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Received for publication 15 January 1970

Salmonella typhosa hybrids able to adsorb lambda were obtained by mating *S. typhosa* recipients with *Escherichia coli* K-12 donors. After adsorption of wild-type λ to these *S. typhosa* hybrids, no plaques or infective centers could be detected. *E. coli* K-12 *gal*⁺ genes carried by the defective phage λ dg were transduced to *S. typhosa* hybrids with HFT lysates derived from *E. coli* heterogenotes. The lysogenic state which resulted in the *S. typhosa* hybrids after *gal*⁺ transduction differed from that of *E. coli*. Ability to produce λ , initially present, was permanently segregated by transductants of the *S. typhosa* hybrid. *S. typhosa* lysogens did not lyse upon treatment for phage induction with mitomycin C, ultraviolet light, or heat in the case of thermoinducible λ . A further difference in the behavior of λ in *Salmonella* hybrids was the absence of zygotic induction of the prophage when transferred from *E. coli* K-12 donors to *S. typhosa*. A new λ mutant class, capable of forming plaques on *S. typhosa* hybrids refractory to wild-type λ , was isolated at low frequency by plating λ on *S. typhosa* hybrid WR4254. Such mutants have been designated as λ xx, and a mutant allele of λ xx was located between the P and Q genes of the λ chromosome. Plaques were formed also on the *S. typhosa* hybrid host with a series of λ ⁱ²¹ hybrid phages which contain the N gene of phage 21. The significance of these results in terms of *Salmonella* species as hosts for λ is discussed.

We have previously reported the conjugal transfer of genetic material from *Escherichia coli* K-12 donors to recipients belonging to the genus *Salmonella* (3, 5, 11). Analysis of progeny derived from *Escherichia coli*-*Salmonella typhosa* matings disclosed that certain of these *S. typhosa* hybrid classes could support the growth of the virulent T phages by virtue of the genetic material acquired from the *E. coli* donor (4, 5, 11). Studies of the interaction of *S. typhosa* hybrids with the temperate bacteriophage lambda (λ) form the subject of the present communication. We report the isolation of *S. typhosa* hybrids which can adsorb λ phage without attendant cellular lysis, and we provide an initial characterization of λ in these "foreign" hosts. We also report the isolation and preliminary characterization of a class of λ mutants, termed λ xx, which can be propagated on certain *E. coli*-*S. typhosa* hybrids.

MATERIALS AND METHODS

Bacterial strains. The strains of *E. coli* K-12 and *S. typhosa* used are listed in Table 1 together with

their pertinent markers and other descriptive information.

Phages. The phages used and the source of the lysates are given in Table 2.

Media. One-per cent Tryptone Broth (TB) containing NaCl (5 g/liter), maltose (2 g/liter), and 0.02 M MgSO₄ was used for phage adsorption experiments and as a general growth medium. Phage assays were performed on TB plates containing 1.5% Difco Noble Agar overlaid with TB soft agar (0.7% Noble Agar). The minimal agar medium for selection of recombinants has been described previously (5, 11). Eosin-Methylene Blue (EMB) and MacConkey Agar base (Difco) supplemented with 1% of a suitable carbohydrate also were employed for selection and purification of recombinants and transductants.

Procedures. The methods used for performing bacterial matings have been reported in detail (5, 11). Phage methods were essentially those described by Adams (1). Procedures for the production of ultraviolet (UV)-induced low-frequency-transducing (LFT) and high-frequency-transducing (HFT) lysates of λ , and transduction methods were similar to those originally devised by Morse et al. (18). Induction of lysogens by using mitomycin C (Calbiochem, Los Angeles, Calif.) or ultraviolet light was performed as described by Korn and Weissbach (14). Induction of strains lysogenic for thermo-inducible λ was per-

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TABLE 1. Characteristics of parent and hybrid strains

Strain no.	Prior designation and source	Description	Relevant genotypic characteristic ^a											
			<i>lac</i>	<i>ara</i>	<i>pil</i>	<i>met</i>	<i>rha</i>	<i>ina</i>	<i>xyi</i>	<i>fuc</i>	<i>trp</i>	<i>gal</i>	<i>λcp</i>	<i>str</i>
WR2001	W1895 (Hfr Cavalli) J. Lederberg (λ ⁺)	<i>E. coli</i> K-12 Hfr O- <i>pur</i> <i>E-lac-ara</i>	+	+	+	-	+	+	+	+	+	+	+	S
WR2002	Hfr Hayes S. E. Luria	<i>E. coli</i> K-12 Hfr O- <i>thr-ara-xyi</i>	+	+	+	+	+	+	+	+	+	+	+	S
WR2003	Hfr Hayes (λ) WR2002 lysogenized with λ	<i>E. coli</i> K-12 Hfr O- <i>thr-ara-xyi</i>	+	+	+	+	+	+	+	+	+	+	+	S
WR2000	W1485 J. Lederberg	<i>E. coli</i> K-12 F ⁺	+	+	+	+	+	+	+	+	+	+	+	S
WR2020	WR2000 lysogenized with λ <i>clI1</i>	<i>E. coli</i> K-12 F ⁺	+	+	+	+	+	+	+	+	+	+	+	S
WR3060	594 M. Lieb	<i>E. coli</i> K-12 F ⁻	+	+	+	+	+	+	+	+	+	-	+	R
WR3061	WR3060 lysogenized with λ	<i>E. coli</i> K-12 F ⁻	+	+	+	+	+	+	+	+	+	-	+	R
WR3062	WR3060 lysogenized with λ ⁺ ₁₄₈₅	<i>E. coli</i> K-12 F ⁻	+	+	+	+	+	+	+	+	+	-	+	R
WR3063	WR3060 lysogenized with λ <i>clI1857</i>	<i>E. coli</i> K-12 F ⁻	+	+	+	+	+	+	+	+	+	-	+	R
WR3064	WR3060 lysogenized with λ <i>clI1</i>	<i>E. coli</i> K-12 F ⁻	+	+	+	+	+	+	+	+	+	-	+	R
WR4200	643 (WRAIR)	<i>S. typhosa</i> recipient	-	-	-	+	-	-	-	-	-	+	-	S
WR4250	643 <i>lac3</i> (WRAIR) mating with W1895	<i>S. typhosa lac</i> ⁺ hybrid	+	-	-	+	-	-	-	-	-	+	-	S
WR4251	X30D (WRAIR) mating between W1895 and 643 <i>lac3</i>	<i>S. typhosa</i> diploid hybrid	+	+	+	-/+	+	+	+/+	-	-	+	+/+	S
WR4252	X30T (WRAIR) from X30D	<i>S. typhosa</i> hybrid segregant	+	-	-	-	+	+	+	-	-	+	+	S
WR4253	X30P (WRAIR) from X30D	<i>S. typhosa</i> hybrid segregant	+	+	+	-	+	+	+	-	-	+	+	S
WR4254	X30W <i>gal</i> ⁻ from X30P; <i>gal</i> ⁻ by mutation	<i>S. typhosa</i> hybrid segregant	+	+	+	-	-	-	-	-	-	-	+	S
WR4255	From mating between W1485 and X30W	<i>S. typhosa</i> hybrid	+	+	+	-	-	-	-	-	-	-	+	R

^a Abbreviations: *lac*, lactose; *ara*, arabinose; *pil*, type I pili; *met*, methionine; *rha*, rhamnose; *ina*, tryptophanase; *xyi*, xylose; *fuc*, fucose; *trp*, tryptophan; *gal*, galactose; *λcp*, receptor site for phage λ; *str*, streptomycin; S, sensitive; R, resistant; +, utilized or present; -, not utilized or absent; +/-, denotes diploidy with numerator indicating diploid allele from K-12 donor and denominator the resident *Salmonella* allele; *, allele from K-12 donor in haploid state.

