A Missing Link to Vitamin B_{12} Metabolism

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Nearly 3% of the human population carries bi-allelic loss-of-function variants in the gene encoding CLYBL. While largely healthy, these individuals exhibit reduced circulating vitamin B_{12} levels. In this issue of *Cell*, Shen and colleagues uncover the metabolic role of CLYBL, linking its function to B_{12} metabolism and the immunomodulatory metabolite, itaconate.

Polymorphic loss-of-function human gene variants (i.e., human gene knockouts) can lend critical insights into gene function and human evolution (Narasimhan et al., 2016). One such gene is citrate lyase subunit beta-like (*CLYBL*), which encodes a ubiquitously expressed mitochondrial enzyme. Individuals with homozygous *CLYBL* mutations resulting in premature stop codon placement and loss of gene function are consistently found to have reduced levels of circulating vitamin B_{12}, but are otherwise healthy. The function of CLYBL in humans is obscure, and how CLYBL contributes to B_{12} deficiency is unknown. In this issue of *Cell*, Shen and colleagues now report that CLYBL functions as a citramalyl-CoA lyase (Shen et al., 2017). The authors demonstrate a direct connection between CLYBL activity and vitamin B_{12} levels that is dependent on catabolism of itaconate, a recently identified antimicrobial and immunomodulatory metabolite. These findings establish a biochemical role for CLYBL in humans and raise intriguing questions about the regulation of vitamin B_{12} metabolism and its interplay with the numerous aspects of physiology and pathophysiology, particularly in the immune system.

Using an array of approaches including X-ray crystallography and metabolomics, the authors elegantly demonstrate CLYBL to have robust citramalyl-CoA lyase activity. Consistently, it was found that the CLYBL substrate citramalyl-CoA accumulated in *Clybl*-deficient murine cells, establishing that the newly discovered CLYBL enzymatic activity occurs in mammalian cells. The authors further found that CLYBL functions in the C5-dicarboxylate mitochondrial metabolic pathway (Figure 1) as citramalyl-CoA can be generated from itaconate, a metabolite known to be produced in macrophages. Further experiments using metabolomics in *Clybl* wild-type and knockout cells revealed that the intermediate in the pathway, itaconyl-CoA, inhibits methylmalonyl-CoA mutase (MUT) by converting the coenzyme form of B_{12} to the inactive form, which is the mechanism leading to reduced B_{12} levels in individuals lacking CLYBL. Finally, the authors demonstrated that activated macrophages endogenously produce sufficient amounts of itaconyl-CoA to cause depletion of B_{12} levels. In all, Shen and colleagues provide conclusive evidence that CLYBL functions in the C5-dicarboxylate pathway to drive itaconate catabolism via citramalyl-CoA lyase activity and in the absence of CLYBL, itaconyl-CoA accumulates, reduces MUT activity, and causes coenzyme B_{12} degradation (Figure 1).

This study raises interesting questions about the biology of vitamin B_{12} metabolism, and this definitive biochemical mechanism should provide important insights into a fundamental intersection of human nutrition and physiology. Previous genetic studies have indicated that B_{12} deficiencies can result from reduced absorption from the gut or dysregulated cellular transport (Grarup et al., 2013). Shen et al. now demonstrate a cell autonomous mechanism for B_{12} reduction that requires the presence of itaconate. It is conceivable that macrophages in contact with the commensal microflora in the gut may have a significant impact on B_{12} absorption by enterocytes. For example, B_{12} deficiency in the elderly is commonly associated with gut inflammation (Baik and Russell, 1999), a finding that could potentially be explained by the newly described pathway.

A remaining question is how CLYBL deficiency can lead to systemic reduction in B_{12} levels, and therefore how this biochemical pathway may interact with diet, gut microbial composition, digestion, and the immune compartment to produce biological phenotypes. For example, are CLYBL-deficient humans susceptible to diet-induced B_{12} deficiency, and if so, is it coupled to inflammation? To date, itaconate appears primarily associated with macrophage activity, and the authors note that they did not detect high levels of itaconate in *Clybl* knockout cells. One possibility is the presence of other cellular sources of citramalyl-CoA that are then converted to itaconyl-CoA in the absence of CLYBL citramalyl-CoA lyase activity to cause B_{12} degradation. Further studies are needed to elucidate cell-type specific sources of citramalyl-CoA and whether the same mechanism of B_{12} reduction occurs in these contexts.

