map but does overlap the distinct sources at declinations of 41.8°, 42.6°, and 42.8° and may be an instrumental effect due to the fan-shaped beam of Kraus's instrument at Ohio State University.

The cluster of sources on the VRO map near 00^h48^m and 42°45′ does not appear to be present on the 1415-Mc/sec map by Kraus (*5*), but an increase of emission near that position is apparent on his 600-Mc/sec map. This difference suggests that these sources may have a steep spectral index. The dwarf galaxy NGC 205 lies in a slight hollow of emission but the depression is not great enough to be shown by another contour.

The radio emission from M 31 does not appear to extend along the major axis much beyond the optical outline. There does seem to be, however, a rather irregular emission structure extending beyond the optical outline along the approximate direction of the minor axis. The general elongation of the weak contours in the east-west direction may be partly due to the difficulty of preserving absolute calibration of the antenna from one scan to another on adjacent declinations. However, some of the effect appears to be real, and a similar elongation is noticeable on the 38-Mc/sec map by Kenderdine and Baldwin (*6*) and also slightly on the 1415-Mc/sec map by Kraus (*5*).

J. R. DICKEL
J. M. MACLEOD*
G. W. SWENSON, JR.
Vermilion River Observatory, University of Illinois, Urbana

References and Notes

1. J. M. MacLeod, *Science* **145**, 389 (1964).
2. H. C. Arp, *Astrophys. J.* **139**, 1045 (1964).
3. ———, *Science* **145**, 952 (1964).
4. M. S. Large, D. S. Mathewson, C. G. T. Haslam, *Nature* **183**, 1250 (1959).
5. J. D. Kraus, *ibid.* **202**, 269 (1964).
6. S. Kenderdine and J. E. Baldwin, *Observatory* **85**, 24 (1965).
7. We thank Prof. G. C. McVittie for his advice and encouragement and K. S. Yang and A. G. Beveridge for assistance in the preparation of the map. Supported by the Office of Naval Research under contract Nonr 1834(22).
* Present address: National Research Council, Ottawa, Ontario, Canada.

15 July 1965

Autocatalytic Synthesis of a Viral RNA in vitro

Abstract. *Experiments with an RNA-dependent RNA polymerase ("replicase") purified from* Escherichia coli *infected with an RNA bacteriophage (Qβ) demonstrate that the enzyme generates a polynucleotide of the same molecular weight as viral RNA; the "replicase" cannot distinguish the polynucleotide from its own RNA genome. By starting reactions at input ratios below the saturation levels of template to enzyme, autocatalytic kinetics of RNA increase are observed. The data are consistent with the conclusion that self-propagation of complete viral genomes is occurring in this simple system.*

We have reported the purification of RNA-dependent polymerases (termed "replicases") from *Escherichia coli* infected with the RNA bacteriophages MS-2 and Qβ. (*1*, *2*). Both enzymes were purified to the point where they exhibited a requirement for added RNA. Since the two bacteriophages are unrelated (*3*) we tested the expectation (*1*) that virus-induced "replicases" would show a preference for their homologous RNA. Comparison of the MS-2 and Qβ "replicase" isolated from the same host confirmed (*2*) the predicted requirement for homology. Neither enzyme can function with the other's RNA, nor can it function with a host of other RNA species, including the host ribosomal and sRNA (*4*) varieties. Each "replicase" recognizes the RNA genome of its origin and requires it as a template for effective synthetic activity.

The discriminating selectivity of the Qβ "replicase" for its homologous RNA is illustrated in Table 1, which records the response to a variety of heterologous RNA species and authentic Qβ-RNA. The second column of the table shows that the heterologous RNA molecules, in addition to being inactive as templates, are unable to interfere significantly with the template function of Qβ-RNA. It is evident that the "replicase" can be used as a diagnostic tool to detect homology to its own genome.

