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Short communication

V(D)J recombination in zebrafish: Normal joining products with accumulation of unresolved coding ends and deleted signal ends

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Abstract

V(D)J recombination proceeds from a site-specific cleavage to an imprecise end joining, *via* generation and resolution of recombination ends. Although rearranged antigen receptor genes isolated from zebrafish (*Danio rerio*) resemble those made in mammals, differences may arise during evolution from lower to higher vertebrates, in regard to efficiency, fidelity and regulation of this recombination. To elucidate the V(D)J recombination reaction in zebrafish, we characterized recombination ends transiently produced by zebrafish lymphocytes, as well as joining products. Similar to their mammalian counterpart, zebrafish lymphocytes make perfect signal joints and normal coding joints, indicating their competent end resolution machinery. However, recombination ends recovered from the same zebrafish lymphoid tissues exhibit some features that are not readily seen in normal mammalian counterpart: deleted signal ends and accumulation of opened coding ends. These results indicate that the recombination reaction in zebrafish lymphocytes is inefficient and less stringently regulated, which may result from unstable post-cleavage complexes, and/or slow transition from cleavage to resolution. Our data suggests that the V(D)J recombination machinery may have undergone evolution selection to become more efficient in higher jawed vertebrates.

Keywords: Non-homologous end joining; Recombination ends; Zebrafish

1. Introduction

The V(D)J recombination reaction consists of two steps, a site-specific cleavage and an imprecise end joining. The cleavage reaction is catalyzed by recombination-activating gene 1 and 2 (RAG1/2) proteins, which are expressed in developing lymphocytes (Gellert, 2002; Jung and Alt, 2004; Schatz, 2004). RAG1/2 proteins initiate recombination by recognizing recombination signal sequences (RSSs), making excision at the border between the RSSs and the coding segments, generating blunt signal ends (SEs) and hairpin coding ends (CEs), which are likely held in a complex containing RAG1/2 proteins (Fugmann et al., 2000). Subsequently, recombination ends are resolved into signal joints (SJs) and coding joints (CJs) *via* ubiquitously expressed non-homologous end joining (NHEJ) proteins, including Ku70, Ku80, XRCC4 and DNA ligase IV, as well as DNA-dependent protein kinase catalytic subunit (DNA-PKcs) and Artemis for processing hairpin CEs before their final joining (Schatz, 2004).

The zebrafish has emerged as a useful vertebrate genetic model for the study of the immune system due to its rapid growth, high genetic conservation with mammals, and most importantly its feasibility for experimental manipulation at earlier developmental stages (Traver et al., 2003). The V(D)J recombination process appears to be highly conserved among all jawed vertebrates, as inferred from the structural similarity of their RAG1 and RAG2 proteins, and from the characteristics of rearranged antigen receptor genes, especially in the CDR3 region (Hansen and McBlane, 2000). Although zebrafish RAG1/2 genes are orthologous with mouse and human in terms of general gene organization, lineage-dependent expression, and the conserved structure of the catalytic domain, zebrafish recombinases are phylogenetically diverged from mammals. There are 60% and 53% amino acids identical for RAG1 and RAG2, respectively,

Abbreviations: CEs, coding ends; CJs, coding joints; DSBs, double strand DNA breaks; LM-PCR, ligation-mediated PCR; NHEJ, non-homologous end joining; SEs, signal ends; SJs, signal joints

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between zebrafish and mammals (Willett et al., 1997). Furthermore, some differences also exist in Ku70 and Ku80 proteins between zebrafish and mammals although many NHEJ genes from zebrafish have not been identified or fully characterized (Bladen et al., 2005) (unpublished analyses). It would be interesting to determine whether these structural differences have any functional significance on recombination activity. Specifically, does the V(D)J recombination reaction in zebrafish proceed differently from that in mammals? To address this question, we set out to analyze recombination intermediates to get a view of ongoing recombination in zebrafish.

In this study, we found that the joining products formed by zebrafish lymphoid cells resemble those made in mammals, i.e. containing junctional diversity at CJs and possessing perfect junction at SJs. However, zebrafish lymphoid tissues exhibit some unusual features in their end resolution: accumulation of opened CEs and nucleotide deletions at SEs. These findings point to an inefficient transition of recombination ends from cleavage to resolution in zebrafish lymphocytes, which is in striking contrast to the one adopted by mammalian lymphocytes, efficient and seamless. Our findings reveal phylogenetic changes in the V(D)J recombination reaction from lower to higher jawed vertebrates.

2. Methods

2.1. Animals and DNA preparation

Adult zebrafish (*Danio rerio*) were bred and maintained at 28.5 °C with a photoperiod of 14 h light and 10 h dark, according to the established guidelines in the Zebrafish Facility set up at the Biodesign Institute of Arizona State University (Westerfield, 1995). All animal protocols were conducted under the supervision and approval of the ASU IACUC committee. Kidneys and spleens were taken from 10 to 15 adult zebrafish after they were euthanized by tricaine medium (Sigma). In some cases, aurintricarboxylic acid (Sigma), a nuclease inhibitor, was included in the buffer (at 1 mM) for preparing cells from zebrafish kidney.

