Somatic hypermutation: activation-induced deaminase for C/G followed by polymerase η for A/T

Michael S. Neuberger and Cristina Rada

Somatic hypermutation (SHM) introduces nucleotide substitutions into immunoglobulin variable (Ig V) region genes at all four bases, but the mutations at C/G and A/T pairs are achieved by distinct mechanisms. Mutations at C/G pairs are a direct consequence of the C→U deamination catalyzed by activation-induced deaminase (AID). Mutations at A/T pairs, however, require a second mutagenic process that occurs during patch repair of the AID-generated U/G mismatch. Several DNA polymerases have been proposed to play a role in SHM, but accumulating evidence indicates that the mutations at A/T are overwhelmingly achieved by recruitment of DNA polymerase η.

The initial response to antigen is provided by IgM antibodies whose binding sites usually exhibit a relatively low affinity for antigen. Over the subsequent days and weeks, the antibody response matures to yield antibodies (typically of the IgG class) that display greatly increased affinity for antigen. This affinity maturation is achieved during B cell expansion in germinal centers by an iterative alternation of SHM and antigen-mediated selection. SHM itself is characterized by the sequential introduction of (typically) single nucleotide substitutions over a region of DNA encompassing the expressed IgVH and IgVL segments. The mutations themselves can occur at either C/G or A/T pairs and can be either transitions (purine–purine or pyrimidine–pyrimidine substitutions) or transversions (a swapping of purine and pyrimidine). Although the entire process of SHM is dependent on AID (1, 2), the mutations at A/T pairs are produced by a substantially different mechanism from those at C/G pairs (3). Thus, whereas AID-catalyzed deamination of C bases can directly explain the mutations at C/G pairs, those at A/T pairs require a second mutagenic process. Accumulating genetic evidence (4–9), including new data from Delbos et al. (on p. 17 of this issue [10]), increasingly points to a pivotal role for DNA polymerase η in this A/T-specific process.

The mechanics of SHM
AID triggers somatic hypermutation by attacking a small number of C residues within the Ig V domains, deaminating them to U and thereby transforming a few C/G pairs into U/G mispairs (for review see reference 11). The presence of this uracil in DNA triggers an ancient pathway of DNA repair (12, 13), in which the uracil is excised from the DNA deoxyribophosphate backbone by the UNG uracil-DNA glycosylase. This yields an abasic site, which when encountered on the DNA template strand, is likely to stall the progression of the DNA replication fork. Such stalling recruits specialized polymerases (14) that are able to insert a dNTP opposite the abasic site, despite the fact that the abasic site is “noninstructional.” Several translesion polymerases appear able to assist in this synthesis, although the evidence supporting a role for the REV1 polymerase (15–17) is especially clear, as its specificity of nucleotide insertion (almost exclusively dCTP) means that its absence alters the mutation spectrum. Mutations at C/G pairs can thus be envisaged as an inevitable consequence of replication over sites of AID-catalyzed C→U deamination and subsequent UNG-mediated uracil excision (Fig. 1A).

The mechanism by which mutations are generated at A/T pairs is less straightforward. These mutations depend on the original AID-catalyzed C-deamination but are clearly not a direct, inevitable consequence of that deamination. Genetic evidence suggests that a second mutagenic process takes place during patch repair of the original AID-generated U/G mispair (18). This mispair is a dual lesion in that it is both a mismatch and also contains a noncanonical DNA base. As a mismatch, it is recognized by the MSH2–MSH6 heterodimer, which conventionally functions to identify single-base mismatches for the purpose of initiating mismatch repair (Fig. 1A). As a foreign base, it is recognized by the UNG uracil-DNA glycosylase. The patch repair process that generates the A/T mutations can be triggered by either MSH2–MSH6-mediated or UNG-mediated recognition of the initiating U/G lesion, although analysis of mutant mice suggests that MSH2–MSH6-mediated recognition is the major pathway (18–22).

