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The Polymerases for V(D)J Recombination

DNA transactions of a wide variety generally require three major types of enzymatic activities: nucleases, polymerases, and ligases. V(D)J recombination is no exception. In this issue, Bertocci et al. (2006) have provided new insight by generating mice deficient in one or more of the polymerases.

Although the roles of ligation and nuclease action in V(D)J recombination are relatively well understood, genetic assessment of the role of specific polymerases has taken longer. A look at the enzymatic steps of V(D)J recombination illustrates the part played by the DNA polymerases (Figure 1). Initially, the recombination activating gene (RAG) complex nicks between the V or J coding segment and the adjacent recombination signal sequence (abbreviated RSS, and containing the heptamer and nonamer elements). The RSSs can have either 12 bp or 23 bp spacers between the heptamer and nonamer, and hence the name 12-RSS or 23-RSS, respectively. A single recombination event requires one 12-RSS and one 23-RSS. The 3′OH at each nick is used as a nucleophile by the RAG complex to create a hairpin at each coding end, thereby creating blunt signal ends at the 12- and 23-RSSs. The hairpins at the coding ends are opened by the nuclease Artemis: DNA-PKcs, and this nuclease is also capable of trimming 5′ and 3′ overhangs (Ma et al., 2002). Hence, this satisfies the nuclease requirement.

The ligation of the coding ends can occur one strand at a time by the XRCC4:DNA ligase IV complex (Ma et al., 2004), and this ligase and the Artemis:DNA-PKcs nuclease are part of the nonhomologous DNA end-joining pathway (NHEJ), which is present in all eukaryotic cells for repairing double-strand breaks.

The template-dependent polymerases for V(D)J recombination and for NHEJ have been the last of the three essential enzymatic activities to be revealed, and the Reynaud and Weill laboratory has provided the key V(D)J recombination junctional analyses in mutant mice to identify roles for polymerases μ and λ. Ironically, the first mammalian DNA polymerase activity ever discovered by biochemists was the template-*independent* polymerase, terminal deoxynucleotidyl transferase, usually called TdT or terminal transferase. TdT turned out to be expressed only in pre-B and pre-T lymphocytes. TdT plays a nonessential role in the V(D)J recombination mechanism, but contributes enormously to the diversity of the immune repertoire by adding in a "random," or template-independent, manner at the coding ends.

There are roughly 15 DNA polymerases currently identified in mammalian cells, and pol β, pol μ, pol λ, and TdT are the members of the Pol X family. Pol β is involved in base excision repair and is the only Pol X member that lacks a BRCT domain. In *S. cerevisiae*, where there is only one Pol X family member, POL4, this polymerase was shown to be quite important for NHEJ repair of ends with 3′ overhangs (Wilson and Lieber, 1999). At that time, the closest known mammalian homolog of POL4 was polymerase β. However, with the subsequent discovery of the large group of lesion bypass polymerases, mammalian polymerases μ and λ are much closer POL4 homologs and, like yeast POL4, possess a BRCA1 C-terminal (BRCT) domain. Pol μ was found to form a physical complex with the key NHEJ proteins, Ku, and the XRCC4:DNA ligase IV complex (Mahajan et al., 2002), providing a physical linkage to NHEJ. And depletion of pol λ affected end joining in extracts, providing a biochemical connection for this sister polymerase (Lee et al., 2003). Finally, human Ku was shown to recruit pol μ and λ via their BRCT domains (Ma et al., 2004). The BRCT domains of pol μ and λ are essential for their participation in an in vitro reconstitution of NHEJ proteins (Ma et al., 2004).

