Evolution of class switch recombination function in fish activation-induced cytidine deaminase, AID

Koshou Wakae1*, Brad G. Magor2, Holly Saunders2, Hitoshi Nagaoka1, Akemi Kawamura1, Kazuo Kinoshita3, Tasuku Honjo1 and Masamichi Muramatsu1*

1Department of Immunology and Genomic Medicine, Graduate School of Medicine, Kyoto University, Yoshida Sakyo-Ku, Kyoto 606-8501, Japan
2Department of Biological Sciences, University of Alberta, Edmonton, Alberta T6G-2E9, Canada
3Department of Evolutionary Genetics, Graduate School of Medicine, Kyoto University, Yoshida Sakyo-Ku, Kyoto 606-8501, Japan

Keywords: APOBEC-1, B cells, class switch recombination, immunoglobulin gene, somatic hypermutation

Abstract

Following activation of mammalian B cells, class switch recombination (CSR) and somatic hypermutation (SHM) of the Ig heavy chain (IgH) gene can improve the functions of the expressed antibodies. Activation-induced cytidine deaminase (AID) is the only known B cell-specific protein required for inducing CSR and SHM in mammals. Lower vertebrates have an AID homologue, and there is some evidence of SHM in vivo. However, there is no evidence of CSR in the cartilaginous or bony fishes, and this may be due in part to a lack of cis-elements in the IgH gene that are the normal targets of AID-mediated recombination. We have tested whether bony fish (zebrafish and catfish) AID can mediate CSR and SHM in mammalian cells. As expected, ectopic expression of fish AID in mouse fibroblasts resulted in mutations in an introduced SHM reporter gene, indicating that fish AID can mediate SHM. Unexpectedly, expression of fish AID in mouse AID−/−/C255−/− B cells induced surface IgG expression as well as switched transcripts from Ig gene loci, clearly indicating that the fish AID protein can mediate CSR, at least in mouse cells. These results suggest that the AID protein acquired the ability to mediate CSR before the IgH locus evolved the additional exon clusters and switch regions that are the targets of recombination. We discuss how pleiotropic functions of specific domains within the AID protein may have facilitated the early evolution of CSR in lower vertebrates.

Introduction

Most of the tetrapods (amphibians, birds and mammals) can alter the genetic information of their Ig genes by class switch recombination (CSR) and either somatic hypermutation (SHM) or gene conversion. SHM introduces non-templated point mutations in the variable region (V) gene, whereas gene conversion uses upstream pseudo-V genes as a template to introduce patchy replacements in the productive V gene. A subsequent selection process ensures that there will be survival and expansion of B cells in which the mutations have improved the antigen-binding affinity of the antibodies. Cartilaginous fishes are the earliest divergent vertebrates to have Ig’s, and there is evidence that they also modify their V genes by SHM (1, 2).

CSR is a region-specific recombination that replaces the exons encoding the μ chain (Cμ) of the Ig heavy chain (IgH) with a downstream constant region exon (CH) cluster such as Cγ or Cα. This results in a change of the expressed antibody isotype from IgM to other isotypes such as IgG or IgA, diversifying their effector functions without changing antigen specificity. CSR takes place between two of the special intronic sequences, called switch (S) regions, that span from 1 to 10 kb upstream of each the CH genes that take part in class switching (3). S regions consist of a tandem array of repetitive units that often form scattered palindromes. Deletion of the S region from mouse endogenous Ig loci clearly demonstrated the importance of S regions for CSR (4–6).

Amphibians are the most primitive vertebrates to use CSR to exchange CH genes (7, 8). The IgH loci of fishes do not have distinguishable S regions or additional CH genes downstream...
of Cμ and Cδ. In fishes, as in mammals, expression of δ-chain transcripts occurs through alternative pre-mRNA splicing and not CSR. Thus, the IgH loci of fishes lack key cis-elements necessary for class switching to occur, and consistent with this, there has been no evidence of CSR in the fishes (1, 2, 9). Moreover, the primary transcriptional enhancer in fishes is located between Cμ and Cδ, not in the JH and Cμ intron as in vertebrates that have CSR. This distally positioned fish IgH enhancer, termed Eμ3′, would be excised during CSR, were it to occur as it does in the tetrapods (10).

