EVIDENCE OF TANDEM DUPLICATION OF GENES IN A MERODIPLOID REGION OF PNEUMOCOCCAL MUTANTS RESISTANT TO SULFONAMIDE

S. V. S. KASHMIRI[1] AND ROLLIN D. HOTCHKISS
The Rockefeller University, New York, New York 10021

Manuscript received March 3, 1975

ABSTRACT

A Pneumococcal mutant, *sul*^{r}-*c*, resistant to sulfonamides, and three transformants bearing associated *d* or *d*$^+$ resistance markers have earlier been reported to be unstable and show distinct patterns and frequencies of segregating stable progeny lacking the *c* marker. Each of the four strains showed a characteristic dosage of the genes involved in the merodiploidy. Complementary strands of DNA's from these stable and unstable strains were resolved and homoduplex and heteroduplex hybrids made from the separated DNA strands were used as donors in genetic transformations. Activities of a normal marker (streptomycin resistance) and those involved in the heterozygosity (*c*, *d* and *d*$^+$) were quantitatively measured. From those heteroduplexes made up of opposite strands derived from a heterozygote and a stable strain, the normal marker is transferred efficiently, but the heterozygous markers are not. On the other hand, if both strands of a heteroduplex are derived from different heterozygotic strains, all markers can be transferred with usual efficiency to a stable recipient strain. The lowered efficiency in the former type of heteroduplex is attributed to an inhomology resulting from a tandem duplication in the merodiploid strains, and a postulated DNA repair process stimulated by it while in the form of the donor duplex. The inhomology probably includes (a) a microheterogeneity between the *c* site and the wild type locus, and (b) a more extensive incompatibility attributable to an extra segment of genome in a tandem duplication covering the *c* and *d* sites. The first of these inhomologies produces a lowered efficiency of transfer from all configurations of the particular *d* allele associated with the mutant *c* marker, and therefore accounts for the characteristic transfer patterns even from the native merodiploid DNA's.

IT was suggested in a recent report that the heterozygosity and instability of a certain mutant of Pneumococcus and four transformants derived from it might be attributed to the tandem duplication of the genes in a region of merodiploidy. The unstable mutant (*sul*^{r}-*c*), a persistent heterozygote, is resistant to sulfonamides and its first progeny contain about 3% drug sensitive cells. In the highly resistant *cd* transformants that arose through the introduction of (*sul*^{r}-*c*) into a stable sulfonamide resistant strain, *sul*^{r}-*d*, the heterozygosity extends to the closely linked locus *d*.

The marker-transfer frequencies from several different strains bearing *c*, *d* and *d*$^+$ markers suggested configurational differences, in the corresponding

[1] Present address: Litton Bionetics, Bethesda, Maryland 20014.

DNA's. However, no physical evidence was found that these markers were in any way different from other markers carried by DNA. Thus, the marker activities themselves, or the relative marker-to-marker ratios of activity underwent no perturbation or fractionation as the DNA's were sedimented, melted, denatured or renatured (KASHMIRI and HOTCHKISS 1975).

In this article we explore the degree of homology and inhomology between the DNA of merodiploid *c*-bearing and of stable strains by measurements of transforming activity of DNA heteroduplexes obtained by hybridization of separated DNA strands.

MATERIALS AND METHODS

Strains of Pneumococcus, transformation, and scoring of markers

A description of the Pneumococcal strains used and their designations has been given in an accompanying article (KASHMIRI and HOTCHKISS 1975). The stable strains RI-26 ("wild") and RF6-7 (*d*) were used as recipients. The unstable merodiploid strains in order of increasing *d* donor marker activity are: RF3-7, or *c* (containing d^+ only, therefore "cd_0"); RF63-10 (cd_1); RF63-13 (cd_2) and RF63-11 (cd_3). Each of these was also available as a streptomycin-and micrococcin-resistant (S,K) doubly transformed strain.

Transformation and scoring of markers were accomplished as outlined in the same article. The final concentration of DNA used for transformation of thawed frozen cultures was 0.45 μg DNA/ml. All scoring here was done in agar media.

