GENETIC ANALYSIS OF DNA REPLICATION IN BACTERIA: *dnaB* MUTATIONS THAT SUPPRESS *dnaC* MUTATIONS AND *dnaQ* MUTATIONS THAT SUPPRESS *dnaE* MUTATIONS IN *SALMONELLA TYPHIMURIUM*

RUSSELL MAURER,[1] BARBARA C. OSMOND AND DAVID BOTSTEIN

Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

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ABSTRACT

We have isolated and characterized extragenic suppressors of mutations in two different target genes that affect DNA replication in *Salmonella typhimurium*. Both the target and the suppressor genes are functional homologues of known replication genes of *E. coli* that were identified in intergeneric complementation tests. Our results point to interactions *in vivo* involving the *dnaB* and *dnaC* proteins in one case and the *dnaQ* and *dnaE* proteins in the other case. The suppressor mutations, which were isolated as derivatives of λ-Salmonella *in vitro* recombinants, were detected by an adaptation of the red plaque complementation assay. This method was applicable even when the locus of suppressor mutations was not chosen in advance.

IN the analysis of complex biological processes it is often useful to study mutations that suppress the phenotype of target mutations affecting the process of interest. Such suppressor mutations can arise in genes encoding proteins that interact physically with the target gene product, as well as in other genes that bear a less obvious relationship to the target gene (reviewed in HARTMAN and ROTH 1973; BOTSTEIN and MAURER 1982). Suppressor analysis has the potential to uncover new genes affecting the process of interest and to call attention to the possible interaction of particular proteins *in vivo*. In this paper we report the isolation and characterization of extragenic suppressors that affect DNA replication mutants (in *dnaC* and *dnaE*) in *Salmonella typhimurium*.

The design of these experiments is illustrated in Table 1, which shows the expected responses when λ recombinant phage clones carrying bacterial genes infect and lysogenize mutant bacteria. The response is assessed conveniently on Petri plates using the red plaque assay described in the preceding paper (Maurer *et al.* 1984): this assay indicates whether the prophage can aid the growth of the bacterium by complementation and/or suppression of the bacterial defect.

[1] Present address: Department of Molecular Biology and Microbiology, Case Western Reserve University School of Medicine, 2119 Abington Road, Cleveland, Ohio 44106.

TABLE 1

Complementation and suppression analysis by red plaque assay

	Genotype			
	Target gene	Potential suppressor gene	Red plaque formed	Interpretation
Test I: complementation of target gene mutations				
Bacteria	*ts*	*wt*	Yes	*ts* is complemented by the *wt* allele
λ prophage	*wt*			
Test II: control for suppression of target gene mutations				
Bacteria	*ts*	*wt*	No	No complementation
λ prophage		*wt*		No suppression
Test III: suppression of target gene mutations				
Bacteria	*ts*	*wt*	Yes	*ts* is suppressed by the *sp* allele
λ prophage		*sp*		
Test IV: complementation of suppressor gene mutations				
Bacteria	*wt*	*ts*	Yes	*ts* is complemented by the *sp* allele
λ prophage		*sp*		

The first example (test I) in Table 1 recapitulates the complementation test of the preceding paper (MAURER *et al.* 1984). A recessive bacterial temperature-sensitive (*ts*) mutant in a target gene is complemented by a phage carrying the corresponding wild-type allele and a red plaque is formed. In the case shown, any genes that might potentially mutate to suppress the *ts* defect are in their wild-type state, which is active for normal gene function and inactive for suppression.

The second example (test II) shows that an increase in the dosage of a potential suppressor gene (or the placing of it in a λ phage) does not result in suppression of the target gene mutation: thus, the plaques are not red. This is a control for the third case (test III), which shows that some genes can be mutated, in their λ context, to a state that causes suppression of the *ts* mutation in the original target gene, as indicated by the red plaque response. This is, then, a "red plaque *suppression* test." It should be borne in mind, however, that the suppression by the mutant gene on the phage takes place in the presence of the corresponding wild-type allele on the bacterial chromosome. Therefore, the suppressor mutation (*sp*) is dominant to its wild-type allele. Thus, the suppression test (vis-à-vis the target *ts* mutation in the target gene) is also a *complementation* test (vis-à-vis the gene giving rise to the *sp* mutation).

The fourth case in Table 1 (test IV) shows a complementation test in which a λ phage carrying an *sp* mutation complements a bacterial strain bearing a defective (*ts*) allele at the suppressor gene locus. In contrast to the first three

TABLE 2

Strains used

Designation	Genotype	Source or reference
Salmonella typhimurium:		
DB9005	*thyA deo* (fels free, plasmid free)	MAURER *et al.* (1984)
DB9144	DB9005 *dnaE229*(Ts)	Ethyl methanesulfonate mutagenesis (MAURER *et al.* 1984)
DB9178	DB9144 *hisD9426*::Tn*10*	P22(DB7126)[a]
DB9179	DB9178 *hisD*$^+$ *hisC527*(Am)	P22(DB7136)[a]
DB9180	DB9179 *supE20*	P22(DB7155)[a]
DB9181	DB9005 *hisD9426*::Tn*10*	P22(DB7126)[a]
DB9182	DB9181 *hisD*$^+$ *hisC527*(Am)	P22(DB7136)[a]
DB9183	DB9182 *supE20*	P22(DB7155)[a]
DB9184	DB9180 *dnaE11*(Am) *zxx1251*::Tn*10*Δ*16*Δ*17*	See text
DB9185	DB9183 *dnaE11*(Am) *zxx1251*::Tn*10*Δ*16*Δ*17*	See text

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

tests, the target gene locus is wild type in test IV. Although nothing in the experimental design demands *a priori* that the suppressor mutation retain ability to complement defective alleles of the same gene, this was the universal result. This kind of complementation test becomes the basis for identifying the normal function of the genes in which the suppressor mutations arise.

