Modeling of a possible evolutional process from a ribozyme to a catalytic RNP

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ABSTRACT
A model process for molecular evolution from an RNA enzyme to a catalytic RNA-protein complex (RNP) is proposed. In the model, one RNA-RNA interaction in the enzyme is replaced by an RNA-protein interaction via an intermediary state where the original RNA-RNA and newly introduced RNA-protein interaction co-exist. To test the model, a catalytic RNP was designed and examined by employing the *Tetrahymena* ribozyme.

INTRODUCTION
According to the RNA world hypothesis, the initial 'world' consisting of only RNAs has been proposed to evolve into the 'RNA-protein world' via gradual replacements of RNA elements with protein components (fig. 1). The hypothesis is supported by the recent findings that certain RNA elements in a group I ribozyme (1,2) and the translation (3) can be replaced by protein components. In the transition from an RNA to an RNP (RNA-protein complexes), functional property and/or activity of the original RNA must be maintained in its intermediary form(s) because loss of the function must be disadvantageous or fatal for the cells possessing the functional RNA.

RESULTS AND DISCUSSION
An artificial RNP consisting of a modified *Tetrahymena* ribozyme and an artificial protein(2) was designed for testing the model for the evolution from an RNA enzyme to a catalytic RNP. The model consists of three stages that are the 'initial' ribozyme stage, the 'intermediary' stage and the 'final' RNP stage (fig. 2). In the process, the replacement of an RNA-RNA interaction with an RNA-protein interaction should proceed without sacrificing the activity of the ribozyme.

As the replaceable RNA-RNA interaction with an RNA-protein interaction (fig.2), P5b X P6a interaction in the P4-P6 domain was employed because the RNA-RNA interaction consisting of a GAAA loop in P5b region and its specific receptor (11ntR) in P6a region element has been shown to
play an important role for correct folding of the active 3D ribozyme structure. In the model for the ‘intermediary’ stage, P5b and P6 region were replaced with the peptide binding unit boxB and RRE, respectively. The box B is known to bind to both the peptide and the 11ntR so that the ‘intermediary’ molecule is expected to form the active structure by using the RNA-RNA and RNA-protein-RNA bridge interaction (fig. 2). The ‘final’ RNP molecule whose activity is solely dependent on a protein cofactor was also designed by disrupting the 11 nt receptor motif of the ‘intermediary’ molecule.

The activity of the designed molecules was investigated w/ or w/o the protein. The ‘intermediary’ molecules, that showed 2-fold less activity than the ‘initial’ ribozyme without protein, was activated dramatically by adding the protein cofactor. The ‘final’ RNA molecule that was virtually inactive by itself was as active as the ‘initial’ ribozyme in the presence of the protein. These results support the model evolitional process.

REFERENCES