Bioconjugate Chemistry

Article

Cite This: *Bioconjugate Chem.* 2019, 30, 826−832

pubs.acs.org/bc

Genetically Encoded FapR-NLuc as a Biosensor to Determine Malonyl-CoA in Situ at Subcellular Scales

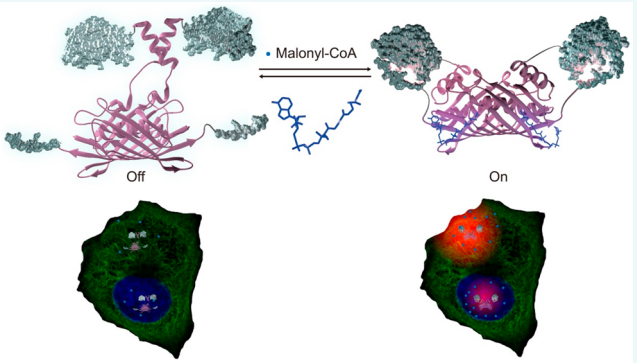
Yipeng Du,[†] Hao Hu,[†,‡] Xiaoxia Pei,[†,‡] Kang Du,[†,‡] and Taotao Wei*[,†,‡]

[†]National Laboratory of Biomacromolecules, Institute of Biophysics, Chinese Academy of Sciences, Beijing 100101, China

[‡]University of Chinese Academy of Sciences, Beijing 100049, China

Supporting Information

ABSTRACT: Malonyl-CoA is one of the key metabolic intermediates in fatty acid metabolism as well as a key player in protein post-translational modifications. Detection of malonyl-CoA in live cells is challenging because of the lack of effective measuring tools. Here we developed a genetically encoded biosensor, FapR-NLuc, by combining a malonyl-CoA responsive bacterial transcriptional factor, FapR, with an engineered luciferase, NanoLuciferase (NLuc). FapR-NLuc specifically responds to malonyl-CoA and enables the rapid detection of malonyl-CoA at the micromolar level. More importantly, it is reflective of the fluctuations of malonyl-CoA in live cells. Upon being targeted to subcellular compartments, this biosensor can detect the changes of malonyl-CoA in situ within organelles. Thus, FapR-NLuc can potentially be used as a tool to study the kinetics of malonyl-CoA in live cells, which will shed light on the underlying mechanisms of malonyl-CoA-mediated biological processes.



INTRODUCTION

Malonyl-CoA was originally identified as a metabolic intermediate involved in the biosynthesis pathway of fatty acids.[1] Twenty years later, investigators recognized malonyl-CoA as the key molecule in regulating fatty acid oxidation by reversibly inhibiting mitochondrial carnitine palmitoyltransferase 1 (CPT1) and controlling the entrance of fatty acids into the mitochondria.[2] It has recently been reported that malonyl-CoA can spontaneously modify proteins to form a new type of post-translational modification, named malonylation, which plays potential roles in multiple metabolic pathways.[3−6] We previously found that the level of protein malonylation was elevated in liver tissues of type 2 diabetic animals[5] and that reducing malonylation attenuated metabolic abnormalities in ob/ob mice.[7] Thus, malonyl-CoA is a crucial biomolecule and its kinetics within tissues and cells requires extensive investigation. However, accurate measurement of malonyl-CoA is still limited due to its relatively small molecular weight, low abundance, and short half-life nature.

Since malonyl-CoA is hydrophilic and can readily be extracted from cultured cells or tissues, several methods have been developed to measure it in vitro. A spectrophotometric method was first used to measure malonyl-CoA in liver extracts by detection of NAPDH during fatty acid synthesis.[8] A more sensitive radioisotopic assay was then developed by measuring incorporation of labeled acetyl-CoA into palmitic acid.[9] Besides these indirect assays, malonyl-CoA can be detected directly by high-performance liquid chromatography (HPLC) or gas chromatography-coupled mass spectrometry (GC-MS) methods.[10−12] Although the assays described above have been successfully applied to measuring concentrations of malonyl-CoA in various mammalian tissues under normal, fasted, high-fat diet, or refeed conditions, they are unsuitable to detect malonyl-CoA in situ in live cells or tissues.

An efficient approach to measuring small molecules in live cells is the development of various biosensors. For example, circularly permuted fluorescent protein (cpFP)-based sensors have been generated for the detection of intracellular NADH.[13] Fluorescence resonance energy transfer (FRET)-based sensors have been developed for visualizing ATP levels inside single live cell.[14] Recently, genetically encoded fluorescent sensors have been developed for detection of dopamine and acetylcholine.[15,16] The key for the successful development of a biosensor for small bioactive molecules is finding its sensor proteins. For instance, bacterial transcription regulation factor Rex is a sensor protein of NADH, while the ε subunit of bacterial F_0F_1-ATP synthase can bind and sense ATP.[13,14] To develop a biosensor for malonyl-CoA, it is necessary to find the proper sensor protein for malonyl-CoA. FapR, a bacterial transcriptional factor involved in lipid biosynthesis, has been shown to specifically and reversibly bind malonyl-CoA.[17] FapR initially binds its bacterial DNA operator as a homodimer and

