Lipoic acid metabolism and mitochondrial redox regulation

Ashley Solmonson and Ralph J. DeBerardinis*

From the Children’s Medical Center Research Institute, UT Southwestern Medical Center, Dallas, Texas 75390, USA

Running Title: Lipoic acid and mitochondrial redox regulation

*To whom correspondence should be addressed: Children’s Medical Center Research Institute, University of Texas Southwestern, 5323 Harry Hines Blvd. Dallas, Texas 75390-8502. Tel.: 214-648-2587; E-mail: Ralph.Deberardinis@UTSouthwestern.edu

Keywords: lipoic acid, cell metabolism, mitochondria, cofactors, redox regulation

Abstract
Lipoic acid is an essential cofactor for mitochondrial metabolism and is synthesized de novo using intermediates from mitochondrial fatty acid synthesis type II, S-adenosylmethionine and iron-sulfur clusters. This cofactor is required for catalysis by multiple mitochondrial 2-ketoacid dehydrogenase complexes, including pyruvate dehydrogenase, alpha-ketoglutarate dehydrogenase, and branched-chain ketoacid dehydrogenase. Lipoic acid also plays a critical role in stabilizing and regulating these multi-enzyme complexes. Many of these dehydrogenases are regulated by reactive oxygen species, mediated through the disulfide bond of the prosthetic lipoyl moiety. Collectively, its functions explain why lipoic acid is required for cell growth, mitochondrial activity and coordination of fuel metabolism.

Introduction
Lipoic acid (6,8-dithiooctanoic acid) was first identified by Lester Reed and colleagues in the 1950s (1-3), and over the last several decades much has been learned about the chemical properties and biological functions of this unique cofactor. The disulfide bond within the molecule provides a source of reductive potential that is required for catalysis by mitochondrial 2-ketoacid dehydrogenases, and participates in stabilization and redox dependent regulation of these multi-enzyme complexes. These functions make lipoic acid essential for cell growth, oxidation of carbohydrates, amino acids and other fuels, and regulating mitochondrial redox balance. Herein, we discuss the intricacies of lipoic acid synthesis in various organisms, the role lipoylation plays in 2-ketoacid dehydrogenase activities and regulation, and how these enzymes are influenced by reactive oxygen species through this covalently bound lipoic acid cofactor.

Lipoic Acid Synthesis
Lipoic acid (LA) metabolism has been intensely investigated by Lester Reed (1), John Cronan (4, 5), and others through genetic and biochemical studies using prokaryotes and S.cerevisiae (6). Homologous enzymes have been identified in plants (7, 8) and mammalian systems (9). That being said, distinct differences in LA metabolism among various organisms are relevant to the regulation of mitochondrial 2-ketoacid dehydrogenases. LA metabolism in E. coli consists of a de novo biosynthetic pathway and a salvage pathway capable of using exogenous LA as a substrate, with both pathways leading to the covalent modification of the ε-amino group on conserved lysine residues within the lipoyl domain of the E2 subunits of mitochondrial 2-ketoacid dehydrogenase complexes (4) (Figure 1A).
The biosynthetic pathway in *E. coli* is initiated by an octanoyltransferase enzyme (octanoyl-ACP:protein-N-octanoyltransferase), LipB, that transfers octanoic acid derived from mitochondrial fatty acid synthesis type II (FASII) from the acyl carrier protein (ACP) to the lipoyl domain of a target enzyme. LipB transfers octanoate directly onto the lipoyl domain (LD) on the E2-subunit of 2-ketoacid dehydrogenases and on to the H-protein of the glycine cleavage system (GCS) (Figure 1A - reaction B1) (10-14). *E. coli* tolerates loss of this enzyme when exogenous lipoate is present through activity of the salvage pathway (discussed below), but in the absence of exogenous lipoate, growth is suppressed and no lipoylation of pyruvate dehydrogenase (PDH-E2) or alpha-ketoglutarate dehydrogenase (OGDH-E2) is observed (15).

In the second step of LA biosynthesis two sulfur atoms are inserted at the C6 and C8 positions by an iron-sulfur lipoic acid synthase enzyme, LipA (Figure 1A – reaction B2) (15-17). This step is similar to biotin synthesis in that the reaction mechanism requires sulfur donation from an [4Fe-4S] cluster within the enzyme and reductive cleavage of s-adenosylmethionine (SAM) to generate 5’deoxyadenosyl 5’-radicals (5’-dA*) that remove a hydrogen atom from C6 and C8 and facilitate insertion of the sulfur atoms (5, 18, 19). The requirements for this enzyme are similar to that of LipB in that *E. coli* can utilize the LA salvage pathway to compensate for loss of LipA in lipoate containing media (15). Importantly, donation of sulfur from the [4Fe-4S] cluster within the enzyme suggests that LipA may be a self-sacrificing protein rather than performing true enzyme catalysis (5). This is supported by the small amount of LA generated per molecule of LipA in *E. coli*, which can be enhanced by co-expression of LipA with iron-sulfur cluster proteins (5, 20). Recently, the *E. coli* iron-sulfur cluster proteins NfuA and IscU were shown to reinstall the [4Fe-4S] cluster of LipA in order to facilitate additional turns of the enzyme (21). This mechanism is consistent with deficiencies in NFU1 resulting in phenotypes associated with lipoic acid deficiency and suggests that this mechanism may be conserved in mammals (22).

The LA salvage pathway in *E. coli* consists of a single lipoyl-protein ligase enzyme, LplA, that first conjugates exogenous lipoic acid to an adenylate intermediate (lipoyl-AMP) followed by ligation to the lipoyl domain (LD) of E2 subunits and H-protein (Figure 1A – reaction S1) (23-25). LplA can use both lipoic acid and octanoate to modify E2 subunits in intact cells, with the latter substrate requiring the activity of LipA to insert sulfur atoms and generate the lipoic acid moiety directly on target enzymes (23, 26). *E. coli* can tolerate the loss of this enzyme, but loss of LplA and LipB result in no lipoylated proteins whereas loss of LplA and LipA results in the accumulation of octanoyl-proteins (26). These data indicate that there are two distinct LA metabolism pathways in *E. coli* that ensure growth in both lipoate containing and deficient environments (11, 26).

LA metabolism in *S. cerevisiae* is different from *E. coli* primarily in that these two pathways are interdependent and cannot fully compensate for one another (27), and lipoylation of PDH-E2 (Lat1) and OGDH-E2 (Kgd2) requires initial lipoylation of the H-protein of the glycine cleavage system (Gcv3) (Figure 1B – reaction 1) (6). This pathway is similar to *Bacillus subtilis*, where the octanoyltransferase in *S. cerevisiae*, Lip2, transfers octanoate (derived from FASII) to Gcv3, and this step is required for lipoylation of Lat1 and Kgd2 (6). Importantly, activity of the glycine cleavage system (GCS) is not required for downstream lipoylation events since loss of other GCS subunits has no impact on Lat1 or Kgd2 lipoylation (6). The lipoate synthase enzyme in *S. cerevisiae* is Lip5, which functions similarly to LipA in *E. coli* through iron-sulfur cluster mediated insertion of the disulfide at C6 and C8. (Figure 1B – reaction 2) (28). Yeast cannot tolerate the loss of Lip2 or Lip5 unless grown in media containing ethanol and succinate, which bypasses Kgd2 in the TCA cycle (6).

