JOURNAL OF BACTERIOLOGY, Aug. 1967, p. 388-395
Vol. 94, No. 2

Suppression of Radiation Sensitivity and Capsular Polysaccharide Synthesis in *Escherichia coli* K-12 by Ochre Suppressors

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Received for publication 26 May 1967

A class of suppressors effective on nonsense polarity mutations suppresses several independent mutations in a gene (*capR*) that controls ultraviolet-radiation sensitivity and capsular polysaccharide synthesis. Since these ochre suppressors function at the level of translation of messenger ribonucleic acid into protein, the results provide evidence that the product of the *capR* gene is a protein.

The sensitivity of *Escherichia coli* K-12 to ultraviolet (UV) and X rays is controlled by several genetic loci. One of these, designated *lon* by Howard-Flanders, Simson, and Theriot (20), also controls capsular polysaccharide (mucoid) synthesis and was designated as R_1 by Markovitz (26). Hereafter, R_1 will be designated *capR*. (Other changes in allele and strain designation are described in Table 1.) Two types of mucoid strains caused by mutation at the *capR* locus have been isolated (26, 27). One, designated *capR6*, is recessive to $capR^+$ (nonmucoid) in heterozygous partial diploids regardless of their relative positions on the F'13 episome and chromosome. A second, designated *capR9* or *capR66*, is dominant to $capR^+$ when it is on the F'13 episome (F'13 $capR9/capR^+$ = mucoid) but recessive when on the chromosome (F'13 $capR^+/capR9$ = nonmucoid) (27); i.e., episomal dominance of *capR9* or $capR^+$. An additional difference is that, although the haploid *capR6* strain is sensitive to UV, the haploid *capR9* strain is even more sensitive to UV (Uretz and Markovitz, *in preparation;* Fig. 1). Radiation resistance is dominant in partial diploids containing $capR^+$ and *capR9* (F'13 $capR9/capR^+$ or F'13 $capR^+/capR9$ = radiation-resistant) (Uretz and Markovitz, *in preparation*) although, as noted above, F'13 $capR9/capR^+$ is mucoid but F'13 $capR^+/capR9$ is nonmucoid.

The results demonstrating episomal dominance of *capR9* or $capR^+$ alleles with respect to mucoid synthesis led to the hypothesis that the product of the *capR* gene that controls radiation sensitivity and capsular polysaccharide synthesis is a protein composed of subunits (27). Recombination analysis mediated by transducing bacteriophage P1 demonstrated that (i) *capR6* and *capR9* are linked to *proC* on the side opposite to *lac;* (ii) small numbers of nonmucoid recombinants are obtained by crossing *capR6* and *capR9;* and (iii) these nonmucoid recombinants are $capR^+$ and are UV-resistant like the wild type. From these results, it was concluded that mucoidness and UV-radiation sensitivity are controlled by one cistron or, if the mutations are nonsense polar mutations, two cistrons in the same operon (27).

Observations of Adler and Hardigree showed that, after X-ray radiation, *lon* (radiation-sensitive, mucoid) and *capR6* strains plated on rich medium form long filaments that are nonseptate (2, 3). The *lon* and *capR9* strains reactivate UV-treated bacteriophage T1 or T7 to the same extent as the wild type (lon^+ or $capR^+$) (20; Uretz, *unpublished data*) and thus are not deficient in repair of UV-treated deoxyribonucleic acid (DNA). The mechanism by which *capR* (*lon*) controls radiation sensitivity is unknown at the present time.

Ochre suppressors function at the level of translation of messenger ribonucleic acid (mRNA) into protein (8, 9, 31). This report shows that UV-radiation sensitivity and capsular polysaccharide synthesis are suppressed by ochre suppressors. The results, therefore, provide further evidence that the *capR* gene product involved in both of these processes is a protein. A preliminary report of this work has been presented (A. Markovitz and B. Baker, Abstr. Intern. Congr. Biochem., 7th, 1967).