MATERIALS AND METHODS

Most materials and methods were described in MAURER *et al.* (1984). Additional bacteria and λ phage are listed in Tables 2–5.

Strain DB9184 was constructed by cotransduction of the *dnaE11*(Am) mutation and a tetracycline resistance insertion (Tn*10*Δ*16*Δ*17*) from λRM325 into strain DB9180. Since strain DB9180 was not itself λ sensitive, this construction required an intermediate step in λ-sensitive Salmonella. Therefore, strain DB4673 [prepared by growth in λ broth + 0.2% (w/v) maltose and 1 mM $MgSO_4$] was infected with λRM325 at a multiplicity of approximately 100. After 15 min at 37°, 0.1 ml of infected cells was diluted into 2 ml of LB containing 0.05% galactose and 10^7 P22intHT12/4. Incubation was continued at 37° overnight. The resulting P22 lysate contained particles that transduced tetracycline resistance into the *supE20*, *dnaE229*(Ts) recipient strain, DB9180. Sixteen of 37 of these transductants were temperature resistant, indicating that they had also acquired a temperature-resistant *dnaE* gene. This gene might have been either a true *dnaE*$^+$ gene or the (suppressed) *dnaE11*(Am) gene. These alternatives were distinguished by a backcross to the *sup*$^+$, *dnaE229*(Ts) recipient, DB9179. Five of ten temperature-resistant strains so tested were proficient donors of temperature resistance (approximately 50% cotransduction with tetracycline resistance), indicating that they carried the *dnaE*$^+$ gene. The other five strains were poor donors of temperature resistance (<5% cotransduction with tetracycline resistance), suggesting that these strains carried the *dnaE11*(Am) gene. One of these latter strains was designated DB9184.

The *dnaE11*(Am) mutation was then transduced from strain DB9184 into the unmutagenized *supE20* strain, DB9183, by selecting tetracycline resistance. Since both the donor and recipient *dnaE* genotypes produced a temperature-resistant phenotype in strain DB9183, transductants carrying *dnaE11*(Am) were again identified by backcross to DB9179. Four of six tetracycline-resistant transductants of DB9183 were found to be *dnaE11*(Am) by this test; one of these was designated DB9185. *Sau*3A partial digest fragments of DNA from strain DB9185 were cloned in the vector λ1059 (KARN *et al.* 1980; MAURER *et al.* 1984).

Suppression assays: Suppressors were isolated and characterized by the red plaque complementation assay, carried out as described in MAURER *et al.* (1984) according to the strategy outlined in Table 1 (see Introduction). For suppressor isolation from genomic libraries, phage plaques were screened at a density of 2.5×10^4 per plate. For red plaque spot tests of purified phage isolates, dilutions at a concentration of 10^6/ml were used.

In addition, λ phage bearing suppressor mutations were sometimes characterized by spotting phage lysates (10^{10}/ml) onto a λ plate seeded with lysogenic *dna-ts* bacteria. As soon as the lysate droplets were absorbed into the agar, the plates were shifted to the nonpermissive temperature and incubated overnight. Against a general background of bacterial revertant colonies, lysates of *bona fide* suppressor phage produced a spot of denser growth. Compared with the red plaque assay, the colony assay was generally more sensitive. For example, suppression usually was detectable at a more extreme nonpermissive temperature using the colony assay than was possible with the red plaque assay.

RESULTS

dnaB *mutations that suppress* dnaC: As a first test of the approach described, we sought suppressors in a gene, *dnaB*, whose product is known to interact *in vitro* with the product of the target gene, *dnaC* (KOBORI and KORNBERG 1982b; WICKNER and HURWITZ 1975). λRM113 carries the Salmonella *dnaB* gene, as judged by the ability of this phage to complement *E. coli* strains bearing mutations *dnaB22* or *dnaB107* but not *E. coli* or Salmonella strains mutant in other replication genes (MAURER *et al.* 1984). In particular, this phage makes a colorless plaque on each of four different Salmonella *dnaC* strains. From a stock of λRM113 mutagenized with hydroxylamine we obtained 13 mutants that make red plaques on at least one of the *dnaC* strains in the red plaque suppression test. The frequency of suppressor mutants depended on both the *dnaC* strain used and the temperature. The latter seemed to be the more important factor since many of the suppressors isolated at 40.5° do not make red plaques on the same *dnaC* strain at 42°. In the more sensitive colony assay, most of the suppressors show some degree of activity at 42°. If only screens at the lower temperature are taken into account, the overall frequency of suppressors was about 10^{-3}.

