Commentary

The Promotion of V Region Hypermutation

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Higher organisms have evolved unusual molecular and genetic mechanisms to generate high affinity and highly specific antibodies to a seemingly infinite number of foreign antigens (1). Even though a highly diverse germline encoded antibody repertoire is created, most of the antibodies have low affinities and the organism must find a way to modify those antibodies so that they will bind with high affinity and neutralize viruses and toxins. In mice and humans this is done during the course of the T dependent antibody response by introducing large numbers of point mutations (2) into the variable (V) region genes that encode the antigen binding site. B cells expressing antibodies with amino acid substitutions that result in a higher affinity are selectively stimulated to proliferate and differentiate by antigen and helper T cells and these higher affinity antibodies come to dominate the antibody response (3–5). Somatic V region hypermutation occurs primarily in B cells, though there is one report of V region mutation in T cell receptors in germinal centers (6).

Even though somatic V region mutation was the first of the many unusual molecular events that occur during B cell differentiation to be documented (2), and the sequences of hundreds of antibodies that are the products of this process have been determined, less is known about the molecular and biochemical mechanisms responsible for V region hypermutation than for other processes involved in the generation of antibody diversity such as V(D)J rearrangement and isotype switching. This is due in part to the lack of cultured cell systems in which the process can be studied (7, 8). Insights are now beginning to be gained from the study of transfected genes in mice (9–13 and reviewed in 14–16) and cultured cell systems that can carry out V region hypermutation of transfected genes have recently been reported (17–20).

In this issue Tumas-Brundage and Manser (21) have reported on the use of transgenic mice to examine the role of the heavy chain promoter in the location and rate of V region hypermutation. As these authors point out, the analysis of the sequences of both endogenous heavy and light chain genes and of transgenes that have undergone somatic mutation have led to the belief that proteins that are recruited to the transcriptional apparatus are involved in V region hypermutation (12, 22–25). The salient characteristics of the mutational process (26) are: (*a*) it is due primarily to point mutations that arise at rates that are estimated to be 10^{-5} to 10^{-3}/base pair/generation and are 4–6 orders of magnitude higher than the rate of mutation of housekeeping genes in higher organisms. This results in the accumulation of 5–15 base changes in the V regions of most antibodies that have been selected for during the late primary and secondary response. However, large numbers of mutations are also seen in passenger transgenes (27) and in the 3′ untranslated regions immediately flanking the V region (28), so the high frequency of point mutations is not an artifact of selection. There is even a report of 40–70 point mutations in V regions associated with δ constant regions, but these antibodies do not appear to play a role in the normal response (29); (*b*) high rates of mutation occur in already rearranged heavy and light chain variable region genes and their immediate flanking sequences. Few mutations are found 5′ to the promoter and the mutational process extends 3′ from the promoter for 1–1.5 kb with the maximum accumulation of mutations in the coding exon and its immediate 3′ flanking region (24, 25, 28, 30–32). The fact that the mutational process begins at the site of initiation of transcription and extends in the direction of transcription for a limited distance has suggested to many that transcription is involved in V region hypermutation (12, 22–25); (*c*) the highest frequency of somatic V region mutations is found in centroblasts in the dark zone of the germinal center (reviewed in reference 33). V region hypermutation appears to occur at about the same time or just before isotype switching but is distinct from and does not depend on that process (34–37). It is unclear whether somatic mutation is limited to one stage in B cell differentiation or if it can occur at lower rates in pre-B cells or in more differentiated plasma cells since most of the relevant studies have used sequencing techniques that have sufficiently high error rates so that mutation below a rate of 10^{-5} could not be detected: (*d*) although point mutations are found throughout the V region and its immediate flanking sequences, there are triplets such as the AGC and TAC and their inverted repeats, GTA and GCT that are preferred targets for the mutational process (27, 28, 38), and more extended versions of some of those triplets such as a purine, a G, a pyrimidine and an A or T (RGYW) have been recognized (27, 28, 38). These hot spots for mutation are not an artifact of selection since they can be deduced from silent base changes and from mutations in untranslated regions (27, 28). In addition, there appears to have been evolutionary selection for hot spot motifs in the complementary determining regions of the V region that encode the contact residues with antigen (39 and reviewed in reference 15). The mutational process results in transi-

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tions more often than transversions and appears to have a bias for the transcribed strand (27, 28, 40).

Experiments first with light chain (reviewed in reference 14) and more recently with heavy chain (reviewed in reference 16) transgenic mice have provided additional information that has focused attention on the interrelationship between transcription and V region hypermutation. The first transgenic experiments by O'Brien et al. (9) showed that transgenes located outside of the Ig locus could undergo what appeared to be the normal process of V region hypermutation and suggested that the Ig gene and its immediate flanking sequences have all of the information that is necessary to target and regulate the process. Subsequent studies revealed that non-Ig genes such as hemoglobin, GPT, the neomycin resistance gene or CAT could replace all or part of the V region and still undergo hypermutation (41, 42), proving that the coding sequence of the light chain V region did not contain any specific signals that targeted mutation to it. This suggested that the sequences flanking the V region were responsible for targeting and regulating the process. The essential role of flanking cis-acting sequences was confirmed by showing that both the intronic and 3′ transcriptional enhancers of the light chain were required for mutation (12). This requirement for these transcriptional regulatory elements drew additional attention to the potential role of transcription in V region hypermutation. This was reinforced by the growing appreciation that factors such as TFIIH, that were part of the basal transcriptional apparatus, also play a role in excision repair in eukaryotic cells in general (43, 44). Peters and Storb (13) provided a dramatic illustration of the importance of the initiation of transcription in V region mutation by introducing a promoter in front of the C region, which is thought not to undergo mutation normally, and showing that the initiation of transcription proximal to the C region resulted in C region mutations in transgenic mice.

