

# Identification and expression of adenosine deaminases acting on tRNA (ADAT) during early tail regeneration of the earthworm

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# Abstract

**Background** RNA editing is a widespread phenomenon in all metazoans. One of the common RNA editing event is the chemical conversion of adenosine to inosine (A-to-I) catalyzed by adenosine deaminases acting on tRNA (ADAT). During *D*. *melanogaster* development, the ADAT1 transcript was found to localize mainly to the central nervous system including brain and ventral nerve cord during brain development. Although an earthworm adenosine deaminases acting on mRNA (ADAR) has been identified and its possible implication in earthworm regeneration has been investigated, there is little accumulated information on ADAT and tRNA editing in the annelid including terrestrial earthworms.

**Objective** This study aimed to investigate the molecular characteristics and the expression pattern of earthworm *ADAT* during tail regeneration to understand its physiological significance.

**Methods** Nucleotide sequence of *Ean-ADAT* was retrieved from the genome assembly of *Eisenia andrei* via Basic Local Alignment Search Tool (BLAST). The genome assembly of *Eisenia andrei* was downloaded from National Genomics Data Center (http://bigd.big.ac.cn/gwh/). The alignment and phylogenetic relationship of the core deaminase domains of ADATs and ADARs were analyzed. Its temporal expression during early tail regeneration was measured using real-time PCR.

**Results** The open reading frame of *Ean-ADAT* consists of 1719 nucleotides encoding 573 amino acids. Domain analysis indicates that Ean-ADAT has a deaminase domain composed of 498 amino acids and a predicted nuclear localization signal at the N-terminal. Its subcellular localization was predicted to be nuclear. The core deaminase region of Ean-ADAT encompasses the three active-site motifs, including zinc-chelating residues and a glutamate residue for catalytic activity. In addition, Ean-ADAT shares highly conserved RNA recognition region flanking the third cysteine of the deaminase motif with other ADAT1s even from the yeast. Multiple sequence alignment and phylogenetic analysis indicate that Ean-ADAT shows greater similarity to vertebrate ADARs than to yeast Tad1p. *Ean-ADAT* mRNA expression began to remarkably decrease before 12 h post-amputation, showing a tendency to gradual decrease until 7 dpa and then it slightly rebounded at 10 dpa. **Conclusions** Our results demonstrate that *Ean-ADAT* belongs to a class of ADAT1s and support the hypothesis of a common evolutionary origin for ADARs and ADATs. The temporal expression of Ean-ADAT could suggest that its activity is unrelated to the molecular mechanisms of dedifferentiation.

Keywords Earthworm · Tail regeneration · Adenosine deaminase acting on tRNA · mRNA expression

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# Introduction

RNA editing, a post-transcriptional modification of RNA molecules, is a widespread phenomenon in all metazoans. The most common RNA editing event is the chemical conversion of adenosine to inosine (A-to-I) catalyzed by adenosine deaminases acting on RNA enzymes, which act on dsRNA or tRNA substrates (Yoon et al. 2020).

Inosine (6-deaminated adenosine) is a non-canonical nucleoside found in all fields of life. Chemically, it is a guanosine analogue and it only differs from the latter by the lack of the N2 amino group. Inosine is rarely present in DNA but is often observed in different types of RNAs including double-stranded RNAs, tRNAs and viral RNAs (Grosjean et al. 1996). In RNA, inosine is produced by the deamination of adenosine. Generally, two types of RNA adenosine deaminases exist: adenosine deaminases acting on mRNAs (ADARs) and adenosine deaminases acting on tRNAs (ADATs), the enzymes of each group being specific for specific modification sites (Torres et al. 2014; Gerber and Keller 1999). Inosine is found in tRNAs in all domains of life. It is present mainly at three positions on tRNAs: position 34, which is the first nucleotide of the anticodon (wobble-position), position 37 (following the anticodon), and position 57 (at the T $\psi$ C-loop) (Auxilien et al. 1996; Torres et al. 2014).

