THE GENETIC BASIS FOR MUCOIDY AND RADIATION SENSITIVITY IN *cap*R (*lon*) MUTANTS OF *E. coli* K-12*

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ABSTRACT

CapR mutants of *E. coli* K-12 overproduce capsular polysaccharide (mucoid phenotype) and enzymes involved in capsular polysaccharide synthesis, and they are sensitive to radiation. It has been uncertain whether both properties are mediated by damage to a single cistron or by a polar effect on a second cistron in the same operon. Introduction of a polarity suppressor caused no change in the overproduction of polysaccharide, in the enzymes of polysaccharide synthesis or in radiation sensitivity of the *capR* mutant. Thus mucoidy and radiation sensitivity resulting from *capR* (*lon*) mutations are both the consequences of impairment of the same cistron. The experiments demonstrate the advantage of the use of polarity suppressors (over conventional nonsense suppressors) in determining whether pleiotropic effects of a mutation are the result of polarity.

INDEPENDENT discoveries have shown that overproduction of capsular polysaccharide and a certain type of radiation sensitivity associated with filament formation in *Escherichia coli* K-12 result from lesions in the *cap*R gene and *lon* gene, respectively (ADLER and HARDIGREE 1964; MARKOVITZ 1964; HOWARD-FLANDERS, SIMON and THERIOT 1964; HOWARD-FLANDERS and BOYCE 1966; MARKOVITZ and BAKER 1967). It is now well established that the phenotypes of *cap*R and *lon* mutants are identical, i.e., mucoid and radiation-sensitive (ADLER and HARDIGREE 1964; HOWARD-FLANDERS, SIMON and THERIOT 1964; MARKOVITZ 1964; MARKOVITZ and ROSENBAUM 1965; ADLER *et al.* 1966; DONCH and GREENBERG 1968) and arise from the same mutational event(MARKOVITZ and ROSENBAUM 1965; DONCH and GREENBERG 1968). There has been uncertainty concerning whether one or more cistrons regulate the two functions (MARKOVITZ and ROSENBAUM 1965), but genetic experiments have not been successful in separating them.

The defects in some strains have been precisely mapped (MARKOVITZ and ROSENBAUM 1965). These strains all exhibit mucoid character and varying degrees of sensitivity to radiation (MARKOVITZ and BAKER 1967). The order of mutations on the genetic map may define a gradient of radiation sensitivity (Figure 1) (MARKOVITZ and ROSENBAUM 1965; MARKOVITZ and BAKER 1967). This is particularly interesting because all are known to be nonsense mutations

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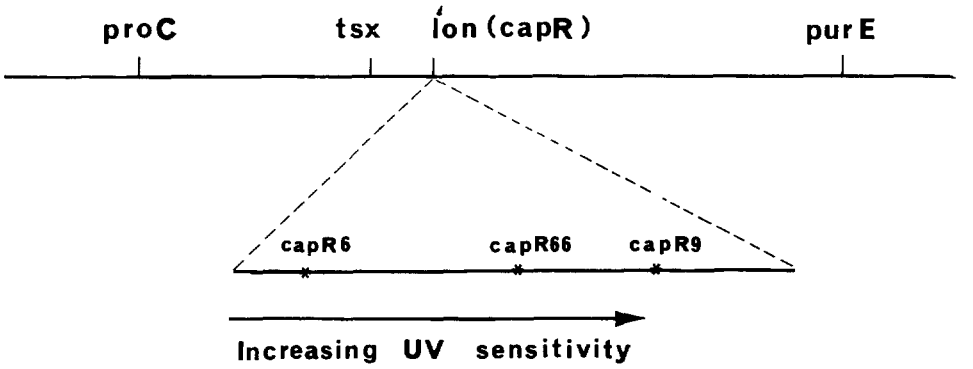

FIGURE 1.—Section of genetic map of *E. coli* (TAYLOR 1970) showing order of *cap*R mutations and their relative radiation sensitivity. (Distances between *cap*R mutations are not drawn to scale).