The Reynaud and Weill laboratory has generated mutant mice of many of the bypass polymerases, among them pol μ and λ. These mutants were a key test of the accumulating genetic and biochemical implication of these two polymerases. Their laboratory had already reported that lack of pol μ resulted in shorter immunoglobulin light-chain junctions by about 6 bp (Bertocci et al., 2003). Surprisingly, the heavy-chain-coding junctions were unaffected in the pol-μ-deficient mouse. This lack of an effect at the Ig heavy-chain junctions and the biochemical observation that pol μ might be better than pol λ at joining 3′ overhangs raised the possibility that only pol μ would be involved in V(D)J recombination and that pol λ might be more relevant to NHEJ of radiation-induced double-strand breaks (Nick McElhinny et al., 2005).

In this issue of *Immunity*, Bertocci et al. report that, in fact, pol λ is also important for V(D)J recombination. Unlike pol μ's role at the Ig light-chain junctions, however, the role of pol λ is important at the Ig heavy-chain junctions. Specifically, the Ig heavy-chain junctions are shorter by about 5 bp in the mutant mouse. Intriguingly, the pol-λ-deficient mice are normal at the Ig light-chain junctions (Bertocci et al., 2006). This reciprocal genetic relationship between pol μ and pol λ is intriguing. It is known that both pol μ and λ are expressed ubiquitously, but the ratio of the two proteins is not known. Bertocci et al. have used quantitative RT-PCR to measure the levels of the mRNAs and found that although pol μ and pol λ have similar gene-expression levels at the pro-B stage, expression of pol μ rises more than 10-fold, and pol λ drops 2-fold, such that the pol μ mRNA is now over 25-fold more abundant than that of pol λ at the pre-B stage, when light-chain rearrangement occurs. The authors suggest that pol λ may be more effective than pol μ at the heavy-chain stage when expression of the two polymerases is similar, but when the level of pol μ reaches a critically high threshold level, then pol μ is the key factor. Exploring this possibility and others must wait until more is known about the relative action of the pol μ and pol λ enyzmes at various DNA end configurations and a determination of the actual protein levels.

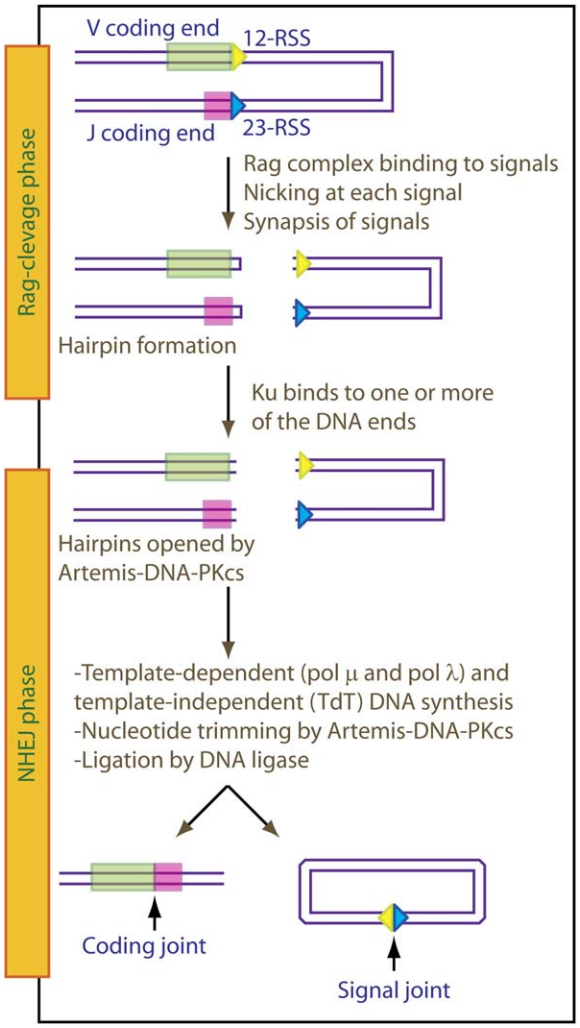

Figure 1. Steps in V(D)J Recombination

The RAG complex acts at recombination signal sequences (RSS). A single V(D)J recombination reaction requires a pair of RSSs, one with a 12 bp spacer between the heptamer and nonamer and the other with a 23 bp spacer. The 12-RSS is shown in yellow, and the 23-RSS is shown in blue. The RAG complex generates double-strand breaks, resulting in hairpinned coding ends. The hairpins are opened by Artemis:DNA-PKcs. The coding ends are then subject to nucleolytic processing by Artemis:DNA-PKcs. Template-dependent synthesis by polymerase μ or polymerase λ can also occur at these coding ends, along with template-independent synthesis by TdT. Finally, XRCC4:DNA ligase IV, along with XLF (also called Cernunnos) carries out the ligation.

