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In bacteria, double-strand DNA break (DSB) repair involves an exonuclease/helicase (exo/hel) and a short regulatory DNA sequence (Chi) that attenuates exonuclease activity and stimulates DNA repair. Despite their key role in cell survival, these DSB repair components show surprisingly little conservation. The best-studied exo/hel and recBCD of Escherichia coli is composed of three subunits. In contrast, RexAB of Lactococcus lactis and exo/hel enzymes of other low-guanine-plus-cytosine branch gram-positive bacteria contain two subunits. We report that RexAB functions via a novel mechanism compared to that of the RecBCD model. Two potential nuclease motifs are present in RexAB compared with a single nuclease in RecBCD. Site-specific mutagenesis of the RexA nuclease motif abolished all nucleolytic activity. In contrast, the RexB nuclease motif mutants displayed strongly reduced nucleolytic activity but maintained Chi recognition and had a Chi-stimulated hyper-recombination phenotype. The distinct phenotypes resulting from RexA or RexB nuclease inactivation lead us to suggest that each of the identified active nuclease sites in RexAB is involved in the degradation of one DNA strand. In RecBCD, the single RecB nuclease degrades both DNA strands and is presumably positioned by RecD. The presence of two nuclease sites would suggest that this RecD function is dispensable in RexAB.

In Vivo Evidence for Two Active Nuclease Motifs in the Double-Strand Break Repair Enzyme RexAB of Lactococcus lactis

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In bacteria, double-stranded DNA breaks (DSB) are frequent events that may be provoked, for example, by pauses in the replication fork (36, 43). Such genomic disruptions are lethal in the absence of DNA repair. In Escherichia coli DSB repair requires the activity of a large enzyme complex, known as RecBCD, that has ATP-dependent helicase and exonuclease activities (see reference 32 for a review). The enzyme degrades both strands, starting from the DNA break until it reaches an octanucleotide sequence, known as Chi, that attenuates degradation and stimulates recombination (44, 46). The enzyme exhibits helicase activity and residual exonuclease activity with an altered polarity after Chi (4, 16); the remaining activity provides a single-stranded DNA substrate for recombinational enzymes to mediate repair.

Organization of the three-subunit exonuclease/helicase (exo/hel) RecBCD. Structure-functional studies of RecBCD have revealed some of the roles of each subunit. RecB seems to possess two key activities of the enzyme. The N-terminal 929 amino acids (out of 1,180 total) have confirmed ATPase and helicase activities (13, 54); this region is similar to that of UvrD helicase. RecBCD helicase activity was recently proposed to function via a mechanism similar to that determined for UvrD (6). Nuclease activity was recently localized to the C-terminal 251 amino acids of RecB and is associated with the presence of a conserved motif, G-i-D-x(12)-D-Y-K-t-d (amino acids in small letters show less conservation) (51, 53, 54). This motif is present in numerous bacterial and eukaryotic enzymes (5). RecBCD was shown to have a single nuclease catalytic center in RecB that works on both DNA strands (51). Little is known about the roles of RecC, except that it appears to greatly enhance activities and processivity of RecB (11, 38); mutations in the RecC gene can also result in loss or modification of Chi recognition, as do mutations in genes of all subunits (1). RecD is an ATPase with similarity to a helicase involved in conjugal transfer of an enteric bacterial plasmid; its homologues seem to be broadly distributed in bacteria (determined by BLAST comparisons; http://www.ncbi.nlm.nih.gov/BLAST/). As part of RecBCD, RecD appears to regulate exonuclease activity. Recent data suggest that RecD maintains RecBCD incompetent for homologous recombination prior to Chi; at Chi, RecD is suggested to undergo a conformational change that attenuates exonuclease activity and stimulates recombination (2, 3, 12, 33, 48). A swing model was proposed in which RecD assures proximity of the RecB nuclease with both DNA strands prior to Chi and a repositioning of the nuclease after Chi (51, 54).

