FUNCTIONAL INTERCHANGEABILITY OF DNA REPLICATION GENES IN *SALMONELLA TYPHIMURIUM* AND *ESCHERICHIA COLI* DEMONSTRATED BY A GENERAL COMPLEMENTATION PROCEDURE

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ABSTRACT

Twenty-four genes from *Salmonella typhimurium* that affect DNA replication were isolated from a λ-Salmonella genomic library by lysogenic complementation of temperature-sensitive mutants of Salmonella or *E. coli*, using a new plaque complementation assay. The complementing λ clones, which make red plaques in this assay, and noncomplementing mutant derivatives, which make uncolored plaques, were used to further characterize the temperature-sensitive Salmonella mutants and to establish the functional similarity of *E. coli* and Salmonella DNA replication genes. For 17 of 18 *E. coli* mutants representing distinct loci, a Salmonella gene that complemented the mutant was found. This result indicates that single Salmonella replication proteins are able to function in otherwise all *E. coli* replication complexes and suggests that the detailed properties of Salmonella and *E. coli* replication proteins are very similar. The other seven Salmonella genes that were cloned were unrelated functionally to any *E. coli* genes examined. ——As an aid to the derivation of chromosomal mutations affecting some of the cloned genes, a general method was developed for placing a transposon in the Salmonella chromosome in a segment corresponding to cloned DNA. Chromosomal mutations were derived in Salmonella affecting a gene (*dnaA*) that was cloned by complementation of an *E. coli* mutant by using the transposon-encoded drug resistance as a selectable marker in local mutagenesis.

WE have been studying DNA replication functions of *Salmonella typhimurium*, a bacterial species similar to *E. coli* both genetically and biochemically (MAURER, OSMOND and BOTSTEIN 1981; BOTSTEIN and MAURER 1982). These studies were undertaken with the knowledge that at least in some respects the replication machinery is similar in the two organisms, as judged by the ability of phage DNA (of λ and P22) and plasmids such as F and pBR322 to be replicated in either host (FRIEDMAN and BARON 1974; BOTSTEIN and HERSKOWITZ 1974; ZINDER 1960; CHISOLM *et al.* 1980). These and other similar observations (see MAURER, OSMOND and BOTSTEIN 1981) can be explained by the substitution of entire sets of replication proteins of one organism

for those of the other and do not address the similarity of the individual proteins. Therefore, in addition to isolation and characterization of Salmonella replication mutants, another goal of our experiments has been a systematic comparison of individual *E. coli* and Salmonella replication functions.

Our approach to several aspects of this genetic analysis relies on molecular cloning of Salmonella genes in phage λ and on a versatile assay (based on the method of AUERBACH and HOWARD-FLANDERS 1981) in which a λ plaque turns red when phage lysogenize and thereby complement mutant bacteria. Initially, this assay is used to isolate, from a genomic library, phage capable of complementing known bacterial mutants. Later, phage derivatives are isolated that form uncolored plaques because the cloned replication gene is mutant. The ability or inability to form a red plaque is a joint property of the phage and the bacterial strain used. Therefore, different bacterial mutants, be they *E. coli* or Salmonella, can be shown to harbor functionally similar mutations by their similar response to a test panel of wild-type and mutant phage clones in the red plaque assay.

By this approach we have isolated and characterized a total of 24 Salmonella DNA replication genes. Of these genes, 17 are functionally interchangeable with previously described replication genes of *E. coli*. Our results imply that the biochemical pathway of DNA replication must be virtually identical in the two species. The seven remaining Salmonella genes have no identified equivalents in *E. coli*. In view of the close similarity of the replication functions, these seven genes may represent replication functions that are present in *E. coli* but which have been missed previously despite exhaustive mutant hunts (most recently, see SEVASTOPOULOS, WEHR and GLASER 1977).

These studies as well as those in the following paper (MAURER, OSMOND and BOTSTEIN 1984) illustrate powerful ways in which molecular clones can be used to analyze gene function. Indeed, in many cases the genes have been studied only in the form of molecular clones. Ultimately, however, a full description of any of the genes studied here requires the isolation of bacterial mutants affected in the gene. To this end we also present a generally applicable, directed method for obtaining such mutants in Salmonella, based on prior molecular cloning of the gene and the principle of local mutagenesis (HONG and AMES 1971). To obtain a suitable linked genetic marker for the mutagenesis procedure, a transposon is placed in the chromosome in a position determined by an intermediate that is the corresponding molecular clone carrying the transposon. This procedure was applied to obtain the first Salmonella mutations affecting a gene that was cloned by complementation of an *E. coli* mutant.

MATERIALS AND METHODS

Bacterial and phage strains: Principal bacterial and phage strains are listed in Table 1. *Dna-ts* mutations were introduced into various Salmonella strains by cotransduction with Tn*10*, selecting tetracycline resistance.

Complementation studies using phage λ in Salmonella hosts were of necessity carried out using derivatives of a unique, λ-sensitive strain, DB4673. Several uncertainties about the genotype of this strain and its derivatives have yet to be resolved. According to PALVA, LILJESTROM and HARAYAMA (1981), strain TS736 was prepared by introducing the *E. coli* episome F′112 (*malB*$^+$)

TABLE 1

Bacterial and phage strains used

Strain (synonym)[a]	Genotype or description	Origin
S. typhimurium		
DB1098	DB4673 *zxx1251*::Tn*10Δ16Δ17 dnaE305*	This work, made by the method of Figure 2
DB4641 (TB37)	F⁻ *"dnaA1" his-3594 thy-1152*	BAGDASARIAN *et al.* (1975)
DB4647 (11G)	F⁻ *dnaC1 met trp thy rpsL*	SPRATT and ROWBURY (1970)
DB4673 (TS736)	?F ?mal *trpB2 ilv-452 metA22 metE551 rpsL120 flaA66 xyl-404 galE496 hsdL6* (r^-m^+) *hsdSA29* (r^-m^+); cold-sensitive growth	PALVA, LILJESTROM and HARAYAMA (1981). See text.
DB4707	DB4673(λ112)	This laboratory
DB4817	DB4707 cold resistant (17°)	Spontaneous
DB4827	DB4817 *hisD9426*::Tn*10*	P22(DB7126)[b]
DB4835	DB4827 *hisD*⁺ *hisC527*(Am)	P22(DB7155)[b]
DB4839	DB4835 *supE20*	P22(DB7155)[b]
DB7126	F⁻*leuA414*(Am) *hisD9426*::Tn*10* (fels free)	WINSTON, BOTSTEIN and MILLER (1979)
DB7155	F⁻*leuA414*(Am) *hisC527*(Am) *supE20* (fels free)	WINSTON, BOTSTEIN and MILLER (1979)
DB9005	F⁻*thyA deo* (fels free, plasmid free)	This laboratory[c]
DB9185	DB9005 *hisC527*(Am) *supE20 dnaE11*(Am) *zxx1251*::Tn*10Δ16Δ17*	MAURER, OSMOND and BOTSTEIN (1984)
NK337	F⁻*hisC527*(Am) *leuA414*(Am) *supE20* (P22 *c2ts29 12amN11 13amH101 int3* Tn*10*)	KLECKNER *et al.* (1975)
E. coli		
DB4548 (BNN45)	F⁻ *hsdR supE44 supF thi met lacY*	R. DAVIS
DB4740 (Q359)	F⁻ *hsdR supE* $\phi 80^R$ (P2)	KARN *et al.* (1980)
DB4755 (E101)	F⁻ *nrdA101 thr-1 leuB6 thi-1 lacY1 tonA21 thyA6 deoC1 rpsL67 supE44* (λ112)	WECHSLER and GROSS (1971)
DB4756 (E107)	F⁻ *dnaB107 thr-1 leuB6 thi-1 lacY1 tonA21 thyA6 deoC1 rpsL67 supE44* (λ112)	WECHSLER and GROSS (1971)
DB4757 (E486)	F⁻ *dnaE486 thr-1 leuB6 thi-1 lacY1 tonA21 thyA6 deoCl rpsL67 supE44* (λ112)	WECHSLER and GROSS (1971)
DB4758 (PC2)	F⁻ *dnaC2 leuB6 thyA47 deoC3 rpsL153* (λ112)	CARL (1970)
DB4760 (N2668)	F⁻ *lig-7 rpsL* (λ112)	GOTTESMAN, HICKS and GELLERT (1973)
DB4671 (AX727)	F⁻ *dnaZ2016 thi lac rpsL* (λ112)	FILIP *et al.* (1974)
DB4883 (E177)	F⁻ *dnaA177 thr-1 leuB6 thi-1 thyA6 deoC1 lacY1 rpsL67 tonA21 supE44* (λ112)	WECHSLER and GROSS (1971)
DB4884	F⁻ *dnaG399 thr-1 leuB6 thi-1 thyA6 deoC1 lacY1 rpsL67 tonA21 supE44* (λ112)	MARINUS and ADELBERG (1970)
DB4894 (HC194)	HfrC *dnaN59 metB thy* (λ⁺)	SAKAKIBARA and MIZUKAMI (1980)

