The Role of DNA Repair in Somatic Hypermutation of Immunoglobulin Genes

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Immunoglobulin (Ig) genes are first modified through the V(D)J (V, variable; D, diversity; J, joining) recombination process in pre–B cells. The Rag1/Rag2-dependent V(D)J recombination generates a large repertoire of B lymphocytes, each expressing a unique antibody molecule. Rearranged Ig genes are further modified by the somatic hypermutation process in activated germinal center B lymphocytes. Through somatic hypermutation, point mutations are introduced into Ig genes at the average frequency of one mutation/10^2 base pairs. After the mutation process, B cells with nucleotide substitutions resulting in higher affinity antibody are selected for proliferation and differentiation into memory or plasma cells.

Several molecular features are characteristic of somatic hypermutation (1). The mutations are mostly single substitutions, very rarely deletions or insertions. Hot spots of mutation have been observed and of these most notable are the AGY trinucleotides. Somatic mutation of Ig genes is confined to a region of ~1.5 kb downstream of the variable region gene promoter. The constant region exon, which is separated from the variable region exon by several kb of intron, is usually not targeted for mutation. There also appears to be a strand bias and A > T bias (2). Which strand is the preferential substrate of the mutation process has, however, not been determined. Further insights into the mechanism of somatic hypermutation were made possible by the use of transgenic mice bearing Ig genes with various modifications. A transgenic construct with deletion of the Igκ intronic enhancer no longer mutates (3). Surprisingly, insertion of an Ig promoter just upstream of the constant region exon results in mutations in the constant region in addition to the variable region, but sparing of the region in between (4). The link between promoter proximity and mutation targeting, as well as the evidence of strand bias and the need for enhancers, strongly suggests that there is a link between transcription and Ig gene somatic hypermutation (4). However, how a mechanism linked to RNA transcription introduces point mutations into Ig genes is still largely a mystery, although a role for RNA polymerase pausing and deposition of a mutator factor with sequence preferences is suggested by recent findings (Storb, U., E. Klotz, J. Hackett, K. Kage, G. Bozek, and T.E. Martin, manuscritp submitted for publication).

Several proposals have been made of how DNA repair might be involved in the somatic hypermutation process. On the one hand, DNA repair may be used to induce the mutations. The transcription-linked model proposed by Peters and Storb (2, 4) suggested the involvement of gratuitous transcription-coupled DNA repair where point mutations accumulate through error-prone DNA repair (5, 6). Cascalho et al. propose that DNA mismatch repair might be exploited to cause mutations by correcting the unmutated base to be complementary to the mutated base (7). Cascalho et al. propose no mechanism for the primary introduction of mutations. According to these two models, diminished DNA repair will result in a decrease in somatic hypermutation. An opposing view is the proposal that somatic mutations of Ig genes cannot be fixed without deficiency in DNA repair (8). In this consideration, somatic hypermutation could be due to suppressing normal DNA repair. A proposal made by Gearhart and coworkers takes a slightly different view of the role of DNA repair, suggesting that DNA mismatch repair in hypermutating cells must function not to induce mutations but to correct them (9, 10). The authors submit that mutations are introduced into Ig genes by an error prone DNA polymerase and then some mutations are repaired by normally functioning DNA repair mechanisms.

So far, the effort to determine the link between DNA repair and somatic hypermutation of Ig genes has been mainly focused on two DNA repair systems, nucleotide excision repair (NER) and DNA mismatch repair (MMR). NER is responsible for repairing UV-damaged DNA (11, 12). It was previously shown that NER preferentially repairs the transcribed DNA strand of highly expressed genes and also that many of the proteins essential for NER function are also components of the general transcription factor TFIIH. These observations led to a model of NER that is coupled to RNA transcription, namely, transcription-coupled repair (TCR; references 5, 6, 13). In TCR, the RNA polymerase complex, arrested due to a DNA lesion, signals for recruitment of a DNA repair complex to the site of arrest. Peters and Storb first proposed that such TCR can be exploited in germinal center B lymphocytes to induce mutation in Ig genes (4). However, experiments with B cells as well as EBV-transformed human lymphocyte cultures from patients with defects in NER showed that somatic mutation appears to be normal despite the severe defect in NER (8, 14). Human subjects studied include individuals with mutation in xeroderma pigmentosum (XP)D (8, 14),

