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Transformation of *Rhodopseudomonas sphaeroides* with Deoxyribonucleic Acid Isolated from Bacteriophage Rϕ6P

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The transformation of the photosynthetic bacterium *Rhodopseudomonas sphaeroides* with the circular genome of the penicillinase-encoding, temperate bacteriophage Rϕ6P was demonstrated. The transformation was dependent on the infection of the recipient by another, apparently closely related, temperate bacteriophage, Rϕ9. Optimum transformation occurred in the recipient cells already lysogenic for Rϕ9 when superinfected with that bacteriophage at multiplicities of infection between 1 and 10 Rϕ9 particles per recipient cell.

Transformation, as a means of genetic analysis, has been demonstrated in a variety of both gram-negative and gram-positive bacterial species (2, 3, 13, 17, 25). In some cases, competence of the recipient cell for the transforming DNA occurs at a particular phase in its growth cycle, whereas in other cases, competence may be induced by a range of chemical treatments (6, 14, 18, 25).

Kaiser and Hogness (8) first demonstrated the transformation of *Escherichia coli* K-12 with DNA extracted from the defective phage λdg with a wild-type λ phage as a helper. Temperate bacteriophages have been implicated in the development of competence in other bacterial species (16, 23). Sjöström et al. (16) demonstrated that lysogeny with phage P11 was a requirement for competence in *Staphylococcus aureus* and that superinfection of the lysogen in the presence of the transforming viral DNA increased the level of transformation. Yasbin et al. (23) showed that strains of *Bacillus subtilis* 168, lysogenic for the bacteriophage ϕ105, had an increased level of transformation with viral nucleic acid, as compared with nonlysogenic strains. However, the transformation of chromosomal markers was decreased by lysogeny, and superinfection could not increase the level of this transformation.

The photosynthetic bacterium *Rhodopseudomonas sphaeroides* is now being used in attempts to analyze the genetic aspects of bacterial photosynthesis. A number of workers have reported that Inc P-1 (10, 15, 20) and Inc W (20) group R plasmids are stably maintained in this organism and have been used as cloning vectors (S. Kaplan, personal communication). A transformation system for *R. sphaeroides* would eliminate some of the problems arising from the need to transform cloned *R. sphaeroides* DNA into *E. coli* before its reintroduction into *R. sphaeroides* with conjugative plasmids.

In previous communications, we reported the isolation and characterization of a viral R plasmid from *R. sphaeroides* (12, 19). This temperate bacteriophage, Rϕ6P, exists as a covalently closed, circular molecule in both the extracellular and prophage states. In addition, the phage encodes the production of a β-lactamase, giving the lysogenized cells the readily identifiable phenotype of penicillin resistance. The circular genome and the penicillinase gene should make Rϕ6P a suitable cloning vector for *R. sphaeroides* DNA.

Here we report the transformation of *R. sphaeroides* with Rϕ6P DNA. This transformation directly involves the action of another temperate bacteriophage, Rϕ9.

MATERIALS AND METHODS

Bacterial and bacteriophage strains. *R. sphaeroides* RS6143 (*ade-3 car-1*) has been described previously (20). Strain RS6143(Rϕ9) was isolated by picking from the center of a turbid Rϕ9 plaque grown on RS6143 and purified twice for single colonies. Exponential-phase cultures of this strain (about 10^9 colony forming units [CFU] per ml) contain about 10^4 to 10^5 plaque-forming units (PFU) per ml of Rϕ9. Strain RS6143(Rϕ6P) was isolated as a penicillin-resistant clone of RS6143 infected with Rϕ6P and purified as described above. Strain RS901 is a prototrophic, penicillin-sensitive, wild-type strain of *R. sphaeroides* isolated in the St. Lucia area. It is lysogenic for Rϕ9.

The isolation and characterization of Rϕ6P has been described previously (12, 19). Bacteriophage Rϕ9 was isolated by plating a chloroform-treated supernatant of an exponential-phase culture of RS901 grown in Z broth (21) onto a lawn of RS6143. A single turbid plaque was picked and used to produce a high-titer lysate by the confluent lysis technique described previously (19).

