Mismatch Repair Co-opted by Hypermutation

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Mice homozygous for a disrupted allele of the mismatch repair gene *Pms2* have a mutator phenotype. When this allele is crossed into quasi-monoclonal (QM) mice, which have a very limited B cell repertoire, homozygotes have fewer somatic mutations at the immunoglobulin heavy chain and λ chain loci than do heterozygotes or wild-type QM mice. That is, mismatch repair seems to contribute to somatic hypermutation rather than stifling it. It is suggested that at immunoglobulin loci in hypermutable B cells, mismatched base pairs are "corrected" according to the newly synthesized DNA strand, thereby fixing incipient mutations instead of eliminating them.

Somatic point mutations are introduced at a high rate into the exon encoding the variable (V) region of an immunoglobulin (Ig) and into its flanking sequences (*1*). The process of somatic hypermutation at the Ig loci is a site-specific, differentiation stage–specific, and lineage-specific phenomenon that contributes to the generation of antibody diversity and affinity maturation (*2*). It has often been speculated that DNA repair has something to do with hypermutation (*3*). In one sense, this speculation is tautological; clearly, if all introduced mutations were repaired, no mutations would be seen. One possibility is that the Ig mutator system might deactivate some or all of the error-free repair systems that normally function in eukaryotic cells. A second possibility, however, is that some error-free repair systems might be modified in such a way that they actually introduce mutations rather than correct them. Here we report experiments that demonstrate that the latter hypothesis is true for at least one repair system.

Using the working hypothesis that the Ig mutator system initially introduces mismatches during the replication of one DNA strand, we have studied the role of mismatch repair in hypermutation. The *mutS-mutL* mismatch repair pathway in *Escherichia coli* has a broad specificity, and its inactivation results in a mutator phenotype (*4*). Human homologs of *mutS* and *mutL* have been implicated in hereditary nonpolyposis colorectal cancer (*5*). Mice homozygous for a gene-targeted defective allele of *Pms2*, a *mutL* homolog, develop tumors more frequently than do wild-type mice, and homozygous males exhibit abnormal chromosome synapsis in meiosis (*6*).

We developed the quasi-monoclonal (QM) mouse for studying Ig diversification by somatic hypermutation as well as by other known and unknown mechanisms (*7*). QM mice are heterozygous at the heavy (H) chain locus: On one allele, the stretch of DNA containing the J_H segments has been replaced by a targeted, rearranged V_HDJ_H exon encoding an H-chain V region bearing the 17.2.25 idiotype (*7*); the other allele is nonfunctional because of a targeted deletion of all J_H gene segments (*8*). Because QM mice are also homozygous for a gene-targeted deletion of J_κ (*9*), they produce only λ-type light (L) chains. When combined with any λ chain, H chains with an unmodified V exon can be detected by an antibody to the idiotype (*7*, *10*).

To study the role of the mismatch repair gene *Pms2* in somatic hypermutation, we first crossed the targeted "knockout" allele $Pms2^{ko}$ (*6*) into mice with the QM genotype. We will refer to these mice by their configuration at the *Pms2* locus: $Pms2^{ko}/Pms2^{ko}$ (or *ko/ko*) for homozygous, DNA-mismatch repair–deficient, QM mice, +/+ for standard repair–proficient QM mice, and $Pms2^{ko}/Pms^{+}$ (or *ko*/+) for heterozygous QM mice. The configurations at all Ig loci are the same for all three types of mice.

