

Chris Anthony

HOME

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MUSIC

FAVOURITES shopping, friends, DIY, entertainment, Southampton etc

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Solution of the problem of the incomplete Serine Cycle

NOTE: I have published a review of this in Science Progress. It is in press.

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Figures illustrating this work are placed beneath this summary, followed by References to the original literature.

The Serine cycle was proposed by Quayle and colleagues [especially David Peel and Peter Large] as the route for methanol assimilation in the pink facultative methylotroph *Methylobacterium extorquens* [Refs 1-4]. An unsolved part of the pathway required acetyl-CoA to be oxidised to glyoxylate. In some bacteria this is achieved by the Glyoxylate cycle; this is the main route for bacterial assimilation of acetate or ethanol, first proposed by Hans Kornberg.

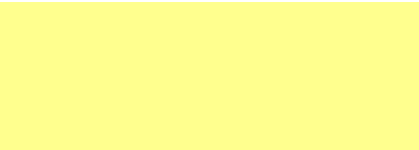
The key enzyme for this pathway [isocitrate lyase] is absent from *M. extorquens* during growth on methanol or ethanol. Pat Dunstan [now Pat Goodwin] in my lab showed that in this organism there is an alternative route for oxidation of acetyl-CoA to glyoxylate which operates during growth on both C1 and C2 compounds [Refs 5-7]. This was achieved by characterisation of mutants unable to grow on these compounds and by short-term C14 labelling experiments of the sort that were used to conclusively confirm the Calvin cycle, the glyoxylate cycle and the serine cycle. The key mutants that first showed that there is a route for oxidation of acetyl-coA to glyoxylate that is essential for growth on C1 and C2 compounds [eg mutant PCT48] were later shown to lack a novel acyl-CoA mutase [encoded by *meaA*; Refs 11,15]. Refs 8-10 cover the work on assimilation of C3 compounds that also use this pathway; this is described in more detail on a separate page [[click here](#)] and it is also summarised in [The Biochemistry of Methylotrophs](#)].

The nature of the pathway for regeneration of glyoxylate from acetyl-coA was eventually determined by Mary Lidstrom, Mila Chistersodova and their colleagues [Refs 12, 13]. Their Glyoxylate Regeneration Cycle [GRC] proposed a large number of intermediates [coenzyme A esters] and novel reactions. This 'long' pathway [in my opinion] did not fit our observations of rapid incorporation of label from radioactive acetate into glycine in methanol-grown cells, but clearly the basic principle of the pathway was likely to be correct.

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The solution to the pathway [with many features in common with the GRC] has been achieved by the group of Georg Fuchs in Freiburg by way of their study of the growth of *Rhodobacter sphaeroides* on acetate; this organism also lacks a glyoxylate cycle [Refs 14, 15]. Key features of the pathway are two key enzymes. These enzymes are:

