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Genetics

Homeologous recombination and mismatch repair during transformation in *Streptococcus pneumoniae*: Saturation of the Hex mismatch repair system

(genetic stability/interspecies recombination/mosaic genes/speciation)

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ABSTRACT **The ability of the Hex generalized mismatch repair system to prevent recombination between partially divergent (also called homeologous) sequences during transformation in *Streptococcus pneumoniae* was investigated. By using as donor in transformation cloned fragments 1.7–17.5% divergent in DNA sequence from the recipient, it was observed that the Hex system prevents chromosomal integration of the least and the most divergent fragments but frequently fails to do so for other fragments. In the latter case, the Hex system becomes saturated (inhibited) due to an excess of mismatches: it is unable to repair a single mismatch located elsewhere on the chromosome. Further investigation with chromosomal donor DNA, carrying only one genetically marked divergent region, revealed that a single divergent fragment can lead to saturation of the Hex system. Increase in cellular concentration of either HexA, the MutS homologue that binds mismatches, or HexB, the MutL homologue for which the essential role in repair as yet remains obscure, was shown to restore repair ability in previously saturating conditions. Investigation of heterospecific transformation by chromosomal DNA from two related streptococcal species, *Streptococcus oralis* and *Streptococcus mitis*, also revealed complete saturation of the Hex system. Therefore the Hex system is not a barrier to interspecies recombination in *S. pneumoniae*. These results are discussed in light of those described for the Mut system of *Escherichia coli*.**

Genetic evidence for the presence in *Streptococcus pneumoniae* of a generalized mismatch repair system acting on recombination intermediates first came from investigation of variations in marker transformation efficiencies (1). It was soon recognized that this mismatch repair system, called Hex, is a DNA replication editor correcting potentially mutagenic mismatches. Two *hex* genes, *hexA* and *hexB*, have been identified (2). Inactivation of either gene confers a mutator phenotype and abolishes mismatch repair in transformation. The Hex system was found to repair different base–base mismatches with different efficiencies (3). *Transition* mutations exhibit a low efficiency of transformation (LE markers) because the Hex system is very efficient in repairing both types of transition mismatches (i.e., G/T and A/C) at the donor–recipient heteroduplex stage during the transformation process. *Transversion* mutations, on the other hand, generally exhibit a 10- to 20-fold higher efficiency of transformation (HE markers) because either one or both transversion mismatches are not corrected by Hex. Genetic and physical (1, 4) evidence for removal of the entire donor strand in transformation has been obtained. A strand-specific repair process in which strand discrimination is nick-directed—i.e., based on the availability of single-strand ends—operating before ligation of the donor (invading) strand to the recipient chromosome would lead to such a removal.

Since the suggestion that the Hex system and its *Escherichia coli* counterpart, the Mut system (5), are evolutionarily related (1), and strengthened by the observation of homologies existing between HexA and MutS (6) and between HexB and MutL (7), evidence is accumulating that generalized mismatch repair systems evolved from a common ancestor and are widespread throughout nature. Human homologs of these mismatch repair proteins have been found at loci associated with hereditary nonpolyposis colorectal carcinoma (HNPCC), and mutations in these genes have been found in HNPCC patients (8) and in human ovarian cancer cell lines (9). In addition, extracts of HNPCC tumor cells are found defective in mismatch repair *in vitro* (10). These defects correlate with genomic instability in simple repeated sequences.

Hex and Mut, the two bacterial mismatch repair systems, have been found to eliminate potential recombinants during homologous recombination between nonidentical sequences that produces heteroduplex DNA containing one or a few mismatches (2, 11). Inhibition of recombination between more diverged sequences by mismatch repair (12) could prevent chromosomal rearrangements by limiting exchanges between dispersed repetitive sequences and favor speciation (13). The observation that *mut* mutations can increase intergeneric recombination between *E. coli* and *Salmonella typhimurium* provided support to the latter hypothesis (13). However, the situation appeared more complex in the case of the Hex system. By using nonisogenic *S. pneumoniae* DNA as donor in transformation of a Hex$^+$ recipient, it has been shown that relatedness of the donor to the recipient strain could influence the transforming efficiency of a LE marker (14). It was suggested that nonisogenic DNA forms mismatched heteroduplexes that compete for the Hex system and that Hex is easily saturated with substrate, leading to escape from correction of the LE marker being scored. Complete saturation (inhibition) of the Hex system was even obtained in transformation with a mixture of isogenic DNA carrying the LE marker and a competitor DNA from a *S. pneumoniae* strain of different serotype (15). Heterospecific DNA such as *Bacillus subtilis* DNA did not compete for the Hex system, whereas *Streptococcus gordonii* (formerly *Streptococcus sanguis* Wicky) DNA

