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This feature is supported by an unrestricted educational grant from AstraZeneca LP

The DDE recombinases: diverse roles in acquired and innate immunity

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Background: The RAG proteins required for V(D)J recombination of immunoglobulin and T-cell receptor genes in the acquired immune response contain a magnesium ion-binding site termed a DDE site, composed of D (aspartic acid) and E (glutamic acid) amino acids. A similar DDE-like magnesium binding site also is present in transposases, retroviral integrases, and the innate antiviral response enzymes RNase H and RNA-induced silencing complex (RISC).

Objective: To help clinicians understand immunodeficiency that results from deficiencies of RAG protein functions, such as severe combined immunodeficiency disorders, Omenn syndrome, and ataxia telangiectasia, and to be familiar with the diverse roles of other DDE enzymes.

Methods: Literature published in peer-reviewed journals during the past 2 decades that identified and characterized DDE enzymes, including RAG proteins, RISC and RNA silencing, RNase H, retroviral integrases, transposases, and a putative DDE recombinase required for herpes virus replication, was selectively reviewed and summarized by the author.

Results: DDE enzymes play a critical role in acquired immunity through RAG-mediated immunoglobulin and T-cell receptor V(D)J recombination in innate immunity through RISC and RNase H. Paradoxically, DDE enzymes are critical components of pathogen-specific enzymes such as retroviral integrase and other pathogen-encoded proteins.

Conclusion: Because of their critical role in acquired and innate immunity, the DDE recombinases are attractive targets for novel pharmacologic therapies. Currently, retroviral integrase inhibitors in clinical trial for human immunodeficiency virus infection appear to be safe and effective and could provide a paradigm for inactivating DDE sites in other viral pathogens, as well as RAG and RISC.

Ann Allergy Asthma Immunol. 2006;97:567–576.

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INTRODUCTION

The discovery that immunoglobulin genes recombine in the genome of developing lymphocytes was an important advance in immunology.¹ Genes termed V(D)J that encode B- and T-lymphocyte receptors recombine in developing lymphocytes using a conserved recombination signal sequence (RSS) shared with the termini of mobile DNA sequences termed transposons.^{2–4} The identification of 2 closely linked

genes termed *RAG-1* and *RAG-2* (recombination activating genes 1 and 2)^{5,6} permitted detailed molecular analysis of the process of immunoglobulin and T-cell receptor recombination and the eventual proof that *RAG-1* and *RAG-2* form a recombinase similar to the recombinase of transposons and retroviral integrases as reviewed in detail elsewhere.^{7–9} This article reviews the mechanism of V(D)J recombination and similar recombination pathways in acquired and innate immunity, with particular emphasis on the clinical importance of this mechanism as a target for new pharmacologic agents.

Both the RAG proteins^{10–12} and transposons and retroviral integrases^{13,14} use a magnesium ion (Mg⁺⁺) binding site composed of D (aspartate) and E (glutamate) acidic amino

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Received for publication April 25, 2006.

Accepted for publication in revised form September 26, 2006.

acids to break and rejoin DNA. Like transposons and retroviral integrases, the RAG proteins can both insert and remove DNA from target sites.^{15,16} These functional similarities suggest that all DDE enzymes may be descendants of a primordial DDE recombinase that splices together and cuts nucleic acids at specific signal sequences.²⁻⁴ Alternatively, a similar recombination mechanism may have arisen independently rather than through descent from a common primordial enzyme. In either case, because the recombination mechanism of V(D)J recombination is mechanistically similar to retroviral insertion and DNA breaks caused by transposable elements, it is possible that similar pharmacologic agents could affect all of these pathways. Thus, new immunosuppressive drugs that block the RAG proteins and V(D)J recombination might be adapted from existing drugs that target the retroviral integrases, or conversely pharmacologic agents that increase retroviral integration could be used to restore RAG function in the case of hereditary immunodeficiency that results from RAG mutations.

