Proc. Nat. Acad. Sci. USA
Vol. 73, No. 3, pp. 697–701, March 1976
Biochemistry

Cloning of *Escherichia coli* DNA that controls cell division and capsular polysaccharide synthesis

[hybrid plasmid/*capR*(*lon*)/restriction endonuclease/radiation sensitivity/insertions]

PATRICIA E. BERG, RANDALL GAYDA, HANNA AVNI, BARBARA ZEHNBAUER, AND ALVIN MARKOVITZ

Department of Microbiology, The University of Chicago, Chicago, Illinois 60637

Communicated by Albert Dorfman, November 14, 1975

ABSTRACT **A 2×10^6 dalton DNA fragment that controls cell division, capsular polysaccharide synthesis, and enzymes of capsular polysaccharide synthesis has been cloned.**

capR(*lon*) mutants of *Escherichia coli* K12 overproduce capsular polysaccharide (colanic acid) and enzymes involved in capsular polysaccharide synthesis, and they are sensitive to ultraviolet (UV) light and ionizing radiation (1–6). After irradiation, *capR* strains form nonseptate filaments that die, although no effect has been detected on mass increase, cell elongation, induced enzyme synthesis, or DNA synthesis during several hours of growth after irradiation (4–7). It has recently been discovered in our laboratory (R. Gayda and A. Markovitz, in preparation) that *capR9* is a conditional lethal mutation even in the *absence* of ultraviolet irradiation. Thus, *capR9* cells grown in minimal medium are nonviable (3% survival) when assayed for colony-forming units on complex medium (yeast extract-tryptone agar, YET) as compared to minimal agar (100% survival). Furthermore, microscopic observations reveal that *capR9* cells grown in minimal liquid medium are of uniform short rod shape, and transfer of such cells to YET broth results in filamentation (inhibition of cell division) of a large percentage of the cells. We designate the phenomenon described above as complex medium-induced killing. The complex medium-induced killing is prevented by the same treatments that rescue *capR9* mutants after UV irradiation; thus pantoyl lactone in YET agar or anaerobic growth rescue essentially all of the colony forming units. The relationship among increased capsular polysaccharide synthesis, UV sensitivity, and complex medium-induced killing is not known. Isolation of the *capR* gene DNA and its protein is an important step in understanding how this gene controls such apparently diverse functions and in understanding cell division.

S. Cohen and coworkers have isolated an efficient molecular vehicle for cloning DNA, the tetracycline resistance plasmid pSC101. It has a molecular weight of 5.8×10^6 and one site sensitive to the restriction endonuclease *Eco*RI (8, 9). We have used pSC101 to clone a 2×10^6 dalton DNA fragment that prevents both complex medium-induced killing and capsular polysaccharide synthesis but does not prevent UV sensitivity in a *capR*(*lon*) strain.

MATERIALS AND METHODS

Bacterial Strains and Plasmids. The bacterial strains used are described in Table 1. The strain containing the plasmid pSC101 was the generous gift of S. Cohen.

Media. Complex media used were L(12) or YET (10 g of tryptone, 5 g of yeast extract, 10 g of NaCl, and water to 1 liter; ref. 13) and the minimal medium was M9 (14) supplemented with 0.6% glucose, thiamine, amino acids, and adenine as required.

DNA Preparations. Covalently closed circular DNA was isolated from a cleared lysate (15) by CsCl–ethidium bromide centrifugation (16), using 1 mg/ml of ethidium bromide.

Restriction and Ligation Reactions. *Eco*RI restriction endonuclease (17) was purified from *E. coli* strain RY13 (kindly provided by L. Brown) through the phosphocellulose step (18) by G. Hayward. The exonuclease inhibitor *p*-chloromercuriphenyl sulfonic acid (final concentration, 0.05 mM) was preincubated with the enzyme for 20 min before addition of DNA. The enzyme reaction has been described (19). *E coli* DNA ligase (20) was the generous gift of P. Modrich. The reaction mixture for ligation was as described (19) except that 3 mM $MgCl_2$ and 0.1 mM NAD were used.

