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Immunoglobulin Class Switch Recombination: Will Genetics Provide New Clues to Mechanism?

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Programmed changes in genomic structure are essential to development of the immune system. The antigen receptors of mature B and T lymphocytes are produced by a site-specific recombination process that rearranges *V, D,* and *J* gene segments into the V(D)J regions encoding the variable domains. Later, after activation of a mature B cell by its specific antigen, gene structure and variable region sequence undergo further alteration. Class switch recombination joins a new constant (C) region to an expressed variable (V[D]J) domain, to allow efficient removal of antigen from the body. Somatic hypermutation introduces single base changes into the variable regions of immunoglobulin genes and then is coupled with selection, which results in an increase in antibody affinity for antigen.

Here, I provide a brief overview of three processes that create and fine-tune immunoglobulin genes as the immune system develops and responds to foreign antigens: V(D)J recombination, class switch recombination, and somatic hypermutation. I then discuss class switch recombination in greater detail, focusing on recent results that show that class switch recombination depends significantly on genes essential in other contexts.

Because development of the immune system relies on recombination, one might predict that genes that function in recombination and/or repair in the immune system would be identified as genes that, when mutated, cause immundeficiency diseases. It is therefore not surprising that several immunodeficiencies have been correlated with deficiencies in enzymes essential to recombination/repair. For example, Omenn syndrome is a combined B and T cell immunodeficiency that, just in the last year, was shown to result from mutations in *RAG1* or *RAG2,* whose products carry out the cleavage step in V(D)J recombination (Villa et al. 1998). Severe combined immunodeficiency (*scid*) mutant mice provide another example, as they almost completely lack mature B cells and T cells because of mutation in the gene that encodes the catalytic subunit of DNA protein kinase ($DNA\text{-}PK_{cs}$), an essential component of the nonhomologous end-joining pathway for repair of double-strand breaks (Petrini 1999 [in this issue]). As discussed below, this repair pathway processes the products of recombination V(D)J and class switch recombination, and $DNA\text{-}PK_{cs}$ deficiency impairs both of these regulated recombination events. Another molecule that may be involved in both V(D)J and class-switch recombination is ATM, a component of the cellular network for sensing DNA damage (reviewed by Canman and Lim 1998). *ATM* is mutated in ataxia telangiectasia, an autosomal recessive disease characterized by loss of cerebellar functions, development of tumors, and deficient T and B cell responses.

In many recombination/repair processes, genetics has provided abundant clues to mechanism. However, at least thus far, the genetic diseases with the most profound impact on switch recombination are caused by defective cell-cell signaling and intracellular regulation, rather than by defective recombination; and individuals with partially impaired somatic hypermutation have been identified only recently (Levy et al. 1998). Could class-switch recombination and somatic hypermutation depend on ubiquitous activities that are essential in other aspects of DNA metabolism? Could the activities essential to these processes be backed up by enzymes with redundant capabilities? Or is it possible that, as more immunodeficiencies are characterized at the molecular level, we will come to recognize more genetic diseases associated with these processes? One purpose of this review is to invite human geneticists and biochemists alike to be on the lookout for such defects.

Three Steps to Create and Perfect an Immunoglobulin Molecule: V(D)J Recombination, Class Switch Recombination, and Somatic Hypermutation

V(D)J recombination is the site-specific process of recombination that occurs in B cells to generate variable

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regions of immunoglobulin molecules or, in T cells, to create the variable regions of the antigen receptor (reviewed by Willerford et al. 1996; Gellert 1997; Schatz 1997). Like other types of recombination, V(D)J recombination can be thought of as comprising sequential DNA cleavage, synapsis, and religation. The cleavage reaction is carried out by a pair of proteins, RAG1 and RAG2, which are produced only in lymphocytes. After cleavage, nucleotides may be added by terminal transferase, and the ends are then sealed in a reaction that depends on ubiquitous factors involved in nonhomologous end joining: Ku70, Ku80, DNA-PK_{cs}, DNA ligase IV, and XRCC4.

After successful completion of V(D)J recombination, a newly fledged B cell displays its antigen receptor on the cell surface and joins the surveillance force of B cells. Antigen binding activates these cells and initiates the next phase of their development. Some B cells are stimulated to secrete immunoglobulin immediately, whereas others proceed along a defined developmental pathway, in which genomic structure is altered by class-switch recombination and somatic hypermutation of V regions. Switch recombination results in the joining of a V(D)J region to a new downstream C region. Each C-region class mediates interactions of the immunoglobulin with specific cellular or soluble factors that allow bound antigens to be cleared from the body.

