[9] *Ibid.*, 1029.
[10] Bhuyan, B. K., and F. J. Simpson, *Can. J. Microbiol.*, **8**, 737 (1962).
[11] Fromm, H. J., *J. Biol. Chem.*, **233**, 1049 (1958).
[12] Wood, W. A., M. J. McDonough, and L. B. Jacobs, *J. Biol. Chem.*, **236**, 2190 (1963).
[13] Hulley, S. B., S. E. Jorgensen, and E. C. C. Lin, *Biochim. Biophys. Acta*, **67**, 219 (1963).
[14] Lampen, J. O., *Abstracts*, National Meeting of American Chemical Society, 1954, p. 44c.
[15] Simpson, F. J., M. J. Wolin, and W. A. Wood, *J. Biol. Chem.*, **230**, 457 (1958).
[16] Simpson, F. J., and W. A. Wood, *J. Biol. Chem.*, **230**, 473 (1958).
[17] Neish, A. C., and F. J. Simpson, *Can. J. Biochem. Physiol.*, **32**, 147 (1954).
[18] Altermatt, H. A., F. J. Simpson, and A. C. Neish, *Can. J. Biochem. Physiol.*, **33**, 615 (1955).
[19] Wolin, M. J., F. J. Simpson, and W. A. Wood, *J. Biol. Chem.*, **232**, 559 (1958).
[20] Cabib, E., in *Annual Review of Biochemistry*, ed. J. M. Luck (1963), p. 346.
[21] Mortlock, R. P., and W. A. Wood, *Bacteriol. Proc.*, 110 (1963).
[22] Mortlock, R. P., and W. A. Wood, *J. Bacteriol.*, **88**, 845 (1964).
[23] Lin, E. C. C., S. A. Lerner, and S. E. Jorgensen, *Biochim. Biophys. Acta*, **60**, 422 (1962).
[24] Mortlock, R. P., D. Fossitt, D. H. Petering, and W. A. Wood, *J. Bacteriol.*, **89**, 129 (1965).
[25] Bonnichsen, R. K., and H. Theorell, *Scand. J. Clin. Lab. Invest.*, **3**, 58 (1951).
[26] Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall, *J. Biol. Chem.*, **193**, 265 (1951).
[27] Dische, Z., and E. Borenfreund, *J. Biol. Chem.*, **192**, 583 (1951).
[28] Mortlock, R. P., D. Fossitt, and W. A. Wood, *Bacteriol. Proc.*, 95 (1964).
[29] Fossitt, D., R. P. Mortlock, and W. A. Wood, *Bacteriol. Proc.*, 82 (1965).
[30] Dowd, J. E., and D. S. Riggs, *J. Biol. Chem.*, **240**, 863 (1965).
[31] Moore, R. O., and Claud A. Villee, *Science*, **142**, 389 (1963).
[32] Camyre, K. P., and R. P. Mortlock, *J. Bacteriol.*, in press.
[33] Lerner, S. A., T. T. Wu, and E. C. C. Lin, *Science*, **146**, 1313 (1964).
[34] Anderson, R. L., and D. P. Allison, *J. Biol. Chem.*, **240**, 2367 (1965).

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*SPECIFIC TEMPLATE REQUIREMENTS OF RNA REPLICASES**

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We have previously[1] reported the isolation of an RNA-dependent RNA polymerase (termed a "replicase" for brevity) from *E. coli* infected with the RNA bacteriophage MS-2. The purified enzyme showed a mandatory requirement for added RNA and, furthermore, exhibited a unique preference for its homologous RNA. Ribosomal and sRNA of the host could not substitute as a template and neither of these cellular RNA types showed any ability to interfere with the template function of the viral RNA.

We pointed out[1] that the ability of the replicase to discriminate solved a crucial problem for an RNA virus attempting to direct its own duplication in an environment replete with other RNA molecules. By producing a polymerase which ignores the mass of pre-existent cellular RNA, a guarantee is provided that replication is *focused on the single strand of incoming viral RNA*, the ultimate origin of progeny.

