*REGULATORY MECHANISMS FOR SYNTHESIS OF CAPSULAR POLYSACCHARIDE IN MUCOID MUTANTS OF ESCHERICHIA COLI K12**

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Jacob and Monod have proposed that regulator genes may control the syntheses of several related enzymes in *Escherichia coli* K12.[1] The present paper provides evidence for a regulator gene (designated R_1)[2] which controls capsular polysaccharide synthesis in *E. coli* K12. Certain mutants of *E. coli* produce large mucoid colonies which are characterized by overproduction of polysaccharide. The polysaccharide from such mucoid mutants of *E. coli* strain W contains galactose, fucose, and uronic acid.[3] Serological studies indicated that the capsular material of two mucoid mutants *and of the wild type grown at 15°C are identical.*[3] The chemical composition and infrared spectrum of the capsular polysaccharide isolated in the present study are similar to that reported for the polysaccharide isolated from a colicinogenic strain of *E. coli* (K235).[4, 5]

Materials and Methods.—The cultures used in this study are described in Table 1, and abbreviations are explained in reference 2. There are at least two nonallelic *Pro*$^-$ loci; *Pro*$_1$$^-$ (strain AB1899), *Pro*$_2$$^-$ (strains X-156 and 156-2)[6] and Pro$_{\neq 2}$$^-$ (Pro$^-_{\text{nonallelic to 2}}$; strains AB60a and F'13).

Media: M-9 minimal medium[7] was supplemented as required for selection and growth of original strains, transductants, and recombinants. Streptomycin was used at a final concentration of 200 μg per ml.

Genetic procedures: Standard procedures for transduction with P1[8], infection of F$^-$ strains with the episome from F'13,[9] and recombination with Hfr strains[10] have been described.

Scoring of transductants and recombinants for immunity to P1 and for maleness with phage MS2: Strains were classified as P1^s or P1^i as described by Luria *et al.*[11] using phage P1 *vir k* or P1. Phage MS2 infects F+, F', and Hfr strains, but not F$^-$ strains. The same test[11] is employed for determining maleness using about 10^9 phage MS2 instead of P1.

Selection of mutants of strain 3.300: Eight spontaneous mucoid mutants (including M25) were selected from colonies on minimal agar plates incubated at 37°C. Eight mucoid mutants (including M6, M12, and M15) were obtained after treatment with hydroxylamine.[12] Two other mucoid mutants were obtained after growth in broth containing 0.1 mg/ml 5-bromodeoxyuridine.

Chemicals: Nucleotides and G-6-P (Sigma Chemical Co.) and F-6-P (Calbiochem) were purchased. GDP-4-keto-D-rhamnose was prepared enzymatically.[13]

Chemical analyses: Methods used for the determination of uronic acid, methylpentose, "total carbohydrate," reducing sugar, and hexosamine appear in a previous communication.[14] Protein was measured by the method of Lowry[15] and keto sugars by a colorimetric method[16] with L-rhamnulose as a standard. Guanosine in nucleotides was calculated from UV absorption data assuming an E_{260} of 11.7.[17]

Enzymes: G-6-P dehydrogenase (Type V) and UDP-glucose dehydrogenase (Type III) (Sigma Chemical Co.), glucostat (Worthington Biochemicals), and Glucose Calsuls (Calbiochem) were purchased.

Enzymatic analyses of carbohydrates isolated from polysaccharide: D-glucose was determined with glucostat and with Glucose Calsuls. D-galactose was measured by the use of crude D-galactose dehydrogenase.[18] L-fucose was estimated with a crude L-fucose, isomerase preparation.[19] Keto sugar, produced by the action of L-fucose isomerase on L-fucose, was measured as described above.[16]

