



Optogenetic Control for Investigating Subcellular Localization of Fyn Kinase Activity in Single Live Cells

Ziliang Huang, Mingxing Ouyang, Shaoying Lu, Yingxiao Wang and Qin Peng

Department of Bioengineering, University of California, San Diego, La Jolla, CA 92093, USA

Institute of Engineering in Medicine, University of California, San Diego, La Jolla, CA 92093, USA

Correspondence to Yingxiao Wang and Qin Peng: Corresponding authors. Department of Bioengineering, University of California, San Diego, La Jolla, CA 92093, USA. yiw015@eng.ucsd.edu, q3peng@eng.ucsd.edu
<https://doi.org/10.1016/j.jmb.2020.03.015>

Edited by Kai Zhang.

Abstract

Previous studies with various Src family kinase biosensors showed that the nuclear kinase activities are much suppressed compared to those in the cytosol, suggesting that these kinases are regulated differently in the nucleus and in the cytosol. In this study, using Fyn as an example, we first engineered a Fyn biosensor with a light-inducible nuclear localization signal to demonstrate that the Fyn kinase activity is significantly lower in the nucleus than in the cytosol. To understand how different equilibrium states between Fyn and the corresponding phosphatases are maintained in the cytosol and nucleus, we further engineered a Fyn kinase domain with light-inducible nuclear localization signal. The results revealed that the Fyn kinase can be actively transported into the nucleus upon light activation and upregulate the biosensor signals in the nucleus. Our results suggest that there is limited transport or diffusion of Fyn kinase between the cytosol and nucleus in the cells, which is important for the maintenance of different equilibrium states of Fyn *in situ*.

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Introduction

Protein phosphorylation, a reversible posttranslational modification, controls many aspects of cell fate. It is often regulated through phosphorylation by kinases and dephosphorylation by phosphatases. In pathological conditions, protein phosphorylation is deregulated [1]. Non-receptor tyrosine kinases, a subfamily of protein kinases, can be anchored to the plasma membrane through their N-terminal modifications and transduce diverse receptor signals to phosphorylate downstream proteins [2]. Src family kinases, the largest subfamily of non-receptor tyrosine kinases including Src, Fyn, and Lck, play critical roles in a variety of pathophysiological processes including cell migration, cell adhesion, T-cell regulation [3–5].

Src family kinases have different activity levels in regulating downstream signals, depending on their subcellular localization. Resting-state Src is localized mainly near the perinuclear region in endosomes, while active Src can be trafficked to the plasma membrane *via* actin cytoskeleton [6,7]. Later study showed that after focal adhesion disassembly,

activated FAK and Src accumulate at a perinuclear region to recycle endocytosed integrin for focal adhesion reassembly, which enables directional cell migration [8]. Fyn kinase also showed different activity levels at different membrane microdomain localizations [9].

In the past decade, our group has developed various genetically encoded biosensors based on fluorescence resonance energy transfer (FRET) to visualize Src, FAK, Fyn, and Lck activities in live cells, and investigate the subcellular localization of kinases and their roles in regulating cellular activities and functions. Interestingly, we consistently observed that all the tyrosine kinase biosensors are quenched in the nucleus [9–12]. However, it remains unclear on the activity of Src family kinases in nucleus, largely due to the lack of tools that can directly visualize and compare kinase activities in and outside of nucleus in the same live cells. In this work, we studied Fyn kinase activity in nucleus, because Fyn kinase plays crucial roles in hematology and T-cell signaling, and our recent work revealed that Fyn kinase is fine-tuned and precisely

regulated by its submembrane localization in T cells [9]. Fyn kinase consists of a unique N-terminal SH4 domain, an SH3 domain, an SH2 domain, a proline-rich linker, an SH1 kinase domain, and an inhibitory C-tail containing a regulatory tyrosine site Y528 [13,14]. The N terminus of Fyn (GCVQCKDK) allows myristic (at Gly site) and palmitic (at two Cys sites) fatty acylation, which can target Fyn to the plasmid membrane [15]. Even though Fyn lacks a nuclear localization signal (NLS), previous work showed that there was Fyn activity in the nucleus of a subset of cells during limited period of early development in zebrafish [16]. Later it was reported that palmitate can activate Fyn and re-localize Fyn into the nucleus in macrophages to phosphorylate nuclear factor erythroid-2-related factor 2 (Nrf2) and transport Nrf2 out of nucleus [17]. It remains unclear though how Fyn activity is dynamically regulated in different subcellular compartments.

