LOCALIZED CONVERSION IN *STREPTOCOCCUS PNEUMONIAE* RECOMBINATION: HETERODUPLEX PREFERENCE

MICHEL SICARD, JEAN-CLAUDE LEFEVRE, PEZECHPOUR MOSTACHFI, ANNE-MARIE GASC AND CLAUDINE SARDA

Centre de Recherche de Biochimie et de Génétique Cellulaires du CNRS, 31062 Toulouse, France

Manuscript received December 10, 1984
Revised copy accepted April 22, 1985

ABSTRACT

In pneumococcal transformation the frequency of recombinants between point mutations is generally proportional to distance. We have recently described an aberrant marker in the *amiA* locus that appeared to enhance recombination frequency when crossed with any other allele of this gene. The hyperrecombination that we have observed in two-point crosses could be explained by two hypotheses: the aberrant marker induces frequent crossovers in its vicinity or the mutant is converted to wild type. In this report we present evidence showing that, in suitable three-point crosses, this hyperrecombination does not modify the recombination frequency between outside markers, suggesting that a conversion occurs at the site of this mutation. To estimate the length over which this event occurs, we isolated very closely linked markers and used them in two-point crosses. It appears that the conversion system removes only a few base pairs (from three to 27) around the aberrant marker. This conversion process is quite different from the mismatch-repair system controlled by *hex* genes in pneumococcus, which involves several thousand base pairs. Moreover, we have constructed artificial heteroduplexes using separated DNA strands. It appears that only one of the two heteroduplexes is specifically converted. The conversion system acts upon 5′..ATTAAT..3′/3′.. TAAGTA..5′. A possible role of the palindrome resulting from the mutation is discussed.

IN genetic crosses the recombination frequency strongly depends upon the nature of the marker. This "marker effect" was extensively studied in *Streptococcus pneumoniae*, for which it has been proposed that the reduced integration efficiency of some point mutations results from an excision and repair process (EPHRUSSI-TAYLOR and GRAY 1966). During the integration of these markers, several thousand base pairs are excised (LACKS 1966; SICARD and EPHRUSSI-TAYLOR 1966; MÉJEAN and CLAVERYS 1984) and both DNA strands rapidly become mutant homoduplex (LOUARN and SICARD 1968). Such markers are thus transformed via a long-patch conversion event which is controlled by the *hex* system (LACKS 1970; TIRABY and SICARD 1973; CLAVERYS *et al.* 1984). When deletions or insertions are used in transformation, another effect on recombination is observed. There is a strong recombinational stimulation in the vicinity of the heterology (CLAVERYS, LEFÈVRE and SICARD 1980). In our laboratory, most progress in the analysis of the marker effect was obtained

using mutants in the *amiA* locus. This locus is suitable for genetic analysis because mutants can be easily isolated on agar plates containing 10^{-5} M amethopterin, and wild-type recombinants can be scored on a synthetic medium containing an excess of isoleucine (SICARD 1964). These dual properties of *amiA* mutants are due to an alteration of the membrane potential: chemicals that penetrate via this electric transmembrane potential, such as amethopterin and branched amino acids (valine, leucine and isoleucine), are taken up less efficiently (TROMBE, LANEELLE and SICARD 1984). A reduction of drug uptake explains amethopterin resistance, and reduction of branched amino acid uptake confers sensitivity to an imbalance in the three amino acids. Because pneumococcus requires all three amino acids, starvation will result and only wild-type bacteria can grow in the appropriate selective medium. Many *amiA* mutants were isolated and mapped using this selection (SICARD and EPHRUSSI-TAYLOR 1965). During the course of genetic studies, we have recently described an aberrant marker (*amiA36*) that stimulates recombination frequency when crossed with any other allele of this locus (LEFÈVRE *et al.* 1984). This effect is especially apparent for short distances as small as 27 base pairs, where we observed a 20% recombination frequency instead of the 1% expected. The mutation results from a C/G to A/T transversion in the sequence 5′..ATTCAT..3′, generating 5′..ATTAAT..3′. This hyperrecombination is thus quite different from the recombinational stimulation described for deletions or insertions. It could be explained by short-patch conversion toward the wild type as also observed in some λ crosses (LIEB 1983). It is possible to test this hypothesis in three-factor crosses where the aberrant marker *amiA36* is located between two closely linked outside markers. In this report we will present evidence showing that this hyperrecombination does not modify the recombination frequency between outside markers, which suggests that a conversion occurs at this mutation. We have also prepared heteroduplex molecules by cross-annealing resolved complementary strands of different genetic composition and have used them in transformation. These experiments show that only one of the two heteroduplexes is specifically converted. Moreover, the use of a very closely linked marker indicated that this new mode of genetic conversion in pneumococcal transformation extends over more than 3–5 base pairs and less than 27 base pairs.

