N-Lysine Propionylation Controls the Activity of Propionyl-CoA Synthetase*\(^{\text{S}}\)

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Protein acetylation is a ubiquitous means for the rapid control of diverse cellular processes. Acetyltransferase enzymes transfer the acetyl group from acetyl-CoA to lysine residues, while deacetylase enzymes catalyze removal of the acetyl group by hydrolysis or by an NAD\(^{+}\)-dependent reaction. Propionyl-coenzyme A (CoA), like acetyl-CoA, is a high energy product of fatty acid metabolism and is produced through a similar chemical reaction. Because acetyl-CoA is the donor molecule for protein acetylation, we investigated whether proteins can be propionylated in vivo, using propionyl-CoA as the donor molecule. We report that the Salmonella enterica propionyl-CoA synthetase enzyme PrpE is propionylated in vivo at lysine 592; propionylation inactivates PrpE. The propionyl-lysine modification is introduced by bacterial Gcn-5-related N-acetyltransferase enzymes and can be removed by bacterial and human Sir2 enzymes (sirtuins). Like the sirtuin deacetylation reaction, sirtuin-catalyzed depropionylation is NAD\(^{+}\)-dependent and produces a byproduct, O-propionyl ADP-ribose, analogous to the O-acetyl ADP-ribose sirtuin product of deacetylation. Only a subset of the human sirtuins with deacetylation activity could also depropionylate substrate. The regulation of cellular propionyl-CoA by propionylation of PrpE parallels regulation of acetyl-CoA by acetylation of acetyl-CoA synthetase and raises the possibility that propionylation may serve as a regulatory modification in higher organisms.

Protein acetylation is a ubiquitous means for the rapid control of diverse cellular processes (1, 2). Acetylation occurs at lysine residues, with acetyl-CoA (Ac-CoA)\(^{5}\) serving as the acetyl group donor. In higher organisms, aberrant acetylation of lysine residues in histone tails correlates with diseases such as cancers and developmental disorders and may contribute to modulation of cell life span (3, 4). From bacteria to humans, N-Lys acetylation controls the activity of acetyl-coenzyme A synthetase (AMP-forming; Acs) and potentially that of other members of the AMP-forming family of enzymes (5–7). In Salmonella enterica, Acs is deacetylated by CobB, a member of the Sir2 family of NAD\(^{+}\)-dependent deacetylases (a.k.a. sirtuins) (8). Interestingly, strains of S. enterica lacking CobB deacetylase activity cannot grow on propionate because the propionyl-CoA synthetase (encoded by the \(prpE\) gene) that activates propionyl-CoA is not active (5, 9).

Cells generate propionyl-CoA from several different processes, including the catabolism of odd chain fatty acids, the decarboxylation of succinate, the catabolism of amino acids (e.g. threonine), and the activation of propionate (10–12). Propionate is a powerful inhibitor of cell growth that is widely used as a food preservative. Reports in the literature suggest that propionyl-CoA may be responsible for the cytotoxic effects of propionate. Although the direct effects of propionyl-CoA are unclear, it is clear that cells avoid accumulating this metabolite (13–15). The cell maintains low levels of propionyl-CoA by using it as a source of carbon and energy. The predominant pathway for propionate degradation in prokaryotes and eukaryotes is the 2-methylcitric acid cycle, which converts propionate to pyruvate via reactions similar to those of the Krebs cycle (supplemental Fig. S6) (16, 17).

Propionylated lysine residues were recently identified in histones (18, 19); by analogy with histone acetylation, propionyl-CoA is presumably required as a propionyl donor. However, the physiological significance of lysine propionylation was unknown. We report here that the propionyl-CoA synthetase (PrpE) enzyme of S. enterica is reversibly propionylated in vivo and that this modification regulates enzymatic activity. The modification is removed by the S. enterica sirtuin CobB in an NAD\(^{+}\)-dependent reaction that mirrors the sirtuin deacetylation reaction. Our results suggest that propionylation/depropionylation may be a conserved regulatory mechanism in higher organisms and that acylation/deacetylation systems for the control of acyl-CoA (AMP-forming) ligases may be a general mechanism for maintaining CoA homeostasis in all cells.