Complementation analysis of suppressor mutations: All of the suppressor phage make red plaques at 40.5° and 42° in the *dnaB* complementation assay (test IV in Table 1). Thus, the suppressor mutations do not inactivate *dnaB* function or render it temperature sensitive. This finding made possible an experiment that implicates the cloned *dnaB* gene, rather than any other gene cloned with *dnaB* on the same fragment of DNA, in the activity of one of the suppressor mutations, *sp18*. The demonstration is based on the properties of mutant derivatives of λRM208 (λRM113*sp18*) that lack the ability to complement *dnaB* (test IV) or suppress *dnaC* (test III), or both. The logic of the demonstration is as follows. If two different genes are separately responsible for red plaque formation in test III and test IV, then no single mutation can eliminate both activities. To test the requirements of *sp18*, phage carrying this mutation were treated with hydroxylamine and then screened for colorless plaque mutants using a *dnaC* tester (test III). Seven mutations were obtained exhibiting a variety of effects on suppression and complementation (Table 3). In some cases

TABLE 3

Properties of the sp18 *suppressor*

Phage	Genotype		Tester strain alleles (*sup*$^+$/*supE*)				
			Test IV:	Test III			
			dnaB22	*dnaC141*	*dnaC602*	*dnaC1*	*dnaC601*
λRM113	*dnaB*$^+$		3/3	0 at 42°; generally 0 at 40.5°			
λRM208	*sp18*		3/3	3/3	3/3	*/*	3/3
λRM208	*sp18*	42°	3/3	3/3	3/3	3/3	3/3
λRM231	*sp18, 63*		3/3	3/3	3/3	*/*	3/3
λRM231	*sp18, 63*	42°	3/3	2/1	2/3	0/0	1/0
λRM232	*sp18, 65*		0/1	0/0	0/0	0/0	0/0
λRM233	*sp18, 66*		3/3	0/0	0/0	0/0	1/0
λRM234	*sp18, 67*		0/3	0/0	0/0	0/0	0/0
λRM235	*sp18, 68*		0/1	0/0	0/0	0/0	0/0
λRM236	*sp18, 69*		0/0	0/0	0/0	0/0	0/0
λRM237	*sp18, 70*		0/3	0/3	0/3	0/*	0/3

Phages λRM231–λRM237 were isolated from a mutagenized aliquot of λRM208 on the basis of failure to form a red plaque on a *sup*$^+$, *dnaC601* tester strain at 42°. Shown are the results of red plaque assays conducted at 40.5° unless indicated otherwise. The designations 3, 2, 1 and 0 indicate decreasing strength of response, with grade 1 of doubtful significance and grade 0 indicating no detectable response. Each entry gives paired data for isogenic tester strains that differ only in the absence (*sup*$^+$) or presence (*supE*) of an amber-suppressing tRNA. A positive response in the *dnaC* columns signifies extragenic suppression since the wild-type parent phage, λRM113, generally does not make a red plaque under the same conditions (test II in Table 1). Occasionally, this control, which was present on every assay plate, showed some degree of positive response at 40.5°. An asterisk (*) indicates instances in which the significance of an apparent suppression response could not be determined because the control also was positive. The tester strains used, reading from left to right across the top of the table, were RM84, RM83, DB4712, DB4765, DB4714, DB4738, DB4715, DB4735, DB4725 and DB4737.

the effects are subtle. For example, mutation 63 leaves unchanged the ability of λRM208 to complement *dnaB* mutations but diminishes the suppression of several *dnaC* alleles, particularly at 42°. At the other extreme, mutations 65, 68 and 69 unconditionally abolish complementation and suppression. The most revealing mutation is 70, which abolishes complementation and suppression in strains lacking an amber suppressor but permits complementation and suppression in strains having an amber suppressor. Thus, mutation 70 must be an amber mutation of the *dnaB* gene, and the properties of this mutation, as well as mutations 65, 68 and 69, indicate that suppression by *sp18* depends on the cloned *dnaB* gene. This dependence indicates that *sp18* is either a mutation of the *dnaB* structural gene or a *cis*-acting regulatory mutation that affects the level of *dnaB* expression.

A second group of *dnaC* suppressors was isolated in a way that permitted them all to be examined for *dnaB* dependence without further mutagenesis. These suppressors were derived from a phage that carries an amber mutation in *dnaB*, as judged by ability to complement *supE* but not *sup*$^+$ *dnaB* strains of *E. coli*. A mutagenized lysate of this phage strain was screened by the red plaque suppression assay (test III) using *supE20 dnaC* Salmonella testers. The *dnaB* dependence of 13 suppressors isolated this way was determined by com-

TABLE 4

Derivatives of λdnaB *that suppress* dnaC

Phage	Genotype	Assay	Tempera-ture	Tester strain alleles				
				Test IV:	Test III			
				dnaB22	*dnaC141*	*dnaC602*	*dnaC1*	*dnaC601*
λRM113	*dnaB*$^+$	P, C	42°	3	0	0	0	0
λRM205	*sp15*	C	42°	3	1	3	3	3
λRM206	*sp16*	P	42°	3	2	3	1	2
λRM207	*sp17*	P	42°	3	3	3	2	3
λRM208	*sp18*	P, C	42°	3	3	3	3	3
λRM209	*sp19*	C	42°	3	0	2	3	1
λRM210	*sp20*	C	42°	3	1	3	3	3
λRM211	*sp21*	P	42°	3	1	3	0	1
λRM212	*sp22*	P	42°	3	3	3	2	3
λRM213	*sp23*	P	42°	3	3	3	3	3
λRM214	*sp24*	P	42°	3	3	3	2	1
λRM215	*sp25*	P	42°	3	2	3	0	1
λRM217	*sp27*	P	42°	3	1	3	0	1
λRM218	*sp28*	P	42°	3	0	2	0	1
λRM220	*am34*	P, C	40.5°	3	0	0	0	0
λRM238	*am34, sp71*	C	40.5°	3	2	1	0	2
λRM239	*am34, sp72*	C	40.5°	3	2	1	0	2
λRM241	*am34, sp74*	C	40.5°	3	2	3	0	3
λRM242	*am34, sp75*	C	40.5°	3	2	1	0	2
λRM243	*am34, sp76*	C	40.5°	3	2	1	0	2
λRM244	*am34, sp78*	C	40.5°	3	2	1	1	2
λRM245	*am34, sp80*	C	40.5°	3	2	3	1	3
λRM246	*am34, sp81*	C	40.5°	3	2	3	0	3
λRM247	*am34, sp83*	C	40.5°	3	2	1	0	2
λRM248	*am34, sp85*	C	40.5°	3	3	3	1	3
λRM249	*am34, sp87*	C	40.5°	3	2	1	1	2
λRM250	*am34, sp88*	C	40.5°	3	2	3	1	3
λRM251	*am34, sp90*	C	40.5°	3	2	3	0	2

