Title	部位特異的RNA編集を用いたインビトロでの青色蛍光タンパク質（BFP）から緑色蛍光タンパク質（GFP）への変換に関する研究
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Study on conversion from blue fluorescent protein (BFP) to green fluorescent protein (GFP) *in vitro* by using site-directed chemical RNA editing

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1. Introduction

RNA editing is post-transcriptional process to change one or more nucleotides in the sequences of RNA. This process naturally makes the diversity of protein and various phenotypes [1, 2, 3]. Because the RNA editing is power to recode genetic information of RNA, there are many efforts to control or mimic RNA editing [4, 5]. In this study, we reported a strategy for site-directed non-enzymatic chemical RNA editing that allows application of C-to-U editing. Our method is simple not expensive and non-toxic, and was firstly reported by Prof. Fujimoto. In his protocol, template-directed DNA photoligation was mediated by artificial oligonucleotides, a short single strand 20-mer target was used and the C-to-U substitution was efficient and sequence-specific *in vitro* [6]. In our works, this method was studied and developed to apply toward genetic code restoration.

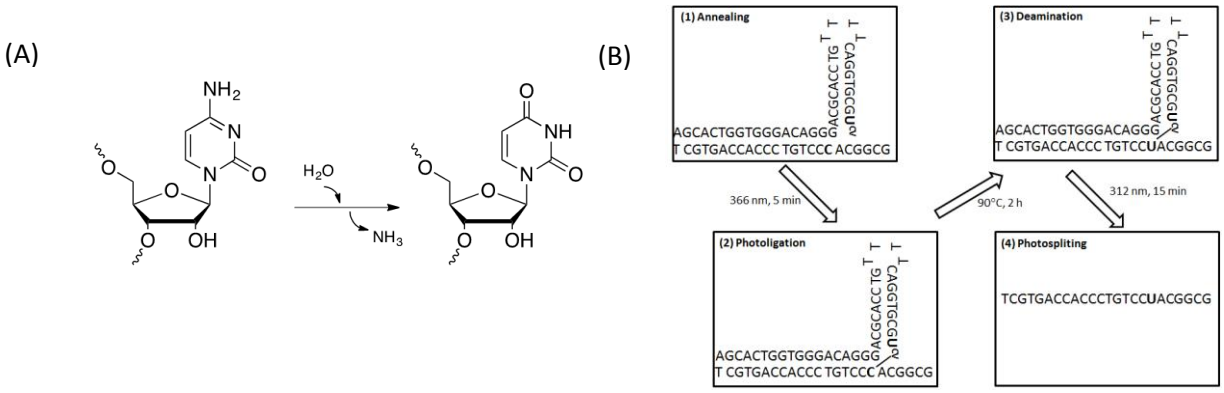

Figure 1: Schematic representation of artificial transition. (A) Deamination of cytidine to uridine. (B) The steps of artificial translation.

2. The application of site-directed chemical RNA editing for treatment of Leigh syndrome

First, a mitochondrial DNA T8993C mutation of Leigh syndrome patient was used as a model. We carried out converted C8993U by artificial site-directed chemical RNA editing.

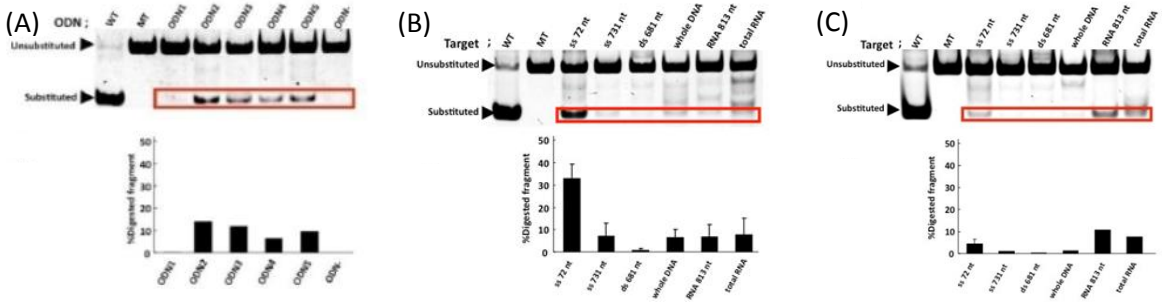

Figure 2: The efficiency of site-directed deamination. (A) 5 ^{CV}U-containing ODNs and a synthetic ss72-nt target at 90 °C for 2 h. (B) ODN2 and various targets at 90 °C for 2 h. (C)) ODN2 and various targets at 37 °C for 3 days.