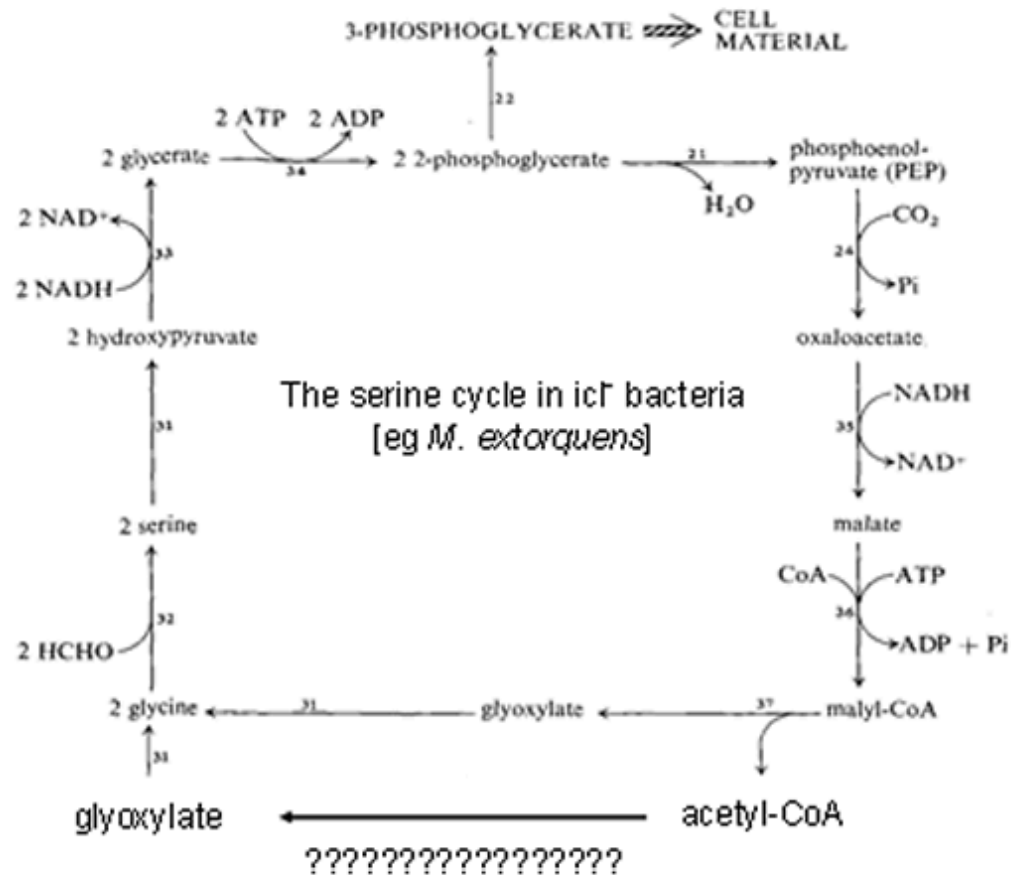
1. Crotonyl-CoA carboxylase/reductase, catalyzing a reductive carboxylation of crotonyl-CoA to ethylmalonyl-CoA using NADPH.

2. Ethylmalonyl-CoA Mutase: a New coenzyme B12-dependent Acyl-CoA Mutase that catalyses the transformation of ethylmalonyl-CoA to methylsuccinyl-CoA.

A third enzyme essential for the conversion of crotonyl-coA to methylsuccinyl-CoA is a non-specific epimerase, converting (2S)-ethylmalonyl-coA to (2R)-ethylmalonyl-coA, the substrate for the mutase.

