CAPSID PROTEIN OF f2 AS TRANSLATIONAL REPRESSOR*

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Earlier experiments by Lodish and Zinder (1966) on the regulation of protein synthesis by f2-infected cells led these authors to propose that f2 capsid protein served as a type of regulator or repressor of virus-specific protein synthesis. We have tested this idea using f2 RNA as polycistronic message for protein synthesis in extracts of E. coli. In agreement with the model of Lodish and Zinder (1966), we find that capsid protein binds to f2 RNA causing inhibition of synthesis of f2-directed proteins; most interesting was the finding that f2-directed histidine incorporation was more strongly affected than arginine incorporation. We assume that much of the arginine is incorporated into capsid peptides while histidine, not found in the capsid of f2, is being incorporated into other f2 proteins (Capecchi, 1966; Nathans et al., 1966). We speculate that f2 capsid may function as a translational repressor in this system; the working model is presented in Fig. 1 and is discussed below.

Results and Experimental

Isolation of f2, f2 capsid and RNA. RNA phage f2 was purified as described by Nathans et al. (1962); f2 RNA was prepared by the phenol method used by these authors and f2 capsid was isolated by their acetic

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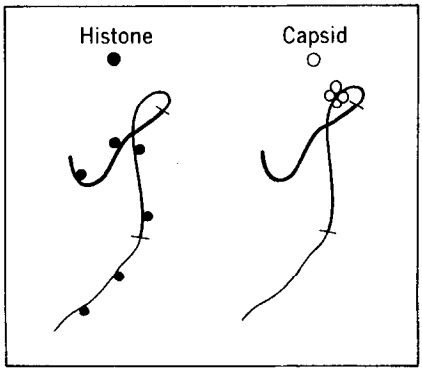

Fig. 1. Capsid as translational repressor. The essence of the model is that f2 capsid protein binds specifically to f2 RNA inhibiting synthesis of histidine containing proteins (early enzymes such as RNA synthetase?) while allowing translation of capsid to proceed normally. The heavy-lined portion of the RNA thread is meant to identify the capsid gene; there is actually no evidence for this gene order.

acid procedure. After dialysis to remove acetic acid the capsid protein fraction was stored at $0^{\circ}C$ at a protein concentration of about 1-2 mg per ml. The protein solution tended to become slightly turbid after several days' storage, but this did not appear to affect its activity. Some variation of activity was noticed with different batches of protein.

Cell-free extracts of E. coli strain A-19 (RNaseless strain obtained from J. D. Watson) were prepared as described by Capecchi (1966). The incorporation assay and conditions were also after Capecchi (1966). L-arginine C^{14} and L-histidine C^{14} were purchased from New England Nuclear Corporation. Purified histone from calf thymus (Kinkade and Cole, 1966) was kindly supplied by Dr. R. D. Cole.

Binding of Capsid to its RNA. We first carried out several experiments on the binding of capsid protein to radioactive viral RNA. A rapid filtration procedure was used. The binding of radioactive viral RNA to cellulose-nitrate filters in the presence of capsid is shown in Fig. 2.

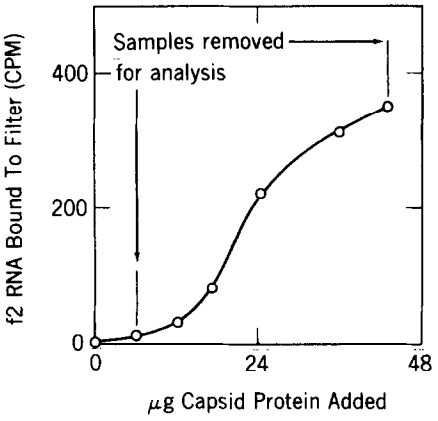

Fig. 2. Binding of f2 capsid to its RNA-a filtration assay. Reaction mixture for binding experiments contained in 0.2 ml 7 μg radioactive f2 RNA (~700 cpm P^{32}), 5 μmoles tris buffer at pH 7.4, and capsid as indicated. After incubation of capsid and RNA for 5 min at 25°C, the tubes were diluted to 10 ml with 0.05 M tris buffer and passed through cellulose nitrate filters (0.45μ). The filters were washed with buffer and counted.

It was of some interest that capsid tended to protect the viral RNA strand from complete digestion by added RN'ase. After RN'ase digestion approximately 10-20 per cent of the radioactive RNA in the complex was still retained by the filter. This experiment also shows that the viral RNA-protein complexes probably do not resemble mature phage particles since the RNA would be fully protected in its capsid. Electron micrographs supported this idea - the RNA-protein complexes were seen as threads rather than spherical-shaped particles. A variety of basic proteins including histone, lysozyme, and spermidine caused RNA binding to the filter.

A sedimentation analysis of the RNA-capsid complexes was next carried out. As shown in Fig. 3, complexing of capsid protein to viral RNA caused a marked shift in the sucrose gradient profiles only when large amounts of capsid were used.

