Discovery of a cis-acting ribozyme conducting new type of reaction by *in vitro* selection

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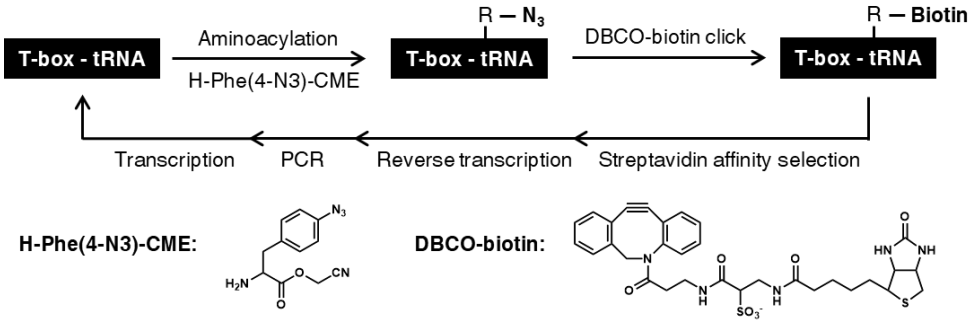
RNA world hypothesis[1] relies on various functional catalytic RNA molecules (ribozymes) which are essential for RNA-based life, and the transition to an RNA-peptide cooperative world[2]. To bridge this transition, ribozymes that can catalyze the conjugation between RNA molecules and amino acids are assumed to be critical.

Previously, the aminoacylation ribozyme based on T-box riboswitch scaffold has been reported to work with Biotin-Phenylalanine-cyanomethyl ester (Biotin-Phe-CME) as its only substrate[3]. To further functionalize it to be capable with N-terminal free substrates, H-Phe(4-N3)-CME was used for *in vitro* selection. After aminoacylation, ideal species would have an azide group, which can be then clicked with Dibenzocyclooctyne (DBCO)-biotin for the following streptavidin-affinity selection.

As a result of the selection, several families of ribozymes were identified. Evaluating the clones of 24 top sequences, we found all of them reacting with H-Phe(4-N3)-CME without T-box riboswitch scaffold. Primer extension assays indicated that modification site can be adenosine or guanosine in different families. In substrate scanning, both the primary amine group and CME leaving group in substrate were important for ribozymes' activity. We also found that RNA product conjugated with H-Phe(4-N3)-CME did not react with biotin-sulfo-NHS, which suggested that reaction product did not have primary amine group.

These results suggested that the primary amine group in the substrate may undergo some reactions. Since RNA amination in current living organisms is limited to cytidine[4] to our knowledge, our results indicated these ribozymes conducting a new type of reaction. We are working on the mass spectrum to identify the product and to decipher the reaction mechanism.



[1] *Nature* **319,** 618 (1986) [2] *Nat Rev Mol Cell Bio.* **12,** 135 (2011) [3] *Nat Chem Biol* **16**, 702-709 (2020) [4] *Nat Struct Mol Biol* **18,** 1268–1274 (2011)