RNase AS versus RNase T: Similar yet Different

Xuhua Tang,1 Siew Choo Lim,1 and Haiwei Song1,*

1Institute of Molecular and Cell Biology, 61 Biopolis Drive, Proteos, Singapore 138673, Singapore
*Correspondence: haiwei@imcb.a-star.edu.sg
http://dx.doi.org/10.1016/j.str.2014.04.006

In this issue of Structure, Romano and colleagues show that RNase AS specifically hydrolyses adenylate-containing RNA and affects mycobacterial virulence. This study reveals the structural basis underlying the substrate specificity of this enzyme.

Tuberculosis (TB) is a disease caused by Mycobacterium tuberculosis. Infections of M. tuberculosis are often asymptomatic, chronic in a clinically symptomatic state, and highly recalcitrant to antibiotics. Because latent TB infection affects about a third of the world population, treatment of both chronic and latent forms of TB are essential in controlling and eliminating this infectious and often fatal disease. The cell-envelope of M. tuberculosis plays a key role in bacterial virulence and antibiotic resistance (Brennan and Nikaido, 1995); hence, targeting the cell-envelope biosynthesis is a promising strategy for TB therapy.

In this issue of Structure, Romano et al. (2014) identified RNase AS, an exonuclease important in mycobacterial virulence. The gene (rv2179c) encoding RNase AS was identified through transposon mutagenesis in the orthologs of M. marinum and M. smegmatis. Mutants lacking this gene display different mycobacterial cell surface properties compared to the wild-type in a double filter assay that measures the amount of capsular α-glucan. Through knockout and rescue experiments in zebra fish embryos, the authors showed that this gene is important for mycobacterial virulence in vivo.

RNase AS belongs to the DEDDh subfamily of the DEDD 3′-to-5′ exonuclease superfamily. This large family includes many essential prokaryotic and eukaryotic exonucleases, and all bear four conserved metal-binding acidic residues and one general base residue in the active site and share a common two-metal ion mechanism of hydrolysis (Zuo and Deutscher, 2001). Through oligonucleotides degradation experiments, Romano et al. (2014) confirmed that RNase AS is a 3′ to 5′ exoribonuclease with strong specificity for adenylate-containing RNA.

Structurally, RNase AS resembles RNase T, another DEDDh exonuclease from E. coli, despite their low sequence identity. Although both RNase AS and RNase T function as a dimer and share a similar dimeric architecture, they have distinct substrate recognition mechanisms (Figure 1). RNase AS exhibits a strong substrate preference of short adenylate-containing RNA over DNA, while RNase T is a more efficient DNase than RNase, which specifically trims the 3′ end of structured DNA (Hsiao et al., 2011; Hsiao et al., 2014). Moreover, RNase T has an unusual substrate specificity in that its exonucleotic activity is blocked by 3′-terminal C residues and double-stranded structures (Hsiao et al., 2011). Structural comparison between RNase AS and RNase T reveals two significant differences in the structures. First, a long α-helix in RNase T is broken into two helices in RNase AS by the insertion of Pro105 to contribute the formation of the cleft hosting AMP(+1) adenine base (Romano et al., 2014). Second, RNase AS and RNase T have different electrostatic potential surfaces (Romano et al., 2014), although they both adopt an opposing dimeric arrangement (Zuo et al., 2007). RNase T has a characteristic positively charged patch (NSB patch) from one monomer juxtaposed with the catalytic DEDD residues from the other monomer, which is involved in the binding of long nucleotides (Hsiao et al., 2011). In contrast, RNase AS has a positively charged patch located just below the catalytic cleft from the same monomer (Romano et al., 2014). These structural differences likely account for their distinct substrate preferences.

Based on the crystal structures of RNase AS with bound AMP or UMP, Romano et al. (2014) proposed that the specificity for adenine moiety at the nucleotide (+1) position is determined by a single hydrogen bond between the NH2 group of the adenine base and the carbonyl oxygen of Met106. To confirm this, the authors experimentally proved that RNase AS is unable to degrade polyinosine, a polynucleotide in which the adenine NH2 group is replaced by carbonyl oxygen, because the latter is unable to act as a donor in a hydrogen bond interaction. This novel and simple substrate selection mechanism is drastically different from the one proposed for RNase T. Structural analyses of RNase T-DNA complexes show that RNase T dimer has an ideal architecture to specifically trim the 3′ end of structured DNAs through an elegant mechanism to screen out 3′-terminal cytosines for cleavage by inducing an inactive
Membrane Interaction and Functional Plasticity of Inositol Polyphosphate 5-Phosphatases

Werner Braun1,2,* and Catherine H. Schein3

1Sealy Center for Structural Biology and Molecular Biophysics, University of Texas Medical Branch, Galveston, TX 77555, USA
2Department of Biochemistry and Molecular Biology, University of Texas Medical Branch, Galveston, TX 77555, USA
3Foundation for Applied Molecular Evolution, 720 SW 2nd Avenue Suite 201, Gainesville, FL 32601, USA
*Correspondence: webraun@utmb.edu
http://dx.doi.org/10.1016/j.str.2014.04.008

In this issue of Structure, Tré saugues and colleagues determined the interaction of membrane-bound phosphoinositides with three clinically significant human inositol polyphosphate 5-phosphatases (I5Ps). A comparison to the structures determined with soluble substrates revealed differences in the binding mode and suggested how the I5Ps and apurinic endonuclease (APE1) activities evolved from the same metal-binding active center.

A complex family of membrane-bound inositol-lipid conjugates plays a central role in cell proliferation, synaptic vesicle recycling, and actin polymerization. Control of these pathways depends on a family of enzymes that control the phosphorylation of insoluble phosphoinositides (PtdIns), i.e., glycerolphospholipids bound to the 1-hydroxyl of myo-inositol. Specific kinases add phosphate groups at the 3, 4, and 5 hydroxyls of the inositol ring, leading to seven different isoforms. These, in turn, are removed by a family of phosphatases. In this issue of Structure, Tré saugues et al. (2014) show the binding site for three human phosphatases that remove the 5-phosphate from PtdIns with two or three phosphates bound to PtdIns. Mutations in the similar 5-phosphatase active site of these enzymes are related to Lowes syndrome and Dent disease (5-phosphatase OCRL), characterized by renal failure, as well as defects in insulin signaling and obesity (5-phosphatase SHIP2). They also determined the structure of INPP5B, whose similarity in substrate specificity to OCRL suggests it may have overlapping function. The details of these new structures could help in the design of treatments for renal syndromes. Inhibitors of SHIP2 might even be novel weight-loss medications, because mice deficient in SHIP2 were resistant to diet-induced obesity.

These structures are most remarkable for the details they can shed on how insoluble inositides bind, because the only structure of a 5-phosphatase catalytic domain previously reported is that of a complex of Schizosaccharomyces pombe synaptojanin (SPsynaptojanin) with a soluble ligand (Tsujishita et al., 2001). They are also important for the details they provide on a unifying mechanism for dephosphorylation of PtdIns with other types of phospho-transfer reactions.

The inositol position is different in the new structures from models of the