A Nitrosoguanidine-induced Cluster of Linked Mutations near the Streptomycin Locus of *Streptococcus*

By ESTHER METZER,* VIRGINIA SCHURMAN
AND A. W. RAVIN

*Departments of Biology and Microbiology, University of Chicago,
Chicago, Illinois 60637, U.S.A.*

(Received 3 October 1973; revised 26 November 1973)

SUMMARY

A mutant induced by nitrosoguanidine in the Challis (group H) strain of *Streptococcus* contains a number of separable mutations linked to each other and to the locus governing sensitivity to streptomycin. One suppresses the level of resistance conferred by mutations in the streptomycin-sensitivity locus, two confer sensitivity to mitomycin and one confers sensitivity to ultraviolet radiation. None of these mutations affects transformability with either homologous or heterologous DNA. However, with the suppressor in the recipient strain there is a loss of streptomycin-resistant transformants, dependent upon the level of resistance conferred by the donor streptomycin-resistance marker and the degree of linkage between the suppressor and that marker.

INTRODUCTION

In transformations of pneumococcal and streptococcal strains, donor genetic markers are integrated with probabilities that depend upon the marker itself and the heterology between recipient and donor genomes. Recently, a class of mutants failing to discriminate between markers has been isolated in pneumococci (Lacks, 1970; Tiraby, 1971). Such mutants continue to discriminate against DNA of heterospecific origin (Tiraby & Ravin, 1973). In *Escherichia coli* and other bacteria, decreased genetic recombination has been found among mutants conferring sensitivity to ultraviolet light or to mitomycin (Clark & Margulies, 1965; Okubo & Romig, 1966; Hoch, Barat & Anagnostopoulos, 1967). To determine whether mutants unable to discriminate against heterospecific DNA occurred among such recombination-deficient mutants, mitomycin-sensitive mutants of the Challis strain of *Streptococcus* were induced by nitrosoguanidine and tested for discrimination. One mutant which appeared to be altered in discrimination subsequently proved to contain, within a cluster of linked mutations, a suppressor of streptomycin resistance. Due to the presence and nature of the suppressor, the results of transforming the mutant with a variety of streptomycin-resistance mutations can be misinterpreted to be the consequences of discrimination defects.

METHODS

The group H Challis strain of *Streptococcus* was used; this was the recipient for homo-specific (Challis) and heterospecific (SM-1 pneumococcus) DNA in previously reported experiments (Chen & Ravin, 1966; Biswas & Ravin, 1971). These reports also described media and detailed methods of obtaining transforming DNA and of transforming bacteria.

* Present address: Department of Molecular Biology, Hebrew University, Hadassah Medical School, Jerusalem, Israel.

In the usual 'short term' transformation, bacteria previously rendered competent and frozen in 10 % glycerin were thawed in ice-water and exposed, at a density of 10^7 bacteria/ml, to donor DNA for 10 to 20 min after which DNase was added. Incubation was continued until the acquired donor markers were phenotypically expressed and the appropriate dilutions were plated in selective and non-selective medium. In 'long term' transformation, bacteria were not previously rendered competent but were grown for 2 h at 37 °C from an inoculum of approximately 10^6 bacteria/ml, after which DNA was added and incubation continued at 37 °C until growth stopped (about 2×10^8 bacteria/ml). At this time plating was made on to selective and non-selective media. 'Long term' transformation does not require exact knowledge of when competence arises, and was especially suitable when the slow-growing mutant strain 204/7 was used as recipient. Unless otherwise indicated, a final concentration of 1 µg DNA/ml was used in both methods.

Growth of a culture was measured by increase in extinction at 650 nm in a Bausch and Lomb Spectronic colorimeter using an uninoculated culture tube as a blank. Ultraviolet sensitivity of wild-type and 204/7 cultures was measured by spotting 0.01 ml amounts of an appropriate dilution of an exponentially-growing culture (E_{650} 0.1) on non-selective agar medium and exposing for various times to irradiation from a Hanovia germicidal lamp (10.8 ergs/mm²/s at 254 nm). The numbers of colonies in the spots were counted after 24 to 48 h incubation at 37 °C, and curves relating survival to dose were plotted. For identification of transformants with respect to u.v.-sensitivity, 0.01 ml spots were tested on the same plate as spots of known wild-type and 204/7 cultures. The spots were then exposed for 60 s (648 ergs/mm²), and examined after 24 h incubation at 37 °C. Wild-type spots contain appreciable growth with over 1000 times more colonies than 204/7 spots.

