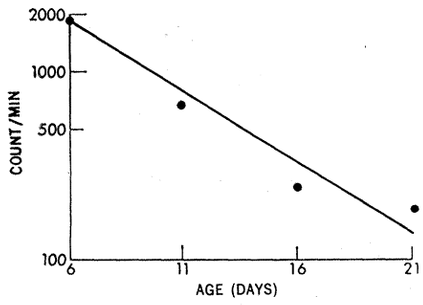


Fig. 2. Turnover of microtubule protein. Mice (5 days old) were injected intracerebrally with 2 $\mu$c of leucine-C$^{14}$; three were killed at each age indicated. The supernatant (100,000$g$) of the brain homogenate was isolated, 0.2 mg of the supernatant was subjected to electrophoresis, and the radioactivity (count/min) in the fractions with a molecular weight of 60,000 was determined. Since the protein content of brain increased during the period studied, corrections were made for dilution of the labeled protein, and these corrected results are shown on the ordinate.

leucine-C$^{14}$ at 1 day, 3 days, 5 days, or 7 days after birth, and killed 1 day after injection. A peak of radioactive protein of identical molecular weight was detected in the particulate component of brain homogenates at 24 hours and at longer times after the administration of labeled amino acid. This is consistent with the finding that a substantial portion of the colchicine-binding activity in brain is in the particulate fraction (*13*). The turnover of the microtubular protein was estimated in mice injected with leucine-C$^{14}$ at 5 days of age (Fig. 2). The half-life was approximately 4 days. In adult mice, the labeled protein with a molecular weight of 60,000 was also found 1 day after intracerebral injection of leucine-C$^{14}$. This peak represented only about 15 to 20 percent of the total labeled soluble protein. Other studies with embryonic axolotl brain grown in vitro and incubated for 3 hours with leucine-C$^{14}$ upon electrophoresis of whole tissue homogenates (*14*) showed a highly labeled peak containing material with a molecular-weight of 60,000.

Because of its abundance, rapid rate of synthesis and turnover, characteristic molecular weight, colchicine-binding activity, and vinblastine precipitability, this brain protein should prove amenable to correlative studies of its metabolism and its role in brain function.

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## Self-Assembly of Q$\beta$ and MS2 Phage Particles: Possible Function of Initiation Complexes

Abstract. *Four kinds of particles were reconstituted with RNA and protein from the genetically unrelated bacteriophages Q$\beta$ and MS2, namely, two homologous and two heterologous, with respect to RNA and protein. However, once Q$\beta$ RNA (or MS2 RNA) reacted with a few molecules of either Q$\beta$ or MS2 protein to form a nucleoprotein complex (initiation complex), it formed a phagelike particle only with subsequent addition of the same protein.*

The small spherical RNA bacteriophages and plant viruses have been useful model systems to verify Crick and Watson's (*1*) suggestion that such small viruses would possess cubic symmetry with the shape specifications residing primarily in a single protein subunit. Self-assembly into virus-like ribonucleoprotein particles is possible in vitro (*2*). It does not appear that there is a great specificity as to the nucleic acid encapsulated in vitro. Within the cell, however, viral nucleic acid and viral protein interact with high specificity and efficiently produce infectious particles. Cellular assembly mechanisms may not involve free interaction between pools of nucleic acid and protein as in the case in vitro; alternatively there could be a highly controlled assembly coupled to mechanisms of synthesis of the component parts. We have sought experimental support for the latter possibility and have separated the process of encapsulation in vitro into an RNA-protein "initiation" stage, followed by a cooperative interaction between the coat-protein subunits. The "initiation complex," which is composed of an RNA strand and a few molecules of coat protein, provides specificity in particle formation. Similar complexes could be natural products of a coupled RNA transcription-translation system and thereby ensure efficiency and specificity in formation of mature infectious particles.

Free interaction of the four possible combinations of RNA and protein from the physically similar but genetically unrelated coliphages Q$\beta$ and MS2 (*3*) gave the expected two homologous and two heterologous phagelike particles. Electron micrographs of the phagelike particles (Fig. 1) show that both the homologous and heterologous species appeared isometric, and approximately the same dimension as authentic phages. Large and distorted particles were more frequent in reconstituted particles. In gel electrophoresis (100 minutes; 4 ma) homologous reconstituted particles migrated as the respective authentic phage (41 mm for MS2; 19 mm for Q$\beta$), and the heterologous particles migrated as the phage from which the coat protein was derived.

