Conversion of Deletions During Recombination in Pneumococcal Transformation

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ABSTRACT

Genetic analysis of 16 deletions obtained in the *amiA* locus of pneumococcus is described. When present on donor DNA, all deletions increased drastically the frequency of wild-type recombinants in two-point crosses. This effect was maximal for deletions longer than 200 bases. It was reduced for heterologies shorter than 76 bases and did not exist for very short deletions. In three-point crosses in which the deletion was localized between two point mutations, we demonstrated that this excess of wild-type recombinants was the result of a genetic conversion. This conversion extended over several scores of bases outside the deletion. Conversion takes place during the heteroduplex stage of recombination. Therefore, in pneumococcal transformation, long heterologies participated in this heteroduplex configuration. As this conversion did not require an active DNA polymerase A gene it is proposed that the mechanism of conversion is not a DNA repair synthesis but involves breakage and ligation between DNA molecules. Conversion of deletions did not require the Hex system of correction of mismatched bases. It differs also from localized conversion. It appears that it is a process that evolved to correct errors of replication which lead to long heterologies and which are not eliminated by other systems.

GENERAL recombination involves the exchange of genetic information between homologous DNA segments. However the molecular mechanism of recombination is not well understood, although many models have been proposed. DNA strand transfer, as postulated by several workers (WHITEHOUSE 1963; HOLLIDAY 1964; MESELSON and RADDING 1975), would create heteroduplex DNA as an intermediate in recombination. In pneumococcal transformation, recombination can be studied easily since genetic transfer is simpler than in most other systems: only one random strand of DNA penetrates the cell and integrates into the chromosome (LACKS 1962, FOX and ALLEN 1964). Therefore recombination between donor DNA and recipient DNA requires a heteroduplex intermediate. The existence of such a heteroduplex structure has been strongly supported by the discovery and subsequent analysis of mismatch repair. Several poorly transformable point mutations are recognized specifically and this process eliminates the genetic material originating from the donor DNA by an excision and repair mechanism (EPHRUSSI-TAYLOR and GRAY 1966; for reviews see SICARD *et al.* 1985; CLAVERYS and LACKS 1986; SICARD 1987). In contrast, highly transformable markers are not excised but are integrated into the chromosome, becoming homoduplex after one round of cell duplication. Therefore, recombination can proceed by two pathways: the first requiring a transfer of genetic information from one DNA strand to the other, the second takes place through a mere integrative process. It was found that very short deletions (*i.e.*, from 5 to 34 base pairs (bp), are very efficiently transformed and are not recognized by the mismatch repair system (GASC *et al.* 1987). On the other hand, long deletions transform less well than point mutations and the reduction of transformation efficiency is proportional to the length of these nonhomologies, most probably as a result of inhibition of pairing by heterologous regions (LACKS 1966). However, the mechanism of recombination of long deletions is unknown. In this paper we present evidence for a novel process quite different from the excision repair of some point mutations.

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MATERIALS AND METHODS

Strain and transformation procedures: The *Streptococcus pneumoniae* strains used in this study are derived from Avery's strain R36A. All markers conferring resistance to 2×10^{-5} M amethopterin belong to the same locus, *amiA* (SICARD 1964). One independent marker (*str41*) conferring resistance to streptomycin is used as a reference for transformation. A polymerase A deficient strain containing a *polA* gene disrupted by the insertion of a cat fragment (*polA::cat 28*) was a generous gift from P. LOPEZ (LOPEZ *et al.* 1988). Culture media, preparation of DNA and transformation

procedures have been described earlier (CLAVERYS, ROGER and SICARD 1980).

Recombination between *amiA-r* mutants: Frequencies of *amiA* transformants are measured by comparison with the frequency of transformants for the reference gene *str41*. Cells bearing an *amiA-r* mutation can be transformed with DNA from cells bearing another *amiA-r* mutation, and wild-type recombinants will be produced provided that the two *amiA* mutations are at different sites. Wild-type recombinants are scored by plating in synthetic medium containing an excess of isoleucine because amethopterin-resistant bacteria are especially sensitive to this excess (SICARD 1964). The number of wild-type recombinants will depend on several factors such as the position of the mutant sites relative to each other, and the efficiency of integration of the amethopterin marker in the recipient cell (LACKS and HOTCHKISS 1960).

The *recombination frequency* between two markers is the ratio of the number of $amiA^+$ transformants to the number of streptomycin-resistant transformants divided by the efficiency of the *amiA* marker in the recipient cells. To determine this efficiency, a strand carrying this *amiA* marker is transformed by a DNA bearing the $amiA^+$ allele and the *strR41* reference gene. The efficiency is the ratio of the number of $amiA^+$ transformants to streptomycin resistant transformants. In this paper we have measured this efficiency in an hex^- recipient strain which does not discriminate point mutations into different classes (CLAVERYS, ROGER and SICARD 1980).

