CoA regulation and metabolic control

All organisms require coenzyme A (CoA) for intermediary metabolism. CoA ushers organic acid substrates, particularly fatty acids, through multiple reactions that supply energy, building blocks for membrane structure, protein modifications that alter subcellular interactions or activities and secondary metabolite production. Acetyl-CoA is an allosteric regulator of key metabolic activities that direct carbon flux. CoA synthesis from pantothenate is dynamic and responsive to nutritional and environmental conditions, with the goal of cell homoeostasis during proliferation and in support of specialized tissue functions. Pantothenate is the unique precursor of CoA, and the pantothenate kinase (PanK) family of enzymes exerts control over the amount of CoA produced. The PanKs are regulated co-ordinately by several mechanisms, and the complexity of CoA regulation is currently unfolding. Little is known about the mechanisms of CoA degradation that work together with biosynthesis to maintain a threshold level of cellular CoA.

CoA is derived from pantothenate

CoA is an essential cofactor in all organisms that functions as the major acyl group carrier in hundreds of reactions in intermediary metabolism. The dynamic regulation of the intracellular CoA level is emerging as a key requirement for metabolic plasticity in mammalian tissues. CoA is synthesized in five steps from pantothenate, cysteine and ATP (Figure 1). The first step in the pathway of CoA biosynthesis is the ATP-dependent phosphorylation of pantothenate by PanK. Bacteria, fungi and plants make pantothenate, but animals cannot and obtain pantothenate from the diet as vitamin B5. PanK activity controls the entry of pantothenate into the CoA biosynthetic pathway which is the only known metabolic fate for pantothenate. CoA and its thioesters are degraded by Nudix hydrolases and vanins, and the pantothenate or pantetheine degradation products are recycled into CoA via PanK (Figure 2).

Feedback regulation of CoA biosynthesis

Almost all organisms share a common feedback inhibition mechanism to regulate the intracellular CoA concentration that was first revealed by the inhibition of *Escherichia coli* PanK by CoA and its thioesters. Thus the cellular CoA concentration is buffered by the self-adjusting biochemical feedback mechanism that governs PanK activity (Figure 2). PanK overexpression in *E. coli* results in an 80-fold higher protein level, but only a 3-fold increase in the steady-state level of CoA, illustrating the stringency of feedback control. Single-copy expression of an engineered feedback-resistant PanK in *E. coli* results in a significantly higher intracellular CoA concentration, and an accelerated release of phosphopantetheine into the culture medium. Thus the expression level of feedback-regulated PanKs defines the upper limit for the concentration of cellular CoA. Bacteria express two other PanKs that are not closely related to the type I *E. coli* enzyme. *Staphylococcus aureus* PanK is an example of a type II PanK which lacks the characteristic feedback-regulation property. *Pseudomonas aeruginosa* expresses the type III bacterial PanK. This isoform requires a monovalent cation for activity and is also not feedback-regulated by CoA or its thioesters.

Most bacteria, fungi and lower metazoans have a single gene encoding a PanK, whereas mammals have
three genes encoding four isoenzymes. The primary sequences of the prokaryotic and the eukaryotic PanK proteins are not homologous, with the exception of the *S. aureus* type II PanK that is distantly related to the eukaryotic proteins. The most abundant and potent feedback regulator of mammalian PanKs is acetyl-CoA, which functions as a competitive inhibitor with respect to ATP. The crystal structure of the PanK–acetyl-CoA binary complex shows the pantetheine moiety of acetyl-CoA bound at the pantothenate-binding site and the adenine ring of CoA locking the ATP-binding domain into an open conformation incapable of binding ATP. The mammalian PanK1α and PanK1β proteins arise from differential splicing of the *Pank1* RNA, and these two isoenzymes are the least sensitive to feedback regulation. PanK1β is relatively resistant to acetyl-CoA with a 25 μM IC₅₀, whereas PanK1α has an IC₅₀ of 5 μM. PanK2 and PanK3 proteins are encoded by distinct genes and are more stringently regulated by acetyl-CoA at the submicromolar range. PanK3 is also inhibited by the less abundant long-chain acyl-CoAs. Thus PanK2 and PanK3 are inhibited under conditions where the PanK1 enzymes are operational.