The analysis of a presumed replicating reaction centers on the nature of the product. If the concern is with the synthesis of a viral nucleic acid, data on base composition and nearest neighbors, while of interest, are hardly decisive. The crucial issue is whether replicas are being produced. To answer this question, information on the sequence of the synthesized RNA is required. Evidence of similarity between template and product would provide as-

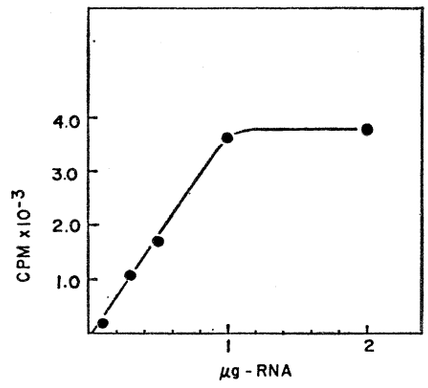

Fig. 1. Template saturation of "replicase." In addition to 40 μg of enzyme protein, each standard reaction volume of 0.25 ml contained (in μmole): tris HCl, *p*H 7.4, 21; $MgCl_2$, 3.2; CTP, ATP, UTP, and GTP, 0.2 each. The reaction mixture was held for 20 minutes at 35°C and cooled in an ice bath, and the reaction was terminated by the addition of 0.15 ml of neutralized saturated pyrophosphate, 0.15 ml of neutralized saturated orthophosphate, and 0.1 ml of 80 percent TCA. The precipitate was transferred to a membrane filter and washed seven times with 5 ml of cold 10 percent TCA. The membrane was then dried, and its radioactivity was counted in a liquid scintillation spectrometer (*2*). The washing procedure yielded zero time values of 80 count/min with input of 1×10^6 count/min. The labeled UTP^{32} was synthesized (*1*) and was used at a concentration of 1×10^6 count/min for each 0.2 μmole. The enzyme was isolated from *E. coli* (Q13) infected with bacteriophage Qβ (*2*).

Table 1. Template selectivity of Qβ-replicase. Standard reaction volumes were 0.25 ml and contained the components in the concentrations specified in the legend for Fig. 1. Each reaction contained 40 μg of enzyme protein and 1 μg of the RNA indicated. When two templates are included, 1 μg of each is present. The reactions were run for 20 minutes at 35°C and then treated as described in Fig. 1. The UTP^{32} was the labeled phosphate; the activity was such that the incorporation of 4000 count/min corresponds to the synthesis of 1.06 μg of RNA. sRNA had been tested in an earlier experiment and showed no effect on synthesis in the presence of Qβ-RNA. The numbers represent the counts incorporated into acid-precipitable nucleic acid in 20 minutes.

RNA	Single template	Single template of column 1 + Qβ-RNA
Qβ	4929	3873
Turnip yellow mosaic virus	146	3383
Coliphage MS-2	35	3584
Ribosomal RNA (*E. coli* Q13)	45	3042
sRNA (*E. coli* Q13)	15	
Satellite virus of tobacco necrosis virus	61	3625
None	30	

surance that the reaction being studied is indeed relevant to an understanding of the replicative process.

The ability of the "replicase" to distinguish one RNA sequence from another provides means to obtain information pertinent to the similarity question. Two sorts of experiments can show whether the product is recognized by the enzyme as a template. One approach is an examination of the kinetics of RNA synthesis at template concentrations which start below those required to saturate the enzyme. If the product can serve as a template, a period of autocatalytic increase of RNA should be observed. Kinetics should be exponential until the product saturates the enzyme, and then the rate of synthesis should become linear.

A second type of experiment is a direct test of the ability of the synthesized product to function as the initiating template. Here, a synthesis of sufficient extent is carried out to insure that the initial input of RNA becomes a quantitatively minor component of the end product. The synthetic RNA can then be purified and examined for template functioning, a property best examined by means of a saturation curve. If the response of the enzyme to variation in concentration of product is the same as that with viral RNA, one would have to conclude that the product generated in the reaction is as effective a template for the "replicase" as is RNA from the mature virus particle.

The successful performance of such experiments imposes rather severe demands on the purity of the enzyme employed. The enzyme must be free from interfering and confounding activities so that the reaction can be studied in a simple mixture containing, in addition to enzyme, only the required ions, substrates, and templates. Further, since normal template function (*5*) requires intact molecules, elimination of nuclease must approach completeness. The necessary purity can be achieved (*2*), and the purified "replicase" in the experiments to be described was prepared by the procedure of Haruna and Spiegelman (*2*) from *E. coli* Q13 (*6*) infected with the RNA bacteriophage Qβ. The replicase has the following distinctive features: (i) freedom from detectable amounts of DNA-dependent RNA polymerase, ribonuclease I (*7*), ribonuclease II (*8*), and RNA phosphorylase; (ii) complete dependence on added RNA for synthetic activity; (iii) competence for prolonged synthesis (more than 5 hours) of RNA; (iv) ability to synthesize many times the input templates; (v) saturation at low ratios of RNA to protein; (vi) virtually exclusive requirement for intact homologous template under optimum ionic conditions.