Several properties of the QM mouse must be kept in mind in order to understand and interpret the experiments to be described here. (i) The number of germline elements contributing to the primary Ig repertoire—the Ig repertoire developed after

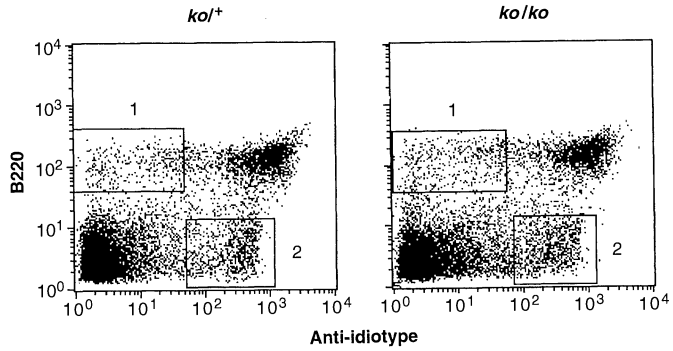

Fig. 1. Flow cytometric analysis of peripheral blood lymphocytes from QM mice heterozygous (*ko/+*, left) or homozygous (*ko/ko*, right) for the disrupted $Pms2^{ko}$ allele. Ordinates, fluorescein isothiocyanate–conjugated rat monoclonal antibody to B220 (Pharmingen 01124A); abscissae, biotin-coupled antibody to 17.2.25 V_HDJ_H idiotype (monoclonal rat antibody R2.438.8), followed by phycoerythrin-coupled streptavidin (Pharmingen 13025D). Peripheral blood lymphocytes were prepared from total blood by hemolysis in a standard NH_4Cl lysis buffer. Incubations with the antibodies were done for 30 min at 4°C before analysis and sorting. Dead cells were excluded by propidium iodide staining. Boxes 1 and 2 indicate the windows used for sorting.

Fig. 2. Somatic mutations in a 21-nt sequence contained in J_H4 alleles derived from B220-positive, idiotype-negative B cells of QM mice (+/+, left) or QM mice homozygous for the disrupted $Pms2^{ko}$ allele (*ko/ko*, right). The sequence starts with the triplet CCT, which represents the N region of the knocked-in gene. Sequences were determined as described (*6*, *7*).

+/+

	Pro	Tyr	Tyr	Ala	Met	Asp	Tyr
	CCT	TAC	TAT	GCT	ATG	GAC	TAC
1	---	C--	---	T--	---	---	---
2	---	---	C--	---	---	---	---
3	---	---	---	A--	---	---	---
4	---	---	---	A--	---	---	---
5	---	---	---	-G-	---	---	---
6	---	---	---	-T-	---	---	---
7	--C	-TT	---	T--	---	---	-T-
8	---	---	---	---	---	---	---
9	---	---	---	---	T-T	---	---
10	---	---	---	---	---	---	---
11	---	---	---	-G-	---	---	---
12	---	---	C--	---	---	---	---
13	--C	---	-T-	-G-	T--	--	
14			-	---	---	---	---
15			-	---	---	---	---
16				--	---	---	---

ko/ko

	Pro	Tyr	Tyr	Ala	Met	Asp	Tyr
	CCT	TAC	TAT	GCT	ATG	GAC	TAC
1	---	---	---	---	---	---	---
2	---	---	---	---	---	---	---
3	---	---	---	---	--		
4	---	---	--C	---	---	---	---
5	---	---	---	---	---	---	---
6	---	---	---	---	---	---	---
7	---	---	---	---	---	---	---
8	---	---	---	---	---	---	---
9	---	---	---	---	---	---	---
10	---	---	---	---	---	---	---
11	---	---	---	---	---	---	---
12	---	---	---	---	---	---	---
13	---	---	---	---	---	---	---
14	--C	---	-T-	-TC	---	---	---
15	---	---	---	---	---	---	---
16	---	---	---	---	---	--	
17	---	---	---	---	---	---	---
18	---	---	---	---	---	---	---
19	---	---	---	---	---	---	---
20	---	---	---	---	---	---	---
21	---	---	---	---	---	---	---
22	---	---	---	---	---	---	---
23	---	---	---	---	---	---	
24						---	---