ADAR family present only in metazoans appears to have evolved from ADAT, a critical protein present in all eukaryotes, via the addition of a double-stranded RNA binding domain (Grice and Degnan 2015). It is thought that ADATs involved in the modification of tRNA have a common evolutionary origin with ADARs involved in pre-mRNA editing (Gerber et al. 1998). Unlike ADARs, only a few ADATs have been identified in eukaryotes including yeasts (Gerber et al. 1998), octopus (XP 029641018), drosophila (Keegan et al. 2000), and human (Maas et al. 1999). The phenotypic consequences of the lack of inosine modifications on tRNAs in metazoans have been almost completely unexplored. During D. melanogaster development, the ADAT1 transcript which is specific for adenosine 37 of tRNA<sup>ala</sup> was found to localize mainly to the central nervous system including brain and ventral nerve cord (Keegan et al. 2000), suggesting a possible role for ADAT1 and I37 modification of tRNA<sup>Ala</sup> during brain development (Torres et al. 2014).

Earthworms show a wide spectrum of regenerative potential capable of re-constructing body parts lost due to injury. Among the earthworm species, Eisenia andrei has powerful regenerating capacity and can completely regenerate an amputated tail about within a month. It is generally believed that earthworm regeneration is an epimorphosis, which is characterized by the dedifferentiation of adult tissue to form a highly proliferating cell mass called a blastema, followed by its re-specification into appropriate cell types (Ribeiro et al. 2018). In E. andrei, blastema forms beneath wounded dermis at 1-3 day post-amputation (dpa), and segmentation occurs within 7 dpa, when redifferentiation is not yet dynamic (Park et al. 2013; Shao et al. 2020). Between 7 and 10 dpa, the blastema continues to grow and elongates, but there are no external signs of segmentation. After 7 dpa, outgrowth of the regenerating tissue was observable under low magnification, after which the redifferentiation of various tissues actively takes place (Park et al. 2013).

Very recently, an earthworm ADAR, *Pex-ADAR*, has been identified and its spatiotemporal expression implies that

this RNA editing enzyme could be implicated in muscle redifferentiation (Yoon et al. 2020). However, in the annelid including terrestrial earthworms, there is little accumulated information on ADAT and tRNA editing. Through the genome sequence of *E. andrei*, we identified a full-length cDNA sequence showing significant homology to mammalian *ADAT1*. To our knowledge, this is the first report on the molecular characterization of an annelid ADAT and its expression analysis in earthworm tail regeneration, which should help to elucidate the physiological significance of ADAT, which is conserved from yeast to human.

# **Materials and methods**

#### Animals and computational sequence analysis

Sexually mature *E. andrei* obtained from a commercial source (Seoul, Korea) were reared by the method previously described (Park et al. 2017). Before being used, the earthworms were placed in Petri dishes lined with filter paper moistened with earthworm saline for 48 h to purge the gut contents. Nucleotide sequence of *Ean-ADAT* was retrieved from the genome assembly of *Eisenia andrei* via Basic Local Alignment Search Tool (BLAST). The genome assembly of *Eisenia andrei* was downloaded from National Genomics Data Center (http://bigd.big.ac.cn/gwh/) under accession code: GWHACBE00000000. The open reading frame (ORF) was determined using the ORF finder on the server of National Center of Biotechnology Information (NCBI). The subcellular localization of Ean-ADAT was predicted by PSORT II (Nakai and Horton 1999).

