Cell Metabolism
Review

Acetyl Coenzyme A: A Central Metabolite and Second Messenger

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Acetyl-coenzyme A (acetyl-CoA) is a central metabolic intermediate. The abundance of acetyl-CoA in distinct subcellular compartments reflects the general energetic state of the cell. Moreover, acetyl-CoA concentrations influence the activity or specificity of multiple enzymes, either in an allosteric manner or by altering substrate availability. Finally, by influencing the acetylation profile of several proteins, including histones, acetyl-CoA controls key cellular processes, including energy metabolism, mitosis, and autophagy, both directly and via the epigenetic regulation of gene expression. Thus, acetyl-CoA determines the balance between cellular catabolism and anabolism by simultaneously operating as a metabolic intermediate and as a second messenger.

Introduction

Acetyl-coenzyme A (acetyl-CoA) is so central for intermediate metabolism that hypothetical reconstructions of the origin of life postulate its involvement in ancestral methanotrophic reactions performed by the last common precursor of prokaryotes (Nitschke and Russell, 2013). From archaebacteria to mammalians, acetyl-CoA occupies a critical position in multiple cellular processes, as a metabolic intermediate, as a precursor of anabolic reactions, as an allosteric regulator of enzymatic activities, and as a key determinant of protein acetylation (Choudhary et al., 2014). Acetyl-CoA is indeed the actual molecule through which glycolytic pyruvate enters the tricarboxylic acid (TCA) cycle, is a key precursor of lipid synthesis, and is the sole donor of the acetyl groups for acetylation (Choudhary et al., 2014).

Acetyl-CoA is a membrane-impermeant molecule constituted by an acetyl moiety (CH_3CO) linked to coenzyme A (CoA), a derivative of vitamin B_5 and cysteine, through a thioester bond (Shi and Tu, 2015). As thioester bonds are energy rich, the chemical structure of acetyl-CoA facilitates the transfer of the acetyl moiety to a variety of acceptor molecules, including amino groups on proteins (Shi and Tu, 2015). Acetylation can occur as a co-translational event. In this setting, N^{α} acetyltransferases (NATs) transfer an acetyl group from acetyl-CoA to the α-amino group of the N-terminal residue of the protein (which generally is serine, alanine, glycine, threonine, valine, or cysteine), once the initiator methionine has been removed by methionine aminopeptidases. N-terminal acetylation affects the vast majority of human proteins, determining their stability, localization, and function (Hollebeke et al., 2012).

Nonetheless, acetylation is mostly studied as a post-translational modification that concerns the N^{ε} amino group of lysine residues, eliminating their positive charge and increasing sterical hindrance (Choudhary et al., 2014). Thus, N^{ε} acetylation can alter the functional profile of a specific protein by influencing its catalytic activity, its capacity to interact with other molecules (including other proteins), its subcellular localization, and/or its stability (Choudhary et al., 2014). These effects can directly originate from the changes in charge and hindrance imposed by N^{ε} acetylation, or they can reflect (1) the binding of proteins that contain acetyl-lysine recognition bromodomains (Zeng et al., 2010) or (2) the occupancy of lysine residues that may have been subjected to other post-translational modifications, including ubiquitination, methylation, formylation, and higher-order acylation reactions (Choudhary et al., 2014).

N^{ε} acetylation can occur through a non-enzymatic mechanism, especially in alkaline environments like the mitochondrial matrix (Weinert et al., 2014), or can be catalyzed by a diverse group of lysine acetyltransferases (KATs) (Choudhary et al., 2014). The human genome encodes no less than 22 distinct KATs, which possess marked substrate specificity (Davenport et al., 2014). Many KATs have a relatively high K_D (low affinity) for acetyl-CoA, meaning that physiological fluctuations in the abundance of acetyl-CoA within the cellular compartment where such KATs are expressed can affect their catalytic activity. For instance, KAT2A (also known as GCN5) has a K_D for acetyl-CoA of 0.56 μM and its yeast counterparts of 8.5 μM (Langer et al., 2002), both of which fall in the concentration range of acetyl-CoA found in human or yeast cells (Cai et al., 2011; Lee et al., 2014). Other KATs have a similar affinity for acetyl-CoA

and CoA, meaning that their activity is regulated by the relative abundance of acetyl-CoA and CoA (the latter can mediate product inhibition). Importantly, the concentration of acetyl-CoA (or the acetyl-CoA/CoA ratio) does not only determine the enzymatic activity of KATs, but also alters their specificity. Thus, KATs like E1A binding protein p300 (EP300) and CREB binding protein (CREBBP, best known as CBP) exhibit distinct Hill values (i.e., different degrees of cooperativity) at varying acetyl-CoA levels, meaning that their selectivity (i.e., their preference to acetylate distinct lysine residues) changes (Denisov and Sligar, 2012; Henry et al., 2015).

In response to a series of physiological or pathological conditions, the abundance and/or distribution of acetyl-CoA in distinct subcellular and/or pericellular compartments changes considerably. Thus, acetyl-CoA can act as a second messenger that relays signals from the extracellular to the intracellular milieu. This may contribute to the elevated variability in lysine acetylation patterns encountered in organs and subcellular fractions in distinct functional states (Lundby et al., 2012).

Here, we discuss the ability of acetyl-CoA to regulate adaptive responses to homeostatic perturbations by controlling the equilibrium between catabolic and anabolic reactions as well as by influencing major cellular processes such as cell cycle progression, mitosis, autophagy, and regulated cell death (RCD).

Acetyl-CoA: A Central Metabolite

Acetyl-CoA is not only the product of multiple catabolic reactions, but also one of the central substrates for anabolic metabolism. For illustrative purposes, we will discuss the mitochondrial and extramitochondrial metabolism of acetyl-CoA separately.

Generation of Mitochondrial Acetyl-CoA

In most mammalian cells, acetyl-CoA is predominantly generated in the mitochondrial matrix by various metabolic circuitries, namely glycolysis, β-oxidation, and the catabolism of branched amino acids (Figure 1A).

Glycolysis culminates in the generation of cytosolic pyruvate, which is imported into mitochondria by the mitochondrial pyruvate carrier (MPC), a heterodimer of MPC1 and MPC2 (Herzig et al., 2012). Mitochondrial pyruvate is decarboxylated to form acetyl-CoA, CO_2, and NADH by the so-called pyruvate dehydrogenase complex (PDC), a large multicomponent system that in humans is composed of (1) three proteins that are directly involved in CoA- and NAD^+-dependent pyruvate decarboxylation, i.e., pyruvate dehydrogenase (lipoamide) (PDH, which in exists in three isoforms), dihydrolipoamide S-acetyltransferase (DLAT), and dihydrolipoamide dehydrogenase (DLD); (2) two regulatory components, i.e., pyruvate dehydrogenase kinase (PDK, which also exists in four isoforms) and pyruvate dehydrogenase phosphatase (PDP, a heterodimer involving either of two catalytic subunits and either of two regulatory subunits); and (3) one non-enzymatic subunit, i.e., pyruvate dehydrogenase complex, component X (PDHX) (Patel et al., 2014). Importantly, both acetyl-CoA and NADH as well as ATP allosterically inhibit PDC as they activate PDK. Conversely, CoA, NAD^+, and ADP promote pyruvate decarboxylation by inhibiting PDK. Finally, acetyl-CoA acts as a potent allosteric activator of pyruvate carboxylase (PC), another pyruvate-consuming enzyme that synthesizes oxaloacetate for anaplerotic reactions (Adina-Zada et al., 2012). These regulation circuitries ensure that the products of glycolysis are employed for ATP generation when cells are in energy-low conditions (characterized by elevated ADP, NAD^+, and CoA levels), but diverted toward anabolic metabolism when the energy stores are repleted (characterized by elevated ATP, NADH, and acetyl-CoA levels).

Alternatively, acetyl-CoA can be generated as the end product of β-oxidation. In this case, one among several members of the acyl-CoA synthetase protein family catalyzes the CoA- and ATP-dependent conversion of cytosolic free fatty acids into acyl-CoA. Acyl-CoA is then condensed with *L*-carnitine to form acylcarnitine and free CoA, a cytosolic reaction catalyzed by carnitine palmitoyltransferase 1 (CPT1). Acylcarnitine is imported into mitochondria through the antiporter solute carrier family 25 (carnitine/acylcarnitine translocase), member 20 (SLC25A20), which exchanges it for free *L*-carnitine. Finally, mitochondrial acylcarnitine is reconverted by carnitine palmitoyltransferase 2 (CPT2) into *L*-carnitine (which drives the so-called ''carnitine shuttle'') and acyl-CoA, and the latter undergo β-oxidation to generate NADH and acetyl-CoA for use as direct and indirect, respectively, respiratory substrates (Rufer et al., 2009).

Branched-chain amino acids (i.e., valine, leucine, and isoleucine) can also be employed to produce acetyl-CoA (Harris et al., 2005), a molecular circuitry that may depend on the mitochondrial deacetylase sirtuin 3 (SIRT3), at least in some tissues (including the brain, liver, kidney, and skeletal muscles) (Dittenhafer-Reed et al., 2015). To this aim, branched amino acids must be first transaminated to branched-chain α-ketoacids, a reaction that can be catalyzed by the cytosolic enzyme branched-chain amino acid transaminase 1 (BCAT1). Upon import into the mitochondrial matrix via the carnitine shuttle (see below) (Violante et al., 2013), branched-chain α-ketoacids are processed via a multi-step reaction comparable to the decarboxylation of pyruvate. This irreversible reaction is catalyzed by the mitochondrial branched-chain α-ketoacid dehydrogenase (BCKD) complex, a large multicomponent enzymatic system yielding NADH, acetyl-CoA, and other acyl-CoA thioesters (which can be processed by β-oxidation or the TCA) as end products (Harris et al., 2005). Of note, some mammalian cells express a mitochondrial variant of BCAT1 (i.e., BCAT2), which is catalytically active (Yennawar et al., 2006). However, how branched-chain amino acids enter the mitochondrial matrix has not been determined yet.