The inability of Lip2 and Lip5 mutants to grow on media supplemented with lipoic acid was the first indication that an independent LA salvage pathway did not exist in *S. cerevisiae* (29). However, identification of an LplA homolog in yeast, Lip3, and the observation that lipoylation of Gcv3 was maintained in Lip3 mutants indicated that Lip3 functions downstream of Lip2 (Figure 1B – reaction 3). *In vitro* studies have demonstrated that Lip3 is an octanoyltransferase, using octanoyl-CoA or octanoyl-Gcv3 as a substrate but the enzyme lacks the ability to utilize
lipoate plus ATP to generate the adenylate intermediate seen with LpA (27). Expression of E. coli LpA in yeast strains lacking Lip2 or Lip5 completely rescues growth in the presence of lipoate indicating that the lack of a true LA salvage pathway in S. cerevisiae is an aspect of the differential activities of LpA and Lip3 (27). Questions as to the true substrate of Lip3 in vivo are still unanswered from these studies. Does Lip3 transfer an octanoyl moiety from Gcv3 to Lat1 and Kgd2, where Lip5 then generates the lipoyl moiety; or does Lip3 act as a lipoyltransferase, transferring a lipoyl moiety from Gcv3? The maintenance of Gcv3 lipoylation in Lip3 mutant strains indicates that octanoyl-Gcv3 is a substrate for Lip5 and that either Lip2 does not transfer an octanoyl moiety from ACP to Lat1 and Kgd2, or that Lip5 cannot act on octanoyl-Lat1/Kgd2. This is particularly interesting since expression of yeast Lip3 in E.coli ΔlipB ΔlpA allows for growth in the presence of octanoate but not lipoate. Furthermore, in octanoate containing media, E.coli ΔlipB ΔlpA expressing Lip3 have lipoylated PDH and OGDH (27). As well, lipoylation was enhanced in the presence of octanoate to a greater degree than with lipoate and on PDH more so than OGDH (27). These data suggest that in an E. coli system, Lip3 is an octanoyltransferase and that LipA can act on octanoyl-PDH/OGDH, but this has not been demonstrated in the native cellular environment of Lip3.

Although less well understood in mammalian systems, the LA biosynthetic pathway in mice and humans is carried out by an octanoyltransferase ortholog of LipB/Lip2 and a lipoic acid synthase ortholog of LipA/Lip5 known as LIPT2 and LIAS, respectively (Figure 1C) (30-32). Deficiencies in either of these enzymes, as well as disruptions in mitochondrial FASII or iron sulfur biogenesis, result in diminished lipoylation of PDH and OGDH and ultimately impaired mitochondrial function (30, 33, 34). The lipoyltransferase ortholog in mammals is LIPT1 and similar to Lip3 in S.cerevisiae, it lacks the ability to generate an activated lipoyl-AMP and therefore is thought to be downstream of LIPT2 (9, 30, 35). There has been a report identifying a mammalian lipoic acid-activating enzyme that could activate exogenous lipoic acid (36); however, this function was ultimately attributed to the mitochondrial medium-chain acyl CoA synthetase (ACSM1) (37, 38). This enzyme can utilize both the (R)- and (S)-enantiomers of LA and primarily uses GTP to activate the natural (R)-lipoic acid, but so far there has been no substantial evidence to support that this enzyme functions in LA metabolism in vivo (36). This is consistent with the inability for exogenous LA to rescue defects in cells derived from LIAS deficient patients, embryonic lethality in LIAS deficient mice, or to ameliorate symptoms in patients with this disease (22, 30, 32). Taken together, this suggests that mammalian LA metabolism is similar to S. cerevisiae where LIPT2 transfers octanoylate from ACP to the H-protein of GCS, LIAS inserts sulfur atoms into the octanoyl group on H-protein, and LIPT1 transfers the lipoyl group from the H-protein to E2 subunits. Similar to Lip3, experiments to show the true in vivo substrate of LIPT1 have not been reported; thus it is unclear if LIPT1 is an octanoyltransferase, a lipoyltransferase, or both.

**Lipoic acid as a cofactor for mitochondrial 2-ketoacid dehydrogenases**

As discussed above, the function of LA metabolism is to provide an essential, enzyme-bound cofactor for mitochondrial 2-ketoacid dehydrogenases and the glycine cleavage system (GCS). The 2-ketoacid dehydrogenases include pyruvate dehydrogenase (PDH), alpha-ketoglutarate dehydrogenase (OGDH), branched chain ketoacid dehydrogenase (BCKDH) and 2-oxoadipate dehydrogenase (OADH) (30, 39). These multi-enzyme complexes are composed of three independent subunits that undergo coupled reactions facilitated by the LA cofactor (Figure 2) (40). The E1 subunit, which provides substrate specificity to the multi-enzyme complex, utilizes the cofactor thiamine pyrophosphate (TPP) to decarboxylate the substrate generating an acyl-TPP intermediate followed by reductive acylation of the lipoyl group on the E2 subunit. The E2 subunit functions as a dihydrolipoyl acyltransferase that transfers the acyl group to CoA, generating an acyl-CoA and dihydrolipoamide. The E3 subunit is a dihydrolipoamide dehydrogenase (DLD) using FAD to oxidize dihydrolipoamide, regenerating the disulfide bond for use in subsequent rounds of catalysis. The E3 subunit oxidizes FADH2 back to
Lipoic acid and mitochondrial redox regulation

FAD using NAD\(^+\) producing NADH in the process (40) (Figure 2). This activity is similar for PDH, OGDH and BCKDH, where the E1 and E2 subunits are specific to the decarboxylation substrate and the E3 protein subunit is shared among all three complexes (4). The activity of 2-OADH has not been investigated to the degree of these other complexes, but elevated 2-oxoadipate has been reported in patients with DLD deficiency, suggesting that this complex uses the same DLD subunit shared by other 2-ketoacid dehydrogenases (41).

Importantly, lipoylated 2-ketoacid dehydrogenases play a key role in carbon entry into the TCA cycle. Therefore, dysfunction in these enzymes may produce aberrant mitochondrial metabolism that can be deleterious (Figure 3). Inborn errors have been reported in each of these enzyme complexes with mutations having been reported in all of the subunits.

Pyruvate dehydrogenase deficiency is rare but hundreds of cases have been reported, most involving mutations in the X-linked gene encoding the E1-alpha 1 subunit (PDHA1). Severe mutations are devastating in boys but cause a more variable spectrum of severity in females due to random X-inactivation. Broadly, symptoms of PDH deficiency include lactic acidosis, hypotonia, seizures, ataxia, and developmental delay. Treatment for PDH deficiency is limited; the use of a ketogenic diet and in some cases treatment with dichloroacetate or thiamine can provide improvement of clinical symptoms (42).

Alpha-ketoglutarate dehydrogenase deficiency has been reported less frequently than PDH deficiency, but the primary clinical manifestations are similar including developmental delay, ataxia, hypotonia, and in some cases encephalopathy (42). The elevation of alpha-ketoglutarate levels may also be associated with an elevation in 2-hydroxyglutarate (2-HG), a potential epigenetic regulator. It is currently unknown whether 2-HG levels contribute to pathology in alpha-ketoglutarate dehydrogenase deficiency (43, 44).

