Heteroduplex DNA Mismatch Repair System of *Streptococcus pneumoniae*: Cloning and Expression of the *hexA* Gene

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Mutations affecting heteroduplex DNA mismatch repair in *Streptococcus pneumoniae* were localized in two genes, *hexA* and *hexB*, by fractionation of restriction fragments carrying mutant alleles. A fragment containing the *hexA4* allele was cloned in the *S. pneumoniae* cloning system, and the *hexA*$^+$ allele was introduced into the recombinant plasmid by chromosomal facilitation of plasmid transfer. Subcloning localized the functional *hexA* gene to a 3.5-kilobase segment of the cloned pneumococcal DNA. The product of this gene was shown in *Bacillus subtilis* minicells to be a polypeptide with an M_r of 86,000. Two mutant alleles of *hexA* showed partial expression of the repair system when present in multicopy plasmids. A model for mismatch repair, which depends on the interaction of two protein components to recognize the mismatched base pair and excise a segment of DNA between strand breaks surrounding the mismatch, is proposed.

As a consequence of the mechanism of DNA uptake in the transformation of *Streptococcus pneumoniae*, single-strand donor DNA segments several kilobases (kb) in length enter the cell (15, 21, 29) and are integrated into the recipient DNA to give a heteroduplex product (11). Evidence for a mismatch repair system in *S. pneumoniae* that acts on such heteroduplex DNA initially came from differences observed in transformation frequencies for markers corresponding to different mutations within the same gene (9, 16, 20). Markers representing transition mutations were integrated much less efficiently than those representing transversions or small deletions (2, 3, 17, 22). The low efficiency appeared to result from exclusion of the donor marker and an adjacent segment of donor DNA several kb long (17). It was proposed that a repair system recognizes the mismatch and excises a portion of one strand in the heteroduplex, which is then restored by repair replication (8). Inasmuch as only the donor marker was eliminated (17), and the repair occurred early in the integration process (12, 31), it was suggested that the target for repair is the incompletely integrated donor segment in the heteroduplex (5, 14, 22).

The isolation of *hex* mutant cells, in which all single-site markers were transformed at high efficiency, supported the hypothesis of a mismatch repair system in normal cells (18). Thus, in a Hex$^+$ recipient, markers that produce mismatches not recognized by the repair system give high transformation efficiency, whereas those that produce recognizable mismatches give low transformation efficiency because the donor marker is "corrected," that is, eliminated, most of the time. In a Hex$^-$ recipient, no mismatches are corrected, so all markers give high transformation efficiency. Furthermore, Hex$^-$ cells showed high rates of spontaneous mutation (22, 33). This indicated that the *hex* repair system also corrected potential mutational mismatches in the replication of chromosomal DNA. In the present work, six independently isolated *hex* mutations were analyzed with respect to their location in the genome. They were found to fall into at least two different genetic loci.

One of the *hex*$^+$ genes and three of its mutant alleles were cloned in a pneumococcal cloning system. Phenotypic expression of the cloned genes was examined in *S. pneumo-*

niae, and the nature of their gene products was analyzed by transfer of the recombinant plasmids to *Bacillus subtilis* minicells. On the basis of these and prior results, a tentative model for the mechanism of heteroduplex DNA base mismatch repair was formulated.

MATERIALS AND METHODS

Bacterial strains and plasmids. Strains of *S. pneumoniae* and their relevant mutant genotype were 175 (*malM567*), 193 (*malDXMP581*), 533 (*nov str sul-d*), 590 (*hex-3 malM515*), 742 (*amiA9 nov str sul-d*), 767 (*hex-1 malDXMP581*), 777 (*hex-Rx malDXMP581*), 782 (*hex-4 malDXMP581 str*), 793 (*hex-6 amiA9 malDXMP581*), and 794 (*hex-7 amiA9 malDXMP581*). Markers that transform with high efficiency are designated HE markers; those that transform with low efficiency are designated LE markers. Plasmid vectors were pLS101, which conferred Mal$^+$ or Tc^r (1); pLS70, partial cleavage of which by *PstI* gave the pMV158 vector that confers Tc^r (32); and pLS69, of which a Tc^r-conferring fragment was used (1).