Isolation of deletions: We have previously reported that hybrid DNA consisting of pneumococcal chromosomal DNA inserted in a vector, is mutagenic for the recipient pneumococcus and that most of these mutations were deletions adjacent to the cloned pneumococcal fragment starting at or near its extremities and extending outside it (CLAVERYS, LEFÈVRE and SICARD 1980). We have isolated deletions by this procedure. The *amiA* locus has been subdivided into several fragments defined by restriction sites (Figure 1). The *Eco*RI fragment $B1^+$ $B2^+$ was cloned in *Escherichia coli* by insertion into phage λ (CLAVERYS, LEFÈVRE and SICARD 1980) and used to transform $amiA^+$ pneumococcal strains. Amethopterin-resistant mutants were recovered and crossed with several strains carrying mutations mapped in the *amiA* locus. A group of *amiA* mutants (shown as wavy lines in Figure 1) were deletions located to the right of fragment B2 since they do not recombine when intercrossed or crossed with mutant *amiA5* and *amiA30*. Another group of deletions are at the left of fragment B1 since they do not yield any wild-type transformants when intercrossed or crossed with *amiA6* and *amiA141* mutants. Some deletions obtained similarly by cloning in pBR325 were isolated by H. VASSEGHI in our laboratory. *AmiA28*, *amiA30*, *amiA40*, and *amiA109* are spontaneous deletions.

Cloning and sequencing procedures: Mutations at the *amiA* locus were cloned by the rapid procedure previously described (MÉJEAN *et al.* 1981; SICARD *et al.* 1985a, b). The nucleotide sequence of the segment carrying the deletions was determined by the method of SANGER, NICKLEN and COULSON (1977).

Isolation of double mutant *amiA22-amiA109*: Both markers confer resistance to the maximum level of amethopterin (2×10^{-5} M). In order to set up a screening procedure we used a strain carrying a pneumococcal suppressor (Su) that reduces the resistance of nonsense mutations at the *amiA* locus (GASC *et al.* 1979). Deletion *amiA109* is not suppressible whereas *amiA22 Su* resists only to 4×10^{-6} M amethopterin. Thus, we transformed *amiA22 Su* strain with DNA bearing *amiA109* mutation and selected transformants resistant to 10^{-5} M amethopterin. Colony sizes were heterogenous. Bacteria in large colonies were highly resistant to amethopterin because they had lost the suppressor. The small colonies had retained the *Su* gene which reduces the growth of strains when present (GASC *et al.* 1979). Several small colonies resistant to 10^{-5} M amethopterin were subcultured and transformed with DNA containing either *amiA22* or *amiA109*. Most of them did not yield any wild-type transformant. They were *amiA22 amiA109* double-mutants. One of these was kept for further genetic analysis.

RESULTS

Physical properties of deletions: In this study, we have used 16 deletions at the *amiA* locus, 4 of spontaneous origin and 12 induced by transformation of wild-type recipient bacteria with a cloned fragment of the wild-type *amiA* locus (Table 1). The spontaneous mutations are dispersed in the locus. We have sequenced two of them. Interestingly these deletions occurred between short repeated sequences, 9 bases (TTACTCAAG) for *amiA40* and 8 bases (CTCGTGAA) for *amiA109*. The induced mutations start at or near the extremities of the cloned fragment used to obtain them and extend outside it with the exception of *amiA578* as already reported (CLAVERYS, LEFÈVRE and SICARD 1980). Altogether they cover a wide spectrum of sizes, from 76 bp to more than 1100 bp at several locations in the locus. Therefore, they will be useful for defining the general properties of recombination of the deletions.

Transformation efficiencies of deletions: Amethopterin-sensitive recipient strains were transformed with DNA from strains bearing deletions conferring resistance to amethopterin and the streptomycin-resistance marker. Transformants resistant to amethopterin or streptomycin were selected and the transformation efficiency was calculated. The efficiencies of the deletions are listed in Table 1. It can be seen that for spontaneous deletions the efficiencies are high. The shortest deletion (76 bp) has the same efficiency as deletions or additions of 5–34 bp (GASC *et al.* 1987) or some point mutations. The longer ones (200–396 bp) are slightly less efficient. The situation is more complicated for induced mutants because it was found that several mutants induced by hybrid λ-phage DNA contain variable insertions of vector DNA (CLAVERYS, LEFÈVRE and SICARD 1980). Indeed their efficiencies are often lower than the efficiency of spontaneous mutants (Table 1). As it is known that the reduction of transformation efficiency is proportional to the size of heterology (LACKS 1966), it is likely that the efficiency of such mutants is a combination of the effect of the size of the deletion and the size of the inserted sequence.