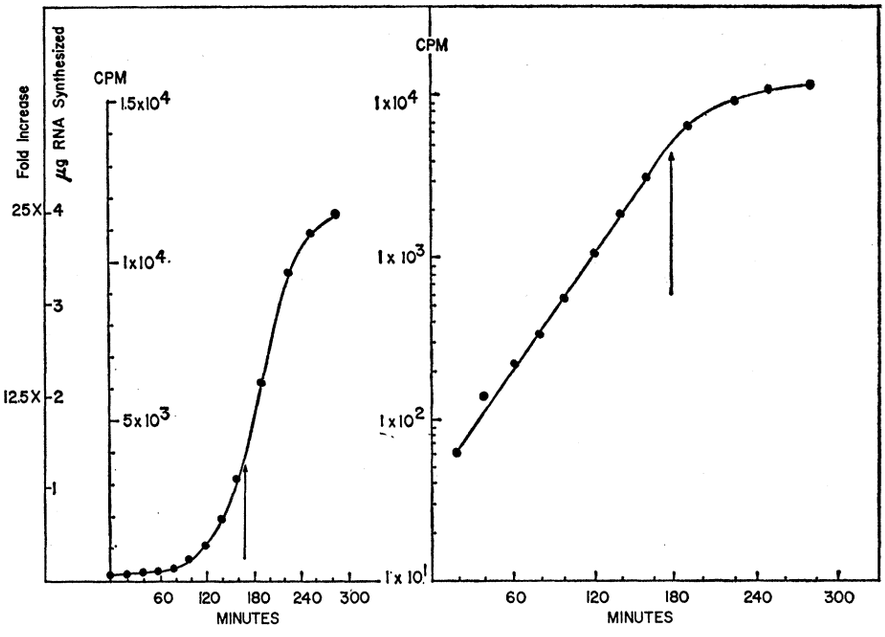

Fig. 2. Kinetics of RNA synthesis. A 2.5-ml reaction mixture was set up containing the components at the concentrations specified in the legend for Fig. 1. The mixture contained 400 μg of enzyme protein and 2 μg of input Qβ-RNA so that the starting ratio of template to enzyme was one-fifth of the saturating amount. At the indicated times, 0.19-ml portions were removed and assayed for radioactive RNA as described in Fig. 1. The ordinates for radioactivity and amounts of RNA synthesized refer to the amount in 0.19-ml samples. The data are plotted against time arithmetically on the right, and semilogarithmically on the left. The arrows indicate change from autocatalytic to linear kinetics.

We now describe experiments with the purified "replicase" which were designed to compare Qβ-RNA and product RNA for their ability to serve as templates. From data we conclude that the enzyme cannot distinguish between the newly synthesized RNA molecules and RNA derived from mature virus particles.

The response of the purified "replicase" induced in *E. coli* by the bacteriophage Qβ to increasing concentrations of Qβ-RNA is given in Fig. 1. In the presence of 40 μg of protein, the enzyme is saturated by approximately 1 μg of template RNA. It is of the utmost importance to ascertain that the RNA used is intact (28*S*) by prior examination of size in a sucrose gradient. Fragmented material can barely function as templates; furthermore, the nature of the reaction is quite different (*5*).

It is evident from Fig. 1 that a search for autocatalytic kinetics must be carried out when the input of template is well below 1 μg of RNA for each 40 μg of enzyme. An experiment was set up in which the ratio of input template to protein was one-fifth of the saturation value. The results are plotted in Fig. 2. There was exponential increase of RNA over a period of approximately 3 hours. The arrows indicate the time at which the kinetics departed from exponential and became linear. Extrapola-