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standard V(D)J rearrangement—is highly limited in QM mice; only one VH, one D, one J_H, three V_λ, and three J_λ are involved. This property is an advantage for identifying mutations, because the germline sequences are known. (ii) Because of the low diversity of the primary repertoire, there is an enormous selective pressure for Ig diversification. Thus, V-gene replacements, which would ordinarily be of little consequence, become frequent. This property is an advantage for studying V-gene replacement, but it negates

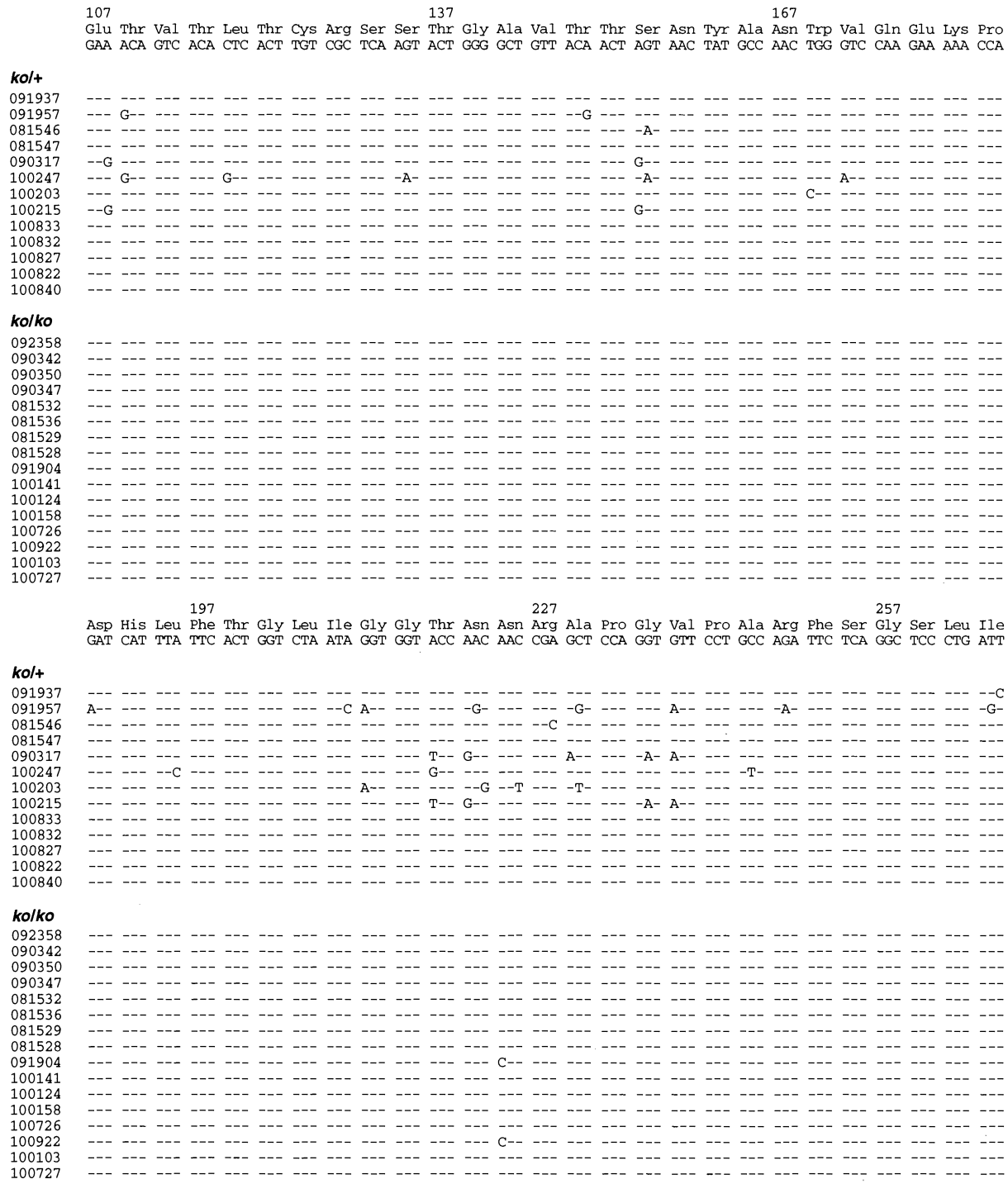

Fig. 3 (above and on facing page). Comparison of the λ_1 alleles derived from B220-negative, idiotype-positive B cells (window 2 in Fig. 1) of QM mice heterozygous (*ko/+*, upper set) or homozygous (*ko/ko*, lower set) for the disrupted *Pms2*^{ko} allele. The 317-nt sequences contain most of Vλ_1. The contiguous cDNA sequence is numbered starting with 1 at the first nucleotide of the ATG start codon. Nucleotide identity is indicated by a dash (-); a blank indicates not read. PCR amplification was done from cDNA with 5 pmol of the Vλ_1 5′ primer 5′-GGAATTCCTGCACTCACCACATCACCTGG and Cλ_1 3′ primer 3′-GGATCCTACCTTCCAGTCCACTGTCACC. The reactions were done in a total volume of 50 μl with 2.5 U of Taq polymerase and 1× PCR buffer with 1.5 mM $MgCl_2$ and 200 μM dNTPs. The program consisted of 40 cycles of 1 min at 94°C, 1 min at 59°C, and 2 min at 72°C. The last cycle included a prolonged extension step (10 min) to favor the A addition necessary for cloning the PCR fragments into the Invitrogen pCR 2.1 vector. Double-stranded DNA was prepared from clones and sequenced.

part of the advantage of the limited primary repertoire for studying hypermutation. (iii) With flow cytometry, we can identify several B cell populations in the peripheral blood of QM mice (*7*, *11*). A naïve IgM-positive population has never been activated, and the Ig loci harbor few if any somatic mutations. Another population, which falls within window 1 (Fig. 1), consists of idiotype-negative cells, most of which have modified their V_HDJ_H exon by V_H gene replacement and somatic hypermutation; this population contains a substantial proportion of