Mutants of *Salmonella anatum* That Block Bacteriophage ϵ^{15} Infection at Early Stages

SHIRO KANEGASAKI* AND TOSHIO TOMITA

The Institute of Medical Science, The University of Tokyo, Tokyo, Japan 108

Received for publication 5 February 1976

A total of six mutants, to which phage ϵ^{15} could adsorb but failed to lyse, were isolated from *Salmonella anatum* and characterized. A significant portion of active phage particles could be recovered from the phage-bacterium complexes before they became irreversibly adsorbed, and adsorbed phage did not kill these mutants at all. These reversibly adsorbed phage had become sensitive to chloroform, at least in some cases. The results indicate that the steps that may be blocked are deoxyribonucleic acid ejection or penetration. The mutants were tentatively classified into three groups by their susceptibility to phages c341 and Felix O. The inhibition of phage infection was overcome by host range mutants of phages ϵ^{15} and c341. The isolated lipopolysaccharide from the parent strain inactivated phages ϵ^{15} , c341, and a host range mutant of ϵ^{15} in vitro. However, neither phage ϵ^{15} nor c341, or the host range mutant of phage ϵ^{15} , was inactivated by incubation with the mutant lipopolysaccharides, even when they were derived from the mutants susceptible to c341 or the host range mutant of ϵ^{15} . These results may suggest that more than the receptor lipopolysaccharide of the bacterial surface is involved in the early stages of phage infection.

The attachment of phage to a specific receptor site on the bacterial surface is an essential step in phage infection. A successful adsorption may be followed by the ejection of nucleic acid from phage and its penetration into the cells. These early steps of phage infection are not yet well understood, although later events concerning the replication of nucleic acid and synthesis of phage particles have been well documented.

To determine the molecular events involved in the early stages of phage infection, we have chosen to study the temperate *Salmonella* phages ϵ^{15} , ϵ^{34} , g341, and P22, which have a well-defined surface receptor. The bacterial receptor is the O antigen, a component of lipopolysaccharide that is an important constituent of the outer membrane of gram-negative organisms (19). Reports from this laboratory (5-7, 9, 11; S. Iwashita and S. Kanegasaki, J. Biol. Chem., in press) have clearly demonstrated that the base plate of each phage consists of an enzyme that cleaves or deacetylates the O polysaccharide of the host bacterium. The host specificity of these phages is believed to be decided by the substrate specificity of their base plate enzymes. The base plate protein is essential for phage adsorption, forming an enzyme-substrate-type complex with the O antigen polysaccharide. However, adsorption (or the enzyme reaction as well) is not sufficient to release phage nucleic acid from phage capsid.

To understand the successive stages in the early phase of infection, we have isolated, from mutagenized *Salmonella anatum*, a series of mutants to which phage ϵ^{15} can attach but fails to kill. We especially focus our attention on the altered structure of the bacterial envelope and tentatively name these bacterial mutants *pep* for penetration of phage deoxyribonucleic acid (DNA). The parent strain *S. anatum* A1 is referred to as *pep*⁺. In this manuscript, the isolation and characterization of *pep* mutants are described. A preliminary account of this work has appeared (S. Kanegasaki, S. Iwashita, and T. Tomita, International Virology III, Abstr., 3rd Int. Cong. Virol., Madrid, 1975, p. 213).

MATERIALS AND METHODS

Bacterial strains and phages. *S. anatum* strain A1 and phages ϵ^{15} , c341, and Felix O were obtained from A. Wright. Phage ϵ^{15} vir was isolated from a hydroxylamine-treated phage stock (4). Host range mutants were isolated from the rare plaques found on plates that contained *pep* bacterium (SK59) and hydroxylamine-treated ϵ^{15} vir or c341. The partial rough mutant (SK36), which contained reduced levels of rhamnose, was isolated from nitrosoguanidine-treated *S. anatum*.

Microbiological techniques. Standard conditions for growth of cells and growth and assay of phage have been described previously (6, 11). Bacteria were grown in L-broth (15) unless otherwise stated.

