Marine Transducing Bacteriophage Attacking a Luminous Bacterium

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The isolation and partial characterization of a marine bacteriophage attacking a strain of luminous bacteria is described, including some physical, biological, and genetic properties. It is a DNA phage of density of 1.52 with a long flexible tail and an apparently icosahedral head. With respect to stability in suspension, it has a rather specific requirement for the sodium ion in high concentration; it is further stabilized by the addition of calcium and magnesium ions. These same ions are likewise all required for both good plating efficiency and plaque uniformity. Although it goes through a typical lytic growth cycle (about 45 min), with a burst size of 100, and no stable lysogens have been isolated, it is nevertheless a transducing phage specifically for the tryptophan region, transducing several, but not all, independently isolated Trp⁻ auxotrophs to prototrophy. No other auxotrophs of a variety of amino acids were transduced by this phage to prototrophy. Phage infection does not change the normal expression of the luminescent system, and light remains at near normal levels until cell lysis occurs.

Luminous bacteria occur ubiquitously in the oceans and may be easily isolated either directly from sea water simply by plating on a seawater nutrient agar medium or by picking bright colonies growing as saprophytes on the skin of dead fish (8). The luminous bacteria also occur as true symbionts, cultured with special light-emitting organs in certain fish and other animals (4, 8). Recent studies suggest that the "free-living" organisms and the symbionts may actually represent two distinct groups (12).

Light emission in the bacteria is blue-green (λ_{max} , about 490 nm) and is continuous; it may be very bright, of the order of 10^3 to 10^4 quanta per second per bacterium. The in vitro bioluminescent reaction involves an enzyme (bacterial luciferase) which reacts with reduced flavine mononucleotide, aldehyde, and oxygen, using energy from this oxidation to populate an excited state (10, 20).

The possibility that a phage system might be of value in studying in vivo control mechanisms in bioluminescence was indicated by the work of M. Baylor (personal communication and reported by Harvey, 1952, p. 17-18) on a bacteriophage attacking a luminous bacterium iso-

lated in Bermuda. This phage had the interesting property of increasing light emission after infection. Attempts to recover Baylor's phage were unsuccessful, probably because the host bacterium was lost. A new phage isolation was made at Woods Hole, Mass., by using *Photobacterium fischeri* (ATTC 7744) as the host (9). The phage obtained failed to exhibit the properties reported by Baylor, but it did have other interesting properties. After infection, phage-resistant bacteria were found which, in all instances, exhibited a greatly decreased light emission and also carried the phage. True lysogeny was never demonstrated, and this strain was also lost before the study was completed.

The phage described here, referred to as hv-1, was isolated in 1964 by using as the host a different species, *Beneckea harveyi* (21). This phage appears to be more virulent than those previously described, and although we have not been able to isolate stable clones of phage-resistant bacteria (after infection with this phage), it can act as a transducing phage.

MATERIALS AND METHODS

Isolation and growth of bacteria and phage. The luminous bacterium, recently classified by Reichelt and Baumann (21) as *Beneckea harveyi* (their strain no. 392), was maintained on slants of nutrient agar

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made with either the sea water or the synthetic medium described below. This species, which has been used for many studies from this laboratory (5, 11, 19), was previously referred to as strain "MAV".

Phage attacking this bacterium were obtained from an enrichment culture in which a dead squid and mud from the Eel Pond and Little Harbor were mixed with a heavy inoculum of the bacteria. After 2 days at room temperature, samples were removed, treated with chloroform, and passed through a Seitz filter. Phage was detected in the filtrate through plating by conventional methods. A pure strain was obtained by repeated isolation from single plaques from petri dishes containing but a single plaque.

