Journal of Biochemistry and Molecular Biology, Vol. 36, No. 2, March 2003, pp. 201-206

JOURNAL OF
Biochemistry and Molecular Biology

© KSBMB & Springer-Verlag 2003

Recombination Activating Gene 1 Product Alone Possesses Endonucleolytic Activity

Deok Ryong Kim*

Department of Biochemistry, College of Medicine, Gyeongsang National University, 92 Chilam-dong, Jinju 660-751, Korea

Received 13 September 2002, Accepted 6 November 2002

Two lymphoid-specific proteins, RAG1 and RAG2, are required for the initiation of the V(D)J recombination *in vitro*. The V(D)J cleavage that is mediated by RAG proteins at the border between the coding and signal sequences results in the production of a hairpin at the coding end and a double-stranded break at the signal end. Two hairpin coding ends are re-opened, modified, and sealed; whereas, the signal ends are directly ligated. Here I report that only RAG1 can carry out a distinct endonucleolytic activity *in vitro* using an oligonucleotide substrate that is tethered by a short single-stranded DNA. The purified RAG1 protein alone formed a nick at the near position to the recombination signal sequence. This endonucleolytic activity was eliminated by immunoprecipitation using the RAG1-specific antibody, and required the 3'-hydroxy group. All of the RAG1 mutants that were incapable of the nick and hairpin formation in the V(D)J cleavage analysis also showed this new endonucleolytic activity. This suggests that the nicking activity that was observed might be functionally different from the nick formation in the V(D)J cleavage.

Keywords: RAG1, V(D)J recombination, Nuclease

The gene that encodes the variable region of the immunoglobulin (Ig) or T-cell receptor (TCR) is assembled by a unique process called the V(D)J recombination from small segments of variable (V), diversity (D), and joining (J) (Tonegawa, 1983). Each segment is flanked by a recombination signal sequence (RSS) that contains a conserved heptamer and nonamer that are separated by a relatively unconserved sequence of either 12 or 23 base pairs. This gene segment rearrangement can provide the diverse Ig or TCR molecules during lymphoid cell development (Sakano *et al*., 1979; Lewis, 1994).

The mechanism of the initial V(D)J cleavage has been extensively studied using two purified RAG1 and RAG2 proteins. The RAG1/RAG2 complex recognizes RSSs and forms a nick at the coding/signal border of the top DNA strand (Hiom and Gellert, 1997; Akamatsu and Oettinger, 1998; Bailin *et al*., 1999; Mo *et al*., 1999). Subsequently, the free hydroxyl group directly attacks the phosphodiester linkage of the other bottom strand through direct-transesterification, which results in a double-strand break (DSB) formation: a covalently sealed-coding end (hairpin) and a blunt signal end (McBlane *et al*., 1995; van Gent *et al*., 1995; Estman *et al*., 1996; Ramsden *et al*., 1996). Each DNA end at the two signals joins together through the action of many DNA repair proteins, such as DNA-PK, XRCC4, DNA ligase IV, or other unknown proteins (Li *et al*., 1995; Grawunder *et al*., 1998; Hammmarsten and Chu, 1998; McElhinny *et al*., 2000). Contrasting the signal ends, the two coding ends are usually further processed by nuclease or TdT before they join together. The process of joining steps in the V(D)J recombination is still unclear.

The recombination activating gene (RAG) 1 and RAG2 are composed of a single polypeptide of 1040 and 527 amino acids (a.a.), respectively (Schatz *et al*., 1989; Oettinger *et al*., 1990). Both proteins are specifically expressed in only lymphocytes (B or T cells), where the V(D)J recombination actively occurs. It is absolutely required to form a synaptic complex with the two recombination signal sequences and complete the V(D)J cleavage at both RSSs (Ramsden *et al*., 1997). According to recent data, the RAG proteins may also be involved in opening hairpins that are produced by the RAG-dependent cleavage (Besmer *et al*., 1998; Shockett and Schatz, 1999). However, their roles in hairpin opening remain unclear. Also, other proteins such as the Artemis/DNA-PK complex or NBS1/RAD50/MRE11 complex have been suggested as hairpin-resolvers in the V(D)J recombination (Paull and Gellert, 1999; Ma *et al*., 2002). The endonuclesase activity of Artemis is acquired when it is phosphorylated by

*To whom correspondence should be addressed.
Tel: 82-055-751-8734; Fax: 82-055-759-8005
E-mail: drkim@nongae.gsnu.ac.kr

DNA-PK; this activity is sufficient to open hairpins that are generated by the RAG complex.

