Development of functional nucleic acids for analysis and control of gene expression in living cells

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Because RNA plays key role in biological system, RNA is most important target for biological research. Biologists study RNA using *in vitro* detection technique. However, *in vivo* detection method such will be required for further research. Hence we have developed new methods for detection or controlling of RNA expression in living cells.

Oligonucleotide-templated reactions are attracting attention as a method for RNA detection in living cells. Previously, a reduction-triggered fluorescence probe has been reported that is based on azide reduction to switch fluorescence on. Recently, we reported a more advanced probe, a reduction-triggered fluorescent amplification probe that is capable of amplifying a target signal. Azido-masked fluorescein on the probe showed a strong turn-on fluorescence signal upon oligonucleotide-templated Staudinger reduction. We successfully detected the 28S rRNA and beta-Actin mRNA signal. The data suggest that a reduction-triggered amplification probe may be a powerful tool in analyzing the localization, transcription, or processing of RNA species in living eukaryotic cells.

Dumbbell-shaped nanocircular RNA were designed and synthesized for RNA interference applications, which consist of a stem and two loops. RNA dumbbells are specifically recognized and cleaved by the human Dicer enzyme, and are thus transformed into double-stranded RNA in cells, although this RNA is resistant to degradation in serum. The structure was optimized to maximize its RNAi activity. The most potent activity was achieved when the stem length was 23 base pairs. The RNAi activity is prolonged by the shape of the molecule, an endless structure, compared with that of normal siRNA.