MATERIALS AND METHODS

Bacteria. All strains of bacteria were derivatives of *E. coli* K-12. Their genotype, source, and derivation 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/ml, 0.6% D-glucose, and other components as required for selection of transductants and growth of strains for measurement of capsular polysaccharide synthesis. Streptomycin (200 μg/ml) was present in M-9 minimal medium. L broth (25) was used to grow strains for transduction. Yeast extract-Nutrient Broth (YEN broth), for growing strains to be UV-radiated, was made up as follows: 8 g of Nutrient Broth (Difco), 1 g of yeast extract (Difco), 5 g of NaCl, and water to 1 liter. The agar used for plating in irradiation experiments (YET agar) was made up as follows: 10 g of tryptone (Difco), 5 g of yeast extract (Difco), 10 g of NaCl, 21 g of agar, and water to 1 liter.

Transduction. The procedures of Lennox (24) were employed. Immunity or sensitivity to P1 was determined as described by Luria et al. (25).

Procedure for irradiation experiments. Bacteria were grown for approximately 16 hr in YEN broth at 37 C with aeration by means of a reciprocal shaker. These cells were in the stationary phase of growth. They were centrifuged at 4 C, washed, and resuspended in buffer (6 g of Na_2HPO_4 and 3 g of KH_2PO_4 in 1 liter) to a concentration of 10^8 to 3×10^8 cells/ml. A 10-ml amount of cell suspension in a plastic petri plate (diameter, 5 cm) was stirred by means of a magnetic stirrer and was UV-irradiated with a 15-w GE germicidal lamp at a distance of 46 cm from the bottom of the UV lamp to the liquid surface (9.5 cm of the lamp was exposed). The UV exposure was 450 ergs per cm^2 per sec, as determined by a YSI-Kettering model 65 Radiometer. After irradiation, all procedures were carried out in semidark conditions (yellow or no light), including incubation of plated cells (37 C).

UV-radiation data presented for strains that contained *supC* are the averages of data from three independent transductions of *supC* into the strains indicated (Fig. 2 and 3). No differences in UV sensitivity between these independent transductants that contained *supC* were noted. All radiation data are the averages of two to seven separate experiments. For spreading experiments (Fig. 4), 0.1 ml of buffer was added and the plates were respread with a bent-glass rod in the usual manner. Nonrespread controls were run in all experiments.

TABLE 1. *Mutants of Escherichia coli K-12 used*

Strain[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
MC100[c]	+	1	1	1	1	1	1	1	N	−	−	−	−	−	−	R
MC101[d]	6	1	1	+	1	1	1	1	M	−	−	+	−	−	−	R
MC102[d]	9	1	+	1	1	1	1	1	M	−	+	−	−	−	−	R
MC103[d]	66	1	+	1	1	1	1	1	M	−	+	−	−	−	−	R
MC104[e]	6	1	1	+	1	1	1	1	N	+	−	+	−	−	−	R
MC114[f]	+	+	+	+	1	+	+	+	N	+	+	+	−	+	+	S
MC115[g]	+	+	+	+	1	+	+	+	N	+	+	+	−	+	+	S

[a] Sources of the strains listed below are given in the succeeding footnotes. Other strains were prepared as follows. Sup-3 was transferred from MC104 to the following strains by selecting the Leu$^+$ phenotype in transductions: P1 (MC104) × MC101 = strain MC105; P1 (MC104) × MC102 = strain MC106; P1 (MC104) × MC103 = strain MC107. SupA and SupC were transferred to the same recipients by selecting the Trp$^+$ phenotype in transductions; P1 (MC114) × MC101, MC102, or MC103 to yield strains MC108, MC109, or MC110, respectively; P1 (MC115) × MC101, MC102, or MC103 to yield strains MC111, MC112, or MC113, respectively. All strains used in this study were sensitive to bacteriophage P1 (24).

[b] Muc, colony morphology; Leu, leucine; Pro, proline; Pur, adenine or guanine; Trp, tryptophan; Lac, lactose; Gal, galactose; Str, streptomycin; N, nonmucoid; M, mucoid; −, absent or required; +, synthesized or utilized; R, resistant; S, sensitive.

[c] From R. Curtiss III, W945. Strain MC100 was previously designated strain X-156 (26, 27).

[d] From this laboratory (26, 27). Strains MC101, MC102, and MC103 were previously designated strains S43-1, T72-C2, and T72-E2 respectively (27). *CapR6*, *capR9*, and *capR66* were previously designated R^-_{1-M6}, R^C_{1-9}, and R^C_{1-66}, respectively (26, 27).

