Visualization of distinct substrate-recruitment pathways in the yeast exosome by EM

With the support by the National Natural Science Foundation of China and the National Basic Research Program of China, Prof. Wang Hongwei's laboratory at the School of Life Sciences, Tsinghua University, published their research findings in an article "Visualization of distinct substrate-recruitment pathways in the yeast exosome by EM" in *Nature Structural & Molecular Biology* (2014, 21: 95—102).

The eukaryotic exosome is a multi-subunit protein complex crucial for RNA maturation, surveillance and turnover. In tune with a similar function of the archaeal exosome, RNA substrates with long 3' single-stranded (ss) overhangs are first channeled through the eukaryotic exosome core before being degraded by exonuclease Rrp44. In this paper, a series of three-dimensional (3D) reconstructions of exosome bound with different ssRNA oligonucleotides illustrate that Rrp44's conformational change is induced by the 3' ss portion of the RNA substrate (Figure A). Biochemical evidence suggests the existence of an additional substrate recruitment pathway, where the RNA avoids the exosome core and reaches Rrp44's exonuclease site directly. Using single particle electron microscopy (EM) analysis, the two distinct RNA recruitment pathways are both observed and reconstructed (Figure B). In the through-exosome route, channeling of the single stranded substrates from the core to Rrp44 induces a characteristic conformational change in Rrp44. In the alternative direct-access route, this conformational change does not take place and the RNA substrate is visualized entering Rrp44's exonuclease site directly. Cryo-EM data also reveal tRNA's interaction with the exosome at the predicted direct-access route (Figure C). The results provide mechanistic explanations for several RNA processing scenarios by the eukaryotic exosome and indicate substrate specific modes of degradation by this complex.

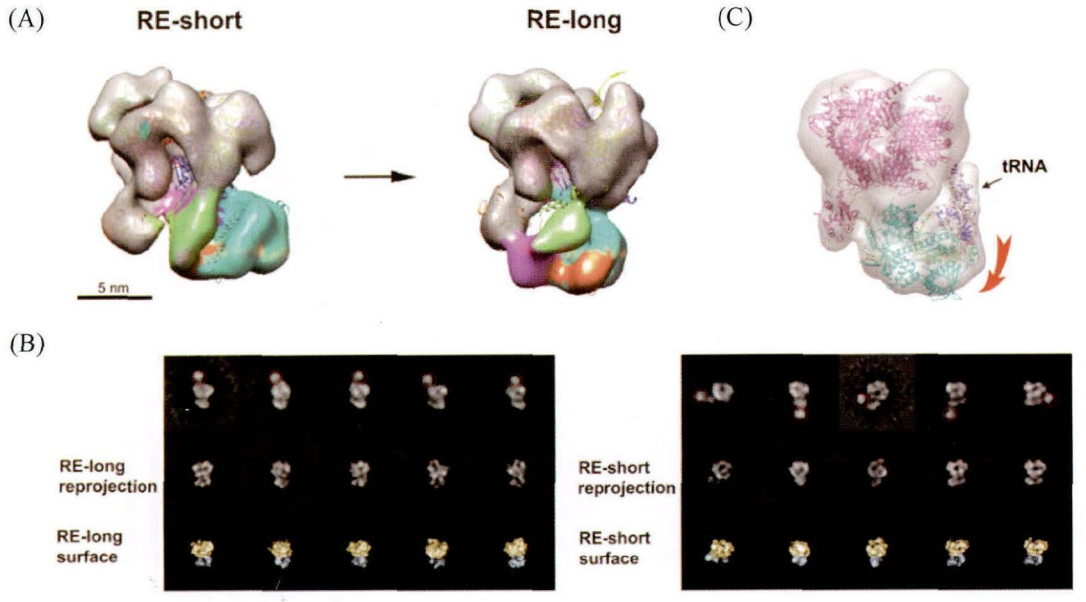

Figure (A) Comparison and analysis of the exosome binding with short ssRNA (RE-short) and exosome binding with long ssRNA (RE-long) 3D models. (B) Representative 2D class averages from samples of exosome incubated with streptavidin-coupled ssRNA. (C) 3D reconstruction of exosome with tRNA.