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P1-mediated Transduction of a Gene That Controls Radiation Sensitivity and Capsular Polysaccharide Synthesis from *Shigella dysenteriae* to *Escherichia coli*

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When *Shigella dysenteriae* strain 60 is used as a donor and *Escherichia coli* K-12 strains that are ultraviolet (UV)-sensitive, mucoid, and proline-requiring (Pro^-) are employed as recipients, selection for Pro^+ yields 2 to 6% nonmucoid clones. All of the nonmucoid clones examined are UV-resistant. Most of the nonmucoid UV-resistant transductants are partial diploids for the genes being studied. When these *Shigella-Escherichia* hybrids are used as donors with the same *E. coli* recipients, the cotransduction of Pro^+ and nonmucoidness is greatly increased (59 to 94% cotransduction). All of these nonmucoid transductants examined were also UV-resistant. The results indicate that *Shigella* contains an allele (designated Sh*proC*$^+$) homologous to *proC* of *E. coli* and a second linked allele (designated Sh*capR*$^+$) homologous to the *capR* allele of *E. coli*. The Sh*capR*$^+$ allele changes the phenotype of certain *E. coli* strains from mucoid UV-sensitive (*capR6*) or very sensitive (*cap*R9) to nonmucoid and UV-resistant. Unanticipated *capR* allele interactions in the partial diploid hybrids are described.

Luria and Burrous first demonstrated that hybrids of *Escherichia coli* and *Shigella dysenteriae* could be formed utilizing F^+ of Hfr strains of *E. coli* K-12 (8). Lennox found that the generalized transducing phage, P1kc (hereafter referred to as P1) can transfer characters between the two genera (7).

CapR [also designated *lon* by Howard-Flanders, Simson, and Theriot (5)] is a regulator gene for polysaccharide synthesis (10, 11) and also controls ultraviolet (UV) and X-ray sensitivity (2, 3, 5, 11, 12; Uretz and Markovitz, *in preparation*). *CapR*$^+$ strains are UV- and X-ray-resistant and nonmucoid, whereas single-step mutants that contain the allele *capR6* are mucoid [i.e., produce large quantities of capsular polysaccharide that contains D-glucose, D-galactose, D-glucuronic acid, and L-fucose (10, 11)] and are UV and X-ray sensitive (2, 3, 5, 11, 12), forming long filaments that are nonseptate after X-ray radiation (3). Strains with the allele *capR9* are mucoid and are even more sensitive to UV than strains with the *capR6* allele (11, 12). Strains that contain the *lon* or *capR9* alleles reactivate UV-treated phage T1 and T7 to the same extent as the wild type (*lon*$^+$ or *capR*$^+$) (5; Uretz, *unpublished data*) and thus are not deficient in repair of UV-treated viral deoxyribonucleic acid (DNA). The mechanism by which *capR* (*lon*) controls radiation sensitivity is unknown at present.

Transduction utilizing phage P1 with *proC*$^+$ as a selective maker permitted recombination studies between *capR6* and *capR9* alleles in *E. coli* K-12 strains (11). Among *proC*$^+$ transductants, a small percentage (0.1 to 0.76%) of nonmucoid (*capR*$^+$) recombinants were detected. All of the *capR*$^+$ clones tested from this and similar crosses (a total of 17) were also UV-resistant (11; and Uretz and Markovitz, *in preparation*). Thus, UV-radiation sensitivity and mucoidness appear to be controlled by one cistron or two cistrons in the same operon (11, 12). In addition, UV sensitivity and capsular polysaccharide synthesis associated with either *capR6* or *capR9* are suppressed by ochre suppressors (12). We have therefore concluded that the *capR* gene product involved in UV sensitivity and capsular polysaccharide synthesis is a protein (6, 12).

The present study demonstrates that *S. dysen-*

teriae strain 60 contains *Shigella proC*$^+$ (Sh*proC*$^+$) linked to *Shigella capR*$^+$ (Sh*capR*$^+$). These genes, after transduction by P1, convert a mucoid UV-sensitive (*capR6*) or very sensitive (*capR9*) Pro$^-$ (*proC1*) strain of *E. coli* K-12 to a nonmucoid, UV-resistant, and proline-independent (Sh*capR*$^+$ Sh*proC*$^+$) strain. Evidence is also presented that partial diploidy occurs frequently when these *S. dysenteriae* 60 genes are transferred to *E. coli* K-12.

MATERIALS AND METHODS

Bacteria. S. dysenteriae strains 60 and 16 were provided by L. Barksdale and S. E. Luria, respectively. Both strains are P1-sensitive (P1-S). *E. coli* K-12 strains used are described in Table 1.

