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J Immunol 2006; 177:5386-5392; doi: 10.4049/jimmunol.177.8.5386
http://www.jimmunol.org/content/177/8/5386
Somatic Hypermutation and Class Switch Recombination in Msh6−/− Ung−/− Double-Knockout Mice

Hong Ming Shen,* Atsushi Tanaka,† Grazyna Bozek,* Dan Nicolae,‡ and Ursula Storb2*†

Somatic hypermutation (SHM) and class switch recombination (CSR) are initiated by activation-induced cytosine deaminase (AID). The uracil, and potentially neighboring bases, are processed by error-prone base excision repair and mismatch repair. Deficiencies in Ung, Msh2, or Msh6 affect SHM and CSR. To determine whether Msh2/Msh6 complexes which recognize single-base mismatches and loops were the only mismatch-recognition complexes required for SHM and CSR, we analyzed these processes in Msh6−/−Ung−/− mice. SHM and CSR were affected in the same degree and fashion as in Msh2−/−Ung−/− mice; mutations were mostly C,G transitions and CSR was greatly reduced, making Msh2/Msh6 contributions unlikely. Inactivating Ung alone reduced mutations from A and T, suggesting that, depending on the DNA sequence, varying proportions of A,T mutations arise by error-prone long-patch base excision repair. Further, in Msh6−/−Ung−/− mice the 5′ end and the 3′ region of Ig genes was spared from mutations as in wild-type mice, confirming that AID does not act in these regions. Finally, because in the absence of both Ung and Msh6, transition mutations from C and G likely are “footprints” of AID, the data show that the activity of AID is restricted drastically in vivo compared with AID in cell-free assays. The Journal of Immunology, 2006, 177: 5386–5392.

Received for publication May 23, 2006. Accepted for publication July 20, 2006.

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*This work was supported by National Institutes of Health Grants AI47380 and AR053130.

†Address correspondence and reprint requests to Dr. Ursula Storb, Department of Molecular Genetics and Cell Biology, University of Chicago, 920 East 58th Street, Chicago, IL 60637. E-mail address: stor@midway.uchicago.edu

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**Materials and Methods**

**Mice**

Ung<sup>−/−</sup> mice were a gift of D. Barnes and T. Lindahl (Imperial Cancer Research Fund, Clare Hall Laboratories, South Mimms, U.K.), and were further bred in our mouse facility on a C57BL/6 background. A male Msh6<sup>−/−</sup> mouse was obtained from the Mouse Repository at the National Cancer Institute (Rockville, MD); the latter mice were originally donated by W. Edelmann (Albert Einstein College of Medicine, Bronx, NY). Both Ung<sup>−/−</sup> and Msh6<sup>−/−</sup> mice had a C57BL/6 × 129 background. The mice were maintained according to National Institutes of Health instructions. (Ung<sup>−/−</sup>Msh6<sup>−/−</sup>)F<sub>1</sub> mice were acquired by mating Ung<sup>−/−</sup> mice with the Msh6<sup>−/−</sup> mouse. Offspring were further crossed twice to generate Ung<sup>−/−</sup>Msh6<sup>−/−</sup>, Ung<sup>−/−</sup>Msh6<sup>−/-</sup>, and Ung<sup>−/−</sup>Msh6<sup>−/-</sup> mice. The experiments with mice have been reviewed and approved by the University of Chicago Institutional Animal Care and Use Committee.

**Cells and DNAs**

Mice (all mice were 2 mo old, except for the Ung<sup>−/−</sup>Msh6<sup>-/-</sup> mice which were 3 mo old; see Table I) were immunized i.p. with 10<sup>8</sup> sheep RBC (SRBC; MP Biomedicals) on day 1, boosted with the same amount of SRBC on day 8, and sacrificed on day 11. Sera were saved for ELISA analysis, while spleens were removed and minced in RPMI 1640. RBC in the spleen were lysed and the remaining spleen cells were stained with fluorescein isothiocyanate-conjugated mAb to B220 (BD Biosciences) at the Immunology Core Facility (University of Chicago). The purified cells were lyzed in lysis buffer containing protease K (500 μg/ml), EDTA (50 mM, pH 8.0), SDS (1%), and Tris (50 mM, pH8.0) overnight at 37°C. DNAs extracted using phenol/chloroform.

