Spot14/Mig12 heterocomplex sequesters polymerization and restrains catalytic function of human acetyl-CoA carboxylase 2†

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INTRODUCTION

Lipid metabolism is vital in supporting dynamic cellular functions ensuring the balance in anabolic/catabolic homeostasis (Wakil and Abu-Elheiga, 2009; Lopaschuk and Abu-Elheiga, 2009; Abu-Elheiga et al., 2010; Folmes et al., 2013; Knoblach et al., 2013). Altered fatty acid metabolism precipitates a spectrum of metabolic dysfunctions including obesity, diabetes and cardiovascular disease. Central in lipid metabolism is the tightly regulated enzyme acetyl-CoA carboxylase (ACC), which catalyzes the carboxylation of acetyl-CoA into malonyl-CoA, a critical metabolic intermediate (Muoio and Newgard, 2006; Saggersen, 2008; Tong, 2005; Wakil and Abu-Elheiga, 2009). Human ACCs are multifunctional enzymes encompassing three distinctive domains, i.e., a biotin carboxylase, a biotin carboxyl carrier protein domain and a carboxyltransferase domain, all essential for stringent regulation of lipid metabolism (Bianchi et al., 1990; Cronan and Waldrop, 2002; Tong, 2005; Brownsey et al., 2006; Tong, 2013). In lipogenic tissue, malonyl-CoA produced by the ACC1 isoform is utilized for fatty acid synthesis targeting ACC1, a distinctive subtype of ACC. Here, we examined whether Spot14/Mig12 modulates ACC2. Nanoscale protein topography mapped putative protein–protein interactions between purified human Spot14/Mig12 and ACC2, validated by functional assays. Human ACC2 displayed consistent enzymatic activity, and homogeneous particle distribution was probed by atomic force microscopy. Citrate-induced polymerization and enzymatic activity of ACC2 were restrained by the addition of the recombinant Spot14/Mig12 heterocomplex but only partially by the oligo-heterocomplex, demonstrating that the heterocomplex is a designated metabolic inhibitor of human ACC2. Moreover, Spot14/Mig12 demonstrated a sequestering role preventing an initial ACC2 nucleation step during filamentous polymer formation. Thus, the Spot14/Mig12 heterocomplex controls human ACC2 polymerization and catalytic function, emerging as a previously unrecognized molecular regulator in catalytic lipid metabolism. © 2013 The Authors. Journal of Molecular Recognition published by John Wiley & Sons, Ltd. Additional supporting information may be found in the online version of this article at the publisher’s web site.

Keywords: Spot14; Mig12; acetyl-CoA carboxylase; protein–protein interactions; atomic force microscopy; Thrsp; silkworm Bombyx mori; fatty acid oxidation

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Spot14 encoded by Thrsp has been recognized as a putative regulator of de novo lipogenesis (Seelig et al., 1981; Kinlaw et al., 1995). Widely expressed in lipogenic tissues including liver, mammary gland and adipose tissue, Thrsp rises rapidly under physiological stimuli and thyroid hormone challenges and is tightly regulated by transcriptional factors, including the sterol regulatory element binding protein and the carbohydrate responsive element binding protein, which activate genes involved in fatty acid synthesis (Mater et al., 1999; Ma et al., 2005; LaFave et al., 2006). Blocking translation of Thrsp reduces the levels of lipogenesis associated with the reduction of lipogenic enzymes, including ATP-citrate lyase, fatty acid synthase, malic enzyme and pyruvate kinase (Kinlaw et al., 1995; Brown et al., 1997). Spot14 knockout, with a deletion of the entire coding sequence, has produced early embryonic lethality, yet less disruptive Spot14 knockout has delivered viable offspring with increased and decreased lipogenesis in liver and lactating mammary glands, respectively (Kinlaw et al., 1995; Kinlaw et al., 2006; Aipoalani et al., 2010). Furthermore, Spot14 knockdown by RNA interference in hepatocytes reduces fatty acid synthesis (Aipoalani et al., 2010), suggesting a multifaceted role of Spot14 with unknown molecular targets.

