PERSPECTIVES: IMMUNOLOGY

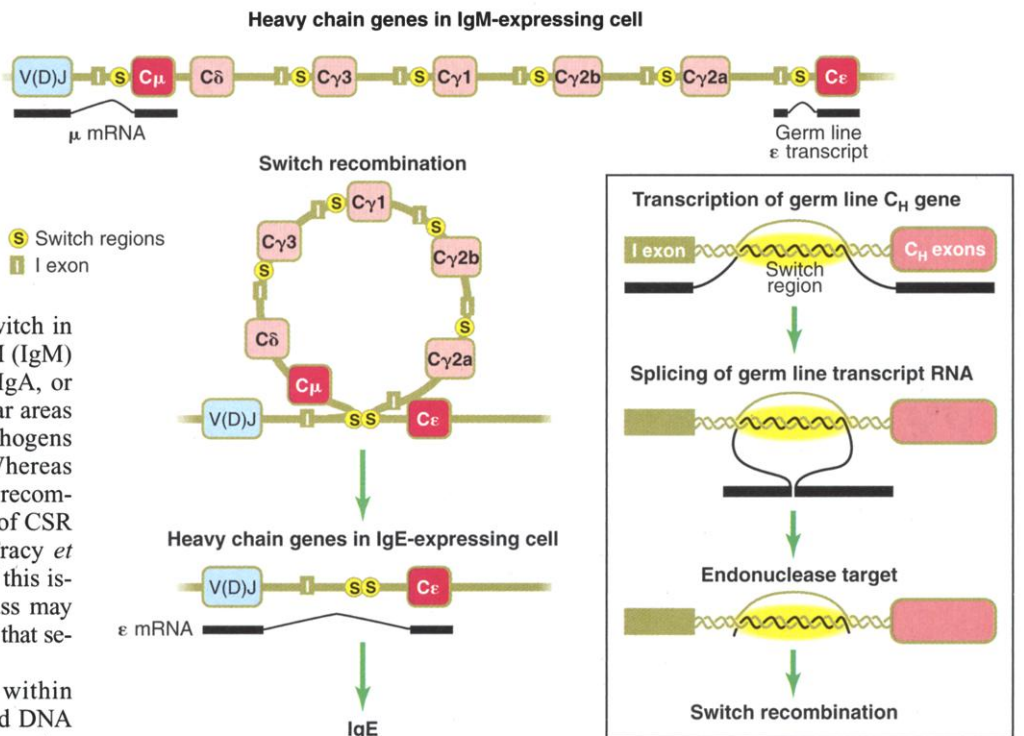
A Touch of Antibody Class

Janet Stavnezer

The cells of the immune system that produce immunoglobulin in response to antigen are called B cells. During their life-span these cells undergo two entirely different DNA recombination events. The first, called V(D)J recombination, creates the antigen specificity of immunoglobulins as the B cells develop in the bone marrow (in the absence of antigen). Later, when B cells are activated by antigen in the spleen or lymph nodes in response to immunization or infection, DNA recombination results in a switch in the class of antibody that the B cells produce. This antibody class switch recombination (CSR) has no effect on the antigen specificity of the antibody but results in expression of a different heavy chain constant region (C_H) gene (see the figure). Thus, there is a switch in production from immunoglobulin M (IgM) to other classes of antibody (IgG, IgA, or IgE), which are targeted to particular areas of the body and eliminate the pathogens inducing the immune response. Whereas the molecular mechanics of V(D)J recombination are well understood, those of CSR have yet to be elucidated. Enter Tracy *et al.* (*1*), who reveal on page 1058 of this issue that the choice of antibody class may be regulated by RNA-DNA hybrids that select the sites of recombination.

Switch recombination occurs within (and also nearby) tandemly repeated DNA sequences called switch (S) regions that are located upstream of each of the C_H genes (except Cδ). Mouse and human S regions are 1 to 10 kb in length and contain repeat sequences 20 to 100 base pairs long that have a high percentage of guanosine bases (G-rich). The nucleotide sequence of each S region is unique, so the mechanism of CSR cannot involve straightforward homologous recombination (recombination between identical or nearly identical DNA sequences); rather, it appears to involve a type of recombination called nonhomologous end joining, in which sequence homology plays little part.