[e] A leucine-positive nonmucoid revertant of MC101 that contains an ochre suppressor designated *sup-3*.

[f] From C. Yanofsky (32) via M. Rachmeler. Strain MC114, previously designated strain W3110-tryp del A-E_1 su_{oc} A, carries a deletion of the *trp* region and contains an ochre suppressor linked to *gal* (32). The suppressor, previously designated su_{oc} A(32), is now designated *sup* A.

[g] From C. Yanofsky (32) via M. Rachmeler. Strain MC115, previously designated strain W3110-tryp del A-E_1 su_{oc} C, carries a deletion of the trp region and contains an ochre suppressor linked to *trp* (32). The suppressor, previously designated su_{oc} C(32) is now designated *supC*.

Release and determination of capsular polysaccharide. Most of the polysaccharide was usually found in the supernatant liquid of centrifuged cultures, but some strains retained more polysaccharide as capsules than others. Therefore, liquid cultures were placed in a boiling-water bath for 15 min before centrifugation. This procedure released essentially all the capsular polysaccharide from the cells. Cells were removed by centrifugation, and the supernatant liquid was dialyzed overnight against running tap water. Methylpentose was determined by the method of Dische and Shettles (10) with L-rhamnose as a standard (10-min boiling period).

RESULTS

Effect of suppressors on polysaccharide synthesis. The first indication that a suppressor could prevent capsular polysaccharide synthesis (due to *capR6*) was the following. Approximately 5×10^7 cells of strain MC101 (*capR6 leu-1*) were plated on media without leucine and a single nonmucoid clone grew. P1 was grown on this strain (designated MC104) and the phage were used to transduce the original strain to Leu^+. All Leu^+ transductants were nonmucoid. P1 phage grown on strain MC104 [P1 (MC104)] were also able to transduce two other *leu-1* independent mucoid mutations at *capR* (*capR9* and *capR66*) to nonmucoid when Leu^+ was selected (all Leu^+ clones were nonmucoid). The quantitative comparison of the effect of the suppressor [designated *sup-3*; this suppressor suppresses amber and ochre mutants of bacteriophage T4 (D. McMahon and R. Haselkorn, *personal communication*)] of *leu-1* and *capR6*, *capR9*, or *capR66* on polysaccharide synthesis in liquid media is shown in Table 2. *Sup-3* prevents polysaccharide synthesis due to *capR6*, *capR9*, and *capR66* almost completely in all three strains. The effect of *supC* on polysaccharide synthesis is duscussed below.

Yanofsky and Ito have constructed derivatives of strain W3110 containing a deletion of the entire *trp* region and each of six suppressors, including three amber and three ochre suppressors (32). We tested all of the suppressors by transduction and found that *supA* and *supC* suppress the *trp-1* mutation in our strains. Each of these two suppressors was transduced into *trp-1* strains that contained *capR6*, *capR9*, or *capR66*. On original transduction plates, all trp^+ clones were still mucoid. Transductants that contained *supA* were not studied further with respect to polysaccharide synthesis. Transductants that contained *supC* in combination with *capR6*, *capR9*, or *capR66* were found to vary with respect to polysaccharide synthesis. For example, when they were grown in minimal medium at 37 C and subsequently plated on minimal agar at 37 C, mucoid and nonmucoid clones were observed (from 69 to 88% of the clones appeared nonmucoid). However, these nonmucoid clones became mucoid when replicated onto the same medium at 37 C and, furthermore, the apparent nonmucoid clones on the original plates that were replicated became mucoid when reincubated at 37 C. Tryptophan did not influence these results. The quantitative comparison of the effect of *supC* on polysaccharide synthesis in liquid media is shown in Table 2. It is apparent from the results described and those presented in Table 2 that *supC* reduces synthesis of capsular polysaccharide, but not to the same extent that *sup-3* does.