Isolation of *pep* mutants. *S. anatum* cells were

mutagenized by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine according to the method of Adelberg et al. (1), and, after overnight incubation at 37 C, the cells were infected with $\epsilon^{15}\text{vir}$ at a multiplicity of 5. The mixture was then diluted into fresh medium, and, after overnight shaking, aliquots were washed and spread on plates and the resulting colonies were picked from the plates and tested for their ability to adsorb phage ϵ^{15} . Among the 240 colonies tested, we found six strains that were resistant to $\epsilon^{15}\text{vir}$ (spotted) but adsorbed radioactive $\epsilon^{15}\text{vir}$ at a level greater than 20% of the value obtained with the sensitive parent.

Adsorption capacity of ^{35}S -labeled ϵ^{15} . Bacteria (5×10^6 to 50×10^6 cells/ml) grown in L-broth and the radioactive phage (5,350 counts/min, 4.7×10^4 plaque-forming units per counts/min) were mixed and incubated for 10 min at 37 C. The mixture was filtered through a membrane filter and the radioactivity retained on the filter was counted.

Preparation of ^{35}S -labeled phage. A1 cells were grown in 50 ml of LST-glucose medium (6) at 37 C to a concentration of 4×10^8 /ml and infected with $\epsilon^{15}\text{vir}$ phage (multiplicity of infection = 5). A 2.5-mCi portion of carrier-free $\text{H}_2^{35}\text{SO}_4$ was added 20 min after infection, and growth was continued for a further 100 min. Cells were lysed with chloroform. The radioactive phage particles were purified in a CsCl gradient.

Rhamnose-protein ratio. A loopful of cells was scraped from a plate and suspended into 1 ml of 0.04 N NaOH. Portions were used for determination of protein by the Lowry method and of rhamnose by the Dische method, with correction made for color development in controls with no cysteine (3).

Extraction of lipopolysaccharide. Ethylenediaminetetraacetate-lysozyme-treated cells (10) or washed cells were extracted by the hot phenol-water procedure of Westphal and Jann (18). The water phase was dialyzed extensively against water. No further purification was done in this experiment to avoid selective loss, which may occur during the purification.

RESULTS

Isolation of ϵ^{15} -insensitive mutants and their susceptibility to various phages. A total of six nitrosoguanidine-induced mutants were selected from *S. anatum* strain A1 in six independent experiments, as described above. These mutants allow the adsorption of phage $\epsilon^{15}\text{vir}$ (clear plaque mutant of ϵ^{15}), yet are not lysed by the phage. The efficiency of plating (EOP) of $\epsilon^{15}\text{vir}$ on these mutants is significantly reduced compared to that on the parent strain (Table 1).

The mutants also adsorb phage c341 (clear plaque mutant of g341), which uses the same receptor polysaccharide as ϵ^{15} , although two kinds of response were observed (Table 1): strains SK19 and 55 were as susceptible to this phage as their parent strain, whereas strains SK312, 316, 510, and 59 were not. Phage Felix O, which infects both smooth and rough strains of *Salmonella*, grew well on all the mutants except strain SK59.

Host range mutants of phages $\epsilon^{15}\text{vir}$ and c341 were obtained from the plaques that formed on strain SK59 with an EOP of 10^{-6} and 10^{-4} , respectively. Phages that form plaques on mutant SK59 plate with high efficiency on this strain even after passage through the parent A1 strain. The EOPs of one of these mutant phages, $\epsilon^{15}\text{hp32}$, on these bacterial mutants were almost 10 times higher than that on the parent strain (Table 2). In contrast, host range mutants of phage c341 formed plaques on these mutant bacteria with high, but not full, efficiency. Mutants SK19 and 55 were more sensitive to the host range mutant phages of c341 than mutants SK312, 316, 510, and 59.

From the results of susceptibility of the mu-

TABLE 1. EOP of various phages on *pep* mutants

Group	Bacteria	Genotype	EOP ^a of:		
			$\epsilon^{15}\text{vir}$	c341	Felix O
I	<i>S. anatum</i> A1	<i>pep</i> +	1.0	1.0	1.0
	SK36		0.6	1.0	2.3
	SK19	<i>pep</i>	1.8×10^{-5}	1.0	1.8
	SK55		1.0×10^{-4}	0.1	1.3
II	SK312	<i>pep</i>	2.1×10^{-4}	— ^b	1.3
	SK316		$<5.0 \times 10^{-6}$	—	2.2
	SK510		1.0×10^{-6}	—	2.4
III	SK59	<i>pep</i>	6.7×10^{-6} ^a	—	$<10^{-5}$

^a Phages were plated on various hosts and incubated at 37 C. EOP was calculated relative to the titer of *S. anatum* A1 (the parent).

