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Review

A-to-I editing on tRNAs: Biochemical, biological and evolutionary implications

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ABSTRACT

Inosine on transfer RNAs (tRNAs) are post-transcriptionally formed by a deamination mechanism of adenosines at positions 34, 37 and 57 of certain tRNAs. Despite its ubiquitous nature, the biological role of inosine in tRNAs remains poorly understood. Recent developments in the study of nucleotide modifications are beginning to indicate that the dynamics of such modifications are used in the control of specific genetic programs. Likewise, the essentiality of inosine-modified tRNAs in genome evolution and animal biology is becoming apparent. Here we review our current understanding on the role of inosine in tRNAs, the enzymes that catalyze the modification and the evolutionary link between such enzymes and other deaminases.

1. Inosine and inosine modification enzymes

Inosine is a non-canonical nucleoside found in all domains 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 [76,63,30]. In RNA, inosine is produced by the deamination of adenosine [31,5]. Generally, 2 groups of RNA adenosine deaminases exist: adenosine deaminases acting on messenger RNAs (ADARs) and adenosine deaminases acting on tRNAs (ADATs), the enzymes of each group being specific for specific modification sites [5,76,24,25].

ADARs are present only in metazoans and, in vertebrates, three genes that encode for different ADAR proteins have been described. ADAR1 and ADAR2 are expressed in most tissues and their deamination activity has been confirmed. ADAR3 on the contrary is only expressed in the central nervous system and its function is currently unknown, as it lacks deamination activity [45]. As mentioned, ADARs deaminate adenosines in mRNAs to inosine. Since inosine (which is derived from adenosines), resembles guanosine, A-to-I editing on mRNAs can result in amino acid substitutions during translation, and alterations of splice sites during mRNA processing [76]. Additionally, ADARs can edit non-coding RNAs and have regulatory functions, for example, by affecting the biogenesis, processing and target selection of siRNAs and miRNAs [76].

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 TΨC-loop) (Fig. 1A). Interestingly, at position 34, inosine is the final modified base, while at positions 37 and 57 inosine is found in a methylated state (m^1I37, m^1I57 or m^1Im57) [40,55].

Methyl-inosine 37 has only been found in eukaryotic $tRNA^{Ala}$ [30,40] and the modification involves two enzymatic reactions. First, A37 is deaminated to I37 by the homodimeric adenosine deaminase acting on tRNA 1 (ADAT1/Tad1) [24,53,54]. This reaction is then followed by a methylation step carried out by the tRNA methyltransferase 5 (Trm5) [8] (Fig. 1C). Inosine at position 57 has only been found in Archaea [74,30,31]. Conversion of A57 into m^1I57 also occurs in two steps. However, during the first step, A57 is methylated by an S-adenosyl-L-methionine-(SAM) dependent tRNA methyltransferase (TrmI) [65,18]. Then, m^1A57 is further deaminated to m^1I57 by an as yet uncharacterized enzyme [31]

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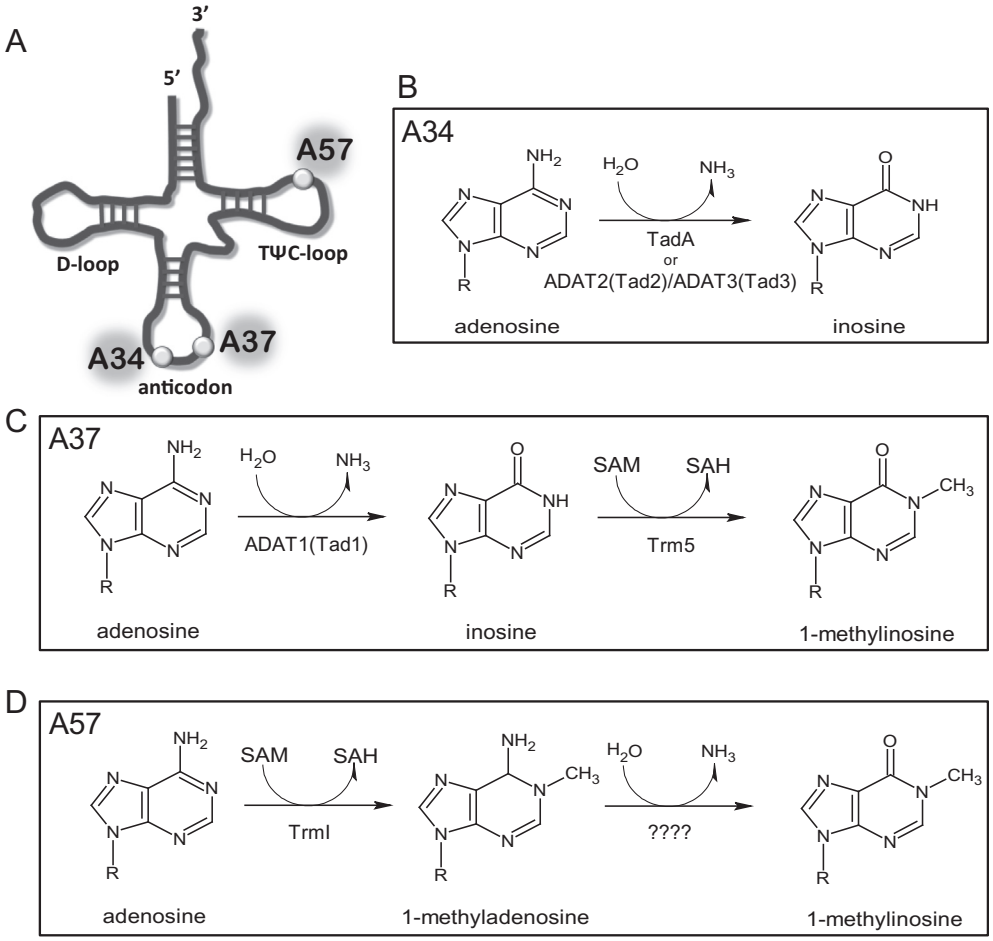

