Electronic Supporting Information (ESI) for

The first successful observation of in-cell NMR signals of DNA and RNA in living human cells

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Materials and Methods

Materials

An oligo DNA (5'-G*C*GAAGC-3', *: phosphorothioate) and its 5′-fluorescein-labeled form were synthesized, purified, and de-salted by FASMAC Co., Ltd. (Kanagawa, Japan). An oligo RNA (5'-GGCACUUCGGUGCC-3', fully 2'-OMe) and its 5′-fluorescein-labeled form were synthesized, purified, and de-salted by Hokkaido System Science Co., Ltd. (Hokkaido, Japan).

Introduction of nucleic acids into HeLa cells by streptolysin O (SLO) treatment

Introduction of DNA and RNA into HeLa cells by SLO was performed as described previously,[1] with some modifications. HeLa cells grown in DMEM medium containing 10% FBS under a 5% CO_2 atmosphere were harvested and then washed twice with PBS. SLO (Bioacademia) to form pores on plasma membranes was added to a final concentration of 0.06 μg/mL, followed by incubation at 4 °C for 10 min with gentle rotation. After washing three times with ice-cold PBS, the cells were incubated with transport buffer (25 mM HEPES-KOH (pH 7.4), 115 mM potassium acetate, and 2.5 mM $MgCl_2$) at 37 °C for 5 min with shaking. The cells were washed with transport buffer, and then incubated with transport buffer that included cytosol prepared from murine liver cells, as described previously,[2] an ATP regenerating system (1 mM ATP, 50 ng/mL creatine kinase, and 2.62 mg/mL creatine phosphate), 1 mg/mL glucose, 1 mM GTP, and either DNA or RNA at 37 °C for 30 min with shaking. The concentration of either DNA or RNA was 10 μM for flow cytometry and microscopy experiments, and 1 mM for in-cell NMR experiments, respectively. For resealing of the plasma membranes, $CaCl_2$ was added to a final concentration of 1 mM and the cells were incubated at 37 °C for a further 5 min with shaking. The cells were then washed three times with HBSS including $CaCl_2$. The resealed cells were layered onto HBSS buffer containing 14% percoll (precentrifuged at 2,000 x g for about 1 hour), and then centrifuged at 400 x g for 3 min. After the centrifugation, the supernatant (the dead cells) was removed and the cell pellet (living cells) was washed three times with HBSS buffer.

Flow cytometry (FCM) analysis

The resealed cells were resuspended in HBSS buffer containing 5 μg/mL propidium iodide (PI). The prepared cells were subjected to flow cytometry using an Attune NxT Flow Cytometer (Thermo Fisher Scientific).

Confocal microscopy

For fluorescent imaging, the resealed and centrifuged cells were resuspended in the culture medium including 8 μM Hoechst 33342, and then incubated for 20 min in a 35-mm poly-lysine-

coated glass bottom dish (IWAKI) at room temperature. All microscopy images were acquired with an Olympus FV1000 confocal scanning laser microscope equipped with a 60× UPlanSApo objective.

In vitro nuclear magnetic resonance (NMR) measurements

DNA and RNA were dialyzed against transport buffer (25 mM HEPES-KOH (pH 7.4), 115 mM potassium acetate, and 2.5 mM magnesium chloride) and then diluted the final concentration of 1.25 mM with the transport buffer (DNA and RNA stock solutions). Each stock solution was incubated at 95 °C for 5 min and then cooled to 18 °C in 30 min using a thermal cycler. Then each stock solution was diluted to 50 μM with transport buffer including 5% D_2O and 10 μM 4,4-dimethyl-4-silapentane-1-sulfonic acid (DSS). NMR spectra were recorded at 18 °C by the Selective Optimized-Flip-Angle Short-Transient (SOFAST)[3] technique with PC9 and rSNOB pulses for water suppression using Bruker BioSpin DRX 600 and AVANCE III HD 600 spectrometers equipped with a cryogenic probe and a Z-gradient.

In-cell NMR measurements

The resealed and centrifuged cells were resuspended in HBSS buffer including 5% D_2O and 10 μM DSS. The cells were transferred to a 4 mm Shigemi tube with an inner tube. NMR spectra were recorded for one hour at 18 °C by the SOFAST technique. After the measurement, the cell suspension was centrifuged, and the supernatant and cell pellet were separately collected. To assess the leakage of DNA and RNA from HeLa cells during recording, an NMR spectrum of the supernatant was measured.

References

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