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Amber Mutant of Bacteriophage Qβ Capable of Causing Overproduction of Qβ Replicase

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Amber mutant *am*B86 of bacteriophage Qβ is capable of causing the production of five to eight times more viral replicase than wild-type phage. Su⁻ bacteria infected with the mutant can carry the viral RNA in a plasmid-like state for many bacterial generations.

Studies of phage Qβ RNA replicase have been expedited by the isolation of phage mutants capable of causing overproduction of the enzyme. The best known example is the mutant *am*12, which contains an amber mutation in the coat cistron (1). Nonpermissive (Su⁻) bacteria infected with *am*12 synthesize viral coat protein fragments which are unable to repress replicase cistron translation in the manner of full-length normal coat protein (4). Viral replicase, therefore, accumulates in infected Su⁻ cells to three to five times the level found during wild-type infection (3).

Ball and Kaesberg (J. Mol. Biol., in press) have shown that amber mutations in the coat cistron of Qβ affect expression of the replicase cistron in an amount dependent upon the codon number of the mutation, i.e., these mutants exhibit a smooth gradient of polarity. The mutant H4, described in that paper, has an amber mutation at codon number 86 (glutamine) and, on that basis, should be expected to induce greater Qβ replicase production than *am*12 which has an amber mutation at codon number 37 (glutamine). Mutant H4, now redesignated more descriptively *am*B86 (amber in the B cistron at codon 86), was isolated after hydroxylamine mutagenesis of wild-type Qβ by Jean Rohrschneider of our laboratory. The purpose of our present communication is to report characteristics of *am*B86 that relate to its production of Qβ replicase.

Figure 1 shows the kinetics of appearance of replicase upon infection of *Escherichia coli* Q13 (RNase I⁻, PNPase⁻ [polynucleotide phosphorylase], met⁻, rel⁻) with wild-type Qβ, with *am*B86, and with *am*12. Up to 20 min after infection, the wild-type Qβ-infected culture shows the most rapid increase in replicase activity, followed in order by the *am*B86 and *am*12 cultures. At later times (before lysis), the production of wild-type phage-directed enzyme slows down probably because the newly synthesized viral coat protein represses further replicase cistron translation. The coat protein fragments made by either mutant-infected culture would not be expected to repress replicase synthesis. Therefore, the enzyme activity of these cultures can continue to increase, although *am*B86, because of its smaller polar effect, produces replicase at a faster rate than *am*12 and results in greater enzyme overproduction. Yields of *am*B86 replicase average five to eight times those obtained by comparable wild-type infection and two to three times those obtained by *am*12 infection.

The total yield of enzyme varies with the Su⁻ bacterial strain infected (Fig. 2). *Am*B86 infection of Q13 or A19 (RNase I⁻, met⁻) gives a higher maximum level of replicase per cell than does infection of K38 or M27. The mutant is also capable of causing some enzyme overproduction upon infection of K110(Su-3), a tyrosine-inserting amber supressor *E. coli* (Fig. 3) (2). This strain is nonpermissive for amber coat protein mutants of Qβ(1), even though full-length viral coat protein is synthesized in this strain. However, this coat protein is aberrant in sequence and possibly is not capable of normal coat protein-like repression of translation.

For maximum replicase yield, wild-type infected cells are usually harvested about 40 min after infection, before the onset of lysis. The mutants *am*B86 and *am*12, by virtue of their inability to lyse Su⁻ cells, can be harvested much later, allowing a larger overall cell yield, as well as more enzyme per cell, than from a wild-type infection (Fig. 4). However, with *am*12, cell lysis due to second round infection by wild-type revertants limits the time at which infected cells can be harvested to about 80 min after infection. Addition of 0.2 M $MgSO_4$ to the growth medium can partially delay this lysis (3), but we find the yield of enzyme per cell is then lower than without $MgSO_4$. Mutant *am*B86