## **Comparative and phylogenetic analyses**

Amino acid sequences of ADAT1s and ADARs were retrieved from the GenPept Database via BLASTP and Uni-Prot (http://www.uniprot.org/). Amino acid sequence alignment was carried out by MUltiple Sequence Comparison by Log-Expectation (MUSCLE) algorithm and phylogenetic analysis was performed by the maximum likelihood method, using MEGA X software (https://www.megasoftware.net/). Bootstrap analysis was performed with 100 replications. The phylogenetic tree was built with ADAT1 orthologs of metazoan animal models and yeasts (Tad1p), [Lophotrochozoa: Eisenia andrei, Crassostrea gigas (XP\_011432605), Octopus sinensis (XP\_029641018), Pomacea canaliculata (XP\_025115922) and Mizuhopecten yessoensis (XP\_021375838); Ecdysozoa: Araneus ventricosus (A0A4Y2QEA4) and Drosophila melanogaster (Q9V3R6); Deuterostomia: Homo sapiens (Q9BUB4), Rattus norvegicus (D4ADL5), Mus musculus (Q9JHI2), Gallus gallus

(Q5ZI16), Xenopus laevis (A0A1L8GF60) and Danio rerio (F1R076); yeasts, Saccharomyces cerevisiae (P53065) and Schizosaccharomyces pombe (O42912)], ADAR1 orthologs [Homo sapiens (P55265) and Mus musculus (Q99MU3)], and ADAR2 orthologs [Homo sapiens (P78563) and Mus musculus (Q91ZS8)].

# **Quantitative real time PCR**

Total RNA was isolated from the tail regenerates of E. andrei during regeneration using TRIzol (Ambion, Austin, TX, USA) at the times indicated. We selected mRNA from total RNA using oligo (dT) primers (Promega, Madison, WI, USA) and then reverse transcribed the mRNA into cDNA (SuperScript II First-Strand Synthesis System for RT-PCR, Invitrogen, Waltham, MA, USA). Quantitative reverse-transcription PCR (qRT-PCR) was performed using WizPure<sup>TM</sup> qPCR Master (SYBR) (Wizbiosolutions, Korea) with specific primer pairs on an Applied Biosystems Stepone plus real-time PCR System. The sequences of primer pairs were as follows: Ean-ADAT (forward) 5'-GCAGCACCGGAT GGAGTTAG-3' and (reverse) 5'-ATCTGGCCTTGGACG AGTCA-3'; and Ean-β-actin (forward) 5'- CATCCATCG TCCACAGGAAGTG-3' and (reverse) 5'-CGTGTTCAT CTCAGGAGGCAGA-3'. Relative quantification of mRNA was conducted using the comparative  $2 - \Delta \Delta Ct$  method with  $\beta$ -actin as the reference gene. All data are expressed as means  $\pm$  SEM and analyzed by using GraphPad Prism 6.01 (GraphPad Software, Inc.). Differences between groups were assessed using a student's t-test.

## **Results and discussion**

#### Sequence and domain analyses

The nucleotide and predicted amino acid sequences of *Ean-ADAT* found in the earthworm *E. andrei* are shown in Fig. 1. The open reading frame of *Ean-ADAT* consists of 1719 nucleotides encoding 573 amino acids with a calculated molecular mass of 62.7 kDa. Domain analysis indicates that Ean-ADAT has a deaminase domain composed of 498 amino acids and a predicted nuclear localization signal at the N-terminal. Its subcellular localization was predicted to be nuclear.

Since it is thought that the differences between the sequences of the deaminase core region is a reliable index to determine the ortholog's relationship to newly sequenced RNA-editing enzymes (Keegan et al. 2004), the alignment of core deaminase domains of Ean-ADAT with other ADAT and ADAR family members is carried out (Fig. 2).

Like the other ADATs, the core region of Ean-ADAT encompasses the three active-site motifs, including zincchelating residues (histidine or cysteine in black boxes) and a glutamate (E) residue in the first active-site motif that is thought to be involved in proton transfer in the deaminase active site. It is also noted that Ean-ADAT shares highly conserved RNA recognition region flanking the third cysteine of the deaminase motif with other ADAT1s even from the yeast, indicating that Ean-ADAT belongs to a class of ADAT1s. However, the predicted Ean-ADAT adenosine deaminase domain shows 49.2% identity and 69.0% similarity to the human ADAR1 protein, and 52.0% identity and 65.9% similarity to the human ADAR2, while it shows 31.7% identity and 54.2% similarity to the S. cerevisiae Tad1 protein. The fact that Ean-ADAT shows greater similarity to vertebrate ADARs than to yeast Tad1p, supporting the hypothesis of a common evolutionary origin for ADARs and ADATs (Gerber et al. 1998; Keegan et al. 2000).