In addition to these nearly ubiquitous metabolic circuitries, there are organ-specific pathways for mitochondrial acetyl-CoA generation. For instance, neurons can employ the ketone body *D*-β-hydroxybutyrate to generate acetyl-CoA (Cahill, 2006). This occurs via a three-step reaction involving the NAD^+-dependent oxidation of *D*-β-hydroxybutyrate to acetoacetate (catalyzed by 3-hydroxybutyrate dehydrogenase, type 1, BDH1), the transfer of CoA from succinyl-CoA to acetoacetate (catalyzed by 3-oxoacid CoA transferase 1, OXCT1, or OXCT2), and the CoA-dependent cleavage of acetoacetyl-CoA into two acetyl-CoA molecules (catalyzed by an enzymatic complex with acetoacetyl-CoA thiolase activity) (Cahill, 2006). Of note, it has recently been demonstrated that the acetylation pattern of mitochondrial proteins, as controlled by SIRT3, is crucial for mice that respond to starvation to increase the hepatic synthesis

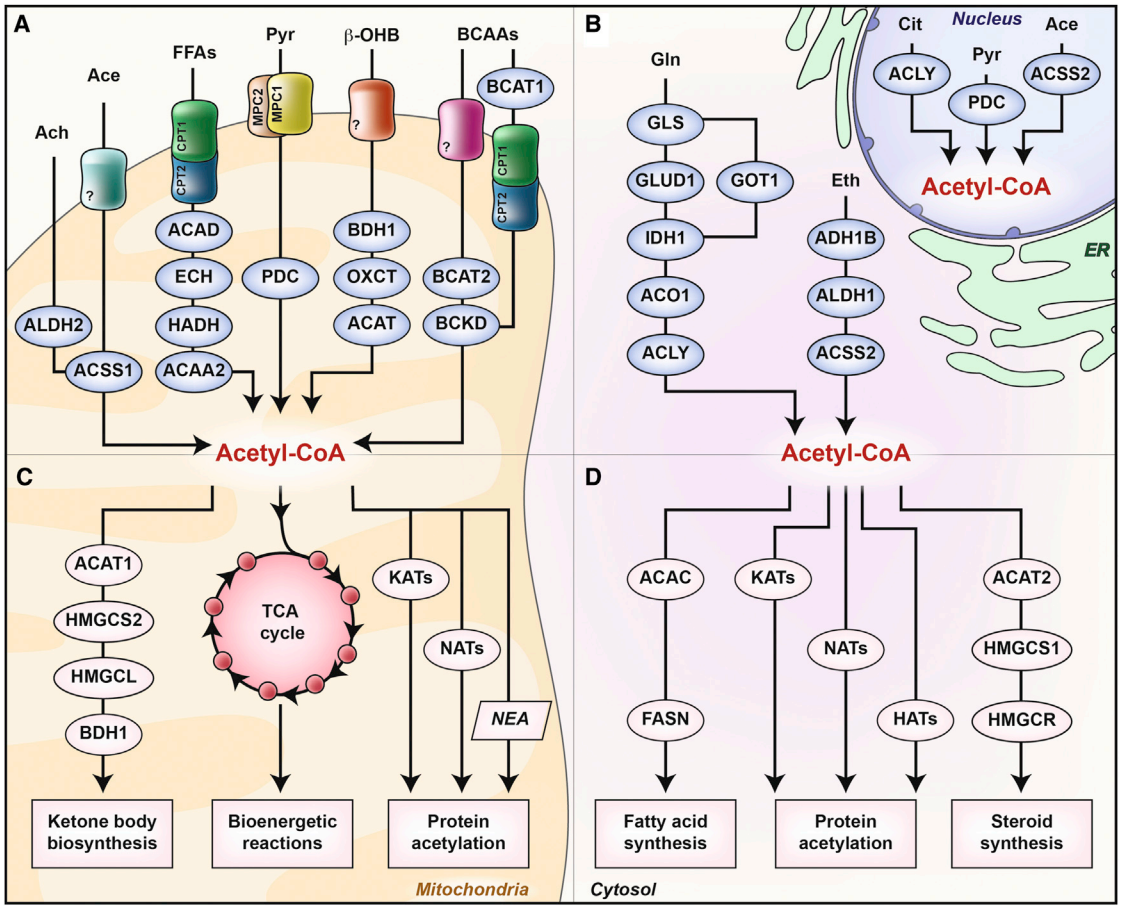

Figure 1. Mitochondrial and Nucleo-Cytosolic Bioenergetic Metabolism of Acetyl-CoA in Mammalian Cells

(A–D) Acetyl coenzyme A (acetyl-CoA) is a key metabolic intermediate. Generally, the majority of cellular acetyl-CoA is generated (A) and consumed (C) in the mitochondrial matrix, in the context of the oxidative metabolism of glycolytic pyruvate (Pyr), free fatty acids (FFAs), branched-chain amino acids (BCAAs), or ketone bodies within the tricarboxylic acid (TCA) cycle. Acetyl-CoA can also be generated in the cytosolic compartment (B), where it supports several anabolic reactions, including lipogenesis, steroidogenesis, and the synthesis of specific amino acids (D). In some malignant cells, the pyruvate dehydrogenase complex (PDC), ATP citrate lyase (ACLY), and acyl- CoA synthetase short-chain family, member 2 (ACSS2) are also found in the nucleus and produce acetyl-CoA therein. Of note, both mitochondrial and nucleo-cytosolic acetyl-CoA pools are critically involved in protein acetylation reactions. ACAA2, acetyl-CoA acyltransferase 2; ACAC, acetyl-CoA carboxylase; ACAD, acyl-CoA dehydrogenase; ACAT1, acetyl-CoA carboxylase 1; ACAT2, acetyl-CoA acetyltransferase 2; Ace, acetate; Ach, acetaldehyde; ACO1, aconitase 1, soluble; ACSS1, acyl-CoA synthetase short-chain family, member 1; ADH1B, alcohol dehydrogenase IB (class I), beta polypeptide; ALDH1A1, aldehyde dehydrogenase 1 family, member A1; ALDH2, aldehyde dehydrogenase 2 family; BCAT1, branched chain amino-acid transaminase 1, cytosolic; BCAT2, branched chain amino-acid transaminase 2, mitochondrial; BCKD, branched-chain α-ketoacid dehydrogenase; BDH1, 3-hydroxybutyrate dehydrogenase, type 1; β-OHB, *D*-β-hydroxybutyrate; Cit, citrate; CPT1, carnitine palmitoyltransferase 1; CPT2, carnitine palmitoyltransferase 2; ECH, enoyl-CoA hydratase; ER, endoplasmic reticulum; Eth, ethanol; FASN, fatty acid synthase; Gln, glutamine; GLS, glutaminase; GLUD1, glutamate dehydrogenase 1; GOT1, glutamic-oxaloacetic transaminase 1, soluble; HADH, hydroxyacyl-CoA dehydrogenase; HAT, histone acetyltransferase; HMGCL, 3-hydroxymethyl-3-methylglutaryl-CoA lyase; HMGCR, 3-hydroxy-3-methyl-glutaryl-CoA reductase; HMGCS1, 3-hydroxy-3-methylglutaryl-CoA synthase 1, HMGCS2, 3-hydroxy-3-methylglutaryl-CoA synthase 2, IDH1, isocitrate dehydrogenase 1; KAT, lysine acetyltransferase; MPC1, mitochondrial pyruvate carrier 1; MPC2, mitochondrial pyruvate carrier 2; NAT, N^{α} acetyltransferase; NEA, non-enzymatic acetylation; OXCT, 3-oxoacid CoA transferase.

of ketone bodies (which relies on acetyl-CoA, see below) while activating bioenergetic pathways that utilize ketone bodies in extrahepatic tissues (Dittenhafer-Reed et al., 2015). Hepatocytes can also obtain acetyl-CoA from ethanol (Cederbaum, 2012; Lundquist et al., 1962). In this case, ethanol is first converted into acetaldehyde in the cytosol by alcohol dehydrogenase IB (class I) beta polypeptide (ADH1B). Acetaldehyde freely diffuses to the mitochondrial matrix, where it is converted into acetate by aldehyde dehydrogenase 2 family (ALDH2). Finally, the mitochondrial enzyme acyl-CoA synthetase short-chain family, member 1 (ACSS1) employs acetate to produce acetyl-CoA (Fujino et al., 2001). This metabolic circuitry may explain why ethanol ingestion causes protein acetylation preferentially in hepatic mitochondria (Fritz et al., 2013).

Generation of Cytosolic Acetyl-CoA

In physiological and normoxic conditions, glycolysis- or β-oxidation-derived mitochondrial acetyl-CoA represents the major source of cytosolic acetyl-CoA upon transportation (see below). That said, there are at least two relatively ubiquitous metabolic circuitries through which cells actually produce acetyl-CoA in the cytosol (Figure 1B).

First, cytosolic acetyl-CoA can originate from glutamine reductive carboxylation, especially when glycolysis is blocked (Yang et al., 2014), in hypoxic conditions (Metallo et al., 2012),

or in the presence of mitochondrial defects (Mullen et al., 2012). Upon uptake from the extracellular milieu (which is mediated by various transporters, depending on cell type), cytosolic glutamine is metabolized by glutaminase (GLS) to generate glutamate, which enters mitochondria through the H^+-dependent glutamate/aspartate antiporter solute carrier family 25 (aspartate/glutamate carrier), member 13 (SLC25A13). Mitochondrial glutamate is converted into α-ketoglutarate (a reaction catalyzed by glutamate dehydrogenase 2, GLUD2, or glutamic-oxaloacetic transaminase 2, mitochondrial, GOT2), which undergoes reductive carboxylation within the TCA cycle to generate citrate. Finally, citrate can be exported back to the cytosol via the dicarboxylate antiporter solute carrier family 25 (mitochondrial carrier; citrate transporter), member 1 (SLC25A1), and converted into oxaloacetate and acetyl-CoA by ATP citrate lyase (ACLY) (Zaidi et al., 2012). Of note, glutamate also can be converted into α-ketoglutarate by GLUD1, a cytosolic variant of GLUD2, at least in some settings (Grassian et al., 2014). Along similar lines, a cytosolic form of GOT2 (i.e., GOT1) can catalyze the reversible interconversion of glutamate and oxaloacetate into α-ketoglutarate and aspartate. Cytosolic α-ketoglutarate can be metabolized by isocitrate dehydrogenase 1 (IDH1) and aconitase 1 soluble (ACO1) to generate citrate for acetyl-CoA synthesis by ACLY (Grassian et al., 2014).