(MARKOVITZ and BAKER 1967). A similar defect in *E. coli* B, which confers on that strain its characteristic radiation sensitivity, may also be a nonsense mutation (DONCH, CHUNG and GREENBERG 1969). Such evidence strongly suggests the possibility of a polar effect on a second cistron (MARKOVITZ and ROSENBAUM 1965). Suppression of the nonsense codon could provide an answer. Unfortunately, work with conventional nonsense suppressors has given inconclusive results (MARKOVITZ and BAKER 1967; MARKOVITZ, unpublished data). BECKWITH (1963) and CARTER and NEWTON (1971) have obtained mutations which suppress polarity while having no effect on the loss of function caused by the mutation itself (KUWANO, SCHLESSINGER and MORSE 1971; MORSE and PRIMAKOFF 1970; MORSE and GUERTIN 1972; SCAIFE and BECKWITH 1966). For example, a (polar) nonsense mutation in the *lacZ* gene prevents a cell from synthesizing any of the proteins of the *lac* operon. Introduction of the polarity suppressor "suA" does not allow the cell to produce β-galactosidase (the product of *lacZ*), but permits both the survival of mRNA for the distal gene, *lacY*, and synthesis of *lac*Y protein. In this paper we assume that "suA" would be capable of suppressing polar effects resulting from nonsense mutations at the *cap*R locus. Therefore the presence of this so-called "suA" (su78) in a cell also bearing a *cap*R mutation should eliminate polar effects involved in the phenotype and provide answers to two questions: (1) Is more than one cistron involved? (2) If more than one cistron is involved, does the genetic locus defined by mutations as *cap*R (*lon*) produce a peptide controlling polysaccharide production or does it produce a peptide affecting radiation senstivity?

MATERIALS AND METHODS

Bacteria: All strains of bacteria utilized in the experiments were derivatives of *E. coli* K-12. The properties and derivation of basic strains employed are given in Table 1.

Media: M9 minimal medium (ADAMS 1959) was supplemented with 2.5×10^{-3} M $CaCl_2$ and 10μg of thiamine hydrochloride/ml. Amino acids and adenine were added at 50 μg/ml and anthranilic acid was added at 100 μg/ml when required. Vitamin-free casamino acids (Difco)

TABLE 1

Bacterial strains

Strain	Mutant alleles important to this study	Phenotype*	Derivation, source and/or genotype
MC171	*cap*R9	M	A. MARKOVITZ;† F⁻, *leu*-6, *pro*C-34, *pur*E38, *trp*E43, *ara*-14, *xyl*-5, *mtl*-1, *thi*-1, *ton*A23, *tsx*-67, *azi*-6, *str*-109, λ⁻, P1^s, *cap*R9
W4597	*gal*U	N	E. LEDERBERG; *gal*U
AB259	None	N	E. ADELBERG; *pro*C+ *cap*R+
2034	*trp*E	N	D. MORSE via D. APIRION (APIRION's strain N473); *leu*⁻ *trp*E9851
2054	*trp*E suA	N	D. MORSE via D. APIRION (APIRION's strain N474); *leu*⁻ *trp*E9851 suA am100
MR2–201	*lac*Z su78	N	A. NEWTON; Δ(*lac*)su78/F′*lac*Z U118+U131 *pro*C+
JBC100	None	N	P1 (*pro*C+ *capR*+) × MC171; sel. pro+, score non-mucoid phenotype
JBC101	*cap*R9*lac*Z	M	P1 (*trp*+ *gal*U) × MC171; sel. *trp*+, score gal⁻, non-mucoid
	*trp*E		P1 (*trp*E9851 *gal*U+) × MC171 *gal*U *trp*+; sel. *gal*+, score *trp*⁻, *anth*^{s}, *mucoid*
			P1 (*lac*Z U118+U131 *proC*+) × MC171 *trp*E9851; sel. *pro*+, score *lac*⁻
JBC102	*cap*R9 *lac*Z *trp*E suA	M	P1 (suA) × JBC101; sel. *anth*^{r} at 42°C, score *trp*⁻, *lac*⁻, *mel*+ at 37°C
JBC103	*cap*R9 *lac*Z *trp*E su78	M	P1 (su78) × JBC101; sel. *anth*^{r} at 42°C, score *trp*⁻, *lac*⁻, *mel*+ at 37°C

* On M9 minimal medium at 37°C. M = mucoid, N = non-mucoid.