Activation-induced cytidine deaminase (AID) is known to be essential for gene conversion, SHM, and CSR, and its expression in mammals is restricted to lymphoid organs that contain activated B cells (11–15). It is known that ectopic expression of AID can trigger CSR and SHM even in fibroblasts when appropriate reporter genes are transcriptionally activated (16, 17). Extensive studies of AID mutants have revealed that AID has at least four functional domains or motifs. The (deoxy)cytidine deaminase motif is a catalytic domain that is strongly conserved within members of the (deoxy)cytidine deaminase gene superfamily (11), and this motif is essential for CSR and SHM activity by AID (13, 18, 19). Replacement mutations at the N-terminus (W20K, V18R, V18S/R19V, G23S, Y13H) of AID disrupt SHM activity without affecting CSR activity (20). On the other hand, deletion of 16 amino acids from the C-terminus of AID results in loss of CSR but has only a minimal effect on SHM activity, therefore the C-terminus is proposed to be a specific domain for CSR (19–21). The C-terminal 16 amino acids contain a functional nuclear export signal (NES) (21–24), however, the functional relationship between CSR and nuclear export by the NES remains to be clarified.

Two models (RNA editing and DNA deamination) are proposed to explain how AID initiates CSR and SHM (3, 14, 25). A common feature between the two models is that AID is involved in a step to create DNA breaks in either the V region or the S region for SHM and CSR, respectively. AID homologues have recently been cloned from bony fishes (zebrafish, catfish, and pufferfish), however functional characterization remains to be done (26–28). The conservation of amino acids between bony fish and mouse AID is >50% identity, and most of the functionally important amino acids in mammalian AID are also conserved in bony fish AID. One intriguing observation is that the C-terminal 16 amino acids, essential for CSR but dispensable for SHM, are also well conserved in fish AID, even though the fish IgH lacks the cis-elements necessary for CSR.

To determine whether fish AID has activity for CSR and SHM, catfish and zebrafish AID cDNAs were expressed in previously established mammalian cell assay systems (17, 19). Our results provide functional evidence that fish AID is capable of driving both CSR and SHM in mouse cells. We discuss how the C-terminal 16 amino acids in fish AID evolved before it functioned for CSR in vivo.

Methods

In vitro assay for SHM and CSR

The SHM assay was done in a mouse fibroblast cell line NIH3T3pl19 that contains a reporter gene of SHM (17). For the CSR assay, spleen B cells from AID-deficient mice were used. Infection of cells with an AID-expressing retrovirus leads to AID over-expression. The SHM reporter is a GFP reversion construct that only generates a functional GFP transcript if an introduced stop codon is reverted to a functional codon by SHM (29). GFP transcription is under the control of a tetracycline-responsive promoter, and the gene is only transcribed once tetracycline is removed from the media. Detection of GFP reversion and sequencing of mutant GFP substrate were done in the same way described previously (17). Sequencing of mutant GFP substrate in this study was done at day 10 after removal of tetracycline from the medium.

Assays of CSR activity by FACS analysis were described previously (19). cDNAs of chicken, catfish (Ictalurus punctatus, GenBank accession number AY436507) (26), catfish JP8Bdel [catfish version of JP8Bdel that is a human AID mutant lacking C-terminal 16 amino acids (22)] and catfish–mouse-chimeric AID (chimeric cDNA between catfish JP8Bdel and mouse C-terminal 16 amino acids) were made by standard PCR methods, and inserted into pFB (Stratagene, California, USA; for SHM assay) and pMX-IRES-GFP (18) (for CSR assay) vectors. The chicken AID cDNA was kindly provided by Dr. H. Arakawa (Heinrich-Pette-Institute, Germany) (12). Detection of post-switch transcripts was done by reverse transcription–PCR as previously described (14). Sequences used in Fig. 1(B) are as follows, GenBank accession number; mouse (AF132979), human (AB040430) and zebrafish, Danio rerio (AY528720) AID. To generate zebrafish AID expression vector, first strand cDNA was synthesized with SuperScript II (Invitrogen, California, USA) from mRNA of zebrafish spleen as recommended by the company’s protocol, and zebrafish AID cDNA was amplified with 5′-ctGAATTCCatgatctgtg-3′ and 5′-gcatGGATCtataaccacaagagc-3′. Zebrafish AID cDNA was sub-cloned into EcoRI/BamHI site of pFB and pFB-IRES-GFP (30) vectors.

