Review

MOLECULAR METABOLISM

Intracellular Citrate/acetyl-CoA flux and endoplasmic reticulum acetylation: Connectivity is the answer

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ABSTRACT

Background: Key cellular metabolites reflecting the immediate activity of metabolic enzymes as well as the functional metabolic state of intracellular organelles can act as powerful signal regulators to ensure the activation of homeostatic responses. The citrate/acetyl-CoA pathway, initially recognized for its role in intermediate metabolism, has emerged as a fundamental branch of this nutrient-sensing homeostatic response. Emerging studies indicate that fluctuations in acetyl-CoA availability within different cellular organelles and compartments provides substrate-level regulation of many biological functions. A fundamental aspect of these regulatory functions involves Nε-lysine acetylation.

Scope of review: Here, we will examine the emerging regulatory functions of the citrate/acetyl-CoA pathway and the specific role of the endoplasmic reticulum (ER) acetylation machinery in the maintenance of intracellular crosstalk and homeostasis. These functions will be analyzed in the context of associated human diseases and specific mouse models of dysfunctional ER acetylation and citrate/acetyl-CoA flux. A primary objective of this review is to highlight the complex yet integrated response of compartment- and organelle-specific Nε-lysine acetylation to the intracellular availability and flux of acetyl-CoA, linking this important post-translational modification to cellular metabolism.

Major conclusions: The ER acetylation machinery regulates the proteostatic functions of the organelle as well as the metabolic crosstalk between different intracellular organelles and compartments. This crosstalk enables the cell to impart adaptive responses within the ER and the secretory pathway. However, it also enables the ER to impart adaptive responses within different cellular organelles and compartments. Defects in the homeostatic balance of acetyl-CoA flux and ER acetylation reflect different but converging disease states in humans as well as converging phenotypes in relevant mouse models. In conclusion, citrate and acetyl-CoA should not only be seen as metabolic substrates of intermediate metabolism but also as signaling molecules that direct functional adaptation of the cell to both intracellular and extracellular messages. Future discoveries in CoA biology and acetylation are likely to yield novel therapeutic approaches.

Keywords CoA; Citrate; Acetyl-CoA; Acetylation; Endoplasmic reticulum

1. INTRODUCTION

In order to adapt to fluctuations in nutrient availability, cells have developed sensing mechanisms and signaling processes that ensure the activation of homeostatic responses [1,2]. Key cellular metabolites reflecting the immediate activity of metabolic enzymes as well as the functional metabolic state of intracellular organelles have emerged as powerful signaling regulators. These metabolites can act by influencing enzymatic kinetics and modulate transcriptional, translational or post-translational events [2–4].

The citrate/acetyl-CoA pathway is a recognized key node in metabolism with substantial functions within several biological regulatory mechanisms. Fluctuations in acetyl-CoA availability within different cellular organelles and compartments provides substrate-level regulation of proteins and pathways. Evidence indicates that protein acetylation across multiple organelles and compartments reflects the metabolic status of the cell [1,4]. Evidence also indicates that many proteins and biochemical pathways are directly modulated by Nε-lysine acetylation through the availability and intracellular compartmentalization of acetyl-CoA [1,4]. Here, we will examine the emerging regulatory functions of the citrate/acetyl-CoA pathway and the specific role of the endoplasmic reticulum (ER) in the maintenance of intracellular crosstalk and homeostasis.

2. PROTEIN ACETYLATION

Protein acetylation is a co- and post-translational modification that involves the addition of an acetyl group on a nitrogen atom usually located either at the N-terminus of a nascent protein or on a lysine side chain. The former is referred to as N-terminal or Nα-acetylation and occurs co-translationally to stabilize the nascent polypeptide after the initiator methionine is removed. N-terminal acetylation can occur on

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different amino acids, although alanine, serine and methionine residues seem to be modified more predominantly. It is found on over 80% of all proteins and is generally considered irreversible. The addition of an acetyl group on the ε-amino group of the lysine residue is referred to as lysine acetylation or Nε-lysine acetylation [5,6]. In contrast to N-terminal acetylation, Nε-lysine acetylation is a post-translational event; it is highly dynamic and it regulates a variety of protein functions including enzymatic activity, stability, and subcellular distribution of targeted proteins. The addition of the acetyl group neutralizes the lysine residue's positive charge, which can induce a conformational change by adding a more "bulky" feature to the local structure as well as impacting local ionic interactions. Lysine acetylation can also interfere with additional post-translational modifications targeting the same residue, such as methylation or ubiquitination.

Both N-terminal and Nε-lysine acetylation use acetyl-CoA as donor of the acetyl group, but they are carried out by different enzymes: N-acetyltransferases (NATs) typically catalyze N-terminal acetylation while Lysine-acetyltransferases (KATs) typically catalyze lysine acetylation. Protein acetylation occurs on multiple subcellular locations, including the cytosol, nucleus, mitochondria, and ER lumen, with specific NATs and KATs identified in different organelles and compartments. Lysine acetylation, which will be the focus of this review, is highly responsive to metabolic shifts, allowing dynamic adaptation to a variety of stimuli and playing a fundamental role in many biological outcomes [7,8]. KATs are divided into three large families based upon phylogenetic sequence similarities as follows: (i) the GCN5-related N-acetyltransferases (GNAT) family, including KAT2A/GCN5, KAT2B/PCAF, and the ER-based ATase1/NAT8B and ATase2/NAT8; (ii) the cAMP-responsive element-binding protein (CREB)-binding protein (CREBBP) family, including CBP and P300/KAT3B; and (iii) the MYST family containing MOZ/KAT6A, YBF2, SAS2 and TIP60 [9]. Lysine deacetylases (KDACs) are subdivided into the following: (i) Class I, containing HDAC1, HDAC2, HDAC3, and HDAC8; (ii) Class IIa, containing HDAC4, HDAC5, HDAC7, and HDAC9; (iii) Class IIb, containing HDAC6 and HDAC10; (iv) Class III, containing all sirtuins; and (v) Class IV, containing HDAC11 [10].

3. COA AND ACETYL-COA

Coenzyme A (CoA) was initially identified in the early '40s by Fritz Lipmann while studying the biochemical process associated with "intermediate metabolism". This discovery led him to being awarded the Nobel Prize in Physiology or Medicine in 1953, which he shared with Hans Krebs. Today, it is estimated that CoA is used as a substrate in approximately 4–5% of all biochemical reactions. The biosynthesis of CoA begins with pantothenic acid (vitamin B5) and involves pantothenate kinase (PANK), phosphopantothenoylcysteine synthetase (PPCS), phosphopantothenoylcysteine decarboxylase (PPCDC), and coenzyme A synthase (COASY). COASY is a bifunctional protein with both phosphopantetheine adenylyltransferase (PPAT) and dephospho-CoA kinase (DPCK) activity (Figure 1A). Although most of CoA is synthesized in the cytosol, the above biosynthetic enzymes are also found in the nucleus and mitochondria suggesting possible contribution of non-cytosolic sources [6].

Defective CoA biosynthesis is associated with specific subtypes of neurodegeneration with brain iron accumulation (NBIA), a heterogeneous group of severe degenerative diseases of the brain that begin in childhood and progress rapidly within 1–2 decades. The most common form of NBIA associated with defective CoA biosynthesis is the pantothenate kinase-associated neurodegeneration (PKAN) with loss-of-function mutations on *PANK2* followed by the COASY protein-associated neurodegeneration (CoPAN) with mutations on *COASY* [11–15].