In studies on repression of translation of Q$\beta$ and MS2 viral RNA it has been reported that a few molecules of phage protein can form complexes with homologous RNA but not with heterologous RNA (*4*). Contrary to these findings, we observed complex formations between homologous as well as heterologous RNA and protein. When radioactive Q$\beta$ protein was allowed to react with Q$\beta$ or MS2 RNA, complexes were readily formed, as revealed by sucrose density-gradient centrifugation. The results of a typical reaction when

the molar ratio of protein to RNA was 16 are shown in Fig. 2. From the specific radioactivity of protein and the absorbancy at 260 nm ($A_{260}$) of RNA, the number of protein molecules per RNA strand was estimated. At the peak tube of Fig. 2A there were about 5 to 6 molecules of Q$\beta$ protein associated with a molecule of Q$\beta$ RNA. Similar studies with MS2 RNA (Fig. 2B) indicated that about 3 to 4 molecules of Q$\beta$ protein sedimented with an MS2 RNA molecule at the peak tube. The association of protein molecules was a cooperative process. Protein became more efficiently incorporated into the nucleoprotein complex as the number of protein molecules in the complex increased. For example, when the initial molar ratios of Q$\beta$ protein to Q$\beta$ RNA were 4, 8, 16, 32, or 64, we observed that 0.4, 1.6, 5.6, 14.4, or 64 molecules of the protein became associated with a strand of RNA at the peak tube in the sucrose density sedimentation. Similar results were also obtained in the reaction between MS2 RNA and Q$\beta$ protein.

Thus a few molecules of phage coat protein will associate with both homologous and heterologous RNA under the described conditions. The nucleoprotein complex, which we term "initiation complex," could be incorporated into phagelike particles only by addition of the same protein. For example, a complex was prepared from radioactive Q$\beta$ protein and Q$\beta$ RNA at an initial molar ratio of 16. This complex in tris-acetate [tris(hydroxymethyl)-aminomethane] buffer was combined in a molar ratio of 260 with nonradioactive Q$\beta$ protein dissolved in 6*M* urea. Most of the complex was incorporated into phagelike particles after dialysis of the resulting 1.0*M* urea solution (Fig. 3A). At tube 12, the molar ratio of protein to RNA was 183. An icosahedral shell of triangulation number 3 requires 180 subunits. On the other hand, when nonradioactive MS2 protein was reacted identically with the complex, the initiation complex around tube 20 was preserved and was not incorporated into particles of mixed coat proteins (Fig. 3B). Some phagelike particles were observed, as shown by the absorbancy peak around tube 12. Contrary to the finding of mixed protein particles in reconstituted plant viruses (*5*), there was no radioactive Q$\beta$ protein associated with the assembled particles. This indicates that the coat proteins of Q$\beta$ and MS2 do not mix. The observed particles were probably formed from free Q$\beta$ RNA arising from partial dissociation of the complex in 1.0*M* urea after the addition of protein. The average ratio of protein to RNA of the preserved complex increased from 5.6 to 7.6, suggesting that complexes with lower protein to RNA ratios were preferentially dissociated. Figure 3 also shows a similar study with the initiation complex of MS2 RNA and radioactive Q$\beta$ protein. The complex was incorporated into phagelike particles when the same protein (Q$\beta$) was used in the cooperative encapsulation step (Fig. 3C). The peak at tube 10 contained particles with 168 being the estimated average ratio of protein to RNA. With a different protein (MS2) most of the initiation complex was preserved, and no radioactive Q$\beta$ protein was found in the particles (Fig. 3D). The molar ratio of the initiation complex increased from 3.7 to 5.1 at tube 20 after the second step. The dissociation of the initiation complex could be avoided during incorporation experiments if freshly prepared protein monomers (*6*) in tris acetate buffer are used instead of in 6*M* urea.

When the heterologous initiation complex of $^{32}$P-labeled Q$\beta$ RNA and MS2 protein was chased by either MS2 or Q$\beta$ protein dissolved in tris-acetate buffer, only MS2 protein was able to move the complex from the position of the 30*S* complex to that of 70*S* particles in sucrose gradient analyses. Although each protein formed initiation complexes with either RNA, encapsulation into particles occurred only with subsequent addition of the same protein. Thus there was a complete specificity for each protein in the cooperative encapsulation phase of assembly.