For mutagenesis with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (NTG), a growing Challis culture was exposed to 0.1 µg NTG/ml for 30 min at 37 °C (about 99 % killing). The culture was washed and incubated for 2 h to permit expression of mutant phenotypes, and often stored for 48 h by freezing at -70 °C after adding glycerin at a final concentration of 10 %.

Mitomycin C was obtained from Nutritional Biochemicals Co., and NTG from the Aldrich Chemical Co.

RESULTS

Isolation and properties of mutant 204/7

A Challis strain carrying an erythromycin-resistance marker (*ery-r2*) originally derived by heterospecific transfer from the SIII-1 strain of *Pneumococcus* was mutagenized with NTG. After thawing, the culture was plated on non-selective medium. Forty-two colonies were streaked on sectors of non-selective agar medium, which served as master plates for replication on to non-selective medium and medium containing 100 µg streptomycin/ml, 0.25 µg erythromycin/ml (selective for *ery-r2* mutants), or 0.015 µg mitomycin C/ml (which permits growth of the wild-type Challis strain). Of these 42 isolates, all of which were streptomycin-sensitive and erythromycin-resistant, 13 failed to grow and 4 grew only slightly on mitomycin agar. Of those not growing on mitomycin agar, one strain, 204/7, was studied in detail. In addition to being erythromycin-resistant, it was sensitive to both mitomycin and u.v. light (Fig. 1), and grew more slowly than either wild-type Challis or *ery-r2* Challis strains (Fig. 2).

Fig. 1

Fig. 2

Fig. 1. Sensitivity of wild-type, 204/7, and derivative Challis strains to u.v. irradiation. The wild-type strain is designated *UV-r su⁻(str)* (●), 204/7 as *UV-s su⁺(str)* (□), and a transformant obtained in the wild-type strain by treatment with DNA from 204/7 is designated as *UV-s su⁻(str)* (○). The wild type and 204/7 also carry the *ery-r2* marker.

Fig. 2. ●, Growth of wild type (36); □, wild type carrying *ery-r2* (34); and ○, 204/7 strain carrying *ery-r2* (52). The figures in parentheses are the generation times in minutes calculated from the exponential phase of the growth curve.

Transformability of strain 204/7

Despite its slower growth, strain 204/7 can be grown to competence in a similar way and with the same levels of transformability as the wild-type Challis strain. Because of its slower growth, the peak of competence usually occurred later during the growth of the 204/7 culture (Fig. 3). The expression of *str-r* markers at 37 °C requires 2 h for 204/7 and 90 min for wild-type recipients, so that 2 h were allotted for phenotypic expression in all experiments. Although Fig. 3 shows a higher peak of transformability for wild type (9 % transformation efficiency) than for 204/7 (2 %), this difference was well within the variability in efficiency observed with different wild-type preparations. Moreover, a transformation efficiency of 10 % was observed with one batch of competent 204/7 cells. Therefore, 204/7 was not defective in genetic recombination.

Using 'long term' transformation, 204/7 was compared with wild type in its response to a number of homospecific DNA preparations differing in *str-r* marker and to a heterospecific DNA carrying the pneumococcal *str-r53* marker (Table 1). While 204/7 discriminated against the pneumococcal marker about as much as did the wild-type Challis strain, transformation by the homospecific *str-r44* and *str-r46* markers was reduced relative to that of the homospecific *str-r43* marker, and considerable heterogeneity was noted in the size

Fig. 3

Fig. 4

Fig. 3. Growth and development of transformability in wild-type and 204/7 Challis strains. Samples removed at various times and tested for transformability by 20 min exposure to 1 μ g homospecific Challis *str-r43* DNA/ml and plated 2 h later in agar containing 100 μ g streptomycin/ml. Efficiency of transformation = no. *str-r* transformed colonies/total no. colony-forming units. (●, ▲), Extinction; (○, □) transformation efficiency.