Optogenetics has risen as a powerful approach to control over protein activity with unprecedented spatiotemporal precision by genetically encoded photoreceptors [2]. Among different types of photoreceptors, the LOV2 domain of *Arabidopsis sativa* phototropin 1 (AsLOV2) is relatively small and well suited for engineering optogenetic tools [18]. In dark state, the C-terminal J α helix is docked on the surface of LOV2 domain, whereas illumination with blue light (~450 nm) leads to its opening and exposure to the solvent [19]. Based on LOV2 domain, a light-inducible nuclear localization signal (LINuS) has been developed to control the nuclear import of a protein of interest in mammalian cells upon blue light illumination [20]. The synthesized protein unit comprises a photosensitive LOV2 domain and an NLS fused into the C-terminal J α helix of LOV2 domain. Before blue light, NLS is concealed by J α helix. After blue light illumination, the J α helix undocks from the core LOV2 domain and opens up to expose the NLS.

In our study, we demonstrated that mCherry can be translocated into nucleus by LINuS after blue light illumination on HEK293T cells. Based on this strategy, we engineered blue light inducible Fyn FRET biosensor by adding a LINuS at C terminus of Fyn biosensor (FynBS-LINuS). Before and after blue light, the FynBS-LINuS can spatiotemporally monitor endogenous Fyn kinase activity in the cytosol and nucleus in the same live cell, respectively. We also fused the Fyn kinase domain in-between mCherry and LINuS to translocate Fyn kinase from cytosol into nucleus upon blue light, with a co-transfected cytosolic Fyn FRET biosensor applied to monitor Fyn kinase activity in the same live cells. Our work establishes a light inducible system to control Fyn kinase activity in cytosol and nucleus, which could provide more mechanistic understanding on the activity and function of Src family kinases, specifically Fyn kinase, in cell nucleus.

Results

Fyn activity is suppressed in the nucleus

We previously developed a highly sensitive Fyn biosensor based on FRET to monitor Fyn kinase activity in live cells, which consists of an ECFP at the N terminus, followed by an SH2 domain from Src as a binding domain, a 15-residue flexible linker, a Fyn substrate peptide, and a YPet at the C terminus [9]. Mouse embryonic fibroblast (MEF) cells expressing the Fyn biosensor were stimulated with growth factor platelet-derived growth factor (PDGF) to induce kinase activation. We observed rapid FRET responses (ECFP/FRET ratio changes from ~0.30 to ~0.55) in the cytosol but very little FRET changes in the nuclear regions (Figure 1(a) and (b)). Because the FRET signal of nuclear regions may include some cytosolic fractions above or below the nuclei, we further engineered a nucleus-localized Fyn biosensor with NLSs and tested in MEF cells. As shown in Figure 1(c) and (d), there was almost no FRET change upon PDGF stimulation in the MEF cells. We also showed that pervanadate (tyrosine phosphatase inhibitor) treatment can cause increase of Fyn activity in the nucleus (Supplementary Figure 1). These results indicate that in PDGF-stimulated MEF cells, Fyn kinase has high activity in the cytosol, but almost no activity in the nucleus.

Light-inducible nuclear translocation of Fyn biosensor

Our Fyn biosensor results showed that the level of Fyn activity is higher in the cytosol than in the nucleus, suggesting that Fyn activity may be regulated separately in these cellular compartments, and hence, there is a different equilibrium state in the nucleus from that in the cytosol. However, this only demonstrated the static distribution of Fyn activity in the cells, and an examination of the dynamic change of ECFP/FRET status of biosensor when translocating between cytosol and nucleus would provide more insights into how cytosolic and nuclear Fyn activities are differentially regulated. Based on our previous observations, we hypothesized that if we can track the change of ECFP/FRET status of Fyn biosensor during its nuclear translocation, we should be able to see a decrease of ECFP/FRET status in the translocated biosensor.

Optogenetics can provide a direct way to test this hypothesis. A LINuS has been reported, in which the AsLOV2 domain is utilized for caging of an NLS fused at the C terminal of LOV2 domain. Upon blue light, the J α helix of the LOV2 domain is unfolded and the caged NLS will be exposed and become functional in localizing the protein into nucleus [20]. To utilize this LINuS system to test our hypothesis, the reported