Acetyl-CoA is the most versatile and most studied CoA derivative. Initial studies linking acetyl-CoA to lipid metabolism led the 1964 Nobel Prize in Medicine or Physiology being awarded to Konrad Bloch and Feodor Lynen. Acetyl-CoA is normally synthesized in the cytoplasm, mitochondria, nucleus and peroxisomes [6]. Cytosolic acetyl-CoA is mainly generated by two different enzymes: (i) ATP Citrate Lyase (ACLY), which uses citrate and CoA, and (ii) Acetyl-CoA Synthetase (AceCS1 or ACSS2), which uses acetate and CoA (Figure 1A). Under normal condition, ACLY is thought to be the main source of cytosolic acetyl-CoA [6]. Cytosolic acetyl-CoA serves as donor of the acetyl group for both N-terminal and Nε-lysine acetylation of targeted proteins within the cytosol. Cytosolic acetyl-CoA has free access to the nucleus where it can diffuse through the nuclear pores and can serve as substrate for nuclear-based protein acetylation [6,16]. Active transport of acetyl-CoA from the cytosol into the lumen of the ER is ensured by a specific ER membrane transporter SLC33A1 (also referred to as AT-1), which exchanges acetyl-CoA for free CoA in an antiporter fashion [17]. Within the ER lumen, acetyl-CoA serves as substrate for ER-based protein acetylation. Both mitochondria and peroxisomes are able to make their own acetyl-CoA and, therefore, do not immediately depend on acetyl-CoA availability in the cytosol. The nucleus is also able to make acetyl-CoA locally [18,19]. Additional details on the different metabolic pathways that feed into the different intracellular pools of acetyl-CoA are described elsewhere [4,6,20,21].

4. SLC25A1, SLC13A5, ACLY AND THE CITRATE/ACETYL-COA PATHWAY

SLC25A1 (also referred to as Citrate/Isocitrate Carrier, CIC, or Citrate Transport protein, CTP) and SLC13A5 (also referred to as Sodium/Citrate co-Transporter, NaCT) are major contributors of cytosolic citrate (Figure 1A).

4.1. The citrate transporter, SLC25A1

SLC25A1 is a mitochondria membrane transporter (Figure 1B); it exchanges mitochondria citrate for cytosolic malate through an antiporter mechanism [22,23]. *In vitro* K_m for malate and citrate are in the low mM range (approx. 0.13 mM for malate and 0.76 mM for citrate). Additional substrates, at least *in vitro*, include phosphoenolpyruvate and isocitrate. When reconstituted *in vitro*, transport was found to be dependent on ΔpH and to be electroneutral with a $H^+ + citrate^{3-}$ exchange for $malate^{2-}$. SLC25A1 is predicted to have six transmembrane domains spanning the mitochondria membrane and to assemble as a dimer. It employs a ternary complex/sequential mechanism, which involves concurrent binding of both substrates, cooperative conformational changes, and exchange of substrates across the mitochondrial membrane [22,23]. The exported citrate derives from immediate engagement of the TCA cycle within the mitochondria and is used by ACLY to feed into the cytosolic acetyl-CoA pathway, which is required for protein acetylation and lipid biosynthesis. As part of its biochemical activity, ACLY also generates oxaloacetate, which can then be converted into malate and reenter the mitochondria through the antiporter mechanism. Therefore, SLC25A1 ensures continuous crosstalk between the mitochondrial and the cytosolic malate/citrate/acetyl-CoA pathways (Figure 2). These two pathways can be genetically separated by targeting SLC25A1 or ACLY [24]. The SLC25A1 system also supplies the malate-aspartate shuttle by providing malate to generate oxaloacetate and aspartate within the mitochondria. Aspartate can then be exchanged with cytosolic glutamine/glutamate through SLC25A12/A13

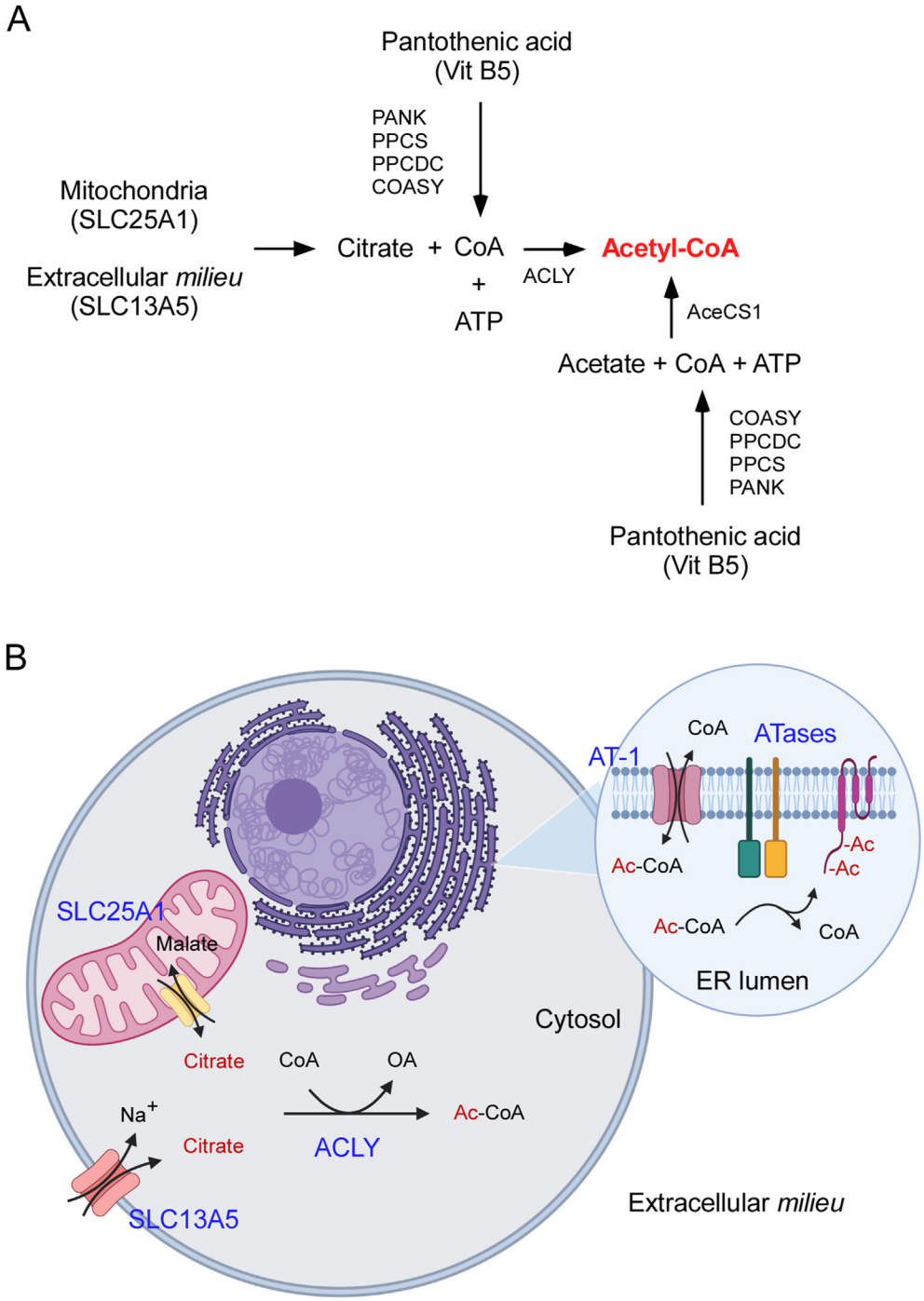

Figure 1: Integration of biosynthetic pathways that ensure the intracellular flux of acetyl-CoA. (**A**) Schematic description of acetyl-CoA biosynthesis in the cytosol. Full description is in the text. (**B**) SLC25A1 and SLC13A5 provide citrate to the cytosol where it is converted into Acetyl-CoA (Ac-CoA) by ACLY. Cytosolic acetyl-CoA is imported into the lumen of the rough ER by AT-1. AT-1 is the only ER membrane acetyl-CoA transporter and acts as an antiporter. Within the ER, acetyl-CoA is used by ATase1 and ATase2 for Nε-lysine acetylation of ER cargo and resident proteins. The acetyltransferase activity of ATase1 and ATase2 generates free CoA, which is exported back to the cytosol by the antiporter mechanism of AT-1.