Second, a cytosolic counterpart of ACSS1, i.e., acyl-CoA synthetase short-chain family, member 2 (ACSS2), employs acetate to produce acetyl-CoA in an ATP-dependent manner (Schug et al., 2015). Cytosolic acetate can derive from the extracellular milieu, upon uptake by various members of the monocarboxylate transporter protein family (Halestrap and Price, 1999), or it can be synthesized from ethanol-derived acetaldehyde by a cytosolic variant of ALDH2, namely aldehyde dehydrogenase 1 family, member A1 (ALDH1A1), at least in hepatocytes (Cederbaum, 2012). Although circulating acetate levels are relatively low in modern humans (as compared to their ancestors, owing to dietary changes) (Frost et al., 2014), the ACSS2-dependent conversion of acetate into acetyl-CoA has been shown to be preponderant in primary and metastatic malignant cells of various origin (Mashimo et al., 2014), especially in hypoxic conditions (Kamphorst et al., 2014; Schug et al., 2015). In line with this notion, hepatocellular carcinomas driven by the expression of simian virus 40 (SV40) T antigen or by *v-myc* avian myelocytomatosis viral oncogene homolog (*Myc*) overexpression combined with phosphatase and tensin homolog (*Pten*) inactivation exhibit reduced growth rate in *Acss2*$^{-/-}$ mice (Comerford et al., 2014). Moreover, elevated ACSS2 levels are associated with high tumor grade and dismal disease outcome in patients with human gliomas (Mashimo et al., 2014) and triple-negative breast carcinomas (Comerford et al., 2014). Thus, the metabolic stress associated with malignant transformation and/or tumor progression (which generally involves decreased nutrient availability and hypoxia) may render cancer cells addicted to ACSS2 activity (which is non-oncogenic per se) (Galluzzi et al., 2013). Further corroborating this notion, breast carcinomas often exhibit *ACSS2* amplifications (Schug et al., 2015).

Ectopic Acetyl-CoA Generation

As malignant cells progress through the S phase of the cell cycle, functional PDC can translocate from mitochondria to the nucleus, catalyzing the ectopic synthesis of acetyl-CoA from pyruvate (Sutendra et al., 2014). Nuclear PDC levels as well as histone 3 (H3) acetylation increase upon the administration of epidermal growth factor (EGF), whereas the inhibition of EGF receptor (EGFR) signaling limits nuclear PDC accumulation concomitant with a reduction in H3 acetylation levels. Also, the inhibition of respiratory complex I with rotenone can promote the mitochondrio-nuclear relocalization of PDC, a process that appears to require heat shock 70 kDa protein 1A (HSPA1A) (Sutendra et al., 2014). However, the exact mechanisms through which PDC is exported from mitochondria and enters the nucleus remain enigmatic. Since PDK is not detectable within nuclei (Sutendra et al., 2014), nuclear PDC may be constitutively derepressed to support histone acetylation and cell cycle progression. Interestingly, two other acetyl-CoA-generating enzymes, namely ACLY and ACSS2, have been localized to the nucleus and linked to cell growth and proliferation (Comerford et al., 2014; Takahashi et al., 2006; Wellen et al., 2009). Taken together, these observations suggest that, even though acetyl-CoA freely diffuses between the cytosol and nucleus, the production of acetyl-CoA in the proximity of histones may favor the activation of epigenetic programs associated with cell growth (see below).

Metabolic Fate of Mitochondrial Acetyl-CoA

Normally, mitochondrial acetyl-CoA is metabolized within the TCA to yield NADH, the main substrate for ATP synthesis via oxidative phosphorylation (Boroughs and DeBerardinis, 2015). Some cells, however, including hepatocytes, can employ mitochondrial acetyl-CoA to synthesize ketone bodies, i.e., acetoacetate, acetone, and *D*-β-hydroxybutyrate (Newman and Verdin, 2014). This metabolic pathway, which is catalyzed by the sequential activity of the acetoacetyl-CoA thiolase complex, 3-hydroxy-3-methylglutaryl-CoA synthase 2 (HMGCS2), 3-hydroxymethyl-3-methylglutaryl-CoA lyase (HMGCL), and BDH1, is particularly active in conditions of fasting or caloric restriction, when the majority of mitochondrial acetyl-CoA derives from β-oxidation (Figure 1C) (Newman and Verdin, 2014). Liver-derived ketone bodies enter the circulation and are readily taken up by neurons and cardiomyocytes, which employ them to produce ATP upon reconversion to acetyl-CoA and entry in the TCA (Cotter et al., 2013). Of note, *D*-β-hydroxybutyrate acts as an endogenous inhibitor of class I histone deacetylases, i.e., HDAC1, HDAC2, and HDAC3 (Shimazu et al., 2013). This may explain why fasting and caloric restriction increase global histone acetylation in some mouse tissues, particularly the liver and kidney (Donohoe et al., 2012; Shimazu et al., 2013).

Metabolic Fate of Cytosolic Acetyl-CoA

Cytosolic acetyl-CoA is the precursor of multiple anabolic reactions that underlie the synthesis of fatty acids and steroids, as well as specific amino acids including glutamate, proline, and arginine (Figure 1D). The rate-limiting step of lipogenesis is catalyzed by acetyl-CoA carboxylase (ACAC), a biotin-dependent enzyme that irreversibly carboxylates acetyl-CoA to malonyl-CoA (Brownsey et al., 2006). The reverse reaction (i.e., the decarboxylation of malonyl-CoA to acetyl-CoA), which is catalyzed by malonyl-CoA decarboxylase (MLYCD), not only inhibits lipogenesis but also stimulates the mitochondrial uptake of free fatty acids for β-oxidation by relieving the malonyl-CoA-mediated inhibition of CPT1 (Koves et al., 2008). Interestingly, the muscle-specific knockout of *Mlycd* prevents the development of

diet-induced glucose intolerance in mice as it inhibits β-oxidation (Koves et al., 2008). The human genome codes for two ACAC isoforms, namely ACACA and ACACB, which are differentially expressed in distinct tissues (Travers and Barber, 1997). In energy-low conditions (low ATP, high cyclic AMP levels), ACAC is inhibited upon phosphorylation by protein kinase, AMP-activated (PRKA, best known as AMPK). In line with this notion, the cytosolic pool of acetyl-CoA increases upon AMPK activation, at least in yeast (Zhang et al., 2013). Whether a similar effect occurs in mammalian cells remains to be confirmed. In mouse hepatocytes, the knockout of both *Acaca* and *Acacb* causes the hyperacetylation of extramitochondrial proteins (but the hypoacetylation of mitochondrial proteins) (Chow et al., 2014), suggesting that the utilization of acetyl-CoA for lipogenesis has a significant impact on acetylation reactions. Spermidine/spermine N(1)-acetyltransferase 1 (SAT1) also consumes cytosolic acetyl-CoA as it acetylates polyamines (i.e., spermidine and spermine) to facilitate their secretion from cells and *in fine* their excretion with urine (Pegg, 2008). This pathway may be important for increasing energy expenditure upon inhibition of nicotinamide N-methyltransferase (NNMT) in adipocytes (Kraus et al., 2014). Extramitochondrial variants of the acetyl-CoA thiolase complex and HMGCS (i.e., HMGCS1) employ three acetyl-CoA molecules to synthesize 3-hydroxy-3-methyglutaryl-CoA (HMGC) (Edwards and Ericsson, 1999). Whereas mitochondrial HMGC generally feeds the synthesis of ketone bodies, its cytosolic counterpart is metabolized by 3-hydroxy-3-methyl-glutaryl-CoA reductase (HMGCR, the pharmacological target of statins) within the mevalonate pathway, which is essential in all cells for the production of sterols, ubiquinone (coenzyme Q_{10}), heme A, and other isoprenoids (Edwards and Ericsson, 1999). Finally, cytosolic acetyl-CoA is required for cell type-specific metabolic circuitries, such as the synthesis of acetylcholine, implying that the survival and activity of cholinergic neurons are particularly dependent on acetyl-CoA (Szutowicz et al., 2013).

Peroxisomal Metabolism of Acetyl-CoA in Yeast

The peroxisomal metabolism of acetyl-CoA has not been studied in detail in the mammalian system (Chen et al., 2012). In yeast, β-oxidation underlies the synthesis of a significant fraction of the peroxisomal acetyl-CoA pool (Choudhary et al., 2014). In addition, yeast cells can produce acetyl-CoA within peroxisomes directly from acetate, a CoA-dependent reaction catalyzed by acetate-CoA ligase 1 (Acs1) (Chen et al., 2012). Finally, yeast cells employ the majority of peroxisomal acetyl-CoA to feed the glyoxylate cycle, a metabolic circuitry that generates succinate for carbohydrate synthesis (Kunze et al., 2006). Of note, recent data suggest that (at least some) human tissues may be endowed with enzymatic activities that are required for the glyoxylate cycle, such as the ability to reversibly convert acetyl-CoA, glyoxylate, and H_2O into malate and CoA (Strittmatter et al., 2014). Nonetheless, the glyoxylate cycle is still considered as an anabolic pathway operating in lower organisms only.

Compartmentalization of Acetyl-CoA Metabolism

Acetyl-CoA exists in separate mitochondrial, peroxisomal, nucleo-cytosolic, and intrareticular pools (Figure 2). Indeed, the inner mitochondrial, peroxisomal, and reticular membranes are impermeant to the highly charged acetyl-CoA molecule. Conversely, nuclear pores allow acetyl-CoA to freely distribute between the cytosol and the nucleus. Of note, the endoplasmic reticulum (ER) does not contain acetyl-CoA-generating enzymes, implying that the acetylation of proteins within the ER lumen critically depends on the import of acetyl-CoA from the cytosol. Taken together, these observations suggest that the bioenergetic and signaling functions of acetyl-CoA are finely regulated by compartmentalization and inter-organellar acetyl-CoA fluxes, which (at least in part) are influenced by the subcellular localization of acetyl-CoA-generating reactions.