AID–GFP fusion protein and western blot

PCR was used to make cDNA without a stop codon for mouse, catfish, catfish JP8Bdel and catfish–mouse-chimeric AID, and then those cDNAs were sub-cloned into pFlag-CMV5a (Invitrogen) for cell transfection and subsequent western blot analyses. To generate the expression vectors for AID–GFP fusion proteins, mouse and catfish AID cDNAs were further transferred to a pFB-GFP2 vector that was made by sub-cloning an EcoRI/Xhol GFP fragment from pGFP2-N2 (Perkin Elmer, Maryland, USA) into the pFB vector. GFP fluorescence was detected with an Axiovert 200 microscope (Zeiss, Munich, Germany) (22). AID expression vectors were transfected together with pEGFP-N2 (Clontech, California, USA) into 293T cells, and protein derived from the equivalent of 20 000 cells was loaded onto a SDS-PAGE gel. Detection of flag epitope and GFP protein on western blots has been described (19). The signal intensity of western blot bands was measured with Image-Gauge version 4.22 (Fujifilm, Tokyo, Japan). All the expression vectors used in this study were sequenced to confirm successful construction. Those sequences are available by request. Production and infection of recombinant retrovirus used in this study were described previously (18).
Fish AID has SHM activity in mouse cells

It was previously reported that expression of mammalian AID triggers SHM in mouse fibroblasts (NIH3T3p19) that stably retain a mutant GFP substrate in the genome (17). To determine whether fish AID can mediate SHM, NIH3T3p19 cells were infected with retroviruses expressing AID or AID mutants from various species. After cell infection, tetracycline was removed from the medium, cells were cultured either at 37°C (left) or 28°C (right) for 7 days, and then reverted GFP+ cells were detected by FACS. Each dot indicates the number of GFP+ cells per 10^5 live cells. (B) Amino acid alignment of AID from indicated five species. The dotted box, open box and gray box indicate domains essential for SHM, (deoxy)cytidine deamination and CSR, respectively. The arrow shows the position of the JP8Bdel truncation. Amino acids in bold indicate the NES. Asterisks and dots represent identical and similar amino acid residues, respectively.

---

**Results**

Fish AID has SHM activity in mouse cells

It was previously reported that expression of mammalian AID triggers SHM in mouse fibroblasts (NIH3T3p19) that stably retain a mutant GFP substrate in the genome (17). To determine whether fish AID can mediate SHM, NIH3T3p19 cells were infected with retroviruses expressing AID or AID mutants from various species. After cell infection, tetracycline was removed from the cell media to allow expression of the tet-repressible GFP reversion reporter (17). Seven days after withdrawal of tetracycline from the medium, cells expressing reverted GFP were detected by FACS analysis. As expected, mouse, human and chicken AID expression causes 666 ± 103, 637 ± 140 and 365 ± 116 GFP reversion per 10^5 cells, respectively (Fig. 1A, left). Since only a single copy of GFP reporter gene is integrated into the NIH3T3p19 cell genome, all mutations of the GFP gene must be due to SHM and not gene conversion, even when the chicken AID is being used. Human H56Y AID (also known as P19) has a replacement mutation on one of the essential amino acid residues in the (deoxy)cytidine deaminase motif, and was previously reported to be a functionally dead mutant (13, 19). Consistent with the previous study (19), cells expressing human H56Y AID, or infected with vector alone, showed almost no reversion of the mutant GFP substrate, indicating that this assay system of...
SHM is almost completely dependent on AID activity. On the other hand, in the case of fish AID, significant GFP reversion (zebrafish, 189 ± 26 GFP reversion per 10^6 cells; catfish 39 ± 9.5 GFP reversion per 10^5 cells) was detected, although at a lower frequency than mouse AID (Fig. 1A, left). Sequencing of the mutant GFP substrate in catfish AID-expressing cells confirmed introduction of point mutations, albeit at a 4.7-fold lower frequency than seen with mouse AID (Table 1). The over-expression of wild-type fish AID in mammalian cells resulted in patterns of mutations similar to those observed with over-expressed mammalian AID, both in this (Table 1) and previous studies (17, 31–34). All of the mutations by catfish AID were targeted to G’s and C’s and there was a strong bias toward transition nucleotide replacements.

