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Partial Nucleotide Sequencing of cDNA of Rat Recombination Activating Gene, RAG-1

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Summary. **The recombination activating gene-1(RAG-1)gene encodes the RAG-1 products, which are thought to regulate the V(D) J recombination of immunoglobulins and T cell receptors on B and T cells, respectively. Although human and mouse RAG-1 genes have been previously analyzed, that in rats remains to be investigated. In this study, the nucleotide sequencing of rat RAG-1 was partially elucidated. Using mouse primers and their rat polymerase chain reaction (PCR) products, 510 bp from the nucleotide position of 304 was characterized. The sequence homology in rat RAG-1 ⟨510bp⟩ was estimated to be 94% based on mouse RAG-1. The present results may be useful for further characterization of rat RAG-1 and for investigation of sites where the expression of the RAG-1 gene takes place in rats.**

INTRODUCTION

The recombination activating gene-1 (RAG-1) gene is known to encode the RAG-1 products, which regulate the V(D)J recombination of immunoglobulins and T cell receptors on B and T cells, respectively.[1)] Although RAG-1 genomic and cDNA clones have been produced and analyzed in humans and mice, those in rats remain to be investigated. Nucleotide sequencing of human and mouse RAG-1 cDNA clones predicts 119 kD proteins of 1,043 and 1,040 amino acids, respectively, with 90% sequence identity. Targeting mice of RAG-1 as well as RAG-2 did not produce either T cells or B cells.[2,3)] Since rats are often used to characterize immune responses under many experimental conditions, including transplantation and autoimmune diseases, it is important to clarify where such V(D)J recombination occurs in rats as an indication of RAG-1 or RAG-2. In the present study, we performed a partial nucleotide sequencing of RAG-1 in rats by using the primers of mouse RAG-1 and its polymerase chain reaction (PCR) products. The results enable a comparison of the sequence homology of RAG-1 genes among rats, mice and humans and provide information on appropriate primers to analyze rat RAG-1.

MATERIALS AND METHODS

Twelve-week-old male Lewis rats were used. Thymocytes and spleen cells were obtained by forcing each

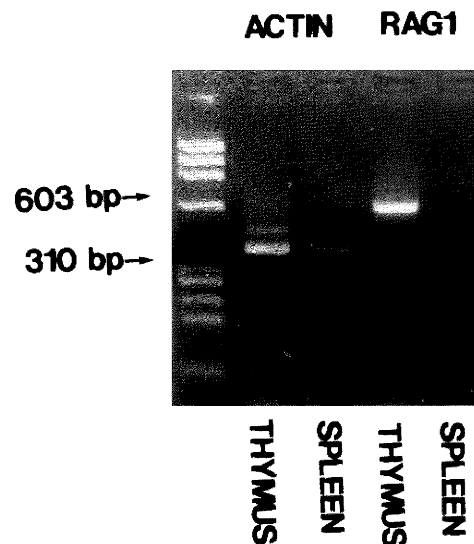

Fig. 1. Rat RAG-1 transcripts produced by mouse RAG-1 primers. PCR was performed by using the primers for β-actin and RAG-1 as described above. PCR products of RAG-1 and β-actin as well as markers were estimated by staining with ethidium bromide. RAG-1 transcripts were detected in the thymus but not the spleen.

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RAT    CAGAATTCAGTTCTGACTCAACGAGCATTGAAACTCCATCCTAAATTTTCAAAGAAATTCCATGTTGATGGGAAGTCA
       Q  N  S  V  L  T  Q  R  A  L  K  L  H  P  K  F  S  K  K  F  H  V  D  G  K  S
MOUSE  CAGAACTCAATTCTGACTCAACGAGCACTGAAACTCCATCCTAAATTTTCAAAGAAATTCCATGCTGATGGGAAGTCA
       Q  N  S  I  L  T  Q  R  A  L  K  L  H  P  K  F  S  K  K  F  H  A  D  G  K  S
HUMAN  CAGAAGCCAGTCCCAACTCAGCCATTGTTAAAAGCCCACCCTAAGTTTTCAAAGAAATTTCACGACAACGAGAAAGCA
       Q  K  P  V  P  T  Q  P  L  L  K  A  H  P  K  F  S  K  K  F  H  D  N  E  K  A