Somatic hypermutation introduces single base changes into an expressed V region at a rate nearly a million-fold higher than the normal mutation rate in mammalian somatic cells, thereby fine-tuning antigen specificity in a process that is intimately coupled with selection (for a collection of recent reviews about this fascinating process, see *Immunological Reviews* Vol. 162, 1998). We know a great deal about regulation of somatic hypermutation, but essentially nothing about its mechanism. Nonetheless, in the past year, there has been considerable progress, both in eliminating genes as potential candidates for function in somatic hypermutation and in identifying genes that may be involved in hypermutation. Currently, ~30 genes—most of them involved in DNA recombination/repair—have been tested for function in somatic hypermutation, by analysis of hypermutation either in strains of mice that carry targeted deletions or in humans with corresponding genetic disease (reviewed by Harris et al. 1999). Although we currently know of no human genetic diseases that result in impaired somatic hypermutation, analyses of knock-out mice have implicated two genes in this process: *Pms2* (Cascalho et al. 1998; Frey et al. 1998; Winter et al. 1998) and *Msh2* (Phung et al. 1998; Rada et al. 1998), both components of the highly conserved eukaryotic mismatch repair system. *Pms2* appears to affect the hypermutation level, whereas *Msh2* influences the spectrum of mutation. An intense ongoing controversy surrounds the question of whether these phenotypes indicate a direct role for mismatch repair in somatic hypermutation, or if they reflect altered selective pressures or the effects of chronic stimulation of B cells in the mutant backgrounds. For a taste of this controversy, see Frey et al. (1998), Kelsoe (1998), Kim and Storb (1998), and Wiesendanger et al. (1998).

Class Switch Recombination

Class switch recombination is a regulated process of DNA deletion. After V(D)J recombination, but prior to switch recombination, the heavy-chain locus is organized for production of IgM antibodies, in which the heavy chain contains a V(D)J region just upstream of the Cμ C region (fig. 1). IgM antibodies efficiently remove antigen by activating the complement cascade. Switch recombination results in production of other classes of antibodies. These include the IgG subclasses, IgG1, IgG2, IgG3, and IgG4, the major serum antibodies that remove antigen via their interaction with receptors on phagocytic cells. IgG antibodies are unique in that they can cross the placental barrier to provide immune defenses to a newborn baby. IgA antibodies, found in secretions that include saliva, tears, milk, and intestinal mucus, coat invading pathogens to remove them from the body. IgE, a very minor class of antibody, sensitizes mast cells and is associated with allergy. In most people, the serum antibody distribution is 10% IgM, 75% IgG, 10–15% IgA, and 0.004% IgE. A number of apparently heritable immunodeficiencies are characterized by altered levels of specific classes or subclasses of serum antibodies; only some of these immunodeficiencies are well understood.

Class switch recombination involves DNA regions, called "switch (S) regions," that are located in the introns upstream of each C region. S regions are 2–10 kb in length and are composed of repetitive sequences, which are G rich on the nontemplate strand. Although the repeats in all the S regions are G rich, the S regions are not homologous to one another. Of the hundreds of switch recombination junctions examined, no two are alike: junctions are heterogeneous in sequence and in the sites at which both donor (upstream) and acceptor (downstream) recombination endpoints are located (Dunnick et al. 1993). Thus, switch recombination does not depend on site-specific or homologous recombination processes. It is also important to stress that switch recombination is completely distinct from V(D)J recombination: it occurs later in B cell development; it rearranges C regions, not variable region segments; it is region-specific but not sequence-specific; and it is independent of two proteins essential for V(D)J recombination, RAG1 and RAG2.