Received: December 24, 2018
Revised: January 9, 2019
Published: January 10, 2019

DOI: 10.1021/acs.bioconjchem.8b00920
Bioconjugate Chem. 2019, 30, 826−832

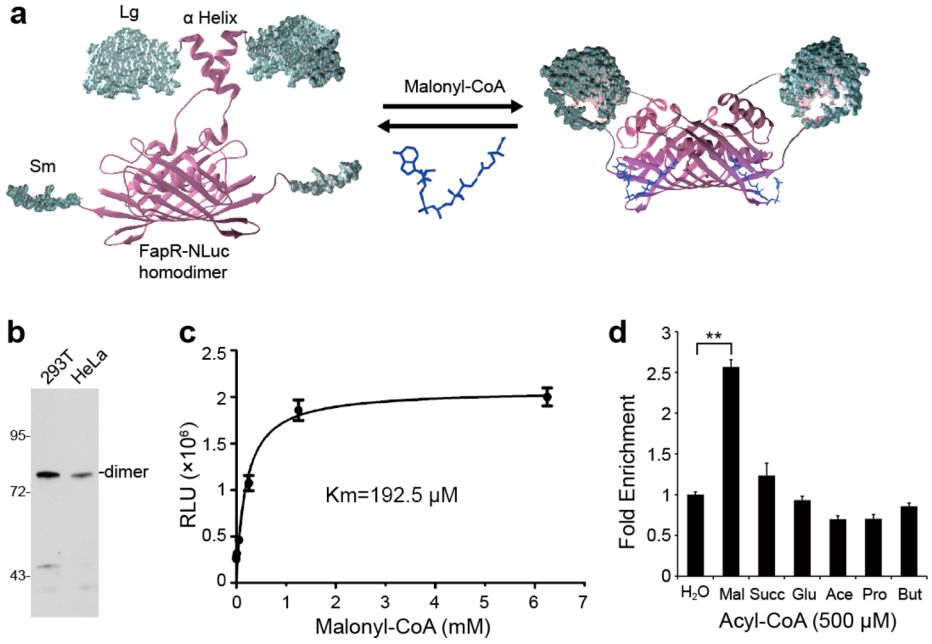


Figure 1. Generation and characterization of FapR-NLuc in vitro. (a) Schematic diagram for FapR-NLuc sensing malonyl-CoA. FapR-NLuc exists as homodimer. Each FapR-NLuc monomer is composed of one FapRΔ43 protein linked by large (Lg) and small (Sm) subunits of NanoLuciferase. Malonyl-CoA binding induces conformational changes, which bring the two subunits of nanoluciferase together and recovery of luciferase activity. (b) Flag-tagged FapR-NLuc expressed in cultured mammalian cells existed as dimers in native-PAGE. The calculated molecular weight of monomer Flag-tagged FapR-NLuc was about 40 kDa. Anti-Flag tag antibody was used for the detection. (c) Michaelis−Menten plot for dose−response of FapR-NLuc to malonyl-CoA in vitro. 1 μg/mL purified FapR-NLuc proteins were used in each reaction. Relative luminescence units (RLU) were measured in 96-well microtiter plates. Solid line represents fit to Michaelis−Menten equation. The calculated K_m was 192.5 μM. All reactions were repeated three times. (d) Response of FapR-NLuc to various types of acyl-CoA including Mal: malonyl-CoA; Succ: succinyl-CoA; Glu: glutaryl-CoA; Ace: acetyl-CoA; Pro: propionyl-CoA; But: butyryl-CoA. **: p-value ≤0.01.

represses the expression of a group of genes involved in lipid synthesis.[17] However, when the intracellular concentration of malonyl-CoA increases, malonyl-CoA gradually binds to FapR dimers, triggering great intramolecular conformational changes and releasing the FapR dimer from the DNA operator.[18] Relieved from FapR, genes repressed by FapR start to undergo transcription. Malonyl-CoA biosensors integrated with FapR operator with reporter genes encoding EGFP, RFP, or luciferases, have been generated and tested in *Escherichia coli*[19] and *Saccharomyces cerevisiae*.[20] By using a similar strategy, a malonyl-CoA biosensor has been developed in mammalian cells.[21] These biosensors translate levels of malonyl-CoA to expression intensity of reporter genes and thus solved the problem of malonyl-CoA detection in live cells; however, no approach has been developed that can measure malonyl-CoA directly at subcellular scales, such as the cytosol, nuclei, and mitochondria.

In the present study, we applied a different strategy for malonyl-CoA biosensor development. We generated a fusion protein FapR-NLuc, which contains FapR and an engineered luciferase NanoLuciferase (NLuc). The luciferase activity of FapR-NLuc was correlated with the malonyl-CoA concentration. We further demonstrated that this new biosensor was suitable for the measurement of malonyl-CoA in vitro and in mammalian cells. More importantly, when it was targeted to different subcellular compartments, it could detect the dynamics of malonyl-CoA concentrations in situ in distinct subcellular compartments, including the cytosol, nuclei, and mitochondria.

RESULTS AND DISCUSSION

To detect malonyl-CoA in living cells, we started with the bacterial malonyl-CoA-binding protein FapR. Binding of malonyl-CoA to FapR induces a global structural change of FapR from a loose to a tense state.[18] We constructed a fusion protein containing FapR and NLuc. NLuc is an engineered luciferase that is composed of two subunits, a 1.3 kDa small subunit and an 18 kDa large subunit.[22] The two subunits associate weakly and little luciferase activity is detected when they are apart. However, when they are put together, they assemble into a functional luciferase with high activity. Based on the properties of the FapR and NLuc proteins, we hypothesized that binding of malonyl-CoA to FapR would be linked to activity of NLuc if FapR and NLuc were properly fused. Structural analysis revealed that FapR contains an N-terminal DNA-binding domain, an α-helix linker, and a C-terminal malonyl-CoA-binding domain. The α-helix stretches out the globular malonyl-CoA-binding domain in the absence of malonyl-CoA.[18] Binding to malonyl-CoA triggers conformational change of the FapR protein, which makes the α-helix shrink back, pulling the N-terminal of the α-helix and the C-terminal of FapR closer (Figure 1a). To take advantage of these attributes, the FapR with a deletion of the DNA-binding domain was fused with the small and large subunits of NLuc on its N- and C-terminal (Figure 1a). The constructed plasmid, named FapR-NLuc, was codon optimized for mammalian expression. FapR-NLuc was readily expressed in HEK293T and HeLa cells and most of the expressed proteins existed as dimers according to molecular weight in a native PAGE gel (Figure 1b).