One could even wonder why mammals have two different evolutionary descendents of the yeast POL4 polymerase. The new Bertocci et al. paper also addresses this by generating mice that are double deficient for pol μ and pol λ (Bertocci et al., 2006). They found that the B cells derived from such mice display defects in both the Ig light and the Ig heavy chain. Interestingly, the cells from such mice were not sensitive to ionizing radiation, whereas cells deficient in all of the other NHEJ components (Ku, Artemis, DNA-PKcs, XLF, XRCC4, and DNA ligase IV) are sensitive. This is consistent with the yeast work, where it is clear that even in the absence of POL4 (the only POL X family member in yeast), fill-in synthesis is only partially reduced in NHEJ (Wilson and Lieber, 1999). This implies that other polymerases, perhaps pol δ or pol ε, can provide fill-in synthesis activity when a Pol X family member is not present. The yeast data indicate that the Pol X family members are particularly important for joining when one end is a 3′ overhang; when neither end is a 3′ overhang, joining is less reliant on the Pol X polymerases (Wilson and Lieber, 1999). It has been proposed that this may be due to a better ability of Pol X polymerases to handle polymerization from a 3′ OH located in the unstable area within the incipient junction. In contrast, 5′ overhangs can be filled by initiating polymerization from the upstream 3′ OH located in the more stable duplex region immediately adjacent to the junction (Daley et al., 2005). In other words, it is easier to polymerize into the junction from the adjacent duplex DNA (5′ overhangs) than it is to polymerize from the junction itself (3′ overhangs), and Pol X polymerases may specialize in the latter.

It is interesting to consider the Bertocci et al. paper in the context of other recent studies. Tom Wilson's laboratory has expressed human pol μ and pol λ in *S. cerevisiae* that are null for POL4 (Daley et al., 2005). They examined four different 3′ overhang configurations with 1 to 4 bp of terminal microhomology (annealing between the two DNA ends). For two of the configurations, pol λ supported more efficient joining, and for the other two, pol μ was slightly more efficient. More in vivo functional data as well as biochemical data will be needed to understand the basis for these differences when both polymerases are present.

The new paper from the Reynaud and Weill laboratory provides further insight into the contribution of TdT in the context of pol μ and λ (Bertocci et al., 2006). They note that Ig heavy chain junctions in pol λ null mice show similar TdT additions (N regions) as wild-type. This important observation helps to order the events during coding end processing because it indicates that pol λ does most of its work prior to the action of TdT and indicates that pol λ is recruited to the site of end processing more effectively than TdT.

In addition to the principle findings highlighted here, the Bertocci et al. paper contains a wealth of key descriptive information about the relative contributions of the pol μ, pol λ, and TdT to the junctional processing in V(D)J recombination as well as the role of pol λ in replicative senescence (Bertocci et al., 2006). These in vivo mammalian data will be the benchmark against which continuing biochemical and functional studies from yeast to humans will now compare their findings. It is likely to take several more years before all of the richness of this important study can be fully extracted.

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Tec Kinases in T Cell Development: A Clue behind the Mask?

In this issue of *Immunity*, studies from Atherly et al. (2006) and Broussard et al. (2006) show that mice deficient in Tec kinases support development of a population of CD8$^+$ T cells with "innate-immune" properties. The similarity of these cells to NKT and MHC-class-Ib-restricted T cells suggests a common developmental pathway.