Increased recombination frequencies when deletions are in donor DNA: We have previously found

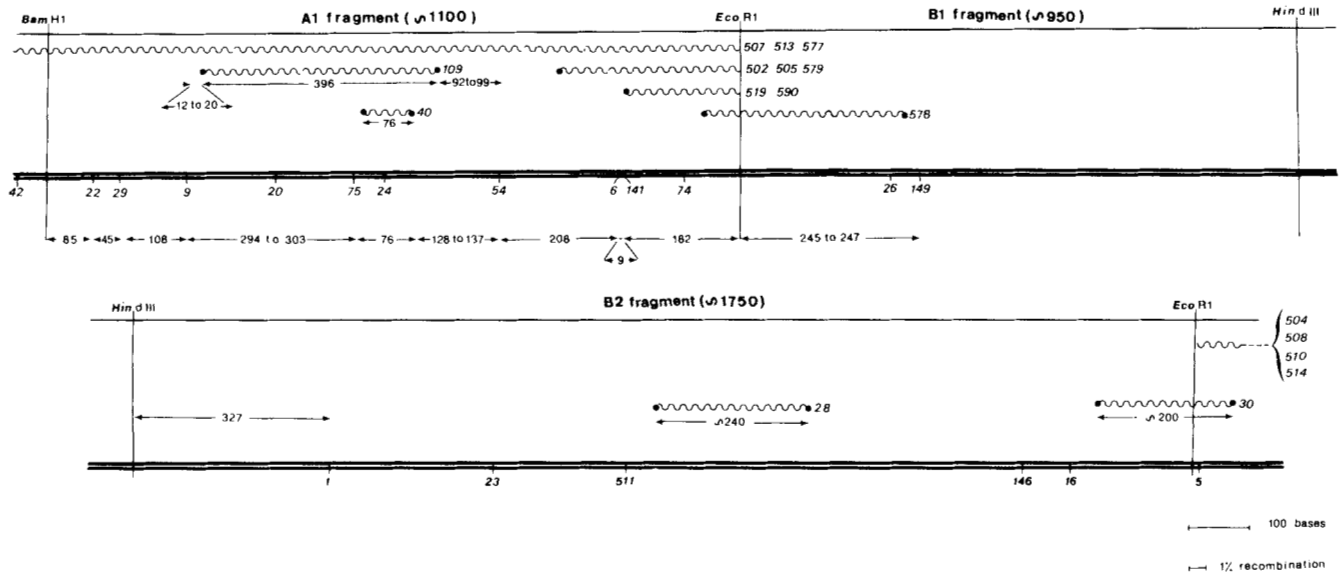

FIGURE 1.— Genetic map of the *amiA* locus. The map is divided in two parts: the upper and lower part are, respectively, the left and the right part of the locus. Restrictions fragments are shown by horizontal *single lines*. The genetic map is marked by a number of alleles (large figure) shown near the *double line* (SICARD and EPHRUSSI-TAYLOR 1965; CLAVERYS *et al.* 1981). Wavy line refer to deletions. Bases separating mutations are indicated by small numbers. When mutations involve a deletion between repeated sequences, distances are given by two numbers due to inaccuracy of the position of interruption in the repeated sequence. When it is possible, sites have been mapped according to their physical distances (number of bases separating the sites). Otherwise they are located from measurement of recombination frequency in two-point crosses (1% corresponding to 27 bp). (LEFÈVRE *et al.* 1984). Very closely linked markers (below 1% of recombination) cannot be accurately mapped by this method.

that, when crosses are done with donor DNA bearing deletions, recombination frequencies are higher than in reciprocal crosses. (CLAVERYS, LEFÈVRE and SICARD 1980). However due to the limited number of deletions it was not possible to know if this hyperrecombination is a general property of deletions. To address this question, deletions were used in reciprocal transformation with other markers of the amiA locus. Wild-type recombinants were scored and recombination frequencies were calculated as described in MATERIALS AND METHODS. These values have been multiplied by 100. Table 2 summarizes the results of some of these crosses. Deletions are underlined. In control experiments we observed that recombination frequencies between point mutations are similar in reciprocal crosses. For example, it was 18% in crosses between *amiA22* and *amiA6*. A similar value was found when the deletion *amiA502* which overlaps the site *amiA6* was transformed with DNA bearing *amiA22* (19.8%). However, when *amiA22* was transformed with DNA bearing the deletion *amiA502*, the recombination frequency was much higher (42.3%). We have chosen to measure this excess as a difference between the reciprocal crosses, *i.e.*, 42.3 minus 19.8 (22.5). Table 2 shows the results of some reciprocal crosses involving deletions. All reciprocal crosses show a discrepancy with the largest differences being in the crosses listed in Table 2A. The average difference was 24 for these crosses. Two factors can influence this value: the distance between the point mutation and the deletion, and the size of the deletion itself.

Distance between the point mutation and the deletion: Differences are smaller in crosses indicated in Table 2B. They are reduced but still visible when the markers are very close. For example, *amiA6* and *amiA519* are 1 to 8 bases apart since this deletion did not recombine with *amiA141* which is separated from *amiA6* by 8 bp. Recombination frequencies found between *amiA6* and *amiA519* were 0.35% in one direction and more than tenfold more (4.3%) in the other direction of the cross. The difference was only 3.95. Below a distance of 99 bp distance, the excess of recombinants fluctuates between from 10.8 and 3.95 (Table 2B). In the cross between *amiA29* and *amiA109* separated by 120 to 128 bp, we observed the maximal difference (24.8) (Table 2A). All the examples of crosses given in Table 2A involves a point mutation distant from the deletion by more than 120 bp (Figure 1). Excess of recombination frequency reaches its maximum for distances over 120 bp. An explanation to account for the effect of the distance between the point mutation and the deletion, will be proposed in the discussion.