Lodish and Zinder (*7*) have suggested a coupled transcription-translation system late in f2 phage infection. In that model, the growing progeny viral RNA strand is pulled off the double-stranded template by immediate association with ribosomes and concomitant initiation of protein synthesis. In studies on repression by coat protein of RNA translation, Robertson *et al.* (*8*) observed that coat protein preferentially associated with double-stranded RNA rather than single-stranded RNA. The com-

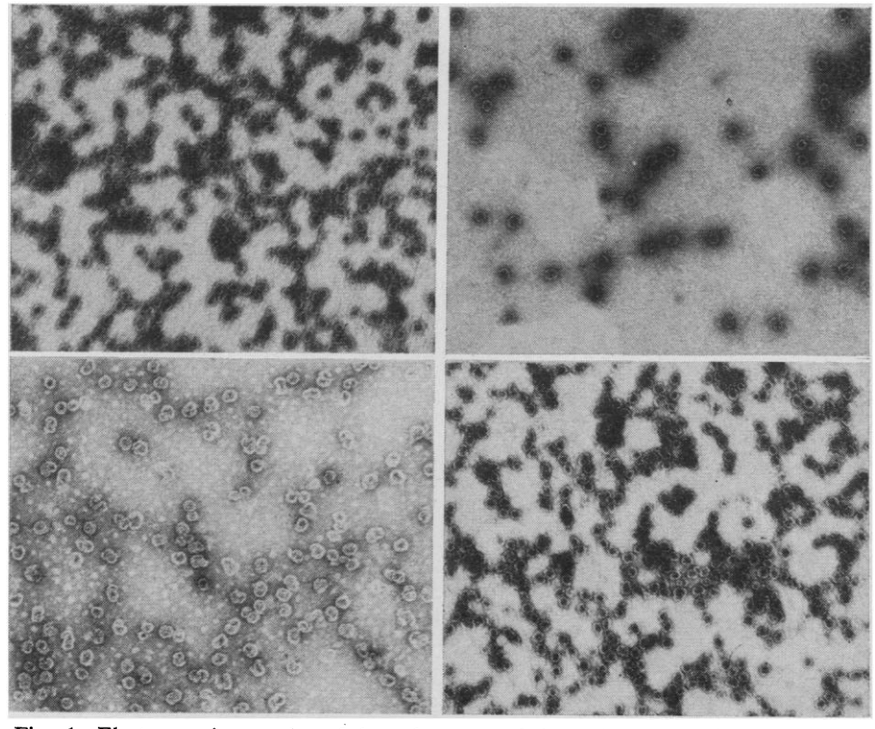

Fig. 1. Electron micrographs of homologous and heterologous particles. Phagelike particles were reconstituted from purified Q$\beta$ or MS2 RNA (*12*) and protein as previously described (*2*). The mixtures were then sedimented into a sucrose gradient (4 to 17 percent) at 4°C with a Spinco SW41 rotor for 110 minutes at 40,000 (rev/min). The RNA and protein were converted into particles which were slightly lighter than authentic phages. The peak containing particles was dialyzed against a tris-acetate buffer (0.1*M* tris *p*H 7.2, 0.05*M* KCl and 0.02*M* magnesium acetate). The particles were then stained with uranyl acetate (*13*) and magnified 150,000 times. (Upper left) Particles made from MS2 RNA and Q$\beta$ protein; (upper right) homologous Q$\beta$ particles; (lower left) homologous MS2 particles; (lower right) particles made from Q$\beta$ RNA and MS2 protein.

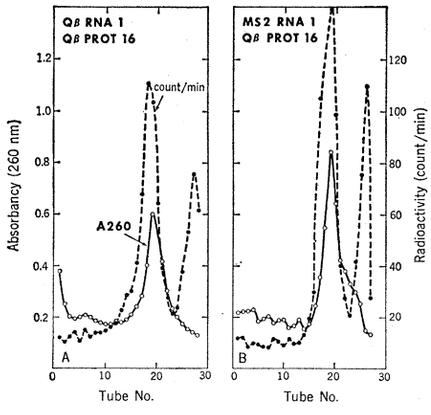


Fig. 2. Formation of nucleoprotein complexes between Qβ protein and Qβ or MS2 RNA. The reaction mixtures in a total volume of 100 μl contained the following components: 80 μg of Qβ or MS2 RNA (dissolved in 10 μl of 0.01*M* tris-HCl, *p*H 7.2, 0.001*M* EDTA); $4.6 \times 10^{-8}$ μc/(20 μg) of $^{14}$C-labeled Qβ protein per 20 μg of Qβ protein (dissolved in 10 μl of 8*M* guanidine-hydrochloride, *p*H 7.2, plus 1 percent mercaptoethanol), and the guanidine hydrochloride solution to make up the final volume. The molar ratio of protein to RNA was 16 to 1. After dialysis twice against tris-acetate buffer, the reaction mixtures were centrifuged in a sucrose gradient (4 to 17 percent) (SW41 rotor, 40,000 rev/min for 110 minutes at 4°C). The amount of RNA was estimated from absorption at 260 nm (25 $A_{260}$ units equal 1 mg of RNA). The protein was precipitated by cold trichloroacetic acid (10 percent) containing 0.2 percent sodium tungstate. The radioactivity was determined in a scintillation counter after the precipitate was filtered and dried on membrane filters. (A) Qβ RNA reacted with Qβ protein. (B) MS2 RNA reacted with Qβ protein. Controls (not shown here) of $^{14}$C-labeled Qβ protein, RNA, and phage showed peaks at tubes 29, 26, and 9, respectively.