DIVERSE ROLES OF RAG AND RELATED DDE RECOMBINASES

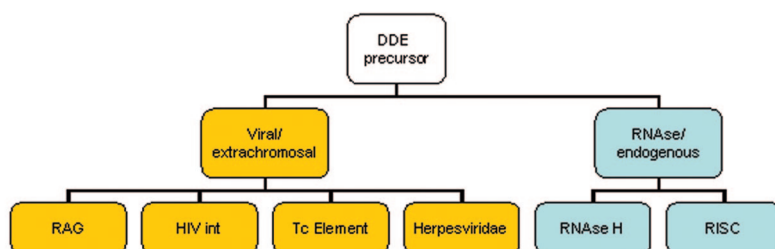
The RAG proteins are required for immunoglobulin and T-cell receptor recombination, which generates the diversity of the acquired immune response, and their absence results in a phenotype of severe combined immunodeficiency (SCID) in both humans and knockout animals.^{5,6,17,18} Partial deficiency of the RAG recombination enzymes results in the Omenn syndrome of immunodeficiency and atopic dermatitis.¹⁹⁻²² Other unusual presentations of selective immunodeficiency due to partial RAG function have been described.²³

RAG proteins required for V(D)J recombination share a common recombination mechanism with diverse proteins important in innate immunity, including RNA-induced silencing complex (RISC) and RNase H, as well as viral recombinases (Fig 1).²⁴⁻²⁶ RISC encodes a DDE nuclease denoted “slicer” that can be programmed to cut and inactivate messenger RNA (mRNA) with short complementary RNA sequences termed *RNAi*.²⁷ For example, the RISC DDE enzyme can be programmed to destroy mRNA encoded by human immunodeficiency virus (HIV) or other viral pathogens.²⁸

Paradoxically, the enzyme family that recombines immunoglobulin and T-cell receptor genes to form an immune repertoire against viral pathogens, including retroviruses such as HIV, is related to enzymes required for the same pathogens to replicate (Fig 1). Thus, retroviral gene vectors that encode a DDE enzyme RAG for therapy of RAG deficiency are also inserted into the genome using a DDE enzyme, the retroviral integrase.²⁹ Additional members of the same family of DDE enzymes may also be required for the recombination of herpes viruses,³⁰ and infection with some pathogens, such as Epstein-Barr virus, activate expression of the *RAG* genes in lymphocytes.³¹

A consequence of the interrelated DDE enzymes is that therapies designed to inactivate one form of DDE recombinase, for example, retroviral integrase inhibitors,³²⁻³⁴ can interfere with the RAG proteins required to generate the immune repertoire.³⁵ Theoretically, the retroviral integrase itself could bind to and interfere with the RAG proteins through a hybrid bimolecular DDE site, and retroviral integrase inhibitors might also be effective against other pathogens, such as herpes viruses.³⁶

DDE* Recombination Superfamily:



Nucleic Acid Recombination

Catalyzed by magnesium or divalent cation

Binding conserved aspartate (D) and glutamate (E) residues

Figure 1. Enzymes with a DDE-like magnesium-binding site include the RAG proteins required for generation of the acquired immune repertoire, recombinases and integrases encoded by various viral pathogens and transposons, and the RNA-induced silencing complex (RISC) complex and RNase H required for innate immunity to viral pathogens. These diverse DDE enzymes may have descended from a DDE-precursor enzyme able to cleave and recombine nucleic acids. HIV rt indicates human immunodeficiency virus reverse transcriptase.

ESSENTIAL ROLE OF RAG PROTEINS IN GENERATION OF THE IMMUNE REPERTOIRE

When the RAG-encoded proteins were first identified through reverse genetics,^{5,6} it was not clear whether RAG proteins were transcription factors that activated a cellular recombinase that subsequently recombined immunoglobulin genes or were the recombinase that recombined the immunoglobulin genes. The work of several laboratories was required to demonstrate that the RAG proteins are recombinases that splice together multiple sections termed V and J in the case of the T-cell receptor and immunoglobulin light chain genes and V, D, and J in the case of the immunoglobulin heavy chain (Fig 2). The sites of immunoglobulin recombination by RAG proteins are termed V(D)J recombination signal sequences (RSS) to indicate that these specific sequences are required for site-specific joining of immunoglobulin V, D, and J regions (Fig 2).

Other host factors are required for efficient V(D)J recombination in vitro and in vivo, including host factors such as the ubiquitous Ku protein and DNA bending proteins.³⁷ A host protein denoted Artemis opens the hairpin ends generated by RAG.^{38,39} After processing by Artemis, the free DNA ends at the splice joint are then repaired by several additional host DNA repair enzymes regulated by the DNA-dependent protein kinase (DNAPK), which is recruited to free DNA ends by Ku.⁴⁰ Deficiency of any of these enzymes, either the RAG proteins or the host-encoded DNA repair proteins such as Artemis^{38,39} or DNAPK (required for phosphorylation-dependent activation of DNA repair enzymes), can thus result in a complete lack of both T and B lymphocytes and a SCID phenotype clinically similar to RAG deficiency.⁴⁰