V(D)J recombinase is quite similar to bacterial transposases, HIV integrase, or other DNA polymerases that require three critical negative-charged amino acids (aspartate or glutamate) for their catalysis, called DD..E motif. These acidic amino acid residues can chelate divalent ions (Mg^{2+}) that stabilize a transient reaction complex in catalysis. In recent papers, these acidic residues of the V(D)J recombinase are found only in RAG1 (Kim *et al*., 1999; Landree *et al*., 1999; Fugmann *et al*., 2000). The mutation on aspartate 600 and 707 residues to other residues on RAG1 completely eliminate the V(D)J recombination activity, although they bind to the RSSs to form a normal synaptic complex together with RAG2. In conclusion, RAG1 plays a very important role in providing some critical residues for catalysis in the V(D)J recombination.

Two signal-tethered substrates have been used to study the mechanism of the coupled cleavage in the V(D)J recombination (Kim and Oettinger, 1998). A small single-stranded DNA tether can provide enough flexibility for the two signal sequences to form the synaptic complex with RAG proteins. In this report, I will explore a novel endonucleolytic activity of RAG1 that is distinct from the V(D)J recombinase activity by the RAG1/RAG2 complex by using this oligonucleotide substrate *in vitro*.

Materials and Methods

Substrate preparation and cleavage assay All of the substrates and assays have been described (Kim and Oettinger, 1998). Labeling at the 3' end of a DNA strand was carried out by either a reaction that contained terminal deoxy-nucleotide transferase (TdT) (New England Biolab, Beverly, USA) and ^{32}P-α-dCTP (NEN, Boston, USA) at 37°C for 30 min, or a reaction with T7 DNA polymerase (Boeringer Mannheim, Mannheim, Germany) and ^{32}P-dCTP at 37°C for 5 min, after two oligonucleotides were annealed. Labeling at the substrate that contained a dideoxynucleotide at the second position from the 3 end was performed by the reaction with ^{32}P-dideoxy-ATP (Amersham Pharmacia Biotech, Piscataway, USA), dCTP (for the first complementary nucleotide), and T7 DNA polymerase. Following the addition of EDTA (final 20 mM), the reaction was extracted by a phenol-chloroform solution and purified by a G-50 spin column.

RAG1 immunoprecipitation The myc-tagged wild-type RAG1 (1.6 μg) protein, purified from Sf9 insect cells using a baculoviral expression system, was incubated with an anti-myc antibody (1.6 μg, Oncogene Science, Cambridge, USA) in a solution (20 mM HEPES, pH 7.5, 20% glycerol, 200 μg/ml BSA, and 0.02% NP-40) for 1 h at 4°C. An anti-MBP antibody (New England Biolab, Beverly, USA) was used as the negative control. The RAG1-antibody mix was added to 15 μl of Immunopure@plus immobilized protein G bead (cross-linked 6% agarose, capacity of 20 μg IgG per μl gel, Pierce, Rockford, USA) that was pre-equilibrated by the above solution, then incubated at 4°C for 3 h with gentle mixing (Bae and Lee, 2001; Kim, 2001). The slurry was spun briefly, and the supernatant was subjected to the cleavage assay that was previously mentioned.

Results

RAG1 alone has nicking activity The RAG1 and RAG2 proteins are absolutely required for the completion of cleavage in the V(D)J recombination *in vitro* or *in vivo*. An oligonucleotide substrate that was tethered by a short single-stranded DNA was used to study the mechanism of the coupled cleavage in the V(D)J recombination using two purified RAG proteins from a previous study (Kim and Oetinger, 1998). This tethered substrate, which was labeled at each position, allowed us to detect various cleavage products from the RAG-dependent cleavage. When labeled at the 5' end position of the bottom strand of the substrate, three different fragments from the RAG-dependent cleavage can be expected (Fig. 1A). A fragment (indicated as *a*) was produced by nicking only at the 12 signal sequence; the *b* fragment came only from the hairpining at the 12 signal sequence without cleavage at the 23 signal sequence. The *c* fragment was a result of the complete cleavage at the 23 signal sequence.