XPB, XPV, or Cockayne syndrome (CS)A (14). XPD and XPB are required for NER as well as components of the transcription factor TFIIH. These human subjects show clear defects in repair of UV-damaged DNA as well as neurological complications (11, 15). XPV appears to be involved in postreplication DNA repair. In cells with mutant CSA, the preferential repair of the transcribed strand attributed to TCR function is abolished (11, 15). The apparently normal frequency of somatic hypermutation in these NER- or TCR-defective human cells leads to the conclusion that NER does not play a major role in the somatic hypermutation process. Experiments carried out in knockout mice with mutant XPC genes (16) or mutant XPA (9, 17), or XPD genes (17), further support the conclusion that somatic hypermutation is normal despite the significant decrease in NER. Analysis of mice with mutant CSB that lack functional TCR but not global repair by the NER machinery (17) again showed that somatic hypermutation was normal in these mice. It thus appears that NER is not required for somatic hypermutation, nor for curtailing excessive mutations.

MMR is best characterized in prokaryotes where MMR is responsible for correcting errors, short tandem mismatches as well as single base pair mismatches, introduced by the DNA polymerase during DNA replication (18). Key factors of MMR include MutS, which recognizes mismatches, MutH, which is a site-specific endonuclease, and MutL whose function is poorly understood. MutH, which recognizes hemimethylated DNA, is responsible for distinguishing the newly synthesized DNA strand from the template strand. The repair of only the newly synthesized strand is effective in suppressing the generation of mutations. Search for human and mouse homologues of the prokaryotic genes have identified multiple homologues of MutS as well as of MutL, which suggests that there may be functional redundancy as well as functional specialization among these homologues. No homologue of MutH has been identified and it is not yet known how new strand and template strand of DNA are recognized and distinguished in eukaryotes. Several lines of mice with deficient MMR have been created by gene targeting and these mutant strains show microsatellite instability and high frequency of tumors (19, 20). The analysis of Ig somatic hypermutation in these mice, however, seems to yield conflicting data. Winter et al. studied $V_\kappa Ox1$ genes in Pms2- (MutL homologue) deficient mice and observed little change in mutation frequency compared with normal mice (9). Similar findings were made in our laboratory showing normal frequencies of somatic hypermutation in Pms2- and Mlh1- (another MutL homologue) deficient mice (Kim, N., and U. Storb, unpublished data). Despite normal frequencies of mutations in Pms2-deficient mice, Winter et al. reported an increase in the occurrence of tandem mutations, supporting their view that DNA repair, MMR in particular, normally functions to correct some of the mutations introduced into Ig genes in hypermutating cells.

In contrast to the data from Winter et al. and our labora-

tory, Cascalho et al., analyzing somatic hypermutation of VH genes as well as Vλ1 genes of the same strain of Pms2-deficient mice, arrived at a different conclusion (7). Their data suggest that deficiency in MMR due to mutation of Pms2 significantly decreases the frequency of somatic hypermutation of Ig genes. In this case, it is proposed that DNA MMR actually functions to fix, rather than correct, the previously introduced mutations by repairing the unmutated strand using the mutated strand as the template. Unlike the experiments of Winter et al. and of our laboratory where the only genetic alteration was in the Pms2 locus, mice analyzed by Cascalho et al. contained, in addition, the disruption of the immunoglobulin heavy chain locus at both alleles, and an insertion into one allele of a functional VDJ_H resulting in a quasimonoclonal mouse. Can this difference account for the striking discrepancy between the data of Winter et al. and Cascalho et al.? Extraordinary circumstances of selective pressure present in the quasimonoclonal mice might partly contribute to the different observations. The fact that somatic mutation is not eliminated in these mice lends support to this idea.

