that indicates that sMepS is bound to only sNlpI, not to Prc (**Fig. 2(c)**). An analysis with anisothermal titration calorimeter (ITC) demonstrated that only sNlpI is involved in sMepS binding (**Fig. 2(d)**). A structural comparison with CtpB (a PDZ-containing protease) indicates that the two conserved hinge residues – L245 and L340 – in Prc might participate in sensing the PDZ ligand. To validate this hypothesis, two single mutants (Prc-L245A and Prc-L340A/G) and one double mutant (Prc-L340G/L245A) were generated, followed by sMepS degradation assays. These results show clearly that all mutants have an impaired activity to degrade sMepS, especially the double mutant that was almost completely inactive (**Fig. 2(e)**).

In summary, these findings not only elucidate the vital role of two lipoproteins – NlpI and MepS – in regulating a cell-wall enzyme (Prc) but also provide a new strategy to design specific antibacterial agents to inhibit the proteolytic activity of Prc. (Reported by Chun-Hsiang Huang)

*This report features the work of Chung-I Chang and his co-workers published in Nat. Commun.* ***8****, 1516 (2017).*

**TLS 15A1 Biopharmaceuticals Protein Crystallography**

- MR, SAD, MAD, SIR, MIR, SIRAS, MIRAS
- Protein Crystallography

**References**


1. S. K. Singh, S. Parveen, L. SaiSree, and M. Reddy, Proc. Natl. Acad. Sci. USA **112**, 10956 (2015).
2. M. Ohara, H. C. Wu, K. Sankaran, and P. D. Rick, J. Bacteriol. **181**, 4318 (1999).
3. M. Y. Su, N. Som, C. Y. Wu, S. C. Su, Y. T. Kuo, L. C. Ke, M. R. Ho, S. R. Tzeng, C. H. Teng, D. Mengin-Lecreulx, M. Reddy, and C. I. Chang, Nat. Commun. **8**, 1516 (2017).


# RNase R: A Proficient Enzyme Involved in the Decay of RNA

*According to the structural and biochemical basis of RNase R, this proficient enzyme is capable of binding, unwinding and degrading structured RNA simultaneously during RNA decay.*

Belonging to the RNase II family of ribonucleases, RNase R is involved in the decay of RNA in all kingdoms of life. Previous work indicated that this family possesses a conserved catalytic domain (referred to as the RNB domain) with 3′-to-5′ exoribonuclease activity to cleave RNA. Six RNase II family proteins, including RNase R and RNase II (from *Escherichia coli*), Rrp44 and DSS1 (from yeast), and Dis3L and Dis3L1 (which are RNase R homologues from human beings), have been characterized to participate in the degradation of RNA. Some human diseases, such as multiple myeloma and Perlman syndrome, result from malfunctions of Dis3L and Dis3L1, indicating that ribonucleases in the RNase II family play vital roles in RNA metabolism.[1]

The roles of RNase R and RNase II in RNA decay have been well studied in *E. coli*. RNase II cleaves only linear RNA, but RNase R is capable of degrading structured RNA with repetitive sequences. RNase R can degrade duplex RNA with 3′-overhang independently, revealing that RNase R is a bifunctional enzyme for RNA unwinding and degrading simultaneously.[2]

About the domain organization, in general, the RNase II family of ribonucleases consists of a RNB exoribonuclease domain, two cold-shock domains (CSD1 and CSD2) and a S1 domain. RNase R has two extra domains – a helix-turn-helix (HTH) domain and a K/R-rich domain, at the N- and C-terminal regions, respectively. According to previous reports on RNase R, the RNB domain is responsible for RNA unwinding and degradation; the remaining auxiliary domains are associated with RNA binding. Two crystal structures of Rrp44 and Dis3l2 in a complex with its single-stranded RNA (referred to as *ssRNA*) have been solved. The crystal structure of ssRNA-bound Rrp44 shows that the ssRNA is located between the CSD1 and RNB domains (referred to as the side channel) for further RNA degradation; a distinct binding mode for RNA decay can be observed in that of Dis3l2, the ssRNA is bound between the CSD1 and S1 domains (referred to as the top channel). It is still elusive how duplex RNA with a 3′-overhang is bound and becomes un-

wound by RNase R. To elucidate how RNase R degrades duplex RNA, a research team led by Hanna S. Yuan (Institute of Molecular Biology, Academia Sinica) solved the crystal structure of RNase R in a truncated form (RNase R DHTH-K -- only this construct can yield crystals) using a molecular replacement method with a 1.85 -Å data set at beamline **TLS 13C1** of NSRRC.[3]