Each suppressor phage is described under conditions that best elicit an allele-specific response for that phage (see DISCUSSION). The entry in the third column indicates the suppression assay used: P, the red plaque assay, or C, the colony assay. The *dnaB* complementation was assayed by red plaque assay in all cases. Symbols are as for Table 3. The responses indicated were observed in both *sup*$^+$ and *supE* bacteria for phages λRM205 through λRM218, which were derived from λRM113 (*dnaB*$^+$). The responses indicated were observed in *supE*, but not in *sup*$^+$, bacteria for phages λRM238 through λRM251, which were derived from λRM220 (*dnaB34*[Am]). The tester strains are listed in the legend to Table 3.

paring suppression of isogenic *dnaC* strains that were either *supE20* or *sup*$^+$. Without exception these *dnaC* suppressors require *supE20* for their expression. Therefore, like *sp18*, these suppressors affect the structure or expression of *dnaB*.

Allele specificity of suppression: The 26 suppressors were tested for ability to suppress each *dnaC* mutation available to us, using four combinations of assay method and test temperature. The spectrum of mutations suppressed depended strongly, in most cases, on the test conditions. Many of the suppressors exhibited allele specificity under at least one set of test conditions (Table 4; see DISCUSSION).

It should be remembered that the suppressors we have detected and studied constitute a biased sample of the suppressors present in the mutagenized lysate. The bias was introduced by screening portions of the lysate with different *dnaC* testers. For example, a suppressor active on only one *dnaC* allele would be missed unless that particular *dnaC* allele were present in the screening strain. In contrast, a less specific suppressor would be detected with most (or all) possible tester strains. In short, the more nonspecific the suppressor, the more likely its detection.

Suppressors of dnaE *isolated from the total genome*: In the preceding section we have described a method for generating and characterizing suppressor mutations in a chosen suppressor gene. More generally, we wish to find suppressor mutations in the genome at large, that is, without choosing a candidate suppressor gene in advance. In principle this can be accomplished by screening mutagenized genomic libraries by the red plaque test, using whatever bacterial tester strain is of interest. However, this approach is compromised by the presence, in a genomic library, of clones carrying the wild-type gene that complements the defect in the tester strain, as in test I of Table 1. The red plaques formed by these complementing clones vastly outnumber the red plaques formed by suppressor clones, even after heavy mutagenesis of the library. Successful screening of a mutagenized genomic library thus requires a way of selectively depleting the library of complementing clones. Our solution was to use a genomic library constructed with DNA from a strain that is mutant in the target gene. This solution is illustrated by the isolation of suppressors of *dnaE* mutations.

The *dnaE* gene encodes the α subunit of DNA polymerase III (GEFTER *et al.* 1971; MCHENRY and KORNBERG 1981). Many other subunits of this complex enzyme have not been identified genetically, and part of the interest in this experiment lay in the possibility that another polymerase III gene would be revealed as the locus of a suppressor of *dnaE*. To obtain suppressors, we used a λ-Salmonella genomic library constructed with DNA from strain DB9185. λ*dnaE* phage isolated from this library by complementation of *supE dnaE*(Ts) bacteria could not complement *sup*$^+$ *dnaE*(Ts) strains, confirming that the *dnaE* gene of strain DB9185 carries an amber mutation. Strain DB9185, and, therefore, the library made from it, is wild type at all other replication loci. A hydroxylamine-treated aliquot of the library was screened for red plaque mutants using two different *sup*$^+$ *dnaE*(Ts) testers. Three mutants were obtained from 10^6 plaques screened with strain DB4713 at 37°, and no mutants were obtained from an equal number of plaques screened with strain DB4722 at 42°.