DOI: 10.1021/acs.bioconjchem.8b00920
Bioconjugate Chem. 2019, 30, 826−832

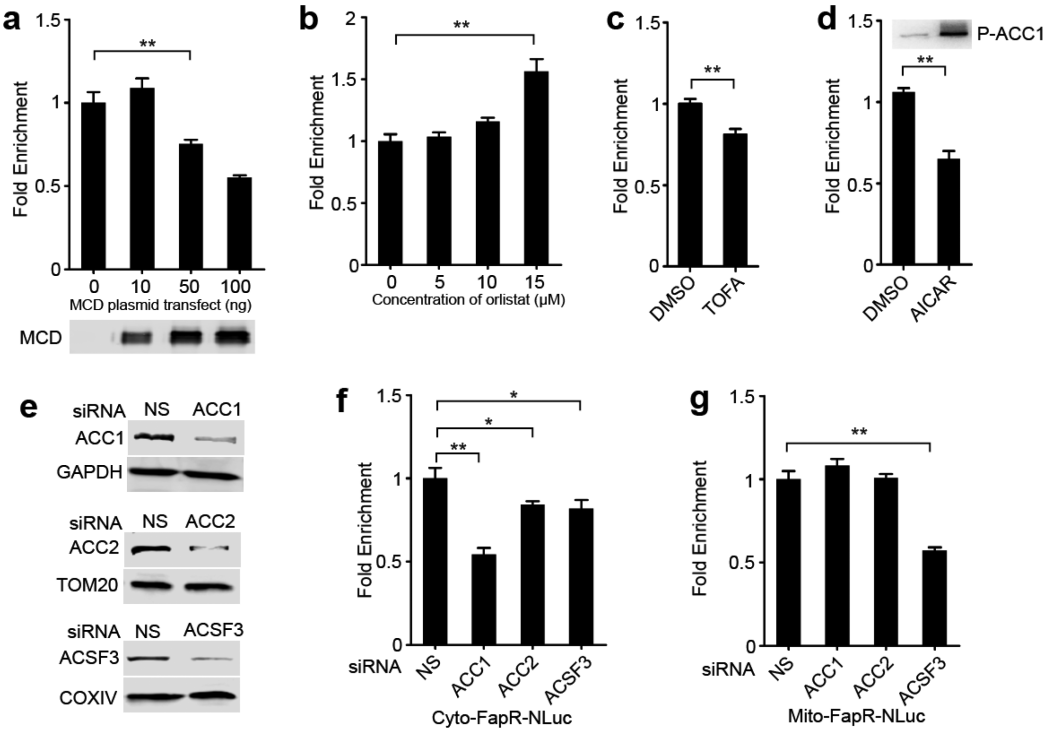


Figure 2. Characterization of FapR-NLuc in cells. (a) Coexpression of equal amounts of FapR-NLuc plasmids (100 ng) with increased amounts of MCD plasmids (0−100 ng) in HeLa cells. Luminescence intensity was measured 24 h after transfection. Expression levels of FapR-NLuc and MCD protein were determined by Western blot. **: p-value ≤0.01. (b) FapR-NLuc was expressed in HeLa cells. Various concentrations of orlistat was added 12 h after transfection. 24 h after orlistat treatment, luminescence intensity was measured. (c and d) Treatment of TOFA (ACC1 inhibitor) and AICAR (ACC1 kinase activator) on HMCC97L cells. FapR-NLuc plasmids were first transfected for 24 h, TOFA or AICAR was then added for another 3 h. Luminescence were immediately measured and normalized as fold enrichment. P-ACC1: Phospho-ACC1 (Ser80). (e) Knockdown of ACC1, ACC2, and ACSF3 by siRNA. Detection of interference effects by immune blotting to ACC1, ACC2, and ACSF3 antibodies. GAPDH, TOM20, and COXIV were used to control loadings. NS: nonspecific. (f and g) Detection of luminescence by cytosolic (f) or mitochondrial (g) FapR-NLuc after knocking down of ACC1, ACC2, or ACSF3. *: p-value ≤0.05. **: p-value ≤0.01.

To determine the response of FapR-NLuc to malonyl-CoA in vitro, we purified the FapR-NLuc proteins from HEK293T cells and incubated equal amounts of FapR-NLuc with increased concentrations of malonyl-CoA. We observed a dramatic increase in relative luminescence (RLU) within the micromolar range of malonyl-CoA, reaching its maximum value at about 2 mmol (Figure 1c). When the values were regressed to a Michaelis−Menten curve, the calculated K_m was 192.5 μM. The K_d of purified FapR protein (without the DNA binding domain) for malonyl-CoA determined by isothermal titration calorimetry (ITC) was 7.1 μM.[17] The K_m for purified NLuc using furimazine as a substrate was 10 μM.[22] The differences of K_m and K_d between FapR-NLuc and FapR or NLuc proteins indicates that the recombinant FapR-NLuc biosensor has completely distinct properties.

