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Control of translational repression by protein–protein interactions

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ABSTRACT

The coat protein of the RNA bacteriophage MS2 is a translational repressor and interacts with a specific RNA stem-loop to inhibit translation of the viral replicase gene. As part of an effort to dissect genetically its RNA binding function, mutations were identified in the coat protein sequence that suppress mutational defects in the translational operator. Each of the mutants displayed a super-repressor phenotype, repressing translation from the wild-type and a variety of mutant operators better than did the wild-type coat protein. At least one mutant probably binds RNA more tightly than wild-type. The other mutants, however, were defective for assembly of virus-like particles, and self-associated predominantly as dimers. It is proposed that this assembly defect accounts for their super-repressor characteristics, since failure to assemble into virus-like particles elevates the effective concentration of repressor dimers. This hypothesis is supported by the observation that deletion of thirteen amino acids known to be important for assembly of dimers into capsids also resulted in the same assembly defect and in super-repressor activity. A second class of assembly defects is also described. Deletion of two amino acids from the C-terminus of coat protein resulted in failure to form capsids, most of the coat protein having the apparent molecular weight expected of trimers. This mutant (dl-8) was competely defective for repressor activity, probably because of an inability to form dimers. These results point out the inter-dependence of the structural and regulatory functions of coat protein.

INTRODUCTION

The RNA bacteriophage, MS2, is composed of 180 molecules of coat protein, one molecule of maturation protein, and one copy of the single-stranded RNA genome. Coat protein is multi-functional. As the major structural protein of the virus, it must engage in the protein-protein interactions that form the virus shell and in the protein-RNA interactions that account for encapsidation of viral RNA. Coat protein also has a genetic regulatory function. The same site-specific interaction of coat protein with MS2 RNA that probably initiates encapsidation of the genome is also responsible for translational repression of viral replicase synthesis (1). It has been shown that the active repressor is a dimer and that one RNA operator molecule is bound by a repressor dimer at saturation (2). Furthermore, at low protein concentrations and low pH, dimers predominate and can serve as precursors to capsids (2). However, the molecular basis of the transition from dimeric repressors to viral particles is poorly understood.

Recently, the three-dimensional structure of the intact MS2 virus was determined at 3.3A resolution (3). Compact coat protein dimers were a prominent feature of the icosahedral T=3 structure, and probably represent the basic building block of the viral shell. Unfortunately, the RNA component was not visible in the electron density map, so that the nature of protein-RNA interactions was not revealed. However, it was possible to identify regions of the protomer which might be critical for dimer stabilization and for the higher order interactions that result in the assembly of dimers into virus particles.

Recently the construction of a two-plasmid system for genetic analysis of the translational repressor activity of coat protein was described (4). In this system coat protein expressed from one plasmid represses synthesis of a replicase-β-galactosidase fusion protein encoded on a second plasmid, permitting the use of simple genetic screening methods for the isolation of coat mutants with altered repressor properties. Since the plasmid-produced coat protein assembles into virus-like particles, it is also possible to monitor the effects of mutations on virus assembly and coat protein dimerization. As part of a study to define amino acid residues important for translational repression, we used this system to isolate and characterize coat protein mutants that suppress the effects of mutations in the translational operator. We reasoned that these suppressors of operator constitutive (O^c) mutations might help identify the RNA binding site. Here we describe the results of these experiments, and the unexpected observation that most of the mutants seem to aquire their suppressor functions not by virtue of new RNA contacts, but by inhibition of protein-protein interactions necessary for virus assembly. This indicates that the structural and genetic regulatory roles of coat protein are inter-related.

It had been shown previously that amino acid residues at the C-terminus are necessary for incorporation of coat protein into viral shells *in vitro* (5). In experiments described below, we confirmed this observation by deletion of nucleotides encoding

the C-terminal two amino acids of coat protein. This mutation resulted in the expected defect in virus assembly, but also caused loss of translational repression. These effects may identify an additional set of protein-protein contacts important both for virus assembly and formation of the RNA binding site.