(Figure 2). SLC25A12/A13 plays a fundamental role in feeding the TCA cycle under certain metabolic and cellular states [24—26] and works to maintain the cytosolic pool of aspartate. It is worth mentioning that many of the studies focused on elucidating the components of the TCA cycle and crosstalk/salvage pathways utilized immortalized/cancer cells. Nonetheless, there is evidence of significant differences in engagement of these pathways as cells switch from proliferative to non-proliferative states, suggesting that post-mitotic cells such as neurons may require different outputs to ensure acetyl-CoA availability [24—26]. Gene duplication events of *SLC25A1* (contained in 22q11.21) are associated with autism spectrum disorder (ASD) [27—29] (see also *SFARI Database*). The association between overexpression of SLC25A1

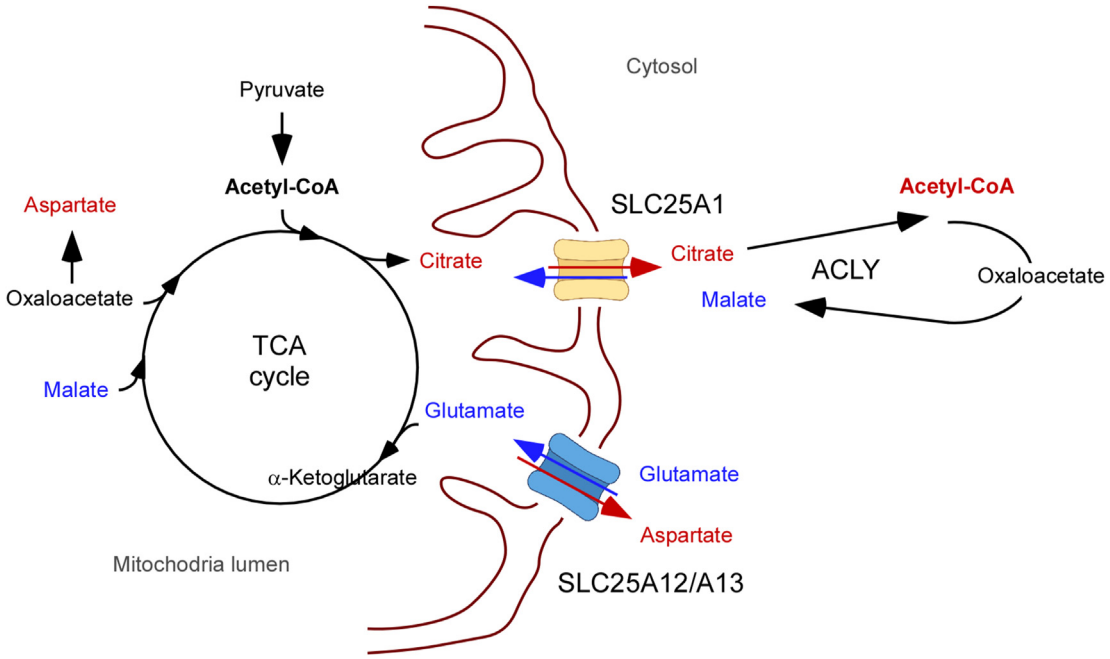

Figure 2: SLC25A1 ensures metabolic crosstalk between mitochondria and cytosol. SLC25A1 integrates metabolic signals between the mitochondria and the cytosol by exchanging citrate for malate through an antiporter mechanism. The SLC25A1 system can also feed into the malate-aspartate shuttle by providing malate for the generation of oxaloacetate and aspartate within the mitochondria. Aspartate can then be exchanged with cytosolic glutamate through SLC25A12/A13. Both SLC25A1 and SLC25A12/A13 feed into the TCA. Full description is in the text.

and ASD is also observed in the mouse where it manifests with altered neuronal morphology and white matter integrity [30]. Reduced gene dosage of *SLC25A1* is associated with DiGeorge syndrome (also referred to as 22q11.2 hemizygous microdeletion syndrome), which manifests with developmental delay, multi-system deficits, dysmorphism and intellectual disability resembling ASD, attention deficit hyperactivity disorder (ADHD) or even schizophrenia [31–33]. Loss-of-function mutations in *SLC25A1* are also associated with neurodevelopmental deficits and intellectual disability, intractable seizures, severe metabolic alterations, and early death [34,35]. Genetic variations affecting *SLC25A12* have been associated with ASD and developmental forms of epileptic encephalopathy [36–40], while genetic variations in *SLC25A13* have been associated with different metabolic disorders that manifest with severe neurological symptoms [41]. These observations show that genetic alterations affecting the two most important systems that control crosstalk between the mitochondrial and the cytosolic malate/citrate/acetyl-CoA pathways appear to be linked to severe neurological disorders, highlighting a fundamental role in normal brain development and function.

4.2. The citrate transporter, SLC13A5

SLC13A5 is a plasma membrane transporter (Figure 1B); it transfers citrate and Na^+ from the extracellular environment to the cytosol. SLC13A5 is predicted to have 11 or 12 membrane domains and to assemble as a homodimer. The calculated Na^+:citrate stoichiometry *in vitro*, under an experimental pH of 7.2, was found to be between 3 and 4. At the same conditions, citrate exists predominantly as a trivalent anion [42,43]. Therefore, Na^+:citrate cotransport can either be electrically silent (3:1) or electrogenic (4:1). The electrogenic properties of a 4:1 stoichiometry might be particularly relevant for the brain where activity-dependent regulation of citrate import is crucial for neuronal physiology. *In vitro* K_m for citrate is in the 20 μM range for rodent Slc13a5 and in the 650–1400 μM range for human SLC13A5 [42–44]. Additional substrates, at least *in vitro*, include isocitrate and a few dicarboxylates. However, at steady-state, citrate concentrations are in the 200 μM range in the blood and in the 500 μM range in the cerebrospinal fluid (CSF) [42,43]. These concentrations are either the second highest (in the blood) or the highest (in the CSF) among monocarboxylates and TCA cycle intermediates. Therefore, it is likely that *in vivo* SLC13A5 only serves as a citrate transporter. Furthermore, in light of its transport properties and the available concentration of citrate in the extracellular *milieu*, SLC13A5 is viewed as a high-capacity transporter, in contrast to SLC25A1, which is viewed as a low capacity transporter.