Defects in DNAPK or another important kinase-sensing damaged DNA termed ATM kinase (ataxia-telangiectasia

mutated)^{41–44} can also result in partial immunodeficiency with a radiation sensitive phenotype due to defective DNA repair in such disorders as ataxia-telangiectasia, because these host proteins are also involved in repair of free DNA ends (Fig 3). Diversity of immunoglobulin and T-cell receptors results from the largely random joining of different V, D, and J segments, the random insertion of nucleotides at the joints,⁴⁰ and the RAG-independent processes of immunoglobulin somatic mutation and class switching reviewed elsewhere.^{45,46} Because rearranged genes express many more immunoglobulin and T-cell receptors than could be encoded by the original gene segments, altering the activity of proteins in the V(D)J recombination pathway such as RAG might thus be useful either in increasing or decreasing the generation of the immune repertoire.

REGULATION OF RAG PROTEIN EXPRESSION AND V(D)J RECOMBINATION

Defects in immune repertoire development may also be related to defective control of expression of V(D)J recombination rather than the absence of V(D)J recombination. RAG protein expression is a dynamic process.^{47–51} Through RAG reactivation termed *receptor editing*, novel immunoglobulin or T-cell receptor genes can be generated to replace autoreactive or nonreactive B cells. Dual receptor T cells in which 2 T-cell receptors of different specificities are expressed on the cell surface of a single T cell can result from V(D)J recombination. A number of models have been suggested regarding the regulation and role of receptor editing in autoimmunity or other abnormalities.^{49,51–57}

Regulatory mechanisms limit the powerful RAG recombination only to recombine genes of immunoglobulin and T-cell receptors and the process does not normally target other genes.^{8,40} First, immunoglobulin and T-cell receptor genes recombine during a portion of the cell cycle termed G0/G1 (growth 0 and 1) at which other time other genes and DNA are not replicating or accessible to recombination.^{58,59} Subsequently, when other genes are replicating and recombining during the synthesis phase of the cell cycle, the RAG recombinase is inactivated by phosphorylation and degradation of the RAG-2 protein.^{58,59}

The DNA ends generated by action of the RAG proteins are also held in a nucleoprotein complex, including the RAG proteins, where they cannot recombine with other genes.⁷ Another checkpoint, the tumor suppressor p53 family of proteins regulating cell growth and apoptosis,⁶⁰ detects any free DNA ends present in the cell. Free DNA ends activate DNAPK and ATM kinase, which in turn phosphorylate a repressor protein mdm2 and cause mdm2 to release p53 to the cell nucleus and stop cell division until the free ends are repaired (Fig 3). Loss of these checkpoints in hereditary disorders such as ataxia-telangiectasia due to mutation of the p53-activating ATM kinase leads to both defective generation of the immune repertoire and elevated rates of cancer in hematopoietic cells and radiation sensitivity.^{41–44,61} Since at least some human cancers appear to result from incorrect

Mechanism of V(D)J recombination

Germ Line V, D, J segments:

V-V-V-V-V-V-----J-J-J-J-J-J-J-J-J-J-conserved Ig light chain, TCR

V-V-V-V-V-V-----D-D-D-D-D-D-----J-J-J-J-J-J-J-J-J-J-conserved Ig heavy chain

Rag Proteins



V-V-V-V-V-V-----J-J-J-J-J-J-J-J-J-J-conserved Ig light chain, TCR

V-V-V-V-V-V-----J-J-J-J-J-J-J-J-J-J-conserved Ig light chain, TCR

Figure 2. RAG proteins RAG-1 and RAG-2 form a DDE enzyme that cuts tandemly repeated germline immunoglobulin genes V, D, and J segments and recombines them to form specific immunoglobulin and T-cell receptor (TCR) genes in hematopoietic cells. Joining of V and J segments (illustrated in colored typeface) to form a mature immunoglobulin chain is shown in more detail in the lower portion of the figure. Noncoding sequences are removed from the transcribed messenger RNA by posttranscriptional splicing as shown.