Furthermore, phylogenetic analysis exhibits that Ean-ADAT could be grouped together with other ADAT1s and it has greater similarity to vertebrate ADAR1s and ADAR2s than to yeast Tad1p (Fig. 3), supporting the evolutionary hypothesis that pre-mRNA editing may have evolved when an original ADAT acquired dsRNA-binding domains and a new set of targets in pre-mRNA (Keegan et al. 2000).

# Temporal expression of *Ean-ADAT* mRNA during early tail regeneration

The expression level of Ean-ADAT mRNA during the early tail regeneration of E. andrei was determined using realtime qRT-PCR (Fig. 4). During early tail regeneration, Ean-ADAT mRNA expression began to remarkably decrease before 12 h post-amputation, showing a tendency to gradual decrease until 7 dpa and then it slightly rebounded (p < 0.01) at 10 dpa. The qRT-PCR analysis of the temporal expression of Ean-ADAT mRNA indicated that its transcription was inactivated in the early stages (1-7 dpa) when blastemal cells proliferate and the central nerve cord is reconstructed. Expression then slightly rebounded at subsequent stages (10 dpa) when diverse cell types or tissues were regenerated in each segment. This could suggest that Ean-ADAT activity is unrelated to the molecular mechanisms of dedifferentiation. Its slight but significant rebound at 10 dpa might be attributable to the partial restoration of amputated region or be implicated in redifferentiation.

In *D. melanogaster*, the expression pattern of *ADAT1* transcript localized mainly to the central nervous system possibly suggests that its activity could be associated with brain and/or ventral nerve cord development (Torres et al. 2014). However, the high level of *ADAT1* expression in the