MATERIALS AND METHODS

Strains and transformation procedures: The strains used in this study are derived from Avery's strain R36A. All recipient strains are *hex*$^-$, *i.e.*, they transform all markers at the same high efficiency (CLAVERYS, ROGER and SICARD 1980). The markers belong either to the *amiA* locus, conferring resistance to 2×10^{-5} M amethopterin (SICARD 1964) or a locus that confers resistance to 2 mg/ml of streptomycin (the *Str41* marker). Culture media, preparation of DNA and transformation procedures have been described earlier (CLAVERYS, ROGER and SICARD 1980).

Quinacrine-induced mutants: Exponentially growing cells were treated with different concentrations of quinacrine in the growth medium and then incubated at 37° in the dark for 1 hr. To score the mutants, aliquots of the culture were plated on complete agar medium. After 2 hr of incubation at 37°, an overlayer of agar containing 2×10^{-5} M amethopterin was poured onto the plate. The optimal mutagenic concentration was selected (30 μg/ml at pH 7.7). The frequency of quinacrine-induced mutants was 2×10^{-3} per surviving bacterium, a 54-fold increase over the

spontaneous mutation frequency. Under these conditions, frameshift mutants can be isolated (GASC and SICARD 1978).

Recombination between amiA-r *mutants*: Cells bearing an *amiA-r* mutation can be transformed with DNA from cells bearing another *amiA* mutation, and *str41* and wild-type recombinants can be produced. These are scored by plating on synthetic medium containing an excess of isoleucine (SICARD 1964), since mutations at the *amiA* locus result from an alteration of the membrane potential which reduces the uptake of branched amino acids (valine, leucine and isoleucine) as well as amethopterin. Since all three amino acids are required for growth, an excess of isoleucine results in starvation for the other two. The frequency of recombination between two *amiA* sites is the ratio of wild-type transformants to streptomycin transformants.

Preparation of heteroduplex DNA: The resolution of complementary DNA strands by means of interaction with poly(U,G) has been reported (GUHA and SZYBALSKI 1968; ROGER 1972; CLAVERYS, ROGER and SICARD 1980). It has been adapted to separate pneumococcal DNA strands. One milliliter of a DNA solution (10 OD units/ml) was denatured by addition of 4 ml of 0.1 M NaOH. After 5 min at room temperature, the solution was neutralized with 0.4 ml of 2 M NaH_2PO_4. Four milliliters of this solution were mixed with 0.4 ml of a poly(U,G) (lot H4 from Biopolymers Inc.) solution (40 OD units/ml) and incubated for 2 hr. To 3.5 ml of this mixture was added 5.3 g of CsCl (optical grade) and water to obtain 9.346 g total weight. This tube was centrifuged in a SW39 rotor at 30,000 rpm for 72 hr at 20°. Fractions (4 drops) were collected from the bottom of the tube and mixed with 0.6 ml water for optical density measurement at 260 nm in a Beckman double-beam spectrophotometer. Peak fractions were pooled and dialyzed for 2 days against phosphate buffer (0.02 M potassium phosphate, pH 6.8, 0.15 M NaCl). DNA concentrations of heavy and light strands were equalized by addition of 0.15 M NaCl to obtain OD 0.5 units at 260 nm. Heavy and light fractions derived from two differently marked DNAs (0.2 ml each) were mixed with 0.04 ml of 5 M NaCl and incubated at 65° for 1 hr. These heteroduplex preparations were used to transform competent pneumococcal strains at a DNA concentration below the saturating level (0.2 μg/ml).

Cloning and sequencing procedure: The mutation *amiA121* has been cloned by the rapid procedure previously described (MÉJEAN *et al.* 1981). The principle of this method is to use an *E. coli* plasmid unable to replicate in pneumococcus, carrying a marker expressed in the latter bacteria and a fragment of pneumococcal chromosome which enables homology-dependent recombination. We have transformed the pneumococcal *amiA121* strain with the *E. coli* hybrid plasmid pR28 carrying an erythromycin resistance marker and the B^+ fragment (Figure 1) from the *amiA* locus. Erythromycin-resistant transformants were selected using 2 μg/ml of antibiotic. They result from an homologous recombination between B^+ fragments. They have integrated into the pneumococcal chromosome the *E. coli* plasmid between a duplication of this B^+ fragment (MÉJEAN *et al.* 1981). The plasmid and neighboring pneumococcal sequences can be reextracted from the chromosome by restriction enzyme: pneumococcal DNA (10 μg) purified from an erythromycin-resistant transformant was digested with *PstI* enzyme which cleaves the plasmid once in the ampicillin gene. The fragments were ligated and used to transform *E. coli* strain 586 as described previously (CLAVERYS, LOUARN and SICARD 1981). Erythromycin-resistant (500 μg/ml) transformants were selected. Their plasmid carries pneumococcal DNA containing the A1 fragment containing the *amiA121* mutation, the erythromycin gene and the plasmid sequence required for its replication in *E. coli*. The nucleotide sequence of the segment carrying the *amiA121* mutation was determined by the method of MAXAM and GILBERT (1980).

