Northeast Structural Genomics Consortium

Structural and Functional Studies of Bacterial MiaA Orthologs. NESG Targets BhR41, SeR100

Bacterial and eukaryotic tRNAs that decode codons starting with uridine have a hydrophobically hypermodified adenosine at position 37 (A(37)) adjacent to the 3'-end of the anticodon (Fig. 1), which is essential for efficient and highly accurate protein translation by the ribosome. However, it remains unclear as to how the corresponding tRNAs are selected to be modified by alkylation at the correct position of the adenosine base (Fig. 1A). In a collaboration with the research groups of Chimnaronk (Mahidol University, Thailand), Tanaka (Hokkaido University, Japan), and Atta/Fontecave (Institut de Recherches en Technologie et Sciences pour le Vivant, France), we have determined crystal structures of three bacterial tRNA isopentenyltransferase (MiaA) in apo- and tRNA-bound forms (Fig. 1C-E), which completely render snapshots of substrate selections during the modification of RNA. A compact evolutionary inserted domain (swinging domain) in MiaA that exhibits as a highly mobile entity moves around the catalytic domain as likely to reach and trap the tRNA substrate (Fig. 1C). Thereby, MiaA clamps the anticodon stem loop of the tRNA substrate between the catalytic and swinging domains, where the two conserved elongated residues from the swinging domain push the two flanking bases out of the tRNA stem loop, thereby causing the flipping of A(37) into the reaction tunnel (Fig. 1D). The site-specific isopentenylation of RNA is thus ensured by a characteristic pinch-and-flip mechanism and by a reaction tunnel to confine the substrate selection (Fig. 1E).

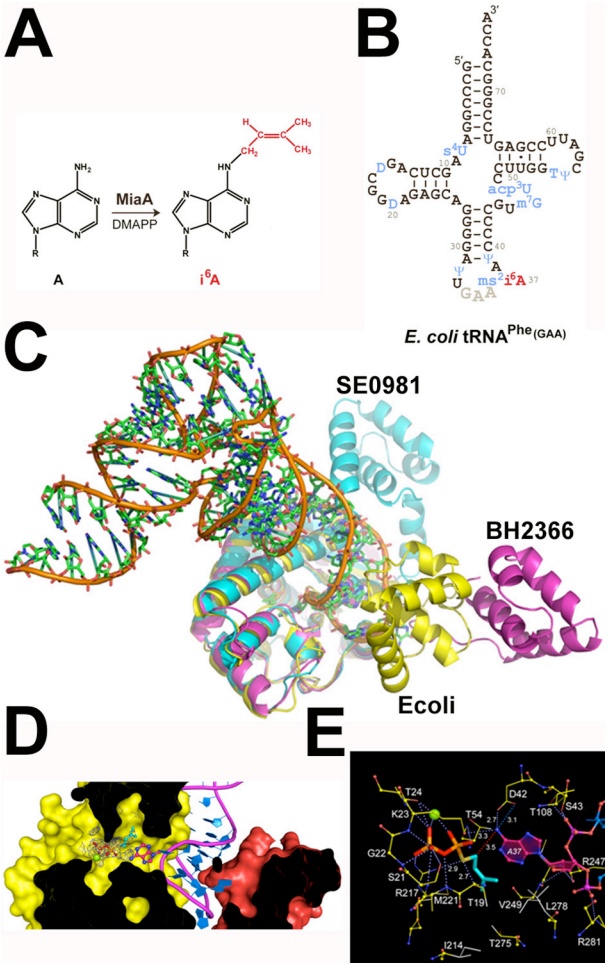

Fig. 1. **(A).** Reaction catalyzed by MiaA and the position of modification is highlighted in red on E. coli $tRNA^{Phe}$ (panel **B**). **(C).** Crystal structures of MiaA orthologs: E. coli (in yellow) in complex with tRNA, and apo Staphylococcus epidermidis SE0981 and Bacillus halodurans BH2366 (in cyan and magenta respectively). The overlaying of the catalytic domain of three MiaAs reveals that the swinging domain is highly mobile in the absence of tRNA. **(D).** The cross section of *E. coli* MiaA in complex with tRNA and IPP, showing that A37 (in red for carbon atoms) is flipped into the channel and positioned near IPP (in cyan). **(C).** The active site of MiaA depicting substrate IPP (in cyan for carbon atoms) and A37 (in magenta).

Chimnaronk S, Forouhar F, Sakai J, Yao M, Tron CM, Atta M, Fontecave M, Hunt JF, Tanaka I. Biochemistry 2009, 48:5057-5065. Snapshots of Dynamics in Synthesizing N^6-isopentenyladenosine at tRNA Anticodon.