It is well established that cytokines (factors stimulating other immune cells) produced by helper T lymphocytes determine the antibody class produced by CSR. For example, if mouse B cells are activated by antigen and by T cells that secrete interleukin-4, they switch from IgM to IgG1 or IgE production. If they are activated by antigen in the presence of T cells that secrete interferon-γ, they switch from IgM to IgG2a. Transforming growth factor–β directs the antibody class switch from IgM to IgG2b or IgA (*2*). These three cytokines are known to induce the transcription of specific RNAs (called germ line or sterile transcripts) from the C_H genes to which the B cells subsequently switch (*3*). The germ line transcripts do not appear to be translated into protein. They share the same overall structure, with transcription starting upstream of the S region and terminating at the polyadenylation sites at the 3′ end of each C_H gene. Splicing of the upstream region (I exon) to the exons of the C_H gene produces the germ line transcript (see the figure). Interestingly, gene targeting experiments have shown that these transcripts are necessary for CSR to occur (*4*). Furthermore, they seem to act only in cis (that is, at the allele from which they were transcribed) and do not seem to influence events at C_H genes on the other chromosome. Because the RNA transcripts must be spliced together for CSR to occur, they cannot be involved in simply remodeling chromatin.



Antibody class switching. Arrangement of immunoglobulin heavy chain (C_H) genes in a B cell that is producing IgM antibody and then switches, through DNA recombination, to synthesis of IgE. Below the Cε gene is shown the germ line ε RNA transcript (the same basic structure is found in all C_H gene transcripts). During switch recombination, the segment of the chromosome residing between the recombining S (switch) regions is excised as a circle and lost from the cell. After switch recombination, the V(D)J segment (encoding the variable region) is unchanged and the recombined Cε gene encodes a mature ε heavy chain mRNA. **(Inset)** S region sequences within the intron of the primary germ line transcript do not dissociate from the template and form stable RNA-DNA hybrids after transcription (*1*). Because splicing occurs concomitantly with transcription, splicing of the germ line transcript might stabilize the RNA-DNA hybrid by removing segments incapable of forming hybrids. The RNA-DNA hybrid and single-stranded nontemplate DNA strand are targets for a hypothetical endonuclease that might initiate switch recombination.

These studies have been complemented by biochemical experiments demonstrating that transcripts of the S regions in immunoglobulin genes remain associated with the template DNA after transcription in vitro (*5*). Although the structure of the RNA-DNA hybrids was not well defined in these early experiments, Tracy and Lieber recently showed that the G-rich transcribed

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RNA forms a hybrid with the cytosine-rich DNA template leaving the other DNA strand (the nontemplate strand) on its own (*6*). The RNA in the hybrid is sensitive to ribonuclease (RNase) H, an enzyme that specifically snips the RNA in DNA-RNA hybrids. In their new work, Tracy *et al.* (*1*) now demonstrate that after B cells from mouse spleen are induced to undergo CSR, RNA-DNA hybrids form within the S regions of the DNA. They also provide data indicating that these RNA-DNA hybrids are important for CSR in vivo.

To isolate S region RNA-DNA hybrids, the investigators used RNase A (which cleaves single-stranded RNA), followed by DNase I (which cleaves all DNA), to digest all of the nucleic acids of B cells activated by cytokines and lipopolysaccharide to induce CSR. In the residual material (which should contain RNA that was present in RNA-DNA hybrids) they detected a heterogeneous RNA population containing S region sequences. The RNA species were found in B cells that had been induced to express the germ line transcripts, and the S region sequences in the RNA-DNA hybrids were derived from the intron regions of these transcripts. Treating activated B cells with RNase H before DNase I eliminated this RNA population.