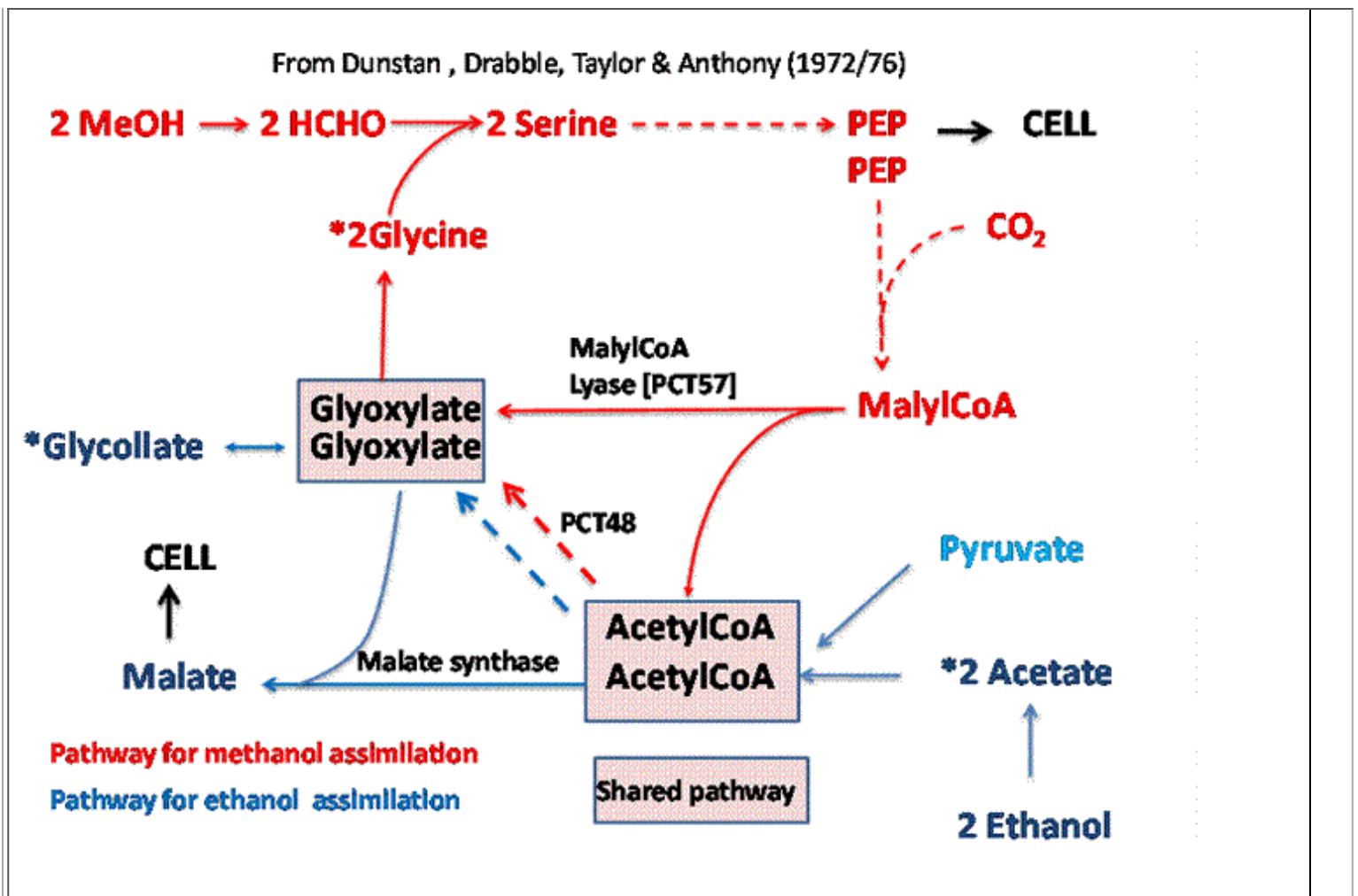
These enzymes also operate during growth of *M. extorquens* on methanol [Ref 14], and the pathway has been further confirmed by Julia Vorholts group in Zurich in a remarkable series of C13 labelling experiments where kinetic isotopomer profiles collected by Liquid chromatography-high resolution mass spectrometry during short term incubation experiments were combined with steady-state isotopomer distributions measured by NMR [Ref 16].

The Serine cycle Taken from the Biochemistry of Methylotrophs.
[click here](#) for complete book



Summary of proposals of Dunstan & Anthony showing a shared pathway for C1 and C2 assimilation [Refs 5-8].