Polymerase η: the prime suspect for mutations at A/T
The first breakthrough in identifying the major DNA polymerase involved in mutagenesis at A/T pairs came with the discovery by Zeng et al. that SHM at A/T (but not C/G) pairs is severely depressed in patients suffering from the variant form of Xeroderma pigmentosum (4). Xeroderma pigmentosum is a disease that renders patients highly susceptible to sun-induced skin cancers because of a deficiency in the cell’s ability to repair ultraviolet-induced DNA damage. These patients carry inactivating mutations in the gene encoding DNA polymerase η, a translesion polymerase that is thought to play a role in allowing the replication fork to bypass cyclobutane pyrimidine dimers, which...
are major products of ultraviolet damage (23). Zeng et al. therefore proposed that mutations at A/T pairs were caused by errors of misincorporation by this low-fidelity polymerase during patch DNA synthesis (4). A similar depression of mutation accumulation at A/T pairs was later observed after disruption of the gene encoding DNA polymerase η in the mouse (6, 8).

Although these studies demonstrated a major role for DNA polymerase η in the generation of mutations at A/T pairs, it is clear that this polymerase is not the only DNA polymerase that can generate the A/T mutations. Although deficiency in polymerase η diminishes mutation accumulation at A/T pairs, it does not abolish it: the mutations at A/T are reduced from ~50% to ~20% of the total. The striking finding reported by Delbos et al. (10) is that mutations at A/T pairs are essentially totally abolished when deficiency in polymerase η is combined with deficiency in MSH2.

How should one interpret this result? Although genetics provides powerful insights into what happens in vivo, it is a dangerous practice to extrapolate from the phenotype of a mutant to deduce what goes on in the wild type. Simple epistasis analysis would suggest that, because mutations at A/T pairs are obliterated by simultaneous disruption of MSH2 and DNA polymerase η, but not by either disruption on its own, then the two proteins should lie on different pathways. The matter, however, is not quite so simple, as single disruptions in either MSH2 or polymerase η each yield a substantial, but not complete, depression in mutation accumulation at A/T pairs. The most likely explanation of the results is that the MSH2–MSH6-mediated recruitment of DNA polymerase η, which was previously thought to be a major pathway to mutations at A/T pairs, is in fact the overwhelming mechanism by which such mutations are generated. In the absence of DNA polymerase η, MSH2–MSH6 recruits a backup polymerase that provides a low background of mutations at A/T. In the absence of MSH2–MSH6, the need to replicate across the UNG-generated abasic site can still result in the recruitment of DNA polymerase η, generating a reduced load of A/T mutations (Fig. 1 B). However, unlike the MSH2-recruited mutagenic patch repair, mutagenesis at A/T pairs as a consequence of replicating across the abasic site is wholly dependent on polymerase η.

These observations result in a pleasing simplification of our view of SHM. Although multiple translesion polymerases have been implicated in antibody hypermutation (for review see references 24–26), DNA polymerase η appears to be the most dominant (if not the only) contributor to A/T mutations under normal conditions. That does not mean that there is no role in SHM for the other translesion polymerases. First, some other polymerase can, at least in the absence of polymerase η, provide
some degree of backup mutations at A/T pairs in the MSH2-triggered pathway. Second, as discussed, the generation of transversion mutations at C/G pairs depends on replication across an abasic site and this process requires a translesion polymerase. Nevertheless, when considering mutation creation as opposed simply to the repair of AID-induced damage, it seems that the dominant polymerase is polymerase \( \eta \).

Unanswered questions

The recruitment of DNA polymerase \( \eta \) for mutagenesis at A/T pairs raises many questions. For example, why (and how) does MSH2 recruit DNA polymerase \( \eta \) to the U/G lesion? The primary role of MSH2–MSH6 is to initiate postreplication mismatch repair, correcting the occasional errors of misincorporation perpetrated by the replicative DNA polymerases \( \alpha \), \( \delta \), and \( \epsilon \) (27). There is no evidence that polymerase \( \eta \) normally plays any role in mismatch repair, which is presumably usually a process of high fidelity. So, although the patch repair that generates mutations at A/T pairs is similar to conventional mismatch repair in that it is triggered by MSH2–MSH6 and likely involves strand degradation by exonuclease 1, it differs from conventional mismatch repair in that it is mutagenic and depends on polymerase \( \eta \). Wilson et al. have provided evidence that MSH2 will associate with polymerase \( \eta \) and stimulate its activity (28). Is this association specific to B cells undergoing SHM? And what prevents polymerase \( \eta \) from being recruited during normal mismatch repair?