Effect of the size of deletions: The length of the deletions that yielded the highest difference were spread out from 200 bp (*amiA30*) to more than 1000 bp (*amiA507, 513*). Beyond 200 bp the length did not seem to influence difference (Table 2A). On the contrary, smaller deletions show smaller differences. When *amiA40* DNA carrying a deletion of 76 bp was used to transform the strain *amiA54*, the difference

TABLE 1

Transforming efficiencies of deletions at the *amiA* locus

Deletions	Size (bp)	Transformation efficiency
Spontaneous		
amiA40	76 (a)	0.96
amiA30	200 (b)	0.79
amiA28	240 (b)	0.91
amiA109	396 (a)	0.83
Induced		
amiA519	From 182 to 191 (c)	0.60
amiA590	From 182 to 191 (c)	0.94
amiA502	From 219 to 399 (c)	0.56
amiA505	From 219 to 399 (c)	0.63
amiA578	From 280 to 304 (c)	1.01
amiA507	>1100	0.34
amiA513	>1100	0.26
amiA577	>1100	0.83
amiA504	>1100	0.41
amiA508	>1100	0.45
amiA510	>1100	0.43
amiA514	>1100	0.23

Deletions were either spontaneous or induced by transformation of wild-type bacteria with a cloned wild-type fragment of the *amiA* locus (CLAVERYS, LEFÉVRE and SICARD 1980). Deletions that were indistinguishable by their sizes were pooled together. Sizes were determined either by (a) sequencing, (b) electrophoretic migration of restriction fragments carrying deletions (the accuracy of this method is estimated at 10%) (LATASTE, CLAVERYS and SICARD 1980), or (c) genetic mapping from two point crosses involving these deletions and sequenced sites at the *amiA* locus. The transformation efficiency of a given marker was determined as described in MATERIALS AND METHODS. Statistical fluctuations and errors in measuring ratios calculated according to KIMBALL (1961) were below 10%.

observed was 7.0 (Table 2B). This low difference cannot be due to the distance between the two markers which is around 130 bp. Indeed we have demonstrated in the previous section that this distance was long enough to yield the maximal excess of recombinants. It is likely that the low difference observed with deletion *ami A40*, about 1/3 the maximal value, is due to about the small size of this deletion. When a 5-bp deletion was used in reciprocal crosses with nearby markers, there was no difference (GASC *et al.* 1987). These results suggest that only extended deletions cause differences in reciprocal crosses.

The size of the deletion as well as the distance between the point mutation and the deletion are probably not the only factors modifying the excess of recombinants. Indeed *amiA28* bears a 240-bp deletion which exhibited a difference of 8.9 or 16 depending upon the point mutation used in the cross. As these markers are a few hundred bases apart, this suggests that the nature of the sequence might influence the excess of recombinants.

Conversion of deletions: The excess of recombination frequency might result from two different processes: 1) the nonhomologous region could sometimes be excluded from pairing whereas the remaining homologous part of the donor molecule would be

TABLE 2

Recombination frequencies from reciprocal transformations involving deletions

A

Recipient strain	Donor DNA	Recombination frequency (×100)	Difference
amiA20	*amiA6*	8.4	
amiA6	*amiA20*	8.2	
amiA22	*amiA6*	18.0	
amiA6	*amiA22*	18.1	
amiA502	*amiA20*	10.8	27.3
amiA20	*amiA502*	38.1	
amiA502	*amiA22*	19.8	22.5
amiA22	*amiA502*	42.3	
amiA502	*amiA29*	18.9	21.7
amiA29	*amiA502*	40.6	
amiA504	*amiA16*	10.5	30.1
amiA16	*amiA504*	40.6	
amiA504	*amiA146*	11.8	28.6
amiA146	*amiA504*	40.4	
amiA109	*amiA6*	2.6	23.8
amiA6	*amiA109*	26.4	
amiA109	*amiA29*	1.6	24.8
amiA29	*amiA109*	26.4	
amiA30	*amiA146*	8.0	19.9
amiA146	*amiA30*	27.9	
amiA26	*amiA507*	25.1	17.7
amiA507	*amiA26*	7.4	
amiA26	*amiA513*	29.2	21.9
amiA513	*amiA26*	7.3	

B

Recipient strain	Distance (bp)	Donor DNA	Recombination frequency (×100)	Difference
amiA6	(1–8)	*amiA519*	4.3	3.95
amiA519		*amiA6*	0.35	
amiA9	(12–20)	*amiA109*	11.3	10.8
amiA109		*amiA9*	0.5	
amiA74	(50)	*amiA578*	11.4	
amiA578		*amiA74*	1.7	9.7
amiA54	(91–99)	*amiA109*	5.1	4.2
amiA109		*amiA54*	0.9	
	Deletion size (bp)			
amiA54	76	*amiA40*	12.0	
amiA40		*amiA54*	5.0	7.0
amiA1	240	*amiA28*	23.0	16.0
amiA28		*amiA1*	7.0	
amiA16	240	*amiA28*	20.8	8.9
amiA28		*amiA16*	11.9	

Recombination frequencies were calculated as described in MATERIALS AND METHODS. Difference in reciprocal crosses is the difference between recombination frequencies multiplied by 100. Deletions are underlined.

