among species and populations can be understood as adaptations. But at some stages of evolution certain characters effectively "click in" and remain fixed in the descendent group of species (*5–7*). For instance, the chorda dorsalis (the embryonic precursor of our vertebral column) is absent in invertebrates, variably present in the relatives of vertebrates (ascidians and related groups) and absolutely fixed in vertebrates. The first who most clearly saw a connection between this pattern and increasing complexity was Rupert Riedl in the 1970s (*6*). He postulated that with increasing complexity some characters become more important because more and more new characters are functionally or developmentally predicated on them. Once such characters have accumulated many "responsibilities," mutational change will be detrimental and thus these characters become evolutionarily fixed. This increasing burden leads to fixation of characters. The problem with this view, however, was that it did not connect well with the then current population genetic theory.

Standard population genetic theory supports a liquid genome metaphor. In the balance between mutation and selection, each population settles into a state in which the most fit genotype is always surrounded by a sizable swarm of mutant genotypes buzzing around the best genotype (*8*), so much so that the concept of wild-type becomes meaningless. Variation is the name of the game. Only the amount of variation depends, in a continuous manner, on the relative strength of stabilizing selection, genetic drift, and mutation. Well, not exactly, according to the report by Waxman and Peck (*1*), which shows that there is a complexity limit beyond which genes can freeze into a fixed state and where the swarm of genetic variation suddenly disappears like fog in the sun. In the Waxman-Peck model, the complexity limit is reached once the genes affect more than two characters that are under simultaneous stabilizing selection.

To be precise, this freezing phenomenon has been described before (*9*), but it was seen as an arcane result of mathematical population genetics of uncertain significance and familiar to only a very few specialists. The significance of the present report is that Waxman and Peck have shown that this obscure property of mutation-selection equations has a connection to a generic property of organisms: complexity. Each gene has many effects and functions, each character is functionally connected to multiple others. Since this is the case, the freezing of genetic and phenotypic states is a necessary outcome, just as many organismal biologists have suspected for more than a century.

This sounds great and simple, but nothing in science is ever really simple. There is always the question whether the models are producing artifacts rather than pointing to fundamental insights. There is also no definite empirical proof as to whether the Bauplan concept is a perceptual artifact or a real pattern. Both are empirical questions that need to be settled. What makes the result by Waxman and Peck nonetheless exciting is that new emergent phenomena can be discovered that are not obvious from the study of simple models. More complexity is not just more of the same, but can lead to qualitatively new phenomena. This has long been know to physicists, but there are only a handful of examples where "complexity effects" were described in population genetic models (*10*). These and the report by Waxman and Peck show the need to study the population genetic theory of complex adaptations as a separate problem.

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IMMUNOLOGY

Fixing Mismatches

Michele Shannon and Martin Weigert

Complex biological processes evolve by co-opting bits and pieces of preexisting cellular machinery and using them for new purposes. On page 1207 of this issue, Cascalho *et al.* (*1*) provide a new example of this strategy in the case of somatic mutation of antibody variable (V) genes. The mutation of V genes is the key mechanism by which the body develops and modifies the antibody repertoire, the huge diversity of antibodies that allows us to keep pace with emerging and antigenically altered pathogens. V gene mutation differs from spontaneous mutation in two ways: The rate is extremely high (six to seven orders of magnitude higher), and luckily, this hypermutation is confined to a region in and around expressed V genes. Cascalho *et al.* have discovered that a protein ordinarily involved in correcting mutations is involved in causing V gene hypermutation.

Somatic hypermutation is not the first immunological process to co-opt ubiquitous proteins. The other key immunological process for generating diversity, V(D)J recombination, also evolved by using this strategy (*2*). Rearrangement of variable (V), diversity (D), and joining (J) gene segments to produce functional antibody and T cell receptors requires the lymphocyte-specific RAG-1 and RAG-2 (the products of recombination activation genes). In and of themselves, though, the RAG proteins cannot complete the process of gene recombination; instead, the RAGs primarily recognize specific sequences and nick the DNA. Ubiquitous factors then take over to complete the recombination process. Many of these co-opted factors have been identified by educated guesswork and experiments with mouse strains impaired in V(D)J recombination. For example, the *scid* mutation renders mice both radiation-sensitive and recombination-deficient (*3*). The connection between these two phenotypes became obvious when the *scid* mutation was shown to affect DNA-dependent protein kinase, part of the machinery for repairing breaks in double-stranded DNA (*4*). Thus, to accomplish V(D)J recombination, the immune system co-opted DNA repair machinery.

The new studies by Cascalho *et al.* demonstrate that DNA repair machinery has been enlisted by the immune system once again—this time for V gene mutation. The authors investigated whether a mouse genetically engineered to lack a DNA repair function is also defective in somatic hypermutation. This candidate gene strategy

