Biological Functions of the Bacteriophage T3 SAMase Gene

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Received for publication 28 February 1975

Certain differences between phage T3 on the one hand and T3sam⁻ and T7 on the other hand indicate that the T3-coded SAMase function is responsible (i) for the development of the pseudolysogenic state by preventing T3 DNA methylation, and (ii) for the partial protection of the phage DNA against restriction by the P system.

The genetically closely related bacteriophages T3 and T7 have many similar structures and functions (1, 2, 8, 9, 10). One of the most striking differences between these phages is that T3, in contrast to T7, codes for an S-adenosylmethionine-cleaving enzyme, called SAMase (4, 5, 6). The corresponding gene assigned to gene 0.3 (15) is located on the left end of the T3 genome, and the SAMase is the first phage-induced protein to appear in T3-infected cells (9). SAMase is not only of interest as one of the functions of phage T3 but also is of general significance since S-adenosylmethionine (SAM) acts as methyl group donor in DNA methylation reactions and as an obligatory or facultative cofactor of some host-controlled restriction systems (for a review see reference 13).

However, the biological functions of the T3-coded SAMase are still totally unknown, since T3 wild type (T3⁺) and T3 mutants unable to synthesize SAMase (T3sam⁻) have identical growth characteristics under a variety of conditions and in a variety of laboratory hosts tested (6, 8). Such a T3sam⁻ phage stock was kindly supplied by R. Hausmann, Freiburg, Germany. Besides confirming all of Hausmann's results concerning the growth characteristics of T3sam⁻, we observed identical serological behavior of T3sam⁻ and T3⁺.

Recently we have found a number of differences between T3 and T7. In each of these cases we subsequently examined the behavior of the T3sam⁻ mutant, in the hope of obtaining some indications on the role of the SAMase.

In starved *Escherichia coli* B wild type (unpublished results) as well as in auxotrophic mutant cells, infection by T3 or T7 and incubation of the complexes in minimal medium is followed by a markedly different behavior of these phages (1). T3 does not perform a lytic cycle but enters in a pseudolysogenic (or temporary lysogenic) state which is maintained over

several hours, even in dividing cells. When the pseudolysogenic cells are transferred into broth medium, they re-enter the lytic cycle and phage multiplication occurs (induction of T3 prophage). In contrast to T3, phage T3sam⁻, like T7, replicates almost normally in starved cells (Fig. 1).

The difference between the pseudolysogenic behavior of T3⁺ and the lytic behavior of

FIG. 1. Replication of T3, T7, and T3sam⁻ in starved host cells. *E. coli* B logarithmic cells were washed, strongly aerated in buffer for 60 to 90 min (starvation), brought to 1.5×10^8 cells/ml of M9 minimal medium, and immediately infected with a multiplicity of 5 purified phages/cell. After 5 min of adsorption free phage were inactivated by T3-specific antiserum. The infected cells were then diluted 10⁴-fold in M9 minimal medium and incubated at 37°C. Triton of plaque-forming units was carried out at the indicated times on broth medium plates (11). Symbols: ○, T3; □, T7; △, T3sam⁻; ⊗, transfer of Te-infected complexes into broth medium (2.0% meat tryptic peptone) at 180 min; and ×, uninfected cell control.

T3sam⁻ does not seem to depend on a possible expression of SAMase activity in starved T3⁺-infected cells. In mixed infections with T3⁺ and T3sam⁻, the T3sam⁻ mutant always determines a lytic reaction (Table 1).

We suppose that the decision between pseudolysogenic and lytic behavior depends on the degree of methylation of the phage genomes. This means that the presence of SAMase in the preceding growth cycle is decisive. Whereas T3 DNA is devoid of methylated bases due to degradation of SAM in the host cells, T7 DNA is methylated (4). It follows that T3sam⁻ DNA should also be methylated. We have tried to prevent the methylation of T3sam⁻ genomes during phage growth in suppressor-negative cells by preinfecting with SAMase-active T3 amber mutants. For experimental details see Table 1. The T3sam⁻ mutants obtained in this manner (designated T3sam⁻⁰) behave like T3⁺ in infection of starved cells and maintain a pseudolysogenic state (Table 1, see also Fig. 1). We cannot explain why the degree of methylation of these phage genomes is important for their behavior in starved but not in normal cells. Possibly DNA methylation plays a role in transcription control (16). Presently we are carrying out molecular studies of the DNAs of the various phage species which should enable an unequivocal distinction between our explanation of the results via methylation patterns and other, less probable hypotheses. SAM acts also as cofactor in the synthesis of spermidine and spermine. It is theoretically not impossible that T7 and T3sam⁻ contain these polyamines, whereas T3 and T3sam⁻⁰ do not due to SAM destruction during phage propagation.