^b Indicates significantly low values (at least a four-order difference). In these cases, the phage form very faint plaques and could not show an exact number.

tant bacteria to various phages, we tentatively classified the mutants into three groups (see Tables 1 and 2): group I (SK19 and 55), group II (SK312, 316, and 510), and group III (SK59). Mutants SK19, 312, and 59 were chosen for further study as representatives of each group.

Adsorption kinetics of phage ϵ^{15} to the *pep* mutants and their parent. Table 3 shows the rhamnose-protein ratio of each *pep* mutant. The levels of rhamnose indicate the levels of O antigen of these mutants, which were reduced. Increased EOP of Felix O on the mutants as compared to that on their parent also shows that the levels of O antigen are reduced in these mutant cells (Table 1). However, the reduction of O antigen itself may not explain the results of phage susceptibility, since the EOP of ϵ^{15} vir on one rough mutant (SK36) which contained lower levels of rhamnose than the *pep* mutants (Table 3) was close to that on the parent cells.

Furthermore, all the mutant cells adsorb ϵ^{15} vir at a lower, but significant, rate compared with the parent strain (Fig. 1). Strains SK19 and 59 showed a relatively fast adsorption rate, and SK59 adsorbed at least 10 phage particles per cell. The adsorption rate of this phage to the partial rough mutant mentioned above is also shown in Fig. 1 and was of a similar level. All

FIG. 1. Adsorption of [^{35}S] ϵ^{15} vir to *pep* and *pep*⁺ bacteria. Cells in 2 ml of L-broth (6.5×10^7 /ml) and 10 μl of the radioactive phage particles (2,850 counts/min, 1.3×10^8 plaque-forming units) were mixed and incubated at 37 C. Samples were filtered through membrane filters at the indicated time, and the radioactivity retained on filters was counted. Symbols: ○, *pep*⁺ bacteria; ●, *pep* mutants.

TABLE 2. EOP of host range mutants of phage ϵ^{15} vir and c341 on *pep* mutant bacteria

Group	Bacteria	EOP ^a of:		
		$\epsilon^{15}\text{hp32}$	c341hp2	c341hp5
	<i>S. anatum</i> A1	1.0	1.0	1.0
I	SK19	7.0	1.0	4.0×10^{-1}
	SK55	6.7	1.0	4.0×10^{-1}
II	SK312	1.1×10	2.1×10^{-1}	3.1×10^{-2}
	SK316	1.1×10	2.6×10^{-1}	1.0×10^{-2}
	SK510	1.1×10	1.5×10^{-1}	1.5×10^{-2}
III	SK59	1.2×10	2.2×10^{-1}	3.0×10^{-2}

^a Phages were plated on the *pep* and *pep*⁺ hosts and incubated at 37 C. EOP was calculated relative to the titer on the *pep*⁺ host (*S. anatum* A1).

TABLE 3. Rhamnose-protein ratio of the *pep* and *pep*⁺ bacteria

Bacteria	Rhamnose (nmol/mg of protein)
<i>S. anatum</i> A1 (<i>pep</i> ⁺)	207
SK36 (<i>pep</i> ⁺)	27
SK19 (<i>pep</i>)	30
SK312 (<i>pep</i>)	31
SK59 (<i>pep</i>)	40

these mutant cells were agglutinated by anti-O 3,10 serum, indicating that these mutants have O antigen similar to that of the parent.

Killing of host cells by phages ϵ^{15} vir and c341. Phage ϵ^{15} vir killed the parent cells efficiently, but there was no detectable killing of the mutants even at a very high multiplicity (Table 4). The results suggest that DNA does not penetrate normally into the cells. Phage c341 did not kill the mutants either, with the exception of strain SK19. This mutant (SK19) and the parent are both susceptible to this phage (Table 1).

