Electronic Supplementary Material (ESI) for Biomaterials Science.

Supplementary Information

Photo-regulation of Constitutive Gene Expression in Living Cells by Using of Ultrafast Photo-cross-linking Oligonucleotides

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Experimental Procedures

Preparation of oligonucleotides: Oligoribonucleotides were purchased from Fasmac (Japan). Phosphorothioate oligodeoxyribonucleotides were synthesized by an automatic DNA synthesizer (3400 DNA synthesizer, Applied BioSystems) and purified by a reversed-phase HPLC (JASCO PU-980, HG-980-31, DG-980-50, UV-970 system equipped with an InertSustain^{TM} C18 column (GL Science, 5 μm, 10 × 150 mm)). Preparation of oligonucleotides was confirmed by MALDI-TOF-MS (see Supporting Information Table S2). Phosphoramidite of ^{CNV}K was synthesized according to a method described in the literature.[1]

Cell culture and transfection of AS-ODNs and siRNA: GFP stable cell line (GFP-HeLa) was purchased from Cell Biolabs, Inc. (CA, USA). Cells were cultured in Dulbecco's MEM (10% fetal bovine serum, 100 U/ml penicillin, 100 μg/ml streptomycin) in a humidified chamber (37ºC, 5% CO_2). Cells were trypsinized and resuspended in Opti-MEM without antibiotics and transferred to 96-well plate at a density of 2×10^4 cells per well in a volume of 100 μl and incubate for 24 h (37ºC, 5% CO_2) before antisense treatment. Transfection of K-ASs was carried out using Lipofectamine RNAi Max (Invitrogen) according to the manufacture's procedure. After the transfection, cells were cultured in Dulbecco's MEM (10% fetal bovine serum, 100 U/ml penicillin, 100 μg/ml streptomycin) and in a humidified chamber (37ºC, 5% CO_2).

Photoirradiation: Before the photoirradiation, cell culture medium was replaced to PBS. Photoirradiation was performed by a UV-LED light source (1600 mW/cm^2, Z-UV, OMRON, Japan). After the photoirradiation, cell culture medium was replaced to Dulbecco's MEM (10% fetal bovine serum, 100 U/ml penicillin, 100 μg/ml streptomycin) and then cultured in a humidified chamber (37ºC, 5% CO_2).

Quantification of mRNA: Two hours after the photoirradiation**,** total RNA was extracted using CellAmp^{TM} Direct RNA Prep Kit (Takara, Japan) and reverse transcription was

performed by the PrimeScript® RT reagent Kit (Takara, Japan) according to the manufacturer's procedure. Resulting cDNA was subjected to real-time PCR using an automated real-time PCR system (Smart Cycler®, Takara, Japan) with SYBR Premix Ex Taq II perfect real time (Takara, Japan) and 0.4 μM of primers (For GFP: forward; ATGGTGAGCAAGGGCGAG, reverse; GTGGTGCAGATGAACTTC (for AS-a), forward; CAACAGCCACAACGTCTATATC, reverse; AACTCCAGCAGGACCATGTGAT (for AS-b,c,d), For β-actin: forward; CTGGCACCACACCTTCTACA, reverse; AGCACAGCCTGGATAGCAAC, For SIN3B: forward; TTCAACAACGCCATCAGCTA, reverse; GGCAGGAACTGTCCAAACTC). Quantity of GFP, β-actin and SIN3B mRNA was estimated from the change in C_T values with the normalization using the amounts of GAPDH mRNA estimated from real-time RT-PCR with a GAPDH primer set (forward; CATGCCAGTGAGCTTCCCGTT, reverse; GTGGAGTCTACTGGCGTCTTC).

Confocal laser scanning fluorescence microscopy: After the transfection of AS-ODN, 10 sec of photoirradiation was performed and the cells were cultured for 24 h. Fluorescence image of the cells were obtained by a confocal laser-scanning microscope (C2Si, Nikon, Japan) with 488 nm laser excitation and 525 nm detection and 30 μm pinhole radius.

Table S1. Sequence and MALDI-TOF-MS analysis of the synthetic oligonucleotides using this study