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Abbreviations: cfu, colony-forming unit; Pen$^{r/s}$, penicillin-resistant/sensitive; Rif^r, rifampicin-resistant; Sm^r, streptomycin-resistant; LE, low efficiency of transformation; HE, high efficiency of transformation.

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was reported either to have no detectable influence (15) or to compete to a small degree (14).

We report our investigation of the action of the Hex system during homeologous transformation using as donor cloned fragments 1.7–17.5% divergent in sequence from the recipient. Saturation of the Hex system has been demonstrated with excess mismatches within a single donor–recipient heteroduplex per cell. Complete saturation of the Hex system was also observed in heterospecific transformation involving DNA from two close relatives of *S. pneumoniae, Streptococcus mitis* and *Streptococcus oralis.* Increase in cellular concentration of either HexA or HexB proteins was shown to restore repair capacity. A preliminary report of this work has appeared (16).

MATERIALS AND METHODS

Strains and Media. *S. pneumoniae* strains were grown, and precompetent cells were prepared and transformed at a cell density of about 5×10^7–10^8 colony-forming units (cfu)/ml following treatment with crude preparations of competence factor (CF), as described (17). Depending on the penicillin-resistance (Pen^r) gene used as donor in transformation, two Hex^+ pneumococcal recipient strains that are derived from R6, a nonencapsulated strain derived from R36A (18), were used as recipients, R800 (MIC) of 0.008 μg [minimum inhibitory concentration of benzypenicillin per ml] (17) or $R6^{R1}$ (MIC of 0.06 μg/ml) (19). R800 could be transformed to the first level of penicillin resistance using a resistant *pbp2x* gene. $R6^{R1}$ is a first-level, Pen^r transformant of strain R6 possessing resistant *pbp2x* and *pbp1a* genes and could be transformed to a higher level of resistance to penicillin (MIC of 0.5 μg/ml) using a resistant *pbp2b* gene. The Hex^- derivatives of R800 and $R6^{R1}$ were R801 (20) and $R6^{R1}\Delta3$, respectively. The former carries a mutation in the *hexB* gene leading to the synthesis of a truncated HexB protein (7). The latter was constructed by introducing the *hexAΔ3* mutation by transformation of the $R6^{R1}$ recipient with DNA from the pSP11Δ3 recombinant plasmid (21). Chromosomal DNA from strain R119 (21) was used as donor of the *str41* streptomycin-resistance marker and of the *rif23* rifampicin-resistance marker. Antibiotic concentrations used were as follows: 2 μg of erythromycin per ml, 2 μg of rifampicin per ml, 200 μg of streptomycin per ml, and 1 μg of tetracycline per ml.

Recombinant Phages and Plasmids. Methods for preparing *S. pneumoniae* chromosomal DNA and for obtaining plasmid DNA from *E. coli* or from *S. pneumoniae* have been described (21). Isolation and cloning into M13mp19 of the *pbp2x* resistant gene fragments (2056 bp) from the Pen^r, clinical isolates strains 669, 29044, and 8249 and from one transformant (8249T) obtained using R6 as acceptor strain and 8249 donor DNA have been described (22). Plasmid pCGD1 (19) carries a 3205-bp (*Mbo* I partial digest) fragment from the Pen^r, clinical isolate strain 64147, containing part of the *pbp2b* gene (coding region for the 486 carboxy-terminal residues) and downstream sequences. Plasmid pCGD10, which carries a 1834-bp insert, was derived from pCGD1 by restriction endonuclease digestion with *Eco*RV, followed by ligation. Plasmid pCGD11 was generated by substituting a *Spe* I–*Eco*RI fragment (containing the 3′ extremity of the *pbp2b* gene and flanking sequences downstream of it) in plasmid pCGD1 by the corresponding region from the penicillin-sensitive (Pen^s) R6 strain. Plasmid pCGD7 carries a hybrid insert obtained by cloning into plasmid pBS9 (23) a 1673-bp *Mbo* I fragment containing the coding region of the transpeptidase domain of *pbp2b* from the Pen^s strain R6 and substituting a 303-bp *Hin*cII–*Xba* I fragment by the corresponding fragment from the Pen^r strain 64147. Isolation and cloning into M13tg130 of the *pbp2b* resistant gene fragment (1505 bp) from the Pen^r, clinical isolate strain 53139/72 have been described (24).