Two complex media were used, the first a synthetic sea water medium (used when we wanted to alter the concentration of specific ions) having the following composition per liter: NaCl (30g), $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (1 g), $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ (1.4 g), $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ (0.7 g), yeast extract (0.5 g), peptone (Difco), (5 g), and glycerol (3 ml). The second was a sea water base medium with the following composition: sea water (750 ml), distilled water (250 ml), yeast extract (0.5 g), peptone (5.0 g), and glycerol (3 ml). The two media were equally effective and optimal for bacterial and phage growth, and were used for plating with either 1.5% agar or, for top agar, with 0.5% agar. Minimal medium contained NaCl (30 g), $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$ (7g), KH_2PO_4 (1 g), $(\text{NH}_4)_2\text{PO}_4$ (0.5 g), MgSO_4 (0.1 g), and glycerol (3 ml) per liter of distilled water.

Bacteria were routinely grown in screw-cap tubes (16 by 125 mm) containing 5 ml of liquid medium. Both growth and luminescence could be directly monitored in these tubes; cell density was monitored with a Coleman Jr. II spectrophotometer (660 nm), where 0.1 cell density units is equal to 5×10^7 viable cells per ml, and bioluminescence was monitored with a photomultiplier photometer of the type described by Mitchell and Hastings (17), where one light unit is equal to 2×10^{10} quanta per s.

Phage were titered by standard methods. Soft agar was kept in a water bath at 45 C until needed, at which time 3 ml was mixed with 5 to 100 μ liters of phage suspension and 0.5 ml of a bacterial suspension (an overnight culture adjusted to a cell density of approximately 0.5), and immediately overlaid onto fresh nutrient plates. With *B. harveyi* (and many marine bacteria with which we have worked), it is important not to let them remain at 45 C for longer than is absolutely necessary. A few minutes at this temperature leads to killing and incomplete lawn formation.

At 20 C, plaques developed overnight (10 to 14 h), whereas at 30 C they could be counted after 6 h. The development and appearance of the plaques was dependent upon the medium, but there was little or no effect of temperature between 20 and 37 C upon plaque morphology.

Phage stocks were prepared either by confluent lysis or in liquid cultures. Plates were inoculated with approximately 10^8 phage and 10^7 bacteria in a soft-agar overlay and were allowed to incubate overnight. Harvesting was accomplished by suspension in complete medium (5 ml per plate), followed by treatment with chloroform and centrifugation at 5,000 $\times g$ for 30

min to remove bacterial debris. The phage were then collected by centrifugation at high speed (39,000 $\times g$) for 1 h, resuspended in filtered sea water (1 ml per plate harvested), and stored at 4 C with a drop of chloroform added. Such preparations had titers of approximately 7×10^{11} . In liquid (5 ml in tubes), exponentially growing cultures were inoculated at an optical density of 0.5 (5×10^8 cells/ml) with 0.1 ml of phage (10^9 /ml). After 8 h the culture was lysed in chloroform and phage were prepared as described above. Such preparations had titers of approximately 5×10^{11} .

A one-step growth experiment was performed according to the method of Adams (2). One milliliter of a rapidly growing culture (5×10^7 cells/ml) of *B. harveyi* was mixed with 5×10^7 phage particles, and 5 min were allowed for adsorption; 1 ml of hv-1 phage antiserum was then added to this suspension to neutralize any unadsorbed phage. After 5 min, 10 μ liters of this mixture was diluted into 200 ml of sea water complete medium (final concentration of 2.5×10^3 cells/ml) in a 500-ml Erlenmeyer flask, and growth with shaking was initiated at 25 C. At 10-min intervals, two 0.1-ml samples were removed. One was used to detect infective centers and so was directly assayed for PFU, whereas the other, used to detect intracellular mature phage particles, was lysed in chloroform (induced), dried, resuspended in sea water, and assayed for PFU. All titers were performed as described above, with *B. harveyi* as the host.

Electron microscopy. Negatively stained specimens were prepared by placing a drop of virus suspension on a carbon-coated grid, washing two times with distilled water, and negatively staining with unbuffered 2% uranyl acetate. All steps were done very quickly to minimize the time of contact with distilled water. A three-focus series was photographed on a Philips 300 electron microscope operating at 80 kV. The microscope was calibrated during the same session by using a standard diffraction grating.