RESULTS

Evidence for conversion of amiA36 *to wild type*: The *amiA36* marker is located 27 base pairs to the right of *amiA36*, whereas *amiA54* is located on the other side at a distance of about 200 base pairs as estimated by genetic analysis (Figure 1) (LEFÈVRE *et al.* 1981). To test whether the *amiA36* marker induces frequent crossing over in its vicinity or whether it is converted to wild type, three-factor crosses must be performed with *amiA36*. This requires the isolation

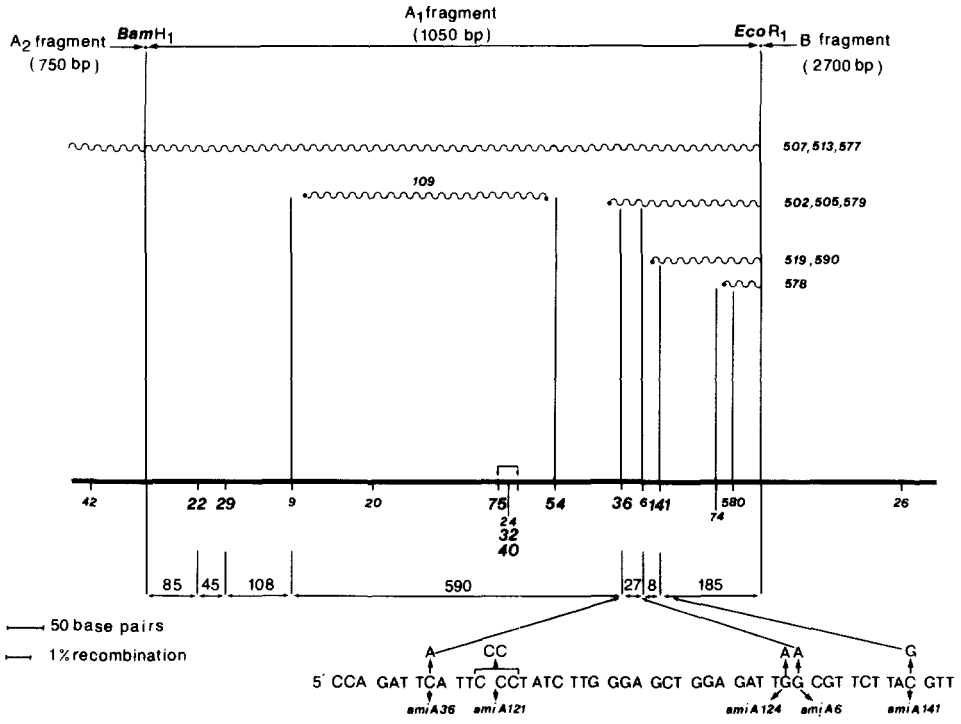

FIGURE 1.—Genetic map of the left side of the *amiA* locus and sequence of the *amiA36-amiA141* region. The restriction fragment A1, bracketed by the neighboring fragments A2 and B, is marked by a number of alleles shown below the heavy line. Wavy lines refer to deletions. Numbers of nucleotides which separate sites are shown above the arrows.

of a double mutant such as *amiA36-amiA6*. To obtain this double mutant, we transformed an *amiA36* strain with DNA carrying the *amiA6* allele cloned on a pBR325 vector (MÉJEAN *et al.* 1981). Because both markers are nonsense mutations, they confer the maximum level of resistance to amethopterin. Since it is likely that the double mutant will be as resistant as the single mutants, thus preventing its direct selection, the transformation mixture was plated on nutrient agar and individual isolated colonies were subjected to further screening. Cultures of these colonies were separately transformed by *amiA36* or *amiA6* DNA. Wild-type transformants were scored as described in MATERIALS AND METHODS. One isolate of 52 did not recombine with either marker but recombined with other markers of the *amiA* locus, indicating that it is a double mutant *amiA36-amiA6*.

To test whether placing *amiA36* between *amiA54* and *amiA6* increases recombination between them, we transformed this double mutant (*amiA36-amiA6*) with DNA carrying *amiA54*, a marker to the left of *amiA36*. The *amiA6* strain was simultaneously transformed by the same DNA preparation. Wild-type recombinants were scored. Reciprocal crosses between the *amiA54* recipient strain and *amiA36-amiA6 str41* or *amiA6 str41* DNAs were also performed. The results of these experiments are shown in Table 1. It can be seen that