Growth media and transformation procedures. Media for culture growth and plating (17), procedures for transformation (1), and conditions for selection of Mal$^+$, Sul^r, Str^r, Nov^r, Ami^r, and Tc^r transformants were described (1, 35).

Tests for Hex$^-$ transforming activity and Hex phenotype. Hex$^-$ transforming activity in restriction fragments or plasmids extracted from gels was tested by first transforming cultures of strain 175 (*hex$^+$*) with the DNA. The cultures were then treated with UV-irradiated DNA from strain 533 (*nov*, LE marker). The DNA was irradiated in solution at 40 μg/ml in a layer 0.5 mm deep with a fluence of 480 J/m^2 from a germicidal lamp. Novr transformants were grown up in bulk culture and transformed with UV-irradiated DNA from strain 742 (*amiA9*, LE marker; *sul-d*, HE marker). The ratio of Ami^r to Sul^r (LE/HE) transformants measured the extent of the original Hex$^-$ transformation.

Mal$^+$ or Tc^r clones to which recombinant plasmids were transferred were tested for their Hex phenotype by transformation with UV-irradiated DNA from strain 533 (*nov*, LE marker; *str*, HE marker). The LE/HE ratio indicated the phenotype.

Preparation and manipulation of DNA. Chromosomal DNA (17), purified plasmids (6), and crude plasmid extracts (1)

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were prepared as described. Enzymes were from commercial sources and used according to the recommendations of the supplier. Restriction fragments were separated by electrophoresis in 0.8% low-melting-point agarose and extracted from gel segments melted at 65°C and cooled to 37°C. The mixtures were treated with Tris-buffered phenol first at 37°C, then at 20°C, and the DNA was precipitated with ethanol. Ligation of fragments was carried out as described (32). Plasmids in crude extracts of transformants were analyzed by electrophoresis in 1% agarose; monomer forms of the plasmids were eluted from gel segments as described (1) and tested for Hex⁻ transforming activity.

Analysis of proteins encoded by plasmids in minicells of _Bacillus subtilis_. Recombinant plasmids were transferred first to strain MB11 (_lys-3 metB10 hisH2_) and then to strain CU403 (_divIVB1 thyA thyB metB_) by transformation to Tcʳ (10). Procedures for the preparation of minicells, labeling of protein with [³⁵S]methionine, analysis of extracts in polyacrylamide gradient gels, and autoradiography have been described (10).

RESULTS

Localization of _hex_ genes on chromosomal restriction fragments. Six independently isolated _hex_ mutations conferred similar phenotypes of high-efficiency transformation for all single-site markers (18) and elevated spontaneous mutation frequency (22, 33). To determine whether one or more genes were affected by these mutations, we sought to assign the various mutant alleles to chromosomal restriction fragments of a particular size. It was possible to assay Hex⁻ transforming activity by a modification of a method used to transfer _hex_ alleles (33). A Hex⁺ recipient population is treated first with DNA carrying the Hex⁻ marker. The extent of Hex⁻ transformation is determined in a subsequent two-step treatment with UV-irradiated tester DNA carrying both an LE and an HE marker. The UV treatment decreases the efficiency of the LE marker (16, 24), so that in the absence of Hex⁻ transforming activity in the initial step the LE/HE ratio is ca. 0.01. The presence of Hex⁻ transforming activity is detected by ratios significantly higher than this, which, depending on the extent of the initial Hex⁻ transformation, can approach 1.0.

To localize the _hex_ alleles on restriction fragments, chromosomal DNA from _hex-1_ and _hex-4_ strains was treated with _Bam_HI, _Eco_RI, and _Hpa_II; DNA from the _hex-Rx_ strain was treated with the two latter enzymes. The cleaved DNA was fractionated by agarose gel electrophoresis, fragments corresponding to various size classes were extracted from segments of the gel, and the extracted DNA was tested for its ability to transfer the _hex_ mutation to a Hex⁺ recipient. The results are shown in Table 1. The _hex-4_ mutation was located on a _Bam_HI fragment 6 to 8 kb in size, whereas _hex-1_ was found on a much larger _Bam_HI fragment. Conversely, with _Eco_RI the peak of _hex-4_ activity migrated more slowly than that of _hex-1_. With _Hpa_II, _hex-1_ appeared on a fragment 6 to 8 kb in size, whereas no activity was observed for _hex-4_ in any fraction, including those smaller than shown in Table 1. This absence of activity indicates that _Hpa_II cuts very close to the _hex-4_ mutation (23). The _hex-Rx_ allele behaved rather similarly to _hex-1_, inasmuch as the slight differences observed may be due to experimental variation in migration of borderline fragments.