† Genealogy of the strain was provided by B. BACHMAN from files of the Coli Genetic Stock Center (CGSC). The allele designations supersede previous designations from this laboratory (KANG and MARKOVITZ 1967; LIEBERMAN, BUCHANAN and MARKOVITZ 1970; LIEBERMAN, SHAPARIS and MARKOVITZ 1970; MARKOVITZ 1964; MARKOVITZ and BAKER 1967; MARKOVITZ and ROSENBAUM 1965).

were added at 0.5% where indicated. Either 0.6% glucose or 1% lactose, galactose, or melibiose was used as a carbon and energy source. These media were solidified by adding 1.5% agar. Streptomycin was used at 200 μg/ml.

EMB plates were made using eosin methylene blue agar (Difco) with 1% sugar added after sterilization. L broth was used to grow strains for transduction. Medium for radiation experiments (YET) has been described (MARKOVITZ and BAKER 1967).

Chemicals: Nicotinamide adenine dinucleotide (NAD), NAD phosphate, ATP, glucose-1, 6-diphosphate, galactose-1-phosphate, glucose-6-phosphate, and uridine diphosphate (UDP)-D-glucose were purchased from Sigma Chemical Co. ^{14}C-galactose was purchased from Amersham-Searle, and DEAE cellulose paper was Whatman DE-81. Omnifluor was obtained from New England Nuclear Corp.

Enzymes: Glucose-6-phosphatate dehydrogenase and phosphoglucomutase were purchased from Sigma Chemical Co.

Transductions were performed using bacteriophage PlKc as described by LENNOX (1955).

Growth of bacteria: Bacteria were maintained on minimal glucose plates. Cells for irradiation experiments were grown overnight in YET broth at 37°C with reciprocal shaking. Cells for determination of capsular polysaccharide and enzyme assays were grown overnight at 23°C with reciprocal shaking in minimal glucose medium.

Procedures for irradiation experiments have been previously described (MARKOVITZ and BAKER 1967).

Preparation of cell-free extracts: These procedures have appeared earlier (HUA and MARKOVITZ 1972). Crude extracts were kept at 4°C and assayed within 24 hrs.

Enzyme assays: Galactokinase was assayed according to the procedure of SHERMAN and ADLER (1963), except that the final volume was adjusted to 0.5 ml. Assay mixtures were incubated for 30 min at 37°C. Galactose and galactose-1-phosphate were separated with the aid of DEAE cellulose (WETEKAM, STAACK and EHRING 1971). Radioactive samples were dried at 70°C and counted in a Packard Tri-carb model 3310 liquid scintillation counter using 7.5 ml of toluene solution of Omniflour (4.33 g/liter) per sample.

The assay of HANSEN *et al.* (Method 1) (1966) as modified by LIEBERMAN, BUCHANAN and MARKOVITZ (1970) was used to measure UDP-glucose pyrophosphorylase.

UDP-glucose dehydrogenase assays followed the procedure of STROMINGER *et al.* (1957).

Assay procedures for glucose-6-phosphate dehydrogenase have been described in detail elsewhere (KANG and MARKOVITZ 1967).

For the three latter enzymes, assays were performed at room temperature and in all three cases changes in optical density were measured at 340 nm with a Gilford recording spectrophotometer model 240 or model 2000. Enzymatic activity for all assays performed was directly proportional to the amount of extract added, and assay values are the average of two assays with different quantities of extract.

Chemical analyses: Protein was determined by the method of LOWERY *et al.* (1951) with bovine serum albumin as a standard. Capsular polysaccharide was estimated as nondialyzable methylpentose in supernatant fractions from boiled cultures (KANG and MARKOVITZ 1967) by the method of DISCHE and SHETTLES (1948). D-fucose was employed as a standard.