TABLE 2. *Suppression of capsular polysaccharide synthesis by ochre suppressors*

Strain	Genotype	Growth[a]	Production of polysaccharide[b]
MC101	*capR6*	0.37	146
MC105	*capR6, sup-3*	0.35	0
MC111	*capR6, supC*	0.29	11
MC102	*capR9*	0.40	185
MC106	*capR9, sup-3*	0.38	9
MC112	*capR9, supC*	0.34	57
MC103	*capR66*	0.32	70
MC107	*capR66, sup-3*	0.36	9
MC113	*capR66, supC*	0.34	32

[a] As optical density at 600 mμ. Bacteria were grown on minimal medium supplemented as required (with and without the amino acid affected by the suppressor; i.e., with and without leucine for *sup-3* or with and without tryptophan for *supC* at 37 C) on a reciprocal shaker. The results are the average of those obtained with and without the amino acid affected by the suppressor, since no effect of that amino acid was noted on growth or polysaccharide synthesis. All cultures harvested were in the stationary phase of growth.

[b] Micrograms of nondialyzable methylpentose per milliliter of medium per unit of optical density.

Effect of suppressors on UV-radiation sensitivity. The response of strains containing $capR^+$, *capR6*, *capR9*, or *capR66* to UV radiation are given in Fig. 1. The *capR9* and *capR66* mutations make the strains very sensitive to UV compared with the strain that contains $capR^+$ (MC100). The *capR6* mutation makes the strain UV-sensitive (intermediate between $capR^+$ and *capR9*). These responses are characteristic of the particular *capR* mutation, since when each is transduced into a number of different strains the same response to UV is observed, as indicated in Fig. 1.

The effects of ochre suppressors on UV sensitivity due to the *capR9* mutation are illustrated in Fig. 2. There is an increase in UV resistance

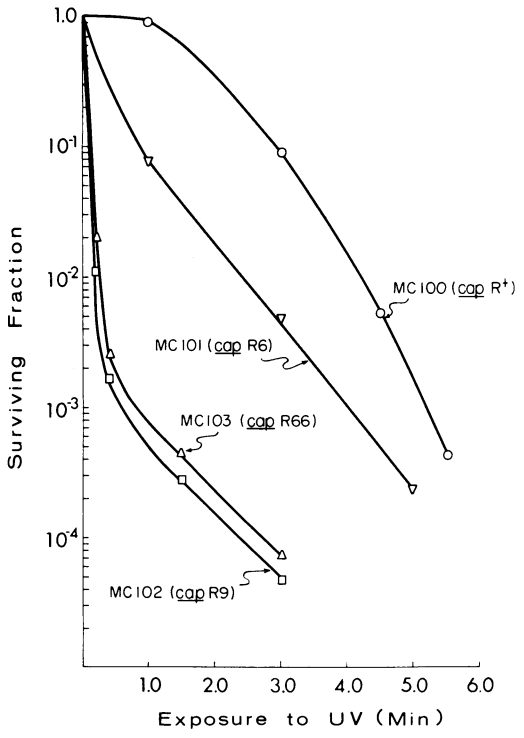

FIG. 1. *Fraction of capR$^+$, capR6, capR66, and capR9 strains that survive UV radiation.*

associated with each of the three suppressors, *supC* being the most effective. Virtually identical results were obtained when the effects of the ochre suppressors on the *capR66* mutation were studied (data not presented). The effects of ochre suppressors on UV sensitivity associated with the *capR6* mutation are presented in Fig. 3. Again, *supC* is the most effective; *capR6 supC* is almost as UV-resistant as *capR$^+$* (compare Fig. 1 and 3). The effects of *supA* and *sup-3* on UV sensitivity associated with *capR6* are relatively small. Other experiments showed that neither *supC* nor *sup-3* affected the radiation resistance or the colony morphology of *capR$^+$* strains.

UV sensitivity associated with *lon* is observable when bacteria are plated on rich media. When *lon* strains are UV-treated and plated on minimal medium, they are as UV resistant as *lon$^+$* strains (20). We also have observed that strains containing *capR6*, *capR9*, or *capR66* are as UV-resistant as *capR$^+$* strains when plated on minimal medium. Therefore, it might be argued that the effect of ochre suppressors on the UV response of *capR* mutants is an indirect effect: reduction of the growth rate on rich media (YET agar). We have attempted to examine this possibility by measuring the time at which division occurs and the generation time under the conditions of the UV experiments as follows. Strains MC102 (*capR9*), MC106 (*capR9 sup-3*), and MC112 (*capR9 supC*) were grown and plated as in other UV studies (with and without UV radiation) and incubated at 37 C. At intervals from 0 to 8 hr, plates were respread and reincubated at 37 C to obtain plate counts. The results, shown in Fig. 4, are complicated by the killing of a fraction of the plated cells after 2 to 3 hr of incubation by the respreading process. This sensitivity to respreading was also observed with the wild type (*capR$^+$*). The loss of colony-forming units due to respreading cells right after they are first spread on the agar surface is minimal, except in the UV-treated *capR9* strain (zero-time, Fig. 4). There does not appear to be any demonstrable effect of *supC* or *sup-3* on the time at which division starts or on the generation time in *capR9* strains (Fig. 4) but the results are not conclusive. Another way of testing whether or not an increase in generation time increases survival on rich media is to grow cells at lower temperature before or after UV treatment, or at both times. Strain MC102 was