TABLE 1

MUTANTS OF *Escherichia coli* K12 USED IN THIS STUDY

Strain*	Mating type	Phenotype	Source and/or derivation
3.300†	HfrH	Sm^s, $P1^s$, Lac^+ (z^+, i^-)	S. E. Luria
AB261	Hfr P4X-6	Met^-, Sm^s, $P1^s$	E. A. Adelberg
AB60a	Hfr P4X-6	$P1^s$, $Pro_{\neq 2}^-$	E. A. Adelberg
F'13	F' (*Lac-Pur*)	Sm^s, $Pro_{\neq 2}^-$	L. S. Baron
AB1899	F$^-$	Pro_1^-, $P1^s$	H. Adler, AB1157
X-156	F$^-$	Leu^-, Pro_2^-, Pur^-, Try^-, Lac^- (z_2^-, i^+), Gal_2^-, Sm^r, $P1^s$	R. Curtis III, W945
156-2	F$^-$	Leu^-, Pro_2^-, Pur^-, Try^-, Lac_G^-,[6] Sm^r, $P1^i$	N. Schwartz, X-156
S18-1	F$^-$	Leu^-, Pro_2^-, Lac^-, Sm^r, $P1^i$	M6 × 156-2
T10-25	F$^-$	Leu^-, Pur^-, Try^-, Lac^-, Sm^r, $P1^s$	P1 (M6) × X-156
S27-1	F'	Leu^-, Sm^r, $P1^i$	F'13 × S18-1
S28-4	F'	Leu^-, Try^-, Sm^r, $P1^s$	F'13 × T10-25
T31-1		Leu^-, Sm^r, $P1^i$	P1 (S28-4) × S18-1

* All strains were grown in the presence of thiamin. Strains S18-12 and S18-13 are independent isolates and are similar to S18-1, except that they are also Try^-. Strains W327 (received from H. V. Rickenberg), W2915, W945, W3805, and W3142 are from the Lederberg collection.

† Mucoid mutants of strain 3.300, designated M6, M12, M15, and M25, were selected as described under *Materials and Methods*.

Paper chromatography: The following solvents were used: (1) neutral 1 *M* ammonium acetate: ethanol (3:7);[20] (2) isobutyric acid:0.5 *N* NH_4OH (5:3); (3) acetic acid:n-butanol:water (1:4:1); (4) 2-butanone saturated with water;[21] (5) pyridine:n-butanol:water (4:6:3); (6) tert.-amyl alcohol:isopropanol:water (8:2:3);[22] (7) pyridine:ethyl acetate:acetic acid:water (5:5:1:3).[4]

Extracts of E. coli K12: Extracts were prepared from washed cells suspended in 0.05 *M* Na_2HPO_4-KH_2PO_4, pH 7.5, by sonic disruption in a Raytheon 9 kc sonic disintegrator for 30 min followed by centrifugation at 34,000 × *g* for 20 min. The supernatant fractions were either assayed the same day or divided into convenient aliquots and stored at −20°C until they were assayed. In order to ascertain the effect of freezing and thawing on enzyme activity, appropriate control experiments were carried out. The results showed that the activity of G-6-P dehydrogenase was decreased in extracts of both mucoid and nonmucoid strains while the other enzyme activities were unaffected.

Enzymatic analyses of E. coli K12 extracts: UDP-galactose-4 epimerase was determined by the two-step procedure.[23] The conversion of GDP-mannose to GDP-fucose (GDP-fucose synthetase system) was measured spectrophotometrically.[24] A typical complete reaction mixture contained the following concentrations of components in a total volume of 0.5 ml: 50 mM Na_2HPO_4-KH_2PO_4, pH 8.0; 2 mM $MgCl_2$; 10 mM EDTA; 1 mM DPN; 0.096 mM GDP-mannose; 0.06 mM TPNH; and enzyme. An identical reaction mixture with the exception of GDP-mannose was used to measure endogenous TPNH oxidation. The conversion of GDP-mannose to GDP-4-keto-D-rhamnose (GDP-mannose dehydrase) was determined spectrophotometrically by the addition of purified GDP-4-keto-D-rhamnose reductase[13] to the above reaction mixture. The conversion of GDP-4-keto-D-rhamnose to GDP-fucose (GDP-4-ketorhamnose-stimulated TPNH oxidation) was measured spectrophotometrically by the substitution of GDP-4-keto-D-rhamnose for GDP-mannose in the reaction mixture described above (without added GDP-4-keto-D-rhamnose reductase). Phosphoglucose isomerase and G-6-P dehydrogenase were determined spectrophotometrically.[25] Activity of extracts of both exponential and stationary cultures are included in the *Results*.

