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# Somatic Hypermutation: How Many Mechanisms Diversify V Region Sequences?

Minireview

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Immunoglobulin variable (V) region sequences are tailored to recognize antigen by the process of somatic hypermutation. Somatic hypermutation of V regions can occur either before or after challenge with antigen. When somatic hypermutation occurs before challenge with antigen, the result is to increase the diversity of the preimmune repertoire. When somatic hypermutation occurs in response to antigen stimulation, it is coupled with selection for antigen binding, and the result is to increase antibody affinity for a specific antigen.

Insights into mechanism can sometimes come from studying related processes in a variety of organsims. Here I review some similarities between antigen-independent and antigen-driven somatic hypermutation and suggest that variations upon a single molecular mechanism might produce the distinct patterns of templated and untemplated mutation that characterize somatic hypermutation in different organisms.

### *Antigen-Driven Somatic Hypermutation: Mutation Coupled with Selection*

Somatic hypermutation has been most intensively studied in mice, where hypermutation occurs after challenge with antigen and targets single base changes to the rearranged V regions. The rate of hypermutation approaches $10^{-3}$ per base pair per generation, some $10^5$-fold higher than the mutation rate for an untargeted locus in the same cell. Hypermutation is coupled with selection for antigen binding, and a 10- to 100-fold increase in affinity for specific antigen distinguishes the very good antibodies of the primary response ($K_d$, $10^{-7}$ M) from the extraordinary antibodies of the secondary response ($K_d$, $10^{-8}$ to $10^{-9}$ M). This dramatic increase in affinity can result from as few as two or three amino acid substitutions in a 100 residue V domain.

Antigen-driven somatic hypermutation occurs in highly organized microenvironments, called germinal centers, where hypermutation is coupled with selection for antigen binding. Germinal centers develop in the follicles of the peripheral lymphoid organs following challenge with antigen (reviewed by Nossal, 1991; MacLennan, 1994). Visualized in sections of lymph node or spleen, germinal centers consist of a mantle surrounding a histologically distinct dark zone and a light zone. Small, resting B cells compose the mantle, and these cells express a large and diverse repertoire of unmutated V region sequences. B cells selected for antigen recognition populate the dark zone, where they proliferate and where somatic hypermutation occurs. Descendants of dark zone B cells migrate to the light zone, cease proliferating, and reveal their newly altered surface immunoglobulin molecules. Antigen displayed on the web of follicular dendritic cells in the light zone can then mediate affinity selection. Distinct surface markers characterize germinal center B cells at different stages of development, and this has recently permitted fractionation of germinal center B cells into distinct populations (Pascual et al., 1994), a critical step in studying both the biology and biochemistry of hypermutation.

In addition to B cells, a few T cells also reside within the germinal centers. These T cells were long thought to regulate B cell hypermutation while being immune to the hypermutation process themselves. Recently, however, Kelsoe and collaborators reported that Vα (but not Vβ) regions of the T cell receptor genes undergo hypermutation in germinal centers (Zheng et al., 1994). T cell receptor hypermutation is surprising and, not surprisingly, controversial (see Bachl et al., 1995; Kelsoe et al., 1995). Although immunoglobulin gene hypermutation can be readily rationalized, T cell hypermutation seems dangerous—if these cells return to the periphery, they may exhibit new and possibly autoreactive specificities.

### *Hotspots for Hypermutation*

In hypermutated murine V region sequences, mutations are not evenly distributed throughout the V region but are concentrated in the complementarity determining regions (CDRs), which encode the amino acids that make contact with antigen (see Figure 1). Clustering of hypermutation in the CDRs was noticed when the first V regions were sequenced. The ready explanation was that affinity selection must enrich for B cells carrying mutations in the regions that encode the antigen-binding site. However, it has recently been shown that targeting of somatic hypermutation to the CDRs is a property of the hypermutation mechanism itself (Betz et al., 1993a, 1993b; González-Fernández and Milstein, 1993; González-Fernández et al., 1994; Yélamos et al., 1995).