cells expressing IgM (*7*, *11*). A third population, which falls within window 2 (Fig. 1), consists of idiotype-positive cells, most of which (>95%) have switched their H-chain isotypes and show numerous somatic mutations in their V_HDJ_H exons (*12*). (iv) These mutations have been selected in response to environmental antigens. As with the V-gene replacements, they result from a strong selective pressure due to the limited primary repertoire—a phenomenon that has been termed hyperselection (*11*). This means that Ig genes encoding antibodies against a whole panoply of antigens are studied, rather than those against a single antigen as in conventional immune response experiments.

With flow cytometry, we compared the cell population profiles of four *ko/ko* and four *ko/+* QM mice with fluorescence-labeled antibodies specific for the following pairs of markers: B220 versus the idiotype, μ^a versus the idiotype, B220 versus λ, and μ^a versus λ. In addition, another four *ko/ko* and four *ko/+* QM mice were analyzed only with the μ^a-versus-idiotype pair of reagents. No appreciable qualitative or quantitative differences among the two genotypes were apparent. Figure 1 shows a typical pattern with the B220-versus-idiotype pair.

In a pilot experiment, we compared the H-chain alleles from sorted idiotype-negative B cells of *ko/ko* and +/+ QM mice. From the sorted cells we isolated mRNA and reverse-transcribed it to get cDNA, then amplified H-chain sequences by polymerase chain reaction (PCR) and cloned and sequenced them. Because the V_H segments from gate 1 cells all differ, and the D segments are too near to the recombination breakpoints resulting from the V_H gene replacement process, we analyzed a short 21-nucleotide (nt) stretch of J_H4 sequence, which is shared by all alleles (Fig. 2). In the +/+ mice, there were 20 mutations in a total of 306 nt, yielding a frequency of 6.5%; in the *ko/ko* mice, there were 5 mutations in 478 nt, yielding a frequency of 1.0%. That is, mice deficient in the mismatch repair enzyme actually had a lower mutation frequency than did wild-type mice. This seemingly paradoxical result suggested to us that mismatch repair is a component rather than an antagonist of the Ig hypermutation system.