Based on the overall structure, RNase R DHTH-K has a unique feature in that it possesses two open channels – a top channel between the S1 and CSD1 domains and a side channel between RNB and CSD1 domains (**Fig. 1(a)**); this structure differs from other reported crystal structures, such as Rrp44-ssRNA and Dis3l2-ssRNA. Comparison with related structures, including Dis3l2, Rrp44 and RNase II, indicates that the tri-helix region in the RNB domain (shown in pink in **Fig. 1(b)**) might be involved in RNA unwinding. To test this hypothesis, the authors constructed two wedge mutants – a tri-helix mutant (RNase R D3H) and a single-helix replacement mutant (RNase R 1H); subsequent analysis of circular dichroism and thermal melting assays demonstrated that the two wedge mutants have overall protein folding similar to that of the wild type for further RNase activity assays. **Figures 1(c) and 1(d)** show clearly that the two wedge mutants can still degrade the ssRNA without a secondary structure, but they could not unwind completely and degrade the structured RNA because of a partial loss of their RNA-unwinding activity. Regarding the RNA binding activity, this group performed binding-affinity assays to the full-length RNase R, RNase R DHTH-K, RNase R D3H and RNase R 1H. Comparison of their dissociation parameters ($K_d$) indicated that the two wedge mutants do not significantly affect the RNA-binding ability of RNase R.

On combining the structural information and bioassay data, the authors provided two possible models to elucidate the mechanism of RNA unwinding and degradation. On comparison of the two models, two similarities were observed: the unwinding process of the duplex region of RNA occurs at the tri-helix wedge region of the RNB domain; the degrading of unwound the 3′-overhang of RNA is conducted at the active site. Which channel is for the duplex region of RNA binding and which channel is for the 5′ non-scissile stand exiting are, however, still unclear (**Figs. 2(a) and 2(b)**). To answer this question, the co-crystallization of RNase R with structured RNA continues. (Reported by Chun-Hsiang Huang)

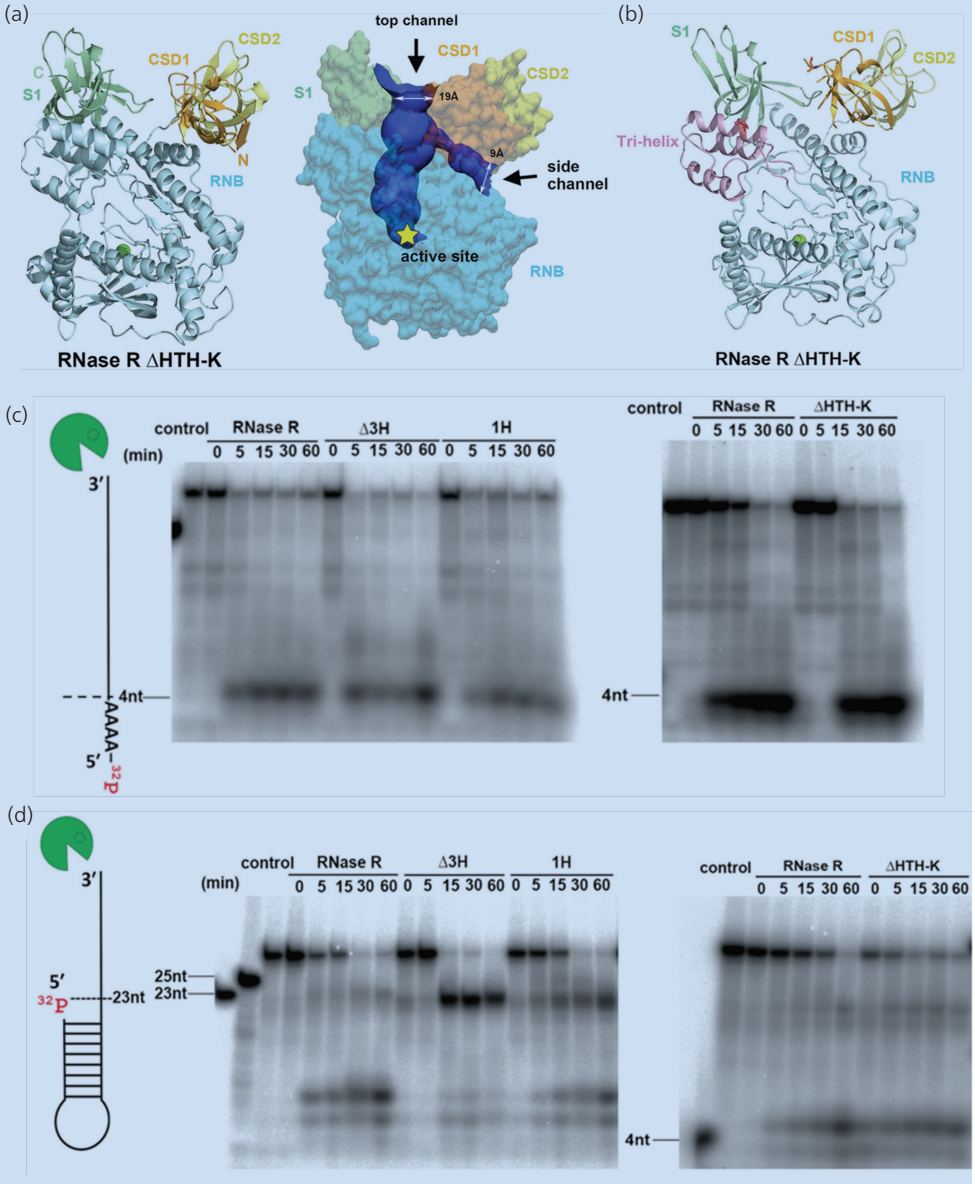
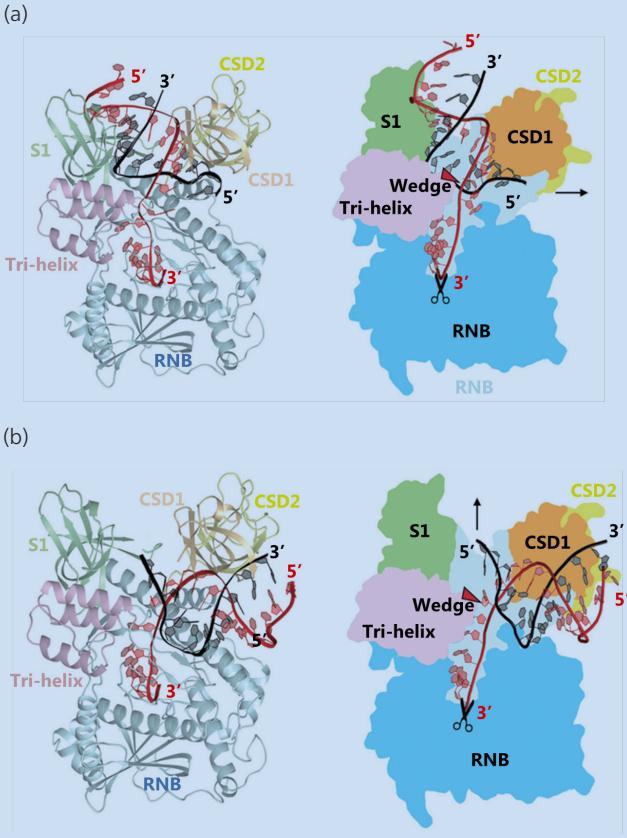



