Lipoic acid metabolism and mitochondrial redox regulation

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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, α-ketoglutarate dehydrogenase, and branched-chain ketoacid dehydrogenase. Lipoic acid also plays a critical role in stabilizing and regulating these multienzyme 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.

Lipoic acid (6,8-dithiooctanoic acid) was first identified by Reed and co-workers (1, 3) and Jukes and co-workers (2) in the 1950s, 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 multienzyme complexes. These functions make lipoic acid essential for cell growth, oxidation of carbohydrates, amino acids, and other fuels, and regulation of 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)[2] metabolism has been intensely investigated by Reed (1), Cronan (4, 5), and others through genetic and bio-

chemical studies using prokaryotes and *Saccharomyces 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 2-ketoacid dehydrogenases. LA metabolism in *Escherichia coli* consists of a *de novo* biosynthetic pathway and a salvage pathway capable of using exogenous LA as a substrate. Both pathways lead to the covalent modification of the ε-amino group on conserved lysine residues within the lipoyl domain of the E2 subunits of 2-ketoacid dehydrogenase complexes (Fig. 1*A*) (4).

The biosynthetic pathway in *E. coli* is initiated by an octanoyltransferase enzyme (octanoyl-(ACP:protein)-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. In the first step of LA biosynthesis, LipB transfers octanoate directly onto the lipoyl domain on the E2 subunit of 2-ketoacid dehydrogenases (Fig. 1*A*, *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 α-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 (Fig. 1*A*, *reaction B2*) (15–17). This step is similar to biotin synthesis in that the reaction mechanism requires sulfur donation from a [4Fe-4S] cluster within the enzyme and reductive cleavage of *S*-adenosylmethionine (SAM) to generate 5′-deoxyadenosyl 5′-radicals 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 originally suggested that LipA may be a self-sacrificing protein rather than performing true enzyme catalysis (5). This had been supported by the small amount of LA generated per molecule of LipA in *E. coli*, which could be enhanced by co-expression of LipA with iron–sulfur
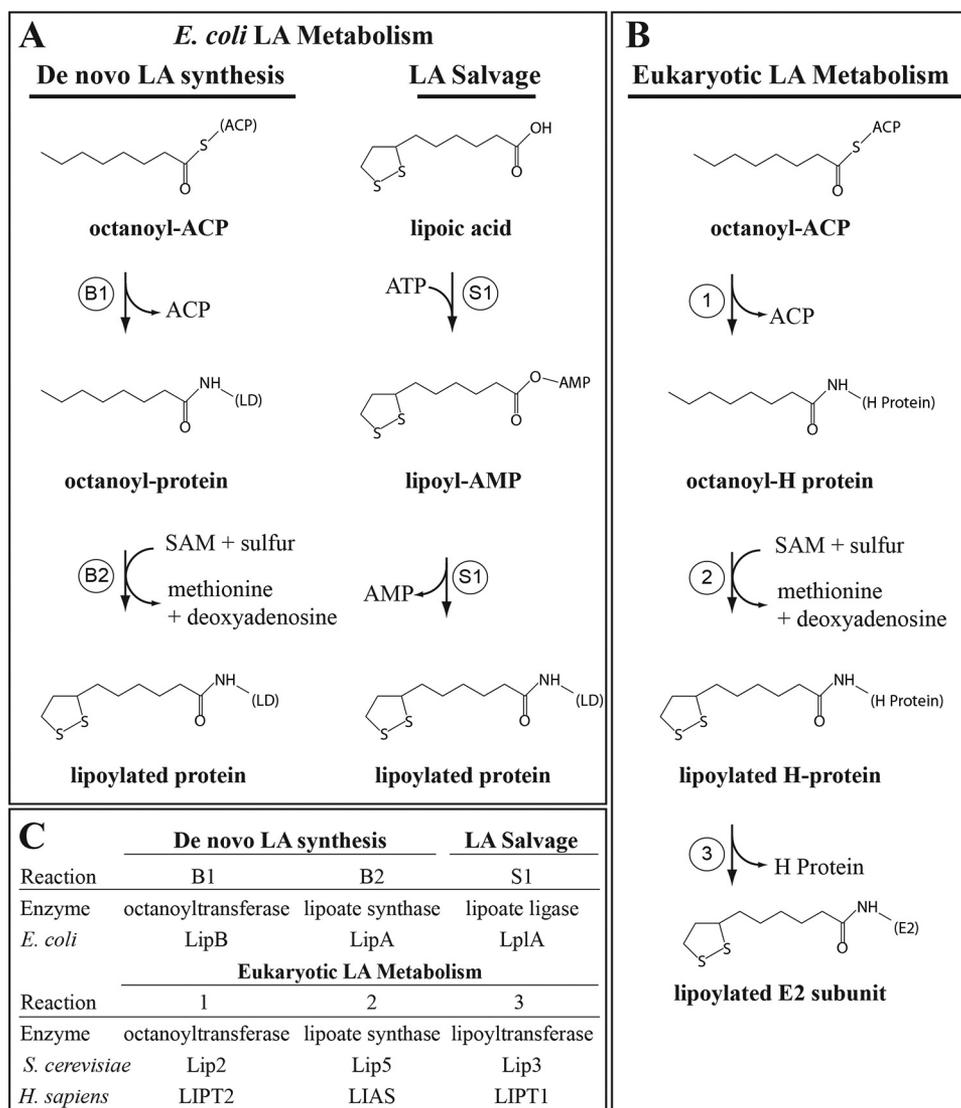
Figure 1. Structures, enzymes, and reaction mechanisms of lipoic acid metabolism. *A, de novo* lipoic acid synthesis and salvage pathways in *E. coli*. *B,* lipoic acid metabolic pathway in *S. cerevisiae* and *Homo sapiens*. *C,* orthologous enzymes associated with lipoic acid metabolism in each organism.