To separate the intrinsic properties of the hypermutation process from the effects of selection for antigen binding, Milstein, Neuberger, and their colleagues began by amassing a sequence database of VκOx1 light chain V regions that had undergone somatic hypermutation without affinity selection. These Vκ regions had escaped selection because they were carried as "passenger" transgenes that did not contribute to an antigen-specific immune response (Betz et al., 1993a, 1993b; González-Fernández and Milstein, 1993). Sequences of many such hypermutated but unselected genes revealed a very strong intrinsic hotspot in CDR1 of the VκOx1 region. Sub-

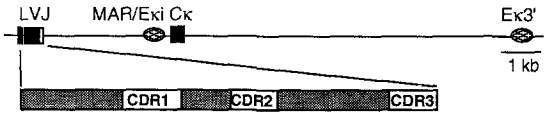


Figure 1. A Rearranged Murine κ Light Chain Locus

Residues within the CDRs of the V region are involved in antigen recognition.

sequently, examination of a collection of hypermutated Vλ regions, which had escaped selection because out-of-frame rearrangements produced nonfunctional coding sequences, revealed several active hotspots in each CDR, as well as clusters of hotspots within the J–C intron (González-Fernández et al., 1994). The hotspots in the intron were especially surprising, as mutation within the intron should not contribute to affinity selection. Most recently, these same laboratories showed that hypermutation could be targeted to passenger transgenes in which nonimmunoglobulin sequences—β-globin, *gpt*, and *neo* genes—were substituted for V region sequences (Yélamos et al., 1995). Remarkably, hypermutation of the nonimmunoglobulin genes occurred at frequencies comparable to V region transgenes in the same cells and was specific to germinal center B cells. Other laboratories had attempted to generate reporter genes for hypermutation that carried non–V region sequences, but none of these genes had been targeted for single base changes at a frequency that approached that of a rearranged V region (reviewed by Hengstschläger et al., 1995).

When hypermutated V region sequences are examined, it appears that the mutation process preferentially alters purines in the coding strand; G to A transitions are particularly abundant. Although there is no single sequence characteristic of all hotspots in the variety of hypermutated genes that have now been examined, the AGYT sequence motif is evident at many of the observed hotspots, in both immunoglobulin and nonimmunoglobulin genes. In some cases, hotspots also correlate with palindromic sequences or the potential for hairpin formation.

### *Somatic Hypermutation to Produce the Preimmune Repertoire*

Two different processes have been shown to diversify V region sequences before challenge with antigen. In some organisms, including mice and humans, a large number of V, D, and J segments undergo rearrangement, and the many combinatorial possibilities generate the diversity of the primary repertoire. In other organisms, a limited number of V gene segments undergo rearrangement, and the rearranged sequences are then diversified by a process of mutation that targets sequence changes to the rearranged V regions. Targeted V region mutation has been shown to generate the preimmune repertoire in chicken (Reynaud et al., 1987, 1989; Thompson and Neiman, 1987), rabbit (Knight and Becker, 1990; Becker and Knight, 1990), and sheep (Reynaud et al., 1991, 1995). In the shark, antigen receptor genes can undergo somatic diversification, but it is not yet known whether this diversification occurs to generate the preimmune repertoire or to modify V region sequence in response to antigen stimulation (Greenberg et al., 1995).

Like antigen-driven hypermutation, preimmune diversification occurs in organized germinal centers within lymphoid tissue. Among mammals, the ileal Peyer's patch in sheep (Reynaud et al., 1991) and the appendix in rabbit (Weinstein et al., 1994) have been shown to be sites of V region hypermutation.

### *Two Mechanisms of Hypermutation: One Templated and One Untemplated?*

Gene conversion, also known as templated mutation, is the mechanism of preimmune diversification in the chicken, where long tracts of sequence changes in the rearranged heavy and light chain genes have been shown to match pseudo–V region donors in the germline (Thompson and Neiman, 1987; Reynaud et al., 1987, 1989). In the rabbit, preimmune diversification also appears to depend largely or completely on templated mutation (Knight and Becker, 1990; Becker and Knight, 1990). In contrast, while there are apparent examples of templated mutation that occur during hypermutation of murine immunoglobulin V regions (Maizels, 1989; David et al., 1992; Xu and Selsing, 1994), templating alone cannot explain all mutation in this organism, particularly since some mutations—for example, mutations in the J–C intron—have no donor sequences in germline DNA. Similarly, in the sheep, the known germline V region sequences do not match the mutations observed to accumulate in ileal Peyer's patches, and preimmune diversification therefore does not appear to involve gene conversion (Reynaud et al., 1991, 1995).

Sheep Vλ regions do exhibit a characteristic pattern of hypermutation that is very similar to that of murine V regions that have undergone hypermutation in the absence of affinity selection: mutations are concentrated in the CDRs, and there is a marked bias toward mutation of purines in the coding strand, especially G to A transitions. In a detailed analysis of the pattern of targeted sequence diversification in sheep ileal Peyer's patches (Reynaud et al., 1995), Weill and collaborators noted these similarities and suggested that this might reflect an identical molecular mechanism at work in both processes. The major difference would then be one of regulation: preimmune diversification occurs in the absence of external antigen, while antigen-driven somatic hypermutation occurs in response to challenge with antigen.