Media. M-9 minimal medium (1) was supplemented with 2.5×10^{-3} M $CaCl_2$, 10 μg of thiamine HCl per ml, 0.6% D-glucose, and other components as required for selection of transductants and growth of original strains. Streptomycin (200 μg/ml) was present in M-9 minimal medium, and L broth (9) was used to grow strains for transduction.

Transduction. The procedures of Lennox (7) were employed. Sensitivity or immunity to phage P1 was determined as described by Luria et al. (9).

Radiation sensitivity. Media and detailed procedures for UV radiation experiments used in these studies have been published (12). The UV exposure was 450 ergs per cm^2 per sec, as determined by a YSI-Kettering model 65 radiometer. The sensitivity to UV of strains carrying different *capR* alleles is illustrated in Fig. 1. The genotypes and corresponding phenotypes with respect to response to UV are designated as follows: *CapR*$^+$ = UV resistant (UV-R); *capR6* = UV sensitive (UV-S); *capR9* = UV very sensitive (UV-VS). The responses of *Shigella-Escherichia* hybrids (strains SE 103 and SE 104) are designated as UV intermediate (UV-I). When the terms UV-R, UV-S, UV-VS, or UV-I are used, they indicate that two or more separate survival curves were determined unless otherwise stated. All survival curves were determined with three or more different doses of UV. Thus, a strain with phenotype UV-R responded to UV as did strain MC100, and a strain with phenotype UV-VS responded as did strain MC128 (Fig. 1). When the recipient used was P1-S, only transductants that were still P1-S were examined for their response to UV.

RESULTS

Transduction of nonmucoidness and Pro$^+$ *from Shigella to E. coli K-12.* When phage P1 grown on *S. dysenteriae* strain 60 or 16 were used to transduce Pro$^-$ (*proC1*) strains of *E. coli* K-12 Pro$^+$ (Sh*proC*$^+$). transductants were obtained at a frequency of transduction (approximately 10^{-8} transductants per plaque-forming unit) similar to that reported by others studying galactose or lactose transduction with *Shigella* as a donor and *E. coli* as a recipient (7, 9) (Table 2). Of particular interest is the fact that 2 to 6% of Sh*proC*$^+$ transductants were also nonmucoid (Sh*capR*$^+$) although the original recipients were mucoid, thus demonstrating cotransduction of Sh*proC*$^+$ and Sh*capR*$^+$. When several nonmucoid *Shigella-Escherichia* hybrids were plated on media with or without proline, it was found that some segregated mucoid clones. The results of these analyses (Table 3) indicate that partial diploids resulted from transduction of the *Shigella* genes Sh*capR*$^+$ Sh*proC*$^+$ to *E. coli* (*capR proC*). For example, many clones that were nonmucoid and Pro$^+$ segregated mucoid clones that were Pro$^-$. This result indicates that the hybrid was a partial diploid of the following genotype: Sh*capR*$^+$ Sh*proC*$^+$ *capR proC*. Other recombinant types of partial diploid were detected (i.e., a mucoid Sh*proC*$^+$ *proC*). By methods previously described (9), we attempted to prepare high-frequency transducing lysates for the *capR proC* region using

TABLE 1. *Mutant strains of E. coli K-12 used in this study*

Strain	Source[a]	Mutant loci and mutation sites								Relevant phenotype[b]								
		capR	*leu*	*proC*	*purE*	*trp*	*lac*	*gal*	*str*	Muc	Leu	Pro	Pur	Trp	Lac	Gal	Str	P1
MC100	A	+	1	1	1	1	1	1	1	N	−	−	−	−	−	−	R	S
MC101	B	6	1	1	+	1	1	1	1	M	−	−	+	−	−	−	R	S
MC127	B	6	1	1	+	1	2	+	1	M	−	−	+	−	−	+	R	Im
MC128	B	9	+	1	1	+	1	+	1	M	+	−	−	+	−	+	R	S

[a] A = R. Curtiss III, W945. Strain MC100 was previously designated strain X-156 (11, 12). B = This laboratory (11, 12). Strains MC101, MC127, and MC128 were previously designated strains S43-1, S18-12, and S50-9-3a, respectively (11, 12). *Cap R*$^+$, *cap R6*, and *cap R9* were previously designated R_1^+, R_{1-M6}^- and R_{1-9}^c, respectively (11, 12).