MATERIALS AND METHODS

Construction of replicase-lacZ fusions possessing mutant translational operators

Construction of the pRZ type plasmids has been described before (4). Plasmid pRZ5 is the prototype and consists of a synthetic version of the wild-type MS2 translational operator fused to a deletion mutant of *lacZ* lacking its initiation codon. The site for translation initiation is supplied by the synthetic operator sequence, and places synthesis of the replicase-β-galactosidase fusion protein under translational control of coat protein. The operator is bounded by *Eco*RI and *Bam*HI sites, facilitating the replacement of the wild-type operator with synthetic sequence variants. The plasmids called pRZ6, pRZ9, and pRZ10 differ from pRZ5 by nucleotide substitutions at the −5 position (see Figure 1). This site was previously shown to be important for binding of coat protein (6). Transcription of the hybrid gene is driven by the *E. coli lac* promoter on a plasmid that also contains a p15A origin of replciation and a chloramphenicol resistance determinant.

Mutagenesis of the coat protein sequence

A collection of mutations in the MS2 coat gene was produced by the method of Myers, et al. (7). The coat sequence had been previously cloned into pUC119 to give pCT119 (4). This plasmid contains a M13 origin of replication for the production of single-stranded DNA useful for mutagenesis and nucleotide sequence determination. JV30 cells containing pCT119 were infected with the helper phage M13KO7 and single stranded plasmid DNA was isolated (8). This was treated with nitrous acid, formic acid, or hydrazine for various times as described (7). After mutagenesis the complementary strand was synthesized with AMV reverse transcriptase primed with the M13 universal sequencing primer. The coat sequence was then excised at the *Hind*III and *Kpn*I sites that flank the coat coding region in pCT119 and was recloned into pUC119. In this way the isolation of mutations in plasmid sequences was avoided. Mutant libraries were constructed in the strain DH5-alpha using DNA treated with each mutagen under conditions where about 10% of colonies gave a repressor-defective phenotype (i.e. blue colonies on X-gal plates) when tested against pRZ5 (the wild-type operator). Each library consisted of about four thousand independent transformants. These colonies were removed from plates by scraping and plasmid DNA was isolated. To isolate suppressors of the O^c defect of pRZ6 (see Figure 1), DNA from each library was introduced by transformation into a F^- derivative of strain CSH41 (9) containing pRZ6 and plated at a density of several hundred colonies per plate on LB medium containing X-gal, ampicillin, and chloramphenicol. The next day colonies displaying a white phenotype were picked for further analysis. Thirteen clones were subjected to DNA sequence analysis and among these, four different mutations were found.

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pRZ5    AAACATGAGGATTACCCATGT
pRZ6    AAACATGAGGAAAACCCATGT
pRZ9    AAACATGAGGATCACCCATGT
pRZ10   AAACATGAGGATGACCCATGT
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Figure 1. The nucleotide sequences of the translational operators of the pRZ plasmids used in this study. Asterisks indicate the sites of nucleotide substitutions relative to the wild-type sequence (pRZ5).

Assays of β-galactosidase activity

The β-galactosidase activities expressed by the various strains were determined by methods described by Miller using ONPG (9).

Chromatographic analysis of coat protein

Fifty ml cultures of CSH41F⁻ containing pRZ6 and pCT119 or one of the mutant derivatives of pCT119 were grown to $A_{600}=2$ and the cells were collected by centrifugation. The cell pellet was resuspended in 10ml of 10mM Tris, 1mM EDTA, pH 7.4 and subjected to six 15 sec. bursts with a sonicator at full power on ice, care being taken to avoid heating of the sample. Debris was removed by centrifugation at 10,000 rpm for 15 min., and proteins were precipitated from the supernatant fraction with ammonium sulfate at 50% of saturation. After collecting the precipitate by centrifugation, the pellet was dissolved in 1 ml column buffer (100mM NaCl, 10mM Tris, 0.1mM $MgSO_4$, 0.01mM EDTA, pH 7.4) and applied to a 2.5×45 cm column of Sepharose CL-4B. Five ml fractions were collected. The presence of coat protein was determined by SDS polyacrylamide gel electrophoresis of a portion of each fraction. Coat protein was visualized by western blot analysis (10) using anti-MS2 serum and ^{125}I-protein A and quantitated with the use of a scanning densitometer. For further analysis of low molecular weight components, proteins from the appropriate fractions of the Sepharose CL-4B column were concentrated by ammonium sulfate precipitation (50% of saturation), redissolved in the same column buffer and applied to a 1.5×45 cm column of Sephadex G75. Two ml fractions were colected and again coat protein was detected by western blotting. The molecular sizes of the various coat protein variants were assessed by comparison to the elution positions of bovine serum albumin, ovalbumin, carbonic anhydrase, and lysozyme.