cluster proteins (5, 20). However, recently, the *E. coli* iron–sulfur cluster proteins NfuA and IscU were shown to reinstall the [4Fe-4S] cluster of LipA 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 of E2 subunits and H-protein (Fig. 1*A, 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 results 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) (Fig. 1*B, 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 because 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 (Fig. 1*B, reaction 2*) (28). Yeast cannot tolerate the loss of Lip2 or Lip5 unless grown in medium containing ethanol and succinate, which bypasses Kgd2 in the TCA cycle (6).

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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 (Fig. 1*B, 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 LplA (27). Expression of *E. coli* LplA 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 LplA 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 because expression of yeast Lip3 in *E. coli* Δ*lipB* Δ*lplA* allows for growth in the presence of octanoate but not lipoate. Furthermore, in octanoate-containing media, *E. coli* Δ*lipB* Δ*lplA* expressing Lip3 has 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 (Fig. 1*C*) (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). LIPT1, the lipoyltransferase ortholog in mammals, is similar to LIP3 in *S. cerevisiae* in that it lacks the ability to generate an activated lipoyl-AMP intermediate 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
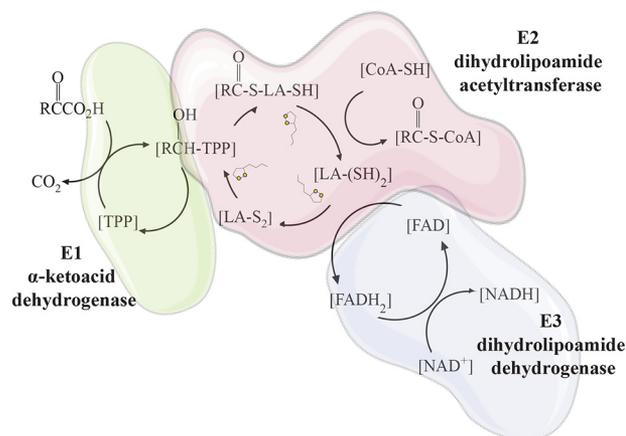

Figure 2. Reaction mechanisms of mitochondrial 2-ketoacid dehydrogenases and redox-dependent regulation. 2-Ketoacid dehydrogenase complexes consist of three enzyme subunits that use coupled reactions to decarboxylate a 2-ketoacid substrate and produce a CoA ester. The E1 subunit is a 2-ketoacid decarboxylase 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 the lipoyl group on the E2 subunit for subsequent rounds of catalysis and generates NADH through coupled oxidation–reduction reactions of $FADH_2$ and NAD^+.

LIPT2 transfers octanoate 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. It is unclear at present whether LIPT1 is an octanoyltransferase, a lipoyltransferase, or both, as its true *in vivo* substrate remains to be clarified.