The optimal temperature for fish B cell culture is reported to be 27°C (35), and an observation by Conticello et al. (28) suggests that an optimal temperature for the enzymatic activity by fish AID may be <37°C. NIH3T3pI19 cells can tolerate culture condition at 28°C without inducing cell death, therefore we also assessed SHM activity of catfish and zebrafish AID at 28°C. As shown in Fig. 1 (right), the reversion frequency by mammalian and chicken AID at 28°C was 6- to 7-fold lower than that at 37°C. Conversely, the reversion frequency of fish AID at 28°C was not greatly diminished, being only 1- to 1.2-fold less effective than that at 37°C. Consequently, zebrafish AID induced GFP reversion (15 ± 16 per 10^5 cells) lower than mouse AID (150 ± 16 per 10^5 cells) at 28°C.

**Fish AID can trigger CSR in mouse B cells**

Past studies of mammalian AID mutants revealed four functional domains within the protein. Shinkura et al. (20) demonstrated that the N-terminal domain (13–23 amino acids) is essential for SHM activity (Fig. 1B). Consistent with this idea, this domain is well conserved in the SHM competent fish AID (Fig. 1 and Table 1). The (deoxy)cytidine deaminase motif of AID forms a single zinc finger and is known to be essential for both CSR and SHM activities (13, 19, 36). Zinc-coordinating amino acid residues (mouse H56, E58, C87 and C90) are completely conserved between mammals and fish AID, except that fish AID has a longer spacer between E58 and C87. Indeed, Conticello et al. (28) demonstrated that another bony fish, pufferfish AID has (deoxy)cytidine deaminase activity when expressed in *Escherichia coli*.

Deletion of the C-terminus from mammalian AID disrupts both cytoplasmic localization and CSR activity, though it has little effect on SHM. Therefore, the C-terminus of AID is thought to be a CSR-specific domain and was proposed to attract CSR-specific cofactors (19, 21, 37). As indicated in Fig. 1(B), fish AID has a reasonably conserved C-terminus, although fish IgH loci do not have the cis-elements (S regions and CH exon clusters) needed for CSR to occur (1, 2, 9). When zebrafish AID was expressed in mouse AID^+/− B cells, class switching to IgG1 was obvious when analyzed by FACS. On the other hand, induction of IgG1 switching by catfish AID occurs at a similar frequency as the human AID mutant 190X (also known as JP41) that was previously reported to induce weak CSR activity in AID^+/− B cells (19). Switching by catfish AID appears to be very limited but it is not an artifact of FACS analysis because the catfish JPBBDel mutant, which lacks the C-terminal 16 amino acids, does not show any switching (Fig. 2A). As shown in Fig. 2(B), CSR by catfish AID was further confirmed by detection of post-switch transcripts transcribed from switched IgH loci. Fish–mouse-chimeric AID, in which the catfish JPBBDel is fused with the mouse C-terminal 16 amino acids, rescued the CSR defect of catfish JPBBDel and induced switching slightly more efficiently than wild-type catfish AID. The stronger CSR activity in cells expressing the fish–mouse-chimeric AID cannot be simply explained by better expression of the chimeric AID protein (Fig. 2C), because the chimeric AID did not show a greater efficiency for SHM than wild-type fish AID (Fig. 1A). We interpret this to mean that the catfish C-terminus contributes to CSR activity significantly in mouse cells, but to a lesser extent than the mouse C-terminus. As was the case for SHM activity,