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GTGCCTAAAgtggcaacccagactgttggtggtgtagagcttcctgtagccgcaTGGCGTTCG
val pro lys val ala thr gln thr val gly gly val glu leu pro val ala ala trp arg ser

GTGCCTAAAggcgccTGGCGTTCG
val pro lys gly ala trp arg ser
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Figure 2. Construction of the dlFG mutation. The upper line shows the coat sequence in the vicinity of the FG loop. Lower case letters indicate the 39 nucleotides encoding the loop and brackets define the 33 nucleotides to be deleted. The lower line shows the sequence of the dlFG mutant constructed by site-directed mutagenesis (see Methods for details). In addition to the deletion, two nucleotide substitutions were introduced as indicated by the asterisks.

Site-directed mutagenesis

Deletion of the so-called FG loop sequence (3) was accomplished by the method of Kunkel, et al. (11) using the mutagenic oligonucleotide, 5'-TACGAACGCCAGGCGCCTTTAGGC-ACCT-3'. The object was to delete the 15 amino acid loop connecting the F and G β-strands, replacing it with two residues capable of forming a β-turn. The resulting sequence changes are shown in Figure 2. To facilitate screening, the mutation was designed to create a *Hae*II site at the deletion junction. The structure of the resulting dl-FG mutant was confirmed by DNA sequence analysis.

Construction of the dl-8 mutation

The plasmid pUCter3 was constructed specifically for the creation of *Bal*31 deletions within protein coding regions. It was derived from pUC18 by the insertion into the polylinker between the *Sma*I and *Eco*RI sites of synthetic DNA containing adjacent translation terminators in all three reading frames. The oligonucleotide reconstructed the *Sma*I site, but eliminated *Eco*RI, repacing it with *Bam*HI (see Figure 3). When a *Bal*31 generated blunt end is joined to the plasmid at the *Sma*I site any open reading frame in the cloned DNA encounters a translation terminator almost immediately. To construct deletions in the C-terminal coding sequences of coat, pCT119 was cleaved at a *Kpn*I site near the 3' end of the coding region, and treated with *Bal*31 exonuclease. After inactivation of the exonuclease by phenol/chloroform extraction, the DNA was digested with *Hind*III which cuts 5' of the coding region. The products were fractionated by agarose gel electrophoresis and fragments of appropriate size were recovered from the gel and cloned into pUCter3 between the *Hind*III and *Sma*I sites. DNA sequence analysis showed that the mutant called dl-8 was the least extensive of the deletions that extended into the coding region, and resulted in the loss of the C-terminal ile-tyr dipeptide.

RESULTS

Screening for coat protein mutants that suppress O^c *mutations*

Each of the pRZ plasmids produces replicase-β-galactosidase fusion enzymes under control of the replicase translational operator. Plasmid pRZ6 contains a mutation in the operator that prevents efficient translational repression by wild-type coat protein. The sequences of the wild-type and several mutant operators are shown in Figure 1. A library of mutations in the coat sequence was screened for mutants that aquired the ability to suppress the operator constitutive phenotype of pRZ6 and produce white or pale blue colonies on plates containing the chromogenic dye X-gal (see Methods). DNA sequence analysis identified four such mutations. The entire coat DNA sequence was determined in all cases and each mutant was shown to result from a single nucleotide substitution. The specific nucleotide changes and the resulting amino acid substitutions are listed in Table I. The mutations were originally designated by numbers (621, 645, 647, and 653), but are hereafter identified by a shorthand nomenclature that identifies the specific amino acid substitution. For example, V29I is a replacement of valine at position 29 with isoleucine. Most of the mutations were isolated more than once. Note that one of the mutants, C101R, has been described previously as part of a series of site-directed mutations constructed to assess the role of cysteine residues (12). It was isolated again independently in this study.

