

Induction of Translational Readthrough across Thalassemia-causing Premature Stop Codon in β -globin-encoding mRNA

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Supporting Information

Detailed Experimental Procedures

Cell culture

HEK 293 cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM, Sigma) and 10% Foetal Bovine Serum (Thermo Fisher Scientific), 100 unit/ml Penicillin, and 100 μ g/ml streptomycin (Lonza) in an incubator (Panasonic) containing humidified atmosphere and 5% CO₂.

Antibodies

Anti-GFP antibody was obtained from BioLegend (902602) and anti-GAPDH from Sigma (G9295). HRP-conjugated secondary mouse antibody was obtained from Thermo Fisher Scientific.

Plasmid constructs

All the constructs were made in pCDNA 3.1 vector backbone. Plasmid expressing full-length *HBB* cDNA was cloned in-frame and upstream of the coding sequence of *GFP* or *FLuc* in between *Hind*III and *Kpn*I sites. UGG to UAA mutation in *HBB* cDNA was achieved by PCR-based site-directed mutagenesis.

Fluorescence-based translational readthrough assay in HEK293 cells

HEK293 cells were seeded in a 24-well plate at 80% confluency. Next day, cells were transfected with 500 ng of *HBB*^{w16*}-GFP plasmid and 5 μ M of oligo using Lipofectamine

2000 (Invitrogen) according to manufacturer's instructions. The GFP fluorescence was captured using Olympus IX73 P2F microscope using 10x objective. For flow cytometry, cells were washed, trypsinized and resuspended in 1x PBS. They were analysed using CytoFLEX Flow Cytometer (Beckman Coulter).

SDS-PAGE and Western blot

Cells were lysed in lysis buffer (10 mM Tris pH 7.4, 150 mM NaCl, 1 mM EDTA, 1mM EGTA and 1% Triton X-100). Lysates were denatured and resolved for 1.5 h in 12.5% SDS polyacrylamide gel. Gels were then soaked in transfer buffer containing 10% methanol and proteins were transferred onto PVDF membrane (Immobilon-P, Merck Millipore) using Trans-Blot semi-dry transfer apparatus (Bio-Rad). After transfer, the blot was blocked with 5% skimmed milk for 1 h. It was then treated with primary antibody (anti-GFP antibody) followed by HRP-conjugated secondary antibody as per the instructions given in the antibody manual. The blot was developed using Clarity ECL reagent (Bio-Rad) and the images were captured using LAS-3000 imager (Fujifilm).

Luciferase-based translational readthrough assay in HEK293 cells

HEK293 cells were seeded in a 24-well plate at 70-80% confluency. Next day, cells were transfected with 500 ng of *HBB^{w16*}*-FLuc plasmid, 100 ng of RLuc plasmid and 5 μ M of oligo using Lipofectamine 2000 (Invitrogen) according to manufacturer's instructions. The cells were lysed 24 hours post transfection and luciferase activity was measured using Dual-Luciferase Reporter Assay System and GloMax Explorer (Promega).

RNA isolation and Reverse transcriptase-PCR:

Total RNA was isolated from the cells using TRI reagent (Sigma-Aldrich) according to manufacturer's instructions. Quality of RNA was checked by running on 1% agarose gel and quantified using nanodrop spectrophotometer. cDNA was synthesized using oligo dT primer and RevertAid Reverse Transcriptase (Thermo Scientific). PCR amplification was done using *GAPDH* and *FLuc*-specific primers.