RAT    AGCGACAAAGCAATTCACCAAGCCAGGCTTAGACACTTCTGCCGCATCTGTGGGAATCACTTCAAGAGTGACGGGCAC
       S  D  K  A  I  H  Q  A  R  L  R  H  F  C  R  I  C  G  N  H  F  K  S  D  G  H
MOUSE  AGCGACAAAGCAGTTCACCAAGCCAGGCTTAGACACTTCTGCCGCATCTGTGGGAATCGTTTCAAGAGTGACGGGCAC
       S  D  K  A  V  H  Q  A  R  L  R  H  F  C  R  I  C  G  N  R  F  K  S  D  G  H
HUMAN  AGAGGCAAAGCGATCCATCAAGCCAACCTTCGACATCTCTGCCGCATCTGTGGGAATTCTTTTAGAGCTGATGAGCAC
       R  G  K  A  I  H  Q  A  N  L  R  H  L  C  R  I  C  G  N  S  F  R  A  D  E  H

RAT    AACCGGAGATACCCAGTCCACGGGCCCGTGGACGCTAAAACTCAAAGCCTTTTCCGAAAGAAGGAAAAAAGAGTCACG
       N  R  R  Y  P  V  H  G  P  V  D  A  K  T  Q  S  L  F  R  K  K  E  K  R  V  T
MOUSE  AGCCGGAGATACCCAGTCCACGGGCCCGTGGACGCTAAAACCCAAAGTCTTTTCCGAAAGAAGGAAAAAAGAGTCACT
       S  R  R  Y  P  V  H  G  P  V  D  A  K  T  Q  S  L  F  R  K  K  E  K  R  V  T
HUMAN  AACAGGAGATATCCAGTCCATGGTCCTGTGGATGGTAAAACCCTAGGCCTTTTACGAAAGAAGGAAAAGAGAGCTACT
       N  R  R  Y  P  V  H  G  P  V  D  G  K  T  L  G  L  L  R  K  K  E  K  R  A  T

RAT    TCCTGGCCAGATCTCATTGCCAGAGTTTTCCGGATTGATGTGAAGTCAGATGTTGACTCCATCCACCCCACTGAATTC
       S  W  P  D  L  I  A  R  V  F  R  I  D  V  K  S  D  V  D  S  I  H  P  T  E  F
MOUSE  TCCTGGCCAGACCTCATTGCCAGGATTTTCCGGATCGACGTGAAGGCAGATGTTGACTCCATCCACCCGACGGAATTC
       S  W  P  D  L  I  A  R  I  F  R  I  D  V  K  A  D  V  D  S  I  H  P  T  E  F
HUMAN  TCCTGGCCGGACCTCATTGCCAAGGTTTTCCGGATCGATGTGAAGGCAGATGTTGACTCGATCCACCCCACTGAGTTC
       S  W  P  D  L  I  A  K  V  F  R  I  D  V  K  A  D  V  D  S  I  H  P  T  E  F

RAT    TGCCATAACTGTTGGAGCATTATGCACAGGAAGTTCGGCAGTGCTCACAGTCAGGTCTACTGCCCAAGGAATGTGACC
       C  H  N  C  W  S  I  M  H  R  K  F  G  S  A  H  S  Q  V  Y  C  P  R  N  V  T
MOUSE  TGCCATGACTGTTGGAGCATCATGCACAGAAAGTTCAGCAGTTCCCACAGTCAGGTCTACTTCCCAAGGAAAGTGACC
       C  H  D  C  W  S  I  M  H  R  K  F  S  S  S  H  S  Q  V  Y  F  P  R  K  V  T
HUNAN  TGCCATAACTGCTGGAGCATCATGCACAGGAAGTTTAGCAGTGCCCCATGTGAGGTTTACTTCCCGAGGAACGTGACC
       C  H  N  C  W  S  I  M  H  R  K  F  S  S  A  P  C  E  V  Y  F  P  R  N  V  T

RAT    GTGGAGTGGCACCCCCACACACCGTCCTGTGACATCTGCTTTACTGCCCATCGGGGACTGAAGAGGAAGAGACATCAG
       V  E  W  H  P  H  T  P  S  C  D  I  C  F  T  A  H  R  G  L  K  R  K  R  H  Q
MOUSE  GTGGAGTGGCACCCCCACACACCGTCCTGTGACATCTGTTTTACTGCCCATCGGGGACTCAAGAGGAAGAGACATCAG
       V  E  W  H  P  H  T  P  S  C  D  I  C  F  T  A  H  R  G  L  K  R  K  R  H  Q
HUMAN  ATGGAGTGGCACCCCCACACACCATCCTGTGACATCTGCAACACTGCCCGTCGGGGACTCAAGAGGAAGAGTCTTCAG
       M  E  W  H  P  H  T  P  S  C  D  I  C  N  T  A  R  R  G  L  K  R  K  S  L  Q