It seems worth noting that sequence recognition by the enzyme can be of value not only to the virus but also to the investigator. The search for viral RNA replicases must perforce be carried out in the midst of a variety of highly active cellular

polymerases capable of synthesizing polyribonucleotides. If the enzyme finally isolated possesses the appropriate template requirement, a comforting assurance is furnished that the effort expended, and the information obtained, are indeed relevant to an understanding of viral replication. Operationally, this view demands that viral RNA be used at all fractionation stages in assaying for polymerase activity.

Our line of reasoning led to the expectation that RNA replicases induced by other RNA viruses would show a similar preference for their homologous templates. An opportunity to test the validity of this prediction came with the isolation of a new and unrelated[3, 4] RNA bacteriophage ($Q\beta$) in the laboratory of Professor I. Watanabe. A modification of the procedure employed to isolate the MS-2 replicase sufficed to yield a highly purified and active $Q\beta$ replicase.

It is the primary purpose of the present paper to detail the pertinent properties of the purified enzyme. Comparison of the MS-2 and $Q\beta$ enzymes isolated from the same host confirms the predicted requirement for homology. Each replicase recognizes the RNA genome of its origin and requires it as a template for normal synthetic activity.

Materials and Methods.—(1) *Bacteria and viruses:* The bacterial viruses employed are MS-2 (originally obtained from Dr. A. J. Clark) and $Q\beta$ (kindly provided by Professor Watanabe). It is essential for any laboratory working with both MS-2 and $Q\beta$ to monitor continually for contamination of one by the other. Fortunately there is virtually no serological cross reaction between the two,[3, 4] so that the appropriate antisera can be used for identification and purity checks. The host and assay organism is a mutant Hfr strain of *E. coli* (Q-13) isolated in the laboratory of W. Gilbert by Diane Vargo. It has the convenient property of lacking[5] ribonuclease I and RNA phosphorylase. Preparation of virus stocks and purified RNA followed the methods of Doi and Spiegelman.[2]

(2) *Preparation of infected cells:* The basic medium employed for growing infected cells and producing virus contained the following in grams per liter: NH_4Cl, 1; $MgSO_4 \cdot 7\ H_2O$, 0.06; gelatin 1×10^{-5}; casamino acids (vitamin-free), 15; glycerol, 30; to this is added after separate autoclaving 7 ml of 0.1 *M* $CaCl_2$ and 10 ml containing 4 gm of $Na_2HPO_4 \cdot 7\ H_2O$ and 0.9 gm KH_2PO_4. Lysates in liter quantities are first prepared to be used for infection of larger volumes of cell suspensions. These are obtained by infecting log phase cultures (OD_{660} of 0.25) with a purified phage preparation at a multiplicity of about 5. They are incubated while shaking at 37°C until lysis is complete and then monitored for titer and purity of the phage. Such lysates can be stored frozen at −17°C indefinitely and thawed just prior to use. In general, 35-liter quantities of cells are grown up in carboys to an OD_{660} of between 0.275 and 0.290. The temperature in the carboys is 34°C, while the temperature of the water bath in which they are immersed is maintained at 37°C. When the cells reach an OD_{660} of 0.275, they are infected with virus at a multiplicity of between 10 and 50 and allowed to aerate for mixing for 1 min. The aeration is interrupted for 10 min for absorption, reinstituted, and the incubation continued. At 25 min sufficient sucrose and magnesium are added to give final concentrations of 18% and 0.01 *M*, respectively. After another 5 min the process is terminated by the addition of crushed ice. The cells are harvested in a Sharples centrifuge and stored at −14°C, at which temperature ability to yield active enzyme is retained for periods exceeding 6 months. Uninfected cells are prepared and stored in the same manner. To provide uniform preparations for enzyme isolation, the cells are thawed sometime prior to use and resuspended (20 gm of packed cells in 100 ml) in a solution containing 0.01 *M* Tris buffer pH 7.4, 0.001 *M* $MgCl_2$, and 0.0005 *M* mercaptoethanol and 5 μg/ml of DNase. After thorough resuspension with a magnetic stirrer at 4°C, the suspension is divided into convenient aliquots in plastic tubes, frozen, and stored at −14°C.

(3) *Radioactive substrates:* P^{32}-labeled ribonucleoside monophosphates were synthesized as described previously.[1] The labeled mononucleotides were converted enzymatically to the corresponding ribonucleoside triphosphates by a kinase preparation isolated[6] from *E. coli*.