**PCR, cloning and mutation analysis**

The PCR method for the H chain gene was described elsewhere (26, 27). Briefly, PFU turbo DNA polymerase (Invitrogen Life Technologies) was used during a touchdown PCR in the presence of a pair of primers which specifically annealed to the rearranged VJH558 region and the J<sub>H</sub>4-C<sub>H</sub> intron (27). Gel electrophoresis was performed to purify a 1.2-kb DNA fragment containing the JC intron of VDJH4-rearranged genes. The uracil DNA glycosylase-deficient mice underwent efficient somatic hypermutation indicated by the mutation frequencies in mutated DNA sequences (3.3 and 2.3/1000 bp, compared with 2.6 and 2.5/1000 bp in wild-type mice) (Table I). However, the overall mutation frequencies in mice with the Msh6<sup>-/-</sup> background and wild-type Ung were reduced (1.4, 1.5, 1.3, and 1.5/1000 bp in mutated DNA sequences) (Table I) and mutated sequences accumulated only few mutations (Fig. 1, pie charts). The unchanged mutation frequencies in Ung<sup>−/−</sup> mice and the reduced mutations in the second response of Msh6<sup>-/-</sup> mice agrees with previous findings in Ung<sup>−/−</sup> mice (5, 26), Msh6<sup>−/−</sup> mice (7, 9), and Msh2<sup>−/-</sup> mice (10), respectively. Thus, production of Msh6, as well as Msh2, which together are involved in mismatch repair of single base mismatches or loops, appears to increase the mutation frequency. Alternatively, Msh2/Msh6 may aid the survival of mutating B cells (10) (see Discussion). Interestingly, in Msh6<sup>−/−</sup>Ung<sup>-/-</sup> double-knockout mice the mutation frequencies in mutated sequences.