TABLE 1

Recombination frequencies in crosses involving amiA36 *and outside markers*

Recipient	Donor	*amiA*$^+$ transformants/ml	*str-r* transformants/ml	*amiA*$^+$/*str-r*
Three-point crosses with the double mutant *amiA36-amiA6*				
amiA36-amiA6	*amiA54 str41*	3.89×10^5	5.71×10^6	0.068
amiA6	*amiA54 str41*	1.27×10^5	1.67×10^6	0.076
amiA36-amiA6	*amiA141 str41*	2.87×10^4	2.71×10^6	0.0106
amiA6	*amiA141 str41*	7.2×10^3	1.06×10^6	0.0068
amiA36-amiA6	*amiA + str41*	1.18×10^6	1.15×10^6	1.03
amiA6	*amiA + str41*	3.37×10^6	3.07×10^6	1.09
amiA54	*amiA36-amiA6 str41*	7.6×10^4	9.7×10^5	0.078
amiA54	*amiA6 str41*	4.61×10^4	6.16×10^6	0.075
amiA54	*amiA*$^+$*str41*	5.57×10^6	4.43×10^6	1.26
Two-point crosses with *amiA36*				
amiA54	*amiA36 str41*	6.35×10^5	2.62×10^6	0.24
amiA6	*amiA36 str41*	4.59×10^5	1.92×10^6	0.24
amiA36	*amiA54 str41*	6.81×10^5	2.92×10^6	0.23
amiA36	*amiA6 str41*	1.80×10^5	6.92×10^5	0.26
amiA36	*amiA*$^+$*str41*	7.10×10^5	6.30×10^5	1.12

Frequencies of wild-type recombinants were measured relative to the frequency of a standard reference gene, *str41*, conferring streptomycin resistance. This procedure corrects for variations in competence from one culture to another. All recipient strains are *hex*$^-$, *i.e.*, they transform all markers at the same high efficiency. The expected recombination frequency between *amiA36* and *amiA6* is 1% (27 base pairs between them) (CLAVERYS, LATASTE and SICARD 1979).

the frequency of recombinants between the outside markers has not been increased in the three-factor cross in which *amiA36* is involved. Thus, it is likely that the *amiA36* mutation is converted to the wild-type sequence without inducing exchanges between outside markers. To further show that *amiA36* does not modify recombination frequencies between markers located in other parts of the locus, the same double mutant was also transformed by DNA carrying the *amiA141* marker located 8 base pairs to the right of *amiA36*. As a control, *amiA36* was also crossed with *amiA141*. The recombination frequency between these closely linked sites was not increased by the presence of the *amiA36* mutation in three-factor cross (Table 1).

Since, there is no effect of the *amiA36* mutation on the frequency of recombination between closely linked outside markers in three-point crosses, the only way to account for the high (20%) frequency of wild-type recombinants in two-point crosses in which *amiA36* is involved is to assume that, in the heterozygote, the mutant is often converted to the wild genotype.

Length of the conversion event: The conversion process involves no more than 27 base pairs (LEFÈVRE *et al.* 1984). To estimate the length over which this event occurs and, more specifically, to determine whether it is limited to 1 base pair at the *amiA36* site, we isolated mutants by a quinacrine treatment

which yields mostly frameshift mutants in pneumococcus. A set of 127 amethopterin-resistant mutants isolated as described in MATERIALS AND METHODS was cultured and separately transformed by *amiA502* or *amiA519* DNA; these markers are deletions that define a short interval around *amiA36* (Figure 1).

Three quinacrine-induced mutants could be located close to *amiA36*. One, *amiA121* was selected for further study. We cloned it using the vector integration procedure described in MATERIALS AND METHODS and sequenced 250 bases of the A1 fragment (Figure 1) from the right-hand *Eco*RI site. The only difference is a C/G deletion in a run of CCC/GGG located 3–5 base pairs to the right of *amiA36* (Figure 1). In transformation between *amiA36* and *amiA121*, the recombination frequency is 0.7%. Since this frequency is much below 20%, the conversion generally extends farther than 5 base pairs to one side of the *amiA36* mutant site. However, since for such a short sequence one would expect from 0.1 to 0.2% recombinants (CLAVERYS, LATASTE and SICARD 1979), some hyperrecombination is observed. In a small proportion of cases the conversion may extend less than 5 base pairs on one side.

Heteroduplex preference: The genetic conversion that we have described is a localized process and differs from the excision-repair system which removes several kilobases around the mismatched bases. Thus, it is not surprising that short-patch conversion acts in *hex*$^-$ as well as *hex*$^+$ recipient cells (LEFÈVRE *et al.* 1984). Since we have previously reported that hyperrecombination (*i.e.*, localized conversion) occurs, as it does in the *Hex* system, at the heteroduplex stage of recombination (LEFÈVRE *et al.* 1984), we address the following question: are the two possible heteroduplexes equally able to induce conversion? We took advantage of the separability of pneumococcal DNA strands using a procedure of denaturation-renaturation in the presence of poly(U,G) and ultracentrifugation in a CsCl gradient (ROGER 1972). We separated DNA strands nealed *amiA36* and *amiA6* mutants (Figure 2). Since single-stranded DNA does not penetrate pneumococcal cells, in order to facilitate uptake we cross-annealed *amiA36*$^+$*-6* strands with the complementary *amiA36-6*$^+$ strands to construct two artificial heteroduplexes. These heteroduplexes were used in transformation at the same DNA concentration (0.2 μg/ml). Since, inside the cell, only single-stranded donor DNA is found (LACKS 1962; FOX and ALLEN 1964), the recipient chromosome will either pair with an *amiA36* strand without any possibility to yield wild-type recombinants or with an *amiA36*$^+$*-amiA6* strand. That may induce a conversion event. Using the two artificial heteroduplexes, we compared transformation of the *amiA36* mutant by the wild-type sequence of this marker when on either L or H strands. The presence of the *amiA6* mutation in the *amiA36*$^+$ strand is required to detect the conversion event by the measure of recombination frequency. In Table 2 we see that only one heteroduplex yields a high frequency of wild-type convertants. A reciprocal experiment was also performed: the *amiA6* recipient was transformed by the two heteroduplex preparations. We obtained many fewer convertants when the L strand carried the wild-type sequence at the *amiA36* site than when the H strands carried it. A better measurement of the heteroduplex preference can be derived from the experimental results, using as a reference the *str41* allele