Complementation analysis: To identify the locus of the *dnaE* suppressors, we tested the three suppressor phage for ability to complement strains representing almost all of the other known replication genes in *E. coli* and Salmonella (*i.e.*, we applied test IV). Each such strain can be complemented by a clone from a wild-type λ-Salmonella library (MAURER *et al.* 1984). When tested with this battery, the *dnaE* suppressor phage were unreactive toward any strains except DB4931, an *E. coli dnaQ49* mutant. This result suggested that the suppressors arose on a λ*dnaQ* phage. To test this interpretation we sought

TABLE 5

Derivatives of λdnaQ *that suppress* dnaE

Phage	Genotype	Tester strain alleles: Test IV: *dnaQ49* (43°)	Test III: *dnaE229* (40.5°)	Test III: *dnaE305* (37°)	Test III: *dnaE693* (40.5°)	Test III: *dnaE698* (40.5°)
λRM354	*dnaQ*$^+$	+	−	−	−	−
λRM370	*sp19*	+	+	+	+	+
λRM371	*sp20*	+	+	+	−	−
λRM372	*sp21*	+	+	+	+	−
λRM366	*zxx1252*::Tn*10*Δ	+	+	+	NT	−
λRM360	*zxx1253*::Tn*10*Δ	+	+	+	−	−

Results of red plaque assays are shown. Distinction between degrees of positive response were not attempted in this experiment. The tests were conducted at a characteristic temperature for each bacterial mutant as indicated in the column headings. +, Plaques were red; −, plaques were not red; NT, not tested.

additional suppressors of *dnaE* starting from λ*dnaQ*$^+$. We obtained two new suppressors from 3×10^4 plaques screened with strain DB4722 at 40.5° and one new suppressor from a similar number of plaques screened with strain DB4713 at 37°. In addition to these hydroxylamine-induced mutants, we also examined plaque-forming mutants derived by Tn*10* insertion mutagenesis of λ*dnaQ*$^+$. Two of 12 independent insertions conferred ability to suppress *dnaE*. The relevant insertions are the two rightmost shown in Figure 1 of MAURER *et al.* (1984). These insertions do not inactivate *dnaQ*, as assayed by complementation, and are positioned 2 to 3 kb away from insertions that do inactivate *dnaQ*.

Like the suppressor mutations derived by Tn*10* insertion, the three new hydroxylamine-induced suppressor mutations do not affect *dnaQ* complementation. To determine the involvement of *dnaQ* in suppression of *dnaE*, three suppressor phage derived from λ*dnaQ*$^+$ (two hydroxylamine-, one Tn*10*-induced) were treated further with hydroxylamine, and a total of eight *dnaQ*$^-$ mutant derivatives were isolated. All eight mutants were now unable to suppress *dnaE*. Thus, the suppressor mutations are either structural or *cis*-acting regulatory mutations affecting *dnaQ*. The Tn*10* insertions are presumably regulatory in nature since they are located outside the *dnaQ* structural gene.

Allele specificity: Table 5 summarizes the allele specificity of the *dnaE* suppressors. In the most common pattern, the suppressors are active on *dnaE305* at 37° and on *dnaE229* at 40.5° but not on *dnaE693* and *dnaE698* at 40.5°. The temperatures cited are the lowest practical nonpermissive temperature for each mutant. The suppressor *sp19* is active on all four *dnaE* strains under these conditions.

DISCUSSION

Suppressors of dnaC*(Ts)*: We have isolated and partially characterized 26 mutations of λ*dnaB* that suppress *dnaC*(Ts) mutations in Salmonella. Fourteen of

these suppressor mutations were shown to reside at, or affect the expression of, the *dnaB* locus. The remainder were not analyzed in the same detail, but it seems highly likely that they, too, reside at *dnaB* because of their similar derivation. These suppressors are unlike suppressors of *dnaC*(Ts) that have been isolated previously in *E. coli*. Such suppressors map in a distinct gene called *dnaT* (LARK and LARK 1978; LARK, RIAZI and LARK 1978). The reciprocal phenomenon, *dnaC* mutations that suppress *dnaB*(Ts), has never been reported, but other interactions involving *dnaB*, *dnaC* and related phage proteins have been observed. For example, certain *E. coli dnaC*(Ts) mutations can be suppressed by a naturally occurring *dnaB*-like protein, the *ban* gene product encoded by phage P1 (SCLAFANI and WECHSLER 1981a). In addition, certain *dnaB* mutations that specifically restrict DNA replication of phage λ can be suppressed by mutations in the phage *P* gene, which encodes a *dnaC*-like protein (GEORGOPOULOS and HERSKOWITZ 1971; SAITO and UCHIDA 1977; WICKNER 1979; KLEIN, LANKA and SCHUSTER 1980). Finally, the unique initiation-defective *dnaB252* mutation of *E. coli* can be suppressed by high gene dosage of $dnaC^+$ (SCLAFANI and WECHSLER 1981b).

These results have been interpreted as supporting the idea that *dnaB* protein (or the P1 analogue) interacts physically, *in vivo*, with the *dnaC* protein (or the λ analogue). This explanation has appeal because *dnaB* and *dnaC* proteins bind to one another *in vitro* to form an active complex (WICKNER and HURWITZ 1975; KOBORI and KORNBERG 1982b). Except for the unique case of *dnaB252* (SCLAFANI and WECHSLER 1981b), our experiments constitute the first genetic test of this idea without complications engendered by the involvement of phage analogue proteins.

Interpretation of our results requires an analysis of the allele specificity exhibited by the various suppressors. This is a complicated matter because the tests used were qualitative, not quantitative; because two tests of differing sensitivity were used; and because tests were done at two different temperatures. For each suppressor mutation, the spectrum of mutations suppressed depended markedly on the test conditions. Lower temperature and use of the colony assay each favored easier detection of suppression. In many cases, suppressors were seen to be allele *nonspecific* under one set of conditions, but allele *specific* under a slightly more stringent set of conditions. We have chosen to consider such suppressors allele specific for the purpose of discussion. At issue is whether a suppressor protein interacts (directly or indirectly) with the mutant target protein or, alternatively, bypasses the mutant target protein altogether. A bypass suppressor, although it might be active only under certain conditions (*e.g.*, of temperature), should suppress all target mutations equally well under a given condition. Using this criterion of allele specificity we have identified 13 suppressors that, at least under one set of conditions, do not operate through a bypass pathway. This categorization of suppressors was reached by considering as positive the grade 3, 2 and 1 responses shown in Table 4. If the ambiguous grade 1 responses are considered negative, the number of allele-specific suppressors increases to 22. Whatever their true number, the activity of these allele-specific suppressors requires some type of interaction of the suppressor *dnaB* protein with the mutant *dnaC* protein.