TABLE 2. Characteristics of bacteriophage preparations employed

Phage	Lysate prepn	Description	Source
λ	UV, mitomycin inductions	Wild-type	K-12 (λ) strains W1895, P4X-6
λ^{434}	UV, mitomycin inductions	Hybrid between λ and 434	K-12 (λ^{434}) strain B345, A. D. Kaiser
λ_{vir}	Lytic infection	Virulent mutant able to lyse λ and λ^{434} lysogens ($v_1v_2v_3$)	A. Weissbach
$\lambda c1857$	Thermal induction	ind ⁻ , thermoinducible due to <i>c1</i> mutation	M. Yarmolinsky, K-12 strain W3350 (λc)
$\lambda c1t1$ $\lambda c1t2$	Thermal induction	Thermoinducible due to <i>c1</i> mutation	M. Lieb
$\lambda b2$	Lytic infection	Deletion of b2 region	A. Weissbach
λ -21hy1 to λ -21hy-10	Lytic infection	Hybrids between λ and 21	A. D. Kaiser

formed by shaking log-phase cultures at 45 C for 15 min, followed by shaking at 37 C, until lysis was complete. After removal of bacterial debris by centrifugation, the lysates were preserved by the addition of a small amount of chloroform and stored at 4 C.

RESULTS

A preliminary survey of several *Salmonella* species showed that they were unable to adsorb λ , a finding similar to that previously observed with *E. coli* B (2). The inability of *E. coli* B to adsorb λ , however, was readily overcome by P1 transduction of the *E. coli* K-12 *malA* genes specifying, in part, the λ phage receptor site (2). Since the transduction of *E. coli* K-12 genes to *Salmonella* was not feasible, we employed intergeneric conjugation procedures to construct *Salmonella* hybrids with the λ receptor locus (λrcp^+).

Initial matings between *E. coli* Hfr Cavalli (WR2001) and *S. typhosa* 643 (WR4200) have produced hybrids such as WR4250, which have acquired only the proximal genes of the Hfr (3, 11). Further crosses between WR2001 and hybrid WR4250, however, can result in the transfer of approximately 30% or more of the *E. coli* K-12 chromosome. The *Salmonella* hybrids isolated from such intergeneric crosses are usually unstable merodiploids which continually segregate clones with the genotype of the *Salmonella* parent and segregants which have stably retained various segments of the *E. coli* genome (4, 11). The available selective marker in WR4250 in reasonably close proximity to the λrcp locus was the marker *xyl* (Fig. 1). We expected that, among hybrids selected for *xyl*⁺, some would be λrcp^+ . Matings were, therefore, performed between WR2001 (*met*⁻, *xyl*⁺, λrcp^+) and WR4250 (*met*⁺, *xyl*⁻, λrcp^-) on minimal xylose-agar. *Salmonella xyl*⁺ hybrids were isolated at a frequency of

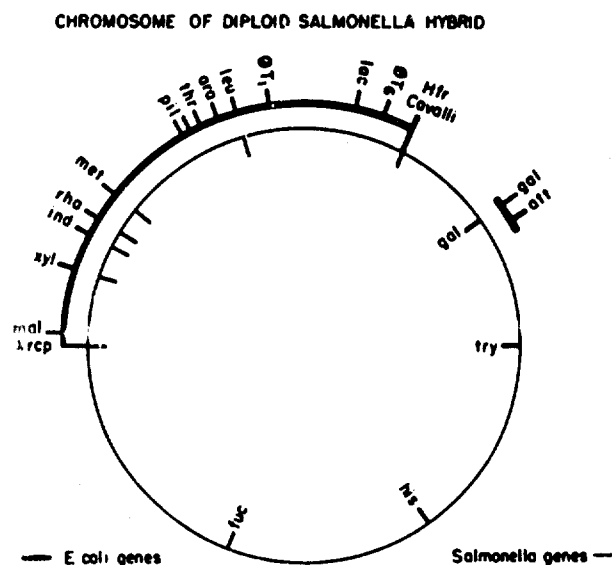


FIG. 1. Schematic representation of *Salmonella* diploid hybrid indicating the approximate extent of chromosomal material acquired from *E. coli* K-12 donors. The transfer of the *E. coli gal* region can be accomplished by using the *E. coli* F⁺ strain WR2000 as the donor.

approximately 10⁻⁵ per donor cell. These hybrids were essentially identical to the merodiploid X30D (WR4251) which we described in detail previously (4, 5, 11), i.e., they have received the *E. coli* genes from the origin of WR2001 extending to the *xyl*⁺ region (and often beyond; see Fig. 1 and 2). Although most diploid hybrids are very unstable and continuously segregate clones which have lost detectable *E. coli* genetic material, occasionally hybrids such as WR4251 are found which yield two distinct segregant types designated X30T (WR4252) and X30P (WR4253) (5, 11). Segregants of the WR4252 type appear stable, but have characteristically lost a segment of the *E. coli* genetic material encompassing the *ara*⁺-

pil⁺ regions. Colonies of the WR4253 type seem to be stable but are different from WR4251 in having lost the *S. typhosa* Vi antigen (4, 5, 11). After repeated cultivation, however, it was found that WR4253 continued to segregate clones which lost the *rha*⁺, *tna*⁺, and *xyl*⁺ *E. coli* loci singly or en bloc. WR4253 clones were usually stable for the *E. coli* *lac*⁺, *ara*⁺, and *pil*⁺ genes. Both the WR4252 and WR4253 segregants appeared to be haploid for at least part of the chromosomal region derived from *E. coli* because they exhibited the recessive *met*⁻ alleles of the *E. coli* parental strain. Hybrids of the WR4251, WR4252, and WR4253 types could be distinguished on ordinary nutrient agar on the basis of their distinctive colonial morphology (5, 11).

Adsorption of λ by *Salmonella* hybrids. A number of *xyl*⁺ merodiploid hybrids were examined to determine whether any could now adsorb λ . Approximately 2×10^6 λ plaque-forming units (PFU) were added to 2×10^9 cells of each hybrid in TB broth containing 0.2% maltose. After 15 min at 37°C, the mixtures were treated with chloroform and centrifuged, and the supernatant fluid was assayed on *E. coli* for unadsorbed phage. A majority of the hybrids tested adsorbed more than 80% of the added phage, indicating the acquisition of the *λrcp*⁺ gene by these hybrids.