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\(^{5}\) The abbreviations used are: Ac-CoA, acetyl-coenzyme A; TFA, trifluoroacetic acid; GST-\(H_{s}\), glutathione S-transferase hexahistidine tag; MBP-\(H_{s}\), maltose-binding protein hexahistidine tag; H\(_{s}\)-rTEV, C-terminal tag, hexahistidine-tagged recombinant tobacco etch virus; HPLC, high pressure liquid chromatography; Pat, protein acetyltransferase; MALDI-TOF, matrix-assisted laser desorption ionization time-of-flight; MS, mass spectrometry; Acs, acetyl-coenzyme A synthetase; OPADPR, O-propionyl-ADP-ribose.
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EXPERIMENTAL PROCEDURES

Protein Purification

Protein Purity Assessment—Proteins were resolved by 12% SDS-PAGE (20) and visualized with Coomassie Blue (21). Purity was assessed by band densitometry using a Fotodyne imaging system with Foto/Analyst v.5.00 software (Fotodyne Inc) for image acquisition and TotalLab v.2005 software for analysis (Nonlinear Dynamics).

*S. enterica* PrpE<sup>WT</sup> and PrpE<sup>P592E</sup> Proteins—These proteins were purified by chitin purification as described (11).

Pat—GST-H<sub>4</sub>-Protein Acetyltransferase (Pat) was overproduced as described (22). The Pat used in all experiments except the propionylation and depropionylation time courses was purified by glutathione affinity chromatography as described (22) and was 32% homogeneous. Pat used for time courses was purified by affinity chromatography using a 5-ml HisTrap HP column on an AKTA FPLC Purifier system (Amersham Biosciences). Cells expressing GST-H<sub>4</sub>-Pat were harvested by centrifugation at 10,500 × g for 12 min in a Beckman Coulter Avanti J-20 XOI refrigerated centrifuge with a JLA-8.1000 rotor. Cells were resuspended in buffer A (sodium phosphate buffer (20 mM, pH 7.5, at 24 °C), NaCl (0.5 M),imidazole (20 mM)) and broken by sonication using a 550 Sonic Dismembrator (Fisher Scientific) for 3 min (50% duty). After equilibration with buffer A and loading of cell-free extract, the column was washed with 50 ml of buffer A, followed by 40 ml of 8% buffer B (sodium phosphate buffer (20 mM, pH 7.5, at 24 °C), NaCl (0.5 M),imidazole (0.5 M)). A 50-ml linear gradient increased buffer B to 100%. GST-H<sub>4</sub>-Pat eluted at ~40% buffer B and was 67% pure. GST-H<sub>4</sub>-Pat was stored in tris(hydroxymethyl)aminomethane buffer (Tris-HCl, 50 mM, pH 7.5, at 25 °C) containing KCl (100 mM) and 50% glycerol (v/v) at 4 °C.

CobB Sirtuin—Synthesis of CobB sirtuin fused to an N-terminal chitin binding tag was directed by plasmid pCOBB31 (supplemental Table S1) in *Escherichia coli* strain ER2566 (New England Biolabs). Cells were grown at 25 °C in two liters of lysogenic broth (23, 24) supplemented with ampicillin (100 μg/ml) and ZnSO<sub>4</sub> (50 μM). Gene expression was induced by addition of isopropyl-β-D-thiogalactoside to 1 mM at 600~0.4.

Cells were grown overnight at 25 °C, harvested by centrifugation at 10,500 × g for 12 min as described above, and broken using a chilled French pressure cell (Spectronic Instruments; two passes at 1.3 kPa). Protein was purified and the tag removed using the standard protocol for the IMPACT-CN Protein Fusion and Purification System (New England Biolabs). Cell-free extract was incubated with chitin beads for 1 h at 4 °C prior to column preparation. After tag removal, CobB protein was stored in Tris-HCl buffer (50 mM, pH 7.5, at 25 °C) containing KCl (100 mM) and 50% glycerol (v/v) at ~80 °C.