RESULTS

Effect of Hex on Recombination Between Partially Divergent Sequences. The cloned *pbp2b* and *pbp2x* DNA fragments used as donor in transformation varied both in size (from 1.5 to 3.2 kb) and in divergence (from 1.7 to 17.5%) as shown in Fig. 1 and in Table 1. Transformation of Hex^- recipient strains to Pen^r occurred at similar frequencies with each of the various cloned fragments, except for the most divergent fragment, 8249, where recombination was reduced 20- to 40-fold (Table 1, column 3). However, transformation of Hex^+ recipients with the very same fragments occurred with widely varying efficiencies. Thus the efficiency of transformation using pCGD7 as donor was ≈20-fold lower than that of pCGD1, suggesting efficient mismatch correction with the former donor. Transformation of the Hex^+ recipient $R6^{R1}$ (Table 1, column 4) with pCGD1 was only 2-fold lower than that obtained of the Hex^- recipient $R6^{R1}\Delta3$, indicating that the Hex system was unable to reject the multiply mismatched pCGD1 donor, in about 50% of the transformed cells. Inhibition of mismatch repair depended more on the total number of potential mismatches than on the size of the donor since pCGD11, which has the same size

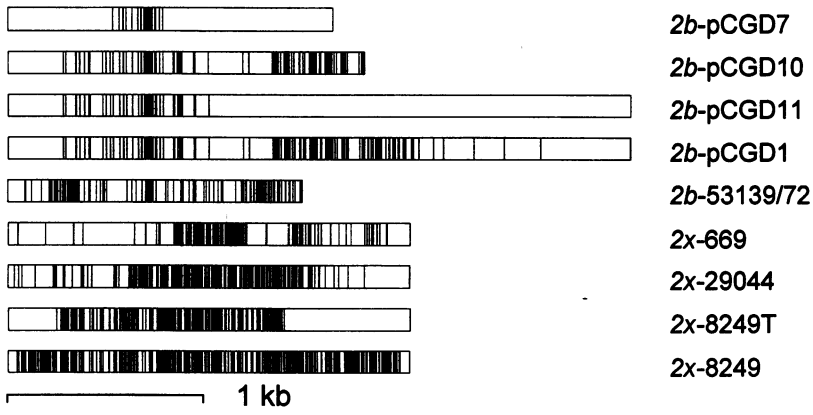

FIG. 1. Cloned *pbp2b* and *pbp2x* DNA fragments used as donor in transformation. Each potential mismatch between Pen^r donor fragments and the corresponding chromosomal region in the Pen^s recipient strain is indicated by a vertical bar. The degree of divergence from the recipient is indicated for each fragment in Table 1. The overall mismatch composition is similar for all fragments: 60–70% transition mismatches, 16–25% A/G or C/T mismatches, 6–18% A/A or T/T mismatches, and 4–9% G/G or C/C mismatches. Fragments carried by plasmids pCGD1–pCGD11 are derived from the same Pen^r fragment cloned from the clinical isolate strain 64147 (see text). With the exception of the downstream sequences of the wild-type and 64147 *pbp2b* genes (C.G.D., unpublished data), sequence data have appeared in the GenBank data base [accession nos. X16367 (2*x*-wild type), X65133 (2*x*-669), X65131 (2*x*-29044), X65132 (2*x*-8249), X13137 (2*b*-wild type), X13136 (2*b*-64147), and M25524 (2*b*-53139/72)].

Table 1. Effect of Hex on recombination between partially divergent sequences and saturation of the Hex system

DNA	% divergence	Hex⁻ recipient* (Pen^r)	Hex⁺*/ Hex⁻* (Pen^r)	Rif^r/Sm^r†
None	—	—	—	0.015 ± 0.005
pCGD7	2.0	2.25	0.029	0.05 ± 0.01
pCGD10	6.3	2.98	0.30	0.47 ± 0.05
pCGD11	1.7	3.28	0.18	0.13 ± 0.04
pCGD1	4.6	3.07	0.50	0.65 ± 0.06
53139/72	9.5	3.05	0.093	0.07 ± 0.01
669	6.9	3.02	0.18	0.30 ± 0.07
29044	10.3	1.94	0.21	0.30 ± 0.06
8249T	9.1	3.96	0.15	0.15 ± 0.04
8249	17.5	0.10	0.019	ND

ND, not determined.