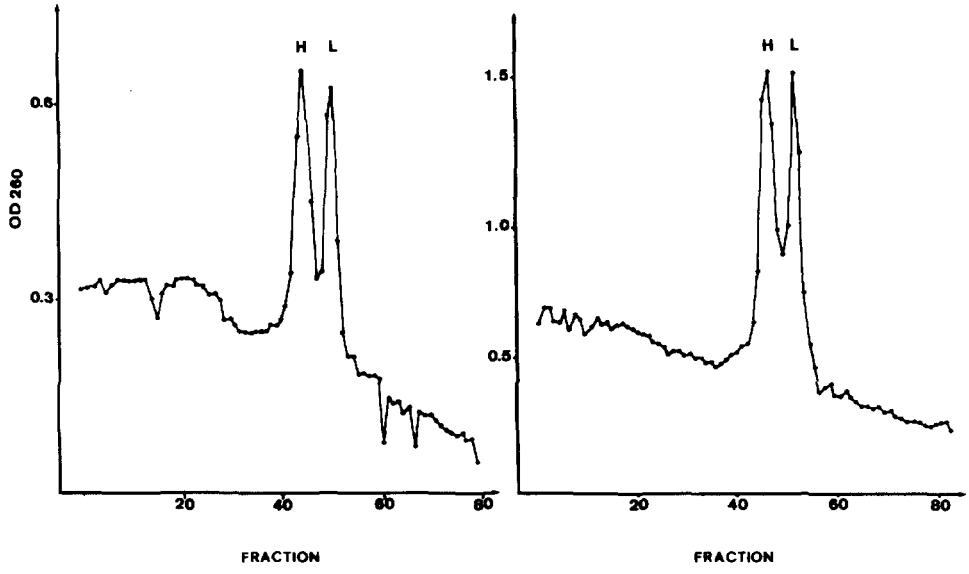

FIGURE 2.—Resolution of H and L strands of denaturated pneumococcal DNA. Equal amounts of alkali-denaturated DNA and poly(U,G) were mixed in a solution containing 0.2 M Na phosphate, pH 7. CsCl was added. A 5-ml aliquot was centrifuged in a SW39 rotor at 30,000 rpm for 72 hr at 20°. Left, DNA from the *amiA36* strain; right, DNA from the *amiA6* strain.

TABLE 2

Heteroduplex specificity of localized conversion

Recipient strain	Donor DNA heteroduplex	Donor-recipient heteroduplex	*amiA*$^+$ transformants/ml	*str-r* transformants/ml	*amiA*$^+$/*str-r* (%)
amiA36	3′ + *6* H 5′ *36* + L	3′ + *6* H 5′ *36* + L	1.63×10^5	9.16×10^5	17.8
	3′ *36* + H 5′ + *6* L	3′ *36* + H 5′ + *6* L	1.30×10^4	7.04×10^5	2.1
amiA	3′ + *6* H 5′ *36* + L	3′ + *6* H 5′ *36* + L	7.93×10^5	6.20×10^6	12.8
	3′ *36* + H 5′ + *6* L	3′ *36* + H 5′ + *6* L	9.60×10^4	5.00×10^6	1.9

which is present in both strands of the transforming DNAs. The ratio of *amiA*$^+$ to *str-r* transformants was eight times larger when the H strand carries the wild-type sequence at the *amiA36* site than when it was on the L strand. The residual (2%) wild-type frequency when the "ineffective" heteroduplex was used in transformation may have resulted from normal recombination between the *amiA36* and *amiA6* sites separated by 27 base pairs, since the average recombination frequency over this interval is about 1% (CLAVERYS, LATASTE

and SICARD 1979). The 2% may also have arisen in part from contamination by *amiA6* H strand in the *amiA6* L preparation. The degree of purity of the strands in the fractions was evaluated by their lack of ability to regain transforming activity when L (or H) strand preparations are self-annealed, compared with recovery of activity if mixed together before annealing. By this test, they were at least 90% resolved.