Surprisingly, a new fragment of about 102 nt (indicated by *d* in Fig. 1B) was detected in the RAG1-dependent manner in the presence of Mn^{2+} as a divalent ion. It was also produced in the presence of both RAG1 and RAG2 (lane 5 in Fig. 1B). This fragment was undetected in either the reaction that was omitted by both RAG1 and RAG2, or the reaction only with RAG2. Additionally, another unexpected fragment in the presence of both RAG1 and RAG2 was detected at a position that was equivalent to about 95 nt (marked by *e*). Three other fragments (indicated by *a, b, c*), which resulted from the RAG1/2-dependent cleavage, also appeared as expected (lane 5, Fig. 1B).

In order to confirm that this nuclease acts as an endonuclesase, the same oligonucleotide substrate was labeled at the 3' end of the bottom strand and subjected to cleavage analysis (Fig. 1C). RAG1 alone produced a 48 nt fragment (indicated as *a* in Fig. 1C) that was complementary to the product (about 100 nt) that was derived from the 5' labeled substrate (total length of the bottom strand is 148 nt). Also, nicking or hairpining by RAG1/RAG2 only at the 12 RSS generated a 36 nt fragment (indicated as *b* in Fig. 1C). Therefore, this RAG1-specific activity was endonucleolytic.

Since we can not rule out the possibility that the RAG1-specific fragment might come from a specific protein preparation, I next tested a few more RAG1 preparations and a different RAG1 protein version that was tagged to MBP (maltose-binding protein). As shown in Fig. 2A, two separately-purified RAG1 proteins had the same activity to generate a RAG1-specific fragment. Also, MBP-RAG1, fully

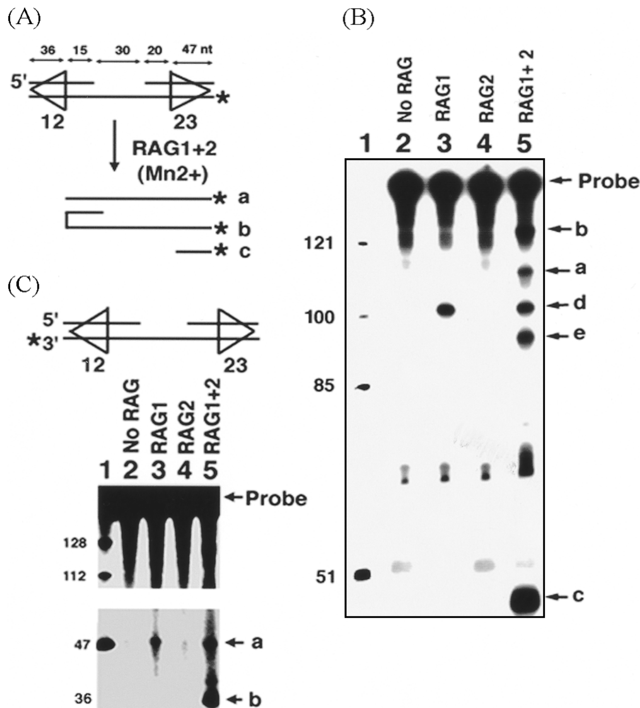

Fig. 1. RAG1 nicking activity. (A) Substrate. This tethered substrate was described by Kim and Oettinger (1998). The 5' end of the bottom strand was labeled by ^{32}P (marked by *). Triangle indicates a recombination signal sequence (RSS). The RAG1 and 2-dependent cleavage in the presence of Mn^{2+} generates three different-sized fragments as follows: the *a* fragment (112 nt) is from nicking at the 12 signal sequence. The *b* fragment (127 nt) is from hairpining at the 12 signal sequence. The *c* fragment (47 nt) from hairpining at the 23 signal sequence. (B) Endonuclesase activity of RAG1. Cleavage reaction was performed (Kim and Oettinger, 1998) in the presence of the oligonucleotide substrate, as previously described. Each product was separated on a 8M-urea and 10% superdenaturing polyacrylamide gel electrophoresis. Lane 1 shows the size markers 121, 100, 85, 51 nt. Lane 2 shows the reaction where both RAG proteins are omitted. Lane 3 shows only RAG1. Lane 4 shows only RAG2. Lane 5 shows a reaction with RAG1 and RAG2. The positions of each reaction product (*a-e*) are indicated by arrows. (C) Labeling at the 3 end of the substrate. The bottom strand of the DNA substrate was labeled by the TdT reaction with ^{32}P-α-dCTP, and annealed with two other oligonucleotides. Labeling at this position creates only two products in the RAG1/RAG2 reaction. One is 36-nt, a product from either nicking or hairpining at the 12 signal (marked as *b*). The other is 121-nt, a product from hairpining at the 23 signal (not indicated on the gel). The fragment *a* is a RAG1-specific product.