Organization of the two-subunit exo/hel enzymes. To date, models of exo/hel activities are based on those of RecBCD. Numerous RecBCD homologues have been identified in gram-negative enterobacteria and in the high-guanine-plus-cytosine-content mycobacteria. However, the functional RecBCD analogue in the low-guanine-plus-cytosine-content branch of gram-positive bacteria is structurally distinct from...
RecBCD. Using Lactococcus lactis as a model, a two-subunit enzyme called RexAB (comprising 1,073- and 1,099-amino-acid subunits, respectively) is necessary and sufficient to confer exo/hel activity and interacts with the L. lactis Chi site (22). RexAB bears homologues in at least six other gram-positive low-guanine-plus-cytosine-content bacteria as well as in the gram-negative bacterium Porphyromonas gingivalis (determined by BLAST comparison; http://www.ncbi.nlm.nih.gov/BLAST/). As studied in L. lactis or in Bacillus subtilis (AddAB), the two-subunit exo/hel enzymes display biological and/or biochemical activities equivalent to those of RecBCD (i.e., ATP-dependent exonuclease, helicase, exonuclease blocking at Chi, and Chi-stimulated recombination; 8, 9, 10, 22, 23, 28, 30, 31). RexA and its analogues in other gram-positive bacteria have homology with PcrA helicase (determined by BLAST comparison; http://www.ncbi.nlm.nih.gov/BLAST/), whose mechanism has been deduced from structural determinations (49). In addition, the nuclease motif described above for RecB is conserved in L. lactis RexA and in all known two-subunit exo/hel enzymes (5, 28, 53). This similarity has led to the hypothesis that all two- and three-subunit exo/hel enzymes function via similar mechanisms.

However, several lines of evidence argue against a common mechanism of DSB repair. The exo/hel-Chi couples show replication and their genome distribution properties differ for each organism (7, 8, 10, 21, 45). This suggests that the Chi features of high frequency and overrepresentation on the genome had to arise independently in each case (7, 21). In addition, although the enzymes have equivalent biological functions, their structures are strikingly different. Similarities between two- and three-subunit enzymes are restricted to ATPase, helicase, and nuclease motifs present, for example, in RecB (of RecBCD) and RexA (of RexAB); no similarity is detected in the other exo/hel subunits. For comparison, RecA proteins of Escherichia coli and L. lactis are 56% identical (19). Recent in vitro studies with the two-subunit AddAB exo/hel may suggest that its activities differ from those of RecBCD (9). Unlike RecBCD, where a Chi encounter affects the degradation pattern of both strands, attenuation at Chi of AddAB-mediated degradation seems to affect only the Chi-containing strand (9, 16); however, exo/hel activities in vitro are very sensitive to assay conditions, which could explain these observations. The above considerations raise the possibility that the two- and three-subunit exo/hel enzymes are programmed differently to carry out their functions.

We examined the divergence between exo/hel enzymes of different microorganisms. Analyses of the L. lactis RexAB enzyme reveal the presence of two potential nuclease activities on the enzyme, one on each subunit. Each nuclease motif was modifed by site-specific mutagenesis. The mutants show clear phenotypic differences in vivo, revealing that each nuclease motif has a key functional role in DNA degradation. Our results lead us to suggest that RexAB exo/hel has two distinct nuclease activities that may each degrade one double-stranded DNA (dsDNA) strand. Differences between the two- and three-subunit enzymes further suggest that the ubiquitous DSB repair strategy may undergo selective pressures that increase divergence.

MATERIALS AND METHODS


textual content
A. RexA nuclease motif

**FIG. 1.** Alignment of nuclease motifs present in each subunit of the two-subunit exo/hel enzymes. (A) RexA subunit homologue alignments are presented for the region surrounding the nuclease motif (corresponding to positions 1085 to 1164 of the 1,173-amino acid L. lactis RexA subunit). (B) RexB subunit homologies in the region surrounding the nuclease motif (corresponding to positions 871 to 949 of the 1,099-amino acid L. lactis RexB subunit). Highly conserved motifs are enclosed in a rectangle, and amino acids that are totally conserved are in bold. The black bar over amino acids in small letters show some variation; in RexB it is G-r-i-D-R-i-D-x(9–12)-v-D-Y-K-S-s. The striking similarities between these motifs lead us to ask whether RexAB may bear two active nuclease sites, one in each subunit.

**Detection of HMW.** High-molecular-weight linear plasmid multimer (HMW) accumulation was detected on whole-cell lysates after agarose gel electrophoresis (14). Plasmid DNA, labeled by chemiluminescence using the ECL system (Amersham), was used as a probe. Southern blot hybridization was performed as recommended by the kit supplier.

