Supplementary Material (ESI) for Photochemical & Photobiological Sciences

Stress-induced environmental changes in a single cell as revealed by fluorescence lifetime imaging

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Material and methods

Cell preparation

HeLa cells were grown in Dulbecco's modified Eagle medium supplemented with 10 % fetal bovine serum, 2×10^5 U/L penicillin G, and 200 mg/L streptomycin sulfate at 37°C in a humidified atmosphere containing 5 % CO_2 at 37°C. HeLa cells were transfected in LAB-TEK 8-well chambered coverslips (Nalge Nunc International) or on 35-mm coverslips (Iwaki) using Lipofectamine 2000 (Invitrogen). The concentration of Lipofectamine 2000 reagent used in the assay was 2 µl/mL and 3.5 µl/mL medium in use of 8-well chambered coverslips and 35-mm coverslips, respectively.

Plasmid construction

The sequence encoding tudor was isolated from an *Oryzias latipes* embryo cDNA library by reverse transcriptase-polymerase chain reaction (RT-PCR) amplification as described previously.[1] The tudor-coding sequence was modified by PCR to include *Bgl*II and *Eco*RI restriction sites using the following primers: 5'-CGAGATCTGAAATGAACGAGCTGCGTATGCCGAA-3' and 5'-GCGAATTCAACACAAGAGTTGTTTTATATTGAACCCA-3'. The PCR product was digested with *Bgl*II and *EcoR*I and ligated into the multiple cloning site of pEGFP-Cl (Clontech), which allows transcription of the enhanced green fluorescent protein-tudor fusion under control of the human cytomegalovirus (CMV) promoter.

Measurements of fluorescence lifetime imaging

Measurements of fluorescence lifetime images were carried out using a four-channel time-gated detection system.[2–4] The second harmonic of an output from a mode-locked Ti:sapphire laser (Tsunami, Spectra Physics) was used as the excitation light source. The pulse duration and the repetition rate of the laser pulse were 80 fs and 81 MHz, respectively. The excitation beam was introduced into the scanner head (C1, Nikon) of a confocal microscope (TE2000-E, Nikon). The excitation beam was focused onto the sample with a 40× or 60× oil objective, and the fluorescence from the sample was collected with the same objective and transmitted into two filters (BA520 and EX510-560, Nikon) to eliminate scattered excitation light. The fluorescence was detected using a photon counting photomultiplier in a LIMO high-speed lifetime imaging module (Nikon Europe BV). The fluorescence decay was measured for each pixel of the confocal microscope image. To minimize the amount of data generated, the lifetime imaging module captures the fluorescence decay trace into four time windows using time-gating electronics. Each reference trigger of the laser-pulse train enables four accumulation registers sequentially, and the detected fluorescence photons are counted and accumulated by one of the four accumulation registers. Each fluorescence lifetime was evaluated by analyzing the four time-window signals with an assumption of a single exponential decay, and the results were converted into a fluorescence lifetime image. The size of the image was 256 × 256 pixels. All of the time windows were set at 2.0 ns. The background was evaluated by the counts at the area where fluorescent cells were not observed. The compensation of the delay of the fluorescence photon signals was adjusted by measuring the fluorescence lifetime images of a standard slide (Molecular Probes) or dye molecules in a polymer film.

Supporting references

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