The *Salmonella* hybrid strains which could adsorb λ were employed as indicator hosts in agar overlays to study the formation of plaques by λ and the λ derivatives λ b2, λ i⁴³⁴, and λ vir. The lysates which were employed contained 2×10^9 to 5×10^9 PFU/ml on the λ -sensitive *E. coli* indicator WR2000. No λ plaques were observed on any of the *Salmonella* hybrid strains even at the lowest phage dilution plated (approximately 10^9 PFU). On retesting, each of the hybrids could still adsorb more than 80% of added λ phage. To study this phenomenon, we concentrated our efforts on strain WR4254, a segregant of WR4253 which lost the *E. coli* *rha*, *tna*, *xyl* chromosomal segment, but retained *λrcp* (see Fig. 2 and Table 1).

Initially, it appeared that, after phage adsorption, there was no discernible effect of the phage on the growth and viability of the hybrid. Figure 3, for example, shows that the growth of WR4254 is not affected by λ vir even at a multiplicity of 20 phage per cell. Under the same conditions, the *E. coli* strain WR2000 was lysed rapidly. In addition, plate counts of WR4254, made at intervals after the addition of λ vir at large multiplicities of infection (MOI), showed essentially the same viability as compared to control cultures without phage. The addition of

<i>E. coli</i> WR2000	λ rcp ⁺ <i>xyl</i> ⁺ <i>tna</i> ⁺ <i>rha</i> ⁺ <i>met</i> ⁻ <i>trp</i> ⁺ <i>gal</i> ⁺ <i>ara</i> ⁺ <i>lac</i> ⁺
<i>S. typhosa</i> WR4200	λ rcp ⁻ <i>xyl</i> ⁻ <i>tna</i> ⁻ <i>rha</i> ⁻ <i>met</i> ⁺ <i>trp</i> ⁻ <i>gal</i> ⁻ <i>ara</i> ⁻ <i>lac</i> ⁻
WR2000 × WR4200 = HYBRID WR4250	λ rcp ⁺ <i>xyl</i> ⁺ <i>tna</i> ⁺ <i>rha</i> ⁺ <i>met</i> ⁺ <i>trp</i> ⁺ <i>gal</i> ⁺ <i>ara</i> ⁺ <i>lac</i> ⁺
WR2000 × WR4250 = DIPLOID WR4251	λ rcp ⁺ <i>xyl</i> ⁺ <i>tna</i> ⁺ <i>rha</i> ⁺ <i>met</i> ⁻ <i>trp</i> ⁺ <i>gal</i> ⁺ <i>ara</i> ⁺ <i>lac</i> ⁺
WR4251 SEGREGANT = HYBRID WR4252	λ rcp ⁺ <i>xyl</i> ⁺ <i>tna</i> ⁺ <i>rha</i> ⁺ <i>met</i> ⁻ <i>trp</i> ⁺ <i>gal</i> ⁺ <i>ara</i> ⁺ <i>lac</i> ⁺
WR4252 SEGREGANT = HYBRID WR4253	λ rcp ⁺ <i>xyl</i> ⁺ <i>tna</i> ⁺ <i>rha</i> ⁺ <i>met</i> ⁻ <i>trp</i> ⁺ <i>gal</i> ⁺ <i>ara</i> ⁺ <i>lac</i> ⁺
WR4253 SEGREGANT = HYBRID WR4254	λ rcp ⁺ <i>xyl</i> ⁺ <i>tna</i> ⁻ <i>rha</i> ⁻ <i>met</i> ⁻ <i>trp</i> ⁺ <i>gal</i> ⁺ <i>ara</i> ⁺ <i>lac</i> ⁺

FIG. 2. Inferred genotypes of *E. coli*-*S. typhosa* hybrid strains. +/—, Diploid state in which the numerator is the allele from the *E. coli* K-12 donor and the denominator is the allele belonging to the resident *Salmonella* chromosome. Diploidy is established by the segregation of the negative phenotype, whereas haploidy is characterized by stable expression of either the positive or negative phenotype. It is not possible to guarantee the haploid nature of strains with positive phenotypes, but such strains when depicted as haploids have not yielded negative segregants since first isolated 10 years ago. (*E. coli* chromosome, —; *S. typhosa* chromosome, —).

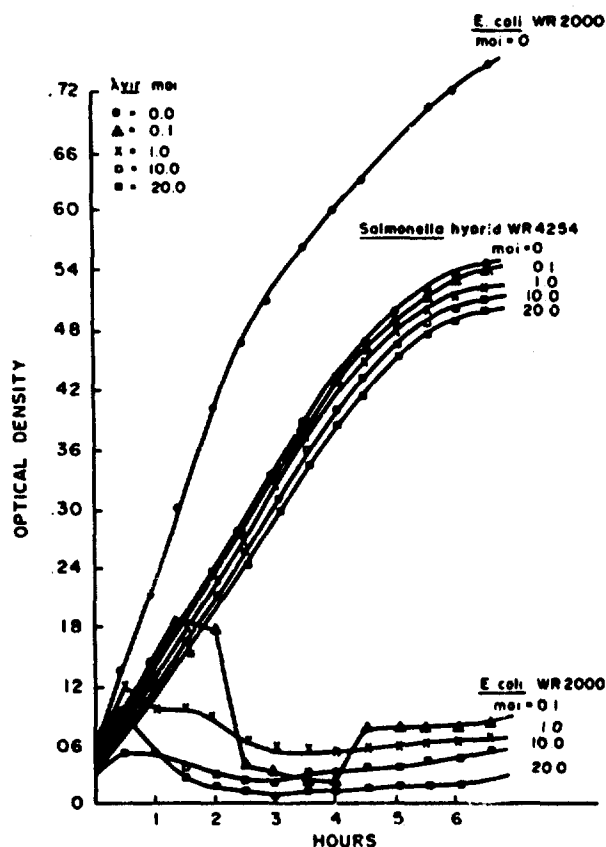


FIG. 3. Log-phase cells of *E. coli* WR2000 and *S. typhosa* hybrid WR4254 were diluted into TB medium prewarmed to 37°C and subjected at zero time to increasing multiplicities of λ vir as indicated. The cultures were placed in a shaking-water bath at 37°C and agitated slowly.

λ phage to TB broth cultures of WR4254 was followed by a marked decrease in the titer of the phage (presumably due to adsorption), but the phage failed to show any evidence of new phage progeny in samples taken hourly or even after overnight incubation. Similar results were obtained with the WR4251 and WR4252 segregant types. All attempts to measure infective centers, to demonstrate the production of progeny phage after the adsorption of λ to WR4254 and other *Salmonella* hybrids, or to detect a lysogenic response ($< 2 \times 10^{-4}$ per adsorbed phage) were fruitless. We considered that λ might be restricted and modified (2) after growth in a *Salmonella* hybrid so that any progeny phage which might be released would have a different host range than that of the input virus. Consequently, all phage titrations were performed on both WR4254 and WR2000. There was no evidence of phage progeny which could plaque on either of these strains after adsorption of λ to the *Salmonella* hybrid.

Thus, although we had isolated hybrids capable of adsorbing λ phage, there was no positive evidence for the replication and maturation of mature phage particles within the *Salmonella* host. The data indicated that either the adsorption of the phage was a phenomenon unrelated to phage infection or that some property of the phage, the host, or both, was interfering with the normal course of λ development.

Transduction of gal^+ to *Salmonella* hybrids. To study the possible fate of λ deoxyribonucleic acid (DNA) more readily in further experiments, we employed the defective derivative λdg in which bacterial galactose (*gal*) genes replace part of the viral DNA (8, 9, 18). Any replication of the gal^+ phage DNA after infection of a gal^- host would, therefore, be betrayed by the presence of phenotypically gal^+ cells (transductants).

