85. *Template Activity of Various Phage RNA for Replicases of Q β and VK Phages*

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Haruna *et al.* have reported^{1),2)} the isolation of two RNA replicases (RNA-dependent RNA polymerase) induced by serologically distinct^{3),4)} RNA phages (MS2 and Q β). Each enzyme exhibits a unique requirement for intact homologous template. This was the first instance of a nucleic acid polymerase (DNA or RNA) exhibiting a clear-cut requirement for a specific template. Weissmann and Feix⁵⁾ have confirmed this property with Q β replicase and August⁶⁾ found that purified Q β replicase which he prepared by a different method also responded only to Q β RNA. Further, Fiers *et al.*⁷⁾ confirmed the specific response of MS2 replicase to MS2 RNA.

We have isolated a number of RNA phages and studied their characteristics. These phages have been classified into three groups (group I, II, and III) by a serological method and a Millipore filtration method.⁸⁾ Results of analyses of inactivation by ultraviolet irradiation, stability at various pH, buoyant density, base ratio of phage RNA and tryptophan content in phage protein indicated that these chemical and physical properties of RNA phages were characteristic of each group.⁹⁾

We attempted to isolate replicases from each phage group and examine the template specificity of enzymes for various phage RNA. For this purpose, we chose several phages from each phage group for the preparation of RNA and used Q β and VK phages for the preparation of replicases. Q β and VK phages were isolated independently and belonged to the same group (group III).⁸⁾ We present the template activity of various RNA from phages which belong to group I, II, and III for replicases of Q β and VK phages which belong to group III.

Materials and methods. The bacterial viruses were isolated in our laboratory,⁸⁾ except for MS2, f2, and R17 which were kindly supplied by Dr. A. Rich, Dr. T. Watanabe, and Dr. T. Nagata, respectively. The host and assay organism is a mutant Hfr strain of *E. coli* (Q13) obtained from Dr. S. Spiegelman. All the methods of preparing infected cells, purifications of the replicase, isolation of RNA from the virus and assay for enzyme activity have been

described previously.^{2),10)} Before Q β and VK phages were used for preparation of infected cells, phage preparations were examined for the contamination of one by the other by the filtration and elution through Millipore filter and the serological tests. The labeled H³-UTP was purchased from Schwarz BioResearch, Inc. and used at a concentration of 5×10^5 cpm for each 0.2 μ moles.

Results and discussion. We isolated the replicases induced by Q β or VK phage and examined the specific template requirements of both enzymes. Table I shows the abilities of various phage RNA molecules to stimulate the Q β and VK replicases. Both enzymes are virtually inactive with RNA molecules from group I and II phages at either level of RNA input. On the other hand, RNA molecules from the homologous phages (group III) are active as templates and equally effective in stimulating the reaction. We could not find the difference in the specific template requirement of Q β and VK replicases. It would appear from these data that the group specificity for templating activity is completely confirmed

Table I. Template specificity of Q β and VK replicases
(H³-UMP incorporation into acid-insoluble fraction)

Enzyme		Q β replicase		VK replicase	
Group	Template RNA	1 μ g	2 μ g	1 μ g	2 μ g
I	R17	7 cpm	0 cpm	0 cpm	32 cpm
	Z R	122	107	17	49
	MS2	6	18	49	32
	f2	17	3	0	28
	MY	42	78	19	0
	G R	29	0	13	44
II	E I	145	132	22	63
	K J	145	62	0	16
	G A	53	84	69	11
	S D	0	0	11	15
	S W	0	18	24	0
III	NH	2645	3092	834	1056
	NM	2440	2956	849	991
	S T	2499	3102	962	1052
	Q β	3247	3366	1109	1159
	VK	2430	3058	859	1015
—		11		19	

Assays were carried out as described previously.²⁾ Thirty μ g of Q β replicase or 15 μ g of VK replicase was added in reaction mixture (0.25 ml) which was run for 20 min at 35°C.

Table II. Comparison between template specificity of replicase and grouping of RNA phages based on serological properties

Group		I						II					III				
Phage		MS2	f2	MY	ZR	GR	R17	GA	SW	EI	KJ	SD	Q β	VK	NM	NH	ST
Anti-serum sensi- tivity*	Anti-MS2	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-
	Anti-GA	-	-	-	-	-	-	+	+	+	+	+	-	-	-	-	-
	Anti-Q β	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+
Tem- plate activi- ty**	Q β replicase	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+
	VK replicase	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+
	MS2 replicase ²⁾	+											-				

* + indicates inactivation by antiserum.

** + indicates that RNA molecule is active as template.

among three groups. Table II shows the group specificity for the template requirement of replicases as compared to the grouping based on the serological property. This table indicates that the group specificity for template requirement is in good accordance with the serological grouping.

The RNA molecules from group I and II phages could not serve as templates for group III enzymes. This fact probably implies that the replicases induced by phages which belong to group I and II are different from enzymes of group III phages. To confirm this, studies on replicases of group I and II phages are now in progress.

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