Name	Target region	Sequences[a]	Calcd. [(M+H)$^+$]	Found
cORN-a	14 – 38	3' UGGUGGGGCCACUUGUCGAGGAGCG 5'		
AS-a		5' ACCACCCCGGTGAACAGCTCCTCGC 3'	7913.73	7913.48
K-AS-a1		5' AXCACCCCGGTGAACAGCTCCTCGC 3'	8020.77	8020.51
K-AS-a2		5' ACCACCCCGGTGAXCAGCTCCTCGC 3'	7996.76	7997.45
K-AS-a3		5' ACCACXCCGGTGAACAGCTCCTCGC 3'	8020.77	8018.87
K-AS inv		5' CGCTCCTCGACAAGTGGCCCCACXA 3'	8020.77	8019.69
cORN-b	471 – 495	3' UCAAGUGGAACUACGGCAAGAAGAC 5'		
AS-b		5' AGTTCACCTTGATGCCGTTCTTCTG 3'	7995.72	7996.88
K-AS-b1		5' AXTTCACCTTGATGCCGTTCTTCTG 3'	8037.74	8037.41
K-AS-b2		5' AGTTCACCTTGAXGCCGTTCTTCTG 3'	8062.75	8063.08
cORN-c	542 – 566	3' UACCCCCACAAGACGACCAUCACCA 5'		
AS-c		5' ATGGGGGTGTTCTGCTGGTAGTGGT 3'	8211.74	8216.00
K-AS-c1		5' AXGGGGGTGTTCTGCTGGTAGTGGT 3'	8303.78	8300.48
cORN-d	592 – 616	3' UGACCCACGAGUCCAUCACCAACAG 5'		
AS-d		5' ACTGGGTGCTCAGGTAGTGGTTGTC 3'	8140.74	8140.82
K-AS-d1		5' AXTGGGTGCTCAGGTAGTGGTTGTC 3'	8247.78	8247.92
K-AS-d2		5' ACTGGGTGCTCAXGTAGTGGTTGTC 3'	8207.77	8209.54

[a] "X" and under bar indicate ^{CNV}K and photo-cross-linking site, respectively.

Table S2. Melting temperature (T_M) of K-ASs with its complementary ORNs.[a]

Duplex	T_M / ºC	Duplex	T_M / ºC	Duplex	T_M / ºC
AS-a/cORN-a	69.4 ± 0.6	AS-b/cORN-b	55.6 ± 0.6	AS-d/cORN-d	59.3 ± 0.6
K-AS-a1/cORN-a	67.0 ± 0.5	K-AS-b1/cORN-b	52.3 ± 0.4	K-AS-d1/cORN-d	58.6 ± 0.4
K-AS-a2/cORN-a	64.5 ± 0.5	K-AS-b2/cORN-b	51.1 ± 0.4	K-AS-d2/cORN-d	57.6 ± 0.6
K-AS-a 3/cORN-a	65.8 ± 0.8	AS-c/cORN-c	67.3 ± 0.0		
K-AS inv/cORN-a	n.d.[b]	K-AS-c1/cORN-c	65.4 ± 0.3		

[a] T_M values were determined from UV melting curves of the duplex ([Duplex] = 0.5 μM in 50 mM soduim cacodylate (pH 7.4) containing 100 mM NaCl). [b] Not determined.

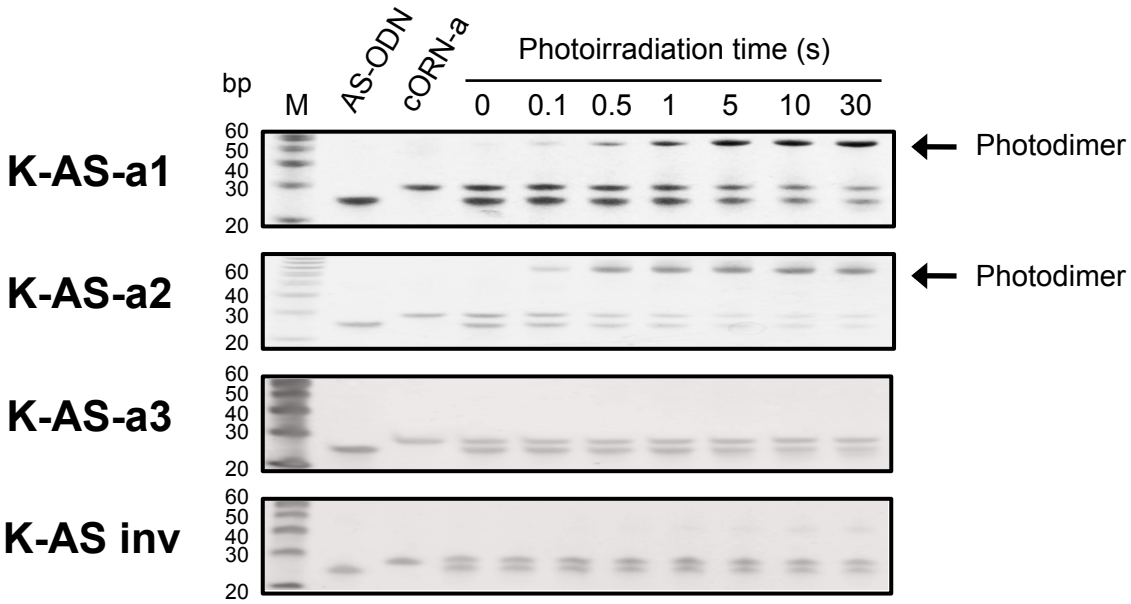

Figure S1. Denaturing PAGE analysis of the photo-cross-linking reaction of K-AS-a1, -a2, -a3 and inv with cORN-a. PAGE analyses were performed on 15% polyacrylamide gel containing 7M urea and 25% formamide. "M" indicates 10 bp ladder marker. Hetero-duplexes ([AS-ODN] = [cORN] = 2 μM in PBS) were irradiated (366 nm) at 37ºC.