Figure 1. Pathway of CoA biosynthesis. The commitment step is the phosphorylation of pantothenate (vitamin B₅) by pantothenate kinase (PANK) to 4′-phosphopantothenate. This is followed by condensation with cysteine catalysed by 4′-phosphopantothenoylcysteine synthase (PPCS) and then decarboxylation to form 4′-phosphopantetheine by 4′-phosphopantothenoylcysteine decarboxylase (PPCDC). 4′-Phosphopantetheine is adenylylated to dephospho-CoA by phosphopantetheine adenylyltransferase (PPAT), then phosphorylated by dephospho-CoA kinase (DPCK) at the 3′-hydroxy group of the ribose to form CoA. In mammals, the last two steps are catalysed by a single bifunctional polypeptide, CoA synthase.

Figure 2. Ins and outs of pantothenate metabolism. Pantothenate is actively transported into cells and committed to CoA biosynthesis by phosphorylation via pantothenate kinase (PanK). The CoA synthase catalyses the last two steps in the pathway in mammals. Acetyl-CoA is the most potent feedback inhibitor of the mammalian PanK enzymes. CoA can be degraded to acyl-phosphopantetheine by the Nudix hydrolases 7 and 19. Alternatively, CoA donates the phosphopantetheine prosthetic group to activate the fatty acid synthase. Prosthetic group turnover releases the phosphopantetheine that can be reincorporated into the CoA biosynthetic pathway. Extracellular pantetheine can be converted into pantothenate by the vanin family of enzymes, or pantetheine can re-enter cells and be phosphorylated by PanK. In mammals, the source of extracellular pantetheine is not known, but it is possible that the phosphopantetheine intermediate may exit from the cell, similar to what is known about bacterial pantothenate metabolism.
Complexity of CoA regulation

Mammalian cells typically express a combination of PanK isoforms. Tissues where PanK1 expression is the dominant isoform, like the liver and heart, exhibit the highest tissue CoA levels. Metabolic ‘activators’ displace the CoA thioester inhibitors from the PanK2 and PanK3 enzymes to counteract the inhibition by acetyl-CoA. Both long-chain acyl-carnitine and acyl-ethanolamide activate PanK2 and PanK3, suggesting that physiological conditions that elevate the concentrations of these metabolites stimulate CoA synthesis. Fasting liver is active in fatty acid β-oxidation, and the elevated acyl-carnitine level activates PanK2/3 and CoA biosynthesis to ensure an adequate supply of CoA for fatty acid catabolism. The critical importance of this regulatory circuit is revealed by the inability of PanK1−/− mice to increase hepatic CoA during fasting. Hepatic triacylglycerol accumulates due to a deficiency in fatty acid oxidation, which in turn limits the capacity of the liver to carry out gluconeogenesis (Figure 3). Acetyl-CoA is a key regulator of intermediary metabolism. For example, when acetyl-CoA increases, it not only inhibits PanK activity, but also binds to pyruvate dehydrogenase to limit entry of carbon into the tricarboxylic acid cycle. The differential responses of the PanK isoenzymes to regulatory ligands and their tissue-specific expression profiles work together to create the unique tissue CoA levels that match the metabolic activity and function of a specialized tissue.

