level of specificity we have observed *in vivo*. However, it is obvious that solution conditions *in vitro* seldom mimic perfectly those encountered *in vivo*. It should also be noted that we do not know the precise concentrations or molar ratios of coat protein and *lac* RNA in our experiments and can't compare them with the *in vitro* experiments in this respect. We also wonder whether specificity of encapsidation is somehow aided by nucleation of coat protein on nascent RNAs during their synthesis.

Although our results show that the translational operator can function as a *pac* site for a heterologous RNA, they do not imply that it is necessarily the sole element required for efficient, specific packaging of viral RNAs in the infected cell. We can't yet rule out the possibility that other sequence or structural elements play a role in packaging. We can think of at least three possibilities: 1. A potential binding site for coat protein has been identified in the MS2 maturase sequence on the basis of structural homology to the translational operator (12). We wonder whether the presence of a second operator-like structure permits cooperative RNA binding, resulting in nucleation of RNA encapsidation at lower RNA concentrations than would be possible with a single site. 2. It should also be noted that the maturase protein interacts specifically with viral RNA at two sites (21) and may, therefore, play a facilitating role in packaging, perhaps by condensing the RNA. Any packaging function performed by maturase must be a secondary one, however, since amber mutants of MS2 defective in maturase synthesis are capable of specific packaging of viral RNA (13). 3. Non-specifc aspects of RNA structure might also play a role. For example, the extensively folded nature of MS2 RNA (5) may make it especially suitable for uptake into the confines of a small capsid. Additional work will be required to determine whether the translational operator is the sole determinant of selective packaging of genomic RNA by bacteriophage MS2.

ACKNOWLEDGEMENT

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