**Table 1. Catfish AID shows similar pattern of mutation with mammalian AID**

<table>
<thead>
<tr>
<th></th>
<th>Mouse</th>
<th>Human</th>
<th>Chicken</th>
<th>Catfish</th>
<th>Catfish JPBBDel</th>
<th>Mock</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total bases sequenced (bp)</td>
<td>9730</td>
<td>7784</td>
<td>9730</td>
<td>13 622</td>
<td>7784</td>
<td>37 947</td>
</tr>
<tr>
<td>Total number of clones sequenced</td>
<td>10</td>
<td>8</td>
<td>10</td>
<td>14</td>
<td>8</td>
<td>39</td>
</tr>
<tr>
<td>Number of clones that have point mutations</td>
<td>10</td>
<td>6</td>
<td>10</td>
<td>7</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Total deletion and insertion number</td>
<td>5</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Number of clones with a deletion or insertion</td>
<td>4</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total number of mutations^a</td>
<td>46</td>
<td>30</td>
<td>44</td>
<td>14</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>Mutation rate (per 1000 bp)^a</td>
<td>4.72</td>
<td>3.85</td>
<td>4.52</td>
<td>1.02</td>
<td>1.02</td>
<td>0.03</td>
</tr>
<tr>
<td>Point mutations at A or T (%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Point mutation at C (%)</td>
<td>58</td>
<td>53</td>
<td>44</td>
<td>57</td>
<td>25</td>
<td>0</td>
</tr>
<tr>
<td>Point mutation at G (%)</td>
<td>42</td>
<td>47</td>
<td>55</td>
<td>43</td>
<td>75</td>
<td>100</td>
</tr>
<tr>
<td>C → T</td>
<td>21</td>
<td>12</td>
<td>16</td>
<td>7</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>G → A</td>
<td>11</td>
<td>13</td>
<td>21</td>
<td>6</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>G → C</td>
<td>5</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>C → G</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>G → T</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>C → A</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Percentage of point mutation at RGYW/WRCY motif^b</td>
<td>68</td>
<td>56</td>
<td>49</td>
<td>64</td>
<td>50</td>
<td>100</td>
</tr>
</tbody>
</table>

^aIncludes point mutations, deletions and insertions. ^bPoint mutation at RGYW and WRCY were divided by total mutation number.
the CSR activity of the zebrafish AID was considerably greater than that observed for the catfish AID (Figs 1A and 2A).

**Intracellular localization of catfish AID**

Another functional domain of AID is the NES that is located within the C-terminal 16 amino acids (Fig. 1B) (21–24). The majority of mammalian AID protein localizes to the cytoplasm because of the NES, although an inhibitor of the nuclear export pathway (leptomycin B) revealed that AID shuttles between the nucleus and cytoplasm (21–24). Figures 1(B) and 3 show complete structural and functional conservations of the NES in fish AID. The catfish AID–GFP fusion protein localized mainly to the cytoplasm, as does the mouse AID–GFP protein (Figs 3A and B). Deletion of the C-terminus from catfish AID disrupted cytoplasmic retention of fish AID (Fig. 3C). These observations indicate that the NES of the catfish AID is functionally operational, at least in mammalian cells. As far as protein expression and intracellular localization are concerned (Figs 2C and 3), catfish and mouse AID behaves similarly.

**Discussion**

**Evolution of CSR activity in fish AID before functional requirement**

In this study, we have demonstrated that fish and mouse AID share most of the functional characteristics of AID reported thus far (intracellular localization and activity of CSR and SHM). There is no evidence that fishes have, or did have, any form of antibody CSR, though this possibility cannot be unequivocally ruled out. Why then is the C-terminus in fish AID conserved to the extent that the fish AID has CSR activity in mouse cells? Our data (Fig. 3) are consistent with the notion that retention of AID outside of the nucleus was an early evolutionary characteristic of AID regulation. It is conceivable that the original NES domain at the C-terminus of AID had evolved to appropriately regulate SHM activity by AID. In agreement with this idea, C-terminal-truncated mutants of mammalian AID were reported to have an increased frequency of SHM (five to seven times more frequent than wild type) (21, 22) and occasionally cause cell death (37). Without the C-terminal 16 amino acids, AID might lose its ability to properly regulate SHM activity. A tight correlation between CSR activity and an intact NES motif suggested that the NES residues themselves, or export of AID from the nucleus to the cytoplasm, might be important for AID to trigger CSR. It is likely that the C-terminus of fish AID is conserved solely for interactions with the nuclear export factor. Ability to induce CSR by fish AID may be a by-product resulting from conservation of NES. Functional overlap in AID C-terminus between controlling nuclear export and CSR might have facilitated the evolution of CSR in the early tetrapods.