[b] (Muc) colony morphology; (Leu) leucine; (Pro) proline; (Pur) adenine or guanine; (Tryp) tryptophan; (Lac) lactose; (Gal) galactose; (Str) streptomycin; (P1) bacteriophage P1kc; (N) nonmucoid; (M) mucoid; (−) absent or required; (+) synthesized or utilized; (R) resistant; (S) sensitive; (Im) immune.

partial diploids that were P1 immune, but the attempts were unsuccessful.

Transduction of nonmucoidness and Pro$^+$ from Shigella-Escherichia hybrids to E. coli. When P1 was grown on nonmucoid Pro$^+$ *Shigella-Escherichia* hybrids of the type Sh*capR*$^+$ Sh*proC*$^+$/ *capR proC* and the phage was used to transduce *E. coli* (*capR proC*), it was found that 59 to 94% of Pro$^+$ transductants were nonmucoid (Table 4). This is to be contrasted with the results in Table 2 where only 2 to 6% joint transduction of Sh*capR*$^+$ and Sh*proC*$^+$ occurred. In addition, the frequency of transductants per plaque-forming unit was approximately 30-fold greater in the transduction of genes from the hybrid to *E. coli* as compared to the transduction of genes from *S. dysenteriae* 60 to *E. coli*.

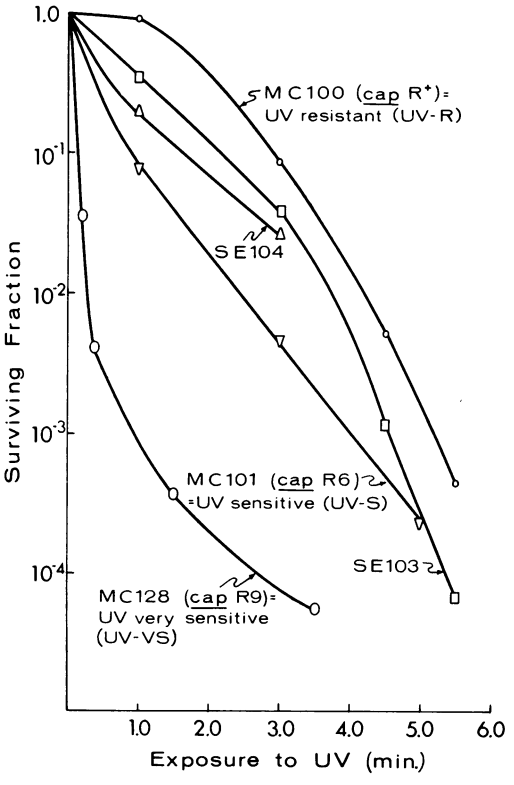

FIG. 1. *Fraction of E. coli (strains MC100, MC101, and MC128) and Shigella-Escherichia hybrids (mucoid strains SE103 and SE104) that survive UV radiation.*

Analysis of UV response of Shigella-Escherichia hybrids. We have examined the response to UV of *Shigella-Escherichia* hybrids and segregants reported in Tables 2, 3, and 4. It was found that all (more than 20) of the nonmucoid Pro$^+$ hybrids tested from independent transduction events were UV-R (a single irradiation experiment for each transductant) as defined in Fig. 1 and Materials and Methods. This was true both of clones that were demonstrated to be partial diploids (experiment 1, Table 5) and of clones in which partial diploidy was not observed.

Two types of unanticipated results have been noted in examining the proline-independent *Shigella-Escherichia* hybrids that were mucoid.

(I) Certain of these mucoid Pro$^+$ strains (SE 103 and SE104) responded to UV intermediate (UV-I) between UV-R and UV-S (Fig. 1 and experiment 2, Table 5). Strain SE103 was used as a donor in transduction experiments with *E. coli* strain MC100 (*proC1*, *capR*$^+$) as a recipient. Out of 956 Pro$^+$ transductants, only 5 were mucoid. When all five mucoid transductants were tested with UV, they gave a UV-I response (single experiment for each clone). Strains SE 103 and SE104 were stable in that they did not segregate nonmucoid clones but did segregate Pro$^-$ clones. The UV response of a mucoid Pro$^-$ clone derived from SE103 was like the original recipient, i.e., UV-S (single irradiation experiment). The UV-I response has also been noted in two of three mucoid transductants when a nonmucoid *Shigella-Escherichia* hybrid (strain SE 101) was used as donor and strain MC101 (*capR6*

TABLE 2. *Transduction of mucoid strains of E. coli K-12 to nonmucoid by phage P1 grown on S. dysenteriae*

Donor strain	MOI[a]	Mucoid recipient strain	Proline-independent		Per cent nonmucoid
			Nonmucoid	Total	
S. dysenteriae 60	5	MC127	10	420	2.4
S. dysenteriae 60	1 to 5	MC101	33	792	4.2
S. dysenteriae 60	1	MC128	6	101	6
S. dysenteriae 16	1 to 5	MC127	1	64	2

[a] MOI (multiplicity of infection) as determined with *E. coli* K-12 as a host. Phage P1 grown on *Shigella dysenteriae* 60 yielded 2.8 times more plaques when titered on *S. dysenteriae* 60 than on *E. coli* K-12 as a host.

