TRANSLATIONAL REPRESSION OF f2 PROTEIN SYNTHESIS*

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Recently, we have proposed a mechanism which may explain how bacteriophage f2 regulates its protein synthesis after infection (Ward et al., 1967). Sugiyama and Nakada (1967) originally proposed a similar system for MS2. The model is shown in Fig. 1. The essence of the model is that virus shell protein subunits made early after infection combine with each other to form an active repressor molecule which binds to the viral RNA genome shutting off phage genes which are no longer needed in the infection. We have called this kind of regulation "translational repression" since it occurs at the messenger level.

In the experiments described below translational repression was studied in extracts using f2 RNA as polycistronic messenger and f2 capsid as repressor. The major findings were: 1) Histidine genes were completely shut off by about six molecules of capsid. 2) Viral capsid but not maturation protein was active as repressor. 3) Capsid from the unrelated bacteriophage Qβ was inactive as repressor, whereas capsid from the related viruses MS2 and fr was fully active. 4) Capsid, as repressor, bound to a region of the RNA protecting about 0.5% of the molecule from ribonuclease digestion.

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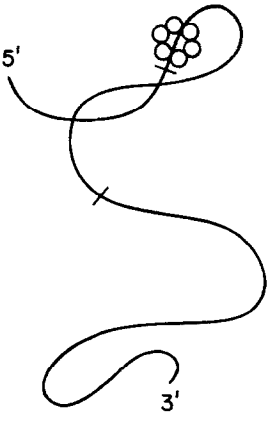

Fig. 1. Proposed model for translational repression. Capsid (circles) binds to RNA genome (thread) shutting off "histidine" genes. Translational repression may work closely with polarity in this system.

Results

Assay for Repression Using the Cell-free System.--The cell-free extract of E. coli and assay mixture for protein synthesis were similar to those described by Capecchi (1966). An RNase-less strain was used (Capecchi, 1966).

The viral messenger RNA and capsid were extracted from purified f2 particles according to the method of Sugiyama and Nakada (1967). The RNA phages Qβ, MS2, and fr were prepared by a similar procedure. All radioactive amino acids were obtained from New England Nuclear Corp. Virus protein concentration was determined by colorimetric and optical density methods as was RNA and cell protein.

In our assay we take advantage of the fact that f2 messenger RNA directs the incorporation of radioactive histidine into all viral induced proteins except the coat which does not contain this amino acid (Capecchi, 1966); capsid synthesis was followed by measuring arginine incorporation into protein. In other words we follow genetic translation of the coat gene versus the "histidine-containing" genes by radioactive incorporation. We have found this to be a suitable assay although by this technique it is not possible to

distinguish the various histidine-containing proteins one from the other.

Strong Repression of Histidine Genes with Capsid.--In an earlier experiment (Ward et al., 1967), we were able to show that capsid differentially shut off histidine incorporation while f2 directed arginine incorporation was less drastically affected.

We wondered if it would be possible to completely shut off the histidine genes with capsid. In certain experiments we had earlier observed a more drastic inhibition of histidine incorporation. We felt that the failure to obtain complete repression in earlier experiments might be due to partially degraded capsid or RNA preparation or to improper Mg^{++} concentrations. To insure faithful translation of the f2 message we used freshly prepared viral RNA and capsid, and carefully checked the Mg^{++} levels of the assay mixtures. Under these conditions little f2-directed histidine incorporation took place when capsid was added as repressor (Fig. 2). A mechanism to account for this very strong repression with capsid is discussed below.