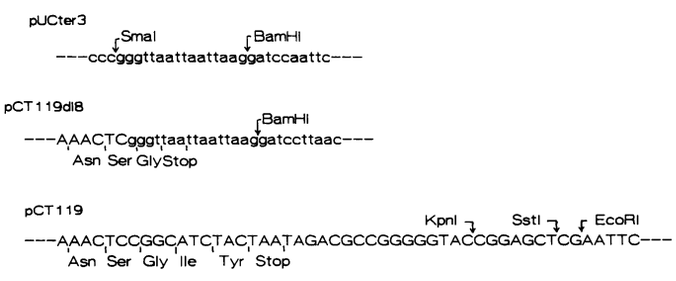

Figure 3. Construction of the C-terminal deletion mutant, dl-8. The two parental plasmids, pUCter3 (shown in lower case) and pCT1 (upper case) were recombined as shown by ligating the blunt end generated by *Bal* 31 treatment of the 3' end of the coat gene to the *Sma* I site of pUCter3 (see Methods for details).

In vivo repressor activities of the mutants

Each pCT119 mutant was introduced into a strain containing either pRZ5 (wild-type operator), or pRZ6, pRZ9, or pRZ10 (mutant operators, see Figure 1. This collection of pRZ plasmids includes all nucleotide substitutions at the critical −5 position. The ability of each of the coat mutants to repress β-galactosidase synthesis was determined by solution assay using ONPG (9). The results are tabulated in Table II. None of the coat variants loses its ability to bind the wild-type operator. In fact, all four coat mutants exhibit a super-repressor phenotype, repressing β-galactosidase synthesis from pRZ5 better than wild-type coat protein. In this experiment coat protein repressed pRZ5 about 50-fold, but each of the four coat muants repressed at levels approaching or exceeding 200-fold. Each coat mutant repressed pRZ6, the plasmid used in their isolation, on the order of 15 to 20-fold, compared to the 5-fold repression conferred by wild-type. As summarized in Table II, each of the mutant coat proteins is a better repressor of all the variant operators tested, and none of them shows any preference for repression of the mutant operator used in their isolation. Consequently, we will hereafter refer to the super-repressor phenotype of these mutations.

Self-association properties of the coat mutants

The elution behavior of variant coat proteins on columns of the gel exclusion chromatography matrix, Sepharose CL-4B is diagnostic of their abilities to assemble into virus-like particles. It has already been reported that plasmid-produced coat protein co-elutes with virus and forms typical capsids as visualized by electron microscopy (4). Mutants which lose the ability to assemble into capsids exhibit different elution profiles. The Sepharose CL-4B profiles for wild-type coat protein and each of the super-repressor mutants are shown in Figure 4. The wild-

Table I. The nucleotide and amino acid substitutions of the various mutants described in the text.

Mutation	Nucleotide Substitution	Amino Acid Substitution
621 (V29I)	GUC to AUC	Val29 to Ile
645 (L77P)	CUU to CCU	Leu77 to pro
647 (W82R)	UGG to CGG	Trp82 to Arg
653 (C101R)	UGC to CGC	Cys101 to Arg

type protein eluted at the same position as MS2, indicating the formation of virus-like particles. This was also true of the mutant called V29I. The other three mutants (L77P, W82R, and C101R) showed a different behavior. Each of these mutant coat proteins was distributed into two major peaks. One was usually a broad peak and eluted only a little later than capsids. The other eluted at a position corresponding to a much lower molecular size. When the material from this second peak was concentrated and applied to a column of Sephadex G75 (see Figure 5), it co-eluted, in each case, with a carbonic anhydrase standard (molecular weight 29,000) suggesting the formation of coat protein dimers (molecular weight 27,400).