In B cells activated for switch recombination, translocations can occur that involve cellular oncogenes. The

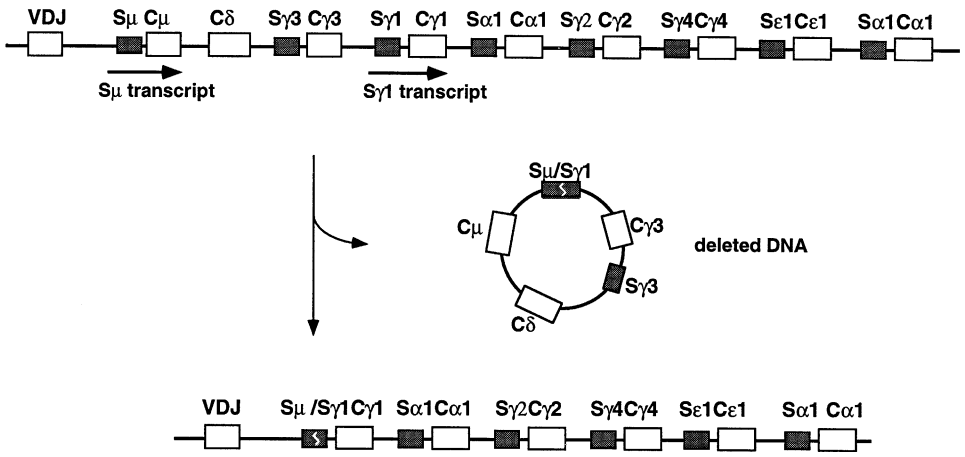

Figure 1 Class switch recombination is a targeted and regulated process of DNA deletion. The top line diagrams the human IgH locus, showing a rearranged VDJ region, S regions, and C regions. The figure illustrates recombination from μ to $\gamma 1$; as a result of this recombination event, the B cell switches from producing IgM to producing IgG1 antibodies. Recombination is activated by simultaneous transcription of the Sμ and Sγ1 regions (arrows). Circular molecules that contain the deleted C region and flanking sequences can be isolated from cells that have recently carried out switch recombination (*middle*). This suggests that, during recombination, distant switch regions are brought together to form a recombination complex, which undergoes cleavage and religation to produce a chromosomal switch junction and the excised switch circle. Switch recombination junctions are heterogeneous in structure and position, but, since the S regions are found within introns, their sequences are excluded from the mature immunoglobulin transcripts.

best-studied of these are the translocations of c-*myc* to the immunoglobulin heavy-chain loci that are characteristic of certain B cell lymphomas. Switch recombination may therefore share aspects of mechanism with aberrant processes of recombination that contribute not only to cancers but also to development of genetic disease.

B cells carry out switch recombination in response to signals from T cells, which stimulate antigen-specific B cells to initiate a signaling cascade. Essential to this cascade is interaction between the B cell CD40 surface receptor and the T cell CD40 ligand, CD40L. Genetic analysis reveals the importance of the CD40/CD40L interaction: mutations in CD40L are responsible for X-linked hyper-IgM immunodeficiency, characterized by the absence or extreme decrease in serum IgG and IgA, but with normal or elevated IgM. Deficient intracellular signaling is also the cause of X-linked agammaglobulinemia (XLA), an immunodeficiency characterized by the absence of mature B cells and low levels of all Ig subclasses. XLA is caused by lack of Bruton tyrosine kinase (Btk), an intracellular Src-related nonreceptor tyrosine kinase. Deficiency of the p85α subunit of phosphoinositide 3-kinase (PI-3 kinase) produces a phenotype in mice similar to that of Btk deficiency (Fruman et al. 1999).

Simultaneous Transcription of Both Targeted S Regions Is Prerequisite to Switch Recombination

An important clue to the mechanism of switch recombination is provided by observations from many laboratories showing that switch recombination requires that both S regions that will carry out recombination be simultaneously transcribed (reviewed by Snapper et al. 1997). Each S region carries its own promoter, and targeted deletion of the promoter renders an S region inactive for recombination. Recombination of specific switch regions is regulated by a signaling cascade that is activated when T cell–derived cytokines or lymphokines bind to specific receptors on the B cell surface. Triggering of these receptors is relayed to the nucleus by a pathway that culminates in binding of transcription factors to elements in S-region promoters, which results in activation of S-region transcription. Among the factors known to regulate switch-region transcription are members of the JAK/STAT family, NF-IL6, and the p50 subunit of NF-κB (reviewed by Henderson and Calame 1998).

Why is transcription prerequisite to switch recombination? There are several possible explanations, which are not mutually exclusive. It is unlikely that the S-region transcripts encode essential polypeptides, because they contain many stop codons. Switch transcripts are spliced after transcription, and the requirement for splicing (Lorenz et al. 1995) may reflect involvement of the mature transcript or the splicing apparatus in switch recombination. Transcription also alters chromatin structure, producing a more open conformation, and this may be necessary to render duplex DNA accessible to enzymes that carry out recombination (e.g., Willerford et al. 1996). Finally, as is discussed below, transcription may

cause S-region DNA to assume unusual and characteristic structures that serve as efficient substrates for a specific class of recombination enzymes.

