THE TOPOISOMERASE ACTIVITY OF T4 *amG39* MUTANT IS RESTORED IN Mu LYSOGENS

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Received 30 October 1981

1. Introduction

Integrative recombination is a basic step in the Mu bacteriophage cycle. This phage has developed a system of random insertion in the *E. coli* K12 genome [1,2], which is accomplished by means of the viral extremities and gives linear and non-permutated integration within a given gene. Two viral genes, *A* and *B*, are known to mediate integration [3], but their biochemical activity is still unclear; recently we have shown that the product of Mu *lig* gene takes part in the integrative process [4] and can complement *E. coli* K12 and T4 ligase$^-$ mutants.

Integrative recombination appears to be mediated by specific topoisomerase activities [5,6]. We postulate that Mu conservative integration [7] can be carried out by a nicking–closing complex. To prove this we tried to demonstrate Mu-specific topoisomerase activity and to clarify if genes *A*, *B* and/or *lig* are responsible for such product(s). Not having succeeded in showing such an activity in crude extracts of Mu lysogens, we tried to demonstrate the possible Mu topoisomerase as a 'complementing action' by Mu on T4 *amG39*, a mutant in T4 topoisomerase II [8–10].

These results show that *su*$^-$ Mu lysogenic strains restore in vivo a normal cycle of T4 *amG39*, and in vitro the T4 DNA topoisomerase II activity measured in cell extracts obtained after T4 *amG39* infection.

2. Materials and methods

2.1. *Bacterial and phage strains*

Escherichia coli K12 derivatives C600 *su*$^+$, RS54 *su*$^-$ and RS54 lysogens for Mu *c*$^+$ (R278), for Mu Δ*c-B*, deleted in the early region (R261) and for Mu *ligts2* (R273) [11]. T4 *amG39*, defective in topoisomerase activity [8].

2.2. *Growth rate of T4* amG39 *on various strains and* 2.3. *Total incorporation of [*methyl-^{3}*H] thymidine into T4* amG39 *DNA*

The experiments were performed at 25°C as described in [8]. [*methyl*-^{3}H]Thymidine was obtained from Amersham.

2.4. *Topoisomerase test*

Strains C600, RS54 and R261 were grown to 5×10^8 cells/ml in LB and infected with T4 *amG39* at m.o.i. of 5 at 37°C. Cells were lysed by sonication 15 min after infection and then centrifuged 15 min at 8000 × *g* in an Eppendorf model 3200 centrifuge.

Protein concentration was measured with the Biorad protein assay kit.

Each supernatant was tested for T4-induced ATP-dependent DNA topoisomerase II activity as in [10]. The substrate was pAT153 DNA, a plasmid derived from pBR322 by deletion of 600 basepairs, at 0.1 μg/ assay mixture.

Gel photographs were analyzed with the Beckman DU-8 gel scanner.

3. Results

3.1. *Growth restoration of T4* amG39 *in Mu lysogenic* su$^-$ *strains*

Table 1 shows that the presence of a Mu prophage can bring back almost to 1 the e.o.p. of a T4 *amG39* infecting phage, even in the presence of viral immunity as in R278. The restoration effect is lost in presence

Published by Elsevier/North-Holland Biomedical Press
00145793/82/0000–0000/$02.75

Table 1
T4 *amG39* growth restoration

Strain	Relevant genotype	Efficiency of plating
C600	*su*$^+$	2.2×10^8
RS54	*su*$^-$	1.0×10^7
R278	*su*$^-$ (Mu *c*$^+$)	2.1×10^8
R273	*su*$^-$ (Mu *ligts2*)	1.1×10^7
R261	*su*$^-$ (Mu Δ*c-B*)	1.8×10^8

of Mu *ligts2* (strain R273), while the involvement of genes *A* and *B* in this phenomenon is excluded by the fact that also strain R261 (Mu Δ*c-B*) restores the growth of T4 *amG39*.

The p.f.u. of T4 *amG39* mutant on *su*$^-$ strain is ~4% compared to that on *su*$^+$ strain. This relatively high value, in agreement with [8,12], depends on the leakiness of this kind of mutant.

In addition, host range-specific factors also appear to influence phage growth, as indicated by the greater burst size on *E. coli* K12 *su*$^-$ compared to *E. coli* B *su*$^-$ [8]. Due to the impossibility to obtain Mu lysogens in *E. coli* B, we had to use an *E. coli* K12 suppressor strain, even if we did not obtain striking differences between *su*$^+$ and *su*$^-$ hosts.