To detect the specificity of FapR-NLuc, six different types of acyl-CoA derivatives, namely, malonyl-CoA, succinyl-CoA, glutaryl-CoA, acetyl-CoA, propionyl-CoA, and butyryl-CoA, were incubated with purified FapR-NLuc protein and the RLU was measured (Figure 1d). Our results indicate that the FapR-NLuc response is specific to malonyl-CoA, but not other acyl-CoA derivatives. This is consistent with previous reports that FapR binds directly and specifically to malonyl-CoA.[17]

To verify that FapR-NLuc is responsive to malonyl-CoA in mammalian cells, we coexpressed FapR-NLuc with malonyl-CoA decarboxylase (MCD) in HeLa cells. MCD could lower the content of cellular malonyl-CoA by catabolizing malonyl-CoA to acetyl-CoA. When the expression level of FapR-NLuc showed little change, the luminescence decreased as the expression of MCD elevated (Figure 2a). Additionally, we treated cells with Orlistat which is an inhibitor of fatty acid synthase (FASN). FASN uses malonyl-CoA as basic units for fatty acid synthesis. Inhibition of FASN increases the intracellular concentration of malonyl-CoA. We found that Orlistat treatment increased luminescence in FapR-NLuc expressing HeLa cells (Figure 2b). We also treated cells with 5-(tetradecyloxy)-2-furoic acid (TOFA), which is an inhibitor of acetyl-CoA carboxylase (ACC). ACC catalyzes carboxylation of acetyl-CoA to produce most of the malonyl-CoA in the cytosol. Consistently, treatment with TOFA reduced luminescence in FapR-NLuc-transfected cells, which indicated a decrease of malonyl-CoA (Figure 2c). Adenosine 5′-monophosphate (AMP)-activated protein kinase (AMPK) has been reported to phosphorylate and inhibit activity of ACC. When cells were treated with AMPK activator 5-aminoimidazole-4-carboxamide1-β-D-ribofuranoside (AICAR), both increased phosphorylation of ACC1 and decreased luminescence were observed (Figure 2d). To avoid the possibility that an increase in luminescence was caused by the change of FapR-NLuc-expressing levels, we determined levels of FapR-NLuc by Western blotting and found that there was little influence of MCD coexpression, orlistat, TOFA, and AICAR treatment on expression of FapR-NLuc (data not shown). These results suggest that FapR-NLuc can reliably detect levels of malonyl-CoA in mammalian cells.