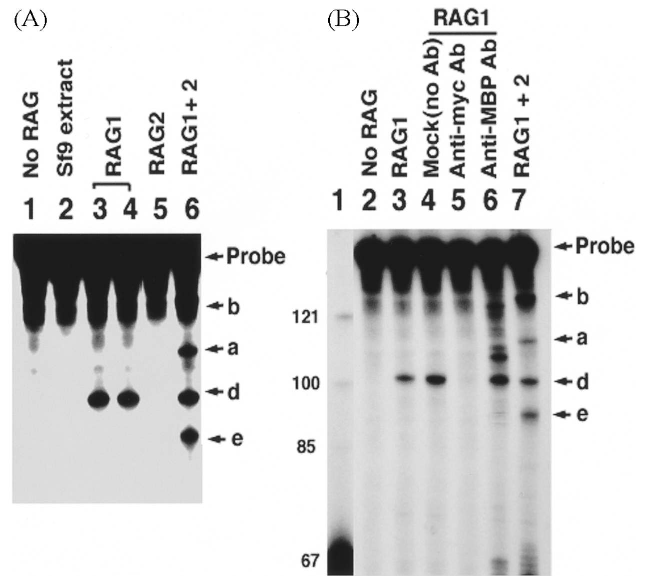

Fig. 2. (A) The Sf9 extract (lane 2) was prepared under the same condition as RAG1. Two RAG1 preparations (lanes 3 and 4) were performed on two different days under the same condition. (B) Immuno-depletion of RAG1. Immunoprecipitation using the RAG1-specific antibody is described under "Material and Methods". Lane 1 size markers 121, 100, 85, and 67 nt. Lane 2, without the RAG proteins. Lanes 3 and 7, reactions with the non-treated RAG1 in the absence or presence of RAG2, respectively. Lanes 4-6, reactions with the treated RAG1 alone: lane 4, without antibody; lane 5, with the RAG1-specific antibody (anti-myc antibody); and lane 6, with the non-specific antibody (anti-MBP antibody). Letters *a, b, d, e* are described in Fig. 1.

active at the *in vitro* cleavage assay, produced the same fragment in the cleavage reaction (data not shown). In addition, because RAG1 is purified from the Sf9 insect cells that are infected by a recombinant baculovirus that carries the *rag*1 gene, this endonuclesase activity might be from a contaminant nuclease that is derived from the Sf9 extract. However, the protein preparation from the Sf9 cells infected with virus that did not carry the *rag*1 gene was unable to produce the RAG1-dependent fragment (lane 2, Fig. 2A).

Immunodepletion of RAG1 abrogates its nicking activity In order to address whether the nicking activity of RAG1 is an intrinsic property or if it is the result of contamination with other proteins, RAG1 from the purified-protein solution was depleted using immunoprecipitation, then subjected to the assay. Since RAG1 is tagged with the myc peptide, then the monoclonal anti-myc antibody was used to specifically precipitate RAG1 out of the protein solution. As expected, the anti-myc antibody removed the nicking activity of RAG1, although the mock immunoprecipitation did not deplete the endonuclesase activity of RAG1 (lanes 4 and 5, Fig. 2B). Also, a non-specific antibody (anti-MBP antibody) failed to eradicate this nicking activity of RAG1 (lane 6, Fig. 2B). An anti-myc antibody in the assay solution did not disturb the nicking activity of RAG1 (data not shown). In conclusion, nicking activity that is shown here is specific to RAG1.