The data presented in this issue of *The Journal of Experimental Medicine* by Jacobs et al. (17) and Phung et al. (10) offer yet more points of contention in the topic of the role of DNA mismatch repair in somatic hypermutation of Ig genes. Jacobs et al. look at the effect of disruption of the Msh2 locus on somatic hypermutations of Vλ1 genes of memory B lymphocytes. Msh2 is a homologue of MutS and an essential factor in MMR in eukaryotes. Msh2 can dimerize with either Msh3 or Msh6 to form a functional complex that recognizes DNA mismatch. Subsequent repair requires a Mlh1/Pms2 dimer as well as other unidentified factors. Analysis of 30 Vλ1 sequences cloned from memory B cells led Jacobs et al. to conclude that somatic mutations found in Msh2 null mice were comparable to those found in wild-type mice. No significant decrease in mutation frequency was detected. Any change in the occurrence of tandem mutations as noted by Winter et al. in their analysis of PMS2 null mice was also not observed. The different findings could be due to the fact that Pms2 acts at a downstream step in MMR from the step Msh2 is involved in as suggested by the authors.

Phung et al. also find that mice with a knockout of the Msh2 genes do somatically mutate their Ig genes at apparently normal frequency (10). Interestingly, they observe a skewing in the pattern of mutations. Normally in mice and humans, somatic mutations from A or T appear about as frequently as those from G or C. In the Msh2 mice, however, ∼90% of the mutations derive from G or C. As Phung et al. point out, a similar pattern is apparent in the Msh2 data from Rajewsky's laboratory (17). This G or C nucleotide preference resembles the mutation pattern seen in Ig genes of cold blooded vertebrates (21, 22). Cold blooded vertebrates, in contrast to mammals, show very little evidence of affinity maturation, i.e., selection of B cells with favorable Ig gene mutation, presumably due to lack of germinal centers (21, 22). Is it possible that the GC nucle-

Table 1. *Nucleotide Changes in Wild-type and Msh2 Mutant Mice**

	Only in wild type		Only in Msh2$^{-/-}$		In both types of mice			Total muts	
	(1) No. of positions	(2) Muts	(3) No. of positions	(4) Muts	(5) No. of positions	(6) Muts wild type	(7) Muts MS$^{-/-}$	(8) Wild type	(9) MS$^{-/-}$
From A/T	25	33 (47)†	4	6 (6)	2	4 (6)	2 (2)	37 (53)	8 (8)
From G/C	8	8 (11)	26	46 (46)	16	25 (36)	46 (46)	33 (47)	92 (92)
Total	33	41 (59)	30	52 (52)	18	29 (42)	48 (48)	70 (100)	100 (100)

Columns *1, 3,* and *5* show the No. of positions within the total sequence of 276 nucleotides that have the particular mutation.
Columns *2* and *4* show the number (and percentage) of mutations at the indicated Nos. of positions occurring only in wild-type mice (*2*) or only in Msh2$^{-/-}$ mice (*4*).
Columns *6* and *7* show the mutations in wild-type mice (*6*) or Msh$^{-/-}$ mice (*7*) at positions that show mutations in both types of mice.
Columns *8* and *9* sum up the mutations in the respective row in wild-type (*8*) or Msh2$^{-/-}$ mice (*9*).
*Summarized from Fig. 2 of ref. 10 (excludes changes in two codons that were clearly selected for high affinity).
‡In parenthesis percent of total mutations in wild-type or Msh2$^{-/-}$ sequences, respectively.

otide preference in Msh2-deficient mice is a matter of lacking B cell selection?

We have analyzed the data of Phung et al. with respect to the changes in sequences from mutant and wild-type mice. The ratio of silent and replacement mutations is about the same. Also, the frequency of conservative changes is about the same in both wild-type and Msh2 mutants. This may suggest that there is no skewing in the selection of B cells in the Msh2 mutant mice. Furthermore, as Phung et al. pointed out, certain codon changes were obviously selected for high affinity to the immunizing antigen as much in the mutant as in the wild-type mice.