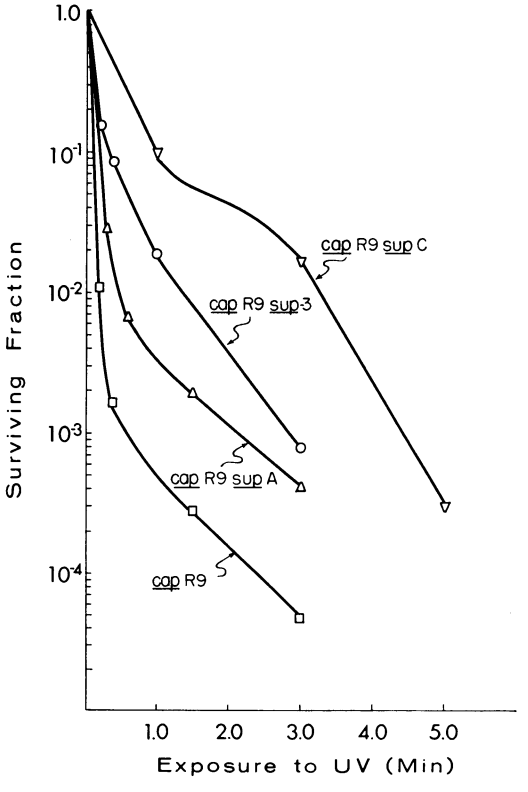

FIG. 2. *Suppression of UV-radiation sensitivity of strain MC102 (capR9) by ochre suppressors.*

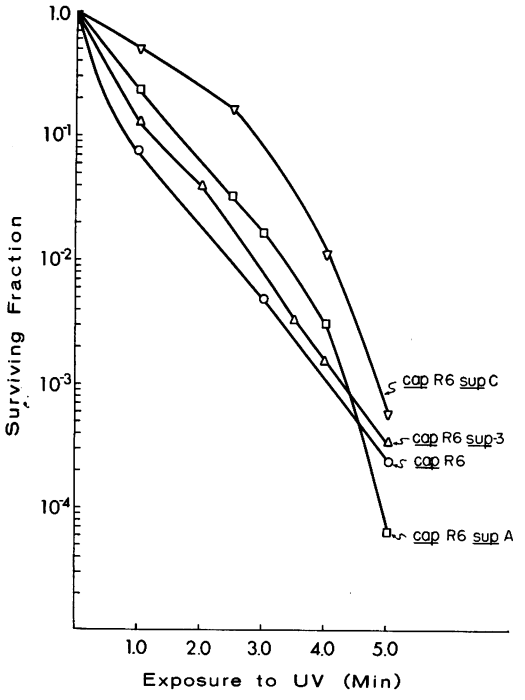

FIG. 3. *Suppression of UV-radiation sensitivity of strain MC101 (capR6) by ochre suppressors.*

grown at 23 and 37 C in YEN broth, washed, and treated with UV. Samples of each culture were then plated on YET agar and incubated at 23 and 37 C (i.e., all possible combinations). The results demonstrated no significant differences in UV sensitivity, despite the fact that it took two to three times as long to obtain maximal colony counts on plates incubated at 23 C. Thus, a substantial reduction of the growth rate on rich medium does not affect the UV sensitivity of strain MC102.