(4) *Chemical and biological reagents:* Unlabeled riboside triphosphates were from P-L Bio-

chemicals, Inc., Milwaukee, Wisconsin. DNase, 2 × recrystallized, was from Worthington Biochemical Company, Freehold, New Jersey; it was further purified on DEAE columns to remove contaminating ribonuclease. Phosphoenolpyruvate (PEP) and the corresponding kinase (PEP kinase) were purchased from Calbiochem, Inc., Los Angeles, California; lysozyme from Armour and Company, Kankakee, Illinois; and protamine sulfate from Eli Lilly, Indianapolis, Indiana. The turnip-yellow-mosaic-virus (TYMV-RNA) and the "satellite virus" of the tobacco-necrosis-virus (STNV-RNA) were both kindly provided by Dr. E. Reichmann of the Botany Department at the University of Illinois.

(5) *Preparation of enzyme:* The following procedure is described for 20 gm of packed cells. The frozen cell suspension (120 ml) is thawed and to this is added 0.5 mg/ml of lysozyme, following which the mixture is frozen and thawed twice, using methanol and dry ice as the freezing mixture. To the lysate are added 0.9 ml of 1 *M* $MgCl_2$ and 2.5 μg/ml DNase, and the resulting mixture is incubated for 10 min in an ice bath. The extract is then centrifuged for 20 min at 30,000 × *g* and the supernate removed. The pellet is transferred to a prechilled mortar, ground for 5 min, and then resuspended in 30 ml of the same buffer as used for the cell suspension except that the magnesium concentration is raised to 0.01 *M* to increase the effectiveness of the DNase digestion. The extract is then centrifuged at 30,000 × *g* for 20 min and the two supernates are combined, adjusted to 0.01 *M* EDTA (previously brought to pH 7.4), and incubated at 0°C for 5 min. Insoluble proteins appear and are removed by centrifugation at 30,000 × *g* for 20 min. At this stage, a typical active infected extract has an OD_{260} of between 150 and 180. Lower values commonly signal a poor infection with a resulting low yield of enzyme. To the cleared supernatant fluid is added 0.01 mg of protamine sulfate for each OD_{260} unit. After 10 min the precipitate, containing virtually all the enzyme activity, is collected by centrifugation at 12,000 × *g* for 10 min. It is dissolved in 12 ml of "standard buffer" (0.01 *M* tris buffer, pH 7.4; 0.005 *M* $MgCl_2$; 0.0005 *M* mercaptoethanol), adjusted to 0.4 *M* $(NH_4)_2SO_4$, and allowed to stand overnight at 0°C. This period of waiting is important for the subsequent fractionation since complete disaggregation was found to be essential for acceptable separation of the replicase from transcriptase. The extract is diluted with 24 ml of standard buffer, and after 20 min is centrifuged at 30,000 × *g* for 20 min; for each 40 ml of supernatant are added 12 ml of a 0.5% solution of protamine sulfate. The precipitate which forms contains virtually all of the DNA-dependent RNA polymerase along with an RNA-independent RNA polymerizing activity. The RNA replicase remains in the supernatant and begins to show good dependence on added RNA. [*Note:* This is one of the critical steps in the fractionation, and any variation in host, medium, time, or temperature of infection modifies the amount of protamine required to achieve separation. It is often safer to titrate small aliquots and determine the amount of protamine needed by appropriate assays.] After 10 min the precipitate is removed by centrifugation at 12,000 × *g* for 10 min. To the resulting supernate is added an equal volume of saturated ammonium sulfate (saturated at 0°C and adjusted to pH 7.0 with ammonium hydroxide). After 10 min at 0°C the precipitate is collected by centrifugation at 12,000 × *g* for 10 min and dissolved in 4 ml of standard buffer containing 0.4 *M* ammonium sulfate. The resulting solution is then dialyzed against 1 liter of standard buffer for 1.5 hr. The dialyzed fraction is adjusted to 0.05 *M* ammonium sulfate with standard buffer and passed through a DEAE column (1.2 × 10 cm) which is washed with 100 ml of standard buffer just prior to use. After loading the protein, the column is washed with 40 ml of standard buffer containing 0.12 *M* NaCl which removes protamine, a poly-A synthetase,[7] and residual K-dependent ribonuclease. The enzyme is then eluted with 35 ml of standard buffer containing 0.20 *M* NaCl. To fractions possessing enzyme activity, saturated ammonium sulfate is added to make the final solution 10% saturation. At this stage, the enzyme preparation has an OD_{280}/OD_{260} ratio of 1.35 and usually contains 1 mg of protein per ml. Under the ionic conditions specified, no loss in activity is observed over a month of storage at 0°C.

