Replication of Viral RNA: RNA Synthetase from Escherichia coli Infected with Phage MS2 or Q_β

Abstract. *An RNA synthetase is formed in* Escherichia coli *after infection with bacteriophages containing RNA. Specific annealing techniques revealed that, from the very outset of the reaction in vitro, the partially purified enzyme-template complex synthesizes parental-type plus strands, namely, MS2-RNA when isolated from cells infected with MS2 phage and Q_β-RNA in the case of cells infected with Q_β phage. This is in contrast to the situation found with Q_β replicase primed with Q_β-RNA, where the initial product is the complementary strand.*

RNA (*1*) synthetase, an enzyme elicited by infection of *Escherichia coli* with MS2 phage (*2*), catalyzes the synthesis of virus-specific RNA (*3–5*). Over 90 percent of the radioactive product synthesized from C^{14}-labeled ribonucleoside triphosphate precursors consisted of parental-type plus strands (*5*). As isolated by phenol extraction, about half of the radioactive RNA was double-stranded, and half was single-stranded (*3*). Specific annealing assays showed that almost all the radioactivity in double-stranded synthetase product was present in viral plus strands (*4*); the minus strands in the duplex were virtually unlabeled. Moreover, the enzyme preparations contained a complex (*4*) of plus and minus viral RNA strands. These and other results (*6–8*) were interpreted to mean that RNA synthetase utilizes the minus strand of the replicating complex as template for the synthesis of progeny RNA by a partly semiconservative mechanism whereby some of the plus strands in the complex are displaced by newly formed plus strands. Possibly, the plus and minus strands of the replicating complex are not hydrogen-bonded in the native state, but give rise to double-stranded RNA during isolation (*6*).

Since phage Q_β differs from MS2 in several respects (*9*), including the RNA base ratios and the amino acid composition of the protein coat (*10*), we wanted to ascertain whether, with the same procedure described previously for the MS2 system (*4*), one could purify from Q_β-infected cells an enzyme-template complex capable of synthesizing Q_β-RNA. This was found to be possible, and we have examined by annealing techniques the product synthesized by both MS2 and Q_β synthetase. We found, as expected, that the MS2 enzyme synthesizes MS2-specific RNA, whereas the Q_β enzyme synthesizes Q_β-specific RNA. In both cases, predominantly plus strands are produced from the outset of the reaction.

The MS2 enzyme was prepared (*4*) with the use of *E. coli* 1113 Fl, a strain derived from *E. coli* 1113 (*11*). The specific activity of the initial extract was 0.12 ($m\mu$mole of nucleotide incorporated from UTP-C^{14} per minute per milligram of protein), and that of the step-4 enzyme was 3.56. The Q_β enzyme was prepared in the same way from Q_β-infected cells. The specific activity of the initial extract was 0.08, and that of the step-4 enzyme was 3.03. The yields of the two enzymes were comparable. Phage Q_β (*11*) was grown and purified as previously described (*12*). The radioactive product of RNA synthetase was prepared (*5*) under the conditions of the standard assay, with 2 mg of enzyme protein and radioactive nucleoside triphosphates with a specific radioactivity of 6900 count/min per millimicromole, and incubated for 5 minutes at 25°C. The total C^{14} radioactivity of the MS2 enzyme product (ribonuclease resistance, 60 percent) was 450,000 count/min, and that of the Q_β enzyme product (ribonuclease resistance, 57.5 percent) was 264,000 count/min. We prepared nonlabeled and P^{32}-labeled MS2- and Q_β-RNA (*7*, *12*) and nonlabeled MS2-specific and Q_β-specific double-stranded RNA (*12*, *13*).

We previously described the methods used in this work for the characterization of the products of RNA synthetase (*4*, *8*, *12*, *14*).