We designed and synthesized 5 ^{CV}U-containing ODNs, and the experimental results revealed that ODN2 could convert C to U most effectively among examined 5 ^{CV}U-ODNs (fig.2A). We succeeded a sequence-specific photochemical base substitution toward ss72-nt, ss731-nt, RNA823-nt and total RNA from patient's cells used as targets at both un-physiological and physiological temperature (fig.2B and 2C). Importantly, we found that almost 10% of full-length RNA was successfully deaminated *in vitro* under physiological conditions (fig.2C).

3. Change from blue fluorescent protein to green fluorescent protein by chemical RNA editing as novel strategy in genetic restoration

Because of some disadvantages of Leigh syndrome cells *in vivo* study such as un-well living, slowing growth, unabsorbed exogenous ODN, in order to apply to further *in vivo* study blue fluorescent protein (BFP), a derivate of green fluorescent protein (GFP) was suggested as new model. BFP differs from GFP by a single nucleotide; a C-to-T change at position 199 transforms the BFP gene into the GFP gene.

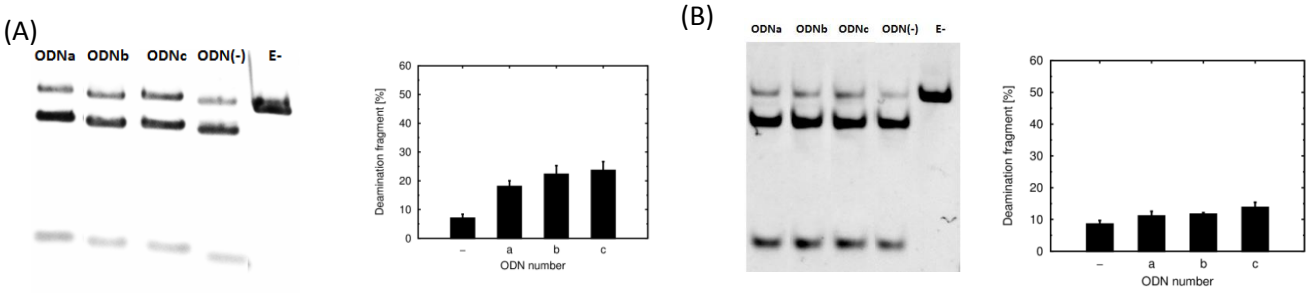

Figure 3: The efficiency of site-directed deamination. (A) 3 ^{CV}U-containing ODNs and a synthetic ss100-nt BFP target at 90 °C for 2 h and their densitometric results. (B) 3 ^{CV}U-containing ODNs and a synthetic ss100-nt BFP target at 37 °C for 3 days and their densitometric results.