Recently, the direct involvement of Spot14 in fatty acid synthesis has been demonstrated (Colbert et al., 2010; Kim et al., 2010). Spot14 alone is unable to bind to and modulate the activity of ACC1, but Mig12, a Spot14 paralog, binds to ACC, inducing polymerization and promoting the catalytic activity of ACC. Co-expression of Spot14 and Mig12, however, leads to assembly of Spot14/Mig12, which attenuates ACC1 polymerization and enzymatic activity, contributing to the regulation of de novo lipogenesis. Although Spot14/Mig12-assisted ACC1 modulation of lipogenesis has been documented, it is unknown whether Spot14/Mig12 affects ACC2, which is mainly expressed in oxidative tissues. Furthermore, the putative molecular mechanism in Spot14/Mig12-dependent ACC2 regulation has not yet been probed.

To address the potential regulatory mechanism defining protein–protein interactions between Spot14/Mig12 and ACC2, we applied here a panel of proteomic methods, including atomic force microscopy (AFM) imaging. We report that the Spot14/Mig12 heterocomplex, not the oligo-heterocomplex, attenuates ACC2 polymerization and demonstrates a sequestering function, providing evidence for a previously unrecognized molecular regulator in lipid metabolism.

MATERIALS AND METHODS

Expression and purification of recombinant Spot14/Mig12 in Escherichia coli

The complementary DNA (cDNA) fragments of human Spot14 (146 amino acids) and Mig12 (183 amino acids) were incorporated into a PETDuet-1 bicistronic expression vector (Novagen), containing an N-terminal (His)6-tag on Mig12 and no tag on Spot14. The identity of fusion constructs was confirmed by DNA sequencing (Reyes et al., 2009). The constructs were transformed in E. coli BL21(DE3) cells and Spot14/Mig12 proteins purified. Recombinant proteins were enriched from a nickel nitritolriacetic acid (Ni-NTA) agarose column and then from a HiLoad Superdex 200 size exclusion column using an AKTA FPLC (GE Healthcare) system (Park et al., 2008; Park and Terzic, 2010). Spot14/Mig12 was concentrated using a Centricon (EMD Millipore) concentrator, and the protein concentration was quantified using an extinction coefficient of 42,400 (Mcm)^{-1} at 280 nm. The purity of Spot14/Mig12 was determined by densitometry.

Size-exclusion chromatography with multangle light scattering, quasi-elastic light scattering and refractive index (SEC/MALS/QELS/RI) detectors

Multidetector SEC/MALS/QELS/RI was performed with an Agilent LC1200 for size exclusion chromatography connected with a Wyatt DAWN HELEOS II multangle static light scattering detector, a Wyatt QELS dynamic light scattering detector, and a Wyatt Optlab rFX differential refractive index detector (Keck Biophysics Facility, Northwestern University) (Sahin and Roberts, 2012; Striegel and Brewer, 2012). The Superdex 200 10/300 GL size-exclusion column (GE Healthcare) was equilibrated for 18 h with column buffer [50 mM phosphate and 300 mM NaCl (pH 7.5)] at a flow rate of 0.5 ml/min. To confirm the functional integrity of the column and the detectors, a bovine serum albumin standard was prepared at 4 mg/ml in column buffer and centrifuged at 10,000 rpm for 10 min, and then 50 μl of the solution was injected onto the column. The Spot14/Mig12 sample was filtered using a 0.1-μm polyvinylidene difluoride (PVDF) membrane centrifugal filter unit (EMD Millipore), and 50 μl of the filtrate was immediately injected onto the column. Data acquisition and analysis were performed using the ASTRA software package (Wyatt Technologies).