Construction and characterization of the FG loop deletion mutant

The failure of the V29I, L77P, and C101R mutants to form capsids may provide an explanation for their superrepressor phenotypes. Once formed, capsids are unable to bind RNA (13). Therefore, any mutation preventing the assembly of coat protein dimers into virus-like partlcles should result in an elevated intracellular concentration of the active repressor species, and lead to increased repression. Inspection of the 3-D structure of MS2 (3) provides a possible explanation for the effects of some of the mutations described above and suggests a means for mimicking their consequences. The structure of the coat protein dimer and the locations of the amino acid substitutions are schematically illustrated in Figure 6A. The L77P substitution falls within a flexible 15 amino acid loop connecting the F and G β-strands of the coat protein monomer. This so-called FG-loop adopts one of three alternative conformations depending on its position in the capsid, and is involved in intersubunit contacts important for capsid formation. The W82R substitution is nearby, affecting the first residue beyond the FG loop. Position 101, although distant from positions 77 and 82 in terms of sequence, lies near them in space. All three of these substitutions occur in sites positioned near the viral 3-fold (i.e., quasi 6-fold) and 5-fold axes in an region involved in the intersubunit contacts that join dimers together into the higher order structure (see Figure 6B). The flexible nature of the FG loop and its apparent lack of direct involvement in monomer folding and dimerization, raised the possibility that deleting most of it might interfere with capsid formation without preventing dimerization.

The derivative called pCT119dl-FG was constructed by site-directed mutagenesis as described in Methods and illustrated in Figure 2. The mutation is the result of the deletion of 13 amino acid residues and the introduction of an amino acid substitution that should permit the formation of a short β-turn to connect the F and G β-strands. This mutant produced white colonies with pRZ5 or pRZ6 on X-gal plates indicating that it possesses suppressor activity. Its repressor function was assessed quantitatively by solution assay of the β-galactosidase activity produced in strains containing the various pRZ derivatives. The results (listed in Table II) show that dl-FG possesses the superrepressor phentoype typical of the other mutants already described.

The ability of the dl-FG coat protein to produce capsids was assessed by its chromatographic behavior. As with the other super-repressor mutants (except V29I) two coat protein peaks were obtained on Sepharose CL-4B (Figure 4). Fractions from the second peak were pooled and analyzed by chromatography on Sephadex G75 (Figure 5). This time coat protein eluted later

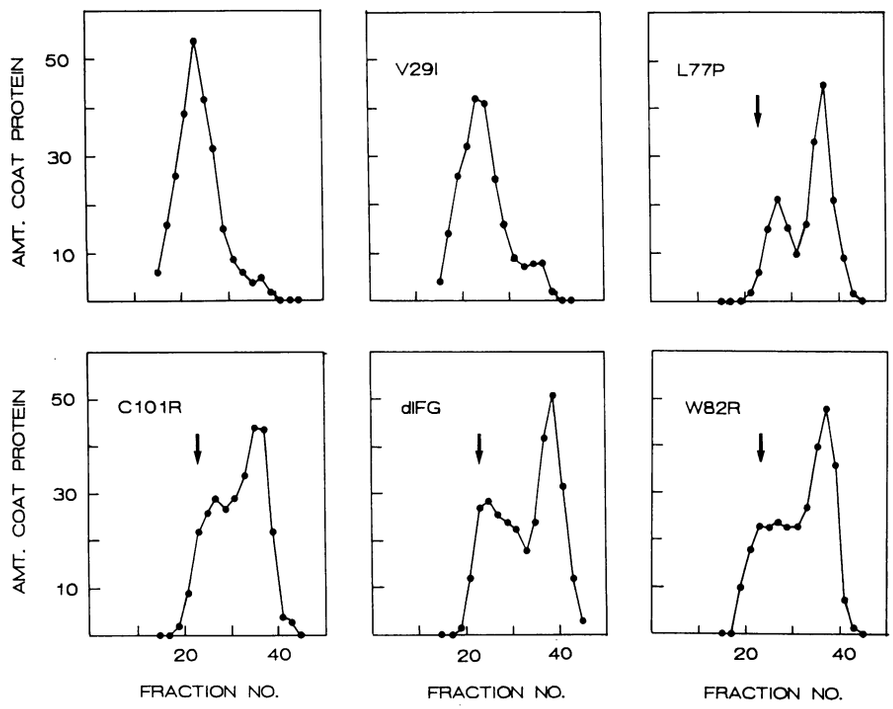

Figure 4. The Sepharose CL-4B elution profiles of wild-type coat protein and the various mutants described in the text. The mutants are identified in the upper left of each panel according to their amino acid substitutions. The amount of coat protein in each fraction is expressed in arbitrary units. About 1 mg of coat protein was present in the crude extracts applied to each column. The arrow indicates the normal elution position capsids.

than the putative dimers of the other suppressor mutants. When compared against a series of molecular weight standards it is evident that this new elution position is that predicted for a coat protein dimer from which 26 amino acids (i.e., 13 per monomer) have been removed.