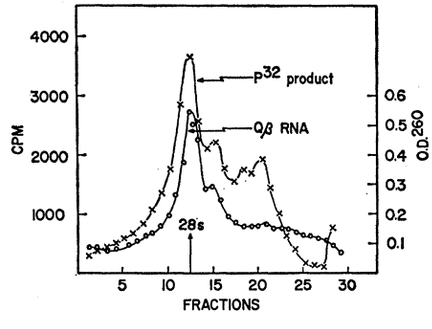

Fig. 3. Size distribution of the synthesized RNA compared to Qβ-RNA. A portion of the material recovered from the 60-fold synthesis (Table 2) was layered on a linear (2.5 to 15 percent) sucrose gradient made up in 0.01*M* tris (*p*H 7.4) buffer and 5×10^{-3}*M* Mg^{++}. It was then centrifuged for 12 hours at 25,000 rev/min and 4°C in an SW 25 Spinco rotor. Samples were removed from the bottom and optical density at 260 mμ and acid-precipitable radioactivity (as for Fig. 1) were measured.

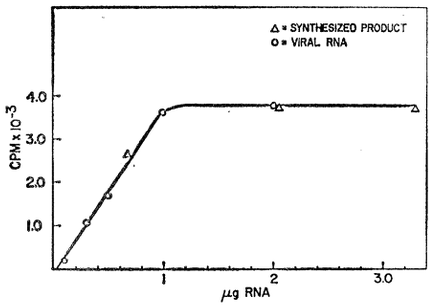

Fig. 4. Saturation of enzyme by synthesized RNA compared to viral RNA. The experiment was carried out as described for Fig. 1. The circles refer to the values obtained with RNA isolated from virus particles in the experiment of Fig. 1, and the triangles to the rates obtained with the RNA synthesized in the experiment of Table 2. Since in the latter case the template was labeled with P^{32}, H^3-UTP at 1×10^6 count/min for each 0.2 μmole was used to follow the synthesis. Preparations and counting as in Fig. 1.

Table 2. Sixty-fold increase in synthesis of viral RNA. A 1-ml reaction mixture contained the following, in micromoles: tris HCl, *p*H 7.4, 84; $MgCl_2$, 12.8; CTP, ATP, UTP, and GTP, 0.8 each. Then 160 μg of enzyme protein and 4 μg of Qβ-RNA as template were added. The specific activity of the UTP^{32} was adjusted so that 1×10^5 count/min are equivalent to 25.4 μg of RNA. The mixture was incubated at 35°C at the indicated intervals, 20 μl were removed, and acid-precipitable RNA was assayed as described in Fig. 1. At the end of the reaction, the RNA was purified as follows: the volume was adjusted to 2 ml with TM buffer ($10^{-2}M$ tris, $5 \times 10^{-3}M$ $MgCl_2$, *p*H 7.5) Water-saturated phenol (2 ml) was then added, and the mixture was shaken in heavy-wall glass centrifuge tubes (Sorvall, 18 by 102 mm) at 5°C for 1 hour. After separation of the water phase from the phenol by centrifugation at 11,000 rev/min for 10 minutes, another 2 ml of TM buffer was added to the phenol, and the mixture was shaken for 15 minutes at 5°C. The phenol and water layers were separated again, and the two water layers were combined. The phenol was eliminated by two extractions with ether; the phenol was removed from the walls of the centrifuge tubes by completely filling the tubes with ether after each extraction. The ether dissolved in the water phase was then removed with a stream of nitrogen. The RNA was precipitated with one-tenth volume of potassium acetate (2*M*) and two volumes of cold absolute ethanol. The samples were kept for 2 hours at −20°C before centrifugation (1 hr, 14,000 rev/min Sorvall SS 34 rotor). The pellets were dried in a vacuum desiccator for 6 to 8 hours at 5°C, and the RNA was then dissolved in 1 ml of buffer ($10^{-2}M$ tris, $10^{-2}M$ $MgCl_2$, *p*H 7.5). The radioactivity in the fraction precipitated with TCA was measured on 20 μl portions of the final product from which the percentage recovery of synthesized RNA can be determined.

Time (min)	Specific activity [count min^{-1} $(20\ \mu l)^{-1}$]	RNA synthesized (μg/ml)	Total RNA
0	80	0	4
60	4,917	63	67
150	15,641	199	203
210	20,113	256	260

tion to the ordinate indicates that the change from autocatalytic to linear synthesis occurred when approximately 1 μg of RNA had accumulated. Synthesis continues for approximately 4 hours and then declines slowly. At the end of the experiment, the RNA synthesized corresponded to approximately 25 times the amount put in at the beginning.

