Biomaterials 48 (2015) 97–107

Contents lists available at ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

Novel near-infrared BiFC systems from a bacterial phytochrome for imaging protein interactions and drug evaluation under physiological conditions

Minghai Chen [a, c], Wei Li [a], Zhiping Zhang [a], Sanying Liu [a, c], Xiaowei Zhang [a], Xian-En Zhang [a, b, *], Zongqiang Cui [a, *]

[a] State Key Laboratory of Virology, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan 430071, China
[b] National Key Laboratory of Macrobiomolecules, Institute of Biophysics, Chinese Academy of Sciences, Beijing 100101, China
[c] Graduate University of Chinese Academy of Sciences, Beijing 100049, China

ARTICLE INFO

Article history:
Received 3 October 2014
Accepted 20 January 2015
Available online 11 February 2015

Keywords:
Near-infrared
BiFC
Bacterial phytochrome iRFP
HIV IN-LEDGF/p75 interaction
Drug evaluation

ABSTRACT

Monitoring protein–protein interactions (PPIs) in live subjects is critical for understanding these fundamental biological processes. Bimolecular fluorescence complementation (BiFC) provides a good technique for imaging PPIs; however, a BiFC system with a long wavelength remains to be pursued for *in vivo* imaging. Here, we conducted systematic screening of split reporters from a bacterial phytochrome-based, near-infrared fluorescent protein (iRFP). Several new near-infrared phytochrome BiFC systems were built based on selected split sites including the amino acids residues 97/98, 99/100, 122/123, and 123/124. These new near-infrared BiFC systems from a bacterial phytochrome were verified as powerful tools for imaging PPIs under physiological conditions in live cells and in live mice. The interaction between HIV-1 integrase (IN) and cellular cofactor protein Lens epithelium-derived growth factor (LEDGF/p75) was visualized in live cells using the newly constructed iRFP BiFC system because of its important roles in HIV-1 integration and replication. Because the HIV IN-LEDGF/p75 interaction is an attractive anti-HIV target, drug evaluation assays to inhibit the HIV IN-LEDGF/p75 interaction were also performed using the newly constructed BiFC system. The results showed that compound 6 and carbidopa inhibit the HIV IN-LEDGF/p75 interaction in a dose-dependent manner under physiological conditions in the BiFC assays. This study provides novel near-infrared BiFC systems for imaging protein interactions under physiological conditions and provides guidance for splitting other bacterial phytochrome-like proteins to construct BiFC systems. The study also provides a new method for drug evaluation in live cells based on iRFP BiFC systems and supplies some new information regarding candidate drugs for anti-HIV therapies.

1. Introduction

Protein–protein interactions (PPIs) are basic biological processes. Monitoring PPIs in living cells is very helpful to understand the mechanisms of physiological and biochemical processes. Several methods are currently available for studying PPIs in living cells, such as fluorescence resonance energy transfer (FRET) [1,2] and protein fragment complementation [3–5]. Bimolecular fluorescence complementation (BiFC) is an attractive and valuable approach to detect PPIs in living cells because of its simplicity, lack of invasion and high sensitivity [6]. The BiFC assay relies on the reconstruction of a fluorescent protein from its two non-fluorescent splits fused with two interacting proteins [7]. BiFC reporters have been developed using many different fluorescent proteins and their mutants to study various PPI events in living cells [7–10]. However, they are almost entirely developed from GFP-like proteins. Most BiFC systems based on GFP-like proteins have short wavelengths that vastly restrict their applications, especially for *in vivo* imaging. Moreover, some of the BiFC systems based on GFP-like proteins, such as our recently constructed mNeptune BiFC system that has the longest wavelength, maturate at low temperatures (below 37 °C) [11]. Therefore, these systems have drawbacks in their applicability under physiological conditions.