The free hydroxyl group at the 3' end is required for RAG1-specific nicking activity After the nick formation by RAG1/RAG2, the free hydroxyl group at the 3' end was

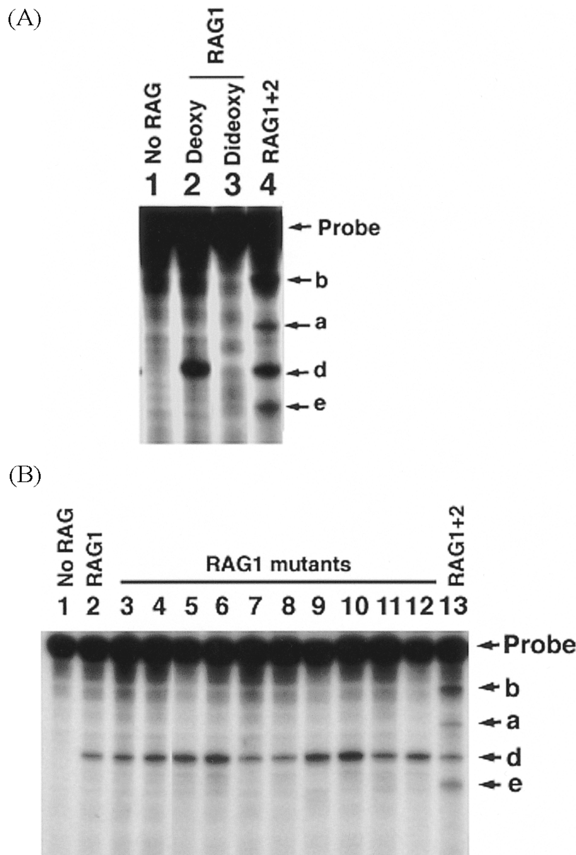

Fig. 3. (A) Effect of the 3'-hydroxyl group on the RAG1 nicking activity. Labeling of the substrate with dideoxyribonucleotide at the 3'- end of the 12 signal (top strand) was done by a T7 DNA polymerase reaction in the presence of ^{32}P-dideoxy-ATP. The labeled substrate was prepared as described in "Methods and Materials". (B) Nicking activity of RAG1 mutants. All of the RAG1 mutants have been described (Sadofsky *et al*., 1993) and purified under the same conditions as the wild-type RAG1 (Kim and Oettinger, 1998). Lanes 2 and 13 show the reactions with the wild-type RAG1 (core domain). Lanes 3 through 13 show the reactions with the mutant RAG1 proteins that were derived from pMS129 (lane 3), pMS130 (lane 4), pMS131 (lane 5), pMS132 (lane 6), pMS133 (lane 7), pMS134 (lane 8), pMS135 (lane 9), pMS136 (lane 10), pMS137 (lane 11), and pMS138 (lane 12), respectively.

specifically used as an electron donor to attack the phosphodiester linkage of the bottom strand, which resulted in the hairpin formation (Ramsden *et al*., 1996). To test whether the hydroxyl group at the 3' end is necessary for the nicking activity by RAG1, a dideoxy-CTP molecule was introduced at the 3' end of the top strand (12 RSS) in the same substrate. The RAG1-dependent nicking activity was completely abolished using a substrate that contained a dideoxy group at the 3' end (Fig. 3A). Furthermore, the absence of a 12 RSS did not affect the nicking activity of RAG1 when the hydroxyl group was provided at the 3' end (data not shown). This result suggests that RAG1 can directly recognize the DNA structure

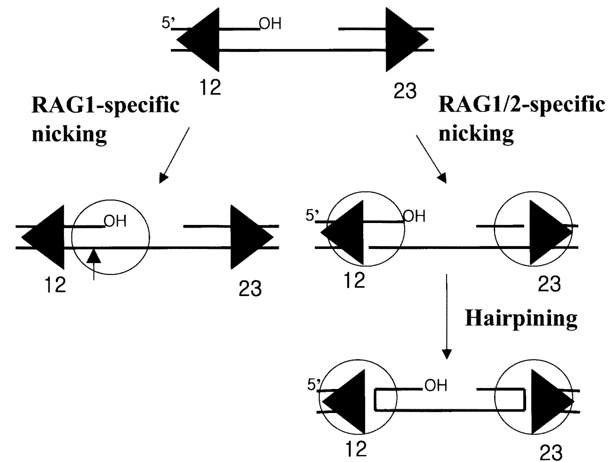

Fig. 4. A model of RAG-dependent cleavages. Triangles indicate the recombination signal sequences (12, 12 RSS; 23, 23 RSS). Circles represent the RAG proteins.

with the 3-hydroxyl group at the edge between the single-stranded DNA and double-stranded DNA, and attack a near phosphodiester bond.

