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An alternative to the glyoxylate shunt

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Summary

A cycle remains a cycle only as long as the spokes of the wheel are not stolen. To keep the citric acid cycle going requires anaplerotic reactions such as the glyoxylate shunt to restore the cycle intermediates that are withdrawn for the biosynthesis of cell constituents, e.g. amino acids and haemin precursors. The article by Erb et al. in this issue of Molecular Microbiology documents an alternative path that replenishes four-carbon intermediates during growth on acetate in the absence of the glyoxylate shunt. The reaction sequence forms malate and succinyl-CoA from three acetyl-CoA, one CO2 and one HCO3 in a linear pathway. This new pathway was discovered in phototrophic anoxygenic bacteria and in few aerobic bacteria, but it is probably widespread among many metabolic groups of bacteria.

The citric acid cycle, discovered by Krebs and Johnson (1937), is one of the central roundabouts in the biochemistry of substrate degradation, catalysing the terminal oxidation of acetyl residues to two CO2 and releasing four pairs of electrons (Thauer, 1988). Nonetheless, this reaction scheme became a cycle only comparably late in biochemical evolution, after the invention of photosynthesis and subsequent respiration of organic matter with sulphate, oxygen or nitrate as electron acceptor. In the early phase of biochemistry, i.e. in the metabolism of fermenting bacteria, most reactions of this cycle already existed to provide precursors for the synthesis of biomass. At that time, the cycle was an open fork rather than a cycle, leading in one branch via citrate to 2-oxoglutarate and subsequently to the biosynthesis of amino acids of the glutamate family, and in the other branch, after carboxylation of pyruvate to oxaloacetate, to malate, fumarate, succinate and succinyl-CoA, leading to the biosynthesis of amino acids in the aspartate family and of haemins for synthesis of cytochromes etc. Closing this open-fork structure by 2-oxoglutarate dehydrogenase allowed the complete oxidation of acetyl residues in a cycle, but the biosynthetic functions of parts of this cycle still persisted, draining off reaction intermediates that would be required to keep the cycle going. To overcome this deficit, the glyoxylate shunt, discovered again by Kornberg and Krebs (1957), operates as a shortcut from isocitrate to succinate and glyoxylate, to form additional oxaloacetate by condensation with acetyl-CoA to malate.

A special problem arises if bacteria grow on acetate or other substrates that are degraded exclusively via acetyl residues: unless these bacteria contain a reversible, ferredoxin-dependent pyruvate:ferredoxin oxidoreductase (as many strict anaerobes do) pyruvate has to be formed from oxaloacetate, which again has to be drawn from the cycle and has to be replenished through the glyoxylate shunt. Thus, the glyoxylate cycle is an indispensable constituent of the citric acid cycle, especially during growth with acetate or substrates that are degraded via acetyl residues.

Nonetheless, there are numerous acetate-metabolizing bacteria that lack isocitrate lyase, the key enzyme of the glyoxylate shunt. Several acetate-degrading anoxygenic phototrophs and a few strict aerobes, such as Methylobacterium extorquens and some streptomycetes, lack this enzyme system. The question of how these bacteria can keep the citric acid cycle going remained open for several decades, and has been solved only now by the work of Tobias Erb, Georg Fuchs and Birgit Alber, with the final experimental evidence presented in this issue. These authors discovered an entirely new pathway for the replenishment of malate and succinyl-CoA from three acetyl-CoA, one bicarbonate and one CO2, which includes a series of specific reactions that were discovered by a creative combination of molecular biological and biochemical studies (Fig. 1). In this sequence, two acetyl-CoA are condensed to acetoacetyl-CoA, reduced to 3-hydroxybutyryl-CoA and dehydrated to form crotonyl-CoA. The latter is carboxylated in a reductive reaction step

that is entirely novel in biochemistry, to form ethylmalonyl-CoA (Erb et al., 2009). This compound is isomerized from the S to the R enantiomer, and rearranged in a coenzyme B12-dependent reaction to form methylsuccinyl-CoA. Dehydrogenation by methylsuccinyl-CoA dehydrogenase leads to mesaconyl-CoA, which, after hydration to methylmalyl-CoA, can be cleaved to glyoxylate and propionyl-CoA. Further condensation of glyoxylate with acetyl-CoA leads to malate, and carboxylation of propionyl-CoA via methylmalonyl-CoA to succinyl-CoA; malate and succinyl-CoA again find their way into the citric acid cycle. The present paper completes this story, which the same authors started in a series of preceding papers (Alber et al., 2006; Erb et al., 2007; 2008; 2009).