DOI: 10.1021/acs.bioconjchem.8b00920
Bioconjugate Chem. 2019, 30, 826−832

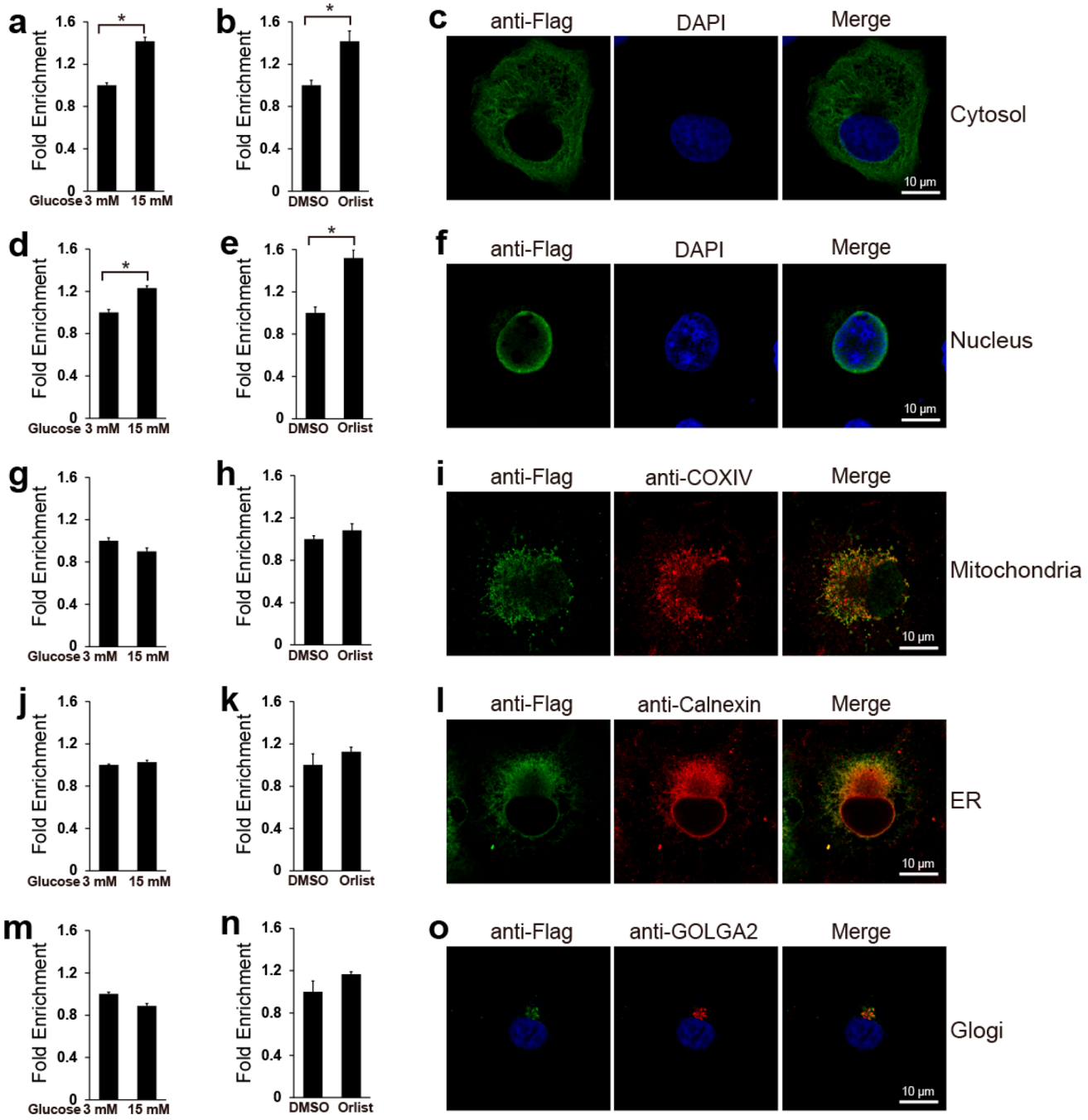


Figure 3. Detection of malonyl-CoA dynamics by FapR-NLuc in different organelles. COS7 cells were transfected with FapR-NLuc plasmids targeting to cytosol (c), nuclei (f), mitochondria (i), ER (l), and Golgi apparatus (o). Response of FapR-NLuc to glucose and orlistat treatment in cytosol (a and b), nucleus (d and e), mitochondria (g and h), ER (j and k), and Golgi apparatus (m and n). Nuclei were stained with DAPI. Makers for mitochondria, ER, and Golgi apparatus were COXIV, Calnexin, and GOLGA2, respectively. *: p-value $\leq$0.05.

To further characterize the ability of FapR-NLuc in sensing intracellular malonyl-CoA, particularly in different cellular compartments, FapR-NLuc was expressed in the cytosol and mitochondria. Levels of malonyl-CoA were downregulated in the cytosol or mitochondria by knocking down ACC1, ACC2, or acyl-CoA synthetase family member 3 (ACSF3) (Figure 2e). ACC1 is a cytosolic protein which is the main source of cytosolic malonyl-CoA for the synthesis of fatty acids. ACC2 interacts with the outer membrane of mitochondria and functions as a fatty acid oxidation regulator by generating local malonyl-CoA. ACSF3 is predominantly found in the mitochondrial matrix and synthesizes most of the malonyl-CoA molecules there. When we targeted FapR-NLuc to the cytosol and measured the luminescence, we detected a significant decrease in luminescence in siACC1 cells. Luminescence were also slightly affected in siACC2 and siACSF3 cells (Figure 2f). When we targeted FapR-NLuc to the mitochondrial matrix and measured the luminescence, we detected a marked decrease inluminescence in siACSF3, but not siACC1 and siACC2 cells (Figure 2g). These results suggested that FapR-NLuc can accurately detect local levels of malonyl-CoA in live cells at subcellular scales.

To further validate whether FapR-NLuc can be used to examine the variability of malonyl-CoA in other subcellular

DOI: 10.1021/acs.bioconjchem.8b00920
Bioconjugate Chem. 2019, 30, 826−832

compartments, FapR-NLuc was localized to different organelles by fusing with proper localization signals and cells were treated with high concentrations of glucose or Orlistat to alter the intracellular levels of malonyl-CoA. Luminescence from cells with cytosol localized FapR-NLuc was elevated when treated with 15 mM glucose or 15 μM orlistat (Figure 3c), which was consistent with cytosolic synthesis of malonyl-CoA (Figure 3a,b). Interestingly, when FapR-NLuc was targeted to the nuclei (Figure 3f), luminescence was similarly elevated (Figure 3d,e). This suggests that malonyl-CoA, originally synthesized in the cytosol, can readily be transported to the nuclei and that the concentrations of malonyl-CoA in the cytosol and nuclei are comparable. It has been reported that histones and many nuclear proteins contain malonylated lysine,[23] which further support the existence of malonyl-CoA in the nucleus. However, when FapR-NLuc was targeted exclusively to mitochondria, endoplasmic reticulum (ER), or Golgi apparatus, we observed little change in luminescence under the same conditions as described above (Figure 3g–o).

It has been estimated from in vitro measurements that the concentration of malonyl-CoA in mammalian cells is in the micromolar range.[24] However, in vivo concentrations of malonyl-CoA have not been reported because of the lack of biological tools. The biosensor used in this study responds to malonyl-CoA at the micromolar range, with a K_m of 192.5 μM. The dynamic response of the biosensor to MCD coexpression, orlistat, TOFA, and AICAR treatment in cultured mammalian cells further confirms the previous estimate of malonyl-CoA concentration in cells.

It has long been recognized that malonyl-CoA is synthesized in the cytosol by ACC1/2. Recent studies demonstrating that malonyl-CoA can also be generated by a malonyl-CoA synthetase ACSF3 in the mitochondria suggest the wide distribution of malonyl-CoA in other subcellular compartments.[25] Unlike previous transcription-based biosensors,[21] the FapR-NLuc biosensor could be targeted to different organelles, enabling the local detection of malonyl-CoA at subcellular scales. In our study, we found that the nucleus-localized FapR-NLuc response was similar to that of cytosolic FapR-NLuc, indicating that concentrations of malonyl-CoA in the nuclei and cytosol are comparable. Interestingly, multiple lysine malonylation sites have been reported on histones.[23] As the group donor for the malonylation reaction, the role of malonyl-CoA in the nuclei and the possible involvement in epigenetic regulation remains to be understood. We also observed decreased cytosolic malonyl-CoA in siACSF3 cells, in which the malonyl-CoA synthesis within the mitochondria was blocked. We speculate that there might be an unknown malonyl-CoA transporter or a shuttle mechanism, which transports malonyl-CoA from cytosol to the mitochondria and thus compensate the reduced malonyl-CoA in the mitochondria.