Only a biochemical analysis can reveal what features of the *dnaB-dnaC* interaction are altered in the mutants described here. The following discussion gives some alternatives that are consistent with our observations. In the simplest case, the mutant *dnaC* protein assumes an altered three-dimensional structure and, consequently, has a lowered affinity for *dnaB* protein. Complex formation and, hence, *dnaC* function are restored by suppressor mutations that may increase the level of *dnaB* protein, thus driving complex formation, or alter the structure of *dnaB* protein, increasing its affinity for the altered *dnaC* protein. Such a mechanism may depend on mixing of suppressor and wild-type *dnaB* subunits since both are present in the suppressed cells and the functional complex has a B_6C_6 composition (KOBORI and KORNBERG 1982b).

A very different idea is based on maintaining a proper balance of *dnaB* and *dnaC* proteins in the cell. If the cell is relatively deficient in *dnaC* protein in the *ts* mutants (*e.g.*, because of rapid degradation) most *dnaC* protein could become trapped in nonfunctional incomplete complexes. The suppressor mutations would restore function by causing a matching reduction in *dnaB* protein levels. Such a mechanism has been invoked to explain the properties of certain morphogenesis mutants of phage T4 and P22 (FLOOR 1970; ISRAEL, ANDERSON and LEVINE 1967). To be compatible with this explanation, the *sp* mutations would have to exert a dominant effect on the expression of both the chromosomal and prophage *dnaB* genes. Moreover, the permissible level of *dnaB* protein would have to be very narrow because elimination of the prophage *dnaB* gene eliminates suppression. This set of circumstances, although not impossible, seems unlikely.

A third possibility is that suppressor *dnaB* protein protects mutant *dnaC* protein from proteolysis, either by binding a protease directly or by binding *dnaC* protein and covering a protease-sensitive site. The latter seems the more likely of the two in view of the known ability of *dnaB* protein to bind *dnaC* protein. On the other hand, KOBORI and KORNBERG (1982a) have argued that wild-type *dnaB* protein does not stabilize wild-type *dnaC* protein.

These hypotheses are amenable to testing *in vitro*. We hope the results of such an analysis with mutant proteins will reveal what features of the normal *dnaB-dnaC* interaction are important *in vivo*.

Suppressors of dnaE*(Ts)*: Three suppressors of *dnaE*(Ts) were obtained from a mutagenized aliquot of a genomic library prepared with DNA from a *dnaE* mutant. Complementation properties of these suppressors suggested that they arose in or near *dnaQ*. Subsequent analysis showed that three additional suppressors derived from $\lambda dnaQ^+$ are indeed structural or regulatory mutations affecting *dnaQ*. Two more suppressors derived from $\lambda dnaQ^+$ were not analyzed in detail but are probably similar. These suppressors are unlike suppressors of *dnaE*(Ts) that have been isolated previously in *E. coli*. Such suppressor mutations map in or near *dnaN*, a gene completely distinct from *dnaQ* (KUWABARA and UCHIDA 1981; NIWA, BRYAN and MOSES 1981; R. E. MOSES, personal communication). However, genetic interaction of *dnaQ* and *dnaE* was suggested previously by the extreme temperature sensitivity of *dnaQ, dnaE* double mutants (HORIUCHI, MAKI and SEKIGUCHI 1981).

Recent experiments have shown that DNA polymerase III isolated from *dnaQ49* and *mutD5* mutant *E. coli* exhibits reduced editing activity (*mutD5* is probably an allele of *dnaQ*) (ECHOLS, LU and BURGERS 1983). Moreover, *dnaQ* product comigrates on two-dimensional gels with authentic ϵ subunit of DNA polymerase III (SCHEUERMANN *et al.* 1983). Thus, the locus of our *dnaE* suppressors does turn out to be an apparent DNA polymerase III gene.

Present evidence suggests that *dnaQ* interacts with *dnaE* in controlling editing function and, hence, spontaneous mutation rate. The critical finding is that only *dnaE* product, of the separated DNA polymerase III subunits, possesses detectable 3′ to 5′ exonuclease activity intrinsic to editing (SPANOS *et al.* 1981). It is noteworthy that the *dnaE* protein (140,000 daltons), ϵ (25,000 daltons) and a third protein (θ, 10,000 daltons) form a tightly associated "core" of DNA polymerase III (MCHENRY and CROW 1979). Thus, there is the potential for *dnaQ* protein to regulate editing by an allosteric effect on *dnaE* protein, among other possibilities. Whether the suppression of *dnaE*(Ts) mutations by mutations in *dnaQ* is a second manifestation of the same mechanism operative in editing control is unknown. In particular, we have not examined the mutation rate of cells harboring *dnaQ*(Sp) mutations in their chromosomes.