Deficiency in the E1 or E2 subunit of branched chain ketoacid dehydrogenase is also known as maple syrup urine disease (MSUD). This disorder includes a classic presentation of a sweet, maple-syrup like odor and an elevation of plasma branched chain amino acids and 2-ketoacids in urine. In the severe neonatal-onset form, patients display metabolic decompensation and neurological distress which may be severe and result in neonatal coma. There are also acute and intermittent forms that may be late-onset and consist of recurrent metabolic decompensation episodes; as well, the chronic and progressive form presents with hypotonia and developmental delay. Treatment of MSUD involves titrating dietary BCAA content to avoid excessive exposure while maintaining sufficient levels to support normal development (42).

2-oxoadipic aciduria has only been reported in about 20 individuals with half of them being asymptomatic. Those individuals that do show symptoms may display psychomotor retardation and hypotonia. Mutations in dehydrogenase E1 and transketolase domain containing 1 (DHTKD1) are thought to be causative in these cases (42).

Mutations have also been reported in dihydrolipoamide dehydrogenase (DLD), LIAS, LIPT2 and LIPT1. These disorders have overlapping phenotypes including lactic acidosis, developmental delay, and seizures. Collectively, dysfunction in these enzymes fall into a larger class of mitochondrial disease known as Leigh syndrome. LIAS patients may be distinguished from LIPT1 patients through presentation of non-ketotic hyperglycinemia associated with dysfunction in the GCS. An excellent review of the clinical manifestations of these disorders was recently described by Mayr et. al (30). Critical to developing treatments for these disorders is intimate knowledge of the regulation of 2-ketoacid dehydrogenase complexes and how their activities may differ in various nutritional states and in individual tissues.

Lipoic acid and ROS generation from mitochondrial 2-ketoacid dehydrogenases

Dihydrolipoamide Dehydrogenase - The ability of the shared E3 subunit to regenerate oxidized lipoic acid for further catalysis in 2-ketoacid dehydrogenase complexes is controlled by the NAD\(^+\)/NADH ratio within the mitochondria (45). When the availability of NAD\(^+\) is diminished, FADH\(_2\) within the E3 subunit can readily be oxidized by \(O_2\) generating a semiquinone (FADH\(^*\)) and superoxide (\(O_2^-\)) (46, 47). The semiquinone may then equilibrate with
the reduced lipoic residue generating a thiol radical within the core complex. This can lead to inactivation of the complex due to reactions between a thiol radical on the E2 subunit and the catalytic region of the E1 subunit (48). This can be seen as a regulatory inactivation to inhibit the catabolism of specific substrates, like pyruvate or alpha-ketoglutarate when NAD\(^+\) is depleted or when ROS production alters the redox balance of the mitochondria (49). It is unclear the degree to which this occurs on each 2-ketoacid complex individually. There is evidence that the E3 subunit may be involved in redox regulation independent of its activities within the 2-ketoacid dehydrogenase complexes (45). Specifically, the flavin can participate in regenerating thioredoxin 2 (Trx2) (50, 51) and has a structure similar to glutathione reductase (52), but it is unclear whether these functions require the entire 2-ketoacid dehydrogenase complex or if the E3 subunit dissociates from the complex for this function.

**Alpha-ketoglutarate dehydrogenase** - The OGDH complex facilitates the decarboxylation of alpha-ketoglutarate (2-oxoglutarate) to form succinyl-CoA and NADH. This complex in mammalian systems is distinct in that the E2 core subunit (DLST) lacks the E1 and E3 binding domains in the prokaryotic protein, suggesting that the overall structure of this complex is organized differently in mammals (53). Multiple studies have demonstrated that the OGDH complex can produce superoxide and H\(_2\)O\(_2\), and many of these studies focus on the activity of the E3 subunit of the complex (46, 48, 54). Experiments in skeletal muscle mitochondria show that OGDH is responsible for a significant portion of mitochondrial superoxide production that is related to the concentrations of alpha-ketoglutarate and free CoA (55). Additionally, the lipoic moiety has been shown to be glutathionylated *in vivo*, which is thought to be a mechanism to reversibly inactivate the complex, preventing ROS-dependent inactivation of the E1 subunit (56, 57). It is not known whether this glutathionylation is enzyme-mediated or occurs spontaneously, but the glutathionylation can be removed through the actions of glutathione reductase 2 (Grx2) (58). Although glutathionylation of the lipoic moiety prevents oxidative damage to the E1 component, it also maintains a reduced flavin on the E3 subunit, and this may contribute to oxidative damage by E3-mediated ROS production (58). Thioredoxin 2 has been shown to protect OGDH from oxidative damage (49-51), and it has been suggested that glutathionylation of the lipoic moiety provides the opportunity for the E3 subunit to participate in redox regulation through ROS scavenging or regeneration of thioredoxin (51). Finally, OGDH has been shown to interact with Complex I of the electron transport chain (ETC), which allows for direct supply of NADH to the NADH-oxidation site of Complex I (Figure 4A) (59-61). Since glutathionylation of Complex I is also regulated by Grx2 (62, 63), it has been suggested that glutathionylation may dissociate the interaction between OGDH and Complex I to limit further electron entry into the ETC until redox homeostasis is reached (Figure 4B) (64). This is particularly important since OGDH activity regulates the rate of NADH production of downstream enzymes within the TCA cycle. Collectively, these findings indicate that OGDH is a key source of ROS in the mitochondria but it may also play a key role in redox balance and regulation through multiple mechanisms.

**Pyruvate dehydrogenase complex** - The pyruvate dehydrogenase complex has been extensively studied as a primary regulatory node for nutrient oxidation in various metabolic states. This enzyme complex irreversibly decarboxylates pyruvate to generate acetyl CoA and CO\(_2\) and is coordinated by three phosphorylation sites on the E1 subunit regulated by a set of kinases (PDKs) and phosphatases (PDP) which interact with lipoyl domains on the E2 subunit and E3 binding protein (65). The PDH complex structure is somewhat different from OGDH in that it includes an E3-binding protein that also contains a lipoic domain. As well, the PDH complex has stable interactions with PDK and PDP (66, 67). The E3-binding protein lipoic domain does undergo lipoylation; however, this lipoylation is not thought to be involved in catalysis but undergoes reductive acetylation as a regulatory mechanism for PDK and PDP (66, 68, 69). As with OGDH, PDH produces superoxide and H\(_2\)O\(_2\), although there is contention around the relative contributions of the E3 and E1 subunits *in vivo* (55, 70). PDH has also been shown to be glutathionylated on the E2 subunit, similar to OGDH s-glutathionylation (70). This glutathionylation can increase ROS
generation from the E3 subunit since FAD cannot be oxidized by the lipoic acid moiety and reduces O₂ by one electron to produce superoxide. Importantly, glutathionylation decreases ROS production when pyruvate is being oxidized, and depletion of glutathione leads to increased ROS production from PDH (71). PDH has also been shown to interact with Grx2 suggesting that the reversible glutathionylation regulates PDH through similar mechanisms to OGDH (70). This common mechanism appears to play a critical regulatory role for mitochondrial metabolism under different nutritional states and likely in various tissues. Finally, recent studies have suggested that Sirtuin 4 (SIRT4) can regulate PDH activity through lipoamidase activity that cleaves the lipoyl moiety from the E2 component (72). This study did not investigate SIRT4 regulation of the other mitochondrial 2-ketoacid dehydrogenases, but these complexes did associate with SIRT4 in their immuneaffinity purification experiment. Subsequent studies demonstrated that this mechanism was conserved in prokaryotic systems and that OGDH and the glycine cleavage system were also targets of the SIRT4 ortholog, CobB (73). Another study also implicated SIRT4 in regulation of leucine catabolism indicating that BCKDH is likely an additional target of this regulation (74).