RESULTS

Construction of strains with suA (su78) and cap*R mutations:* Certain internal controls must be present to be certain that a strain contains a polarity suppressor. For this reason two strongly polar defects were introduced into the strain. The first of these, *trp*E9851 is an ochre mutation in the structural gene for anthranilate synthetase, the first cistron of the tryptophan operon. The polar effect of this mutation renders such mutants unable to grow on high levels of anthranilate (MORSE and GUERTIN 1972). The second polar mutation, *lac*ZU118 + U131, is actually a double mutant having both an ochre and an amber mutation. These defects reside in the first cistron of the *lac* operon. *Lac*Z is the structural gene for β-galactosidase; hence this type of mutant cannot utilize lactose as a carbon and energy source. Moreover, the polar effect caused by this double mutation results in the inability of such mutants to grow on melibiose at temperatures above 25°C since they cannot produce the product of a distal gene in the *lac* operon, *lac*Y (CARTER and NEWTON 1971; PRESTIGE and PARDEE 1965). SuA is known to suppress the polarity of *trp*E9851 (MORSE and GUERTIN 1972) and su78 is able to suppress the polarity of either *trp*E9851 or *lac*ZU118 + U131 (CARTER and NEWTON 1971). In this paper we show that suA also suppresses the polarity effects of the double nonsense mutation in *lac*Z, as might be expected (Table 2). The nature of the mutations, i.e., nonsense mutations, and an amber codon as the cause of the particular suA which we employed (MORSE and GUERTIN 1972) may provide an additional safeguard; the chance occurrence of an ochre suppressor efficient enough to alter the effects of the *cap*R9 mutation in

TABLE 2

Growth characteristics of Polarity suppressor-containing strains

Strain	Genotype	Growth conditions*			
		Lactose, 37°C	Melibiose, 37°C, 24 hrs.	Without tryptophan, 37°C	Anthranilate (without tryptophan), 42°C, 24 hrs.
JBC101	*lac*Z U118+U131 *trp*E9851 su0†	0	0	0	0
JBC102	*lac*Z U118+U131 *trp*E9851 suA	0	+	0	+
JBC103	*lac*Z U118+U131 *trp*E9851 su78	0	+	0	+

* Medium used was M9 minimal with indicated changes in carbon source or amino acid.
† su0 indicates wild-type allele of suA or su78.

the completed strains might also suppress the primary defects in *lac*Z and *trp*E and permit growth on lactose without tryptophan. We might, therefore, have an indicator of spurious results which are the consequence of suppression of nonsense codons rather than suppression of polarity.

The basic strain chosen for the construction of an experimental strain, MC171, contains a *cap*R9 mutation. This allele was chosen because it shows the greatest sensitivity to radiation of any *cap*R mutant available. It is *proClac*$^+$ which permitted the introduction of *lac*ZU118 + U131 by P1Kc transduction. *Pro*$^+$ transductants were selected and screened for *lac*$^-$ clones on EMB-lactose. Introduction of *trp*E9851 required two additional transductions. The strain was first transduced to *trp*$^+$*gal*U by selection on tryptophan-deficient medium and scoring for *gal*U. Next *trp*E9851 and *gal*U$^+$ were introduced by selection on minimal galactose plates and scoring for *trp*$^-$. The suppressors were separately introduced by transduction and selection for anthranilate-resistance at 42°C. Such transductants were screened and clones were isolated which were still *trp*$^-$. Subsequent testing showed that these strains would grow on melibiose at 37°C but not at 42°C. Other experiments were conducted to ascertain the origin of the discrepancy. When the strains were transduced to *cap*R$^+$ or *lac*$^+$ they still grew on melibiose at 37°C but not at 42°C. Thus the absence of growth on melibiose at 42°C is not related to any of the basic requirements for the experiments to follow. Properties of the resulting transductants clearly show that each contains a polarity suppressor and that the suppressors are functioning as expected (Table 2).

Effects of suA and su78 on radiation sensitivity of cap*R mutants:* Radiation sensitivity of the strains was tested and the survival curves are shown (Figure 2). Wild-type and *cap*R9 strains show typical responses; the presence of a polarity suppressor has no apparent effect on the extreme UV sensitivity of the *cap*R9 strain.