Preparation of homo- and hetero-duplex transforming DNA's

Preparation, denaturation and resolution of strands were performed as described in the accompanying article (KASHMIRI and HOTCHKISS 1975) which presents a representative profile of density-fractionated merodiploid DNA. Fractions from two or three independent resolutions were employed for all of the strains utilized.

Several fractions at and near each of the density peaks were pooled and dialyzed against 0.15 M NaCl buffered with 0.02M Na phosphate (pH 6.8) to give heavy (H) and light (L) fractions. Equal amounts of the respective fractions were mixed, sodium ion molarity was adjusted to 0.5M and the mixture, in stoppered tubes, was immersed in refluxing methanol (65°) for an hour followed by chilling in ice. Homoduplexes were made by mixing H and L fractions of the same DNA, whereas complementary-strand fractions derived from two different strains were mixed to make heteroduplexes. For brevity, the heteroduplexes made by mixing complementary-strand fractions of DNA's from a merodiploid and a stable strain will be referred to as *c*/non-*c* heteroduplexes. Likewise, *c*/*c* and non-*c*/non-*c* will be the respective designations of the heteroduplexes that are made by mixing H and L fractions of DNA's from two different heterozygous strains or from two different nonheterozygous strains.

Calculation of the recovery of marker activities from heteroduplexes

The data are presented for each marker in terms of the total activity of that marker summed up for the two reciprocal heteroduplexes relative to the summed activities of the same marker in the two homoduplexes. This way of presentation of the data, rather than the display of the recovery of marker activity in each individual reciprocal heteroduplex, averages the strand-bias effects due to the different transfer efficiencies of the same markers when in their two complementary aspects (GABOR and HOTCHKISS 1966, 1969). Having performed the four different transformations under identical conditions in equivalent aliquots of competent cells, one is permitted to combine and relate the observed activities.

To illustrate the method of calculation: Let *m* and *n* be the respective activities of a certain marker in the homoduplexes ($H_x + L_x$) and ($H_y + L_y$), made up of the complementary strands of the DNA's from any two different strains *x* and *y* respectively. Let *p* and *q* be the respective

activities of the heteroduplexes ($H_x + L_y$) and ($H_y + L_x$); then $\frac{p + q}{m + n} \times 100$ represents the combined-marker activities of the two heteroduplexes as percent of the combined activities of the homoduplexes. In case a particular marker is carried by only one strain, say x, then the relationship is simply $\frac{p + q}{m} \times 100$; but the magnitude remains of the same order.

RESULTS

Low recovery of heterozygous markers in c/non-c heteroduplexes

It has been established that the heterozygous markers (*c*, *d* and d^+) of the unstable Pneumococcal strains are inactivated and recovered on reannealing as well as the str^r, a "stable" marker only loosely linked to the heterozygotic region. It could be expected that the recovery of the activities of these markers and str^r, in a heteroduplex, would be similar. Contrary to this expectation, the results shown in Table 1 reveal that the recoveries of the *c* marker activity and that of the linked pair *cd* are substantially lower (average, 28) than the recovery of the str^r marker activity (average 72). Also, the heteroduplexes cd_0S/d and cd_gS/wild (top two lines of Table 1) in which d^+ and *d* are derived from a merodiploid strain, show lowered recovery for these two markers. Thus markers originating in a heterozygous strain do not efficiently recover their activity when heteroduplexes are made between strands of this strain and opposite strands from a stable strain.

It seems desirable at this point to review in what our test of homogeneity consists. It is a common finding of all workers that denatured DNA, when it is renatured, regains only approximately one-half of its original native transforming activity. In this respect our merodiploid and normal markers proved to be equally renaturable (KASHMIRI and HOTCHKISS 1975). When denatured DNA's are strand-fractionated, and selected fractions are mixed and annealed, there may often be a slightly lower recovery, possibly attributable to incomplete complementarity of the strand fractions chosen. In this respect again, our markers did not show significant differences. However, when opposite strand fractions from differently marked DNA's are mixed and reannealed, there is