CoA biosynthesis is completed in the cytoplasm, but CoA is required for reactions in mitochondria, chloroplasts, the lumen of the endoplasmic reticulum and peroxisomes. To reach these destinations, CoA requires protein-mediated transport across cell membranes. Eukaryotic cells transport CoA into mitochondria for the citric acid cycle and fatty acid oxidation, into chloroplasts for fatty acid synthesis, into the endoplasmic reticulum for glycoprotein acetylation and into peroxisomes for fatty acid oxidation (Figure 4). CoA sequestered in these organelles is thought to reach millimolar concentrations. Acetyl-CoA is also required in the nucleus to support protein acetylation. The four mammalian PanK proteins share a homologous C-terminal catalytic domain, but differ in their N-termini which direct the isoenzymes to different cellular compartments (Figure 4). PanK1α is localized in the nucleus where it is associated with the nucleolus. PanK1α is positioned to sense the acetyl-CoA levels in this cellular compartment. Reduced CoA supply and histone acetylation are observed in the Drosophila fumble mutant, a hypomorph with reduced PanK expression. Human PanK2 primarily resides in the intramitochondrial membrane space following its prior transit into and out of the nucleus. This final destination
places human PanK2 in position to sense the levels of its allosteric regulator, acyl-carnitine. PanK3, rodent PanK2 and PanK1β are found in the cytoplasm, where PanK1β associates with endosomal membranes. Similar to having individual thermostats in separate rooms of the house, PanK sensors at the nucleus, cytosol, endosomes and mitochondria co-operate to fine tune the biosynthesis of CoA to ensure an adequate supply of this cofactor to support subcellular metabolism.

**CoA degradation**

CoA levels in cells are regulated in a cell autonomous manner. CoA or acyl-CoA do not escape from cells, in contrast with carnitines, acyl-carnitines or acyl-ethanolamides that interact with both intracellular and extracellular targets. Pantetheine is actively transported into cells and its phosphorylation by PanK traps this vitamin and funnels it solely down the CoA biosynthetic pathway (Figure 2). In order for the intracellular CoA concentration to dynamically change, both CoA biosynthesis and degradation must be co-ordinately regulated. The CoA degradation pathways and how they are controlled are not as well understood as biosynthesis. CoA turnover in bacteria is mediated by transfer of the phosphopantetheine moiety from CoA to acyl carrier protein. The phosphopantetheine group is then released from the protein and can either be re-incorporated into the CoA biosynthetic pathway or exit the cell. CoA supplies the phosphopantetheine prosthetic group to activate the fatty acid synthase in mammalian cells, and turnover of the prosthetic group occurs independently of protein turnover. CoA degradation in mammalian cells is actively mediated by at least two members of the Nudix hydrolase family of enzymes, Nudix hydrolases 7 and 19. These hydrolases reside in peroxisomes where acyl-CoA substrates are degraded to acyl-phosphopantetheine. The metabolic fate(s) of the acyl group attached to the acyl-phosphopantetheine product is not known. Phosphatases will generate pantetheine. The vanins are secreted proteases that degrade pantetheine to pantothenate and cysteamine. The pantothenate or pantetheine are recycled into CoA by PanK, as both are substrates for this enzyme. There may be additional CoA-degradative mechanisms in mammals that have not yet been discovered.

CoA is often considered as a cofactor that is not limiting in intermediary metabolism. However, the experiments with knockout mice show that the perturbation of CoA homoeostasis by the elimination of a PanK leads to metabolic imbalances. Furthermore, a programme of gene expression changes occur in response to low CoA in liver (Figure 5). PanK1 expression increases significantly and the activities of acyl-CoA thioesterase and carnitine palmitoyltransferase increase to offload acyl groups from acyl-CoA to release more free CoA. The synthesis of acetyl-CoA is limited by inhibition of pyruvate dehydrogenase through up-regulation of pyruvate dehydrogenase kinase 4 expression. And the fatty acid synthase is down-regulated to limit the production of fatty acids that would ligate with CoA. These adaptive changes in gene expression work together to counter a low level of free CoA, pointing out its functional importance. A human neurodegenerative disorder arises from inactivating mutations in the human PANK2 gene. Clearly, chronic reduction of intracellular CoA levels have severe metabolic consequences, thus leading to the conclusion that tissue CoA levels are not maintained at a level that is significantly higher than the metabolic requirement.