(6) *The standard assay—assay of enzyme activity by incorporation of radioactive nucleotides:* The standard reaction volume is 0.25 ml and, unless specified differently, contains the following in μmoles: tris HCl pH 7.4, 21; magnesium chloride, 3.2 (when included, manganese chloride, 0.2); CTP, ATP, UTP, and GTP, 0.2 each. The enzyme is usually assayed at a level of 40 μg of protein in the presence of 1 μg of RNA template. The reaction is run for 20 min at 35°C and terminated in an ice bath by the addition of 0.15 ml of neutralized saturated pyrophosphate, 0.15 ml of neutralized saturated orthophosphate, and 0.1 ml of 80% trichloroacetic acid. The pre-

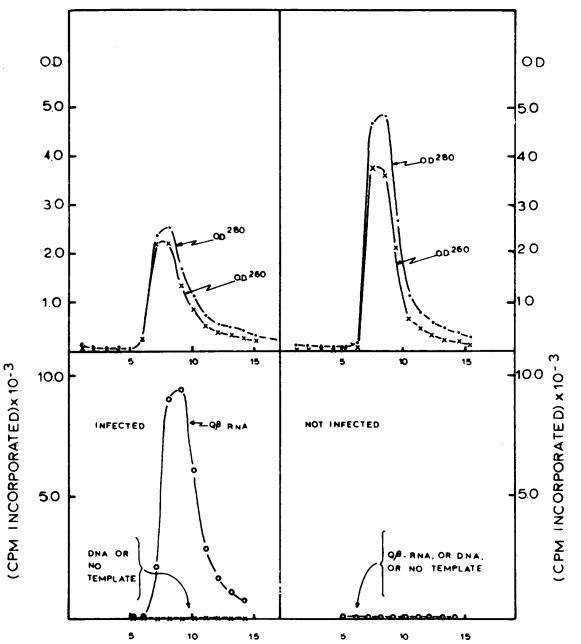

FIG. 1.—Chromatography on DEAE of second protamine supernatant. Just before collection of fraction no. 1, 35 ml of 0.2 *M* NaCl in standard buffer was placed on the column. Prior to this, the column had been washed with 0.15 *M* as described in *Methods*, (5). It will be noted that the peak of enzyme activity is found in the descending shoulder of the OD profile. Rechromatography yields coincidence of the two.

cipitate is transferred to a membrane filter and washed seven times with 5 ml of cold 10% TCA. The membrane is then dried and counted in a liquid scintillation counter as described previously.[1] This washing procedure brings zero time counts to less than 80 cpm with input counts of 1×10^6 cpm. The specific activities of the labeled triphosphates added were adjusted so that with the efficiency employed, 1×10^6 cpm corresponds to 0.2 μmoles of the corresponding triphosphate.

Results—(A) *Properties of the purified Qβ replicase:* Figure 1 compares the elution profiles of protein and enzyme activity of preparations [see *Methods*, (5)] derived from infected and noninfected cells.

Infected preparations exhibit a polymerase activity which elutes with 0.2 *M* NaCl, responds excellently to added Qβ-RNA, and is devoid of the DNA-dependent RNA polymerase. The complete dependence on Qβ-RNA shows that the enzyme contains no nucleic acid which can serve to stimulate the enzyme to activity within the period of assay. A corresponding fraction from uninfected cells shows similar elution properties but possesses no detectable RNA-synthesizing ability.

TABLE 1

ASSAY FOR RIBONUCLEASE AND PHOSPHORYLASE

	C^{14}-ADP incorporation (30 min), cpm	% H^3-RNA Hydrolyzed in 30 Min		
		0.25 *M* K^+	0.25 *M* Na^+	Neither
Before DEAE	40*	41	12	<2
After DEAE	32*	<2	<2	<2

* Similar preparations from wild type incorporate 30,000 cpm (equivalent to 480 μmoles) before DEAE step and about half that after elution from the column.

