The possible involvement of protein synthesis in the injection of PL-1 phage genome into its host, *Lactobacillus casei*

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The process of genome DNA injection, after adsorption, by phage PL-1 into host cells of *Lactobacillus casei* was monitored by using the electron microscope. Injection of DNA was inhibited by the protein-synthesis inhibitors chloramphenicol and erythromycin at concentrations where the colony-forming ability of cells not infected by phage was unaffected. The results suggest that protein synthesis may be involved in some way in the process of genome injection.

Introduction

Information on the initial stages of infection by bacteriophages with a long non-contractile tail, especially on the process of genome injection after adsorption, is much less extensive than that for phages with a contractile tail sheath, such as T-even coliphages. These contractile tail sheaths play an important role in the injection of the phage genome into the host cell (Goldberg, 1980).

We have been investigating the mechanism of genome injection by phage PL-1, which has an icosahedral head with a long non-contractile tail, into the host *Lactobacillus casei* by determining (i) the amounts of ³²P-labelled phage not sheared from the phage-adsorbed cells by blender treatment (Hershey & Chase, 1952), (ii) the number of plaque-forming phage-cell complexes after blending (Watanabe *et al.*, 1979), (iii) the host-killing ability of the phages (Watanabe & Takesue, 1972), and (iv) the number of ghost particles with empty heads observed by electron microscopy (Watanabe *et al.*, 1987). These studies have demonstrated that the process of genome injection is dependent on Ca²⁺ and a high intracellular concentration of high-energy compounds such as ATP.

In the present paper, we have examined by electron microscopy the effects of some protein-synthesis inhibitors on the process of genome injection.

Methods

Phage and bacterial host. Phage PL-1 and its host *Lactobacillus casei* ATCC 27092 have been described previously (Watanabe *et al.*, 1970, 1982).

Phage genome injection. Cells were infected with phages in MR medium (pH 6.0) (Murata *et al.*, 1969) at 37 °C during the exponential growth phase (OD₆₀₀ = 0.35, about 2.5 × 10⁸ c.f.u. ml⁻¹) at a multiplicity of infection of about 100. When the effects of protein-synthesis inhibitors were tested, they were added to the cell suspensions either before, at the same time or after the cells were infected with phages. The extent of PL-1 DNA injection was determined from the increase with time in the number of ghost particles with empty heads among phages already absorbed to the cells. Chloramphenicol was purchased from Sankyo and erythromycin was from Sigma.

Electron microscopy. After fixing the phage-cell complexes with 2% (v/v) glutaraldehyde at 0 °C for 90 min, and washing by centrifugation, the pellets were suspended in TMB-Ca (50 mM-Tris/maleate buffer, pH 6.0, +10 mM-CaCl₂) and negatively stained with 2% (w/v) uranyl acetate for electron microscopy. The electron micrographs were taken with a JEOL-100C electron microscope at 80 kV.

Results and Discussion

Most of the input phages were adsorbed to the cells almost instantly and in a tail-first orientation, irrespective of the presence of antibiotics. After appropriate times, samples of the infected cell suspensions were removed and fixed with glutaraldehyde for electron microscopy. The proportion of ghost particles having an empty head (which were easily distinguished from intact phages with tightly packed DNA) among the total phage particles adsorbed to a cell [ghost/(intact + ghost) × 100] was determined for 20–30 separate cells from each sample. Each cell was usually observed to adsorb on average about 40 phage particles.

Fig. 1 shows the relationship between the concentrations of protein-synthesis inhibitors and the extent of PL-1 DNA injection, which was derived from the number of ghost particles observed 80 min after

Fig. 1

Fig. 1. Effect of the concentrations of protein-synthesis inhibitors on the extent of PL-1 DNA injection into *L. casei*. Cells, which had been pretreated with various concentrations of chloramphenicol (●, ○) or erythromycin (■, □) for 60 min, were mixed with phages at a multiplicity of infection of about 100, and incubated at 37 °C for 80 min in MR medium (pH 6.0). ●, ■, Percentage of PL-1 DNA injected. ○, □, Relative numbers of viable cells. Vertical bars show the standard deviation of the mean.

Fig. 2

Fig. 2. Effect of chloramphenicol on the extent of PL-1 DNA injection. Chloramphenicol (2.0 mg ml⁻¹) was added to the incubation mixture at -60 (■), 0 (●) and 10 min (⊕), and removed from the inhibited mixture at 10 min (⊕), as indicated by the arrows. ○, Control without chloramphenicol. The standard deviation was, on average, about 10%.

infection. In a control experiment without the addition of antibiotics, the extent of PL-1 DNA injection increased with time up to a maximum of about 80%, and was usually completed within 60 min. By contrast, when the host cells were pre-incubated with the drugs for 60 min at 37 °C prior to infection, the extent of DNA injection decreased with increasing drug concentration. The concentrations of drug required to decrease the number of ghost phages by half were about 7 μg ml⁻¹ for chloramphenicol and 1.5 μg ml⁻¹ for erythromycin. Cell suspensions treated for 60 min with these antibiotics, but not subsequently infected with phages, showed no reduction in viability when diluted and plated onto MR agar plates. This confirmed that under the conditions used, these antibiotics have no bactericidal effect and protein synthesis resumes in antibiotic-treated cells.