<table>
<thead>
<tr>
<th>Mouse Type</th>
<th>Age in Months&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Titer&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Mutations*</th>
<th>Mutated Clones</th>
<th>Bases&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Mutations/1,000 bp in Mutated Clones</th>
<th>Mutations/1,000 bp in Sequenced Clones</th>
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<tbody>
<tr>
<td>Wild 1</td>
<td>2</td>
<td>1:256</td>
<td>57</td>
<td>20</td>
<td>58,850</td>
<td>2.6</td>
<td>1.0</td>
</tr>
<tr>
<td>Wild 2</td>
<td>2</td>
<td>1:384</td>
<td>95</td>
<td>34</td>
<td>65,450</td>
<td>2.5</td>
<td>1.5</td>
</tr>
<tr>
<td>Ung&lt;sup&gt;-/−&lt;/sup&gt;Msh6&lt;sup&gt;-/−&lt;/sup&gt; 1</td>
<td>2</td>
<td>1:256</td>
<td>120</td>
<td>33</td>
<td>65,450</td>
<td>3.3</td>
<td>1.8</td>
</tr>
<tr>
<td>Ung&lt;sup&gt;-/−&lt;/sup&gt;Msh6&lt;sup&gt;-/−&lt;/sup&gt; 2</td>
<td>2</td>
<td>1:512</td>
<td>84</td>
<td>33</td>
<td>61,600</td>
<td>2.3</td>
<td>1.4</td>
</tr>
<tr>
<td>Ung&lt;sup&gt;-/−&lt;/sup&gt;Msh6&lt;sup&gt;-/-&lt;/sup&gt; 1</td>
<td>3</td>
<td>1:16</td>
<td>15</td>
<td>10</td>
<td>92,400</td>
<td>1.4</td>
<td>0.2</td>
</tr>
<tr>
<td>Ung&lt;sup&gt;-/−&lt;/sup&gt;Msh6&lt;sup&gt;-/-&lt;/sup&gt; 2</td>
<td>3</td>
<td>1:128</td>
<td>56</td>
<td>33</td>
<td>101,200</td>
<td>1.5</td>
<td>0.6</td>
</tr>
<tr>
<td>Ung&lt;sup&gt;-/−&lt;/sup&gt;Msh6&lt;sup&gt;-/-&lt;/sup&gt; 1</td>
<td>2</td>
<td>1:256</td>
<td>21</td>
<td>15</td>
<td>54,450</td>
<td>1.3</td>
<td>0.4</td>
</tr>
<tr>
<td>Ung&lt;sup&gt;-/−&lt;/sup&gt;Msh6&lt;sup&gt;-/-&lt;/sup&gt; 2</td>
<td>2</td>
<td>1:128</td>
<td>10</td>
<td>6</td>
<td>30,250</td>
<td>1.5</td>
<td>0.3</td>
</tr>
<tr>
<td>Ung&lt;sup&gt;-/−&lt;/sup&gt;Msh6&lt;sup&gt;-/-&lt;/sup&gt; 1</td>
<td>2</td>
<td>1:128</td>
<td>81</td>
<td>27</td>
<td>67,650</td>
<td>2.7</td>
<td>1.2</td>
</tr>
<tr>
<td>Ung&lt;sup&gt;-/−&lt;/sup&gt;Msh6&lt;sup&gt;-/-&lt;/sup&gt; 2</td>
<td>2</td>
<td>1:96</td>
<td>14</td>
<td>6</td>
<td>63,800</td>
<td>2.1</td>
<td>0.2</td>
</tr>
<tr>
<td>Ung&lt;sup&gt;-/−&lt;/sup&gt;Msh6&lt;sup&gt;-/-&lt;/sup&gt; 3</td>
<td>2</td>
<td>1:256</td>
<td>40</td>
<td>16</td>
<td>41,250</td>
<td>2.3</td>
<td>1.0</td>
</tr>
<tr>
<td>Ung&lt;sup&gt;-/−&lt;/sup&gt;Msh6&lt;sup&gt;-/-&lt;/sup&gt; 4</td>
<td>2</td>
<td>1:256</td>
<td>13</td>
<td>11</td>
<td>51,150</td>
<td>2.9</td>
<td>0.8</td>
</tr>
</tbody>
</table>

* Mice were immunized when they were 2 or 3 mo old and sacrificed 11 days later.

**PCR, cloning and mutation analysis**

The mutation frequencies are not changed in Ung<sup>−/−</sup> and Ung<sup>−/−</sup>Msh6<sup>-/-</sup> mice but reduced in Msh6<sup>-/-</sup> mice with wild-type Ung

We analyzed mutations in the H chain locus starting from the first nucleotide of the JC intron of VDJH4-rearranged genes. The uracil DNA glycosylase-deficient mice underwent efficient somatic hypermutation indicated by the mutation frequencies in mutated DNA sequences (3.3 and 2.3/1000 bp, compared with 2.6 and 2.5/1000 bp in wild-type mice) (Table I). However, the overall mutation frequencies in mice with the Msh6<sup>-/-</sup> background and wild-type Ung were reduced (1.4, 1.5, 1.3, and 1.5/1000 bp in mutated DNA sequences) (Table I) and mutated sequences accumulated only few mutations (Fig. 1, pie charts). The unchanged mutation frequencies in Ung<sup>−/−</sup> mice and the reduced mutations in the second response of Msh6<sup>-/-</sup> mice agrees with previous findings in Ung<sup>−/−</sup> mice (5, 26), Msh6<sup>−/−</sup> mice (7, 9), and Msh2<sup>−/-</sup> mice (10), respectively. Thus, production of Msh6, as well as Msh2, which together are involved in mismatch repair of single base mismatches or loops, appears to increase the mutation frequency. Alternatively, Msh2/Msh6 may aid the survival of mutating B cells (10) (see Discussion). Interestingly, in Msh6<sup>−/−</sup>Ung<sup>-/-</sup> double-knockout mice the mutation frequencies in mutated sequences.