Effects of a deletion at the C-terminus on repressor activity and capsid assembly

It has been known for some years that proteolytic removal of the C-terminal tyrosine of the coat protein of phage f2 (a close MS2 relative) results in loss of ability to form virus shells *in vitro* (5). The dl-8 mutant was constructed to reproduce this effect and differs from the wild-type coat protein gene by the loss of sequences encoding the C-terminal dipeptide, ile-tyr (Figure 3). In the colony color assay dl-8 produces blue colonies when tested with the wild-type operator in pRZ5, indicating that it is defective for repressor acitivity. Moreover, no repressor activity is recovered when tested with the pRZ9 recombinant containing a mutant operator with C at position −5, which has been reported to bind coat protein as much as 150-fold better than the wild-type RNA sequence *in vitro* (14). The results of solution assay of the β-galactosidase activity of strains containing these mutants are listed in Table II. This loss of repressor activity is not the result of failure of the mutant to synthesize sufficient quantities of coat protein, since Western blot analysis of cell extracts shows that the dl-8 protein is present in approximately the same quantities as wild-type (not shown).

The capsid-forming potential of the product of the dl-8 mutant was assessed by chromatography on Sepharose CL-4B and on Sephadex G75. As shown in Figure 7, no capsid-sized material was observed. All the coat protein elutes from Sepharose CL-4B in the low molecular weight portion of the chromatogram. On Sephadex G75 the dl-8 protein elutes at a position corresponding to a higher molecular weight than the putative dimers of L77P, W82R, C101R, and dl-FG. Comparison to a series of molecular weight standards indicates an apparent molecular weight of about 40,000, suggesting the formation of trimers (predicted MW=41,100).

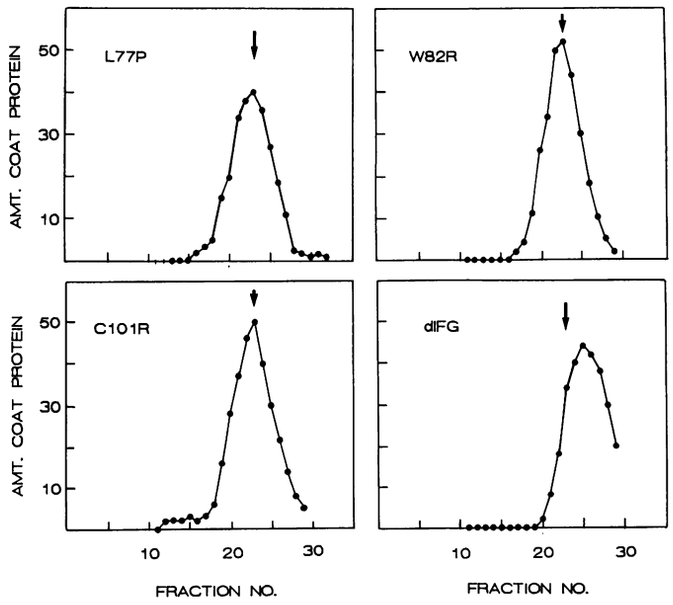

Figure 5. Rechromatography of the low molecular weight peaks from Sepharose CL-4B on Sephadex G-75. Arrows indicate the elution position of carbonic anhydrase (MW 29,000).