Mechanism of V(D)J recombination

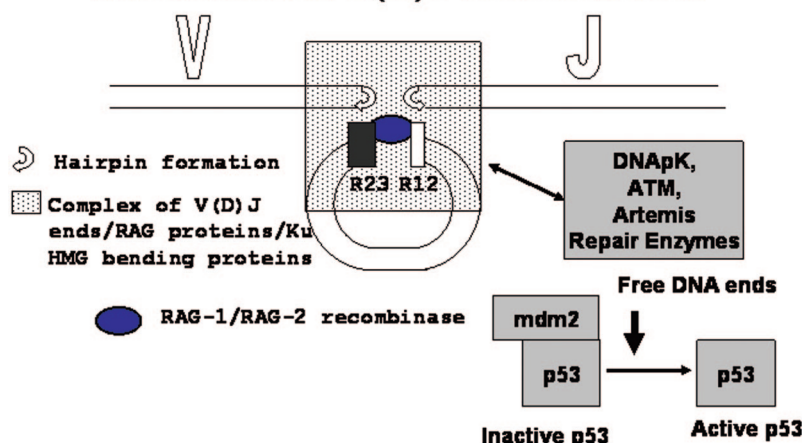


Figure 3. The process of RAG-mediated recombination of the V(D)J recombination signal sequence. Initial cleavage of V and J segments (Fig 2) by RAG proteins and DNA-binding protein such as Ku and high-mobility group (HMG) proteins generates blunt-ended DNA breaks termed *hairpins*. Artemis and other DNA repair proteins form a protein complex that can open and join hairpin DNA ends generated by RAG cleavage to form the variable coding joint required for immunoglobulin and T-cell receptor repertoire development. Proteins that detect DNA damage, such as DNA-dependent protein kinase (DNAPK) and ataxia-telangiectasia mutated (ATM) kinase, are also involved in the recombination process by activating repair factors and arresting cell division until broken DNA ends are repaired. If free DNA ends are not repaired correctly, the tumor suppressor p53 is activated by inactivation of the repressor mdm protein, causing cell death by apoptosis.

joining of V(D)J-like sequences to immunoglobulin or T-cell receptor genes, there remains the possibility that occasional failures of the RAG checkpoints may play a role in cancer mutations.^{8,40}

The RAG proteins also participate in targeted recombination events in generation of the gamma-delta^{62,63} T-cell repertoires in which only a limited repertoire results from regulated nonrandom targeting of the recombinase. A highly restricted T-cell receptor repertoire is also typical of the NK T-cell lineage that plays a critical role in immune regulation and defense against some viral pathogens such as Epstein-Barr virus.^{64,65} Thus, the RAG proteins generate highly diverse repertoires typical of immunoglobulin and $\alpha\beta$ T-cell receptors, as well as receptors with more limited binding specificities. Since some defects related to RAG recombination, such as loss of ATM kinase and DNAPK, result in neurologic abnormalities and defective brain development, the possibility remains that RAG or related genes may play some role in the development of the nervous system, for example, in generation of neuronal diversity via control of neuronal apoptosis or receptor expression.^{42,44,66–68}

ORIGINS OF THE RAG PROTEINS FROM SIMPLER DDE RECOMBINATION PROTEINS IN INVERTEBRATES

Functional similarities are evident between the mechanisms and products of retroviral integration and RAG V(D)J recombination such as the formation of covalently closed circular recombination products (Fig 4). As noted herein, the active site of both the RAG proteins and retroviral integrase binds an Mg^{++} or similar divalent cation required for DNA cleav-

age and rejoining. The active site of all of these recombinases is formed by D (aspartic acid) and E (glutamic acid) residues. These acidic residues bind the positively charged magnesium ion, termed a *DDE site*. In the case of the RAG proteins and transposons or mobile elements, similarities are evident not only in the shared DDE magnesium-binding site these enzymes use to cut and rejoin nucleic acid target sequences but also in the sequences of the target sequences recognized (Fig 5).⁶⁹ Did all of these DDE enzymes result from descent from a common precursor molecule (as suggested in Fig 1) or rather did DDE enzymes arise from a number of different pathways converging to a common recombination mechanism?