Results.—The capsular polysaccharide of strain M25: Capsular polysaccharide was present in dialyzed supernatant fractions of strain M25 grown aerobically at room temperature. All of it was precipitated by cetylpyridinium chloride.[14] Attempts to fractionate the polysaccharide by differential solubility in saline of cetylpyridinium complex or by Dowex-1 Cl^- chromatography[26] indicate only one component. Quantitative precipitin analyses were consistent with the presence of a single antigenic polysaccharide.[27]

Colorimetric analyses of the polysaccharide[14] indicated the presence of uronic acid and methylpentose. The following monosaccharides were identified after acid hydrolysis of the polysaccharide: glucose (solvents 5 and 6); galactose (solvents 5 and 6); fucose (solvents 3, 4, 5, and 6). Enzymatic analyses of monosaccharides indicated that the glucose and galactose were the D isomers and that the fucose was the L isomer. A component with the same mobility as glucuronic acid in solvent 6 was apparent.

Comparison of the capsular polysaccharide of various strains: Capsular polysaccharides from different strains were hydrolyzed and chromatographed in solvent 5 (strains M6, M12, M15, M25, 3.300, S18-1, T31-1, T10-25) or solvent 7 (strains S27-1, S28-4). Glucose, galactose, and fucose were found in the hydrolysate of each in similar proportions as determined by visual inspection of the chromatograms.

Quantitative precipitin analyses were performed with antisera prepared against vaccines of M6, M25, and 3.300 cells. The results suggested that the polysaccharides of M6, M25, and 3.300 behaved as single antigens and were antigenically indistinguishable.[27]

Colony morphology: When grown at 37°C on minimal medium, mucoid clones were 5–15 mm in diameter and appeared white in contrast to the wild type which grew to a diameter of about 2 mm and appeared yellowish. At room temperature, mucoid mutants produced polysaccharide on both minimal agar and EMB-glucose agar (Difco), while at 37° polysaccharide was not produced on EMB-glucose agar. Strain M15 was an exception in that it produced polysaccharide under all conditions of growth. Wild-type strains were somewhat mucoid at room temperature on minimal or EMB-glucose agar but are not mucoid at 37°C.

Conjugation analyses: Throughout these studies, the mucoid character of strain M6 was not observed to mutate to the nonmucoid state. Strain M6 was crossed with X-156 and recombinants for two loci were selected on appropriate media containing streptomycin. The per cent mucoid clones in each recombinant class was: 98 for Pro_2^+-Pur^+, 37 for Pur^+-Gal^+, 20 for Gal^+-Try^+, and 27 for Pro_2^+-Leu^+. Thus, the locus involved in synthesis of polysaccharide appeared to be in the region of the chromosome that included Pro_2^+ to Pur^+ but not Gal^+.

Transduction of nonmucoid to mucoid: The results of a number of transduction experiments demonstrate cotransduction of Pro_2^+-mucoid and cotransduction of Pro_2^+-Lac^+ (see ref. 6 and Table 2). Transductants that have received Pro_2^+-mucoid from the donor did not receive Lac^+, while transductants selected for Pro_2^+ that received Pro_2^+-Lac^+ did not receive the mucoid locus. These results indicate the following sequence of genes: *Lac*-Pro_2-mucoid (or nonmucoid). Other transduction experiments not shown indicate that the mucoid locus is not linked to *Leu*, *Pur*, or *Try*.