Transformation. Transformation was performed using a modification of the procedure of Lederberg and Cohen (21). Cells were grown at 37° in 30 ml of L broth to an optical density at 600 nm of 0.30. After treatment with 0.1 M $MgCl_2$ and 0.1 M $CaCl_2$ (21), wild-type cells were suspended in a final volume of 0.75 ml and *cap*R strains in 0.50 ml of 0.1 M $CaCl_2$. DNA in TEN buffer (21) was added to the treated cells. Immediately after mixing, the mixture was given a 30-sec heat pulse at 37° to increase the transformation frequency (Jacob and Hobbs via M. Mandel, personal communication). After transformation, treated cells were grown 60–90 min at 37° in YET broth containing 10% glycerol and 2 μg/ml of tetracycline before they were plated on either YET agar or minimal agar containing tetracycline (25 μg/ml).

Enzyme and Polysaccharide Assays. The assay of Hansen *et al.*, Method 1 (22), as modified by Lieberman *et al.* (23), was used to measure UDPglucose pyrophosphorylase (EC 2.7.7.9; UTP:α-D-glucose-1-phosphate uridylyltransferase). UDPglucose dehydrogenase (EC 1.1.1.22; UDPglucose: NAD^+ 6-oxidoreductase) assays followed the procedure of Zalitis *et al.* (24). Capsular polysaccharide was estimated as nondialyzable methylpentose in supernatant fractions from boiled cultures (25) by the method of Dische and Shettles (26). Proteins were measured using the Lowry technique (27).

Complex Medium-Induced Killing, UV Irradiation, and Nitrofurantoin Sensitivity. Bacteria were grown to stationary phase in minimal medium. Cells were diluted in phosphate-buffered saline containing gelatin (28) and plated on YET and minimal agar. UV irradiation was performed as described previously (13). Nitrofurantoin sensitivity of purified clones was determined by streaking a loopful of a slightly turbid suspension (about 10^7 bacteria per ml) on YET agar with and without 2 μg of nitrofurantoin/ml. *capR9* strains yield a few isolated clones on YET-nitrofurantoin

Abbreviation: YET broth, yeast extract-tryptone broth.

Table 1. Bacterial strains

Bacterial strains	Relevant alleles	Derivation, source, and/or genotype
MC100	*capR*$^+$	R. Curtiss, III (his strain χ156); F$^-$*leu-6, proC34, purE42, trpE38, thi-1, ara-14, lacY1, galK2, xyl-5, mtl-1, tonA23, tsx-67, azi-6, str-109,* λ$^-$, *pon*$^+$, *capR*$^+$, *capS*$^+$, *capT*$^+$, *supE44.*
RGC105	*capR*$^+$ *thy*$^-$	MC100 sel. as described (3).
KL16-99	*recA thy*$^+$	Hfr, B. Low.
RGC107	*capR9 thy*$^-$	P1 (*proC*$^+$ *capR9*)* × RGC105, sel. Pro$^+$ *capR9.*
RGC108	*capR9 recA*	KL16-99 × RGC107, sel. *thy*$^+$*recA*, score a mucoid clone with increased UV sensitivity and no λ*bio*30-7*nin*5 plaquing.
W3747	F'13 *lac*$^+$ *capR*$^+$ *purE*$^+$	Met$^-$, H. Echols.
RGC111	F'*capR*$^+$/*capR9 recA*	W3747 × RGC108, sel. Pur$^+$Lac$^+$, score a nonmucoid clone.
X7102	*capR*$^+$ *proC*	*proC, trp, lac*ΔX74, *str.* A strain containing no known suppressors. J. Beckwith via D. Court.
RGC121	*capR*$^+$	P1 (*capR*$^+$*proC*$^+$) × X7102, sel. Pro$^+$.
RGC123	*capR9*	P1 (*capR9proC*$^+$) × X7102, sel. Pro$^+$, score mucoid colony that is UV sensitive.
MC120	*capS supE44*	A mucoid mutation (2) that maps near *trp* (10) and has derepressed enzymes of capsular polysaccharide synthesis (6, 11).
MC135	*capR6*	A mucoid mutant of HfrH 3.300 previously designated M6 (1).
MC171	*proC34 capR9*	(Ref. 35)
C600	pSC101	S. Cohen

* The *capR9* mutation was originally obtained on the F'13 episome (2) and is thus a mutation of the *capR*$^+$ allele from F'13.

plates (= nitrofurantoin sensitive) and confluent growth on YET plates, while *capR*$^+$ strains grow equally well on both types of plate (= nitrofurantoin resistant).