1	M <u>E R G G S T F A S C I A N L C F D H Y A K K L T K K G K P Q R R</u> R
1	ATGGAAAGAGGTGGTTCGACATTTGCCAGCTGCATTGCGAATCTCTGCTTTGACCATTACGCA <mark>AAGAAGCTGACGAAGAAGGGAAAGCCGCAGAGAAGGC</mark>
35	W_TT_L_A_A_V_V_E_T_R_A_G_S_D_R_G_A_Q_I_S_S_D_M_R_V_V_[SM_G_T_G_
101	GGGAATGGACAACACTTGCAGCTGTCGTCGAGACGCGGGCTGGTAGCGACAGAGGTGCGCAGATTTCGTCTGATATGAGAGTTGTA
68	S_K_C_I_G_Q_S_Q_M_C_P_Q_G_Q_V_I_N_D_S_H_A_E_V_I_A_R_R_G_F_L_C_Y_L_
201	TTCAAAGTGCATAGGTCAGAGTCAGATGTGCCCTCAGGGTCAGGTCATCAACGACAGCCATGCGGAGGTCATCGCCAGACGGGGTTTTCTCTGCTATCTG
101	<u>R G Q L E E A W T G Q Q D C I F S V A E D G K C S L K N D V R F H L</u>
301	AGGGGTCAGCTGGAGGAAGCCTGGACGGGTCAGCAAGACTGCATCTTCTCAGTTGCTGAGGATGGAAAGTGCTCACTTAAGAATGATGTTCGATTCCATC
135	FTSCI_PCGDASIV_P_KDA_EN_DS_E_S_G_PA_RL_PV_S_HL_
401	TTTTCACATCGTGCATACCATGTGGAGATGCTTCGATTGTTCCAAAAGATGCGGGAGAACGATTCGGAGTCTGGTCCAGCACGTCTTCCTGTTTCACACCCT
168	<u>S E S I G E V T S S R G H A E D N T S Q P I N A E Y N D T G H G P</u>
501	AAGTGAGAGCATCGGCGAGGTTACAAGTTCACGGGGTCATGCGGAGGACAATACCTCCCAACCCATAAACGCTGAATACAATGACACTGGGCATGGACCA
201	F <u>SCQGSNADTSRPGRTHTTADQQKTAGEESTLK</u> A
601	TTTAGTTGTCAGGGTTCCAATGCGGACACCTCTCGCCCTGGGAGGACACATACCACTGCAGATCAACAAAAAACTGCGGGCGAGGAATCAACACTCAAAG
235	<u>SQALKRFERIDSTISIATNVDADGVPAKKMKC</u> T
701	cttcgcaggcgttgaaacgttttgaacgtattgactcaactatatccaatcgcgaccaatgttgacgcggacggtgttcctgcaaagaaaatgaaatgtaccaatgtaccaatgttgacgcgacgatgttcctgcaaagaaaatgaaatgtaccaatgtaccaatgttgacgcgacgatgttcctgcaaagaaaatgaaatgtaccaatgtaccaatgttgacgcgacgatgttcctgcaaagaaaatgaaatgtaccaatgttcctgcaaagaaatgtaccaatgttcctgcaatgttcctgcaaagaaatgtaccaatgttcctgcgacgatgttcctgcaatgttcctgcaaagaaatgtaccaatgttcctgcgacgatgttcctgcaatgttcctgcaatgttcctgcgacgatgttcctgcgacgatgttcctgcaatgttcctgcaatgttcctgcaatgttcctgcgacgatgttcctgcgacgatgttcctgcaatgttcctgcgacgatgttcctgcgacgatgttcctgcaatgttcctgcgacgatgttcctgcgacgatgttcctgcaatgttcctgcgacgatgttcctgcgacgatgttcctgcgacgatgttcctgcgacgatgttcctgcgacgatgttcctgcgacgatgttcctgcgacgatgttcctgcgacgatgttcctgcgacgatgttcctgcgacgatgttcctgcgacgatgttcctgcgacgatgttcctgcgacgatgttcctgcgacgatgttcctgcgacgatgttcctgcgacgatgttcctgcgacgatgttcctgcgacgatgttcctgcgacgatgttcctgcgacgatgttcctgcgacgatgttcctgcgacgatgttcctgcgacgatgttcctgcgacgatgttcctgcgacgatgttcctgcgacgatgttcctgcgacgatgttcctgcgacgatgttcctgcgacgatgttcctgcgacgatgttcctgatgttcctgacgatgttcctgatgttcctgcgacgatgttcctgcgacgatgttcctgcgacgatgttcctgatgttcctgcgacgatgttcctgatgttcctgcgacgatgttcctgcgacgatgttcctgcgacgatgttcctgatgttcctgatgttcctgatgttcctgatgttcctgatgttcctgatgttcctgatgttcctgatgttcctgatgttcctgatgttcctgatgttcctgatgttcctgttcctgatgtttcctgatgtttgtt
268	<u>E Q H E L Q D A A V S S D R P A G N G V E A L H N F R G T E G I L</u>
801	AGAGCAGCATGAATTGCAAGATGCTGCGGTTTCTAGCGATCGACCAGCGGGTAACGGTGTCGAAGCCTTGCACAATTTCCGTGGTACCGAAGGCATCCTG
001	
301	