Little or no response of the FapR-NLuc biosensor to glucose or orlistat treatment was observed in the mitochondria, ER, and Golgi apparatus, suggesting the steady-state of malonyl-CoA concentration to these treatments. It is known that the mitochondria and ER are the compartments for fatty acid elongation, and large amounts of malonyl-CoA are necessary for the biosynthesis of fatty acids. Thus, concentrations of malonyl-CoA in these organelles must be tightly regulated. With FapR-NLuc as a novel tool to study the kinetics of malonyl-CoA in situ at subcellular scales, we will reveal the underlying mechanisms of malonyl-CoA-mediated biological processes within the organelles.

EXPERIMENTAL PROCEDURES

Sensor Construction. FapR cDNA was codon optimized for mammalian expression (GenScript). FapRΔ43, the large (Lg) and small (Sm) subunit of NLuc (Promega), was PCR amplified and cloned into the GV141-3 × FLAG vector (Genechem; vector information and map are shown in Figure S1.) at the EcoR1 and Kpn1 restriction sites. The structure and sequence of the FapR-NLuc biosensor are shown in Figure S2. For subcellular targeting, the original cytosol-expressed FapR-NLuc cDNA was fused with various targeting sequences. For mitochondrion targeting, the peptide sequence of "MSVLTPLLLRGLTGSARRLPVPRAK" from COX8A was fused to the N-terminal of FapR-NLuc. For ER targeting, the peptide sequence of "KLSLVAAMLLLLSAARA" from GRP78 was fused to the N-terminal of FapR-NLuc. For nucleus targeting, the peptide sequence of "PAAKRVKLD" from c-Myc was fused to the C-terminal of FapR-NLuc. For Golgi apparatus targeting, "VGRNSAIAAGVCGALFIGYCIYFDSSSSSDPNFK" was fused to the N-terminal of FapR-NLuc. A mammalian expressing vector for MCD was purchased from Vigene Biosciences. All of the constructs were sequenced and validated for correct expression.

Purification of FapR-NLuc Biosensor. The FapR-NLuc cytosol expression plasmids were transfected into HEK293T or HeLa cells with Lipofectamine 2000 (Thermo Fisher). 24 h after transfection, cells were harvested and incubated in lysis buffer (50 mM Tris HCl, pH 7.4, with 150 mM NaCl, 1 mM EDTA, and 1% (v/v) Triton X-100) for 20 min on a shaker. The cell lysate was centrifuged for 10 min at 12,000 g. The supernatant was immunoprecipitated with anti-FLAG M2 affinity resin (Sigma-Aldrich). The FapR-NLuc proteins were eluted with 150 ng/μL 3 × FLAG peptide. Protein concentrations were estimated by comparison with proteins standards on Coomassie blue stained SDS-PAGE gel. Purified FapR-NLuc proteins were immediately used for experiments.

Assay of the Biosensor Activity. For in vitro biosensor activity measurements, FapR-NLuc proteins with a final concentration of 0.1 μg/mL, and malonyl-CoA (Sigma-Aldrich; M4263) with a series of concentrations (0.1, 1, 10, 50, 250, 1250, and 6250 μM) were first added into the assay buffer. Furimazine (Promega) was then added, mixed, and incubated for 10 min. Luminescence intensity was measured by EnVision Multimode Plate Reader (PerkinElmer). Each measurement was repeated five times. For in vitro specificity experiments, each type of acyl-CoA, including malonyl-CoA, succinyl-CoA (Sigma-Aldrich; S1129), glutaryl-CoA (Sigma-Aldrich; G9510), acetyl-CoA (Sigma-Aldrich; A2056), propionyl-CoA (Sigma-Aldrich; P5397), and butyryl-CoA (Sigma-Aldrich; B1508), with a concentration of 500 μM was added into the assay buffer. Luminescence was measured 10 min after the addition of furimazine. Each measurement was repeated at least 3 times. Fold enrichment was calculated as (sample luminescence − background luminescence)/(control luminescence − background luminescence). For measurements of malonyl-CoA in live cells, 100 ng samples of FapR-NLuc plasmid and different amounts of MCD plasmid (0, 10, 50, 100 ng) were cotransfected into HeLa cells in 96-well plates. 24 h after transfection, luminescence was measured for each well, 10 min after furimazine was added. For Orlistat (Sigma-Aldrich; O4139) treatment, various concentrations of Orlistat (0, 5, 10,

DOI: 10.1021/acs.bioconjchem.8b00920
Bioconjugate Chem. 2019, 30, 826–832

and 15 μM) were added into HeLa cells 12 h after FapR-NLuc transfection. 24 h later, luminescence was measured. For 5-(tetradecyloxy)-2-furoic acid (TOFA; Sigma-Aldrich; T6575) or 5-aminoimidazole-4-carboxamide1-β-D-ribofuranoside (AICAR) (Sigma-Aldrich; A9978) treatment, 20 μM TOFA or 3 mM AICAR was added into HMCC97L cells 24 h after FapR-NLuc transfection. Three hours later, luminescence was measured. Phospho-ACC1 (Ser80) (Absin; Abs131046) was blotted to verify the effects of AICAR. For glucose treatment, cells were incubated with Dulbecco's modified Eagle medium (Thermo Fisher) containing 3 mM glucose overnight and then treated with Hanks' balanced salt solution (HBSS) containing 3 mM glucose for 2 h. Cells were then washed with HBSS containing 3 mM or 15 mM glucose. After culturing for another 1 h, luminescence was measured.