Agarose Gel Electrophoresis. Slab gels in 0.7% agarose (17 × 0.3 cm) were run as described by Helling *et al.* (29) with the following modifications. The gel was run at 3.5 V/cm of gel (60 V) for 18 hr at 10°. Ethidium bromide, 0.5 μg/ml, was present in both the agarose and the buffer; the buffer used was described by Morrow *et al.* (19).

Electron Microscopy. Specimens were prepared by the aqueous technique of Davis *et al.* (30), and were examined on an RCA EMU 3G electron microscope. Photographs of plasmid molecules were projected on an enlarger easel, and contour lengths were measured using a Keuffel and Esser map measuring device. The average molecular length of pSC101 DNA, used as a reference, was determined to be 2.8 μm, which agrees with published values (8, 9).

RESULTS

Isolation of Hybrid Plasmids. The DNA from strain RGC111, which had been transformed to tetracycline resistance with plasmid pSC101, was isolated using CsCl–ethidium bromide gradient centrifugation, as described in *Materials and Methods.* Electron microscopy of the denser band (covalently closed circular DNA) revealed only 5.8 × 10^6 dalton size DNA (pSC101), but not the 254 × 10^6 dalton DNA from F'13 (31). We assumed the less dense band would contain broken fragments of F'13 DNA as well as some chromosomal DNA. Therefore, for the *Eco*RI digest the less dense band of DNA was used and was ligated to *Eco*RI-cut pSC101 DNA.

Transformation of strain RGC108 (*capR9recA*) using the ligated mixture was followed by selection of tetracycline-resistant clones on YET agar containing tetracycline (25 μg/ml). Transformants were scored for mucoidy on minimal plates, and, of approximately 300 scored, two transformants were nonmucoid. The supercoiled plasmids isolated from the two transformants are designated pMC44 and pMC52.

capR9 strains are sensitive to the radiomimetic drug nitrofurantoin (32) as well as to UV. A third plasmid-containing strain was obtained using a *capR9 recA*$^+$ recipient (MC171) and selecting simultaneously for tetracycline (25 μg/ml) and nitrofurantoin resistance (2 μg/ml) on YET agar. One tetracycline-resistant clone obtained was also nonmucoid but neither UV nor nitrofurantoin-resistant on subsequent testing (*Materials and Methods;* also see *Sensitivity to UV light and nitrofurantoin,* below); the plasmid obtained from this clone was designated pMC303.

Inhibition of Polysaccharide Synthesis. DNA from pMC44 or pMC52 was used to transform three different *capR9* strains (RGC123, MC171, and RGC108) and one *capR6* strain (MC135). Selection was on YET agar containing tetracycline (25 μg/ml), and resistant transformants were then scored by streaking on minimal agar. All 20 clones tested from each transformation were nonmucoid, with the exception of one clone from strain MC171 (data not shown). The (control)transformants obtained using pSC101 DNA were not prevented from producing polysaccharide. Further transformation experiments using minimal medium containing tetracycline allowed us to score clones directly for linked transformation, and the results were in agreement with those above; more than 98% of the transformants were nonmucoid using plasmid pMC44 DNA and *capR9* recipients (MC171 and RGC108).

capR9 mutants exhibit derepression of many of the enzymes involved in capsular polysaccharide synthesis (for a review, see ref. 6). Two of these enzymes were assayed in a *capR9* strain (MC171) containing pMC44, pMC52, and pSC101 DNA. The results presented (Table 2) show repression of both UDPglucose pyrophosphorylase and UDPglucose dehydrogenase in cells containing pMC44 and pMC52 DNA but not in those with pSC101 alone. Capsular polysaccharide was also assayed, and, in agreement with our observations on agar plates, strains with pMC44 and pMC52 DNA