TABLE 1—*Continued*

Strain (synonym)[a]	Genotype or description	Origin
DB4900 (JGC155)	F⁻ *ssb-1 gal rpsL* (λ⁺)	GLASSBERG, MEYER and KORNBERG (1979)
DB4903	F⁺ *dnaL708 thi leu his arg met thyA deo lac mal xyl mtl rpsL dnaL708* (λ112)	SEVASTOPOULOS, WEHR and GLASER (1977)
DB4913 (UH52)	F⁻ *dnaX thi lac xyl mtl gal rpsL* (λ⁺)	HUBSCHER and KORNBERG (1979)
DB4915 (KY2750)	F⁻ *dnaP18 thr-1 leuB6 trp-67 his-100 met-99 thi-1 ara-13 lacY1 gal-6 xyl-7 mtl-12 azi-8 rpsL135 tonA2 supE44* (λ112)	WADA and YURA (1974)
DB4918	F⁻ *gyrA43 zei*::Tn*10 argE*(Am) *rif malF*::Tn5 (λ112)	M. SYVANEN; see KREUZER *et al.* (1978)
DB4919 (UH139)	F⁻ *dnaY thi lac xyl mtl gal rpsL* (λ⁺)	HUBSCHER and KORNBERG (1979)
DB4931 (KH1116)	HfrC *dnaQ49 metB1 lac-3 tsx-76 relA1* (λ112)	HORIUCHI, MAKI and SEKIGUCHI (1978)
DB4932	F⁻ Δ*lacX74 rpsL thi tna*::Tn*10*	A. WRIGHT
DB4943	F⁻ *hsdR hsdM* (λAam32)	This laboratory
DB5564	F⁻ *thr leu lacY T1^{R}T5^{R}ϕ80^R supE*	This laboratory
RB132	F⁻ Δ*lac rpsL gal zxx*::Tn*10*Δ4HH104/ pNK217	This laboratory; see FOSTER *et al.* (1981)
RM83 (FA22)	F⁻ *dnaB22 sup* (λ112)	FANGMAN and NOVICK (1968)
RM203 (MF634)	F⁻ *dnaJ259 thr-1 leuB6 thi-1 lacY1 tonA21 supE44* (λ112)	YOCHEM *et al.* (1978)
RM204 (MF746)	F⁻ *dnaK756 thr-1 thi-1 lacY1 tonA21 supE44* (λ112)	YOCHEM *et al.* (1978)
Coliphage λ		
λ112	*imm21 ptrp-lac* W205 carrying cloned *imm*λ *cI* gene; lysogens are immune to 21 and λ	MAURER, MEYER and PTASHNE (1980)
λgt7	*cI⁻ nin5 b522 ara6*; *Eco*RI substitution vector	DAVIS, BOTSTEIN and ROTH (1980)
λ1059	sBam1° *b*189 (*int29, ninL44, cI857, pac129*) Δ(*int-cIII*) KH54 sR14° *nin5 chi3*; Bam and *Sau*3A substitution vector	KARN *et al.* (1980)

[a] For strains that are lysogenic for λ112 (MAURER, MEYER and PTASHNE 1980), the synonyms refer to the nonlysogenic parent.

[b] These strains were constructed by P22 transduction using phage grown on the strain indicated in parentheses.

[c] Salmonella was cured of the LT2 "cryptic" plasmid by a procedure devised by H. WHITFIELD involving forced mating with an F-cryptic cointegrate plasmid that is incompatible with the natural plasmid, followed by spontaneous segregation of the cointegrate plasmid. Details available on request from R. MAURER.

into a *malB*⁻ Salmonella. Strain DB4673, which is an isolate of TS736 obtained through S. KUSTU, exhibits a Mal phenotype that is not characteristic of either *E. coli* or Salmonella wild-type Mal⁺ cells. Specifically, it grows on appropriately supplemented minimal medium with maltose as carbon source but does not convert MacConkey-maltose indicator plates. Moreover, the strain is F⁻ by a variety of criteria, including sensitivity to female-specific phage, inability to donate F′*mal* in a

cross, inability to give rise to Mal$^-$ segregants under the influence of acridine orange and ability to receive another F′ factor (G. SMITH, personal communication). Nonetheless, strain DB4673 exhibits the property of λ sensitivity conferred by the *E. coli malB* region. Possibly, a fragment of *E. coli* DNA including all or part of *malB* has integrated into the Salmonella chromosome in this strain. We also noted that strain DB4673 does not require histidine, even though TS736 is reported to be a histidine auxotroph. Finally, we observed that DB4673 is cold sensitive for growth at 17°. A spontaneous cold-resistant revertant was isolated and used to construct strains DB4835 andDB4839, which have become our standard parental stocks for the construction of Salmonella complementation tester strains.

Genomic libraries: Two λ-*Salmonella* genomic libraries were used. An *Eco*RI complete-digest library, carried in λgt7, was obtained from R. DAVIS (ROWEN, KOBORI and SCHERER 1982). A *Sau*3A partial-digest library was constructed in λ1059 (KARN *et al.* 1980) following the procedure of these authors. Bacterial DNA for this library was isolated from strain DB9005 as described, except that proteinase K was substituted for pronase (EBEL-TSIPIS, BOTSTEIN and FOX 1972). Digested DNA was fractionated on a 0.4% agarose gel in Tris-acetate buffer (DAVIS, BOTSTEIN and ROTH 1980). DNA fragments approximately 15 kb in length were collected by electrophoresis into hydroxyapatite (TABAK and FLAVELL 1978). The DNA was ligated with *Bam*HI-digested λ1059 DNA and packaged *in vitro* (HOHN 1979). Five portions of the packaged mixture, each containing about 5000 recombinants, were amplified separately on strain DB4740. This procedure ensured that overlapping clones isolated from the different amplified portions were of independent origin.

General bacteriological procedures: Bacteriological media and procedures were as described (DAVIS, BOTSTEIN and ROTH 1980), with one modification. Generally, *E. coli* strains were grown inLB + 0.2% (w/v) maltose + 1 mM $MgSO_4$ in preparation for λ infection. Salmonella strains were grown in LB, supplemented with thymine (10 μg/ml) for derivatives of strain DB9005, in preparation for P22 infection. However, strain DB4673 and its derivatives adsorbed λ only if grown in the absence of galactose, including galactose present in yeast extract (a component of LB). Therefore, for λ complementation tests such cells were grown in λ broth + 0.2% (w/v) maltose + 1 mM $MgSO_4$. On the other hand, for use in P22 transductions, either as donor or recipient, such cells were grown in LB + 0.05% (w/v) galactose.