When the radioactive synthetase product is annealed with a large excess of homologous, denatured virus-specific double-stranded RNA, nearly all the labeled RNA becomes resistant to ribonuclease (that is, double-stranded). Hence, the product consists almost entirely of viral plus or minus strands, or both. When this double-stranded material is denatured and annealed again with an excess of nonlabeled, parental plus strands, radioactive viral plus but not minus strands are displaced from the duplex and become sensitive to ribonuclease. This procedure, per-

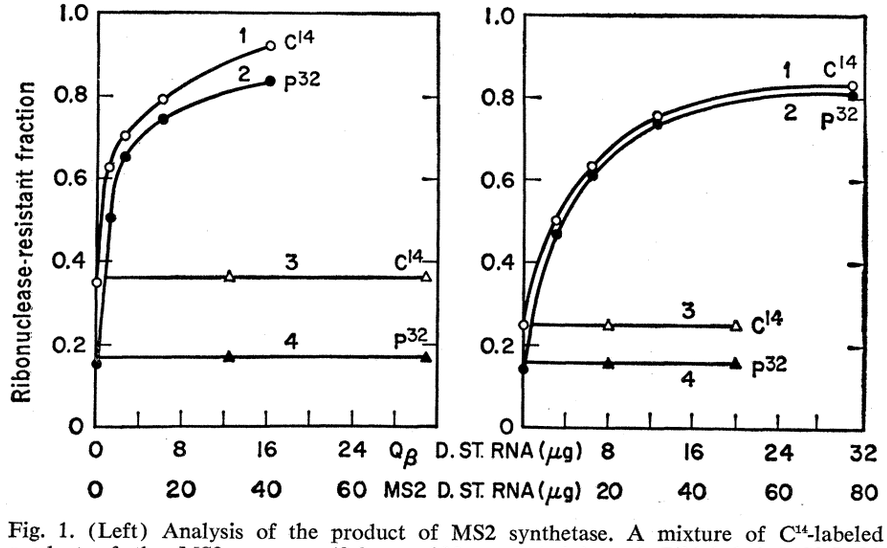

Fig. 1. (Left) Analysis of the product of MS2 synthetase. A mixture of C^{14}-labeled product of the MS2 enzyme (2.8 μg, 890 count/min) and P^{32}-labeled MS2-RNA (0.034 μg, 1250 count/min) in 0.1 ml of 2.5 × SSC, was heat-denatured (3 minutes at 120°C) and reannealed (60 minutes at 85°C) in the presence of increasing amounts of either MS2-specific double-stranded RNA (curves 1 and 2) or Q_β-specific double-stranded RNA (curves 3 and 4). The acid-insoluble radioactivity resistant to ribonuclease was determined and plotted (as fraction of the acid-insoluble radioactivity of a heat-denatured sample not treated with ribonuclease) against the amount of virus-specific double-stranded RNA. (Right) Product of Q_β synthetase. A mixture of C^{14}-labeled product of the Q_β enzyme (7.6 μg, 890 count/min) and P^{32}-labeled Q_β-RNA (0.055 μg, 1250 count/min) in 0.1 ml of 2.5 × SSC was heat-denatured and annealed as above with increasing amounts of either Q_β-specific (curves 1 and 2) or MS2-specific (curves 3 and 4) double-stranded RNA. Other conditions as above. *D.St. RNA*, double-stranded RNA.