Gene duplication events of *SLC13A5* (contained in 17p13.1) are associated with ASD [27–29] (see also *SFARI Database*). The association between overexpression of SLC13A5 and ASD is also observed in the mouse where it manifests with altered neuronal morphology and white matter integrity [45]. Autosomal recessive loss-of-function mutations in *SLC13A5* are associated with developmental/early forms of epileptic encephalopathies with amelogenesis [46,47]. Propensity for epileptic seizures is also observed in the mouse after genetic disruption of *Slc13a5* [48]. Interestingly, heterozygous loss of the *D. melanogaster* version of *Slc13a5* extends lifespan with no apparent deleterious effect. The mutant fly was named INDY for *I am Not Dead Yet* [49]. A similar effect was observed in *Caenorhabditis elegans* [50]. However, the biochemical and biological properties of the fly and worm Slc13a5/NaCT have not been dissected and it is likely that human SLC13A5 has evolved to play significantly different (and more specific) functions. The fact that genetic alterations affecting SLC13A5 are also linked to severe neurological disorders further highlights the fundamental role that citrate import and the consequent citrate/acetyl-CoA pathway plays in normal brain development and functions.

4.3. The ATP Citrate Lyase, ACLY

ACLY is largely a cytosolic protein, although it can shuttle into the nucleus [18,19] (Figure 1B). It catalyzes the formation of acetyl-CoA and oxaloacetate from citrate, free CoA and ATP. It is normally depicted as a six-domain unit with each domain playing important biochemical functions. Specifically, the CoA-binding site is on domain 1, the activating phosphorylation His740 site is on domain 2, the ATP binding sites are on domains 3 and 4, the citrate binding site is largely dependent on domain 5, and the citrate synthase homology domain, which is largely responsible for the catalytic conversion of the intermediate citryl-CoA to acetyl-CoA and oxaloacetate is on domain 6 [51–54]. The active enzyme functions as a homo-tetramer that is dependent on structural fusion of the N-terminal and C-terminal segments [54–56].

ACLY is expressed in every cell and tissue although lipogenic tissues and the brain display higher levels of expression. ACLY appears to be very important for development where the expression levels are very high and appear to variate depending on specific stages. In the brain, the levels of ACLY are much higher during development than during adulthood [57]. The major transcriptional regulator of ACLY is sterol regulatory element binding protein-1 (SREBP-1). Post-translational mechanisms involving phosphorylation at Thr446, Ser450, Ser454 and Ser455 [19,58–60] as well as acetylation/deacetylation at Lys540, Lys546 and Lys554 [61] have also been shown to regulate ACLY under different metabolic conditions [51,62]. Importantly, homozygous disruption of *Acly* in the mouse is lethal [57]. To resolve this limitation, tissue-specific knock-out mice as well as knock-down and biochemical inhibition models were generated and have been used to dissect ACLY functions under different experimental settings, and to study effects on protein acetylation [63–69]. Altered expression and single-nucleotide polymorphisms have been described in several diseases from cancer to steatosis/nonalcoholic fatty liver disease, diabetes, obesity, and neurodevelopmental disorders [51,52]. However, to date no disease-causing mutations have been identified on *ACLY*, which, in sum, highlights the importance of this gene for life.

5. ER ACETYLATION

5.1. The ER acetylation machinery

As mentioned above, the ER imports acetyl-CoA from the cytosol. This is ensured by SLC33A1/AT-1, the only ER-membrane acetyl-CoA transporter [17,70]. AT-1 is a homodimer within the ER membrane and employs an antiporter mechanism by exchanging acetyl-CoA with free CoA [71]. Specifically, acetyl-CoA moves from the cytosol to the ER lumen while free CoA moves in the opposite direction (see Figure 1B). The dimerization is essential for activity and appears to involve interaction of the first intraluminal loop of the two monomers. The disease-associated S113R mutation within the dimerization loop interferes with the ability of AT-1 to form homodimers and inactivates the acetyl-CoA transport activity *in vitro* and *in vivo* [71]. Knock-in mice heterozygous for the S113R mutation display a 50% reduction in acetyl-CoA import into the ER, while mice homozygous for the same mutation do not survive development [71]. These results confirm that AT-1 is the only ER-membrane acetyl-CoA transporter, and that it is essential for life.

So far, only two acetyltransferases have been identified in the ER, acetyl-transferase 1 (ATase1) and 2 (ATase2) (Figure 1B). They are also referred to as N-acetyltransferase 8B (NAT8B) and N-acetyltransferase 8 (NAT8), respectively. Both enzymes are type-II membrane proteins with their catalytic domain facing the lumen of the ER [72]. The acetyl-CoA:lysine acetyltransferase activity of ATase1 and ATase2 has been documented *in vitro* and *in vivo* [72–74]. Currently, there is no biochemical evidence that they can also act as N-terminal/Nα-acetyltransferases. As such, they must be included in the large family of KATs but not NATs. Finally, they retain the highly conserved R/Q-x-x-G-x-G/A acetyl-CoA binding motif of the GNAT superfamily of KATs [5,75–77]. Both ATases act as dimers within the ER membrane through a dimerization domain that is located on the ER luminal C-end [78]. Deletion of the dimerization domain inactivates the enzymatic activity. When co-expressed together in cellular systems, they form homo- and hetero-dimers [78]. However, it is still unclear whether heterodimers can form naturally without overexpression of the two ATases. Both enzymes employ a single displacement bisubstrate ternary complex/sequential mechanism, which requires concurrent binding to both substrates [79]. They share a common acetyl-CoA pocket, which is stabilized by the R149/S183/S190 triad, but differ within the entrance of the putative peptidyl-lysine pocket [79].

There is mounting evidence that AT-1, ATase1, and ATase2 are biochemically coupled and can influence each other in real-time. As mentioned, AT-1 works as an antiporter where the cytosol-to-ER transport of acetyl-CoA occurs in parallel with the ER-to-cytosol exchange for free CoA. As a consequence, the activity of the two ATases can affect the activity of AT-1 by impacting the acetyl-CoA/CoA concentration gradients driving the antiporter mechanism. In other words, the ATases can regulate AT-1 activity by determining the levels of free CoA within the lumen of the ER. Therefore, it was not surprising that adaptive changes in compartments outside of the ER were observed when genetic disruption of either Atase was performed in the mouse, presumably from increased availability of acetyl-CoA in the cytoplasm [74]. It is also important to mention that ATase1, but not ATase2, has a large, disordered loop gating one end of the peptidyl-lysine pocket that allows for allosteric activation by acetyl-CoA through acetylation of K99 [79]. This, therefore, allows for AT-1 to impact allosteric regulation of ATase1, but not ATase2, via modulating the availability of acetyl-CoA in the ER lumen [72,79]. This explains why the upregulation of AT-1 in the mouse can increase the acetylation of ER proteins. In conclusion, AT-1 can regulate ATase1 activity by determining the levels of acetyl-CoA in the ER lumen, while the activity of the ATases can impact AT-1 function and subcellular pools of acetyl-CoA beyond the ER by influencing the antiporter mechanism.

Gene duplication events of *SLC33A1/AT-1* (contained in the 3q25.31 locus) are associated with intellectual disability, dysmorphism and ASD [27–29] (see also *SFARI Database*). Loss-of-function heterozygous mutations in SLC33A1/AT-1 are associated with hereditary spastic paraplegia 42, a peripheral form of neuropathy, while homozygous mutations are associated with severe developmental delay and premature death [80–82]. Importantly, mouse models of reduced or increased AT-1 activity mimic human-associated diseases [71,83,84]. In contrast to AT-1, no disease-associated mutations or gene duplication events have so far been reported for ATase1 or ATase2. Importantly, nonsense and missense changes have been identified on either ATase; however, they do not appear to be associated with diseases, suggesting that one active ATase is sufficient [6]. Consistently, mice with genetic disruption of either Atase (Atase1$^{-/-}$ and Atase2$^{-/-}$) display no disease phenotype [74].

