BIOLOGICAL SCIENCES

Identification of the Terminus of Nascent f2 Bacteriophage RNA

PREVIOUS studies have identified the 5' terminus of several bacteriophage RNAs, including f2, as pppGp¹⁻³. A number of other experiments using phage RNA and fragments derived therefrom as messenger in an *in vitro* amino-acid incorporating system have led to the suggestion that the viral coat protein is encoded near this 5' end⁴⁻⁶. This interpretation depends on assumptions made about the translation of fragmented RNA or the causes of differential rates of protein synthesis from different cistrons *in vitro*.

For some time it has been clear that RNA replication in cells infected with phage involves structures which, when isolated, have been shown to contain double stranded RNA with nascent single strands attached⁷. Engelhardt *et al.*⁸ have shown that these replicating structures, when purified and used to stimulate protein synthesis *in vitro*, cause the nearly exclusive production of f2 coat protein. Further experiments showed that this messenger activity is due to the nascent, single stranded tails on these intermediates. It is clear that the identification of the terminus of the tails with one of the ends of the intact single stranded RNA molecule would allow us not only to ascertain the direction of synthesis of f2 RNA *in vivo* but also to specify the location of the coat protein cistron on the f2 genome. We now present experimental evidence showing that the 5' terminus of the tail is the same as that on the phage RNA (pppGp).

Bacteriophage RNA labelled with ³²P was grown and purified by the method of Webster *et al.*⁸, while ³²P labelled replicative ensemble was prepared by the method of Engelhardt *et al.*⁸. The tails were separated from the double stranded portion of the replicating structures by treatment with the *E. coli* enzyme ribonuclease III, which digests double stranded RNA specifically⁹, followed by phenol extraction and cellulose chromatography as described by Franklin¹⁰ and Robertson *et al.*⁹. Several experiments have been performed to characterize these tails, and we shall report their results elsewhere.

Tails were compared with single stranded f2 RNA for the presence or absence of the pppGp moiety. The RNAs were solubilized in 0.3 M KOH at 37° C and subjected to high voltage electrophoresis (25 V/cm) on Whatman 3MM paper in 0.05 M sodium citrate, pH 3.5. Material with the greatest mobility (faster than GTP) from both f2 and tails was eluted and subjected to a variety of analyses, the details of which we shall report elsewhere. Usually an excess of fast migrating material was isolated from the tails, per mole of internal phosphate, as compared with intact viral RNA. The comparative properties of the two preparations are summarized as follows.

(1) Both adsorb to charcoal, and therefore contain organic phosphate. (2) Both migrate as single peaks of identical mobility when electrophoresis is repeated at pH 4.0. (3) Both show identical *R_F* values when ascending chromatography is carried out on Whatman No. 1 paper in 0.1 M sodium phosphate, pH 6.8, containing 60 per cent ammonium sulphate and 2 per cent *n*-propanol. (4) Both release pGp to a similar extent when treated with venom phosphodiesterase as described by Roblin¹ or when exposed to mild acid hydrolysis as reported by Watanabe and August². The 3', 5' nucleoside diphosphates were separated using two-dimensional paper chromatography as described by Lane and his collaborators^{11,12}. (5) Neither released detectable amounts of pAp, pCp or pUp.

These results show that the tails of the f2 replicating structures contain the same 5' endgroup as f2 RNA. This fact and our failure to find the other three nucleoside tetraphosphates indicate that *in vivo* f2 RNA synthesis occurs in a 5' to 3' direction. Together with the experiments of Engelhardt *et al.*⁸, already mentioned, these

results constitute direct evidence that the coat cistron of f2 RNA is near its 5' terminus.

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Application of High Resolution Nuclear Magnetic Resonance to Conformational Analyses of Polypeptides in Solution

THE considerable interest attached to conformational studies of gramicidin *S* has resulted from the use of this compound as a model for computations of polypeptide conformations. Two conformations have been postulated from energy considerations: Vanderkooi *et al.*¹ have postulated a compact conformation for gramicidin *S* in which two identical pairs (L val-L orn) of the ten amino-acids of this cyclic peptide form a short length of anti-parallel extended β -conformation while the conformation postulated by Liquori *et al.*² is quite different in that the two identical segments (L val-L orn-L leu) form segments of a right handed α -helix. Spectroscopic techniques such as optical rotatory dispersion and infrared which have been applied with success to conformational analysis of synthetic homopolypeptides and proteins have given inconclusive results when applied to gramicidin *S*; this results from the cyclic form of this peptide and the fact that any regular structure, if present, can only involve a very small number of residues. Liquori and Conti³ have attempted to adduce evidence from NMR spectroscopy in favour of the conformation² for gramicidin *S* by comparison of the chemical shifts of the α CH protons with those of synthetic homopolymers. The salient point made by Liquori *et al.* from unpublished results is that "the chemical shift of an α CH proton in a homopolypeptide seems to be strongly dependent on the backbone conformation of the amino-acid residues, to a smaller extent on the nature and conformation of the side chains and it is fairly insensitive to the solvent". Thus by comparison of the chemical shifts of the α CH protons of gramicidin *S* in dimethyl sulphoxide with those of the helical forms of poly L-leucine in trifluoroacetic acid/deuterochloroform (TFA/CDCl₃) mixtures and poly L-ornithine in D₂O, they deduce that there are helical segments in gramicidin *S*. We are not concerned here with the correctness or other-