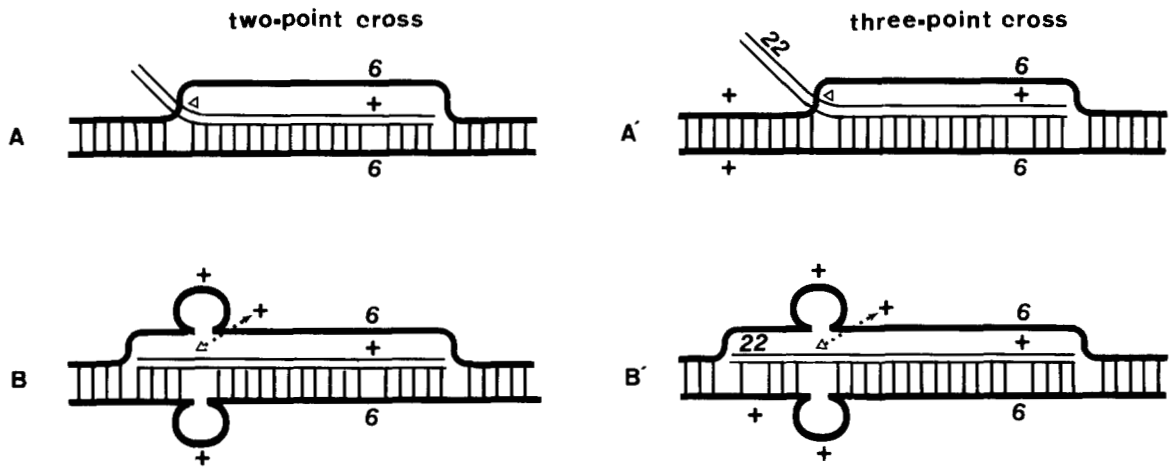

FIGURE 2.—Recombination of deletions in two-and three-point crosses. Strain *amiA6* is transformed by DNA carrying either a deletion (b) (A and B), or the same deletion and the marker *amiA22* (A′ and B′). The upper parts (A and A′) represent the behavior of donor DNA if the deletion was excluded from pairing. In the lower parts (B and B′), the deletion would participate in the recombination: the deletion would be changed into wild-type by gene conversion (arrows).

integrated leading to wild-type recombinants (Figure 2A); and 2) the nonhomologous region could participate in the recombination. The deletion could be changed into wild type which would generate an excess of recombinants. This latter process is basically a gene conversion (Figure 2B). It is possible to test the hypotheses in three-factor crosses where the deletion is located between two closely linked outside markers. This requires the isolation of a double mutant containing a deletion. We have constructed a double mutant bearing the deletion *amiA109* and the point mutation *amiA22* which are 165 to 173 bp apart, as described in MATERIALS AND METHODS. In crosses we have used the mutation *ami A6* which yields high level of recombinants with deletion *amiA109* (Table 2A). We transformed a recipient strain bearing the *amiA6* mutation with DNA carrying the double mutation *amiA109-amiA22.* If the deletion is excluded from recombination (first hypothesis) the outside marker *amiA22* would be also excluded. The frequency of wild type recombinants between *amiA6* and *amiA109 -amiA22* should be close to the value of the excess of recombination' observed between *amiA6* and *amiA109* (Figure 2A′).

If the deletion participates in the recombination and is converted to wild type (second hypothesis) the frequency of wild-type recombinants should be close to the value calculated from the distance between *amiA6* and the deletion *amiA109* and between the two point mutations, when conversion occurs (Figure 2B′). The results of these experiments are shown in Table 3. When *amiA6* is transformed by the double mutant we observed a recombinant frequency of 7.5%. This value is far from the recombination frequency observed when recipient *amiA6* was transformed by donor DNA carrying *amiA109* deletion (26.4%) suggesting that the second process in the most probable. In

TABLE 3

Recombination frequencies in crosses involving deletion *amiA109* and outside markers

Recipient	Donor	Recombination frequency (×100)
amiA109	*amiA6 str41*	2.6
amiA6	*amiA109 str41*	26.4
amiA6	*amiA22 str41*	18.1
amiA6	*amiA109-amiA22 str41*	7.5
amiA109-amiA22	*amiA6 str41*	3.9
amiA109	*amiA54 str41*	0.9
ami54	*amiA109 str41*	5.1
amiA54	*amiA22 str41*	18.1
amiA54	*amiA109-amiA22 str41*	2.4
amiA109-amiA22	*amiA54 str41*	1.0

Recombination frequencies are calculated as indicated in MATERIALS AND METHODS. Deletions are underlined.

that case it is possible to calculate recombination frequency including 1) the effect of the distance between *amiA6* and deletion *amiA109* which is estimated in the cross between the recipient *amiA109* and the DNA carrying *amiA6* (2.6%) and 2) the conversion of the deletion *amiA109* without affecting the *amiA22* marker. The contribution of this conversion of wild-type recombinants can be estimated (Figure 2B′) as the product of the probability of recombination between *amiA6* and *amiA22* (18.1%) (Table 3) by the frequency of conversion of this deletion to wild type (23.8%) (Table 2A) which yields 4.3%. The sum of the two values (2.6% and 4.3%) is 6.9% which is very similar to the experimental value observed for the double mutant (7.5%). Therefore the conversion of the deletion might account for the results of this three-point cross.