Thus, if the difference in mutation pattern is not due to selection, it must be due to a direct effect of the MMR complex, either on the primary mutation mechanism or on differential correction before the mutations are stabilized in the genome. We will return below to the question of an effect of Msh2 on the primary mutation mechanism. Concerning corrections, as Phung et al. note, the data suggest that G or C are preferentially corrected in wild-type cells, but not in the mutant cells. Thus, if mutations from G or C are more frequent in the primary mutation event, they will make up the majority of mutations in the Msh2 mutant mice because they are not corrected. This brings up the burning question of why the overall frequency of mutations is not higher in the Msh2 mutant mice compared with normal mice. We entertain two possibilities. It may be that the mutation frequency in many of the functional Ig genes in the mutant mice is so high that the chance for stop codons and other detrimental changes is much higher than normal. A large proportion of cells would thus be eliminated, making the overall number of surviving B cells with mutations similar to wild type.

However, an analysis of all Ig mutations in the normal and Msh2 mutant mice (10) suggests an interesting alternative explanation, namely that the mutant MMR corrects A- or T-derived mutations much more efficiently than does the normal MMR. The nucleotides targeted in the wild-type versus the Msh2 mutant mice are summarized in Table 1. Clearly, there are very few mutations from A or T that occur only in the Msh2$^{-/-}$ mice (6% of all mutations in the Msh2$^{-/-}$ mice, column *4*) or occur in both the Msh2$^{-/-}$ and wild-type mice (6 and 2%, columns *6* and *7*). The vast majority of mutations from A or T occur only in the wild-type mice (53% of mutations in the wild-type mice; of the 31 A or T positions that are mutated, 27 are mutated in wild-type mice [columns *1* and *5*] and only 6 are mutated in the Msh2$^{-/-}$ mice [columns *3* and *5*]). On the other hand, while 92% of the mutations in the Msh2$^{-/-}$ mice are from G or C (column *9*), a fairly high proportion also occur in the wild type (47% of all mutations in wild-type mice; column *8*). This may suggest the following scenario: the wild-type complex Msh2 + Msh3 or Msh2 + Msh6 may prefer to repair G- or C-derived mismatches (this implies that the MMR complex can identify the germline-derived G or C), and has little or no activity for the repair of A- or T-derived mismatches. The mutant complex (minus Msh2; perhaps another protein can combine with Msh3 and/or Msh6), on the other hand, cannot deal with G- or C-derived mismatches; however, it has an enhanced activity for repairing A- or T-derived mismatches. Since in normal cells the ratio of mutations from A or T versus G or C is ~1, one would postulate an approximately twofold excess of mutations from G or C in the primary step of introducing mutations. Thus, the resulting frequency of overall mutations would be expected to be approximately the same in the wild type and mutant cells. It would be interesting to test if the substrate specificity of MMR in the absence of Msh2 is enhanced for A- or T-derived mismatches. Returning to coldblooded vertebrates, perhaps they lack Msh2.

Based on the scenario where wild-type MMR mainly corrects mutations derived from G or C, and Msh2$^{-/-}$ MMR mainly corrects mutations derived from A or T, the normal frequency of mutations in Msh2$^{-/-}$ mice suggests that MMR plays no significant role in the primary step of

introducing the Ig mutations. This is supported by the finding that the Msh2$^{-/-}$ mice have a normal proportion of mutations in hot spots (of the Ig mutations in the data of Phung et al. [10] 43% in the mutant mice, and 41% in the wild-type mice are in the hot spot AGY). Furthermore, despite the severe reduction in mutations from A or T, the A > T bias and strand bias is preserved in the Msh2$^{-/-}$ mice (10). These conclusions also apply for the Pms2-deficient mice (9). Thus, it appears that MMR is not required for the primary step of introducing mutations into Ig genes, but is involved in correcting certain primary mutations. Furthermore, the presence of hot spots and the A > T and strand biases are apparently properties of the underlying mechanism of somatic hypermutation (5).