The assay for phosphorylase was carried out as described by August *et al.*[11] Each reaction (0.25 ml) contained 40 μg protein and 1 μmole of C^{14}-ADP at 6×10^4 cpm. For the ribonuclease, 10 μg at 1×10^4 cpm were added per reaction mixture.

TABLE 2

EFFECT OF NUCLEASES AND ENERGY GENERATING SYSTEM ON Qβ REPLICASE

Addition	Template	Cpm incorporated
PEP (1.0 μmole) + PEP kinase (10 μg)	Qβ	4803
	—	96
DNase (5 μg)	Qβ	3938
	—	45
RNase (1 μg)	Qβ	19
	—	37
None	Qβ	4142
	—	75

Except for the additions noted, the assays were carried out under the standard conditions described in *Methods*, (6).

TABLE 3

REQUIREMENTS OF Qβ REPLICASE

Assay mixture	UMP (or AMP) incorporation, μμmoles
Complete	390
−Qβ-RNA	7
−GTP	5
−Mg, −Mn	8

Conditions of assay are those described in *Methods*, (6), except that Mn^{++} was included at 0.2 μmoles per 0.25 ml.

Table 1 shows that after the DEAE step the enzyme is completely devoid of ribonuclease I, phosphorylase, and the K^+-stimulated ribonuclease. The presence of the energy-generating system (PEP + PEP kinase) has little effect on the reaction (Table 2), indicating freedom from interfering enzymes which can destroy riboside triphosphates. Finally, DNase has no influence on the reaction, whereas the presence of even small amounts of pancreatic ribonuclease completely eliminates the net synthesis of polyribonucleotide.

The enzyme system requires not only template but, in addition, all four riboside triphosphates (Table 3). The replicase has an absolute requirement for divalent ions, magnesium being the preferred ion with homologous RNA. Manganese substitutes partially (10%) and induces interesting changes in the nature of the reaction, the details of which will be described elsewhere.[17]

Figure 2 shows the kinetics observed in a reaction mixture containing saturating amounts of template (1 μg RNA per 40 μg of protein). Continued synthesis is observed at 35°C for periods exceeding 5 hr. It will be noted that in 2 hr the amount of RNA synthesized corresponds to 5 times the input template. By variation in the amount of RNA added and the time permitted for synthesis, virtually any desired amount of increase of the starting material has been achieved. The cessation of synthesis within 5–10 min reported by others[8–11] for presumably similar preparations has been observed by us only in the early stages of purification. Figure 3 examines the effect of added amounts of protein at a fixed level of template (5 μg RNA/0.25 ml). It is evident that the reaction responds linearly, indicating the absence of interfering contaminants in the purified enzyme.

(B) *Specific template requirements of the replicases:* We now turn our attention to the primary question which the present study sought to resolve. Table 4 records the abilities of various RNA molecules to stimulate the Qβ replicase to synthetic activity at the saturation concentration (1 μg) of homologous RNA and twice this level. The response of the Qβ replicase is in accord with that reported[1] for the MS-2 replicase, the preference being clearly for its own template. The only heterologous RNA showing detectable activity is TYMV and, at the 2-μg level, it supports a synthesis corresponding to 6 per cent of that observed with the homologous Qβ-RNA. Both of the heterologous viral RNA's, MS-2, and STNV are completely inactive and, again, so are the ribosomal and transfer RNA species of the host cell (*E. coli* Q-13). As might be expected, bulk RNA from infected cells shows some

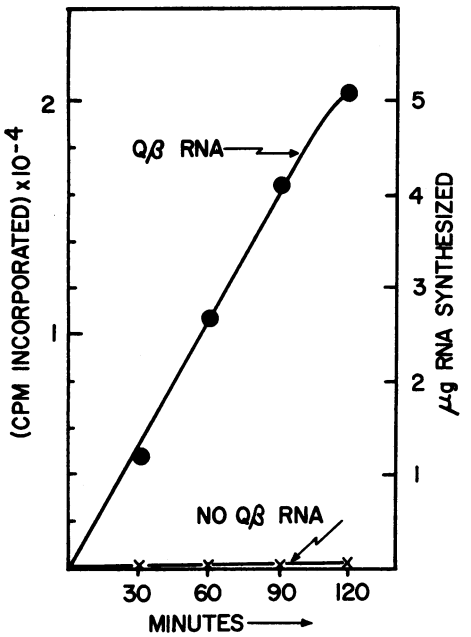

FIG. 2.—Kinetics of replicase activity. Each 0.25 ml contained 40 μg of protein and 1 μg of Qβ-RNA. All other conditions are as specified in *Methods*, (6). The specific activity of the UTP^{32} was such that the incorporation of 4,000 cpm corresponds to the synthesis of 1 μg of RNA.