* Corresponding authors. State Key Laboratory of Virology, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan 430071, China. Tel.: +86 27 8719 9115; fax: +86 27 8719 9492.
E-mail addresses: zhangxe@ibp.ac.cn (X.-E. Zhang), czq@wh.iov.cn (Z. Cui).

http://dx.doi.org/10.1016/j.biomaterials.2015.01.038
0142-9612/

Phytochromes, different from GFP-like proteins, are bacterial or plant photopigments that naturally absorb red and near-infrared light [12]. Recently, phytochromes have been explored for use in *in vivo* imaging with infrared fluorescence spectrum [13,14]. For example, iRFP is a bacterial phytochrome-based near-infrared fluorescent protein, with excitation and emission maxima at 690 nm and 713 nm, respectively [14]. iRFP possesses a high brightness and low cytotoxicity *in vivo* and utilizes physiological levels of biliverdin (BV) chromophore to acquire fluorescence. Considering the superiorities of phytochromes for *in vivo* imaging, new phytochrome-based BiFC systems will be very attractive for PPIs imaging. Phytochromes have a different structure compared with GFP-like proteins, which have a β-barrel structure. The split sites in BiFC systems based on GFP-like proteins are unable to be applied and aligned in phytochrome-based systems. A systematic study of splitting the phytochrome is in crucial for the construction of phytochrome-based BiFC systems, although a polypeptide break between the PAS and GAF domains has been recently reported for the construction of an iRFP BiFC system [15].

Because PPIs often play key roles in pathological processes such as virus infection, targeting of PPIs is also a promising approach for designing novel therapeutic drugs. The visualization of the efficacy of potent compounds to inhibit PPIs in live subjects will provide a good method for drug screening. However, this method remains to be explored. Molecular imaging of PPIs in live cells or live animals treated with drugs will provide a direct evaluation of drugs under physiological conditions. Here, based on the construction of novel near-infrared BiFC system from the bacterial phytochrome iRFP, we performed drug evaluation of the ability to inhibit PPIs in live cells.

Lens epithelium-derived growth factor (LEDGF/p75) is a cellular cofactor of HIV-1 integrase (IN) that is critical for tethering the IN and viral pre-integration complex to the host chromatin [16]. The important roles of the IN-LEDGF/p75 interaction in HIV-1 integration and replication make it an attractive antiviral target [17,18]. Recently, efforts to disrupt the IN-LEDGF/p75 interaction have resulted in the discovery of some new potent inhibitors and drugs for anti-HIV therapies [19–23]. However, these inhibitors were usually evaluated *in vitro*. For example, carbidopa was found to have the capacity to inhibit the IN-LEDGF/p75 interaction with moderate IC50 values (6.54 μM) using AlphaScreen [24]. However, further investigations are needed under physiological conditions.

In this work, we conducted a systematic research on splitting the bacterial phytochrome iRFP for the construction of near-infrared BiFC systems. Twelve sites on iRFP were chosen for splitting, and four sites were found to be useful for constructing novel BiFC systems. Based on the phytochrome iRFP-based BiFC system, the intracellular interaction of IN-LEDGF/p75 was imaged, and compounds to inhibit the IN-LEDGF/p75 interaction were studied in live cells.

2. Materials and methods

2.1. Construction of plasmids

The iRFP protein was split at twelve positions between amino acids 38/39, 46/47, 61/62, 97/98, 99/100, 112/113, 122/123, 123/124, 166/167, 179/180, 193/194, and 248/249. The fragment pairs obtained were named iRN38/iRC39, iRN46/iRC47, iRN61/iRC62, iRN97/iRC98, iRN99/iRC100, iRN112/iRC113, iRN122/iRC123, iRN123/iRC124, iRN166/iRC167, iRN179/iRC180, iRN193/iRC194, and iRN248/iRC249, which correspond to the amino acid sequences of 1–38 and 39–316, 1–46 and 47–316, 1–61 and 62–316, 1–97 and 98–316, 1–99 and 100–316, 1–112 and 113–316, 1–122 and 123–316, 1–123 and 124–316, 1–166 and 167–316, 1–179 and 180–316, 1–193 and 194–316, 1–248 and 249–316, respectively.