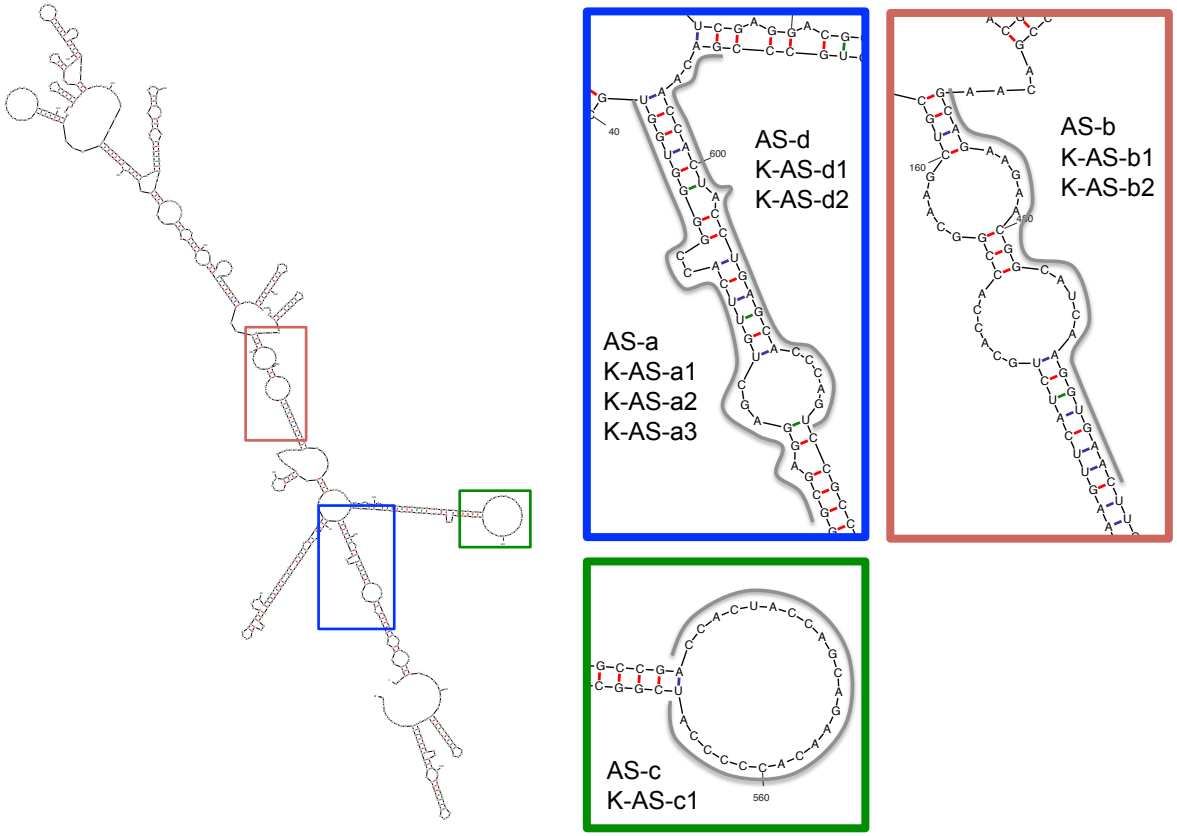

Figure S2. Secondary structure of GFP mRNA calculated by mfold[2] and the target site of the AS-ODNs.