Table 2. Repression of colanic acid synthesis and enzymes of colanic acid synthesis by plasmids pMC44 and pMC52

Strain	Colanic acid*	UDP-G PPase†	UDP-G dehydrogenase†
MC100	15	0.49	<0.02
MC171	790	3.9	0.54
MC120	160	3.1	3.0
MC171/pMC44	20	0.29	<0.02
MC171/pMC52	10	0.60	0.09
MC171/pSC101	840	9.2	1.2
MC120/pMC44	142	2.7	0.74
MC120/pMC52	182	2.3	0.69
MC120/pSC101	155	2.9	1.9

Plasmid-containing strains were grown at 37° in minimal medium, in one experiment without tetracycline and in a second experiment with tetracycline (12.5 μg/ml). Since no significant differences were observed between the two experiments the data were averaged. Strains without plasmids were grown in the absence of tetracycline in two independent experiments. UDP-G PPase, UDPglucose pyrophosphorylase.

* Expressed as μg of nondialyzable methylpentose/ml per unit of cell turbidity (optical density at 600 nm).

† Specific enzymatic activity is expressed as μmol/hr per mg of protein.

exhibit wild-type levels of polysaccharide (Table 2).

An important test of the specificity of the effect of pMC44 and pMC52 DNA is to observe their effect on another polysaccharide-producing strain of *E. coli*, strain MC120. This strain has a mutation in *capS*, causing derepression of polysaccharide; *capS* maps at a different site from *capR* (2, 10). Transformation of a *capS* strain with pMC44 and pMC52 DNA demonstrated that these plasmids prevented neither capsular polysaccharide synthesis nor the enzymes of capsular polysaccharide synthesis (Table 2).

Mucoid segregants from nonmucoid strains containing pMC44 or pMC52 DNA were often observed. One of these, which was also tetracycline sensitive (a rare spontaneous event), was tested for the presence of plasmid DNA by CsCl-ethidium bromide centrifugation and found to be cured. The simultaneous loss of tetracycline resistance and nonmucoidness coincident with loss of the plasmid supports the conclusion that the plasmid contains the gene conferring nonmucoidness. Another mucoid segregant that was tetracycline resistant (strain RGC108/pMC44M1) contained an extra piece of DNA in the non-pSC101 DNA (shown by agarose gel electrophoresis and electron microscopy, below). Therefore, either loss or modification of the plasmid DNA was associated with polysaccharide overproduction in *capR9* strains.

Table 3. Rescue from complex medium-induced killing by plasmids

Strain	Chromosomal genotype	% Survival*
RGC121	*capR*$^+$	95 ± 4.0
RGC123	*capR9*	3.2 ± 3.6
RGC123/pMC44	*capR9*	45 ± 10
RGC123/pMC303	*capR9*	65 ± 24
RGC123/pMC52	*capR9*	0.1 ± 0.1
RGC123/pSC101	*capR9*	0.8 ± 1.1

Results are the average of two to four separate experiments. The data include results using two independent transformants of *capR9* strains containing pMC44, pMC303, and pMC52.

* % Survival is calculated as follows:

$$\frac{\text{colony count on yeast extract-tryptone}}{\text{colony count on minimal medium}} \times 100.$$