Isolation of Salmonella dna *mutants*: Cultures of strain DB9005 were treated with 0.15% ethyl methanesulfonate for 105 min at 30° (MILLER 1972). Mutagenized bacteria were plated at 30° at a density of 300 colonies per plate. Colonies were replica plated to 42° to detect temperature-sensitive mutants. Temperature-sensitive candidates were inoculated in LB broth in duplicate wells of a microtiter dish and allowed to grow to saturation at 30°. DNA synthesis in these isolates was determined by an autoradiographic procedure (WECHSLER *et al.* 1973). First, with a multiprong inoculator, samples from the microtiter dish were printed onto nitrocellulose paper, which in turn was resting on Whatman 3MM paper saturated with M9 glucose medium containing 3 μg/ml of thymine, 5 mg/ml of casamino acids and 100 μg/ml of tryptophan. This was incubated at 30° until bacterial growth was visible at the sites of inoculation (3–4 hr). The entire arrangement was then transferred to 42° for 1 hr to allow replication mutants to complete any residual DNA synthesis. Then the nitrocellulose was transferred to new Whatman paper saturated with the same medium containing 3 μg/ml of cold thymine and 1 μCi/ml of ^{3}H-thymine. Incubation was continued at 42° for 1.5 hr. The nitrocellulose was then transferred sequentially to Whatman paper saturated with 0.1 N NaOH (1 min), two changes of 100 mM Tris, pH 7, containing 100 μg/ml of thymine and 10 mM sodium pyrophosphate (5 min each), then 1 M sodium salicylate (30 min; CHAMBERLAIN 1979). The filter was then dried and exposed to radiographic film (Kodak XR5) for 1–5 days. Isolates in which the duplicate inoculations gave diminished labeling were retained as putative replication mutants.

Some mutants were isolated during trials with variations on this protocol. Variations included use of agar supports instead of Whatman paper for the various incubations, initial transfer of colonies from agar to nitrocellulose instead of printing from microtiter dish cultures, omission of thymine from the first incubation at 42°, variation of the length of the first and/or second incubation at 42° and variation of the label specific activity. None of these variations were judged significant improvements. One useful variation was counterstaining of the nitrocellulose. Prior to treatment with sodium salicylate the filter was floated and then gently submerged in 95% ethanol containing 62 μg/ml of Coomassie blue. After 15 min, the filter was transferred to fresh 95%

ethanol, which was gradually replaced with water over a period of 1 hr. Finally, the filter was treated with salicylate as before. In this procedure the stain was taken up preferentially by the nitrocellulose, so that the colonies appeared white against a blue background. By comparing the stained filter to the autoradiograph we were able to eliminate occasional false negatives resulting from a diminished amount (or loss during processing) of labeled material.

Identification of transposon insertions linked to dna *mutations*: A pool of derivatives of strain DB9005 carrying independent insertions of Tn*10* was generated with strain NK337 as described (KLECKNER *et al.* 1975). Particular insertions of interest were identified within this pool by the property of linkage to a locus conferring temperature-sensitive growth on a mutant. Specifically, P22 grown on the pooled bacteria was used to transduce a *ts* mutant to tetracycline resistance at 30°; the transductants were replica plated to 42° to identify temperature-resistant transductants. Such transductants were used to donate tetracycline resistance to the original mutant strain. A *ts* tetracycline-resistant transductant isolated from this latter cross was subsequently used as a donor with which to introduce the *ts* mutation into other strains. In addition, each Tn*10* was backcrossed into strain DB9005 to form a temperature-resistant reference strain used in the mapping studies described next.

Transductional mapping: A panel of Tn*10*-bearing reference strains was assembled, comprising 12 strains obtained as previously described and 56 additional strains contributed by several investigators. Many of the latter strains are described in Tables 3 and 4 of SANDERSON and ROTH (1983). P22 transducing lysates were prepared separately on each reference strain and adjusted to a concentration of 2×10^9 plaque-forming units/ml. With an inoculating device a drop of each lysate (up to 32 lysates per plate) was delivered onto an LB + tetracycline (20 μg/ml) agar plate seeded with 5×10^8 tetracycline-sensitive *dna* cells (*i.e.*, the original mutant isolates). After incubation at the nonpermissive temperature the plates were scored for growth of cells in a patch, indicating cotransduction of dna^+ and Tn*10* mediated by a particular lysate. Cotransduction was confirmed by repeating the transduction at low multiplicity, selecting tetracycline resistance and scoring *dna* as an unselected marker. A similar method was independently devised by ROSENFELD and BRENCHLEY (1979).

Complementation tests: Complementation was assessed in partial diploids formed by lysogenizing mutant bacteria with λ clones carrying wild-type or mutant DNA replication genes. In these tests, phage were first allowed to form plaques on an ordinary indicator strain, and then temperature-sensitive tester bacteria were added for lysogenization *in situ* (see AUERBACH and HOWARD-FLANDERS 1981). Complementation was observed as the growth of tester bacteria, at the nonpermissive temperature, in the vicinity of a plaque. Detection of this response was aided by the inclusion of a sugar (maltose) and a tetrazolium dye which is reduced to an insoluble red formazan by growing cells.

The detailed protocol was as follows: phage to be tested were allowed to form plaques on a lawn of strain DB5564, using LB agar plates formed in glass Petri dishes. The next day, the plates were sterilized by inversion over chloroform for 30 min. Residual chloroform in the agar was allowed to dissipate by airing plates for at least 10 min. The plates were then irradiated with UV to a dose of 340 ergs/mm^2. Finally, the plates were overlaid with a mixture of 2 ml of soft agar, 0.3 ml of a 20% (w/v) maltose solution, 0.1 ml of a 0.4% (w/v) 2,3,5-triphenyl tetrazolium HCl solution (sterilized by filtration) and 0.3 ml of logarithmic phase tester cells. After 1–2 days of incubation at the nonpermissive temperature, generally 42°, the plates were scored. Either free phage or complemented bacteria, as required, were recovered from red plaques for further analysis.

In various experiments either *E. coli* or Salmonella derivatives were used as tester cells. Salmonella testers were derivatives of a specially constructed strain, DB4673, that is able to adsorb λ phage (PALVA, LILJESTROM and HARAYAMA 1981). For complementation tests, these cells were lysogenized with λ112 (MAURER, MEYER and PTASHNE 1980) and made *dna-ts* by cotransduction with an appropriate Tn*10*. *E. coli dna-ts* mutants were in some cases already lysogenic for λ^+; if not, they were lysogenized with λ112 (see Table 1). The appearance of red plaques was the same whether *E. coli* or Salmonella tester strains were used, even though the Salmonella strains express a restriction system (SB; COULSON and VAN PEL 1974) that restricts *E. coli*-grown λ by a factor of about 100.

The use of immune lysogens as tester cells has several implications which all relate to the fact that phage promoters are turned off by the prophage repressor and hence no phage genes are expressed. These implications will be discussed in general terms, followed by a quantitative assessment of some of the issues raised.

The first point is that integration of phage DNA depends on host recombination functions and the presence of homologous DNA in the tester cell chromosome. There is both λ homology provided by the tester cell prophage, and in Salmonella tester cells, homology with the cloned fragment. For the *Sau*3A clones, the length of λ homology is about twice that of the Salmonella homology; hence, most integrations probably occur within the prophage.

The red plaque assay depends on the ability of UV irradiation to stimulate homologous recombination in *E. coli* (JACOB and WOLLMAN 1955). This effect has been demonstrated for phage-prophage crosses carried out using irradiated λ phage in unirradiated homoimmune lysogens (HOWARD-FLANDERS and LIN 1973). Similarly, in the red plaque assay the phage, but not the tester cells, are irradiated. This treatment strengthens the response and enhances the reproducibility of the assay. Quantitative analysis (see following data) indicates that the important effect of UV irradiation is to enhance integration. The dose of UV employed is not significantly mutagenic for λ because the tester cells are not irradiated (DEFAIS *et al.* 1971).

The presence of active homologous recombination functions under the conditions of the red plaque assay raises the possibility that some part of the red plaque response might result from marker rescue rather than complementation. Quantitative analysis of a case in which a wild-type clone is assayed with temperature-sensitive bacteria indicates that most "complemented" cells are true partial diploids, that is, the presence of the temperature-sensitive allele can be demonstrated in these cells. The situation may be somewhat different when a clone is used bearing a mutation in the gene of interest; this is considered further in DISCUSSION.