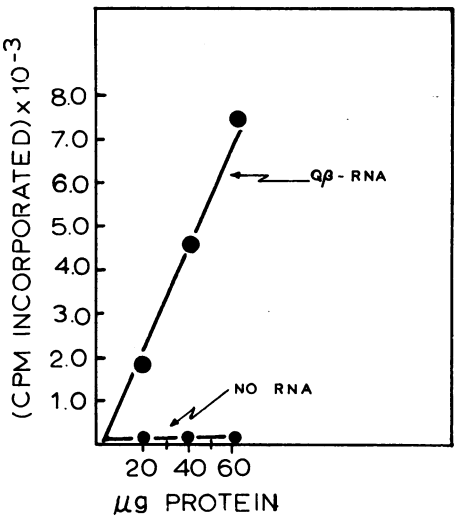

FIG. 3.—Response to added protein. Assays were run for 20 min at 35°C under conditions specified in *Methods*, (6). Again, the incorporation of 4,000 cpm corresponds to the synthesis of 1 μg of RNA.

templating activity which increases as the infection is allowed to progress. There is no detectable DNA-dependent RNA polymerase activity.

To permit a definitive comparison of the two replicases derived from the same host, the MS-2 enzyme was isolated from appropriately infected Q-13. The purification of the MS-2 replicase followed precisely the same protocol as described for the Qβ enzyme [*Methods*, (5)] except that 0.22 M NaCl is used for elution from the DEAE column.

The results of the comparison between the two replicases are shown in Table 5 and they are satisfyingly clear-cut. The MS-2 replicase shows no evidence of accepting the Qβ-RNA as a template at either level of RNA input. Similarly, the Qβ replicase completely ignores the MS-2 RNA while functioning quite well on its own template. It would appear from these data that the prediction of template specificity is completely confirmed.

Discussion.—(1) *State of the enzyme and the method of purification:* It is possible to isolate comparatively pure replicase from suitably infected wild-type Hfr strains.[1] However, the use of the mutant Q-13 offered an obvious advantage for the purification of the replicase since the crude extract was already free of ribonuclease I and phosphorylase. The two remaining interfering activities were due to the DNA-dependent RNA polymerase (transcriptase) and a potassium-stimulated ribonuclease.

It is important to emphasize that the complete removal of the transcriptase is

TABLE 4

RESPONSE OF Qβ REPLICASE TO DIFFERENT TEMPLATES

Template	Input Levels of RNA 1 μg	2 μg
Qβ	4929	4945
TYMV	146	312
MS-2	35	26
Ribosomal RNA	45	9
sRNA	15	57
Bulk RNA from infected cells	146	263
Satellite virus	61	51
DNA (10 μg)	36	

Conditions of assay are those specified in *Methods*, (6). However, as in all cases, assay for DNA-dependent activity is carried out at 10 μg of DNA per 0.25 ml of reaction mixture. Control reactions containing no template yielded an average of 30 cpm.

TABLE 5

TEMPLATE SPECIFICITY OF TWO RNA REPLICASES

Enzyme	RNA Templates: MS-2 RNA 1 μg	MS-2 RNA 2 μg	Qβ-RNA 1 μg	Qβ-RNA 2 μg
MS-2	4742	4366	0	56
Qβ	36	65	2871	3731

Conditions of assay are those specified in *Methods*, (6), with Mn^{++} present at 0.2 μmoles per 0.25 ml.