In a similar experiment, we analyzed the preformed V_HDJ_H exon in idiotype-positive cells. Because this population has not undergone V_H replacement, we could analyze a longer sequence per allele—310 nt covering part of V_H, all of D, and part of J_H (*7*). Alleles from the +/+ mice had 112 mutations in a total of 3086 nt, yielding a frequency of 3.6%; the *ko/ko* mice had 2 mutations in 1240 nt, or 0.16%.

We also investigated the λ_1 L-chain locus of *ko/ko* and *ko/+* QM mice in the idiotype-positive cell population defined by window 2 (Fig. 1). We sorted peripheral blood B cells from pools of six mice of each genotype, isolated mRNA, reverse-transcribed it into cDNA, then amplified λ_1 L-chain sequences, which were cloned and sequenced (Fig. 3). The sequences cover a stretch of 237 nt covering most of $V\lambda_1$; they have been truncated well short of the VJ junction to avoid any chance of confusion with junctional diversity (*13*). In *ko/+* QM mice, there were 49 mutations in 3056 nt, yielding a frequency of 1.6%. In *ko/ko* QM littermates, there were only 7 mutations in a total of 3675 nt, yielding a frequency of 0.2%. Thus, the absence of mismatch repair also leads to a decrease in hypermutation at the λ L-chain locus.

In other similar experiments we also examined λ_1 sequences obtained from idiotype-negative cells. In the same 237 nt stretch of $V\lambda_1$ used above, there were 21 mutations in 4977 nt (a frequency of 0.4%) in *ko/+* QM mice and only 2 mutations in 4392 nt (a frequency of 0.04%) in *ko/ko* QM mice. The lower frequency of mutations in the idiotype-negative cells was not unexpected. Because the idiotype-positive cells have retained the gene-targeted V_HDJ_H exon, antigen selection pressure ought to be stronger on the λ gene and thus result in a higher mutation frequency; indeed, this seems to be the case.

As stated above, it is obvious that if all pre-mutations introduced by the Ig mutator system were repaired, there would be no

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                                        287                                                 317
          Gly Asp Lys Ala Ala Leu Thr Ile Thr Gly Ala Gln Thr Glu Asp Glu Ala Ile Tyr Phe Cys Ala Leu Trp Tyr
          GGA GAC AAG GCT GCC CTC ACC ATC ACA GGG GCA CAG ACT GAG GAT GAG GCA ATA TAT TTC TGT GCT CTA TGG TAC
ko/+
091937    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
091957    --- --- --- --- --- --- --- --- --- --- --- -G- --- --- --- --- --- --- C-T --- --- --- --- --- --- ---
081546    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
081547    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- -C- --- --- --- --- ---
090317    --- -G- G-- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
100247    --- --- --- --- --- -A- --- --- --- --- --- --- --- --- --- --- --- --
100203    --- --- --- --- --- --- --- --- --G --- --T --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
100215    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --A --- --- --- --- --- --- --- --- --- ---
100833    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
100832    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
100827    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
100822    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
100840    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
ko/ko
092358    --- --- --- --- A-- --- --- G-- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
090342    --- --- --- --- --- --- --- --- --- --- --- --- --A --- --- --- --- --- --- --- --- --- --C --- --- --- ---
090350    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
090347    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
081532    --- --- --- --- --- --- --- --- -
081536    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
081529    --- --- --- --- --- --- --- --- --- --- ---
081528    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
091904    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
100141    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
100124    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
100158    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
100726    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
100922    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
100103    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --
100727    -A- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
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somatic hypermutation. Perhaps the simplest way for the Ig mutator system to avoid that scenario would be to overwhelm the DNA repair system with so many mutations that they could not all be repaired before becoming fixed. But if that were the case, then $Pms2^{ko}/Pms2^{ko}$ mice should have a higher frequency of mutations at the Ig loci, as they have at other loci (*6*, *14*); in fact, the frequency is lower at the Ig loci. Another simple strategy for the Ig mutator system would be to throw a monkey wrench into the works to turn off or otherwise ensure that DNA repair was ineffective in hypermutable B cells. But if that were the case, then $Pms2^{ko}/Pms2^{ko}$ mice should have the same frequency of mutations at the Ig loci; in fact, the frequency is lower. Yet another strategy would be for the Ig mutator system to co-opt the DNA repair system to subvert it to create rather than prevent mutations. The third strategy would seem to be the only one that would explain the results of the experiments described here. Of course, the above argument applies only to a mismatch repair system requiring *Pms2*. How the Ig mutator system deals with other DNA repair pathways can only be discovered by experimentation.