In order to determine whether or not nonmucoid clones from primary transduction plates were heterogenotes, a number were streaked or replicated *on the medium used for primary selection.* Ninety-four nonmucoid-Pro_2^+ transductants from several experiments [P1(M6) × 156-2, Table 2] were streaked, and 7 segregated large numbers of mucoids. Some of the nonmucoid clones from the first streakings again yielded large numbers of mucoid clones when restreaked. A much larger number of nonmucoid-Pro_2^+ transductants (997) from the same experiments were simply

TABLE 2

TRANSDUCTION OF NONMUCOID STRAINS BY P1 GROWN ON MUCOID STRAIN M6

Donor	Recipient	Selected marker	Colony type	Non-selected marker	Number with non-selected marker	No. of colonies tested	Per cent containing non-selected marker
M6 (mucoid)	156-2 (nonmucoid)	*Pro*$_2$+	Both*	Mucoid	463	3,793	12.2 (20.1)†
		Pro$_2$+	Mucoid	*Lac*+	1	107	0.9
		Pro$_2$+	Nonmucoid	*Lac*+	55	239	23
		Lac+	Nonmucoid	*Pro*$_2$+	114	626	18.2
M6 (mucoid)	X-156 (nonmucoid)	*Pro*$_2$+	Both	Mucoid	255	2,012	12.7 (18.4)†
		Pro$_2$+	Mucoid	*Lac*+	1	73	1
		Pro$_2$+	Nonmucoid	*Lac*+	9	54	17
3.300 (nonmucoid)	156-2 (nonmucoid)	*Pro*$_2$+	Both	*Lac*+	164	586	28
		Lac+	Both	*Pro*$_2$+	164	742	22.1
		Pro$_2$+	Both	Mucoid	2	1,239	0.2
		Lac+	Both	Mucoid	0	1,203	0

* The designation "both" includes both mucoid and nonmucoid clones.
† Numbers in parentheses have been corrected for nonmucoid clones on primary transduction plates that segregated many mucoids on subsequent streaking or replica plating.

replicated on the same medium, and again mucoid clones (97) were obtained. Segregation of nonmucoid clones to yield mucoid clones was specific for nonmucoid-*Pro*$_2$+ transductants as compared to nonmucoid-*Lac*+ transductants. Similar results were obtained for nonmucoid-*Pro*$_2$+ transductants from the cross, P1(M6) × X-156. On the basis of these results and studies with the F'13 episome (see below) it is concluded that capsular polysaccharide synthesis associated with mutations of a locus near *Pro*$_2$− (designated R_1) is recessive in heterogenotes. The wild type (strain 3.300; nonmucoid on minimal agar at 37°C) is designated R_1+ and the mutant (mucoid under the same conditions) is designated R_1−.

Transduction of mucoid strains by P1 (3.300): The data of Table 3 show that P1 (3.300) transduced R_1− (mucoid) strains to R_1+ (nonmucoid) in 22% of *Pro*$_2$+ transductants. The frequency of joint transduction of *Pro*$_2$+-R_1+ was similar to the corrected figures for joint transduction of *Pro*$_2$+-R_1− (see Table 2), thus indicating that reciprocal crosses give similar results. No correction is necessary when scoring *Pro*$_2$+ -R_1+ joint transduction since heterogenotic clones for R_1 (R_1+/R_1−) would be scored as R_1+. It was of interest to determine whether or not any of these nonmucoid-*Pro*$_2$+ transductants were heterogenotes. When 91 nonmucoid-*Pro*$_2$+ transductants were streaked, 7 yielded large numbers of mucoids.

Occurrence of R_1+ and R_1−: Transduction tests similar to those indicated in Tables 2 and 3 demonstrated that nonmucoid strains AB261, AB60a, W3142, W3805, and W327 contained R_1+ while mucoid strain AB1899 contained R_1−.

TABLE 3

TRANSDUCTION OF MUCOID STRAINS BY P1 GROWN ON NONMUCOID STRAIN 3.300

Selected marker	Colony type	Nonselected marker	Number with nonselected marker	Number of colonies tested	% Containing nonselected marker
Pro$_2$+	Both*	R_1+	280	1,253	22.3
Pro$_2$+	Mucoid (R_1−)	*Lac*+	31	103	30
Pro$_2$+	Nonmucoid (R_1+)	*Lac*+	2	90	2
Lac+	Both	R_1+	5	1,357	0.4

* As in footnote of Table 2.
Donor: 3.300 (R_1+).
Recipients: S18-1, S18-12, S18-13 (all are mucoid (R_1−)).