Lambda lysates were prepared by the UV induction of *E. coli* K-12 WR2004 and tested for transduction of gal^+ to WR4254. For comparative purposes, the gal^- *E. coli* K-12 strains WR3060, WR3061 (λ), and WR3062 (λ^{i434}) were also tested as recipients. Although gal^+ transductants were observed with the *E. coli* recipients at a frequency of about 10^{-6} /PFU, the lysates were ineffective when used with the WR4254 hybrid under identical conditions. Heterogenote transductants (gal^-/gal^+) were isolated from *E. coli* strains, and $\lambda + \lambda dg$, as well as $\lambda^{i434} + \lambda dg$ HFT lysates, were prepared from gal^-/gal^+ transductants of WR3061 and WR3062 by mitomycin C induction. The HFT lysates showed transduction frequencies of 2×10^{-2} to 5×10^{-2} /PFU with λ -immune *E. coli* hosts and about 10 to 30-fold lower with λ -sensitive *E. coli* hosts.

When these HFT lysates were tested on WR4254, it was found (Table 3) that gal^+ transductants could indeed be isolated at a frequency of 10^{-3} to 3×10^{-3} /PFU. This represented a frequency of about 0.01 to 0.05 that of WR3061 (λ) and about 0.5 that of WR3060, a λ -sensitive strain of *E. coli*. In the experiments presented in Table 3, the MOI was adjusted to about 1 PFU per recipient cell. Increases in the MOI resulted in proportional increases in the number of transductants up to the MOI of 10 PFU which showed a reduction in frequency.

Gal^+ transductants of WR4254 were isolated after exposure to an HFT lysate at increasing multiplicities of 0.3, 1.0, 3.0, and 10 PFU per bacterium. At the lower multiplicities of 0.3 and 1.0, a large percentage of the transductants proved to be stable gal^+ clones, suggesting that transduction was a replacement presumably with elimination of the phage genome. When the input ratio of phage was increased to 10, however, the percentage of gal^-/gal^+ unstable heterogenotes increased, so that up to 95% of the transductants were heterogenotes (Table 4). Unstable heterogenotes formed by transduction of *E. coli* are the result of an addition of the transducing phage to the host genome with or without an active (nondefective) phage as well. These data indicated that, after adsorption of λ to the *Salmonella* hybrid WR4254, gal^+ DNA did enter the host bacterium and was capable of being replicated. It was not clear, however, whether the transductants solely represented the "rescue" of bacterial gal^+ genes by the host, or if the gal^+ genes were still maintained in association and replicated with the phage genome.

It was necessary, therefore, to determine whether the gal^+ transductants of WR4254 had become λ lysogens. None of more than 100 of the stable gal^+ clones showed any detectable release of mature λ or λ^{i434} into the culture medium. Among the unstable WR4254 gal^-/gal^+ heterogenotes, however, about 20% released phage into the supernatant fluid. Heterogenotes prepared as a result of transduction with ($\lambda + \lambda dg$) HFT

TABLE 3. Transduction of galactose utilization by HFT lysates of phage lambda

Strain	HFT lysate
<i>Escherichia coli</i> WR3061 (λ)	1
<i>E. coli</i> WR3060	0.03-0.1
<i>Salmonella</i> hybrid WR4254	0.01-0.05

* MOI was adjusted to approximately 1 PFU/bacterium by adding 2×10^9 phage to an equal volume containing 2×10^9 recipient bacteria. After incubation for 25 min at 37 C, the mixtures were plated on EMB agar containing 1% galactose.

TABLE 4. Analysis of transductants of *Salmonella* hybrid WR4254 at increasing multiplicities of infection

MOI ^a	Stable <i>gal</i> ⁺ % ^b	Heterogenotes per cent <i>gal</i> ⁺ / <i>gal</i> ⁺	Heterogenotes per cent <i>gal</i> ⁺ / <i>gal</i> ⁺ producing λ
0.3	60	40	7
1.0	47	53	8
3.0	35	65	10
10.0	5	95	38

^a The phage lysate used was prepared by thermal induction of a heterogenote derived by transduction of *gal*⁺ to WR3064 using an LFT lysate obtained by thermal induction of WR2020.

^b Percentages are based on a minimum of 100 tested transductants which were purified by re-streaking on FMB agar containing 1% galactose. To test for phage production, colonies of each transductant were picked into TB and incubated for 18 hr at 37 C before being spotted on overlays of WR2001.

lysates showed only λ phage, whereas heterogenotes prepared from $\lambda^{i434} + \lambda dg$ HFT lysates released λ^{i434} , mature λ , or, in most instances, both phages. The phages liberated by these heterogenotes still failed to produce plaques on *S. typhosa* hybrid WR4254, being detected only on *E. coli* WR2000 and other suitable *E. coli* strains. The percentage of heterogenotes releasing mature phage into the supernatant fluid increased with higher multiplicities of phage used for transduction (Table 4). The possibility of external phage contamination was eliminated by treating phage-producing clones with λ antiserum and demonstrating that phage could still be found in the supernatant fluid of overnight cultures. The number of PFU spontaneously produced by the *Salmonella* hybrid was, however, considerably lower (about 10^2 to 10^4 /ml) as compared to *E. coli* heterogenotes (about 10^6 /ml). The spontaneous production of phage by these hybrids was interpreted as a presumptive indication of lysogeny.

Segregation of phage production from *Salmonella* heterogenotes. The stability of the presumed lysogenic state was tested in six independent heterogenote isolates of WR4254. Each clone initially identified as a phage-producer was re-isolated three times, grown in broth, diluted, and plated. Individual colonies were then scored for the presence or absence of mature phage particles in the culture fluid. The percentage of progeny among the series of different WR4254 heterogenotes varied from 2 to 90% (Table 5), indicating that phage production (and presumably phage persistence) was an unstable trait

rather than the very stable property exhibited by lysogenic *E. coli* K-12. The possibility of external phage contamination was again excluded by treatment with λ antiserum. Clones treated with antiserum were streaked and examined for phage-producing ability throughout four cycles of single-colony reisolation. The ability to produce phage segregated independently of the presence of the *gal*⁺ genes (Table 6). In no instance have we observed any reappearance of phage production once this ability was lost. Clones with the presumptive genotype ($\lambda + \lambda dg$) were exposed to λ^{i434} , but in no instance were plaques or infectious centers demonstrated. Similarly, heterogenotes which retained the *gal*⁺ phenotype, but had lost phage-producing ability, could not be stimulated to produce phage once more by λ^{i434} superinfection. It should be noted that clones which had lost the *gal*⁺ phenotype and phage-producing ability were not superior to the original WR4254 parental strain as a recipient

TABLE 5. Phage distribution in *Salmonella* hybrid WR4254 heterogenotes with lambda^a

Heterogenote WR4254 strains	No. of colonies tested	Colonies releasing phage %
1	202	44
2	230	8
3	155	16
4	112	2
5	220	90
6	179	83

^a *Gal*⁺ heterogenote colonies were picked into TB and incubated for 18 hr at 37 C before being spotted on WR2000.