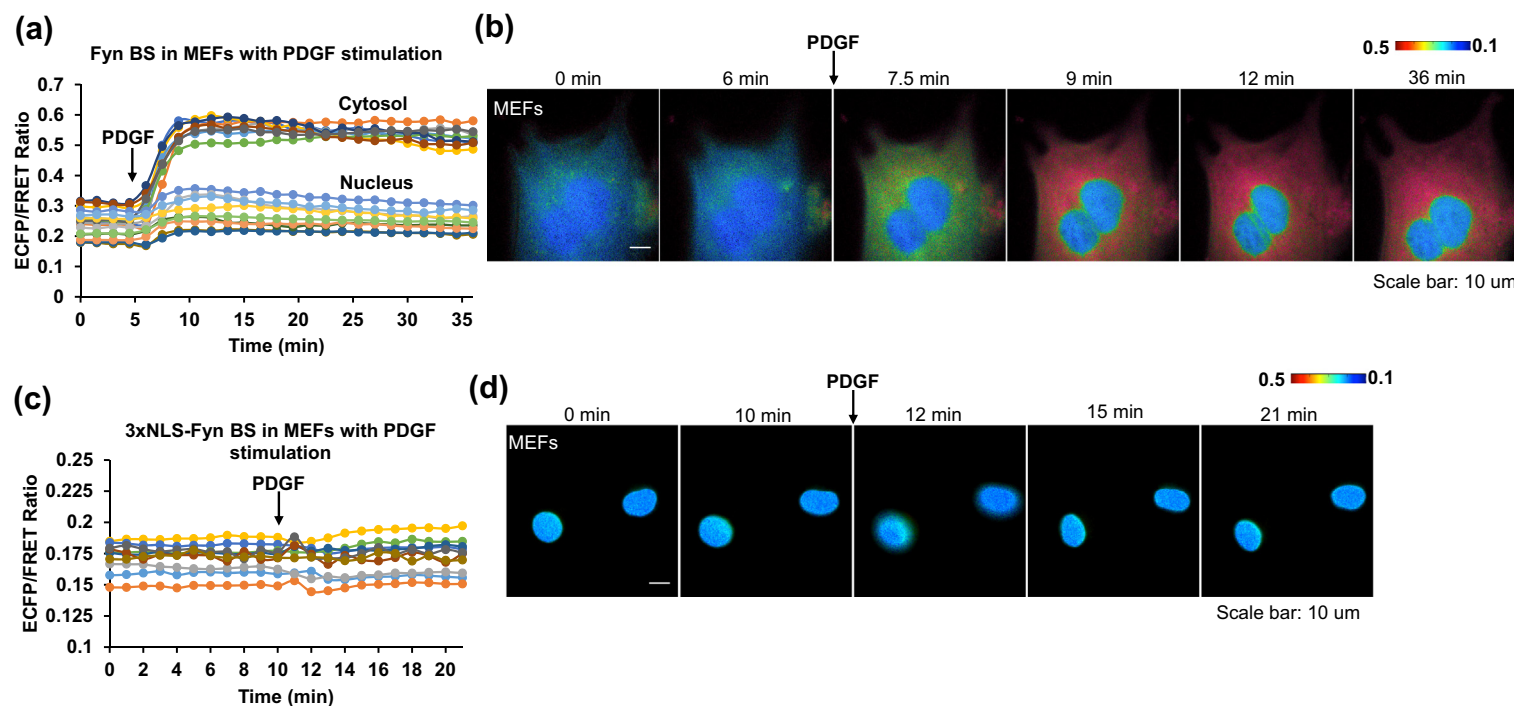


Figure 1. Fyn kinase showed low activity in the nucleus. (a) Time courses of the ECFP/FRET ratio change in cytosolic and nuclear regions of MEF cells transfected with Fyn biosensor. Upon PDGF stimulation (50 ng/ml), the ECFP/FRET ratio of Fyn biosensor in cytosol increased, whereas the Fyn biosensor did not show much response in the nucleus ($n = 9$ for both cytosol and nucleus groups). (b) The representative ECFP/FRET ratio images of MEF cells transfected with Fyn biosensor and treated with PDGF at different time points. The color bar from blue to red represents the ECFP/FRET ratio of Fyn biosensor from low to high, respectively. The scale bar represents 10 μ m. (c) The time courses of the ECFP/FRET ratio change in the nucleus of MEF cells transfected with Fyn biosensor with three NLSs (3xNLS-FynBS). There wasn't detectable response of the Fyn biosensor upon PDGF stimulation (50 ng/ml, $n = 8$). (d) Representative ECFP/FRET ratio images of MEF cells transfected with 3xNLS-FynBS and treated with PDGF. The color bar from blue to red represents the ECFP/FRET ratio of the biosensor from low to high, respectively. The scale bar represents 10 μ m.