Branched chain ketoacid dehydrogenase - The branched chain ketoacid dehydrogenase complex is downstream of the branched chain aminotransferase enzyme (BCAT), which catalyzes the breakdown of valine, leucine and isoleucine to the ketoacids, 3-methyl-2-oxobutyanoate (KIV), 4-methyl-2-oxopentanoate (KIC), and 3-methyl-2-oxopentanoate, respectively (75) (Figure 3). BCKDH then decarboxylates these ketoacids releasing CO₂ and generating an acyl-CoA. This complex has a similar structure and regulation to PDH with stably associated kinase and phosphatases (76, 77). Structural analysis of BCKDH demonstrated that the phosphorylation loop of the E1 subunit undergoes a disordered-to-ordered conformational change that regulates binding of the lipoyl group on the E2 subunit near the catalytic site of the E1 subunit. In the dephosphorylated (active) state, the lipoyl group is positioned near the active site of the E1 subunit to facilitate reductive acylation immediately after the decarboxylation reaction. During the phosphorylated (inactive) state, the ordered conformation of the phosphorylation loop prevents lipoyl domain positioning near the E1 active site. (78). Subsequent studies demonstrated a similar role for the lipoyl domain in the E2 subunit that involves a substrate gating mechanism. The E2 subunit undergoes a conformational change upon CoA binding, which allows for the lipoyl-acyl intermediate to enter the acyltransferase catalytic site (79). This mechanism prevents the reduced lipoamide from entering the E2 catalytic site when free CoA levels are reduced. BCKDH has been shown to produce superoxide and H₂O₂ to a lesser degree than OGDH and PDH (55). The overall details of redox-regulation of BCKDH are less clear compared to the other 2-ketoacid dehydrogenases with most of the literature focused on the E3 subunit that is shared by the other complexes.

2-oxoadipate dehydrogenase - Catabolism of tryptophan and lysine lead to the production of 2-oxoadipate (2-OA), which is converted to glutaryl-CoA by 2-oxoadipate dehydrogenase (OADH) (80). Glutaryl-CoA has recently been shown to post-translationally modify lysine residues on mitochondrial proteins (81). Thus, dysfunction in this enzyme could impact mitochondrial function through mechanisms independent of 2-OA per se. Mutations in dehydrogenase E1 and transketolase domain containing 1 (DHTKD1) lead to accumulation of 2-OA, however reports have indicated that OGDH can also decarboxylate 2-OA which is just slightly larger than its conventional substrate, alpha-ketoglutarate. It is unclear whether DHTKD1 is associated with an independent dehydrogenase complex or if it shares activity with OGDH. Recent studies have indicated that ROS production can be attributed to the DHTKD1 enzyme (82). Paradoxically, ROS production seems to increase when the enzyme is either suppressed or overexpressed, although the mechanisms regulating this phenomenon are not understood (83). Expression data indicate that DHTKD1 is highly expressed in liver and kidney with comparable protein levels observed in human skeletal muscle (82), indicating that these tissues would be the appropriate systems to investigate these open questions.

Concluding Remarks
Lipoic acid is an often overlooked, essential cofactor for mitochondrial oxidative metabolism, which participates in catalysis and regulation of multiple enzyme complexes. Biosynthesis of LA links mitochondrial fatty acid synthesis, SAM and iron-sulfur cluster biosynthesis with TCA cycle oxidative capacity. This information is particularly important for diagnosis of inborn errors of metabolism since defects in lipoic acid metabolism can promote a myriad of clinical symptoms associated with dysfunction in a number of enzyme complexes (22, 30, 31, 84). Additionally, because lipoic acid plays a regulatory role, symptoms of some inborn errors may only manifest, or may worsen, in nutritional states associated with high flux through enzymes requiring lipoylation. The lack of an independent salvage pathway in humans abrogates the use of LA supplementation as a therapeutic option; thus, further investigation of how LA metabolism is regulated and functions in humans will be necessary to treat inborn errors of this pathway.

Conflict of interest

The authors declare that they have no conflicts of interest with the contents of this article.

References

1. REED, L. J. (2006) The Chemistry and Function of Lipoic Acid. in Advances in Enzymology and Related Areas of Molecular Biology, pp. 319–347, Nord/Advances, John Wiley & Sons, Inc., Hoboken, NJ, USA, 77, 319–347
2. Patterson, E. L., Brockman, J. A., Jr., Day, F. P., Pierce, J. V., Macchi, M. E., Hoffman, C. E., Fong, C. T. O., Stokstad, E. L. R., and Jukes, T. H. (1951) CRYSTALLIZATION OF A DERIVATIVE OF PROTOGEN-B. J. Am. Chem. Soc. 73, 5919–5920
3. Reed, L. J., DeBUSK, B. G., GUNSLALUS, I. C., and HORNBERGER, C. S. (1951) Crystalline alpha-lipoic acid; a catalytic agent associated with pyruvate dehydrogenase. Science. 114, 93–94
4. Cronan, J. E. (2016) Assembly of Lipoic Acid on Its Cognate Enzymes: an Extraordinary and Essential Biosynthetic Pathway. Microbiol. Mol. Biol. Rev. 80, 429–450
5. Cronan, J. E. (2014) Biotin and Lipoic Acid: Synthesis, Attachment, and Regulation. EcoSal Plus. 10.1128/ecosalplus.ESP-0001-2012
6. Schonauer, M. S., Kastaniotis, A. J., Kursu, V. A. S., Hiltunen, J. K., and Dieckmann, C. L. (2009) Lipoic acid synthesis and attachment in yeast mitochondria. J. Biol. Chem. 284, 23234–23242
7. Ewald, R., Hoffmann, C., Florian, A., Neuhaus, E., Fernie, A. R., and Bauwe, H. (2014) Lipoate-Protein Ligase and Octanoyltransferase Are Essential for Protein Lipoylation in Mitochondria of Arabidopsis. Plant Physiol. 165, 978–990
8. Kang, S. G., Jeong, H. K., Lee, E., and Natarajan, S. (2007) Characterization of a lipoate-protein ligase A gene of rice (Oryza sativa L.). Gene. 393, 53–61
9. Fujiwara, K., Okamura-Ikeda, K., and Motokawa, Y. (1996) Lipoylation of acyltransferase components of alpha-ketoacid dehydrogenase complexes. J. Biol. Chem. 271, 12932–12936
10. Jordan, S. W., and Cronan, J. E., Jr. (1997) A New Metabolic Link. J. Biol. Chem. 272, 17903–17906
11. Jordan, S. W., and Cronan, J. E. (2003) The Escherichia coli lipB gene encodes lipoyl (octanoyl)-acyl carrier protein:protein transferase. J. Bacteriol. 185, 1582–1589
12. Jordan, S. W., and Cronan, J. E. (1997) Biosynthesis of lipoic acid and posttranslational modification with lipoic acid in Escherichia coli. Meth. Enzymol. 279, 176–183
13. Parry, R. J. (1977) Biosynthesis of lipoic acid. 1. Incorporation of specifically tritiated octanoic acid into lipoic acid. J. Am. Chem. Soc. 99, 6464–6466
14. White, R. H. (2002) Stable isotope studies on the biosynthesis of lipoic acid in Escherichia coli. Biochemistry. 19, 15–19
15. Reed, K. E., and Cronan, J. E. (1993) Lipoic acid metabolism in Escherichia coli: sequencing and
functional characterization of the lipA and lipB genes. *J. Bacteriol.* **175**, 1325–1336