The heavy strand is the strand that is transcribed since, in the absence of excision repair, a marker is more rapidly expressed when carried on this strand (CLAVERYS, ROGER and SICARD 1980). The sequence of the *amiA* locus indicated only one open reading frame. The transcribed (H) strand appears to be the 3′...5′ strand of this locus which is transcribed from left to right (CLAVERYS *et al.* 1983). Indeed, four nonsense mutations defined by suppressor analysis (GASC *et al.* 1979) are stop codons in the corresponding messenger. Thus, sequence determinations have shown that the *amiA* mutant H strand contains T instead of G in the wild-type allele (LEFÈVRE *et al.* 1984). Thus, the conversion system acts preferentially upon (L)5′..A..3′/3′..G..5′(H) when the H strand carries the wild-type allele at the *amiA36* site.

DISCUSSION

Enhanced recombination around the *amiA36* site does not seem to induce a high frequency of exchanges between outside markers. Thus, the most likely explanation is that *amiA36* is often converted to the wild-type sequence (once out of every five transformation events). The results reported here do not yield any information about the reciprocal possibility, *i.e.*, the conversion of $amiA^+$ to *amiA36* in a suitable transformation test. Experiments are in progress to answer this question.

The conversion process was shown previously to be localized, since it does not extend over a marker located 27 base pairs to the right of *amiA36*. We have isolated and studied the behavior of a closer marker, *amiA121*, 3–5 bases away from *amiA36*. As we could detect little or no hyperrecombination between these sites, it appears that this process generally converts more than 3–5 base pairs. The fact that *amiA121* is a 1-base pair deletion may interfere with the pairing process and, therefore, with the conversion event. However, this is unlikely since frameshift mutations are well repaired by the *Hex* system which acts on heterozygotes (GASC and SICARD 1978). Moreover, hyperrecombination has been observed in crosses between *amiA36* and another 1-base pair deletion located 160 base pairs away (M. SICARD, unpublished data).

The conversion process that we have described is quite different from the mismatch repair system controlled by *hex* genes in pneumococcus. First of all, conversion involves only a few base pairs near the mutation (fewer than 27), whereas mismatch repair excised several thousand base pairs. Second, localized conversion acts equally well on donor or recipient strands, since hyperrecombination is observed in reciprocal crosses (LEFÈVRE *et al.* 1984, Table 1), whereas donor DNA is preferentially excised by the *Hex* system. Third, conversion does not require functional *hex* genes. Fourth, the only heteroduplex that can be converted in pneumococcus by the localized process, *i.e.*, 5′..A..3′/

