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SOMATIC HYPERMUTATION OF IMMUNOGLOBULIN GENES

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ABSTRACT

The relationship between somatic hypermutation and affinity maturation in the mouse is delineated. Recent work on the anatomical and cellular site of this process is surveyed. The molecular characteristics of somatic hypermutation are described in terms of the region mutated and the distinctive patterns of nucleotide changes that are observed. The results of experiments utilizing transgenic mice to find out the minimum *cis*-acting sequences required to recruit hypermutation are summarized. The hypothesis that V gene sequences have evolved in order to target mutation to certain sites but not others is discussed. The use that different species make of somatic hypermutation to generate either the primary or secondary B cell repertoire is considered. Possible molecular mechanisms for the hypermutation process and future goals of research are outlined.

INTRODUCTION

To find out the structural basis for the great functional diversity of antibodies (1) was an early focus of research in immunology. The answer was gradually revealed. The elucidation of the four chain structure (2) led to the question of how individual heavy and light chain molecules could vary so as to be able to recognize different antigens and yet maintain the same basic structure. Sequencing of monoclonal immunoglobulins obtained from patients with myeloma (3, 4, 5) showed that the amino-terminal domains of the polypeptide chains were heterogeneous, whereas the carboxy-terminal domains fell into a restricted number of classes. How could the genetic information for heavy and light chain molecules

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be encoded so as to produce such a structure? Was every antibody sequence represented in the germline or were there a limited number of germline elements that underwent somatic diversification?

Somatic hypermutation, which is the alteration of a germline immunoglobulin sequence by introduction of nucleotide changes during the lifetime of a B cell, was originally postulated to provide an answer to such questions and was conceived to be a mechanism that could contribute, in part or in entirety, to antibody diversity (6, 7).

The major outcome of the initial characterization of the immunoglobulin gene loci by molecular cloning was that the somatic recombination of different germline-encoded gene segments constituted a principal source of immunoglobulin diversity (8, 9). The first indications that antibodies contained differences from each other came from sequencing of ten λ light chains from mouse myeloma proteins (10). This revealed that six were the same (the putative germline sequence), whereas the others bore one or two differences. Cloning of the mouse $V_\lambda 1$ germline gene (11) allowed the definitive demonstration that these $V_\lambda 1$ sequences from myelomas contained differences from the germline and that somatic hypermutation had occurred. Other studies analyzing immunoglobulins produced by various mouse myelomas and plasmacytomas (12–15) reached similar conclusions.

Much of the early evidence that somatic hypermutation accompanied the affinity maturation of antibodies came from studies in the mouse, and this is the species on which most of this review is focused.

SOMATIC HYPERMUTATION AND AFFINITY MATURATION

During the course of an immune response, the affinity of serum immunglobulin for antigen typically increases (16–18). Early work showed that structural alterations occurred in the antibodies produced during an immune response (19), but their exact nature was not known.

Somatic hypermutation plays a central role in antibody affinity maturation and is in large part responsible for the production of the secondary repertoire (20–30). This secondary repertoire is constituted by cells, which show improved antigen binding characteristics, and which are themselves descended from B lymphocytes triggered by the initial antigen challenge. The nature of these changes has been systematically investigated by immunizing with several different model haptens, e.g. *p*-azophenylarsonate (23, 24), 2-phenyl-5-oxazolone (25–28), phosphorylcholine (29, 30), and 4-hydroxy-3-nitrophenylacetyl (31, 32).

Sequencing of the V genes from hybridomas producing antigen-specific antibody revealed two types of changes during the immune response. First, the immunoglobulin genes were usually expressed in mutated form with the degree of mutation increasing during the course of the response. For example, following immunization with 2-phenyl-5-oxazolone (27, 28), there were essentially no mutations at day 7 (following primary immunization), but the extent of mutation was such that by day 14 no identical sequences were found. Second, the repertoire of germline V genes used in the response shifted. Thus, at day 7 and to a lesser extent at day 14, a V_HOx1/V_κOx1 gene combination predominated. Following secondary immunization, V_H/V_L gene combinations other than V_HOx1/V_κOx1 were found with greater frequency. This phenomenon, designated a repertoire shift, has also been found in other systems (31–33).