TABLE 1

A Comparison of the Recovery of Markers from DNA Hybrids of the Heterozygous and Nonheterozygous Strains

Heteroduplex DNA	Recipient	Relative marker recovery				
		S	*c*	*d*	d^+	cd^+
cd_0S/d	*d*	77	35	—	51	32
cd_gS/wild	wild	85	48	59	—	—
cd_0S/wild	*d*	65	22	—	—	18
$cd_0S/$ *d*	wild	50	19	85	—	(19)
wild/ *d*	*d*	—	—	—	78	—
wild/ *d*	wild	—	—	92	—	—

Figure in parenthesis is derived from the value for *c* taking into account the known genotypes.
Relative marker recovery represents the activity in heteroduplex pairs in percent of that from homoduplex pairs (see MATERIALS AND METHODS).

characteristically some additional deficit, in heteroduplexes compared to homoduplexes, even when the marked strains are closely related as are ours. It is in this last test that *c*-related markers show a substantial difference in Table 1 being less than one-half as well recovered as stable markers. The deficit in the recovery of *c*-related markers is observed for each individual reciprocal heteroduplex, even though Table 1 displays the deficit only in the total recovery for the two reciprocal heteroduplexes.

For the purpose of these comparisons, individual strand-marker efficiencies (GABOR and HOTCHKISS 1966, 1969) are eliminated by averaging of the reciprocal heteroduplexes. Other factors reflecting the choice of fractions, the conditions of fractionation or renaturation, etc., are balanced out by making the heteroduplexes and homoduplexes in the same experiment and from the identical two pairs of strand fractions. Thus, it will be seen that although in different experiments (horizontal lines in all of the Tables herewith) recoveries in heteroduplexes versus homoduplexes show moderate variation, wherever a relatively low value is obtained for str^r marker, the merodiploid markers always show correspondingly still lower values.

The low recovery of the activities of the *d* and d^+ markers, in these heteroduplexes, might be attributable to: (1) an intrinsic property of these markers, (2) the type of the heteroduplex (*c*/*c*; *c*/non-*c*, non-*c*/non-*c*) in which they are measured, (3) the genetic nature (composition, structure) of the strains that carry these markers, or (4) a combination of two or more of these factors. Two aspects of *d* and d^+ markers make them useful for analyzing these possibilities. First, the markers *d* and d^+, unlike *c*, can be carried also by nonheterozygous stable strains, and secondly, two of the merodiploids, cd_oS and cd_sS, carry only the d^+ or the *d* allele, respectively.

That the *d* and d^+ markers of stable strains are not intrinsically incapable of efficient recovery is evident from the figures in the bottom two lines of Table 1. It is also obvious that the *d* marker carried in the stable or non-*c* component is efficiently reactivated in a *c*/non-*c* heteroduplex (cd_oS/d). By contrast, as already mentioned, when the *d* or d^+ markers come from the *c* component of a *c*/non-*c* heteroduplex they are less efficiently recovered.

These results clearly point out that the low recovery of activity of the merodiploid markers in heteroduplexes must be partly attributable to the genetic nature of the unstable strains. It remains to be settled whether this genetic property manifests itself only in a particular type of heteroduplex.

Normal recovery of heterozygous markers in c/c heteroduplexes

The results presented in Table 2 indicate the role of the nature of the heteroduplex as a determinant of the efficiency. The first four lines of this table reaffirm the finding that the recovery of the activities of the *d* and d^+ markers are low, and that of *c* marker and the linked pairs *cd* and cd^+ still lower, in comparison to the recovery of str^r. In these first cases the markers assayed are derived from the *c* component of a *c*/non-*c* type of heteroduplex. The results given in the last two lines of the table show that the activities of the *c*, *d* and d^+ markers and their

TABLE 2

Comparison of Recovery of Markers of Merodiploid Strains when the c *Region is Present in Only One or in Both of the Strands of Heteroduplex DNA*

Heteroduplex DNA	Recipient	Relative marker recovery					
		S	*c*	*d*	*d*$^+$	*cd*	*cd*$^+$
cd_0S/wild	wild	68	26	—	—	—	(26)
cd_0S/ *d*	*d*	64	31	—	49	—	(31)
cd_3S/wild	wild	85	48	59	—	35	—
cd_3S/ *d*	*d*	91	41	—	—	(41)	—
cd_0S/cd_3S	wild	70	71	105	—	78	68*
cd_0S/cd_3S	*d*	78	79	—	78	87†	68

Figures in parenthesis are assumed from the value for *c* taking into account the known genotypes.