The article by Jacobs et al. (17) also discusses the analysis of somatic hypermutation in mouse strains lacking functional Rad54-dependent double-strand break repair (DSBR) or 3-alkyladenine glycosylase (AAG)-mediated base excision repair (BER). Both mutant strains of mice show normal frequency and pattern of mutation in their memory B cell Vλ1 sequences. However, the possibility remains that an alternate pathway of BER that does not require AAG might be involved in somatic hypermutation. Interestingly, a homologue of bacterial 8-oxoguanine DNA glycosylase that is involved in BER is highly expressed in germinal centers and may play a role in somatic mutation (23). Another possibility is that PCNA-dependent long patch BER is involved (12).

Also discussed in the article by Jacobs et al. are mice lacking poly ADP-ribose polymerase (PARP), which may bind to a single-strand nick in DNA and is involved in certain types of DNA repair (12). Even though PARP mutant mice show sensitivity to ionizing radiation and other DNA damaging reagents, a "nick sensor" function of PARP must be redundant (12) and the normal Ig gene mutation frequency and pattern found in PARP mutant mice are not a clear indication that repair of a single-strand break is not involved in somatic hypermutation.

Identification of *cis*-acting factors in the somatic hypermutation process has seen great success through the use of Ig transgenes (for review see reference 24). Identification of *trans*-acting factors, however, has proven to be much more difficult. The lack of an in vitro system has been the main hurdle in this pursuit. So far, little detail is known about the molecular mechanism of somatic hypermutation. The report of altered mutation frequency or mutation pattern in Pms2 and Msh2 mutant mice is the first evidence of *trans*-acting factors involved in somatic hypermutation. A very careful analysis to reconcile the conflicting results and to identify the exact nature of the effect Pms2 or Msh2 on somatic hypermutation will be necessary. Using a passenger transgene that is not under selective pressure (25, and Storb, U., E. Klotz, J. Hackett, K. Kage, G. Bozek, and T.E. Martin, manuscript submitted for publication) will help greatly in deciphering the role of Pms2 and Msh2 in Ig hypermutation.

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References

1. Storb, U. 1996. Molecular mechanism of somatic hypermutation of immunoglobulin genes. *Curr. Opin. Immunol.* 8:206–214.

2. Storb, U., A. Peters, E. Klotz, N. Kim, H.M. Shen, K. Kage, B. Rogerson, and T.E. Martin. 1998. Somatic hypermutation of immunoglobulin genes is linked to transcription. *Curr. Top. Microbiol. Immunol.* 229:11–19.

3. Betz, A., C. Milstein, R. Gonzalez-Fernandes, R. Pannell, T. Larson, and M. Neuberger. 1994. Elements regulating somatic hypermutation of an immunoglobulin κ gene: critical role for the intron enhancer/matrix attachment region. *Cell.* 77:239–248.

4. Peters, A., and U. Storb. 1996. Somatic hypermutation of immunoglobulin genes is linked to transcription initiation. *Immunity.* 4:57–65.

5. Friedberg, E. 1996. Relationships between DNA repair and transcription. *Annu. Rev. Biochem.* 65:15–42.

6. Hanawalt, P.C. 1994. Transcription-coupled repair and human disease. *Science.* 266:1957–1958.

7. Cascalho, M., J. Wong, C. Steinberg, and M. Wabl. 1998. Mismatch repair co-opted by hypermutation. *Science.* 279:1207–1210.

8. Wagner, S., J. Elvin, P. Norris, J. McGregor, and M. Neuberger. 1996. Somatic hypermutation of Ig genes in patients with xeroderma pigmentosum (XP-D). *Int. Immunol.* 8:701–705.

9. Winter, D.B., Q.H. Phung, A. Umar, S. Baker, R.E. Tarone, K. Tanaka, R.M. Liskay, T.A. Kunkel, V.A. Bohr, and P.J. Gearhart. 1998. Altered spectra of hypermutation in antibodies from mice deficient for the DNA mismatch repair protein PMS2. *Proc. Natl. Acad. Sci. USA.* In press.