essential if questions of mechanism and replicase specificity are to be answered without ambiguity. The transcriptase *can employ any RNA as templates for RNA synthesis*[13, 14] and, in the process, forms a ribonuclease-resistant structure. Consequently, the use of DNase or actinomycin D does not ensure against confusion with transcriptase activity. In the fractionation procedure described [*Methods*, (5)], most of the transcriptase is removed in the precipitate fraction of the second protamine step. The remainder is left behind as a late component in the DEAE column. The potassium-dependent nuclease is tightly bound to cell membrane fragments which are discarded in the low-speed fraction by our comparatively gentle freeze-thaw method of cell rupture. The small amount of ribonuclease that does contaminate the extract is removed from the DEAE column by washing with 0.12 *M* NaCl which at the same time eliminates protamine and a poly-A-synthesizing enzyme.[7] The resulting freedom from interfering and confounding activities makes it possible to study the replicase in a simple mixture containing only the required ions, substrates, and template.

(2) *Comparison with other viral-induced enzymes:* We may summarize the distinctive properties of the purified replicases described above as follows: (*a*) complete dependence on added RNA; (*b*) competence for prolonged (more than 5 hr) synthesis of RNA; (*c*) ability to synthesize many times the input template; (*d*) saturation at low levels of RNA (1 μg RNA per 40 μg protein); and (*e*) virtually exclusive requirement for homologous template under optimal ionic conditions.

In view of the very different states of purity, it is difficult to interpret the differences in the properties observed with the enzymes reported here as compared with those detected[15] or isolated[8–12] by others. Activity independent of added RNA[8, 9] suggests that the purification has not achieved removal of contaminating RNA and, in any case, precludes examination of template specificity. August *et al.*[10–12] isolated an enzyme from *E. coli* infected with a mutant of f2 which is stimulated by a variety of RNA species, including host ribosomal and sRNA. It is conceivable that the f2-replicase is nonspecific. However, the August preparation requires 20 μg of RNA for each μg of protein, whereas the enzymes described here are fully saturated at 0.025 μg per μg of enzyme protein. The authors point out[11] that the inordinately large amounts of RNA required may be due to the detectable con-

tamination of their preparation with ribonuclease. Under the circumstances, one should withhold judgment on the significance of the apparent lack of specificity since it is open as yet to a rather trivial explanation.

(3) *Implications of template specificity requirement:* The comparison of the two purified replicases reported establishes that each requires its homologous template. The experiment with the satellite-RNA (STNV) was a particularly interesting challenge. Reichmann[16] showed that the satellite virus contains only enough RNA to code for its own coat protein, which suggests that it must employ the replicase of the companion virus (TNV) for its multiplication. This implies either that the satellite is related in sequence to the TNV virus, or that it possesses a feature permitting it to employ any viral RNA replicase. The fact that STNV-RNA did not serve as a template for either one of the two purified replicases implies that the answer will be found in at least partial sequence homology between STNV and TNV genomes, a prediction open to experimental test.

The specificity relations exhibited raise the question of the mechanism used by the replicase to distinguish its template from other RNA molecules. The involvement of a beginning sequence is an obvious possibility. However, as will be shown,[17] the recognition mechanism is even more subtle, being designed to avoid replication of fragments of its own genome even if they contain the beginning sequence.

It should be evident that the replicases are approaching a state of purity permitting the performance of unambiguous experiments which can hope to illuminate the mechanism[17] of the RNA replicative process.

Summary.—Two RNA replicases induced in the same host by unrelated RNA bacteriophages have been purified and their responses to various RNA molecules examined. Under optimal ionic conditions both are virtually inactive with heterologous RNA, including ribosomal and sRNA of the host. Neither replicase can function with the other's RNA. Each recognizes the RNA genome of its origin and requires it as a template for synthetic activity. This discriminating selectivity of its replicase is of obvious advantage to a virus attempting to direct its own duplication in a cellular environment replete with other RNA molecules.

We should like to express our deep appreciation to Dr. I. Watanabe for making available to us the Qβ virus which made possible the informative comparison reported. Similarly our profound thanks are due to Drs. W. Gilbert and J. D. Watson and to Miss Diane Vargo for providing the mutant Q-13. Finally, we are grateful to Mrs. Louise Jordan for skillful assistance in the performance of the experiments.

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[1] Haruna, I., K. Nozu, Y. Ohtaka, and S. Spiegelman, these PROCEEDINGS, **50**, 905–911 (1963).