It can be concluded that the _hex_ system involves at least two genes that are not closely linked in the genome, neither of which have vital functions. One gene, represented by

hex-4, will be called _hexA_; the other, represented by _hex-1_ and possibly _hex-Rx_, will be called _hexB_.

Cloning of the _hexA4_ gene. The gene with the _hex-4_ mutation was cloned in the vector plasmid pLS101 (1), which carries _tet_ and _malM_ gene markers. The 6- to 8-kb fraction of _Bam_HI-cut _hex-4_ chromosomal DNA was ligated with _Bcl_I-cut pLS101 and countercut with _Bcl_I to destroy reconstituted vector, and the ligation mixture was used to transform strain 767 to Mal⁺. Crude plasmid extracts were prepared from 15 Mal⁺ transformants and analyzed by gel electrophoresis. They all contained recombinant plasmids with inserts in the range of 6 to 8 kb. When the plasmids were eluted from the gel and tested for Hex⁻ transforming activity, two of them showed activity. One of these was taken as the _hex-4_ recombinant plasmid and called pLS120. The rather high yield of _hex-4_ recombinant plasmids (2 of 15) may be attributable to countercutting with _Bcl_I, which would have destroyed plasmids with inserts containing _Bcl_I sites. Plasmid pLS120 contains a 6.7-kb chromosomal insert that encompasses a functional _hexA_ gene, as shown below. Its restriction map is presented in Fig. 1.

Introduction of the _hexA⁺_ allele into pLS120. The wild-type allele was introduced into the _hexA4_-containing plasmid in place of the mutant allele by the process of chromosomal facilitation of plasmid transfer (26), in which chromosomal markers frequently enter the plasmid molecule during its establishment. The _hex⁺_ strain 193 was transformed with pLS120, and the plasmids from 19 Mal⁺ transformants were tested for Hex⁻ transforming activity. Of the 19, 2 carried the original _hexA4_ allele; their plasmids gave LE/HE ratios in the test of 0.42 and 0.50. However, 17 of the 19 appeared to have plasmids that incorporated the _hexA⁺_ allele from the chromosome; they gave LE/HE ratios of 0.03 to 0.07 in the test. Such high frequencies of chromosomal marker incorporation during plasmid transfer (i.e., 17 of 19 or 89%) were previously found for _malM_ markers that were centrally located in the plasmid insert homologous to the chromosome (26). One of the _hexA⁺_ plasmids was designated pLS121. As shown below, this plasmid confers a Hex⁺ phenotype to _hex-4_ but not to _hex-1_ cells.

Expression of cloned _hexA_ alleles. The ability of the _hexA_ genes in plasmids pLS120 and pLS121 to complement various _hex_ mutations in the chromosome is shown in Table 2. In all cases (except for _hex-3_, in which a Tcʳ-conferring plasmid was used) the recipient strain contained a large _mal_ deletion in its chromosome, and transfer of the plasmid was accom-

TABLE 1. Hex⁻ transforming activity in restriction fragments of chromosomal DNA containing various _hex_ mutations

DNA fragment size[a] (kb)	Ratio of LE/HE transformants in test[b] with DNA cut by:							
	_Bam_HI		_Eco_RI			_Hpa_II		
	hex-1	hex-4	hex-Rx	hex-1	hex-4	hex-Rx	hex-1	hex-4
12–30	**0.15**	0.01	**0.20**	**0.18**	**0.14**	0.06	0.03	0.01
8–12	0.09	0.01	**0.12**	**0.41**	0.07	0.09	0.04	0.01
6–8	0.03	**0.17**	0.04	0.05	0.03	**0.16**	**0.37**	0.01
4.5–6	0.01	0.01	0.03	0.03	0.01	**0.15**	0.04	0.01
3.5–4.5	0.02	0.04	0.01	NT[c]	0.03	0.03	0.04	0.01

[a] _hex⁻_ DNA was prepared from _S. pneumoniae_ strains 767 (_hex-1_), 782 (_hex-4_), and 777 (_hex-Rx_) and digested with the indicated restriction enzymes. The DNA fragments were separated by electrophoresis in agarose and extracted from the gel.