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 GCS. The 2-ketoacid dehydrogenases include pyruvate dehydrogenase (PDH), α-ketoglutarate dehydrogenase (OGDH), branched-chain ketoacid dehydrogenase (BCKDH), and 2-oxoadipate dehydrogenase (OADH) (30, 39). These multienzyme complexes are composed of three independent subunits that undergo coupled reactions facilitated by the LA cofactor (Fig. 2) (40). The E1 subunit, which provides substrate specificity to the multienzyme 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 $FADH_2$ back to FAD using NAD^+ producing NADH in the process (40) (Fig. 2*A*). 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
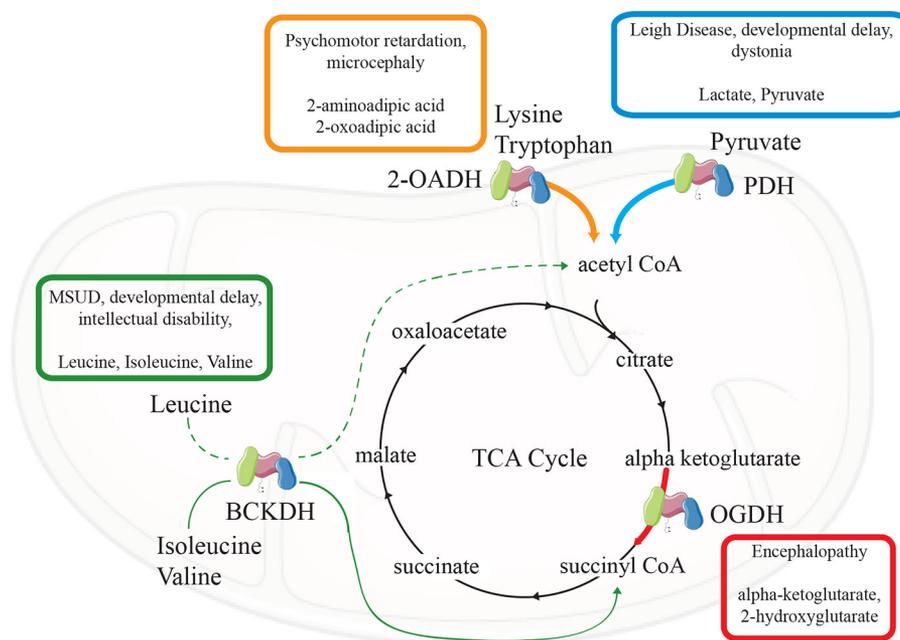
Figure 3. Mitochondrial 2-ketoacid dehydrogenases and the TCA cycle. Mitochondrial lipoylated enzymes individually contribute to pathways that generate products that can participate in the TCA cycle. Inborn errors in these dehydrogenases can be deleterious, with clinical symptoms including developmental delay (*PDH* and *BCKDH*), encephalopathy (*OGDH*), and microcephaly (*2-OADH*). Deficiencies in these enzymes can accumulate metabolites, including pyruvate and lactate (*PDH*), α-ketoglutarate and 2-hydroxyglutarate, branched-chain amino acids and their corresponding 2-ketoacids, and 2-oxoadipic acid. 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.

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 (Fig. 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-α1 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).

α-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 α-ketoglutarate levels may also be associated with an elevation in 2-hydroxyglutarate, a potential epigenetic regulator. It is currently unknown whether 2-hydroxyglutarate levels contribute to pathology in α-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. 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 maple syrup urine disease involves titrating dietary branched-chain amino acid 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 DLD, LIAS, LIPT2, and LIPT1. These disorders have overlapping phenotypes, including lactic acidosis, developmental delay, and seizures. Collectively, dysfunction in these enzymes falls into a larger class of mitochondrial disease known as Leigh syndrome. LIAS patients may be distinguished from LIPT1 patients through presentation of nonketotic 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.

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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 lipoyl residue generating a thioyl radical within the core complex. This can lead to inactivation of the complex due to reactions between a thioyl radical on the E2 subunit and the catalytic region of the E1 subunit (48). This can been seen as a regulatory inactivation to inhibit the catabolism of specific substrates, like pyruvate or α-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 whether the E3 subunit dissociates from the complex for this function.