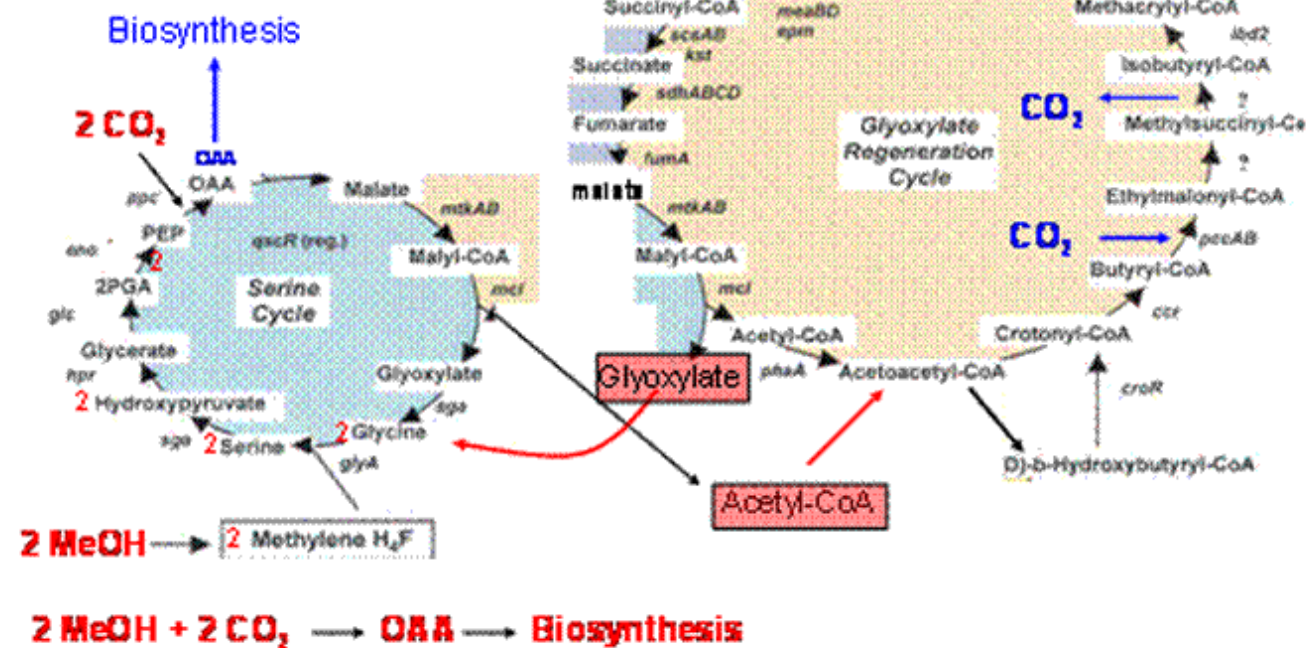
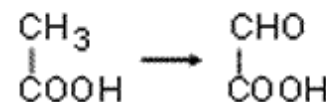
The mutants of the PCT48 type [*meaA*; Ref 11] were later shown to lack a novel mutase, subsequently identified as ethylmalonylCoA mutase [Ref 15].