The second point is that expression of the cloned gene depends on bacterial promoters because phage promoters are turned off. In a typical case, the normal promoter for the gene of interest is present in the λ clone. In such a case complementation can begin as soon as the phage DNA enters the cell. This might be considered transient complementation since formation of a stable diploid requires integration of the phage DNA. Unintegrated phage DNA cannot be replicated in these immune cells and is lost from one daughter at cell division. Transient complementation may play an important part in the red plaque assay, particularly if the relevant gene product is synthesized in excess of the minimum required level. Another pertinent consideration is the effective multiplicity of infection obtained during lysogenization *in situ*, since high multiplicity would lead to longer persistence of unreplicated phage DNA in complemented cells and their descendants. The true multiplicity is difficult to measure, but an approximate calculation indicates there are about 100 phage for every tester cell in the vicinity of a plaque.

The contributions of complementation and recombination to the red plaque assay, as well as the effect of UV irradiation, were measured in an experiment in which temperature-resistant cells isolated from red plaques were tested for the presence of the original temperature-sensitive mutation. This experiment utilized a Salmonella tester strain, DB4711, which carries a tetracycline resistance marker, Tn*10*, 50% linked to the temperature-sensitive mutation, *ts288*. Phage λRM441, which carries a fragment of wild-type Salmonella DNA and which forms red plaques on this tester, was allowed to do so, using the standard (+UV) protocol. Cells from 20 different red plaques were purified by single-colony isolation at a permissive temperature (30°), and then five to ten isolated colonies derived from each plaque were tested for temperature sensitivity. Forty-six of 170 colonies were temperature sensitive and are presumed to be segregants derived from transiently complemented cells. Thirty-two of the remaining, temperature-resistant cells were used as donors in a P22-mediated transduction of wild-type cells to tetracycline resistance, with transductants selected at 30°. In 28 of the 32 cases, including at least one representative from each of the original 20 plaques, approximately equal numbers of temperature-sensitive and temperature-resistant colonies were found among the transductants. This result, together with the frequent segregation of temperature-sensitive daughters from temperature-resistant ancestors, shows that the majority of temperature-resistant cells retained their original temperature-sensitive mutation. Therefore, the red plaque response was attributable to complementation in this case. The structure of the four exceptional donors, which produced exclusively temperature-resistant transductants,

was not investigated further. Their existence is not necessarily indicative of marker rescue, because integration of the λ clone genome within the *ts*-Tn*10* interval could have produced a *ts*/*ts*$^+$ partial diploid in which Tn*10* and *ts* were no longer linked.

When the same experiment was conducted omitting the UV irradiation, 95% of the single colonies obtained after one round of purification at 30° were temperature sensitive. The eight remaining temperature-resistant isolates served as donors of temperature sensitivity in the transductional test. Thus, UV treatment of the phage increased the proportion of stable temperature-resistant transductants among cells recovered from complementation plaques. Since formation of a red plaque by λRM441 in the absence of UV apparently resulted mainly from the growth of unstable transductants, we inferred that the failure of most clones to form a red plaque without UV reflects a requirement for stable transduction (*i.e.*, integration) in the general case.

Isolation of mutations in λ clones: λ clones were subjected to both chemical and transposon mutagenesis. Chemical mutagenesis involved treatment of phage lysates with 0.4 M hydroxylamine for 24 hr (MILLER 1972). There was no convenient plaque morphology marker with which to monitor the extent of mutagenesis, but survival, measured as plaque-forming units, was typically 0.1–1%. Mutagenized lysates were screened for mutants that make uncolored plaques, using the red plaque assay with an appropriate tester strain.

Transposon mutagenesis involved a 2.95-kb element derived from Tn*10* (Tn*10*Δ*16*Δ*17* or more simply Tn*10*Δ; FOSTER *et al.* 1981). This deleted Tn*10* transposes only when provided with transposition functions by another Tn*10* in the same cell. Mutagenesis was by lytic passage of phage in strain RB132. This strain contains multiple copies of Tn*10*Δ carried on a plasmid and a source of "high hopper" transposition functions provided by a chromosomal element. Specifically, 10^8 phage were incubated for 10 min at 37° with 0.1 ml of fresh bacterial culture of strain RB132. The mixture was diluted with 5 ml of LB and aerated for 2 hr at 37°, and then growth was terminated by treatment with chloroform. Under these conditions, λ multiplied about 50-fold. Much less than 1% of the total lysate was screened for λ::Tn*10*Δ derivatives, a precaution that virtually ensured independence of the phage isolates. Lysates of λ1059 clones passaged in strain RB132 contained λ::Tn*10*Δ phage at a frequency of $1–5 \times 10^{-5}$ assayed as will be described.

Two alternative plaque assays were used to detect λ::Tn*10*Δ. These assays are equivalent in that the same phage are detected, but the selective plaque assay is now the method of choice. In the MacConkey assay, phage were adsorbed to strain DB4932, mixed with strain DB4943 and plated in half-strength MacConkey lactose agar (without drug) on MacConkey lactose plates containing 8 μg/ml of tetracycline HCl. In this assay, λ1059 clones make clear, colorless plaques, whereas derivatives carrying Tn*10*Δ make plaques with turbid, red centers. Reconstruction experiments show that λ::Tn*10*Δ is detected with an efficiency of 0.5 or more in the presence of 10^6 λ phage on the same plate.

In the selective plaque assay, phage were adsorbed to 0.1 ml of a fresh overnight culture of strain DB5564, and then plated in 3 ml of soft agar (without drug) on LB plates containing 8 μg/ml of tetracycline HCl. Under these conditions the bacteria make a faint lawn in which λ::Tn*10*Δ makes plaques but λ does not. This assay is at least as efficient as the MacConkey assay and has the advantage that the selected plaques are nearly pure.

Either of the assays for λ::Tn*10*Δ requires the phage to grow lytically. In the case of λ1059 clones, virtually all nonessential phage DNA has been deleted, so most insertion phages that are detected would be expected to carry their insertion in the cloned Salmonella fragment. This was in fact observed, as illustrated for derivatives of λRM354 (*dnaQ*$^+$) in Figure 1. Rarely, a viable insertion phage was detected with a site of insertion in λ DNA (between gene *R* and *cos*; data not shown).

*Transfer of Tn*10Δ *between phage and bacteria*: A fortuitous feature of λ growth in Salmonella permits the detection of recombination (resulting in the transfer of Tn*10*Δ) in both directions between λ1059 clones and Salmonella. Normally, propagation of λ depends on transcriptional antitermination mediated by the phage-encoded *N* protein in cooperation with several host proteins including the *nusA* product (see HERSKOWITZ and HAGEN 1980). Salmonella *nusA* protein does not support antitermination by λ *N* protein (FRIEDMAN and BARON 1974), resulting in abortion of the λ growth cycle at an early step in Salmonella. However, λ1059 contains a mutation, *nin5*, that allows slight growth of λ in the absence of *N* function (COURT and SATO 1969). In Salmonella,

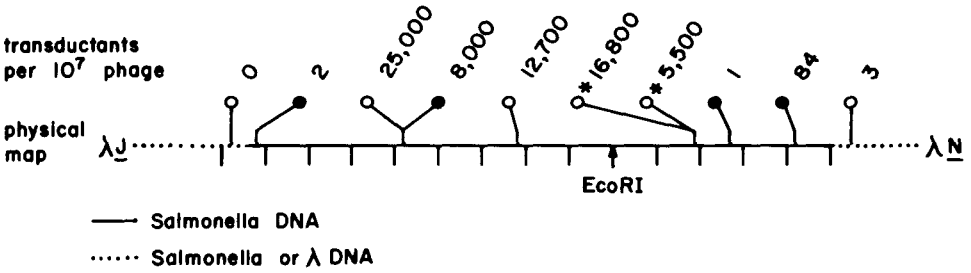

FIGURE 1.—Map position and transduction of Tn*10*Δ insertions in λRM354. The line illustrates the physical map of the cloned DNA present in λRM354 (*dnaQ*$^+$) and flanking λ DNA. Hatch marks indicate intervals of one kilobase pair starting from the unique *Eco*RI cleavage site in this cloned DNA. Positions of Tn*10*Δ insertions deduced from restriction enzyme analysis of phage DNA are shown (±200 base pairs). The positions of the λ-Salmonella joints (*Bam*HI-*Sau*3A fusions) are not known with precision, and it is possible, therefore, that the leftmost and/or rightmost insertions shown are actually in adjacent λ DNA. Filled or open circles indicate the orientation of the insertion relative to λ sequences: the orientation of insertions indicated by filled circles is such that IS10-Left sequences (FOSTER *et al.* 1981) are at the left end of the insertions (λ in the conventional orientation, as shown). The numbers above the insertions indicate the number of tetracycline-resistant transductants obtained per 10^7 plaque-forming units when each phage was used to infect strain DB4673 at low multiplicity (0.1 or less). By red plaque complementation assays, all of the insertions except the two indicated by an asterisk are *dnaQ*$^+$. The transductants formed by infection with the *dnaQ*$^-$ phage have a marked mutant phenotype that will be described in a future publication.

the factors affecting growth of λ1059 clones are balanced so that both surviving cells and phage are produced from populations of infected cells. Surviving cells are invariably phage-free segregants rather than lysogens, since these phage have no repressor gene and cannot form viable lysogens.