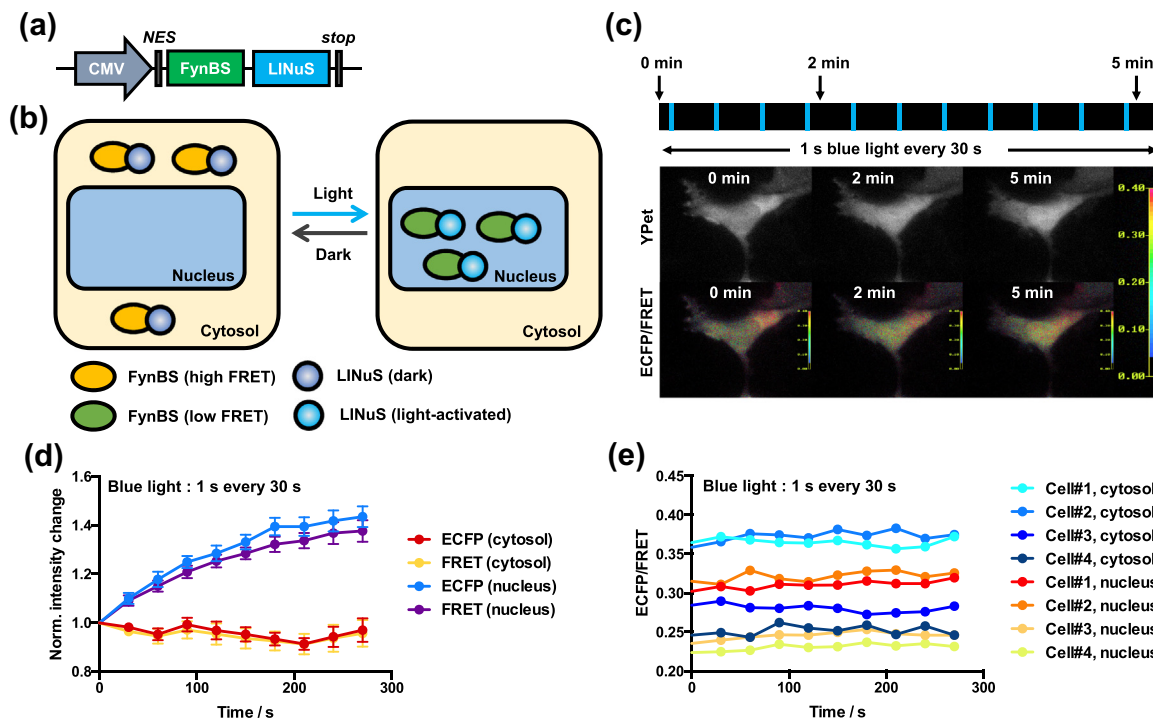


Figure 2. Light-inducible nuclear translocation of Fyn biosensor. (a) Construct for light-inducible translocation of Fyn biosensor. The Fyn biosensor is fused with an NES at the N terminus, and a light-inducible NLS at the C terminus. (b) Scheme of light-inducible nuclear translocation of Fyn biosensor. Before light stimulation, the Fyn biosensor (with NES) is localized in the cytosol, where Fyn kinase activity is expected to be relatively high (left). Upon blue light, the light-inducible NLS will be triggered to cause translocation of Fyn biosensor into nucleus, where Fyn kinase activity is expected to be low (right), resulting in a change of ECFP/FRET ratio of the biosensor. (c) Live cell imaging of light-inducible nuclear translocation of Fyn biosensor in HEK 293T cells. Intensity was quantified from the images which was taken from YPet only channel. (d) Change of cytosolic and nuclear ECFP and FRET intensities during light-induced nuclear translocation of Fyn biosensor in HEK 293T cells. $n = 4$. The error bar represents SEM. Time 0 s refers to the first frame of the imaging where no blue light stimulation was applied to the system. Blue light stimulation was conducted after the first acquisition of FRET images with the pattern of 1 s every 30 s during the entire imaging process. (e) Change of cytosolic and nuclear ECFP/FRET ratios during the light-induced nuclear translocation of Fyn biosensor as shown in (d).

construct with LINuS was first tested with our microscope settings (Supplementary Figure 2). The results clearly showed that the LINuS can be activated by blue light (460 nm, 1 s per 30 s) and subsequently lead cargo protein reporter into the nucleus.