16. Parry, R. J., and Trainor, D. A. (1978) Biosynthesis of lipoic acid. 2. Stereochemistry of sulfur introduction at C-6 of octanoic acid. *J. Am. Chem. Soc.* **100**, 5243–5244

17. Miller, J. R., Busby, R. W., Jordan, S. W., Cheek, J., Henshaw, T. F., Ashley, G. W., Broderick, J. B., Cronan, J. E., and Marletta, M. A. (2000) *Escherichia coli* LipA Is a Lipoyl Synthase: In Vitro Biosynthesis of Lipoylated Pyruvate Dehydrogenase Complex from Octanoyl-Acyl Carrier Protein †. *Biochemistry*. **39**, 15166–15178

18. Wang, S. C., and Frey, P. A. (2007) S-adenosylmethionine as an oxidant: the radical SAM superfamily. *Trends in Biochemical Sciences*. **32**, 101–110

19. Vanden Boom, T. J., Reed, K. E., and Cronan, J. E. (1991) Lipoic acid metabolism in *Escherichia coli*: isolation of null mutants defective in lipoic acid biosynthesis, molecular cloning and characterization of the *E. coli* lip locus, and identification of the lipoylated protein of the glycine cleavage system. *J. Bacteriol.* **173**, 6411–6420

20. Kriek, M., Peters, L., Takahashi, Y., and Roach, P. L. (2003) Effect of iron–sulfur cluster assembly proteins on the expression of *Escherichia coli* lipoic acid synthase. *Protein Expression and Purification*. **28**, 241–245

21. McCarthy, E. L., and Booker, S. J. (2017) Destruction and reformation of an iron cluster during catalysis by lipoate synthase. *Science*. **358**, 373–377

22. Baker, P. R., II, Friederich, M. W., Swanson, M. A., Shaikh, T., Bhattacharya, K., Scharer, G. H., Aicher, J., Creadon-Swindell, G., Geiger, E., MacLean, K. N., Lee, W.-T., Deshpande, C., Freckmann, M.-L., Shih, L.-Y., Wasserstein, M., Rasmussen, M. B., Lund, A. M., Procopis, P., Cameron, J. M., Robinson, B. H., Brown, G. K., Brown, R. M., Compton, A. G., Dieckmann, C. L., Collard, R., Coughlin, C. R., II, Spector, E., Wempe, M. F., and Van Hove, J. L. K. (2013) Variant non ketotic hyperglycinemia is caused by mutations in GLRX5. *Brain*. **137**, 366–379

23. Morris, T. W., Reed, K. E., and Cronan, J. E. (1994) Identification of the gene encoding lipoate-protein ligase A of *Escherichia coli*. Molecular cloning and characterization of the *lplA* gene and gene product. *J. Biol. Chem.*

24. Green, D. E., Morris, T. W., Green, J., Cronan, J. E., and Guest, J. R. (1995) Purification and properties of the lipoate protein ligase of *Escherichia coli*. *Biochemical Journal*. **309** (Pt 3), 853–862

25. Reed, L. J., LEACH, F. R., and Koike, M. (1958) Studies on a lipoic acid-activating system. *J. Biol. Chem.* **232**, 123–142

26. Morris, T. W., Reed, K. E., and Cronan, J. E. (1995) Lipoic acid metabolism in *Escherichia coli*: the *lplA* and *lipB* genes define redundant pathways for ligation of lipoate groups to apoprotein. *J. Bacteriol.* **177**, 1–10

27. Hermes, F. A., and Cronan, J. E. (2013) The role of the *Saccharomyces cerevisiae* lipoate protein ligase homologue, Lip3, in lipoic acid synthesis. *Yeast*. **30**, 415–427

28. Sulo, P., and Martin, N. C. (1993) Isolation and characterization of LIP5. A lipoate biosynthetic locus of *Saccharomyces cerevisiae*. *J. Biol. Chem.* **268**, 17634–17639

29. Marvin, M. E., Williams, P. H., and Cashmore, A. M. (2001) The isolation and characterisation of a *Saccharomyces cerevisiae* gene (LIP2) involved in the attachment of lipoic acid groups to mitochondrial enzymes. *FEMS Microbiol. Lett.* **199**, 131–136

30. Mayr, J. A., Feichtinger, R. G., Tort, F., Ribes, A., and Sperl, W. (2014) Lipoic acid biosynthesis defects. *Journal of Inherited Metabolic Disease*. **37**, 553–563

31. Habarou, F., Hamel, Y., Haack, T. B., Feichtinger, R. G., Lebigot, E., Marquardt, I., Busiah, K., Laroche, C., Madrange, M., Grisel, C., Pontoizeau, C., Eisermann, M., Boutron, A., Chrétien, D., Chadeaux-Vekemens, B., Barouki, R., Bole-Feyssot, C., Nitschke, P., Goudin, N., Boddaert, N., Nemazanyy, I., Delahodde, A., Kölker, S., Rodenburg, R. J., Korenke, G. C., Meitinger, T., Strom, T. M., Prokisch, H., Rotig, A., Ottolenghi, C., Mayr, J. A., and de Lonlay, P. (2017) Biallelic Mutations in LIPT2 Cause a Mitochondrial Lipoylation Defect Associated with Severe Neonatal
Lipoic acid and mitochondrial redox regulation