[b] _hex⁺_ cells of strain 175 were first treated with _hex⁻_ DNA fragments and then transformed with UV-irradiated _nov_ (LE) DNA; the _nov_ population was grown and tested with UV-irradiated _amiA9_ (LE), _sul-d_ (HE) DNA. Boldface values were significantly higher than the background.
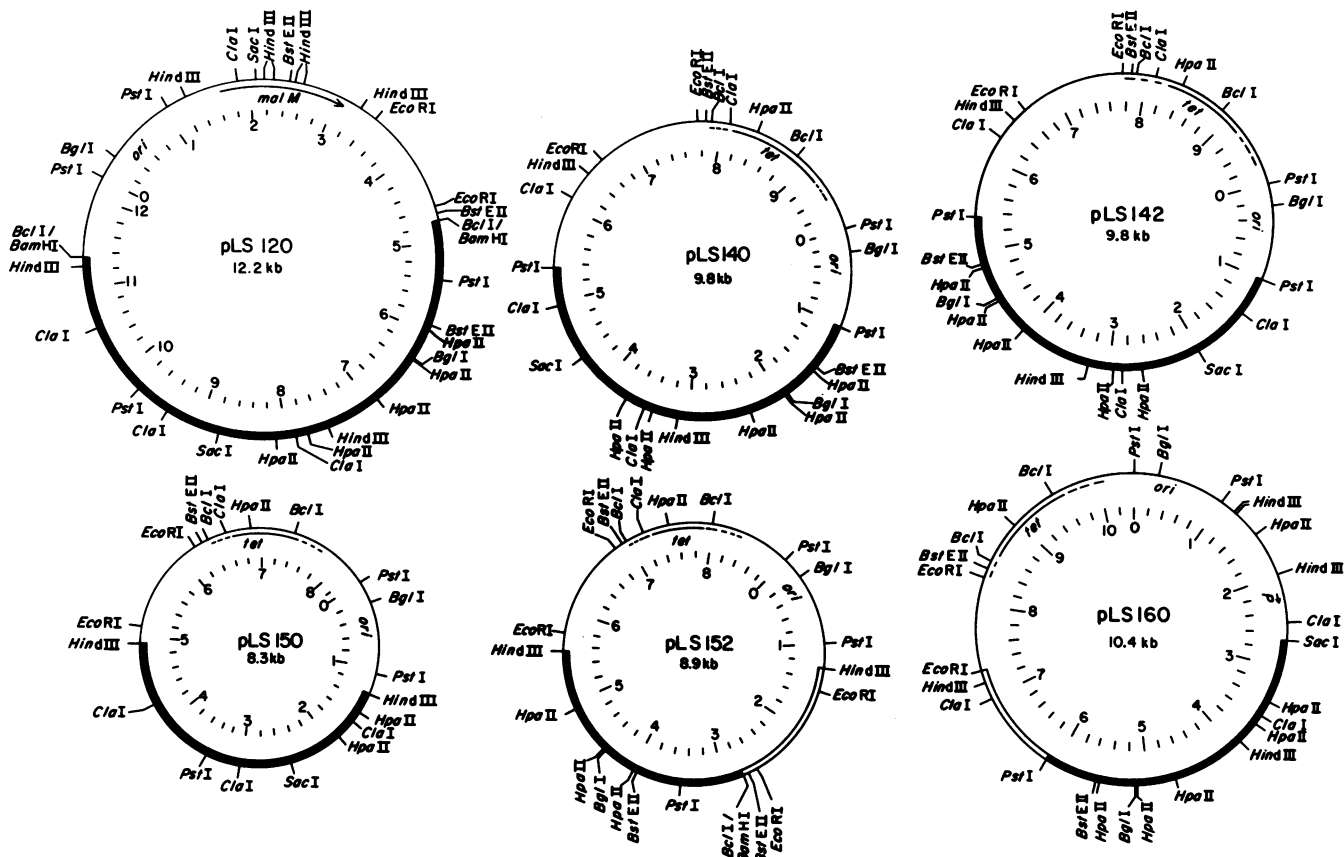
[c] NT, Not tested.