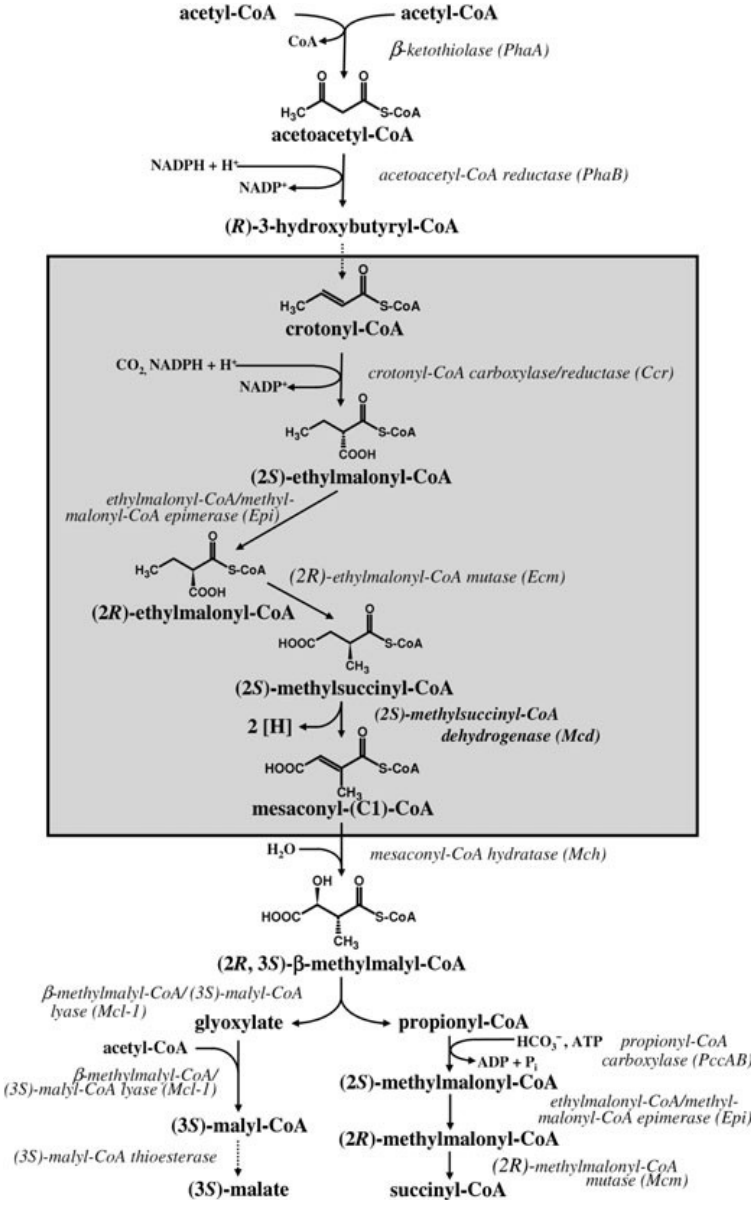

Fig. 1. The ethylmalonyl-CoA pathway as suggested by Erb *et al.* (2009).

The fascination of this study derives from the elegant combination of classical hard-core biochemistry and molecular biological techniques. Who wants to synthesize a radioactively labelled substrate for an enzyme assay in a series of biochemical reactions catalysed by enzymes that first have to be produced in recombinant bacteria and then purified?

Physiological experiments with a methylsuccinyl-CoA dehydrogenase-negative mutant, purification of the key enzyme and its spectroscopic characterization, and finally sequence comparison with other acyl-CoA dehydrogenases add to the diversity of methods used in this study, which establishes a new anaplerotic sequence to the citric acid cycle that is essential for acetate utilization not only in phototrophs but probably in many other aerobic bacteria.

The discovery of the new anaplerotic sequence via ethylmalonyl-CoA adds another example to the fascinating metabolic diversity of prokaryotes. Another example is the fact that until the late 1970s, there was only one known pathway for autotrophic CO2 fixation known in biochemistry, the Calvin–Bassham cycle that is used by green plants and many bacteria. Today, we know of a further five pathways for autotrophic CO2 fixation that were found preferentially in anaerobic bacteria and in Archaea. The group of Georg Fuchs has made major contributions to nearly all of these discoveries. One alternative pathway, by the way, is a reversal of the citric acid cycle that, in this function, synthesizes an acetyl residue from two CO2 and four electron pairs, and is used by anoxygenic green phototrophs and few sulphate-reducing bacteria. To turn the citric acid cycle backwards requires a few modifications in the cycle to circumvent irreversible reaction steps. Another pathway of autotrophic CO2 fixation, the CO-dehydrogenase pathway (Wood–Ljungdahl pathway), also represents an alternative pathway for acetate oxidation that does not interfere with the intermediates of the citric acid cycle (Thauer, 1988). This astonishing multitude of biochemical alternatives, especially in the biochemistry of anaerobes, was discovered only in the last 30 years and documents how many different biochemical options were played through in the early evolution of life and were maintained in different branches of the tree of life, sometimes with rather different functions.

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