Encephalopathy. Am. J. Hum. Genet. 101, 283–290
32. Yi, X., and Maeda, N. (2005) Endogenous Production of Lipoic Acid Is Essential for Mouse Development. Molecular and Cellular Biology. 25, 8387–8392
33. Smith, S., Witkowski, A., Moghul, A., Yoshinaga, Y., Nefedov, M., de Jong, P., Feng, D., Fong, L., Tu, Y., Hu, Y., Young, S. G., Pham, T., Cheung, C., Katzman, S. M., Brand, M. D., Quinlan, C. L., Fens, M., Kuyper, F., Misquitta, S., Griffey, S. M., Tran, S., Gharib, A., Knudsen, J., Hannibal-Bach, H. K., Wang, G., Larkin, S., Thweatt, J., and Pasta, S. (2012) Compromised Mitochondrial Fatty Acid Synthesis in Transgenic Mice Results in Defective Protein Lipoylation and Energy Disequilibrium. PLoS ONE. 7, e47196–15
34. Heimer, G., Kerätär, J. M., Riley, L. G., Balasubramaniam, S., Eyal, E., Pietikäinen, L. P., Hiltunen, J. K., Marek-Yagel, D., Hamada, J., Gregory, A., Rogers, C., Hogarth, P., Nance, M. A., Shalva, N., Veber, A., Tzadok, M., Nissenkorn, A., Tonduti, D., Renaldo, F., University of Washington Center for Mendelian Genomics, Bamshad, M. J., Leal, S. M., Nickerson, D. A., Anderson, P., Annable, M., Blue, E. M., Buckingham, K. J., Chin, J., Chong, J. X., Cornejo, R., Jr, Davis, C. P., Frazier, C., He, Z., Jarvik, G. P., Jimenez, G., Johanson, E., Kolar, T., Krauter, S. A., Luksic, D., Marvin, C. T., McGee, S., McGoldrick, D. J., Patterson, K., Perez, M., Phillips, S. W., Pijalan, J., Robertson, P. D., Santos-Cortez, R., Shankar, A., Slattery, K., Shively, K. M., Siegel, D. L., Smith, J. D., Tackett, M., Wang, G., Wegener, M., Weiss, J. M., Wernick, R. I., Wheeler, M. M., Yi, Q., Kraoua, I., Panteghini, C., Valletta, L., Garavaglia, B., Cowley, M. J., Gayevskiy, V., Roscioli, T., Silberstein, J. M., Hoffmann, C., Raas-Rothschild, A., Tiranti, V., Anikster, Y., Christodoulou, J., Kastaniotis, A. J., Ben-Zeev, B., and Hayflick, S. J. (2016) MECR Mutations Cause Childhood-Onset Dystonia and Optic Atrophy, a Mitochondrial Fatty Acid Synthesis Disorder. The American Journal of Human Genetics. 99, 1229–1244
35. Fujiwara, K., Okamura-Ikedu, K., and Motokawa, Y. (1994) Purification and characterization of lipoam-AMP:N epsilon-lysine lipoamyltransferase from bovine liver mitochondria. J. Biol. Chem. 269, 16605–16609
36. Fujiwara, K., Takeuchi, S., Okamura-Ikedu, K., and Motokawa, Y. (2001) Purification, characterization, and cDNA cloning of lipoate-activating enzyme from bovine liver. J. Biol. Chem. 276, 28819–28823
37. Fujino, T., Takei, Y. A., Sone, H., Ioka, R. X., Kamataki, A., Magoori, K., Takahashi, S., Sakai, J., and Yamamoto, T. T. (2001) Molecular Identification and Characterization of Two Medium-chain Acyl-CoA Synthetases, MACS1 and the SaGene Product. J. Biol. Chem. 276, 35961–35966
38. Vessey, D. A., Lau, E., Kelley, M., and Warren, R. S. (2003) Isolation, sequencing, and expression of a cDNA for the HXM-A form of xenobiotic/medium-chain fatty acid:CoA ligase from human liver mitochondria. Journal of Biochemical and Molecular Toxicology. 17, 1–6
39. REED, L. J. (2001) A Trail of Research from Lipoic Acid to α-Keto Acid Dehydrogenase Complexes. J. Biol. Chem. 276, 38329–38336
40. REED, L. J. (2002) Multienzyme complexes. Acc. Chem. Res. 7, 40–46
41. Shaag, A., Saada, A., Berger, I., Mandel, H., Joseph, A., Feigenbaum, A., and Elpeleg, O. N. (1999) Molecular basis of lipoamide dehydrogenase deficiency in Ashkenazi Jews. Am. J. Med. Genet. 82, 177–182
42. Saudubray, J.-M., Baumgartner, M. R., and Walter, J. (2016) Inborn Metabolic Diseases, Springer. 10.1007/978-3-662-49771-5
43. Intlekofer, A. M., Dematteo, R. G., Venneti, S., Finley, L. W. S., Lu, C., Judkins, A. R., Rustenburg, A. S., Grinaway, P. B., Chodera, J. D., Cross, J. R., and Thompson, C. B. (2015) Hypoxia Induces Production of L-2-Hydroxyglutarate. Cell Metabolism. 22, 304–311
44. Burr, S. P., Costa, A. S. H., Grice, G. L., Timms, R. T., Lobb, I. T., Freisinger, P., Dodd, R. B., Dougan, G., Lehner, P. J., Frezza, C., and Nathan, J. A. (2016) Mitochondrial Protein Lipoylation and the 2-Oxoglutarate Dehydrogenase Complex Controls HIF1? Stability in Aerobic Conditions. Cell Metabolism. 24, 740–752
45. Ambrus, A., and Adam-Vizi, V. (2017) Human dihydrolipoamide dehydrogenase (E3) deficiency:
Lipoic acid and mitochondrial redox regulation

Novel insights into the structural basis and molecular pathomechanism. *Neurochem. Int.* 10.1016/j.neuint.2017.05.018

46. Starkov, A. A., Fiskum, G., Chinopoulos, C., Lorenzo, B. J., Browne, S. E., Patel, M. S., and Beal, M. F. (2004) Mitochondrial alpha-ketoglutarate dehydrogenase complex generates reactive oxygen species. *J. Neurosci.* 24, 7779–7788

47. Bunik, V. I., and Pavlova, O. G. (2001) Inactivation of α-ketoglutarate dehydrogenase during oxidative decarboxylation of α-ketoacidic acid. *FEBS Lett.* 323, 166–170

48. Bunik, V. I., and Sievers, C. (2002) Inactivation of the 2-oxo acid dehydrogenase complexes upon generation of intrinsic radical species. *Eur. J. Biochem.* 269, 5004–5015

49. Bunik, V. I. (2003) 2-Oxo acid dehydrogenase complexes in redox regulation. *Eur. J. Biochem.* 270, 1036–1042

50. Bunik, V., Follmann, H., and Bisswanger, H. (1997) Activation of Mitochondrial 2-Oxoacid Dehydrogenases by Thioredoxin. *Biological Chemistry.* 378, 694

51. Bunik, V., and Follmann, H. (2001) Thioredoxin reduction dependent on α-ketoacid oxidation by α-ketoacid dehydrogenase complexes. *FEBS Lett.* 336, 197–200

52. Williams, C. H., Arscott, L. D., and Schulz, G. E. (1982) Amino acid sequence homology between pig heart lipoamide dehydrogenase and human erythrocyte glutathione reductase. *Proc. Natl. Acad. Sci. U.S.A.* 79, 2199–2201

53. Nakano, K., Takase, C., Sakamoto, T., Nakagawa, S., Inazawa, J., Ohta, S., and Matuda, S. (1994) Isolation, Characterization and Structural Organization of the Gene and Pseudogene for the Dihydrolipoamide Succinyldtransferase Component of the Human 2- Oxoglutarate Dehydrogenase Complex. *The FEBS Journal.* 224, 179–189

54. Ambrus, A., Tretter, L., and Adam-Vizi, V. (2010) Inhibition of the α-ketoglutarate dehydrogenase-mediated reactive oxygen species generation by lipoic acid. *Biochimica et Biophysica Acta (BBA) - Bioenergetics.* 1797, 57

55. Quinlan, C. L., Goncalves, R. L. S., Hey-Mogensen, M., Yadava, N., Bunik, V. I., and Brand, M. D. (2014) The 2-oxoacid dehydrogenase complexes in mitochondria can produce superoxide/hydrogen peroxide at much higher rates than complex I. *J. Biol. Chem.* 289, 8312–8325

56. Applegate, M. A. B., Humphries, K. M., and Szweda, L. I. (2008) Reversible Inhibition of α-Ketoglutarate Dehydrogenase by Hydrogen Peroxide: Glutathionylation and Protection of Lipoic Acid. *Biochemistry.* 47, 473–478

57. Nulton-Persson, A., Szweda, L., and Humphries, K. (2009) Inactivation and Inhibition of Alpha-Ketoglutarate Dehydrogenase. in *Lipoic Acid, Oxidative Modification of Lipoic Acid*, CRC Press, 10.1201/9781420045390.ch8

58. Mailloux, R. J., Craig Ayre, D., and Christian, S. L. (2016) Induction of mitochondrial reactive oxygen species production by GSH mediated S-glutathionylation of 2-oxoglutarate dehydrogenase. *Redox Biol.* 8, 285–297