To transfer Tn*10*Δ from phage to bacteria, strain DB4673 was infected with λ::Tn*10*Δ, incubated at 30° or 37° for 30 min, and then plated for tetracycline-resistant transductants.

To transfer Tn*10*Δ from bacteria to phage, a tetracycline-resistant derivative of strain DB4673 was infected with the appropriate λ clone at a multiplicity of ten and incubated for 10 min at 30°; then, 2×10^7 infected cells were diluted into 5 ml of LB broth, and phage growth was allowed to continue at 30° for an additional 90 min. The resulting phage lysate was introduced at low multiplicity into the nonrestricting host strain, DB4548, and infectious centers were assayed for production of λ::Tn*10*Δ using the MacConkey assay.

In these Tn*10*Δ transfer experiments, the DNA segment bearing Tn*10*Δ replaces the corresponding DNA in the recipient molecule. A genetic experiment that strongly supports this conclusion involved cotransduction of another mutation (Figure 2). In this experiment, an insertion derived in λ*dnaE* (λRM303) was sequentially introduced into Salmonella, rescued back into λ*dnaE* together with a *dnaE*(Ts) mutation, *dnaE305* (Figure 2A), and then reintroduced into *dnaE*$^+$ Salmonella to produce a strain that was tetracycline resistant *and* temperature sensitive (Figure 2B). Moreover, the original *dnaE305* mutant and the ultimate temperature-sensitive transductants exhibited the identical extreme sensitivity to temperatures as low as 35°. This observation indicated that the transductants carried *dnaE305*, not some other mutation. Because *dnaE305* is recessive to *dnaE*$^+$ (Table 2), the formation of a *dna*$^-$ λ phage in Figure 2A and the acquisition of temperature sensitivity by the ultimate transductants in Figure 2B required that the preexisting normal segment in the recipient molecule be replaced by the corresponding Tn*10*Δ-bearing segment.

RESULTS

Salmonella DNA replication mutants: Most mutants described here were obtained by screening among temperature-sensitive mutants for those that exhib-

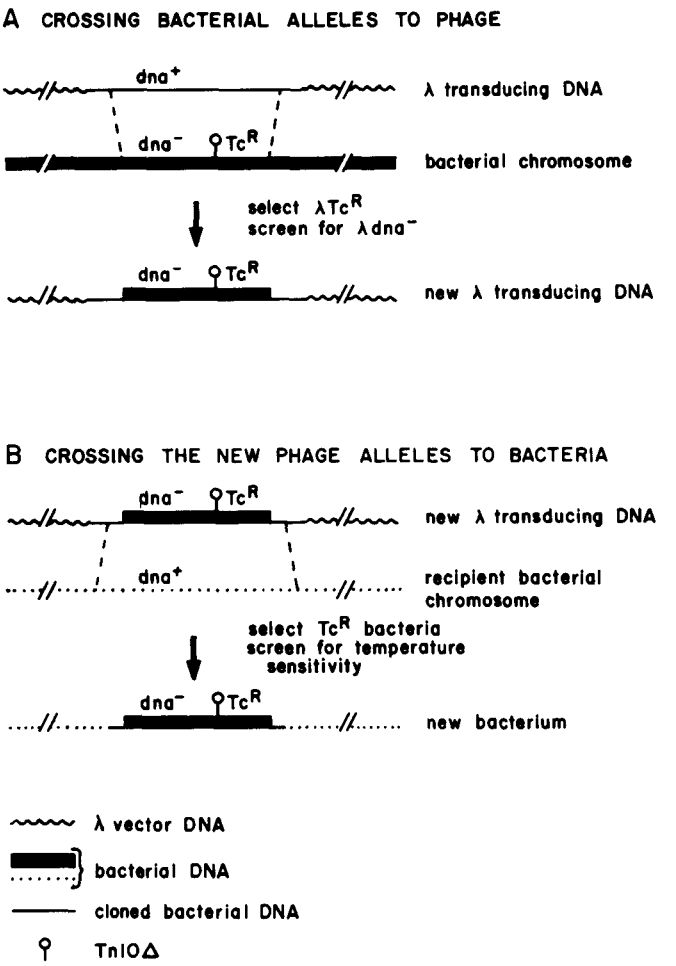

FIGURE 2.—Cotransduction of two mutations between bacterial and bacteriophage chromosomes. Shown are the recombination events implicated in the exchange of genetic markers between λ phage and Salmonella. The positions shown for *dnaE* and Tn*10*Δ (Tc^R) within the cloned DNA segment are arbitrary. A, λRM303 (*dnaE*$^+$) was passaged lytically in strain DB1098 (*dnaE305*, Tc^R), and a Tc^R, *dnaE*$^-$ progeny phage was produced. B, Salmonella strain DB4673 (*dnaE*$^+$) was infected with the new phage and a Tc^R, *dnaE*(Ts) bacterium indistinguishable from strain DB1098 was produced. In both A and B, genetic evidence clearly indicated that the *dnaE*$^+$ gene of the recipient DNA molecule was lost, implicating homologous recombination as illustrated.

ited a defect in DNA replication. The primary criterion for a replication defect was diminished ^{3}H-thymine incorporation in colony autoradiography. After treatment of strain DB9005 with ethyl methanesulfonate, approximately 1% of the surviving cells were temperature sensitive for growth. Of these, 5–10% exhibited a defect in ^{3}H-thymine incorporation; 77 such strains were retained for further study. The independence of most of these mutants was inferred from their derivation from different independently mutagenized cultures or from genetic linkage studies.

Genetic mapping: A genetic test was used to distinguish mutations associated

with distinct loci. Each mutation was examined for transductional linkage to a series of diagnostic Tn*10* (tetracycline resistance) insertions scattered around the chromosome. Two mutations were considered to affect different genes if they were not both linked in common to any Tn*10*. As little as 5% cotransduction, corresponding to about 0.5 min on the Salmonella genetic map, would have been detected; thus, each diagnostic Tn*10* defined a region of chromosome about 1 min in length. The analysis has defined 11 distinct loci at which temperature-sensitive, replication-defective mutations were found. The mapping procedures simultaneously identified a selectable marker (Tn*10*) appropriate for manipulation of each locus for further strain construction or local mutagenesis.

Many of the temperature-sensitive mutations were unlinked to any of the Tn*10* insertions. This result reflects the distribution of the available Tn*10* insertions, which, together, cover about 40% of the Salmonella genetic map. Consequently, complementation analysis, which required a different strain background, was restricted to 16 mutations for which a linked Tn*10* was identified.

Cloning of Salmonella replication genes by complementation: Phage λ *in vitro* recombinants carrying wild-type Salmonella replication genes were isolated from genomic libraries on the basis of lysogenic complementation of bacterial replication mutants. An amplified complementation signal was obtained by first allowing the phage to form plaques on a lawn of ordinary *E. coli* and then adding the tester bacteria for lysogenization *in situ*: the red plaque assay. The plaques of interest were marked by adjacent growth of the bacterial tester strain and the ensuing production of reduced (red) tetrazolium. *E. coli* mutants in most known replication loci as well as the 16 Salmonella mutants described earlier were screened in this way. Clones that were obtained were reexamined with all of the *E. coli* and Salmonella tester strains to determine the full spectrum of activity of each clone. The results of these tests are presented in Table 2.