Media and cultural conditions. Z broth (21) was routinely used in all experiments. Where necessary, it was solidified with 1.5% (wt/vol) agar. Z agar overlays consisted of Z broth plus 0.8% (wt/vol) agar. The selection of penicillin-resistant transformants was

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made on Z agar supplemented with 1 μg of penicillin G per ml (PZ agar). All dilutions were made in TM buffer (19).

All cultures were grown at 35°C unless otherwise indicated. Liquid cultures were aerated by shaking as described previously (19).

Bacteriophage techniques. The soft layer overlay technique of Adams (1) as described previously (19) was used for bacteriophage titration. An exponential-phase, Z broth culture of RS6143 was used as the indicator bacterium. High-titer lysates of the phages were produced by the confluent lysis technique (19). The virus particles were purified by pelleting the agar and cellular debris by centrifugation at 12,000 × *g* for 20 min. The virus particles were pelleted from the supernatant by centrifugation at 100,000 × *g* for 3 h and then banded in cesium chloride as described by Miller et al. (9). Purified virus (10^{12} to 10^{13} PFU/ml) was suspended in TM buffer and stored at 4°C.

Extraction of viral nucleic acid. Purified virus particles were extracted twice with TM buffer-saturated phenol as described previously (19). Viral nucleic acid was banded in an isopycnic ethidium bromide-cesium chloride density gradient by the method of Pemberton and Clark (11). The fluorescent band of nucleic acid was removed and extracted twice with *n*-butanol to remove the ethidium bromide. Cesium chloride was removed by dialysis against two changes of 0.1× SSC (15 mM NaCl plus 1.5 mM sodium citrate [pH 7.0]).

Electron microscopy. The method used for the electron microscopic examination of virus particles and viral nucleic acid has been described previously (19).

RESULTS

Characterization of Rϕ9. The characteristics of bacteriophage Rϕ9 were determined with the procedures described for the characterization of Rϕ6P (19). Bacteriophage Rϕ9 was morphologically indistinguishable from Rϕ6P when examined by electron microscopy and also possessed a circular encapsidated genome whose molecular length was 15 ± 0.5 μm, as compared with Rϕ6P, which had a molecular length of 16.5 μm. Unlike Rϕ6P, phage Rϕ9 did not encode a diffusible penicillinase and formed larger (2-mm), easily distinguished, turbid plaques on RS6143 after 24 h, as compared with the smaller (0.5-mm), rather indistinct plaques formed by Rϕ6P under the same conditions. The two phages were heteroimmune, with each forming plaques on hosts lysogenic for the other phage (Table 1). The efficiency of plating of Rϕ9 on strains lysogenic for Rϕ6P was reduced 100- to 1,000-fold, and this may be due to a weak restriction and modification system encoded by Rϕ6P, as this drop in plating efficiency was not observed when Rϕ9 was propagated on Rϕ6P lysogens (Table 1).

Detection of transformation. Initially, transformation was detected after the addition of 1 to 2 μg of Rϕ6P DNA to 200 μl of an exponential Z broth culture of RS6143 infected with Rϕ9 at a multiplicity of infection of about 10. The mixture was incubated at 35°C for 3 h, diluted into 5 ml of Z broth, and incubated overnight at 35°C. Penicillin-resistant transformants were recovered at a frequency of 2.5 × 10^{-5} per survivor when the culture was plated on PZ agar. Transformants were not recovered when the recipient was either infected with Rϕ9 or treated with Rϕ6P DNA independently. An identical experiment with an Rϕ9 lysogen, RS6143(Rϕ9), as the recipient cell yielded penicillin-resistant transformants at the higher frequency of 1.6 × 10^{-3} per survivor. When this lysogenic recipient strain was used, a low level of transformants was recovered from the culture incubated with Rϕ6P DNA only. The lysogenic culture supernatant contained a free Rϕ9 virus titer of 10^4 to 10^5 PFU/ml due to the spontaneous release of the prophage. The comparison of the lysogenic and nonlysogenic recipient strains is given in Table 2. The lower frequency of transformants with RS6143 was probably due, in part, to the lysis of the nonlysogenic recipient strain by the infecting Rϕ9 particles, as shown by the drop in the viable count for this strain (Table 2) which does not occur with the Rϕ9 lysogenic (immune) recipient strain.