The prototypic mismatch repair system in *E. coli* corrects the newly synthesized DNA strand, which is transiently unmethylated (*15*), using the old methylated DNA strand as a template. In eukaryotic cells, the basis for strand repair bias is not well understood, although it may involve single-strand breaks (*16*). We propose the following mechanism for the action of *Pms2* at the Ig loci: After mismatches have been introduced at an Ig locus in hypermutable cells by an unknown mechanism, the mismatch repair system identifies the "wrong," mutated strand as a template and thus fixes the mutations. In mice without mismatch repair, this model, in its simplest version, predicts that at the next replication the old strand will give rise to a nonmutant allele, whereas the new strand will give rise to an allele with one or more mutations. Thus, the frequency of mutations would be reduced by one-half. Because we found rather lower mutation frequencies in the absence of repair than would be predicted by this basic model, it might require some elaboration. For example, other repair mechanisms might correct mismatches in the absence of the repair system involving *Pms2*, and this would reduce the mutation frequency to less than one-half.

It has been reported that many human tumors exhibit high mutation rates (*17*). We envisage that the co-option of mismatch repair that we have described here for Ig hypermutation may also play a role in some of these tumor phenotypes. That would require, however, that the co-option not be limited to Ig genes but have a broad scope of action.

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Pleiotropy and the Preservation of Perfection

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A mathematical model is presented in which a single mutation can affect multiple phenotypic characters, each of which is subject to stabilizing selection. A wide range of mutations is allowed, including ones that produce extremely small phenotypic changes. The analysis shows that, when three or more characters are affected by each mutation, a single optimal genetic sequence may become common. This result provides a hypothesis to explain the low levels of variation and low rates of substitution that are observed at some loci.

Many continuously varying phenotypic characters are subject to stabilizing selection, so that the optimal phenotypic value lies between the minimum and maximum possible values (*1–7*). These phenotypic characters can be anything from the circumference of the stem of a plant to the distance between two subunits within a protein. Models of stabilizing selection often allow for a continuous range of mutations, so that some mutations have very small effects, whereas others have substantial effects (*2*, *3*, *8–14*). We follow this approach in the present study. Analyses of stabilizing selection have concentrated on models for which any given mutation affects only one phenotypic character. Nevertheless, mutations that affect multiple characters are well known and are commonly regarded as ubiquitous (*5*, *14–25*). Here, we show that, when three or more characters are affected by each mutation, a single optimal genetic sequence may become predominant. This finding contrasts sharply with the usual finding that, in equilibrium, the optimal sequence is rare and many slightly suboptimal sequences are present.

Consider a simple nonpleiotropic model of viability selection in a very large population of haploid and asexual organisms (the results are expected to generalize to sex and diploidy). Parents produce offspring and then die, so that generations are discrete. After birth, offspring undergo viability selection, and the probability that an individual will survive depends on phenotype, which is described by measurements on k different characters. An individual's measurement on the ith character is denoted by z_i (where $-\infty < z_i < \infty$). These characters are chosen so that they affect fitness independently, and $z_i = 0$ is the optimal value for each character.

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