Is the Injection of DNA Enough to Cause Bacteriophage P22-
Induced Changes in the Cellular Transport Process of
Salmonella typhimurium?

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It was demonstrated earlier in this laboratory that phage P22 induces a transient depression in the cellular transport processes of the host Salmonella typhimurium immediately after infection and that an effective injection process is enough to cause the depression. By using defective phage particles that contain host DNA instead of phage DNA for infection, it has been demonstrated that the injection of phage-specific DNA is essential for this. The defective particles adsorbed to the host and injected their DNA, but the cellular transport processes of the host were not altered. Thus, the injection of host DNA by the phage fails to affect the transport process. Insensitivity of the phage DNA-induced depression in transport to chloramphenicol rules out the involvement of newly synthesized protein in this change and indirectly suggests the possible role of phage DNA-associated internal proteins of P22.

It has been reported from this laboratory that infection of Salmonella typhimurium with phage P22 leads to an immediate depression in the cellular transport process (11). This phenomenon has been extensively studied (1, 5, 8, 11, 15). Such depression is, however, not observed after infection with a 16°ts mutant at the non-permissive temperature or of superinfection of a sie+ (superinfection exclusion-positive) lysogen with P22 C; (3, 11). In these cases the injection of DNA does not take place normally (6, 14). It has been shown in an earlier publication that injection of DNA is enough for transient depression (8), but it will be demonstrated here that injection of phage-specific DNA is essential for that purpose.

The defective phage particles produced by the induction of int- lysogen (13) were used to demonstrate this. The int- mutant infects sensitive cells and produces normal phage particles but is defective in the integration gene and hence cannot lysogenize the host. However, stable lysogens can be constructed by complementation with int+ phage. Such lysogens can be induced, but defective phage particles containing mostly host DNA molecules having the length of phage DNA are produced (13, 16).

As will be shown below, the defective particles adsorb to the host and inject their DNA, but there is no depression in the cellular transport processes. It should be pointed out that an m3int- strain instead of m int- has been used in these studies, as the m3 mutant of P22 is known to produce a severe depression (15).

MATERIALS AND METHODS

Chemicals. [3H]uridine (5,000 Ci/mol) and [3H]-
leucine (5,200 Ci/mol) were obtained from Bhabha
Atomic Research Centre, Bombay, India. PPO (2,5-
diphenyloxazole) and dimethyl POPOP [1,4-bis-(5-
phenyloxazolyl)benzene] were purchased from Amer-
sham-Searle Corp., Arlington Heights, Ill. Nitrocellu-
lose membrane filters (0.45 μm) were obtained from
Schleicher & Schuell Co., ARLINGTON, N.H. Chloramphen-
icol was a product of Parke, Davis Ltd., Bombay. Mitomycin C was obtained as a gift from Y. Takagi,
Kyushu University, Fukuoka, Japan. Cesium chloride
was obtained from Harsaw Chemical Co. All other
chemicals were commercial preparations of analytical
grade.

Bacterial and phage strains. S. typhimurium
(LT2), its sieA - sieB - lysogens, and the two auxo-
trophs proA15 and proC90 (9) used in transduction
experiments were obtained from M. Levine, Depart-
ment of Human Genetics, University of Michigan, Ann
Arbor, Mich. The phage strains P22 C, and P22 m3int-
were also obtained from M. Levine. The P22 m3int-
lysogen was prepared as described by Smith (13).

The defective particles produced by the induction of m3int- lysogen will be referred to as defective par-
ticles. m3int- will designate the particles produced by
infesting cells with the m3int- mutant of the phage.

Preparation of defective particles. The defec-
tive particles were prepared by inducing P22 m3int-
lysogen with mitomycin C and were purified by differential centrifugation and finally by CsCl gradient centrifugation (13). For the preparation of 32P-labeled defective particles, m3int+ lysogen was grown in minimal medium. Exponentially growing cells were harvested, washed, and suspended in low-phosphate medium as described by Botstein and Levine (2). Mitomycin C was added at a concentration of 10 μg/ml. Carrier-free 32P (1 μCi/ml) was added 10 min after the addition of mitomycin. The particles were purified from the lysates described earlier (13). For comparative transport studies, unlabelled defective particles were simultaneously prepared in low-phosphate medium.

Measurement of growth and transport. The cells were grown in minimal medium as described before (3). The rate of uridine uptake into the soluble pool was measured as described by Rao et al. (11). Amino acid transport was studied according to the method described by Khandekar et al. (8).

