Synthesis of Bacteriophage φ6 Double-stranded Ribonucleic Acid

(Accepted 14 July 1976)

SUMMARY

Uracil was incorporated into all three bacteriophage φ6 dsRNA segments throughout the infection cycle; the rates of incorporation into each of the three segments were approx. constant for the first 15 to 20 min and then increased rapidly until 50 min after infection. The medium and small dsRNA segments were produced in greater amounts than the large dsRNA segment at all times in the infection cycle. Inhibition of host RNA and protein synthesis with rifampin and chloramphenicol revealed that virus dsRNA synthesis immediately after infection was independent of either host function.

Whereas several plant and animal viruses contain a genome consisting of segmented double-stranded ribonucleic acids (dsRNA), φ6 of Pseudomonas phaseolicola is the only known dsRNA-containing bacteriophage (Wood, 1973). The φ6 genome consists of three unique dsRNA segments of approx. 2.3, 3.1, and 5.0 × 10^6 daltons which are present in equimolar concentrations in the mature phage (Semancik, Vidaver & Van Etten, 1973; Van Etten et al. 1974). The present report describes the incorporation of uracil into the three dsRNA segments during the infection cycle of φ6.

Procedures for growing the phage, synchronously infecting the host by allowing the phage to pre-adsorb to the bacteria at 0 °C for 30 min and then terminating the incubation were identical to those described previously (Coplin et al. 1975). The infected cells were pulsed or continuously labelled with 3H-uracil as indicated in the table and figure. Nucleic acids were extracted with 0.05 M-tris, 0.1 M-NaCl, 0.005 M-EDTA, 2.5 % sodium N-lauroyl sarcosine buffer, pH 8.0, and phenol–cresol at 65 °C as previously described (Coplin et al. 1975). After precipitation of the nucleic acids with 3 vol. of 95 % ethanol, the precipitate was dissolved in 0.5 ml of STM buffer (0.01 M-tris; 0.3 M-NaCl; and 0.01 M-magnesium acetate, pH 7.2) containing 2.5 µg of RNase A and 5 µg of DNase and incubated for 30 min at 32 °C. Five hundred µg of bentonite were added and the samples were centrifuged at low speed to remove the bentonite. The dsRNA was precipitated with 3 vol. of ethanol in the presence of 20 µg of bentonite. The removal of DNA was necessary because it was not easily separated from dsRNA by gel electrophoresis, and the removal of single-stranded RNA by RNase treatment allowed greater amounts of sample to be electrophoresed without overloading the gels. Radioactive RNA was electrophoresed on 2.5 % polyacrylamide gels containing 0.5 % agarose at 6 mA/gel for 8 to 9 h. The gels were scanned, sliced, eluted, and counted as previously described (Van Etten et al. 1973).

The rates of uracil incorporation into each of the three dsRNA segments in a pulse labelling experiment are shown in Fig. 1(a). Incorporation into all three segments was detected within the first 6 min after infection. The rates of uracil incorporation into each of the three dsRNA segments remained approx. constant for the first 15 to 20 min, increased rapidly until about 50 min after infection, and then decreased slightly. The slight decrease in the rate of dsRNA synthesis observed 50 min after infection may be due to the assembly of at least some of the dsRNAs into virus particles; phage release occurs between 60 and 80 min after infection.
**Short communications**

Table I. Distribution of uracil into the three φ6 dsRNA segments at various times after infection*

<table>
<thead>
<tr>
<th>Time of sample (min)</th>
<th>Large</th>
<th>Medium</th>
<th>Small</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>23</td>
<td>35</td>
<td>42</td>
</tr>
<tr>
<td>30</td>
<td>25</td>
<td>36</td>
<td>39</td>
</tr>
<tr>
<td>40</td>
<td>32</td>
<td>36</td>
<td>32</td>
</tr>
<tr>
<td>50</td>
<td>34</td>
<td>36</td>
<td>30</td>
</tr>
<tr>
<td>90 (mature φ6)</td>
<td>46</td>
<td>31</td>
<td>23</td>
</tr>
<tr>
<td>Expected†</td>
<td>48</td>
<td>30</td>
<td>22</td>
</tr>
</tbody>
</table>

* Ten μCi/ml of ³H-uracil (50 μCi/μmol) were added to a master culture at the time of infection. At the times indicated, 2.5 ml samples were removed and the nucleic acids extracted, treated with DNase and RNase in STM buffer, and electrophoresed on polyacrylamide gels. The values at 90 min after infection were obtained from mature phage particles released during the incubation. The values are the average of three separate experiments. The standard deviation was ±2 calculated on the assumption that the variance for each value was identical.

† Equimolar distribution calculated from the mol. wt. of the φ6 dsRNA.