Novel aspects of methodology: Our method for isolating extragenic suppressors has demonstrable advantages compared with previous methods. First, there is no requirement that suppressors confer on cells any phenotype other than suppression. Therefore, a much broader range of suppressors can be studied than with methods such as the cs-ts method (JARVIK and BOTSTEIN 1975), which depends on accessory phenotypes associated with a small fraction of suppressor mutations. Second, the suppressor mutations are isolated on a λ DNA molecule, and, thus, the step of separating target and suppressor mutations is trivialized. Moreover, further studies on suppression of other target mutations or molecular analysis of the suppressor gene are easily approached using the λ clone.

The most useful feature of our approach is the ability to determine the locus of a suppressor mutation by genetic complementation tests. In these tests the suppressor (*sp*) allelle functions as a "wild type" that complements a standard temperature-sensitive mutation of the suppressor gene. It is remarkable that every suppressor mutation we have studied exhibits this complementation behavior since to do so requires compatibility of the suppressor mutation with the wild-type allele of the target gene. This would not have been expected if the suppressor mutations alter protein conformation so as to recognize specifically an altered conformation in the mutant target protein. Yet, for reasons that are not clear, compatability with the wild-type target protein has been the universal rule, as far as we can ascertain, for "interaction" suppressors isolated by a variety of methods in numerous organisms and gene systems (*e.g.*, SAITO and UCHIDA 1977; JARVIK and BOTSTEIN 1975; MOIR *et al.* 1982; KUWABARA and UCHIDA 1981; WALKER, RAMSEY and HALDENWANG 1982; AMES and SPUDICH 1976). Perhaps, the easiest pathway for formation of a suppressor protein is through loss of specific determinants (bulky or charged side groups) that contribute to the physical interaction with the wild-type target protein but

interfere with the physical interaction with the mutant target protein. Such suppressors would then be characterized by a less specific (more broadly compatible) interaction with various mutant target proteins.

Two classes of suppressor mutants would not be detected by the novel procedures we have used. The first class consists of recessive suppressors, that is, suppressor mutations that do not suppress in a heterozygous diploid with the wild-type allele of the suppressor gene. Protease-deficient (*lon*$^-$) mutations are a specific example of this class (GOTTESMAN and ZIPSER 1978). Evidence that other recessive suppressors might be scarce comes from yeast. MOIR *et al.* (1982) isolated suppressors of cell cycle (*cdc*) mutants. The suppressors were isolated in a haploid strain and, when tested in a heterozygous diploid, were found to be dominant (for suppression) in every case. Similar results have been obtained with bacterial and bacteriophage suppressor mutations (KUWABARA and UCHIDA 1981; SAITO and UCHIDA 1977).

The second class consists of suppressors that, in a wild-type genetic background, exhibit dominant lethality. λ phage carrying mutations in this class might fail to propagate on wild-type *E. coli* and thus would be lost from mutagenized phage lysates. We have no way of evaluating the frequency of mutations in this class. With the exception of this one uncertain class and protease mutants, it appears that our novel method for isolating suppressors detects essentially the same suppressors as are obtained by other methods.

One further point regarding our strategy for isolating *dnaE* suppressors requires comment. We chose to prepare a library from a *dnaE*(Am) strain rather than from a more readily available *dnaE*(Ts) strain because we were unsure that a λ*dnaE*(Ts) phage would exhibit a satisfactory negative phenotype in the red plaque complementation assay. This fear subsequently proved unfounded at least for *dnaE305* (MAURER *et al.* 1984). More recently, intragenic complementation of two *dnaA*(Ts) mutations in the red plaque complementation assay has been noted (E. SHEKHTMAN and R. MAURER, unpublished data). Thus, it appears that some, but not all, *dna*(Ts) strains are suitable DNA donors for the approach we have described.

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LITERATURE CITED

AMES, G. F.-L. and E. N. SPUDICH, 1976 Protein-protein interaction in transport: periplasmic histidine-binding protein J interacts with P protein. Proc. Natl. Acad. Sci USA **73:** 1877–1881.

BOTSTEIN, D. and R. MAURER, 1982 Genetic approaches to the analysis of microbial development. Annu. Rev. Genet. **16:** 61–83.

ECHOLS, H., C. LU and P. M. J. BURGERS, 1983 Mutator strains of *Escherichia coli, mutD* and *dnaQ*, with defective exonucleolytic editing by DNA polymerase III holoenzyme. Proc. Natl. Acad. Sci. USA **80:** 2189–2192.

FLOOR, E., 1970 Interaction of morphogenetic genes of bacteriophage T4. J. Mol. Biol. **47:** 293–306

GEFTER, M., Y. HIROTA, T. KORNBERG, J. WECHSLER and C. BARNOUX, 1971 Analysis of DNA polymerases II and III in mutants of *Escherichia coli* thermosensitive for DNA synthesis. Proc. Natl. Acad. Sci. USA **68:** 3150–3153.

GEORGOPOULOS, C. P. and I. HERSKOWITZ, 1971 *Escherichia coli* mutants blocked in Lambda DNA synthesis. pp. 553–564. In: *The Bacteriophage Lambda*, Edited by A. D. HERSHEY. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

GOTTESMAN, S. and D. ZIPSER, 1978 Deg phenotype of *Escherichia coli lon* mutants. J. Bacteriol. **133:** 844–851.

HARTMAN, P. E. and J. R. ROTH, 1973 Mechanisms of suppression. Adv. Genet. **17:** 1–105.