plex with single-stranded RNA could be incorporated into particles with additional coat protein, while the double-stranded complex was stable. Moreover, Engelhardt *et al.* (*9*) found that the multi-stranded intermediates of replication contained only translatable copies of the coat gene as compared with other genes. A nucleoprotein complex has been observed by Capecchi and Gussin (*10*) during the synthesis in vitro of coat protein under the direction of R17 RNA. They suggested that the formation of this complex might be the initial step in the maturation of the virus. Godson (*11*) found in phage-infected cells most of the progeny single-stranded RNA was present in polysomes. He suggested a slightly modified model of the coupled transcription-translation in which a completed viral RNA strand is released as viral polysomes which in turn continue to synthesize viral coat protein.

The observation of highly specific initiation complexes described in this report suggests the possibility of a further coupling of assembly to RNA transcription and translation. The strands taking part in translation could be captured immediately by a few molecules of homologous coat protein and thus be designated for encapsulation into mature particles. The model has intrinsic advantages from the viral point of view of maximizing encapsulation of infectious viral RNA. Each encapsulated strand would have been tested in both transcription and translation. Defective or nontranslatable molecules would thus be excluded from encapsulation. Support of this model should come from proof that each RNA of progeny phage has been involved in translation.

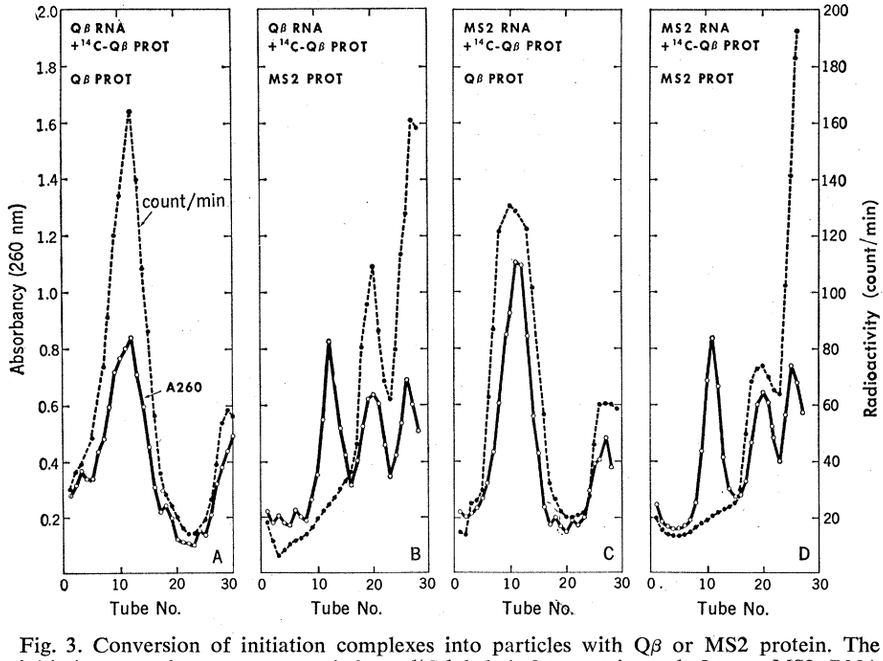


Fig. 3. Conversion of initiation complexes into particles with Qβ or MS2 protein. The initiation complex was prepared from $^{14}$C-labeled Qβ protein and Qβ or MS2 RNA at a ratio of protein to RNA of 16 as described in Fig. 2. After dialysis, a solution (RNA, 135 μg; protein $1.7 \times 10^{-3}$ μc) containing the complex of Qβ RNA and Qβ protein was diluted to 1.0 ml with tris acetate buffer, and Qβ or MS2 protein (540 μg) dissolved in 0.2 ml of 6*M* urea was added. The mixture was dialyzed immediately against tris acetate buffer at room temperature for 2 hours and analyzed by the sucrose density centrifugation. Similarly, a solution (RNA, 145 μg; protein, $2.5 \times 10^{-3}$ μc) containing the complex of MS2 RNA and Qβ protein was reacted with Qβ or MS2 protein (580 μg). (A) Initiation complex of Qβ RNA-Qβ protein reacted with Qβ protein in the second step. (B) The same complex reacted with MS2 protein. (C) Initiation complex of MS2 RNA and Qβ protein reacted with Qβ protein. (D) The same complex reacted with MS2 protein.

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