For the construction of plasmids containing the fusion proteins EGFP-iRN and iRC-EGFP, the N- and C-terminal coding regions of iRFP were amplified by PCR from plasmid pcDNA3.1-iRFP. iRN38, iRN46, iRN61, iRN97, iRN99, iRN112, iRN122, iRN123, iRN166, iRN179, iRN193 and iRN248 shared the same forward primer: 5′-GAA*GATCT*ATGGCTGAAG GATCCGTC-3′ (BglII included). The reverse primers were 5′-AA*GGTACC*GTCGGCGGCGAGGGCGAG CAGCAG-3′ (for iRN38), 5′-AA*GGTACC*G TCGCTGCCGGCAACGATCGTCATG-3′ (for iRN46), 5′-AA*GGTACC*GCCGATCAGGGCGCC GATCG-3′ (for iRN61), 5′-AA*GGTACC*CATCGTGAAGCCGACAGTG-3′ (for iRN97), 5′-AA*GGTACC*CTTTCGCATCGTGAAGCCGACAGTG-3′ (for iRN99), 5′-AA*GGTACC* CTGATCATGGCGATGCCAGGAG-3′ (for iRN112), 5′-AA*GGTACC*CTGGGGAGGCTCGAGCTCGAGG-3′ (for iRN122), 5′-AA*GGTACC*CCGCTGGGGAGGCTCGAGCTCGAGGAAGATG-3′ (for iRN123), 5′-AA*GGTACC*ATCGAAGCCGGTAATC-3′ (for iRN166), 5′-AA*GGTACC*GCCGCTGAAGTCGGAGG-3′ (for iRN179), 5′-AA*GGTACC*TTTTGACTCGACCTCGG-3′(for iRN193), and 5′-AA*GGTACC*CAGGATGGCGAAGCTAAG-3′ (for iRN248) (the KpnI site was contained in each reverse primer). Each fragment was cut by BglII and KpnI and then inserted into the same pEGFP-C1 sites.

The forward primers for the iRCs were 5′-G*AAGATCT*ATGACGATCGTTGCCGG-3′ (for iRC39), 5′-G*AAGATCT*ATGAACCTTCCCGAACTCACCGGACTGGC-3′ (for iRC47), 5′-G*AAGATCT* ATGCGCTCTGCGGCCGATGTC-3′ (for iRC62), 5′-G*AAGATCT*ATGA TGCGAAAGGACG-3′ (for iRC98), 5′-G*AAGATCT*ATGGACGCAGGCTTCATCGGCTCCTG-3′ (for iRC100), 5′-G*AAGATCT* ATGCTCATCTTCCTCGAGCTCGAGCCTC-3′ (for iRC113), 5′-G*AAGATCT* ATGCGGGACGTCGCCGAGCCG-3′ (for iRC123), 5′-G*AAGATCT*ATGGACGT CGCCGAGCCGC-3′ (for iRC124), 5′-G*AAGATCT*ATGCGGGTGATGATCTATCGC-3′ (for iRC167), 5′-G*AAGATCT*ATGGAAGTGATCGCAGAGG-3′ (for iRC180), 5′-G*AAGATCT*A TGCTAGGCCTGCACTATCCTGCCTC-3′ (for iRC194), and 5′-G*AAGATCT*ATGCG CAGCGTCTCGCCCGTCC-3′ (for iRC248) (the BglII site was included in each forward primer). The same reverse primer 5′-CCC*AAGCTT*CTCTTCCATCACGCCGATCTGC-CAGG-3′ was used for iRC39, iRC47, iRC62, iRC98, iRC100, iRC113, iRC123, iRC124, iRC167, iRC180, iRC194, and iRC248 (HindIII contained). Each iRC fragment was digested by BglII and HindIII and then inserted into the corresponding sites of pEGFP-N1.