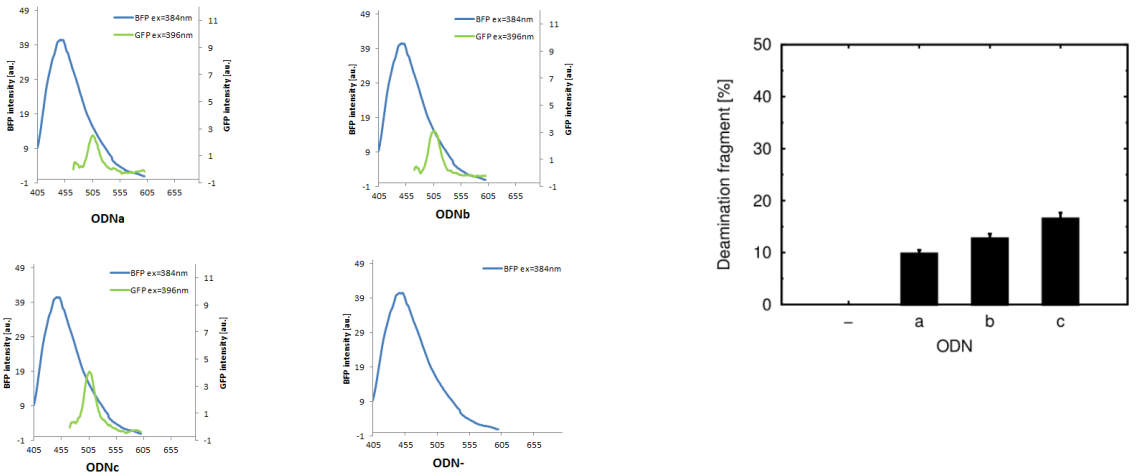

Figure 4: Efficiency of site-directed deamination between 3 ^{CV}U-containing ODNs and a synthetic full-length mRNA BFP target was obtained by measurement of BFP and GFP emission spectra after photochemical deamination at 60 °C for 4 h.

We successfully performed site-directed photochemical base substitution in synthetic ss100-nt and *in vitro*-synthesized full-length BFP mRNA targets. ODNc exhibited most effective C199U transition under both unphysiological and physiological temperature among the three tested ^{CV}U-containing ODNs (fig.3, fig.4 and fig.5). ODNc contains longer hairpin sequences than do ODNa and ODNb; this appears to work effectively

because long sequences increase the stability of ODNs. The C199U transition was more effective in the case of ODNb than in the case of ODNa because the comparatively longer complementary sequence of ODNb will bind more strongly to the target. The relationship between ODN sequences and deamination efficiency is crucial and need further study. We determined that approximate 10% of the full-length mRNA was deaminated in *in vitro* deamination under physiological temperature (fig.5)

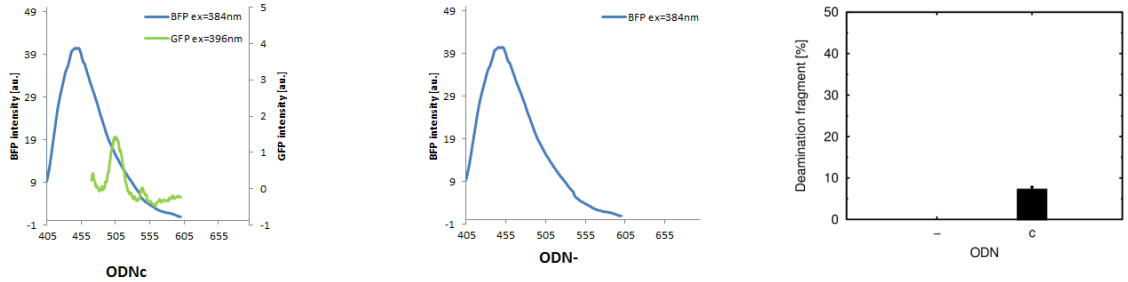

Figure 5: Efficiency of site-directed deamination between 3 ^{CV}U-containing ODNs and a synthetic full-length mRNA BFP target was obtained by measurement of BFP and GFP emission spectra after photochemical deamination at 37°C for 10 days.

4. The relationships between structures and deamination efficiency of carboxyvinyldeoxyuridine ODNs on chemical RNA editing

The structure and sequence of ^{CV}U-containing ODNs are key features of ODNs on its biological functions. To investigate the relationship between the ^{CV}U sequences and deamination efficiency, a series of oligodeoxynucleotides (ODNs) were designed and subjected to site-directed non-enzymatic editing.