301 901	S <u>SSSPLNSDLRPVVEGFDVHRTGAKCAPGERED</u> M AGCTCTTCATCGCCATTGAACTCTGACCTTAGGCCAGTTGTGGGAGGGGCTTTGATGTTCATCGCACCGGGGCTAAATGTGCTCCTGGGGAGAGGGAGG
301 901 335	S_S_S_P_L_N_S_D_L_R_P_V_V_E_G_F_D_V_H_R_T_G_A_K_C_A_P_G_E_R_E_D_M AGCTCTTCATCGCCATTGAACTCTGACCTTAGGCCAGTTGTGGAGGGGCTTTGATGTTCATCGCACCGGGGCTAAATGTGCTCCTGGGGAGAGGGAGG
301 901 335 1001	S_S_S_S_P_L_N_S_D_L_R_P_V_V_E_G_F_D_V_H_R_T_G_A_K_C_A_P_G_E_R_E_D_M   AGCTCTTCATCGCCATTGAACTCTGACCTTAGGCCAGTTGTGGGGGGGCTTTGATGTCATCGCACCGGGGCTAAATGTGCTCCTGGGGGGGG
301 901 335 1001 368	S_S_S_S_P_L_N_S_D_L_R_P_V_V_E_G_F_D_V_H_R_T_G_A_K_C_A_P_G_E_R_E_D_M   AGCTCTTCATCGCCATTGAACTCTGACCTTAGGCCAGTTGTGGGGGGGG
301 901 335 1001 368 1101	S_S_S_S_P_L_N_S_D_L_R_P_V_V_E_G_F_D_V_H_R_T_G_A_K_C_A_P_G_E_R_E_D_M   AGCTCTTCATCGCCATTGAACTCTGACCTTAGGCCAGTTGTGGGGGGGG
301 901 335 1001 368 1101 401	S_S_S_S_P_L_N_S_D_L_R_P_V_V_E_G_F_D_V_H_R_T_G_A_K_C_A_P_G_E_R_E_D_M   AGCTCTTCATCGCCATTGAACTCTGACCTTAGGCCAGTTGTGGGGGGGG
301 901 335 1001 368 1101 401 1201	S_S_S_S_P_L_N_S_D_L_R_P_V_V_E_G_F_D_V_H_R_T_G_A_K_C_A_P_G_E_R_E_D_M   AGCTCTTCATCGCCATTGAACTCTGACCTTAGGCCAGTGTGGGGAGGGGGGGCTTTGATGTCATCGCACCGGGGCCTAAATGTGCTCCTGGGGGAGGGGGGGG
301 901 335 1001 368 1101 401 1201 435	S S S S P L N P V E G F D H R T G A K C A P G E R E D M   AGCTCTTCATCGCCATTGAACTCTGACCTTAGGCCAGTTGTGGGAGGGGCTTTGATGTCATCGCACCGGGGGGGG
301 901 335 1001 368 1101 401 1201 435 1301	S_S_S_S_P_L_N_S_D_L_R_P_V_V_E_G_F_D_V_H_R_T_G_A_K_C_A_P_G_E_R_E_D_M   AGCTCTTCATCGCCATTGAACTCTGACCTTAGGCCAGTTGTGGGAGGGGGGGG
301 901 335 1001 368 1101 401 1201 435 1301 468	S S S S S P L N S D L R P V E G F D V H R T G A K C A P G E R E D M   AGCTCTTCATCGCCATTGAACTCTGACCTTAGGCCAGTTGTGGGAGGGGCTTTGATGTCATCGCACCGGGGGGGG
301 901 335 1001 368 1101 401 1201 435 1301 468 1401	S S S S S P L N S D L R P V E G F D V H R T G A K C A P G E R E D M   AGCTCTTCATCGCCATTGAACTCTGACCTTAGGCCAGTTGTGGGAGGGGCTTTGATGTCATCGCACCGGGGGGGG
301 901 335 1001 368 1101 401 1201 435 1301 468 1401 501	S S S S S P L N S D L R P V E G F D V H R T G A K C A P G E R E D M   AGCTCTTCATCGCCATTGAACTCTGACCTTAGGCCAGTTGTGGGAGGGGCTTTGATGTCATCGCACCGGGGGGGG
301 901 335 1001 368 1101 401 1201 435 1301 468 1401 501 1501	S S S S P L N P V E G F D V H R T G A K C A P G E R E D M   AGCTCTTCATCGCCATTGAACTCTGACCTTAGGCCAGTTGTGGGAGGGGCTTTGATGTCATCGCACCGGGGGGGG
301 901 335 1001 368 1101 401 1201 435 1301 468 1401 501 1501 535	S S S S P L N S D L R P V E G F D V H R T G A K C A P G E R D M   AGCTCTTCATCGCCATTGAACTCTGACCTTAGGCCAGTTGTGGAGGGGGGCTTTGATGTCATCGCACCGGGGGGGCCCTCTCCCTGGGGGAGGGGAGGGGAGGGGGGGG
301 901 335 1001 368 1101 401 1201 435 1301 468 1401 501 1501 535 1601	S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S
301 901 335 1001 368 1101 401 1201 435 1301 468 1401 501 1501 535 1601 568	S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S S