Improvements in antibody–antigen binding affinity can be brought about by alterations to the kinetic parameters of binding, namely on-rate and off-rate. In the 2-phenyl-5-oxazolone system, improvements to the V_HOx1/V_κOx1 pair are frequently achieved by decreasing the off-rate. By contrast, the repertoire shift away from V_HOx1/V_κOx1 is characterized by moving to antibodies that exhibit a high on-rate. It is these latter antibodies and their derivatives that dominate the later stages of the response (34), although the combination of V_κOx1 with alternative V_H genes is also found. It may be that the deep antigen binding pocket in V_HOx1/V_κOx1 limits the effective on-rate and that antibodies with a different geometry would not be so hampered and could give rise to further improvements. However, many secondary response antibodies use V_κOx1 with different V_H genes, e.g. members of the MOPC21 or J558 gene families (28). Generalization as to what germline genes will constitute a potential high affinity antibody is, therefore, not possible because on-going mutation may alter, i.e. either improve or worsen, the binding.

The course of the response to 2-phenyl-5-oxazolone is accompanied by a 100-fold increase in the antibody affinity for the antigen (28). Despite the influence of repertoire shift mentioned above, a striking correlation emerges between stage of immunization (primary, secondary, or tertiary), degree of somatic hypermutation and increase in affinity. Direct proof that somatic hypermutation alone can be responsible for alterations in binding affinity has come from several different lines of evidence. V_κOx1 light chains bearing single mutations were allowed to combine with the unmutated V_HOx1 heavy chains (35), and affinities were measured. The substitution of His34 by glutamine or asparagine increases affinity by about tenfold. In an alternative approach, decrease in affinity for antigen was directly attributed to somatic hypermutation (36). Hybridomas producing an antibody of specific idiotype (normally with a high affinity for p-azophenylarsonate) were established, and those that had barely detectable

affinity for this antigen were selected. Extensive somatic hypermutation of the canonical sequence implies that this process is responsible for the abolition of antigen binding. A third line of evidence was derived from mutagenesis experiments. The germline gene V_H186.2 encodes most antibodies against the hapten 4-hydroxy-3-nitrophenyl acetyl in C57BL/6 mice. A point mutation that causes Trp33 to be changed to leucine occurs in most high affinity antibodies. This nucleotide substitution was introduced into a germline gene, and the affinity was thereby raised tenfold (37). Conversely when an antibody bearing this mutation as well as many others was mutagenized in order to restore the wild-type sequence, the affinity dropped.

CELLULAR ASPECTS

Germinal centers are located in the secondary lymphoid follicles in the peripheral lymph nodes of human and mouse. Their involvement in somatic hypermutation was suspected from the temporal association between their development and the maturation of the immune response.

About 4 days following immunization, B lymphocytes migrate to primary lymphoid follicles, where they proliferate. Germinal centers then develop during the ensuing 3–6 days (38). Histologically, germinal centers can be divided into a basal dark zone of proliferating centroblasts and an apical light zone containing centrocytes (the progeny of centroblasts) as well as a network of follicular dendritic cells able to hold antigen on their surface (39, 40). By 3 to 4 weeks, centroblasts and centrocytes can no longer be found.

Proof that mutation occurs in germinal centers has come from immunohistology (38), sequencing of antibody genes from B cells within and without germinal centers (41), and sequencing of antibody genes from individual germinal centers (42). The mutating population of human B cells has been further characterized by sorting of tonsillar cells for expression of various surface markers and then sequencing antibody genes within these populations (43). From this study, it has been inferred that hypermutation occurs in surface IgD^-, $CD23^-$ centroblasts, but not in their precursor cells which express these two markers.

Peripheral blood contains B cells of differing origins and maturity. Both newly produced lymphocytes that have just been selected from the bone marrow into the peripheral blood and memory B cells will be present. Analysis of human peripheral blood B cells fractionated according to surface expression of IgM and IgD (44) shows, as expected, that naive IgM^+IgD^+ cells express V_κ genes with virtually no somatic hypermutation, whereas memory cells (IgM^-IgD^-) bear highly mutated genes. The effect of the age of the animal on the degree of somatic hypermutation has been investigated in two systems. In mouse Peyer's patches, the proportion of lymphocytes that bear mutations increases gradually

from birth and peaks at 5 months of age (45). In the case of humans, mutated V gene sequences have been recovered from the tonsils of a four-year-old child (46).