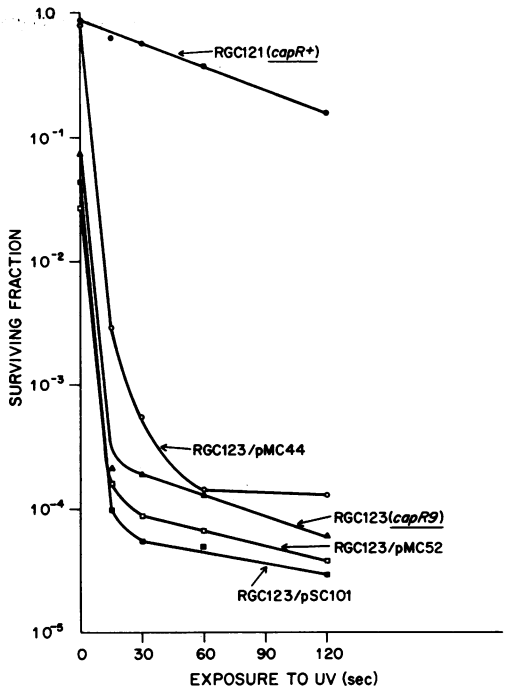

FIG. 1. Effect of plasmids on UV sensitivity of *capR9* strains. Bacteria were grown to stationary phase in minimal medium. Irradiation techniques were as previously described (13). The number of colonies on minimal agar at zero time is taken as 1.0. The surviving fraction is calculated as the number of viable cells per ml on YET agar divided by the number on minimal agar at zero time.

Complex Medium-Induced Killing. When *capR* mutants are grown in minimal broth and then plated on YET plates, many filaments are formed, and over 95% of the cells do not form clones (R. Gayda and A. Markovitz, in preparation; Table 3). This phenomenon is also observed when *capR* mutants are grown in YET broth and then plated on YET agar, but the killing effect is not as reproducible. In contrast, a *capR9* strain (RGC123) containing pMC44 formed few filaments when such transfer experiments were performed compared with cells containing pSC101 (data not shown). In addition, viability was partially restored by pMC44 and pMC303 (Table 3). Thus, both pMC44 and pMC303 affect cell division in *capR* mutants. pMC52, although reducing the number of filaments after the shift from minimal to complex medium, does not rescue cells from complex medium-induced killing (Table 3).

Sensitivity to UV Light and Nitrofurantoin. Radiation viability studies were performed on strains RGC121 (*capR*$^+$), RGC123 (*capR9*), RGC123 containing pSC101 (RGC123/pSC101), RGC123/pMC44, and RGC123/pMC52. None of the plasmids prevented radiation sensitivity (Fig. 1) or sensitivity to nitrofurantoin. However, pMC44 prevented complex medium-induced killing, and thus survival at time zero was almost complete in strain RGC123/pMC44 although there was no increased resistance to killing by UV light (Fig. 1). The plasmid pMC303 was tested in the original *capR9* strain in which it was isolated (strain MC171/pMC303) and also did not confer radiation or nitro-

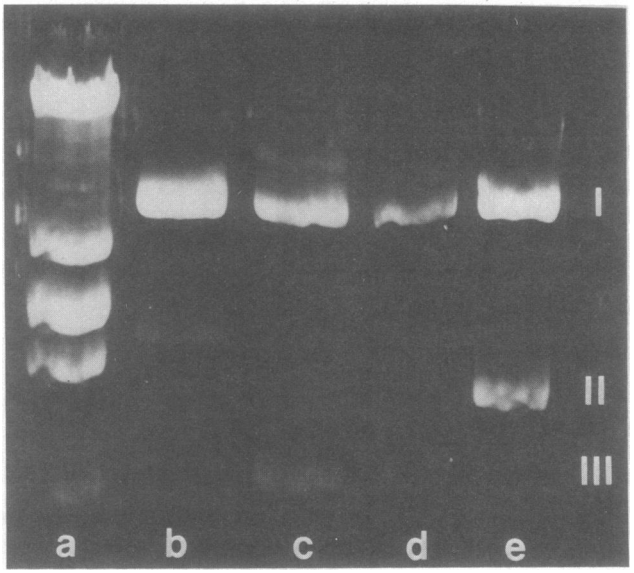

FIG. 2. Agarose gel electrophoresis of *Eco*RI digests of plasmid DNA. (a) λ DNA, (b) pSC101 DNA, (c) pMC44 DNA, (d) pMC52 DNA, and (e) pMC44M1 DNA. Bands I, II, and III have molecular weights of 5.8×10^6, 2.8×10^6, and 2.0×10^6, respectively, using λ restriction fragments as the reference (33). pSC101 DNA (b) exhibits a rapidly migrating minor band that has the same mobility as supercoiled pSC101 DNA. pMC44 DNA (c) exhibits a minor band of higher molecular weight than band I. This may be linear pMC44 DNA, cut only once. Migration was from top (cathode) to bottom.