* Value of *cd*$^+$ as *c* not linked to *d*

† Value of *cd* as *c* not linked to *d*$^+$

combinations *cd* and *cd*$^+$ are recovered as efficiently, even from the merodiploid strains, as the activity of the *str*^{r} marker, if the heteroduplex is made from the complementary strands of two merodiploids.

Recovery of the marker activities from the strains heterozygous for the alleles of d

The results discussed thus far have dealt only with those heterozygotic strains that carry exclusively either the *d* or *d*$^+$ marker. Two other merodiploids, RF63SK-10 (cd_1S) and RF63SK-13 (cd_2S), carry not only the *c* and *c*$^+$ markers but they are "intermediate" in that they carry both alleles of *d* as well. For a full comparison of the efficiency of recovery of the merodiploid markers from these strains, in *c*/*c* and *c*/non-*c* types of hybrids, another type of strain heterozygotic in the sulfonamide resistance region of the genome would be needed: one carrying exclusively the *c* allele. Heterozygotes of this type have never been found. However the strains cd_0S and cd_3S, which have already been dealt with, by virtue of carrying *c* in the form of either the linked pair *cd*$^+$ or *cd* only, can also partly satisfy the need. Since cd_0S carries *c* only in the *cd*$^+$ combination, its complementary strands can be used to analyze heteroduplexes of the c/c type carrying the *d* marker from the intermediate strains, cd_1 and cd_2. In this *d*$^+$ background, the *d* stands out as a label to identify the rescue of strands from a particular source in a heteroduplex. Likewise, the purity of *d* in the cd_3S permits the *cd*$^+$ component of equivalent heteroduplexes with the cd_1 and cd_2 DNA to be identified.

Table 3 is a summary of the results of the experiments dealing with the "intermediate" merodiploids, cd_1S and cd_2S. The *c*/non-*c* type of heteroduplexes, as already established, shows low activities of the merodiploid markers, but the same DNA fractions when put together in heteroduplexes of the c/c type show higher or normal reconstitution of activity of these markers when tested in the same transformation experiments. The *c*, *d*, *d*$^+$, as well as the *cd* and *cd*$^+$, activities

TABLE 3

Normal Recovery of Merodiploid Markers from c/c Heteroduplexes

Heteroduplex DNA	Recipient	Relative marker recovery					
		S	*c*	*d*	d^+	*cd*	cd^+
cd_1S/wild	wild	80	40	59	—	42	36*
cd_0S/wild	wild	67	25	—	—	—	(25)
cd_1S/cd_0S	wild	64	89	103	—	89	88*
cd_1S/ d	d	81	46	—	72	47†	—
cd_3S/ d	d	72	30	—	—	(30)	—
cd_1S/cd_3S	d	89	77	—	114	74†	—
cd_2S/wild	wild	82	30	65	—	27	31*
cd_9S/wild	wild	68	31	—	—	—	(31)
cd_2S/cd_0S	wild	75	71	99	—	102	66*
cd_2S/ d	d	69	34	—	47	34†	33
cd_3S/ d	d	72	45	—	—	(45)	—
cd_2S/cd_3S	d	77	78	—	87	81†	69

Figures in parenthesis are derived from the value for *c* taking into account the known genotypes.

* Value of cd^+ as *c* not linked to *d*

† Value of *cd* as *c* not linked to d^+

in the four *c*/*c* heteroduplexes shown are more than twice as great as recoveries from the eight *c*/non-*c* hybrids, and fully equal to those of all of the str^r recoveries.