DISCUSSION

Ochre suppressors are active in reversing the effects of nonsense (8, 32) but not missense (32) mutations. Since two ochre suppressors suppress UV radiation sensitivity and capsular polysaccharide synthesis associated with *capR9*, *capR66*, and *capR6*, these mutations may be considered as nonsense mutations [either amber or ochre (8, 32)] in *capR*. Evidence from numerous studies indicates that suppression of nonsense mutations takes place at the level of translation of mRNA into protein (6, 9, 16, 30, 31). Suppression of nonsense mutations in regulator genes of the β-galactosidase (7, 28) and alkaline phosphatase systems (14) by suppressors of the amber-ochre class has been taken as evidence that these regulator genes specify proteins. Subsequent studies

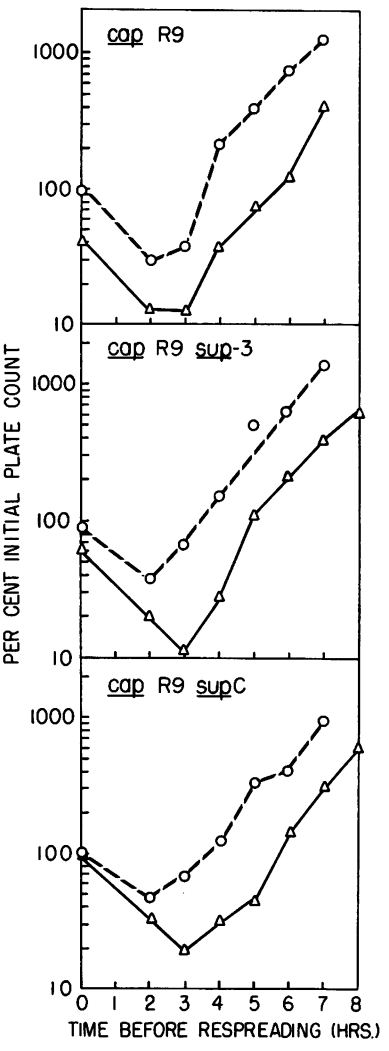

FIG. 4. *Effect of respreading plated bacteria at various times after plating (incubation temperature, 37 C) on the final plate count. The initial plate counts at time zero (not respread) for the unirradiated and irradiated bacteria are each set equal to 100%. (a) Strain MC102 (capR9); ○, unirradiated control; △, UV-irradiated before plating to a survival of 4.4% (30 sec). (b) Strain MC106 (capR9 sup-3); ○, unirradiated control; △, UV-irradiated before plating to a survival of 29% (60 sec). (c) Strain MC112 (capR9 supC); ○, unirradiated control; △, UV-irradiated before plating to a survival of 55% (60 sec).*

have confirmed that the *i* gene product in the β-galactosidase system (18) and the product of one of the regulator genes of alkaline phosphatase (15) are proteins. Similarly, the suppression of both UV radiation sensitivity and capsular polysaccharide synthesis by ochre suppressors pro-

vides evidence that the *capR* gene product involved in these processes is a protein. This is consistent with previous genetic (27) and biochemical (22) studies which suggested that the *capR* gene product was a protein composed of subunits.

At least four enzymes involved in capsular polysaccharide synthesis are elevated in *capR* mucoid mutants as compared with *capR*$^+$ strains (26; Markovitz, *unpublished data*), but a mucoid mutation at another locus [*capS* linked to *trp*; *capS* was previously designated R_2-mucoid (22, 26, 27)] does not lead to elevation of any of these enzymes (with the possible exception of the last enzyme in guanosine-5′-diphosphate-fucose synthesis; Markovitz and Lieberman, *unpublished data*). Thus, polysaccharide synthesis is not necessarily equated with derepression of all the enzymes of polysaccharide synthesis. If the suppression of the *capR* mucoid mutations leads to synthesis of an altered protein product of the *capR* gene similar to the wild type, then some, or perhaps only one, of the enzymes involved in polysaccharide synthesis should approach the *capR*$^+$ level (repressed level). This remains to be demonstrated.

The quantitative effect of a particular ochre suppressor on UV-radiation sensitivity does not allow one to predict the effect that suppressor will have on capsular polysaccharide synthesis. Consider, for example, the effect of suppressors on the *capR9* mutation. *SupC* suppresses UV sensitivity (Fig. 2), but has a mild effect on polysaccharide synthesis (Table 2). In contrast, *sup-3* has much less effect in suppressing UV sensitivity than *supC*, but completely prevents polysaccharide synthesis (Fig. 2 and Table 2).