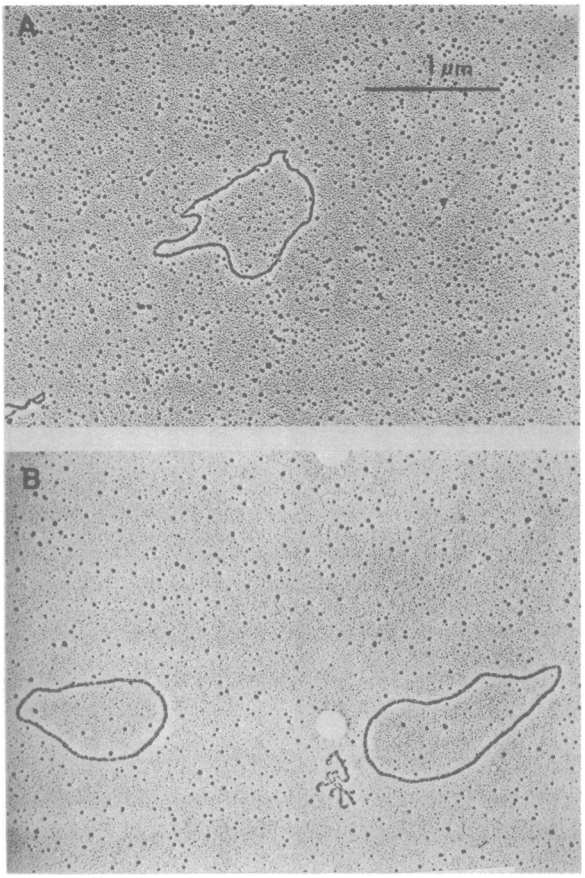

FIG. 3. Electron photomicrographs of pMC44, pMC52, and pSC101 DNA. (a) pMC44. The contour length is 3.7 μm. (b) pMC52 (right open circle) and pSC101 (left open circle). The contour length of pCM52 DNA is 3.8 μm, while that of pSC101 DNA is 2.8 μm.

furantoin resistance (data not shown).

Analysis of Hybrid Plasmid DNA. Agarose gel electrophoresis of plasmid DNA, after cutting with *Eco*RI enzyme, was performed using pSC101 DNA (Fig. 2b) and λ DNA (Fig. 2a; ref. 33) as standards. Plasmids pMC44 (Fig. 2c), pMC52 (Fig. 2d), and pMC303 (not shown) all exhibited two bands of DNA, I and III. Band I corresponds to pSC101 DNA, with a molecular weight of 5.8×10^6. Band III has a molecular weight of 2.0×10^6. Plasmid DNA from the mucoid, tetracycline-resistant segregant previously mentioned (strain RGC108/pMC44M1) exhibited two bands after the restriction enzyme reaction, bands I and II. The molecular weight of band II is 2.8×10^6, i.e., the molecular weight of band II is 800,000 greater than that of band III.

Electron micrographs of plasmid DNA are shown in Fig. 3. Length measurements on at least 25 molecules of pSC101, pMC44, and pMC52 DNA reveal an apparent difference in size distribution of the molecules between pMC44 and pMC52. The average molecular weight of pMC44 DNA by this technique is $7.8 \pm 0.3 \times 10^6$ and of pMC52 is $8.3 \pm 0.4 \times 10^6$. Measurements on the plasmid DNA from strain RGC108/pMC44M1 gave an average molecular weight of $8.8 \pm 0.5 \times 10^6$, or 1.0×10^6 greater than pMC44 (data not shown). For each plasmid the electron microscopy measurements agree with those obtained by summing the molecular weights of the two major restriction fragments as determined by agarose gel electrophoresis.