**Recombination test.** We used a previously described strategy to measure Chi-stimulated homologous recombination using short dsDNA substrates (15). The plasmid target (named pBla) is a pBR322 derivative with a 111-bp deletion in the β-lactamase gene (bla). The intact bla gene is restored via a double exchange event with a linear DNA fragment (see reference 22 for details of its construction). In brief, primers were designed to PCR amplify a flaing homology to ChiLl) was compared in this way. Although the reason why RexAB activates wild-type RecBCD exonuclease is unknown, it is possible that the electric shock induces the SOS response, which is known to diminish exonuclease activity (37). In the RexA subunit, a consensus is G-i-i-D-x(12)-D-Y-K-t-d (amino acids in small letters show some variation); in RexB it is G-i-i-D-R-i-D-x(9–12)-v-D-Y-K-S-s. The striking similarities between these motifs lead us to ask whether RexAB may bear two active nuclease sites, one in each subunit.

**Site-specific mutagenesis of putative nuclease motifs in RexA and RexB.** The rexAB operon (rexB is followed by rexA) was previously cloned on a low-copy-number plasmid and shown to fulfill the biological roles of RecBCD in an E. coli ΔrecBCD strain (22). The putative RexA nuclease motif (RexA\textsuperscript{Nuc}) was modified by a 3-amino-acid-deletion removing the tripeptide DYK in positions 1114 to 1116 (called RexA\textsuperscript{ΔDYK}) (Fig. 1). The putative RexB nuclease motif (RexB\textsuperscript{Nuc}) was modified by alteration of D\textsuperscript{910} to A (called RexB\textsuperscript{D910A}) or by a 3-amino-acid-deletion, removing the tripeptide DYK in positions 910 to 912 (called RexB\textsuperscript{ΔDYK}) (Fig. 1). In addition, a 293-amino-acid in-frame deletion of RexB that removed C-terminal amino acids 771 to 1063 (called RexBNuc) was modified by alteration of D\textsuperscript{910} to A (called RexBNuc\textsuperscript{D910A}) (Fig. 1). The cloned rexAB mutated genes gave rise to pRex plasmids bearing the name of the mutation and were established in an E. coli ΔrecBCD strain (37).

**RESULTS**

Conserved regions of two- versus three-subunit exo/hel enzymes. Alignments of several two-subunit exo/hel enzymes reveal that these enzymes are poorly homologous, even in closely related species. They bear very little homology with the three-subunit exo/hel. Nevertheless, a short nuclease motif was previously revealed in the AddA subunit of the B. subtilis AddAB enzyme and in the RecB subunit of RecBCD (28, 53). In RecBCD, this motif corresponds to the sole nuclease activity of the exo/hel enzymes (51). We found that this motif is actually present in both subunits of the two-subunit enzymes (Fig. 1). In the RexA subunit, a consensus is G-i-i-D-x(12)-D-Y-K-t-d (amino acids in small letters show some variation); in RexB it is G-i-i-D-R-i-D-x(9–12)-v-D-Y-K-S-s. The striking similarities between these motifs lead us to ask whether RexAB may bear two active nuclease sites, one in each subunit.

UV resistance conferred by the different pRexAB mutants was examined (Table 1). The ΔrecBCD strain shows greater UV resistance in the presence of pRexAB (22) or pRexAB\textsuperscript{D910A}. However, UV resistance of the ΔrecBCD strain was not at all or was only very slightly improved in the presence of pRexA\textsuperscript{ΔDYK}, pRexAB\textsuperscript{771–1063}, or pRexB\textsuperscript{ΔDYK} compared to that of the control strains. These results indicate that the introduced mutations affect the DNA repair capacity of RexAB exo/hel.
TABLE 1. Capacity of RexAB nuclease mutants to complement a recBCD strain

<table>
<thead>
<tr>
<th>E. coli strain (plasmid)</th>
<th>Relative survival after UV irradiation&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Relative PFU of T4g&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>recBCD (pRexAB)</td>
<td>1</td>
<td>0.000062</td>
</tr>
<tr>
<td>recBCD (pRexAB&lt;sup&gt;D910A&lt;/sup&gt;)</td>
<td>0.4</td>
<td>0.0014</td>
</tr>
<tr>
<td>recBCD (pRexAB&lt;sup&gt;ADYK&lt;/sup&gt;)</td>
<td>0.09</td>
<td>0.015</td>
</tr>
<tr>
<td>recBCD (pRexAB&lt;sup&gt;5771-1063&lt;/sup&gt;)</td>
<td>0.05</td>
<td>0.17</td>
</tr>
<tr>
<td>recBCD (pRexA&lt;sup&gt;ADYKB&lt;/sup&gt;)</td>
<td>0.01</td>
<td>0.2</td>
</tr>
<tr>
<td>recBCD (pGB2)</td>
<td>0.01</td>
<td>1</td>
</tr>
</tbody>
</table>

