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Developmental and Comparative Immunology 29 (2005) 61–71
Developmental & Comparative Immunology
www.elsevier.com/locate/devcompimm

# Identification of the activation-induced cytidine deaminase gene from zebrafish: an evolutionary analysis☆

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Received 28 March 2004; revised 2 May 2004; accepted 15 May 2004

Available online 2 July 2004

**Abstract**

In the present study, we report the identification of the activation-induced cytidine deaminase (AID) encoding gene in frog, dog and chimpanzee, where both somatic hypermutation and class switch recombination (CSR) occurs and in zebrafish and fugu, species lacking CSR. The cDNA sequence of the zebrafish AID reported here suggests both N and C ends of the previously predicted protein sequence are incorrect. A comparison of AID sequences among mammals, birds, amphibians and fish revealed conserved aa residues which may be essential for AID activity, although the cytidine deaminase active motif in the latter is nine amino acids longer. Furthermore, an aa deletion, and extensive substitutions in the C terminal end of AID from bony fish indicate that the molecule may not yet have developed a capacity to recruit the specific cofactor(s) needed to initiate CSR.


*Keywords:* Activation-induced cytidine deaminase; Class switch recombination; C-terminal; Deaminase motif; Evolution; Genomic structure; Somatic hypermutation; Zebrafish

## 1. Introduction

Somatic hypermutation (SHM), gene conversion and class switch recombination (CSR) are essential for generation of immunoglobulin diversity. Gene conversion is the dominant mechanism for V region diversification in chickens, cows and pigs. Humans and mice in contrast, rely predominantly on SHM [1], and rabbits use both processes [2–4]. CSR, on the other hand, is a region specific, deletional, recombination process utilized by species with multiple immunoglobulin classes such as amphibians, birds and mammals. It involves cutting and re-ligation of switch regions located upstream of the constant region encoding genes, resulting in a switch in immunoglobulin isotype expression without altering the antibody specificity.

Although, all three mechanisms have been intensively studied during the past decades, the precise

*Abbreviations:* AID(AICDA), activation-induced cytidine deaminase; CSR, class switch recombination; HIGM, hyper-IgM syndrome; RACE, rapid amplification of cDNA ends; SHM, somatic hypermutation.

☆ The sequences reported in this study have been deposited in GenBank under accession numbers: AY528720, TPA BK005129–BK005133.

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doi:10.1016/j.dci.2004.05.005

steps involved are still not clearly understood. Until recently, they have been considered to be mechanistically distinct processes. Many *trans*-acting proteins have been found to be differentially involved in the two processes where DNA-PKcs, Ku70/80, ATM (mutated in Ataxia-Telangiectasia) and H2AX all influence CSR but not SHM [5–10]. Mismatch repair proteins such as MSH2, PMS2 and MLH1 on the other hand, have been shown to be involved both in SHM and CSR [11–17]. Recently, a B cell specific factor involved in both SHM and CSR, activation-induced cytidine deaminase (AID), was identified [18,19]. AID deficient mice are devoid of both SHM and CSR [19], as are patients with an autosomal recessive form of the hyper-IgM syndrome (HIGM2), caused by mutations in the human AID encoding gene [20]. Ectopic expression of AID in fibroblasts is sufficient to induce both SHM and CSR, suggesting that it is the only B cell specific factor needed for these processes [21,22]. AID is also essential for gene conversion in chickens [23], thus linking all three mechanisms involved in diversification of the antibody repertoire.

The deamination of cytidine by AID results in the generation of C to U mismatches. These are resolved by different DNA repair systems, resulting either in SHM, gene conversion or CSR [24,25]. According to this model, the DNA deaminating activity of AID is sufficient to initiate both SHM and CSR. AID proteins derived from HIGM2 patients containing insertions, replacements or truncations in the C-terminal region of the molecule, retain strong SHM activity whereas the ability to mediate CSR is almost completely lost [26]. Furthermore, a deletion mutant of mouse $AID^{\Delta 189-198}$ that is more active in catalyzing cytidine deamination than wild-type AID when expressed in *E. coli* [27], promotes both gene conversion and SHM but fails to induce CSR in eukaryotic cells [27]. These two studies strongly suggest that the C-terminal of AID interacts physically with a cofactor that is required for CSR, and that the essential function of AID in CSR is not limited to its cytidine deaminase activity [26,27].

In fish, CSR does not occur although SHM has been clearly demonstrated [28–31], suggesting the presence of an AID homologue. Indeed, while our work was in progress, the catfish equivalent of AID was described [32]. We have identified the AID homologue in a variety of species in order to try to delineate the evolution of this molecule.

## 2. Materials and methods

### 2.1. RNA isolation and first-strand cDNA synthesis

Total RNA was purified using the RNeasy® minikit (QIAGEN, Valencia, CA) from whole adult zebrafish. About 5 μg total RNA was reverse transcribed into first-strand cDNA using a First-Strand cDNA Synthesis Kit (Amersham Pharmacia Biotech, Uppsala, Sweden).

### 2.2. Rapid amplification of the zebrafish AID cDNA 3′ end (3′ RACE)

The first PCR amplification was carried out using 3 μl of the synthesized first-strand cDNA as a template and the primers Zebra-AIDs1 (5′ GAG CTT CTC TTT CTG CGT CAC TTG G 3′) and cDN (designed based on the sequence of the *Not* I-d$(T)_{18}$ primer supplied in the First-Strand cDNA Synthesis Kit, 5′ AAC TGG AAG AAT TCG CGG CC 3′). The cycling parameters were 92 °C 2 min, then 30 cycles of 92 °C 10 s, 60 °C 30 s, 68 °C 90 s. The first PCR reaction was subsequently diluted 40 times using distilled water and 5 μl of this mixture was used as a template for the second PCR amplification using the primers Zebra-AIDs2 (5′ ACT CCG CTC CCT CCT CGC AAC AAA C 3′) and Fs-common (designed based on the sequence of the *Not* I-d$(T)_{18}$ primer supplied in the First-Strand cDNA Synthesis Kit, 5′ TGG AAG AAT TCG CGG CCG CAG GAA 3′). The amplifying conditions were 92 °C 2 min, then 30 cycles of 92 °C 10 s, 62 °C 30 s, 68 °C 40 s. The resulting PCR product was cloned into a pGEM-T vector (Promega, Madison, WI) for sequencing. For both PCR reactions, an Expand™ Long Template PCR system Kit (Roche) was used to ensure amplification fidelity.

### 2.3. Rapid amplification of the zebrafish AID cDNA 5′ end (5′ RACE)

A BD SMART rapid amplification of cDNA ends (RACE) cDNA amplification kit (BD Biosciences