DISCUSSION

A hybrid plasmid containing the *capR*$^+$ gene should be phenotypically identifiable in a *capR9* genetic background by at least two criteria, repression of mucoidy and an increase in UV resistance. Markovitz and Rosenbaum (2), in studies using F'13, showed episomal dominance in *capR9*/*capR*$^+$ partial diploids, i.e., the gene on the episome determines whether a cell is mucoid or nonmucoid. This dominance is reflected in the level of GDPmannose pyrophosphorylase and UDPglucose pyrophosphorylase (23). Partial diploids are radiation resistant in all cases (34). Thus, we expect a *capR9* strain with a *capR*$^+$ plasmid to be nonmucoid and radiation resistant.

As shown in Table 2, *capR9* strains containing the hybrid plasmids pMC44 and pMC52 do indeed show wild-type levels of the two enzymes assayed, UDPglucose pyrophosphorylase and UDPglucose dehydrogenase. The low polysaccharide content of these strains is correlated with decreased enzymes.

However, the constructed plasmids do not affect the UV sensitivity of *capR9* cells. This apparent discrepancy could be explained in several ways. (*i*) There are two cistrons in a single operon involved in the *capR* phenotype, one which determines derepression of enzymes for polysaccharide synthesis the other causing UV sensitivity, and we have only one functional cistron (*capR*$^+$) in the hybrid plasmids. A recent paper by Bush and Markovitz (35), using two alleles of the polarity suppressor *suA*, refutes this hypothesis since introduction of this suppressor caused no change in the overproduction of polysaccharide or in radiation sensitivity of *capR* mutants. (*ii*) The restriction enzyme used, *Eco*RI cut the *capR* gene so that an incomplete polypeptide is being made which restores only repression of polysaccharide synthesis

and cell division ability. This hypothesis is plausible. (*iii*) Neither plasmid contains the *capR* gene. (*a*) A suppressor on the plasmid is responsible for the phenotypic changes. Growth tests with mutants of bacteriophage T4 and λ that contain mutations suppressible by amber and ochre suppressors indicated the absence of suppression in plasmid-bearing derivatives of strain RGC123 (a *capR9* strain). (*b*) The phenotypic effects are a result of expression of both the pSC101 plasmid genome and the new piece of DNA joined to it. The major objection to this hypothesis is that the plasmids do not turn off polysaccharide synthesis in a mucoid *capS* strain when MC120/pMC44 or MC120/pMC52 is grown on minimal medium (Table 2). The fact that pMC44 and pMC303 not only repress polysaccharide synthesis but also overcome the cell division effect strongly suggests that these plasmids contain part of the *capR* gene (hypothesis *ii*). It is worth noting that the isolation of plasmids pMC44 and pMC303 has allowed us to separate two phenomena not previously separated, conditional lethality caused by a complex plating medium and UV-induced filamentous death, which is also conditionally lethal and dependent on the complex plating medium (4).

Polyacrylamide gel electrophoresis of the polypeptides coded for in minicells containing pMC44 or pMC52 revealed two major radioactive peptides, in addition to the polypeptides made by pSC101 DNA, but no differences between pMC44 and pMC52 (R. Gayda, P. E. Berg, and A. Markovitz, in preparation). However, as shown above, pMC44 rescues *capR* mutants from complex medium-induced killing and pMC52 does not. It seems important to identify the difference between these two plasmids since it may lead to identification of a factor regulating cell division.