Immunofluorescence. Immunofluorescence experiments for subcellular localization validation of each FapR-NLuc targeting were performed as previously described.[26] Markers for the mitochondria, ER, and Golgi apparatus were anti-COXIV (ProteinTech; 11242-1-AP), anti-Calnexin (ProteinTech; 10427-2-AP), and anti-GOLGA2 (ProteinTech; 11308-1-AP), respectively.

Knockdown of ACC1, ACC2, and ACSF3 Genes by Small Interference RNA (siRNA). The sequences of siRNA (GenePharma) used for knockdown of genes were a pool of three sequences targeting different sites of mRNA. The siRNA sequences for ACC1 were 5-GCUUCUACUUUCUGGAAUUTT-3, 5-GCUCAUACACUUCUGAAUATT-3, and 5-GCAGCUAUGUUCAGAGAAUTT-3. The siRNA sequences for ACC2 were 5-GCUGAAGGAUAUCCGGCUUTT-3, 5-GCACCUGCGUGGUAGAAUUTT-3, and 5-GCGUUCAGAUCAUGCAUUATT-3. The siRNA sequences for ACSF3 were 5-CCACACGUACAGGGAGCUUTT-3, 5-GCGCUAACGAUGCCUCCUATT-3, and 5-GGAGGAACAAGGGCGCCAUTT-3. The siRNA sequence for the nonspecific control was 5-UUCUCCGAACGUGUCACGUTT-3. Transfection was performed using standard procedures.[27] Immunoblotting to ACC1 (ProteinTech; 21923-1-AP), ACC2 (Absin; Abs137457), and ACSF3 (ProteinTech; 25484-1-AP) antibodies were performed to determine interference results. GAPDH (ProteinTech 60004-1-Ig), TOM20 (Biosciences; 612278), and COXIV antibodies were used to control loading for cytosol and mitochondrial subcellular compartments. 24 h after siRNA transfection, FapR-NLuc plasmids were transfected. Luminescence was measured after another 24 h.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.bioconjchem.8b00920.

Sequence of FapR-NLuc biosensor (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: weitt@ibp.ac.cn.

ORCID

Yipeng Du: 0000-0002-0000-623X

Taotao Wei: 0000-0002-5145-6222

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported by grants from the National Key R&D Program of China (2017YFA0205501), the Strategic Priority Research Programs (Category A) of the Chinese Academy of Sciences (XDA12030207), the National Natural Science Foundation of China (31671175, 31771257), and the National Laboratory of Biomacromolecules.

■ REFERENCES

(1) Wakil, S. J. (1962) Lipid metabolism. *Annu. Rev. Biochem. 31*, 369−406.

(2) McGarry, J. D., and Foster, D. W. (1980) Regulation of hepatic fatty acid oxidation and ketone body production. *Annu. Rev. Biochem. 49*, 395−420.

(3) Peng, C., Lu, Z., Xie, Z., Cheng, Z., Chen, Y., Tan, M., Luo, H., Zhang, Y., He, W., Yang, K., et al. (2011) The first identification of lysine malonylation substrates and its regulatory enzyme. *Mol. Cell. Proteomics 10*, M111.

(4) Colak, G., Pougovkina, O., Dai, L., Tan, M., Te Brinke, H., Huang, H., Cheng, Z., Park, J., Wan, X., Liu, X., et al. (2015) Proteomic and biochemical studies of lysine malonylation suggest its malonic aciduria-associated regulatory role in mitochondrial function and fatty acid oxidation. *Mol. Cell. Proteomics 14*, 3056−3071.

(5) Du, Y., Cai, T., Li, T., Xue, P., Zhou, B., He, X., Wei, P., Liu, P., Yang, F., and Wei, T. (2015) Lysine malonylation is elevated in type 2 diabetic mouse models and enriched in metabolic associated proteins. *Mol. Cell. Proteomics 14*, 227−236.

(6) Nishida, Y., Rardin, M. J., Carrico, C., He, W., Sahu, A. K., Gut, P., Najjar, R., Fitch, M., Hellerstein, M., Gibson, B. W., et al. (2015) SIRT5 regulates both cytosolic and mitochondrial protein malonylation with glycolysis as a major target. *Mol. Cell 59*, 321−332.

(7) Du, Y., Hu, H., Qu, S., Wang, J., Hua, C., Zhang, J., Wei, P., He, X., Hao, J., Liu, P., et al. (2018) SIRT5 deacylates metabolism-related proteins and attenuates hepatic steatosis in ob/ob mice. *EBioMedicine 36*, 347−357.

(8) Guynn, R. W., Veloso, D., and Veech, R. L. (1972) The concentration of malonyl-coenzyme A and the control of fatty acid synthesis in vivo. *J. Biol. Chem. 247*, 7325−7331.

(9) McGarry, J. D., Stark, M. J., and Foster, D. W. (1978) Hepatic malonyl-CoA levels of fed, fasted and diabetic rats as measured using a simple radioisotopic assay. *J. Biol. Chem. 253*, 8291−8293.

(10) Hosokawa, Y., Shimomura, Y., Harris, R. A., and Ozawa, T. (1986) Determination of short-chain acyl-coenzyme A esters by high-performance liquid chromatography. *Anal. Biochem. 153*, 45−49.