Export of Acetyl-CoA from Mitochondria

The transport of acetyl-CoA from the mitochondrial matrix to the cytosol heavily relies on the so-called "citrate-malate-pyruvate shuttle." In this context, mitochondrial acetyl-CoA is condensed with oxaloacetate by citrate synthase (CS), the first enzyme of the TCA cycle, generating citrate and free CoA. Citrate can be exported to the cytosol through SLC25A1 (also known as citrate carrier), followed by the regeneration of oxaloacetate and acetyl-CoA upon the ATP- and CoA-dependent reaction catalyzed by ACLY (Zaidi et al., 2012). Cytosolic oxaloacetate is the substrate of malate dehydrogenase 1, NAD (soluble) (MDH1), catalyzing the NADH-dependent synthesis of malate, which can be transported back to the mitochondrial matrix via solute carrier family 25 (mitochondrial carrier; dicarboxylate transporter), member 10 (SLC25A10), an inorganic phosphate/dicarboxylate antiporter (Mizuarai et al., 2005), or solute carrier family 25 (mitochondrial carrier; oxoglutarate carrier), member 11 (SLC25A11), an α-ketoglutarate/malate antiporter (Wallace, 2012). Alternatively, malic enzyme 1, NADP(+)-dependent, cytosolic (ME1) can convert malate into pyruvate, which can re-enter the mitochondrial matrix via the MPC (Bender et al., 2015). Acetyl-CoA is also exported from mitochondria via the carnitine shuttle. As mentioned above, fatty acids enter mitochondria in the form of acylcarnitine via the antiporter SLC25A20, which generally exchanges them with free *L*-carnitine (Rebouche and Seim, 1998). Mitochondrial *L*-carnitine can also be converted by carnitine O-acetyltransferase (CRAT) into acetyl-*L*-carnitine, and the latter shares with free *L*-carnitine the ability to drive the SLC25A20 antiporter. Finally, cytosolic acetyl-*L*-carnitine can regenerate acetyl-CoA and *L*-carnitine via the CoA-dependent reaction catalyzed by a cytosolic variant of CRAT (Madiraju et al., 2009). Thus, the citrate-malate-pyruvate and the carnitine shuttle may ensure the continuous transfer of acetyl-CoA from mitochondria to the cytosol. Of note, the enzymatic activity of CRAT in skeletal muscles appears to be critical for whole-body glucose tolerance and metabolic fitness (Muoio et al., 2012). Most likely, this reflects the ability of CRAT to drive the export of excess acetyl-CoA from the mitochondrial matrix, hence preventing PDC substrate inhibition (Muoio et al., 2012). Taken together with the data obtained with muscle-specific *Mlycd*$^{-/-}$ mice (Koves et al., 2008), these observations connect insulin (INS) resistance with a situation of mitochondrial overload characterized by excess β-oxidation and high acetyl-CoA levels.

The acetyl-CoA concentration gradient across the inner mitochondrial membrane is influenced not only by the rate of acetyl-CoA synthesis and consumption in the cytosol and within mitochondria, but also by the activity of: (1) the citrate carrier (SLC25A1), which can be increased by pro-inflammatory transcription factors like NF-κB and signal transducer and activator

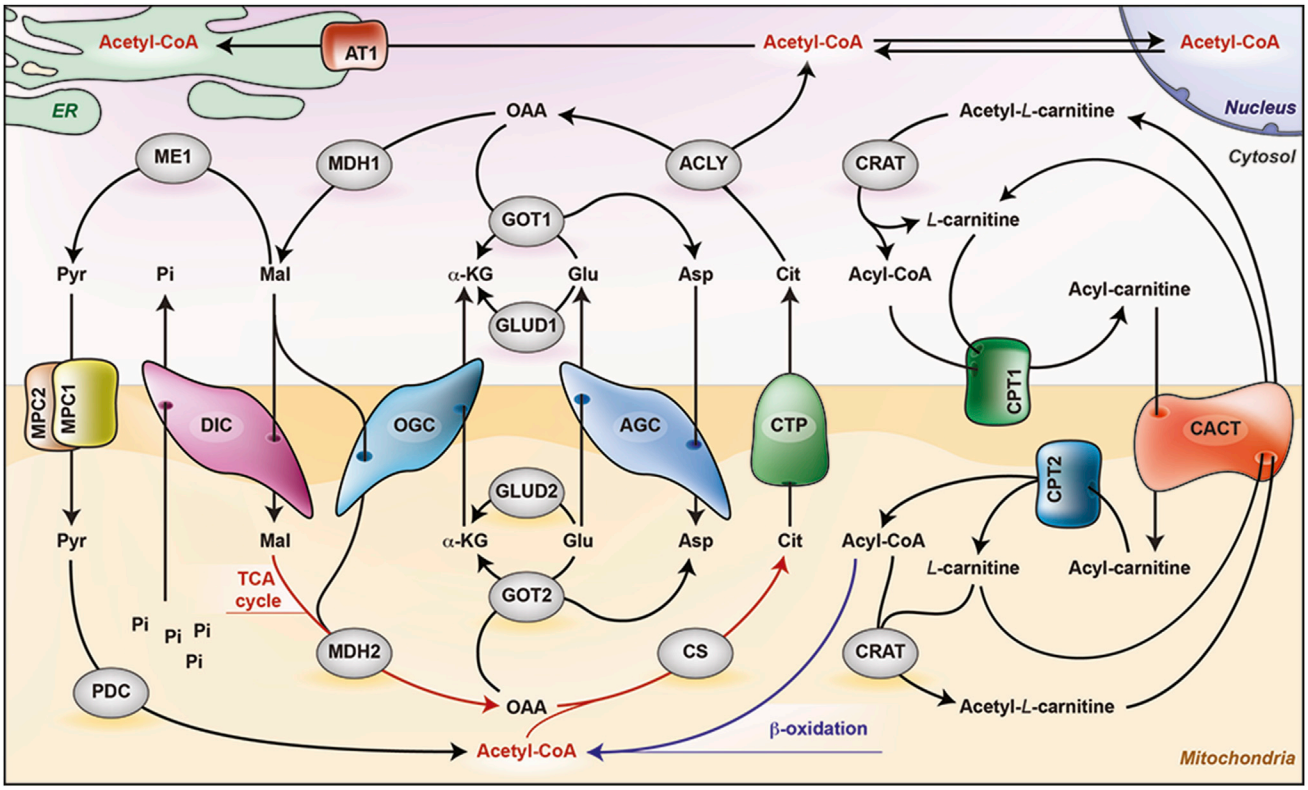

Figure 2. Compartmentalization of Acetyl-CoA Metabolism

The subcellular fluxes of acetyl coenzyme A (acetyl-CoA) are tightly regulated. Two multicomponent systems ensure the exchange of acetyl-CoA between the nucleo-cytosolic compartment and mitochondria: the so-called "carnitine shuttle" and the so-called "citrate-malate-pyruvate shuttle." The former plays a key role in β-oxidation and branched-chain amino acid (BCAA) mitochondrial catabolism, as it allows cytosolic acyl-CoA (derived from free fatty acids, FFAs) and α-ketoacids (derived from BCAAs) to enter the mitochondrial matrix upon conjugation with *L*-carnitine. The actual import of these species into mitochondria is coupled to the export of *L*-carnitine or acetyl-*L*-carnitine, and acetyl-*L*-carnitine can be metabolized by a cytosolic variant of carnitine O-acetyltransferase (CRAT) to generate acetyl-CoA (and free *L*-carnitine). The latter allows for the exchange of several metabolic intermediates between the cytosol and mitochondria, including citrate, oxaloacetate, α-ketoglutarate, glutamate, and aspartate. In doing so, the citrate-malate-pyruvate shuttle provides substrates for anabolic metabolism (in energy-high conditions) or supports bioenergetic metabolism (in energy-low conditions). AT1 (official name SLC33A1) is responsible for the import of acetyl-CoA into the endoplasmic reticulum (ER) lumen. α-KG, α-ketoglutarate; ACLY, ATP citrate lyase; AGC, aspartate/glutamate carrier (SLC25A13); Asp, aspartate; CACT, carnitine/acylcarnitine translocase (SLC25A20); Cit, citrate; CS, citrate synthase; CTP, citrate transporter (SLC25A1); DIC, dicarboxylate carrier (SLC25A10); Glu, glutamate; GLUD1, glutamate dehydrogenase 1; GLUD2, glutamate dehydrogenase 2; GOT1, glutamic-oxaloacetic transaminase 1, soluble; GOT2, glutamic-oxaloacetic transaminase 2, mitochondrial; Mal, malate; MDH1, malate dehydrogenase 1, NAD (soluble); MDH2, malate dehydrogenase 2, NAD (mitochondrial); ME1, malic enzyme 1, NADP(+)-dependent, cytosolic; MPC1, mitochondrial pyruvate carrier 1; MPC2, mitochondrial pyruvate carrier 2; OAA, oxaloacetate; OGC, oxoglutarate carrier (SLC25A11); PDC, pyruvate decarboxylase; Pi, inorganic phosphate; Pyr, pyruvate.

of transcription 1 (STAT1) (Infantino et al., 2014); and (2) ACLY, which is regulated by several signal transducers, including Kirsten rat sarcoma viral oncogene homolog (KRAS) and *v-akt* murine thymoma viral oncogene homolog 1 (AKT1) (Berwick et al., 2002; Lee et al., 2014). Of note, the export of citrate from mitochondria creates the need for the anaplerotic replenishment of TCA cycle intermediates that regenerate oxaloacetate, meaning that the extracellular availability of glutamine and the metabolic flux through glutaminolysis also influence the relative abundance of mitochondrial and cytosolic acetyl-CoA (Yang et al., 2014). Finally, acetyl-CoA might be non-specifically released from the mitochondrial matrix upon transient openings of the so-called "permeability transition pore complex," a multicomponent channel built across the inner and outer mitochondrial membranes permeant to solutes < 1.5 kDa and involved in the regulation of cell death (Galluzzi et al., 2015a). This possible mechanism of acetyl-CoA release has not been investigated in detail thus far.