FIG. 1. Maps of recombinant plasmids used to localize the *hexA* gene. Thin line, vector portion; solid bar, chromosomal insert; open bar, subcloned vector segment. Vector genes: *malM*, amylomaltase; *tet*, tetracycline resistance; *ori*, presumed origin of replication; *P*, promoter for *malM* transcription. Arrows indicate the direction of transcription. All plasmids shown except pLS150 and pLS152 expressed *hexA4* gene function.

plished by selecting for Mal⁺ clones. Individual clones were tested for their Hex phenotype by transformation with UV-irradiated DNA containing *nov* (LE) and *str* (HE) markers. The chromosomal mutant strains gave LE/HE ratios of ca. 1.0, typical of the Hex⁻ phenotype. Strains with the Hex⁺ phenotype typically gave ratios of ca. 0.003. When the *hexA4* plasmid pLS120 was introduced into a *hexA4* recipi-

TABLE 2. Complementation of *hex* alleles

Recipient strain	Recipient chromosome allele	Donor plasmid	Donor plasmid allele	Total no. of clones tested	No. of clones among plamid-containing transformants tested showing phenotype[a]:		
					Hex⁻	Hex⁺	Partial Hex⁺
782	*hexA4*	pLS120	*hexA4*	9	0	0	9[b]
782	*hexA4*	pLS121	*hexA⁺*	36	0	4	32[b]
793	*hex-6*	pLS121	*hexA⁺*	15	13	2	0
794	*hex-7*	pLS121	*hexA⁺*	19	0	2	17[c]
767	*hex-1*	pLS121	*hexA⁺*	22	22	0	0
777	*hex-Rx*	pLS121	*hexA⁺*	19	19	0	0
590	*hex-3*	pLS141	*hexA⁺*	18	18	0	0

[a] Phenotype in terms of LE/HE (Nov^r/Str^r) range with UV-irradiated donor DNA: Hex⁻, 0.8 to 1.0; Hex⁺, 0.003 to 0.008; partial Hex⁺ as indicated below.

[b] LE/HE range, 0.2 to 0.3.

[c] LE/HE range, 0.5 to 0.7.

ent, however, none of the clones were completely Hex⁻. Rather, they were all partially Hex⁺, with LE/HE ratios in the range of 0.2 to 0.3. This weak Hex⁺ phenotype may reflect the multicopy nature of the plasmid. Estimates of copy number for similar plasmids range from 10 to 30 (25). Presumably, *hexA4* is a leaky mutation, and its residual activity becomes apparent as a result of increased gene dosage with the multicopy plasmid. If this is correct, then *hexA4* makes a poorly functional product, the activity of which is detectable only when it is made in large amounts. So the function of the *hexA* product in the mismatch repair system would appear to depend on its concentration in the cell.

The ability of the *hexA⁺* allele carried by pLS121 to complement the *hexA4* allele in the recipient chromosome to restore the full Hex⁺ phenotype is evident from the second line of Table 2. Of the 36 tested Mal⁺ transformants, 4 were Hex⁺. The other 32, that is 88%, apparently resulted from chromosomal facilitation in which the plasmid took up the *hexA4* allele from the chromosome during its establishment. They showed the partial Hex⁺ phenotype conferred by the multicopy *hexA4* plasmid. Plasmids were isolated from eight of these clones, and they all exhibited Hex⁻ transforming activity. The high levels observed for recipient marker introduction by chromosomal facilitation suggest that the *hexA4* mutation is located at least 1.5 kb from the vector border. Previous studies of *mal* markers in pLS70 showed that markers at this distance were introduced at frequencies

as high as 86%, whereas those closer to the border entered less frequently (26).

Recipients with hex-6 in their chromosome were also complemented by the hexA⁺ plasmid (Table 2). In this case 2 of 15 transformants tested were fully Hex⁺, which means that hex-6 is an allele of the hexA gene. The other 13, or 87%, which resulted from introduction of the chromosomal allele into the plasmid, were completely Hex⁻. It thus appears that the hexA6 mutation is not leaky like hexA4. The hex-7 recipient behaved similarly to hexA4, except that it gave a partial Hex⁺ phenotype closer to Hex⁻, so the hexA7 mutation seems to be less leaky than hexA4. None of the other mutations, hex-1, hex-Rx, or hex-3, were complemented by a hexA⁺ plasmid. They must be alleles of one or more hex genes other than hexA. This is consistent with the results of the analysis of restriction fragments.