In contrast to retroviral integrases and transposon transposases, which are relatively small molecules of approximately 300 to 400 amino acids, the RAG-1 protein is much larger, almost 1,000 amino acids. The RAG-2 protein may be a completely unrelated protein to RAG-1 and serves to stabilize the DDE site in RAG-1.⁷⁰ Some insights into the origins of RAG proteins have been provided recently through the study of nonvertebrate organisms such as sea urchins that lack RAG proteins and V(D)J recombination but have transposons with some similarity to RAG-1 and structural analysis of the RAG proteins.^{69–71} Invertebrate genomes such as the sea urchin and fruit flies lack V(D)J recombination but contain transib transposons with a carboxyl terminus similar to the RAG-1 carboxyl terminus, including a DDE-like site but lack the RAG-1 amino terminus of approximately 300 amino acids.⁶⁹ Some transib transposons in the sea urchin seem to have fused at their amino terminus with another apparently unrelated sea urchin protein, leading to a larger protein sim-

Retroviral Integration/V(D)J Recombination

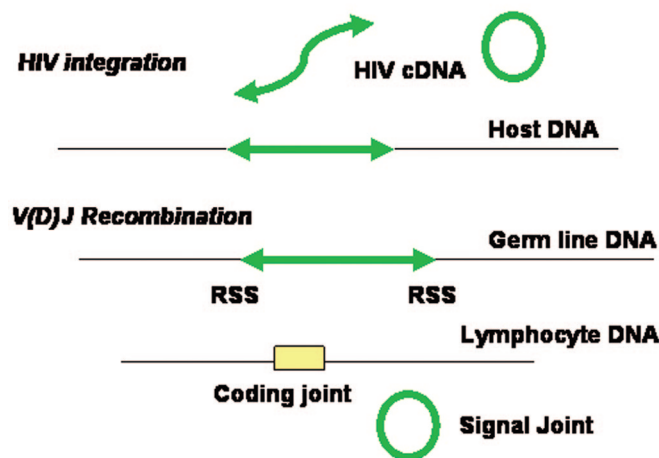


Figure 4. An inverse functional relationship is evident between the process of retroviral integration, which inserts reverse transcribed proviral DNA into the genome and also forms covalently closed extrachromosomal circles of the viral genome (top), and V(D)J recombination, which removes covalently closed extrachromosomal circles of V(D)J excision products and leaves behind variable coding joints. HIV indicates human immunodeficiency virus; cDNA, complementary DNA; RSS, recombination signal sequence.

DDE Recombination Signals

- Tc1: **CAGTGCTGGCCAAAAGATATCCACTTTTG**
- Tc3: **CAGTGTGGGAAAGTTCTATAGGACCCCC**
- EBV: **CACAGGCAACCCTGACAAAGCCCCCAGGAAAGA**
- HSUb: **CACACCCCCCGGGGTGCGCGCGCGCCCTTTAAAG**
- HSUc: **CAGGCCGCCCGGACCGCGCCCGCCTTTTTTG**
- R12: **CACAGTGCTACAGACTGGAACAAAAACC**
- R23: **CACAGTGGTAGTACTCCACTGTCTGGCTGTACAAAAAC**
- TS1b: **CACAGTGGCAATTGGCCGAATTTTTTTGGAAAAAGCAA**

Figure 5. Known signal sequences for DDE recombinases all have a terminal CA dinucleotide (C = cytidine, A = adenine, G = guanine, and T = thymidine in this figure) and tracts of multiple A and T bases associated with DNA bending. Sequences shown include the termini of DNA transposons, such as Tc1 and Tc3, and the transib family, which most closely resemble the V(D)J recombination signal sequence (RSS). The V(D)J RSS shown as consensus sequences denoted R12 and R23, since the RSS may have either 12 or 23 nucleotides between the terminal CA dinucleotide and the A/T-rich sequences termed nonamers. Recombining regions of herpes viruses, such as Epstein-Barr virus (EBV) and herpes simplex virus, may also recombine by a DDE mechanism, since they have sequences that resemble known DDE signals as shown and also may encode a conserved DDE like protein (see text and cited references, Dreyfus et al).

ilar to RAG-1.⁶⁹ Thus, RAG-1 seems to be 2 separate proteins that are fused together, a carboxyl terminus with a DDE site from a transib transposon and an amino terminus from an unrelated protein of unknown function. Furthermore, at one genomic site the sea urchin RAG-1 like protein appears to be

located next to a RAG-2 like protein just as it is in the vertebrate genome.⁷¹ These observations support the hypothesis that diverse DDE enzymes, including the RAG proteins, retroviral integrases, and transposases, all have arisen from a primordial transposase-like molecule encoded by a transposable element or transposon.