(11) King, M. T., Reiss, P. D., and Cornell, N. W. (1988) Determination of short-chain coenzyme A compounds by reversed-phase high-performance liquid chromatography. *Methods Enzymol. 166*, 70−79.

(12) Reszko, A. E., Kasumov, T., Comte, B., Pierce, B. A., David, F., Bederman, I. R., Deutsch, J., Des Rosiers, C., and Brunengraber, H. (2001) Assay of the concentration and ^{13}C-isotopic enrichment of malonyl-coenzyme A by gas chromatography-mass spectrometry. *Anal. Biochem. 298*, 69−75.

(13) Hung, Y. P., Albeck, J. G., Tantama, M., and Yellen, G. (2011) Imaging cytosolic NADH-NAD$^+$ redox state with a genetically encoded fluorescent biosensor. *Cell Metab. 14*, 545−554.

(14) Imamura, H., Nhat, K. P., Togawa, H., Saito, K., Iino, R., Kato-Yamada, Y., Nagai, T., and Noji, H. (2009) Visualization of ATP levels inside single living cells with fluorescence resonance energy transfer-based genetically encoded indicators. *Proc. Natl. Acad. Sci. U. S. A. 106*, 15651−15656.

(15) Jing, M., Zhang, P., Wang, G., Feng, J., Mesik, L., Zeng, J., Jiang, H., Wang, S., Looby, J. C., Guagliardo, N. A., et al. (2018) A genetically encoded fluorescent acetylcholine indicator for in vitro and in vivo studies. *Nat. Biotechnol. 36*, 726−737.

(16) Sun, F., Zeng, J., Jing, M., Zhou, J., Feng, J., Owen, S. F., Luo, Y., Li, F., Wang, H., Yamaguchi, T., et al. (2018) A genetically

DOI: 10.1021/acs.bioconjchem.8b00920
Bioconjugate Chem. 2019, 30, 826−832

encoded fluorescent sensor enables rapid and specific detection of dopamine in flies, fish, and mice. *Cell 174*, 481−496.
(17) Schujman, G. E., Guerin, M., Buschiazzo, A., Schaeffer, F., Llarrull, L. I., Reh, G., Vila, A. J., Alzari, P. M., and de Mendoza, D. (2006) Structural basis of lipid biosynthesis regulation in Gram-positive bacteria. *EMBO J. 25*, 4074−4083.
(18) Albanesi, D., Reh, G., Guerin, M. E., Schaeffer, F., Debarbouille, M., Buschiazzo, A., Schujman, G. E., de Mendoza, D., and Alzari, P. M. (2013) Structural basis for feed-forward transcriptional regulation of membrane lipid homeostasis in Staphylococcus aureus. *PLoS Pathog. 9*, No. e1003108.
(19) Xu, P., Wang, W., Li, L., Bhan, N., Zhang, F., and Koffas, M. A. (2014) Design and kinetic analysis of a hybrid promoter-regulator system for malonyl-CoA sensing in *Escherichia coli*. *ACS Chem. Biol. 9*, 451−458.
(20) Li, S., Si, T., Wang, M., and Zhao, H. (2015) Development of a synthetic malonyl-CoA sensor in *Saccharomyces cerevisiae* for intracellular metabolite monitoring and genetic screening. *ACS Synth. Biol. 4*, 1308−1315.
(21) Ellis, J. M., and Wolfgang, M. J. (2012) A genetically encoded metabolite sensor for malonyl-CoA. *Chem. Biol. 19*, 1333−1339.
(22) Dixon, A. S., Schwinn, M. K., Hall, M. P., Zimmerman, K., Otto, P., Lubben, T. H., Butler, B. L., Binkowski, B. F., Machleidt, T., Kirkland, T. A., et al. (2016) NanoLuc complementation reporter optimized for accurate measurement of protein interactions in cells. *ACS Chem. Biol. 11*, 400−408.
(23) Xie, Z., Dai, J., Dai, L., Tan, M., Cheng, Z., Wu, Y., Boeke, J. D., and Zhao, Y. (2012) Lysine succinylation and lysine malonylation in histones. *Mol. Cell. Proteomics 11*, 100−107.
(24) Saggerson, D. (2008) Malonyl-CoA, a key signaling molecule in mammalian cells. *Annu. Rev. Nutr. 28*, 253−272.
(25) Bowman, C. E., Rodriguez, S., Selen Alpergin, E. S., Acoba, M. G., Zhao, L., Hartung, T., Claypool, S. M., Watkins, P. A., and Wolfgang, M. J. (2017) The mammalian malonyl-CoA synthetase ACSF3 is required for mitochondrial protein malonylation and metabolic efficiency. *Cell Chem. Biol. 24*, 673−684.
(26) Chen, Q., Zhang, J., Zhao, K., Li, W., Miao, Q., Sun, Y., Zhao, X., Wei, T., and Yang, F. (2014) Lysosomal chymotrypsin induces mitochondrial fission in apoptotic cells by proteolytic activation of calcineurin. *Protein Cell 5*, 643−647.
(27) Wong, C. Y., Jiang, H., Abel, P. W., Scofield, M. A., Xie, Y., Wei, T., and Tu, Y. (2016) Phorbol myristate acetate suppresses breast cancer cell growth via down-regulation of P-Rex1 expression. *Protein Cell 7*, 445−449.

DOI: 10.1021/acs.bioconjchem.8b00920
Bioconjugate Chem. 2019, 30, 826−832