All of the mucoid strains isolated from strain 3.300, except M15, were similarly tested for R_1^- or R_1^+. Of a total of 17 mucoid strains, 16 were R_1^-. P1 (M15) could not be prepared.

The mucoid locus of M12: Strain M12, *although mucoid*, contained R_1^+ since 37% of Pro_2^+ transductants were nonmucoid in the cross, P1(M12) × S18-1 (a mucoid (R_1^-)). In addition, P1 (M12) did not transduce 156-2 (a nonmucoid) to mucoid when Pro_2^+ was selected. M12 is an unstable mucoid strain. Repeated dilution plating of mucoid clones indicated that about 2% of the cells of such a clone are nonmucoid. The frequency of nonmucoid cells increased during overnight growth in complex media but AO (20 μg per ml) did not change the proportion of nonmucoid cells. Such results provide no evidence for participation of an F′ episome.[28] The mucoid locus of M12 was transferred to X-156 by conjugation. Four isolated mucoid recombinants were tested by repeated dilution plating and were found to be unstable as was M12. On the basis of these results we designate the mucoid locus of M12 as R_2. Transduction analyses indicate R_2 is not linked to *Lac*, Pro_2, or *Leu*.

Episomal analysis of the R_1 locus: F′13 was mated[9] with two different mucoid strains containing R_1^- (Table 4). When Pro_2^+ recombinants were selected, 97.1%

TABLE 4

EPISOMAL ANALYSIS OF THE R_1^- LOCUS

Donor	Recipient	Selected marker(s)	Nonselected marker	Number with nonselected marker(s)	Number of colonies tested	% Containing nonselected marker
F′13 (nonmucoid)	S18-1 (mucoid)	Pro_2^+	Nonmucoid	702*	723	97.1
		Lac^+	Nonmucoid	259†	263	98.5
F′13 (nonmucoid)	T10-25 (mucoid)	Lac^+-Pur^+	Nonmucoid	2,701	2,830	95.4

* All clones were also Lac^+ by replica plating.
† All clones were also Pro_2^+ by replica plating.

were nonmucoid. When Lac^+ recombinants were selected, 98.5% were nonmucoid. In the second mating shown in Table 4, 95.4% of selected Lac^+-Pur^+ recombinants were nonmucoid. The small percentage of mucoid clones have been examined for other episomal markers including the sex factor and represent examples of chromosomal integration and gene conversion similar to the findings of Pittard *et al.*[29] These results (and those presented in the following paragraph) indicate that the episome of F′13 contains R_1^+ which functions in its episomal position to prevent capsular polysaccharide synthesis in strains containing the R_1^- locus on the chromosome.

Hirota has shown that a high percentage of F^+ episome-infected cells can be cured by growth in AO.[28] Thus, growth of nonmucoid (R_1^+/R_1^-), Lac^+, Pro_2^+ cells (Table 4, F′13 × S18-1) in AO should yield mucoid (R_1^-), Lac^-, Pro_2^- clones if the positive alleles are on the episome. Twenty Pro_2^+-nonmucoid (Table 4, F′13 × S18-1) recombinants were isolated, grown with and without AO and tested for loss of the episomal markers. Growth in Penassay broth (Difco) which contained AO greatly increased the number of mucoid clones. Furthermore, mucoid clones lost the other episomal markers (Pro_2^+, Lac^+) simultaneously. Nonmucoid clones still contained Pro_2^+ and Lac^+. Few exceptions were noted. Similar results were obtained with nonmucoid clones isolated from the second mating of Table 4.

Additional evidence that the episome from strain F'13 contained Pro_2^+-R_1^+ was obtained by using an episome-infected nonmucoid clone (S28-4) as donor in transduction studies similar to those described in Table 2 and 3. P1 (S28-4) transduced S18-1 to nonmucoid when Pro_2^+ transductants were selected. P1 (S28-4) also transduced 156-2 to the mucoid state when Pro_2^+ transductants were selected, indicating that the chromosome of S28-4 still contained Pro_2^+-R_1^-. Thus, both R_1^+ and R_1^- were recovered from strain S28-4, and it may be designated R_1^+/R_1^-.

