

Simple RNA polymerase from a thermo-phage with possible pre-LUCA structural features

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The modern cellular RNA polymerases have huge structures to achieve precise regulations. Main parts of their structure are conserved in all domains of life, which indicates RNA polymerase of the last universal common ancestor, LUCA, was already a huge protein complex with more than 2000 amino acid residues. Thus, there should have been long evolutionary processes of RNA polymerase before LUCA. However, standard phylogenetic analysis cannot investigate evolutionary processes of a specific protein before LUCA because there are no sequences to compare. To understand such ancient processes, we have been trying to reconstruct pre-LUCA evolutionary intermediates by structural and synthetic biology methods.

For example, our group reconstructed the ancient beta-barrel conserved at the core of RNA polymerase by homodimerization of a 43 a.a. peptide with only seven amino acid types, suggesting the core fold of RNA polymerase could have been synthesized by a primitive translation system [1]. However, we could not successfully install an RNA polymerase activity to the reconstructed beta-barrel. Thus, we have been searching for model proteins for pre-LUCA RNA polymerase with catalytic activities.

Recently, we have solved the crystal structure of a simpler RNA polymerase newly identified from a thermophilic bacteriophage [2]. Structure based alignment with cellular RNA polymerases suggested it has pre-LUCA structural features. Interestingly, its core domain also has higher sequential and structural similarities to the previously reconstructed ancient beta-barrel mentioned above. Our current trials to further engineer evolutionary intermediates between them will also be discussed.

[1] Yagi S, et al. Seven Amino Acid Types Suffice to Create the Core Fold of RNA Polymerase. *Journal of the American Chemical Society* (2021) doi: 10.1021/jacs.1c05367

[2] Chaban A, et al. Tail-tape-fused virion and non-virion RNA polymerases of a thermophilic virus with an extremely long tail. *bioRxiv* (2022) doi: 10.1101/2022.12.01.518664