Export of Acetyl-CoA from Peroxisomes

In yeast cells, peroxisomal acetyl-CoA can be exported to the nucleo-cytosolic compartment by at least two distinct mechanisms: (1) via a carnitine shuttle, relying on the generation of peroxisomal acetyl-*L*-carnitine by the yeast ortholog of CRAT (Franken et al., 2008); and (2) in the form of succinate, one of the products of the glyoxylate cycle (which consumes acetyl-CoA) (Kunze et al., 2006). Of note, the release of succinate does entail a reduction in peroxisomal pool acetyl-CoA, but not a direct increase in its nucleo-cytosolic counterpart.

Import of Acetyl-CoA into the Endoplasmic Reticulum

The transfer of acetyl-CoA from the cytosol to the ER lumen is mediated by solute carrier family 33, member 1 (SLC33A1, also known as AT1) (Kanamori et al., 1997), a protein that is mutated in spastic paraplegia 42, an autosomal dominant neurodegenerative disease characterized by muscle wasting and progressive weakness of the inferior limbs (Lin et al., 2008). Importantly, *SLC33A1* is transactivated by X-box binding protein 1 (XBP1),

an ER stress-responsive factor (Damiano et al., 2015). Thus, reticular acetyl-CoA levels are expected to increase (at the expense of the nucleo-cytosolic pool) in the course of ER stress responses (Galluzzi et al., 2014). In the ER lumen, acetyl-CoA is the substrate of a few acetyltransferases including members of the N-acetyltransferase 8 (NAT8) protein family and calreticulin (CALR, a chaperone with acetyltransferase enzymatic activity) (Ding et al., 2014). Acetylation reactions in the ER lumen may allow correctly folded proteins to progress to the Golgi apparatus for secretion, implying that the reticular pool of acetyl-CoA could play an important role in protein quality control (Pehar and Puglielli, 2013). Interestingly, N-acetyltransferase 8-like (NAT8L) is specifically expressed by neurons, where it plays a key role as it catalyzes the synthesis of *N*-acetylaspartate, the most prominent storage and transport form of acetyl groups in the brain (Moffett et al., 2013).

Compartmentalized Regulation of Protein Acetylation

The compartmentalization of acetyl-CoA pools has a major impact on protein acetylation, be it non-enzymatic or be it catalyzed by NATs or KATs. The rate of non-enzymatic acetylation, which presumably relies on the highly reactive intermediate acetyl-phosphate (functioning as the donor of acetyl groups), is strongly influenced by the physicochemical properties of the microenvironment (Kuhn et al., 2014). This implies that intracellular pH gradients, such as the one built across the inner mitochondrial membrane by respiratory chain complexes I–IV, may influence acetylation reactions (Wagner and Payne, 2013). Moreover, spermidine can compete with acetyl-CoA for binding to EP300 (and perhaps other acetyltransferases), hence increasing the acetyl-CoA concentration required for optimal acetyltransferase activity (Pietrocola et al., 2015). Since spermidine levels vary in different organelles (Casero and Marton, 2007), this phenomenon may contribute to the compartmentalized regulation of protein acetylation. Specific acetyltransferases and deacetylases are also subjected to subcellular compartmentalization. This applies, for instance, to EP300 as well as to the deacetylase SIRT1, both of which shuttle between the nucleus and the cytosol (Chang and Guarente, 2014). Another deacetylase of the sirtuin family, i.e., SIRT3, localizes for the most part to the mitochondrial matrix, and its expression levels have been correlated with differential patterns of mitochondrial protein acetylation both in baseline conditions (Dittenhafer-Reed et al., 2015) and in the course of chronic caloric restriction (Hebert et al., 2013). Importantly, the deacetylase activity of sirtuins obligatorily relies on NAD^+ as a cofactor, establishing yet another link between metabolism and protein acetylation levels.

Fluctuations of Acetyl-CoA Levels

At odds with ATP levels, which are relatively stable (to support bioenergetic homeostasis), the abundance of acetyl-CoA fluctuates in response to both intracellular and extracellular cues. Thus, acetyl-CoA levels have been shown to vary even in the course of embryonic development (Tsuchiya et al., 2014). Unfortunately, however, acetyl-CoA is generally quantified in whole-cell or whole-organ extracts, and very few studies have measured changing acetyl-CoA concentrations at the subcellular level.

Changing Acetyl-CoA levels in Cultured Cells

When human cancer cells are switched from normal culture media to serum- and nutrient-free conditions, intracellular acetyl-CoA levels fall well before the concentration of ATP and NADH diminishes. Such a drop is more pronounced in the cytosolic fraction than in whole-cell extracts, suggesting (but not demonstrating unequivocally) that the mitochondrial pool of acetyl-CoA is less susceptible to variations than it nucleo-cytosolic counterpart (Mariño et al., 2014). The reduction in intracellular acetyl-CoA levels can be detected as early as 30 min after the switch of culture conditions and can be avoided by providing various acetyl-CoA precursors, including dimethyl-α-ketoglutarate (a cell-permeant form of α-ketoglutarate), leucine or its derivative α-ketoisocaproic acid, pyruvate, and PDC stimulators (like lipoic acid and DCA) (Mariño et al., 2014). Conversely, when cancer cells are cultured in rich media, various selective manipulations of acetyl-CoA metabolism effectively can reduce intracellular acetyl-CoA levels. These interventions include the pharmacological or genetic inhibition of the MPC heterodimer, CPT1, the BCKD complex, the citrate carrier (SLC25A1), ACLY, and ACSS2 (Mariño et al., 2014). Thus, all these pathways appear to contribute to the maintenance of optimal acetyl-CoA levels, at least in human malignant cells cultured in standard conditions.

Also, oncogenes may affect acetyl-CoA concentrations. The overexpression of the anti-apoptotic protein BCL2-like 1 (BCL2L1, best known as BCL-X_L) appears to reduce the amounts of citrate, acetyl-CoA, and N^{α}-acetylated proteins in the cytoplasm, and this would etiologically contribute to oncogenesis (Yi et al., 2011). To the best of our knowledge, however, this report has not been confirmed by independent investigators yet. The knockout of *Myc* in rat fibroblasts results in decreased acetyl-CoA levels (Edmunds et al., 2014), corroborating the notion that Myc family members normally stimulate mitochondrial acetyl-CoA production (and histone acetylation) (Morrish et al., 2010). Similar observations (i.e., increased acetyl-CoA/CoA ratio and/or histone acetylation levels) have been made: (1) upon the transgenic expression of a myristoylated, constitutively active variant of AKT1 (myrAKT1) in cultured human glioblastoma cells; (2) upon the constitutive expression of oncogenic KRAS, stimulating accrued AKT1 signaling, in pancreatic cells in vivo; as well as (3) following the doxycycline-inducible expression of myrAKT1 in mammary epithelial cells in vivo (Lee et al., 2014). At least in part, the ability of AKT1 to promote the accumulation of acetyl-CoA originates from its ability to phosphorylate ACLY on S455, hence stimulating its catalytic activity (Lee et al., 2014). These data lend further support to the notion that increased levels of acetyl-CoA and acetylated histones may be required to sustain the accelerated proliferation of cancer cells.

Changing Acetyl-CoA Levels In Vivo

Mice deprived of food (but with access to water ad libitum) for 24 hr exhibit a significant reduction in total acetyl-CoA levels in several organs, including the heart and muscles, corresponding to a decrease in protein acetylation levels (Mariño et al., 2014). However, the same experimental conditions have no major effects on acetyl-CoA concentrations in the brain (Mariño et al., 2014) and actually increase hepatic acetyl-CoA and protein acetylation levels (Chow et al., 2014). This latter phenomenon has been causally linked to the mobilization of subcutaneous fat stores, one of the first effects of short-term fasting (Browning et al., 2012). In fasting conditions, indeed, fatty acids are released by adipocytes and employed (at least in part) as

substrate for β-oxidation (which produces acetyl-CoA) within hepatocytes (which are particularly rich in mitochondria) (Browning et al., 2012). Repeated intraperitoneal injections of dimethyl-α-ketoglutarate or dichloroacetate (DCA) efficiently prevent the drop of acetyl-CoA levels provoked by starvation in the heart and muscle of mice (Mariño et al., 2014). Along similar lines, the provision of excess acetate has been shown to increase acetyl-CoA levels in the hypothalamus upon the activation of ACAC, eventually resulting in the synthesis of anorectic neuropeptides and appetite suppression (Frost et al., 2014). Finally, ethanol intake augments acetyl-CoA levels in hepatic mitochondria (Fritz et al., 2013).

The aforementioned findings indicate that alterations in food and alcohol intake have a direct impact on intracellular acetyl-CoA levels. However, it appears unlikely that the starvation-induced decrease of acetyl-CoA in the heart and muscles solely and directly results from reduced glucose availability. Indeed, intracellular acetyl-CoA concentrations drop well before glycemia decreases, at the same time as circulating triglycerides increase. This phenomenon may therefore reflect the ability of starvation to cause a diminution in the plasma levels of various cytokines and growth factors, including INS and insulin-like growth factor 1 (IGF1), coupled to an increase in the circulating amounts of the IGF1 antagonist insulin-like growth factor binding protein 1 (IGFBP1) (Cheng et al., 2014). Limited growth factor signaling results indeed in reduced AKT1 activation, which has (at least) two negative consequences for acetyl-CoA metabolism. First, AKT1 signaling is required for normal glucose uptake through plasma membrane glucose transporters (Wieman et al., 2007). Second, AKT1 normally phosphorylates ACLY on S455, hence stimulating acetyl-CoA production (Lee et al., 2014). Although this hypothetical pathway linking starvation to dwindling acetyl-CoA levels has not been formally explored in vivo, signs of AKT1 activation (i.e., AKT1 phosphorylation on S473) positively correlate with histone acetylation levels in human gliomas and prostate cancers (Lee et al., 2014). Another major pathway that may link starvation to dropping acetyl-CoA levels in some cells involves SIRT1, which is activated in energy-low conditions (high NAD^+ levels) (Morselli et al., 2010) and inhibits ACSS2 (Sahar et al., 2014). This latter effect may explain the circadian rhythmicity of hepatic acetyl-CoA levels in mice (Sahar et al., 2014). Finally, some tissues may experience intracellular acetyl-CoA depletion in response to organismal starvation as a consequence of limited glutamine availability, at least hypothetically (Pozefsky et al., 1976). This possibility awaits experimental verification. Irrespective of this open issue, it seems likely that several mechanisms operating in parallel connect organismal starvation with the decrease in intracellular acetyl-CoA levels observed in some tissues.