Expression and purification of recombinant ACC2 in silkworm Bombyx mori

Heterologous human ACC2 was bioengineered using a B. mori nucleopolyhedrovirus (BmNPV) bacmid, an E. coli and B. mori hybrid shuttle vector, in silkworms. The cDNA fragment of ACC2, excluding the first N-terminal 27 hydrophobic amino acids for membrane attachment sequence and the following stretch of 28 to 148 responsible for mitochondrial targeting sequence, was cloned along with a C-terminal Flag tag into a pFastbac1 (Invitrogen) expression vector. Constructs confirmed by DNA sequencing were transformed into E. coli B. mori DH10Bac-CP competent cells containing the cysteine protease-deficient BmNPV bacmid (Hiyoshi et al., 2007; Motohashi et al., 2005), following a standard protocol of the Bac-to-Bac Baculovirus Expression System (Invitrogen) (Figure 1) (Luckow et al., 1993). Kanamycin-, tetracycline- and gentamicin-resistant white colonies on LB-agar plates were selected, and then the recombinant BmNPV bacmid containing human ACC2 was purified using the QIAGEN Large-Construct Kit. Identity of recombinant bacmids was confirmed by polymerase chain reaction using M13 forward and reverse sequencing primers and bacmid DNA sequencing. Recombinant BmNPV bacmid was injected into silkworm larvae using a DMRIE-C (Invitrogen) transfection reagent, using a 26-gauge needle. At 7 days post-injection, silkworm fat body was collected and diluted with 1 ml cold Tris-0.1 M NaCl buffer [50 mM Tris-HCl and 150 mM NaCl (pH 7.5)] containing 0.02% Triton X-100 per fat body. After brief sonication on ice, the crude fat body extracts were centrifuged at 6000 g for 20 min, 4 °C, to remove cellular debris following an additional centrifugation at 30,000 g for 30 min, 4 °C. The supernatant was then passed through an anti-Flag M2 affinity agarose gel column (Sigma-Aldrich), washed, and eluted with Tris-0.1 M NaCl buffer, containing 0 and 100 μg/ml Flag peptide, respectively. Eluted fractions containing ACC2 were pooled.
and stored at −80°C. Protein concentration was quantified by the Bradford protein assay (Bio-Rad). The purity and integrity of recombinant ACC2 were evaluated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), and Western blot analysis was performed using a monoclonal anti-Flag antibody (Sigma-Aldrich).

Atomic force microscopy

Tapping mode AFM imaging was performed using a Nanoscope IV PicoForce Multimode AFM equipped with an E-scanner and a rectangular-shaped silicon cantilever with a 42 N/m spring constant (Bruker) and a resonant frequency of ~300 kHz at ambient environment (Park et al., 2008; Park and Terzic, 2010). Recombinant human ACC2 (10–25 μg/ml) was incubated with or without 10 mM citrate for 20 min at 37°C. Separately, ACC2 (10–25 μg/ml) was co-incubated with 10 mM citrate and Spot14/Mig12 (5 μg/ml) for 20 min at 37°C. This incubation mixture was deposited on the freshly cleaved mica surface, incubated for 2 h in a moisture chamber, washed three times with phosphate-buffered saline (PBS) and three times with water, and gently dried under nitrogen stream. Images (512 × 512 pixels/image) were obtained from each sample with a maximum image size of 2 × 2 μm. All displayed images represent flattened raw data. Height images were used for measurements of lateral dimensions of proteins, and analysis was performed using the Nanoscope Analysis version 1.40 software (Bruker). For particle analysis, particles were visually inspected to set the threshold height in order to quantitate diameter, area, total number of particles, and a display correlation histogram of particles above the set point value. The absolute in x-axis setting was selected in depth histograms.

Acetyl-CoA carboxylase assays

Human ACCs are multifunctional enzymes harboring three distinctive domains, i.e., a biotin carboxylase, a biotin carboxyl carrier protein domain and a carboxyltransferase domain (Bianchi et al., 1990; Cronan and Waldrop, 2002; Tong, 2005; Brownsey et al., 2006; Tong, 2013). Biotin carboxylase catalyzes the adenosine diphosphate (ADP)-dependent carboxylation of biotin using bicarbonate as the donor of the carboxyl group (Tong, 2005; Chou et al., 2009). We measured ACC2 activity based on ADP-dependent biotin carboxylase activity, which catalyzed ATP into adenosine diphosphate (ADP) and inorganic phosphate. Assays were performed in 190 μl of 50 mM HEPES, 10 mM MgCl₂, 5 mM NaHCO₃, 10 mM sodium citrate, 0.2 mM acetyl-CoA, 1 mM biotin, and 2 mM ATP (pH 7.5). Reactions were initiated by the addition of 10 μl of purified ACC2, continued for 30 min at 37°C, and then terminated by the addition of 200 μl of 12% SDS. Produced organic phosphate was detected by colorimetry (Park et al., 2008; Park and Terzic, 2010). In a separate set of experiments, ACC2 activity was measured in the presence of recombinant Spot14/Mig12.