### *A Unified Model for Somatic Hypermutation*

Do the templated and untemplated processes of hypermutation depend on mechanisms that are completely distinct? While it is possible that different mechanisms are responsible for templated and untemplated hypermutation of immunoglobulin genes, this has a troubling evolutionary and phylogenetic implication—namely, that one (templated) mechanism is shared by chicken and rabbits, and a different (untemplated) mechanism is shared by sheep and mice. An attractive alternative is that a single molecular mechanism may be able to generate mutations that in some instances show clear evidence of templating and in others do not.

Figure 2 outlines a scheme for hypermutation that accommodates both templated and untemplated mutation within a larger pathway that may be conserved in evolution. The first step would be a lesion, possibly targeted to a site that scores as an intrinsic hotspot. The lesion could be a single-stranded nick (shown in Figure 2 for simplicity) or a double-stranded break, analogous to the break that initiates mating-type switching in yeast. A site that scores

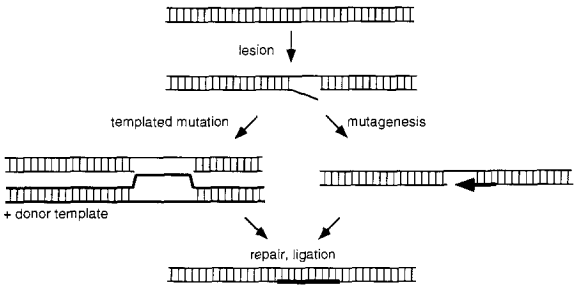


Figure 2. A Unified Model for Somatic Hypermutation

A single initiating lesion could lead to either templated (left) or untemplated (right) hypermutation, depending on the balance of cellular factors.

as a hotspot for hypermutation is an obvious candidate for the site of the initiating lesion. The second step would be sequence alteration either by templated mutation (requiring interaction with a donor sequence) or by a pathway of targeted mutagenesis. A variety of parameters could determine whether mutation that occurred in the second step was templated or template independent. These include the length or site of the initial lesion, the possibility that the DNA at the lesion was prone to forming structures (for example, hairpins or cruciforms) that might influence its ability to undergo pairing or repair, and the relative abundance of factors that promote heteroduplex formation versus untemplated single base changes. The third step would be repair, followed by ligation. The accuracy and rapidity of repair could influence the level of mutation, and mutation coupled with repair (step 3) could further alter V regions that had already undergone templated mutation (step 2).

### *The Transcription/Repair Machinery: Part of the Problem or Part of the Solution?*

A variety of evidence implicates the transcriptional machinery in the hypermutation process. First, the promoter is the upstream boundary for hypermutation. Nonetheless, hypermutation does not depend on the presence of an immunoglobulin promoter, as a β-globin promoter can also support hypermutation of an adjacent rearranged κ transgene (Betz et al., 1994). Second, rearrangement juxtaposes a V region with transcriptional regulatory elements, and rearrangement is prerequisite for hypermutation. Third, two transcriptional enhancers have been identified at the murine κ locus, Eκ3′ and Eκi–MAR (see Figure 1), and deletion of either enhancer diminishes κ hypermutation (Betz et al., 1994). Fourth, immunoglobulin genes that cannot be expressed in antibody-producing cells appear not to undergo hypermutation (although it has not been shown whether immunoglobulin genes are actively transcribed in B cells undergoing hypermutation in the light zone of the germinal center). Nonetheless, the elements that activate immunoglobulin gene transcription in antibody-producing cells are not identical to those that activate hypermutation. Deletion of Eκi–MAR abolishes hypermutation without diminishing transgene expression (Betz et al., 1994), and, similarly, a λ light chain transgene under control of the heavy chain intron enhancer does not hypermutate, although it is actively expressed (Hengstschläger et al., 1994).

Recently, it has emerged that some of the DNA repair enzymes are themselves part of the RNA polymerase II transcription initiation complex (reviewed by Drapkin et al., 1994). This unexpected discovery suggests a new possibility—hypermutation may depend on a repair step that is coupled to transcription. This would explain why hypermutation depends on transcriptional regulatory elements and why the promoter is the upstream boundary for hypermutation. Ironically, then, the repair machinery might be the cause of mutation.

One perplexing aspect of somatic hypermutation has been the fact that no activities have been identified biochemically that are likely participants in a targeted, V region–specific pathway of mutation. Obtaining sufficient starting material was once a difficulty, but, as noted above, enriching for hypermutating B cells has become increasingly feasible. It is a chilling thought, but some long hours in the cold room may now be the key to understanding the mechanism of somatic hypermutation.

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