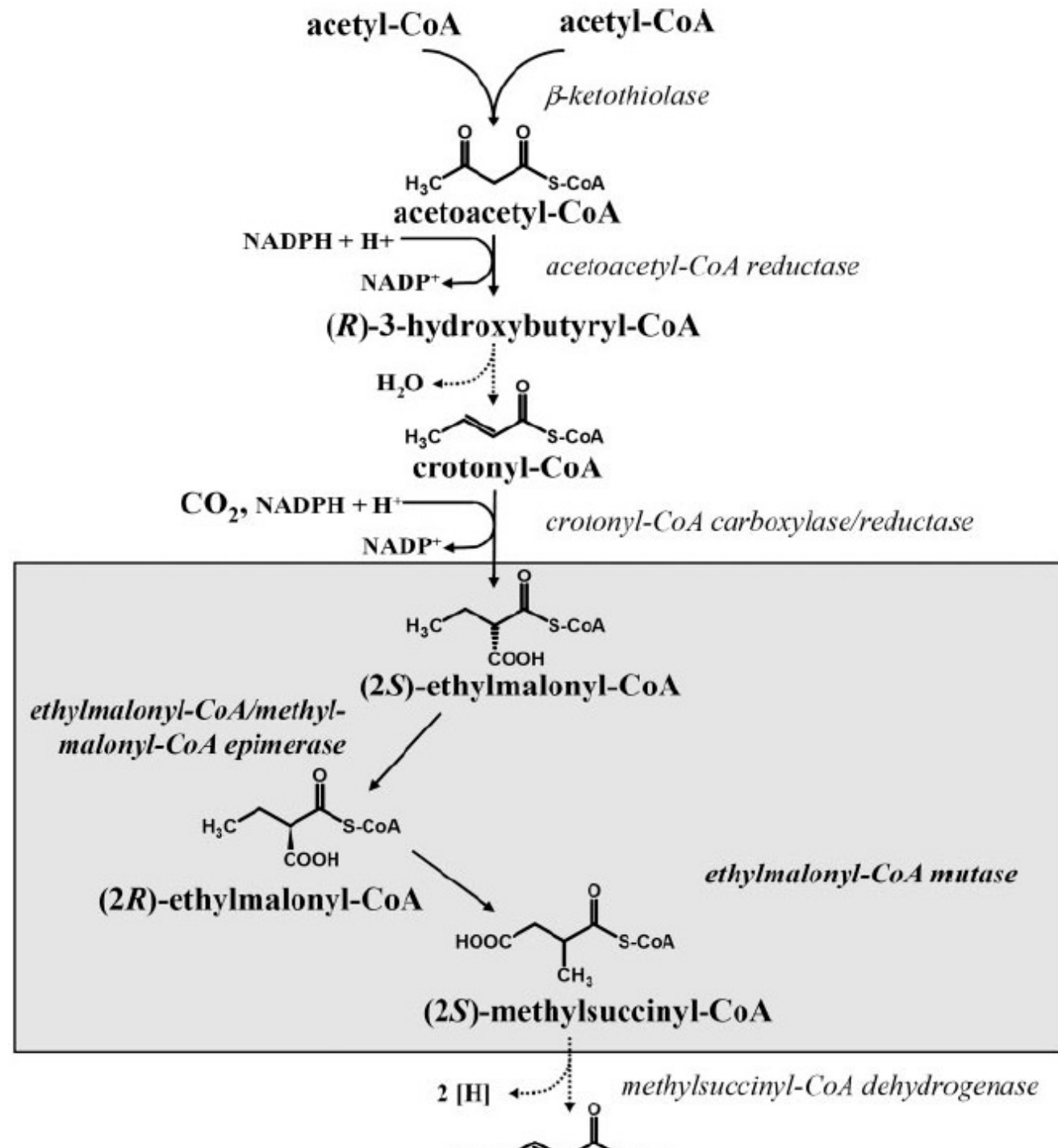
The Glyoxylate Regeneration Cycle proposed by Chistoserdova, Lidstrom & colleagues [Refs 12, 13]. I have modified this slightly for clarity.