TABLE 3. *Examination of transductants for partial diploidy of capR and proC among proC⁺ transductants*

Donor	*E. coli* recipient	P1[a]	No. tested	Muc	Transductant clones		
					Stable[b] with or without proline	Unstable[b]	
						With proline	Without proline
Shigella 60	MC127 (*capR6*)	—	8	N	4	2[c]	2
		Im	3	N	1	1	1[d]
Shigella 60	MC101 (*capR6*)	S	22	N	13[e]	4	2[d], 3
		—	10	M[f]	10	0	0
		Im	1	N	0	0	1
Shigella 60	MC128 (*capR9*)	S	5	N	5	0	0
		—	10	M	10	0	0
Shigella 16	MC127 (*capR6*)	—	1	N	0	1[c]	0

[a] Im = immune to P1; S = sensitive to P1; — = not tested.

[b] Stable nonmucoid clones (N) are defined as those that yield no more than two mucoid clones (M) on a streak plate. Unstable N clones are defined as those that yield many M clones on a streak plate.

[c] Some mucoid segregants were demonstrated to be *proC*.

[d] More mucoid segregants were observed with proline.

[e] Some of these stable clones, as defined in footnote *b* above, yielded one or two mucoid segregants on streak plates, as do most wild-type *E. coli* strains. However, mucoid segregants from two were tested and found to be *proC*. Thus, some stable clones are also partial diploids (Sh*capR*⁺ Sh*proC*⁺ *capR6 proC*).

[f] Stability of M clones refers to detection of N clones. Although all M clones were stable with respect to mucoidness, one was found to yield *proC* clones. Thus, partial diploidy for *proC* (Sh*proC*⁺ *proC*) was observed.

TABLE 4. *Transduction of proline-requiring mucoid strains of E. coli by P1 grown on Shigella-Escherichia hybrids that are proline-independent and nonmucoid*

Donor[a] (nonmucoid)	Origin of donor	Recipient (mucoid)	Proline-independent		Nonmucoid
			Nonmucoid	Total	
					%
SE100	P1(Sh 60) × MC128	MC101	154	164	94
SE101	P1(Sh 60) × MC101	MC101	159	211	75
SE102	P1(Sh 60) × MC101	MC101	142	241	59

[a] The multiplicity of infection was less than 1.

TABLE 5. *Genotypes and phenotypes of Shigella-Escherichia hybrids*

Expt	Strain	Origin	Genotype		Phenotype		
			proC	*capR*	Pro	Muc	UV[a]
1	SE100[b]	P1 (*Shigella* 60) × MC128	Sh+/1	Sh+/9	+	N	R
	SE101[b]	P1 (*Shigella* 60) × MC101	Sh+/1	Sh+/6	+	N	R
2	SE103	Same as SE101	Sh+/1	Sh+/6	+	M	I
	SE104	Same as SE101	Sh+/1	Sh+/6	+	M	I
3	SE105	Same as SE100	Sh+/1	Sh+/9	+	M	R
	SE106	Nonmucoid segregant of SE105	Sh+/1	Sh+/9	+	N	R
	SE107	Mucoid segregant of SE106	Sh+/1	Sh+/9	+	M	R
	SE108	Proline-requiring mucoid segregant of SE106	1	9	−	M	VS

[a] The determination of the response to UV is described in Materials and Methods. UV-R, UV-I, and UV-VS are defined as giving survival curves similar to strains MC100, SE103, and MC128, respectively (Fig. 1).

[b] Partial diploidy was determined by the observation that recessive phenotypes segregated, i.e., Pro⁻ mucoid clones that were UV-VS (*capR9*) or UV-S (*capR6*) and Pro⁺ mucoid clones that were UV-S (*capR6*).

proC) was the recipient (Table 4). Thus, it appears that Sh*capR*$^+$ can interact with *capR6* so that the resultant phenotype is mucoid and UV-I (Table 5, experiment 2) or nonmucoid and UV-R (Table 5, experiment 1).