Fig. 1. Adenosine deaminase reactions on tRNAs. (A) Schematic representation of the clover-leaf tRNA secondary structure. Locations of residues modifiable to inosine are shown in light-gray circles (A34, A37 and A57). D-loop: dihydrouridine-loop. TΨC-loop: ribothymine-pseudouracyl-cytosine-loop. (B) The formation of inosine 34. The one-step hydrolytic deamination reaction is catalyzed by the TadA homodimer in prokaryotes or the ADAT2(Tad2)/ADAT3(Tad3) heterodimer in eukaryotes. (C) The formation of methylinosine 37. The reaction proceeds via two steps: hydrolytic deamination of adenosine catalyzed by the ADAT1(Tad1) homodimer and methylation of inosine catalyzed by Trm5. (D) The formation of methylinosine 57. The reaction proceeds via two steps: methylation of adenosine catalyzed by TrmI and subsequent hydrolytic deamination of methyladenosine (catalyzing enzymes are not known).

(Fig. 1D). In hyperthermophilic organisms, A57 was also found to be modified into di-methylated inosine (1,2′-O-dimethylinosine: m^1Im57) [19].

In contrast, I34 is not present in Archaea but is present in Bacteria and Eukarya. In prokaryotes, only $tRNA^{Arg}$ is inosine-modified and the A34-to-I34 conversion is catalyzed by the homodimeric tRNA adenosine deaminase A (TadA) [73]. In eukaryotes, I34 is present in 7–8 different cytosolic tRNAs [40,55] and the deamination reaction is catalyzed by the heterodimeric adenosine deaminase acting on tRNA (hetADAT) composed of two subunits named ADAT2/Tad2 and ADAT3/Tad3 [25] (Fig. 1B). Additionally, I34 can be found on chloroplastic $tRNA^{Arg}$ and the enzyme responsible for the deamination reaction is the tRNA adenosine deaminase arginine (TADA), that shares sequence similarities to the prokaryotic TadA [17,42].

2. Structures of ADATs

The first ADAT to be crystalized was the prokaryotic enzyme TadA [46,20,52]. TadA contains the consensus motif (C/H)XEX_n PCXXC, typically present in cytidine deaminases, that has Zn-binding regions and forms the catalytically active domain of the protein [73]. TadA was shown to form homodimers in order to be active but the tRNA substrate was not necessary for dimer formation [73]. It is thus not surprising that the first crystal structures were obtained in the absence of tRNA substrates [46,20]. Each TadA monomer is composed of five β-sheets located in the center of the molecule and flanked by five α-helices. Both monomers cooperate to form a dimer of an overall globular shape presenting the catalytic domain at the dimer interface. As expected, this catalytic domain contains Zn atoms bound to it and a conserved glutamate residue that mediates the proton transfer necessary for the deamination reaction [46,20,47].

Interestingly, the crystal structure of *Staphylococcus aureus* TadA in complex with the anticodon stem-loop of $tRNA^{Arg}$ (bearing nebularine at the wobble position) revealed a dramatic RNA conformation switch in order to accommodate the RNA into the TadA binding pocket [52]. The RNA stem conformation remained practically unchanged, however residues 33–37 in the anticodon loop no longer base stacked and were instead widely spread in order to maximize the surface available for RNA:protein interaction. Apart from these nucleotides, the C32:A38 base pair that caps the anticodon loop of $tRNA^{Arg}$ was also shown to interact strongly with TadA in the crystal structure. As expected, the adenosine analogue nebularine at position 34 was found extruded from the anticodon stem-loop and deeply inserted into the catalytic pocket of TadA, stabilized by various hydrogen bonds and Van der Waals interactions [52].