STRUCTURE OF DDE SITES IN RETROVIRAL INTEGRASES AND RNase H

In contrast to the RAG proteins, DDE proteins, including the retroviral integrases, have been crystallized and their structures solved, thus providing some important hints as to RAG and DDE enzyme mechanisms of action. The crystal structure of the retroviral integrase was the first of this family of enzymes to be solved and showed a folded region of proteins strands termed an *RNase H fold* surrounding the DDE magnesium site,^{13,14} suggesting that retroviral integrase was a distant relative of RNase H (Fig 6). RNase H, a much simpler enzyme structure than the RAG-1 protein or retroviral integrase, plays a more limited role in degrading hybrids between DNA and RNA that occur during viral and genomic replication.⁷²⁻⁷⁷

Retroviruses encode their own copy of RNase H in addition to retroviral integrase; thus, a variety of small molecules, including thioamides, aminoglycosides, neomycin, and N-hydroxyimides,⁷⁷⁻⁸¹ have been characterized that block RNase H rather than or in addition to retroviral integrase. As in the retroviral integrase and RAG proteins, a DDE-bound magnesium ion is responsible for the cleavage of the DNA/RNA hybrid by RNase H.^{73,76}

Unlike RNase H, RAG proteins and the retroviral integrases can participate in both integration events in which

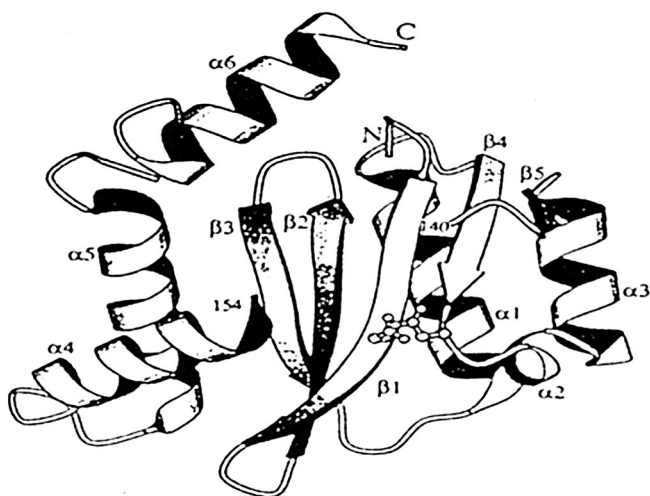


Figure 6. The RNase fold structure first identified in RNase H is shown from the crystal structure of human immunodeficiency virus 1 (HIV-1) integrase. The RNase fold contains amino acids that form a central plane of folded β -pleated sheets flanked by flexible α -helices forming a pocket that can position a divalent magnesium ion (Mg^{++}) in proximity to a DNA double-stranded helix, causing breakage of the DNA strand. Reversal of the process results in DNA joining. The reader is directed to additional references cited in the text for a more detailed discussion of the mechanism of RNase H and RAG protein nucleic acid cleavage, for example, Nowotny et al.⁷⁶

DNA is inserted at a novel site, as well as disintegration events in which the reverse occurs and DNA is removed from sites in the genome. DDE enzymes must be able to hold onto the separated ends of cut DNA in a stable complex to permit rejoining. Because of the possibility of both integration and

disintegration by the same enzyme, it seems that retroviral integrases could in principle also be used to disintegrate retroviruses or other genetic material from the genome, whereas the RAG proteins normally disintegrating V(D)J RSS circles from between immunoglobulin genes (Fig 4) could conversely be used to integrate genomic sequences into the host chromosome if the mechanisms were fully understood. The first generation of retroviral gene therapy vectors insert randomly or at unpredictable preferred sites into the genome.²⁹ These obstacles may be overcome in the future through a combination of new vectors that are more site specific in their integration sites and also vectors that are less likely to unpredictably activate nearby genes. A more immediate benefit that results from the solution of the retroviral integrase DDE site is the generation of retroviral integrase inhibitors, some of which are currently in clinical trials,^{32–34,48} as well as other potential HIV therapies based on inactivation of RNase H.^{77–81}

THE RISC FAMILY OF DDE-LIKE ENZYMES

Another type of DDE-like enzyme is a related family of proteins termed RISC. RISC is a component of innate immunity to viral pathogens that operates on single- and double-stranded RNA templates that are cut and inactivated (Fig 7).^{25,26} RISC has a function termed “slicer” that cuts double-stranded RNA (Fig 8), whereas the RAG proteins and retroviral integrases are active on double-stranded DNA, which they cut and rejoin (Figs 3 and 4). RISC is a site-specific enzyme and can be used to specifically turn off genes,^{27,28} termed *gene silencing* or *RNA interference*.

