STRUCTURE AND MECHANISM OF *E. coli* RNASE T

Yuhong Zuo and Murray P. Deutscher

Department of Biochemistry and Molecular Biology, University of Miami School of Medicine,
P.O. Box 016129, Miami, FL 33101

INTRODUCTION. RNase T is one of eight distinct 3' to 5' exoribonucleases identified in *Escherichia coli*[1]. It belongs to the DEDD exonuclease superfamily[2] characterized by common motifs consisting of four invariant acidic residues, which in DNA polymerases were shown to form the exonuclease active site[3]. RNase T plays an important role in stable RNA metabolism, including tRNA end turnover and 3' maturation of many stable RNAs[1]. RNase T proteins are small polypeptides of about 220 amino acids. They are closely related to the proof-reading domains/subunits of bacterial DNA polymerase III, and interestingly, *E. coli* RNase T also displays strong DNA exonuclease activity[4,5]. *E. coli* RNase T must form a homodimer in order to function[1].

METHODS. Site-directed mutageneses were PCR-based using synthetic oligonucleotides. RNase T activity against tRNA was measured by an acid-soluble assay, whereas RNase T processing of oligonucleotides was analyzed by denaturing polyacrylamide gel electrophoresis. The NCBI nonredundant protein and nucleic acid databases were searched by **BLAST** programs for RNase T orthologs. Multiple sequence alignment was generated by ClustalX with some editing based on BLAST searches and predicted secondary structures, which were predicted by **3D-PSSM** and **PredictProtein**. Homology modeling was carried out by **Modeller4** installed on PC Linux system.

RESULTS. To investigate the catalytic mechanism of RNase T and its requirement for dimerization, we carried out a systematic mutagenic study. Based on this study, the conserved acidic residues along with some other residues of the three DEDD exonuclease motifs were shown to be important for RNase T catalytic activity, consistent with a possible common catalytic mechanism of all DEDD members. In addition, we also identified in RNase T orthologs three other conserved sequence segments that contain a high level of positively-charged and aromatic residues. Structural modeling by threading the RNase T sequence through the *E. coli* exonuclease I crystal structure (PDB: **1FXX**) indicates that these three segments cluster together to form a putative nucleic acid-binding patch, which locates on the opposite side of the DEDD catalytic center in the RNase T monomer. Kinetic analyses of the corresponding mutants confirmed the involvement of this positively-charged/aromatic patch in nucleic acid-binding. By complementing two inactive RNase T mutants which lost either catalytic activity or nucleic-acid binding capability, RNase T activity could be partially reconstituted (Fig. 1). This result supports a structural model in which the catalytic center is derived from one subunit and the major substrate-

binding site comes from the other subunit, and it provides an explanation for the dimerization requirement of *E. coli* RNase T.

Fig.1 Reconstitution of RNase T activity from two inactive mutants

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