Obviously, metabolic disorders also affect acetyl-CoA concentrations. For example, type 1 diabetes causes a major increase in hepatic acetyl-CoA concentrations (Perry et al., 2014). Interestingly, this effect can be mimicked by a 3-day high-fat dietary regimen combined with an artificial elevation of circulating corticosterone levels (which occur spontaneously in animal models of type 1 diabetes) and can be reverted by the administration of mifepristone, an antagonist of glucocorticoid receptors (Perry et al., 2014). Thus, the increased turnover of fatty acids and acetate associated with type I diabetes may elevate mitochondrial acetyl-CoA levels, ultimately promoting hepatic gluconeogenesis by virtue of its capacity to allosterically activate PC and inhibit the PDC. Further supporting this notion, mice genetically endowed with a muscle-specific defect in β-oxidation are significantly less prone to develop diet-induced INS resistance than their wild-type counterparts (see above) (Koves et al., 2008).

Acetyl-CoA and Gene Expression

In multiple cell types, histone acetylation is highly sensitive to the availability of acetyl-CoA (Cai et al., 2011; Donohoe et al., 2012; Lee et al., 2014; Moussaieff et al., 2015; Takahashi et al., 2006; Wellen et al., 2009). Acetyl-CoA is indeed the obligate cofactor for histone acetyltransferases (HATs), meaning that the abundance of the nucleo-cytosolic pool may have a direct impact on the enzymatic activity of HATs, especially those with a relatively high K_D for acetyl-CoA. Thus, drops in nucleo-cytosolic acetyl-CoA levels below the K_D of specific HATs may directly reduce their activity. Moreover, several HATs including GCN5 are subjected to product inhibition by free CoA (Tanner et al., 2000). This indicates that the acetyl-CoA/CoA ratio may be the relevant regulator of the enzymatic activity of HATs, rather than the absolute levels of acetyl-CoA (Lee et al., 2013). Irrespective of the underlying mechanisms, this establishes a direct link between the nucleo-cytosolic abundance of acetyl-CoA and the epigenetic control of gene expression. Indeed, high levels of histone acetylation are required not only to support global transcription (Moussaieff et al., 2015; Takahashi et al., 2006), but also for the preferential transactivation of genes involved in cell growth and replication (Cai et al., 2011; Donohoe et al., 2012; Lee et al., 2014; Shi and Tu, 2013), glycolysis (Wellen et al., 2009), and resistance to oxidative stress (Shimazu et al., 2013).

Fluctuations in metabolites other than acetyl-CoA also affect histone acetylation, hence impacting transcription both quantitatively and qualitatively. Besides feeding the TCA (and hence stimulating the synthesis of acetyl-CoA) (Donohoe et al., 2012), *D*-β-hydroxybutyrate inhibits HDAC1, HDAC2, and HDAC3 (Shimazu et al., 2013). Along similar lines, *L*-carnitine and sphingosine-1-phosphate favor histone acetylation by operating as HDAC1 and HDAC2 inhibitors (Hait et al., 2009; Huang et al., 2012). Conversely, increases in the NAD^+/NADH ratio (which accompany starvation) promote histone deacetylation by stimulating the activity of sirtuins (Guarente, 2013). Also variations in intracellular pH (pH_i) have a major impact on histone acetylation. Intracellular acidification (i.e., decreased pH_i) promotes the activity of histone deacetylases (as well as the export of protonated acetate via monocarboxylate transporter as a pH_i buffer mechanism), whereas intracellular alkalinization (i.e., increased pH_i) augments global histone acetylation (McBrian et al., 2013). Thus, histone acetylation appears to function as a rheostat to regulate pH_i. Of note, the chromatin of mammalian cells contains at least 10^9 potential acetylation sites, meaning that massive histone acetylation may cause a sizeable depletion of the nucleo-cytosolic acetyl-CoA pool, hence impacting cellular metabolism. Conversely, the acetyl residues stored in histones may be mobilized to generate acetate as an energy source (Martinez-Pastor et al., 2013). All these examples underscore the intricate links between metabolism and histone acetylation levels, which in turn influence gene expression both quantitatively and qualitatively.

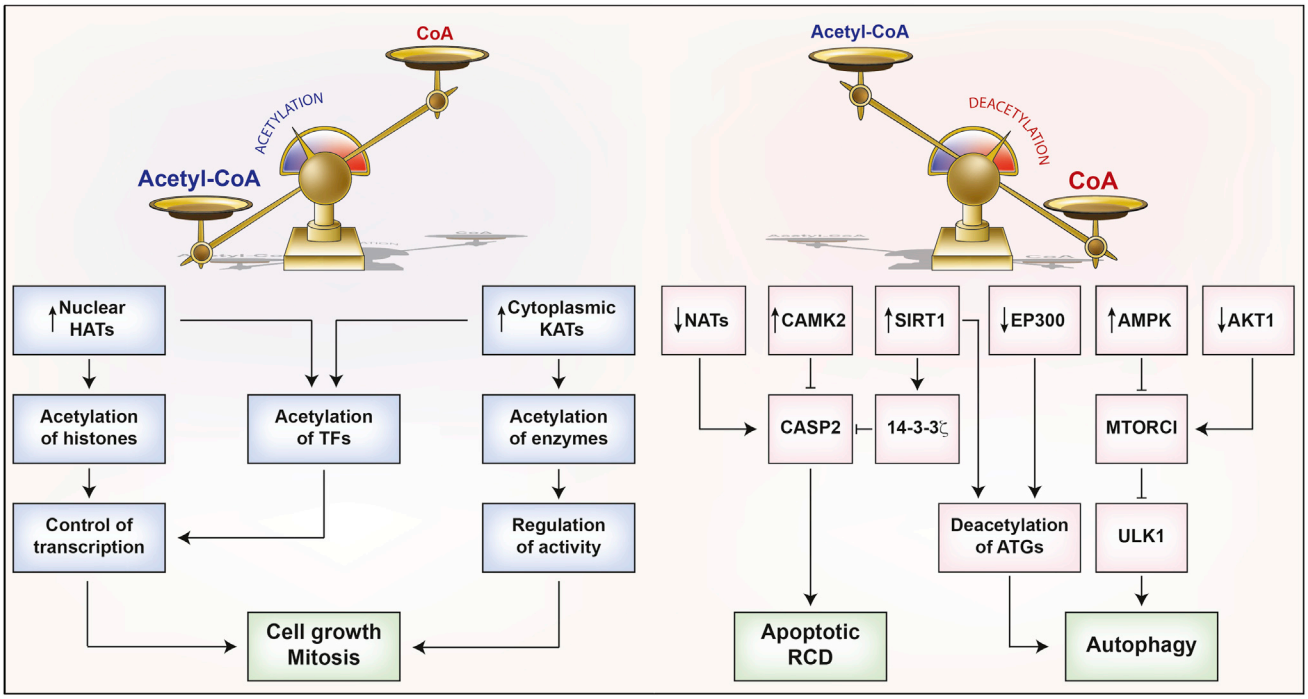

Figure 3. Impact of the Acetyl-CoA/CoA Ratio on Global Cellular Functions

The relative abundance of acetyl coenzyme A (acetyl-CoA) and coenzyme A (CoA) controls various cellular processes, including cell growth and mitosis, regulated cell death (RCD), and autophagy. For instance, elevated acylation levels of histones, transcription factors (TFs), and various enzymes have been associated with intensive growth and proliferation. The pro-apoptotic activity of caspase-2 (CASP2), which appears to require N^{α}-acetylation, is controlled upon physical sequestration by 14-3-3ζ, and this inhibitory interaction is promoted by the calcium/calmodulin-dependent protein kinase II (CAMK2)-dependent phosphorylation of CASP2 as well as by the sirtuin 1 (SIRT1)-dependent deacetylation of 14-3-3ζ (two processes that are favored by low acetyl-CoA/CoA ratios). Finally, the accumulation of CoA at the expense of acetyl-CoA favors the SIRT1- and E1A binding protein p300 (EP300)-dependent deacetylation of various ATG proteins, promotes the activation of AMPK, and inhibits *v-akt* murine thymoma viral oncogene homolog 1 (AKT1) signaling, globally stimulating the autophagic flux. HAT, histone acetyltransferase; KAT, lysine acetyltransferase; MTORC1, mechanistic target of rapamycin complex I; NAT, N^{α} acetyltransferase; ULK1, unc-51-like autophagy activating kinase 1.

Fluctuations in the nucleo-cytosolic pool of acetyl-CoA also modulate gene expression by altering the acetylation state of transcription factors. Thus, acetate stimulates erythropoiesis in multiple mouse models of anemia by activating a signaling pathway that involves (1) the conversion of acetate into acetyl-CoA (by ACSS2); (2) the acetylation of endothelial PAS domain protein 1 (EPAS1) by CBP; and (3) the recruitment of a transcriptionally active EPAS1-containing complex to the promoter of the gene encoding erythropoietin (Xu et al., 2014). Other prominent transcription factors that are regulated by CBP- or EP300-dependent acetylation as well as by SIRT1- or HDAC1-mediated deacetylation are forkhead box O (FOXO) proteins (van der Horst and Burgering, 2007) and tumor protein p53 (TP53) (Xu, 2003). FOXO1 plays an important role in linking caloric restriction to lifespan extension in several model organisms (Kim et al., 2015). TP53 is a key regulator of homeostasis in physiological and pathological conditions, and its activity is controlled by several post-translational modifications, including acetylation at multiple (at least seven) lysine residues (Tang et al., 2008). Besides EP300 and CBP, at least four other KATs can acetylate TP53, namely, KAT2B (also known as PCAF), KAT6A (also known as MOZ), KAT5 (also known as TIP60), and KAT8 (also known as MOF). Acetylation opens the conformation of TP53, alters its ability to bind specific response elements on DNA, and generally increases its transcriptional activity (Tang et al., 2008). Of note, the enzymatic activity of CBP responds to physiological variations in acetyl-CoA levels, at least in some cell types (Xu et al., 2014). It has not yet been demonstrated whether this holds true for other KATs operating on transcription factors. In spite of this uncertainty, these examples illustrate the broad effects of (de)acetylation reactions on the control of gene expression.