Subsequently, we have engineered a light-inducible Fyn biosensor, of which the nuclear translocation can be controlled by blue light (Figure 2(a)). The NES tag can keep the biosensor outside of nucleus before light activation, while LINuS can enable nuclear translocation upon blue light (Figure 2(b)). HEK293T cells expressing the light-inducible Fyn biosensor showed cytosolic expression before light stimulation. We were able to observe rapid nuclear translocation of the biosensor upon blue light stimulation, which did not change ECFP/FRET ratio in both cytosol and nucleus (Figure 2(c)). Further quantification of intensity changes in ECFP and FRET channels verified the nuclear translocation of biosensor (Figure 2(d), Supplementary Figure 3a). Quantification of ECFP/FRET ratio during the light-induced nuclear translocation also showed no signifi-

cant change of ECFP/FRET ratio of the Fyn biosensor in both cytosol and nucleus (Figure 2(e)), suggesting that the biosensor molecules with high ECFP/FRET ratio being translocated from the cytosol into the nucleus did not cause increase of overall ECFP/FRET ratio in the nucleus, but rather these biosensors became dephosphorylated quickly by the higher levels of phosphatases present in the nucleus. To investigate whether change of ECFP/FRET could be due to change of the environment in the nucleus, we constructed a mutated version of Fyn biosensor-LINuS with a single tyrosine (Y) mutation to unphosphorylatable phenylalanine (F) in the biosensor peptide substrate [2]. We observed that the difference of FRET signals between cytosol and nucleus in the cells is less than 5% (Supplementary Figure 3b), suggesting that the change of FRET signal is mainly due to the de-phosphorylation action but not to the change in the environment in the nucleus. Taken together, our results clearly showed that the Fyn activity in nucleus is suppressed compared to that in the cytosol.