Transformants recovered from either RS6143 or RS6143(Rϕ9) were immune to both Rϕ6P and

TABLE 1. *Heteroimmunity and restriction between Rϕ9 and Rϕ6P*[a]

Phage	Host strain	Titer for: RS6143	RS6143(Rϕ9)	RS6143(Rϕ6P)
Rϕ6P	RS6143	10^{10}	7.2 × 10^{10}	<10^2
Rϕ6P	RS6143(Rϕ9)	5.6 × 10^{10}	3 × 10^{10}	1.75 × 10^8[b]
Rϕ9	RS6143	3 × 10^{10}	<10^2	7.5 × 10^7
Rϕ9	RS6143(Rϕ6P)	7.8 × 10^9	10^8[c]	8 × 10^9

[a] High-titer preparations of each phage were made by the confluent lysis technique on the indicated host strain. Dilutions of each preparation in TM buffer were plated on Z layers seeded with the appropriate indicator strain and incubated at 35°C.

[b] Titer represents Rϕ9 spontaneously released from the host strain.

[c] Titer represents Rϕ6P spontaneously released from the host strain.

TABLE 2. *Effect of lysogeny with Rφ9 on the frequency of Rφ9-mediated transformation*[a]

Recipient strain	Viable count (CFU/ml) for:		Penicillin-resistant transformants (CFU/ml)	Frequency of penicillin-resistant transformants per survivor
	No Rφ9	DNA + Rφ9		
RS6143	6×10^9	4×10^8	10^4	2.5×10^{-5}
RS6143(Rφ9)	5×10^9	3×10^9	10^6	1.6×10^{-3}

[a] A 200-μl amount of an exponential-phase culture of the recipient strain (about 10^8 CFU/ml) was mixed with 20 μl of Rφ6P DNA (1 to 2 μg) and 10 μl of Rφ9 (10^{10} PFU/ml) and incubated at 35°C for 3 h. The mixture was diluted into 5 ml of Z broth and incubated overnight at 35°C and then plated for single colonies on Z and PZ agar.

Rφ9 as detected by cross-streaking against both phages and were presumably lysogenic for both. Spontaneously released phages from such transformants were found to produce two plaque types. The Rφ6P plaque type produced penicillin-resistant lysogens immune to Rφ6P alone, whereas the Rφ9 plaque type produced pencillin-sensitive lysogens immune to Rφ9 alone. These observations indicated that the transformed cells were lysogenic for both phages, rather than harboring a recombinant prophage. Whether recombination could occur between the two phages was not determined.

The addition of pancreatic DNase destroyed any transformation, but transformation was insensitive to RNase or protease (Table 3). The variation in the level of transformants observed in the RNase- and protease-treated mixtures was paralleled by the changes in the free virus titer, possibly due to the action of the enzyme on the virus particle.

Expression of transforming DNA. The appearance of transformants after the initial infection with the phage was studied as follows: a 200-μl portion of an exponential-phase culture of RS6143(Rφ9) (10^9 CFU/ml), 2 μg of Rφ6P DNA, and 10 μl of Rφ9 (10^{10} PFU/ml) were incubated at 35°C for 2 h. This mixture was diluted into 5 ml of Z broth and incubated at 35°C. Samples were removed at various times and assayed for penicillin-resistant clones. These began to appear after 180 min, and the ratio of transformants per viable cell remained constant for 8 to 10 h (Table 4). From this time onward, the ratio began to increase, presumably due to reinfection of the nontransformed cells in the population by Rφ6P released from transformed cells. This Rφ6P release was confirmed when a chloroform-sterilized, 8-h transformation mixture produced a titer of about 10^6 PFU/ml on RS6143(Rφ9).

Effect of DNA and Rφ9 concentrations on transformation. The results depicted in Fig. 1 show that there was a linear relationship between the DNA concentration and the level of transformation over the range tested (1 ng to 1 μg).