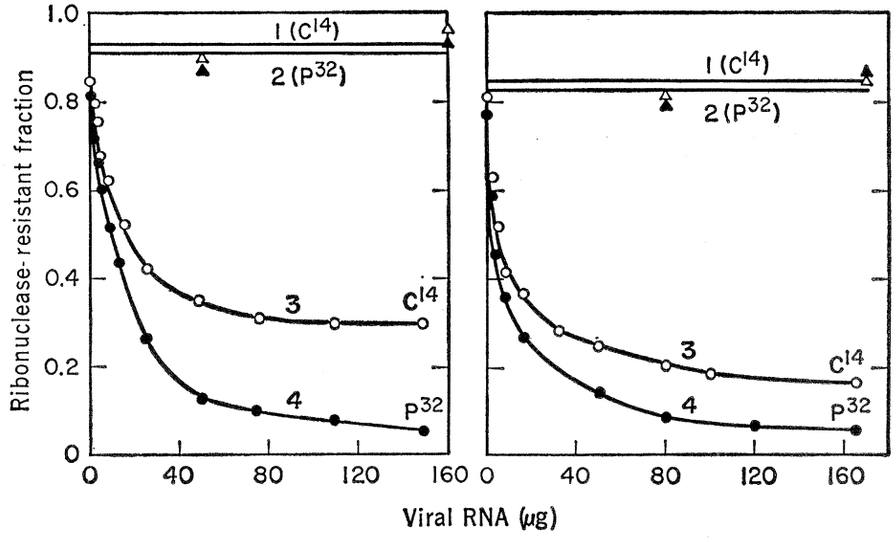

Fig. 2. Analysis of the products of MS2 and Q_β synthetase by the double isotope-specific dilution assay. (Left) A mixture of C^{14}-labeled MS2 enzyme product (2.8 μg, 900 count/min), P^{32}-labeled MS2-RNA (0.034 μg, 1250 count/min), and excess of nonlabeled, MS2-specific double-stranded RNA (20 μg) was heated (3 minutes at 120°C) and annealed (60 minutes at 85°C) in the presence of increasing amounts of either Q_β-RNA (curves 1 and 2) or MS2-RNA (curves 3 and 4) and the acid-insoluble radioactivity resistant to ribonuclease was determined and plotted as in Fig. 1. (Right) The same experiment was performed with C^{14}-labeled Q_β synthetase product (7.6 μg, 900 count/min), P^{32}-labeled Q_β-RNA (0.055 μg, 1250 count/min), and excess of Q_β-specific double-stranded RNA (20 μg), with increasing amounts of either MS2-RNA (curves 1 and 2) or Q_β-RNA (curves 3 and 4).

formed in the presence of homologous P^{32}-labeled viral RNA as an internal standard, is referred to as the double isotope-specific dilution assay; it identifies the RNA formed by the synthetase and determines the distribution of radioactivity between plus and minus strands.

The MS2- and Q_β-RNA have similar molecular weights but differ significantly in base composition (*10*). Moreover, whereas MS2-RNA completely displaces its labeled counterpart from labeled, MS2-specific double-stranded RNA after thermal denaturation and reannealing, Q_β-RNA has no effect in this system (*14*, *15*). Furthermore, P^{32}-labeled MS2-RNA becomes double-stranded (Table 1) and hence resistant to ribonuclease, when heated and annealed with an excess of unlabeled MS2-specific, but not of Q_β-specific, double-stranded RNA; the reverse is true of P^{32}-labeled Q_β-RNA. These results indicate that there is no base sequence homology between the two RNA's.

The following experiments show that the product of MS2 synthetase is MS2-RNA and that the product of Q_β synthetase is Q_β-RNA. When the C^{14}-labeled product of the MS2 enzyme is heated and annealed, in the presence of P^{32}-labeled MS2-RNA as an internal standard, with increasing amounts of MS2-specific double-stranded RNA, the bulk of both the C^{14} and P^{32} radioactivity becomes resistant to ribonuclease (Fig. 1, left). The figure shows further that no such annealing occurs when Q_β-specific double-stranded RNA is substituted for the MS2-specific duplex. Figure 1 (right), with P^{32}-labeled Q_β-RNA as internal standard, shows that the opposite relations hold for the C^{14}-labeled product of the Q_β enzyme. In view of the fact that the synthetase product, as isolated, contains unlabeled virus-specific double-stranded RNA, there is a substantial amount of C^{14} radioactivity resistant to ribonuclease upon annealing in the absence of added homologous double-stranded RNA. Under the same conditions, P^{32} radioactivity resistant to ribonuclease is also due to annealing of some of the added viral P^{32}-RNA with the minus strands in the synthetase product.