PncA Nicotinamidase—*S. enterica* pncA was amplified using primers to add 5′-KpnI and 3′-HindIII restriction sites and cloned into plasmid pTEV6 cut with the same enzymes to yield plasmid pPNC2 (supplemental Table S1), which encodes PncA protein with an N-terminal maltose-binding protein-hexahistidine (MBP-H<sub>6</sub>) tag. Plasmid pPNC2 was moved into *E. coli* strain BL21(DE3) by electroporation (25). The resulting strain was grown overnight and subcultured 1:100 (v/v) into 2 liters of lysogenic broth containing ampicillin (100 μg/ml). The culture was grown shaking at 37 °C to 600~0.7, and MBP-H<sub>6</sub>-PncA synthesis was induced with isopropyl-1-thio-β-D-galactopyranoside (1 mM). The culture was grown overnight at 25 °C. Cells were harvested and MBP-H<sub>6</sub>-PncA puriﬁed as described for purification of GST-H<sub>4</sub>-Pat. PncA eluted at ~30% buffer B. MBP-H<sub>6</sub>-PncA-containing fractions were pooled and H<sub>4</sub>-rTEV protease (26) added to reach a 1:50 H<sub>4</sub>-rTEV protease:MBP-H<sub>6</sub>-PncA ratio; H<sub>4</sub>-rTEV protease was puriﬁed as described (27). The cleavage reaction mixture was incubated at room temperature for 3 h and dialyzed overnight against two liters of buffer A at 4 °C. Tagless PncA protein (83% pure) was resolved from the reaction mixture using the 5-ml HisTrap HP column, which did not bind tagless PncA. Protein was stored in Tris-HCl (50 mM, pH 7.5, at 25 °C) containing KCl (100 mM) and 20% (v/v) glycerol at ~80 °C.

*S. enterica* Acs Peptide—Peptide consisting of the C-terminal 52 amino acids of *S. enterica* Acs was synthesized by the Peptide Synthesis Facility of the University of Wisconsin-Madison Bio-technology Center. Peptide was purified by preparative scale HPLC using a Dynamax C18 column (22×250 mm). Peptide eluted at 36.5% acetonitrile with a final chromatographic purity of 89%.

Human Proteins—Human SIRT1 protein was a gift from John Denu. Human SIRT2 and SIRT3 were overexpressed in *E. coli* strain BL21(DE3) transformed with plasmids SIRT2-pHEX and SIRT3-pQE-80, respectively (6, 28). Cells were grown in 1 liter of lysogenic broth supplemented with ampicillin (100 μg/ml) and ZnSO<sub>4</sub> (50 μM) at 37 °C to 600~0.7. Protein expression was induced with isopropyl-1-thio-β-D-galactopyranoside (1 mM), and cultures were grown overnight at 20 °C. Cells were harvested by centrifugation and broken by sonication, and hSirT2 and hSirT3 were purified using His-Bind Quick 900 cartridges (Novagen) according to the manufacturer’s instructions. Proteins were dialyzed into storage buffer (Tris-HCl (50 mM, pH 7.5, at 25 °C) containing 1 mM dithiothreitol and 20% glycerol (v/v)) and stored at ~20 °C. hSirT2 protein was 52% pure, and hSirT3 protein was 49% pure.

Other Proteins—*Bacillus subtilis* AcuA, *Thermotoga maritima* Sir2, human SIRT4, and murine SIRT1 proteins were purified as described (29–31).

Enzyme Activity Assays

Acylation/Decylation Assays—Conditions for protein acylation and deacetylation have been described (22). PrpE (62.5 pmol) was incubated at 37 °C with GST-H<sub>4</sub>-Pat (62.5 pmol) and [1-<sup>14</sup>C]Ac-CoA (20 μM, 54 mCi/mmol) or [1-<sup>14</sup>C]Pr-CoA (20 μM, 53 mCi/mmol) (Moravek) in 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES) buffer (50 mM, pH 7.5, at 24 °C) containing tris(2-carboxyethyl)phosphine hydrochloride (200 μM); final volume was 100 μl. After 2 h, GST-H<sub>4</sub>-Pat protein was removed using GST-Mag<sup>®</sup>-agarose beads (Novagen). CobB sirtuin (15.6 pmol), NAD<sup>+</sup> (1 μM), and PncA (50 pmol, when noted) were added and reactions incubated at 37 °C for an additional 2 h. Reactions were stopped with trichloroacetic acid (final concentration 0.5%) or gel-loading buffer (Tris-HCl buffer (50 mM, pH 6.8, at 24 °C), SDS (2%, w/v), bromphenol blue (0.1%, w/v), glycerol (10%, v/v), dithiothreitol (100...
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MALDI-TOF Peptide Fingerprinting