1701 GGAAGCAGATTCACTTCGATGA

**Fig. 1** Nucleotide and deduced amino acid sequences of earthworm *Ean-ADAT*. The open reading frame of *Ean-ADAT* gene consists of 1719 nucleotides encoding 573 amino acids. Predicted nuclear localization signal and deaminase domain are presented in the red and

nervous system is controversial. Considering the high degree of homology between dADAT1 and the ADAR enzymes, it is possible that the embryonic expression of *dAdat1* is found in the central nervous system and is reminiscent of rRED2 (a brain specific member of the RNA-specific adenosine deaminase family) expression which is confined to the brain green boxes, respectively. The stop codon is indicated by an asterisk. Residue numbers for nucleotides and amino acids are indicated to the left row (colour figure online)

(Melcher et al. 1996; Keegan et al. 2000). As the function of ADATs during development and differentiation has been explored to a limited extent, the earthworm regeneration system would be helpful to elucidate the molecular mechanism behind how ADATs activities are involved in the nervous system.

Eisenia andrei ADAT Crassostrea_gigas ADAT1 XP 011432605 Octopus_sinensis ADAT1 XP 028641018 Pomacea_canalicultat ADAT1_XP_025115922 Mizuhopecten yessoensis ADAT1 AV4208A4 Drosophila melanogaster ADAT1 044208A4 Drosophila melanogaster ADAT1 0497386 Homo sapiens ADAT1_028UB4 Rattus norvegicus ADAT1_04ADL5 Mus musculus ADAT1 052T16 Xenopus laevis ADAT1 052T16 Xenopus laevis ADAT1 052T16 Saccharomyces_cerevisiase Tad1p_042015 Homo sapiens ADAT1_058506 Schizosaccharomyces pombe Tad1p_042912 Homo sapiens_ADAT1_098M03 Homo sapiens_ADAT1_098M03 Homo sapiens_ADAT2_091258	: *:*:::::::::::::::::::::::::::::::	AWTGQODCIFSVAEDG-KCSLKNUVFFHLFTSG -NHGGKSEIFT-SARDSTNNKCRLKPGVKFHLFTSG -VINGESSEVFVLPASKAHNQCLLKNNVEFQFPMSS -VINGESSEVFTS9NKAAHNQCLLKNNVEFQFPMSS -VIDGESKVLE-VINNC-RVKVKAGVHFHFSS -VITDGESKVLE-VTINC-RVKVKAGVHFHFSS DRIFH-NNSTLS-TTINDEHVEFHFLST DRIFH-NNSTLS-TTINDEHVEFHFLST DRIFH-NNSTLS-TTINDEHVEFHFLST DRIFH-NNSTLS-TTINDEHVEFHFLST DRIFH-NNSTLS-TTINDEHVEFHFLST DRIFH-NSSLS-HVRLKPDLSFVFFSS DRIFH-NSSLS-HVRLKPDLSFVFFSS 	**** : IPGODAS VVPKDAEN-DSESGPA TTPGDASI FPKDAIS TTPGDASI FPKDAIS TTPGDASI FPKTEVHSETPLIKD TTPGDASI FPKTEVHSETPLIKD TTPGDASI FPKNSED TTPGDASI FWNSED TTPGDASI FWNEFF TTPGDASI FWNEFF TTPGDASI FWNEFF TTPGDASI FVIEFF TTPGDASI	RLPVSHLSESIGE 