Figure 2 shows that there was a variation in the level of transformation with the variation in the multiplicity of infection with Rφ9. At multiplicities greater than about 10, the level of transformation declined, probably due to lysis from without by the superinfecting Rφ9 particles.

Sensitivity to DNase treatment with

TABLE 3. *Effect of DNase, RNase, and protease on Rφ9-mediated transformation*[a]

Treatment	Penicillin-resistant transformants (CFU/ml)	Free virus titer (PFU/ml)
None	3×10^4	8×10^8
DNase	$<10^1$	4×10^8
RNase	10^5	1.2×10^9
Protease	5×10^3	1.5×10^8

[a] A 50-μl amount of an exponential-phase culture in RS6143(Rφ9) (about 10^9 CFU/ml) was incubated for 2 h at 35°C with 20 μl of Rφ9 (10^{10} PFU/ml), 10 μl of Rφ6P DNA (500 μg/ml), and 20 μl of DNase (Sigma; 2 mg/ml in TM buffer), RNase (Sigma; 0.4 mg/ml in TM buffer, heated to 80°C for 10 min to inactivate any DNase), or protease (Sigma type VI; 10 mg/ml in TM buffer). A control in which Z broth replaced the enzyme preparations was included. After this incubation, 50 μl was removed, diluted into chloroform-saturated TM buffer, left for 20 min to kill all cells, and titrated for free virus on RS6143. A 200-μl amount of Z broth was added to the remaining transformation mixture, and incubation was continued for 8 h at 32°C. The mixture was then plated for penicillin-resistant transformants on PZ agar.

TABLE 4. *Expression of transforming DNA*[a]

Time after infection (min)	Viable count (CFU/ml)	Penicillin-resistant transformants (CFU/ml)	Ratio of penicillin-resistant transformants per survivor
120	1.5×10^8	ND[b]	
180	1.5×10^8	$<10^1$	6×10^{-8}
255	2.0×10^8	9×10^1	4.5×10^{-7}
300	3.5×10^8	2.1×10^2	6×10^{-7}
420	7.5×10^8	5.2×10^2	7×10^{-7}
480	1.25×10^9	9.6×10^2	7.7×10^{-7}
600	4×10^9	1.5×10^3	3.8×10^{-7}

[a] A 200-μl amount of RS6143(Rφ9) (about 10^8 CFU/ml), 10 μl of Rφ9 (10^{10} PFU/ml), and 2 μg of Rφ6P DNA were incubated at 35°C for 2 h. This mixture was diluted into 5 ml of Z broth, and the incubation was continued. Samples were removed at various times and assayed for the total viable count and for penicillin-resistant clones.

[b] ND, None detected.

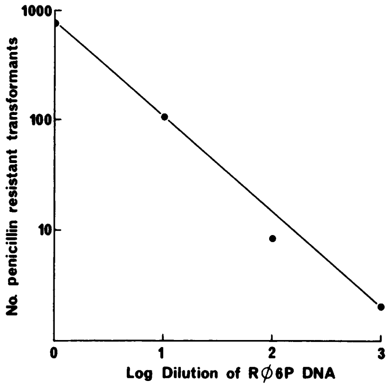

FIG. 1. *Effect of DNA concentration on transformation. A 25-μl amount of RS6143(Rφ9) (10^9 CFU/ml), 5 μl of Rφ9 (10^{10} PFU/ml), and 10 μl of a series of 10-fold dilutions of Rφ6P DNA (initial concentration, about 100 μg/ml) in a total volume of 50 μl were incubated for 3 h at 35°C. A 200-μl amount of Z broth was then added, and the reaction mixture was incubated for a further 6 h. Samples were then diluted and plated on PZ agar.*