As seen in Figs 1(A) and 2(B), the efficiency of SHM and CSR by zebrafish AID is much better than that of catfish AID. Zebrafish AID shares 130 amino acids with mouse AID, while catfish AID shares only 118 amino acids. Fifteen amino acids of zebrafish AID are conserved in mouse but not in catfish AID. Conversely, only three amino acids (Y13, S38 and Y63) of...
catfish AID are conserved in mouse AID but not in zebrafish AID. Therefore, the amino acid sequence of zebrafish AID is more similar to the sequence of mouse AID than is the catfish AID. This may be the reason why the zebrafish AID shows better CSR and SHM activity than catfish AID. The observed differences in the ability of zebrafish and catfish AID proteins to mediate both SHM and CSR in mouse cells may be an example of how subtle amino acid differences can affect AID function. Again, we might anticipate selection for mutations within the early tetrapod AID gene that would improve CSR capabilities.

Recently, it has been noted that zebrafish (38) and trout (39) have upstream constant domain exons encoding the heavy chains for the isotypes referred to as IgZ and IgT, respectively. In these instances, the ‘isotype switch’ appears to result from alternative use of D- and J-element clusters during VDJ recombination. Thus, these distinct isotypes have distinct antigen specificities, unlike the case in CSR. The fact that multiple isotypes have been retained in these organisms suggests some selective advantage in expressing multiple isotypes, even in the earliest vertebrates having humoral immunity. By extension early tetrapods, which by chance acquired S regions and duplicated CH genes in their IgH locus, may have had a selective advantage if they could use their pre-existing AID to generate additional isotypes by CSR. Indeed, the next divergent lineage after the bony fishes, the amphibians, do have such an IgH locus and CSR (7, 8) as well as AID (28).

**Acknowledgements**

We thank T. Toyoshima and A. Takano for technical support; Reiko Shinkura and Sidonia Fagarasan for critical reading of the manuscript. This study was supported by the Takeda Science Foundation and the Inoue Foundation for Science, Center of Excellence (COE) formation for Genomic Analysis of Disease Model Animals with Multiple Genetic Alterations and COE grants from the Minister of Education, Culture, Sports, Science and Technology. B.G.M. is supported by an operating grant from the Natural Sciences and Engineering Research Council (NSERC) of Canada. H.S. is supported by a NSERC pre-doctoral fellowship.

**Abbreviations**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>AID</td>
<td>activation-induced cytidine deaminase</td>
</tr>
<tr>
<td>COE</td>
<td>Center of Excellence</td>
</tr>
<tr>
<td>CSR</td>
<td>class switch recombination</td>
</tr>
<tr>
<td>IgH</td>
<td>Ig heavy chain</td>
</tr>
<tr>
<td>NES</td>
<td>nuclear export signal</td>
</tr>
<tr>
<td>NSERC</td>
<td>Natural Sciences and Engineering Research Council</td>
</tr>
<tr>
<td>S</td>
<td>region switch region</td>
</tr>
<tr>
<td>SHM</td>
<td>somatic hypermutation</td>
</tr>
<tr>
<td>V region</td>
<td>variable region</td>
</tr>
</tbody>
</table>

**Fig. 3.** Intracellular localization of catfish AID–GFP fusion protein. One day after transfection of COS7 cells, GFP signal was observed by fluorescence microscopy. The major GFP signals for both the mouse (A) and catfish AID–GFP (B) proteins were cytoplasmic, whereas GFP alone distributed evenly through cell (D). The catfish JP8Bdel–GFP fusion protein distributed mainly to nucleus, but the GFP signal was occasionally seen in cytoplasm (C).
Fish AID has class switch activity


References