59. Fukushima, T., Decker, R. V., Anderson, W. M., and Spivey, H. O. (1989) Substrate channeling of NADH and binding of dehydrogenases to complex I. *J. Biol. Chem.* 264, 16483–16488

60. Sumegi, B., and Srere, P. A. (1984) Complex I binds several mitochondrial NAD-coupled dehydrogenases. *J. Biol. Chem.* 259, 15040–15045

61. Porpaczy, Z., Sumegi, B., and Alkonyi, I. (1987) Interaction between NAD-dependent isocitrate dehydrogenase, alpha-ketoglutarate dehydrogenase complex, and NADH:ubiquinone oxidoreductase. *J. Biol. Chem.* 262, 9509–9514

62. Wu, H., Xing, K., and Lou, M. F. (2010) Glutaredoxin 2 prevents H(2)O(2)-induced cell apoptosis by protecting complex I activity in the mitochondria. *Biochim. Biophys. Acta.* 1797, 1705–1715

63. Beer, S. M., Taylor, E. R., Brown, S. E., Dahm, C. C., Costa, N. J., Runswick, M. J., and Murphy, M. P. (2004) Glutaredoxin 2 Catalyzes the Reversible Oxidation and Glutathionylation of Mitochondrial Membrane Thiol Proteins. *J. Biol. Chem.* 279, 47939–47951

64. McLain, A. L., Cormier, P. J., Kinter, M., and Szweda, L. I. (2013) Glutathionylation of α-
ketoglutarate dehydrogenase: The chemical nature and relative susceptibility of the cofactor lipoic acid to modification. *Free Radical Biology and Medicine.* 61, 161–169

65. Wieland, O. H. (1983) The mammalian pyruvate dehydrogenase complex: Structure and regulation. In *Reviews of Physiology, Biochemistry and Pharmacology, Volume 96,* pp. 123–170, Reviews of Physiology, Biochemistry and Pharmacology, Springer, Berlin, Heidelberg, Berlin, Heidelberg, 96, 123–170

66. Powers-Greenwood, S. L., Rahmatullah, M., Radke, G. A., and Roche, T. E. (1989) Separation of protein X from the dihydrolipoyl transacetylase component of the mammalian pyruvate dehydrogenase complex and function of protein X. *J. Biol. Chem.* 264, 3655–3657

67. Gopalakrishnan, S., Rahmatullah, M., Radke, G. A., Powers-Greenwood, S., and Roche, T. E. (1989) Role of protein X in the function of the mammalian pyruvate dehydrogenase complex. *Biochemical and Biophysical Research Communications.* 160, 715–721

68. Rahmatullah, M., and Roche, T. E. (1987) The catalytic requirements for reduction and acetylation of protein X and the related regulation of various forms of resolved pyruvate dehydrogenase kinase. *J. Biol. Chem.* 262, 10265–10271

69. Neagle, J., De Marcucci, O., Dunbar, B., and Lindsay, J. G. (2001) Component X of mammalian pyruvate dehydrogenase complex: Structural and functional relationship to the lipoate acetyltransferase (E2) component. *FEBS Lett.* 253, 11–15

70. O’Brien, M., Chalker, J., Slade, L., Gardiner, D., and Mailoux, R. J. (2017) Protein S-glutathionylation alters superoxide/hydrogen peroxide emission from pyruvate dehydrogenase complex. *Free Radical Biology and Medicine.* 106, 302–314

71. Fisher-Wellman, K. H., Gilliam, L. A. A., Lin, C.-T., Cathey, B. L., Lark, D. S., and Neufer, P. D. (2013) Mitochondrial glutathione depletion reveals a novel role for the pyruvate dehydrogenase complex as a key H2O2-emitting source under conditions of nutrient overload. *Free Radical Biology and Medicine.* 65, 1201–1208

72. Mathias, R. A., Greco, T. M., Oberstein, A., Budayeva, H. G., Chakrabarti, R., Rowland, E. A., Kang, Y., Shenk, T., and Cristea, I. M. (2014) Sirtuin 4 is a lipoamidase regulating pyruvate dehydrogenase complex activity. *Cell.* 159, 1615–1625

73. Rowland, E. A., Greco, T. M., Snowden, C. K., McCabe, A. L., Silhavy, T. J., and Cristea, I. M. (2017) Sirtuin Lipoamidase Activity Is Conserved in Bacteria as a Regulator of Metabolic Enzyme Complexes. *MBio.* 8, e01096–17

74. Anderson, K. A., Huynh, F. K., Fisher-Wellman, K., Stuart, J. D., Peterson, B. S., Douros, J. D., Wagner, G. R., Thompson, J. W., Madsen, A. S., Green, M. F., Sivley, R. M., Ilkayeva, O. R., Stevens, R. D., Backos, D. S., Capra, J. A., Olsen, C. A., Campbell, J. E., Muoio, D. M., Grimsrud, P. A., and Hirschy, M. D. (2017) SIRT4 Is a Lysine Decaycle that Controls Leucine Metabolism and Insulin Secretion. *Cell Metabolism.* 25, 838–855.e15

75. Faure, M., Glomot, F., Bledsoe, R., Hutson, S., and Papet, I. (1999) Purification and cloning of the mitochondrial branched-chain amino acid aminotransferase from sheep placenta. *Eur. J. Biochem.* 259, 104–111

76. Wynn, R. M., Kato, M., Machius, M., Chuang, J. L., Li, J., Tomchick, D. R., and Chuang, D. T. (2004) Molecular Mechanism for Regulation of the Human Mitochondrial Branched-Chain α-Ketoacid Dehydrogenase Complex by Phosphorylation. *Structure.* 12, 2185–2196

77. Chang, C.-F., Chou, H.-T., Chuang, J. L., Chuang, D. T., and Huang, T.-H. (2002) Solution structure and dynamics of the lipoic acid-bearing domain of human mitochondrial branched-chain alpha-keto acid dehydrogenase complex. *J. Biol. Chem.* 277, 15865–15873

78. Machius, M., Wynn, R. M., Chuang, J. L., Li, J., Kluger, R., Yu, D., Tomchick, D. R., Brautigam, C. A., and Chuang, D. T. (2006) A versatile conformational switch regulates reactivity in human branched-chain alpha-ketoacid dehydrogenase. *Structure.* 14, 287–298

79. Kato, M., Wynn, R. M., Chuang, J. L., Brautigam, C. A., Custorio, M., and Chuang, D. T. (2006) A synchronized substrate-gating mechanism revealed by cubic-core structure of the bovine branched-chain alpha-ketoacid dehydrogenase complex. *EMBO J.* 25, 5983–5994
Lipoic acid and mitochondrial redox regulation

80. Danhauser, K., Sauer, S. W., Haack, T. B., Wieland, T., Staufner, C., Graf, E., Zschocke, J., Strom, T. M., Traub, T., Okun, J. G., Meitinger, T., Hoffmann, G. F., Prokisch, H., and Kölker, S. (2012) DHTKD1 mutations cause 2-aminoacidic and 2-oxoacidic aciduria. *Am. J. Hum. Genet.* **91**, 1082–1087