Characterization of the phage adsorbed to the *pep* mutants. To characterize phage ϵ^{15} or c341, which adsorbed to the mutant cells, the following experiments were performed. After incubation of ϵ^{15} vir or c341 with the mutant or the parent cells, the mixture was divided into three portions; to one portion, a drop of chloroform was added, to a second portion nothing was added, and both fractions were serially diluted. The diluted fractions were plated on an *S. anatum* indicator plate to assay the surviv-

ing phage particles. At the same time, the incubation mixture (the third portion) was centrifuged, and the phage in the supernatant fraction was assayed. Most (92 to 100%) of the phages had adsorbed to and cosedimented with the cells under these conditions (Table 5).

TABLE 4. Cell survival after phage infection

Bacteria	Phage	MOI ^a	Surviving cells ^b
<i>S. anatum</i> A1	$\epsilon^{15}vir$	50	<0.01
		500	<0.01
SK19		50	0.9
		500	1.0
SK312		50	0.9
		500	1.0
SK59		50	1.0
		500	1.1
<i>S. anatum</i> A1	c341	25	0.01
		250	<0.01
SK19		25	0.1
		250	0.07
SK312		25	1.0
		250	1.1
SK59		25	1.0
		250	1.2

^a MOI, Multiplicity of infection.

^b Cells (at 4.4×10^8 /ml) were infected with the indicated multiplicities of the phages listed. After 10 min of incubation at 37 C, the number of surviving cells was determined by diluting aliquots in $\phi 80$ buffer and plating them on nutrient broth.

In Table 5, the number of surviving phage with no chloroform treatment includes the number of infected bacteria (infective centers) and that of reversibly adsorbed phage that can be released from the phage-bacterium complexes. By the addition of chloroform after incubation with the A1 parent cells, the number of surviving phage (ϵ^{15} or c341) was reduced to less than 0.04, indicating that most of the phage were killed with the infected cells (i.e., most of phage had infected). This is also the case with SK19 and phage c341. In contrast, phage ϵ^{15} was not inactivated by the addition of chloroform after incubation with the mutant SK59, showing that the phage ϵ^{15} had reversibly adsorbed to this mutant.

In all other cases (SK19 and ϵ^{15} /SK312 and ϵ^{15} or c341/SK59 and c341), however, a significant portion of phage did not form plaques when chloroform was added to the incubation mixture. The results do not mean that the adsorbed phage had infected, since none of the mutants was killed by the phage (see Table 4). After incubation with cells (no chloroform addition), these phages were still capable of forming plaques if the parent cells were used as indicator. Therefore, these phages must have adsorbed to the mutants in a reversible manner. The results suggest that some modification had taken place in these phages through the interaction with the cell surface component of the mutant cells.

TABLE 5. Inactivation of phage $\epsilon^{15}vir$ or c341 by the parent or the pep mutant cells

Bacteria	Phage	Treatment with $CHCl_3$	Surviving phage ^a	% Phage inactivated ^b	% Phage adsorbed ^c
<i>S. anatum</i> A1	$\epsilon^{15}vir$	—	4.4×10^6		
		+	1.8×10^5	96.3	99.7
SK19		—	5.8×10^6		
		+	2.5×10^6	56.8	98.9
SK312		—	6.2×10^6		
		+	4.8×10^6	31.6	92.1
SK59		—	4.9×10^6		
		+	4.8×10^6	0.0	99.1
<i>S. anatum</i> A1	c341	—	1.0×10^7		
		+	1.8×10^5	98.2	98.8
SK19		—	1.4×10^7		
		+	2.0×10^6	86.4	99.3
SK312		—	1.7×10^7		
		+	6.5×10^6	61.7	96.3
SK59		—	1.6×10^7		
		+	4.3×10^6	73.2	98.8

^a Phage $\epsilon^{15}vir$ (7.8×10^6 /ml; multiplicity of infection = 0.02) or c341 (2.1×10^7 /ml; multiplicity of infection = 0.06) was incubated for 10 min at 37 C with the parent or the mutant cells (3.4×10^8 /ml). Surviving phage were assayed by means of plaque counts. Significant numbers of phage were inactivated by the incubation with cell fractions even without addition of chloroform for an unknown reason.