Enzymatic analyses: Bacteria were grown in M-9 media-0.6% glucose at room temperature with shaking, since R_1^- strains produced polysaccharide at room temperature but not at 37°.

The results in Table 5 demonstrate that when the mutant strains, M6 and M25 (R_1^-), are compared with the wild-type strain, 3.300 (R_1^+), not only do the former

TABLE 5

EFFECT OF R_1 ALLELES ON SYNTHESIS OF CAPSULAR POLYSACCHARIDE AND ON ENZYMATIC ACTIVITIES

Strains	Relevant genes	Poly-saccharide*	UDP-galactose 4-epimerase (specific enzymatic activity)†	GDP-Fucose Synthetase System‡		
				Complete	GDP-mannose dehydrase	GDP-4-ketorhamnose-stimulated TPNH oxidation
3.300	R_1^+	1.7	0.81 ± 0.06	−	−	−
M6 & M25	R_1^-	34	10.3 ± 4.4	+	++	++
S18-1	R_1^-	49	7.8	+	++	++
T31-1	R_1^+	1.4	3.1	−	−	−
S27-1	R_1^+/R_1^-	1.7	3.3	−	+	+

* μg nondialyzable methylpentose per 10 μg hot trichloroacetic acid-insoluble *N* during the exponential phase of growth. Cultures were grown in M-9 minimal medium-0.6% glucose with shaking at room temperature.

† μmoles UDP-glucose/hr/mg protein; the number of separate cultures analyzed were: for 3.300, 4; M6 and M25, 5; S18-1, 1; T31-1, 2; S27-1, 2.

‡ − indicates not detected; + indicates easily detected < ++. Assays were performed on two or more cultures of each strain, and freshly prepared, as well as frozen and thawed, extracts of strains 3.300 and T31-1 were tested.

show marked polysaccharide production, but likewise they show higher levels of UDP-galactose 4-epimerase (=epimerase) as well as GDP-fucose synthetase. In contrast, other data indicate that the levels of G-6-P dehydrogenase and phosphoglucose isomerase were similar in all three strains.

In *Aerobacter aerogenes* the conversion of GDP-mannose to GDP-fucose via GDP-4-keto-D-rhamnose requires at least two enzymes.[30] Net synthesis of methylpentose was demonstrated starting with GDP-4-ketorhamnose, TPNH, and extracts of a mucoid strain. The nucleotide was hydrolyzed, and the methylpentose was identified as fucose in solvents 3 and 4.

Transfer of R_1^- (strain M6) to 156-2 by recombination (S18-1) or to X-156 by transduction (T10-25, data not shown) yielded mucoid strains that had enzymatic activities similar to strains M6 and M25, although the parental strains 156-2 and X-156 had activities similar to strain 3.300.

It was desirable to compare the R_1^+ derived from strain 3.300 and the R_1^+ originally derived from strain F'13 in an otherwise identical genome. Therefore, P1 (3.300) and P1 (S28-4) were employed to transduce strain S18-1 (Pro_2^-, R_1^-). Pro_2^+ clones that were R_1^+ were selected and purified. Polysaccharide synthesis by these nonmucoid strains was similar.

A heterozygote (R_1^+/R_1^-, S27-1) and an isogenic transductant containing R_1^+ (T31-1) were compared. The heterozygote and strain T31-1 produced very little

polysaccharide during the exponential phase of growth (Table 5). However, strains S27-1 and T31-1 produced substantial quantities of polysaccharide 24–28 hr after growth had stopped, indicating that the regulatory system eventually became inoperative. In contrast, strain 3.300 never behaved in this fashion.

Both strains S27-1 and T31-1 produced levels of epimerase that were intermediate between strain 3.300 and R_1^- strains. GDP-mannose dehydrase and enzyme(s) catalyzing GDP-4-ketorhamnose-stimulated TPNH oxidation were detected individually in S27-1 but not in T31-1. However, G-6-P dehydrogenase and phosphoglucose isomerase activities were similar in strains T31-1 and S27-1.