Eisenia andrei ADAT Crassostrea gigas ADATI XP 011432605 Octopus sinensis ADATI XP 029641018 Pomacea canalicultad ADATI XP 025115922 Mizuhopecten yessoensis ADATI 044720584 Drosophila melanogaster ADATI 044720584 Homo sapiens ADATI 098U54 Rattus norvegicus ADATI 044015 Mus musculus ADATI 095U16 Xenopus laevis ADATI 045216 Xenopus laevis ADATI 045216 Saccharomyces cerevisiae Tadip D53065 Schizosaccharomyces pombe Tadip_042912 Homo sapiens ADATI 098W3 Homo sapiens ADARI 098W3	VTSSRGHAEDNTSQP INAEYNDTGHGPFSCQGSNADTSRPGRTHTTAD GEBEUDMDSFSWQYGKKRKSDTKSDIEPKCLKVESSITSSDYNSPNSD 	2QKTAGEESTLKASQALKRFERIDSTISJ LDLQDLHSNCPKQSEQCVSDLVLPPKETDTEKVT KFKTDDGI- SVSPVEGK	IATNVDADGVPAKKMKCTEQHELG SD5NNLKTDOEGKYSESSNILTN JTHAVDRNDHVSDTSRWBHHEDVL JDETDKQGGDEARKLTEDLHEPN 	2DAAVSSDRPAGNG RACLICNKHSKDG .PLGQSDAQTLSVR NNCNIREDRNPGNL .HHQSFGKQKSGPI HGTQSSGPV RAVQQVFAKPEGNV 230240
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Homo_sapiens_ADAR2_P78563 Mus_musculus_ADAR2_Q91ZS8	GEGTI PVRSNAS 	IQTWDGVLQGERLLTMSCSDKIAF IQTWDGVL	RWNVVGIQGSLLSIFVE-PIYF 5 RWNVVGIQGSLLSIFVE-PIYF 330340	52.0 65.6

Fig. 2 Alignment of core deaminase domains from ADAT1 and ADAR family, showing relatively well conserved RNA recognition region of ADATs compared to vertebrate ADARs. The three active site motifs are bracketed, within which zinc-binding residues (histidine or cysteines) are represented in closed black boxes. A glutamate residue in the first active-site motif for catalytic activity is presented in the red box. A potential RNA recognition region flanking the third cysteine of the deaminase motif is presented in green box. The accession number of each sequence is denoted after the species name. Conserved residues are indicated with an asterisk (\*), while (:) and (.) indicate conservative and semi-conservative substitutions. Sequence identity (%) and similarity (%) were expressed in comparison with human ADAR1, ADAR2 and S. cerevisiae Tad1p (colour figure online)



**Fig.3** Phylogenetic relationship among deaminase domains of ADAT1s, ADARs and Tad1ps based on the Maximum likelihood method. Phylogenetic analysis indicates that Ean-ADAT could be clustered with ADAT1s from other animal species employed, separately grouped from ADAR1s and ADAR2s of vertebrates (*Homo* 



**Fig. 4** Temporal expression analysis of *Ean-ADAT* mRNA using real time qRT-PCR during early tail regeneration of *E. andrei*. In the regenerating tail, *Ean-ADAT* mRNA expression began to decrease before 12 h post-amputation, showing the minimal expression around 7 dpa. The relative level was normalized to  $\beta$ -actin. The data, obtained from three independent experiments, are expressed as mean ± SEM. \*Indicates statistical significance (*p* < 0.01) compared with un-amputated control

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#### **Compliance with ethical standards**

**Conflict of interest** The authors declare that there is no conflict of interest regarding the publication of this.

Ethics approval and consent to participate Not applicable.

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