**Statistical analysis**

Pairwise comparisons of Ig levels in different transgenic mice were performed using a rank-based nonparametric testing procedure. For each experiment, the Ig levels of the mice were ranked, and the sum of ranks for one of the strains was calculated across experiments. An empirical distribution of this statistic under the null hypothesis of no difference in Ig levels was constructed using simulations, and two-sided p values were calculated from this distribution. We performed 10,000 simulations, and each simulated rank was drawn by assuming that the Ig levels of the two strains of mice are independent identically distributed variables within each experiment. The statistically significant differences are indicated in Fig. 2.
were as in wild-type mice (2.7, 2.1, 2.3, and 2.9/1000 bp of mutated sequences) (Table I) and many mutated sequences accumulated large numbers of mutations (Fig. 1, pie charts) (see Discussion).

Ung−/− mice can mutate A and T, but at a reduced frequency
In Ung−/− mice, as described before (5, 26), most of the mutations from C and G were transitions. The Ung−/− and wild-type mice showed a similar ratio of mutations at A over those at T, however, the A,T mutation frequency was reduced in the Ung−/− mice (Fig. 1). The percentage of A,T mutations in the 1100 nt 3′ of JH4 was 34%, compared with 51% in the wild-type mice (Table II, no. 6), a reduction in Ung−/− by 33%. This is a similar reduction of A,T mutations as previously seen near JH4 in the same Ung−/− mice (5, 32) which had 43% A,T mutations, compared with 57% in wild-type mice (a reduction by 25%) (Table II, no. 4). Our findings here are also similar to the A,T mutations in the 1145 nt 3′ of JH4 in another study (26). There, wild-type mice had 47% A,T mutations, and Ung−/− mice only 36% (Table II, no. 3). The least reduction of A,T mutations by only 11% was seen in the most highly mutated region of a κ transgene (Table II, no. 1) (26). The highest reduction of A,T mutations (by 52%) was found toward the

Table II.  Ung is responsible for various proportions of mutations at A and T depending on the target sequence

<table>
<thead>
<tr>
<th>Sequence*</th>
<th>% Mutations from A and T</th>
<th>Mutation Frequency</th>
<th>Hot Spots/100 nt</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. 917-bp center k-TG</td>
<td>53/47</td>
<td>0.89 Ung−/−</td>
<td>Ung/Wt</td>
</tr>
<tr>
<td>2. 864-bp 5′ k-TG</td>
<td>52/43</td>
<td>0.83 Ung−/−</td>
<td>Ung/Wt</td>
</tr>
<tr>
<td>3. 1145-bp Jα4C-μ int</td>
<td>47/36</td>
<td>0.77 Ung−/−</td>
<td>Ung/Wt</td>
</tr>
<tr>
<td>4. Vκ 3′ flank</td>
<td>57/43</td>
<td>0.75 Ung−/−</td>
<td>Ung/Wt</td>
</tr>
<tr>
<td>5. 338-bp 5′ VJ λ1c</td>
<td>45/30</td>
<td>0.67 Ung−/−</td>
<td>Ung/Wt</td>
</tr>
<tr>
<td>6. 1100-bp Jα4C-μ int</td>
<td>51/34</td>
<td>0.67 Ung−/−</td>
<td>Ung/Wt</td>
</tr>
<tr>
<td>7. 1321-bp JC-int k-TG</td>
<td>65/31</td>
<td>0.48 Ung−/−</td>
<td>Ung/Wt</td>
</tr>
</tbody>
</table>