The germinal center is the site of both B cell proliferation and selection. The interplay between these processes must be regulated to generate efficiently B cells that produce high affinity antibodies. It has been inferred from mathematical modeling that cycling between distinct phases of proliferation and selection is the best way to achieve this outcome (47). The rationale is that a large pool of cells is produced during the proliferative phase which will increase the likelihood that an advantageous mutation will be produced and subsequently selected. If this theory is correct, then roles can speculatively be assigned to the populations of B cells found in actual germinal centers (48). It may be that centroblasts in the basal dark zone represent the proliferating population in which there is ongoing somatic hypermutation and that selection occurs following the interaction between centrocytes and follicular dendritic cells in the apical light zone. A corollary to this view of events within a single germinal center is that B cells can go through multiple cycles of proliferation and selection. One would predict that the evolution of highly mutated clones from those bearing fewer mutations could then be discerned. This has been substantiated (49–51) by analysis of B cells picked individually from germinal centers.

THE MUTATIONS

The Mutation Domain

Extensive analysis of somatic mutations in antibodies has revealed that mutations are largely confined to the variable domains and are rarely found in the constant domains (51–55). While they are detected throughout the variable domain, it is mutations in the complementarity-determining regions (CDRs) (28, 56) that have most frequently been implicated in improved antigen binding—a finding consistent with the fact that it is the CDRs that play the major role in direct antigen contact.

Mutations largely occur over a region of one to two kilobases, around the rearranged V-J gene segments (52, 57). They do not usually extend into the C-region, although rare mutations in the mouse Cλ1 gene have been described (55). On this point it may be relevant that, at the mouse λ loci, the C genes are quite close to the J clusters.

The mutation domain extends from a 5′ site within the leader intron (58–61), although sequences upstream of the transcriptional start site do mutate at a lower frequency (57–59), and continues through the V gene well into the J-C intron (62). Mutations occur in the framework regions as well as the hypervariable

regions of the rearranged V gene segments (14). Support for the concept that the process is targeted to a specific region of DNA came from the finding that nonfunctional V genes also bear mutations (63). Although most nucleotides in or close to the V segment can mutate, the mutations tend to be found in the complementarity determining regions (58, 59, and see below) rather than being randomly spread out.

Nature of the Mutations

The nature of the mutations observed in antigen-selected B cells will be determined by (i) the mutations that were originally generated in the germinal center, together with (ii) selection by antigen for those B cells carrying mutations that confer a selective advantage. The effect of skewing by antigenic selection can be considerable. For example, most day 14 hybridomas producing 2-phenyl-5-oxazolone-specific antibodies and that use the V_HOx1/V_κOx1 combination carry mutations in the His34 codon of V_κOx1 (27). A similarly dominant mutation of Trp33 to leucine is found in the V_H genes of B cells selected in response to 4-hydroxy-3-nitrophenylacetyl (31). These mutations, which characteristically are both in complementarity determining regions, are rarely found when these V genes are selected by other antigens (62, 64, 65).

How can the intrinsic specificity of the mutational mechanism (i.e. removed from the skewing effects of antigen selection) be studied? There have been studies of mutations in V-region flanking sequences (58–62) where antigenic selection does not operate, and the presence of hotspots that do not cause amino acid replacements has been documented (28). More recently, the fact that the immunoglobulin transgenes can act as substrates for hypermutation (66) has been exploited to analyze the intrinsic features of the hypermutation mechanism. Information has been gleaned from experiments in which either the transgene does not contribute to the expressed antibody of the cell [and is acting as a silent passenger target for mutation (67)] or in which the transgene is contributing to the antibodies directed against a wide variety of antigens and in which there is, therefore, no dominant skewing by a single antigen (64, 65). Here we focus on those aspects of mutation that appear intrinsic as they may give clues to the molecular mechanism responsible for the mutational process.

Nucleotide Substitution Preferences

The mutations introduced during hypermutation are predominantly single nucleotide substitutions (rather than insertions or deletions) (14, 15, 52), although they show a slightly increased tendency to occur in clusters (68). Transitions are commoner than transversions and, furthermore, as assessed on the coding strand, G bases are mutated more often than C bases and A's more than T's. This implies that the hypermutation machinery can discriminate between the

two DNA strands—i.e. there is strand polarity (67). This pattern of base substitutions is not restricted to transgenes but has also been found in human V_H genes from splenic B cells (69)—a population selected by many antigens so that the effects of selection by individual antigens can be ignored. However, heterologous genes, i.e. nonimmunoglobulin, behave in a more complicated fashion (70). A bacterial *neo* gene showed the characteristic polarity observed for immunoglobulin genes, but bacterial *gpt* and human β globin genes did not.