Localization of the hexA gene by subcloning. The 4.4-kb PstI fragment contained entirely within the hexA4-containing chromosomal segment of pLS120 was inserted in both directions into the PstI cloning site of the vector pMV158 (32) to give pLS140 and pLS142 (Fig. 1). These plasmids also conferred the partial Hex⁺ phenotype of multicopy hexA4. When pLS140 was transferred to the hexA⁺ strain 175, it picked up the hexA⁺ allele by chromosomal facilitation to give pLS141, which conferred the full Hex⁺ phenotype. Plasmid pLS160 was constructed by inserting the 4.7-kb SacI-EcoRI fragment of pLS142 into the vector pLS69 from which a SacI-EcoRI segment was removed. Cells containing pLS160 also showed the partial Hex⁺ phenotype indicative of a functional hexA4 gene in the plasmid.

Several subcloned fragments did not give expression of the hexA4 allele. Plasmid pLS139 (data not shown) was obtained by removing the ClaI segments at 3.5 to 6.1 kb from pLS140 (Fig. 1). Plasmids pLS150 and pLS152 were formed by inserting HindIII fragments from pLS120, positioned at 7.5 to 11.2 kb and 3.3 to 7.5 kb on the map shown in Fig. 1, respectively, into the HindIII cloning site of the pLS101 vector. None of these plasmids conferred the partial Hex⁺ phenotype of cells with a functional hexA4 plasmid. Thus, the hexA gene lies entirely within the 3.4-kb segment bordered by the SacI and PstI sites, and it includes the ClaI and HindIII sites located within that segment.

Identification of hexA protein in minicells. Plasmids pLS140, pLS141, pLS142, and pLS160 were transferred to the minicell-producing strain of B. subtilis. Proteins produced by minicells containing these plasmids were labeled with [³⁵S]methionine and analyzed by electrophoresis in polyacrylamide gradient gels in the presence of sodium dodecyl sulfate. The pattern of labeled proteins resulting from the hexA-carrying plasmids was compared with that obtained from minicells containing pLS1 and pLS69 (Fig. 2). Plasmid pLS1 contains all but a 1.1-kb EcoRI fragment of the vector pMV158; pLS69 contains all of pMV158 and, in addition, a pneumococcal mal insert, which encodes the amylomaltase polypeptide (32). All of the plasmids gave labeled proteins with apparent M_rs of 39,000, 59,000, 68,000, and 91,000, which are presumably either vector products (10) or minicell host proteins for which mRNA was stabilized by the presence of the plasmid; they were absent from labeled minicells that contain no plasmid (10). These bands were all seen with the vector pLS1 (Fig. 2, lane 2). Minicells containing pLS69 also produced an intensely labeled amylomaltase band at the position of M_r 70,000 (Fig. 2, lane 5). The amylomaltase polypeptide migrates more slowly than expected from its M_r of 58,000 (22).

Only one new polypeptide band was seen in minicells with plasmids carrying the hexA gene (Fig. 2, lanes 1, 3, 4, and 6). This band corresponds to an M_r of 86,000. Its intensity varied in the different minicell preparations, but it was present at the same position with both hexA⁺ (Fig. 2, lane 1) and hexA4 plasmids. Inasmuch as the hexA gene lies within the 3.4-kb segment from SacI to PstI in pLS160 and is interrupted by the HindIII site in that segment, the M_r-86,000 protein, which requires 2.4 kb of coding capacity, is most likely the product of the hexA gene. This conclusion is consistent with observations made above that the hexA4 mutation is located near a HpaII site and at least 1.5 kb from the vector-insert border in pLS120.