Centrifugation. Preparative density gradient centrifugation of the phage was carried out in a Spinco model L-2 ultracentrifuge with an SW 39L rotor. CsCl (2.6 g) was dissolved in 3.7 ml of stock solution and centrifuged at 35,000 rpm for 36 h. The CsCl concentration of the phage-containing fraction was determined with the Abbe refractometer, and the density of that fraction was read from an empiric table.

Analytical density gradient centrifugation of the extracted nucleic acid was carried out in the Spinco model E ultracentrifuge at 20 C for 20 h at 44,700 rpm in a standard cell. The nucleic acid was extracted according to Mandell and Hershey (16). UV absorption photographs were taken at the end of the centrifugation period.

Antisera. New Zealand white rabbits were used to prepare antisera against the phage. Subcutaneous injection (1.0 ml of a purified phage preparation at a titer of 10^{12} PFU/ml) in the neck were administered at 3-day intervals for 6 weeks or until a high titer of antibody was obtained, as determined by phage inhibition test.

Auxotrophs. Auxotrophic mutants of the wild type (*B. harveyi*) were isolated after treatment with the

chemical mutagen *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine, obtained from Sigma Chemical Co., St. Louis, Mo. The mutagenesis procedure was identical with that described by Cline and Hastings (5), with identification accomplished by replica plating to mixtures of nutrients.

Transduction. A stock culture of phage, prepared as described above, was diluted into sea water to a titer of 2.5×10^{10} PFU/ml. Transduction was attempted by mixing 1 ml of an overnight culture of an auxotrophic mutant of the luminescent bacterium, adjusting to a cell density of 5×10^8 cells per ml followed by the addition of 10 μ liters of the phage suspension. This resulted in a final multiplicity of infection of 0.5 phage per bacterium. This suspension was mixed, allowed to stand for 10 min, and then plated onto minimal medium. In all cases, controls of phage and bacteria individually were also plated.

RESULTS

Some properties of the phage are summarized in Table 1, details of which will be considered below.

Host range. When tested on different isolates of marine luminous bacteria, the host range of phage *hv*-1 was found to be very limited. One hundred and seven different strains, 39 symbiotic and 68 "free-living," were tested for sensitivity to this phage using the "spot test" (strains were grown in soft overlay agar, and a drop containing 10^7 phage particles was put on the agar). Only four strains were found to be sensitive to the phage, and all were of the "free-living" type. These four strains exhibited similar properties in all other tests, and may in fact be the same.

Physical characteristics. As seen by the electron microscope in negatively stained preparations, the phage head appeared hexagonal and was most likely icosohedral (Fig. 1). It had a long, flexible tail of the kind that has been described by Bradley (3) as noncontractile, and it appeared to fall into his group B. The apparent length of the edge of the head was 40

nm and the corresponding diameter was 70 nm. The subunits of the head were apparent in intact heads and often appeared as projections in collapsed heads. The tail was about 220 nm long and 12 nm wide and had distinct annuli. Many phage were observed with broken tails. The fragility of the tail and mace-like projections in collapsed heads may both have been due to osmotic stress, as this phage is more stable in high salt. Ghosted phage that have lost their DNA have a groove in their tail, indicating that stain has penetrated the tail (arrows, Fig. 1). Eiserling and Boy de la Tour (7) have suggested that this groove results from the loss of DNA from the tail. A more detailed morphological examination of *hv*-1 and other marine phage is in progress.

The density of the intact phage is 1.515 g/cm^3 , measured by that of the peak tube from a preparative density gradient centrifugation of the phage in cesium chloride. The nucleic acid was extracted, and its density was estimated to be approximately 1.70 g/cm^3 , determined by comparison with that of a reference DNA obtained from a *Pseudomonas* phage having a density of 1.72 g/cm^3 . This measurement characterizes the nucleic acid of our phage as DNA. The size of the DNA was determined to be 55×10^6 daltons by electron microscopy with the use of a T-7 phage standard.