DISCUSSION

The MS2 coat protein performs both structural and genetic regulatory functions, serving as the major protein of the viral capsid and as a translational repressor of replicase synthesis in infected cells. Our studies point out the inter-relatedness of these functions and show how disruption of intersubunit contacts important to one function can have important consequences for the performance of the other function. Three of the four mutants that we characterized as suppressors of mutations in the translational operator are probably not affected directly in the manner in which they contact RNA. Instead, these mutations (L77P, W82R, and C101R) disrupt the ability of coat protein dimers to assemble into viral particles. Once assembled into capsids, coat protein is incapable of binding RNA (13). Therefore, we propose a mechanism of super-repression in which inhibition of capsid formation results in an increase in the effective intracellular concentration of repressor, facilitating repression at a variety of variant operators. This interpretation is supported by the observation that a substantial region of the protein (the FG loop) can be deleted with effects that mimic those of L77P, W82R, and C101R. However, we must emphasize that our results do not necessarily exclude the possibility that the substituted residues play a role in RNA contact. Direct measurement of the

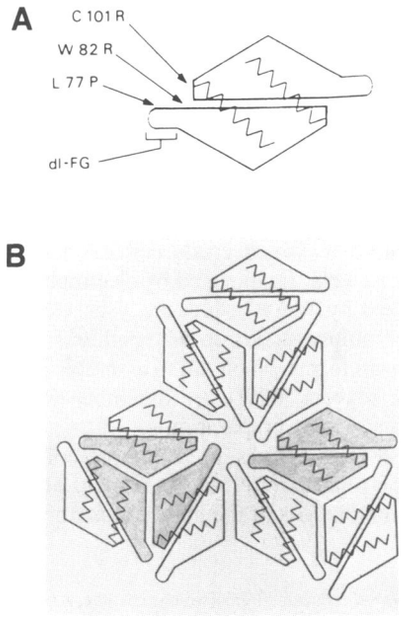

Figure 6. (A) A schematic representation of the coat protein dimer. This drawing is derived from the published crystal structure of the viral particle (Valegard, et al., 1990). Each monomer is roughly triangular in shape and extends an alpha-helical arm over the β-sheet of the adjacent monomer. The FG loop is shown as a short loop at one end of the triangle. The approximate positions of amino acid residues affected by the various mutations are indicated. Although the mutations are shown only once, each occurs twice in the symmetric dimer. (B) A schematic view of a portion of the viral particle as viewed down a 3-fold (i.e. quasi 6-fold) axis, illustrating how dimers are joined together to form the virus shell. One of the dimers is shaded. Also shaded is one of the trimers we propose may be formed in the dl-8 mutant as a result of the disruption of the two-fold contacts that make dimers.

intrinsic RNA-binding affinities of the mutant coat proteins *in vitro* should resolve this issue.

Inspection of the three-dimensional structure of MS2 rationalizes the effects of the mutations on capsid assembly. Figure 6 illustrates how the L77P, W82R, and C101R amino acid substitutions affect portions of the coat molecule that lie near the viral 3-fold (i.e. quasi 6-fold) and 5-fold axes. This region is involved in the interactions that join dimers together into capsids. The altered amino acid residues must either correspond to sites of intersubunit contact, or influence the conformations of such sites. The FG loop, in particular, is directly involved in contacts between dimers, consistent with the effects of the dlFG mutation. On the other hand, the V29I substitution affects a region of the molecule not obviously implicated in intersubunit contacts and does not inhibit assembly. Therefore, it is likely that V29I super-represses by binding RNA more tightly than wild-type coat protein.

We do not know whether these mutations completely eliminate the capacity for capsid assembly. Each Sepharose CL-4B profile shows the presence of species smaller than capsids but larger than dimers. It is not possible from these data to know whether these represent unstable capsids that are undergoing disassembly as they are diluted during chromatography, or whether they are non-specific aggregates, or some other aberrant structure. It is clear, however, that when dimers are concentrated for rechromatography on Sephadex G75 they do not regenerate these higher molecular weight species.

We should emphasize that our proposed mechanism for super-repression does not require that capsid assembly be completely blocked by the mutations; only that assembly occur at a higher than normal coat protein concentration, resulting in an increased intracellular pool of repressor molecules. If such mutant capsids are formed, they may in fact be less stable than wild-type and disaggregate in the high-salt conditions of ammonium sulfate precipitation, or because of dilution during chromatography. However, observation of the failure of these mutants to form capsids is not unique to the chromatographic methods we describe here. The phenomenon has also been observed using an alternative method in which crude cell extracts were applied directly to agarose gels, fractionated by electrophoresis, and coat protein visualized by Western blot analysis. Capsids possess a characteristic mobility under these condtions and are easily distinguished from forms which fail to assemble. All the mutants described here (except V29I) are assembly-defective by this criterion (manuscript in preparation).