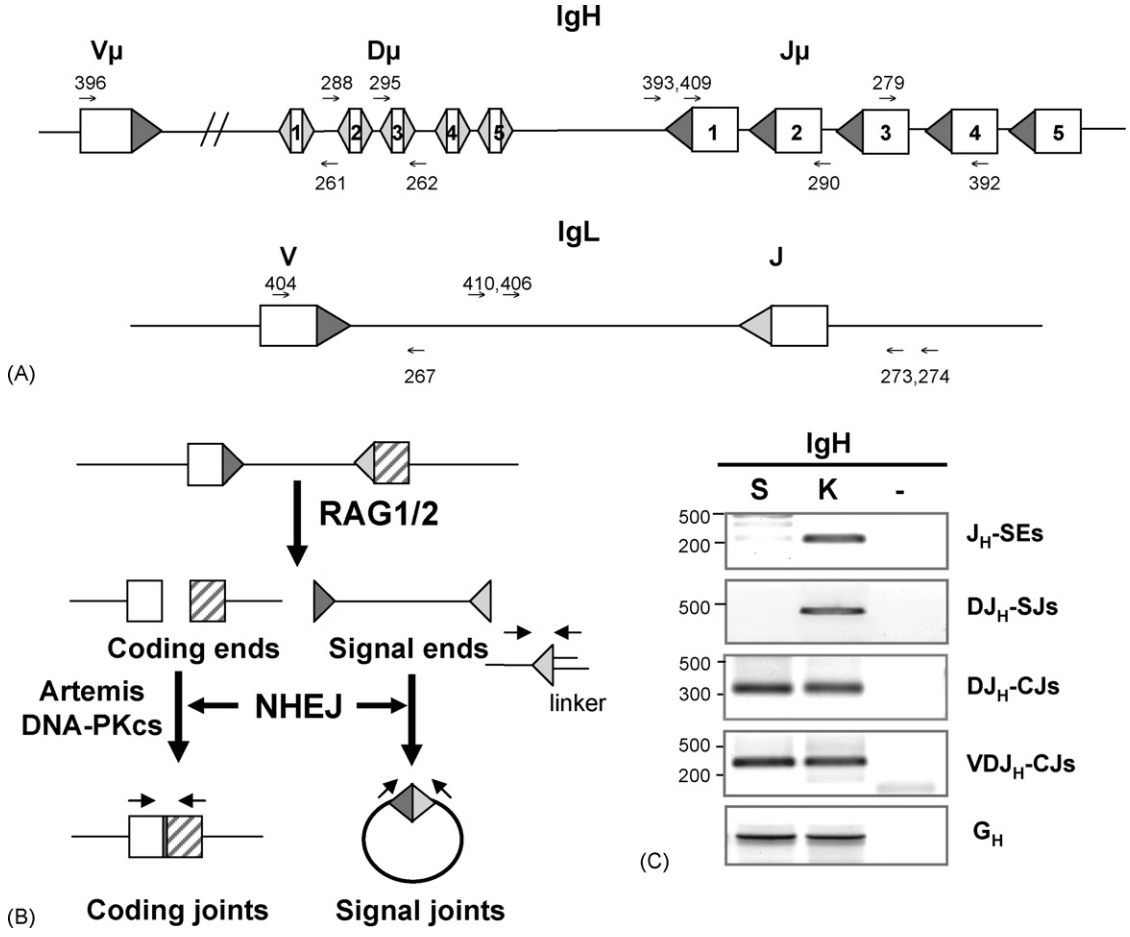

Fig. 1. Rearrangement of immunoglobulin (Ig) genes in zebrafish lymphoid tissues. (A) Outline of zebrafish IgH and IgL gene loci, as well as location of primers that are listed in Table 1. These primers, denoted as small arrows, are utilized in PCR amplification of recombination ends and joints, as shown in Table 2. (B) Diagram illustrating the production of signal ends (SEs), coding ends (CEs), coding joints (CJs) and signal joints (SJs), as well as their detection by genomic PCR (for CJs and SJs) and ligation-mediated PCR (LM-PCR for SEs and CEs). The V, D or J gene segments (rectangular boxes) are flanked by recombination signal sequences (RSSs, triangles). (C) Analysis of IgH recombination status. Genomic DNA was prepared from spleen (S) and kidney (K), J_H-SEs were examined by a semi-nested LM-PCR (see Table 2 for primer selection). Rearranged VDJ_H- and DJ_H-CJs or DJ_H-SJs were amplified by genomic PCR. Germline heavy chain (G_H) was included as a control for DNA input.