Fig. 4. Sensitivity to streptomycin of wild type carrying *ery-r2* marker (●) and of 204/7 (○). Samples containing about 10^8 exponentially-growing cells were plated at suitable dilutions in media containing the concentrations of streptomycin indicated.

Table 1. Comparison of wild-type Challis and 204/7 in frequency of *str-r* transformants selected on 100 μ g streptomycin/ml

Strain	Donor <i>str-r</i> marker	Transformant frequency	Relative efficiency of <i>str-r</i> transformation*
Wild-type	Challis <i>r43</i>	4.03×10^{-2}	1.0
	<i>r44</i>	3.93×10^{-2}	1.0
	<i>r45</i>	2.65×10^{-2}	0.6
	<i>r46</i>	3.37×10^{-2}	0.8
	pneumo <i>r53</i>	2.0×10^{-3}	0.05
204/7	Challis <i>r43</i>	1.11×10^{-2}	1.0
	<i>r44</i>	1.07×10^{-3}	0.1
	<i>r45</i>	5.03×10^{-3}	0.5
	<i>r46</i>	1.35×10^{-3}	0.1
	pneumo <i>r53</i>	6.62×10^{-4}	0.06

* Efficiencies normalized to that of *str-r43* transformation, which was arbitrarily set at 1.0.

Table 2. *Frequencies of str-r transformants in the 204/7 recipient strain selected at different concentrations of streptomycin*

Donor <i>str-r</i> marker*	Selective streptomycin concentration	Transformant frequency ($\times 10^{-2}$)	Relative efficiency
<i>str-r43</i>	100	2.27	1.0
	25	2.32	1.0
	10	2.20	1.0
<i>str-r44</i>	100	0.51	0.2
	25	2.24	1.0
	10	2.55	1.1
<i>str-r46</i>	100	1.39	0.6
	25	3.29	1.4
	10	4.52	2.0
<i>str-r53</i>	100	2.24	1.0
	25	2.37	1.0
	10	2.87	1.2

* All markers, including *str-r53* which was originally introduced by heterospecific transformation, were contained in homospecific Challis donor DNA (5 $\mu\text{g/ml}$).

of the colonies of *str-r43* and *str-r53* transformants selected in agar containing 100 μg streptomycin/ml. No heterogeneity was seen in transformations of the wild type.

Challis strains carrying the *str-r44* and *str-r46* markers ordinarily have 100 % plating efficiency in media containing up to 100 μg streptomycin/ml, while those carrying the *str-r43*, *str-r45* and *str-r53* markers (the latter introduced via pneumococcal DNA) resist over 1000 μg streptomycin/ml. Consequently, the relatively low frequency of *str-r44* and *str-r46* transformants obtained in 204/7, as well as the heterogeneity in size of transformed colonies, could be attributed to a suppressor of streptomycin resistance in the 204/7 recipient cells. This suppressor would be like those previously observed in pneumococcus (Bryan, 1961; Mishra & Ravin, 1966; Ravin, Rotheim & Coulter, 1969). The suppressor in 204/7 could be reducing the level of resistance of cells bearing *str-r44* or *str-r46* mutations, so as to cause a loss of newly produced transformants containing these markers when selected in media containing 100 μg streptomycin/ml.

The first indication that this was the correct explanation for the low frequency of *str-r44* and *str-r46* transformants was that 204/7 was clearly more sensitive to streptomycin than wild type (Fig. 4). Further support for the suppressor hypothesis came from the results of experiments in which Challis DNA preparations containing the *str-r43*, *str-r44*, *str-r46* or *str-r53* markers were used to transform 204/7, with selection at streptomycin concentrations ranging from 10 to 100 $\mu\text{g/ml}$ (Table 2). (Selection at 10 $\mu\text{g/ml}$ was possible only in the 204/7 strain, which has a very low streptomycin tolerance; appreciable growth of the wild-type Challis strain occurred on streptomycin concentrations below 35 $\mu\text{g/ml}$.) Upon decreasing the selective streptomycin concentration, *str-r44* and *str-r46* transformation frequencies in 204/7 increased, and reached or exceeded the levels for *str-r43* and *str-r53* markers. The frequency of *str-r43* or *str-r53* transformants did not change significantly when the selective concentration was reduced.