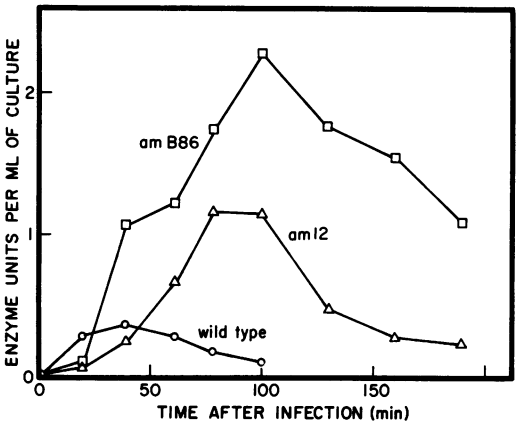

FIG. 1. *Kinetics of replicase activity recovered following viral infection. Q13 bacteria (6 × 10⁷/ml) in tryptone broth (10 g of tryptone [Difco], 8 g of NaCl, 1 g of glucose, 1 g of yeast extract, 0.22 g of $CaCl_2$ per liter) at 37 C were infected at a multiplicity of infection of 15 by wild-type Qβ (○), amB86 (□) (0.03% revertants), or am12 (△) (0.02% revertants). At appropriate times, samples (5 ml) were removed and chilled, the cells were pelleted by centrifugation and then suspended in 0.2 ml of buffer containing: 0.01 M Tris-hydrochloride, 0.001 M EDTA, 0.01 M 2-mercaptoethanol, 25% glycerol, pH 7.5. The cells were lysed by freezing and thawing five times and then incubating 1 h at 15 C with lysozyme (100 μg/ml). DNase (5 μg/ml) and $MgCl_2$ (0.01 M) were added, and incubation was continued for 15 min. Qβ replicase activity was assayed in the cell extracts by addition of a 50-μliter sample to a 0.2-ml reaction mixture containing: 0.0125 M Tris-hydrochloride, pH 8.0, 0.025 M 2-mercaptoethanol, 0.01 M $MgCl_2$, 0.005 M phosphoenolpyruvate, 10 μg of pyruvate kinase per ml, 50 μg of DNase per ml, 25 μg of Qβ RNA per ml, 1.5 μg of rifampin per ml, 8.1 × 10^{-4} M ATP, GTP, and CTP, 1.62 × 10^{-4} M UTP, and 25.0 μCi of ^{3}H-UTP per ml. After incubation for 20 min at 37 C, the reaction was stopped by the addition of 0.15 ml of cold 0.15 M sodium pyrophosphate and 0.1 ml of cold 80% trichloroacetic acid. The precipitates were collected on membrane filters (Millipore type HA), washed exhaustively with cold 10% trichloroacetic acid containing 1% sodium pyrophosphate, before being dried and assayed for radioactivity by liquid scintillation counting. Enzyme activities are expressed in nanomoles of UMP incorporated in 20 min at 37 C.*

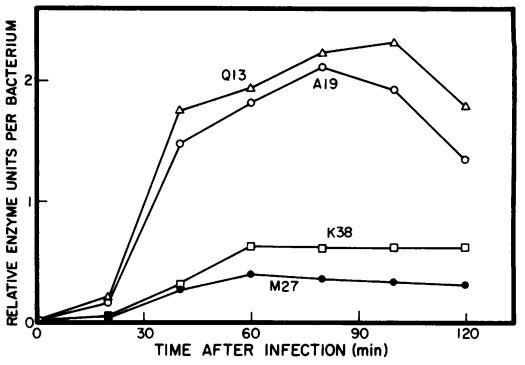

FIG. 2. *Relative replicase activity recoverable from several bacterial strains. Q13 (△), A19 (○), K38 (□), or M27 (●) bacteria were infected with amB86 at a multiplicity of infection of 15. Samples were removed and assayed for replicase activity as described in Fig. 1. A relative measure of enzyme activity is obtained by dividing the enzyme units assayed per milliliter of culture by the absorbancy at 590 nm of the culture at the time the sample was taken.*

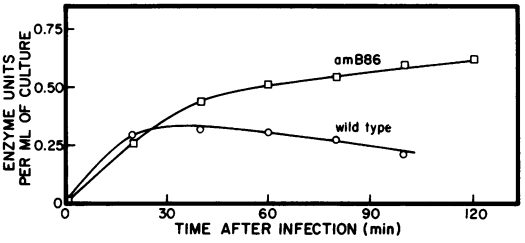

FIG. 3. *Kinetics of replicase activity recovered from bacterial strain K110. K110 (Su-3) bacteria (6 × 10⁷/ml) were infected at a multiplicity of infection of 15 by wild-type Qβ (○) or amB86 (□). At appropriate times samples were removed and assayed for enzyme activity as described in Fig. 1.*

infection requires no $MgSO_4$ because no detectable lysis by wild-type revertants is evident even as long as 3 h after infection. Maximum replicase yields are achieved with *am*B86 by harvesting the cells 100 to 120 min after infection.