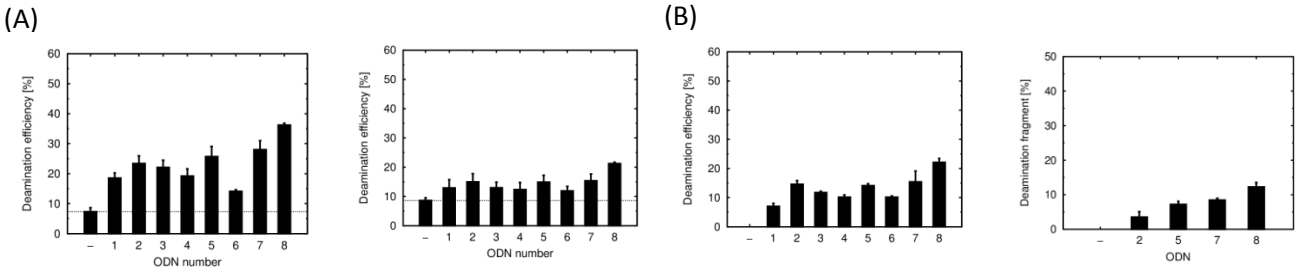

Figure 6: Efficiency of site-directed deamination between 8 ^{CV}U-containing ODNs and (A) synthetic ss100-nt BFP target or (B) a synthetic full-length mRNA BFP target was obtained by measurement densitometric measurements or measurement of BFP and GFP emission spectra after photochemical deamination at 90 °C for 2h and 37 °C for 10 days respectively.

From these experimental results, we showed that there are strong relationship between the deamination efficiency, and the sequence complementary length and hairpin loop length. The optimal deamination efficiency was achieved with ODNs having a sequence complementary length slightly more than 15nt and a hairpin length of 8nt. In order to confirm our conclusions on the optimum conditions for the ODNs, we designed, synthesized, and surveyed a new ODN with these conditions, i.e., an ODN has a sequence complementary length of 16nt and a hairpin length of 8nt (ODN8). The results were shown that ODN8 has best deamination efficiency comparing to other ODNs (fig.6).

5. Conclusion

We successfully performed site-directed photochemical base substitution to restore the mutated mRNA to a “healthy RNA” under physiological temperature by using photochemical base substitution. We believe that the site-directed photochemical deamination technology could serve as a new approach for genetic restoration.

Here, we designed and studied site-directed chemical deamination for genetic restoration *in vitro*. *In vivo* studies that include cultured cells and model animals will be conducted in the near future because of the requirement of relatively more complex technology.

References

1. Gray MW. *Biochemistry* **2012**, 51(26): 5235-5242
2. Decher N, Netter MF, Streit AK. *Chemical Biology & Drug Design* **2013**, 81(1): 13-21.
3. Simpson L, Emeson RB. *Annual Review of Neuroscience* **1996**, 19: 27-52.
4. Montiel-Gonzalez MF, Vallecillo-Viejo I, Yudowski GA, Rosenthal JJC. *Proc. Natl. Acad. Sci. U.S.A.* **2013**, *110*: 18285–18290.
5. Volgel P, Schneider MF, Wettengel J, Stafforst T. *Angew. Chem. Int. Ed. Engl.* **2014**, *53*: 6267–6271.
6. Fujimoto K, Konishi-Hiratsuka K, Sakamoto T, Yoshimura Y. *Chem. Commun.* **2010**, 46: 7545-7547.

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List of publications

1. Vu TL, Ooka Y, Alam S, Suzuki H, Fujimoto K, and Tsukahara T: Chemical RNA editing as a possibility novel therapy for genetic disorders. *IJAC* **2012**, 2(6): 237-241.
2. Suzuki H, Takeuchi M, Sugiyama A, Alam AHMK, Vu TL, Sekiyama Y, Dam HC, Ohki S, and Tsukahara T: Alternative splicing produces structural and functional changes in CUGBP2. *BMC Biochemistry* **2012**, 13:6.
3. Vu TL, Nguyen TKT, Alam S, Sakamoto T, Fujimoto K, Suzuki H and Tsukahara T: Change from Blue Fluorescent Protein to Green Fluorescent protein by Chemical RNA Editing as Novel Strategy in Genetic Restoration. *Chemical Biology & Drug Design*. doi: 10.1111/cbdd.12592
4. Vu TL, Nguyen TKT, Alam S, Md Thoufic AA, Sakamoto T, Fujimoto K, Suzuki H and Tsukahara T: The Relationship between Structures and Deamination Efficiency of Carboxyvinyldeoxyuridine ODNs on Chemical RNA Editing for Genetic Restoration. In preparation.

Keywords

Chemical RNA editing, deamination, photochemical reaction, carboxyvinyldeoxyuridine ODNs (^{CV}U-ODNs), C-to-U transition.