To determine if the three dsRNA segments were present throughout the infection cycle in equimolar concentrations, continuously labelled virus dsRNA was extracted at various times after infection (Table I). DsRNA was also extracted from mature virus particles isolated at 90 min after infection. Since the three dsRNA segments have similar base compositions (Van Etten et al. 1974), the distribution of labelled uracil among them should be proportional to their relative concentrations. Therefore, if they occur in equimolar concentrations, the expected distribution would be 48, 30, and 22% for the large, medium, and small segments, respectively. This distribution was observed for dsRNA isolated from virus particles but not for dsRNA extracted from infected cells. Over the interval from 20 to 50 min after infection, the large segment increased from 23 to 34%, the medium segment was constant at about 36%, and the small segment decreased from 42 to 30%. Thus, more medium and small dsRNA segments are produced in the infected cell than can be incorporated into mature phage. These results are consistent with our previous observation that medium and small single-stranded RNAs (presumed to be plus strands) are also produced in greater quantities than the large plus strand (Coplin et al. 1975).

Since uracil incorporation into dsRNA was detected as early as 6 min after infection (Fig. 1a), we determined whether this early incorporation was dependent on host RNA and protein synthesis. Cultures were treated with either 200 μg of rifampin/ml or 100 μg of chloramphenicol/ml for 10 min before they were infected with φ6. At these concentrations, rifampin and chloramphenicol inhibit host RNA and protein synthesis by more than 95% within 10 min (D. L. Coplin, J. L. Van Etten & A. K. Vidaver, unpublished results). The cells were then incubated with ³H-uracil from 0 to 10 min after infection. Gel electrophoresis of the radioactivity in the dsRNA regions is shown in Fig. 1 (b to d). All three segments of the virus dsRNA were labelled in the untreated culture (Fig. 1b) as well as those from the cultures treated with chloramphenicol (Fig. 1c) and rifampin (Fig. 1d). As a control, cells were incubated with ³H-uracil during the 30 min adsorption period at 0 °C and RNA extracted without warming the culture. No incorporation of uracil into dsRNA was detected during this period. The increased uracil incorporation into φ6 dsRNAs in the presence of rifampin, as compared to the control, probably results from more ³H-uracil being available for incorporation since host RNA synthesis is prevented by the drug.
Short communications

Fig. 1. (a) Pulse labelling of φ6 dsRNA during the infection cycle. At intervals after infection, 4 ml samples were removed from a master culture and pulsed with 4 μCi/ml of 3H-uracil (18 Ci/mmol) for 5 min. The nucleic acids were extracted, treated with DNase and RNase in STM buffer, and electrophoresed on polyacrylamide gels. The radioactivity which migrated in the regions of the large (Δ—Δ), medium (■—■), and small (○—○) φ6 dsRNA segments is plotted versus time. The times represent the mid-points of the 5 min pulses. The arrow indicates when phage is first released. (b to d) The effect of chloramphenicol and rifampin on uracil incorporation into φ6 dsRNA during the first 10 min after infection. Rifampin (200 μg/ml) or chloramphenicol (100 μg/ml) were added to 8 ml cultures 10 min before phage addition. Two μCi/ml of 3H-uracil (20-4 Ci/mmol) were added at zero time and the samples chilled at 10 min after infection. The nucleic acids were extracted and treated with DNase and RNase in STM buffer prior to electrophoresis on polyacrylamide gels. Radioactive profiles of each gel are shown: (b) untreated infected control, (c) chloramphenicol treated, and (d) rifampin treated. The three peaks correspond to the region where large (L), medium (M), and small (S) dsRNA segments migrate.

Since the host DNA-directed RNA polymerase is sensitive to rifampin and host de novo protein synthesis is not required, it is likely that the initial uracil incorporation into dsRNA is due to either the modification of a host enzyme by cellular or virus proteins or an enzyme associated with the virion such as the RNA polymerase present in purified preparations of φ6 (Van Etten et al. 1973). Although we originally suggested that the RNA polymerase found in φ6 might complete regions complementary to single-stranded RNA tails present in some copies of the virion genome (Van Etten et al. 1973), more recent experiments indicate that it might be involved in the synthesis of a replicative intermediate-like structure (J. L. Van Etten, D. L. Coplin & A. K. Vidaver, unpublished data). Our current hypothesis is that the uracil incorporated in vivo into dsRNA in the first 15 min after infection is due to the synthesis of plus strands via a semiconservative mechanism; thus the nascent plus strands displace the parental plus strands from the duplex.

We thank G. A. Vidaver and M. K. Brakke for their helpful advice and R. K. Koski for
his technical assistance. This investigation was supported in part by Public Health Service Grant AI 10638 from the National Institute of Allergy and Infectious Diseases. This paper is published with the approval of the director as paper No. 3947, Journal Series, Nebraska Agricultural Experiment Station. This work was conducted under Nebraska Agricultural Experiment Station Project No. 21-21.

Department of Plant Pathology
University of Nebraska
Lincoln, Nebraska 68583
U.S.A.

REFERENCES


(Received 11 May 1976)

* Present address: Department of Plant Pathology, Ohio Agricultural Research and Development Center, Wooster, Ohio 44691, U.S.A.