Blue native-PAGE (BN-PAGE) and Western blotting

Recombinant Flag-tagged ACC2 (2–5 μg) was incubated at 37°C for 10 min with 1 μg of Spot14/Mig12 in the presence and absence of 1 mM citrate in Tris-NaCl buffer with 5 mM dithiothreitol. In separate experiments, recombinant ACC2 was incubated with 1 mM citrate at 37°C for 5 min, followed by an additional 10 min of incubation with an addition of Spot14/Mig12. After incubation, 3 μl of loading buffer containing 5% Coomassie G-250, 1 M 6-aminohexanoic acid, and 10 mM Bis-Tris pH 7.5 was added to each 12 μl of reaction mixture, which was subjected to non-denaturing BN-PAGE using a 5%–12% gradient NativePAGE Novex Bis-Tris Gel (Invitrogen). Native gel uses Coomassie G-250 as a charge-shift molecule (Wittig et al., 2006). Resolved proteins were transferred by electrophoresis onto 0.2-μM Immun-Blot PVDF membranes (Bio-Rad), and excess Coomassie G-250 was removed from the membranes by sequential washing with methanol and TBS-T (Tris-NAc buffer with 0.1% Tween 20). The membranes were incubated with 5% non-fat dried milk in TBS-T, and using a monoclonal anti-Flag antibody (Sigma-Aldrich), immunoblot analysis was performed. Antibody-bound proteins were counterstained using horseradish peroxidase–conjugated secondary antibody and then detected using SuperSignal West Pico or West Femto (Pierce) (Karger et al., 2008; Olson et al., 2006).

Production of lenti-Spot14 and lenti-Mig12 viruses

The human cDNA of Mig12 and Spot14 were cloned into a multiple attenuated human immunodeficiency virus (HIV)-based lentiviral vector for HEK293T cell transfection to produce virus particles. In order to ensure biosafety when handling HIV-based lentiviruses and to prevent viral replication in vivo, a three-component plasmid method was utilized composed of a bicistronic plasmid carrying...
the gene of interest and enhanced green fluorescent protein (eGFP) as a marker (pHR-SIN-BX-IRES-Em), a helper plasmid expressing the vesicular stomatitis virus glycoprotein (VSV-G) (pMD-G) and a packaging plasmid (pCMV/R8.91) expressing GAG and POL (Ikeda et al., 2003; Ahmed et al., 2008; Sakuma et al., 2012). HEK293T cells were transfected with plasmids to generate replication-deficient and self-inactivating lentiviral particles using the FuGENE 6 transfection reagent (Roche) following standard protocols (Naldini et al., 1996; Zufferey et al., 1997; Ikeda et al., 2003). Strep-tagged Spot14 or Flag-tagged Mig12 and eGFP proteins were co-expressed in the same cells using the bicistronic pHR-SIN-BX-IRES-Spot14 (or -Mig12) expression vector. As a control, bicistronic vector (pHR-SIN-BX-IRES-Em) expressing eGFP alone was used. On day 3 or 4 after transfection, VSV-G–pseudotyped high-titer lentiviruses were collected from culture supernatants and concentrated about 1000-fold by ultracentrifugation. Viral titer was determined by counting the number of GFP-positive HEK293T cells infected through a series of dilution of the concentrated lentiviruses.

Stable expression of Spot14 and Mig12 proteins using lentiviruses

HEK293T cells were infected with high-titer lentiviruses (multiplicity of infection (MOI) of 5) that produced Spot14, Mig12 or Spot14/ Mig12 in the presence of 5 μg/ml of polybrene. After overnight culture with lentivirus, cells were washed and incubated in fresh culture medium. At 3 days post-infection, transduction efficiency was evaluated using GFP. To check for stable expression of Spot14 and Mig12 from the transduced cells, Western blot analysis was performed. Cultured cells were washed two times with PBS, incubated with cell lysis buffer (Cell Signaling) for 5 min on ice, and then scraped to collect cells. After sonication, crude extracts were centrifuged at 14,000 g in a cold table top centrifuge, and the resulting supernatant was collected and stored at −80 °C. ACC2 expression and polymerization, and Spot14 or Mig12 incorporation into ACC were probed using immunoblot analyses using a monoclonal antibody of acetyl-CoA carboxylase 2 (Cell Signaling), anti-Flag (Sigma-Aldrich), Strep•Tag (EMD Millipore) and Spot14 (Santa Cruz Biotech). Typically, 10 μg of the total protein from HEK293T cell extracts was used for gradient BN-PAGE.