To test whether the RNA-DNA hybrids are required for CSR, Tracy *et al.* (*1*) created transgenic mice that constitutively express RNase H in splenic B cells (and presumably in many other cells). The immune system of these mice is normal in a variety of tests, although they have greatly reduced levels of S region RNA-DNA hybrids. When the mice were immunized, their B cells showed an impaired capacity for class switching relative to the B cells of wild-type mice, as assessed by levels of antigen-specific serum IgG and examination of DNA recombination within S regions.

These results raise several interesting questions. What structural features of the S region are required for formation of the stable hybrid during transcription? Although mouse and human S regions are G-rich, and runs of guanosines are known to form unusual structures, this may not be a crucial feature because the S region upstream of the Ig Cμ gene in the frog *Xenopus* is A-T rich (*7*). It is possible that the stretches of purine-pyrimidine asymmetry, the repeated sequences, and the numerous palindromes (sequences that read identically in both directions) in the S regions of mouse, human, and *Xenopus* are essential for CSR.

How does the RNA-DNA hybrid regulate class switching? Tracy and co-workers suggest that it could serve as the recognition target for a hypothetical endonuclease that initiates CSR by creating double-strand breaks (*8*) (see the figure). This is an interesting hypothesis, although none of the available data indicate that germ line transcripts are required for formation of double-strand breaks in S regions.

Although the authors do not determine whether splicing of germ line transcripts is required for formation of RNA-DNA hybrids, it is possible that splicing may stabilize the hybrids by removing extra nucleotides incapable of participating in their formation. Answers to these and other questions raised by the Tracy *et al.* study will bring us to a much better understanding of the mechanism of CSR.

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PERSPECTIVES: ECOLOGY

Black-Footed Ferret Recovery

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The black-footed ferret, *Mustela nigripes*, is a nocturnal carnivore indigenous to the western United States (see the figure) that dines almost exclusively on prairie dogs. By the 1970s the black-footed ferret was believed to be extinct, the initial decline in its numbers matching that of prairie dogs, which were poisoned, shot, and otherwise extirpated to improve range-land habitat for cattle. The discovery of a remnant population of black-footed ferrets in Wyoming in 1981 launched a struggle to save the species that brought together an interdisciplinary team of veterinarians, zoo-biologists, wildlife managers, and behavioral ecologists. The cooperative efforts of at least 35 private and government agencies have succeeded in rebuilding a healthy captive population of ferrets large enough to provide recruits to seed new wild colonies.

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At the dawn of the 21st century, an important conservation goal has been achieved: The black-footed ferret has been returned to the wild and is now breeding in at least four locations in the Rocky Mountain region of the western United States. The successful restoration of black-footed ferrets (as well as gray wolves) means that the mammalian fauna of this region is as diverse now as it was 100 years ago. Obviously, formidable challenges remain in the restoration of this region. In particular, invasive plant species and agriculture have completely altered the flora and invertebrate fauna of vast areas; similarly, huge demands for freshwater have severely damaged riverbank habitats. The struggle to save the black-footed ferret has yielded fascinating lessons about captive breeding, behavioral conditioning for reintroduction, disease management, and prairie dog politics that can be applied to saving other endangered animals.

The captive breeding program for black-footed ferrets began in October 1985 with six ferrets from the last known wild population at Meeteetse in Wyoming. Two of these ferrets were infected with canine distemper; this spread to the other four, and all six died. Intense efforts were then launched to capture as many of the last remaining free-living ferrets as possible. These were vaccinated against distemper soon after capture, and all 18 captured ferrets survived to form the sole captive breeding population for the species. No young were born in the population's first year in captivity, but in the second year two females produced a total of eight kittens, of which seven survived. These were followed by 34 kittens in 1988, 58 in 1989, and 66 in 1990.

The captive breeding program aims to manage a captive population of 240 ferrets (90 males and 150 females) of prime breeding age (1 to 3 years old) housed in several facilities to reduce the risk of a catastrophe such as a fatal disease outbreak. One of the main goals of this program is to maintain 80% of the genetic