Like the retroviral integrases (Fig 6) and RNase H, the RISC structure has been crystallized and solved.^{25,26} RISC has

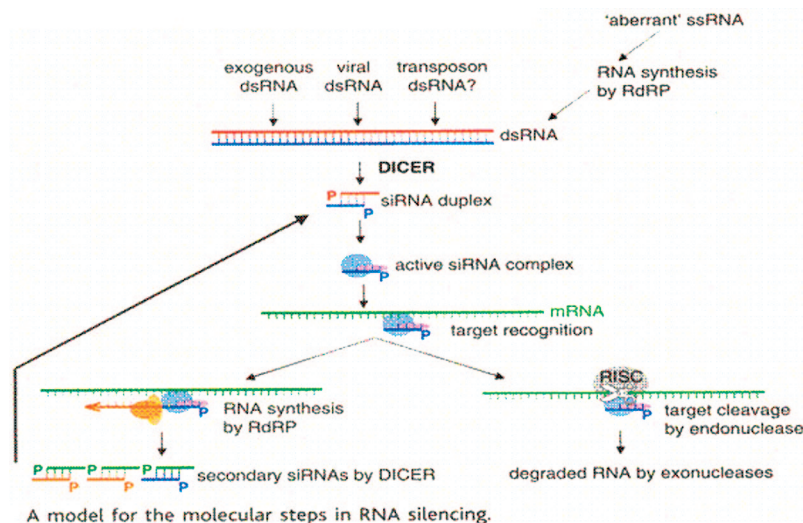


Figure 7. RNA silencing by the RNA-induced silencing complex was discovered initially during studies of DNA transposons but is a general form of innate antiviral immunity in vertebrates, invertebrates, and plants. siRNA indicates double-stranded silencing RNA (also termed RNAi) bound to RISC; ss, single stranded; ds, double stranded. Amplification of silencing RNA by RNA-dependent RNA polymerase (RdRP), an amplification of siRNA by host polymerases shown in this figure, does not occur in vertebrates.

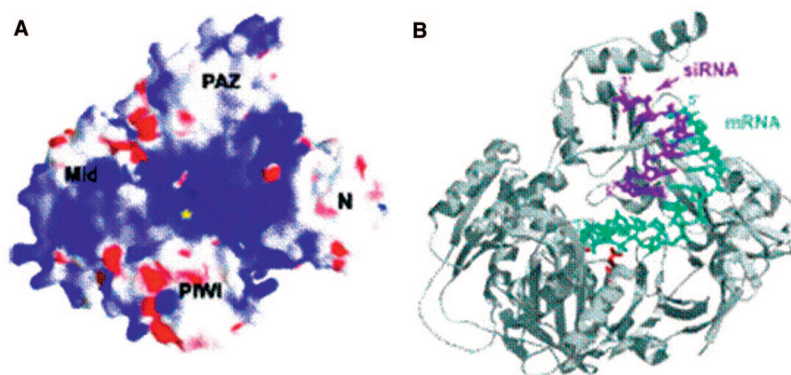


Figure 8. The structure of the RNA-induced silencing complex (RISC) determined by x-ray crystallography demonstrates that subunits PAZ (top of left structure model) and PIWI (bottom of left structure model) form a groove that binds double-stranded RNA analogous to the binding of double-stranded DNA by the retroviral integrases (Fig 6). As in the retroviral integrases which cleave nucleic acids by a magnesium binding DDE site, cleavage of the double-stranded nucleic acid by RISC occurs by a magnesium ion bound in a DDE-like pocket formed by an RNase H-like fold in the PIWI region of RISC. siRNA indicates double-stranded silencing RNA.

an amino terminal sequence (PAZ) that binds single-stranded RNA guide sequences and a carboxyl RNase H-like domain termed *PIWI* that contains a DDE-like site (Fig 8). In contrast to other described DDE sites, the RISC magnesium-binding site may contain a histidine (H) amino acid at one of the magnesium-binding positions instead of a D or E, an observation of unknown significance.

A single-stranded RNA guide sequence bound to PAZ can then bind to a complementary target mRNA and cut the target using the DDE-like site present in another portion of the enzyme, PIWI. The result is that the target mRNA is destroyed and released from the RISC, which then binds and inactivates more mRNA, binding the guide sequence.^{24,26} Because this is a catalytic process, a single RNA guide sequence complementary to a target RNA can potentially destroy hundreds or thousands of target mRNA, resulting in gene silencing. The RISC complex also seems to compose a multigene family in vertebrates with multiple roles in both antiviral response and gene expression and development.