Phage stability. One criterion by which it has been proposed that marine phage can be distinguished from those of other origins is that of their specific ion requirements (22). We therefore investigated the stability of *hv*-1 phage in aqueous media as a function of both composition and concentration of salts. The results of experiments on survival of phage in various solutions are given in Table 2. The phage was rapidly inactivated in distilled water, and this inactivation was considerably slower in any solution in which relatively high concentrations of monovalent ions were present. Furthermore, the component which had the greatest stabilizing effect was the Na^+ ion in high concentrations. This effect of sodium may, in fact, serve as a criterion for distinguishing viruses of marine origin from those of nonmarine origin, as proposed by MacLeod and Onofrey (14, 15) for marine bacteria.

The phage is thus stable in natural and artificial sea water, and although NaCl in a 3% concentration is the most important phage-stabilizing factor of sea water, it can not entirely replace it. Stability in any NaCl solution is improved by the addition of Ca^{2+} and Mg^{2+} which, by themselves, in low concentration, do not stabilize the phage suspension.

Another feature proposed by Spencer (22) to

TABLE 1. Characteristics of bacteriophage *hv*-1

Determination	Description
Shape	Head: appears hexagonal, probably icosohedral Tail: long, flexible, noncontractile
Density	1.515 g/cm^3
DNA	1.70 g/cm^3 55×10^6 daltons
Adsorption time	Complete in 5 min
Burst time	45 min
Burst size	100 phage/bacterium infected
Thermal inactivation	50% activity lost in 18 min at 62 C; 40 s at 70 C

FIG. 1. Negatively stained *hu-1*. (A) Ghosted phage; (B) Intact phage; Arrows: groove in the tail which appears in ghosted particles.

be distinctive for marine phages is that they are more heat labile than nonmarine forms. Since *hu-1* has an 18-min half-time for inactivation at 62 C, it does not appear to support this proposition.

Relatively high concentrations of calcium and magnesium were required to give a good plating efficiency and uniform plaques, but the absence of these ions had no deleterious effect on the

attachment of the phage to the bacterium (Table 3). The requirement evidently relates either to injection of the phage DNA or to some stage in its maturation. It is not required only for phage release, since no additional yield was observed by chloroform treatment of a liquid culture infected in the absence of calcium and magnesium.

The formation of plaques was closely linked

TABLE 2. Stability of phage *hv-1* with time in different dilute solutions at 4 C°

Solution	PFU remaining (%)		
	2 h	8 h	24 h
Sea Water	100	100	100
Synthetic sea water	100	100	100
Distilled water	<1		
3% NaCl	74 ± 12	64 ± 4	27 ± 9
3% KCl	40 ± 10		15 ± 3
2% NaCl	63 ± 5	66 ± 12	23 ± 6
1% KCl	8 ± 3		
1% NaCl	67 ± 13	53 ± 9	9 ± 3
0.5% NaCl	49 ± 30	15 ± 5	7 ± 2
15 mM Ca ²⁺ + Mg ²⁺	<1		
1 mM Ca ²⁺ + Mg ²⁺	<1		
3% NaCl + 15 mM Ca ²⁺ + Mg ²⁺	100	100	65 ± 5
2% NaCl + 15 mM Ca ²⁺ + Mg ²⁺	100	100	65 ± 0
1% NaCl + 15 mM Ca ²⁺ + Mg ²⁺	76 ± 8	43 ± 5	14 ± 5
25% Sucrose			
0.5% Yeast extract	50	30	20 ± 9
0.5% peptone	20		

^a Phage (10¹¹/ml) in sea water was diluted into each of the above solutions to a final concentration of 10⁵ phage/ml and plated for PFU at the times indicated.

to the growth of the host bacterium. Since the host bacterium is an obligate marine form, its growth rate may be readily manipulated by varying the NaCl concentration in the nutrient medium (8). At concentrations below 0.5% NaCl, growth is virtually nil, while at 0.6% it is very slow and plaques form very badly. Plaques form well with 1% NaCl, but a turbid ring forms in the plaque. The nature of this phenomenon is not understood; bacteria isolated from such turbid rings are not resistant to phage. The growth rate of the bacteria declines at 3.5% NaCl and above, but plaques in this case appear normal.