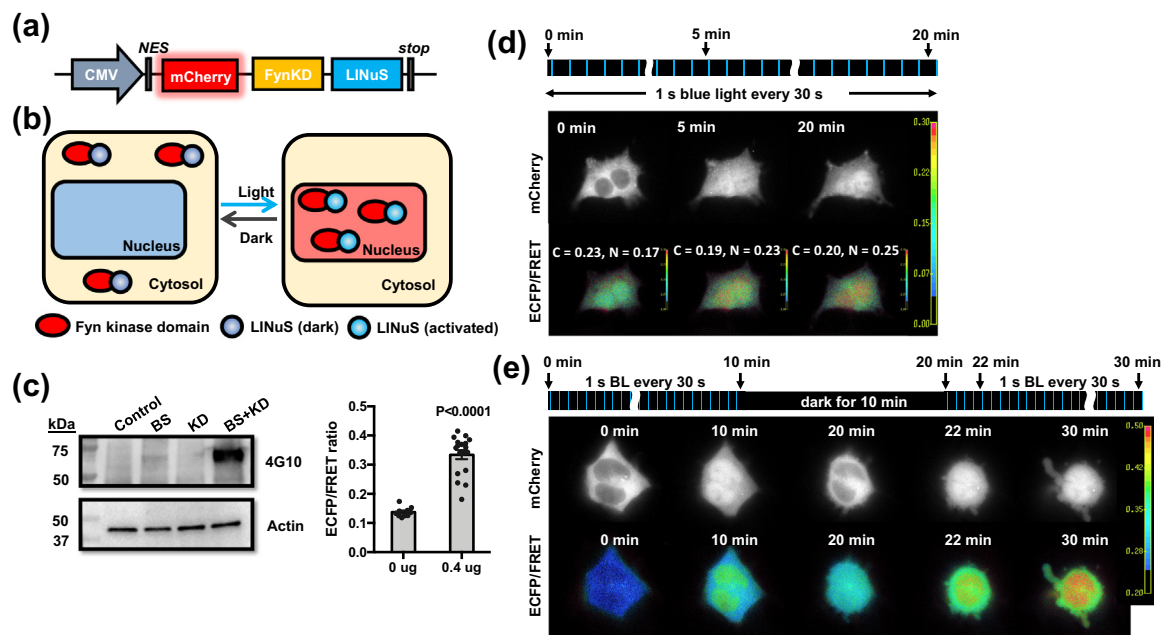


Figure 3. Light-inducible nuclear translocation of kinase can cause change of subcellular kinase activity. (a) Construct for light-inducible nuclear translocation of Fyn kinase domain (FynKD). The fused fluorescent protein mCherry is used as indicator of the subcellular localization of FynKD. A LINuS is fused at the C terminus of FynKD. (b) Scheme of light-inducible nuclear translocation of Fyn kinase domain. The Fyn kinase domain (with NES) is localized in cytosol before blue light stimulation (left). Upon blue light stimulation, LINuS will be activated and thus the kinase domain can be translocated into nucleus (right). Due to the reversibility of LINuS, kinase domain can be translocated back to cytosol without blue light. (c) Fyn kinase domain can phosphorylate Fyn biosensor and cause FRET change in live cells. Left, Western blots showing phosphorylation of Fyn biosensor by Fyn kinase domain. Control, non-transfected cells. BS, cells transfected with Fyn biosensor. KD, cells transfected with Fyn kinase domain. BS + KD: cells co-transfected with Fyn biosensor and Fyn kinase domain. 4G10, anti-phosphotyrosine antibody. Actin, protein loading control. Molecular weight markers were provided on the left side of each blot. Western blot results showing the amounts of biosensor in the total cell lysates and in immunoprecipitated samples were provided in Supplementary Figure 4. Right, HEK 293T cells were (i) transfected with Fyn biosensor only or (ii) co-transfected with biosensor and Fyn kinase domain. At 36 h after transfection, ECFP/FRET ratios in the cytosolic region of the transfected cells in these two groups were quantified and compared. Statistical analysis was performed with two-tailed Student's *t*-test. (d) Translocation of Fyn kinase domain can cause changes of cytosolic and nuclear Fyn activity. HEK 293T cells were co-transfected with NES-mCherry-FynKD-LINuS and Fyn biosensor. Blue light can trigger translocation of FynKD (mCherry channel) and cause increase and decrease of Fyn activity in nucleus and cytosol, respectively (reflected by the ECFP/FRET ratio of Fyn biosensor). The label "C" represents the ECFP/FRET ratio value in the cytosolic region of the cell shown in the image, and the label "N" represents the ECFP/FRET ratio value in the nuclear region. (e) The light-induced nuclear translocation of KD is reversible. HEK 293T cells transfected with NES-mCherry-FynKD-LINuS and Fyn biosensor were first stimulated with blue light, then rested in dark for 10 min, and re-stimulated.

Light-inducible nuclear translocation of Fyn kinase domain

Many studies have shown that kinase activities and phosphatase activities in the cells are at equilibrium to maintain homeostasis [21,22]. Our results with the light-inducible Fyn biosensor suggest that the equilibrium of Fyn kinase and the corresponding phosphatases in the cytosol is at a different state from that in the nucleus. However, it is still unclear that how the Fyn kinase activities in these cellular compartments are regulated separately. One possible mechanism is that there is controlled transportation or diffusion of Fyn kinase between cytosol and nucleus. Alternatively, it is possible that there is relatively free transpor-

tation or diffusion of Fyn kinase between cytosol and nucleus, but the Fyn kinase transported from cytosol into nucleus quickly becomes suppressed by the phosphatases present in the nucleus. The integration of FRET biosensor and LINuS technologies can provide a direct way of testing these hypotheses, enabling us to observe the change of Fyn kinase activity in the nucleus during its nuclear translocation from the cytosol. To this end, we have engineered a Fyn kinase domain with NES and LINuS, of which the nuclear translocation can be controlled with blue light (Figure 3(a)). With this construct, the kinase domain would stay outside of nucleus before blue light stimulation due to the NES fused at the N terminus. Upon blue light, the fused LINuS can be activated and

enable nuclear translocation of the kinase domain (Figure 3(b)), which has been verified to be able to phosphorylate the tyrosine substrate in the Fyn biosensor and cause ECFP/FRET change of the biosensor (Figure 3(c) and Supplementary Figure 4). Indeed, the nuclear translocation of the FynKD can be triggered by blue light illumination, as revealed by the fused mCherry fluorescent protein (Figure 3(d) and Supplementary Figure 5). More importantly, the Fyn biosensor expressed in the same cells clearly indicate that the nuclear translocation of Fyn kinase domain can lead to increase of the Fyn activity in the nucleus. Furthermore, we also observed that after nuclear translocation, the FynKD can translocate back to cytosol due to the reversibility of LINuS (Figure 3(e)). As we expected, this reversed translocation caused the decrease of nuclear Fyn kinase level, which can be re-stimulated with blue light. Thus, our results demonstrated that the nuclear translocation of Fyn kinase domain indeed can cause corresponding increase of Fyn activity level. Considering that different equilibrium states exist in the cytosol and nucleus, it is possible that no or very limited transportation or diffusion of active Fyn between nucleus and cytosol in the cells.

Discussion

The subcellular localization of Fyn kinase is well regulated by biochemical and biophysical signals [23–25]. Our results showed that nuclear Fyn activity is much lower in nucleus than in cytoplasm (Figure 1), suggesting different regulatory mechanisms for Fyn at different subcellular compartments. It will be interesting in the future to further explore the physiological importance of low Fyn activity in the nucleus. Indeed, abnormal Fyn activity has been reported to be related to multiple diseases such as cancers, neuronal degeneration diseases, and immunological disorders [26–29].