In an attempt to learn more about the detailed mechanisms responsible for V region mutation, Betz et al. (12) showed that the hemoglobin promoter could be substituted for the light chain promoter in light chain transgenes without having a dramatic effect on the rate of mutation. This was an important finding since it suggested that the light chain promoter was not required for either B cell specificity or the restriction of mutation to the Ig gene. Tumas-Brundage and Manser (21) have investigated the role of the promoter further using a heavy chain V region transgene that is not active in its ectopic location (16). However, on rare occasions the V region transgene that they have introduced rearranges or undergoes gene conversion so that it is now located in the heavy chain locus (16). B cells expressing this V region, now in association with the endogenous C region, are stimulated by antigen and accessory cells to replicate and differentiate presumably in a relatively normal manner. Because of positive selection, there are enough of these B cells expressing the modified transgenic V region to be recovered as hybridomas. This system has the benefit of allowing Tumas-Brundage and Manser, and Selsing and his colleagues who have used a similar approach (10), to analyze the behavior of modified variable region genes that are now in the correct chromosomal location and associated with all of the 3′ elements that are normally present. In addition, the B cells expressing this transgene are in a mouse that also has B cells expressing unmodified endogenous V regions so that V regions under the control of variant and wild type promoters can be compared in the same mouse. The studies described in this paper (21) and in their previous work (16) indicate that the sequences 3′ to the V region are important in targeting and regulating V region hypermutation. This is consistent with the findings of others that there are important regulatory sequences in the intron between J and C (29) and associated with the C regions (19).

Tumas-Brundage and Manser have studied transgenes with a minimal TATA-containing heavy chain promoter or a non-Ig promoter that is from the gene that encodes the Ig-β polypeptide (45), a part of the complex through which membrane Ig receptors signal. This B29 promoter lacks a TATA box but shares at least six transcriptional regulatory elements, including one for Oct 2, with the Ig promoter and confers B cell specific expression, so it could recruit many of the same transcriptional factors as the Ig promoter (45). There is no evidence, however, that Ig-β chains undergo somatic mutation. With both the minimal Ig and the B29 promoters, V region somatic mutation occurs, though perhaps at a lower frequency (21). Tumas-Brundage and Manser also suggest that in the studies by Betz at al. (12) there may have been a lower frequency of mutation in the transgenes under control of the hemoglobin promoter. It may be difficult to make reliable quantitative comparisons between transgenes, or even between the transgenes and a comparable endogenous gene, because differences in expression could result in differences in selection and mutation. In fact, differences in expression could explain why B cells expressing the modified transgenic V region do not participate fully in the memory response (21). The important point is that mutation occurs in Ig genes under the control of a variety of promoters. We cannot be certain that a transcriptional factor, or factors, that normally interact with the Ig promoter is not also interacting with these variant promoters and recruiting some B cell and Ig gene specific factor that is required for mutation. Nevertheless, these studies, along with those of Betz et al. (12), suggest that the role of the promoter is non-specific in that transcription is required but there is not a particular regulatory sequence in the promoter that is necessary for mutation.

Tumas-Brundage and Manser have also placed a Drosophila intron as a spacer between the promoter and the V region exon to examine whether the 3′ border of mutation is determined by its distance from the promoter. Previous studies with endogenous V regions with different distances between the promoter and the V region suggested that the distance from the promoter determined the 3′ border of mutation (25). Tumas-Brundage and Manser found the highest frequency of mutations in the spacer and a some-

what lower frequency in its immediate 3′ region, which is now the displaced coding region of V (21). These findings confirm that mutation can occur in non-Ig sequences (41, 42) and suggest that the 3′ border of mutation is determined by its distance from the promoter.

The authors conclude that their results support an important role for transcription in V region hypermutation. However, it is still not obvious how transcription actually contributes to mutation. For example, although it is true that TFIIH is part of both the transcriptional initiation apparatus and of the apparatus that carries out nucleotide excision repair (reviewed in 43 and 44), the available evidence indicates that TFIIH is lost from the transcriptional process within 30–70 bases after initiation (44). It is possible that there is pausing of transcription at certain places in the V region, perhaps marked by the hot spot motifs, and that TFIIH, or a similar group of proteins, are then recruited to that site and carry out a process that is error prone because of some B cell– and Ig-specific factor (23). However, it still seems equally possible that the high rate of transcription is merely making the V region accessible to other factors that are not directly connected to transcription since a role for error prone replication has not been ruled out. This sort of argument is reminiscent of the discussions of the role of germline transcripts of the C region in isotype switching (46) which is still unresolved, though recent transgenic experiments suggest splicing of the germline transcript is a critical event in this process (47). It is clear that high rates of transcription per se are not sufficient for V region hypermutation since there is little mutation in T independent responses even though large amounts of Ig are produced (48).

The experiments described by Tumas-Brundage and Manser (21) demonstrate the benefits of using an in vivo system but also illustrate why it will be difficult to resolve the questions that are raised if only transgenic systems are used. These are very demanding experiments and it is virtually impossible in transgenic systems to do all of the controls and to examine the many different constructs that are required to identify the essential elements in these *cis*-acting sequences. Nevertheless, it is just those sorts of experiments that will be required to dissect out the role of particular *cis*-acting elements in transcription and mutation. This will require in vitro systems in which V region mutation occurs at the same high rates and through the same mechanisms as in vivo (19, 20). In the meantime, studies such as those reported by Tumas-Brundage and Manser (21) are contributing important information on normal mechanisms of V region hypermutation against which all in vitro systems will have to validated.

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