3.2. *Replication of T4* amG39 *in Mu lysogenic* su$^-$ *strains*

The kinetics of incorporation of [^{3}H]thymidine in T4 *amG39* after infection of R261 and R273 are shown in fig.1. After 75 min the amount of incorporation into T4 *amG39* infecting strain R261 is ~5-times greater than when infecting R273 and RS54, the non-lysogenic control. Restoration is insensitive to nalidixic acid (fig.1B).

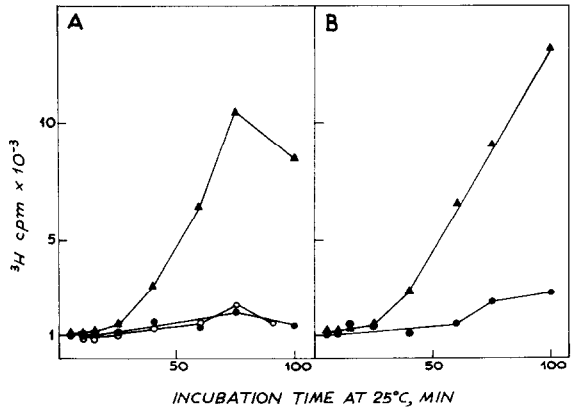

Fig.1. DNA synthesis of T4 *amG39* in Mu lysogens: (A) without nalidixic acid; (B) with nalidixic acid; (•) RS54; (○) R273; (▲) R261.

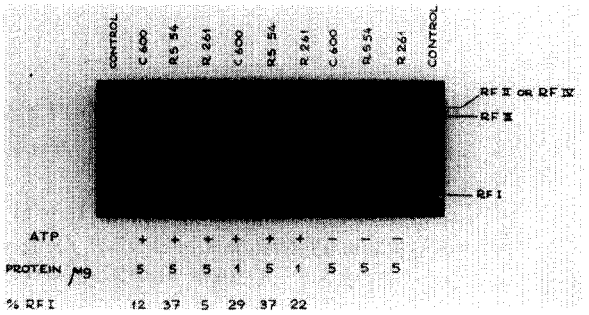

Fig.2. Restoration of topoisomerase activity of T4 *amG39*. ATP was at 0.5 mM, when added. The incubation mixture for each strain contained a different quantity in μg of crude extract: (RFI) supertwisted; (RFII) nicked; (RFIII) linear; (RFIV) relaxed DNA.

3.3. *In vitro reactivation of the topoisomerase activity of T4* amG39

Strains C600, RS54 and R261 were infected with T4 *amG39*. Sonically disrupted cell extracts, diluted to the same protein concentration (5 mg/ml), were tested for T4-induced topoisomerase II activity [10] (fig.2).

The topoisomerase test measures the conversion of the pAT153 supercoiled form, RFI, into the relaxed form RFIV through the formation of the intermediate topoisomers. This DNA supercoil relaxation is ATP-dependent and, in the absence of ATP, unit length linear DNA can be observed easily upon the addition of SDS and proteinase K [10,13].

It is evident that for the same amount of extract for each infected strain there is a different amount of activity, measured as RFI disappearance. Looking at the *su*$^-$ strains, we note that Mu presence brings the activity to the level of the *su*$^+$ control. This increase in topoisomerase activity is ⩾5-times higher than that in the non-lysogenic *su*$^-$ host, as can be seen comparing the activity of RS54 to the 1:5 dilution of R261.

We must point out that it is not easy to quantify correctly the topoisomerase activity, especially in crude extracts, since the test measures the reduction of the substrate and not the product of the reaction itself. The reduction of the substrate can be ascribed