Suppressed and unsuppressed str-r transformants

Large and small colonies of *str-r43* and *str-r53* transformants were observed on 100 μg streptomycin/ml, but not on 25 $\mu\text{g/ml}$. Many of these large and small colonies were streaked

Table 3. *Separability of su⁺(str) from mit-s and str-s mutations during str-r transformations of the 204/7 strain*

Number in parentheses is the percentage of *str-r* transformants having the indicated phenotype.

Donor DNA*	No. <i>str-r</i> transformants tested	su ⁻ (str)				su ⁺ (str)			
		mit-R	mit-I	mit-S	Total	mit-R	mit-I	mit-S	Total
Challis <i>str-r43</i> <i>su</i> ⁻ (<i>str</i>) (ex Ch)	284	53 (18.6)	26 (9.1)	3 (1.1)	82 (28.8)	22 (7.7)	117 (41.2)	63 (22.2)	202 (71.1)
Pneumo <i>str-r53</i> <i>su</i> ⁻ (<i>str</i>) (ex pn)	316	7 (2.2)	148 (46.8)	158 (50.0)	313 (99.0)	0 (0)	0 (0)	3 (0.9)	3 (0.9)
Challis <i>str-r53</i> <i>su</i> ⁻ (<i>str</i>) (ex pn)	520	n.t.	n.t.	n.t.	516 (99.2)	n.t.	n.t.	n.t.	4 (0.8)

* Origin of wild type allele of suppressor [*su*⁻(*str*)] is indicated in parentheses: (ex Ch) and (ex pn) = 'from Challis' and 'from SM-1 pneumococcus', respectively. n.t. = Not tested.

on non-selective agar plates. On replication to a series of agar plates containing increasing concentrations of streptomycin, the large colonies were shown to contain bacteria resistant to at least 1000 µg streptomycin/ml whereas the small colonies contained bacteria unable to grow on 500 µg streptomycin/ml. Each phenotypic class was uniform in its resistance level and distinct from the other. The higher resistance class was similar to *str-r43* transformants induced in wild type, and its genotype was therefore designated *str-r43 su*⁻(*str*). The genotype of the transformants with suppressed resistance was designated *str-r43 su*⁺(*str*), that of 204/7 *str-s su*⁺(*str*), and of wild type *str-s su*⁻(*str*).

Separation of the mutant sites of 204/7

To determine whether the suppressor was separable from the mutations causing mitomycin sensitivity, strain 204/7 was transformed with DNA from two sources: a Challis donor containing the *str-r43* marker of Challis origin, and a SM-1 pneumococcal donor carrying the pneumococcal *str-r53* marker. Transformants were selected on 25 µg streptomycin/ml, and the colonies transferred to non-selective agar media for subsequent replication to agar containing 500 and 25 (or 100) µg streptomycin/ml to distinguish *su*⁺(*str*) from *su*⁻(*str*) bacteria, as well as to agar containing 0.015 µg mitomycin/ml to determine mitomycin sensitivity (Table 3). Three types of response to mitomycin were detected: mitomycin-resistant like the wild-type strain (mit-R), mitomycin-sensitive like the 204/7 mutant (mit-S), and an intermediate response (mit-I). The three phenotypic classes were distinct and reproducible: mit-R cells showed vigorous, uniform growth, mit-S cells failed to grow, and mit-I cells grew feebly with scattered papillae. Thus, in all, six phenotypic classes were recognized.

The results demonstrate that: (1) The *su*⁺(*str*) site in the 204/7 strain can be separated from the *str-s* marker when the latter is replaced by an *str-r* marker during transformation. (2) Linkage of the suppressor to the *str* locus it modifies is close, for in almost 30 % of the transformations by Challis *str-r43* DNA, the non-suppressing allele (*su*⁻(*str*)) of the suppressor accompanies the *str-r43* marker. (3) Linkage of the suppressor site to the *str-r53* marker in the pneumococcal genome appears to be even closer, since the non-suppressing allele accompanies *str-r53* 99 % of the time. (4) The mutated sites causing mitomycin sensitivity are separable from the suppressor of streptomycin resistance. (5) Mit-I transformants are about twice as common as mit-S transformants when homospecific *str-r43*

Table 4. Test of DNA from suppressed and unsuppressed *str-r43* transformants on wild-type Challis [*str-s su⁻(str)*] recipients