RESULTS

Structural integrity of Spot14/Mig12

Using a bicistronic vector, Spot14/Mig12 was co-expressed in E. coli, and purified with a Ni-NTA column and then with a Superdex 200 size-exclusion column chromatography. Based on molecular weight standards in gel filtration chromatography, recombinant Spot14/Mig12 was produced as a heterocomplex (αβ)n and an oligo-heterocomplex (αβ)n, displaying >93% purity and migrated at the corresponding molecular weight on SDS-PAGE (Figure 2A). No equilibrium shift was detected between the heterocomplex and the oligo-heterocomplex analyzed in size-exclusion chromatography.

Multidetector SEC/MALS/QELS/RI was utilized to evaluate the molecular composition of recombinant Spot14/Mig12 eluted from a Superdex 200 column. SEC/MALS/QELS/RI determines

Figure 2. Structural integrity of ACC2 and Spot14/Mig12. (A) Human Spot14/Mig12 was expressed in E. coli with an amino-terminal 6x histidine tag and purified by Ni-affinity and size exclusion chromatography, producing oligo-heterocomplex and heterocomplex proteins verified by molecular weight standards. Mig12 (21.8 kDa) and Spot14 (16.5 kDa) migrated at their expected molecular weights on SDS-PAGE. (B) Human ACC2 was expressed in silkworm B. mori fat body with a carboxyl-terminal Flag tag and purified from an anti-Flag M2 affinity agarose gel column. SDS-PAGE of Coomassie blue-stained ACC2 (261 kDa) migrated at a corresponding molecular weight. The identity of ACC2 was confirmed by immunoblot analysis using a monoclonal anti-Flag antibody. C, crude extract of silkworm fat body; F, flow through from an anti-Flag affinity column; E, elution fractions.
the absolute molar mass and average size of particles in solution by measuring the characteristics of scattered light from the sample (Sahin and Roberts, 2012; Striegel and Brewer, 2012). The heterocomplex resolved monodisperse and homogeneous particles whereas the oligo-heterocomplex separated into four different components (Figure S1 in the Supplementary Material). The observed molar mass at the apex of the heterocomplex peak was 39.01 kDa, which is less than 2% off from the theoretical molecular mass of the Spot14/Mig12 heterocomplex, confirming the molecular composition. In contrast, the oligo-heterocomplex resolved into dodeca-, hexa-, tetra-, and di-heterocomplexes, where the predominant species was a hexa-heterocomplex. Based on these analyses, we designated Spot14/Mig12 components into heterocomplex (αβ) and hexa-heterocomplex (αβ)₆.

Human acetyl-CoA carboxylase production in silkworm

Human acetyl-CoA carboxylase 2 (ACC2), an ACC isoform abundantly present in the heart and skeletal muscle, was expressed in silkworm B. mori with a Flag purification tag (Figure 2B). Binding to an anti-Flag M2 affinity agarose gel column (Sigma-Aldrich) was targeted by a Flag tag, with Flag peptide elution yielding enriched purity of ACC2 (>93%) compared to fat body extracts and washed fractions. Detected at corresponding molecular weight on SDS-PAGE, the expressed ACC2 was validated with immunoblotting using an anti-Flag antibody. In addition, silkworm-based protein expression provided a high yield of purified ACC2 (0.15 mg/silkworm) with a specific activity of 21.4 ± 1.4 nmol/mg/min (n = 5), similar to reported values (Kim et al., 2007). Thus, human ACC2 expressed in silkworm harbors consistent enzymatic activity, underscoring that the silkworm-based protein expression system provides a reliable platform for eukaryotic ACC2 production.

ACC2 and Spot14/Mig12 interactions

Acetyl-CoA carboxylase is modulated by multiple regulatory factors (Thampy and Wakil, 1988; Wakil and Abu-Elheiga, 2009). Phosphorylation of ACC by adenosine monophosphate (AMP)—activated protein kinase inactivates enzymatic activity whereas dephosphorylation activates the enzyme promoting polymerization. ACC is also allosterically activated by a high concentration of citrate, which induces the polymerization of an inactive protomeric form into an active filamentous form with increased functional activity (Beaty and Lane, 1983; Gregolin et al., 1966; Kim et al., 2007). Without citrate, recombinant ACC bioengineered in a baculovirus/Sf9 cell expression system or ACC isolated from tissue does not show any detectable catalytic activity (Thampy and Wakil, 1988; Cheng et al., 2007).