* k-TG, kappa transgene; int, intron.

b Only the mutable region starting at +100 nt is included.

c The only gene in this list whose expression depended on B cell selection.
3’ end of the JC intron of the same κ transgene (Table II, no. 7) (26). Because in this study (26), all regions were sequenced from PNA\(^{\text{high}}\) B cells of the same mice (Table II, nos. 1, 2, 3, 5, 7), but the proportion of A,T mutations in \(\text{Ung}^{+/-}\) vs wild-type mice vary from 0.48 to 0.89, the differences are unlikely due to immunization, but rather may depend on the primary sequence. Taken together, these findings suggest that Ung, and thus presumably BER, plays a role in A,T mutations (see Discussion).

Most mutations in Msh6\(^{-/-}\) and Ung\(^{-/-}\)Msh6\(^{-/-}\) mice are transitions from C and G

A previous study showed that C,G mutations were mainly transitions and A,T mutations were greatly diminished in Ung\(^{-/-}\)Msh2\(^{-/-}\) double-knockout mice (23). To further determine whether the Msh2/6 complex is essential and whether there is any contribution by the Msh2/Msh3 complex, or Msh2 alone, to generate transversions from C,G or mutations from A,T, we analyzed Ung\(^{-/-}\)Msh6\(^{-/-}\) mice along with Ung\(^{+/-}\)Msh6\(^{-/-}\) mice as controls (Fig. 1). Only 2% of the mutations in the Ung\(^{-/-}\)Msh6\(^{-/-}\) mice were at A or T, the rest were C,G mutations, all of them being C>T and G>A transitions. Thus, the findings in the Ung\(^{-/-}\)Msh6\(^{-/-}\) are very similar to the mutation profile in the Ung\(^{-/-}\)Msh2\(^{-/-}\) mice, indicating that the Msh2/6 complex, but not the Msh2/Msh3 complex, is required for generating high levels of A,T mutations. A less severe reduction of A,T mutations was seen in Msh6\(^{-/-}\) mice with wild-type Ung supporting the notion that some mutations at A and T can arise by Ung-dependent BER without the help of MMR (but see Discussion) (Fig. 1).

CSR is diminished in Ung\(^{-/-}\)Msh6\(^{-/-}\) mice

To ascertain whether CSR was impaired in the Ung\(^{-/-}\)Msh6\(^{-/-}\) mice, we conducted ELISA analyses of the sera of SRBC-immunized mice. Data in Fig. 2 are based on the pool of two sets of ELISA data from two groups of mice (each group has a wild-type, an Ung\(^{+/-}\)Msh6\(^{+/+}\) +/+ , an Ung\(^{+/-}\)Msh6\(^{-/-}\) +/- , and an Ung\(^{-/-}\)Msh6\(^{-/-}\) mouse). Compared with the wild-type mice, Ung\(^{-/-}\)Msh6\(^{-/-}\) mice had significantly lower concentrations of switched Igs (IgG1, IgG2a, IgG3 and IgA; \(p < 0.0002\)), except for IgG2b (\(p < 0.1376\)), indicating that CSR in the Ung\(^{-/-}\)Msh6\(^{-/-}\) mice was significantly impaired (Fig. 2). In Ung\(^{-/-}\)Msh6\(^{-/-}\) mice serum IgG and IgA were essentially absent. Compared with wild-type mice, in the Ung\(^{-/-}\)Msh6\(^{-/-}\) double-knockout mice the IgG1, 2a, 2b, 3, and IgA levels were 12–120 times lower (\(p < 0.0002\)) (Fig. 2), indicating that CSR is greatly diminished. CSR in the Ung\(^{+/-}\)Msh6\(^{-/-}\) mice, however, is not severely impaired, except for IgA (\(p < 0.0002\)). This differs from previous data that showed that both IgG1 and IgG3 in Msh6\(^{-/-}\) mouse sera were significantly reduced (7). The mice in that study were 3–6 mo old, our Msh6\(^{-/-}\)Ung\(^{-/-}\) mice were 3 mo old (Table I). Because at these ages residual maternal Abs are unlikely, perhaps unknown background genes are involved in the different findings. The higher IgM titers in Ung\(^{-/-}\)Msh6\(^{-/-}\), Ung\(^{-/-}\)Msh6\(^{-/-}\), and Ung\(^{-/-}\)Msh6\(^{-/-}\) mice were expected (Fig. 2) to compensate for the reduced or absent IgGs and IgA by overexpressing IgM. Interestingly, although CSR in the Ung\(^{-/-}\)Msh6\(^{+/+}\) was significantly reduced compared with wild-type mice, it was higher than in Ung\(^{-/-}\)Msh6\(^{-/-}\) mice (\(p < 0.0002\)) (Fig. 2), indicating that both BER and MMR are responsible for CSR and that their roles in CSR may be somewhat overlapping.