**Fig. 1**: (a) The crystal structure of RNase RΔHTH-K shows two open channels and a $M^{g2+}$-bound active site. RNB domain (sky blue), S1 domain (green), CSD1 domain (orange yellow), CSD2 domain (yellow) and $Mg^{2+}$ (green sphere). (b) A tri-helix wedge region in the RNB domain (pink). (c) & (d) RNase activity assay. [Reproduced from Ref. 3]




*This report features the work of Hanna S. Yuan and co-workers published in Nucleic Acid Res.* ***45****, 12015 (2017).*

### TLS 13C1 SW60 – Protein Crystallography

- MR, SIR, MIR
- Protein Crystallography

### References

1. R. Tomecki, K. Drazkowska, I. Kucinski, K. Stodus, R. J. Szczesny, J. Gruchota, E. P. Owczarek, K. Kalisiak, and A. Dziembowski, Nucleic Acid Res. **42**, 1270 (2014).
2. N. Awano, V. Rajagopal, M. Arbing, S. Patel, J. Hunt, M. Inouye, and S. Phadtare, J. Bacteriol. **192**, 1344 (2010).
3. L. Y. Chu, T. J. Hsieh, B. Golzarroshan, Y. P. Chen, S. Agrawal, and H. S. Yuan, Nucleic Acid Res. **45**, 12015 (2017).

**Fig. 2**: (a) & (b) Two possible working models for RNA binding, unwinding and degradation for RNase R. [Reproduced from Ref. 3]

# Preserved Collagen in an Early Jurassic Sauropodomorph Dinosaur

*Protein preservation in a terrestrial vertebrate is revealed inside the Haversian canals of a rib of a 195-million-year-old Lufengosaurus. This study was selected as one of the Discover's 100 top stories of 2017.*

The opportunity to reveal a genomic connection between extinct ancient animals and extant animals is strongly dependent on the DNA species in the fossil; fossilized organic remains are therefore crucial sources of possible genomic information to relate biological and evolutionary information.[1] The half-life of DNA after an animal death is predicted to be ~521 years, based on the statistics of bone fossil from moa; it is quite rare to extract the DNA molecules from a multimillion-year-old fossil. Yao-Chang Lee (NSRRC) and Robert Reisz (University of Toronto) together with their co-workers reported SR-FTIR spectral evidence of protein preservation in a terrestrial vertebrate found inside the Haversian canals of a rib of a 195-million-year-old Lufengosaurus, in which the blood vessels and nerves would normally have been present in a living organism.[2] The FTIR spectra acquired on utilizing synchrotron radiation-based Fourier-transform infrared (SR-FTIR) measurements *in situ* revealed the characteristic IR absorption bands of amides A and B, amides I, II and III of collagen. Using a confocal Raman microscope, aggregated hematite particles ($\alpha$-$Fe_2O_3$) of diameter about 6–8 mm were also identified inside the Haversian canals, in which the collagen and protein remains were preserved. These authors proposed that iron(II) ions likely had an antioxidant role in the preservation of the proteins before the formation of the micrometre-sized hematite particle, and might be remnants partially contributed from hemoglobin and other iron-rich proteins from the original blood.

Rib fossils of an adult Lufengosaurus were collected and studied (specimens housed in the ChuXiong Prefectural Museum, catalogue CXPM Z4644). Rare or no evidence of soft tissue preservation exists for