5.2. ER acetylation, proteostasis and metabolic crosstalk

George Palade (Nobel Prize in Physiology or Medicine, 1974) first recognized that the majority of ribosomes are attached to the ER membrane. Indeed, in non-polarized cells, about 75% of all mRNAs and ribosomes are attached to the ER where the bulk of protein biosynthesis occurs [85]. About half of these newly-synthesized proteins will be released into the cytosol while the other half will insert into

the ER and proceed in the secretory pathway [85]. Both the cytosol and the ER/secretory pathway contain mechanisms that ensure protein quality control and efficient removal of misfolded/unfolded polypeptides to maintain protein homeostasis (also referred to as proteostasis) [86,87]. Maintenance of proteostasis in the ER/secretory pathway is particularly important for polarized cells such as neurons, which heavily rely upon the secretory pathway for neurotransmission and synaptic plasticity [88,89].

It is now evident that the ER also plays an essential role in metabolic cross-communication between intracellular organelles and compartments. This is achieved through a series of key metabolites and signaling events, which reflect the immediate activity of metabolic enzymes as well as the functional metabolic state of a given compartment [90–92]. In this way, the levels and availability of signaling molecules and/or metabolites allow for the implementation of an appropriate adaptive response. This general nutrient-signaling-pathway enables rapid modulation and reprogramming of the secretory pathway (and other intracellular activities) upon fluctuation of metabolites/nutrients [2,3,93].

The ER acetylation machinery, namely the acetyl-CoA transporter AT-1 and acetyltransferase enzymes ATase1 and ATase2, has emerged as a major regulatory point for both proteostasis within the ER/secretory pathway and metabolic crosstalk among different intracellular organelles and compartments (see Figure 3).

5.2.1. Proteostatic functions of the ER acetylation machinery

The proteostatic functions of the ER acetylation machinery require two separate but highly connected events: (i) selection of correctly folded glycoproteins so that they can engage the secretory pathway, and (ii) induction of ER-specific autophagy (referred to as reticulophagy) to dispose of toxic protein aggregates [71,73,74,78,83,84,94–100].

5.2.1.1. Selection of correctly folded glycoproteins. This function is achieved in partnership with the SEC61/SEC62/SEC63 translocon and the oligosaccharyltransferase (OST) complex [78]. Specifically, correctly folded glycoproteins are recognized by the ATases and acetylated, while unfolded/misfolded glycoproteins are not. Acetylated glycoproteins are then allowed to engage the secretory pathway, while non-acetylated are not [73,78,95,100]. In essence, the acetyl group appears to serve as a "positive marker" for correctly folded glycoproteins (see Figure 3). The acetyl group that is added in the lumen of the ER is then removed when the acetylated protein reaches the Golgi apparatus by a Golgi-based deacetylase (yet to be fully identified and characterized) [94,101]. Studies performed with two different type I membrane proteins, BACE1 and CD133, indicate that removal of the acetylated lysine residues reduces the ability of the nascent glycoprotein to leave the ER and engage the secretory pathway [78,94,102]. In both cases, the effect was not absolute indicating that the acetyl group is not the only signal used by the cell to determine whether the nascent polypeptide can (or cannot) leave the ER. Removal of the N-glycosylation sites prevents both glycosylation and acetylation while removal of the acetylation sites prevents acetylation but not glycosylation of the nascent polypeptide [78]. Therefore, N-glycosylation occurs first and is independent of Nε-lysine acetylation, while Nε-lysine acetylation occurs after and depends on N-glycosylation. Finally, disruption of the normal folding of the nascent glycoprotein prevents acetylation, thus proving that only correctly folded polypeptides are recognized by the ATases and acetylated [78]. The resolution of the

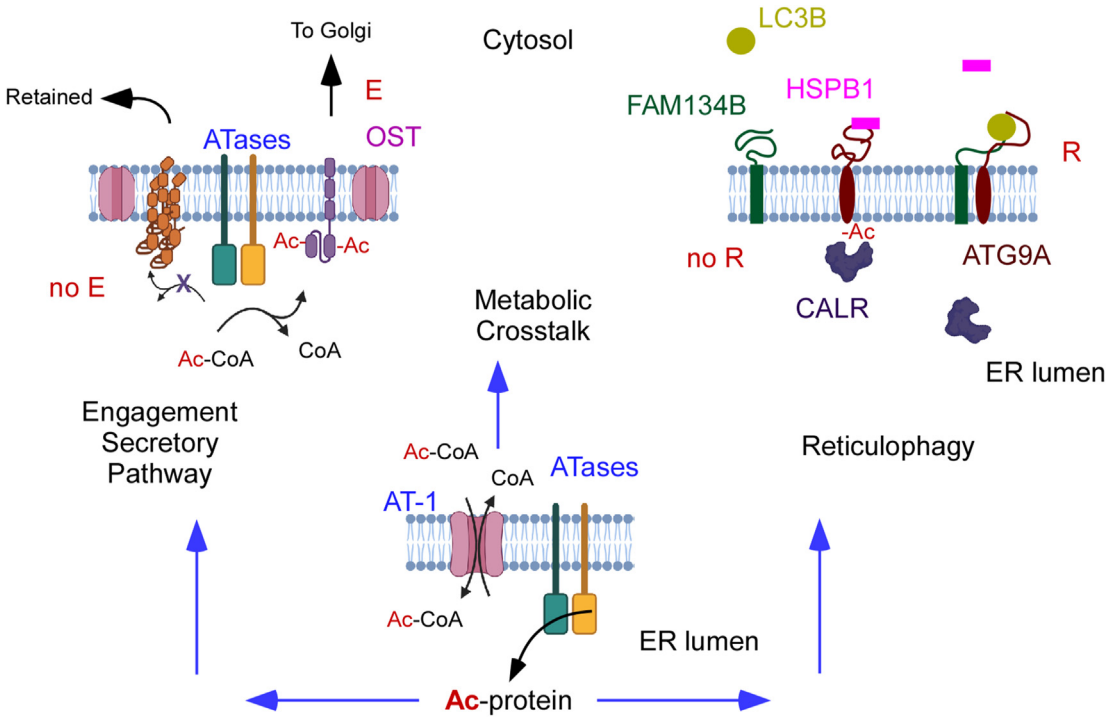

Figure 3: The ER acetylation machinery regulates three essential aspects of cell biology. The ER acetylation machinery regulates ER proteostasis by maintaining a tight balance between (i) the engagement of the secretory pathway by correctly folded (acetylated) glycoproteins and (ii) the disposal of unfolded/misfolded (non-acetylated) protein aggregates through reticulophagy. The ER acetylation machinery also ensures intracellular metabolic crosstalk between different intracellular organelles and compartments (see Table 1). Full description of each function is in the text. Ac, acetyl group; E, engagement; no E, no engagement; R, reticulophagy; no R, no reticulophagy.

first ER acetylome revealed that about 60% of acetylated proteins were ER-cargo/transiting proteins that travel through the secretory pathway; the remaining 40% were ER-resident proteins [97]. They included chaperones, or enzymes involved with folding and post-translational modification of nascent proteins. They also included proteins that are not N-glycosylated [97]. Therefore, Nε-lysine acetylation is not limited to ER-transiting proteins or to glycoproteins.

