Effect of an *Escherichia coli* traD (ts) Mutation on MS2 RNA Replication

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**SUMMARY**

We have continued our studies on the effect of high temperature (42 °C) on the development of RNA bacteriophage MS2 in the temperature-sensitive conjugational transfer-deficient mutant *Escherichia coli* JCFL39 carrying a traD (ts) mutation. At 42 °C, mutant cells permit the penetration and translation of phage MS2 RNA but do not permit MS2 RNA replication. We suggest a role for the traD (ts) mutation in MS2 RNA replication.

Achtman *et al.* (1971) and Achtman (1973) have shown that the *Escherichia coli* gene traD is involved in DNA transfer during bacterial conjugation and that the traD− mutants are resistant to the RNA phage f2. Studies with the *E. coli* traD− mutant JCFL39, which is temperature-sensitive for conjugal transfer because it carries a traD (ts) mutation (Achtman *et al.*, 1971), have shown that this mutant is also temperature-sensitive for the growth of group I RNA phages (MS2, f2, and R17) (Schoulaker & Engelberg-Kulka, 1978). Furthermore, the assembly of group I RNA phage MS2 is inhibited when the temperature is shifted up to 42 °C at the time of infection (Schoulaker-Schwarz & Engelberg-Kulka, 1981). In this report, we show that in mutant cells grown for one generation at 42 °C before infection at the same temperature, an additional step in the development of RNA phage MS2 is affected: MS2 RNA replication is inhibited. We discuss the role of gene traD in phage MS2 replication.

The bacterial strains used here were the parent strain *E. coli* M176, and its traD (ts) derivative JCFL39, both kindly supplied by Dr N. S. Willetts. M176 is a derivative of JC3272 (lac− Gal− His− Lys− Str8 Spc8) which carries the wild type Flac element JCFLO. Strain JCFL39 carries the Flac element JCFL39, which is traD (ts), in place of JCFLO. Strain F− JC3272 Lac− was derived from strain M176 Lac+ by curing with acridine orange (Miller, 1972) and picking up Lac− colonies on MacConkey agar plates. Phage MS2 was kindly supplied by Dr R. Sinsheimer and phage Qβ by Dr J. T. August.

To study the penetration of MS2 RNA into mutant cells grown for one generation at 42 °C, cells were infected at 42 °C with MS2 containing 3H-labelled RNA and subsequently washed 4 times with phosphate-buffered saline at 4 °C in order to remove phages which did not inject their RNA. As shown previously (Engelberg & Artman, 1970), this method removes all such particles. Thus, the radioactivity that remains with the cells reflects the amount of RNA injected. It was found that the same amount of radioactivity remained associated with the mutant cells whether they were grown and infected at 35 °C or at 42 °C. This indicates that MS2 RNA penetration into the mutant is not inhibited at high temperatures. Control experiments with JC3272, which lacks the phage receptors, showed no penetration whatsoever. MS2 RNA penetration into the mutant at 42 °C was further confirmed by our experiments by showing the synthesis of MS2 coat protein in mutant cells grown and infected at 42 °C (Fig. 1). However, although MS2 RNA penetrates and is translated in the mutant at 42 °C, MS2 RNA does not replicate under these conditions as shown by analysis of the fate of 3H-labelled MS2 RNA extracted with phenol at various times from mutant cells grown and infected at 42 °C. As previously reported (Pace *et al.*, 1967; Weissmann *et al.*, 1964, 1968) when phage-infected cells are deproteinized by phenol for RNA isolation, the first step of RNA replication, the synthesis of minus strands, appears as the conversion of parental RNA into a double-stranded form; the second step, the synthesis of progeny strands, appears as the displacement of the parental RNA from the duplex. The appearance of parental RNA in the double-stranded form was determined by the method of Franklin (1966), which is based on the finding that single-stranded RNA
Coat

Fig. 1. SDS-polyacrylamide gels of MS2-specified proteins synthesized in rifampin-treated cells of mutant JCFL39. The cells were grown in TPG medium (Sinsheimer et al., 1962) at 42 °C to a density of 2 x 10^8 cells/ml, centrifuged, resuspended in TPG medium with a low concentration of lysine (2 µg/ml), shaken for an additional 5 min and infected with MS2 at a multiplicity of 50. The addition of rifampin (100 µg/ml) 5 min after infection completely inhibited host protein synthesis. 30 min after infection, the cells were labelled by the addition of 3H-lysine (5 µCi/ml) and at 70 min after infection, the cells were harvested by centrifugation at 4 °C. The cells were lysed and samples were denatured and dialysed as described by Jockush et al. (1970). The proteins were analysed by gel electrophoresis as described previously (Schoulaker-Schwarz & Engelberg-Kulka, 1981). The position in the gels of the labelled MS2 coat protein was determined by its mobility relative to the purified protein (mol. wt. 14000).