<sup>a</sup> Cells were irradiated at 100 J/m². Note that survival of the wild-type strain (AB1157) was 4 × 10⁻⁴, while that of recBCD (pRexAB) was 3 × 10⁻⁵. Results are means of two experiments.

<sup>b</sup>The values given are relative to a plaque titer of ~4 × 10¹⁰ PFU/ml on the recBCD strain; AB1157 gave a relative plaque titer of 0.0001. Results are means of at least three experiments.

<sup>c</sup>pGB2 is the vector used to clone recAB genes. For both experiments, determination values were within twofold of presented values.

Changes in RexA or RexB nuclease motifs affect RexAB exonuclease activities. Phage T4g2 is susceptible to exo/hel degradation. As nuclease activity inhibits plaque-forming ability, plaque formation would reflect diminished nuclease activity in the recAB mutants (Table 1). The recBCD mutant containing pRexAB essentially inhibits phage multiplication, whereas this strain lacking recAB genes is totally permissive (22). The strain containing pRexA<sup>ADYKB</sup> allowed efficient phage multiplication. Thus, inactivation of the nuclease motif of RexA essentially abolishes all DNA degradation activity by RexAB.

For strains containing pRexAB<sup>D910A</sup> and pRexAB<sup>ADYK</sup>, T4g2 infectivity was increased 10- and 100-fold, respectively (Table 2), indicating that nuclease activity is significantly reduced in these strains. Note that nuclease inactivation may even be underestimated in this assay, as unwinding activity alone may have a modest inhibitory effect on T4g2 multiplication (40). This result shows that the RexB nuclease motif DYK is a functionally active component of the exo/hel enzyme. The strain containing pRexAB<sup>5771-1063</sup> is totally permissive for phage multiplication, indicating that a large deletion in RexB abolishes in vivo nuclease activity.

These data show unambiguously that both the DYK motif in RexB (positions 910 to 912) as well as that in RexA (positions 1114 to 1116) are involved in nuclease activities of RexAB. Thus, the RexAB enzyme appears to differ from RecBCD, in which a single nuclease locus is involved.

**RexB<sub>Nuc</sub> mutants recognize Chi.** We previously developed an in vivo test to detect Chi attenuation of exo/hel exonuclease activity by using an RC plasmid as substrate. A σ-formed replication intermediate of RC plasmids provides a dsDNA end as an entry point for exo/hel enzyme. If the RC plasmid contains a Chi site in the orientation recognized by exo/hel, degradation is attenuated and σ-form replication results in accumulation of HMW (14, 26). In the absence of Chi on the plasmid, HMW do not accumulate as long as exo/hel is active. However, if exo/hel is nuclease defective, any RC plasmid will accumulate HMW (e.g., RC plasmids accumulate large amounts of HMW in an E. coli recD mutant; unpublished data).

We examined the activity of rexAB mutants in the E. coli ΔrecBCD background on RC plasmids with or without an L. lactis Chi site (called Chi<sub>Ll</sub>) (Fig. 2). In the presence of pRexAB, HMW accumulation was observed only if Chi<sub>Ll</sub> was present on the RC plasmid. In the absence of any exo/hel enzyme, HMW accumulated regardless of the presence of Chi<sub>Ll</sub> on the plasmid (Fig. 2) (22). The strain containing pRexA<sup>ADYKB</sup> behaved like the exo/hel-deficient strain. These results are consistent with the nuclease-negative phenotype conferred by RexA<sup>ADYKB</sup> in the phage infection test. Other mutated rex4 alleles in which the nuclease motif was deleted gave rise to similar phenotypes (L. Rezaı¨ki and A. Gruss, unpublished observations). We also observed Chi<sub>Ll</sub>-independent HMW accumulation in the strain expressing RexAB<sup>5771-1063</sup>, confirming that nuclease activity is deficient in this enzyme.