The structural properties of human ACC2 modulated by citrate were evaluated by mapping the architecture of purified proteins derived from silkworm B. mori using AFM at a nanoscale resolution (Figure 3). ACC2 alone demonstrated almost homogeneous island-like topographies, whereas when citrate was added to

![Figure 3](image-url)
ACC2, filamentous polymers of ACC2 were produced. To quantify structural changes of ACC2 induced by citrate, two distinctive structural properties, diameter and area, were evaluated using the Nanoscope particle analysis software (Figure 4 and Figure S2 in the Supplementary Material). ACC2 alone demonstrated particle distribution that was 58 ± 4 nm in diameter and 3395 ± 365 nm² (n = 152) in area, whereas citrate-induced ACC2 produced filamentous polymeric particles that were 129 ± 9 nm in diameter and 19 659 ± 4851 nm² (n = 70) in area, revealing significant topographic changes of ACC2 produced by citrate (Figures 3 and 4; Table 1). Thus, high-resolution AFM imaging and citrate-induced ACC2 polymerization validated the functional integrity of recombinant ACC2 generated with high fidelity in silkworm B. mori.

To resolve the putative protein–protein interactions between Spot14/Mig12 and ACC2, AFM imaging and functional assays were employed (Figures 3 and 4). As Spot14/Mig12 was expressed as heterocomplexes and hexa-heterocomplexes, these proteins were incubated with citrate and ACC2. The ensuing microarchitecture revealed completely and partially dispersed polymeric ACC2 nanostructures, much larger than the Spot14/Mig12 hexa-heterocomplex alone (Figure S3 in the Supplementary Material and Table 1). When a Spot14/Mig12 hexa-heterocomplex was further evaluated using gradient BN-PAGE (Figure 5). As Spot14/Mig12 inhibits ACC2 polymerization, the addition of Spot14/Mig12 to ACC2 would immobilize the polymerization process regardless of the ACC2 polymerization steps. Incubation of recombinant ACC2 with citrate induced the formation of high-molecular-weight ACC2 polymers, but incubation of ACC2 with citrate and the Spot14/Mig12 heterocomplex reduced ACC2 polymer formation, supporting AFM studies (Figure 5A). However, Spot14/Mig12 addition to the nucleated ACC2 polymers induced by citrate during pre-incubation could neither arrest the polymerization process nor depolymerize polymeric ACC2 back to lower molecular weight components (Figure 5B). These findings demonstrate a capping function of the Spot14/Mig12 heterocomplex, preventing ACC2 nucleation during filamentous polymer formation.

The sequestering function of Spot14/Mig12 in ACC2 polymerization was further validated in cell culture using a lentiviral protein expression system. Strep-tagged Spot14 and Flag-tagged Mig12 were produced individually or co-produced in the HEK293T cells using bicistronic pHR-SIN-BX-IRE-Spot14 (or -Mig12) expression lentivirus vector. Bicistronic vector (pHR-SIN-BX-IRE-Em) expressing eGFP alone was used as a control expression

Figure 4. Diameter (A) and area (B) analyses of AFM height images produced by ACC2 alone, ACC2 + citrate, ACC2 + citrate + Spot14/Mig12 (αβ), and ACC2 + citrate + Spot14/Mig12 (αβ)₆. Cit, citrate; (αβ), Spot14/Mig12 heterocomplex; (αβ)₆, Spot14/Mig12 hexa-heterocomplex.