The S’ region of Ig genes is not accessible to AID

In a previous study, we showed that, as in wild-type mice, mutations in Ung\(^{-/-}\) mice start 100–200 bp downstream of the Ig promoter (26). This finding suggested that AID did not gain access to the very S’ end of Ig genes, because, if it did, in the absence of Ung one would have expected an AID “footprint” of C:G to T:A transitions (26). However, the possibility remained that C,G transition mutations were not seen because mismatch repair recognized potential AID-created uracils and resulted in faithful, error-free restitutions of cytosines. To address this question, we analyzed mutations in the S’ end of Igλ genes in the Ung\(^{-/-}\)Msh6\(^{-/-}\) mice. Of 57 mutations in the Igλ chain gene from 61 clones, no mutations occurred in the first 97 bp and full mutation activity started only ~200 bp downstream of the promoter (Fig. 3). This distribution of mutations is the same as in wild-type or Ung\(^{-/-}\) mice (26). Because BER and MMR, the major apparent mechanisms responsible for posturacil SHM events, were absent in these mice, we conclude that AID, indeed, is unable to access the S’ region of Ig genes in vivo.

AID activity declines significantly within a 1.1-kb sequence 3’ of J\(_{53}

Mutations are also absent in the S’ end of Igλ genes, both in wild-type and Ung\(^{-/-}\) mice, suggesting that AID does not act beyond ~1.5–2 kb from the promoter (26). To assess the possibility that high fidelity mismatch repair may eliminate uracils in this region, we compared the mutation distributions in the 1.1-kb sequence 3’
of JH4. In both the Ung−/−Msh6−/− and wild-type mice, the highest density of mutations was at the 5' end of the JC intron; more 3' the density of mutations was gradually reduced so that mutations at the 3' end of the 1.1-kb region 3' of JH4 were scarce, both in Msh6−/−Ung−/− and wild-type mice (Fig. 4, C and D). Two factors might contribute to the mutation distribution in this 1.1-kb DNA sequence: the distribution of cytosines and the occurrence of the WRC/GYW hotspots. Comparing the 5' end to the 3' end of the 1.1-kb DNA fragment, the frequency of cytosines is similar (Fig. 4A), while the frequency of WRC/GYW hotspots is actually slightly higher 3' (Fig. 4B), indicating that the distribution of mutations is independent of either the distribution of cytosines or the occurrence of AID hotspots. Thus, increasing scarcity of AID-induced deaminations appears responsible for the waning mutagenesis toward the 3' end, suggesting that, indeed, AID does not access or does not act at the 3' end of Ig genes.

