GENE $\underline{m}_3$ MEDIATED EFFLUX OF INTRACELLULAR LEUCINE FROM BACTERIOPHAGE P22 INFECTED *SALMONELLA TYPHIMURIUM*

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SUMMARY

Infection of *Salmonella typhimurium* with the $\underline{m}_3$ mutant of bacteriophage P22 leads to a rapid and severe efflux of intracellular leucine. The superinfection exclusion (*sie*) genes of P22 interfere with the function of m_3 gene, the product(s) of which is speculated to be an internal protein of phage P22.

INTRODUCTION

Although $\underline{m}_3$ mutants of bacteriophage P22 have been used as morphology markers for some time (1) the biological function of the $\underline{m}_3$ gene was unknown. It has been observed in this laboratory that $\underline{m}_3$ mutants do not grow well at high multiplicities of infection when compared with $\underline{m}^+$ at similar multiplicities. The $\underline{m}_3$ mutants cause permanent inhibition of the rate of incorporation of exogenous uridine into the host (2,3). It was also shown earlier that bacteriophage P22 infection results in transient depression of the rate of transport (uptake) across the cell membrane of the host (4). Further, the transient inhibition of the rate of macromolecular synthesis in P22 infected *S.typhimurium* (5) seems to be related to the transient inhibition of the net influx. Efflux of solute molecules from the pool of phage infected host has been reported by a number of workers (6-10).

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It may be due to physical damage of the membrane but the sealing is most probably a phage gene mediated phenomenon (9,10). Infection with phage P22 also results in transient efflux of intracellular solutes from S.typhimurium (11). The present report will show that in contrast to transient efflux infection with the m_3 mutants leads to a severe efflux of solutes with the permanent loss of the capacity to transport solutes inside the cell.

MATERIALS AND METHODS

^{3}H-L-leucine (5200 mC/mM) was obtained from the Bhabha Atomic Research Centre, Trombay, India. All other chemicals were commercial preparations of high purity. Nitrocellulose filters (0.45μ) were obtained from Schleicher and Schüll Co. of U.S.A.

S.typhimurium LT2, the sensitive strain, its two lysogens, sie A^+ sie B^+ and sie A^- sie B^- (12), the wild type phage P22 (C^+) and its clear plaque forming mutants, C_1, C_1h_{21}, C_2 (1) were kindly supply by M.Levine of the University of Michigan, Ann Arbor, Michigan U.S.A. The m_3 mutants of phage P22 were isolated from the corresponding m^+ strains obtained from M.Levine's laboratory and were tested with the standard strains of M.Levine. The other two lysogens, sie A^+ sie B^- and sie A^- sie B^+ (13) were obtained from D.Botstein of Massachusetts Institute of Technology, Massachusetts, U.S.A. The sie A^+ sie B^+ and sie A^- sie B^- lysogens are referred to as sie$^+$ and sie$^-$ lysogens. All the phage stocks were purified from lysates by differential centrifugation and finally suspended in mineral base media (14).

Amino acid transport was measured as described before (11).

RESULTS AND DISCUSSION

Efflux of intracellular leucine following infection with m_3 mutants:

LT2 exhibits transient change in the rate of transport of solutes following infection with "C" mutants of phage P22 (4) whereas m_3 mutants permanently block the transport process (3). Therefore the efflux of intracellular leucine from the infected cells was studied (Fig.1). When the transport of leucine attains the maximum rate the cells were infected with m_3C_1 and the amount of leucine retained by the cells was measured at different times. Uninfected cells and C_1 infected cells were kept as control. There is rapid

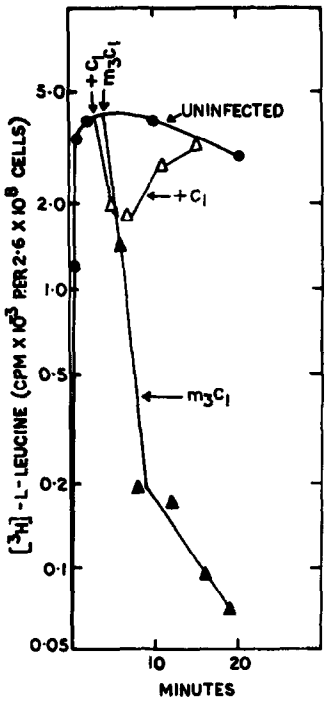

Fig.1. Effect of phage infection on leucine transport. Exponentially growing cells were infected with either +$\underline{C}_1$ or $\underline{m}_3\underline{C}_1$ strains (as indicated) at an m.o.i. of 10. The experimental procedure was as described before (11).

efflux of leucine following infection with $\underline{m}_3\underline{C}_1$ whereas the efflux is small and transient in case of $\underline{C}_1$ infection. Similar results were obtained with a number of $\underline{m}_3$ mutants carrying other markers, e.g., $\underline{m}_3\underline{C}_2$, $\underline{m}_3\underline{C}_1\underline{h}_{21}$, $\underline{m}_3\underline{C}_2\underline{h}_{21}$ etc., (results not presented), indicating that $\underline{m}_3$ gene is most probably involved in the efflux process.