Acetyl-CoA and Cellular Anabolism versus Catabolism

Increased nucleo-cytosolic acetyl-CoA levels shift cellular metabolism toward anabolic reactions as they shut off catabolic circuitries. This effect is not limited to basic biochemical circuitries, but involves complex cellular (and organismal) programs. Thus, acetyl-CoA levels affect the propensity of cells to grow, progress along the cell cycle, mount autophagic responses to stress, and succumb to RCD (Figure 3). In addition, the abundance of acetyl-CoA in defined cell types influences the metabolic relationship between different organs, as well as behavioral cues such as appetite control.

Cell Growth and Mitosis

By favoring histone acetylation, elevated acetyl-CoA levels stimulate the expression of multiple genes that are required for cell growth and proliferation (Cai et al., 2011; Donohoe et al., 2012; Lee et al., 2014; Shi and Tu, 2013; Wellen et al., 2009). In yeast, the Acs1-dependent synthesis of acetyl-CoA does not only support global transcription, but it also allows for the preferential

transactivation of various genes involved in cell growth and replication, like those encoding cyclin CLN3, several ribosomal subunits, and glycolytic enzymes (Cai et al., 2011; Shi and Tu, 2013). In contrast, when yeast cells enter the stationary phase during the so-called "diauxic shift" (i.e., the transition of cultured yeast from a fermentative to a respiratory metabolism), acetyl-CoA levels and global histone acetylation decrease. In this context, a minority of so-called histone hypoacetylation-activated genes (which are repressed during the exponential growth phase) become activated (Mehrotra et al., 2014). In the mammalian system, high acetyl-CoA concentrations also favor cell growth and replication at the expenses of differentiation (Moussaieff et al., 2015; Shan et al., 2014; Sutendra et al., 2014). Embryonic stem cells quickly lose pluripotency along with a metabolic shift involving decreased glycolytic activity, lowered acetyl-CoA availability, and histone deacetylation (Moussaieff et al., 2015). In line with this notion, artificially preserving acetyl-CoA levels though the exogenous supply of acetate efficiently delays embryonic stem cell differentiation as it preserves histone acetylation (Moussaieff et al., 2015). Cancer cells, which are characterized by increased proliferation rates, ectopically synthesize acetyl-CoA in the nucleus (perhaps in an S phase-specific manner), resulting in increased histone acetylation levels (Comerford et al., 2014; Sutendra et al., 2014; Takahashi et al., 2006; Wellen et al., 2009). Transcriptomic studies revealed that a significant proportion of the genes that are responsive to acetyl-CoA (and hence histone acetylation) levels are involved in DNA replication, cell cycle progression, and general anabolism (Lee et al., 2014). Of note, the acetylation of phosphogluconate dehydrogenase (PDG) by a cytosolic variant of DLAT and acetyl-CoA acetyltransferase 2 (ACAT2) augments its enzymatic activity, hence promoting cellular anabolism as a consequence of an increased metabolic flux through the pentose phosphate pathway (PPP) (Shan et al., 2014). Altogether, these observations suggest that increased acetyl-CoA concentrations promote cell growth and replication via transcriptional and non-transcriptional mechanisms.

Catabolism and Autophagy

The depletion of nucleo-cytosolic acetyl-CoA activates AMPK and inhibits the mechanistic target of rapamycin (MTOR) complex I (MTORCI), hence arresting anabolic reactions and stimulating autophagy (Galluzzi et al., 2015b; Mariño et al., 2014). Histone deacetylation caused by the shortage of acetyl-CoA favors the expression of pro-autophagic genes (Eisenberg et al., 2014), whereas cytoplasmic deacetylation reactions activate various proteins that are required for autophagy (Mariño et al., 2014). In line with this notion, replenishing the nucleo-cytosolic pool of acetyl-CoA by the microinjection of acetyl-CoA into cultured human cells or by the systemic administration of dimethlyl-α-ketoglutarate to mice suppresses starvation-induced autophagy (Mariño et al., 2014). In human cells, low acetyl-CoA levels restrain the enzymatic activity of EP300, and epistatic analyses suggest that the inhibition of EP300 is largely responsible for autophagy induction in these conditions (Mariño et al., 2014). Various proteins of the autophagic machinery, including ATG5, ATG7, and ATG12, are acetylated (and hence inhibited) by EP300 or deacetylated (and hence activated) by SIRT1 (Madeo et al., 2014) Moreover, microtubule-associated protein 1 light chain 3 (MAP1LC3, best known as LC3) is deacetylated by SIRT1 in the nucleus, allowing it to shuttle back to the cytoplasm (in complex with tumor protein p53 inducible nuclear protein 2, TP53INP2) and participate in the assembly of autophagosomes (Huang et al., 2015). ATG9A, a protein that shuttles between the juxta-nuclear *trans*-Golgi compartment and late endosomes, must also be deacetylated for autophagy to proceed normally (Pehar et al., 2012). In line with this notion, loss-of-function mutations of *SLC33A1*, resulting in a depletion of reticular acetyl-CoA, cause excessive autophagy (Peng et al., 2014).

The knockdown of biogenesis of lysosomal organelle complex-1, subunit 1 (BLOC1S1, a mitochondrial KAT also known as GCN5L1) favors the deacetylation of mitochondrial proteins while stimulating mitophagy (Webster et al., 2013), an organelle-specific type of autophagy targeting mitochondria (Green et al., 2011). Beyond putative direct effects on mitochondria, BLOC1S1 may control the activity of the pro-autophagic transcription factor EB (TFEB) (Pietrocola et al., 2013). *Bloc1s1*$^{-/-}$ mouse embryonic fibroblasts indeed exhibit increased expression levels of TFEB and its downstream targets (Scott et al., 2014), providing yet another link between deacetylation and the activation of a pro-autophagic transcriptional program. These examples illustrate how the depletion of acetyl-CoA in distinct subcellular compartments can ignite autophagy via a multipronged effect on various autophagy-regulatory processes.

Several inducers of autophagy, including (but not limited to) caloric restriction and spermidine, have lifespan-extending effects in various model organisms, including yeast, worms, flies, and mice (Madeo et al., 2014), as they lower global protein acetylation levels (Mariño et al., 2014). Intriguingly, reduced levels of Myc also increase the lifespan of flies (Greer et al., 2013) and mice (Hofmann et al., 2015) and are associated with lowered nucleo-cytosolic acetyl-CoA levels (Edmunds et al., 2014; Morrish et al., 2010). Along similar lines, nucleo-cytosolic acetyl-CoA metabolism has been genetically linked to autophagy regulation and lifespan in yeast and *Drosophila melanogaster* (Eisenberg et al., 2014). Yeast cells overexpressing the ortholog of mammalian ACSS2 (i.e., Acs2p) indeed exhibit reduced lifespan as compared to their wild-type counterparts. Moreover, the brain-specific knockout of *AcCoAS* (the sole ortholog of mammalian ACSS1 and ACSS2 in *Drosophila*) suffices to extend fly lifespan (Eisenberg et al., 2014). It has not been tested yet whether direct genetic manipulations of acetyl-CoA metabolism may have lifespan-extending effects in mammals as well. Moreover, it remains to be determined whether the autophagy-inducing and antidiabetic effects of endurance training (He et al., 2012) may be ultimately linked to decreased mitochondrial protein acetylation levels, as it is the case for rats selectively bred for a high running capacity (Overmyer et al., 2015).

Regulated Cell Death

The acetyl-CoA/CoA ratio appears to influence the propensity of cells to undergo regulated variants of cell death, including caspase-dependent apoptosis and regulated necrosis (Green et al., 2014). To mention one example, the acetyl-CoA/CoA ratio appears to be involved in a complex system of regulation of caspase-2 (CASP2), an apoptotic protease. CoA (but less so acetyl-CoA) interacts with calcium/calmodulin-dependent protein kinase II (CAMK2), hence reducing its threshold for activation by Ca^{2+} ions (McCoy et al., 2013). Active CAMK2 can

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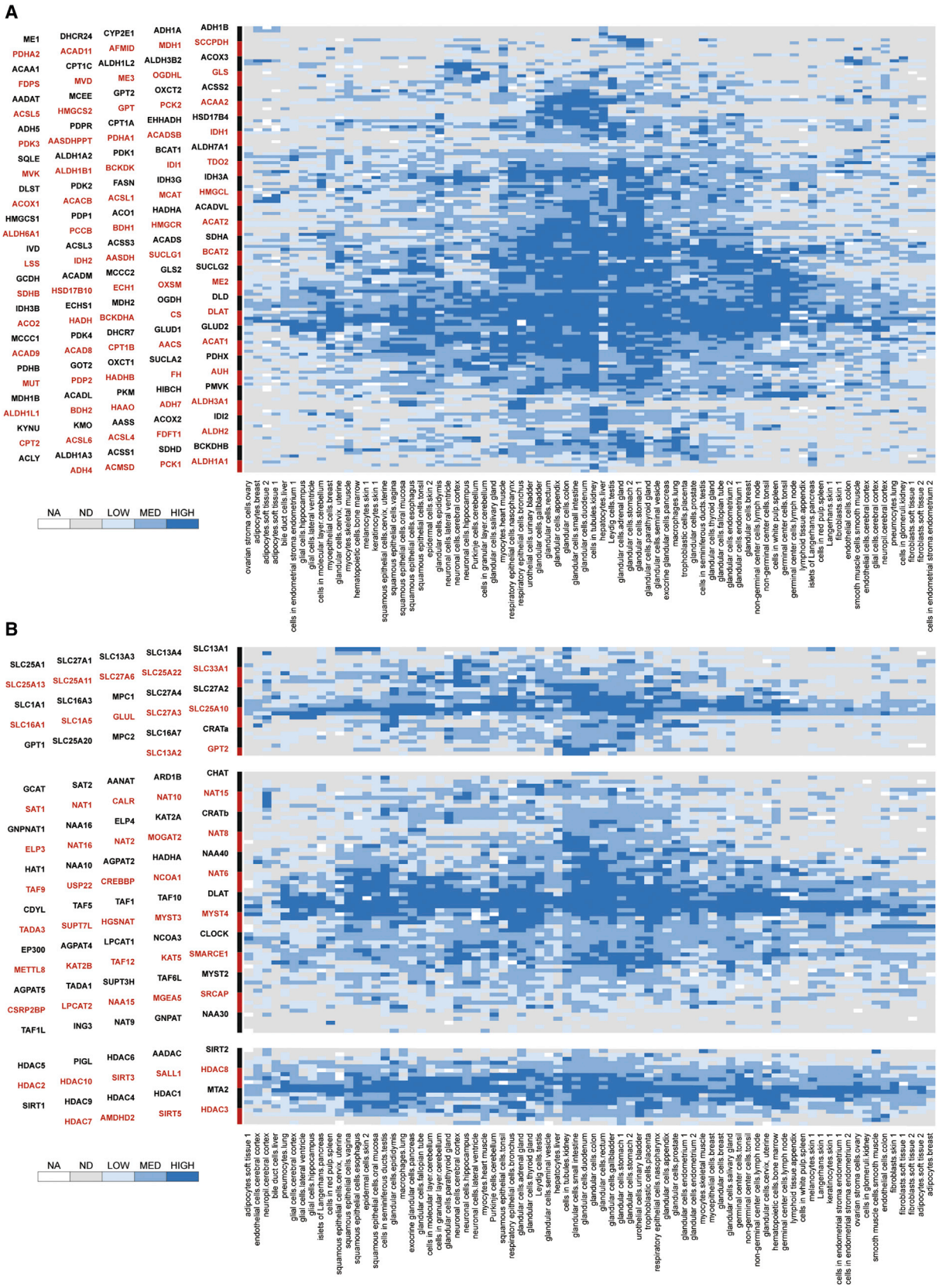