For every *E. coli* replication mutant in our possession, except *dnaP18*, we found clones from the Salmonella genomic library that complement the mutation. In addition, all 16 Salmonella mutants could be complemented. Some λ clones complement mutations in two different *E. coli* genes. In every such instance the genes are sufficiently linked in *E. coli* that we could reasonably expect their Salmonella homologues to be present on a single 15- to 20-kb piece of DNA (*dnaA-dnaN*, *dnaJ-dnaK*, *dnaX-dnaZ*, *gyrA-nrdA*; SAKO and SAKAKIBARA 1980; SAITO and UCHIDA 1978; HENSON *et al.* 1979; FUCHS and KARLSTROM 1976). At this gross level, then, the organization of replication genes in Salmonella and *E. coli* is the same.

Allelism tests and gene identification: As shown in Table 2, certain λ clones complement multiple mutants, sometimes including both Salmonella and *E. coli* derivatives. In these instances it is not clear how many different gene loci are involved. The plaque complementation test can be used to answer this question, that is, to determine whether different chromosomal mutations are allelic (or functionally homologous, in the case of intergeneric comparisons). The

TABLE 2

Cloning of Salmonella replication genes

Representative clone	Strains complemented (alleles)	
	Salmonella	*E. coli*
λRM180 (15)	RM28 (*ts727*)[a] RM29 (*ts728*)[a] RM30 (*ts729*)[a]	DB4883 (*dnaA177*) DB4894 (*dnaN59*)
λRM113 (3)		DB4756 (*dnaB107*) RM83 (*dnaB22*)
λRM287 (5)[b]	DB4712 (*ts141*) DB4715 (*ts1*)[c] DB4714 (*ts602*) DB4725 (*ts601*)	DB4758 (*dnaC2*)
λRM303 (10)	DB4713 (*ts305*) DB4722 (*ts229*) RM165 (*ts693*)[a] RM166 (*ts698*)[a]	DB4757 (*dnaE486*)
λRM328 (2)		DB4884 (*dnaG399*)
λRM335 (1) λRM333 (8)		RM203 (*dnaJ259*) RM203 (*dnaJ259*) RM204 (*dnaK756*)
λRM345 (6)		DB4903 (*dnaL708*)
λRM354 (4)		DB4931 (*dnaQ49*)
λRM417 (8)	DB4719 (*ts660*)	DB4913 (*dnaX*) DB4761 (*dnaZ2016*)
λRM408 (8)		DB4919 (*dnaY*)
λRM116 (1)		DB4760 (*lig-7*)
λRM419 (4)		DB4900 (*ssb-1*)
λRM431 (5) λRM439 (5)	DB4708 (*ts98*)	DB4918 (*gyrA43*) DB4918 (*gyrA43*) DB4755 (*nrdA101*)
λRM441 (7)[d]	DB4710 (*ts262*) DB4711 (*ts288*)	
λRM451 (4)	DB4716 (*ts653*) DB4724 (*ts611*)	

TABLE 2—*Continued*

Representative clone	Strains complemented (alleles)	
	Salmonella	*E. coli*
λRM454 (2)	DB4717 (*ts603*)	
λRM456 (2)	DB4718 (*ts645*)	
λRM458 (4)	DB4720 (*ts662*)	
λRM462 (1)	DB4721 (*ts663*)	
λRM463 (15)	DB4723 (*ts598*)	

The numbers in parentheses following the representative phage designations indicate the number of isolates that exhibited the complementation behavior shown. Phage connected by a brace had partially overlapping complementation properties. Unless indicated otherwise, phage isolates were from the *Sau*3A partial-digest libraries. Salmonella strains were tetracycline-resistant, temperature-sensitive derivatives of DB4707, DB4835 or DB4839.

[a] These strains were derived by local mutagenesis (see Tables 4 and 5).

[b] All five isolates were from the λgt7 library, and each phage DNA contained a single *Eco*RI fragment of identical size. Thus, at most, two phage types (alternative orientation of the insert) were represented.

[c] Mutant allele of SPRATT and ROWBURY (1970).

[d] λRM441 was from the λgt7 library. The other six isolates were from the *Sau*3A libraries.

principle employed is to ask whether a single gene in the λ clone is responsible for all of the observed complementation properties. This is done by deriving mutants of the λ clone that are defective in a cloned replication gene as judged by failure to complement one bacterial mutant. The ability of these mutant λ clones to complement the other bacterial mutants is then assessed. The results of such tests clearly show that *dna-1*, *dna-141*, *dna-601* and *dna-602* are mutations of the Salmonella *dnaC* homologue (Table 3), whereas *dna-229*, *dna-305*, *dna-693* and *dna-698* are mutations of the Salmonella *dnaE* homologue (Table 4). For consistency of nomenclature we designate these Salmonella genes *dnaC* and *dnaE*, respectively.

Local mutagenesis of a cloned locus: It is clear from the complementation data presented in Tables 2–4 that Salmonella possesses replication genes functionally equivalent to those of *E. coli*, yet chromosomal mutations affecting many of these genes were not among the initial group analyzed. To obtain mutations in these genes we turned to local mutagenesis. This is a powerful method for inducing mutations in a limited, defined region of a bacterial chromosome by *in vitro* mutagenesis of generalized transducing particles (HONG and AMES 1971). Transductants are necessarily mutagenized only in the immediate vicinity of the selected marker.

The main requirement for application of this technique is a selectable marker near the gene of interest. Meeting this requirement is not trivial when the gene of interest is identified first through molecular cloning. We reasoned that a suitable selectable marker could be obtained for any of the cloned genes by introducing a drug resistance transposon into the cloned DNA and then de-

TABLE 3

Complementation analysis of dnaC *mutations*

	Bacterial mutations				
	ts1	*ts141*	*ts601*	*ts602*	*dnaC2*
λRM287	+	+	+	+	+
λRM287 *dnaC*⁻ (7 isolates)	−	−	−	−	−

Three *dna* mutations from our collection (*ts141*, *ts601* and *ts602*), the "*dnaC*" mutation of SPRATT and ROWBURY (1970) (*ts1*) and the *E. coli dnaC2* mutation were analyzed by red plaque complementation tests using one wild-type clone and seven hydroxylamine-induced mutant clones derived from the wild-type clone. These tests were carried out at 40.5°, the highest permissive temperature for the *dnaC*⁺ parent of the *E. coli* mutant. +, plaques were red; −, plaques were not red.

TABLE 4

Complementation analysis of mutations in and near dnaE

	Bacterial mutations						
	ts229	*ts305*	*ts680*	*ts693*	*ts698*	*ts712*	*dnaE486*
λRM307 (2)	+	+	+	+	+	+	+
λRM311 (4)	+	+	−	+	+	+	+
λRM303 (4)	+	+	−	+	+	−	+
λRM303 *dnaE*⁻ (7)	−	−	−	−	−	−	−

Two *dna* mutations (*ts229* and *ts305*), four uncharacterized *ts* mutations derived in the same region of the Salmonella chromosome and the *E. coli dnaE486* mutation were analyzed by red plaque complementation tests using ten overlapping wild-type clones and seven hydroxylamine-induced mutant clones derived from one of the wild-type clones. The numbers in parentheses following the representative phage designations indicate the total number of phage isolates giving the same complementation pattern. +, plaques were red; −, plaques were not red.

manding recombination of the transposon into the homologous position in the bacterial chromosome. This approach required a transposon that is small (because of size constraints imposed by λ packaging) and the transposition of which could be controlled experimentally. A deletion derivative of Tn*10*, Tn*10Δ16Δ17* (or simply Tn*10*Δ) was found suitable (FOSTER *et al.* 1981). This deleted element is incapable of independent transposition but transposes efficiently if provided with transposition functions *in trans*, as in strain RB132. When subsequently moved into a strain lacking transposition functions, insertions of Tn*10*Δ do not undergo further transposition and, thus, they behave as stable genetic markers.