[2] Doi, Roy H., and S. Spiegelman, these PROCEEDINGS, **49**, 353–360 (1963).

[3] Watanabe, I., *Nihon Rinsho*, **22**, 243 (1964).

[4] Overby, L., G. H. Barlow, R. H. Doi, M. Jacob, and S. Spiegelman, *J. Bacteriol.*, in press.

[5] Gesteland, R. F., *Federation Proc.*, **24**, 293 (1965). (Q-13 is a derivative of A-19, a RNase negative mutant reported here.)

[6] Bresler, A. E., *Biochim. Biophys. Acta*, **61**, 29–33 (1962).

[7] August, J. T., P. J. Artiz, and J. Hurwitz, *J. Biol. Chem.*, **237**, 3786–3793 (1962).

[8] Weissmann, C., P. Borst, R. H. Burdon, M. A. Billeter, and S. Ochoa, these PROCEEDINGS, **51**, 682 (1964).

[9] Weissmann, C., L. Simon, and S. Ochoa, these PROCEEDINGS, **49**, 407–414 (1963).

[10] August, J. T., S. Cooper, L. Shapiro, and N. D. Zinder, in *Cold Spring Harbor Symposia on Quantitative Biology*, vol. 28 (1963), p. 99.
[11] August, J. T., L. Shapiro, and L. Eoyang, *J. Mol. Biol.*, **11**, 257–271 (1965).
[12] Shapiro, L., and J. T. August, *J. Mol. Biol.*, **11**, 272–284 (1965).
[13] Krakow, J. S., and S. Ochoa, these PROCEEDINGS, **49**, 88–94 (1963).
[14] Nakamoto, T., and S. B. Weiss, these PROCEEDINGS, **48**, 880–887 (1962).
[15] Baltimore, D., and R. M. Franklin, *Biochem. Biophys. Res. Commun.*, **9**, 388–392 (1963).
[16] Reichmann, M. E., these PROCEEDINGS, **52**, 1009–1017 (1964).
[17] Haruna, I., and S. Spiegelman, manuscript in preparation.

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*INCORPORATION OF GLYCINE INTO THE CELL WALL GLYCOPEPTIDE IN STAPHYLOCOCCUS AUREUS: ROLE OF sRNA AND LIPID INTERMEDIATES**

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The synthesis of the cell wall glycopeptide in *Staphylococcus aureus* and *Micrococcus lysodeikticus* from its precursors, UDP-acetylglucosamine (UDP-GlcNAc) and UDP-acetylmuramyl·L-ala·D-glu·L-lys·D-ala·D-ala (UDP-MurNAc-pentapeptide), has been under investigation in several laboratories.[1–4] The product of this reaction is a linear polymer consisting of alternating GlcNAc- and MurNAc-pentapeptide residues. A unique feature of this reaction mechanism is the fact that UMP and Pi are formed from one of the substrates while UDP is formed from the other.[2, 3] This finding led to the observation that MurNAc(-pentapeptide)-P-lipid and GlcNAc-MurNAc(-pentapeptide)-P-lipid are intermediates in the reaction.[3] This sequence is presumably a means by which the intracellular nucleotides are transported through the membrane for the synthesis of an extracellular product, the cell wall. It also ensures the alternation of the sugars in the glycopeptide product.

The biosynthesis of the cell wall of *S. aureus* also requires the formation of polyglycine cross bridges which link glycopeptide chains in a two- or three-dimensional network, attached at one end to the ϵ-amino groups of lysine and at the other to D-alanine, the carboxyl terminus of the cell wall tetrapeptide.[5–7] An important clue to the mechanism of formation of these bridges was the observation that an ATP and uridine nucleotide-requiring system which incorporated glycine into an acid-precipitable product was RNase-sensitive.[1] The product was incompletely characterized, however, and appeared to be lysozyme-insensitive.

With enzyme particles prepared after disintegration of cells of *S. aureus* with alumina and by incubation at low temperature, an extremely efficient synthesis of glycopeptide is obtained.[3] Under these conditions polyglycine chains can also be added to the ϵ-amino groups of lysine in the glycopeptide. In the present paper the mechanism of this reaction will be described. Glycine is activated as glycyl-sRNA which then transfers glycine to the recently described lipid intermediates in