α-Ketoglutarate dehydrogenase

The OGDH complex facilitates the decarboxylation of α-ketoglutarate (2-oxoglutarate) to form succinyl-CoA and NADH. This complex in mammalian systems is distinct in that the E2 core subunit (dihydrolipoyl succinyltransferase) 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_2O_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 α-ketoglutarate and free CoA (55). Additionally, the lipoyl 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 glutathione modification can be removed through the actions of glutathione reductase 2 (Grx2) (58). Although glutathionylation of the lipoyl 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 lipoyl 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
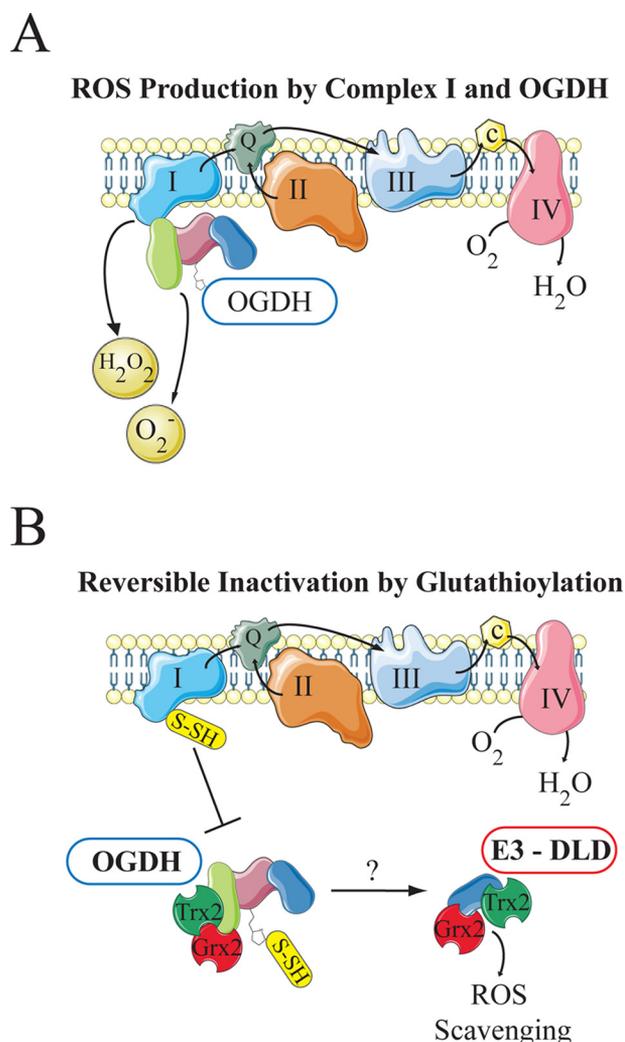
A

ROS Production by Complex I and OGDH

B

Reversible Inactivation by Glutathioylation

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.

shown to interact with complex I of the electron transport chain, which allows for direct supply of NADH to the NADH-oxidation site of complex I (Fig. 4A) (59–61). Because 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 electron transport chain until redox homeostasis is reached (Fig. 4B) (64). This is particularly important because 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 lipoyl domain. As well, the PDH complex has stable interactions with PDK and PDP (66, 67). The E3-binding protein lipoyl 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_2O_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 because $FADH_2$ cannot be oxidized by the lipoic acid moiety and reduces O_2 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 immunoaffinity 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, which catalyzes the breakdown of valine, leucine, and isoleucine to the ketoacids, 3-methyl-2-oxobytanoate (KIV), 4-methyl-2-oxopentanoate (KIC), and (*S*)-3-methyl-2-oxopentanoate (KMV), respectively (Fig. 2*B*) (75). BCKDH then decarboxylates these ketoacids releasing CO_2 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 reac-

tion. 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 H_2O_2 to a lesser degree than OGDH and PDH (55). The overall details of redox regulation of BCKDH are less clear compared with 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 leads 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. Thus, dysfunction in this enzyme could impact mitochondrial function through mechanisms independent of 2-OA *per se* (81). 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, α-ketoglutarate. It is unclear whether DHTKD1 is associated with an independent dehydrogenase complex or whether it shares activity with OGDH and which enzyme deficiency may be causative in 2-oxoadipate aciduria. 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 because 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 investi-

gation of how LA metabolism is regulated and functions in humans will be necessary to treat inborn errors of this pathway.

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