The mechanism by which polymerase \( \eta \) generates the mutations at A/T pairs also remains uncertain. The widely favored view is that these mutations are simply attributable to errors of base pairing by polymerase \( \eta \) during the patch DNA synthesis. This requires that the errors made by polymerase \( \eta \) occur largely opposite A or T on the template strand (and not opposite C or G)—a requirement that is well supported by the extensive in vitro analysis of the error spectrum of purified polymerase \( \eta \) from the Kunkel lab (9). This would predict that the nature of the nucleotide substitutions introduced during this second phase of SHM could be modified by altering the error spectrum of polymerase \( \eta \).

Thus, antibody hypermutation involves two distinct mutagenic processes. Mutations at C/G pairs are a direct consequence of an active assault on the DNA molecule itself: these deviations from the parental DNA sequence can therefore be viewed as essentially a sin of commission. The mutations at A/T pairs, however, depend on a subsequent and distinct mutagenic process that involves polymerase \( \eta \). If these mutations at A/T pairs are indeed simply errors resulting from a lack of fidelity in polymerase \( \eta \), then these mutations can be considered a sin of omission. But the sin of omission would never have happened were it not for the original sin of commission (29).

REFERENCES

1. Muramatsu M., K. Kinoshita, S. Fagarasan, S. Yamada, Y. Shinkai, and T. Hongyo. 2000. Class switch recombination and hypermutation require activation-induced cytidine deaminase (AID), a potential RNA editing enzyme. Cell. 102:553–563.
2. Revy, P., T. Muto, Y. Levy, F. Geissmann, A. Plebani, O. Sanal, N. Catalan, M. Forveille, R. Dufourcq-Libelouse, A. Genery, et al. 2000. Activation-induced cytidine deaminase (AID) deficiency causes the autosomal recessive form of the Hyper-IgM syndrome (HIGM2). Cell. 102:565–575.
3. Rada, C., M.R. Ehrenstein, M.S. Neuberger, and C. Malsen. 1998. Hot spot focusing of somatic hypermutation in MSH2-deficient mice suggests two stages of mutational targeting. Immunity. 9:135–141.
4. Zeng, X., D.B. Winter, C. Kasmer, K.H. Kraemer, A.R. Lehmann, and P.J. Gearhart. 2001. DNA polymerase eta is an A-T mutator in somatic hypermutation of immunoglobulin variable genes. Nat. Immunol. 2:537–541.
5. Faili, A., S. Aoufouchi, S. Weller, F. Vuillier, A. Stary, A. Sarasin, C.A. Reynaud, and J.C. Weill. 2003. DNA polymerase eta contributes to hypermutation of immunoglobulin gene hypermutation in the mouse. J. Exp. Med. 203:259–267.
6. Delbos, F., A. De Smet, A. Faili, S. Aoufouchi, J.C. Weill, and C.A. Reynaud. 2005. Contribution of DNA polymerase eta to immunoglobulin gene hypermutation in the mouse. J. Exp. Med. 201:1191–1196.
7. Mayorov, V.I., I.B. Rogozin, L.R. Adkisson, and P.J. Gearhart. 2003. DNA polymerase eta contributes to strand bias of mutations of A versus T in immunoglobulin genes. J. Immunol. 174:7781–7786.
8. Martomo, S.A., W.W. Yang, R.P. Wersto, T. Ohkumo, Y. Kondo, M. Yokoi, C. Maunatani, F. Hanasaka, and P.J. Gearhart. 2005. Different mutation signatures in DNA polymerase eta- and MSH6-deficient mice suggest separate roles in antibody diversification. Proc. Natl. Acad. Sci. USA. 102:8658–8663.
9. Rogozin, I.B., Y.I. Pavlov, K. Bebenek, T. Matsuda, and T.A. Kunkel. 2001. Somatic mutation hotspots correlate with DNA polymerase eta error spectrum. Nat. Immunol. 2:530–536.
10. F. Delbos, S. Aoufouchi, A. Faili, J.-C. Weill, and C.-A. Reynaud. DNA polymerase eta is the sole contributor of A/T modifications during immunoglobulin gene hypermutation in the mouse. J. Exp. Med. 203:17–23.
11. Neuberger, M.S., R.S. Harris, J. Di Noia, and S.K. Petersen-Mahrt. 2003. Immunity through DNA deamination. Trends Biochem. Sci. 28:305–312.
12. Poole, A., D. Penny, and B.M. Sjoberg. 2001. Confounded cytosine! Tinkering and the evolution of DNA. Nat. Rev. Mol. Cell Biol. 2:147–151.
13. Barnes, D.E., and T. Lindahl. 2004. Repair and genetic consequences of endogenous DNA base damage in mammalian cells. Annu. Rev. Genet. 38:445–476.
14. Prakash, S., R.E. Johnson, and L. Prakash. 2005. Eukaryotic translesion synthesis DNA polymerases: specificity of structure and function. Annu. Rev. Biochem. 74:317–353.
15. Sjoberg, E., J.P. Langerak, A. Tasabhi-Shiblyk, P. van den Berk, H. Jacobs, and N. de Wind. 2006. Strand-biased defect in C/G transversions in hypermutating immunoglobulin genes in Rev1-deficient mice. J. Exp. Med. 203:319–323.
16. Ross, A.L., and J.E. Sale. 2006. The catalytic activity of REV1 is employed during immunoglobulin gene diversification in DT40. Mol. Immunol. 43:1587–1594.
17. Rada, C., J.M. Di Noia, and M.S. Neuberger. 2004. Mismatch recognition and uracil excision provide complementary paths to both Ig switching and the A/T-focused phase of somatic mutation. Mol. Cell. 16:163–171.
18. Martin, A., Z. Li, D.P. Lin, P.D. Bardwell, M.D. Iglewski-Usel, W. Edelmann, and M.D. Schaffr. 2003. Msh2ATPase activity is essential for somatic hypermutation at A-T basepairs and for efficient class switch recombination. J. Exp. Med. 198:1171–1178.
19. Martomo, S.A., W.W. Yang, and P.J. Gearhart. 2004. A role for Msh6 but not Msh3 in somatic hypermutation and class switch recombination. J. Exp. Med. 200:61–68.
21. Bardwell, P.D., C.J. Woo, K. Wei, Z. Li, A. Martin, S.Z. Sack, T. Parris, W. Edelmann, and M.D. Scharff. 2004. Altered somatic hypermutation and reduced class-switch recombination in exonuclease 1-mutant mice. *Nat. Immunol.* 5:224–229.