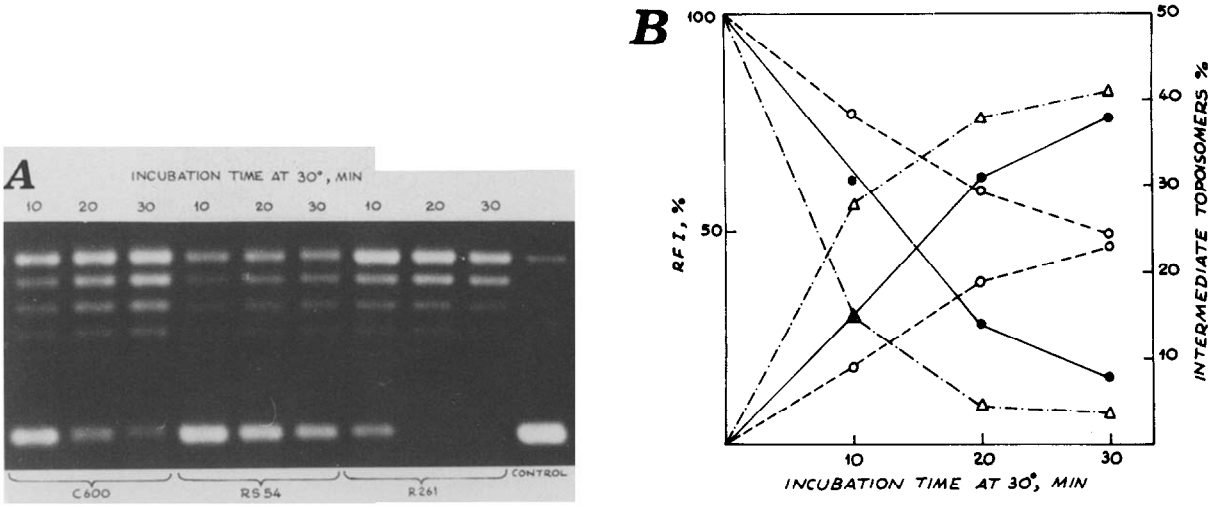

Fig.3. Kinetics of restoration of T4 *amG39* topoisomerase activity. (A) Gel electrophoresis: Supercoiled DNA of pAT153 was incubated at 30°C for various times with 5 μg protein obtained from each strain infected with T4 *amG39*. (B) Gel photograph scanning: Fraction of DNA in supercoiled and intermediate forms from each assay after scanning of gel photograph (A): (•) C600; (○) RS54; (△) R261.

also to contaminating endonuclease activities, normally present in *E. coli* crude extracts.

To overcome these difficulties, we followed the kinetics of both the RFI disappearance and the intermediate topoisomers formation, which are the specific products of this kind of enzyme. With the same amount of protein from each of the 3 extracts, the RFI reduction and the corresponding increase in topoisomers are evidently higher in C600 and R261 than in RS54 (fig.3A).

The values obtained by scanning of the gel photograph, expressed as % of RFI left after treatment of the plasmid, are plotted in fig.3B. The % itself corresponds to the ratio 'area of the RFI peak/total DNA' in each slot.

It is possible to conclude that the presence of Mu in the *su*$^-$ strain R261 is responsible for an increased activity which can be even higher than that observed in the *su*$^+$ control, C600.

4. Discussion

Topoisomerases are primarily involved in DNA initiation [10,14] or in legitimate or illegitimate recombination [5,6].

We show that Mu can restore the topoisomerase activity deficient in a T4 mutant in gene *39*, that such an activity is expressed in lysogens even in the presence of viral immunity, and that is independent from Mu *A* and *B* genes. This complementing activity was measured in vivo as recovery of normal T4 *amG39* growth rate and DNA synthesis and, in vitro, as restoration of a normal level of topoisomerase activity.

The in vivo data show that the presence of Mu brings back to 1 the growth rate of T4 *amG39* infecting *su*$^-$ lysogenic strains. Comparison of the growth rate and DNA synthesis values between a *su*$^+$ and a *su*$^-$ strain are in agreement with [8]: these amber mutants grow on cells lacking the suppressor and produce a substantial yield of progeny phage [8]. This may be due to host gyrase subunit that can compensate for the loss of topoisomerase gene functions. In fact, T4 DNA replication, normally only weakly sensitive to gyrase inhibitors, becomes more sensitive to these drugs in topoisomerase-defective mutants [9,12]. The *nal*-resistant DNA synthesis, measured in R261 strain (fig.1B), is a good indication that the recovery of normal T4 growth is due to Mu functions expressed in *su*$^-$ lysogenic strains.

The in vivo results are supported by the observation that also the T4-induced topoisomerase activity, reduced in *39* amber mutants [10], is restored in Mu lysogenic strains.

However, we must consider that a real quantification of the topoisomerase activity restoration due to

Mu is impaired by the presence of a residual activity in RS54, an *E. coli* K12 *su*$^-$ strain. This residual activity, not detected in [9,10], is due to *E. coli* K12–*E. coli* B host range differences, as seen in vivo [8,12]. On the other hand, *E. coli* B strains cannot be used as Mu does not grow on them.

As a consequence, these data are most probably an underestimation of the effective Mu ability to reactivate the topoisomerase defect in T4 *amG39* mutant.

It is very interesting that Mu *ligts2* has lost the ability to complement a T4 *amG39* superinfecting phage (see table 1, strain R273). More Mu *lig* mutants will be analyzed to clarify whether Mu *lig* gene is the only one responsible for the T4 *amG39* complementing effect, or a cooperation between two or more products is necessary.

Our results exclude that genes *A* and *B* are involved in the process.

Acknowledgements

We thank Dr C. Marchelli and Professor E. Calef for their criticism.

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