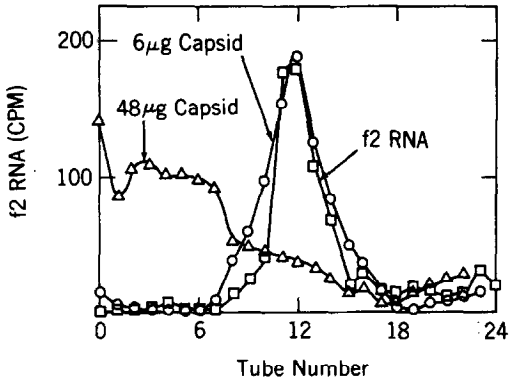

Fig. 3. Capsid-RNA complexes-sedimentation profiles. Samples of capsid-RNA complexes (see Fig. 2) were layered on sucrose gradients (5-20 per cent) and centrifuged for 3 hrs at 38,000 rpm in the SW-39 rotor. Samples were collected and counted for radioactivity. Note that with 6 μg capsid the sedimentation rate was not changed appreciably.

When 6 μg of capsid was used the RNA profile was similar to RNA alone. Higher protein concentrations (48 μg) caused smearing of the sedimentation profile. The heavy sedimenting material was not investigated but might be partially completed particles.

The purpose of these two experiments was to show that viral capsid protein readily binds to its RNA. It is of some importance, as will be discussed below, that small amounts of capsid while not appreciably affecting the sedimentation profile or causing RNA binding nevertheless appear to inhibit protein synthesis.

f2 Capsid as Repressor. We next tested the effect of capsid on protein biosynthesis directed by f2 RNA (Fig. 4). It should be mentioned that f2 RNA codes for at least three phage proteins - capsid, RNA polymerase, and maturation protein (Capecchi, 1966; Nathans et al., 1966). Usually two classes of f2-directed proteins are recognized - capsid which does not contain histidine and the histidine-containing peptides thought to include RNA polymerase and maturation protein (Capecchi, 1966). For the experiment described in Fig. 4, we have used arginine-C^{14} to label capsid

protein and histidine-C^{14} for the histidine class of proteins.

As shown in Fig. 4, addition of capsid protein inhibited the synthesis of the histidine class of peptides more severely than the arginine class. This experiment has been repeated several times and 2-7 fold relative reduction in histidine incorporation over arginine incorporation was observed in each case. In control experiments (Fig. 4) histone from calf thymus was found to show little specificity - being a strong inhibitor of both classes of protein. These results are discussed below.

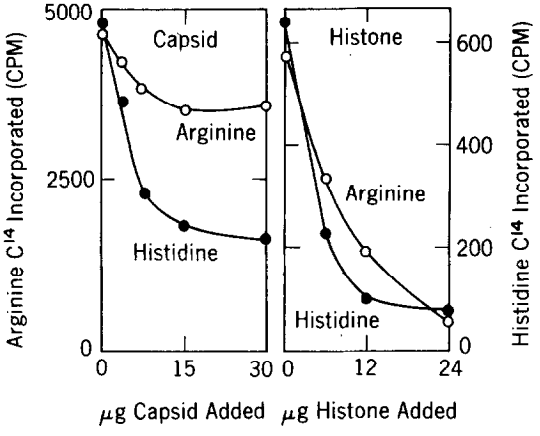

Fig. 4. Effect of capsid and histone on f2-directed protein synthesis. Incorporation assay and conditions after Capecchi, 1966. f2 RNA (9 μg) was used as template. Capsid and histone pre-incubated for 5 min at 25° with f2 RNA. Note the stronger inhibition of histidine incorporation by f2 capsid.

Discussion

The key experiment is the finding (Fig. 4) that f2 capsid protein shows a specific inhibitory effect on biosynthesis of the histidine class of proteins. We feel that this experiment supports the model for translational repression described in Fig. 1. Similar experiments have recently been reported (Eggen and Nathans (1967) and Sugiyama and Nakada (1967)).

It is certainly an intriguing idea that virus-specific protein

synthesis in f2-infected cells may be a self-regulating process. The essence of the proposed model (Fig. 1) is that capsid protein (a late viral function) shuts off early proteins such as RNA polymerase. This regulatory switch is attractive from the standpoint of economy of protein synthesis - for example, when enough RNA polymerase is produced it is switched off and capsid protein needed in very large amounts becomes the major synthetic product. A virus mutant missing this regulatory switch has been isolated by Lodish and Zinder (1966). The pattern of synthesis by this mutant was dramatically altered. In fact, it was the study of this mutant which first focused attention on f2 capsid as translational repressor. Our studies are presently concerned with the nature of the capsid-RNA interaction. The specificity of the capsid-RNA reaction is very interesting.

References

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