22. Shen, H.M., A. Tanaka, G. Bozek, D. Nicolae, and U. Storb. 2006. Somatic hypermutation and class switch recombination in *Msh6(−/−)* *Ung(−/−)* double-knockout mice. *J. Immunol.* 177:5386–5392.

23. Johnson, R.E., S. Prakash, and L. Prakash. 1999. Efficient bypass of a thymine-thymine dimer by yeast DNA polymerase, Poleta. *Science.* 283:1001–1004.

24. Seki, M., P.J. Gearhart, and R.D. Wood. 2005. DNA polymerases and somatic hypermutation of immunoglobulin genes. *EMBO Rep.* 6:1143–1148.

25. Diaz, M., and C. Lawrence. 2005. An update on the role of translesion synthesis DNA polymerases in Ig hypermutation. *Trends Immunol.* 26:215–220.

26. Casali, P., Z. Pal, Z. Xu, and H. Zan. 2006. DNA repair in antibody somatic hypermutation. *Trends Immunol.* 27:313–321.

27. Jiricny, J. 2006. The multifaceted mismatch-repair system. *Nat. Rev. Mol. Cell Biol.* 7:335–346.

28. Wilson, T.M., A. Vaisman, S.A. Martomo, P. Sullivan, L. Lai, F. Hanaoka, A. Yasui, R. Woodgate, and P.J. Gearhart. 2005. MSH2-MSH6 stimulates DNA polymerase eta, suggesting a role for A:T mutations in antibody genes. *J. Exp. Med.* 201:637–645.

29. Aquinas, T. 1947. Of the distinction of sins. In *The Summa Theologica: Treatise on Habits (Question 72, Article 6).* Benziger Bros edition.