In-gel digest and MALDI-TOF analysis of PrpE proteins were performed at the Mass Spectrometry Facility of the University of Wisconsin-Madison Biotechnology Center. PrpE proteins were excised, destained, and dehydrated and then reduced with dithiothreitol (25 mM) for 30 min at 56 °C, alkylated with iodoacetamide (55 mM) in darkness at room temperature for 30 min, and digested with 0.4 μg of trypsin (Promega Sequence Grade Modified) overnight at 37 °C. All steps were performed in (NH₄)HCO₃ (25 mM, pH 8). Resulting peptides were extracted with 0.1% trifluoroacetic acid (TFA) followed by acetonitrile/H₂O/TFA (70:25:5%, v/v/v). Peptides were dried in a SpeedVac concentrator (Thermo), resuspended in 20 μl of TFA (0.1%, v/v), and solid phase extracted using ZipTips® μC18 pipette tips (Millipore). Peptides were eluted off the C18 column with acetonitrile/H₂O/TFA (70:25:0.2%, v/v/v) onto an AnchorChip™ plate (Bruker Daltonics) and recrystallized with 1 μl of matrix (20 mg/ml of α-cyano-4-hydroxycinnamic acid in acetonitrile/H₂O/TFA (70:25:0.2%, v/v/v)). Peptide fingerprint analysis was performed on a Bruker BIFLEX III MALDI-TOF mass spectrometer (Bruker Daltonics). Peptide mapping analysis was performed with the Mascot search engine (Matrix Science).

MS/MS analysis was performed at the Mass Spectrometry Facility of the University of Wisconsin-Madison Biotechnology Center using a 4800 MALDI TOF/TOF mass spectrometer (Applied Biosystems) in positive ion mode. Fragment ions were assigned using the Protein Prospector MS-Product utility (University of California, San Francisco Mass Spectrometry Facility).

OPADPR Production

In situ O-propionyl-ADP-ribose (OPADPR) generation reactions contained (per 125 μl): Acs peptide (62.5 μM), AcuA (62.5 μM), CobB (62.5 μM), PncA (3.13 μM), Pr-CoA (1 mM), NAD⁺ (1 mM), HEPES (50 mM, pH 7.5, at 25 °C), and tris(2-carboxyethyl)phosphine hydrochloride (200 μM). CobB protein was omitted from the control reaction. Reactions were incubated overnight at 37 °C, stopped by the addition of TFA to 1% (v/v), and filtered through 0.45-μm Spin-X® Centrifuge Tube Filters (Corning Inc.) prior to HPLC analysis.

Reaction components were separated using a Beckman Coulter System Gold HPLC system equipped with an Altima HP C18 AQ column (4.6 × 150-mm, 5-μm pore size; Alltech). The protocol used was based on those described for the purification of O-acetyl-ADP-ribose (37, 38). The system was run at 25 °C at a flow rate of 1 ml/min and monitored at 260 and 214 nm. Following injection of a 50-μl sample, the column was developed isocratically in solvent A (0.05% TFA/H₂O) for 6 min. The gradient was increased linearly to 40% solvent B (0.02% TFA/acetonitrile) over 40 min. Fractions of interest were collected and frozen at −80 °C prior to mass spectrometry analysis.

Enhanced mass spectrometry of fractions of interest was performed using an ABI 3200 Q Trap linear ion trap quadrupole liquid chromatography/MS/MS mass spectrometer (AB Sciex). Sample was directly infused with an infusion
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RESULTS

Previously published work (9) suggested that the Pat acetyltransferase and CobB deacetylase modulated the activity of PrpE in vivo. When tested in vitro, the S. enterica Pat enzyme acetylated or propionylated PrpE, whereas the CobB sirtuin deacetylated PrpE (Fig. 1A, lane 2, rows II and IV). Because both PrpE and Acs contain a P-X4-GK motif in which the lysine residue is critical for function (5, 11), we reasoned that this lysine could be the modified residue, by analogy with Acs (5). Failure of Pat to acetylate a PrpE K592E mutant (Fig. 1A, lane 1, rows II and IV) was consistent with the hypothesis that Lys-592 was indeed the modification site of PrpE.

PAGE gel and phosphorimage of PrpE K592 protein incubated with [1-14C]Pr-CoA showed that propionylation of PrpE resulted in 70% loss of specific activity (1.5 pmol PrpE/1011 counts). Activity assays showed that propionylation of PrpE resulted in 30% loss of specific activity (1.5 pmol PrpE/1011 counts) relative to that of unmodified PrpE enzyme (5 pmol PrpE/1011 counts). The above-mentioned peptides were not observed.