Tec kinases play an important role in signal transduction downstream of the T cell receptor. Mice deficient in the Tec family members *Itk* and *Rlk* are reported to have grossly normal T cell development, in that they make and export CD4$^+$ and CD8$^+$ T cells. In this issue of *Immunity*, the Berg (Atherly et al., 2006) and Schwartzberg (Broussard et al., 2006) labs provide evidence that behind this mask of normalcy, CD8$^+$ T cells in these mice are in fact highly unusual. They are NK1.1$^+$, rapidly produce interferon-γ (IFN-γ), and are selected on bone marrow-derived MHC class I. These characteristics are shared by a numerically tiny population of cells in normal mice—NKT cells and class-Ib-restricted T cells. These reports provide a classic example of the need to look beyond simple phenotypic analysis when studying the developmental impact of gene deficiency in T cells. They also provide an intriguing clue about how NKT and class-Ib T cells may develop in normal mice.

The two studies analyzed *Itk* or *Itk*- and *Rlk*-deficient T cells and report similar findings in both strains (Atherly et al., 2006; Broussard et al., 2006). The numerically normal CD8$^+$ T cells from deficient mice express a markedly different phenotype from wild-type naive CD8$^+$ T cells; they are CD122^{hi}, CD44^{hi}, CD3^{int}, NK1.1$^+$, and β7integrin^{lo}. Furthermore, they make high amounts of IFN-γ when stimulated directly ex vivo. Such characteristics can normally be found in memory CD8$^+$ T cells and in CD8$^+$ T cells undergoing homeostatic expansion. Thus, it is possible that this phenotype is secondary to a severe developmental defect in conventional T cells and represents a small number of memory T cells that expanded in the periphery and migrated back to the thymus. This possibility is ruled out by the lack of thymic re-entry in adoptive-transfer experiments and by the fact that these unusual cells develop even in fetal thymic organ cultures. Thus, it would appear that Tec-kinase deficiency results in the generation of abnormal CD8$^+$ T cells in a T cell-instrinsic fashion.

Interestingly, NKT cells and class-Ib-restricted T cells also exhibit these phenotypic and functional properties, although they are normally found in quite small numbers in the body and are positively selected on hematopoetic progenitors (Bendelac et al., 1994; Urdahl et al., 2002) (Figure 1). Because they produce IFN-γ rapidly, they have been called "innate-like" T cells and are suggested to function early on in immune responses. Surprisingly, Broussard et al. reported that Tec-kinase-deficient CD8$^+$ T cells can also be selected when class I is expressed only on hematopoetic cells. Furthermore, some Tec-kinase-deficient CD8$^+$ T cells could be found in Kb- and Db-deficient mice, lacking class Ia molecules. Is it possible that the only T cells that develop and expand in *Itk*$^{-/-}$*Rlk*$^{-/-}$ mice are NKT or class-1b T cells? This seems unlikely because the CD8$^+$ T cells in *Itk*$^{-/-}$*Rlk*$^{-/-}$ mice fail to stain with CD1d tetramers, and only a small population develops in class-Ia-deficient mice. Rather, the data suggest that class I ligands that normally lead to other fates (e.g., positive or negative selection) are leading to the generation of this unusual population of "innate-like" T cells in Tec-kinase-deficient mice.

In this scenario, one might imagine that Tec kinases are merely shifting the strength or duration of T cell receptor (TCR) signaling in the thymus and that such a shift leads to an altered threshold for different selection events. Indeed, it was previously suggested that Tec-kinase deficiency results in a shift in signaling thresholds in the thymus (Schaeffer et al., 2000). Here, one would propose that the generation of innate-like T cells requires a slightly lower threshold than clonal deletion but a slightly higher threshold than positive selection. Thus, the ligands that normally cause clonal deletion would generate innate-like cells in Tec-kinase-deficient mice. Although there is no direct evidence for this hypothesis, high-affinity ligands were shown to induce HY TCR transgenic T cells in organ cultures to