To Cut or Not to Cut?

This report features the work of Hanna S.Yuan and her co-workers published in Nucl. Acids Res. ***40****, 4146 (2012).*

The traditional view of RNA is that it serves as an intermediate between genes in DNA and the biological machinery in protein. DNA is first transcribed into RNA in a complementary form in nucleus. After being transported into cytoplasm, these copied blueprints are used by ribosomes to make proteins, which comprise the biological machines in cells and living organisms. These RNA are called coding RNA because they encode information to make proteins. The kinds and amount of genes transcribed into RNA determine the composition of the proteins in cells, thus, regulate the functions and the destiny of cells. The used RNA are degraded, which makes RNA a brief life in a cell. Other than coding RNA, there are many noncoding RNA in cells. They play important roles as regulators and catalysts. The Nobel Prize in Chemistry, 1989, was awarded to scientists who discovered these roles of RNA. Many human diseases directly involve RNA.[1] The regulation of RNA biogenesis is thus an important topic in understanding how life functions.

The synthesis and degradation of RNA determines its half live. Many proteins are involved in RNA processing and degradation, including ribonuclease (RNase), polynucleotide phosphorylase (PNPase) and exosome.[2] PNPase is a highly conserved enzyme which digests RNA from 3' to 5' end in most of the prokaryotes and eukaryotes. PNPase and exosome share the same core of six RNase PH domains and they also contain similar KH and S1 domains for RNA binding. Therefore, PNPase and exosome share similar domain architecture and are the main biological machines for RNA degradation.

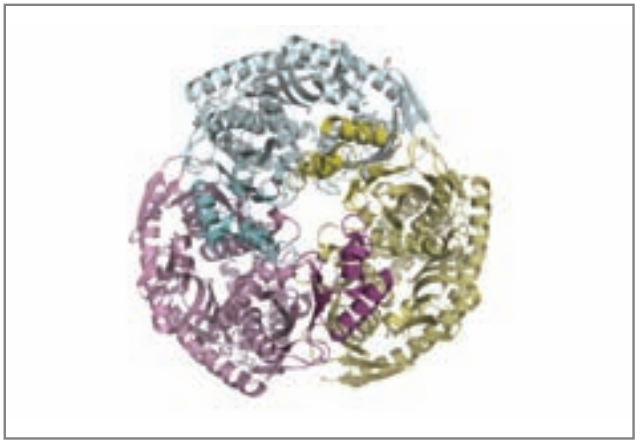

Fig. 1: Top view of human PNPase showing trimer of PNPase. PH domains are in grey colors (grey yellow, grey cyan and grey pink) and KH domains are in bright color (yellow, cyna and pink). Six PH domains form the core of phosphorylase and three KH domains form the pore on top of it.

Interestingly, human PNPase is located in mitochondria, not only playing a role in RNA degradation but also responsible for transporting RNA from cytoplasm into mitochondria. Recently, it is shown that mutations in human PNPase are directly linked to mitochondrial diseases, making it important to elucidate the structural basis for its role in RNA degradation and transport.

It is intriguing that PNPase on the one hand digests RNA, but on the other hand, it transports RNA. What is unclear is how PNPase carries RNA into mitochondria but does not digest them. To answer this question, Dr. H. Yuan and her co-workers in Academia Sinica have solved the structure of human PNPase without a S1 domain (ΔS1 hPNPase) at resolution 2.1 Å.[3] This work used beamline **BL13C** in NSRRC (Fig. 1).

Unlike previously solved structures of bacterial PNPase lacking the S1 and KH domains, human PNPase structure contains a KH domain. It forms a doughnut shape as a trimer of PNPase, in which each PNPase contributes two RNase PH domains to form a hexamer ring similar to that of exosome. The KH domain of each PNPase forms a pore of trimer on top of this PH domain ring, whereas exosome uses the S1 domain to form such a pore. Both KH and S1 domains bind RNA, indicating that this pore recruits RNA into the catalytic PH domain for degradation. Dr. Yuan's research team found that the GXXG motif on the KH domain is essential for RNA binding. On comparing the KH pore of PNPase and the S1 pore in exosome, they found also that the more constricted is the pore, the more efficient is the RNA binding and cleavage. The KH pore of human PNPase is so narrow that a conformational change must occur to accommodate the entrance of the single-stranded RNA into the catalytic chamber of PNPase.

Based on their structure, the distance from the opening of the KH pore to the catalytic site in the PH domain is about 6 nm, enough for accommodating 8 nucleotides. The research team found that human PNPase can efficiently cleave RNA having 3' overhanging longer than 12 nucleotides. The RNA transported by PNPase all have 3' overhang less than that. This result explains why these carried RNA can tag along with PNPase into mitochondria and chloroplast without being digested. This smart protein thus knows to cut or not to cut RNA when it encounters various RNA in cells (Fig. 2).

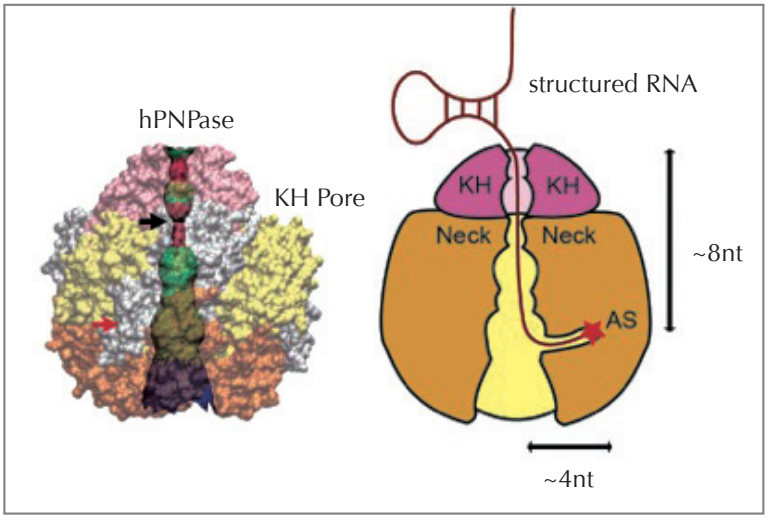

Fig. 2: Cut-open view of human PNPase (left) and model of RNA binding and digestion (right). KH pore form the narrow entrance for RNA (black arrow, left) and active site locates inside the core (red arrow, left). RNA need to be longer than 12 nuleotides to reach the active site (AS, right). Shorter RNA will bind with human PNPase and be transported into mitochondria without being digested. (courtesy of Dr. Hanna S. Yuan)

References

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2. S. Lin-Chao, N.-T. Chiou, and G. Schuster, J. Biomed. Sci. **14**, 523 (2007).
3. C. Lin, Y.-T. Wang, W.-Z. Yang, Y.-Y. Hsiao, and H. Yuan, Nucl. Acids Res. **40**, 4146 (2012).