Finally, this landmark study has important implications for understanding human health and disease. B_{12} deficiency commonly manifests in anemia and neuropathy, but the mechanistic and regulatory links between these conditions and CLYBL deficiency are unknown. The B_{12} regulation described in the current study may have important implications for other metabolic pathways and pathophysiological contexts. Serine, glycine, and one-carbon (SGOC) metabolism, which requires B_{12} to couple the methionine and folate cycles, is important for nucleotide biosynthesis, redox homeostasis, and methylation reactions (Locasale, 2013). Thus, there exists the possibility in some

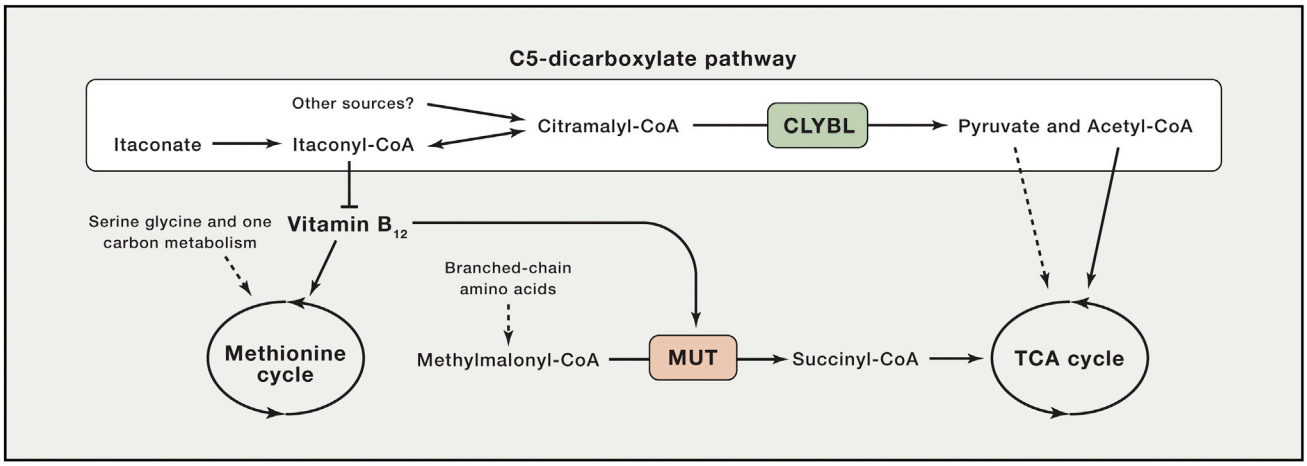

Figure 1. A Novel Pathway Linked to Vitamin B_{12} Metabolism

Itaconate, a recently described immunomodulatory metabolite, is converted to itaconyl-CoA and citramalyl-CoA in mitochondria. Citramalyl-CoA is then catabolized by citrate lyase subunit beta-like (CLYBL) to produce pyruvate and acetyl-CoA and fuel the tricarboxylic acid (TCA) cycle (C5-dicarboxylate pathway; white box). When CLYBL is absent, a condition that occurs in roughly 3% of the human population, itaconyl-CoA accumulates, leading to inactivation of vitamin B_{12} and subsequent methylmalonyl-CoA mutase (MUT) inhibition. The inactivation of vitamin B_{12} may have broad consequences such as reduced serine, glycine, one-carbon and folate metabolism through inhibition of the methionine cycle and inhibition of branched-chain amino acid catabolism in the TCA cycle. Solid arrows represent direct biochemical actions. Dotted arrows represent indirect biochemical actions.

contexts for inhibition of methionine synthase by B_{12} degradation. Intriguingly, recent reports indicate dysfunctional mitochondria contribute to defects in SGOC metabolism (Bao et al., 2016; Nikkanen et al., 2016). It will be interesting to explore the effects of CLYBL status and itaconate metabolism on the SGOC network. Clinically, diabetics treated with metformin, a compound that targets the mitochondria (Liu et al., 2016), are susceptible to B_{12}-deficiency (de Jager et al., 2010). One possible explanation is that disruption of normal mitochondrial function may impact the C5-dicarboxylate metabolic network through the mechanism described by Shen et al. In addition, the levels of *S*-adenosylmethionine from B_{12}-coupled methionine and folate cycles affect key histone methylation marks (Mentch et al., 2015). Thus, this novel mechanism may also provide a further link to gene expression changes through epigenetic alterations. Finally, knowledge of endogenous metabolite itaconyl-coA as an inhibitor of MUT may promote the development of small molecule-based rescue strategies of B_{12} degradation, which might help a subset of patients with B_{12} deficiency-induced neuropathy and anemia. In light of these numerous areas of future study, the biochemistry of a human knockout gene demonstrates the power of human genetics to open new areas of biology and places vitamin B_{12} metabolism at the interface of immune and nutritional physiology.

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