Phage mutants carrying insertions of Tn*10*Δ were isolated from lysates passaged on strain RB132. Recombination of these insertions from their position in cloned Salmonella DNA to the homologous position in the Salmonella chromosome was achieved by low multiplicity infection of nonlysogenic Salmonella (DB4673) with λ clones carrying Tn*10*Δ, followed by selection for tetracycline-resistant surviving cells. This protocol takes advantage of different genetic blocks affecting both lytic and lysogenic propagation of these λ phage in Salmonella (see MATERIALS AND METHODS). Tetracycline-resistant survivors were obtained at frequencies that were characteristic for each insertion and were as

high as 10^{-3}/plaque-forming unit. This is illustrated for λ*dnaQ* derivatives in Figure 1.

Local mutagenesis with Tn*10* as the selected marker was used to derive new *ts* mutations in and around *dnaE* and *dnaA*. The Tn*10* in the latter case was obtained by phage-chromosome recombination as described. Complementation tests of four locally derived *ts* mutations near *dnaE* revealed that two are alleles of *dnaE* and the other two are not (Table 4). Similar analysis of six locally derived *ts* mutations near *dnaA* revealed that three are alleles of *dnaA* and the remainder are in genes other than *dnaA* or *dnaN* (Table 5). Thus, beginning with just a reference mutation in *E. coli* and the cloned, complementing Salmonella gene, chromosomal mutations in the Salmonella gene were efficiently obtained and characterized.

The gene assignments of the locally derived mutations were corroborated by overlapping clone analysis, also shown in Tables 4 and 5. The λ1059 library contains Salmonella DNA fragments that were prepared by partial digestion of high molecular weight DNA with *Sau*3A. Thus, independently formed clones containing *dnaE* vary in the extent of flanking material to each side of *dnaE*. As expected, the new mutations assigned to *dnaE* were complemented by every λ*dnaE*$^+$ phage tested. In contrast, the other mutations from the *dnaE* experiment were complemented by only a subset of the λ*dnaE*$^+$ phage. Analogous results were obtained in the *dnaA* experiment.

Overlapping clone analysis proved a useful adjunct to complementation analysis using wild-type and mutant λ clones. The former type of analysis gives a minimum estimate of the number of different gene loci represented in a group of mutants (three in the case of the *dnaE* experiment; see Table 4), whereas the latter gives direct evidence for gene assignment. For example, overlapping clone analysis could not distinguish *dnaA* from *dnaN* chromosomal mutations since every λ*dnaA* phage also carried *dnaN*. In contrast, the combination of wild-type and mutant λ clones provided a specific complementation assay for a single gene, permitting the unambiguous assignment of chromosomal mutations to *dnaA*.

DISCUSSION

Functional interchangeability of individual replication proteins: One important conclusion arising out of the complementation results presented here is the functional similarity of replication proteins of *E. coli* and Salmonella. Virtually every *E. coli* replication mutant is saved by introducing a gene from Salmonella; thus, each Salmonella protein is able to interact and function appropriately with partners from *E. coli*. Clearly, replication in these two organisms occurs through the same biochemical pathway. The same conclusion has been reached by ROWEN, KOBORI and SCHERER (1982) with reference specifically to *dnaC*, *dnaG* and *dnaZ* on the basis of genetic complementation tests, and by KOBORI and KORNBERG (1982) with reference to *dnaC* on the basis of extensive characterization of the purified Salmonella protein.

Of *E. coli* mutants used to screen λ-Salmonella libraries, only strain DB4915 (*dnaP18*) failed to reveal a complementing clone. This exception may reflect

TABLE 5

Complementation analysis of mutations in and near dnaA

	Bacterial mutations							
	ts722	*ts723*	*ts725*	*ts727*	*ts728*	*ts729*	*dnaA177*	*dnaN59*
λRM181 (5)	+	p	+	+	+	+	+	+
λRM180 (4)	−	−	p	+	+	+	+	+
λRM182 (5)	−	−	−	+	+	+	+	+
λRM180 *dnaA*⁻ (6)	NT	NT	NT	−	−	−	−	+
λRM180 *dnaN*⁻ (3)	NT	NT	NT	+	+	+	+	−
λRM180 *dnaA*⁻*N*⁻ (1)	NT	NT	NT	−	−	−	−	−

Six uncharacterized *ts* mutations derived in the *dnaA* region of the Salmonella chromosome and the *E. coli dnaA177* and *dnaN59* mutations were analyzed by red plaque complementation tests using 14 overlapping wild-type clones and ten hydroxylamine-induced mutant clones derived from one of the wild-type clones. The numbers in parentheses following the representative phage designations indicate the total number of phage isolates giving the same complementation pattern. +, plaques are uniformly red; −, plaques are not red; p, plaques show many red papillae. The significance of the latter response is unknown. Perhaps it reflects marker rescue by a clone carrying only part of a gene. NT, not tested.

a species-specific requirement for *dnaP* or failure of a Salmonella *dnaP* gene to be expressed in *E. coli*, but other possibilities also need to be considered. On trivial possibility, that *dnaP18* is dominant over *dnaP*⁺, can be ruled out based on complementation studies using an F′ factor (WADA and YURA 1974). Another possible explanation rises from consideration of the pleiotropic effects of *dnaP18* on DNA replication and cell division and the differential reversibility of these effects. The defect in DNA replication is readily reversed when cells are returned to a permissive temperature after several hours at the nonpermissive temperature. However, the cell division defect is not reversible after the same treatment (WADA and YURA 1974). If complementation of the cell division defect of *dnaP18* follows a long phenotypic lag, it might not have been detected by the red plaque assay because no time is allowed for phenotypic expression.

In recent attempts to clone the *dnaA* gene of Salmonella or *E. coli* by complementation, plasmids or phage clones were obtained which carried, not *dnaA*, but other genes that suppressed *dnaA* (PROJAN and WECHLSER 1981; TAKEDA and HIROTA 1982; ROWEN, KOBORI and SCHERER 1982). In the experiments reported here, only bona fide *dnaA* clones were obtained, as judged by the presence of complementing activity toward a tightly linked gene, *dnaN*, on all such clones. Whether our success can be attributed to the use of *dnaA177* as the reference mutation, or to some aspect of methodology, is unclear. It is also unclear whether our *dnaA* mutations are allelic with the Salmonella "*dnaA*" initiation mutation of BAGDASARIAN *et al.* (1975). A λ-sensitive Salmonella strain carrying this mutation was unsuitable as a red plaque tester strain because it aggregated extensively in liquid culture.

Other studies of functional interchangeability: Functional interchangeability of *E. coli* and *S. typhimurium* genes has been examined in two other constellations of genes involved in cellular processes with the potential for multiple protein-protein interactions. Comparison of these results with our own is instructive.

In one case (DEFRANCO, PARKINSON and KOSHLAND 1979), an F′ factor carrying six wild-type *E. coli* chemotaxis (*che*) genes was able to complement Salmonella *che* mutants in any of six different genes. For two of these *E. coli* genes, a clear genetic correspondence and functional interchangeability with a Salmonella analogue were established by complementation tests using derivative F′ factors bearing mutations in the different *che* genes. Correspondences for other *che* genes could be determined only by consideration of both genetic and phenotypic properties of mutants because the complementation data alone were ambiguous. For example, *cheB* and *cheZ* of *E. coli* were deduced to correspond to *cheX* and *cheT* of Salmonella, respectively, but efficient complementation of *cheX* or *cheT* mutants required the presence of both wild-type *cheB* and *cheZ*. To explain these results, the authors proposed that *cheB* and *cheZ* proteins form a functional complex that can work with other components specified by either *E. coli* or Salmonella; however, interspecific complexes (*e.g.*, *cheB* with *cheT*) cannot be formed or are marginally functional at best. Thus, although individual chemotaxis functions may not be freely interchangeable, larger groups of chemotaxis functions clearly are. In this study, the level at which functional interchangeability could occur could not be determined readily because the F′ factors used contained most of the cell's *che* genes, no more than one of which was mutant in a given experiment.

Study of *fla* genes (involved in flagellar synthesis) produced similar conclusions (KUTSUKAKE *et al.* 1980). *Fla* genes are found in three widely separated clusters in both *E. coli* and Salmonella. The clusters are generally interchangeable between the two organisms, as assayed by complementation using P1-mediated abortive transduction, but there are some restrictions on the interchangeability at the level of individual genes. These restrictions seem to involve components of the basal body, the structure upon which the hook protein normally assembles. The restrictions are most easily explained by postulating that the Salmonella hook protein is unable to assemble on a chimeric basal body.