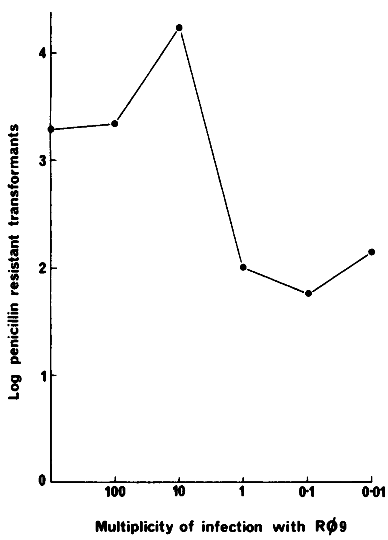

FIG. 2. *Effect of Rφ9 concentration on transformation. A 10-μl amount of a series of 10-fold dilutions of an Rφ9 preparation (initial titer, 2 × 10^{10} PFU/ml) was added to reaction cocktails containing 25 μl of RS6143(Rφ9) (2.5 × 10^7 CFU/ml) and 5 μl of Rφ6P DNA (100 μg/ml) in a total volume of 50 μl. These mixtures were incubated for 3 h at 35°C, after which 200 μl of Z broth was added, and the incubation was continued for 6 h before plating for penicillin-resistant colonies on PZ agar.*

time. The sensitivity of the transformation to DNase was tested, and the results of this experiment are depicted in Fig. 3. The transformation was initially sensitive to DNase, but by 60 min, it had become insensitive. The drop in transformants recovered at 180 min probably reflects the lysis of the transformed cells akin to zygotic induction. The increase in the recovery of penicillin-resistant cells at 240 min is presumably due to the reinfection of sensitive recipients in the population by free Rφ6P particles.

Competence after infection with Rφ9. The results of an experiment to study the uptake of transforming DNA after periods of preincubation of the recipient with Rφ9 are shown in Fig. 4. The level of transformation declined as the period of preincubation with the phage increased.

Kinetics of adsorption of Rφ9. Figure 5 shows the comparative rates of adsorption of Rφ9 to nonlysogenic (RS6143) and lysogenic [RS6143(Rφ9)] strains of *R. sphaeroides*. Rφ9 adsorbed very rapidly to RS6143 but more slowly to RS6143(Rφ9). It should be noted that by 80 min after mixing, most of the Rφ9 particles had adsorbed, and the level of transformation began to decline noticeably at about this time.

Effect of UV-inactivated Rφ9 on transformation. The role of the helper phage was investigated by studying the effect of UV inactivation on the ability of the viral preparation to enhance the transformation with Rφ6P DNA. The results of this experiment are shown in Table 5. It is clear that severe UV inactivation of the helper had no effect on the ability of the preparation to mediate the transformation.

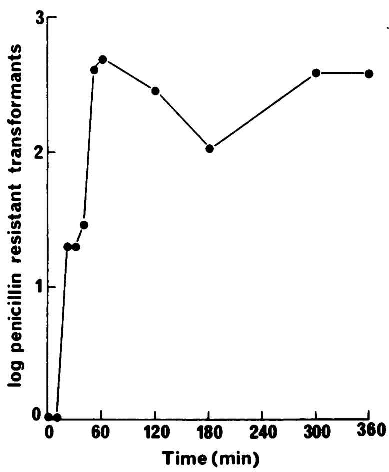

FIG. 3. *Sensitivity of transformation to DNase. A 1.5-ml amount of RS6143(Rφ9) (about 10^9 CFU/ml in Z broth) was incubated, with shaking, with 0.2 ml of Rφ9 (10^{10} PFU/ml) and 0.1 ml of Rφ6P DNA (50 μg/ml). At various times after infection, 50-μl samples were removed and added to 10 μl of DNase (2 mg/ml in TM buffer) plus 10 μl of Z broth, and the incubation was continued for a total of 8 h at 35°C. These mixtures were then diluted and plated on PZ agar.*

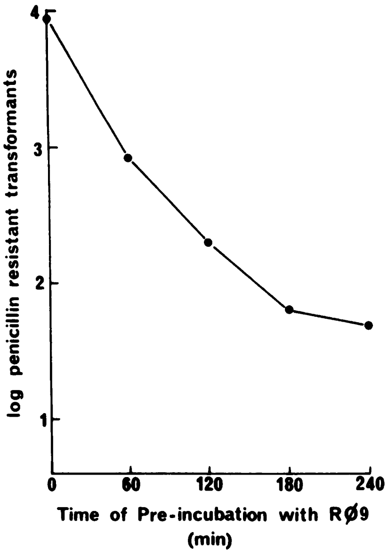

FIG. 4. *Competence after infection with Rφ9. Samples (50-μl each) of an incubation mixture containing 1.5 ml of RS6143(Rφ9) (10^9 CFU/ml in Z broth) and 0.2 ml of Rφ9 (10^{10} PFU/ml) were added to reaction cocktails containing 10 μl of Rφ6P DNA (50 μg/ml) and 10 μl of Z broth at various times after the initial mixing of the cells and the phage. These reaction mixtures were incubated for 8 h after the addition of the DNA before being diluted and plated on PZ agar.*