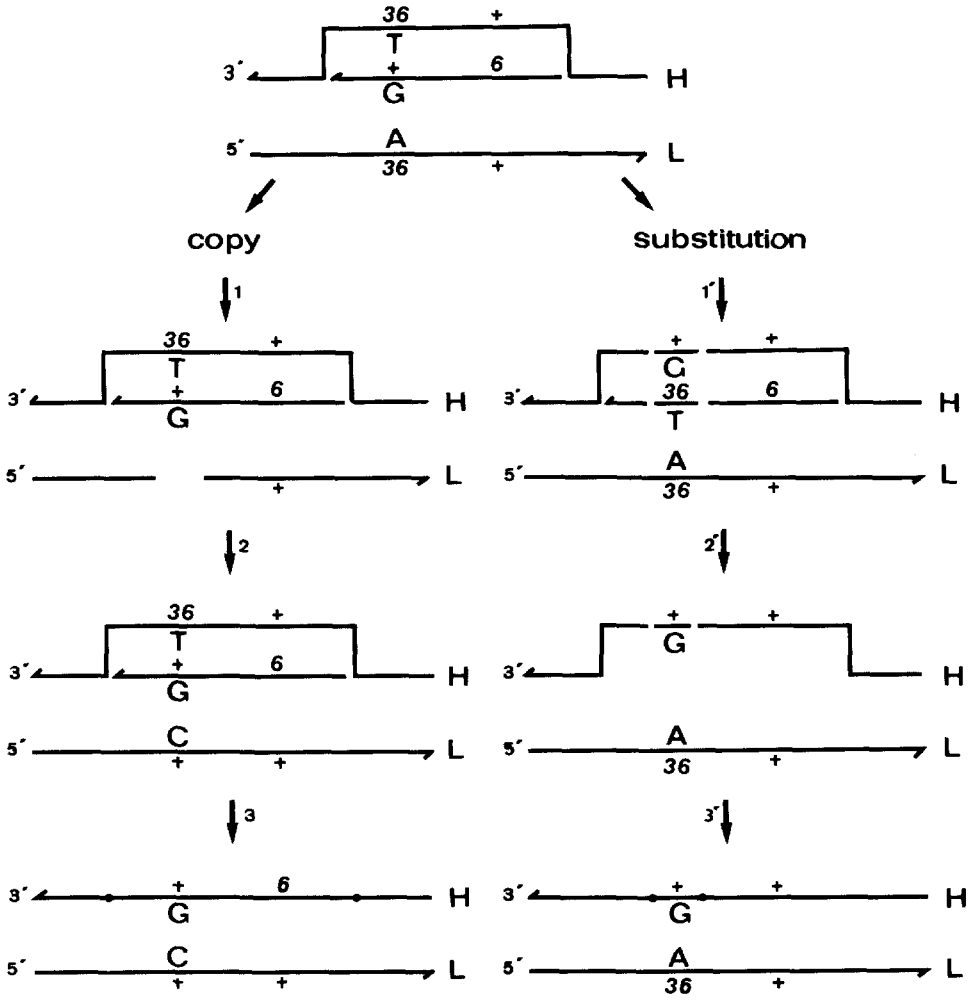

FIGURE 3.—Model for localized conversion. In the upper part is diagrammed the only heteroduplex that is converted at the pairing step between a single-stranded donor DNA carrying the *amiA*$^+$ allele and the recipient chromosome. Left, Copy hypothesis. Step 1: 5′..A..3′ (*amiA36*) is excised in the recipient chromosome. Step 2: The gap is filled by DNA synthesis taking the 3′..G..5′ (+) strand as template. Step 3: The other chromosomal DNA strand is degraded and nicks are ligated. Right, Substitution hypothesis. Step 1′: Breaks occur on both sides of *amiA36*, allowing an interchange of a small sequence between the incoming donor single-stranded DNA and the chromosomal DNA strand of the same polarity. Step 2′: Donor DNA is lost. Step 3′: Nicks are ligated.

3′..G..5′, is recognized by the *Hex* system in one sequence environment (LACKS, DUNN and GREENBERG 1982) but very poorly in others (CLAVERYS *et al.* 1983). This mismatch is also repaired with variable efficiencies (KRAMER, KRAMER and FRITZ 1984; WAGNER *et al.*, 1984) in a methyl-directed repair system of *E. coli* which shares many properties with the *Hex* system of *S. pneumoniae*.

The heteroduplex preference and the very special base sequence that is required for this conversion suggest that a specific enzymatic system is able to recognize this structure. It is interesting to point out that the A:G mispaired bases are surrounded by three A:T pairs which may facilitate an opening of DNA strands to allow an enzymatic conversion complex to recognize this structure. Obviously, more data are required to test the effect upon conversion of the sequences at or near a mutation. The palindrome of 6 A:T base pairs found at the *amiA36* mutation could be mere coincidence or it may be a structure related to the localized conversion process. An argument for this possibility is that, in λ phage, two mutations involving short palindromes also induce localized conversions (LIEB 1983). However, much more information is required to test these hypotheses.

The mechanism of conversion is still unknown. It has been postulated that limited DNA degradation and DNA synthesis may occur to produce extra copies of a given allele in gene conversion (WHITEHOUSE 1963; HOTCHKISS 1974; RADDING 1978). Indeed, the localized conversion induced by the *amiA36* mutation can be explained by this hypothesis. In this copy model (Figure 3), a short sequence around the mutation would be excised from the recipient DNA and resynthesized using as a template of the 3′..G..5′ wild-type sequence. This model is similar to the excision-repair hypothesis proposed to explain the long-patch conversion directed by the *hex* genes (EPHRUSSI-TAYLOR and GRAY 1966), which has been recently supported by biochemical evidence for the excision process (MÉJEAN and CLAVERYS 1984). One can also propose an alternative model, the replacement of a recipient mutant strand by the wild-type strand of the same polarity (Figure 3). This substitution would require endonucleolytic activities to cut donor and recipient strands near the mutation. There is no experimental evidence at the moment to support either hypothesis.

This work was supported by research grants from the University Paul Sabatier. Technical assistance for part of the experiments was provided by CATHERINE GASC.

LITERATURE CITED

CLAVERYS, J. P., H. LATASTE and A. M. SICARD, 1979 Localization of two *Eco*RI restriction sites within the *amiA* locus in pneumococcus: relationship between the physical and the genetic map. pp. 161–169. In: *Transformation 1978*, Edited by S. W. GLOVER and L. O. BUTLER. Costwold Press, Oxford.

CLAVERYS, J. P., J. C. LEFÈVRE and A. M. SICARD, 1980 Transformation of *Streptococcus pneumoniae* with *S. pneumoniae*-λ phage hybrid DNA: induction of deletions. Proc. Natl. Acad. Sci. USA **77:** 3534–3538.

CLAVERYS, J. P., J. M. LOUARN and A. M. SICARD, 1981 Cloning of *Streptococcus pneumoniae* DNA: its use in pneumococcal transformation and in studies of mismatch repair. Gene **13:** 65–73.

CLAVERYS, J. P., V. MÉJEAN, A. M. GASC and A. M. SICARD, 1983 Mismatch repair in *Streptococcus pneumoniae*: relationship between base mismatches and transformation efficiencies. Proc. Natl. Acad. Sci. USA **80:** 5956–5960.

CLAVERYS, J. P., H. PRATS, H. VASSEGHI and M. GHERARDI, 1984 Identification of *Streptococcus pneumoniae* mismatch repair genes by an additive transformation approach. Mol. Gen. Genet. **196:** 91–96.

CLAVERYS, J. P., M. ROGER and A. M. SICARD, 1980 Excision and repair of mismatched base pairs in transformation of *Streptococcus pneumoniae*. Mol. Gen. Genet. **178:** 191–201.