Donor DNA	Expt. no.	Frequency <i>ery-r</i> transformants ($\times 10^{-2}$)	Frequency <i>str-r</i> transformants ($\times 10^{-2}$) selected on streptomycin ($\mu\text{g/ml}$):			<i>str-r</i> transformants tested		
			35	100	500	Total isolated	No. <i>su⁻(str)</i>	No. <i>su⁺(str)</i>
<i>str-r43 su⁻(str)</i> <i>ery-r2</i>	1	1.5	n.t.	2.2	2.2	72	72	0
	3	0.4	0.5	n.t.	n.t.	234	234	0
					Total	306	306	0
<i>str-r43 su⁺(str)</i> <i>ery-r2</i>	1	1.3	n.t.	1.9	< 0.01	70	0	70
	2	n.t.	3.1	n.t.	n.t.	126	3	123
	3	0.2	0.3	n.t.	n.t.	234	0	234
					Total	430	3	427

n.t. = Not tested.

DNA is used, which suggests that more than one site conferring mitomycin sensitivity is present in 204/7, and possession of only one of these sites produces the mit-I phenotype. (6) The mitomycin-sensitivity sites are linked both to the suppressor and the streptomycin-resistance locus. In the cross with Challis *str-r43* DNA, only about 4 % (3/82) of the unsuppressed (*str-r43 su⁻(str)*) transformants retained the mitomycin sensitivity (mit-S) of the recipient. Moreover, the mitomycin-resistance sites of the donor strain accompanied the *str-r43* marker about 25 % of the time [(53 + 22)/284].

To determine whether the heterology involved in the Challis 204/7 $\times$ pneumococcus *str-r53* cross is responsible for the close linkage between the suppressor and the *str* marker, a *str-r53 su⁻(str)* transformant was isolated and DNA prepared from it. [Although the transformant has primarily a Challis genome, both the *str-r53* and *su⁻(str)* alleles are of pneumococcal origin since the recipient was originally *str-s su⁺(str)*.] This DNA was used to transform 204/7 (Table 3) and 520 *str-r53* transformants selected and tested only for the presence of the *su⁺(str)* allele. Only four contained the suppressor allele; there was 99 % linkage between *str-r53* and *su⁻(str)*. This means that it was not the gross heterology in the Challis $\times$ pneumococcus cross that caused the observed increase in linkage between the suppressor and *str* sites. Possibly the *str* to *su* region is physically shorter in the pneumococcal genome or it contains a sequence that increases genetic linkage between the two loci.

Test of DNA extracted from suppressed and unsuppressed str-r43 transformants of 204/7

As a check of the genotypic distinction between the two phenotypic classes of streptomycin-resistant transformants induced in 204/7 by *str-r43* DNA, a DNA preparation obtained from a transformant of each type was tested on the wild-type Challis strain and the results compared (Table 4). In addition to the *ery-r2* marker, each DNA bears *ex hypothesi* the *str-r43* marker, but only DNA from the suppressed transformant should be able to produce two types of resistant transformants (suppressed and unsuppressed) in wild-type bacteria, while DNA from the unsuppressed transformant should produce only the unsuppressed type. These expectations were confirmed. Comparison of Tables 3 and 4 shows, however, that there is greater linkage between the sites of the *str-r43* and *su⁺(str)* markers when the latter are in the *cis* or coupling configuration (< 1 % separation) than when they are in the *trans* or repulsion configuration (70 % separation).

Fig. 5. Summary of crosses involving transfer of mitomycin sensitivity.

Analysis of mutations conferring mitomycin sensitivity

Evidence has been given suggesting that two separate but linked mutations confer the mitomycin sensitivity of 204/7, and that the presence of only one of the sites in the genome confers a level of mitomycin resistance intermediate between that of 204/7 and the wild type. Since all mutants in the intermediate class are the same, each mit-S mutation is assumed to confer a similar degree of sensitivity.