The results are consistent with the implication that the product of the reaction can stimulate new enzyme molecules to activity. Thus enzyme recognizes the product as homologous to its own genome. To test this conclusion more directly, a 1-ml reaction mixture was set up as described in Table 2. The synthesis was allowed to proceed for 3.5 hours, by which time the increase of input material was more than 60-fold. The reaction was then terminated, and the RNA was purified by the phenol method, which yielded 55 percent of the synthesized product.

Figure 3 shows the distribution in a linear sucrose gradient of the synthesized product (judged by P^{32}) as compared with Qβ-RNA freshly isolated from virus. Much of the product has the 28*S* size characteristics of Qβ-RNA. There is evidence of some smaller fragments which may represent either abortive or incomplete syntheses.

Since the product was labeled with P^{32}, it was necessary to examine its template function with another identifying isotope (H^3-UTP). This examination was carried out as described in the legend for Fig. 4, which illustrates the response of the "replicase" to various input levels of the product (triangles) as compared with the original viral RNA (circles). The RNA synthesized in the course of the 60-fold increase is as effective in serving as a template as the original viral RNA.

The data support the assertions that the reaction generated a polynucleotide of the same molecular weight (1×10^6) as viral RNA and that the "replicase" cannot distinguish this polynucleotide from its homologous genome. It is clear that the enzyme is faithfully copying the recognition sequence employed by the "replicase" to distinguish one RNA molecule from another.

The questions of whether identical replicas have been in fact produced, and whether the product contains all the information required to program the synthesis of complete virus particles are considered in a separate communication (*9*).

I. HARUNA
S. SPIEGELMAN
Department of Microbiology, University of Illinois, Urbana

References and Notes

1. I. Haruna, K. Nozu, Y. Ohtaka, S. Spiegelman, *Proc. Nat. Acad. Sci. U.S.* **50**, 905 (1963).
2. I. Haruna and S. Spiegelman, *ibid.* **54**, 579 (1965).
3. I. Watanabe, *Nihon Rinsho* **22**, 243 (1964); L. Overby, G. H. Barlow, R. H. Doi, M. Jacob, S. Spiegelman, *J. Bacteriol.*, in press.
4. Abbreviations used: sRNA—soluble RNA; ATP, CTP, GTP, and UTP—adenosine cytidine, guanosine, and uridine triphosphate; UTP^{32}—UTP labeled with P^{32}; TCA—trichloroacetic acid.
5. I. Haruna and S. Spiegelman, in preparation.
6. R. F. Gesteland, *Fed. Proc.* **24**, 293 (1965). Q13 is a derivative of A19, a ribonuclease-negative mutant reported in this reference.
7. P. F. Spahr and B. R. Hollingworth, *J. Biol. Chem.* **236**, 823 (1961).
8. P. F. Spahr, *J. Biol. Chem.* **239**, 3716 (1964).
9. S. Spiegelman, I. Haruna, I. B. Holland, G. Beaudreau, D. Mills, *Proc. Nat. Acad. Sci. U.S.* **54**, 919 (1965).
10. Supported by PHS research grant CA-01094 and NSF grant 2169.

2 August 1965

Potassium, Rubidium, Strontium, Thorium, Uranium, and the Ratio of Strontium-87 to Strontium-86 in Oceanic Tholeiitic Basalt

Abstract. *The average concentrations of potassium, rubidium, strontium, thorium, and uranium in oceanic tholeiitic basalt are (in parts per million) K, 1400; Rb, 1.2; Sr, 120; Th, 0.2; and U, 0.1. The ratio Sr^{87} to Sr^{86} is about 0.702, that of K to U is 1.4×10^4, and of Th to U is 1.8. These amounts of K, Th, U, and radiogenic Sr^{87} are less than in other common igneous rocks. The ratios of Th to U and Sr^{87} to Sr^{86} suggest that the source region of the oceanic tholeiites was differentiated from the original mantle material some time in the geologic past.*

Studies of submarine volcanics by Engel and Engel (*1*) suggest that low potassium tholeiites are a predominant primary magma erupted from the mantle and that alkalic basalts are magmatic differentiation products of the tholeiites. The chemical properties of the tholeiites are fundamental to all hypotheses of terrestrial differentiation, heat flow of the earth, and crustal evolution.

Questions have arisen as to the