OUTLOOK

Evolving adaptive immunity

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Generation of a diverse repertoire of antigen receptor specificities via DNA recombination underpins adaptive immunity. In this issue of *Genes & Development*, Carmo and colleagues (pp. 909–917) provide novel insights into the origin and function of recombination-activating gene 1 (RAG1) and RAG2, the lymphocyte-specific components of the recombinase involved in the process.

"It appears therefore that immunologically competent cells have evolved a pattern of somatic genetic behavior which is radically different from anything normally found in modern molecular genetics."

—Dreyer and Bennett (1965).

We now know that the "radically different" behavior of immune cells involves assembling antigen receptor genes of B and T lymphocytes via a DNA cut-and-paste mechanism known as V(D)J recombination (Jung et al. 2006). For immunoglobulin (Ig) heavy chain and T-cell receptor (TCR) β -chain genes, this involves two somatic recombination events that put together variable (V), diversity (D), and joining (J) gene segments that are widely separated in the germline. Ig light chains and TCR α chain genes are assembled by one recombination event that juxtaposes V and J gene segments. A random assortment of recombining gene segments and the imprecise nature of V(D)J recombination ensure that millions of antigenic specificities are generated prior to antigen exposure.

Central to this process are the recombination-activating gene 1 (RAG1) and RAG2. RAG1 and RAG2 proteins initiate V(D)J recombination by introducing DNA double-strand breaks at specialized recombination signal sequences (RSSs) that flank antigen receptor gene segments. These breaks are processed and religated by ubiquitously expressed enzymes of the nonhomologous end-joining (NHEJ) pathway. RSSs have a very well-defined organization, consisting of a highly conserved heptanucleotide sequence and a somewhat less conserved nonamer sequence that are separated by either 12 or 23 nucleotides (referred to as 12RSS and 23RSS, respectively). RAG1/2-initiated DNA breaks require recognition and synapsis between a 12RSS and 23RSS but not between 12/12RSSs or 23/23RSSs. This feature of asymmetry is referred to as

the 12/23 rule. An interesting consequence is that gene segments with the same RSS configuration can undergo evolutionary amplification without adversely affecting recombination. Thus, the hundreds of V gene segments present in the genome (each with either a 12RSS or 23RSS) will only participate in functional V-to-D (or V-to-J) rearrangements but not in nonproductive V-to-V rearrangements. The mechanistic basis of the 12/23 rule and its origin are therefore of fundamental importance to adaptive immunity. Another enigmatic aspect of V(D)J recombination reaction is that RSS recognition and catalytic residues that cleave DNA are both located in RAG1. However, both RAG1 and RAG2 are required to assemble antigen receptor genes. The molecular mechanisms by which RAG2 "reveals" RAG1 functionality are not known.

A critical insight as to the origins of the V(D)J recombinase came from the hypothesis that the RAG1/RAG2 combination is a "disassembled transposon" (Thompson 1995). Soon thereafter, it was shown that vertebrate RAG1/RAG2 could carry out transposition reactions in vitro (Agrawal et al. 1998; Hiom et al. 1998). This idea gained further traction with the identification of a RAG1-like domain in a transposase used by the *Transib* transposon (Kapitonov and Jurka 2005). Remarkably, the DNA sequence recognized by the *Transib* transposase is very similar to the highly conserved heptamer sequence (CACAGTC) of vertebrate RSSs, suggesting that the *Transib* transposase and RAG1/RAG2 share target specificity. In other words, target specificity of these proteins has not changed substantially over 1000 million years of evolution (Fig. 1). Once a functional transposon that worked with a RAG1-like transposase had been identified, the next question was whether RAG2 was ever a part of a functional transposon or whether RAG2 is a relatively new non-transposon-associated component coopted by the immune system.

The first RAG1-like (*RAG1L*) and RAG2-like (*RAG2L*) genes outside the immune system were identified in the purple sea urchin *Strongylocentrotus purpuratus* (Fugmann et al. 2006). While the genomic organization of *spRAG1L* and *spRAG2L* (*RAG1L* genes in *S. purpuratus*)

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In this issue of *Genes & Development*, Carmona et al. (2016) provide insights on several outstanding questions regarding the origin and function of RAG1 and RAG2. First, they demonstrate that the vertebrate RAG1 by itself can carry out the complete V(D)J recombination reaction, albeit at much lower efficiency compared with the combination of RAG1 and RAG2. These observations provide direct experimental evidence that RAG1 can function alone, as would have been predicted from the occurrence of a RAG1L protein in the *Transib* transposon. Interestingly, recombination mediated by RAG1 alone does not follow the 12/23 rule, as reflected in comparable levels of recombination between substrates carrying two 12RSSs or a 12RSS and 23RSS. Substrates with only two 23RSSs did not recombine detectably, perhaps reflecting lower affinity of RAG1 for 23RSS. Thus, RAG2 not only

Having detected functional activity of vertebrate RAG1 alone, Carmona et al. (2016) were prompted to test the distantly related RAG1L proteins from *Transib* (Hztransib) and *S. purpuratus* (spRAG1L). Neither of these proteins alone activated 12/23-dependent recombination of substrates integrated into fibroblasts. However, cotransfection of vertebrate RAG2 with either RAG1L gene produced detectable levels of 12/23-dependent recombination. Moreover, this activity was abolished by mutating residues in the RAG1L genes predicted to catalyze DNA strand scission. These observations are interesting because they reveal (1) enzymatic activity of RAG1L proteins from these organisms, (2) the ability of RAG1L proteins to recognize and work with vertebrate RSSs, and (3) that these proteins can be functionally assisted by vertebrate RAG2. Carmona et al. (2016) took these studies a step further by assaying in vitro transposition potential of Hztransib in the presence or absence of vertebrate RAG2. They found that Hztransib alone transposed 12RSS-containing donors but not 23RSS-containing donors. Transposition using 12/23RSS or 23RSS substrates required coincubation with vertebrate RAG2, leading the investigators to propose that acquisition of a RAG2L gene may have permitted an ancestral *Transib* element to accommodate 23RSS recognition. Since asymmetry of the V(D)J recombination reaction is an essential ingredient of adaptive immunity (as discussed above), these

observations illuminate the origin and function of each component of the V(D)J recombinase.

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