Grouping of RNA Phages Based on the Template Specificity of Their RNA Replicases

(serological correlation)

T. MIYAKE, I. HARUNA, T. SHIBA, Y. H. ITOH, K. YAMANE, AND I. WATANABE

Department of Molecular Biology, School of Medicine, Keio University, Tokyo, Japan

Communicated by Hitoshi Kihara, May 18, 1971

ABSTRACT

We attempted to apply a new criterion, the template specificity of RNA replicases of *Escherichia coli* RNA phages, for the grouping of these phages. Based on the template specificity, it was shown that (a) Qβ, VK, and ST belonged to one group (group III), and SP and FI to another group (group IV), and (b) some similarity existed between groups III and IV. Considering such template specificity in addition to previously reported properties of phage particles, we could separate RNA phages into four major groups (I–IV) with subgroups a, b, and c in group III, and subgroups a and b in group IV.

In 1961, Loeb and Zinder first isolated an *Escherichia coli* RNA phage (1). Since then, similar phages that contain RNA as their genetic material have been isolated in several laboratories (2–9). We have looked for RNA phages from various sources in Japan and succeeded in isolating more than 50 strains, including phages with unique properties such as VK, ST, SP, and FI. Furthermore, we made several attempts to group these phages, based mainly on their particle properties, and proposed that there were three (I–III) or five (I–V) groups among the *E. coli* RNA phages (10–20). On the other hand, Haruna et al. found that the RNA replicates of RNA phages showed a unique template specificity for the “in vitro” synthesis of phage RNA, that is, the replicase could exclusively utilize either homologous RNA or closely related phage RNA as its template (21). Based on these findings, we started to group RNA phages based on the template specificity of their RNA replicases and to compare the results with previous groupings based on particle properties alone.

In this paper, we present results that show the efficiencies of the replicases from various RNA phages to utilize the RNA of their own or of other phages, and also a schema of grouping based on such specificities.

MATERIALS AND METHODS

RNA phages

MS2, GA, Qβ, VK, ST, SP, and FI phages were used. Strains other than MS2 (2) and Qβ (11) were isolated in our laboratory and their basic properties have been published elsewhere (10–20). According to our previous grouping experiments, MS2 belonged to group I, GA belonged to group II, Qβ, VK, and ST belonged to group III, SP belonged to either group III or IV, and FI belonged to group III or V. Furthermore, group III was divided into three subgroups (a, b, c) (22); Qβ, VK, and ST represented subgroups a, b, and c, respectively.

Bacterial strains

A strain of *E. coli* K12, Hfr Q13 (23), was used to prepare infected cells from which the RNA replicase of RNA phage...
was isolated. This strain was kindly supplied by Dr. S. Spiegelman of Columbia University, U.S.A.; it was lysogenic for λ and lacked both ribonuclease I and phosphorylase activities and also carried a *met* gene as a nutritional marker. To propagate RNA phages as the source for template RNA, *E. coli* K12 A/λ was used. This strain was a derivative of the *E. coli* K12 wild-type strain and carried both *F*<sup>+</sup> and λ-resistant characters.

The methods for preparing infected cells and template RNA, isolating and purifying replicase, assaying enzyme activity, and liquid scintillation counting on membrane filters have been reported (21).