DISCUSSION

The transformation of *R. sphaeroides* strain RS6143 with DNA extracted from the bacteriophage Rφ6P is clearly linked with the infection of the recipient bacterium by the temperate bacteriophage Rφ9. That the penicillin-resistant clones do in fact result from the transformation with Rφ6P DNA is verified by the sensitivity of the process to DNase and the insensitivity to protease and RNase. This conclusion is supported by the linear relationship observed between the Rφ6P DNA concentration and the level of transformation. In accordance with these data is the observation that all the penicillin-resistant transformants are resistant to Rφ6P and release Rφ6P virus particles.

The nature of the enhancing effect of the helper phage remains to be considered. It would appear that the multiplicity of superinfection with Rφ9 is critical to the frequency of recovery of transformants. The optimum level for transformation appears to be between 1 and 10 PFU/CFU, and the level of transformation decreases as the multiplicity of infection drops to the point where the level of added Rφ9 is approximately that of the spontaneously released virus in the culture supernatant. The reduction in the frequency of transformation observed at multiplicities of infection greater than 10 probably reflects lysis from without by the adsorption of the helper Rφ9 virus to the recipient cells.

The uptake of transforming DNA is correlated to the adsorption by the superinfecting helper phage. The adsorption of Rφ9 to RS6143(Rφ9) is largely complete after 80 min, and the addition of transforming DNA after this period results in a decline in the level of transformants obtained.

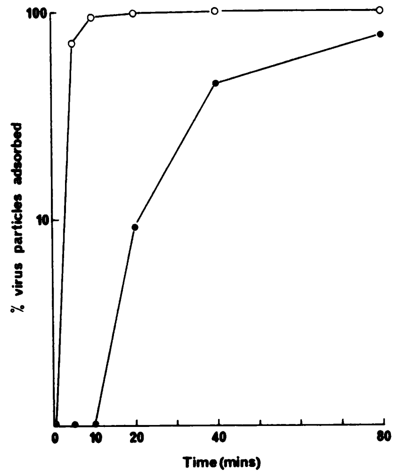

FIG. 5. *Kinetics of adsorption of Rφ9. An exponential-phase culture of the host bacterium (0.9 ml; about 10^8 CFU/ml) was infected with 0.1 ml of Rφ9 (10^9 PFU/ml) and incubated at 35°C. At various times, 50-μl samples were removed and diluted into 5 ml of TM buffer saturated with chloroform and left to stand for 20 min, sufficient to kill all cells. These dilutions were then titered for free phages on layers seeded with RS6143. Symbols: ○, RS6143; ●, RS6143(Rφ9).*

TABLE 5. *Effect of UV-inactivated helper phage on transformation*[a]

UV dose (min)	Virus titer (PFU/ml)	Penicillin-resistant transformants (CFU/ml)
0	2.5×10^{10}	2.5×10^3
1	8.0×10^8	4.0×10^3
2	1.4×10^7	2.5×10^3
5	10^5	2.0×10^3
10	1.8×10^4	1.5×10^3

[a] Samples (1.0 ml each) of a high-titer preparation of Rφ9 diluted in TM buffer were exposed for the indicated times at 12 cm from twin Gelman G15T8 UV lamps. A 10-μl amount of each irradiated preparation was included in transformation mixtures of 25 μl of RS6143(Rφ9) (10^9 CFU/ml), 5 μl of Rφ6P DNA (500 μg/ml), and 10 μl of Z broth. These were incubated at 35°C for 2 h, 200 μl of Z broth was added, and the incubation was continued for 6 h before being plated for transformants on PZ agar. Each irradiated phage preparation was also assayed for the plaque-forming titer on Z layers seeded with RS6143.