CONCLUSION

Clinicians practicing in clinical immunology should be aware of both the function of RAG proteins and the recent discovery that RAG proteins share a common recombination mechanism with a much larger family of proteins termed *DDE recombinases* (Figs 1 to 5). Any combined T- and B-cell immunodeficiency or immunodeficiency with atopic features should be considered as a possible defect in the RAG proteins or V(D)J recombination (Figs 2 and 3) for further genetic counseling and gene therapy should that option be available in the future.²⁹

Members of the DDE family of enzymes whose structures have been solved all contain a common structure termed an RNase H fold and a bound Mg^{++} ion or ions at a DDE-like site (Figs 6 to 8). Thus, a similar enzymatic reaction may serve multiple and even opposing roles in immunity and

infection. Possibly, all of these enzymes are descended from a common precursor recombinase capable of cutting and rejoining nucleic acids at a bound Mg^{++} ion (Fig 1). It is interesting to speculate that drugs developed to target one form of DDE enzyme, for example, retroviral integration or RNase H, may also with some modification be effective in suppressing or activating other forms of DDE enzymes. DDE-blocking molecules that bind and inactivate RNase H or the retroviral integrases such as thioaptamers, aminoglycosides, neomycin, and N-hydroxyimides thus might be modified to bind and either block or stimulate RAG or RISC protein functions. Alternatively, RAG activation could stimulate immune responses or correct immunodeficiency such as SCID or Omenn syndrome related to loss of RAG function, whereas RISC activation could augment antiviral or anticancer responses.

Targeted inactivation of V(D)J recombination could also be used to prevent generation of novel T- and B-cell receptors without affecting existing T- and B-cell repertoires, for example, in tissue transplantation. Other gene silencing- or RNA interference-based therapies that use RISC and other endogenous RNA processing pathways (Figs 7 and 8) are anticipated for immunological diseases and other chronic diseases if technical obstacles, such as delivery to specific tissues⁸² and drug-related toxic effects,⁸³ can be overcome.

ACKNOWLEDGMENTS

Dr Dreyfus has been supported in part by National Institutes of Health training grants T32-GM07288 and T32-AI07365. Dr Dreyfus is currently a member of the Clinical Faculty at the Yale School of Medicine, Department of Pediatrics, New Haven, CT, and is also the founder and medical director of a biotechnology company, Keren Pharmaceutical Inc, located in New Haven, CT. Keren is devoted to the development of novel gene targeting-based therapies of infectious and immunological diseases. Neither Dr Dreyfus nor Keren Pharmaceutical advocate the use

of existing therapies targeting DDE recombination, such as the retroviral integrase for therapy of other specific disease, and this possibility is discussed only as a theoretical consideration for future nonclinical basic research.

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Objectives: After reading this article, participants should be able to demonstrate an increased understanding of their knowledge of allergy/asthma/immunology clinical treatment and how this new information can be applied to their own practices.

Participants: This program is designed for physicians who are involved in providing patient care and who wish to advance their current knowledge in the field of allergy/asthma/immunology.

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CME Examination

1–6, Dreyfus D. 2006;97:567–576.

CME Test Questions

1. Substrates for DDE recombinases include:
 - a. double-stranded RNA
 - b. double-stranded DNA
 - c. DNA/RNA hybrids
 - d. amino acids
 - e. all except D
2. Mutations in the following can lead to severe combined immunodeficiency:
 - a. Artemis
 - b. RAG-1
 - c. RAG-2
 - d. DNA protein kinase and DNA-dependent protein kinase-activated DNA repair proteins
 - e. all of the above
3. Omenn syndrome consists of all except:
 - a. immunodeficiency
 - b. abnormal teeth
 - c. eczema
 - d. limited T-cell repertoire
 - e. abnormal function of RAG proteins
4. True statements about ataxia-telangiectasia include all except:
 - a. RAG-mediated V(D)J recombination results in abnormal joints
 - b. RAG genes are mutated
 - c. DNA has abnormal sensitivity to radiation damage
 - d. tumor suppressor p53 is not activated correctly
 - e. Neurologic defects are present
5. The active site of RNA-induced silencing complex binds magnesium ion and is termed
 - a. Artemis
 - b. dicer
 - c. slicer
 - d. Zap-70
 - e. SLAM
6. Inhibitors of RNAase H required for human immunodeficiency virus replication include all except:
 - a. thioaptamers
 - b. aminoglycosides
 - c. cephalosporins
 - d. neomycin
 - e. N-hydroxyimide

Answers on page 602.