81. Tan, M., Peng, C., Anderson, K. A., Chhoy, P., Xie, Z., Dai, L., Park, J., Chen, Y., Huang, H., Zhang, Y., Ro, J., Wagner, G. R., Green, M. F., Madsen, A. S., Schmiesing, J., Peterson, B. S., Xu, G., Ilkayeva, O. R., Muehlbauer, M. J., Braulke, T., Mühlhausen, C., Backos, D. S., Olsen, C. A., McGuire, P. J., Pletcher, S. D., Lombard, D. B., Hirschev, M. D., and Zhao, Y. (2014) Lysine glutarylation is a protein posttranslational modification regulated by SIRT5. *Cell Metabolism.* **19**, 605–617

82. Goncalves, R. L. S., Bunik, V. I., and Brand, M. D. (2016) Production of superoxide/hydrogen peroxide by the mitochondrial 2-oxoadipate dehydrogenase complex. *Free Radical Biology and Medicine.* **91**, 247–255

83. Xu, W., Zhu, H., Gu, M., Luo, Q., Ding, J., Yao, Y., Chen, F., and Wang, Z. (2013) DHTKD1 is essential for mitochondrial biogenesis and function maintenance. *FEBS Lett.* **587**, 3587–3592

84. Soreze, Y., Boutron, A., Habarou, F., Barnerias, C., Nonnenmacher, L., Delpech, H. L. N., Mamoune, A., tien, D. C., Hubert, L., Boles-Feyset, C., Nitschke, P., Correia, I., Sardet, C., Boddart, N., Hamel, Y., Delahodde, A. S., Ottolenghi, C., and de Lonlay, P. (2013) Mutations in human lipoyltransferase gene LIPT1 cause a Leigh disease with secondary deficiency for pyruvate and alpha-ketoglutarate dehydrogenase. *Orphanet Journal of Rare Diseases.* **8**, 1–9

Footnotes

This work was supported by grants to R.J.D. from the N.I.H. (R35 CA220449) and the Howard Hughes Medical Institute (Faculty Scholars Program), and by a gift from the Once Upon a Time Foundation. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

The abbreviations used are: LA, Lipoic acid; FASII, fatty acid synthesis type II; ACP, acyl carrier protein; GCS, glycine cleavage system; PDH, pyruvate dehydrogenase; OGDH, alpha-ketoglutarate dehydrogenase; BCKDH, branched chain ketoacid dehydrogenase; SAM, s-adenosylmethionine; S′-dA, 5′-deoxyadenosyl; TCA, tricarboxylic acid; TPP, thiamine pyrophosphate; DLD, dihydrolipoamide dehydrogenase; DLST, dihydrolipoyl succinyltransferase; Grx2, glutathione reductase 2; PDK, pyruvate dehydrogenase kinase; PDP, pyruvate dehydrogenase phosphatase; SIRT4, sirtuin 4; BCAT, branched chain aminotransferase; DHTKD1, dehydrogenase E1 and transketolase domain containing 1;
Lipoic acid and mitochondrial redox regulation

Figures

FIGURE 1. Structures, enzymes and reaction mechanisms of lipoic acid metabolism. A. The de novo lipoic acid synthesis and salvage pathways in *E. coli*. B. The lipoic acid metabolic pathway in *S. cerevisiae* and *H. sapiens*. C. Orthologous enzymes associated with lipoic acid metabolism in each organism.

FIGURE 2. Reaction mechanisms of mitochondrial 2-ketoacid dehydrogenases. 2-ketoacid dehydrogenase complexes consist of three enzyme subunits that use coupled reactions to decarboxylate a 2-ketoacid substrate and produce a CoA ester. The E1 subunit is a 2-ketoacid decarboxylase that uses a covalently bound thymine pyrophosphate (TPP) cofactor to decarboxylate the 2-ketoacid substrate followed by reductive acylation of a lipoyl moiety on the E2 subunit. The E2 subunit is a dihydrolipoamide acyltransferase that transfers the acyl intermediate from the E1 subunit to CoA generating an acyl-CoA and dihydrolipoamide. The E3 subunit is a dihydrolipoamide dehydrogenase that uses FAD to oxidize lipoyl group on the E2 subunit for subsequent rounds of catalysis and generates NADH through coupled oxidation-reduction reactions of FADH₂ and NAD⁺.

FIGURE 3. Mitochondrial 2-ketoacid dehydrogenases and the tricarboxylic acid (TCA) cycle. Mitochondrial lipoated enzymes individually contribute to pathways that generate products that can participate in the TCA cycle. Inborn errors in these dehydrogenases can be deleterious with clinical symptoms including, developmental delay (PDH and BCKDH), encephalopathy (OGDH), and microcephaly (2-OADH). Deficiencies in these enzymes can accumulate metabolites including, pyruvate and lactate (PDH), alpha-ketoglutarate and 2-hydroxyglutarate (OGDH), branched chain amino acids and their corresponding 2-ketoacids (BCKDH), and 2-oxoadipic acid (2-OADH). Deficiencies in lipoic acid metabolism can phenocopy multiple simultaneous 2-ketoacid dehydrogenase deficiencies, and can limit the incorporation of carbon into the TCA cycle from various sources.

FIGURE 4. Regulation of OGDH by reversible glutathionylation. A. 2-oxoglutarate dehydrogenase (OGDH) interacts with Complex I of the mitochondrial electron transport chain and both complexes can generate ROS. B. When ROS levels increase, both complexes are glutathionylated, which is thought to dissociate the interaction between the two complexes and reversibly inactivate OGDH. The OGDH complex is protected from oxidative damage by thioredoxin (Trx2) and the glutathionylation is regulated by glutathione reductase (Grx2). Glutathionylation of the lipoyl moiety on the E2 subunit of OGDH may allow for ROS scavenging by the E3 subunit through interactions with Grx2 and Trx2.
Figure 1

**A**  
*E. coli* LA Metabolism

| De novo LA synthesis | LA Salvage |
|----------------------|------------|
| octanoyl-ACP         | lipoic acid|

**B**  
Eukaryotic LA Metabolism

| octanoyl-H protein | lipoylated H-protein | lipoylated E2 subunit |

**C**  

| De novo LA synthesis | LA Salvage |
|----------------------|------------|
| **Reaction**         | **B1**     | **B2**     | **S1**     |
| **Enzyme**           | octanoyltransferase | lipoate synthase | lipoate ligase |
| *E. coli*            | LipB        | LipA        | LpIA        |

| Reaction | **1** | **2** | **3** |
|----------|-------|-------|-------|
| **Enzyme** | octanoyltransferase | lipoate synthase | lipoyltransferase |
| *S. cerevisiae* | Lip2     | Lip5    | Lip3   |
| *H. sapiens*    | LIPT2    | LIAS    | LIPT1  |
Figure 2

E1 α-ketoacid dehydrogenase

E2 dihydrolipoamide acetyltransferase

E3 dihydrolipoamide dehydrogenase
Psychomotor retardation, microcephaly
2-aminoadipic acid
2-oxoadipic acid
Leigh Disease, developmental delay, dystonia
Lactate, Pyruvate

Lysine
Tryptophan

2-OADH

Leucine, Isoleucine, Valine
MSUD, developmental delay, intellectual disability, metabolic acidosis

BCKDH
Isoleucine Valine

PDH

Pyruvate

PDH

acetyl-CoA

oxaloacetate
citrate

TCA Cycle

malate

succinate

succinyl CoA

alpha-ketoglutarate

OGDH

Leucine

Encephalopathy
alpha-ketoglutarate, 2-hydroxyglutarate

Figure 3
Figure 4

A

ROS Production by Complex I and OGDH

B

Reversible Inactivation by Glutathioylation