1. Markovitz, A. (1964) *Proc. Nat. Acad. Sci. USA* **51**, 239–246.
2. Markovitz, A. & Rosenbaum, N. (1965) *Proc. Nat. Acad. Sci. USA* **54**, 1084–1091.
3. Hua, S. & Markovitz, A. (1972) *J. Bacteriol.* **110**, 1089–1091.
4. Adler, H. I. & Hardigee, A. A. (1964) *J. Bacteriol.* **87**, 720–726.
5. Howard-Flanders, P., Simson, E. & Theriot, L. (1964) *Genetics* **49**, 237–246.
6. Markovitz, A. (1976) in *Surface Carbohydrates of Procaryotic Cells*, ed. Sutherland, I. (Academic Press, New York), in press.
7. Walker, J. R. & Pardee, A. B. (1967) *J. Bacteriol.* **93**, 107–114.
8. Cohen, S. N., Chang, A. C. Y., Boyer, G. W. & Helling, R. B. (1973) *Proc. Nat. Acad. Sci. USA* **70**, 3240–3244.
9. Cohen, S. N. & Chang, A. C. Y. (1973) *Proc. Nat. Acad. Sci. USA* **70**, 1293–1297.
10. Markovitz, A., Lieberman, M. M. & Rosenbaum, N. (1967) *J. Bacteriol.* **94**, 1497–1501.
11. Lieberman, M. M. & Markovitz, A. (1970) *J. Bacteriol.* **101**, 965–972.
12. Lennox, E. S. (1955) *Virology* **1**, 190–206.
13. Markovitz, A. & Baker, B. (1967) *J. Bacteriol.* **94**, 388–395.
14. Adams, M. H. (1959) in *Bacteriophages*, (Interscience Publishers, Inc., New York), pp. 445–447.
15. Guerry, P., LeBlanc, D. J. & Falkow, S. (1973) *J. Bacteriol.* **116**, 1064–1066.
16. Radloff, R., Bauer, W. & Vinograd, J. (1967) *Proc. Nat. Acad. Sci. USA* **57**, 1514–1522.
17. Hedgepeth, J., Goodman, H. M. & Boyer, H. W. (1972) *Proc. Nat. Acad. Sci. USA* **69**, 3448–3452.
18. Greene, P. J., Betlach, M. D., Goodman, H. M. & Boyer, H. W. (1974) in *Methods in Molecular Biology*, ed. Wickner, R. B. (Marcel Dekker, Inc., New York), Vol. 9, pp. 87–103.
19. Morrow, J. F., Cohen, S. N., Chang, A. C. Y., Boyer, H. W., Goodman, H. M. & Helling, R. B. (1974) *Proc. Nat. Acad. Sci. USA* **71**, 1743–1747.
20. Modrich, P., Anraku, V. & Lehman, I. R. (1973) *J. Biol. Chem.* **248**, 7495–7501.
21. Lederberg, E. M. & Cohen, S. N. (1974) *J. Bacteriol.* **119**, 1072–1074.
22. Hansen, R. G., Albrecht, G. J., Bass, S. T. & Seifert, L. L. (1966) in *Methods in Enzymology*, eds., Neufeld, E. F. & Ginsburg, V. (Academic Press, New York), Vol. 8, pp. 248–253.
23. Lieberman, M. M., Buchanan, C. E. & Markovitz, A. (1970) *Proc. Nat. Acad. Sci. USA* **65**, 625–632.
24. Zalitis, J., Uram, M., Bowser, A. M. & Feingold, D. S. (1972) in *Methods in Enzymology*, ed. Ginsberg, V. (Academic Press, New York), Vol. 28, p. 430.
25. Kang, S. & Markovitz, A. (1967) *J. Bacteriol.* **93**, 584–591.
26. Dische, Z. & Shettles, L. B. (1948) *J. Biol. Chem.* **175**, 595–603.
27. Lowry, O. H., Rosenbrough, N. J., Farr, A. L. & Randall, R. F. (1951) *J. Biol. Chem.* **193**, 265–275.
28. Curtiss, R., III (1965) *J. Bacteriol.* **89**, 28–40.
29. Helling, R. B., Goodman, G. M. & Boyer, H. W. (1974) *J. Virol.* **14**, 1235–1244.
30. Davis, R. W., Simon, M. & Davidson, N. (1971) in *Methods in Enzymology*, eds. Grossman, L. & Moldave, K. (Academic Press, New York), Vol 21, pp. 413–428.
31. Hu, S., Ohtsubo, E. & Davidson, N. (1975) *J. Bacteriol.* **122**, 749–763.
32. Kirby, E. P., Ruff, W. L. & Goldthwait, D. A. (1972) *J. Bacteriol.* **111**, 447–453.
33. Thomas, M. & Davis, R. W. (1975) *J. Mol. Biol.* **91**, 315–328.
34. Uretz, R. B. & Markovitz, A. (1969) *J. Bacteriol.* **100**, 1118–1120.
35. Bush, J. W. & Markovitz, A. (1973) *Genetics* **74**, 215–225.