**Future directions**

An important issue for future research will be to address whether the level of CoA and/or its thioesters control metabolism. The intricate regulatory network for CoA homoeostasis in mammals suggests that that quantitative changes in the CoA or acetyl-CoA levels can actually determine the directions of metabolic flux that are intrinsic to energy production. The ob/ob or leptin-deficient mouse has abnormally high hepatic CoA levels compared with its wild-type counterpart. The high CoA is associated with abnormally low expression of Nudix hydrolase 7. Constitutive gluconeogenesis in the leptin-deficient liver results in hyperglycaemia and hyperinsulinaemia. The idea that the elevated CoA...
is driving this metabolic imbalance is supported by the normalization of serum glucose and insulin levels when hepatic CoA levels are decreased in the ob/ob Pank1−/− double knockout mouse. Key experiments to critically test the role of CoA in controlling metabolism will be the tissue-specific elevation of CoA levels by selective overexpressing PanKs or PanK mutants that are engineered to be refractory to feedback regulation. The transcription factors that control PanK expression are largely unknown, and the significance of the post-translational phosphorylation and acetylation sites identified in proteomic screens remain to be elucidated. What is clear is that mammals have invested in a diversity of transcriptional, post-transcriptional and biochemical regulatory mechanisms for CoA homoeostasis, suggesting that the control of the cellular levels of this cofactor is critical to support tissue-specific intermediary metabolism. ■

We thank the American Heart Association, US National Institutes of Health (grant number GM062896) and the American Lebanese Syrian Associated Charities for financial support.

References
1. Leonardi, R., Zhang, Y.M., Rock, C.O. and Jackowski, S. (2005) Prog. Lipid Res. 44, 125–153
2. Leonardi, R., Chohnan, S., Zhang, Y.M. et al. (2005) J. Biol. Chem. 280, 3314–3322
3. Hong, B.S., Yun, M.K., Zhang, Y.M. et al. (2006) Structure 14, 1251–1261
4. Hong, B.S., Senisterra, G., Rabeh, W.M. et al. (2007) J. Biol. Chem. 282, 27984–27993
5. Leonardi, R., Rock, C.O., Jackowski, S. et al. (2007) Proc. Natl. Acad. Sci. U.S.A. 104, 1494–1499
6. Leonardi, R., Zhang, Y.M., Yun, M.K. et al. (2010) Chem. Biol. 17, 892–902
7. Leonardi, R., Rehg, J.E., Rock, C.O. and Jackowski, S. (2010) PLoS ONE 5, e11107
8. Zallot, R., Agrimi, G., Lerma-Ortiz, C. et al. (2013) Plant Physiol. 162, 581–588
9. Alfonso-Pechio, A., Garcia, M., Leonardi, R. and Jackowski, S. (2012) PLoS ONE 7, e49509
10. Siudeja, K., Srinivasan, B., Xu, L. et al. (2011) EMBO Mol. Med. 3, 755–766
11. Zhang, Y.M., Chohnan, S., Virga, K.G. et al. (2007) Chem. Biol. 14, 291–302
12. Zhou, B., Westaway, S.K., Levinson, B., Johnson, M.A., Gitschier, J. and Hayflick, S.J. (2001) Nat. Genet. 28, 345–349
13. Leonardi, R., Rock, C.O. and Jackowski, S. (2014) Diabetologia 57, 1466–1475

Suzanne Jackowski is a member of the faculty at St. Jude Children’s Research Hospital, Memphis, Tennessee, USA, in the Department of Infectious Diseases. She obtained her PhD in Biomedical Science at the University of Tennessee-Oak Ridge National Laboratory, and was a postdoctoral fellow first in the Department of Physiology at the University of Connecticut Health Center and following in the Department of Microbiology at the University of Illinois, Champaign-Urbana. email: Suzanne.Jackowski@stjude.org

Charles Rock is a Member of the faculty at St. Jude Children’s Research Hospital, Memphis, Tennessee, USA, in the Department of Infectious Diseases. He received his PhD in Biochemistry from the University of Tennessee-Oak Ridge National Laboratory and then did postdoctoral training in the Department of Molecular Biophysics and Biochemistry at Yale University and in the Department of Microbiology at the University of Illinois, Champaign-Urbana. email: charles.rock@stjude.org