In experiments to increase the number of infected cells and thereby increase replicase yields, we attempted to grow infected bacteria by cloning them on agar plates and using these clones to inoculate fresh medium. Though we have since abandoned this method as a means of preparing large quantities of replicase, we succeeded in propagating the mutant RNA in a plasmid-like state for many bacterial generations. Only about 1% of the bacteria present 120 min after infection of Q13 by *am*B86 are capable of forming clones. When these clones are picked and used to inoculate liquid medium, 60 to 70% of the resulting cultures show no detectable viral-specific replicase activity. The percentage depends heavily upon the level of Qβ-resistant bacteria present during the initial infection. The remaining 30 to 40% of the cultures generally show viral replicase levels per cell of one-fourth to one-third that maximally obtained by an initial wild-type infection, but

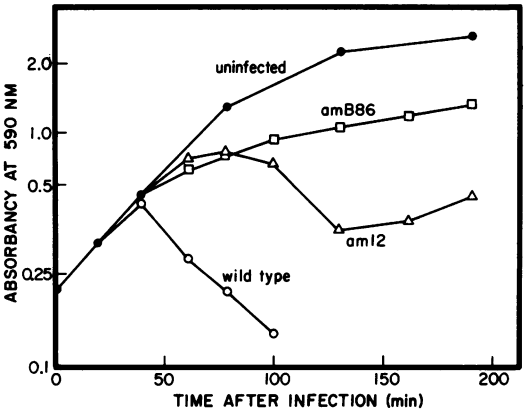

FIG. 4. *Cell density of E. Coli after viral infection. Q13 bacteria were infected by wild-type Qβ* (○), *amB86* (□), *am12* (△), *or uninfected* (●) *as described in Fig. 1. The cell density was determined by measuring the absorbancy at 590 nm. An absorbancy at 590 nm of 0.3 corresponds to* 10^8 *Q13 cells/ml.*

we have observed some cultures which assay at almost the maximum wild-type level per cell. There is a rough correlation between the level at which a culture carries replicase activity and the doubling time of its cells. Uninfected or resistant cultures double in 30 min in our incubators, while those showing replicase activity double in 40 to 80 minutes, with the higher replicase levels generally reflected by the longer doubling times. It is possible that we have failed to observe cultures carrying replicase activity at levels approaching those of an initial *am*B86 infection because the bacteria containing these abnormally high amounts of virus divide too slowly to form clones.

Since all the bacteria in a clone and its resulting culture arise from one infected cell, the viral contents of the primary bacterium are diluted at least 10^{10}-fold by the time the sample is taken for assay, thereby eliminating the possibility that the replicase activity observed in these cultures is a residue from the initial infection. Within a culture, the replicase level per cell is fairly constant throughout log-phase growth. When a culture is itself recloned, the average replicase level per cell of the resulting progeny cultures is equal to that of the parental culture, thus indicating the occurrence of new replicase synthesis. Cultures carrying replicase activity also synthesize low but detectable amounts of the amber coat protein fragment, as well as full-length viral RNA, detectable by rifampin-resistant uracil incorporation and by gel electrophoresis. We have followed some bacteria through serial cloning and culturing for more than 100 generations without observing loss of replicase activity. However, if culture carrying the activity is allowed to reach stationary phase, is chilled, or is otherwise prevented from continuous growth, the replicase activity per cell gradually decreases. Cultures can be cured of activity by allowing them to stand several days at 4 C. These results indicate that during infection of nonpermissive bacteria the *am*B86 RNA can behave as a self-replicating plasmid, continuing to serve as a message for viral protein synthesis and template for its own replication for many bacterial generations after initial infection.

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