(II) The second unanticipated result was detected in a mucoid clone that produced a complete nonmucoid border. This clone yielded a mucoid UV-R strain (designated SE105, Table 5) when plated. Strain SE105 segregated nonmucoid clones that were also UV-R, designated SE106; strain SE106 segregated mucoid clones that were UV-R (just like SE105); strain SE106 *also* segregated Pro$^-$ mucoid clones that were UV-VS (the survival curves of strains SE 105 through SE108 were determined in single experiments). This latter phenotype was identical to the original recipient, strain MC128. Thus, the presence of Sh*capR*$^+$ and *capR9* can result in either a mucoid or nonmucoid phenotype and a characteristic UV-R response.

DISCUSSION

The results of this study demonstrate that *Shigella dysenteriae* strain 60 contains genes homologous to *capR* and *proC* of *E. coli* K-12. These genes, designated Sh*capR*$^+$ and Sh*proC*$^+$, when transduced to a mucoid UV-VS (*capR9*) Pro$^-$ (*proC1*) *E. coli* strain, yield a nonmucoid, UV-R Pro$^+$ phenotype. Most of these strains were found to be partial diploids, a common result when *Shigella-Escherichia* hybrids are formed (8, 9).

In the first transduction with *Shigella* as a donor and *E. coli* strain MC101 (*capR6 proC1*) as a recipient, 4% of Pro$^+$ transductants were nonmucoid. In contrast, when the nonmucoid *Shigella-Escherichia* hybrid from the above cross was the donor and *E. coli* strain MC101 was again the recipient, 75% of Pro$^+$ transductants were nonmucoid. A similar increase in linkage was found by Wilson for contransduction of the threonine-leucine region when he obtained lysogenic bacteria that liberated high-frequency transducing phage (13). However, our hybrids with increased linkage were sensitive to P1 and were nonlysogenic, whereas his strains were immune to P1. Our frequency of transduction is also too low (1 transduction per 10^5 to 10^6 plaque-forming units) to be considered as high-frenquency transduction. The increase in linkage of Sh*capR*$^+$ and Sh*proC*$^+$ requires further investigation.

Two unanticipated results have been noted in examining Pro$^+$ *Shigella-Escherichia* hybrids that were mucoid. First, an intermediate response to UV was noted (Fig. 1), and strain SE 103 (Table 5) was shown to segregate Pro$^-$ clones that were still mucoid but with the original UV phenotype (UV-S); this result indicated that both Sh*capR*$^+$ and *capR6* genes were present. In comparison, the partial diploid from the same cross that was nonmucoid and UV-R (SE101, Table 5) also segregated mucoid UV-S (*capR6*) clones. Thus, the same partial diploid genotype (Sh*capR*$^+$ Sh*proC*$^+$/*capR6 proC1*) yielded two different phenotypes: mucoid UV-I and nonmucoid UV-R.

The second unanticipated result was that the same genotype (Sh*capR*$^+$ *capR9*) resulted in strains that were mucoid (SE105) or nonmucoid (SE106), although both were UV-R (Table 5). It seems worthwhile to recount previous results that may bear on the results reported above. Previously, *capR* alleles have been obtained on an episome designated F′ 13 (10, 11). Studies with partial diploids exclusively derived from *E. coli* K-12 showed that UV-R was dominant to UV-VS in partial diploids (either F′ 13 *capR9*/*capR*$^+$ or F′ 13 *capR*$^+$/*capR9*) (Uretz and Markovitz, *in preparation*), although F′ 13 *capR9*/*capR*$^+$ was mucoid and F′ 13 *capR*$^+$/*capR9* was nonmucoid (11). The dominance with respect to mucoid synthesis of the allele of *capR* on the F′ 13 episome led to the hypothesis that the active product of the *capR* gene was a protein oligomer composed of several monomers. Mucoidness would be the result of a greater number of monomers produced by the *capR9* allele as compared to the *capR*$^+$ allele; nonmucoidness would be the result of more monomers designated by *capR*$^+$ as compared to *capR9* (11). However, all combinations of monomers specified by *capR*$^+$ or *capR9* would yield oligomers that lead to UV-R (Uretz and Markovitz, *in preparation*). The unusual phenotypes described in this paper can be explained using the interaction of monomers specified by Sh*capR*$^+$ and *capR9* or *capR6* to make oligomers with different regulatory properties with respect to mucoidness and UV sensitivity. However, we have no proof, with the *Shigella-Escherichia* hybrids, as to the presence or absence of episomes. We have attempted to cure some of the rather stable partial diploid strains with acridine orange (4) but have noted no increase in segregation due to growth in this compound. Thus, tandem insertions to form partial diploids must also be considered, and certain types of polarity effects caused by the position of the tandem insertion could also explain the unusual phenotypes found.

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