These results, together with those in the last lines of Table 2, amply demonstrate that markers (*c*, *d* and d^+) of the merodiploid region are as readily reactivated in *c*/*c* heteroduplexes as the *d* and d^+ *markers of* non-*c*/non-*c*, or the str^r marker in any of the three types of heteroduplex. It is only in the *c*/non-*c* heteroduplexes that the merodiploid markers remain low in activity.

This clarification permits us to inquire more particularly into the precise degree of reactivation observed for *d* and d^+ in the *c*/non-*c* heteroduplexes, where it falls between the high values for str^r and the low values for *c*. In the hybrids made from cd_1S with wild or *d* DNA, the d^+ marker appears to be more nearly normally reactivated (72%) than does the *d* (59%). In the cd_2S heteroduplexes the *d* appears to be the more fully reactivated allele.

DISCUSSION

The heterozygous markers of the unstable Pneumococcal merodiploids differ from the normal marker, str^r, carried by the same strains, in their low efficiency of recovery in the *c*/non-*c* heteroduplexes. The activities of these same markers are recovered normally in the hybrid molecules consisting of the complementary strands derived from the DNA of two heterozygotes. This difference of behaviour of the merodiploid markers can be explained on the basis of configurational difference in the structures of the *c*/*c* and *c*/non-*c* duplexes. In the former hybrids, it seems that the degree of perfection of double stranded structure achieved by the heterozygotic region is equivalent to that attained by the regions carrying

the normal markers, or to that of any of the markers in non-*c*/non-*c* hybrids from stable strains.

On the other hand, it appears that some type of inhomology between the complementary strands of DNA from a heterozygote and a wild-type strain interferes with the effective pairing of the heterozygotic region with the corresponding loci of the wild type. Consequently, the *c*/non-*c* duplex may have distortions in the region of heterozygosity. In transformation, such heteroduplex DNA might undergo excision and repair before integration into the genome of the recipient. This trimming-off process would be selectively detrimental to the frequency of the integration of the heterozygous markers because of their frequent removal during this process.

There is some precedent and support for such a possibility. Contrary to the postulate that one strand of donor DNA is destroyed during cell penetration (LACKS 1962), it has recently been pointed out by ROGER (1972) that the complete degradation of one strand of the donor DNA is unlikely in Pneumococcal transformation. Her findings suggest that a process of excision repair may operate prior to the integration of the donor DNA into the recipient genome. In the context of single strand penetration, it is conceivable that the recombinant structure formed on transformation does not undergo excision and repair prior to DNA replication (GUERRINI and FOX 1968) and the screening of the strands takes place during uptake of the donor DNA strands. Penetration of one of the strands may be interfered with if the distortions in the *c*/non-*c* duplex are such as to cause a loop-out of the region of heterozygosity in the strand bearing the *c* mutation.

The inhomology that involves the *c* marker may be related to the nature of this marker itself. Since the *c* marker has not been found in any stable strain, we could not test whether its inefficient recovery in the heteroduplexes is an intrinsic property of this marker. LEDBETTER and HOTCHKISS (1975) concluded that the low frequency of transfer of the *c* marker, in transformations into non-discriminating recipient strains, indicates that it does not belong to the class of markers designated as "low efficiency" (LE) by EPHRUSSI-TAYLOR, SICARD and KAMEN (1965). The low frequency of the transfer of the *c* marker may be attributed to some other type of heterogeneity between the mutant DNA and the wild-type sites of recipient cells. It is obvious that the corresponding incompatibility would exist between the two strands of the *c*/non-*c* heteroduplex prepared *in vitro*.

The low recovery of the *c*, *d* and d^+ marker activities of a double heterozygote, in the particular case of the *c*/non-*c* heteroduplex, indicates an incompatibility of an extensive type between the two strands of such hybrids. The magnitude of this inhomology would be consistent with one of the principal models for the heterozygotic region—an extra segment in the genome of the heterozygote, acquired by it through a linear amplification of the *c* and *d* loci (KASHMIRI and HOTCHKISS 1975). In the *c*/non-*c* duplex formation, the strand of the DNA from the merodiploid for this extra segment would fail to find its match on the complementary strand of DNA from the wild type, and hence, have to loop out in order to allow the neighboring loci to pair effectively with the corresponding loci

of the wild-type strain. Linear amplification of genes has been proposed to account for the properties of the unstable merodiploids for a certain tryptophan marker of *Bacillus subtilis* (AUDIT and ANAGNASTOPOLOUS 1973), for specific str^r and ery^r loci (RAVIN and TAKAHASHI 1970), and for a certain binary capsule marker (BERNHEIMER and WERMUNDSEN 1969) of Pneumococcus.