In both of these cases, differences between the related genes at the nucleic acid sequence level are implied by the absence of detectable recombination, but the extent of amino acid sequence divergence is unknown. Moreover, detailed information on the biochemical activity of these proteins and their postulated interactions is largely absent. A third example, rich in just such details, is provided by the enzyme, tryptophan synthetase. In this case, two kinds of polypeptide chains, encoded by the *trpA* and *trpB* genes, must interact in a precise manner to create fully active protein. If holoenzyme activity is compared with the activity of isolated *trpA* and *trpB* subunits using suitable assays, the quality of the subunit interaction can be assessed [see YANOFSKY and CRAWFORD (1972) for overview]. The *E. coli* and Salmonella versions of *trpA* and *trpB* are freely interchangeable *in vivo* and *in vitro* with little detectable effect on subunit interaction (CREIGHTON 1974). This is true despite substantial divergence at the nucleotide and amino acid sequence level for both genes. *TrpA* differs in 199 of 804 nucleotides and 40 of 268 amino acids (NICHOLS and YANOFSKY 1979), whereas *trpB* differs in 187 of 1191 nucleotides and 14 of 397 amino acids (CRAWFORD 1980). Even more striking, hybrid

trpA proteins consisting of variable lengths of amino-terminal sequence from Salmonella fused to carboxy-terminal *E. coli* sequences are invariably fully functional, both *in vivo* and *in vitro*, even though the hybrid genes encoding these proteins were isolated without requiring $trpA^+$ activity (SCHNEIDER, NICHOLS and YANOFSKY 1981).

The interchangeability of individual *dna* genes suggests that, as in tryptophan synthetase, nucleotide and amino acid differences between *E. coli* and Salmonella are neutral for protein function. The significance of this finding will become more apparent once the extent of divergence of *dna* genes is known. It has been estimated that *E. coli* and Salmonella differ at about 15% of their DNA sequence, based on hybridization and denaturation studies of total DNA (SANDERSON 1971), but *dna* genes have not been studied in this regard except in two cases. *DnaC* genes specifically cross-hybridize, as do *dnaZ* genes; however, the stringency of the hybridization was not reported in either case (KOBORI and KORNBERG 1982; ROWEN, KOBORI and SCHERER 1982).

New replication genes in Salmonella: Reciprocal complementation tests show that seven Salmonella loci represented in our collection of putative replication mutants are not equivalent to any of the known *E. coli* replication genes. That is, clones that complement the *E. coli* mutants fail to complement the novel Salmonella mutants, and clones that do complement the Salmonella mutants fail to complement the *E. coli* mutants. These novel Salmonella mutants await detailed characterization, but the fact of their isolation by a method that also produced bona fide replication mutants in *dnaC* and *dnaE* lends credence to the possibility that different subsets of replication genes would be most easily mutable in Salmonella and *E. coli*.

Red plaque complementation assay: Our results were obtained using a novel red plaque complementation method with *ts*-lethal mutants of Salmonella and *E. coli*. This method relies on the formation of partial diploids by lysogenization of mutant bacteria with specialized λ transducing phage, but the complementation signal is amplified by first allowing the phage to form a plaque, and only later adding tester bacteria for lysogenization *in situ*. Compared with commonly used methods of lysogenic complementation, the red plaque assay has several useful features. First, the assay has an efficiency close to one (every plaque of the appropriate phage is red). Second, complementation is easily distinguished from reversion of the tester bacteria. Third, either free phage or lysogenic bacteria can be recovered from a red complementation plaque for further analysis. Fourth, and most significantly, the plaque assay permits detection of rare mutant phage that have lost their ability to complement: these make plaques that are not red. Thus, it is possible to obtain and characterize a variety of mutations in the λ clone. Here, such phage mutations were used to determine the allelism of independently derived chromosomal *ts* mutations.

These allelism experiments were interpreted assuming that uncolored plaque mutants are affected in the cloned DNA rather than in a λ gene. The following arguments support this view. First, Tn*10*Δ insertions that produce an uncolored plaque phenotype map in the Salmonella segment (Figure 1 and our unpublished results). Second, hydroxylamine-induced mutations that produce

this phenotype can be transferred to the Salmonella chromosome if they are compatible with cell viability (*e.g.*, *dnaE11*(Am); MAURER, OSMOND and BOTSTEIN 1984). Third, the *dnaE305*(Ts) mutation, originally isolated in Salmonella, produces an uncolored plaque phenotype when transferred to λ*dnaE* (Figure 2). Fourth, for phage carrying two replication genes, such as *dnaA* and *dnaN*, each gene can be independently inactivated by mutation (Table 3). This would not be an expected property of λ mutations producing a general defect in complementation. Indeed, it is difficult to see which λ mutations could result in a general complementation defect since no λ sites or genes are involved in either lysogenization *in situ* or expression of cloned genes.

Close inspection of plaques formed by uncolored plaque mutants sometimes reveals a variable but low number of red microcolonies. The basis for this reaction was not investigated, but it most likely reflects recombination between two mutations of the same gene (one mutation carried on λ, the other in the chromosome) to produce a wild-type gene upon integration of the phage DNA. This event ought to be relatively rare among all possible integration events because it requires that integration occur between the positions of the two mutations. Several incidental observations point to this explanation. The microcolonies are not seen when derivatives of λ-Salmonella hybrids are assayed in *E. coli* hosts, suggesting that near-perfect DNA sequence homology is required. In addition, the microcolonies can be seen using phage bearing mutations such as Tn*10*Δ insertions or unsuppressed amber mutations; therefore, it is unlikely that the microcolonies reflect leaky complementation by the mutant phage. Consistent with these observations, microcolonies are not seen when a temperature-sensitive mutation, *dnaE305*, is present in both the phage and the tester strain. If the microcolonies indeed reflect recombination, then presumably recombination is occurring at a comparable frequency in red plaque assays using wild-type clones. Here, however, the relatively few recombinants are greatly outnumbered by complemented cells in which there has been no recombination of the *dna* genes.

*Uses of Tn*10Δ: The various manipulations of the deleted Tn*10* were developed in response to a need to mark chromosomal regions corresponding to cloned DNA. This method is generally applicable to all λ-Salmonella hybrid phage. These manipulations offer the possibility of saturating a small piece of cloned DNA with Tn*10*Δ insertions. These insertions can be individually tested by transduction into the chromosome to assess the null phenotype of the insertion target. The insertions and their associated phenotypes when present in the chromosome form the basis for both a physical and genetic map of the region in question. This procedure also produces a set of mutant phage clones suitable for complementation analysis of *ts*-lethal mutations derived in the corresponding region of the chromosome.

The characteristic frequency of transduction from phage to chromosome observed for each Tn*10*Δ insertion may indicate whether the insertion target is an essential gene of the cell. Insertions in essential targets should not be transducible into the chromosome except in a minority of cells carrying preexisting duplications of the target sequence (ANDERSON and ROTH 1977). Indeed,

we observed that the characteristic phage to chromosome transduction frequencies for insertions in λ*dnaQ* fall into two classes: readily transduced (5×10^{-4} or more transductants per plaque-forming unit) and poorly transduced (10^{-5} or fewer). However, it is not known to what extent these characteristic transduction frequencies are also influenced by the position of the insertion within the clone (*i.e.*, proximity to the λ joint), and it is striking in Figure 1 that the poorly transduced insertions are clustered near the ends of the cloned fragment. We have no independent evidence that shows which insertions in λ*dnaQ* are in essential genes. On the other hand, using unrelated clones bearing essential genes *dnaC* and *dnaZ*, we have found that insertions in these genes map away from the ends of the cloned fragment and are transduced into the chromosome at frequencies similar to the poorly transduced class in λ*dnaQ* (J. ENGSTROM, A. WONG and R. MAURER, unpublished results).

Together, the various methods presented here allow each genetic locus affecting DNA replication to be characterized. In the following paper (MAURER, OSMOND and BOTSTEIN 1984) the approaches presented here will be applied to the analysis of second-site suppressors of DNA replication mutants.

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