The phage infection may inhibit influx and as a result leucine accumulated inside the cells against concentration gradient may leak out by passive diffusion process. Alternatively, phage infection not only blocks influx but promotes efflux as well. In order to decide between the two possibilities the cells were allowed

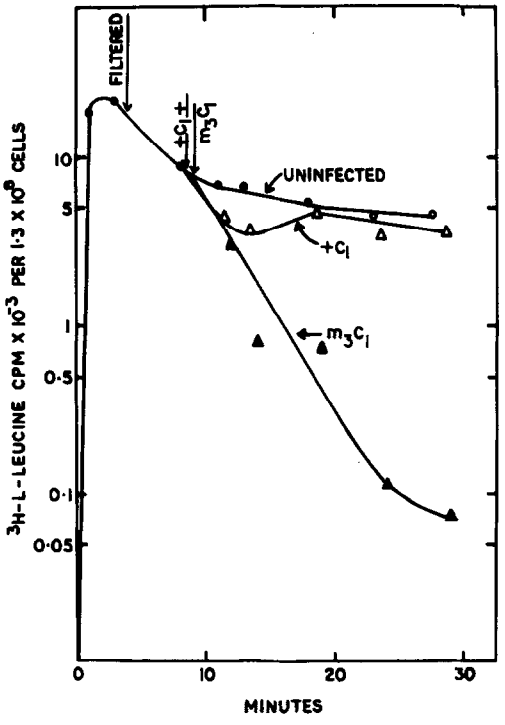

Fig.2. Efflux of intracellular leucine following infection with m_3 mutants. The experimental condition was the same as in Fig.1 except the modifications that when the intracellular pool attains maximum radioactivity the cells were quickly washed free of the exogenous radioactive leucine and resuspended in cold medium as described in the text.

to incorporate ^{3}H-leucine to the maximum level, then quickly filtered through millipore, washed and suspended in fresh medium containing cold leucine and the rate of efflux was measured as before (Fig.2). Under the experimental condition the efflux of ^{3}H-leucine from both uninfected and C_1 infected cells is comparatively small whereas the rate of efflux following m_3 infection is quite rapid. Thus it appears that infection with m_3 mutants results in the promotion of efflux.

Efflux of intracellular leucine following infection of lysogens with m_3 mutants :

To investigate whether the $\underline{m}_3$ gene of the invading phage has to be expressed in the infected cell or not, the $\underline{m}_3$ gene mediated efflux was studied in lysogens infected with $\underline{m}_3$ mutants. Besides the immunity repressor which prevents the expression of the superinfecting phage, the wild type P22 lysogen has additional mechanism for the exclusion of the superinfecting phage genome. There are two superinfection exclusion systems, *sie* A and *sie* B (15,16). The efflux of intracellular leucine was studied in four

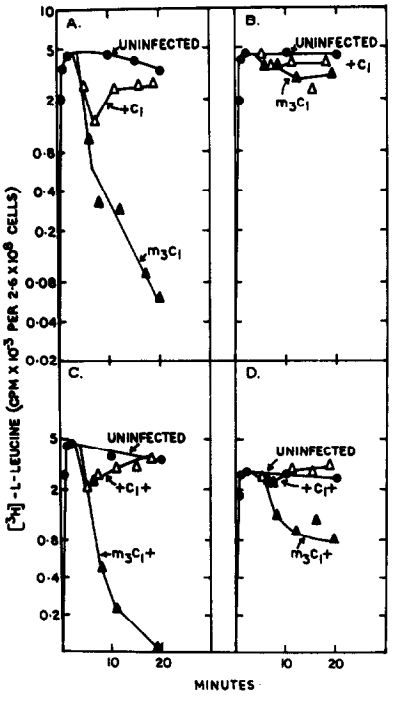

Fig.3. *The sie gene mediated interference with the m_3-mediated efflux process.* The experimental conditions were the same as in Fig.1 except that the different lysogens were used instead of the sensitive cells. (A) *sie* A^- *sie* B^- (B) *sie* A^+ *sie* B^+, (C) *sie* A^- *sie* B^+ (D) *sie* A^+ *sie* B^-

different types of *sie* lysogens (Fig.3). The *sie* A^- *sie* B^- lysogen behaved in the same way as the sensitive cell (Figs. 1 and 3A). Practically no efflux is observed in *sie* A^+ *sie* B^+ lysogen following infection with either m_3C_1 or C_1 (Fig.3B). Although both *sie* A^- *sie* B^+ and *sie* A^+ *sie* B^- lysogens exhibit the efflux following infection with m_3C_1 there is considerable quantitative difference in the extent of efflux in the two strains (Figs. 3C and D). In case of *sie* A^- *sie* B^+ strain practically all of the intracellular leucine diffuses out whereas at least 25-30% of leucine is retained in *sie* A^+ *sie* B^- strain even after 20 min following infection. These results suggest that both *sie* A and *sie* B genes interfere with the efflux of intracellular leucine induced by the m_3 gene. *Sie* A, however, is much more efficient than *sie* B in this respect.

The above results indicate that the phage infection leads to the net efflux of intracellular solutes. This efflux is temporary in nature and may be due to inhibition of influx process (3). Soon after the rate of influx increases except in case of infection with m_3 mutants. As the effect of the m_3 gene is observed even in a lysogen, the m_3 gene product is speculated to be an internal protein of the phage which enters the cellular interior along with the invading phage DNA.

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