EPHRUSSI-TAYLOR, H. and T. C. GRAY, 1966 Genetic studies of recombining DNA in pneumococcal transformation. J. Gen. Physiol. **49** (Part 2): 211–231.

FOX, M. S. and M. K. ALLEN, 1964 On the mechanism of deoxyribonucleate integration in pneumococcal transformation. Proc. Natl. Acad. Sci. USA **52:** 412–419.

GASC, A. M. and A. M. SICARD, 1978 Genetic studies of acridine-induced mutants in *Streptococcus pneumoniae*. Genetics **90:** 1–18.

GASC, A. M., J. VACHER, R. BUCKINGHAM and A. M. SICARD, 1979 Characterization of an amber suppressor in Pneumococcus. Mol. Gen. Genet. **172:** 295–301.

GUHA, A. and W. SZYBALSKI, 1968 Fractionation of the complementary strands of coliphage T4 DNA based on the asymmetric distribution of the poly U and poly U.G. binding sites. Virology **34:** 608–616.

HOTCHKISS, R. D., 1974 Models of genetic recombination. Annu. Rev. Microbiol. **28:** 445–468.

KRAMER, B., W. KRAMER and H. J. FRITZ, 1984 Different base/base mismatches are corrected with different efficiencies by the methyl-directed DNA mismatch-repair system of *E. coli*. Cell **38:** 879–887.

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

LACKS, S., 1966 Integration efficiency and genetic recombination in pneumococcal transformation. Genetics **53:** 207–235.

LACKS, S., 1970 Mutants of *Diplococcus pneumoniae* that lack deoxyribonucleases and other activities possibly pertinent to genetic transformation. J. Bacteriol. **101:** 373–383.

LACKS, S. A., J. J. DUNN and B. GREENBERG, 1982 Identification of base mismatches recognized by the heteroduplex-DNA repair system of *Streptococcus pneumoniae*. Cell **31:** 327–336.

LEFÉVRE, J. C., A. M. GASC, A. C. BURGER, P. MOSTACHFI and A. M. SICARD, 1984 Hyperrecombination at a specific DNA sequence in pneumococcal transformation. Proc. Natl. Acad. Sci. USA **81:** 5184–5188.

LIEB, M., 1983 Specific mismatch correction in bacteriophage lambda crosses by very short patch repair. Mol. Gen. Genet. **191:** 118–125.

LOUARN, J. P. and A. M. SICARD, 1968 Transmission of genetic information during transformation in *Diplococcus pneumoniae*. Biochem. Biophys. Res. Commun. **30:** 683–689.

MAXAM, A. M. and W. GILBERT, 1980 Sequencing end-labelled DNA with base-specific chemical cleavages. Methods Enzymol. **65:** 499–560.

MÉJEAN, V. and J. P. CLAVERYS, 1984 Effect of mismatched base pairs on the fate of donor DNA in transformation of *Streptococcus pneumoniae*. Mol. Gen. Genet. **197:** 467–471.

MÉJEAN, V., J. P. CLAVERYS, H. VASSEGHI and A. M. SICARD, 1981 Rapid cloning of specific DNA fragments of *Streptococcus pneumoniae* by vector integration into the chromosome followed by endonucleolytic excision. Gene **15:** 289–293.

RADDING, C. M., 1978 Genetic recombination: strand transfer and mismatch repair. Annu. Rev. Biochem. **47:** 847–880.

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

SICARD, A. M., 1964 A new synthetic medium for *Diplococcus pneumoniae* and its use for the study of reciprocal transformation at the *amiA* locus. Genetics **50:** 31–44.

SICARD, A. M. and H. EPHRUSSI-TAYLOR, 1965 Genetic recombination in DNA-induced transformation of pneumococcus. II. Mapping of the amiA region. Genetics **52:** 1207–1227.

SICARD, A. M. and H. EPHRUSSI-TAYLOR, 1966 Recombinaison génétique dans la transformation chez le pneumocoque: étude des réversions au locus *amiA*. C.R. Acad. Sci. [D] (Paris) **262:** 2305–2308.

TIRABY, G. and A. M. SICARD, 1973 Integration efficiencies in DNA-induced transformation of pneumococcus. II. Genetic studies of mutants integrating all the markers with a high efficiency. Genetics **75:** 35–48.

TROMBE, M. C., G. LANEELLE and A. M. SICARD, 1984 Characterization of a *Streptococcus pneumoniae* mutant with altered electric transmembrane potential. J. Bacteriol. **158:** 1109–1114.

WAGNER, R., C. DOHET, M. JONES, M. P. DOUTRIAUX, F. HUTCHINSON and M. RADMAN, 1984 Involvement of *E. coli* mismatch repair in DNA replication and recombination. Cold Spring Harbor Symp. Quant. Biol. **49:** 611–615.

WHITEHOUSE, H. L. K., 1963 A theory of crossing-over by means of hybrid deoxyribonucleic acid. Nature **199:** 1034–1040.

Communicating editor: G. MOSIG