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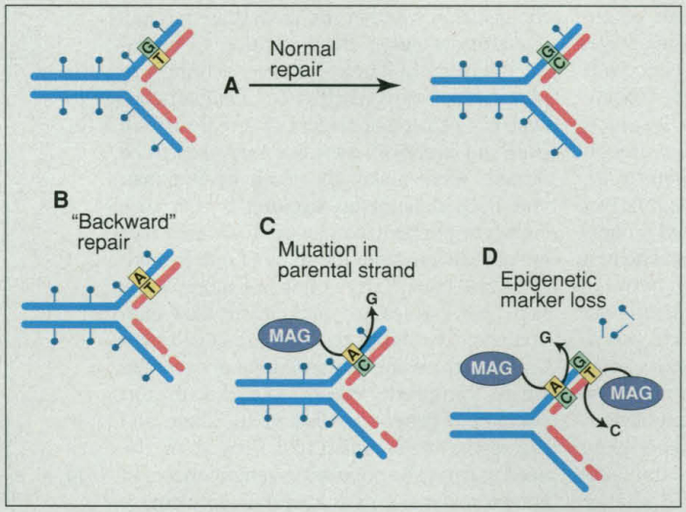
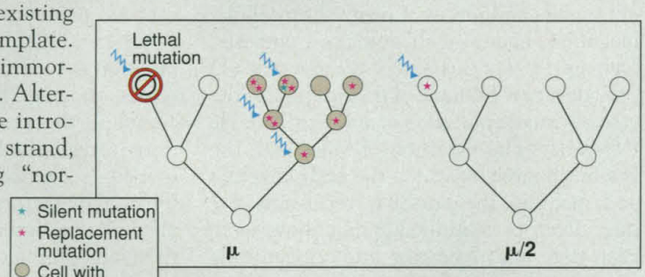
yielded the exciting finding that the somatic hypermutation frequency in the mismatch repair–deficient mouse, the Pms2-/- mouse, is substantially reduced compared to mice with full Pms2 function.

At first glance, this finding is somewhat surprising, given that a mismatch repair mutant would be expected to have increased mutation rates, not decreased rates. Indeed, the mutation frequency in non-V genes in Pms2$^{-/-}$ mice is increased. How is it that Pms2 which usually corrects mismatches and thereby prevents mutation, has been redirected to create mutations? Pms2 is designed to correct errors introduced during DNA replication; hence, it repairs the mismatch in the newly synthesized strand by using the preexisting strand as a template (see **A** in upper figure). However, if the goal is diversification through point mutation, as is the case for V genes, mismatch mutations must be immortalized (fixed) rather than repaired. As Cascalho *et al.* perceive, which strand contains the "mismatch" is in the eye of the beholder. They suggest that Pms2 may be coerced into correcting mismatches "backwards" in V genes—using the newly synthesized strand, rather than the preexisting strand, as a repair template. This would successfully immortalize the mutations (**B**). Alternatively, if mutations are introduced into the parental strand, then Pms2 functioning "normally" would now be working to create mutations (**C**). And still a third mechanism is possible: If epigenetic marks distinguishing parental from newly synthesized strands are removed, mismatch repair would be forced to choose randomly between the two strands for its template (**D**). In that case, a base substitution stands a 50:50 chance of being immortalized.



Turncoat. (**A**) Pms2 ordinarily corrects mismatches generated in newly synthesized, unmethylated DNA (red) according to the fully methylated parental strand (blue). Cascalho *et al.* (*1*) demonstrate that the mechanism of somatic hypermutation has adopted Pms2 to perform the seemingly opposite function of immortalizing mutations. (**B** through **D**) show how Pms2 might fix instead of repair a mismatch. MAGs initiate this process.

Obviously, this is just the beginning of this story. Now that Pms2 has been identified as a ubiquitous component of the V gene mutator, the search for RAGs' counterparts, the mutation activation genes (MAGs), can begin. And the impact of repair machinery on the immune response needs clarification. Indeed, there is a discrepancy between the observed mutation frequency and that expected in the absence of Pms2 activity. Loss of Pms2 function could at most cause the mutation rate to be reduced by half, yet Cascalho *et al.* observe a much more acute reduction (by a factor of 8 to 10) in the absence of Pms2. This discrepancy could mean, as the authors suggest, that other repair systems step in when Pms2 is absent. It could also mean that Pms2 has two functions: one as part of the mismatch repair machinery, and another as part of the MAG machinery.



Mutation frequency is not proportional to mutation rate. V gene mutations that create or modify antibody specificity are selected by antigen leading to increases in the size of the population with advantageous mutations.

It is important to keep in mind, however, that because of the effects of selection, mutation rate and mutation frequency are not proportional to each other. As a result, what seems to be a discrepancy in observed and expected mutation frequencies may not be a discrepancy at all. The nature and pattern of mutation in V genes shows that selection has a profound effect on the rate of clonal expansion. Point mutations accumulate sequentially during clonal expansion, and the influence of selection is seen by the distribution of mutations leading to amino acid substitutions, known as replacement (R) mutations. V genes expressed in expanded clones are enriched for R mutations in the regions coding for the combining site. Conversely, R mutations are underrepresented in the framework regions coding for V gene structure (5). The extent to which R mutations in the framework regions are underrepresented reflects the extent of negative selection: At the estimated mutation rate, 10^{-3} base pairs per division (about one V gene mutation per division)(6), one mutating B cell in four is lost because of deleterious mutation (see lower figure). In fact, if the mutation rate were any higher, B cell clones could not expand.

The overrepresentation of R mutations in the combining site reflects selection for mutants that have created or modified specificities. Positive selection leads to increased B cell division rates (or life-span); in either case, the population with the favorable mutations grows faster. Because the likelihood of observing a mutation is determined by both the rate and the size of the population, the accumulation of mutations will be greater in those B cells in which a mutation has provided a selective advantage. Thus, it would not be unusual for a twofold difference in mutation rate, assumed for the Pms2-/- mouse and its wild-type counterpart, to lead to greater than twofold differences in mutation frequencies.

The rate of mutation is ultimately set by the MAGs. A key feature of MAGs is known: They act on rearranged genes, not on the unrearranged gene segments leftover after V(D)J recombination (7). Thus, V genes per se are not the substrate for hypermutation, and there must be an intimate connection between rearrangement and hypermutation (one also seen in some B cell tumors) at the translocation junctions. RAGs are able to nick DNA (8), making RAGs or RAG-like proteins potential MAG candidates. Thus, V gene mutation may have co-opted not only ubiquitous factors like Pms2, but B cell–specific processes as well.

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