Hotspots

Mutations are not targeted randomly along the length of the V gene. Some residues are frequently targeted (hotspots), others rarely (coldspots). Hotspots can be defined, at least in part, by local DNA sequence. A consensus sequence Pu-G-Py- (where Pu = [A or G] and Py = [C or T]) has been proposed (71) as a favored target for mutation. Indeed, three of the major intrinsic hotspots that have been identified in the V_κOx1 gene are all located in serine codons (Ser26, 31 and 77) that are encoded by AGPy triplets and conform to this consensus. The Pu-G-Py-$\frac{A}{T}$ consensus can of course be translated in different reading frames, and both glutamine and alanine codons have been found to be hotspots when they occur in the context of this sequence. More recently, analysis of intrinsic hotspots in transgenes that carry nonimmunoglobulin sequences in place of the V segment (70) has lent further support to the proposal that the Pu-G-Py-$\frac{A}{T}$ consensus sequences are favored targets for mutation. However, not all Pu-G-Py-$\frac{A}{T}$ sequences are mutational hotspots, and this consensus is clearly insufficient to define a mutational hotspot. Local DNA features such as palindromes or inverted repeats may well play an important role (28, 72, 73).

GERMLINE V GENE SEQUENCES HAVE EVOLVED TO ENCOURAGE TARGETING OF SOMATIC MUTATION

Given that the hypermutation mechanism is intrinsically nonrandom and given also that it is likely to be more useful to target mutations during antibody affinity maturation to some parts of the antibody molecule (e.g. CDRs) rather than others (e.g. frameworks), it might be that V gene sequences have evolved so that hotspots are strategically located. This appears to be the case. The amino acid serine is unusual in that it is encoded by two types of triplet AGPy (that is, AGC and AGT) and TCN (that is, TCA, TCC, TCG, and TCT). The sequence AGPy conforms to the hotspot consensus; TCN does not. This provides a test of whether codon usage has evolved so as to favor local targeting of hypermutation. Combining these pieces of information suggests the hypothesis that one way of ensuring that serine codons in CDRs are more frequent targets for mutation than serine codons in frameworks would be to favor AGPy triplets for CDR

serines and TCN triplets for framework serines. This is indeed the case (74). Significantly, in the case of T cell receptor V genes, no equivalent skewed usage of serine codons is observed (74). (Although mutation may occur at low frequency in T cell receptor Vβ (but not Vα) genes (75), there is no evidence of somatic hypermutation playing any role in their functional diversification.) Thus, it appears that the codon usage of immunoglobulin genes has been selected during the course of evolution so that the most mutable residues are in positions where alterations will be most likely to lead to improved affinity.

Interestingly, there are conserved AGPy serines codons in framework 3 of both V_H and V_κ genes. The significance of this is uncertain, but given that experimental alterations to sites outside the CDRs can alter antibody affinity (76, 77), an intriguing possibility is that the rare conserved AGPy triplets in the framework regions have similarly been selected for their mutability. It appears that biased serine codon usage may be a general feature of antibody molecule design, because such a usage is also found in *Xenopus* V_H genes (78).

DNA SEQUENCES REQUIRED FOR RECRUITING HYPERMUTATION

Somatic hypermutation is restricted to the region around the rearranged V gene. Mutation is rarely observed in unrearranged V genes (14, 79), and only a low frequency of mutations has been observed in incompletely rearranged heavy chain loci bearing DH-JH integrations (80). Most of what is known about the sequences necessary to recruit hypermutation to the immunoglobulin V gene comes from studies of modified immunoglobulin transgenes.