In this study, we applied LINuSs to study the change of Fyn biosensor during its nuclear translocation in single cells and demonstrated that Fyn showed much lower activity when translocated into nucleus compared to that in the cytosol (Figure 2). This dynamic tracking result is consistent with previous observations that the nuclear portion of Src family kinase biosensors shows significantly lower response than those from cytosol upon stimulation [9–11]. Despite this dramatic difference of Fyn activity in the cytosol and nucleus, it is unclear whether there is free transportation or diffusion of Fyn kinase between cytosol and nucleus at rest. Through fusing LINuS to the Fyn kinase domain, we clearly demonstrated that the FRET signals of Fyn biosensor in the nucleus are upregulated upon the light-induced nuclear translocation of Fyn kinase domain, suggesting that active Fyn kinase can cause more phosphorylation of nuclear Fyn biosensor (Figure 3). Based on these

results, we hypothesized that there is a barrier to constrain the transportation or diffusion of active Fyn from cytosol to nucleus in the cells.

The nuclear envelope (NE) can be one possible mechanism for blocking kinase diffusion between cytosol and nucleus [30]. Previous studies showed that inactivation of kinases, such as cyclin-dependent kinase 1 and polo-like kinase 1, and re-activation of protein phosphatases allow the reassembly of NE and nuclear structures [31]. Lamina proteins as the inner skeleton and support layer of NE need to be dephosphorylated to become polymerization and assembled [32]. Active phosphatases must be maintained at a high level inside the nucleus to maintain an intact NE structure to physically separate the nucleus and cytoplasm. This is consistent with the observations that our kinase biosensors typically showed low signals in the nucleus. As such, our light-inducible biosensor and kinase domain should allow the investigation of molecular regulations at different subcellular compartments, providing mechanistic insights underlying the functions of kinases in regulating cellular physiology.

Fyn kinase does not have an NLS, so it cannot directly bind to importin for the transportation across the nuclear pore [16,33]. Nevertheless, transient nuclear Fyn was observed in subsets of cells during a limited period of early development in zebrafish [16]. It has also been reported that GSK-3 β acts as the upstream regulatory molecule of Fyn. Under stressful conditions, such as antioxidants and xenobiotics, the activated GSK-3 β can phosphorylate Fyn at threonine residue(s), leading to nuclear localization of Fyn [34]. The major function of Fyn translocating into the nucleus is to downregulate Nrf2, which is an important transcription factor regulating anti-oxidative defensive gene expression. Specifically, nuclear Fyn can phosphorylate tyrosine 568 of Nrf2, resulting in the nuclear export and degradation of Nrf2 [17,34–36]. Nuclear Fyn can also negatively regulate NOX4 through phosphorylation of its Y566 and prevent cell apoptosis in cardiac remodeling [37]. Our light-activatable Fyn biosensors and kinases should provide new tools for the investigations of spatial separation of molecular regulations at different subcellular compartments. The results should allow the gain of more mechanistic insights underlying the functions of Fyn kinase in regulating cellular physiology and pathology.

Materials and Methods

Cloning

The cloning of cytosolic Fyn FRET biosensor without any specific subcellular-targeting signal was described in our previous work [9], and the sequence

of Fyn biosensor was provided in Supplementary Materials. The nuclear-targeting 3xNLS-Fyn biosensor was constructed by adding triple nuclear localized signal peptide "PKKKRKVED" at the C terminus of the cytosolic biosensor by using Sall site. DNA sequence for 3xNLS (3xPKKKRKVED): CCCAAGAAGAAACGCAAAGTCGAGGATCCAAAG AAGAAAAGGAAGGTTGAAGACCCCAAG AAAAAGAGGAAGGTGGATGGG. Primers for introducing the 3xNLS are provided in Supplementary Materials.

The construct NES-FynBS-LINuS was generated by Gibson assembly of fragments NES-FynBS and LINuS. The construct NES-mCherry-FynKD-LINuS was generated by Gibson assembly (New England Biolab) of fragments NES-mCherry, FynKD, and LINuS following the manufacturer's instructions. DNA fragments were amplified by PCR using Q5 DNA polymerase (New England Biolab). Construct and primer sequences are provided in Supplementary Materials. Primers were synthesized by Integrated DNA Technologies. PCR products were purified by agarose gel electrophoresis method (Zymo Research, D4001). Purified DNA fragments were cloned into pcDNA3.1 vector (digested with BamHI and EcoRI) using Gibson Assembly. All constructs were confirmed by Sanger sequencing (Genewiz).