The plasmids of piRN and piRC were generated through the deletion of EGFP from plasmids of pEGFP-iRN and pEGFP-iRC. For the construction of pbJun-iRN97, pbJun-iRN99, pbJun-iRN122 and pbJun-iRN123 plasmids, bJun was amplified by PCR from plasmid pcDNA3.1-Jun (kindly provided by Professor Luo Qingming, Huazhong University of Science and Technology, Wuhan 430074). The forward primer was: 5′-CG*GCTAGC*GCCACCATGAAGGCGGAGAGGAAGCG-3′ (NheI and Kozak sequences included) and the reverse primer was 5′-CCC*AAGCTT*AAACGTTTGCAACTGC-3′ (HindIII included). iRN97, iRN99, iRN122 and iRN123 shared the same forward primer: 5′-GG*GGTACC*GAGCCACCCCCTCCTATGGCTGAAGGATCCGTC-3′ (KpnI included). The reverse primers were 5′-ATTT*GCGGCCGC*TTACATCGTGAAGCCGACAG-3′ (for iRN97), 5′-ATTT*GCGGCCGC*TTACTTTCGCATCGTGAAGCCGACAG-3′ (for iRN99), 5′-ATTT*GCGGCCG C*TTACTGGGGAGGCTCGAGCTC-3′ (for iRN122), and 5′-ATTT*GCGGCCGC*TTACCGCTGGG-GAGGCTCGAGCTC-3′ (for iRN123) (the NotI site was included in each reverse primer) and they were inserted into the corresponding sites of pcDNA3.1. The plasmids of piRC98-bFos, piRC100-bFos, piRC123-bFos and piRC124-bFos were constructed as follows: bFos was amplified by PCR from plasmid pcDNA3.1-Fos (kindly provided by Professor Luo Qingming) and the forward primer was 5′-GG*GGTACC*GAGCCACCCCCT CCTGGTCGTGCGCAGTCC-3′ (KpnI included). The reverse primer was 5′-ATTT*GCGGCCGC*TTAACCCAGGTCGTTCGGGATTTTGC-3′ (NotI included). The forward primers for iRC98, iRC100, iRC123 and iRC124 were 5′-CG*GCTAGC*GCCACCATGC-GAAAGGACGCAGGCTTCATC-3′ (for iRC98), 5′-CG*GCTAGC*GCCACCATGGACGCAGGCTT-CATCGG-3′ (for iRC100), 5′-CG*GCTAGC*GCCACCATGCGGGACGTCGCCGAGCCG-3′ (for iRC123), and 5′-CG*GCTAGC*GCCACCATGGACGTCGCCGAGCCGC-3′ (for iRC124) (the NheI site and Kozak sequence were included in each forward primer). The same reverse primer 5′-CCC*AAGCTT*CTCTTCCATCACGCCGATCTGCC-3′ was used for iRC98, iRC100, iRC123, and iRC124 (HindIII contained) and then inserted into the corresponding sites of pcDNA3.1.

The plasmids of piRC98-mbFos, piRC100-mbFos, piRC123-mbFos and piRC124-mbFos were constructed by replacing bFos with mbFos at the same sites of piRC98-bFos, piRC100-bFos, piRC123-bFos and piRC124-bFos. mbFos was amplified by PCR from plasmid pcDNA3.1-mbFos (kindly provided by Professor Luo Qingming). The forward primer was 5′-GG*GGTACC*GAGCCACCCCCTCCTATGGGTCGTGCG-CAGTC-3′ (KpnI included) and the reverse primer was 5′-ATTT*GCGGCCGC*TTAA CCCAGGTCGTTCGGGATTTTGC-3′ (NotI included).

The construction of plasmids of pp75-iRN97 and piRC98-IN were conducted as follows: p75 was PCR-amplified from random-primed HeLa cDNA using the following primers: 5′-CG*GCTAGC*GCCACCATGACTCGCGATTTCAAACCTGGAGACC-3′ (NheI and Kozak sequences included), 5′-CCC*AAGCTT*GTTATCTAGTGTA-GAATCCTTCAGAGATATTTCAG-3′ (HindIII included) and bJun was substituted with pbJun-iRN97 at the same position. IN was amplified by PCR from the plasmids of pcDNA3.1-AD8 of HIV-1. The forward primer was 5′-GG*GGTACC*GAGC-CACCCCCTCCTTTTTTGGATGGAATAGAT-3′ (KpnI included) and the reverse primer was 5′-ATTT*GCGGCCGC*TTAATCCTCATCCTGTCTACTTGC-3′ (NotI included). bFos was replaced with piRC98-bFos at the same position.

In the western blot analysis and immunostaining assays, HA or Flag tag gene was inserted into the plasmids of piRN97, pEGFP-iRN97, piRC98, pEGFP-iRC98, piRC98-bFos, piRC98-mbFos, piRC98-IN and piRC98-IN(R166A) to construct the plasmids piRN97-HA, pEGFP-iRN97-HA, piRC98-Flag, pEGFP-iRC98-Flag, piRC98-bFos-HA, piRC98-mbFos-HA, piRC98-IN-HA and piRC98-IN(R166A)-HA.

All of the sequences were verified by DNA sequencing.

2.2. Cell culture and transfection

HeLa and 293T cells were grown in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal calf serum, 100 U/ml penicillin, and 100 μg/ml streptomycin at 37 °C and 5% CO_2 in a humidified incubator. The cells were seeded the day