Given this model of tandem duplication of the genes, accounting for the heterozygosity, a number of other properties of these unstable strains can be reasonably explained:

The genotypes of the heterozygotes

Heterozygotic regions of the four unstable strains are indicated in Table 4. The heterozygosity of the *c* locus is formally attributed to the coexistence of alternative alleles, *c* and c^+, arrayed linearly and in tandem. Those unstable strains homozygous for either the *d* or d^+, have duplicated sites occupied by either the corresponding *d* or d^+ allele. The double heterozygotes carry both of these alleles, in tandem on the same strand.

This portrayal provides a basis for the difference between the recovery of the *d* and d^+ markers from RF63SK-10 (or cd_1S) and RF63SK-13 (or cd_2S), in c/non-c heteroduplexes. This is accounted for on the basis of the linkage of these markers to the *c* or the c^+ allele. In the cd_1S strain, the *d* allele is in normal coupling with the *c* marker and the d^+ is associated in the same way with the c^+ allele. The *d* marker, therefore, suffers the consequences of both the extensive incompatibility between the strands of the heteroduplex, as well as that of the microheterogeneity between the *c* and the c^+ alleles. On the other hand, the recovery of the d^+ marker activity is affected only by the extensive inhomology between the strands in the *c*/non-*c* duplex. Similarly, the higher recovery of the *d* marker activity over that of the d^+ from the cd_2S can be explained.

The stable segregants from the unstable strains

The same figure provides a simple basis for a looping-out mechanism according to which these heterozygotes occasionally segregate out the stable descendants. Occasional self-pairing between the tandem-duplicated regions would permit a single exchange crossover at one or another point in the duplication to give rise to either the stable wild type or the *d* segregants from both the cd_1 and the cd_2 merodiploids. Only one of these could be formed from the other strains, no matter where the crossover occurred.

The characteristic marker dosages of the heterozygotes

Taking the normal dosages as 2 for the *d* and the d^+ alleles present in the respective nonheterozygous strains, the heterozygous strains, have given yields of *d* and d^+ transformants totaling 3 (HOTCHKISS, ABE and LANE 1967, 1971). Their designations as cd_0 to cd_3 signify the relative *d* transformation yields from their DNA's. Although the tandem duplications would provide a total dosage of 4 for *d* and d^+ together, the deficit in transfer can be explained by the proximity of

TABLE 4

Tandem Duplication Model for the Structures of the c Merodiploids

Strain designation	Marker transfer in relation to stable strain: d^+	Marker transfer in relation to stable strain: d	Marker showing deficit when coming from: native homoduplex	Marker showing deficit when coming from: c/non-c heteroduplex	Proposed map of marker region
d S (stable)	0	1.0	—	—	c^+ d S
cd_0S	1.5	0	d^+	*c*, d^+, cd^+	c^+ d^+ *c* d^+ *S*
cd_1S	1.0	0.5	*d*	*c*, *d*, *cd* and cd^+	c^+ d^+ *c* *d* *S*
cd_2S	0.5	1.0	d^+	*c*, d^+, *cd*, and cd^+	c^+ *d* *c* d^+ *S*
cd_3S	0	1.5	*d*	*c*, *d*, *cd*	c^+ *d* *c* *d* *S*

one of the markers in each case to the merodiploid-associated *c* marker. In each case the deficiency (about 50% efficiency of transfer from this site) is detected for that marker indicated as to the right of the *c* insertion.