The nature of the interaction between the transforming DNA and the helper phage remains unknown, but it is clear from the use of a UV-inactivated helper that a viable helper phage is not essential for the observed effect.

The phenomenon of "helped" transformation was originally reported by Kaiser and Hogness (8) with a wild-type λ bacteriophage to enhance the transformation of λ*dg*DNA. Unger and Clark (22) have shown that the infection of *E. coli* with λ interferes with the expression of the *recBC* gene product, exonuclease V, in the recipient. Cosloy and Oishi (5) found that *E. coli* could be transformed with linear DNA if *recBC sbc* mutants were used as recipients. These results suggest that either certain nucleases may play a role in the degradation of transforming DNA and the prevention of transformation or that alternate pathways for recombination need to be derepressed to allow transformational recombination to occur.

Sjöström et al. (16) demonstrated that in *S. aureus,* the recipient must be lysogenic for phage P11 for transformation to occur, and they offered three alternative explanations for the observed effect. The first involved the prevention of degradation of the transforming DNA by products of phage transcription and translation. The second was that the helper phage acted at the cell envelope to somehow enhance penetration of the transforming DNA, and the third relied on the lysogenic conversion of the cell envelope to aid the uptake of the DNA. Because of their results showing that the transformation increased after superinfection of the lysogen and decreased after treatment with anti-P11 antiserum, they favored the second hypothesis. Yasbin et al. (23), while discussing their own findings with *B. subtilis* and the temperate phage φ105, proposed that Sjöström's results were due to the inactivation of an enzyme system akin to that in λ-infected *E. coli* or in *Salmonella* lysogenic for bacteriophage P22 (7). Their conclusions were based on the findings that certain nucleases had been inhibited in competent cells of *B. subtilis.*

Helper phage-mediated transformation in *R. sphaeroides* appears to result from an interaction between virus particles and the surface of the recipient cell. This explanation is favored because of the apparent relationship between the number of transformants recovered and the level of unadsorbed phage in the culture and the ability of UV-inactivated preparations of Rφ9 to mediate transformation. In lysogens, without the addition of superinfecting phages, the level of free virus is sufficient to explain the low level of transformation observed.

The relationship between the two phages Rφ6P and Rφ9 is also noteworthy. Both have very similar morphology and possess the unusual covalently closed, encapsidated genome, but they are heteroimmune. Bacteriophage P1 and bacteriophage P7, which also encodes a diffusible β-lactamase, have been shown to be closely related, yet they too are heteroimmune (4, 24). The exact molecular relationship between Rφ6P and Rφ9 has yet to be determined.

This report of the transformation in *R. sphaeroides* may allow the application of in vitro recombinant DNA techniques to the analysis of the photosynthetic properties and other properties of this organism. Even though transformation with cloning vectors such as RSF1010 has yet to be demonstrated for *R. sphaeroides,* the possibility of using Rφ6P itself as the cloning vector remains. Rφ6P does not appear to have an *Eco*RI site (unpublished data), and it may be possible to introduce such a site by mutation. The discovery of bacteriophage-enhanced transformation in *E. coli* led to the development of a more general transformation system for the organism. The way is now open for a similar development of a more generalized transformation system in *R. sphaeroides.*