We investigated whether other acetyltransferase enzymes could propionylate PrpE. The Gcn-5-related N-acetyltransferase enzyme AcuA from B. subtilis (29) displayed propionylation activity with the specific activity of AcuA (78.4 pmol PrpE propionylated min$^{-1}$ μmol$^{-1}$) 5.5 times higher than that of Pat (14.3 pmol PrpE propionylated min$^{-1}$ μmol$^{-1}$) (Fig. 2).

To determine whether PrpE was posttranslational modification in vivo, we isolated PrpE protein from strains grown on prpE. The strains used in these experiments were JE9221 (Δacs ΔcobB pat)$^+$ and JE9225 (Δacs ΔcobB pat)$^+$ (supplemental Table S1). Our strategy was to overproduce PrpE in the absence of the CobB sirtuin to allow Pat-dependent acetylation and subsequent accumulation of PrpE in its acylated form. Both strains grew on propionate, albeit poorly, because the acetate kinase/phosphotransacylase system was functional (9). We did not expect PrpE to be modified in strain JE9225 because the pat gene was inactive in this strain; hence PrpE from strain JE9225 was used as a negative control. In agreement with this prediction, strain JE9225 grew much better on propionate (doubling time = 5.5 h) than did strain JE9221 (doubling time = 15.8 h), suggesting that Pat inactivated PrpE in strain JE9221 (supplemental Fig. S8).

The MALDI-TOF mass spectrum of tryptic peptides of PrpE isolated from strain JE9221 (Δacs ΔcobB pat)$^+$ contained a signal at m/z = 747.4 atomic mass units (Fig. 1B). The MS/MS fragmentation pattern of the m/z = 747.4 peptide matched that expected for S$^{590}$GK$^{592}$MLR (supplemental Table S3) as well as that observed for a synthetic SGK$^{592}$MLR peptide (supplemental Fig. S9). The peptide fingerprint of PrpE protein isolated from strain JE9225 (Δacs ΔcobB pat)$^+$ matched that of PrpE treated with CoA and did not contain any signals for modified peptides (Fig. 1C). Although a very small peak was seen at m/z = 747.4, MS/MS fragmentation analysis showed it to be an isolate of the m/z = 745.4 peptide (supplemental Fig. S10).

These results demonstrated that Pat was responsible for the propionylation of PrpE in vivo.

We investigated whether other acetyltransferase enzymes could propionylate PrpE. The Gcn-5-related N-acetyltransferase enzyme AcuA from B. subtilis (29) displayed propionylation activity with the specific activity of AcuA (78.4 pmol PrpE propionylated min$^{-1}$ μmol$^{-1}$) 5.5 times higher than that of Pat (14.3 pmol PrpE propionylated min$^{-1}$ μmol$^{-1}$) (Fig. 2).
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Because deletion of the cobB gene encoding the S. enterica sirtuin inactivates PrpE in vivo, we asked whether the CobB sirtuin depropionylates substrates in vitro. As predicted, the S. enterica CobB sirtuin was able to remove the propionyl modification from PrpE (specific activity = 3.1 pmol PrpE depropionylated min⁻¹ μmol⁻¹ CobB) (Fig. 1A, lane 1, row IV, and Fig. 3B). We next asked whether other bacterial and eukaryotic sirtuins had depropionylation activity. The human SIRT2 (hSIRT2) and SIRT3 (hSIRT3) and the bacterial T. maritima Sir2 (Sir2_maritima IV) proteins depropionylated PrpE within 1 h (Fig. 3, C–E). Depropionylation activity was not observed with human (hSirt1) or murine SIRT1 (mSirt1) or human SIRT4 (hSIRT4) (Fig. 3A, lanes 3, 4, 7). However, mSirt1 displayed robust deacetylase activity (17.6 ± 0.4 pmol PrpE deacetylated min⁻¹ μmol⁻¹ mSirt1), suggesting that the lack of depropionylase activity of mSirt1 arises from specific discrimination between acetyl and propionyl lysine rather than being due simply to absence of any enzymatic activity (data not shown).

By analogy with the deacetylation reaction, we predicted that sirtuin-mediated depropionylation would produce OPADPR. OPADPR has been shown to be the product of the sirtuin-catalyzed reaction when synthetic propionylated peptides were used as substrates (39).