TABLE 6. Persistence of phage in a *Salmonella* heterogenote producing phage^a

Transfer no.	<i>gal</i> ⁺ Clones	Colonies releasing phage %	<i>gal</i> ⁺ Clones	Colonies releasing phage %
1st	187	75	121	52
2nd	189	83	40	51
3rd	107	90	47	14
4th	133	79	28	21

^a Colonies of a heterogenote strain with persistent phage production were examined during four serial transfers of single colonies. A single *gal*⁺ colony was picked into broth, diluted, and plated on MacConkey Agar containing 1% galactose. Individual colonies were picked into broth and spotted on overlays containing WR2000. This procedure was repeated for four successive single-colony transfers.

in subsequent transduction experiments. Despite the observation of instability, however, *gal*⁺ phage-producing clones continue to remain in most populations. It seems fair to conclude that λ can lysogenize *S. typhosa* carrying the λ rec⁺ locus. Clearly, however, the infection and lysogenization of *Salmonella* by λ is at considerable variance with the situation in *E. coli*.

Attempts to induce *Salmonella* lysogens. The establishment of lysogeny in *Salmonella* permitted us to test whether the mechanism of prophage induction was operative in this host. Viral development in lytic infection and after release from prophage repression (8, 9) is generally similar. Since the lytic response to external infection was clearly blocked in some manner in *Salmonella* hybrids, it seemed important to determine whether this was still the case once the prophage had become established. The parental *Salmonella* hybrid WR4254, a heterogenetic phage-producing derivative WR4254 *Het29* ($\lambda^{i434} + \lambda$ dg) and *E. coli* WR2001 (λ), was treated with the effective prophage-inducing agent mitomycin C. WR4254 and WR4254 *Het29* were unaffected by growth in 2 μ g of mitomycin per ml, whereas the cells of WR2001 (λ) lysed within 180 min. The concentration of mitomycin C was increased to rule out any differential sensitivity of the two species to the inducing effects of the drug. Although a difference in optical density was discernible between WR4254 and WR4254 *Het29* at a concentration of 20 μ g/ml of mitomycin C, there was no major change in the titer of phage produced by WR4254 *Het29* over the course of the experiments (Fig. 4). The phage titer of the *E. coli* strain increased by about a million-fold. Similar experiments were performed with more than 10 separate heterogenotes derived from WR4254 with similar results. When another inducing agent, UV light, was employed, there was similarly no effect on the *Salmonella* lysogen.

The induction of prophage by growth in mitomycin C and after UV irradiation is most likely an indirect consequence of the inhibition of host-DNA replication. Another treatment which more directly causes repression release was attempted. In this case, the treatment employed was thermal induction of temperature-sensitive λ mutants. Thermal induction probably involves the direct inactivation of the repressor responsible for the maintenance of the prophage state (8, 9, 15). HFT lysates of the thermoinducible mutant λ cI857 were prepared in *E. coli* and used to transduce WR4254. Heterogenotes containing λ cI857 + λ cI857 dg were treated at temperatures ranging from 40 to 48 C. There was no observed lysis nor phage release in the *Salmonella* hybrid

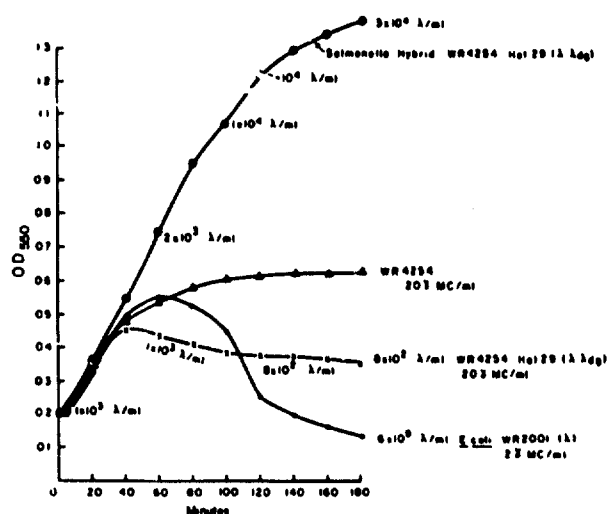


FIG. 4. Log-phase cells of *E. coli* WR2001 and *S. typhosa* hybrid WR4254 and the *Het29* derivative of WR4254 were diluted into TB medium prewarmed to 37 C and treated with mitomycin C (MC) at the concentrations indicated. The cultures were incubated in a shaking-water bath at 37 C and agitated vigorously for maximum aeration. Phage titrations were performed at the points indicated by assaying samples of the supernatant fluid from the growth media on WR2000.

under conditions which resulted in efficient prophage induction in *E. coli* (Fig. 5). Further experiments of a similar nature with two other thermoinducible mutants, λ cI1 and λ cI2 (15), gave identical results. Moreover, the *Salmonella* heterogenotes carrying the thermoinducible mutants were equally stable at both high (45 C) and low (30 C) temperatures. Thus, even under conditions which could have inactivated the immunity repressor, irreversibly in the case of λ cI2, the phage did not successfully enter into lytic growth nor give significant cell killing. There was also no evidence of curing of the phage as occurs in *E. coli* cells which have survived treatment (8, 9, 15).

Isolation of λ mutants capable of forming plaques on *Salmonella* hybrids. Failure to demonstrate any kind of lytic response by λ -infected *Salmonella* cells prompted us to determine whether exceptional members of the phage population could be isolated which lysed the *Salmonella* host. We felt that the isolation of such mutants would provide some clue to the nature of the block which prevented normal phage maturation as well as simplifying an examination of the internal state of λ in the heterogenotes. Phage lysates of λ , λ^{i434} , and λ vir exceeding 10^{11} PFU/ml were plated with WR4254 in agar overlays and carefully examined for plaque formation. A few indistinct plaques resulted from the initial plating of all three phages on WR4254. These

plaques, which appeared at a frequency of about 10^{-10} , were picked and cloned by repeated plating on WR4254 to eliminate the wild-type phages. High-titer lysates of the mutant phages were prepared by the confluent lysis method. Mutants of λ lysing WR4254 were designated as λ_{sx} . The isolation of λ_{sx} permitted us to determine the immunity specificity of WR4254 heterogenotes. The pattern of immunity of the lysogenic *Salmonella* (Table 7) fits the classical picture of *E. coli* K-12. Clearly, repressor was being produced by lysogenic *Salmonella*, and λ_{sx} mutants were sensitive to repression.