Additional crosses were performed to test this hypothesis, and the qualitative results of the various experiments are summarized in Fig. 5. In most of the experiments *str-r* transformants were selected and tested for mitomycin sensitivity, but sometimes unselected clones were isolated from transformed populations and these tested. In either case, the results confirmed the hypothesis. It should be noted that DNA from mit-I strains could confer only an intermediate level of sensitivity on mit-R or mit-S strains. Yet mit-R strains could be converted to mit-I or to mit-S by DNA from mit-S, while mit-S strains could be converted to mit-R or mit-I by DNA from mit-R. The two mutations in the 204/7 genome were designated *mit-s1* and *mit-s2*.

Analysis of the mutation to u.v. radiation sensitivity

When wild-type Challis bacteria were transformed with DNA from 204/7, one mit-S and four mit-I transformants were isolated. The mit-S and one of the mit-I transformants were u.v.-resistant like wild type, while three mit-I transformants were u.v.-sensitive like 204/7. None of the transformants contained *su⁺(str)* as tested by level of streptomycin resistance. Thus, u.v. sensitivity was not a property conferred by the mit-S mutations or by the suppressor allele *su⁺(str)*. That it is due to a single transferable mutation was concluded from a similar cross in which the transformed population was plated in non-selective media and in erythromycin agar. The frequency of *ery-r2* transformants was 7.5%. One hundred colonies were isolated from non-selective media and tested. Nine of these possessed the u.v. sensitivity of the donor and seven also contained the *su⁺(str)* donor allele. Five had either the mit-I or mit-S level of mitomycin sensitivity. The u.v. sensitivity of one of the mit-R *su⁻(str)* transformants is shown in Fig. 1. We concluded that the u.v.-sensitivity marker (*UV-s*) is separate from, but closely linked to, *su⁺(str)* and the two *mit-s* markers.

Other properties of su⁺(str) strains

The 204/7 genome contains, in addition to the NTG-induced markers, the *ery-r2* mutation which originally arose spontaneously in SM-I pneumococcus and was transferred to Challis. *Ery-r2* is weakly linked to the *str* locus (Ravin & Chen, 1967). We found that the NTG-induced mutations in 204/7, while closely linked to the *str* locus, were either unlinked or loosely linked to the *ery* locus. For example, when *str-r43 su⁻(str) ery-s* bacteria were

transformed with DNA from *str-s su⁺(str) ery-r2* cells, about 0.5 % *ery-r2* transformants were produced, and of 120 such transformants picked and tested, all proved to lack the suppressor [i.e. they were *str-r43 su⁻(str)*]. Moreover, we also found that *su⁺(str)* does not affect the level of erythromycin resistance conferred by *ery-r2*.

DISCUSSION

Strain 204/7 has the genotype *str-s su⁺(str) UV-s mit-s1 mit-s2 ery-r2*, in which all but the *str-s* and *ery-r2* markers were induced by a single brief treatment with NTG. It is significant that all of the detected mutations were closely linked. This is undoubtedly the consequence of the production by NTG of clusters of mutations near replicating points in the genome (Guerola, Ingraham & Cerda-Olmeda, 1971). Our results re-emphasize the caution necessary when using NTG as a mutagen. Nevertheless, NTG can prove useful, particularly when markers linked to a locus of special interest are desired. For example, we found that loci conferring sensitivity to u.v. radiation and to mitomycin were located near the streptomycin locus, which affects a 30S ribosomal protein (Traub & Nomura, 1968).

None of the mutations conferring mitomycin sensitivity or u.v.-sensitivity upon the 204/7 strain affected significantly its ability to undergo transformation or to transform with DNA from a heterospecific source. The suppressor of streptomycin resistance, however, can cause spurious effects. If *su⁺(str)* recipients are transformed with *str-r* markers and transformants selected at concentrations of streptomycin that are marginal or sub-optimal for the growth of suppressed *str-r* transformants, low numbers of transformants will be obtained. The loss of *str-r* transformants will depend upon the linkage between the sites of the *str-r* mutation and the wild-type allele of the suppressor in the donor genome: the smaller this linkage the greater the loss. If 204/7 recipients are transformed by homologous DNA carrying the *str-r43* marker and by heterospecific DNA carrying the *str-r53* marker, and *str-r* transformants are selected on 500 µg streptomycin/ml, suppressed *str-r* transformants will be lost, but the loss will be greater with homospecific DNA since linkage of the *str-r* marker to the wild-type allele of the suppressor is much looser than with heterospecific DNA. Since the ratio of heterospecific to homospecific transformants would be higher than for wild-type recipients, strain 204/7 might appear to be discriminating less against heterospecific DNA, but this would be entirely spurious.

