Constructing a ribosome library with random mutations in peptidyl transferase center and screening ribosome sequences to incorporate noncanonical amino acids

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The ribosome is a molecular machinery which can translate mRNAs into amino acid chains. However, wild-type ribosomes cannot efficiently incorporate amino acids with non-canonical backbone structures, such as D-, β-, γ-, and δ-amino acids. To efficiently incorporate D- and β-amino acids, mutant ribosomes have been rationally designed by introducing rRNA mutations[1-3]; however, they are not applicable to *in vitro* translation[4]. In this report, we aimed to evolve *in vitro* active ribosome that can incorporate a variety of non-canonical amino acids (ncAA), such as D-Ala and *cis*-4-aminocyclohexanecarboxylic acid (*cis*-4-ACHC) (Fig. 1, representatives of D- and δ-amino acids, respectively).

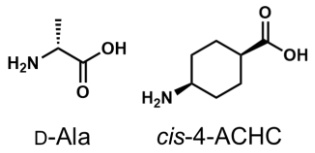

Fig. 1 Targeted ncAA

To this end, a ribosome library with random mutations was constructed and applied to screening of active ribosomes based on the efficiency of D-Ala or *cis*-4-ACHC incorporation. A plasmid encoding the wild-type rRNA gene was first prepared, and random mutations were introduced around the peptidyl transferase center of 23S rRNA by site-directed mutagenesis. The ribosome library was expressed from the plasmids in *E. coli*, purified, and subjected to translation of a model peptide containing an N-terminal biotin tag followed by D-Ala or *cis*-4-ACHC (Fig. 2). Only the ribosomes with high incorporation efficiency of D-Ala or *cis*-4-ACHC were able to synthesize the full-length peptide, thereby displaying the biotin tag, which were selectively captured by streptavidin beads. The rRNA of the selected ribosomes were reverse-transcribed, amplified by PCR, and analyzed by DNA sequencing. In this presentation, we do not discuss the actual mutant information.

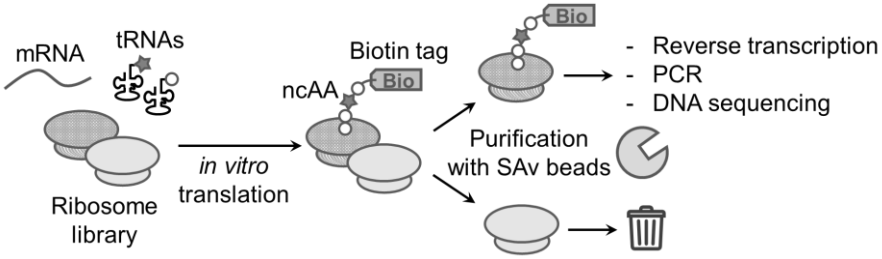

Fig. 2 Scheme of the ribosome selection

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