HORIUCHI, T., H. MAKI and M. SEKIGUCHI, 1981 Conditional lethality of *Escherichia coli* strains carrying *dnaE* and *dnaQ* mutations. Mol. Gen. Genet. **181:** 24–28.

ISRAEL, J. V., T. F. ANDERSON and M. LEVINE, 1967 In vitro morphogenesis of phage P22 from heads and base-plate parts. Proc. Natl. Acad. Sci. USA **57:** 284–291.

JARVIK, J. and D. BOTSTEIN, 1975 Conditional-lethal mutations that suppress genetic defects in morphogenesis by altering structural proteins. Proc. Natl. Acad. Sci. USA **72:** 2738–2742.

KARN, J., S. BRENNER, L. BARNETT and G. CESARENI, 1980 Novel bacteriophage λ cloning vehicle. Proc. Natl. Acad. Sci. USA **77:** 5172–5176.

KLEIN, A., E. LANKA and H. SCHUSTER, 1980 Isolation of a complex between the P protein of phage λ and the *dnaB* protein of *Escherichia coli*. Eur. J. Biochem. **105:** 1–6.

KOBORI, J. A. and A. KORNBERG, 1982a The *Escherichia coli dnaC* gene product. I. Overproduction of the *dnaC* proteins of *Escherichia coli* and *Salmonella typhimurium* by cloning into a high copy number plasmid. J. Biol. Chem. **257:** 13757–13762.

KOBORI, J. A. and A. KORNBERG, 1982b The *Escherichia coli dnaC* gene product. III. Properties of the *dnaB-dnaC* protein complex. J. Biol. Chem. **257:** 13770–13775.

KUWABARA, N. and H. UCHIDA, 1981 Functional cooperation of the *dnaE* and *dnaN* gene products in *Escherichia coli*. Proc. Natl. Acad. Sci USA **78:** 5764–5767.

LARK, K. G. and C. LARK, 1978 *RecA*-dependent DNA replication in the absence of protein synthesis: characteristics of a dominant lethal replication mutation, *dnaT*, and requirement for *recA*$^+$ function. Cold Spring Harbor Symp. Quant. Biol. **43:** 537–550.

LARK, C. A., J. RIAZI and K. G. LARK, 1978 *dnaT*, dominant conditional-lethal mutation affecting DNA replication in *Escherichia coli*. J. Bacteriol. **136:** 1008–1017.

MAURER, R., B. C. OSMOND, E. SHEKHTMAN, A. WONG and D. BOTSTEIN, 1984 Functional interchangeability of DNA replication genes in *Salmonella typhimurium* and *Escherichia coli* demonstrated by a general complementation procedure. Genetics **108:** 1–23.

MCHENRY, C. S. and W. CROW, 1979 DNA polymerase III of *Escherichia coli*: purification and identification of subunits. J. Biol. Chem. **254:** 1748–1753.

MCHENRY, C. and A. KORNBERG, 1981 DNA polymerase III holoenzyme. pp. 39–50. In: *The Enzymes*, Vol. XIV, Edited by P. D. BOYER. Academic Press, New York.

MOIR, D., S. E. STEWART, B. C. OSMOND and D. BOTSTEIN, 1982 Cold-sensitive cell-division-cycle mutants of yeast: isolation, properties, and pseudoreversion studies. Genetics **100:** 547–563.

NIWA, O., S. K. BRYAN and R. E. MOSES, 1981 Alternate pathways of DNA replication: DNA polymerase I-dependent replication. Proc. Natl. Acad. Sci. USA **78:** 7024–7027.

SAITO, H. and H. UCHIDA, 1977 Initiation of the DNA replication of bacteriophage Lambda in *Escherichia coli* K12. J. Mol. Biol. **113:** 1–25.

SCHEUERMANN, R., S. TAM, P. M. J. BURGERS, C. LU and H. ECHOLS, 1983 Identification of the ε subunit of *Escherichia coli* DNA polymerase III holoenzyme as the *dnaQ* gene product: a fidelity subunit for DNA replication. Proc. Natl. Acad. Sci. USA **80:** 7085–7089.

SCLAFANI, R. A. and J. A. WECHSLER, 1981a Suppression of *dnaC* alleles by the *dnaB* analog (*ban* protein) of bacteriophage P1. J. Bacteriol. **146:** 321–324.

SCLAFANI, R. A. and J. A. WECHSLER, 1981b Deoxyribonucleic acid initiation mutation *dnaB252* is suppressed by elevated $dnaC^+$ gene dosage. J. Bacteriol. **146:** 418–421.

SPANOS, A., S. G. SEDGWICK, G. T. YARRANTON, U. HUBSCHER and G. R. BANKS, 1981 Detection of the catalytic activities of DNA polymerases and their associated exonucleases following SDS-polyacrylamide gel electrophoresis. Nucleic Acids Res. **9:** 1825–1839.

WALKER, J. R., J. A. RAMSEY and W. G. HALDENWANG, 1982 Interaction of the *Escherichia coli dnaA* initiation protein with the *dnaZ* polymerization protein *in vivo*. Proc. Natl. Acad. Sci. USA **79:** 3340–3344.

WICKNER, S. and J. HURWITZ, 1975 Interaction of *Escherichia coli dnaB* and *dnaC(D)* gene products *in vitro*. Proc. Natl. Acad. Sci. USA **72:** 921–925.

WICKNER, S. H., 1979 DNA replication proteins of *Escherichia coli* and phage λ. Cold Spring Harbor Symp. Quant. Biol. **43:** 303–310.

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