Using RAG1 mutant proteins, I also examined whether the RAG1-dependent nicking activity that was observed here is the same activity as the one by the RAG1/RAG2 complex for the initial cleavage in the V(D)J recombination. Previously, Sadosky screened the RAG1 mutants that were defective in the V(D)J recombination activity *in vivo* (Sadosky *et al*., 1993). These mutant RAG1 proteins were tested here for their activity in the oligonucleotide-based assay. All of the purified RAG1 mutants showed RAG1-dependent nicking activity (Fig. 3B), suggesting that this RAG1-dependent nicking activity might be different from the RAG1/RAG2-dependent V(D)J cleavage.

Discussion

The V(D)J recombination is a gene rearrangement process that occurs only at the earlier step of lymphocyte development. This process requires DNA double-strand breaks and the repair of two broken DNA ends (Kim *et al*., 2000). The initial cleavage of the V(D)J recombination is mediated by two lymphoid-specific proteins, RAG1 and RAG2. If either RAG1 or RAG2 is absent in the reaction, then the V(D)J cleavage cannot be completed *in vitro* or *in vivo* (Mombaerts *et al*., 1992; van Gent *et al*., 1996). How both the RAG1 and RAG2 proteins are coordinately working in the presence of the two signal sequences was studied using an oligonucleotide substrate that is tethered by short single-stranded DNA (Kim and Oettinger, 1998). In this work, I demonstrated that RAG1 alone contained distinct endonucleolytic activity from the RAG1/RAG2 cleavage activity using the same oligonucleotide substrate. Analyses of

all of the cleavage products of the substrates that were labeled at the different positions showed that the RAG1-dependent fragment (about 100 nt) was produced by specific nicking at the near border between the double-strand and single-strand (Fig. 4).

This endonucleolytic activity is quite specific to RAG1. Immunoprecipitation of the RAG1 protein solution using the RAG1-specific antibody depleted this activity from the supernatant of the protein solution. If this activity comes from a contaminant nuclease that is co-purified with RAG1, then the immunodepletion with a RAG1-specific antibody can not remove this activity. In fact, immunoprecipitation with a non-specific antibody (anti-MBP Ab) did not eradicate this endonucleolytic activity from the protein solution (Fig. 2B). In addition, the SF9 extract without the recombinant RAG1-baculovirus that was purified under the same condition as the RAG1 protein showed no nicking activity. This suggests that this activity is not from the nuclease contamination during the protein purification.

What does this activity imply? Hairpin opening is one possible explanation for this RAG1-nicking activity that we observed. After the hairpin formation by the RAG proteins at both signal sequences, another endonucleolytic activity is required to open hairpins at the two coding DNA ends before being sealed. There are several proposed mechanisms for opening the hairpin in the V(D)J recombination. First, the RAG proteins are directly involved in this process. According to previous reports, the RAG proteins were able to nick the oligonucleotide-based hairpin structure in the presence of manganese as a divalent ion (Besmer *et al*., 1998; Schockett and Schatz, 1999). However, the reaction with magnesium, a more plausible condition in the cell, barely showed the hairpin opening by the RAG proteins. Also, the nicking activity by RAG1 was dependent on manganese ions rather than magnesium ions. In context of the usage of the divalent ion, these two endonuclesase activities might come from the same origin. In fact, a single polypeptide in the Tn 10 transposition reaction performs a dual work of hairpin formation and hairpin resolving (Kennedy *et al*., 1998). Additionally, the hairpin opening in the V(D)J recombination has also been suggested by other nucleases such as the MRE11/NBS1/RAD50 complex and Atemis (Paull and Gellert, 1999; Lee, 2001; Ma *et al*., 2002). In particular, the Atemis nuclease has been implicated for its important role in the V(D)J recombination. Atemis switches its exonucleolytic property to endonucleolytic one upon its phosphorylation by DNA-PK, and makes a nick for the hairpin structure that is produced by the RAG proteins. Although this suggested mechanism might be true in the cell, the hairpin opening by the RAG proteins is still one of the possible mechanisms. Further experiments are required to confirm whether this new nicking activity by RAG1 is directly involved in the resolving hairpins in the V(D)J recombination.