Enhancer Elements

Most work has been carried out on the mouse κ light chain locus. Immunoglobulin κ transgenes mutate at a low rate (66). This suggested that transgenic animals would provide a useful approach to finding the minimal *cis*-acting sequences necessary for full hypermutation. Certain immunoglobulin κ transgenes hypermutate in a way similar to endogenous κ genes as regards both the type and the frequency of mutation (67, 81, 82). Both the transcription enhancer elements in the κ locus (the 3′-enhancer and the intron-enhancer/matrix attachment region) are necessary for effective hypermutation. The intron-enhancer/matrix attachment region may be the more important of the two because its removal abolishes detectable mutation, whereas constructs lacking the 3′-enhancer continue to mutate although at greatly reduced frequency (64). The other transcriptional regulatory element in the locus, the V_κ promoter, does not appear to contain specific sequences necessary for recruiting hypermutation. Transgenes carrying a β-globin promoter in place of the V_κ promoter continued to mutate (64).

However, such studies do not exclude the possibility that an active promoter is required for hypermutation.

Less is known about the regulatory elements in the heavy chain and λ light chain loci. Although mutation is readily observed in V_H (29, 31, 69) and Vλ (62) genes encoded by the endogenous loci, little success has been obtained so far in obtaining hypermutation from constructs bearing rearranged IgH or λ genes (83–85). Presumably, this reflects an absence of necessary *cis*-acting DNA sequences from the constructs that have so far been tested, although the presence of inhibitory sequences obviously cannot be excluded. In the case of one of the rearranged IgH transgenes (84), mutation of the transgenic V_H segment was detected in hybridomas in which *trans*-switching appeared to have recombined the transgenic V_H into the endogenous IgH locus. This observation supports the idea that the transgene may lack sequences located toward the 3′-end of the IgH locus necessary for proper hypermutation. In contrast to the failure with rearranged IgH transgenes, mutation (albeit probably at low frequency) has been obtained using transgenic IgH mini-loci in which the transgenic V_H segments undergo productive rearrangement to yield an antibody repertoire (86, 87). The reason why limited success is obtained here in contrast to failure with the rearranged IgH genes is unresolved.

The V Domain

Although the V gene is of course the target of hypermutation, several experiments have been conducted to ascertain whether it is required for recruiting hypermutation or whether the hypermutation mechanism can target heterologous sequences in place of the V (88–90). Recent experiments (70) have clearly revealed that the bulk of the V segment is not necessary for recruiting hypermutation. Different constructs lacking the leader, the leader-V intron, or the majority of the V gene segment up to the J segment were all effective as targets for hypermutation. Furthermore, heterologous sequences used to replace the V (a segment of the human β-globin gene or bacterial *neo* or *gpt* genes) all acted as substrates hypermutation. Indeed, the pattern of mutation (i.e. a predominance of nucleotide substitutions and a preference for transitions) in these heterologous targets was similar to that obtained with a V gene although polarity was less marked (70). These heterologous sequences also showed the characteristic nucleotide hotspots (conforming to the Pu-G-Py-$\frac{A}{T}$ consensus). Thus, although the V domain has evolved to act as a hypermutation substrate (see above), it does not actually contain sequences necessary for the recruitment of hypermutation.

In conclusion, the only elements in the immunoglobulin loci known to be required for recruiting hypermutation are in the region of the transcription enhancers. No evidence exists, however, that these elements are sufficient; and

the identification of the minimum DNA essential for recruiting hypermutation to a heterologous gene awaits definition.

PHYLOGENETIC ASPECTS

In human and mouse, somatic hypermutation seems to be implicated only in the production of the secondary, affinity-matured, antibody repertoire. However, this does not imply that hypermutation arrived late in evolution. Indeed, it contributes to antibody diversification in other mammals, e.g. in sheep (91), probably in rabbits (92), and in frogs (78, 93). There are also strong indications of its importance in even more primitive organisms such as members of the shark family (94).

The involvement of hypermutation in the generation of antibody repertoires in chicken is not fully resolved; the analysis is complicated by the extensive use of gene conversion in this species (97, 98). [Although the possible involvement of gene conversion in antibody diversification in human and mouse has been discussed (97, 98), there is evidence that it does not play a significant role (64, 99). For example, the variability seen in the germline V_κOx family is quite distinct from that seen in mutated V_κOx1 genes (64). An exception, however, is the case of a transgene, designed to favor gene conversion, which contains two closely linked V genes (100).]