5.2.1.2. Induction of ER-specific autophagy. Autophagy from the ER is achieved by regulating the acetylation status of the autophagy protein 9A (ATG9A) within the ER lumen [96]. If acetylated, ATG9A is "sequestered" by Calreticulin (CALR) and prevented from engaging FAM134B, one of several important reticulophagy receptors that resides within the ER membrane. Under this condition, the cytosolic tail of ATG9A is unable to activate the autophagy core machinery through LC3β and is stabilized by the heat-shock protein HSPB1. In contrast, when non-acetylated, ATG9A is released by CALR and able to engage FAM134B. The formation of the ATG9A-FAM134B complex forces dislodgement of HSPB1 allowing binding of LC3β and activation of the autophagy machinery [74,84,103]. In essence, ATG9A appears to act as a sensor for ER acetylation while FAM134B acts as an ER-based receptor for the general autophagy machinery (see Figure 3). The ability of FAM134B to engage ATG9A and LC3β appears to depend on Ca^{2+} dynamics through two opposing helix-loop-helix EF hands [103]. In addition to FAM134B, ATG9A can also interact with SEC62, another identified reticulophagy receptor [84,103]. Even in this case, the interaction appears to depend on the acetylation status of ATG9A [103]. The fact that the acetylation status of ATG9A can direct the formation of ATG9A-FAM134B and ATG9A-SEC62 complexes, and the consequent engagement of the cytosolic autophagy machinery through LC3β, suggests a coordination of events that ultimately ensure translation of an ER-luminal event (acetylation) to the cytosol (engagement of LC3β). Both FAM134B and SEC62 are preferentially located on the rough ER where the bulk of protein biosynthesis normally occurs [104]. Furthermore, SEC62 is an integral member of the ER translocon machinery and appears to couple the insertion of newly synthesized proteins into the ER with the machinery that detects and disposes of unfolded/misfolded polypeptides [105]. SEC62 has also been implicated in guiding reticulophagy/ER-phagy during recovery from ER stress, termed recovER-phagy, revealing its importance in maintaining ER homeostasis [106]. Isolation of ATG9A from the autophagosome yields a cocktail of proteins involved with the formation of the autophagosome [107]. Isolation of ATG9A from the ER surface, in contrast, yields integral components of the ER quality control machinery as well as ERES and COPII structural components [103]. Finally, BioID proteomics resolves several component of the vesicle-trafficking system [108]. These findings suggest that ATG9A can interact both with the machinery that regulates the trafficking of nascent polypeptides out of the ER and through the secretory pathway, and the machinery that regulates autophagy-mediated disposal of protein aggregates, reinforcing the concept of vicinity and cross talk of the essential biochemical machineries that maintain proteostasis within the ER.

As mentioned above, loss-of-function heterozygous mutations in *SLC33A1/AT-1* are associated with a peripheral form of neuropathy, within the spastic paraplegia (SPG) subgroup [80]. Mutations in *HSBP1* are associated with peripheral forms of neuropathies, within the type 2 Charcot-Marie-Tooth (CMT) disease and the hereditary motor neuropathy (HMN) subgroups [109—119]. Finally, mutations in *FAM134B* are associated with peripheral forms of neuropathy within the general family of hereditary sensory autonomic and motor neuropathy (HSAN/HMN) [120—124]. In conclusion, defects in induction and progression of reticulophagy, as caused by mutations that affect the same molecular pathway, might underlie similar forms of hereditary human diseases.

Table 1 — Intracellular crosstalk functions that are regulated by the ER acetylation machinery.

Cytosol	Nucleus	Mitochondria
Acetyl-CoA metabolism	Gene expression	Acetyl-CoA metabolism
Lipid metabolism	transcription factors	TCA
Glucose metabolism	histone proteins	Oxidative phosphorylation
Amino acid metabolism		Respiratory chain
Protein synthesis and folding		Fusion and fission
Proteasome		
Cell signaling		
Cell migration and growth		
Endocytosis		
Exocytosis		

The antiporter activity of AT-1 ensures intracellular metabolic crosstalk between different organelles and compartments (see Figure 3). Biochemical and biological functions that are influenced by changes in AT-1 activity in mouse models of dysfunctional ER acetylation are schematically represented within each organelle and compartment. Full description and associated references can be found in the text.

5.2.2. Metabolic crosstalk functions of the ER acetylation machinery

The metabolic crosstalk functions of the ER acetylation machinery rest in the ability of AT-1, which acts as an antiporter, to maintain bi-directional flux of acetyl-CoA (from the cytosol to the ER) and CoA (from the ER to the cytosol; see Figure 3). Reduced AT-1 activity leads to accumulation of acetyl-CoA in the cytosol, while increased AT-1 activity has the opposite effect. In either case, changes in acetyl-CoA flux from the cytosol to the ER lumen, as caused by reduced or increased AT-1 activity, cause significant metabolic adaptation [74,83,100,125]. This includes changes in the proteome, glycoproteome and acetyl-proteome in a manner that appears to be highly coordinated, and occurs within different cellular organelles and compartments [74,83,100,125]. Of note is the fact that the mitochondrial adaptive response includes changes in stoichiometry of lysine acetylation, which -due to the high levels of acetyl-CoA present in the mitochondria lumen- was expected to be buffered from cytosolic acetyl-CoA [125]. The mitochondrial adaptive response is also revealed at the morphological level with AT-1 overexpressing animals displaying an expansion of the mitochondrial network [125]. Dissection of the metabolic phenotype of AT-1 overexpressing animals revealed increased mRNA levels of both *Slc25a1* and *Acly*, probably linked to increased need to supply the cytosol with new citrate and replenish the pool of cytosolic acetyl-CoA [83,125]. Finally, proteomic studies revealed significant changes within biochemical pathways related to acetyl-CoA metabolism, tricarboxylate acid (TCA) cycle, oxidative phosphorylation and the electron respiratory chain, as well as the general process of mitochondrial fusion and fission [83,125]. The AT-1 adaptive response also includes changes in the acetylation profile of histone proteins as well as in lipid metabolism within the cytosol, which is somewhat expected given the importance of acetyl-CoA in epigenetic regulation of transcription and fatty acid biosynthesis [74,125].

Globally, the adaptive response within the cytosol, mitochondria and nucleus, as documented by the analysis of the proteome and acetyl-proteome, includes several essential biochemical and biological pathways, from gene expression in the nucleus to metabolism, cell signaling, migration and growth, endo- and exocytosis in the cytosol,

engagement of the TCA, oxidative phosphorylation, respiratory chain and fusion/fission in the mitochondria (Table 1). Interestingly, although well-coordinated and integrated, this adaptive response emerged as quite complex at the single protein level. Specifically, some proteins responded only at the proteome level (increased or reduced protein levels), some only at the acetyl-proteome level (changes in stoichiometry of acetylation as well as pattern of acetylation), and some at both proteome and acetyl-proteome level [30,45,74,83,125]. These findings clearly highlight the ability of the cell to integrate different signals to coordinate the response within a certain pathway.

