Natural minus-strand RNAs of alfalfa mosaic virus as in vitro templates for viral RNA polymerase. 3’-Terminal non-coded guanosine and coat protein are insufficient factors for full-size plus-strand synthesis

C. J. Houwing, M. Huis in ’t Veld, D. Zuidema, M. de Graaff, and E. M. J. Jaspars†

Institute of Molecular Plant Sciences, Gorlaeus Laboratories, Leiden University, The Netherlands

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Summary. Replication complexes of alfalfa mosaic virus produce in vivo large quantities of plus-strand RNAs, but this production is fully dependent on the presence of coat protein. In order to study this process of RNA-dependent and coat protein-regulated RNA synthesis we have isolated the three natural minus-strand RNAs (containing any posttranscriptional modification that might have occurred) and have tested them for coat protein binding sites and template activity in an in vitro system with the viral RNA polymerase. The enzyme was prepared by an advanced isolation procedure. All three minus strands had a single non-coded G at their 3’ terminus. They were not able to withdraw coat protein subunits from virions as free virion RNAs do. No sites protected by coat protein against ribonuclease T1 degradation were found. Two large T1 oligonucleotides from minus RNA 1 and one from minus RNA 3 were bound by coat protein to Millipore filters. Except for minus RNA 3 which caused a minute amount of full-size plus strand to be synthesized, the minus strands did not function as templates for full-size complementary strands. On the other hand, they gave rise to a number of well-defined shorter products, the synthesis of which was stimulated by the addition of coat protein. These products could not be elongated by a chase treatment and were probably the result of internal initiations. It is concluded that, although posttranscriptional modifications of the template and the presence of coat protein may be necessary factors for plus-strand RNA synthesis, they are certainly not sufficient. Our purified in vitro system needs further sophistication.

†Deceased.
Introduction

During the life cycle of plus-stranded RNA viruses minus strands, present in the infected cell in minute quantities, are used at high frequency as templates for viral messenger RNAs and for virion RNAs. In alfalfa mosaic virus (genus *Alfamovirus*, family *Bromoviridae*) the RNA transcription process is strongly regulated by the viral coat protein. In the absence of coat protein the synthesis of viral single-stranded plus RNA is almost completely shut off. This regulatory key function in plus-RNA synthesis most probably also provides the explanation for the unique phenomenon exhibited by the genera *Alfamovirus* and *Ilarvirus*, both of the family of the *Bromoviridae*, namely that the mixture of the three genome RNAs, despite their messenger polarity, is virtually not infectious, but needs a few subunits of coat protein to be “activated” (for reviews on genome activation see [3, 26]).

In order to understand the mechanism of regulation of plus-strand synthesis by coat protein we have been searching for binding sites of the coat protein on natural minus strand RNAs. On the other hand we have optimized our in vitro system for RNA-dependent RNA synthesis by improving the isolation of the viral RNA polymerase and by using as templates natural minus strands instead of transcripts of cloned DNA. By doing the latter we were able to study the templates as present in vivo in the replication complexes with any posttranscriptional modification they might have undergone.

Among the three sites that had affinity for coat protein, one was localized in the promoter for the subgenomic coat protein messenger on minus RNA 3. Further it appeared that the same viral RNA polymerase interacted quite differently with plus and minus RNAs. The enzyme made mainly full-size minus strands on plus-strand templates, the synthesis of which was inhibited by coat protein. On minus-strand templates it started on internal sites giving rise to partial transcripts of well-defined lengths, the synthesis of which was stimulated by coat protein.

Materials and methods

Preparation of virions, virion RNAs and coat protein

In the early experiments on coat protein binding sites traditional methods for cultivation and infection of plants and for preparation of virions were followed [43, 44]. In later experiments virus was grown under more standardized conditions. The virions were purified with a less elaborate and time-consuming method, as reported recently [22].

Coat protein was prepared essentially as described [29], but was stored in small portions at −20°C instead of being lyophilized.

Virion RNAs were extracted with SDS/phenol [5]. Their quality was judged from the appearance upon electrophoresis in 1% agarose minigels [27].

Alfalfa mosaic virus strain 425L (the Leiden variant, see [30]) was used throughout.

Molecular data and extinction coefficients are taken from Jaspars [24], but the correct number of nucleotides of RNA 3 is given by Langereis et al. [30].

Fragments of virion RNAs were obtained by degrading a total virion RNA extract at a concentration of 3.5 mg/ml in 0.02 M NaOH for 75 min at 20°C. The solution was neutralized by adding 1/56 volume of 3 M Na-acetate, pH 5.0, loaded on a 5 to 20% (w/v) sucrose gradient.