Discussion.—The results presented indicate that a regulator gene,[1] R_1 (linked to Pro_2), controls the synthesis of capsular polysaccharide in *E. coli* K12. Partial heterozygotes, obtained by transduction (assumed to be R_1^+/R_1^- and R_1^-/R_1^+) or by episomal infection (R_1^+/R_1^-), are nonmucoid at 37°C on minimal agar plates, indicating that the nonmucoid state is dominant. Therefore, R_1^+ may be thought of as producing a regulatory substance, and R_1^- as producing either a defective regulatory substance or none. R_1 also controls the activity of several enzymes that are probably involved in synthesis of precursors of polysaccharide (epimerase and the GDP-fucose synthetase system) but does not effect G-6-P dehydrogenase or phosphoglucose isomerase activities. The structural gene for epimerase is in the *Gal* region[31] and not adjacent to the R_1 locus. Nevertheless, R_1 regulates the activity of this enzyme from a chromosomal (3.300 versus M6 and M25) and probably from an episomal (T31-1 versus S27-1) position. However, the position of R_1^+ or the presence of unknown markers on the F'13 episome (structural genes for GDP-fucose synthetase system?) or the presence of R_1^- may be important in the enzymatic activity of the GDP-fucose synthetase system (Table 5). Of the enzymes investigated, only the chromosomal locus of the structural gene for epimerase is known. Regulator genes for epimerase have been described that are located in the *Gal* region and the lysine region of the chromosome,[32] but no effect of these regulators on polysaccharide synthesis was reported.

A second gene controlling the synthesis of capsular polysaccharide has also been reported (R_2), but heterogenotes have not been prepared, and therefore it is not certain that it is a regulator gene (i.e., recessive to R_2 nonmucoid in heterozygous condition). Instability of R_2 type mucoids was demonstrated, but it is not known which of several explanations of instability[33] might apply. It is noteworthy that R_1^- is epistatic to R_2 nonmucoid, and R_2 mucoid is epistatic to R_1^+ since M6 (R_1^-, R_2 nonmucoid) and M12 (R_1^+, R_2 mucoid) are both mucoid mutants. A third type of mutant (M15) produced polysaccharide under all conditions of growth. This may be an R^- type of mutant or an O^c (operator constitutive) type.[1]

The present finding of a regulator gene for polysaccharide synthesis in *E. coli* K12 raises the question as to whether or not capsular and cell-wall polysaccharide syntheses in other bacteria are controlled in a similar fashion. A regulatory gene could explain certain observations on transformation of pneumococcal types,[34] but dominance relationships cannot be determined.[35]

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[2] Abbreviations: Hfr, male donor giving rise to high frequency of recombination; F′, male donor containing an episome that carries genetic information for certain markers donated at high frequency; F^-, female recipient strain; HfrH, Hfr (Hayes type); Hfr (P4X-6), Hfr (P4X-6 type) with markers donated in the order, *Pro-Leu-Ara-Lac; Sm*, streptomycin; P1, bacteriophage P1kc; P1 (strain A), P1kc grown on *E. coli* K12 host strain A, etc.; AO, acridine orange; EMB, eosinmethylene blue; superscripts ^{s} or ^{r} or ^{i} indicate sensitivity, resistance, or immunity, respectively. A genetic locus controlling a function is italicized. A superscript minus sign indicates lack of a function normally present in the wild-type strain, while a superscript plus sign indicates presence of a function. Other abbreviations used are the following: G-6-P, glucose-6-phosphate; F-6-P, fructose-6-phosphate; tris, tris (hydroxymethyl), aminomethane; *Leu*, leucine; *Met*, methionine; *Pro*, proline; *Pur*, purine (adenine or guanine); *Try*, tryptophan; *Gal*, galactose; *Lac*, lactose (*z*, the gene controlling the structure of β-galactosidase; *i*, the gene controlling a repressor of the *z* gene; for a complete description of *z* and *i*, see Jacob and Monod[1]). R_1 or R_2, used in conjunction with synthesis of capsular polysaccharide, are explained in the text. Episomal gene(s) are indicated first followed by/and then the chromosomal gene; thus, R_1^+/R_1^- indicates R_1^+ is episomal and R_1^- chromosomal.

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