RAT    CCCAACGTGCAGCTCAGCAAGAAACTAAAAACTGTGCTCAAC
       P  N  V  Q  L  S  K  K  L  K  T  V  L  N
MOUSE  CCCAATGTGCAGCTCAGCAAGAAACTAAAAACTGTGCTCAAC
       P  N  V  Q  L  S  K  K  L  K  T  V  L  N
MOUSE  CCAAACTTGCAGCTCAGCAAAAAACTCAAAACTGTGCTTGAC
       P  N  L  Q  L  S  K  K  L  K  T  V  L· D
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Fig. 2. Comparison of nucleotide sequence and anticipated amino acid sequence of RAG-1 among rats, mice and humans. 510 bp from the position of 304 in RAG-1 nucleotide are represented. Nucleotides and amino acids of rats and humans distinct from those of mice are marked with underlines.

organ through 200-gauge stainless steel mesh.[4] Spleen cells were hemolyzed in 0.17 M Tris-HCl (pH 7.65) supplemented with 0.83% NH_4Cl. Total RNA from 5×10^6 cells was isolated by the acid guanidium phenol chloroform method described by Chomczynski and Sacchi.[5] RNA (3-5 μg) was converted to cDNA using Moloney Murine Leukemia virus reverse transcriptase (Gibco BRL, Grand Island, NY, U.S.A.) and random primer (Takara Shuzo Co., Kyoto, Japan) at 42℃ for 1 h in a 20 μl reaction mixture. One-tenth of the cDNA reaction was then individually mixed with the RAG-1 primer set [5′primer, 5′-GTCTCCAGTAGTTCCAGA; 3′primer, 5′-CTAGCCTGAGTTCTCTTG, 10 pmole][6] and the β actin set [5′primer, 5′-CGTGACATCAAAGAGAAGCTGGTGC; 3′primer, 5′-GCTCAGGAGGAGCAATGATCTTGAT].[7] PCR was performed with 200 μM dNTP and 2.5 u Taq DNA polymerase (Toyobo Co., Osaka, Japan) for 30 cycles (94℃ for 50 s, 55℃ for 30 s, 72℃ for 2 min) in a Perkin-Elmer/Cetus thermocycler. The PCR reaction buffer was modified to achieve a final $MgCl_2$ concentration of 3 mM. Part of the reaction mixture was separated on 3% agarose gel and stained with ethidium bromide. PCR products were purified by Centricon-100(Amicon, Beverly, MA, U.S.A.) and sequenced by the TaqDyeDeoxy Terminator Cycle Sequencing Kit and the 373A DNA sequencing system (Applied Biosystems Inc., Foster, CA, U.S.A).

RESULTS

Total RNA was extracted from thymocytes and spleen cells in normal rats and PCR was performed by using mouse RAG-1 primers. RAG-1 transcripts showing the expected length (580 bp) were detected in the thymus but not in the spleen (Fig. 1). The transcripts of β-actin in a control experiment were demonstrated in both organs.

The RAG-1 transcripts from thymocytes were then analyzed by an automatized squencing analyzer (Fig. 2). As shown in the figure, the nucleotide sequence of 510 bp of rat RAG-1 was characterized. The sequence homology in rat RAG-1 〈510 bp〉 was estimated to be 94% based on RAG-1 in mice; the homology of the anticipated amino acid sequence in rat RAG-1 in this portion was 93%. As is already known, the sequence homology of human RAG-1〈510 bp〉 is 81% and the homology of amino acid sequence in the human RAG-1 in this portion is 76%.

DISCUSSION

The V(D)J recombination gene, RAG-1, is expected to be highly conserved among different species.[1] In this regard, the primers for mouse RAG-1 gene were used to analyze rat RAG-1. As expected, rat RAG-1 transcripts were demonstrated in the thymus, where T cell differentiation intensively takes place.[8] The sequence analysis confirmed that such transcripts are actually rat RAG-1, and revealed that the homology of RAG-1 between rats and mice is greater than that between humans and mice.

In recent studies, not only intrathymic T cell differentiation but also extrathymic T cell differentiation have been shown to be accompanied by the expression of RAG-1 and RAG-2.[6,9,10] Since the extrathymic pathways of T cell differentiation occur at several sites in the body, including in the liver,[11-16] intestine,[6,9] and omentum,[17,18] as shown by the experiments using humans, mice and rats, the present results might be useful in subsequent studies to further analyze such sites.

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