The two rex<sub>B</sub><sup>Nuc</sup> mutants exhibit a markedly different phenotype (Fig. 2). As expected for a nuclease-defective enzyme, the presence of pRexAB<sup>D910A</sup> resulted in more HMW than did pRexAB. However, HMW accumulation remained Chi<sub>Ll</sub>-dependent. Greater amounts of HMW were also observed when pRexAB<sup>ADYK</sup> was present; a modest effect of Chi<sub>Ll</sub> in increasing accumulation was still observed. These results are consistent with our hypothesis that the RexB<sub>Nuc</sub> motif is necessary

TABLE 2. Recombination efficiency in the presence of RexAB nuclease mutants

<table>
<thead>
<tr>
<th>pRexAB derivatives in E. coli</th>
<th>Transformation efficiency with pACYC184 (1 μg)</th>
<th>No. of ampicillin-resistant transformants with&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Chi&lt;sub&gt;Ll&lt;/sub&gt; stimulation&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Chi&lt;sub&gt;Ll&lt;/sub&gt;&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Chi&lt;sub&gt;Ll&lt;/sub&gt;&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>pRexAB</td>
<td>2 × 10&lt;sup&gt;6&lt;/sup&gt;</td>
<td>10</td>
<td>199</td>
</tr>
<tr>
<td>pRexAB&lt;sup&gt;D910A&lt;/sup&gt;</td>
<td>2 × 10&lt;sup&gt;6&lt;/sup&gt;</td>
<td>482</td>
<td>3,012</td>
</tr>
<tr>
<td>pRexAB&lt;sup&gt;ADYK&lt;/sup&gt;</td>
<td>1 × 10&lt;sup&gt;6&lt;/sup&gt;</td>
<td>130</td>
<td>309</td>
</tr>
<tr>
<td>pRexAB&lt;sup&gt;5771-1063&lt;/sup&gt;</td>
<td>8 × 10&lt;sup&gt;3&lt;/sup&gt;</td>
<td>63</td>
<td>112</td>
</tr>
<tr>
<td>pRexA&lt;sup&gt;ADYKB&lt;/sup&gt;</td>
<td>5 × 10&lt;sup&gt;6&lt;/sup&gt;</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>pGB2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2 × 10&lt;sup&gt;6&lt;/sup&gt;</td>
<td>30</td>
<td>19</td>
</tr>
<tr>
<td>TGI-control</td>
<td>1.5 × 10&lt;sup&gt;7&lt;/sup&gt;</td>
<td>416</td>
<td>272</td>
</tr>
</tbody>
</table>

<sup>a</sup> Values represent the total number of transformants obtained in three transformation experiments. For each experiment 200 ng of the appropriate linear DNA was used.

<sup>b</sup> Chi<sub>Ll</sub> stimulation was determined as the ratio of ampicillin-resistant transformants obtained with linear DNA fragments containing Chi<sub>Ll</sub> compared to those with no Chi<sub>Ll</sub> (designated Chi<sub>Ll</sub><sup>c</sup>). To determine the capacity of each fragment to effect homologous recombination, we used conditions of TGI electroporation that abolish nuclease activity; the ratio obtained in TGI was used to correct for Chi activity ratios in the test strains (20) (see Materials and Methods).

<sup>c</sup> Chi<sub>Ll</sub> and Chi<sub>Ll</sub><sup>c</sup> correspond to the linear fragments used for gene conversion, which do and do not, respectively, contain the double Chi<sub>Ll</sub> sites flanking the region of bla homology.

<sup>d</sup>pGB2 was the vector used to clone rexA genes.
The presence of ChiLl stimulates homologous recombination frequencies like those of pRexAB, except that ChiLl recognizes the arrow tail. Wavy lines represent heterologous dsDNA tails. Double-cross-over homologous recombination is required to convert cells to being ampicillin resistant (bla\(^{-}\)). Hatched rectangles represent bla DNA outside the regions present on linear DNA. The figure is essentially the same as one published in reference 22, with permission from the National Academy of Sciences.