The role of somatic hypermutation may have diverged in different species. In human and mouse, hypermutation is involved in the formation of the secondary antibody repertoire, whereas in sheep it appears to play a major role in the formation of the primary repertoire (91). This process occurs in the ileal Peyer's patches, and the extent of accumulated mutations is not reduced by attempts to exclude external antigen (91). Thus, somatic hypermutation may not always be intimately linked to antigen selection. Indeed, in primitive vertebrates such as *Xenopus* (93) and the cartilaginous fish (94, 101), hypermutation appears to occur, but the selection of B cells producing high affinity antibodies seems less developed than in mouse. It is noteworthy that germinal centers have not yet been identified in these species.

Hypermutation may first have arisen to diversify the primary immunoglobulin repertoire when only limited combinatorial diversity was available. For example, a member of the shark family, the little skate (*Raja erinacea*), contains light chain genes that are already joined (i.e. V-J integrated) in the germline. There is obviously no combinatorial or junctional diversity in this system, and yet hypermutation may well occur (102). Further support for the antiquity of the hypermutation mechanism is provided by the novel (non-immunoglobulin and non-T cell receptor) antigen receptor genes found in the nurse shark, which undergo extensive somatic hypermutation (103).

It has been noted (104), in a comparison of the protein coding sequences of human and mouse, that (similar to the hotspots associated with somatic hypermutation—see Germline V Gene Sequences Have Evolved To Encourage Targeting of Somatic Mutation), AGPy codons are more mutable than TCNs. This suggests that there are features common to evolutionary and somatic hypermutation. One can synthesize the following argument: Somatic hypermutation was initially adapted from a basic cellular mechanism in order to diversify the primary antibody repertoire, which was restricted by lack of combinatorial diversity (105). In such a situation, a strong selective pressure existed for immunoglobulin V gene sequences to evolve in order to ensure that mutation was preferentially targeted to regions whose alteration would be likely to lead to useful diversification. In some more recently evolved species, the hypermutation mechanism has a minor or no role in primary diversification but is more important in affinity maturation, where it occurs in cooperation with a specialized cellular selection machinery.

MECHANISMS FOR SOMATIC HYPERMUTATION

Little is known of the mechanism of somatic hypermutation. The features that must be accounted for in any model of the process are: (*a*) its occurrence during only a short period of B cell development; (*b*) targeting to the rearranged V gene; (*c*) dependence on both the 3′ and intron enhancers; (*d*) the mutation domain starting downstream of the promoter and extending well into the J-C intron; (*e*) base substitution preferences, and (*f*) strand polarity.

Various mechanisms for somatic hypermutation have been proposed, for example, gene conversion (97, 98), replication (106) and lag strand DNA synthesis (107). We focus on a further proposal that envisages a role for error-prone DNA synthesis (7) in somatic hypermutation. Indeed, several of the DNA polymerases involved in DNA repair lack the proofreading 3′-to-5′ exonuclease activity and therefore manifest a higher degree of misincorporation. Clearly, any model that involves the introduction of mutations not only requires errors/changes to be made to the DNA, but, if these errors are made on only one strand (as, for example, in DNA synthesis or repair), then the mismatches generated must not be repaired back to the original i.e. germline, sequence.

The requirement for the immunoglobulin κ enhancers for full hypermutation draws attention to a possible linkage between hypermutation and transcription. A further association between transcription and a specific form of DNA repair, i.e. nucleotide excision repair, suggests a model for somatic hypermutation involving the transcription factor complex, TFIIH, whose normal function is the recruitment of RNA polymerase II (108, 109). If, for example, sequences in the immunoglobulin locus (e.g. in the intron enhancer/matrix attachment region)

initiate local or regional single strand nicking, one can envision the TFIIH/RNA polymerase II complex detecting these nicks on the template strand and recruiting an error-prone DNA polymerase. Such linkage with transcription could explain not only the requirement for the enhancer elements, but also the strand polarity and the fact that the hypermutation domain starts downstream of the promoter.

One of the chief problems in elucidating the mechanism of somatic hypermutation has been the absence of an in vitro system for analysis. Ongoing mutation of immunoglobulin V genes has been observed in B cell follicular lymphomas (110), although hypermutating cell lines derived from such lymphomas have not yet been described. Mutations have been detected during propagation of some mouse myelomas and pre–B cell lymphomas (111, 112), and it will be intriguing to ascertain whether the mutations detected in these tumors arose through a process akin to antibody somatic hypermutation. It may be that the recent improvements in the techniques of culturing and differentiating B lymphocytes in vitro (113, 114) will lead to effective systems for the study of hypermutation.

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