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LITERATURE CITED

1. **Adams, M. H.** 1959. Bacteriophages. Wiley Interscience Publishers, New York.
2. **Archer, L. J.** (ed.). 1973. Bacterial transformation. Academic Press, Inc., New York.
3. **Benzinger, R.** 1978. Transfection of *Enterobacteriaceae* and its applications. Microbiol. Rev. **42:**194–236.
4. **Chesney, R. H., and J. R. Scott.** 1975. Superinfection immunity and prophage repression in phage P1. II. Mapping of the immunity difference and ampicillin resistance loci of P1 and φamp. Virology **67:**375–384.
5. **Cosloy, S. D., and M. Oishi.** 1973. Genetic transformation in *Escherichia coli* K12. Proc. Natl. Acad. Sci. U.S.A. **70:**84–87.
6. **Dagert, M., and S. D. Ehrlich.** 1979. Prolonged incubation in calcium chloride improves the competence of *Escherichia coli* cells. Gene **6:**23–28.
7. **Israel, V., M. Woodworth-Gutai, and M. Levine.** 1972. Inhibitory effect of bacteriophage P22 infection on host cell deoxyribonuclease activity. J. Virol. **9:**752–757.
8. **Kaiser, A. D., and D. S. Hogness.** 1960. Transformation of *Escherichia coli* with deoxyribonucleic acid isolated from bacteriophage λ dg. J. Mol. Biol. **2:**392–415.
9. **Miller, R. V., J. M. Pemberton, and K. E. Richards.** 1974. F116, D3 and G101, temperate bacteriophages of *Pseudomonas aeruginosa.* Virology **59:**566–569.
10. **Olsen, R. H., and P. Shipley.** 1973. Host range and properties of the *Pseudomonas aeruginosa* R factor R1822. J. Bacteriol. **113:**772–780.
11. **Pemberton, J. M., and A. J. Clark.** 1973. Detection and characterization of plasmids in *Pseudomonas aerugi-*

nosa strain PAO. J. Bacteriol. **114**:424–433.

12. **Pemberton, J. M., and W. T. Tucker.** 1977. Naturally occurring viral R-plasmid with a circular, supercoiled genome in the extracellular state. Nature (London) **266**: 50–51.
13. **Portolés, A., R. López, and M. Espinosa.** (ed.). 1977. Modern trends in bacterial transformation and transfection: proceedings of the third European meeting on transformation and transfection, Spain, September 1976. Elsevier/North Holland Publishing Co., Amsterdam.
14. **Riggs, H. G., Jr., and E. D. Rosenblum.** 1969. Transfection of lysostaphin-treated cells of *Staphylococcus aureus*. J. Virol. **3**:33–37.
15. **Sistrom, W. R.** 1977. Transfer of chromosomal genes mediated by plasmid R68.45 in *Rhodopseudomonas sphaeroides*. J. Bacteriol. **131**:526–532.
16. **Sjöström, J.-E., M. Lindberg, and L. Philipson.** 1973. Competence for transfection in *Staphylococcus aureus*. J. Bacteriol. **113**:576–585.
17. **Spizizen, J. B., E. Reilly, and A. H. Evans.** 1966. Microbial transformation and transfection. Annu. Rev. Microbiol. **20**:371–400.
18. **Tomasz, A.** 1969. Some aspects of the competent state in genetic transformation. Annu. Rev. Genet. **3**:217–232.
19. **Tucker, W. T., and J. M. Pemberton.** 1978. Viral R plasmid Rϕ6P: properties of the penicillinase plasmid prophage and the supercoiled, circular encapsidated genome. J. Bacteriol. **135**:207–214.
20. **Tucker, W. T., and J. M. Pemberton.** 1979. Conjugation and chromosome transfer in *Rhodopseudomonas sphaeroides* mediated by P and W group plasmids. FEMS Microbiol. Lett. **5**:173–176.
21. **Tucker, W. T., and J. M. Pemberton.** 1979. The introduction of RP4:Mu *cts* 62 into *Rhodopseudomonas sphaeroides*. FEMS Microbiol. Lett. **5**:215–217.
22. **Unger, R. C., and A. J. Clark.** 1972. Interaction of recombination pathways of bacteriophage λ and its host *Escherichia coli* K12: effects on exonuclease V activity. J. Mol. Biol. **70**:539–548.
23. **Yasbin, R. E., G. A. Wilson, and F. E. Young.** 1975. Effect of lysogeny on transfection and transfection enhancement in *Bacillus subtilis*. J. Bacteriol. **121**:305–312.
24. **Yun, T., and D. Vapnek.** 1977. Electron microscopic analysis of bacteriophages P1, P1Cm, and P7. Determination of genome sizes, sequence homology, and location of antibiotic resistance determinants. Virology **77**:376–385.
25. **Zhdanov, V. M.** 1977. Transfection methods, p. 283–321. *In* K. Maramorosch and H. Koprowski (ed.), Methods in virology, vol. 6. Academic Press, Inc., New York.