We developed a system for the in situ generation of OPADPR through iterative propionylation and depropionylation of protein substrate. Reaction mixtures contained AcuA_Bs in lieu of Pat because of the former’s higher propionylase activity and because its smaller size allowed us to use higher enzyme concentrations. A peptide consisting of the C-terminal 52 amino acids of AcsSe was used as protein substrate because it could be added at higher concentration than PrpE. Results of a control experiment verified that AcuA_Bs propionylated the 52-amino acid peptide (supplemental Fig. S11). To prevent inhibition of the sirtuin activity by nicotinamide, all reaction mixtures contained PncA nicotinamidase (supplemental Fig. S12). Reaction mixtures were resolved by C18 reverse-phase HPLC. The large NAD⁺ peak (t = 29.5 min) observed in the chromatogram of control mixture lacking CobB sirtuin was absent in the chromatogram of the mixture containing CobB (Fig. 4A, dashed line). The compound eluting 36.5 min post-injection was OPADPR ((M-H)⁻ ion; m/z = 614.1 atomic mass units) as determined by mass spectrometry (Fig. 4B). Reaction byproducts were identified using authentic standards (nicotinic acid, 5.8 min; ADP-ribose, 3.8 min). To catalyze lysine depropionylation in a manner analogous to the sirtuin deacetylation reaction, the enzyme must be able to accommodate the additional methyl group in the Michaelis complex formed with NAD⁺ and the propionylated peptide. We therefore modeled the complex based on the structure of NAD⁺ and an acetylated peptide bound to Sir2_maritima (40), a bacterial sirtuin that exhibits depropionylation activity (Fig. 3C). As shown in Fig. 5, the structure can readily accommodate the additional methyl of propionyl-lysine in a hydrophobic pocket in the enzyme active site. A model constructed with no adjustments produced minimal clashes (van der Waals distances of 3.2 Å or greater) that were completely eliminated during energy minimization by minor side-chain rearrangements, with individual atomic shifts of less than 0.2 Å, in the vicinity of the propionyl-lysine.

DISCUSSION

Taken together, the data presented here support the conclusion that N-Lys propionylation is a physiologically relevant posttranslational modification for the control of protein function. Because propionylase activity was detectable in two bacterial Gcn-5-related N-acetyltransferase enzymes (S. enterica Pat, B. subtilis AcuA), we suggest that members of this family of enzymes in higher organisms may also have propionylase activity. Similarly, robust depropionylase activity is found among sirtuins from bacteria and higher organisms (Fig. 3), suggesting that other members of this enzyme family may also catalyze this activity. However, because this activity was not detected in human or murine SIRT1 or human SIRT4 enzymes, the ability to accommodate the larger propionyl modification in the
enzyme active site appears to be enzyme-specific. Consistent with the current understanding of the CobB sirtuin-catalyzed deacetylase reaction, the product of the depropionylase activity of CobB was O-PADPR (Fig. 4). It is unclear whether O-PADPR plays any physiological role as has been suggested for the related molecule O-acetyl-ADP-ribose (41–43).

From a physiological standpoint, propionylation is a logical means of posttranslational regulation because it allows the cell to rapidly modulate activity of propionyl-CoA synthetase in direct response to potentially deleterious levels of its product, propionyl-CoA. In contrast, control of propionyl-CoA synthetase activity by acetylation would require conversion of propionyl-CoA to acetyl-CoA via rate-limiting steps of the 2-methylcitric acid cycle (supplemental Fig. S6) (16). This strategy would make inactivation of propionyl-CoA synthetase too slow to prevent any imbalance in CoA homeostasis or to prevent other toxic effects caused by high levels of propionyl-CoA (13). The cell therefore senses propionyl-CoA imbalance directly using the same enzyme pair used to control acetyl-CoA homeostasis.

From a broader physiological perspective, all cells must control their pools of acyl-CoAs to avoid depletion of the pool of free CoA and/or synthesis of toxic metabolites (13, 44). This suggests, by analogy with the work reported here and with earlier findings regarding acetyl CoA homeostasis (8), that there might well be other acyltransferase/deacylase systems that cells from all domains of life use to control the activity of acyl-CoA synthetases. For example, a succinyl-CoA:protein succinyltransferase enzyme might use succinyl-CoA to inactivate the succinate:CoA ligase, while a cognate desuccinylase would reactivate succinyl-CoA ligase. Whether these modifications indeed exist in the cell and, like acetylation, are used to regulate other processes is an intriguing possibility under active investigation.

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