Effects of polarity suppressors on overproduction of polysaccharide: The possibility that *cap*R mutations lie in a cistron related to radiation sensitivity and that overproduction of capsular polysaccharide occurs as a consequence of polar effects can be accurately tested. The presence of a *cap*R mutation causes derepression of several enzymes involved in the production of the capsular material. In

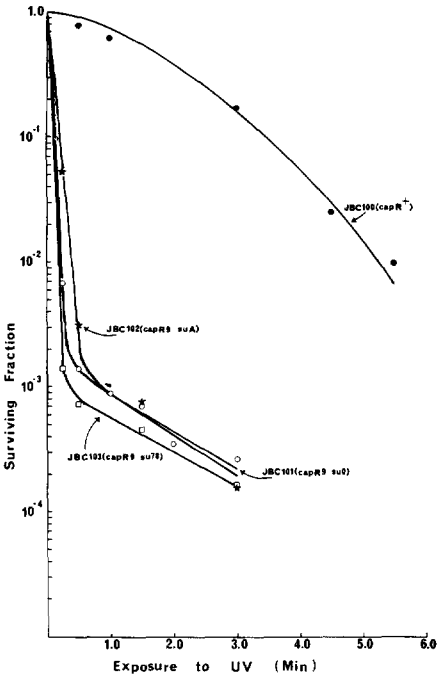

FIGURE 2.—Effect of polarity suppressors on UV sensitivity of *cap*R9 strains.

particular, the levels of uridine diphosphate-glucose (UDPG) dehydrogenase (EC1.1.1.22), UDPG pyrophosphorylase (EC2.7.7.9), and UDP-galactose-4-epimerase (epimerase; EC5.1.3.2) are all elevated in *cap*R strains (LIEBERMAN, BUCHANAN and MARKOVITZ 1970; LIEBERMAN, SHAPARIS and MARKOVITZ 1970; MARKOVITZ 1964). The first two are easily assayable.

Since HUA and MARKOVITZ (1972) and MACKIE and WILSON (1972) have demonstrated that the entire galactose operon is regulated by the *cap*R gene, effects on the epimerase can be equated with those on the more readily assayable kinase [ATP: D-galactose phosphotransferase (Kinase) EC2.7.1.6]. Assays of these enzymes and measurement of capsular polysaccharide were carried out and results are presented in Table 3. Two separately isolated transductants for each type suppressor were tested. These data are consistent with all previous work in our laboratory and, as in the case of radiation sensitivity, the presence of a polarity suppressor has no effect on any of the enzymes of the *cap*R9 strains. The values obtained for polysaccharide seem to show reduction in the suppressor-containing strains. We have found this property to be sufficiently variable that the two-fold difference observed is not considered significant.

Finally, CARTER and NEWTON (1971) have presented evidence that the su78 mutation tends to stabilize total messenger RNA in a cell, to some extent. It might be argued that in our strains the repressive function of the *cap*R gene is restored by su78 (suA) but that stabilization of basal mRNA for the enzymes involved results in the increased activities observed. For this reason a constitutive enzyme,

TABLE 3

*Level of enzymes and polysaccharide produced by bacterial strains**

Strain	UDPG dehydrogenase	UDPG pyrophosphorylase	Galactokinase	Glucose-6-phosphate dehydrogenase	Polysaccharide‡
JBC100	0.29†	0.93	0.38	5.61	49
JBC101	9.72	3.50	2.12	8.84	1105
JBC102–1	8.02	3.34	1.70	9.06	682
JBC102–2	9.28	3.27	1.95	8.05	719
JBC103–1	7.10	2.51	1.40	8.13	528
JBC103–2	8.36	2.34	1.88	7.89	713

* Results are the average of two separate experiments.
† All specific activities for enzymes expressed as micromoles of product formed per hour per milligram of protein.
‡ Expressed as micrograms of nondialyzable methylpentose per milliliter per unit of cell turbidity (optical density at 600 nm.).

glucose-6-phosphate dehydrogenase (D-glucose-6-phosphate: NADP oxidoreductase, EC1.1.4.9), was assayed. If the preceding argument concerning the stabilization of mRNA were valid, a constitutive enzyme unaffected by *cap*R might be expected to show greater activity in a cell containing suA or su78. The activities found are the same as previously reported from our laboratory (MARKOVITZ, LIEBERMAN and ROSENBAUM 1967) and no increase over *cap*R9 levels is associated with the presence of the polarity suppressors.