RESULTS

Frequency of transduction by int- defective particles. Weaver and Levine (16) analyzed the DNA of the int- defective particles with the help of a restriction endonuclease. Digestion of DNA obtained from defective particles with EcoRI restriction endonuclease results in fragments obtained from only one side (right-hand side) of the prophage which is near the proC region of the host. Due to such polarity of encapsulation and the fact that such encapsulation does not proceed with high frequency beyond the third or fourth headful, these defective particles transduce the proC marker at a much higher frequency than the proA marker, which is situated at the other end of the prophage map (16). To confirm that the defective particles isolated in the present investigations could inject their DNA into the host, the transducibility of proC and proA markers by defective phage particles was tested as described by Smith (13). The results presented in Table 1 confirm the transducing capacity of these defective particles.

The antiserum-neutralizing capacity of the defective phage particles was also found to be comparable to that of normal wild-type phage (data not presented).

Estimation of the number of tailless particles. In spite of the fact that the defective particles are capable of transducing certain markers, the question may be raised as to how many of these are capable of adsorbing to the host, especially because it is known that the defective particles produced on induction of int- lysogen are often tailless (13). Since the depression of the cellular transport processes is dependent on the multiplicity of infection (3), it was necessary to determine the extent of adsorption to the host of the particles in the preparation. For this, the cells were incubated with 32P-labeled defective particles for 10 min and then collected by centrifugation. The fraction of radioactivity associated with the cells is an approximate measure of the extent of adsorption of the particles to the host. The results presented in Table 2 indicate that 60 to 75% of the phages adsorb to the host. The results of two experiments presented in Table 2 are the extremes of five experiments.

Effects of defective phage particles on cellular transport processes. The rate of uptake of certain solutes into the soluble pool of S. typhimurium undergoes transient depression as a result of infection with phage P22 (11). The m3 mutant, however, causes a permanent depression of the rate of uridine uptake (15). Therefore, the cells growing exponentially in minimal medium were infected with either P22 m3int− or the defective particles (Fig. 1). Uninfected cells and C1-infected cells both served as controls. As the defective particle preparation contains a number of tailless particles, 100 defective particles were added per cell to ensure the injection of DNA and to maximize the effect, if any. Infection with m3int− causes a permanent inhibition of the transport, as expected (15), and C1 infection leads to transient depression, as demonstrated earlier (11). However, infection with the defective particles does not lead to any change in the rate of uptake of extracellular uridine.

The effect on transport was measured in another way. The m3 mutants are known to cause a severe efflux of intracellular leucine (1); therefore, the efflux of this amino acid was studied in cells infected with either P22 m3int− or defective particles.

<p>| Table 1. Frequency of transduction of proA and proC markers by defective particles |
|----------------------------------|----------------------------------|----------------------------------|</p>
<table>
<thead>
<tr>
<th>Marker</th>
<th>Transductants/10^6 particles</th>
<th>Defective particles</th>
<th>m3int+</th>
</tr>
</thead>
<tbody>
<tr>
<td>proC90</td>
<td>20–50</td>
<td>0.3–0.4</td>
<td></td>
</tr>
<tr>
<td>proA15</td>
<td>1–2</td>
<td>0.27–0.40</td>
<td></td>
</tr>
</tbody>
</table>

* Freshly grown cells (2.5 × 10^6) were infected with 32P-labeled defective particles at a multiplicity of infection of 16.
particles. The defective particles do not cause any efflux of intracellular leucine, whereas there is severe efflux on infection with the m3int- mutant (Fig. 2), as expected.

DISCUSSION

As mentioned above, our earlier studies indicated that the injection of DNA by phage P22 is enough to cause transient depression in the cellular transport process observed in S. typhimurium after infection. The question may arise of whether the physical damage caused to the membrane of the host due to such an injection process is responsible for the observed phenomenon, especially when there are reports in the literature that there is leakage of cellular materials after infection with some phages (10, 12). The present investigations completely rule out this possibility in the case of P22. The defective phage particles containing host DNA inject the DNA, but the cellular transport remains unaltered. This also demonstrates that the injection of phage-specific DNA is essential for transient depression. In this connection it is interesting to recall an earlier observation made in this laboratory that the alteration in the cellular transport is observed even when infection is carried out in the presence of a high concentration of chloramphenicol (4). Although chloramphenicol-insensitive protein synthesis cannot be completely ruled out, the chance of such synthesis is low. It is more likely that some protein(s) associated and encapsulated with phage DNA, such as the proteins reported by Israel (7), causes such change after its injection along with the phage DNA. This, however, remains a speculation in the absence of any direct evidence.

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LITERATURE CITED