This experiment was repeated using the mutant *amiA54* instead of *amiA6* with similar results. The recombination frequency was not strongly increased

TABLE 4

Inhibition of conversion of deletions by excision-repair of mismatched bases

Recipient	Donor	Recombination frequency (×100)
hex⁻amiA24	*amiA502*	26.9
hex⁺amiA24	*amiA502*	7.8
hex⁺amiA502	*amiA24*	8.1
hex⁻amiA74	*amiA578*	11.4
hex⁺amiA74	*amiA578*	2.8
hex⁺amiA578	*amiA74*	1.7
hex⁻amiA6	*amiA109*	22.1
hex⁺amiA6	*amiA109*	2.9
hex⁺amiA109	*amiA6*	3.4
hex⁻amiA6	*amiA519*	4.3
hex⁺amiA6	*amiA519*	0.24
hex⁺amiA519	*amiA6*	0.35

Mutations *amiA6, amiA24, amiA74* are low efficiency that are excised by the Hex-mediated repair system. *amiA109, amiA502, amiA519* and *amiA578* are deletions. Recombination frequencies are calculated as in MATERIALS AND METHODS. Deletions are underlined.

and its value was that predicted if the deletion was converted to wild-type (Table 3).

Therefore the only way to account for the high frequency (23%) of wild-type recombinants in two factor crosses in which a deletion is on the donor DNA, is to assume that, in the heteroduplex DNA, the deletion was converted to wild-type.

Inhibition of conversion of deletions by excision-repair of mismatched bases: The results presented above were obtained using *hex⁻* recipient strains. This means that conversion of deletions does not require a functioning Hex-mediated repair system. A question that one might ask is whether the conversion is affected by the presence of a closely linked low efficiency marker that will trigger the excision-repair system in a *hex⁺* recipient. To answer this question, several low efficiency markers were introduced in a *hex⁺* recipient and crossed with a set of neighboring deletions (Table 4). The recombination frequencies observed showed that differences between reciprocal crosses disappear. Since the Hex system acts at the heteroduplex stage between mismatched bases, this suggests that conversion of deletions occurs also at this stage.

Lack of effect of temperature on the conversion process: When the recipient strain *amiA16* was transformed with DNA carrying deletion *amiA28* at 37° instead of at 30°, which was the optimal temperature, the conversion frequency was not modified (Table 5) although the number of transformants was strongly reduced. If enzymatic activities are required for conversion, they are not specifically affected at these temperatures.

Conversion of deletions does not require polymerase I: Conversion of deletions could be explained by a DNA degradation and DNA synthesis leading to the wild-type sequence in the recipient chromosome. According to this model, one might expect that repair enzymes would be involved in the synthesis of such short patches of DNA sequences. Polymerase I would be a candidate for such a repair. We have introduced an amethopterin-resistant point-mutation *amiA6* in a polymerase I-deficient strain (*polA⁻*) obtained by P. LOPEZ and co-workers (1988). We have transformed this new strain with DNA carrying deletion *amiA109*. It can be seen that the conversion frequency has not been reduced by the *polA⁻* mutation as compared with the results obtained in a *polA⁺* strain (Table 6). Therefore, the mechanism of conversion of deletions does not seem to require DNA repair by the pneumococcal polymerase I.

DISCUSSION

The purpose of this investigation was to study the mode of recombination of deletions in *Streptococcus pneumoniae*. We have obtained 16 deletions. The size of these deletions varies from 76 bp to more than 1100 bp at several locations in the locus. Some were induced by an insertion-deletion procedure using cloned segments of the *amiA* locus, the others were of spontaneous origin. By sequencing, we have shown that spontaneous deletions occurred between short repeated sequences. This extends to streptococci the importance of short repeated sequences in the generation of large deletions originally observed in *Escherichia coli* (FARABAUGH *et al.* 1978; ALBERTINI *et al.* 1982). During the genetic analysis of these deletions we always observed a considerable increase of wild-type recombinants when the deletions were present on donor DNA in transformation of strains carrying neighboring markers. This increase has been measured as a difference between the frequency of wild-type recombinants when the deletions were carried on donor DNA and the reciprocal cross. In the latter type of transformation, the frequencies of wild-type recombinants resulting from genetic exchanges between markers were directly proportional to their physical distances. The frequency of these recombinants was similar to the frequency obtained when only point mutations separated by the same distances were crossed. In other words, the frequency of wild-type recombinants when the deletions are carried on donor DNA is the sum of the frequency of recombinants resulting from the physical distances between the markers and a value related to the presence of the deletion on donor DNA. In this study, we have shown that this value is constant for deletions longer than 200 bp in crosses with a point mutation that is more than 120 bp away. The average value is 24%.