10. Phung, Q.H., D.B. Winter, A. Cranston, R.E. Tarone, V.A. Bohr, R. Fishel, and P.J. Gearhart. 1998. Increased hypermutation at G and C nucleotides in immunoglobulin variable genes

from mice deficient for the MSH2 mismatch repair protein. *J. Exp. Med.* 187:1745–1751.

11. Hoeijmakers, J.H.J. 1993. Nucleotide excision repair II: from yeast to mammals. *Trends Genet.* 9:211–217.

12. Friedberg, E., G. Walker, and W. Siede. 1995. DNA Repair and Mutagenesis. American Society for Microbiology, Washington, DC. pp. 283–316, 135–190, 306–310.

13. Lommel, L., C. Carswell-Crumpton, and P.C. Hanawalt. 1995. Preferential repair of the transcribed DNA strand in the dihydrofolate reductase gene throughout the cell cycle in UV-irradiated human cells. *Mutat. Res.* 336:181–192.

14. Kim, N., K. Kage, F. Matsuda, M.-P. Lefranc, and U. Storb. 1997. B lymphocytes of xeroderma pigmentosum or Cockayne syndrome patients with inherited defects in nucleotide excision repair are fully capable of somatic hypermutation of immunoglobulin genes. *J. Exp. Med.* 186:413–419.

15. Lambert, W., H. Kuo, and M. Lambert. 1995. Xeroderma pigmentosum. *Dermatol. Clinics.* 13:169–209.

16. Shen, H.M., D.L. Cheo, E. Friedberg, and U. Storb. 1997. The inactivation of the XP-C gene does not affect somatic hypermutation or class switch recombination of immunoglobulin genes. *Mol. Immunol.* 34:527–533.

17. Jacobs, H., Y. Fukita, G.T.J. van der Horst, J. de Boer, G. Weeda, J. Essers, N. de Wind, B.P. Engelward, L. Samson, S. Verbeek, et al. 1998. Hypermutation of immunoglobulin genes in memory B cells of DNA repair–deficient mice. *J. Exp. Med.* 187:1735–1743.

18. Modrich, P. 1991. Mechanisms and biological effects of mismatch repair. *Annu. Rev. Genet.* 25:229–253.

19. Baker, S., E. Bronner, L. Zhang, A. Plug, M. Robatzek, G. Warren, E. Elliott, J. Yu, T. Ashley, N. Arnheim, et al. 1995. Male mice defective in the DNA mismatch repair gene PMS2 exhibit abnormal chromosome synapsis in meiosis. *Cell.* 82:309–319.

20. Baker, S., A. Plug, T. Prolla, E. Bronner, A. Harris, X. Yao, D. Christie, C. Monell, N. Arnheim, A. Bradley, et al. 1996. Involvement of mouse Mlh1 in DNA mismatch repair and meiotic crossing over. *Nat. Genet.* 13:336–342.

21. Diaz, M., and M. Flajnik. 1998. Evolution of somatic hypermutation and gene conversion in adaptive immunity. *Immunol. Rev.* In press.

22. Hsu, E. 1998. Mutation, selection, and memory in B lymphocytes of exothermic vertebrates. *Immunol. Rev.* In press.

23. Kuo, F., and J. Sklar. 1997. Augmented expression of a human gene for 8-oxoguanine DNA glycosylase (MutM) in B lymphocytes of the dark zone in lymph node germinal centers. *J. Exp. Med.* 186:1547–1556.

24. Storb, U. 1998. Somatic hypermutation of immunoglobulin genes. *In* The Biology of Germinal Centers in Lymphoid Tissue. G.J.T.A.V.K. Tsiagbe, editor. R.G. Landes Co. Biomed. Publications, Austin, TX. In press.

25. Klotz, E., J. Hackett, and U. Storb. 1998. Somatic hypermutation of an artificial test substrate within an Igk transgene. *J. Immunol.* In press.