Table 1. Particle analysis by AFM

|                          | Diameter (nm) | Area (nm²) |
|--------------------------|--------------|-----------|
| Human ACC2               | 58 ± 4       | 3395 ± 365 | (n = 152) |
| Human ACC2 + citrate     | 129 ± 9      | 19 659 ± 4851 | (n = 70) |
| Human ACC2 + citrate + Spot14/Mig12, (αβ) | 49 ± 3 | 2269 ± 150 | (n = 165) |
| Human ACC2 + citrate + Spot14/Mig12, (αβ)₆ | 127 ± 13 | 19 981 ± 4564 | (n = 50) |
| Spot14/Mig12, (αβ)₆      | 28 ± 2       | 822 ± 125  | (n = 51) |
Figure 5. Spot14/Mig12 heterocomplex prevents ACC2 polymerization. ACC2 polymerization with citrate or citrate + Spot14/Mig12 αβ was initially separated by gradient BN-PAGE and analyzed by Western blot using an anti-Flag antibody. (A) Incubation of ACC2 with citrate induced formation of high-molecular-weight ACC2 polymers, but addition of Spot14/Mig12 αβ diminished the generation of ACC2 polymers. (B) ACC2 and citrate were pre-incubated to produce nucleated ACC2 polymers, followed by additional incubation with Spot14/Mig12 αβ. Spot14/Mig12 heterocomplex addition could not further halt the polymerization process of nucleated ACC2 polymers. S/M αβ, Spot14/Mig12 heterocomplex.

DISCUSSION

Lipid metabolism is an integral metabolic process to maintain dynamic cellular functions (Wakil and Abu-Elheiga, 2009; Lopaschuk et al., 2010; Folmes et al., 2013; Knobloch et al., 2013). Acetyl-CoA carboxylase regulates a first and rate-limiting step in lipid metabolism by catalyzing the carboxylation of acetyl-CoA to produce malonyl-CoA, a crucial metabolite in modulating anabolic lipogenesis and catabolic fatty acid β-oxidation (Muio and Newgard, 2006; Saggerson, 2008; Wakil and Abu-Elheiga, 2009). Two different isoforms of acetyl-CoA carboxylase, ACC1 and ACC2, partake in fatty acid metabolism—ACC1 for lipogenesis and ACC2 for fatty acid oxidation (Abu-Elheiga et al., 1995; Saggerson, 2008; Wakil and Abu-Elheiga, 2009). In particular, the regulation of ACC2 has been associated with metabolic dysfunctions and metabolic remodeling (Abu-Elheiga et al., 2001; Abu-Elheiga et al., 2003; Abu-Elheiga et al., 2012; Kolwicz et al., 2012). Here, high-resolution AFM imaging and biochemical analysis were employed to resolve the molecular mechanism underlying protein–protein interactions between Spot14/Mig12 and ACC2. The Spot14/Mig12 heterocomplex-induced suppression of ACC2 polymerization and function unveils a molecular regulator of ACC2 and associated lipid metabolism.

Malonyl-CoA is recognized as a molecular rheostat adjusting de novo lipogenesis and oxidative mitochondrial energy metabolism (Tong, 2005; Saggerson, 2008; Wakil and Abu-Elheiga, 2009; Reyes et al., 2010; Wolfgang and Lane, 2011; Folmes et al., 2013). In non-lipogenic tissue such as the skeletal and heart muscle, where ACC2 is abundantly expressed, malonyl-CoA allosterically inhibits carnitine palmitoyltransferase (CPT1), an integral membrane protein associated with the mitochondrial outer membrane catalyzing the transfer of long-chain fatty acid into the mitochondria as the rate-limiting step of fatty acid β-oxidation. Thus, operating as a suppressing molecule of ACC2 polymerization and function, although speculative, Spot14/Mig12 might regulate energy metabolism in oxidative tissues.

Atomic force microscopy imaging at a nanoscale resolution and functional catalytic assays provided an approach to reveal the molecular functional unit of Spot14/Mig12 responsible for ACC2 regulation. Incubation of ACC2, citrate and the Spot14/Mig12 heterocomplex produced an inclusive suppression of citrate-induced ACC2 polymerization in contrast with the less efficient Spot14/Mig12 hexa-heterocomplex incubation (Figure 7). However, the nucleated ACC2 polymers assembled during pre-incubation produced microfilaments despite Spot14/Mig12 addition. Moreover, Spot14 incorporation into polymerized ACC was not detected in cultured cells, demonstrating that the Spot14/Mig12 heterocomplex is a designated capping molecule of ACC2 thereby preventing further polymerization.

Crystallographic symmetry of Spot14 crystals suggests an assembly of higher-order oligomers (Krispin and Henrick, 2007; Colbert et al., 2010). In light of this, a recombinant Spot14/Mig12 hexa-heterocomplex was produced, which was less effective in modulating ACC2 function and polymerization. Although the distinctive function of Spot14 oligomer has not been identified, Spot14 oligomer might be involved in the regulation of fatty acid movement or function as a trafficking protein, physically linking different steps of lipogenesis (LaFave et al., 2006). Thus, the main function of Spot14/Mig12 oligomers could be in the modulation of upstream lipogenesis mechanisms, in addition to partial ACC2 regulation.