The morphology of the plaque is dependent not only on the sodium chloride concentration, but also on the presence or absence of calcium and magnesium. The uniformity of the plaque diameter and the regularity of the plaque shape are strongly favored by higher concentrations of these ions. The absence of these ions results in quite hazy or turbid plaques with phage-sensitive bacteria growing in the centers.

Phage lytic cycle. To study the lytic cycle of the phage, we conducted a one-step growth experiment according to Adams (2). A liquid suspension of bacteria was infected with approximately one phage particle per bacterium and was allowed a 5-min adsorption. Antiphage antibody was then added to remove any unad-

sorbed phage particles, and after 10 min the cells were diluted into sea water broth to give a final concentration of 10³ bacteria/ml. At various times after addition of phage, the infected bacteria were assayed by the soft-agar overlay method, with *B. harveyi* as the test strain. The results are illustrated in Fig. 2, which shows the time course for the appearance of both spontaneously produced phage (in the supernatant) and of intracellular mature phage (i.e., those

TABLE 3. Ionic effects on the adsorption of phage *hv-1* to *B. harveyi*^a

NaCl	Mg ²⁺ Ca ²⁺	EDTA	Bacteria	Unadsorbed phage	Percent unadsorbed
SW			—	420,511,480	100
SW			+	2,0,0	0.1
DW			—	360,310,372	74
DW			+	10,5,8	0.5
3%			—	441,387,460	91
3%			+	0,5,0	0.25
3%	15 mM		—	460,401,427	91
3%	15 mM		+	0,0,0	0
3%	15 mM	10 ⁻³ M	—	497,410,465	97
3%	15 mM	10 ⁻³ M	+	2,2,1	0.25

^a Bacteria were diluted into the indicated media to a concentration of 10⁷/ml. To 1-ml samples of these suspensions was added 5 × 10⁶ PFU of phage *hv-1*. After 10 min were allowed for adsorption, 0.1 ml of the mixtures was membrane filtered (Millipore Corp.) washed with 10 ml of the identical ionic solution, and then washed with 90 ml of sea water to stabilize any phage which washed through. A 0.1-ml volume of the filtrates was then titered. For each ionic solution, a control with no bacteria was included. Abbreviations: SW, sea water; DW, distilled water.

FIG. 2. One-step growth with phage *hv-1* and its host. Both curves indicate a burst size of 100.

which are demonstrable by chloroform lysis of infected cells). At 25 C, the first free infective particles appeared spontaneously at about 45 min after infection, and the burst size was approximately 100 phage per bacterium.

The influence of phage infection on luminescence. There was little or no increase in bioluminescence during growth until a cell density of about 0.15 OD units was reached (Fig. 3). Subsequently, while the cell mass doubled, the luminescence increased by a factor of about 100. This has been shown to be due to an "inducer" produced by the cells themselves, acting at the level of the synthesis of luciferase (6, 18). But Baylor's results (8) indicated that phage infection during the early stages of growth could cause an immediate increase in light emission, suggesting the presence of luciferase whose activity was not expressed. It was therefore of interest to investigate the effects, or lack thereof, on light emission of phage infection at different multiplicities and different times during the bacterial growth curve (Fig. 3). Here it can be seen that neither differential inhibition nor differential enhancement of light emission by the bacteria occurred after phage infection. The ultimate decrease in light emission was the result of the lysis of the bacteria by the phage, the time lag being a function of the multiplicity of infection. At high multiplicities, the time at which lysis occurred was approximately 45 min after infection, which agreed well with the latent period seen in the one-step growth curve of Fig. 2. At lower multiplicities, the time to light diminution is longer, requiring more than one round of phage replication before mass lysis occurs. This experiment was repeated several times, adding phage at many times and multiplicities. In no case was an effect on luminescence noted until lysis occurred.