Fig. 2. Percentage of parental MS2 and Qβ RNAs in the double-stranded form during infection of mutant and parental strain grown and infected at 33 °C (O) or 42 °C (■). Cells were grown in tryptone broth at 33 °C or 42 °C to a density of 2 x 10^8 cells/ml and infected at the respective temperature with MS2 or Qβ containing 3H-labelled RNA at a multiplicity of 5. At different times after infection, the cells were harvested and washed four times with phosphate-buffered saline at 4 °C. The temperature of infection did not affect the radioactivity remaining with cells infected with either 3H-MS2 or 3H-Qβ. The isolation of RNA and the determination of the amount of parental RNA in the double-stranded form by chromatography on cellulose columns was done according to Franklin (1966) as described previously (Engelberg et al., 1975). The samples of RNA applied to the column contained about 4000 ct/min. (a) Parental strain M176 infected with MS2, (b) mutant strain JCFL39 infected with MS2, (c) mutant strain JCFL39 infected with Qβ.

adsorbed to cellulose columns can be eluted with 15% ethanol/Na/Tris/EDTA buffer (0.1 M-NaCl, 0.001 M-EDTA, 0.05 M-Tris–HCl pH 6.85), whereas RNA in the double-stranded form is eluted from cellulose columns with buffer alone. We observed both steps of RNA replication in both parental and mutant cells grown and infected at 33 °C, and in parental cells grown and infected at 42 °C (Fig. 2). In contrast, there was no conversion of parental MS2 RNA into double-stranded form in mutant cells grown and infected at 42 °C (Fig. 2b), indicating that
under these conditions the first step of RNA replication is inhibited. Moreover, there was no MS2 RNA replicase activity in crude extracts of infected mutant cells grown at 42 °C (data not shown). Parallel experiments with Qβ showed that RNA replication proceeded normally at 42 °C.

Our results may help explain the involvement of host proteins in the replication of MS2 RNA. Until now, phage RNA replication has been mainly studied in Qβ. The core of Qβ RNA polymerase contains one phage-specified protein and three bacterial proteins (Kamen, 1970; Kondo et al., 1970; Blumenthal et al., 1972; Inouye et al., 1974). An additional protein, called host factor (HF) has been implicated in Qβ RNA replication in vitro (Shapiro et al., 1968; Carmichael et al., 1975). In contrast, very little is known about the enzyme catalysing the RNA replication of the group I RNA phages MS2 and f2. The group I RNA polymerases are unstable and difficult to purify: only f2 poly G polymerase has been extensively purified (Federoff & Zinder, 1971). This enzyme contains proteins of comparable molecular size to the three bacterial proteins of Qβ core RNA polymerase. With either f2 or f2-complementary strands as template, activity of the purified f2 poly G polymerase requires the presence of an unidentified additional factor(s) (Federoff & Zinder, 1973) which cannot be replaced by the host factor required for Qβ RNA replication. Furthermore, in vivo, rifampin selectively inhibits group I RNA phage replication and not that of Qβ (Engelberg et al., 1975), indicating that different bacterial proteins are required by the two types of phages. Our present results, showing that the traD (ts) mutant JCFL39 inhibits replication of MS2 but not that of Qβ at 42 °C, further confirm the requirement of different bacterial proteins in the replication of group I and Qβ RNA phages.

We have previously reported (Schoulaker-Schwarz & Engelberg-Kulka, 1981) that the membranes of mutant JCFL39 are markedly altered at 42 °C. These changes might be responsible for the inhibition of MS2 RNA replication in the mutant at 42 °C. This idea is supported by previous studies showing that MS2 replicative intermediates are tightly bound to the membrane fraction (Haywood, 1973). We suggest that group I RNA replication, in contrast to that of Qβ, takes place on a membrane structure in which the traD gene product is either directly or indirectly involved. This association with the membrane might also account for the lability of group I polymerases and the difficulties in their purification.

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REFERENCES


Short communications


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