The other possibility is that this nicking is the same activity as one by the V(D)J recombinase. RAG1 with RAG2 has an endonucleolytic activity to initiate the V(D)J cleavage (Cuomo *et al*., 1996). According to recent observations, the catalytic residues of the V(D)J recombinase are exclusively located on RAG1 (Kim *et al*., 1999; Landree *et al*., 1999; Fugmann *et al*., 2000). In other words, RAG1 alone can perform catalysis by the assistance of RAG2 in binding to the RSS DNA or complex stability. If this is correct, then why do catalytically-defective RAG1 mutants still possess this nicking activity? The RAG1 mutants that were tested here have no changes at the catalytic residues (D600, D708, and E962). Therefore, if these mutants recognize DNA substrates like the oligonucleotide-tethered DNA, then they might carry out the catalytic reaction under certain conditions. Further experiments will be necessary in order to make a definite conclusion about this RAG1-specific endonuclesase activity.

Acknowledgments This study was supported in part by a Korean Research Foundation Grant, KRF-2000-041-D00286.

References

Akamatsu, Y. and Oettinger, M. A. (1998) Distinct roles of RAG1 and RAG2 in binding the V(D)J recombination signal sequences. *Mol. Cell. Biol.* **18**, 4670-4678.

Bailin, T., Mo, X. and Sadofsky, M. (1999) RAG1 and RAG2 tetramer complex is active in cleavage in V(D)J recombination. *Mol. Cell. Biol.* **19**, 4664-4671.

Bae, J. and Lee, S. (2001) The Human PTK6 Interacts with a 23-kDa Tyrosine-Phosphorylated Protein and is localized in Cytoplasm in Breast Carcinoma T-47D Cells. *J. Biochem. Mol. Biol.* **34**, 33-38.

Besmer, E., Mansilla-Soto, J., Cassard, S., Sawchuk, D. J., Brown, G., Sadofsky, M., Lewis, S. M., Nussenzweig, M. C. and Cortes, P. (1998) Hairpin coding end opening is mediated by RAG1 and RAG2 proteins. *Mol. Cell* **2**, 817-828.

Cuomo, C. A., Mundy, C. L. and Oettinger, M. A. (1996) DNA sequence and structure requirements for cleavage of V(D)J recombination signal sequences. *Mol. Cell. Biol.* **16**, 5683-5690.

Eastman, Q. M., Leu, T. M. and Schatz, D. G. (1996) Initiation of V(D)J recombination *in vitro* obeying the 12/23 rule. *Nature* **380**, 85-88.

Fugmann, S. and Schatz, D. (2001) Identification of basic residues in RAG2 critical for DNA binding by the RAG1-RAG2 complex. *Mol. Cell* **8**, 889-910.

Grawunder, U., Zimmer, D., Fugmann, S., Schwarz, K. and Lieber, M. R. (1998) DNA ligase IV is essential for V(D)J recombination and DNA double-strand break repair in human precursor lymphocytes. *Mol. Cell* **2**, 477-484.

Hammarsten, O. and Chu, G. (1998) DNA-dependent protein kinase: DNA binding and activation in the absence of Ku. *Proc. Natl. Acad. Sci. USA* **95**, 525-530.

Hiom, K. and Gellert, M. (1997) A stable RAG1-RAG2-DNA complex that is active in V(D)J cleavage. *Cell* **88**, 65-72.

Kennedy, A., Guhathakurta, A., Kleckner, N., and Haniford, D. (1998) Tn 10 transposition via a DNA hairpin intermediate. *Cell* **95**, 125-134.

Kim, D. R. (2001) Determination of monoclonal antibodies capable of recognizing the native protein using surface plasma resonance. *J. Biochem. Mol. Biol*. **34**, 452-456.

Kim, D. R., Dai, Y., Mundy, C. L., Yang, W. and Oettinger, M. A. (1999) Mutations of acidic residues in RAG1 define the active site of the V(D)J recombinase. *Genes Dev.* **13**, 3070-3080.

Kim, D. R. and Oettinger, M. A. (1998) Functional analysis of coordinated cleavage in V(D)J recombination. *Mol. Cell Biol.* **18**, 4679-4688.