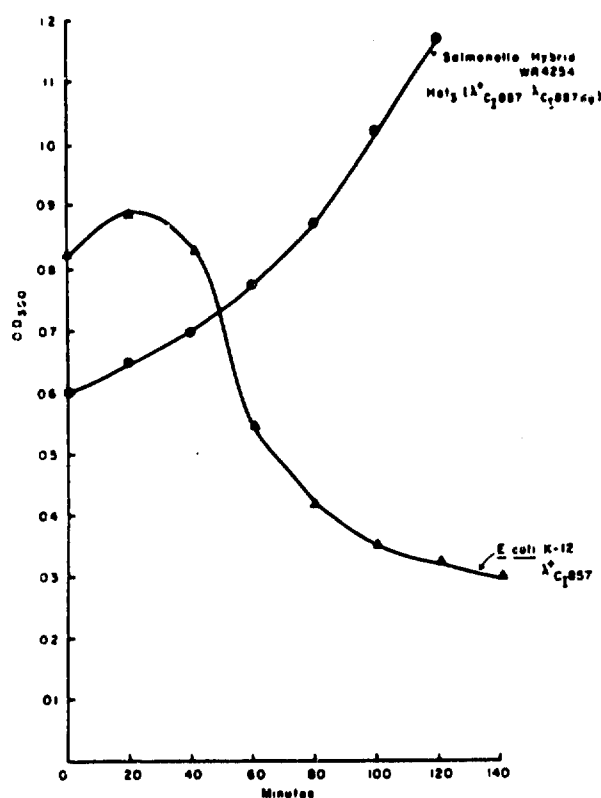


FIG. 5. TB cultures of the Het3 derivative of *S. typhosa* hybrid WR4254 and *E. coli* WR3063 were incubated at 45°C for 15 min and then incubated at 37°C for 140 min in a shaking-water bath with vigorous shaking for maximum aeration throughout the experiment.

The plaques of λ_{sx} were clearer and smaller in size than the typical turbid plaques of λ or λ^{i434} . λ_{sx} Derivatives (except of λ_{vir}) cannot be classified as virulent, as they were repressed by homoimmune phages. Moreover, it was possible to lysogenize *E. coli* strains with λ_{sx} , albeit at a frequency at least 100-fold lower than observed with λ . All attempts to demonstrate a lysogenic response of WR4254 to λ_{sx} have been unsuccessful except by transduction with ($\lambda_{sx} + \lambda_{sxdg}$) HFT lysates prepared by induction of K-12 heterogenotes. The titer of λ_{sx} lysates on WR4254 and WR200C was approximately the same no matter which host was used to propagate the phage. The λ_{sx} phage grown on *E. coli* and the *Salmonella* hybrid were restricted equally by *mal*⁺ strains of *E. coli* B.

To define the region of the λ genome which had been altered to permit plaque formation by λ_{sx} mutants on *Salmonella* hybrid WR4254, a series of crosses were performed between λ_{sx} and cells carrying isolates of λ_{db} lysogens. The λ_{db} prophages employed were derivatives of λ which carried biotin (*bio*) genes (13). As *bio* is on the opposite side of λ from *gal*, the gene content of defective biotin-transducing phage is different from that of λ_{dg} . Whereas λ_{dg} is deleted for viral genes affecting "late" phage functions, λ_{db} possess deletions affecting "early" and regulatory gene functions (8, 9, 13). The λ_{sx} was employed to infect cells harboring various λ_{db} derivatives. By using λ_{db} with deletions extending various distances into the phage chromosome, it was determined that wild-type λ recombinants, i.e., λ which plated on *E. coli* but not on *Salmonella* strain WR4254, could be obtained so long as the λ_{db} derivative included a region between the λ genes P and Q (Table 8). Thus, the mutant λ_{sx} phage owes its ability to plate on *Salmonella* WR4254 (at least in part) to a gene (or genes) which maps between the P and Q markers in λ .

An unexpected finding with the λ_{sx} mutants was the marked difference between WR4254 and its parental merodiploid WR4251 and the segregant WR4252. Although all of these strains

TABLE 7. Pattern of response to cross-infection shown by λ and λ_{sx} phages^a

Superinfecting phage	<i>E. coli</i>	<i>E. coli</i> (λ)	<i>E. coli</i> (λ^{i434})	<i>S. typhosa</i> hybrid WR4254	<i>S. typhosa</i> hybrid WR4254 ($\lambda + \lambda_{dg}$)	<i>S. typhosa</i> hybrid WR4254 ($\lambda^{i434} + \lambda_{dg}$)
λ	+	—	+	—	—	—
λ^{i434}	+	+	—	—	—	—
λ_{vir}	+	+	+	—	—	—
λ_{sx}	+	—	+	+	—	—
λ^{i434}_{sx}	+	+	—	+	+	—
λ_{virsx}	+	+	+	+	+	+

^a Symbols: +, lysis; —, no lysis.

could adsorb λ and were transduced to *gal*⁺ by HFT lysates of λ , WR4251 and WR4252 did not permit the lytic growth of λ ssx. This observation suggested that the state of the *E. coli* genes in *Salmonella* may be a critical factor and further, that in addition to *λrep*, the *E. coli* genetic contribution to the *Salmonella* hybrids has an important bearing on the fate of λ after infection. Based on these results, we have attempted to find a class of *Salmonella* hybrids which would permit plaque formation by wild-type λ . Subsequent mating experiments have resulted in the isolation of such a class of *Escherichia-Salmonella* hybrids. It has also been possible to isolate nitrosoguanidine-induced mutant hosts from WR4254 which now allow normal lytic growth of λ vir and λ ¹⁴³⁴ though not of λ . The properties of these mutants as well as the class of hybrids which support lytic growth of wild type λ will be reported in detail in future publications.

Growth of λ derivatives on *Salmonella* hybrid WR4254. The successful isolation of λ mutants which could undergo at least some degree of normal lytic development on *S. typhosa* prompted us to reexamine some of the many laboratory derivatives of λ phage which were available. It was possible to obtain plaque formation on WR4254 with phage recombinants selected in crosses between λ and phage 21 (Table 9). These recombinants studied by Liedke-Kulke and Kaiser (16, 17) could form plaques on WR4254 if they contained the immunity region of phage 21 (λ ¹²¹). Phage containing the immunity region of phage 434 could not form plaques on the *Salmonella* hybrid. The region of λ which is replaced by the homologous immunity region of phage 434 and by phage 21 is shown in Fig. 6. The *Salmonella* hybrid type WR4252 was not

lysed by any phage derivative tested, but rare mutant plaques could be seen in the case of two of the λ ¹²¹ phage hybrids. These mutant phages have been isolated and are currently being studied to determine how they differ from λ ssx which forms plaques on WR4253 and WR4254.

We also tested a λ mutant, λ c17 (20) which is insensitive to replication inhibition and shows constitutive synthesis of genes O and P whose functions are required for replication of λ DNA. This phage mutant did not show plaque formation on either WR4253 or WR4254.

Absence of zygotic induction in *Salmonella* hybrids. In *E. coli* conjugation experiments, the results of a mating may be dramatically influenced owing to the induction of λ prophage on transfer to a sensitive recipient (22). Two consequences of this zygotic induction are that the numbers of recombinants recovered are markedly reduced and λ lysogeny is not observed in those hybrids which do appear. Since *E. coli-Salmonella* genetic hybrids did not support the vegetative multiplication of wild-type λ , nor could we induce λ lysogenic *E. coli-Salmonella* genetic hybrids, we performed experiments designed to determine whether zygotic induction would occur or was similarly inhibited. For these experiments, Hfr H (WR2002) was lysogenized with wild-type λ so that the resulting strain Hfr H (λ) could be compared with its nonlysogenic parent in matings with WR4254. Both the lysogenic and nonlysogenic Hfr H derivatives were mated with WR4254 on a medium which could select *gal*⁺ *Salmonella* hybrids. In our initial experiments, the frequency of recombination was so extremely low that a valid comparison was not possible (Table 10). We supposed that the low frequency of recombination observed with *Salmonella* and both *E.*

TABLE 8. Mapping of λ ssx mutation by growth in λ db lysogens^a

λ bio ^a genome	N7, 53	Δ	c147	c1	c1168	OS, 29	O125	P72	P3	P116	O21, 73, 117 A11 R5, 16, 54, 60, 76	Bio-0, 4, 24	Formation of recom- binant wild-type λ	
M37-1	—	—	—	+	+	+	+	+	+	+	+	+	+	+
R24-2	—	—	—	—	—	+	+	+	+	+	+	+	+	+
M20-5	—	N	N	N	N	+	+	+	+	+	+	+	+	+
R30H-20	—	N	N	N	N	—	+	+	+	+	+	+	+	+
ESA20	N	N	N	N	N	—	—	—	—	+	+	+	+	+
R39-3	N	N	N	N	N	—	—	—	—	—	+	+	+	+
M34-3	N	N	N	N	N	—	—	—	—	—	+	+	+	—
M55-3	—	N	N	N	N	—	—	—	—	—	—	+	—	—
Nonlyso- genic														—

^a Symbols: N, not known; +, presence of marker; —, absence of marker.