*Each recipient was transformed with recombinant plasmid DNA at a concentration of 1 μg/ml. To correct for variations in competence of the different recipients, numbers of Pen^r transformants were normalized to streptomycin-resistant (Sm^r) transformants obtained with R119 chromosomal DNA in a parallel transformation. To take into account the fact that one of the two mismatches generated upon pairing of the *str41* marker is efficiently corrected out by the Hex system (3), the number of Pen^r transformants obtained in the Hex⁺ recipient was further divided by 2 before making the ratio to Hex⁻. Transformations of Hex⁺ and Hex⁻ recipients with a given donor fragment were always run in parallel, allowing direct comparison. Transformations with different fragments were not run in parallel, making comparison of absolute frequencies less reliable.

†R119 chromosomal DNA was irradiated with ultraviolet light, a treatment known to increase the efficiency of correction by the Hex system (1), and used at a concentration of 0.15 μg/ml to transform the wild-type recipient strain R800. Competitor (unirradiated) DNA from recombinant plasmids was used at a concentration of 1 μg/ml. Aliquots of the transformed culture were plated to determine the number for Sm^r and rifampicin-resistant (Rif^r) transformants.

as pCGD1 but is only 1.7% divergent, exhibited an intermediate transforming efficiency. All other fragments, except 8249, were also only partially corrected out by the Hex system, giving 3- (53139/72) to 10- (pCGD10) fold more transformants than expected in a fully proficient mismatch repair background. Fragment 8249 constituted a noticeable exception since this donor was rejected at least as efficiently as pCGD7—i.e., as efficiently as a point LE marker would be.

Saturation of the Hex System Is Not a Local Effect. To investigate whether a homeologous fragment such as pCGD1 only had a local inhibitory effect on the Hex system or was inhibiting mismatch repair over the whole chromosome, we transformed a Hex⁺ recipient with a mixture of chromosomal DNA carrying the *rif23* LE marker and the *str41* HE reference marker and pCGD1 or other competitor DNAs. Results presented in Table 1 (column 5) demonstrated a 40-fold increase in the number of Rif^r transformants relative to the number of Sm^r transformants in the presence of pCGD1 DNA. Therefore the cloned divergent DNA fragment induced inhibition (saturation) of the Hex system, allowing escape from correction of the LE marker. Saturation was also observed with pCGD10 DNA and other fragments, although to a lesser extent. A plot of the efficiency of the LE marker in the presence of each cloned fragment against the Hex⁺/Hex⁻ relative transforming efficiency of these fragments indicated a linear relationship between the saturation capacity of each fragment and its relative efficiency (not shown).

A Single Divergent Fragment Can Be Refractory to Correction by the Hex System. It was, however, difficult to conclude from the data described above using plasmid DNAs that a single divergent fragment can lead to saturation of the Hex system. Since competent cells of *S. pneumoniae* take up several donor molecules (25), it was possible that saturation occurred as a consequence of several rounds of heteroduplex formation, followed by mismatch repair. To check this hypothesis, we decided to use chromosomal derivatives of pCGD1, pCGD7, and pCGD10 as donor in transformation. Given the number of chromosomal fragments taken up by competent cells (four 35- to 40-kb molecules of a typical chromosomal donor DNA) (25), 90–95% of cells in a transformed culture will take up only a single Pen^r fragment. Chromosomal derivatives of pCGD1, pCGD7, and pCGD10 were constructed by transformation of an R6^{R1}Δ3 recipient strain containing the *str41* reference marker with cloned DNA as donor. A combination of restriction digests, hybridization with specific oligonucleotides, and DNA sequencing was used to confirm the integration of an intact donor fragment in each of these derivatives (O.H., C.G.D., and J.-P.C., unpublished data). The transforming efficiency of the chromosomal pCGD1 derivative in the Hex⁺ recipient (Pen^r/Sm^r = 0.65 ± 0.06) demonstrated that the Hex system can be saturated by a single fragment harboring 149 potential mismatches. The chromosomal pCGD10 derivative possessing 115 base changes exhibited an intermediate efficiency (0.47 ± 0.03), indicating that a substantial fraction of cells in the transformed culture was unable to correct multiple mismatches in the donor–recipient heteroduplex. The chromosomal pCGD7 derivative with 33 potential mismatches exhibited an efficiency (0.17 ± 0.04) similar to that observed for single point mismatches (LE markers) that are efficiently corrected by the Hex system.

