

## Intracellular mRNA imaging with a hybridization sensitive fluorescent nucleotide

Takeshi Kubota, Shuji Ikeda and Akimitsu Okamoto\*

Advanced Science Institute, RIKEN, 2-1 Hirosawa, Wako, Saitama 351-0198, Japan

### ABSTRACT

We reported a technique for detecting intracellular RNA with a newly developed functional probe. To detect mRNAs, a polyA tail targeting probe was synthesized. This probe showed quite large enhancement in the presence of its complementary RNA. In living cultured cells, the probe also showed strong fluorescence. This technique was effective for intracellular mRNA detection.

### INTRODUCTION

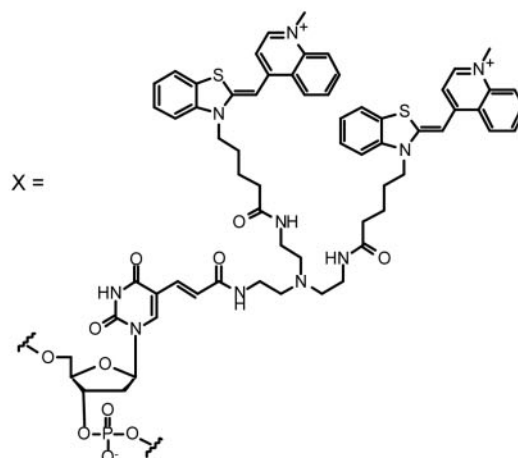
RNA is deeply concerned in the gene regulation such as differentiation and cancerization.<sup>1</sup> To understand when and where the RNAs work in a living cell is highly significant. However the first choice for detecting RNAs is RT-PCR, the result lacks temporal and spatial information. Therefore, microscope imaging is effective to obtain the spatial-temporal information about RNAs in living cells. Intracellular RNAs are detectable with fluorescent labeled complementary nucleotides (probes). The most popular hybridization probe has a quencher and a stem sequence.<sup>2</sup> However, this complicated structure makes the synthetic process troublesome and causes false signal.

To improve this, we have developed a novel hybridization dependent probe.<sup>3</sup> The structure of this probe is very simple and the probe is able to synthesize easily. The probe has a modified uridine in its sequence, and the base has closely located two fluorochromes. Because of the fluorescence suppressing effect, the probe shows little fluorescence at single strand state. The fluorescence liberates when the probe makes double strand with the complementary nucleotide by a conformational change of the fluorochromes.

In this study, we report RNA detecting technique using this newly functional probe in living cells.

### RESULTS AND DISCUSSION

A probe for recognizing a polyA tail of mRNA was designed and synthesized (ODN1). The sequence was 5'-d(TTTTTXTTTTT)-3', where X was an uridine derivative containing two fluorochromes (Fig. 1). The fluorescence of ODN1 at single strand state was quite weak, this means the fluorescent suppression worked strongly. A large enhancement of fluorescence was observed after mixing the probe with the complementary RNA oligomer,

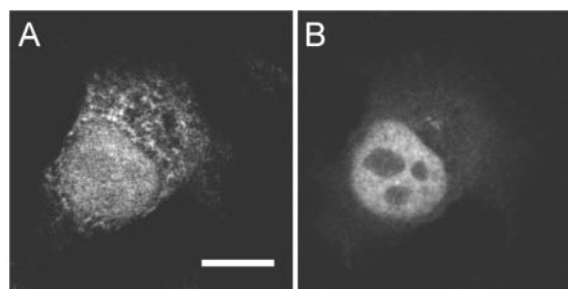


**Fig. 1** Structure of the modified uridine derivative. This nucleotide is abbreviated as X in the sequences.

5'-r(AAAAAAAAAAAAA)-3'. The fluorescent ratio at the peak wavelength between the two states reached more than 100. This significant change in fluorescence intensity became a powerful tool for detecting intracellular mRNAs.

An acute increase of the fluorescence of ODN1 was observed just after the addition of the complementary RNA. In another experiment, the fluorescence of ODN1 increased linearly according to the amount of the complementary RNA and saturated with the equivalent amount of the RNA.

Another probe, which had no target in cells, was also synthesized for a control experiment. The sequence was 5'-d(CGCAATXTAACGC)-3' (ODN2). The ODN2 showed also fluorescent increase in the presence of its



**Fig. 2** Images from the same cell. (A) Excited at 488 nm and detected with 505-550 band pass filter. (B) Excited at 633 nm and detected around 660 nm emission. Bar 10  $\mu$ m.

complementary RNA, 5'-r(GCGUAAAUGCG)-3'.

To living HeLa cells, ODN1 was microinjected with a glass capillary in appropriate concentrations. Bright yellow-green fluorescence was observed from ODN1 injected cells (Ex 488 nm, Em 505-550 nm). ODN2 injected cells showed little fluorescence in the same experimental condition. This result showed that this probe worked properly even in living cells.

To trace the probe itself, we also synthesized a Cy5 conjugated mRNA targeting probe: 5'-Cy5-d(TTTTTXTTTTT)-3' (ODN3). The emission of the probe for detecting mRNA was around 540 nm, and that derived from Cy5 was longer than 650 nm. Therefore, it is easily distinguishable each fluorescence. ODN3 injected cells showed a unique fluorescent image (Fig. 2). The distributions of the fluorescences were partly different in each detecting channel. Strong fluorescence of Cy5 was observed in nucleus, and weak in cytoplasm. Green fluorescence was observed in both nucleus and cytoplasm area. This result indicates that this probe can detect mRNAs with no influence of the distribution of the probe itself.

## CONCLUSION

We applied newly developed fluorescent probes for intracellular mRNA detection. The fluorescent enhancement was quite large and easily detectable. In living cells, we also detected mRNAs successfully.

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\*Corresponding Author. E-mail: [aki-okamoto@riken.jp](mailto:aki-okamoto@riken.jp)