^b Symbols: + **, recovery of turbid wild-type λ plaques which are unable to plaque on *Salmonella* hybrid; +, recovery of only λ ssx plaques.

TABLE 9. Sensitivity of *E. coli* WR2000 and *Salmonella* hybrids WR4254 and WR4252 to λ derivatives^a

λ Derivative	Frequency of plating on		
	<i>E. coli</i> WR2000	<i>S. typhosa</i> hybrid WR4251 (X30W)	<i>S. typhosa</i> WR4252 (X30T)
λ	1.0	$<10^{-9}$	$<10^{-9}$
λb_2	1.0	$<10^{-9}$	$<10^{-9}$
λvir	1.0	$<10^{-9}$	$<10^{-9}$
$\lambda c17$	1.0	$<10^{-9}$	$<10^{-9}$
λ^{434}	1.0	$<10^{-9}$	$<10^{-9}$
λsx	1.0	1.0	$<10^{-9}$
21hy1 $\lambda c111^+$ 34	1.0	1.0	8×10^{-6}
21hy2 $\lambda c111^+$ 34 ^{imm21} 21c11	1.0	1.0	1.6×10^{-6}
21hy4 21h ⁺ imm ²¹ 21c11	1.0	1.0	$<10^{-9}$
21hy5 λh imm ²¹ 21c11	1.0	1.0	$<10^{-9}$
21hy6 21h ⁺ $\lambda c111$ imm ²¹	1.0	0.25	$<10^{-9}$
21hy7 λh 21c11 imm ²¹	1.0	0.5 1.0	$<10^{-9}$
21hy8 λh imm λ	1.0	$<10^{-9}$	$<10^{-9}$
21hy9 imm λ $\lambda c11$	1.0	$<10^{-9}$	$<10^{-9}$
21hy10 imm ⁴³⁴ $\lambda c11$	1.0	$<10^{-9}$	$<10^{-9}$

^a Phage lysates containing about 2×10^8 PFU were prepared on *E. coli* strain W1485 and assayed on WR4254 and WR4252. The number of PFU on the *Salmonella* hybrids were expressed relative to the *E. coli* strain which was taken as 1.0. The extent of imm²¹ and imm⁴³⁴ are shown in Fig. 6.

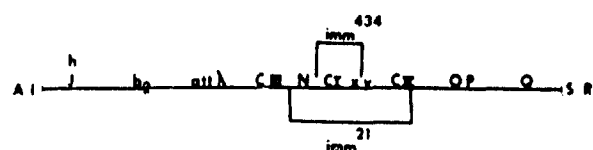


FIG. 6. Genetic map of λ showing the approximate location of markers mentioned in the text (8, 9). The regions of λ deleted in phage λ^{434} and phage λ^{21} which have been replaced by the homologous immunity regions of phage 434 and phage 21 are also indicated.

coli donor strains was probably due to differences in genetic fine structure (11). To overcome this difficulty, the *gal*⁺ region of *E. coli* was introduced in WR4254 by a mating between the F⁺ strain WR2000 and WR4254. A stable *gal*⁺ hybrid of WR4254 was isolated from this mating and treated with the mutagen *N,N'*-methyl nitrosoguanidine to produce a suitable *gal*⁻ derivative. This strain, WR4255, was considered to have the *E. coli* K-12 *gal* region and was employed as a recipient in matings with the λ -lysogenic and λ -sensitive Hfr H strains. There is a high frequency of WR4255 *gal*⁺ recombinants isolated from matings with both the λ -lysogenic and λ -sensitive *E. coli* donors (Table 10). On the other hand, in comparison to control matings with the λ -sensitive *E. coli* K-12 recipient, the λ -lysogenic donor caused a far more dramatic decrease in number of recombinants due to zygotic induction. Since the frequency of recombination measured with the Hfr H λ -sensitive strain was of the same order of magnitude with

TABLE 10. Frequency of recombination for *gal*⁺ in crosses between Hfr H (λ) or Hfr H and *Salmonella* hybrids

Donor	Recipient	Frequency of <i>gal</i> ⁺ recombinants per 100 donors
WR2002 Hfr H	WR4255	4.0
WR2003 Hfr H (λ)	WR4255	3.6
WR2002 Hfr H	WR3060	4.5
WR2003 Hfr H (λ)	WR3060	0.08
WR2002 Hfr H	WR4254	0.000039
WR2003 Hfr H (λ)	WR4254	0.000033

both the *E. coli* λ -sensitive recipient and WR4255, it can probably be concluded that the comparison of recombination frequencies is valid. Thus, if some degree of zygotic induction did occur in the *E. coli*-*Salmonella* genetic hybrid, the effect is markedly reduced as compared to that seen in *E. coli*.

It has been established that the recovery of *gal*⁺ λ -lysogenic progeny from an *E. coli* Hfr H (λ) $\times$ *E. coli* F⁻ mating is virtually nil and this was the case in our experiments (Table 10). Since we now had at our disposal λsx derivatives, we could apply the rule that the presence of immunity to λsx in the WR4255 *gal*⁺ hybrids would indicate the acquisition of λ prophage from the Hfr H (λ) donor strain. More than 30% of the *gal*⁺ *Salmonella* recombinants of WR4255 isolated from the mating with Hfr H (λ) had ac-

quired immunity to λ_{sx} infection, but not to λ^{1434} λ_{sx} infection. These data again support the notion that the vegetative multiplication of wild-type λ is inhibited in *Salmonella*.

DISCUSSION

Our data show that some *E. coli-S. typhosa* genetic hybrids can adsorb and be lysogenized by the temperate coliphage λ . Wild-type λ phage did not, however, multiply lytically to any discernible extent nor kill the bacterial host. Furthermore, λ prophage once established in *Salmonella* could not be induced to follow the normal lytic development cycle even after treatment which normally inactivates specific phage repressor. This behavior of λ in *Salmonella* hosts necessitates a consideration of the nature of the inhibition by *S. typhosa* on the usual expression of the λ lytic response.