Investigation of the Limiting Hex Component. Its ability to be saturated by substrate implies that at least one component of the Hex system is present in only a small quantity per cell. To determine which is the limiting component, we transformed with divergent DNA recipient strains containing a recombinant plasmid carrying either the *hexA* or the *hexB* gene since the presence of recombinants derived from the pMV158 streptococcal plasmid whose copy number has been estimated to be 22 per cell (26) could increase *hex* gene expression. Both recipients appeared more resistant to saturation by excess mismatches when transformed either with the chromosomal pCGD1 derivative (Table 2, column 2) or with a mixture of homologous chromosomal DNA and a cloned divergent fragment in a competitor-type of experiment (Table 2, columns 3–5). Therefore, an increase in either *hexA* or *hexB* gene copy number increased mismatch repair ability.

Investigation of *hexA* and *hexB* Gene Expression. Since positive control of the *hexB* gene by the HexA protein, or *vice versa*, might account for the above observation, the Hex protein content of each recipient was investigated using antibodies raised against HexA or HexB. Western blotting experiments revealed a 5- to 10-fold increase in HexA or HexB protein concentration only in exponentially growing cells containing multiple copies of *hexA* or *hexB*, respectively (data

Table 2. Investigation on the nature of the limiting component of the Hex system

Recipient	Donor DNA: pCGD1-chr. Pen^r/Sm^r*	— Rif^r/Sm^r†	pCGD1 Rif^r/Sm^r†	pCGD10 Rif^r/Sm^r†
Hex⁺	0.65	0.02	0.60	0.47
[phexB⁺]‡	0.20	0.007	0.32	0.25
[phexA⁺]‡	0.26	0.005	0.27	0.15

*Each recipient was transformed with chromosomal (chr.) DNA carrying the homeologous pCGD1 region and the *str41* reference marker, at a concentration of 1 μg/ml. Aliquots of the transformed culture were plated to determine the number for Pen^r and Sm^r transformants.

†Experimental conditions were essentially similar to those described in the legend to Table 1.

‡Strain R6^{R1} contained either the recombinant plasmid pSP41 (20), which carries the *hexB* gene, or the recombinant plasmid pSP11 (21), which carries the *hexA* gene.

not shown). Expression of the *hexB* gene remained unaffected no matter the number of copies of the *hexA* gene, from none to about 20–25 per cell. A similar conclusion was obtained for *hexA* gene expression. Comparison of extracts from competent and noncompetent cultures showed no change in the amount of HexA and HexB proteins, suggesting that *hexA* and *hexB* genes are not induced at competence (data not shown). In addition, no increase in the amount of the two *hex* transcripts was detected in competent cells exhibiting strong competence-specific induction of the *recA* mRNA (data not shown). Thus, unlike *recA* (17), *hexA* and *hexB* genes are not induced at competence.

Effect of Hex on Heterospecific Transformation. By using chromosomal DNA of *S. mitis, S. oralis, S. gordonii,* and *S. sanguis* as competitors, we observed that *S. mitis* and *S. oralis* DNAs competed for the Hex system much more efficiently than *S. gordonii* DNA (Table 3). The small but significant effect of various *S. gordonii* DNAs on mismatch repair efficiency was in agreement with a previous conclusion (14), whereas it was not clear whether *S. sanguis* DNA had any effect on Hex. *S. mitis* and *S. oralis* DNAs were almost as efficient as DNA from the *S. pneumoniae* strain of serotype 18 to saturate the Hex system, which suggests that these two streptococcal species are closely related to *S. pneumoniae.*