Kim, D. R., Park, S. J. and Oettinger, M. A. (2000) V(D)J recombination: site-specific cleavage and repair. *Mol. Cells* **10**, 367-374.

Landree, M. A., Wibbenmeyer, J. A. and Roth, D. B. (1999) Mutational analysis of RAG1 and RAG2 identifies three catalytic amino acids in RAG1 critical for both cleavage steps of V(D)J recombination. *Genes Dev.* **13**, 3059-3069.

Lee, S. (2001) Recognition of DNA damage in mammals *J. Biochem. Mol. Biol.* **34**, 489-495.

Lewis, S. M. (1994) The mechanism of V(D)J joining: lessons from molecular, immunological, and comparative analysis. *Adv. Immunol*. **56**, 27-50.

Li, Z., Otevrel, T., Gao, Y., Cheng, H., Seed, B., Stamato, T. D., Taccioli, G. E. and Alt, F. W. (1995) The XRCC4 gene encodes a novel protein involved in DNA double-strand break repair and V(D)J recombination. *Cell* **83**, 1079-1089.

Ma, Y., Pannicke, U., Schwarz, K. and Lieber, M. R. (2002) Hairpin opening and overhang processing by an Artemis/DNA-dependent protein kinase complex in nonhomologous end joining and V(D)J recombination. *Cell* **108**, 781-794.

McBlane, J. F., van Gent, D. C., Ramsden, D. A., Romeo, C., Cuomo, C. A. and Gellert, M. (1995) Cleavage at a V(D)J recombination signal requires only RAG1 and RAG2 proteins and occurs in two steps. *Cell* **83**, 387-395.

McElhinny, S. A., Snowden, C. M., McCarville, J. and Ramsden, D. A. (2000) Ku recruits the XRCC4-LigaseIV complex to DNA ends. *Mol. Cell. Biol*. **20**, 2996-3003.

Mo, X., Bailin, T. and Sadofsky, M. J. (1999) RAG1 and RAG2 cooperate in specific binding to the recombination signal sequence *in vitro. J. Biol. Chem*. **274**, 7025-7031.

Mombaerts, P., Iacomini, J., Johnson, R. S., Herrup, K., Furley, A. J. W. and Papaioannou, V. E. (1992) RAG-1 deficient mice have no mature B and T lymphocytes. *Cell* **68**, 869-877.

Oettinger, M. A., Schatz, D. G., Gorka, C. and Baltimore, D. (1990) RAG-1 and RAG-2, adjacent genes that synergistically activate V(D)J recombination. *Science* **248**, 1517-1523.

Paull and Gellert (1999) Nbs1 potentiates ATP-driven DNA unwinding and endonuclesase cleavage by the Mre11/Rad 50 complex. *Genes Dev*. **13**, 1276-1288.

Ramsden, D. A., McBlane, J. F., van Gent, D. C. and Gellert, M. (1996) Distinct DNA sequence and structure requirements for the two steps of V(D)J recombination signal cleavage. *EMBO J*. **15**, 3197-3206.

Ramsden, D. A., Paull, T. T. and Gellert, M. (1997) Cell-free V(D)J recombination. *Nature* **388**, 488-491.

Sakano, H., Huppi, K., Heinrich, G. and Tonegawa, S. (1979) Sequences at the somatic recombination sites of immunoglobulin light chain genes. *Nature* **280**, 288-294.

Schatz, D. G., Oettinger, M. A. and Baltimore, D. (1989) The V(D)J recombination activating gene, RAG-1. *Cell* **59**, 1035-1048.

Shockett and Schatz (1999) DNA hairpin opening mediated by the RAG1 and RAG2 proteins. *Mol. Cell. Biol.* **19**, 4159-4166

Tonegawa, S. (1983) Somatic generation of antibody diversity. *Nature* **302**, 575-581.

Van Gent, D. C., McBlane, J. F., Ramsden, D. A., Sadofsky, M. J., Hesse, J. E. and Gellert, M. (1995) Initiation of V(D)J recombination in a cell-free system. *Cell* **81**, 925-934.

Van Gent, D. C., Ramsden, D. A. and Gellert, M. (1996) The RAG1 and RAG2 proteins establish the 12/23 rule in V(D)J recombination. *Cell* **85**, 107-113.