**Discussion**

Compared with Msh2−/−Ung−/− mice, SHM and CSR are affected in the same way in Msh6−/−Ung−/− mice. This suggests that MMR in these processes is induced by recognition of the U/G mismatch by the MutS-α complex, Msh2/Msh6, that is responsible for recognizing single-base mismatches and loops, and not the MutS-β complex, Msh2/Msh3, that recognizes larger mismatches and loops. The fact that almost all mutations at C and G are transitions suggests that they arise by direct replication of the AID created uracils and that, besides BER and MMR, no other repair mechanism may recognize and process the uracils. It had been shown, by us and others, that nucleotide excision repair deficiency does not alter SHM (28–31). The findings here confirm this. Some mutations at A and T still arise in the double-knockout mice, ~2% in the current study (Fig. 1). This is unlikely due to MutS-β (Msh2/ Msh3) activity, because a similar proportion is seen in Msh2/Ung double-knockout mice (23). Thus, perhaps other uracil glycosylases can get involved (32) and, in rare occasions, their action during SHM may attract error-prone polymerases.

The mutation frequencies in mutated DNA clones are the same in Msh6−/−Ung−/− mice as in wild-type mice (Table I). In contrast, in Msh6−/− mice with wild-type Ung, the mutation frequencies are reduced (bold in Table I). Reduction in mutation frequencies has also been observed in mice with Msh2 deficiency (10, 11) but not in mice with Msh2/Ung double deficiency (23). Rada et al. (23) suggested that, in the presence of Ung, CSR was initiated but when MutS-α was absent, CSR was not completed and thus the cells may have died, while in the absence of Ung, CSR would not be initiated. There may also be an interesting alternative explanation, because CSR is not severely compromised in Msh6−/− mice. Perhaps, in the presence of Ung but absence of mismatch repair, many of the uracils created by AID may be repaired to cytosine by the normal base excision repair with polymerase β, thus reducing mutations.

CSR is greatly curtailed in the double-knockout mice, however, it is not eliminated (Fig. 2). There clearly are switched Ig's for IgG1, IgG2b, and IgA. Perhaps IgG2a and IgG3 were also present at levels not detectable, because these Ig isotopes are also rare in wild-type mice. Presumably, switch recombination in these cases results from spontaneous single-strand breaks near each other, one on each DNA strand, in Sμ and a downstream S region. If this were the case, it would suggest that in normal CSR the recruitment of nonhomologous end joining complexes and the synapsing of Sμ and another S region would not require participation of AID. In fact, in the absence of AID some IgG1 and IgG2α was observed in AID−/− mice (33).

**FIGURE 4.** Distribution of mutations in wild-type and Msh6−/−Ung−/− mice in the 1.1-kb JC intron region 3' of JH4. A, The distribution of cytosines. Bars above the horizontal axis represent Cs on both DNA strands. B, The occurrence of WRC/GYW hotspots. Bars above and below the horizontal axis are hotspots in the top and bottom DNA strands, respectively. C, The mutation distribution in the wild-type mice. D, The mutation distribution in the Ung−/−/Msh6−/− mice. E, Map of H chain gene. Bent arrow, start of transcription; VDJ, V region; C, C region; dotted lines, the sequence of interest. The map is not to scale.
interacting with MMR in SHM in the absence of Ung, although it has been reported that pol η can interact with Msh2/Msh6 (35).

In the absence of Msh6 but the presence of wild-type Ung, there are very few mutations from A and T, although more than when both Ung and Msh6 are inactivated (Fig. 1; 7, 9). This scarcity of A,T mutations in mice deficient for Msh6 alone disagrees with the conclusion that Ung is responsible for a proportion of up to 52% of mutations from A and T. However, this finding hints at a collaboration between Ung and MMR (26). Ung is more active on ssDNA than dsDNA (36, 37). Presumably, in normal SHM, Ung acts efficiently on ssDNA created by Msh2/Msh6 induced excision of either the U or G containing strand. However, because Ung is less active in removing the U in dsDNA, long patch base excision repair at a T-base pair and for efficient class switch recombination. J. Exp. Med. 198: 1171–1178.