**FIG. 3.** Strategy to test ChiLl effect on RexAB mutant-mediated homologous recombination. The recombination target plasmid pΔBla bears an internal deletion of bla (Δbla). Linear transforming DNA contains an internal fragment of bla which spans the bla deletion (black rectangle) plus an additional 360 bp of flanking homology (dark grey rectangle) with pΔBla (called bla\(^{int}\)). Where present, double ChiLl sites on the linear fragments are oriented for recognition to enhance recombination and are represented by XX (RexAB recognizes the arrow tail). Wavy lines represent heterologous dsDNA tails. Double-cross-over homologous recombination is required to convert cells to being ampicillin resistant (bla\(^{-}\)).
approximately threefold Chi stimulation effect was observed. These results suggest that the prxAB<sup>D771–1063</sup> enzyme retains some Chi<sub>Ll</sub> recognition activity despite a large C-terminal RexB deletion.

Taken together, these results show that RexA and RexB subunits both contribute to the observed nuclease activity of RexAB. The RexA<sup>D771–1063</sup> mutant abolishes all detectable in vivo activity of the enzyme, including homologous recombination. The RexAB<sup>D910A</sup> and RexB<sup>D771–1063</sup> mutants reduce exonuclease activity but retain Chi<sub>Ll</sub> recognition and display a Chi<sub>Ll</sub>-stimulated hyperrecombination phenotype when using short dsDNA fragments as substrates.

**DISCUSSION**

RexAB exo/hel enzyme function involves two active nuclease sites. The *E. coli* RecBCD-Chi couple has served as the prototype for bacterial DSB repair. Indeed, exo/hel-Chi couples identified in other bacteria were confirmed to fulfill the biological or biochemical functions established in *E. coli* (9, 22, 42, 52). However, RecBCD and the *L. lactis* exo/hel enzyme, RexAB, differ operationally: RecBCD relies on a single nuclease to effect DNA degradation (51, 53). In contrast, we have shown that RexAB contains two nuclease motifs, one in each subunit, both of which are required for full nuclease activity. Inactivation of the RexA nuclease motif results in total loss of exo/hel functions in vivo, whereas inactivation of the RexB nuclease motif reduces degradation while retaining Chi activity. As these two nuclease motifs are present in all identified (or predicted) two-subunit exo/hel enzymes, we predict that these enzymes will have properties similar to those described here.

We propose a model for RexAB activity based on our in vivo results (Fig. 4A). The two major features of the model are the following. (i) RexA, like RecB, has helicase and nuclease activities and drives the enzyme. Unwinding of the double helix is assured by RexA, which has significant homology with PcrA helicases. The RexB subunit could enhance activities of RexA helicase, just as RecC appears to increase RecB processivity and activities (11, 38). (ii) The RexA nuclease cleaves just one strand, from the 3′ end, while the RexB nuclease is positioned to degrade from a 5′ end. This model is consistent with our results showing that RexB<sup>Nuc</sup> mutants degrade DNA but maintain Chi recognition and with recent in vitro studies reported for AddAB in which degradation is attenuated at Chi but only on the Chi-containing strand; degradation of the “bottom” strand continues after Chi (9). This model may be useful in understanding the absence of a third, RecD-like component in the two-subunit exo/hel enzymes. RecD is proposed to position the single nuclease of the RecBCD enzyme so that it degrades the two DNA strands (51); if, as proposed, the nuclease of RexAB each act on a DNA strand, this RecD function would not be needed. Alternatively, the two established nuclease motifs are both needed to degrade each strand. We consider this possibility unlikely, as our results show that the RexA nuclease is active even if RexB<sup>Nuc</sup> is mutated. In vitro analyses will confirm whether this model is valid.

The above model can explain simply the behavior of the RexB<sup>Nuc</sup> mutants (Fig. 4B). As mentioned above, we propose that RexA drives the enzyme. Our data suggest that inactiva-

**FIG. 4. Model for RexAB activity.** (A) Normal RexAB. RexAB advances from an end on its dsDNA linear substrate via the RexA-driven helicase. RexB is proposed here to increase processivity, as does RecC of RecBCD (38). The RexA nuclease motif degrades from the 3′ end until the exo/hel enzyme reaches a Chi site. The conformational change at Chi attenuates RexA nuclease activity and thereby stimulates homologous recombination. The RexB nuclease degrades the dsDNA substrate from the 5′ end (bottom strand). At Chi<sub>Ll</sub>, degradation is essentially unchanged or possibly enhanced, as reported, in vitro for the bottom strand after RecBCD encounters an *E. coli* Chi site (3).