Matthews and Cole (5) observed that proteolytic removal of the C-terminal tyrosine from the coat protein of a related RNA phage, f2, resulted in loss of the ability to form virus-like shells *in vitro*. Our results with the dl-8 mutant, which lacks the C-terminal ile-tyr residues, are consistent with their work. The dl-8 mutant failed to form capsids, producing instead, a species whose apparent molecular weight is close to that expected of a trimer. We speculate that this could be due to disruption of the interactions required for dimerization. Inspection of the crystal structure of MS2 (3) reveals that dimers are apparently stabilized, in part, by the interaction of the C-terminus of one monomer with sequences at the N-terminus of the other (see Figure 6). Since many additional interactions are involved in dimer stabilization, however, it is not an obvious prediction from the crystal structure that dl-8 should be defective for dimerization. Still, a failure to form dimers could conceivably explain both the apparent formation of trimers and the inability of dl-8 to repress translation. The active repressor is a dimer which binds only one molecule of operator RNA (2) and dimerization may be required for formation of a complete RNA binding site. The structure of MS2 indicates the existence of quasi-threefold interactions that normally occur only after dimers are already formed (3). Blocking the twofold interactions necessary for dimerization could, perhaps, reveal the existence of these threefold contacts by the formation of trimers. Figure 6B shows how this might occur. Other interpretations are possible, though, and further experimentation will be necessary to determine whether these speculations resemble reality.

Our results may have implications for control of viral gene expression in MS2 infected cells. Encapsidation of the genomic RNA is presumably initiated by the same event that accomplishes translational repression, i.e., binding of a coat protein dimer to the translational operator/packaging site. Formation of the complete viral particle is accomplished by the subsequent addition of 89 coat protein dimers, and is a highly cooperative process, occurring within a narrow range of coat protein concentrations. Factors that influence the minimum concentration of coat protein necessary to trigger capsid formation correspondingly influence the steady state concentration of repressor molecules. This, in turn, determines the level of replicase translation and genome replication. Our assembly-defective, super-repressor mutants may represent one extreme, where poor assembly prevents, or delays the production of virus particles, and translational repression is too efficient to allow much replicase synthesis. At the other end of the spectrum we imagine coat proteins that assemble into

Table II. Repression of replicase-β-galactosidase synthesis by the wild-type and mutant coat proteins. The β-galactosidase activities of strains containing the indicated coat sequences were divided into the activities produced by strains that didn't contain a coat sequence to obtain the values shown.

Coat Sequence	pRZ Derivative			
	pRZ5	pRZ6	pRZ9	pRZ10
wild-type	53	5	78	2.5
V29I	225	22	94	4
L77P	192	16	157	5
W82R	225	17	133	4
C101R	216	16	58	5
dlFG	152	13	176	n.d.

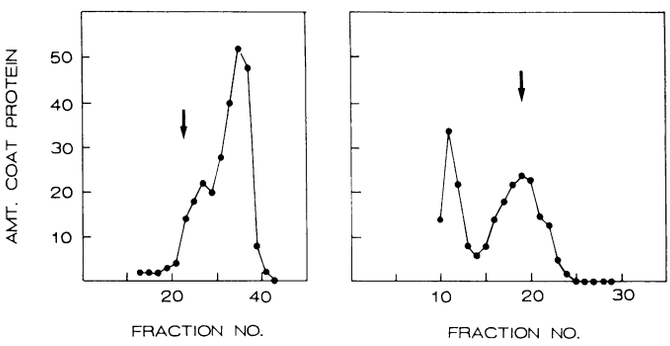

Figure 7. Behavior on Sepharose Cl-4B (left panel) and Sephadex G-75 (right panel) of the coat protein produced by the dl-8 mutant. Whole cell extracts were applied to each column and the coat protein was detected by Western blot analysis of fractions. In the left panel the arrow indicates the elution position of capsids. In the right panel the arrow shows where ovalbumin elutes (MW 45,000).

capsids so readily that dimer concentrations are never high enough to permit the efficient RNA binding required for repression and genome encapsidation.

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