Ordinarily the lytic response involves the orderly temporal expression of "early genes" concerned with DNA replication as well as regulation followed by the expression of "late genes" which specify phage structural proteins (8, 9). The alternative lysogenic response requires the repression of the lytic developmental cycle and results ordinarily in the establishment of prophage at a specific site on the host chromosomal DNA. We have shown that the block to lytic development of λ in *S. typhosa* hybrids can be overcome by either the selection of phage mutants, which we have termed λ_{sx} , or by the isolation of suitable host mutants. The study of lysogenic *Salmonella* hybrids and λ_{sx} derivatives indicates that some host effect on the properties of "early genes," specifically the N, O, P, and Q genes, is involved in the inability of wild-type λ to multiply lytically in *E. coli-Salmonella* genetic hybrids.

It seems valid to conclude that the DNA replication genes (O and P) do act to some extent, since we can demonstrate that λ_{dg} prophage can be maintained indefinitely in *Salmonella*. By the same token, however, the instability of λ_{dg} and the limited ability to produce phage in *Salmonella* hybrids suggest that this replication might be slower than that of the bacterial chromosome. It may be noted that even constitutive λ DNA synthesis as characterized by $\lambda c17$ (Table 9) was not sufficient in itself to permit normal λ development. The λ_{sx} mutants which do grow lytically on *Salmonella* hybrids are a class of clear mutants which were mapped at a site between P and Q genes. Gene Q is a regulatory gene involved in the activation of the "late" λ genes governing head and tail proteins as well as lysis. It has been suggested that N probably carries

out regulation of late proteins through activation of gene Q; the N gene products also regulate DNA replication and recombination (8, 9). One reasonable hypothesis for the ability of λ_{sx} to bring about lysis of *Salmonella* would be that the need for the N product to activate the Q gene is obviated.

Thus the mutant site in λ_{sx} between P and Q could represent a locus which ordinarily interacts with the N gene product and controls expression of gene Q. Presumably the N product of wild-type λ in *S. typhosa* hybrids is somehow blocked from activating Q, whereas in λ_{sx} this requirement is by-passed. A locus termed *byp* has indeed been identified in λ between P and Q, which permits relatively normal lytic response in *E. coli* in the absence of the N gene product (6, 7). We have not confirmed that *byp* and the λ_{sx} mutations are in fact identical, although it would seem quite likely.

The postulated important role of the N gene on the ability of wild-type λ to grow lytically in *Salmonella* receives support from the observations with λ^{121} . In contrast to all other λ derivatives that we have studied, λ^{121} shows relatively normal lytic development in the *Salmonella* hybrid. The recombinant phages λ^{121} and λ^{1434} were selected in crosses between λ and phages 21 and 434. Recent experiments have delineated the 434 and 21 immunity regions substituted by the λ genome (8, 9). Recombinant phage λ^{1434} , which cannot plate on the *Salmonella* hybrid has incorporated the phage 434 genes for *cl* but retains the λ N gene. Recombinant phage λ^{121} , however, has incorporated the phage 21 N, *cl*, and *cII* genes. The N gene, therefore, seems a likely prospect for the difference in behavior of these two phages, since the N gene of phage 21 does not complement the N gene of λ . At any rate, certainly a critical region determining lysis of the *Salmonella* hybrid is defined by the λ^{121} immunity region.

The behavior of λ_{NN^-} mutants (λ having two *susN* mutations) in *E. coli* shows some striking similarities to the behavior of wild-type λ in *Salmonella*. λ_{NN^-} mutants show a pleiotropic defect in production of late structural proteins but can lysogenize the host (8, 9, 21). λ_{NN^-} mutants are also defective in DNA replication although at least some basal transcription of O and P is detectable. The rate-limiting step appears to be initiation of replication rather than DNA synthesis itself (19). Signer (21) recently reported that a λ_{NN^-} mutant replicates as a plasmid in *E. coli* and can effectively transduce but not grow lytically in this host. The replication of wild-type λ in *Salmonella* as a plasmid as well as the

elimination of λ prophage from hybrid *Salmonella* by agents which "cure" plasmids is reported in an accompanying paper (10). A major difference between the behavior of λ NN⁻ mutants in *E. coli* and wild-type λ in *Salmonella* is in their apparent ability to establish repression. Signer (21) reported that NN⁻ λ phage cannot efficiently establish the normal level of repression after infecting a nonimmune cell. Lysogenic *Salmonella* are, however, immune to infection with λ ssx, which implies that either repression can be established or that, as in the case of *E. coli* (8, 9), the super-infecting phage initiates repressor synthesis in the resident prophage. Recent experiments have shown that the product of the *rex* gene, an independent measure of *cl* function which acts in the lysogenic state to block the growth of *rII* mutants of T-even phages (12), is active in λ -lysogenic *S. typhosa* hybrids (Baron, unpublished observation), suggesting that λ prophage in *Salmonella* may establish an effective level of repression. The role of repressor in *Salmonella* lysogens will be reported in a subsequent communication. At any rate, it does seem that a reasonable first hypothesis is that the primary function of λ which is blocked in *Salmonella* hybrids is either the production or the function, or both, of the N gene product. A corollary to this hypothesis is that the "blocking substance" is produced by the host *Salmonella* hybrid.

It seems remarkable that *Salmonella* hybrids can synthesize a cellular product which can so effectively block the normal lytic development of wild-type λ . It should be noted that this ability is not solely a property of *S. typhosa* hybrids. Identical results with λ have been obtained with *S. typhimurium* hybrids, so that this may well be a general property of *Salmonella* species (Penido and Baron, Bacteriol. Proc., p. 30, 1966; Penido, Ryman, Falkow, and Baron, Bacteriol. Proc., p. 30, 1966). In addition, the inhibition of lytic phage development seems to exhibit some degree of specificity. For example, the lytic growth of phage 434 is inhibited to roughly the same extent as λ , whereas ϕ 80, another lambdoid *E. coli* temperate phage, can both effectively lyse and lysogenize *Salmonella* hybrids as can the generalized transducing phage P1 (Penido, Ryman, Falkow, and Baron, Bacteriol. Proc., p. 30, 1966). It is not yet clear, however, whether the varying responses of *Salmonella* hybrids to different *E. coli* temperate phages represents a qualitative or quantitative difference.

The specific nature and cellular location of the *Salmonella* cellular product remains unknown. The hypothesis we have suggested requires that this cellular product actively interact or, alterna-

tively, fails to interact, with the λ N gene or its product(s). We hope that a continued study of both phage and host mutants which permit the normal lytic development of λ in *Salmonella* will provide a novel and useful approach to the study of host-controlled steps in λ infection.

ACKNOWLEDGMENTS

We are deeply indebted to Michael Yarmolinsky and David Friedman for their interest, suggestions, and help in the preparation of this manuscript. We give special thanks to A. D. Kaiser for suggesting the use of his λ 121 hybrids to examine their behavior on *S. typhosa* hybrid strains. Special thanks are due also to our colleagues Peter Gemski, Jr., J. A. Wohlhieter, and E. M. Johnson for their assistance with our interpretation of the data and to A. Weissbach and D. J. Brenner for their critical examination of the manuscript. We also acknowledge the contribution of bacterial strains and phage by M. Yarmolinsky, A. Weissbach, M. Lieb, A. D. Kaiser, and G. Kayajanian.

The work performed at Georgetown University by one of us (S. F.) was supported by grant GE1305 from the National Science Foundation and by Public Health Service grant FR5360.

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