**RESULTS**

*Template specificity of RNA replicases of various RNA phages*

To compare groupings based on the template specificity of RNA replicases with previous groupings, mainly based on the properties of phage particles, it was at first planned to isolate and determine the template specificity of replicases of group I, II, III, IV, and V phages. Because of technical reasons, however, replicases of group I and II phages were found to be difficult to isolate and purify; hence, replicases of the remaining three groups were tested for their template specificities with RNA of groups I–V phages as template. As representative phages of each group, MS2 (I), GA (II), Q<sub>8</sub> (III), ST (III), SP (III or IV), and FI (III or V) were used, and the ability of Q<sub>8</sub>, SP, and FI replicase to utilize these RNAs as template was measured by [3H]UMP incorporation into an acid-insoluble fraction. For easy comparison, the activity thus obtained was normalized to the relative activity in homologous combination (e.g., Q<sub>8</sub> replicase–Q<sub>8</sub> RNA). As shown in Fig. 1, Q<sub>8</sub> replicase utilized its own RNA as template at a high efficiency, but it could not utilize the RNA of MS2 and GA. RNA from SP and FI were certainly utilized by Q<sub>8</sub> replicase, but to a significantly less extent. The replicases of SP and FI could not utilize MS2 and GA RNA, but could utilize SP and FI RNA at a similarly high efficiency. RNA from group III phages (Q<sub>8</sub> and ST) were certainly utilized by these enzymes, but the efficiency was much lower than that with their own RNA. Importantly, no difference in the template specificity of SP and FI replicases was observed, although the properties of SP and FI particles are remarkably different. This result suggests that SP and FI have RNA of similar structure regardless of the differences in their particle properties, and can be classified into one group (IV) with subgroups a (SP) and b (FI). Similar results were observed also in group III. As reported previously, group III phages were divided into three subgroups (a, b, and c), based on their serological properties, their filtration and elution patterns, and their sensitivity to the enzyme Pronase, but the template specificity of Q<sub>8</sub> (IIa), VK (IIb), and ST (IIIc) replicases was similar, as shown in Table 1.

**Table 1. Activity of Q<sub>8</sub>(IIIa), VK(IIIb), and ST (IIIc) replicases to utilize RNA from Q<sub>8</sub>, VK, and ST**

<table>
<thead>
<tr>
<th>RNA</th>
<th>Q&lt;sub&gt;8&lt;/sub&gt;(IIIa)</th>
<th>VK(IIIb)</th>
<th>ST(IIIc)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q&lt;sub&gt;8&lt;/sub&gt;(IIIa)</td>
<td>100</td>
<td>114.2</td>
<td>96.3</td>
</tr>
<tr>
<td>VK(IIIb)</td>
<td>90.8</td>
<td>100</td>
<td>92.9</td>
</tr>
<tr>
<td>ST(IIIc)</td>
<td>92.2</td>
<td>103.6</td>
<td>100</td>
</tr>
</tbody>
</table>

*Activity is shown as the relative activity ([activity in heterologous RNA–enzyme combination/activity in homologous RNA–enzyme combination] × 100)*

**Fig. 2. A schema of grouping of *E. coli* RNA phages.** This schema is based on properties of phage particles and the template specificities of RNA replicases. *Broken lines* indicate a similarity between the groups.

**DISCUSSION**

So far, RNA phages have been grouped mainly on the basis of their serological properties. Since grouping should be based on the quality and quantity of genetic information on their genetic material, however, a new criterion that reflected the structure and function of RNA more directly would be desirable for the grouping of RNA phages. From this point of view, we started to apply the template specificity of RNA replicases for the grouping of RNA phages. The results revealed two points to be considered. The first point was that RNA phages Q<sub>8</sub>, VK, and ST belonged to one group, and SP and FI belonged to another. Previous serological groupings and grouping by template specificity agreed well for Q<sub>8</sub>, VK, and ST phages, but SP and FI were grouped in different groups according to their serological properties and in the same group according to template specificity. Such discrepancies, however, can be explained if we assume that the structure of the RNA is similar in SP and FI (because a replicase can utilize either RNA as the template with almost equal efficiency) but that the structure of the coat protein is quite different in these two phages. Of course, such differences in coat protein should be related to the structure of their RNA; however, big differences in the structure of coat protein do not necessarily require big differences in the RNA, because even a point mutation in the coat-protein cistron could strongly affect neutralization by an antibody if the mutated site was related to an amino acid that played a critical role in the neutralization reaction. From these considerations, we tentatively grouped SP and FI in the same
group, with subgroups a and b. To confirm the similarity in the structure of SP RNA and FI RNA, however, hybridization experiments using these RNAs or, more directly, biochemical analysis to determine the nucleotide sequences of these RNAs should be done.

Additionally, a similarity between groups III and IV was observed with respect to template specificity. Since the molecular mechanism of template specificity is still unknown, we cannot offer a conclusive explanation of these results.

This investigation has been aided in part by grants from the Scientific Research Fund of the Ministry of Education of Japan, the Jane Coffin Childs Memorial Fund for Medical Research, the Takeda Science Foundation, and the Waksman Foundation of Japan.