(B) Mutant affected in the RexB nuclease motif. Activities are as in panel A, except that the RexB nuclease is inactive. As such, although the 3′ end is degraded by RexA nuclease, a 5′ end is generated that can act as a substrate in homologous recombination, as shown from previous in vitro and in vivo data (35, 39). This can explain elevated levels of recombination in the assay using linear DNA fragments lacking Chi<sub>Ll</sub> (Table 2). Upon a Chi<sub>Ll</sub> encounter, RexA nuclease is attenuated, thus making both DNA strands accessible for recombination. A and B refer to RexA and RexB subunits, respectively, ANuc and BNuc correspond to the nuclease domains surrounding the motifs presented in Fig. 1. An open mouth has an active nuclease, while a barred mouth represents an inactive nuclease. AHel corresponds to the RexA helicase domain (deduced from BLAST homologies with PcrA helicase).
tion of the RexB nuclease motif does not abolish other enzyme functions. As such, DNA strands would be unwound and the 3′ end degraded by RexA nuclease. The protruding 5′ end could act as a substrate for homologous recombination, as suggested by previous in vitro and in vivo data (35, 39), consistent with the hyperrecombination phenotype seen for the RexB^Nuc^ mutants in the absence of ChiL stimulation (Table 2). Upon ChiL, encounter, degradation from the 3′ end is inhibited and both strands are available for recombination.

In contrast to RexB^Nuc^ mutants, the RexA^ΔDYK^B mutant exhibited no in vivo biological activity and may thus correspond to a null mutant. Interestingly, in B. subtilis and in E. coli, mutations affecting nuclease motifs in RexA analogous AddA and RecB, respectively, retain helicase activities. For example, an AddAB mutant in which the C-terminal end of AddA is deleted lacks nuclease activity but retains some helicase proficiency in vivo and in vitro (28), and an E. coli RexB^{D1080A}CD gene mutation inactivates the RecB nuclease (53); in vivo, this enzyme is nonfunctional (2). It was reasoned that RexB^{D1080A}CD is unable to undergo a conformational change at Chi and thus remains locked in the “antirecombinase” position; this effect is alleviated by removing RecD (2, 12). Our preliminary results suggest that recombination is restored in a RexA^ΔDYK^ RexB^ΔDYK^ double mutant (data not shown), suggesting that the conformational change observed at ChiL might involve interactions between RexB and RexA nucleic domains. Applying the above reasoning, we speculate that RexA^ΔDYK^ could be locked in a nonrecombinogenic state that blocks the conformational change at ChiL needed to render it recombinogenic. The alternative hypotheses concerning the RexA^Nuc^ mutant will be examined by further genetic and biochemical tests.

**Why is exo/hel so poorly conserved?** The primordial need for an intact genome suggests that DNA genome repair mechanisms have been present early in evolution. Accordingly, generalized homologous recombination proteins such as RecA, SSB, and RecF are rather highly conserved (19) (comparisons analyzed using BLAST; http://www.ncbi.nlm.nih.gov/BLAST/).

It is surprising that components of an important survival function like DNA repair are so markedly divergent. The diversity in DSB repair enzymes may be related to genome plasticity: genome rearrangements are common events that may occur via intrachromosomal transposition, gene duplications and inversions, or entry of exogenous DNA. In L. lactis, there is a 4:1 orientation bias of Chi distribution with respect to the direction of DNA replication (exo/hel recognizes Chi in one orientation) (14,21,23,29,47). Inversion of a large DNA segment could considerably reduce the number of Chi sites available to stimulate repair if a replication fork break occurs in that region (36). Such rearrangements could impose selective pressure for exo/hel divergence. The constant need for the exo/hel enzyme to adapt to altered distributions of Chi on the genome (e.g., due to chromosomal inversions or mutations) could explain why these enzymes are so divergent, even in closely related species.

Is E. coli the right DSB repair model? The E. coli RecBCD exo/hel enzyme has been the paradigm for DSB repair over the last 30 years. But E. coli is a relatively young bacterium in terms of evolution; it seems to have evolved well after oxygen became abundant (27). In contrast, L. lactis, which thrives under low- or no-oxygen conditions, appears to have preceded E. coli evolutionarily (24, 27). To follow the evolution of the DSB repair system, we suggest that comparison with an older microorganism like L. lactis will be informative and may reveal minimum requirements for DSB repair.

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