The linkage between the suppressor site and the *str-r* sites requires further investigation. It would be useful to determine whether pneumococcal genetic elements in the *su(str)* region affect this linkage, and whether the linkage of *su(str)* to the *str* locus is a function of the mutated configuration of the latter, as our results indicated. Effects of the mutated pattern in the *str* locus on genetic linkage in this region were also suggested by Ravin & Iyer (1962) and Rotheim & Ravin (1968).

This work was supported by research grants from the National Institute for Allergy and Infectious Diseases (AI-09117) and the National Science Foundation (GB-18511) and by a pre-doctoral training award to V.S. from the National Institute for General Medical Sciences (GM-603).

REFERENCES

- BISWAS, G. D. & RAVIN, A. W. (1971). Heterospecific transformation of *Pneumococcus* and *Streptococcus*. IV. Variations in hybrid DNA produced by recombination. *Molecular and General Genetics* **110**, 1-22.
- BRYAN, B. E. (1961). Genetic modifiers of streptomycin resistance in *Pneumococcus*. *Journal of Bacteriology* **82**, 461-470.
- CHEN, K. C. & RAVIN, A. W. (1966). Heterospecific transformation of *Pneumococcus* and *Streptococcus*. I. Relative efficiency and specificity of DNA helping effect. *Journal of Molecular Biology* **22**, 109-121.
- CLARK, A. J. & MARGULIES, A. D. (1965). Isolation and characterization of recombination-deficient mutants of *Escherichia coli* K12. *Proceedings of the National Academy of Sciences of the United States of America* **53**, 451-459.
- GUEROLA, N., INGRAHAM, J. L. & CERDA-OLMEDA, E. (1971). Induction of closely linked multiple mutations by nitrosoguanidine. *Nature New Biology* **230**, 122-125.
- HOCH, J. A., BARAT, M. & ANAGNOSTOPOULOS, C. (1967). Transformation and transduction in recombination-defective mutants of *B. subtilis*. *Journal of Bacteriology* **93**, 1925-1937.
- LACKS, S. (1970). Mutants of *Diplococcus pneumoniae* that lack deoxyribonucleases and activities possibly pertinent to genetic transformation. *Journal of Bacteriology* **101**, 373-383.
- MISHRA, A. K. & RAVIN, A. W. (1966). Genetic linkage of streptomycin-dependence and resistance in *Pneumococcus*. *Genetics* **53**, 1197-1213.
- OKUBO, S. & ROMIG, W. R. (1966). Impaired transformability of a *B. subtilis* mutant sensitive to mitomycin C and ultraviolet radiation. *Journal of Molecular Biology* **15**, 440-454.
- RAVIN, A. W. & CHEN, K. C. (1967). Heterospecific transformation of pneumococcus and streptococcus. III. Reduction of linkage. *Genetics* **57**, 851-864.
- RAVIN, A. W. & IYER, V. N. (1962). Genetic mapping of DNA: influence of the mutated configuration on the frequency of recombination along the length of the molecule. *Genetics* **47**, 1369-1384.
- RAVIN, A. W., ROTHEIM, M. B. & COULTER, D. M. (1969). Genetic and biochemical studies of suppression of ribosomal resistance to streptomycin and erythromycin in pneumococcus. *Genetics* **61**, 23-40.
- ROTHERM, M. B. & RAVIN, A. W. (1968). Inactivation and reversion of multisite streptomycin resistance mutations of *Pneumococcus*. *Molecular and General Genetics* **103**, 159-175.
- TIRABY, G. (1971). *Études des mécanismes responsables des efficacités de transformation chez Diplococcus pneumoniae*. Thèse, Docteur de spécialité, Université Paul Sabatier, Toulouse, France.
- TIRABY, G. & RAVIN, A. W. (1973). Recognition of streptococcal DNA by a mutant pneumococcus unable to discriminate among markers in pneumococcal DNA. *Genetics* **74**, 557-562.
- TRAUB, P. & NOMURA, M. (1968). Streptomycin resistance mutation in *Escherichia coli*: altered ribosomal protein. *Science New York* **160**, 198-199.