^b Number of surviving phage after incubation with cells and addition of chloroform versus the number without addition.

^c Based on the number of phage remaining in the supernatant fraction after centrifugation.

Inactivation of phages ϵ^{15} and c341 by lipopolysaccharide extracted from the A1 parent and the *pep* mutant cells. Several reports have shown that isolated lipopolysaccharide can adsorb certain bacteriophages (2, 13) and, in some cases, cause them to release their DNA in vitro (8, 12, 17), although nothing is known about the relationship between the DNA release observed in in vitro experiments and the ejection of phage nucleic acid in vivo. Therefore, it is important to study whether the lipopolysaccharide of the mutants can inactivate the phage ϵ^{15} vir. We have isolated the lipopolysaccharides from *pep* mutants as well as *pep*⁺ parent cells, and the rate of phage inactivation by these lipopolysaccharides was assayed. The lipopolysaccharide obtained from the parent cells converted ϵ^{15} vir into a noninfectious state, probably due to the release of DNA (Fig. 2). In contrast, the lipopolysaccharide fractions obtained from the mutants inactivated ϵ^{15} vir very poorly.

FIG. 2. Inactivation of phage ϵ^{15} vir by the lipopolysaccharide from *pep* or *pep*⁺ strains of *S. anatum*. Phage ϵ^{15} vir (5.0×10^7 in 0.5 ml) was mixed with the lipopolysaccharide fraction (5 μ g of rhamnose in 0.5 ml) and with 10 μ l of 10 mM $MgSO_4$, and the mixture was incubated at 37 C. Aliquots were withdrawn at the indicated periods, immediately diluted with cold ϕ 80 buffer, and plated on *S. anatum* indicator plates. Fractions of survivors at indicated times were plotted. The lipopolysaccharides in this experiment were extracted from ethylenediamine-tetraacetate-lysozyme cells, but similar results were obtained with the lipopolysaccharide from intact cells.

The results of a similar experiment with c341 show that the extent of inactivation by the lipopolysaccharide from the parent cells was significant, but lower, compared to the case of ϵ^{15} vir (Fig. 3). Interestingly, the lipopolysaccharide from SK19, which is susceptible to phage c341, did not inactivate this phage under the condition used. Similar disagreement between intact cell and lipopolysaccharide experiments was observed with host range mutants of phage ϵ^{15} vir (see below). The results may suggest the presence of a component other than the lipopolysaccharides that triggers DNA ejection in intact cells (see Discussion).

Inactivation of phage ϵ^{15} hp32 by lipopolysaccharides and intact cells of a *pep* mutant and the A1 parent. Since the lipopolysaccharide obtained from the A1 parent inactivated phage ϵ^{15} vir but those from the mutant cells did not, it was interesting to test whether these lipopolysaccharides could inactivate the host range mutant phage that can infect the *pep* mutants. The host range mutant phage ϵ^{15} hp32 was inactivated by incubation with the parental lipopolysaccharide but only very poorly by the lipopolysaccharide from mutant SK59 (Fig. 4a).

In contrast, a converse situation was found with the adsorption of ϵ^{15} vir to intact cells (Fig. 4b). In this experiment, after incubation of the host range phage with the bacterial cells, a

FIG. 3. Phage c341 inactivation by the lipopolysaccharide from the mutant or the parent cells. Conditions were the same as in the legend of Fig. 2, except phage c341 was used instead of ϵ^{15} vir.

FIG. 4. Survival of host range mutant phage $\epsilon^{15}hp32$ after incubation with lipopolysaccharide (a) or intact cells (b). Host range phage $\epsilon^{15}hp32$ (10^7 plaque-forming units in 0.5 ml) was mixed with the intact cells (2.0×10^8 in 0.5 ml) or the lipopolysaccharide (5 μ g of rhamnose in 0.5 ml), together with 10 μ l of 10 mM $MgSO_4$, and the mixture was incubated at 37 C. At the indicated periods, a drop of chloroform (b) or nothing (a) was added, and the samples were diluted and plated for counting plaques with *S. anatum* indicator. Relative numbers at the indicated time to that at 0 time are shown.

drop of chloroform was added to kill the bacteria and irreversibly adsorbed phage, and remaining phage were assayed after serial dilutions. Although a significant number of phage did not form plaques after incubation with the parent or the mutant cells, a larger number of phages were inactivated by the mutant cells than by the parent cells.