Studies on the mechanism and frequency by which the unstable duplications are introduced into stable recipients remain to be completed. It should be pointed out that the lowered efficiencies mentioned are encountered with wild-type cells as recipients. Because of their own instability, it is not feasible to do highly quantitative work with the merodiploid strains as recipients. The use of heteroduplexes of *c*/non-*c* donors as reported here, provides a test for hybrid structures prepared *in vitro* rather than as intermediates of transformation, and has shown, we believe, the nature of the inhomology between the stable and unstable types. This inhomology—the result of the duplication—would presumably in turn be recreated as a donor-recipient complex in the transformation of wild-type recipients by *c* DNA, and it will be recalled that this transfer shows a relative efficiency of about 20% whether the *c* DNA is native, denatured, renatured, homoduplex or heteroduplex.

In conclusion, such inhomology as exists between *c*/non-*c* (probably duplicated/non-dupicated) tracts of DNA appears to be subject to two distinct processes during transformation which monitor it for molecular fitness, or complementarity: first, a screening of the presenting donor DNA duplex; secondly, a screening of some form of donor-recipient complex.

DR. MURIEL ROGER is gratefully thanked for advice in the factionation of denatured DNA strands. It is a pleasure to thank ESMERALDA PRAT for help in some of the measurements of biological activity reported. The work was supported by Grant AI-03170 from the National Institutes of Health.

LITERATURE CITED

AUDIT, C. and C. ANAGNOSTOPOLOUS, 1973 Genetic studies relating to the production of transformed clones diploid in the tryptophan region of the *Bacillus subtilis* genome. J. Bacteriol. **114:** 18–27.

BERNHEIMER, H. P. and I. E. WERMUNDSEN, 1969 Unstable binary capsulated transformants in Pneumococcus. J. Bacteriol. **98:** 1073–1079.

EPHRUSSI-TAYLOR, H., A. M. SICARD and R. KAMEN, 1965 Genetic recombination in DNA-induced transformation of Pneumococcus. I. The problem of relative efficiency of transforming factors. Genetics **51:** 455–475.

GABOR, M. and R. D. HOTCHKISS, 1966 Manifestation of linear organization in molecules of Pneumococcal transforming DNA. Proc. Nat. Acad. Sci. USA **56:** 1441–1448, ——, 1969 Differences in rate of phenotypic expression in separated strands of Pneumococcal transforming DNA and evidence for change of reading direction. Genetics suppl. **61:** 15–25.

GUERRINI, F. and M. S. FOX, 1968 Genetic heterozygosity in Pneumococcal transformation. Proc. Nat. Acad. Sci. USA **59:** 429–436.

HOTCHKISS, R. D. and M. ABE, 1968 A heterozygosis present in Pneumococcus and transferable by DNA in transformation. Proc. XII Cong. Genet. **1:** 36.

HOTCHKISS, R. D., M. ABE and D. LANE, 1967 Analysis of a heterozygotic region in Pneumococcal transforming DNA. Genetics suppl. **56:** 566, ——, 1971 Transfer of heterozygotic properties by DNA in Pneumococcus. pp. 21–27. In: *Informative Molecules in Biological Systems.* Edited by L. G. H. LEDOUX, North-Holland Publishing Co.

KASHMIRI, S. V. S. and R. D. HOTCHKISS, 1975 A study of Pneumococcal merodiploids at the molecular level. Genetics **81**: 9–19.

LACKS, S., 1962 Molecular fate of DNA in genetic transformation of Pneumococcus. J. Mol. Biol. **5**: 119–131.

LEDBETTER, M. L. S. and R. D. HOTCHKISS, 1975 Chromosomal basis of the heterozygosity in a partially diploid mutant of Pneumococcus. Genetics **80**: ——, ——.

RAVIN, A. W. and E. A. TAKAHASHI, 1970 Merodiploid ribosomal loci arising by transformation and mutation in Pneumococcus. J. Bacteriol. **101**: 38–52.

ROGER, M. 1972 Evidence for conversion of heteroduplex transforming DNAs to homoduplexes by recipient Pneumococcal cells. Proc. Nat. Acad. Sci. USA **69**: 466–470.

Corresponding editor: F. W. STAHL