Alternative DNA Structures and Class Switch Recombination

Molecular understanding of how triplet-repeat expansion contributes to human genetic disease has shown that DNA has considerable potential to assume structures distinct from the standard Watson-Crick duplex, as was recently reviewed in this journal by Sinden (1999). Analogously, S-region sequences can form alternative DNA structures during transcription in vitro. When S regions are transcribed, the newly synthesized, G-rich RNA forms a stable hybrid with the C-rich template DNA strand (e.g., Reaban and Griffin 1990), leaving the G-rich DNA strand unpaired and free to form intra- or interstrand structures that are stabilized by guanine-guanine (G-G) pairing (e.g., Sen and Gilbert 1988).

Genetic evidence for the potential importance of G-G–paired DNA in switch recombination comes from analysis of the human genetic disease, Bloom syndrome. Bloom syndrome results from mutations in *BLM,* which encodes a DNA helicase of the RecQ family. Affected individuals display a variety of symptoms, including malignancies, growth retardation, sunlight sensitivity, impaired fertility, and immunodeficiency. The *BLM* helicase has ATP-dependent 3′ to 5′ unwinding activity on duplex DNA substrates (Karow et al. 1997), but it preferentially unwinds G-G paired DNA (Sun et al. 1998), a property shared by other RecQ family helicases (Sun et al. 1999). These observations suggest that the failure to unwind G-G–paired substrates may contribute to the immunodeficiency that is characteristic of Bloom syndrome. Additional evidence for the importance of G-G–paired DNA in switching comes from analysis of LR1, a B cell-specific heterodimeric factor. The two subunits of LR1, nucleolin and a specific isoform of hnRNP D, each bind tightly and specifically to standard, B-form DNA duplex sites in the S regions (Hanakahi et al. 1997; Dempsey et al. 1998). The LR1 heterodimer and both its subunits bind with still higher affinity to G-G–paired DNA, suggesting a working model for switch recombination in which the subunits of LR1 mediate synapsis by juxtaposing G-G–paired DNA from two different switch regions (Dempsey et al. 1999).

Other Enzymes that Participate in Class Switch Recombination

Some of the activities involved in class switch recombination are part of the general repair/recombination apparatus. Rad51, the eukaryotic homologue of the crucial prokaryotic recombination protein, RecA, is essential for proliferation of mammalian cells; Rad51 may also have specific functions in switch recombination (Li et al. 1996; Peakman and Maizels 1998). The final steps of the switch-recombination reaction depend on at least three gene products in the nonhomologous end-joining pathway for repair of double-strand breaks: DNA-PK_{cs} (Rolink et al. 1996), Ku70 (Manis et al. 1998), and Ku80 (Casellas et al. 1998). These three proteins also function in the final stages of V(D)J recombination, along with DNA ligase IV and XRCC4 (Nussenzweig et al. 1996; Zhu et al 1996; Frank et al. 1998; Gao et al. 1998; Grawunder et al. 1998), raising the possibility that the latter factors are also involved in switch recombination.

Might other proteins that function in general pathways of recombination and repair prove to have specific roles in switch recombination? One candidate is ATM, a putative protein kinase that is involved in sensing DNA damage (reviewed by Brown et al. 1999). The ATM kinase is deficient in ataxia telangiectasia, a genetic disease characterized by neurodegeneration and telangiectasia, predisposition to cancer, and immunodeficiencies evident, in part, as variably decreased levels of certain classes of serum antibodies. As the mechanism and regulation of switch recombination are worked out in greater detail, the function of ATM in these pathways should become apparent.

Recombination requires DNA cleavage, but, despite considerable interest, no enzyme has yet been identified that specifically cleaves duplex switch region DNA. In V(D)J recombination, cleavage is carried out by RAG1 and RAG2. Mutations in *RAG1* or *RAG2* were only recently found to result in a previously recognized combined B and T cell immunodeficiency, Omenn syndrome (Villa et al. 1998). This highlights the possibility that there may be immunodeficiencies, yet to be defined in molecular terms, that result from impairment of a cleavage activity involved in switch recombination. Alternatively, an enzyme that functions in another context may be put to use by the switch recombination pathway.

Acknowledgments

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