Development of DNA and RNA manipulation using ultrafast photo-cross-linking toward for nucleic acid editing

Abstract

Yasuha Watanabe
1920044
Fujimoto Laboratory

Introduction

The Human Genome Project was completed in 2003, allowing all diseases to be analyzed at the genetic level. Gene therapy has attracted attention as a method for treating genetic diseases such as cancer and familial Alzheimer's disease. The characteristic of gene therapy is expected to be applied to the ability to radically treat diseases at the genetic level.

Recently, the most actively studied gene therapy is the CRISPR system. Announced in 2012, CRISPR-Cas9[1] won the Nobel Prize as a tool for easily cleaving genomic DNA. The CRISPR system is improving day by day, allowing CRISPR-Cas13 to specifically cleave RNA. In the fields of genetic engineering and gene therapy, cutting tools are indispensable for utilizing nucleic acids. However, the CRISPR system has major problems such as off-target effect. I was interested in editing nucleic acids with simpler and easier operations, and decided to research new nucleic acid editing tools.

Results and Discussion

[Chapter 2]

We aimed to develop a method for manipulating DNAzyme activity with light spatiotemporally using ^{CNV}K [2]. A mask strand complementary to itself was extended from the 3' end of the DNAzyme, and ^{CNV}K was introduced into a part of the mask strand. The DNAzyme in which the mask strand and the substrate region were photo-cross-linking succeeded in completely inhibiting the invasion of the substrate strand by covalent bonding. It was also clarified that the covalent bond by photo-cross-linking can completely block the invasion of exonuclease, so that it also has resistance to enzymatic degradation. When ^{CNV}K was photolyzed by irradiating the photo-cross-linked DNAzyme with photo at 312 nm, the substrate chain was invaded to the catalyst core, and 38% RNA cleavage was confirmed in a reaction of 10 equal doses and 15 min. As described above, ^{CNV}K-introduced DNAzyme is expected to be applied to nucleic acid therapy because it is easy and

accurate to switch the activity and has enzyme resistance[3].

[Chapter 3]

We have reported thermal irreversible DDI by antigene probe with ^{CNV}K and self-photo-cross-linking inhibitor (5-cyanouracil, ^{CN}U) [4]. In this chapter, we newly examined inhibitors (Spacer, dSpacer) that can suppress self-photo-cross-linking more than ^{CN}U, and aimed to improve DDI efficiency. The self-photo-cross-link inhibition rates of Spacer (S) and dSpacer (dS) were obtained as 89.1% and 86.0%, respectively, which were (79.0%) higher than those of ^{CN}U. However, the DDI photo-cross-linking rates of Spacer and dSpacer were calculated to be 4.1% and 3.5%, respectively, which were lower than the photo-cross-linking rate (26.1%) of ^{CN}U. DDI photo-cross-linking is thought to consist of several equilibrium reactions, and the thermodynamic parameters of the probe were newly measured. The Tm values of each inhibitor were calculated as 37.3 ℃ for T, 33.5 ℃ for ^{CN}U, 24.9 ℃ for dS, and 25.2 ℃ for S. Comparing these results, it was found that DDI photo-cross-linking consists of a balance between self-photo-cross-linking between probes and stability with target DNA [5].

[Chapter 4]

DNA carries the genetic information of living organisms and has a double helix structure that maintains a very stable state. In addition, the longer the DNA, the more stable it becomes, and it may form complex secondary or tertiary structures. Antigene methods such as PNA and LNA are verified with a model sequence of about 200-400 mer for genomic DNA editing.

We prepared a sequence obtained by extending the model sequence used in Chapter 3 to 400 mer and verified DDI photo-cross-linking. An antigene probe with Cy3 (11bp-5nt) confirmed a band derived from the photo-cross-linker with the target sequence. When 11bp-5nt was used as the DDI probe, a maximum photocrosslinking rate of 78% with the target DNA was observed. The optimal reaction conditions were 1 min, 1 h, and 100 eq. for light irradiation time, incubation time, and equivalent dose, respectively.

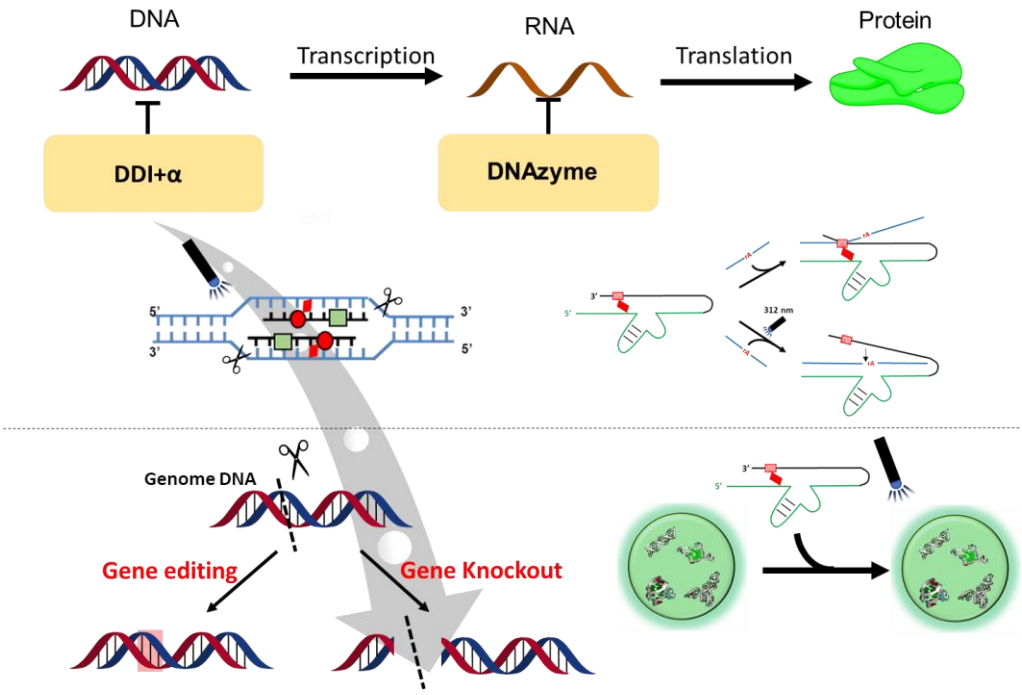

Figure. Overview of future work using ribozymes and DDI

Keywords

Genome editing, Photo-cross-linking, 3-cyanovinylcarbazole, DNAzyme, Double duplex invasion

References

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