DISCUSSION

Our restriction fragment analysis of hex⁻ transforming activity indicated that at least two genes function in the mismatch repair system. This conclusion was corroborated by the cloning of one of the genes and its demonstrated complementation of one set but not another set of hex mutations. Using a different approach, Claverys et al. (4) recently presented evidence also showing more than one gene in the system. By insertional mutagenesis with a plasmid from Escherichia coli, they obtained hex mutants, and they were able to clone a small portion of one gene in E. coli to use as a probe for hybridization analysis. Southern blotting with chromosomal DNA gave a partial restriction map for that gene, which they called hexA. Their map corresponds to our map of the gene in pLS120 from the PstI site at 5.4 kb to the HindIII site at 11.2 kb, which we also called hexA. Some of their insertion mutations did not alter the BglI pattern for hexA and were thus taken to have occurred in another hex locus.
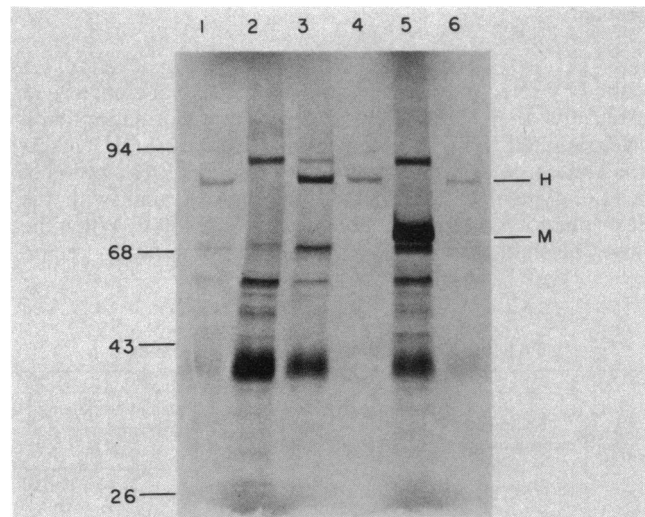

FIG. 2. Synthesis of hexA protein product in minicells of B. subtilis. Minicells of strain CU403 carrying various plasmids were labeled with [³⁵S]methionine. Extracts containing ca. 5 μg of protein were applied to polyacrylamide gels, which after electrophoresis in the presence of sodium dodecyl sulfate were dried and subjected to autoradiography. Plasmids in minicells: pLS141 (hexA⁺) (lane 1); pLS1 (vector) (lane 2); pLS140 (hexA4) (lane 3); pLS142 (hexA4) (lane 4); pLS69 (vector with malM) (lane 5); pLS160 (hexA4) (lane 6). At the left is shown the migration of standard proteins, rabbit phosphorylase, bovine albumin, chicken ovalbumin, and bovine chymotrypsinogen, with M_rs (×10³) indicated. At the right are shown the positions of amylomaltase (M) and hexA protein (H).

The finding of two unlinked genes in the Hex system implies the existence of two biochemical functions; one could be recognition of the mismatch and the other could be excision of the target strand segment. The cloning of *hexA* and, eventually, of *hexB* should help determine these functions. The mutant *hex* alleles investigated here were produced by mutagenesis with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (18) and, therefore, probably resulted from base-change mutations. It is not surprising, then, that *hexA4* and *hexA7* appear to be leaky mutations. The partial expression of these alleles only in multicopy dose shows that higher concentrations of a *hexA* product can overcome its qualitative defect. This finding restricts the possible modes of interaction between the *hexA* and *hexB* components of the system.

A number of prior observations must be included in a model for Hex action. The extent of the excluded donor strand corresponds to the length that would normally be integrated and includes material on both sides of the LE marker (17). It has been shown that this donor material is physically degraded (28). Recognition of the target strand for elimination appears to depend on the presence of strand breaks (5, 14, 22). In transformation the initial integration product is a heteroduplex in which the donor strand segment is not yet covalently joined (7). Mismatch repair, in fact, was shown to occur very early in the integration process (12, 31). In DNA replication the nascent strand would be interrupted at the ends of Okazaki pieces produced either by the process of lagging strand synthesis or by the removal of incorporated uracil residues (34). Ligation of the target strand before mismatch recognition in either transformation or replication may limit the amount of correction. Enhancement of Hex system action by UV irradiation of donor DNA (16, 24) presumably results from the introduction of new breaks by excision repair of the UV lesions.