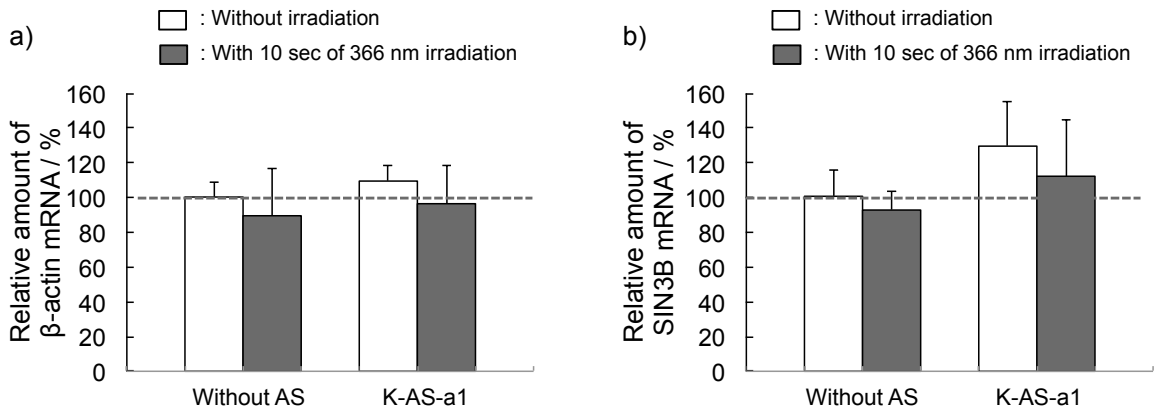

Figure S3. Relative amount of β-actin (a) and SIN3B (b) mRNA in GFP-HeLa cells before and after the photoirradiation with K-AS-a1 treatment ([antisense] = 100 nM, photoirradiation: 366 nm, 10 sec)

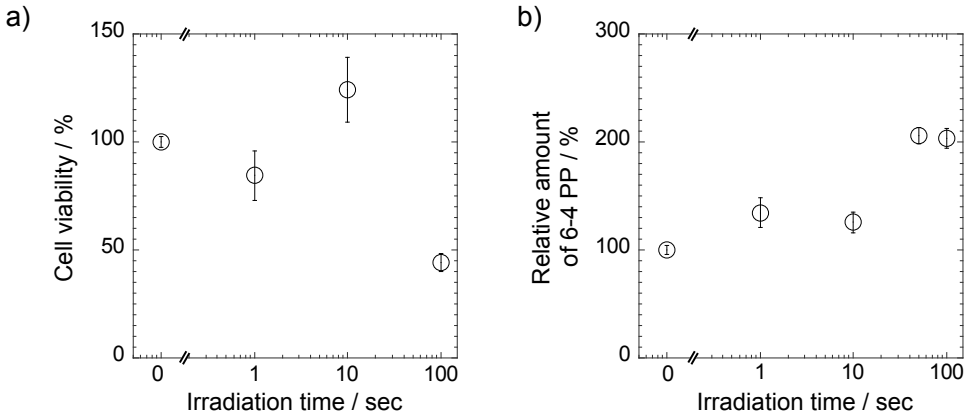

Figure S4. Cell viability (a) and relative amount of pyrimidine (6-4) pyrimidone photoproducts (6-4 PP) (b) in GFP-HeLa cells as a function of irradiation time. Cell viability was estimated by WST-1 assay 48 h after the photoirradiation. The amount of 6-4 PP was measured by ELISA kit (Cell Biolabs, CA) 48 h after the photoirradiation according to a manufacture's protocol.

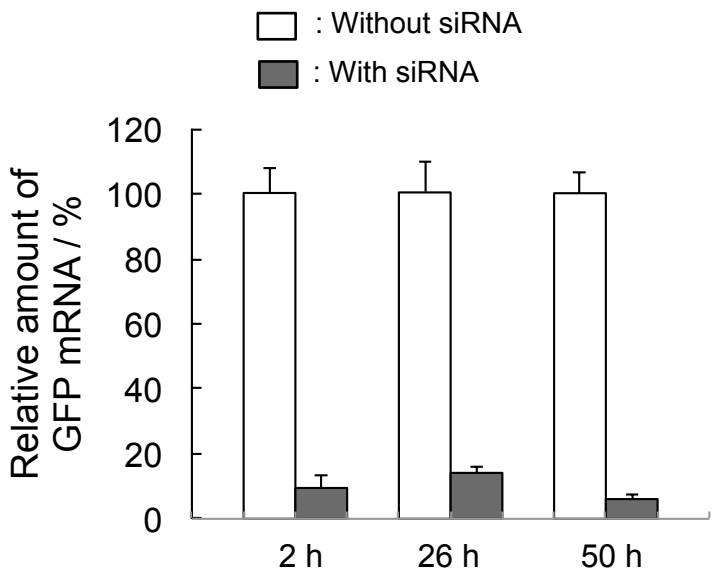

Figure S5. Relative amount of GFP mRNA in GFP-HeLa cells after the treatment of siRNA targeting GFP mRNA ([siRNA] = 100 nM, siRNA (sense); 5'-GCAAGCUGACCCUGAAGUUCA U-3', siRNA (antisense); 5'-GAACUUCAGGGUCAGCUUGCCG-3')

References

1. Y. Yoshimura, K. Fujimoto, *Org. Lett.*, 2008, **10**, 3227.
2. M. Zuker, *Nucleic Acids Res*., 2003, **31**, 3406.