DISCUSSION

Recombination of cloned homeologous *pbp2b* and *pbp2x* fragments into the chromosome of mismatch repair deficient strains of *S. pneumoniae* appeared essentially unaffected by nucleotide divergence of between 1.7% and 10.3%. However, recombination of the most divergent fragment 8249 (17.5%) was drastically reduced (Table 1). This observation could be explained assuming that the minimal efficient processing segment (MEPS) for recombination in *S. pneumoniae* is similar to that of *E. coli* (*ca.* 30 nucleotides) (27), since upon pairing 8249 would generate only two stretches of complete homology (44 and 47 nucleotides) longer than 1 MEPS, one at each end of the fragment (Fig. 1). Recombination of the very same homeologous fragments occurred with widely varying efficiencies in mismatch repair proficient strains. Transformant yields for the fragment harboring the lowest number of potential mismatches and the most divergent fragment were reduced 30- to 50-fold as compared to Hex$^-$ recipients, indicating efficient abortion of recombinants by mismatch repair. Inhibition of recombination of the most divergent fragment by the Hex system may occur at the pairing step, as shown for *in vitro* RecA-catalyzed strand transfer between diverged DNAs in the presence of MutLS proteins (28). All other fragments exhibited intermediate transforming efficiencies indicative of partially inefficient mismatch correction. Simultaneous transformation of a cloned divergent DNA fragment and of a LE marker—i.e., a point mutation normally susceptible to Hex—revealed that inhibition of mismatch correction was not limited to the divergent fragment. The Hex system became saturated by excess mismatches, allowing escape from correction of the LE marker being scored. This saturation is reminiscent of that observed in transformation with nonisogenic *S. pneumoniae* DNA (14, 15). The most diverged fragment could have failed to inhibit mismatch repair because regions of complete homology were very short (see above), thus limiting heteroduplex length and reducing the number of mismatches that can be seen by Hex.

Table 3. Saturation of the Hex system in heterospecific transformation

Competitor DNA	Rif^r/Sm^r
—	0.015 ± 0.005
S. pneumoniae, serotype 18	1.24 ± 0.16
S. mitis, NCTC 12261*	0.84 ± 0.08
S. oralis, NCTC 11427*	0.83 ± 0.08
S. gordonii biovar 2, ATCC 10558*	0.07 ± 0.04
S. gordonii biovar 2, OB11 (Challis)	0.052 ± 0.022
S. gordonii biovar 3, NCTC 3165	0.034 ± 0.03
S. sanguis biovar 1, NCTC 7863*	0.033 ± 0.018

The wild-type strain R800 was transformed under conditions essentially similar to those described in the legend to Table 1 (UV-irradiated R119 chromosomal DNA at a concentration of 100 ng/ml), except that (unirradiated) chromosomal DNA (at a concentration of 7.5 μg/ml) from the various sources indicated was used as competitor, instead of DNA from recombinant plasmids.
*Indicates type strain.

An intermediate transforming efficiency was observed using as donor a chromosomal DNA containing a single 4.6% divergent region, thus demonstrating inhibition of mismatch repair in 30–40% of the cells in the culture, with a unique fragment per cell. Assuming the diverged fragment pairs over its entire length, the total number of mismatches leading to saturation would be 149/2459 nucleotides. However, this number is probably an overestimate as potential mismatches are not randomly distributed along the fragment (Fig. 1). A cluster of mismatches may not be readily incorporated into the heteroduplex and therefore would not be recognized by mismatch repair proteins. In addition, it has been shown that some combinations of mismatches, especially those including C/C, are not efficiently recognized by the Hex system (3). That many mismatches within a single donor-recipient heteroduplex can lead to saturation of the Hex system is quite paradoxical in view of the fact that a single mismatch within a donor fragment of identical size can result in rejection of the entire donor strand (1, 4). We suggest that inhibition could take place during HexA-dependent bidirectional search for nicks that follows the binding to mismatches (24). Ligation events occurring before recognition of a nick by tracking complexes would remove strand-targeting signals required for efficient mismatch correction by Hex, thus preserving the donor strand from repair. Therefore, any event reducing the rate of tracking, such as collision between independent tracking complexes, would reduce repair efficiency.