A tentative model for Hex mismatch correction is shown in Fig. 3. Some features of the model were suggested by Guild and Shoemaker (14). The two *hex* genes are designated *hexX* and *hexY* because it is not yet possible to specify the biochemical functions of *hexA* and *hexB*. The *X* product would monitor DNA, perhaps after entry at the 5' end of the interrupted segment. It may remain anchored at this point and reel in the DNA (14), or it may move along the DNA (Fig. 3). When it encounters a recognizable mismatch, a conformational change is triggered in the *X* protein. It continues to move in the same direction until it encounters the 3' end of the segment. At that point the *Y* product interacts with the complex of *X* and DNA, and it digests the interrupted segment in the reverse direction. A defective *X* product could fail to enter the DNA or fail to recognize the mismatch when passing over it. Increasing its amount would increase the number of passes and allow limited Hex action. If the *Y* product were defective in its association with the triggered *X*-DNA complex, increasing its amount would also partially restore, by mass action, Hex system activity. On completion of strand segment excision, the DNA would be restored by repair replication of the gap.

Mismatch repair has been observed with heteroduplex lambda phage DNA in *E. coli* (30, 36). In this species the repair has also been implicated in mutation avoidance, since it is blocked by mutations in the genes *mutL* and *mutH*, which confer a mutator phenotype. These genes may be analogous to *hexA* and *hexB* in *S. pneumoniae*. Mutations in the *dam* gene, which methylates GATC sites in the DNA of *E. coli* (19), confer a partial mutator phenotype that is ca. 10% of the level of *mutL* and *mutH* (13, 27), and it has been
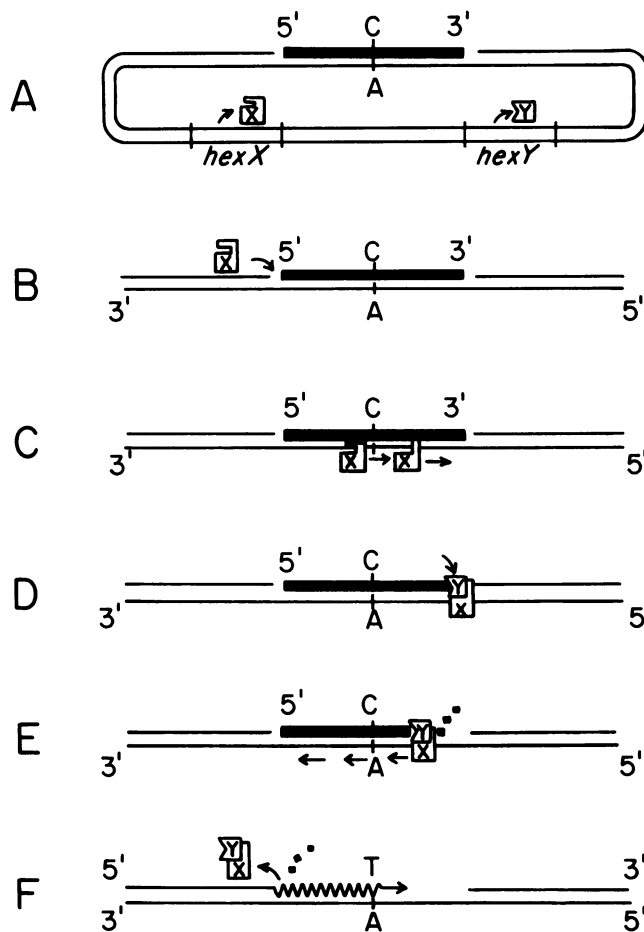

FIG. 3. Model for *hex* mismatch repair. (A) Integration product with cytosine-adenine mismatch before covalent joining of donor segment. (B) Entry of *hex* system monitor at the 5' break. (C) Recognition of mismatch triggers monitor. (D) Monitor halts at 3' break and binds exonuclease. (E) Exonuclease acts in reverse direction. (F) Completion of *hex* action and replication repair of gap. Thin line, chromosomal DNA; solid bar, donor DNA; sawtooth line, repair replication on chromosomal template. *X* and *Y*, monitor and exonuclease, are the hypothetical products of *hexX* and *hexY* genes.

proposed that the absence of methylation normally directs repair to the unmethylated strand (13, 30). However, methylation-related repair might depend for strand targeting on strand breaks produced at unmethylated sites in *E. coli* DNA (22). No methylation is implicated in mismatch repair in *S. pneumoniae*. In transformation both strands of the heteroduplex represent mature DNA and would be fully methylated, so methylation deficiency could not serve to target donor DNA. Accordingly, a unifying perspective would predict that heteroduplex mismatch repair in general depends on strand interruptions in the targeted DNA.

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