DISCUSSION

We have isolated bacterial mutants (*pep*) of *S. anatum* in which the early process of infection by bacteriophage ϵ^{15} is blocked. The mutants adsorb the phage reversibly without being killed; in some cases the adsorbed phage become sensitive to chloroform, suggesting that some interaction between phage and mutant host cell has taken place. These results indicate that the steps that may be blocked in these mutants are DNA ejection or penetration. A similar phenomenon was observed in the case of phage c341.

Requirement of chloroform, in addition to isolated bacterial receptor, for triggering the release of DNA in vitro has been reported in phage λ (14), although the receptor of this phage is known to be a protein (14), in contrast to the receptor of the *Salmonella* phages we

used, which is lipopolysaccharide. An *Escherichia coli* mutant that inhibits the injection of phage λ DNA has been isolated (16). In this case, also, the phage adsorbs to this mutant normally, but DNA seems to remain in the phage head.

Although the *pep* mutants we obtained were partially rough, the roughness does not explain the low levels of EPO, since we have a partial rough mutant, which shows EOP similar to that of the parent. Furthermore these *pep* mutants showed an adsorption rate similar to that of this partial rough mutant.

Isolated lipopolysaccharide from *S. anatum* caused ϵ^{15} inactivation, probably due to the release of DNA. Our results confirm the previous observation of Takeda and Uetake (17) and, in addition, show that the same lipopolysaccharide also causes phage c341 inactivation, although the efficiency is lower. In contrast, the lipopolysaccharide fraction extracted from the *pep* mutants by an identical procedure inactivated phage $\epsilon^{15}vir$ or c341 very poorly. Among these lipopolysaccharide fractions, that obtained from SK59 had poor but significant activity and that from SK19 had little activity.

The results obtained with the isolated lipopolysaccharides are not consistent, however, with the results obtained with the intact cells in the following cases. Phage c341 was poorly inactivated by the lipopolysaccharide from SK19, and, similarly, the host range mutant of phage ϵ^{15} was not inactivated by lipopolysaccharide from SK59. Intact cells of these mutant bacteria were susceptible to, and could inactivate, the phages mentioned above. These results show, at least in some cases, that the inactivation of phage by the isolated lipopolysaccharide does not reflect the ability of the intact cells to allow DNA ejection during the infection process. One explanation of these results is that more than the lipopolysaccharide receptor component of the bacterial surface is involved in the DNA ejection. One of the other possibilities is that lipopolysaccharide is selectively extracted or changed its behavior by the extraction, especially when it is rough.

Since the adsorption and enzymatic cleavage of the receptor polysaccharide by the base plate of smooth-specific *Salmonella* phages (5-7, 9, 11) is not sufficient to cause the release of phage nucleic acid (17; unpublished data) and since there exists a similar level of O antigen cleavage activity in the base plates of the host range mutant phage, which can infect *pep* mutants, and in those of wild-type phage, which cannot (S. Iwashita and S. Kanegasaki, unpublished data), an interaction other than the one be-

tween phage base plate and O polysaccharide seems to be responsible for DNA ejection. Ejected DNA has to penetrate into the cytoplasm to accomplish the infection. It is not certain at present whether the DNA penetrates directly into the cytoplasm or not.

In any case, changes in lipopolysaccharide structure seem to have taken place in these *pep* mutants. Preliminary experiments show that the lipopolysaccharide fractions obtained from the mutants are different from that of the parent cells in several criteria and that even the lipopolysaccharide from the parent seems to be heterogeneous in terms of its capacity to inactivate phages. Further investigation of the properties of the outer membrane, including the lipopolysaccharides of these mutants and their interaction with phages, may yield some insight into the process of DNA ejection and penetration.