Reagents and cell culture

Fetal bovine serum, DMEM, L-glutamine, penicillin/streptomycin, and sodium pyruvate were purchased from Gibco. Phosphate-buffered saline was prepared from phosphate-buffered saline powder (Sigma-Aldrich). Cell lines MEF and HEK 293T (human embryonic kidney 293T) was from American Tissue Culture Collection (ATCC, Manassas, VA), with the authentication and verification of the absence of mycoplasma contamination. Cells were cultured in ATCC-recommended conditions in a humidified incubator of 95% air and 5% CO₂ at 37 °C.

Live cell imaging of Fyn biosensor

Fyn biosensor and the nucleus-targeting 3xNLS-Fyn biosensor constructs were transfected into MEF cells with Lipofectamine 3000 (Invitrogen). Transfected cells were cultured in low serum (0.5%) medium for 24–36 h and then seeded onto fibronectin-coated glass-bottom dishes for 12–16 h before FRET imaging. The FRET imaging of Fyn biosensor was described in our previous work [9]. Briefly, live cell imaging was performed at 37 °C with a Nikon Ti inverted microscope equipped with a ×100/NA 1.4 objective, a cooled charge-coupled device camera (Cascade 512 B, Photometrics), a 420DF20 excitation filter, a 450DRLP dichroic mirror, and two emission filters 480DF30 for ECFP and 535DF35 for YPet. Time-lapse images were acquired by Meta-

Morph 7.8.6.0 software (Molecular Devices, Sunnyvale, CA). The ECFP and FRET images were processed and quantified by image analysis software package *Fluocell* (<http://github.com/lu6007/fluocell>) [38].

The cytosolic and nuclear portions of biosensor signal were quantified by MetaFluor 6.2 software (Molecular Devices, Sunnyvale, CA) by selecting region of interest in cytosol and nucleus specifically.

Live cell imaging of light-activated protein nuclear translocation

A total of 0.8 million HEK 293T cells were seeded in a 3.5-mm dish, transfected at 70% confluency with 2.0 µg of NES-FynKD-LINuS or NES-FynBS-LINuS, and cultured in dark. Twenty-four hours after transfection, medium was replaced with phenol red free DMEM with 10% fetal bovine serum for imaging. Live cell imaging was performed at 37 °C with a Nikon Ti inverted microscope equipped with a ×100/NA 1.4 objective and a cooled charge-coupled device camera (Cascade 512 B) using MetaFluor. The dynamic nuclear translocation of mCherry in the fusion protein was monitored by an excitation filter 580/20 nm and an emission filter 630/20 nm, together with a dichroic mirror 595 nm (Chroma). Blue light stimulation was delivered with an excitation filter 465/30 nm.

Data analysis

Imaging analysis was performed using MetaFluor 6.2 software (Universal Imaging) or ImageJ. The numbers of samples and experiments in each figure are indicated in the corresponding figure legend. Error bars are shown as standard error of the mean (Figure 2(d)) or standard deviation (Figure 3(c)). For statistical analysis, two-tailed Student's *t*-test was applied (Figure 3(c)).

Acknowledgments

This work was supported in part by grants from National Institutes of Health (HL121365, GM125379, GM126016, CA204704, CA209629 (Y. Wang); CA238042 (P. Ghosh)), the Galvanizing Engineering in Medicine program under the Institute of Engineering in Medicine, and Altman Clinical and Translational Research Institute at UC San Diego (Y. Wang).

Author Contributions

Z.H., Q.P., and Y.W. designed research; Z.H. and O.M. performed research; Z.H., Q.P., and O.M.

analyzed data; Z.H., Q.P., O.M., S.L., and Y.W. wrote the paper.

Declaration of Interest

Yingxiao Wang and Shaoying Lu are scientific co-founders of Cell E&G Inc. However, these financial interests do not affect the design, conduct, or reporting of this research.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jmb.2020.03.015>.

Received 2 December 2019;

Received in revised form 27 February 2020;

Accepted 9 March 2020

Available online xxxx

Keywords:

Fyn kinase;

LINuS;

light-inducible kinase;

FRET biosensor;

live cell imaging

Abbreviations used:

FRET, fluorescence resonance energy transfer; NLS, nuclear localization signal; Nrf2, nuclear factor erythroid-2-related factor 2; FynBS-LINuS, LINuS at C terminus of Fyn biosensor; LINuS, light-inducible nuclear localization signal; MEF, mouse embryonic fibroblast; PDGF, platelet-derived growth factor; NE, nuclear envelope.

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