At various times after the infection of cells with phages, chloramphenicol was added to a final concentration of 2.0 mg ml⁻¹, and, at intervals, the phage-cell complexes were observed by electron microscopy. Fig. 2 shows the changes with time in the number of ghost phages, i.e. the extent of PL-1 DNA injection, with or without chloramphenicol. When chloramphenicol was added immediately after mixing the cells with phage suspension, the extent of DNA injection was similar to that seen when chloramphenicol was added to the cells 60 min before infection. However, when chloramphenicol was added to the cells 10 min after the phages (which had already started to inject their DNA), injection was not affected. Thus the inhibitory effect of chloramphenicol on DNA injection was observed only when added before DNA injection. These results suggest

that protein synthesis in the host cells may be required only for initiation of PL-1 DNA injection and not injection itself, although there is the alternative possibility that the process possibly driven by transcription, as suggested for other systems, may require protein synthesis.

Then, 10 min after cells had been infected with phages in the presence of chloramphenicol, the stabilized cell-phage complexes were freed from chloramphenicol by centrifugation and resuspension in fresh MR medium. Incubation was continued. The resuspended cells underwent infection at the same rate as control cells which had not been exposed to chloramphenicol (Fig. 2).

Transfer of the genomes of phages lacking a contractile tail sheath from the phage head to the host cytoplasm has been reported (Roessner *et al.*, 1983; Filali Maltouf & Lebedan, 1983; Letellier & Labedan, 1985; Sabelnikov, 1989) to be independent of membrane potential (Mitchell, 1976). The synthesis of one or more proteins in the newly infected cells has been demonstrated to be required for infection by phage T5 (Lanni, 1965). It was reported for phage T7 that transfer of the first 60% of the DNA was tightly coupled with its transcription by the host RNA polymerase (Zavriev & Vorob'ev, 1984), although the influence of protein-synthesis inhibitors was not investigated. Since, in our experiments, both chloramphenicol and erythromycin inhibited the injection of PL-1 DNA, it is likely that proteins synthesized during the early stages of infection and prior to complete DNA transfer itself are required for transfer of DNA from phage PL-1 to cells of *L. casei*. Further investigations on the participation of protein

synthesis in this process are now being carried out, since chloramphenicol has been reported to inhibit a wide range of biochemical processes besides protein synthesis.

References

- FILALI MALTOUF, A. & LABEDAN, B. (1983). Host cell metabolic energy is not required for injection of bacteriophage T5 DNA. *Journal of Bacteriology* **153**, 124–133.
- GOLDBERG, E. (1980). Bacteriophage nucleic acid penetration. In *Receptors and Recognition*, series B, vol. 7, part 1, pp. 115–141. Edited by L. L. Randall & L. Philipson. London & New York: Chapman and Hall.
- HERSHEY, A. D. & CHASE, M. (1952). Independent functions of viral protein and nucleic acid in growth of bacteriophage. *Journal of General Physiology* **36**, 39–56.
- LANNI, Y. T. (1965). DNA transfer from phage T5 to host cells: Dependence on intercurrent protein synthesis. *Proceedings of the National Academy of Sciences of the United States of America* **53**, 969–973.
- LETELLIER, L. & LABEDAN, B. (1985). Release of respiratory control in *Escherichia coli* after bacteriophage adsorption: process independent of DNA injection. *Journal of Bacteriology* **161**, 179–182.
- MITCHELL, P. (1976). Vectorial chemistry and the molecular mechanics of chemiosmotic coupling: power transmission by proticity. *Biochemical Society Transactions* **4**, 399–430.
- MURATA, A., SOEDA, E. & SARUNO, R. (1969). Factors influencing plaque formation by bacteriophages of *Lactobacillus acidophilus*. *Nippon Nogeikagaku Kaishi* **43**, 311–316.
- ROESSNER, C. A., STRUCK, D. K. & IHLER, G. M. (1983). Injection of DNA into liposomes by bacteriophage λ . *Journal of Biological Chemistry* **258**, 643–648.
- SABELNIKOV, A. G. (1989). DNA transfer through cell membranes in bacteria. *Bioelectrochemistry and Bioenergetics* **22**, 271–288.
- WATANABE, K. & TAKESUE, S. (1972). The requirement for calcium in infection with *Lactobacillus* phage. *Journal of General Virology* **17**, 19–30.
- WATANABE, K., TAKESUE, S., JIN-NAI, K. & YOSHIKAWA, T. (1970). Bacteriophage active against the lactic acid beverage-producing bacterium *Lactobacillus casei*. *Applied Microbiology* **20**, 409–415.
- WATANABE, K., TAKESUE, S. & ISHIBASHI, K. (1979). Adenosine triphosphate content in *Lactobacillus casei* and the blender-resistant phage-cell complex-forming ability of cells on infection with PL-1 phage. *Journal of General Virology* **42**, 27–36.
- WATANABE, K., TAKESUE, S., ISHIBASHI, K., IWAMOTO, T. & KONDO, J. (1982). Fine structure of PL-1 phage active against *Lactobacillus casei*. *Journal of General and Applied Microbiology* **28**, 1–6.
- WATANABE, K., FUKUZAKI, T., HAYASHIDA, M. & NAKASHIMA, Y. (1987). Electron microscopic study of the process of DNA ejection from the head of PL-1, a *Lactobacillus casei* phage. *Journal of General Virology* **68**, 569–572.
- ZAVRIEV, S. K. & VOROB'EV, S. M. (1984). Evidence for the coupling of T7 DNA injection with its transcription during infection. *FEBS Letters* **165**, 31–34.