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Table 1. Main Pharmacological Modulators of Acetyl-CoA Metabolism

Family	Molecule	Indication(s)	Development Status	References
ACAC inhibitors	TOFA	Metabolic disorders	Preclinical	Harwood, 2005
ACLY inhibitors	BMS-303141	Cancer	Preclinical	Hatzivassiliou et al., 2005; Madeo et al., 2014
	Hydroxycitrate	Cancer, obesity	Phase IV clinical development	
	SB-204990	Cancer	Preclinical	
ACSS2 inhibitors	1-(2,3-di(thiophen-2-yl)quinoxalin-6-yl)-3-(2-methoxyethyl)urea	Cancer	Preclinical	Comerford et al., 2014; Mashimo et al., 2014
CPT1 inhibitors	Etomoxir	Cardiac disorders	Terminated	Abozguia et al., 2006
	Perhexiline	Cardiac disorders	Phase II clinical development	
	Trimetazidine	Cardiac disorders	Phase IV clinical development	
CRAT inhibitors	Mildronate	Cardiac disorders	Approved	Makrecka et al., 2014
GLS inhibitors	BC-839	Cancer	Preclinical	Durán et al., 2012
	DON	Cancer	Preclinical	
HDAC inhibitors	Belinostat	Cancer	Phase II clinical development	Falkenberg and Johnstone, 2014
	Parabinostat	Cancer	Phase III clinical development	
	Romidepsin	Cancer	Approved	
	Valproic acid	Cancer	Phase III clinical development	
	Vorinostat	Cancer	Approved	
HMGCR inhibitors	Statins	Cancer, metabolic disorders	Approved	Brautbar and Ballantyne, 2011; Demierre et al., 2005
KAT inhibitors	Anacardic acid	Cancer	Preclinical	Harwood, 2005
	C646	Cancer	Preclinical	
	Curcumin	Cancer, metabolic disorders	Phase II clinical development	
	Garcinol	Cancer	Preclinical	
	MB-3	Cancer	Preclinical	
MPC inhibitors	UK5099	Cancer	Preclinical	Mariño et al., 2014
PDC activators	DCA	Cancer Metabolic disorders	Approved (for lactic acidosis)	Galluzzi et al., 2013
PDC inhibitors	CPI-613	Cancer	Phase II clinical development	Pardee et al., 2014
SIRT1 activators	Resveratrol	Aging, cancer, metabolic disorders	Phase III clinical development	Athar et al., 2007; Baur and Sinclair, 2006
	SRT1720	Aging, metabolic disorders	Preclinical	
SLC25A1 inhibitors	1,2,3-BTC	Cancer	Preclinical	Aluvila et al., 2010

1,2,3-BTC, 1,2,3-benzenetricarboxylate; ACAC, acetyl-CoA carboxylase; ACLY, ATP citrate lyase; ACSS2, acyl-CoA synthetase short-chain family member 2; CPT1, carnitine palmitoyltransferase 1; CRAT, carnitine O-acetyltransferase; DON, 6-diazo-5-oxo-*L*-norleucine; GLS, glutaminase; HDAC, histone deacetylase; HMGCR, 3-hydroxy-3-methyl-glutaryl-CoA reductase; KAT, lysine acetyl transferase; MPC, mitochondrial pyruvate carrier; PDC, pyruvate dehydrogenase complex; SIRT1, sirtuin 1; TOFA, 5-(tetradecyloxy)-2-furoic acid).

phosphorylate CASP2, which favors the inhibitory interaction of the latter with tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta (YWHAZ, best known as 14-3-3ζ) (Nutt et al., 2009). Moreover, SIRT1 can promote the anti-apoptotic functions of 14-3-3ζ by deacetylating it, a reaction that is also favored by the accumulation of CoA at the expenses of acetyl-CoA (Andersen et al., 2011). Finally, the full-blown apoptotic activity of CASP2 may rely on N^{α}-acetylation (Yi et al., 2011), which is supported by high acetyl-CoA levels. Thus, a decreased acetyl-CoA/CoA ratio would promote cell survival by multiple mechanisms impinging on the activity of CASP2. Of note, the phosphorylation of CASP2 by CAMK2 requires NADPH, a key antioxidant (and co-enzymatic reducing agent) mainly generated by the PPP (Galluzzi et al., 2013). Hence, oxidative stress can stimulate cell death by releasing CASP2 from inhibition by 14-3-3ζ (Nutt et al., 2009). Apparently

Figure 4. Expression Levels of Proteins Involved in Acetyl-CoA Metabolism

(A and B) The expression levels of proteins that participate in acetyl coenzyme A (acetyl-CoA) metabolism in the indicated cell types were retrieved from The Human Protein Atlas (http://www.proteinatlas.org/). Proteins were arbitrarily subdivided according to function in "metabolic enzymes" (A) and "transporters," "acetyltransferases," and "deacetylases" (B) and subjected to hierarchical clustering. NA, not assessed; MED, medium; ND, not detected.

at odds with this notion, the activity of SIRT1 depends on NAD^+, which also accumulates in the course of oxidative stress responses (Houtkooper et al., 2012). A model that reconciles these findings is missing. Since CASP2 can be found both in the nucleus and in the cytoplasm, subcellular compartmentalization may play a hitherto unrecognized role in this process. Irrespective of such a conundrum, these findings suggest a tight link between acetyl-CoA metabolism, acetylation reactions, and RCD.

Conclusions and Perspectives

Acetyl-CoA regulates several cellular processes as it controls the balance between anabolic and catabolic reactions, both as a central metabolic intermediate and as a signal transducer. Perhaps only ATP and NADH share with acetyl-CoA such a key position in cell biology. Indeed, acetyl-CoA not only is the substrate, intermediate, or product of vital bioenergetic circuitries, but also regulates several enzymes allosterically and influences the activity of a wide panel of processes (including gene expression) via (de)acetylation reactions. In this respect, three features render acetyl-CoA unique, namely that (1) its abundance dictates one of the quantitatively and functionally most important post-translational modifications that affect eukaryotic proteins, (2) it is subjected to a rigorous subcellular compartmentalization, adding refinement to its regulatory impact, and (3) its concentration and subcellular fluxes are subjected to strong quantitative changes upon variations in its synthesis and use. Thus, acetyl-CoA levels in different subcellular compartments may constitute an intracellular gauge of changing microenvironmental conditions and ultimately tune the cellular programs of adaptation to such perturbations. In line with this latter notion, the expression levels of proteins involved in acetyl-CoA metabolism and transport, as well as in (de)acetylation reactions, exhibit remarkable variations in cells of different histological origin (Figure 4). Thus, the cellular differentiation and activation state might also affect the relative abundance of multiple acetyl-CoA-relevant enzymes, adding yet another layer of complexity to this regulatory network.

Pharmaceutical agents that influence the acetyl-CoA metabolism are being developed at an accelerating pace (Table 1). Some HDAC inhibitors including suberanilohydroxamic acid (SAHA, best known as vorinostat) and romidepsin are approved by international regulatory agencies for use in cancer patients (Falkenberg and Johnstone, 2014). Inhibitors of acetyl-CoA-consuming enzymes like ACACs and KATs are in preclinical development, mostly for the treatment of metabolic syndromes (Harwood, 2005). The PDC-activating agent DCA is licensed for the treatment of lactic acidosis (as it favors the mitochondrial metabolism of pyruvate at the expense of cytosolic reduction to lactate) and is being intensively investigated for its antineoplastic activity (Galluzzi et al., 2013). Inhibitors of acetyl-CoA-generating enzymes such as ACLY are in clinical development as weight loss-promoting or anticancer agents (Hatzivassiliou et al., 2005; Madeo et al., 2014). Along similar lines, ACSS2 inhibitors have been proposed to mediate selective antineoplastic effects, reflecting the dependency of malignant cells on acetate as acetyl-CoA precursor (Comerford et al., 2014). The future will tell whether these agents are associated with unsustainable toxicities due to their disruptive effects on acetyl-CoA metabolism or whether the plasticity of the acetyl-CoA regulatory network can accommodate successful therapeutic interventions.

AUTHOR CONTRIBUTIONS

F.P. and L.G. wrote the review based on the initial draft by F.M. and G.K., designed the table, and supervised the generation of figures; J.M.B.-S.P. created figures and helped with the finalization of the text; F.M. and G.K. conceived the review, wrote a preliminary draft of it, and provided senior supervision to its preparation.

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