5.3. The mice of dysfunctional ER acetylation

The first mouse of dysfunctional ER acetylation to be generated was the AT-1$^{S113R/+}$ knock-in (KI), which was engineered to model the S113R mutation associated with SPG42 [71]. Mice homozygous for the mutation (AT-1$^{S113R/S113R}$) were embryonically lethal while mice heterozygous for the mutation (AT-1$^{S113R/+}$) were born with Mendelian ratio. When housed in a pathogen-free facility, they developed a neurodegenerative phenotype within both the central and peripheral nervous systems. When housed in the presence of mouse pathogens, they also developed propensity to infections, systemic inflammation and cancer [71]. Mechanistically, AT-1$^{S113R/+}$ mice displayed a 50% reduction in acetyl-CoA import into the ER lumen, which was accompanied by reduced acetylation of ATG9A and hyperactivation of reticulophagy. The reduced cytosol-to-ER flux of acetyl-CoA also resulted in expansion of the cytosolic pool of acetyl-CoA with buildup of lipid droplets [125]. Proteomic assessment revealed substantial adaptation across multiple cellular organelles and compartments [71,125]. The phenotype of AT-1$^{S113R/+}$ mice also included spontaneous chronic pancreatitis with severe fibrosis and glad atrophy [126]. An acinar-specific At-1 knock-out (KO) model also developed spontaneous chronic pancreatitis with inflammation, fibrosis and glad atrophy [126]. In essence, the effects of reduced AT-1 activity and reduced acetyl-CoA import into the ER lumen were more evident in cells and tissues that more heavily depend on the efficiency of the secretory pathway, with an immediate effect on neurons, exocrine pancreatic cells, and immune cells.

The generation of Atase1$^{-/-}$ and Atase2$^{-/-}$ mice confirmed that the two acetyltransferases have partially overlapping functions and can compensate for each other [74]. They also confirmed that the ATases can be targeted for therapeutics. The animals displayed no disease-associated phenotype with normal lifespan. Interestingly, Atase1$^{-/-}$ and Atase2$^{-/-}$ mice differed in the activation of reticulophagy and macroautophagy, as well as in the adaptive response within the acetylome and acetyl-CoA metabolism. Both mice had reduced engagement of the AT-1 antiporter mechanism with consequent expansion of the cytosolic pool of acetyl-CoA. However, the excess acetyl-CoA was primarily used to acetylate proteins in the Atase1$^{-/-}$ model and buildup lipid droplets in the Atase2$^{-/-}$ model [74].

Mice with overexpression of human AT-1 displayed autistic-like features when the overexpression was neuron-specific (AT-1 nTg) and a segmental form of progeria when the overexpression was systemic (AT-1 sTg). AT-1 nTg mice displayed altered synaptic plasticity with expansion of dendritic spines and branches [83]. The expanded dendritic system was observed with primary neuronal cultures as well as in the brain. Interestingly, the morphology and the biochemical composition of the spines was preserved. Proteomic assessment demonstrated a widespread upregulation of almost 500 proteins [83]. Pathways affected included the machinery that organizes the exit sites on the ER surface, the transport of vesicles out of the ER and across the secretory pathway, as well as the assembly of synaptic structures at the neuronal periphery. Also affected where many cytosolic proteins that are necessary for protein biosynthesis, and proteins that are necessary for supporting synaptic plasticity through scaffolding and adaptor transducing functions. The AT-1 sTg phenotype included delayed growth, short lifespan, skin lesions and alopecia, osteoporosis and lordokyphosis, rectal prolapse, reduced fertility, systemic inflammation, and accumulation of senescent cells [84]. Mechanistically, the phenotype of AT-1 sTg mice was linked to increased acetylation of ATG9A and a severe block in the ability of the ER to activate reticulophagy. The phenotype was rescued entirely by inhibiting the ATases [84,127].

The use of mice with reduced (AT-1$^{S113R/+}$) and increased (AT-1 sTg) AT-1 activity allowed a systematic analysis of changes imparted upon the ER/secretory pathway as well as the adaptive cellular response [100,125], confirming the different functions of the ER acetylation machinery elaborated and discussed in the previous section. The use of AT-1$^{S113R/+}$ and AT-1 sTg mice also allowed a comprehensive analysis of dynamics of the secretory pathway. Indeed, changes in engagement of the secretory pathway by ER-transiting proteins, as caused by dysfunctional ER acetylation, resulted is significant changes in the N-glycosylation profile of nascent glycoproteins. This was specifically linked to defects in ER-to-Golgi transit resulting in altered Golgi-based glycosylation [100]. A remaining point is to study the effects imparted upon by the overexpression of ATase1 and ATase2 under the neuron-specific and systemic models. A list of currently available mouse models of dysfunctional ER acetylation is found in Table 2.

Table 2 — Mouse models of dysfunctional ER acetylation.

Mouse	Main Phenotype	Main Biological Features
AT-1$^{S113R/S113R}$	Lethal	
AT-1$^{S113R/+}$	Neurodegeneration with propensity to infections, inflammation and cancer	Reduced cytosol-to-ER flux of acetyl-CoA
At-1$^{-/-}$ acinar specific	Inflammation and pancreatitis	
AT-1 nTg	ASD	Increased cytosol-to-ER flux of acetyl-CoA
AT-1 sTg	Segmental progeria	
Atase1$^{-/-}$	No disease phenotype	No Atase1 activity in the ER lumen
Atase2$^{-/-}$	No disease phenotype	No Atase2 activity in the ER lumen
SLC25A1 nTg	ASD	Increased mitochondria- to-cytosol flux of citrate; increased acetyl-CoA biosynthesis
SLC13A5 nTg	ASD	Increased extracellular *milieu*-to-cytosol flux of citrate; increased acetyl-CoA biosynthesis

5.4. The mice of dysfunctional citrate/acetyl-CoA flux

Mice with reduced (AT-1$^{S113R/+}$) and increased (AT-1 nTg and AT-1 sTg) AT-1 activity displayed an adaptive response beyond the ER and secretory pathway, which was tightly linked to changes in the availability of acetyl-CoA in the cytosol [71,83,84,100,125]. This effect translated to changes in the proteome and acetyl-proteome within the cytosol, mitochondria, and nucleus. It also translated in adaptive responses within the epigenetic code and the intermediate metabolism [71,83,84,100,125]. In essence, the ER acetylation machinery emerged as a novel branch of the more general nutrient-signaling pathway, which integrates the availability of specific

metabolites to the modulation of different intracellular activities. The proteomic analysis connected the availability of acetyl-CoA in the cytosol to SLC25A1, SLC13A5 and ACLY, and to the consequent citrate/acetyl-CoA flux [83,125]. As such, the specific roles of SLC25A1 and SLC13A5 were explored by generating overexpressing mouse models.

Neuron-specific overexpression of either SLC25A1 (SLC25A1 nTg) or SLC13A5 (SLC13A5 nTg) yielded an ASD-like phenotype with changes in white matter integrity, altered synaptic plasticity and morphology [30,45]. The fact that AT-1 nTg, SLC25A1 nTg and SLC13A5 nTg display a similar ASD-like phenotype supports the genetic association with ASD (discussed above in a previous section) and the connectivity of the citrate/acetyl-CoA flux. A closer look also revealed some phenotypic differences across models [30,45,83]. Specifically, SLC25A1 nTg and SLC13A5 nTg mice displayed repetitive jumping stereotypy while AT-1 nTg mice did not. SLC25A1 nTg and SLC13A5 nTg mice displayed altered spine morphology with normal numbers while AT-1 nTg mice displayed increased number of dendritic spines with normal morphology. Finally, AT-1 nTg mice displayed changes in both long-term potentiation and long-term depression while SLC25A1 nTg only had abnormal long-term potentiation and SLC13A5 nTg mice only had abnormal long-term depression. These differences might reflect different biochemical outputs with increased citrate/acetyl-CoA availability in the cytosol in the SLC25A1 nTg and SLC13A5 nTg models and reduced citrate/acetyl-CoA availability in the cytosol in the AT-1 nTg model [30,45,83]. Apart from these minor differences, which remain to be fully dissected, the phenotypic similarities across models provides strong support for the connectivity of the citrate/acetyl-CoA flux and the common down-stream output, increased acetyl-CoA flux into the ER with altered dynamics of the secretory pathway. Indeed, the central core of the ASD-like phenotype of AT-1 nTg, SLC25A1 nTg and SLC13A5 nTg mice resides in the marked dysregulation of ER and secretory pathway [30,45,83]. These studies also reinforce the connection between aberrant cytosol-to-ER flux of acetyl-CoA and ASD (see Table 2). A remaining point is to study the effects imparted upon by the systemic overexpression of SLC25A1 and SLC13A5. Interestingly, several attempts to generate ACLY overexpressing models in our laboratory (unpublished) failed to yield mice with increased levels of the protein suggesting that the cell has translational mechanisms in place to ensure tight regulation of ACLY protein levels.