ACKNOWLEDGMENTS

We wish to thank Hiroshi Nikaido, Masanosuke Yoshikawa, and Shintaro Iwashita for their criticism and help in preparing the manuscript. We also thank Yukinori Tsunematsu and Yoshito Kaziro for their interest and encouragement.

This work was supported by a grant-in-aid for scientific research from the Ministry of Education, Science and Culture, Japan.

LITERATURE CITED

- Adelberg, E. A., M. Mandel, and G. C. C. Chen. 1965. Optimal conditions for mutagenesis by N-methyl-N'-nitro-N-nitrosoguanidine in *Escherichia coli* K12. *Biochem. Biophys. Res. Commun.* 18:788-795.
- Boman, H. G., and D. A. Monner. 1975. Characterization of lipopolysaccharides from *Escherichia coli* K-12 mutants. *J. Bacteriol.* 121:455-464.
- Bray, D., and P. W. Robbins. 1967. Mechanism of ϵ^{15} conversion studied with bacteriophage mutants. *J. Mol. Biol.* 30:457-475.
- Hall, D. H., and I. Tessman. 1966. T₄ mutants unable to induce deoxycytidylate deaminase activity. *Virology* 29:339-345.
- Iwashita, S., and S. Kanegasaki. 1973. Smooth specific phage adsorption: endorhamnosidase activity of tail parts of P22. *Biochem. Biophys. Res. Commun.* 55:403-409.
- Iwashita, S., and S. Kanegasaki. 1975. Release of O antigen polysaccharide from *Salmonella newington* by phage ϵ^{34} . *Virology* 68:27-34.
- Iwashita, S., and S. Kanegasaki. 1976. Enzymic and molecular properties of base-plate parts of bacteriophage P22. *Eur. J. Biochem.* 65:87-94.
- Jazwinski, S. M., A. A. Lindberg, and A. Kornberg. 1975. The lipopolysaccharide receptor for bacteriophages ϕ X174 and S13. *Virology* 66:268-282.
- Kanegasaki, S., and S. Iwashita. 1975. The role of tail parts of phage infection, p. 180-184. In T. Hasegawa (ed.), *Proceedings of the 1st intersectoral congress of the International Association of Microbiological Societies*, vol. 2. University of Tokyo Press, Tokyo.
- Kanegasaki, S., and A. Wright. 1970. Mechanism of polymerization of the *Salmonella* O-antigen: utilization of lipid-linked intermediates. *Proc. Natl. Acad. Sci. U.S.A.* 67:951-958.
- Kanegasaki, S., and A. Wright. 1973. Studies on the mechanism of phage adsorption: interaction between phage ϵ^{15} and its cellular receptor. *Virology* 52:160-173.
- Lindberg, A. A. 1967. Studies of a receptor for Felix O-1 phage in *Salmonella minnesota*. *J. Gen. Microbiol.* 48:225-233.
- Losick, R., and P. W. Robbins. 1969. The receptor site for a bacterial virus. *Sci. Am.* 221:120-124.
- Randall-Hazelbauer, L., and M. Schwartz. 1973. Isolation of the bacteriophage lambda receptor from *Escherichia coli*. *J. Bacteriol.* 116:1436-1446.
- Robbins, P. W., and T. Uchida. 1962. Studies on the chemical basis of the phage conversion of O-antigens in the E group *Salmonellae*. *Biochemistry* 1:323-335.
- Scandella, D., and W. Arber. 1974. An *Escherichia coli* mutant which inhibits the infection of phage λ DNA. *Virology* 58:504-513.
- Takeda, K., and H. Uetake. 1973. *In vitro* interaction between phage and receptor lipopolysaccharide: a novel glycosidase associated with *Salmonella* phage ϵ^{15} . *Virology* 52:148-159.
- Westphal, O., and K. Jann. 1965. Bacterial lipopolysaccharides: extraction with phenol water and further applications of the procedure, p. 83-91. In R. L. Whister (ed.), *Methods in carbohydrate chemistry*, vol. V. Academic Press Inc., New York.
- Wright, A., and S. Kanegasaki. 1971. Molecular aspects of lipopolysaccharides. *Physiol. Rev.* 51:748-784.