It is puzzling that an increase of either HexA or HexB protein concentration results in enhanced repair capacity, whereas an oversupply of HexA cannot complement a *hexB* mutation, and *vice versa* (our unpublished data). We suggest that an increase in HexA cellular content could *directly* affect repair, by increasing the number of tracking complexes and, consequently, the probability to initiate tracking nearby extremities of donor fragments. To account for the positive effect of increasing HexB concentration, we suggest that this protein is activated to bind mismatches through specific interaction with HexA-mismatched DNA complexes. This would be consistent with the observation that MutL, the HexB homologue, increases the length of the region protected from DNase I in MutS-mismatched DNA complexes (29). An important consequence of such a binding of HexB would be to prevent the abortive binding of HexA within tracking loops. Thus, an increase in HexB cellular content would *indirectly* increase the amount of free HexA protein available for repair.

Our observations that the Hex system of *S. pneumoniae* was completely saturated with chromosomal DNA from two related streptococcal species, *S. oralis* and *S. mitis*, did not provide further support to the hypothesis that mismatch repair systems can act as a barrier to recombination between DNAs of different species (13). However, the Hex system could represent a special case among generalized mismatch repair systems, related to the fact *S. pneumoniae* is a naturally transformable species. Transformation is a highly regulated process that could have evolved in this organism toward the acquisition of new genetic traits to rapidly adapt to adverse growth conditions. In addition, the finding that excess mis-

matches within a single donor–recipient heteroduplex led to saturation of Hex, whereas single mismatches were corrected, does not fit in with the idea that the system evolved to prevent recombination between diverged DNAs. It is more likely that Hex has been evolved as a postreplication repair system only and that its action during transformation is fortuitous. Consistent with this hypothesis, our observation that the *hex* genes, unlike *recA* (17), are not induced in transforming cells, whereas an increase in Hex protein concentration would result in enhanced repair capacity, further suggests that the Hex system has not been tuned to cope with excess mismatches in heteroduplex regions. The saturation of Hex by DNA from *S. mitis* and *S. oralis* suggests that horizontal transfer of genetic information from these species could occur by natural transformation of a Hex^+ recipient. Our observations provide support to the proposal (24) that alteration of *pbp* genes in Pen^r clinical isolates of *S. pneumoniae* has involved the replacement, presumably by transformation, of (part of) the original sensitive *pbp* genes by homeologous sequences from unknown related species. In agreement with this proposal, it has been shown recently that *S. mitis* and *S. oralis* are likely donors for some of these sequences (30, 31).

The Mut system of *E. coli* and *S. typhimurium* differs from Hex with respect to homeologous recombination in two ways: the lack of saturation and the extent of antirecombinogenic action in intergeneric crosses. Since Mut can, like Hex, be saturated with excess mismatches (see below), the absence of saturation of Mut in homeologous conjugation could indicate that much shorter heteroduplex regions are made than during *S. pneumoniae* transformation. Indeed, it can be inferred from the amount of donor DNA taken up in *S. pneumoniae* that regions of heteroduplex DNA amounting to 100–150 kb could be made, requiring for formation full induction of the *recA* gene (B. Martin and J.-P.C., unpublished observations). The Mut system has been shown to reduce recombination between bacteriophage λ and a plasmid sharing in common a homeologous (11% divergence) 405-bp DNA fragment by about 25-fold (32). Quite remarkably, we observed a similar reduction (about 50-fold) by Hex of recombinants with the most diverged Pen^r fragment. On the other hand, a 1000-fold increase in recombinant production in Mut^- strains as compared to Mut^+ has been reported in conjugational crosses between *S. typhimurium* and *E. coli* (13). The much larger effect of Mut in the latter case could be explained by the fact that conjugative recombination involves the formation of two heteroduplex regions, one at each extremity of the Hfr donor DNA that is otherwise mostly double stranded (33), which could be processed independently by Mut. A 625-fold (25×25) reduction in recombinants would then be predicted.

Transient saturation of the generalized mismatch repair system can occur not only in *S. pneumoniae* but also in *E. coli*. Saturation has been observed in a *mutD*, proofreading deficient, strain that accumulates excessive DNA replication errors (34, 35), during treatments with mutagens whose mutagenic intermediates are subject to mismatch repair (36), or in the presence of multicopy single-stranded DNAs with mismatches (37). Given the biological consequences of mutational inactivation of mismatch repair genes in humans (8), even transient saturation could be highly detrimental. The presence of only a single functional copy of a mismatch repair gene in the germ line of individuals predisposed to some cancers may affect the concentration of the corresponding protein and result in reduced mismatch repair ability (10). This would increase the probability of transient saturation that may favor mutagenic inactivation of the remaining functional allele or the activation of some oncogene.

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