Acetyl-CoA carboxylase is regulated by multiple factors, including covalent modification by phosphorylation/dephosphorylation, whereby phosphorylation inactivates ACC activity whereas dephosphorylation activates the enzyme (Shen et al., 2004; Wakil and Abu-Elheiga, 2009). Based on the crystal structure of the biotin carboxylase domain of ACC2, phosphorylation-regulated polymerization has been proposed (Cho et al., 2010). Phosphorylated Ser222 binds to the putative dimer interface of the biotin carboxylase domain, disrupting polymerization by inhibiting the interactions between the ACC2 dimer interfaces of the neighboring
Figure 6. Spot14/Mig12 sequesters ACC2 polymerization in cultured cells. (A) Cultured HEK293T cells were imaged by bright and fluorescence microscopy, alone or following transfection with control or bicistronic pHR-SIN-BX-IRES-Spot14 (or -Mig12) lentiviruses, co-expressing eGFP. (B) HEK293T cell lysates were resolved by SDS-PAGE and analyzed by Western blot using a monoclonal antibody of anti-Flag (Mig12) and Spot14. (C) HEK293T cell lysates were resolved by gradient BN-PAGE and analyzed by Western blot using a monoclonal antibody of ACC2 and anti-Flag (Mig12). Transfection of Spot14 or co-transfection of Spot14 and Mig12 decreased ACC2 expression. In addition, integration of Mig12 into ACC2 polymers was significantly suppressed when Spot14 and Mig12 was co-transfected. C, control transfection; S, Spot14 transfection; M, Mig12 transfection; S+M, Spot14+Mig12 co-transfection.

Figure 7. A schematic summary of ACC regulation by Spot14/Mig12. Polymerization of ACC by citrate or Mig12 increases catalytic activity. ACC polymerization and its catalytic function are inhibited by the Spot14/Mig12 heterocomplex, more effective than the oligo-heterocomplex.
biont carboxylase domains. Thus, it is worth noting that a Spot14/Mig12 heterocomplex might bind to a biont carboxylase domain, resulting in conformational and structural hindrance for polymerization.

Silkworm B. mori, recognized as a natural bioreactor to produce silk, is an emerging host for recombinant protein production. (Maeda et al., 1985; Tamura et al., 2000; Tomita et al., 2003 Kato et al., 2010; Teule et al., 2012). Silkworm provides a post-translational modification machinery including glycosylation, phosphorylation and disulfide bond formation, all essential for soluble and correctly folded recombinant eukaryotic proteins. (Park et al., 2007; Dojima et al., 2009; Kato et al., 2010; Vandeborre et al., 2011). Using a BmNPV bacmid, an E. coli and B. mori hybrid shuttle vector, authentic recombinant proteins are readily expressed by direct injection of recombinant bacmid into silkworm pupae and larvae, reducing the time required for pre-production virus amplification in cultured cells. Moreover, protein expression levels in silkworms are consistently high (Dojima et al., 2009; Ogata et al., 2009; Park et al., 2009 Kato et al., 2010). To exploit the advantages of silkworm-based protein expression system, human ACC2, a multifunctional enzyme composed of several distinctive domains, was expressed. Indeed, silkworm-based protein production exhibits a high yield of purified protein (0.15 mg/silkworm), which retains catalytic activity and citrate-induced polymerization, underscoring that silkworm serves as a protein expression host to generate high-fidelity recombinant eukaryotic protein.

In summary, regulation of a multifunctional ACC2 by Spot14/Mig12 was resolved using nanoscale AFM imaging and biochemical assays. Recombinant ACC2 from silkworm displayed authentic polymerization and functional activity, which were inhibited by the Spot14/Mig12 heterocomplex. Moreover, as a molecular substrate for ACC2, the Spot14/Mig12 heterocomplex revealed a sequestering function in preventing the function and initial polymerization of ACC2. Thus, deconvoluted protein–protein interactions shed light on a previously unrecognized molecular regulator potentially useful in modulating catalytic lipid metabolism.

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