TABLE 5

Independence of conversion from temperature change

Recipient	Donor	Temperature	*amiA*$^+$ transformants per ml	*str-r* transformants per ml	*amiA*$^+$/*str*
hex$^-$*amiA16*	*amiA28str41*	30°	5.60×10^5	2.77×10^6	0.20
hex$^-$*amiA16*	*amiA*$^+$*str41*	30°	5.62×10^6	5.08×10^6	1.10
hex$^-$*amiA16*	*amiA28str41*	37°	6.70×10^3	3.53×10^4	0.19
hex$^-$*amiA16*	*amiA*$^+$*str41*	37°	6.93×10^5	5.84×10^5	1.18

TABLE 6

Lack of requirement of polymerase I activity in conversion

Recipient	Donor	*amiA*$^+$ transformants per ml	*str* transformants per ml	*amiA*$^+$/*str*
hex$^-$*amiA6*	*amiA109str41*	1.26×10^5	6.32×10^5	0.20
hex$^-$*amiA6*	*amiA*$^+$*str41*	2.16×10^6	2.14×10^5	1.01
hex$^-$*polA*$^-$*amiA6*	*amiA109str41*	3.29×10^4	1.10×10^5	0.29
hex$^-$*polA*$^-$*amiA6*	*amiA*$^+$*str41*	7.70×10^4	7.30×10^4	1.05

We have found that the size of the deletions (when it is smaller than 200 bp) is an important factor for this effect since the excess of wild-type recombinants for a 76-bp deletion is only one-third of the average value for long deletions; 5-bp deletions have no effect (GASC *et al.* 1987).

When the markers are very close, the excess of wild-type recombinants is reduced but still detectable. We suggest that the conversion is not limited to the heterology but extends outside the deletion; in this case close markers on the donor DNA might be included in the conversion resulting in a co-conversion that changes the wild type linked allele into an aminopterin-resistant allele. This will eventually reduce the frequency of wild-type transformants. In the molecular model that we will present later, the size of conversion process will be larger than the size of the heterology (Figure 3). The length of this co-conversion is quite variable upon the deletion studied. Thus it is quite likely that the sequences near the heterology can influence the probability of obtaining a high frequency of wild-type recombinants. It is also consistant with the observation that recA protein is able to catalyse also branch migration of heterologies (DASGUPTA and RADDING 1982).

The mode of integration of deletion has been investigated in three-point crosses in which a deletion was flanked by two outside markers. It was found that the frequency of recombination between these outside markers was not increased by the presence of the deletion. This result might be explained by a conversion of the deletion localized to the heterology and its short neighboring sequences (Figure 3). It does not support the alternative hypothesis previously proposed (SODERGREN and FOX 1979; CLAVERYS, LEFÈVRE and SICARD 1980) according to which, after entry single stranded DNA would initiate pairing to chromosomal DNA between homologous regions, with the heterology further impeding the process of strand migration (Figure 2A). This hypothesis would predict that, in a three factor cross in which the deletion is between two outside markers, the frequency of recombination between these outside markers will be much higher than the frequency observed as a result of exclusion of the deletion and the second marker in donor DNA. Therefore deletions are integrated by a mechanism different from the integration of point mutations by DNA repair. In fact, it is known that kinetics of integration was much slower for deletions than for point mutations and that the delay in integration was not well correlated to the length of the deletions (GHEI and LACKS 1967). This delay may be the time required for the conversion of the deletions into the recipient chromosome.

In reciprocal crosses we did not observe an excess of recombination frequencies, We do not know whether there was no conversion or convertants were not detected. Conversion of a deletion carried on the recipient chromosomes to wild-type sequence will reduce the efficiency of transformation of these deletions and will influence the transformation efficiency. It has been reported that long deletions give a continuous and inverse relationship between transformation efficiency and the length of the heterology (LACKS 1966). It is very likely that this effect results from the inhibition of pairing ability of extended heterologies. On the other hand, short heterologies, *i.e.*, a 93-bp deletion at the *malM* locus (LACKS, DUNN and GREENBERG 1982) a 76-bp deletion (*amiA40*), a 34-bp deletion (*amiA29 rev*) (CLAVERYS *et al.* 1981), a 6-bp