Figure 2 illustrates the results of double isotope-specific dilution assays. As seen above, the bulk of the radioactive RNA in a mixture of C^{14}-labeled MS2 synthetase product and P^{32}-labeled MS2-RNA becomes resistant to ribonuclease, that is, double-stranded, by annealing with an excess of MS2-specific double-stranded RNA (Fig. 2, left). The same is true when the annealing is carried out in the presence of increasing amounts of Q_β but not of MS2-RNA. In the latter case, both the C^{14}-labeled plus strands in the MS2 synthetase product and the P^{32}-labeled MS2-RNA are diluted out and do not become double-stranded. Conversely (Fig. 2, right), the radioactive RNA in a mixture of C^{14}-labeled Q_β synthetase product and P^{32}-labeled Q_β-RNA, which becomes double-stranded when annealed with excess Q_β-specific double-stranded RNA in the absence or presence of MS2-RNA, does so to a much lesser extent when Q_β-RNA is present. Viral RNA competes with homologous viral plus but not minus strands. Thus, the radioactivity that remains resistant to ribonuclease, in the presence of an excess of homologous viral RNA, is in "minus" strands (*8*, *14*). To

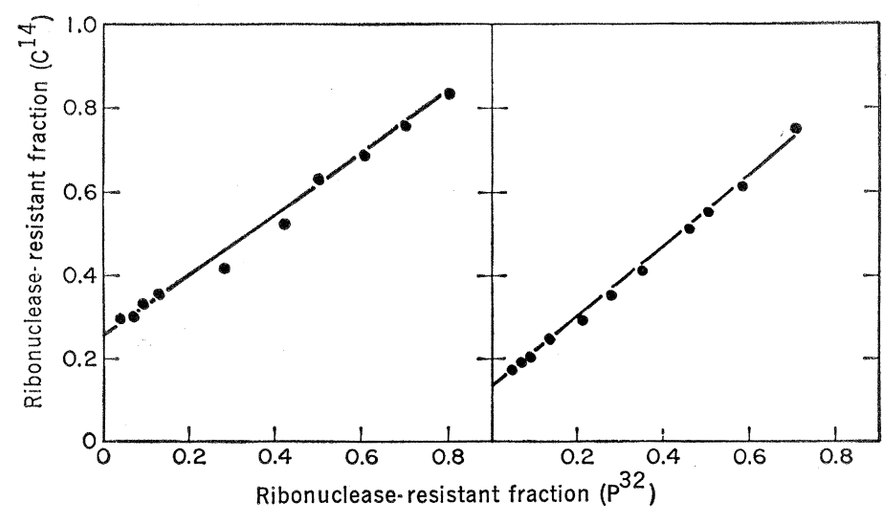

Fig. 3. Determination of plus and minus strands in MS2 (left) and Q_β (right) synthetase products. Data of the double isotope-specific dilution assay of Fig. 2. replotted. The fraction of C^{14} radioactivity resistant to ribonuclease is plotted against that of P^{32} radioactivity.

Table 1. Lack of base sequence homology between MS2- and Q_β-RNA as shown by annealing experiments. The ribonuclease sensitivity of mixtures of P^{32}-labeled MS2- or Q_β-RNA with unlabeled MS2- or Q_β-specific double-stranded RNA was determined after thermal denaturation and reannealing. Conditions: P^{32}-MS2-RNA, 0.15 μg; P^{32}-Q_β-RNA, 0.24 μg; MS2-specific double-stranded RNA, 8.7 μg; Q_β-specific double-stranded RNA, 6.2 μg. Heating for 3 minutes at 120°C in 0.02 ml of 2.5 × SSC was followed by annealing for 60 minutes at 85°C. Sensitivity to ribonuclease was measured (*13*).