Strains resistant to phage. A number of attempts were made to obtain bacterial variants similar to those found by Hastings et al. (9), which were reported to be immune, phage carrying, and dim. These variants had been detected as bacteria which overgrew the clear area of the plaques. Extensive overgrowth does not occur with phage $h\nu$ -1, but variants were isolated by simply picking from the center of the plaques after 1 to 3 days. Although some dim and some immune strains were found among the isolates, these properties were not correlated in our experiments.

Transduction. To use this phage for genetic studies, we tested for its transducing ability. To a suspension of each of the auxotrophic mutants, the phage grown on the parent strain was added at a low multiplicity (0.1 to 0.5); after

FIG. 3. Effect of multiplicities and times of infection by phage $h\nu$ -1 on bioluminescence of *B. harveyi*. An overnight culture was inoculated at low density into 50 ml of sea water complete medium. Volumes of 5 ml were dispensed into each of seven growth tubes, and growth was allowed to proceed at 25 C with shaking. At two different times (A and B), three different multiplicities of phage were added (see insert). Cell density and bioluminescence were measured as described in Materials and Methods. When lysis occurred, accurate cell density determinations became impossible, and the point of maximum cell density obtained in each tube was used from that time on.

adsorption the cells were plated on a minimal medium to screen for prototrophic transductants. Bacteria at the same concentration not infected with phage, and phage alone, were also plated on minimal media to measure spontaneous back mutation and phage contamination, respectively.

A tabulation of the results of the transduction experiments (Table 4) show that of all the auxotrophic mutants tested, only one type, namely tryptophan requiring, was positively transduced, and not even all of these. Only Trp-1, Trp-5, Trp-7, and Trp-8 were transduced.

Using Trp-1, we then examined the effect of multiplicity of infection on transduction efficiency. Maximum transduction occurs at a multiplicity of 0.5; at multiplicities of 0.1 and 1.5 the efficiency was less by a factor of about 3 (Fig. 4). Outside of these multiplicity limits, transduction efficiency fell off rapidly.

Genetic recombination experiments with this phage were attempted. The various Trp⁻ mutants capable of being transduced were grown in full media and used as host strains for phage production, and the phage was screened for its ability to transduce the other (transducible) Trp⁻ mutants. In no case were any transductants to prototrophy found (Table 5A), which indicates that the Trp⁻ lesions in the different

TABLE 4. Summary of transduction data for phage *hv-1* and *B. harveyi*^a

Auxotroph	Transd/ plate	Rev/ plate	Auxotroph	Transd/ plate	Rev/ plate
Met-1	10	5	Pro-1	108	105
Met-2	TM	TM	Pro-3	0	0.3
*Trp-1	69	0	Pro-4	0.3	0.6
Trp-2	0	0	His-1	0.6	1
Trp-3	TM	TM	His-2	0.3	0
Trp-4	1.0	0.5	His-3	1.6	0
*Trp-5	34	3	His-4	0	0
*Trp-7	27	3.5	Asp-1	0	0.6
*Trp-8	39	2	Asp-2	TM	TM
Trp-9	0	0	Lys-1	0.6	0
Trp-10	12	17	Leu	0	0.6
Trp-11	8	30	Thy-1	0	0
Ade-1	0	0	Thy-2	1	2
Ade-2	0	0	Rib-1	0.3	0
Ade-3	0	0	Rib-2	9	11.5
Ade-4	0	0	Thr-1	0	0
Arg-1	0.3	0.3	Thr-2	6	4.5
Arg-3	0	0.3	Ser-1	0.3	1
Ilv-1	1	0.3	Ser-2	TM	TM
Ilv-2	0	0	Ala-1	0.6	1
Ilv-3	0.3	0.3	Ala-2	0	0
Ilv-4	0	0	Asn	0	0

^a After auxotrophic bacteria were mixed with *hv-1* at a multiplicity of 0.25, mixtures were plated onto minimal medium and scored for colonies after 3 days, given as the average of several experiments and plates. Revertants (rev) per plate are given in the second column. TM indicates that there were too many colonies to count. In no case were colonies seen when phage were plated alone. Positive results with respect to transduction (transd) are indicated by *

FIG. 4. Effect of multiplicity of infection on transduction frequency. Using the auxotrophic Trp⁻ mutant of *B. harveyi*, Trp-1, transduction was performed with various multiplicities of phage. Five min were allowed for phage adsorption, and then 0.1-ml samples were plated onto minimal medium. Transductants were counted after 3 days.

mutants are very similar, perhaps identical.