5.5. ER acetylation and therapeutic regulation of proteostasis

Autophagy is an essential component of the cell degrading machinery. It helps dispose of large toxic protein aggregates that form within the secretory pathway as well as in the cytosol. Malfunction of autophagy contributes to the progression of many diseases across lifespan [6,128–131]. In addition, many progressive degenerative diseases are characterized by the aberrant accumulation of toxic protein aggregates. Compelling data indicate that increased levels of autophagy can be beneficial in mouse models of diseases characterized by increased accumulation of toxic protein aggregates [6,84,98,132–136]. As such, improving normal proteostatic mechanisms is an active target for biomedical research [128–131].

A major limitation for autophagy-based translational approaches is the ability to selectively target autophagy to a specific cellular location without disrupting similar on-going processes elsewhere. As an example, toxic protein aggregates that form in the ER should ideally be dealt with by targeting ER-specific autophagy, while similar aggregates that form in the cytosol should be dealt with by targeting cytosol-specific forms of autophagy. The identification of the ER acetylation machinery, and -more specifically-the ATases, provide us a way to target diseases where the accumulation of aggregated (and potentially toxic) species of the nascent polypeptides within the ER and early secretory pathway contribute to the pathogenesis of the disease.

We previously discussed the fact that nonsense and missense changes in the absence of disease manifestations have been identified on both ATases [6]. We also discussed the fact that mice with genetic disruption of either Atase (Atase1$^{-/-}$ and Atase2$^{-/-}$) display no disease phenotype [74]. Therefore, sufficient evidence suggests that therapeutic approaches targeting the ATases are viable and have the potential to be disease-modifying. Using a combination of *in vitro* high throughput screening and *in silico* docking, potential ATase inhibitors with drug-like properties were identified [73,127]. They displayed slightly different binding placements within the ATase models. Specifically, most of them appear to target either the acetyl-CoA or the peptidyl-lysine pocket on ATase1, or the interspace between the two pockets on ATase2, suggesting that the identification and/or synthesis of ATase-specific inhibitors is viable [127]. When tested with AT-1 sTg mice, three compounds displayed strong disease-modifying properties and were able to rescue all progeria-associated manifestations, including the short lifespan [84,127]. When tested on two mouse models of Alzheimer's disease, the $APP_{695/swe}$ and the $APP_{695/swe}$/PS1-dE9, they also displayed disease-modifying properties [98,127]. Interestingly, when tested in mouse models of cytosolic proteotoxicity, the $mHtt^{Q160}$ and the $hSOD1^{G93A}$, they failed to rescue disease manifestations [98]. Therefore, ATase-specific inhibitors can be readily identified, and they can be expected to be specific for proteotoxic states affecting the ER and secretory pathway, such as in Alzheimer's disease.

6. CONCLUDING REMARKS

Coenzyme A, which is derived from vitamin B5 (pantothenic acid), plays a central role in carbohydrate and lipid metabolism as it participates in approximately 4–5% of all biochemical reactions within the cell. Acetyl-CoA, the acetyl group donor for protein acetylation, is produced in the nucleus, cytosol, mitochondria, and peroxisomes, and variations in these different subcellular pools affect both local protein acetylation and metabolic crosstalk to maintain intracellular homeostasis.

Proteins are acetylated either at the N-terminus, termed Nα-acetylation, or on lysine side chains, termed Nε-lysine acetylation, with the latter being critically important in regulating multiple cellular functions including gene expression, proteostasis, and cytoskeletal dynamics. The identification of the ER acetylation machinery, namely the acetyl-CoA transporter AT-1 and acetyltransferases ATase1 and ATase2, has transformed the landscape of Nε-lysine acetylation and revealed new regulatory and translational functions for this -relatively young- post-translational modification. Recent developments demonstrate that Nε-lysine acetylation coordinates the activity of many proteins situated in different cellular organelles and compartments, helping coordinate complex biological events. Fluctuations in acetyl-CoA input and/or utilization in the ER can force adaptation within the cytosol, nucleus and mitochondria; similarly, changes in mitochondria bioenergetics or TCA engagement may redirect biochemical events in the cytosol as well as nucleus and ER. Therefore, Nε-lysine acetylation has evolved as a sensor of the local and global cellular metabolic state to ensure homeostatic balance and functional adaptation to the environment.

Defects in the homeostatic balance of acetyl-CoA flux and acetylation appear to reflect different but converging disease states, and future discoveries in CoA/acetyl-CoA biology and acetylation are likely to yield

novel therapeutic approaches. In conclusion, citrate and acetyl-CoA should not only be seen as metabolic substrates that are key for intermediate metabolism but also as signaling molecules with "second messenger-like" functions that direct adaptation of the cell to both intracellular and extracellular messages.

AUTHOR CONTRIBUTIONS

All authors contributed to the writing of this manuscript.

DATA AVAILABILITY

No data was used for the research described in the article.

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CONFLICT OF INTEREST

The authors have no competing interests to disclose.

ABBREVIATIONS

AceCS1	acetyl-CoA synthetase
ACLY	ATP citrate lyase
ADHD	attention deficit hyperactivity disorder
ASD	autism spectrum disorder
AT-1	acetyl-CoA transporter
ATase1	acetyltransferase 1
ATase2	acetyltransferase 2
ATG9A	autophagy protein 9A
CALR	Calreticulin
CIC	citrate/isocitrate carrier
CMT	Charcot-Marie-Tooth
CoA	coenzyme A
COASY	coenzyme A synthase
CSF	cerebrospinal fluid
CTP	citrate transport protein
DPCK	dephospho-CoA kinase
ER	endoplasmic reticulum
ERES	endoplasmic reticulum exit sites
GNAT	GCN5-related N-acetyltransferases
HMN	hereditary motor neuropathy
HSAN	hereditary sensory autonomic neuropathy
KAT	lysine-acetyltransferase
NaCT	sodium/citrate co-transporter
NAT	N-acetyltransferase
NBIA	neurodegeneration with brain iron accumulation
OST	oligosaccharyltransferase
PANK	pantothenate kinase
PKAN	pantothenate kinase-associated neurodegeneration
PPAT	phosphopantetheine adenylyltransferase
PPCDC	phosphopantothenoylcysteine decarboxylase
PPCS	phosphopantothenoylcysteine synthetase
SLC13A5	solute carrier family 13 member 5
SLC25A1	solute carrier family 25 member 1
SLC33A1	solute carrier family 33 member 1
TCA	tricarboxylic acid

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