Annealing mixture		Acid-insoluble radioactivity (count/min)	
Type of P^{32}-RNA	Type of double-stranded RNA	No ribonuclease	Ribonuclease
Experiment 1			
MS2	MS2	9,900	8209
MS2	Q_β		45
MS2	None		32
Experiment 2			
Q_β	Q_β	11,375	9050
Q_β	MS2		120
Q_β	None		45

determine the proportion of radioactive plus and minus strands in each synthetase product, the C^{14} radioactivity was plotted against P^{32} radioactivity, and the slope and intercept of the resulting straight line (Fig. 3) were computed by the least-squares method. The slope corresponds to the fraction of C^{14} radioactivity in plus strands while the intercept gives that of C^{14} radioactivity in minus strands (*13*). Three closely agreeing experiments with the MS2 and two with the Q_β synthetase product showed that the former contained an average of 75 percent plus and 25 percent minus strands, whereas the latter had 85 percent plus and 12 percent minus strands.

Phage RNA replication occurs in two steps (*14*): (i) synthesis of complementary minus strands, with viral plus strands as template, resulting in the formation of a virus-specific replicating complex and (ii) synthesis of viral progeny plus strands with the minus strands of the replicating complex as template. Although the two steps are genetically separable (*16*), no separate enzymes for each step have yet been isolated. Spiegelman's RNA replicase preparations (*17*), which specifically require homologous viral RNA for activity and synthesize infectious RNA (*18*), catalyze both steps. During the first few minutes of incubation of Q_β replicase with radioactive nucleoside triphosphates, there is predominant synthesis of minus strands, despite reports to the contrary (*19*); later on, plus strands are synthesized in much larger amounts (*12*). One of the main differences between replicase and synthetase preparations is apparently that the former are free of RNA primer and require the addition of homologous RNA, whereas the latter contain viral minus strands (possibly protein-bound), and require no addition of template. Furthermore, in the initial phase of the reaction, Q_β replicase produces almost exclusively viral minus strands which are then presumably used as template, for the synthesis of Q_β-RNA, whereas synthetase produces predominantly plus strands from the outset of the reaction.

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References and Notes

1. Abbreviations: RNA, ribonucleic acid; SSC, 0.15*M* sodium chloride, 0.015*M* sodium citrate; "plus" strands are the viral parental-type strands; "minus" strands are those with a complementary base sequence; UTP, uridine triphosphate.

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Oncogenicity by DNA Tumor Viruses: Enhancement after Ultraviolet and Cobalt-60 Radiations

Abstract. *Simian virus-40, polyoma, and LLE46 virus preparations were treated with ultraviolet or gamma radiations (cobalt-60) in a frozen state. Infectivity and induction of complement-fixing antigen and DNA synthesis declined as a logarithmic function of dose, the latter two properties being more resistant than infectivity to radiation by a factor of 2 to 5. Oncogenicity of all three viruses did not decrease with progressive amounts of both types of irradiation, but actually increased in absolute and relative terms (per infectious unit), even at the maximum dose of irradiation used (24,000 ergs per square millimeter per minute and 2.7 × 10^6 rads).*

Some functions of the genome of tumor viruses are more resistant to physical or chemical inactivation than the function of replication. Thus, the induction of complement-fixing antigens by polyoma, SV40, and LLE46 (a hybrid of adenovirus 7 and SV40) viruses (*1*), the induction of DNA synthesis by SV40 and polyoma (*1, 2*), the induction of thymidine kinase by SV40 (*3*), and the transformation of cells by polyoma (*4, 5*) can be dissociated from the capacity to produce infectious progeny. It is conceivable that the oncogenic interaction of the virus with the target cells requires the expression of only a limited number of functions of the viral genome.

We now report the increase rather than decline of tumorigenicity in vivo per infectious unit of LLE46, polyoma, and SV40 after ultraviolet or cobalt-60 irradiation.

The method of irradiation has been reported (*1*). Briefly, for Co^{60} irradiation, viruses, after separation from cellular debris, were suspended in 1 percent tryptone solution at a final concentration of 10^7 to 10^8 $TCID_{50}$ (tissue culture infective doses, 50 percent effective) or plaque-forming units (PFU) per milliliter. Irradiation was performed in a Co^{60} irradiation source at the Pennsylvania State University (by W. Ginoza).

In order to minimize secondary