On the other hand, when phage grown on the nontransducible Trp⁻ mutants (Trp-2 and Trp-4) was used, good transduction was obtained (Table 5B), indicating that the lesions are distinctly different.

DISCUSSION

Although only a few phages attacking luminescent bacteria have been isolated and studied, and although all their properties are not known in detail, it appears that they may be quite different from each other. The phage described here appears to be a specialized transducing phage since of all the auxotrophic mutants screened, only the Trp⁻ locus was able to be transduced, and at a fairly high frequency. On the other hand, several other properties of the phage seem to be inconsistent with present

TABLE 5. Transduction of Trp auxotrophs with phage prepared on other Trp auxotrophs^a

Source of phage	Auxotrophs transduced	Transd/ plate	Rev/ plate
A	Wild type	*Trp-5	210
		*Trp-7	130
		*Trp-8	240
	Trp-1	Trp-5	7
		Trp-7	7
		Trp-8	7
	Trp-5	Trp-5	14
		Trp-7	10
		Trp-8	7
	Trp-7	Trp-5	0
		Trp-7	0
		Trp-8	11
	Trp-8	Trp-5	11
		Trp-7	5
		Trp-8	6
B	Wild type	*Trp-1	150
		*Trp-5	180
		*Trp-7	75
	Trp-2	*Trp-1	50
		*Trp-5	110
		*Trp-7	75
	Trp-4	*Trp-1	112
		*Trp-5	113
		*Trp-7	90
			0

^a Transduction was performed by using a multiplicity of infection of 0.5, as described in the Materials and Methods, except that phage stocks were prepared also on different Trp auxotrophs. The first four Trp⁻ auxotrophs used as a source of phage were transducible ones, and the last two were nontransducible. For both experiments, control with phage grown on the wild type was included. *, Positive for transduction. Transd, transductions; rev, revertants.

models of specialized transduction, which is usually considered a property of temperate phage. All of our experiments were done with a single strain of bacteria, on which the phage was lytic. It is possible that under some different conditions the phage could also grow as a temperate phage so that stable lysogens could be isolated. But on the basis of our results, it appears that a rigid classification of specialized versus generalized transduction is not applicable to this phage, in line with the suggestion of Luria and Darnell (13).

All of the transductants to Trp^+ so far tested (>100) retained sensitivity to infection and lysis by phage $h\nu$ -1. In no case was immunity conferred simultaneously with transduction to prototrophy (Nealson, unpublished data).

The system is also unusual because not all of the Trp^- auxotrophs isolated showed positive results in transduction experiments. This may have been due to the fact that the *trp* genes in the MAV chromosome are not localized, but distributed over a wide area of the genome. If this is the case, we would expect that if $h\nu$ -1 is grown on Trp^- mutants which cannot be transduced by $h\nu$ -1, the resulting phage would be able to transform the transformable Trp^- mutants. This was in fact the case (Table 5).

With regard to the lesions responsible for Trp^- auxotrophy, we tested the four transducible Trp^- auxotrophs and found that none would grow on any of the tryptophan precursors (anthranilic acid, indole, indolyl-3-glycerol phosphate, or serine) or combinations of them. Two of the nontransducible Trp^- auxotrophs showed positive growth on anthranilate, and four grew on none of the precursors. This may, of course, be because the compounds cannot get into the cells, or because each one of the mutants is altered in the tryptophan synthetase itself or in its control. A theory that all transducible mutants have the same (or very similar) lesion would be consistent with the observation that phage grown on each of the transducible auxotrophic strains will not transduce to Trp^+ any of the other auxotrophs (see Table 3). The nature of tryptophan auxotrophy and its repair by transduction by phage $h\nu$ -1 is thus not yet understood.

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