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A genetically encoded bioluminescent indicator for illuminating proinflammatory cytokines

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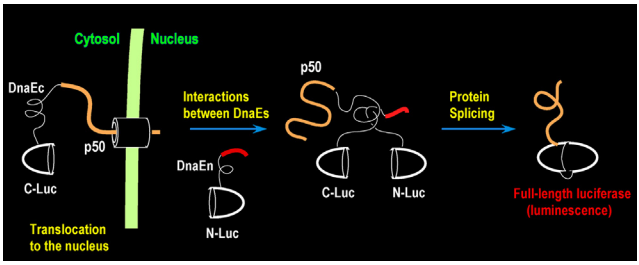
GRAPHICAL ABSTRACT



ABSTRACT

We introduce a method to evaluate the activities of cytokines based on the nuclear transport of NF-κB. A pair of bioluminescent indicators was made for conferring cytokine sensitivity to cervical carcinoma-derived HeLa cells. The principle is based on reconstitution of split fragments of *Renilla reniformis* luciferase (RLuc) by protein splicing with a DnaE intein from *Synechocystis* sp. PCC6803. The bioluminescence intensity of thus reconstituted RLuc in the HeLa cells was used as a measure of the activities for cytokines. With the present method, we evaluated the activities of various cytokines based on the nuclear transport of NF-κB in human cervical carcinoma-derived HeLa cells carrying the indicators. The present approach to evaluating the activities of cytokines may provide a potential clinical value in monitoring drug activity and directing treatment for various diseases related with NF-κB. The method highlights the experimental procedure from our original publications, *Anal. Biochem.* 2006, 359, 147–149 and *Proc. Natl. Acad. Sci. U. S. A.* 2004, 101, 11542.

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The summary of the method is:
- Cytokine activities are determined within 2 h after stimulation.
- Temporarily inactivated split-luciferase fragments are reconstituted by protein splicing.
- Nucleartrafficking of NF-κB was illuminated for gauging the ligand-driven activity.

ARTICLE INFO
Method name: Genetically encoded bioluminescent indicator based on protein splicing
Keywords: Bioluminescence, Protein splicing assay (PSA), Luciferase, Cytokine, nuclear factor-κB (NF-κB), Tumor necrosis factor-α (TNF-α), Nuclear trafficking
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Method details

Introduction

The pro-inflammatory cytokines bind with their specific receptors on the plasma membrane of cells and finally activate nuclear factor-kappa B (NF-κB), which is deeply related to various human diseases including cancers, aging, diabetes, neurodegenerative disorders, and inflammatory diseases [1–5]. In this method, we describe a method to quantitatively determine inflammatory activities of cytokines within 3 h, based on the cytokine-induced nuclear transport of nuclear factor-kappa B (NF-κB) as illustrated in Fig. 1. Upon cytokine stimulation, the subunit of NF-κB, p50, is translocated into the nucleus, where the N- and C-terminal fragments of split-RLuc are spontaneously reconstituted with the help of a naturally split DnaE intein (a catalytic subunit of DNA polymerase III from the genome of *Synechocystis* sp. PCC6803 (*Ssp* genome); 15 kD), called a protein-fragment splicing assay (PSA). The optical intensity reflects the activities of cytokines. This imaging strategy highlights the experimental procedure of our precedent publications [6,7].

Materials

- Cytokines: Human tumor necrosis factor-α (TNF-α); Human oncostatin M (OSM); Human interleukin-1β (IL-1β); Human leukemia inhibitory factor (LIF) (Sigma); IL-1 receptor antagonist (IL-1RA).
- Reagents for genetic engineering: a lipofection reagent (TransIT-LT1, Mirus); a mammalian expression vector (pcDNA 3.1(+)); cDNA encoding p50 of NF-κB; cDNA encoding RLuc.
- Immunocytochemistry-related reagents: mouse anti-Flag antibody (Sigma); mouse anti-NF-κB P50 antibody (Abcam); Cy-5-conjugated secondary antibody (Jackson); paraformaldehyde (PFA) (Sigma); fish skin gelatin (FSG) (Sigma); Mowiol solution (Aldrich); Sytox Green (Molecular Probes).
- Buffers: a TBST buffer (Tris–HCl buffer with saline and Triton X-100); a phosphate buffered saline (PBS) buffer.
- Cell culture and assay reagents: human uterine cervical carcinoma-derived HeLa cells; Dulbecco's modified eagle's medium (DMEM; Sigma); cholesterol-free fetal bovine serum (FBS; Gibco); penicillin-streptmycin (P/S); *Renilla* luciferase assay kit (Promega), a Bradford reagent (Pierce).

Design of the cDNA constructs

The unique bioluminescent indicator is designed as follows (Figs. 1 and 2).

1. Download the amino acid sequence of RLuc (Protein Databank assess number: 2PSD) from a public database, National Center for Biotechnology Information (NCBI).