DISCUSSION

The design of the foregoing experiments depends on the assumption that polarity is the result of mRNA degradation and that the "suA" gene product is responsible for such degradation. We know at least that suA suppresses known polar effects in our strains. Moreover, we know that the mutations in *cap*R with which we work are in segments of the genome which are transcribed to mRNA (as opposed to controlling segments such as operators or promoters) because the mutations are suppressible at the level of translation. We know also that nonsense suppressors partially restore the defective function and reduce one or both of the phenotypic properties associated with *cap*R mutants (MARKOVITZ and BAKER 1967). Although the exact action of the "suA" protein is unproven, our controls and accumulating evidence on suA tend to make the validity of our assumption less and less questionable. Accordingly, the following discussion is based on a model whereby polar effects and suppression of polarity occur as part of mRNA metabolism.

Three possible models can be postulated which might represent the source of the divergent phenotypic traits (mucoidy and radiation sensitivity) associated with the *cap*R syndrome (Figure 3). The first of these (model A) simply involves a single cistron affecting both abnormal properties. The other two models demand a mulitple-cistron arrangement in which one of the phenotypic traits is the result of a genetic lesion in one cistron and the second trait the result of polar effects on

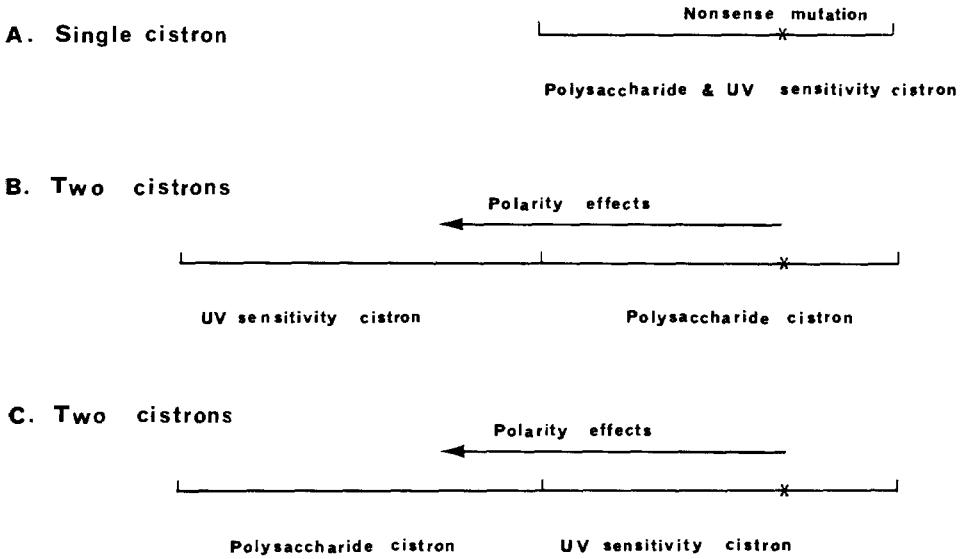

FIGURE 3.—Alternative models for the genetic basis of mucoidy and radiation sensitivity in *cap*R mutants.

a separate cistron in the same operon. In model B, mutations lie in a cistron controlling polysaccharide production and polarity effects are manifested in a distal cistron related to radiation sensitivity. If this model were correct we would have expected an increase in resistance to UV when a polarity suppressor was present in a *cap*R9 strain. Data in Figure 2 indicate that the polarity suppressors, suA and su78, have virtually no effect on the UV sensitivity of the *cap*R9 strain. Other experiments examining effects of exposure to UV for 10 to 30 seconds minimize the disparity among the strains shown in Figure 2. In contrast, previous results from this laboratory demonstrated that suppression of radiation sensitivity by three different ochre suppressors was readily detected (MARKOVITZ and BAKER 1967) even though ochre suppressors are usually less efficient than amber suppressors (GAREN 1968). Thus model B seems very unlikely. Model C has the mutations in the radiation cistron with the polysaccharide cistron impaired by polarity. If model C were correct we would have expected a decrease in the levels of enzymes when a polarity suppressor was present in a *cap*R strain. The data of Table 3 indicate that the suA and su78 alleles did not reduce the derepression of the enzymes of capsular polysaccharide synthesis caused by the *cap*R9 allele, including UDP-glucose dehydrogenase, UDP-glucose pyrophosphorylase and galactokinase. Evidence from previous gene dosage studies comparing *cap*R$^+$ and *cap*R9 indicate that we can detect small variations in the amount of active *cap*R$^+$ product (repressor) by measuring polysaccharide and enzymes of capsular polysaccharide synthesis (MARKOVITZ and ROSENBAUM 1965; LIEBERMAN, BUCHANAN and MARKOVITZ 1970). Thus model C is also not attractive at this time. These data are, however, all consistent with model A and represent the strongest evidence yet available that the very dissimilar properties resulting from