On the basis of previous genetic studies, we proposed that mucoidness and UV-radiation sensitivity were controlled by one cistron or, if the mutations studied were polar (13, 21), two cistrons in the same operon (27). Recent studies have established that nonsense mutations of the amber-ochre class (23, 31) can have polarity effects (19, 29), and Yanofsky and Ito showed that amber and ochre nonsense alterations *invariably* had polar effects in the tryptophan operon (32). Furthermore, suppressors of nonsense mutations partially relieve the polarity effects (5, 17, 19, 29, 32). Therefore, it is most likely that the *capR9*, *capR66*, and *capR6* nonsense mutations have polarity effects. Suppression of UV sensitivity and polysaccharide synthesis observed in strains with ochre suppressors is thus compatible with the original proposal. Usually, similar functions are controlled by the same operon (4, 21, 32), and it is thus rather surprising that the cistron or cistrons controlling UV radiation sensitivity and capsular polysaccharide synthesis, if not identical are, at the very least, in the same operon. Since we have not measured the effects of the ochre suppressors on enzymes of polysaccharide synthesis, the suppression of polysaccharide synthesis may be an indirect effect and not due to suppression of the nonsense mutations in *capR* alleles. However, *sup-3* did not change the mucoid colony type of the *capS* mutant, although two other suppressors have been isolated that prevent polysaccharide synthesis by the *capS* mutant (Markovitz, *to be published*). These results indicate a considerable degree of specificity in the polysaccharide synthesis. It might be argued that the effect of ochre suppressors on UV sensitivity is also indirect and could be explained by a slower division time among survivors of UV radiation. However, respreading experiments after UV radiation (Fig. 4) demonstrated no difference in division time among survivors of UV radiation, whether or not they contain ochre suppressors. More significantly, the UV sensitivity of the *capR9* strain on rich medium was not affected by a slower division time produced by incubation at low temperature (23 C).

Previously, we explained the fact that partial diploids of the type F′13 *capR9*/*capR*$^+$ were mucoid by assuming that when *capR9* is episomal it must produce an excess of protein monomers that interact with the protein monomers specified by the *capR*$^+$ gene to form an inactive oligomer of mixed composition [(27), i.e., negative complementation (11)]. Since *capR9* appears to be a nonsense mutation, it is likely that only part of the monomer specified by *capR9* is synthesized (30). Usually nonsense mutants do not produce immunologically detectable material. Nevertheless, Fowler and Zabin have shown that nonsense mutants in the β-galactosidase structural gene produce cross-reacting material (CRM) that forms precipitin lines when tested with anti-β-galactosidase antiserum (12). It is important to note here that the selection procedure by which the *capR9* nonsense mutant was isolated was designed to yield a mutation dominant to *capR*$^+$ A partial diploid of the type F′13 *capR*$^+$/*capR*$^+$ (nonmucoid) was treated with UV, and mucoid clones were isolated. Three of 29 of these mucoid clones (one of which was designated *capR9*) could also transfer mucoidness with F′13 to a recipient that still contained *capR*$^+$ on the chromosome (27). Such a mutant at *capR* would have to produce a *capR* gene product with biological activity of some sort. Thus, the present studies in which ochre suppressors were used remain compatible with and extend earlier conceptions of the *capR* gene mutations (26, 27).

Adler has stated that a nonmucoid mutant of *lon* (UV-sensitive, mucoid) was isolated that re-

tained its UV sensitivity (Adler, Radiation Res. **22**:165, 1964). We have noted similar mutations and have suggested they might be suppressors of *capR* mutants or mutations in structural genes involved in polysaccharide synthesis (27). The data presented in this paper demonstrate that all of the suppressors we were able to transduce into our strains measurably suppress UV sensitivity (Fig. 2), and *sup-3* does make the clones nonmucoid. In other experiments, we have produced *capR9* strains with structural gene mutations in polysaccharide synthesis (phosphomannose isomerase-negative) that are still very sensitive to UV and are nonmucoid on glucose-minimal medium, but *are mucoid* on minimal medium that contains both glucose and mannose (Markovitz, Sydiskis, and Lieberman, *unpublished data*).

ACKNOWLEDGMENTS

This investigation was supported by a grant from the National Foundation and by Public Health Service grant AI-06966 from the National Institute of Allergy and Infectious Diseases.

We acknowledge the excellent technical assistance of Phyllis Zinser.

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