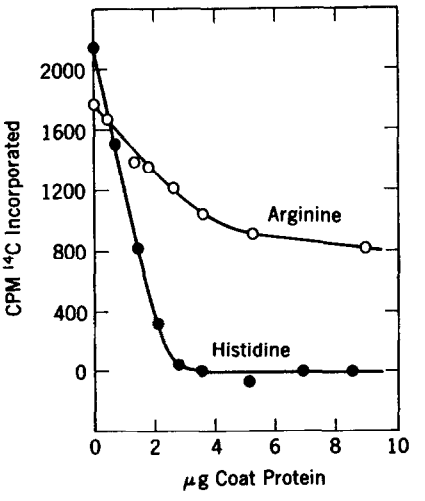

Fig. 2. Shutting off the histidine genes with capsid. Each assay tube in this experiment contained 28.0 μg of RNA and either 2.8 x 10^5 cpm C^{14}-histidine or 1.4 x 10^5 cpm C^{14}-arginine. Coat protein was freshly prepared. The tubes were incubated at 37° C for 11 minutes and precipitated with cold 5% trichloroacetic acid. The radioactive protein samples were collected by filtration on cellulose-nitrate filter pads and counted using a gas flow counter.

As shown in Fig. 2 very small amounts of f2 capsid were active. It was of interest to calculate the number of capsid subunits per RNA molecule which must be added to shut off the histidine genes. We used molecular weight values of 1.1×10^6 for RNA and 15,800 for capsid. In the experiment shown (Fig. 2) approximately 6 subunits of capsid per RNA were needed to completely shut off the histidine genes. We propose that the active repressor species is a capsid hexamer, although more information is needed on this point (see Sugiyama and Nakada, 1967).

Capsid Not Maturation Protein is Repressor.--We next attempted to rule out the possibility that maturation protein (Steitz, 1967) was involved in repression. This possibility existed since maturation protein was carried by intact virus as a minor capsid component (Roberts and Steitz, 1967).

Our "capsid" preparation thus contained this protein in addition to the major shell protein. We prepared capsid from maturation deficient mutant particles (Sus-1) and found that it was fully active as repressor (Table 1). Since Steitz (1967) has recently shown that mutant particles of this type do not contain maturation protein we concluded that capsid itself was the repressor species.

Specific Nature of the Repressor.--A number of experiments were carried out to see if the interaction of viral capsid with viral RNA was a specific one. We wondered whether capsid from one species of phage would repress RNA of another virus. Viruses were chosen which are known to have different coat proteins (Weber and Konigsberg, 1967). Several combinations of capsid and RNA were tested. The results are shown in Table 2.

In summarizing the data of Table 2 we see that f2 and serologically related viruses (Scott, 1965) (MS2 and fr) show strong cross-reactivity. For example, shell from phage MS2 reacts strongly with RNA from f2 and vice versa. It was particularly interesting that the unrelated virus Qβ (Overbey et al., 1966) showed no cross reaction - its coat showed no repressor activity for the unrelated RNA. A similar type of species specificity

Table 1

Capsid From Mutant Particles (Sus 1) Missing The Maturation Protein Was Active

	Capsid μg	Histidine Incorporated* CPM
f2	0	1020
	0.5	840
	1.2	730
	2.4	440
	4.8	40
	12.0	0
Sus 1**	0.71	820
	1.75	620
	3.5	160
	7.0	0

*Protocol as for Fig. 2, except each assay tube contained 37.6 μg of f2 RNA.

**Sus 1 defective particles prepared and purified according to the procedure of Lodish et al. (1965).

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Table 2

Specificity of Repressor

RNA (μg)		Source of Protein (μg)		% Inhibition of C^{14} Histidine Incorporation
f2	(28.0)	f2	(3.5)	100
f2	(28.0)	Ms-2	(5.0)	100
f2	(28.5)	fr	(3.0)	93
f2	(26.0)	Qβ	(10.0)	9
Ms-2	(14.5)	Ms-2	(2.8)	100
Ms-2	(14.5)	f2	(3.4)	100
Qβ	(33.5)	f2	(16.0)	4
fr	(29.5)	fr	(3.0)	77
fr	(29.5)	f2	(3.3)	77
TMV	(31.5)	f2	(17.0)	0*
Poly U	(13.0)	f2	(17.0)	0*

*No inhibition of C^{14} phenylalanine incorporation was observed.

has been shown for the RNA polymerases of these viruses (Haruna and Spiegelman, 1965). We feel that these results plus those obtained by

Sugiyama and Nakada (1967) establish the specific nature of capsid-RNA interaction.