a *cap*R mutation are mediated, at least in part, by the loss of function of a single polypeptide from a single cistron.

It is true that suppression of polarity by suA or su78 is somewhat variable depending on cultural conditions. Under starvation conditions, MORSE and GUERTIN (1972) demonstrate essentially complete suppression of polarity in the tryptophan operon by suA. Under somewhat less severe conditions CARTER and NEWTON (1971) obtain approximately 20–40% efficiency in restoring fully derepressed levels of mRNA for the cistrons of the *lac* operon with su78. Furthermore, all work conducted with polarity suppressors to date has involved structural proteins. We have no way of knowing what conditions might maximize the synthesis of a regulatory protein nor the precise effect of restoring only 20–40% of the mRNA for that protein, if in fact these were the levels we had attained in our strains. However, as noted above, previous work concerning dominance (LIEBERMAN, BUCHANAN and MARKOVITZ 1970; MARKOVITZ and ROSENBAUM 1965) provides convincing evidence that we can detect small variations in the effect of *cap*R mutations on polysaccharide and enzyme synthesis.

The best characterized of the ochre suppressors tested by MARKOVITZ and BAKER (1967), *supC* (=su4$^+$), was shown to have a 12% efficiency of suppression (GAREN 1968). The presence of this suppressor in a *cap*R9 mutant resulted in a requirement of approximately 7.5 times the UV dose to kill 90% of a population of cells, as compared to the dose required to kill the same fraction of a *cap*R9 culture lacking the suppressor (MARKOVITZ and BAKER 1967). Previously unpublished data on the effect of two of those same suppressors on enzyme and polysaccharide production are interesting and demonstrate the variability of suppressor action (MARKOVITZ, BUCHANAN and SHAPARIS, unpublished results). In a *cap*R9 strain the specific activity of UDPG dehydrogenase was elevated 18-fold and UDPG pyrophosphorylase was elevated 17-fold as compared to a *cap*R$^+$ strain. Polysaccharide production was similarly increased. *Sup*C reduced the level of the pyrophosphorylase substantially in cells grown at any temperature; it had little effect on the dehydrogenase. *Sup*3 had almost no effect on either enzyme in cells grown at 23°C, but caused a pronounced reduction in the synthesis of both enzymes in cells grown at 37°C. Both *sup*C and *sup*3 reduced polysaccharide production to only a four- to five-fold excess at either temperature. For the purpose of the present experiments it is important that suppressor effects could be observed at all temperatures tested. In contrast to the results obtained with suA and su78, the results obtained from work with ochre suppressors do not permit a clear-cut choice among the three possible models proposed.

There is another possibility that we cannot exclude at this time. It may be that the inseparability of mucoidy and radiation sensitivity is the result of a novel genetic property. Certain data suggest that both strands of DNA of the *cro* gene of phage λ are transcribed (REICHARDT and KAISER 1971; SPIEGELMAN *et al.* 1972). It is conceivable that a similar situation exists with *cap*R and that each strand of DNA codes for a separate polypeptide, one regulating polysaccharide production and the other affecting radiation sensitivity. A mutation occurring in one DNA strand would always be mirrored in the other and the two mutations

would be inseparable. The means for exploring such possibilities do not presently exist. The eventual isolation of the *cap*R protein should help to elucidate the bifunctional nature of the gene.

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