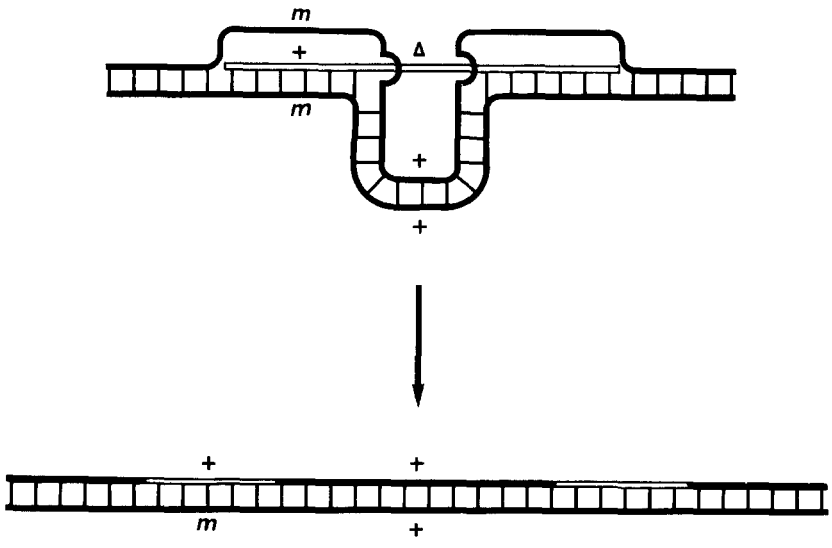

FIGURE 3.—Conversion of deletions. Double strand chromosomal DNA carrying an amethopterin-resistant point mutation (m) is unpaired when a single strand donor molecule invades the recipient molecule (double line). Maximum pairing involves not only regions outside the deletion (b) but also the displaced strand of recipient DNA and the homologous recipient DNA. Breakage and ligation of single strand DNA in the region of both intersections between donor and recipient molecules will lead to converted wild type recombinant. This recombinant molecule will be heteroduplex at the site of the point mutation and wild-type homoduplex at the site of the deletion. After one cycle of chromosomal replication, wild-type colonies will be obtained.

insertion at the *sul-d* locus (LOPEZ *et al.* 1987) and a 5-bp deletion (*amiA134*) (GASC *et al.* 1987), are transformed with an efficiency of 1 in a *hex*$^-$ recipient. They are not recognized by the Hex system. We have found that the efficiency of deletions of intermediate length (200–396 bp) are slightly smaller than 1 in *hex*$^-$ recipients. It is possible that this reduction is due to a loss of donor information. However, it is not excluded that the reduction of transformability by conversion of intermediate length deletion results from pairing inhibition. Studies of a large set of deletions and insertions at the same site would allow testing of this hypothesis.

Conversion of a deletion is inhibited by the Hex-mediated excision repair system only if a susceptible heteroduplex mismatch has been generated nearby. This might be explained by the mode of action of this system when a low efficiency mismatched base pair occurs at the heteroduplex stage, several kilobases of donor DNA sequences are excised (LACKS 1966, SICARD and EPHRUSSI-TAYLOR 1966; MÉJEAN and CLAVERYS 1984). The transformants are the results of a transfer of information to the chromosome by DNA resynthesis (LOUARN and SICARD 1968). In a cross between a low efficiency point mutation carried in a *hex*$^+$ recipient and a donor DNA containing a deletion, the transfer of information leads to an homoduplex recipient DNA carrying the low efficiency integrated marker and the wild-type sequence at the site of deletion, a result of cocorrection. The only wild-type transformants would result from exchanges or breaks between the two sites. This interpretation requires that the elimination of the donor DNA is a faster process than conversion directed by deletion. Indeed it has been shown that this elimination takes less than 2 min at 30° (Méjean and CLAVERYS 1984) whereas the deletion is converted more slowly (GHEI and LACKS 1967).

Moreover, the effect of the mismatch repair system on the conversion of deletions suggests that the conversion occurs at the heteroduplex stage. Therefore a zone of heterology will be present in the heteroduplex DNA. As in phage recombination, heterologies would participate in the formation of the heteroduplex DNA (LICHTEN and FOX 1983a, b). We propose that during branch migration of donor DNA, recipient homoduplex DNA is reconstituted at the site of the deletion, thus yielding maximum pairing (Figure 3). Recipient and donor strands of identical polarity would be in contact, eventually leading to wild-type donor DNA by breakage and reunion. The absence of a requirement of DNA polymerase I for conversion of deletions to wild types supports this hypothesis. This is in contrast to the requirement of *polI* for another type of conversion which is localized to twelve bases in pneumococcus (PASTA, GARCIA and SICARD 1988).

Conversion of deletions has also been reported in eucaryotes such as *Ascobolus immersus* (ROSSIGNOL *et al.* 1984) and it may also be the cause of the stimulation of recombination in lambda phage crosses in which a heterology is involved (LIEB, TSAI and DEONIER 1984). Large heterologies are also converted to wild types or to mutants in lambda phage transfection (DOHET *et al.* 1987) and in plasmid recombination in *E. coli* (YAMAMOTO *et al.* 1988). Preferential conversion of heterology depends upon the microorganism. In *A. immersus* the correction mechanism is able to discriminate the recipient strand as a preferential target, independant of loop position (ROSSIGNOL *et al.* 1984). In lambda phage conversions are observed either toward wild-type or mutant alleles in heteroduplex DNA containing large insertions (DOHET *et al.* 1987). In phage T4 wild type is preferentially converted to deletion (MOSIG 1987). In pneumococcus further studies are warranted to look for any prefer-

ential conversion. Conversion of fairly long heterologies may be a general process. It may be one of the several mechanisms able to eliminate spontaneous mutations such as the Hex system in pneumococcus and the methyl-directed mismatch repair system in *E. coli* which prevent point mutations such as transitions small deletions or insertions (1–3 bases) and some transversions. In addition, the localized conversion system in pneumococcus corrects some transversions not recognized by the other systems. However, none of these systems acts on deletions. It is possible that conversion of deletions that we have described, eliminates a fraction of potential deletions occuring by slipping mispairing during DNA synthesis (ALBERTINI *et al.* 1982). It is likely that this process of correction has been created and preserved during evolution to stabilize the genome.

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