Viral RNA Binding Site for Repressor.--We hoped to isolate the RNA binding site of the repressor by extensive degradation of the RNA in the presence of its repressor. We reasoned that the bound repressor would protect this region from digestion by RNase. It seemed most convenient to use labelled RNA (P^{32}) as substrate and follow digestion in the presence and absence of capsid. In one experiment 30 µg of f2 capsid was incubated with 300 µg radioactive viral RNA (1.2×10^5 CPM). The sample was digested with pancreatic RNase (40 µg) for 30 min at 37° C and assayed for TCA precipitable counts. About 1.1% of the total counts remained precipitable with TCA indicating their polynucleotide nature. A portion of the capsid protected sample was sedimented in a sucrose gradient and samples were assayed for radioactivity. A peak sedimenting at about 5 S was observed. We are exploring this peak to see if it contains the repressor-RNA complex. In a control experiment an identical radioactive RNA sample was processed as above except the RNA was digested with RNase for 30 min before capsid was added. An RNase "core" of approximately 0.5% of the whole molecule was observed (Sober et al., 1966). In several experiments we have found that prior addition of capsid protected a significantly larger portion of the RNA. We chose an RNA/capsid ratio that would minimize reconstitution of intact virus (Hohn, 1967) particles whose RNA would be protected in their coat.

It should also be pointed out that the RNA/capsid ratio we used in the protection experiment gave maximal repression when assayed in the protein synthesis assay above. We therefore argue that repression protection is closely associated with repressor binding to the RNA. We propose that viral capsid as repressor binds to a specific region of the RNA we have tentatively called the repon (see Fig. 1). The nature of the repon is under investigation.

Discussion.--In one sense the infective cycle of the minute phage f2

may be regarded as a biosynthetic pathway with virus components and finished particles as ultimate products of the pathway. Treatment of the infective cycle in this context leads to some interesting speculation regarding the nature of the control mechanisms which operate to insure a balanced infective cycle. We have described what we consider to be an important viral regulatory mechanism which operates in some respects like a "feedback" switch. Capsid, a viral biosynthetic product, serves as an inhibitor to regulate synthesis of other viral components. We call this regulatory switch translational repression since the control is exerted at the messenger RNA or translational level.

There might be other levels of control of the infection such as control due to polarization or polarized reading of the phage message (see Zinder et al., 1966 and other articles in CSH Symposium, Vol. 31, 1966). These workers have studied polarity of translation in vitro using amber mutants of f2. Strong polarity effects were reported. Translational repression appears to work closely with the polarity mechanism. For example, in the case of the capsid mutants of Zinder et al. (1966) translation of the histidine genes was almost completely blocked by the nonsense codon in the capsid gene - translation did not proceed beyond this point. Our data (Fig. 2) indicate that all of the viral genes (except shell) respond to capsid as repressor. If this is the case and the repressor is specifically located as we think on only one of the genes, then strong polarization effects must occur in neighboring genes. The net effect is that repression of one gene shuts off all of the remaining genes on the RNA strand as the result of the strong polarization of the message.

Translational repression, as a regulatory mechanism, seems attractive from several viewpoints. It endows the virus with dynamic self-regulatory powers - it allows certain genes to be shut off when they are no longer needed. The "timing" of the switch is related to the amount of translation or number of times the viral message is read, a fact which allows regulation

to occur even when the infective cycle may be slowed down because of environmental conditions of the infected host cell. Compared to the life cycle of higher cells and even higher phages this type of regulation at first seems primitive. It is interesting to speculate though that translational repression of protein synthesis such as observed here may occur in higher forms although as far as we're aware there are as yet no other examples of this method of regulation.

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