A further difference between behavior of T3 and T7 is observed in P1-lysogenic cells. In the P system these phages are restricted to a different degree, no matter on which of the commonly used *E. coli* hosts the phages were previously grown (3; Krueger and Presber, manuscript in preparation). The efficiency of plating of T3 is 10⁻⁶, and the efficiency of plating of T7 is less than 10⁻¹⁰. T3sam⁻ is restricted to the same extent as T7 (Table 2). Although T3 is restricted by P1-lysogenic cells, the SAMase production takes place as usual after T3 infection (7). Our results seem to show that T3 is partially protected from P restriction by its SAMase due to the degradation of intracellular SAM. This restriction does not consist in a breakdown of phage DNA by SAM-dependent endonuclease since the fate of parental T7 DNA is identical in the permissive *E. coli* B host and in the nonpermissive P1-lysogenic host (7). The

TABLE 1. Burst size after single and mixed infections of starved *E. coli* B cells^a

Phage	Burst size
T3 ⁺ ^b	≤ 1
T3sam ⁻ ^c	51
T3 ⁺ /T3sam ⁻ ^{de}	52
T3 ⁺ /T3sam ⁻ ^{df}	45
T3 ⁺ /T3 ⁺ ^{df}	≤ 1
T3sam ⁻⁰ ^g	≤ 1

^a Preparation and infection of starved cells is described in the legend to Fig. 1. Burst sizes were determined 30 min after infection.

^b SAMase active during preceding propagations; DNA nonmethylated.

^c SAMase inactive during preceding propagations; DNA methylated.

^d Multiplicity of infection of the first infection 20 phage/cell, and of the second infection 2 phage/cell.

^e Simultaneous infection with both phage.

^f Addition of the second phage 10 min after preinfection with T3⁺.

^g Preceding propagations of T3sam⁻ in suppressor-negative cells in the presence of active SAMase of a T3 amber mutant; DNA nonmethylated (T3sam⁻⁰). Two different samples of logarithmic *E. coli* B cells were infected with T3 gene 6 (am A67) or T3 gene 4 (am H272) amber mutants (20 phages/cell). The T3 amber mutants were kindly supplied by R. Hausmann, Freiburg, Germany. Five minutes after this preinfection the cells were infected with T3sam⁻ (1 phage/cell), incubated at 37°C for 20 min, and lysed with chloroform. To ensure that all phage genomes are nonmethylated, the whole procedure was repeated. The T3sam⁻ mutants obtained in this manner (T3sam⁻⁰) were used to infect starved *E. coli* B cells. (No T3sam⁺am⁺ recombinants were identified in the T3sam⁻⁰ lysate; refer to Table 2 for the degree of restriction of T3sam⁻⁰ by the P system.)

TABLE 2. Efficiency of plating of the phages T3, T7, and T3sam⁻ on P1-lysogenic cells^a

Phage	EOP ^b on <i>E. coli</i> 2945
T3	10 ⁻⁶
T7	< 10 ⁻¹⁰
T3sam ⁻	< 10 ⁻¹⁰
T3sam ⁻⁰ ^c	< 10 ⁻¹⁰

^a The phages were grown on *E. coli* 921 (r_K⁻m_K⁻) and plated simultaneously on *E. coli* 921 and P1-lysogenic *E. coli* 2945 (r_K⁻m_K⁻r_{P1}⁺m_{P1}⁺). Both *E. coli* strains were kindly supplied by W. Arber, Basle.

^b EOP, Efficiency of plating.

^c For the preparation of T3sam⁻⁰ see Table 1.

mechanism of restriction of T7 and T3 in the P system as well as the role of SAMase in the partial protection of phage DNA against this

restriction can only be speculated about. Possibly restriction endonucleases also perform single-strand breaks of the type normally required at the origin of phage and plasmid DNA replication (12). The P1 nuclease could induce such nicks on other sites of the phage DNA and in this way inactivate the phage template without destroying it by double-strand breaks. This explanation is in good agreement with results of Skogman and Björk (personal communication), who have found an involvement of P1 endonuclease in T7 restriction without degradation of restricted DNA. Since the P1 restriction endonuclease is partially SAM dependent (13), T3 is more resistant towards this kind of restriction than U7 and T3sam⁻.

There are other differences between the behavior of T3 and T7 which cannot be explained on the basis of the T3 SAMase function and therefore do not help to define the role of this gene. For example, we have found that T7 replicates in *E. coli* W cells normally whereas T3 infection is abortive (14). Mutants of T3, called T3_w, were isolated which were able to grow on *E. coli* W just like T7. The T3_w mutation is not located in the SAMase gene (D. H. Krueger, D. Scholz, W. Presber, and H. A. Rosenthal, manuscript in preparation).

In summary it can be concluded that the SAMase does not only protect T3 DNA against SAM-dependent restriction but also performs regulatory functions via prevention of T3 DNA methylation, for example, in the case of pseudolysogeny. Our results show that both biochemical functions of SAM, to serve as a methyl group donor in DNA modification processes and as a cofactor of DNA restriction, seem to play an important role in the interactions of phage T3 with its host's cells. It is highly probable that SAM and SAM-dependent enzymes are also involved in DNA methylation and cutting processes other than those usually termed modification/restriction.

We thank C. Schroeder, Berlin, German Democratic Republic, for inspiring discussions and help in preparing the manuscript and M. Gold, Toronto, Canada, for critically reading the manuscript.

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