Pathway based on Chistoserdova,
Lidstrom and colleagues

18 steps between acetyl-CoA and glyoxylate; how does ^{14}C -acetate glyoxylate so rapidly?



The EthylmalonylCoA pathway for growth of *R. sphaeroides* on C2 compounds [Refs 14, 15]



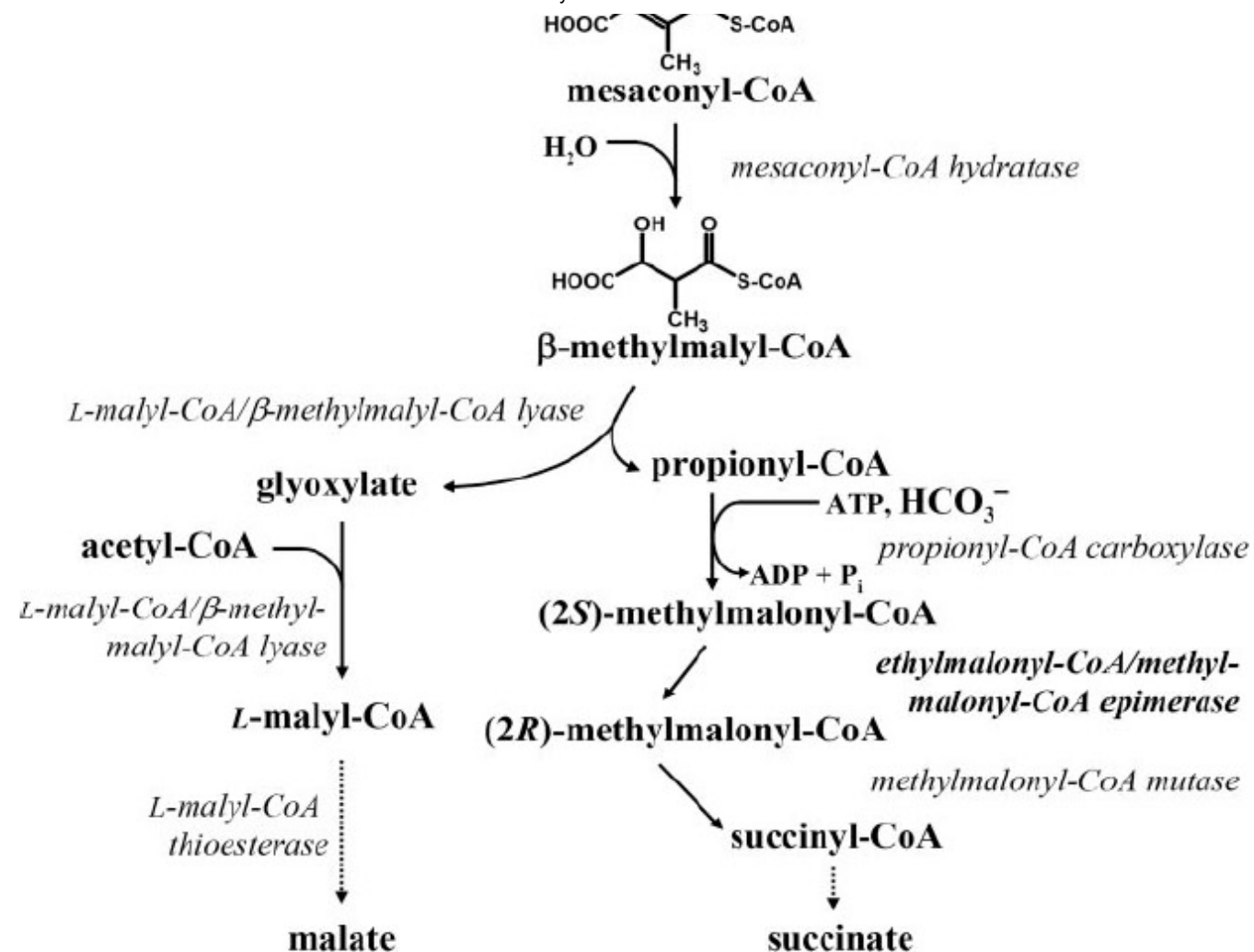
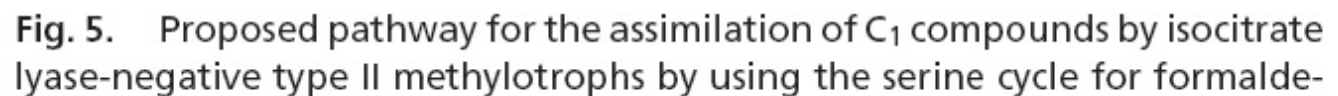


FIGURE 1. The ethylmalonyl-CoA pathway for acetyl-CoA assimilation as proposed for the phototrophic bacterium *R. sphaeroides*. The conversion of the C₄ compound crotonyl-CoA to the C₅ compound β -methylmalyl-CoA involves unique enzymes: crotonyl-CoA carboxylase/reductase (4), mesaconyl-CoA hydratase (3, 5), and a proposed enzyme catalyzing the oxidation of methylsuccinyl-CoA to mesaconyl-CoA (methylsuccinyl-CoA dehydrogenase). Ethylmalonyl-CoA mutase and epimerase are described in this work and catalyze the conversion of ethylmalonyl-CoA to methylsuccinyl-CoA (*highlighted*). The epimerase is a promiscuous enzyme and is also involved in the conversion of propionyl-CoA to succinyl-CoA later in the pathway.

The EthylmalonylCoA pathway for growth on C1 compounds [Ref 14]



hyde fixation. The ethylmalonyl-CoA pathway described here (excluding the condensation of acetyl-CoA and glyoxylate) is integrated in the serine cycle (upper part) and is involved in assimilation of acetyl-CoA and regeneration of glyoxylate during growth on C₁ compounds. It is assumed that during growth on C₂ compounds the ethylmalonyl-CoA pathway (Fig. 4) is used exclusively. Dotted lines indicate more than one reaction step.

The EthylmalonylCoA pathway for growth on methanol [adapted from Ref 14]

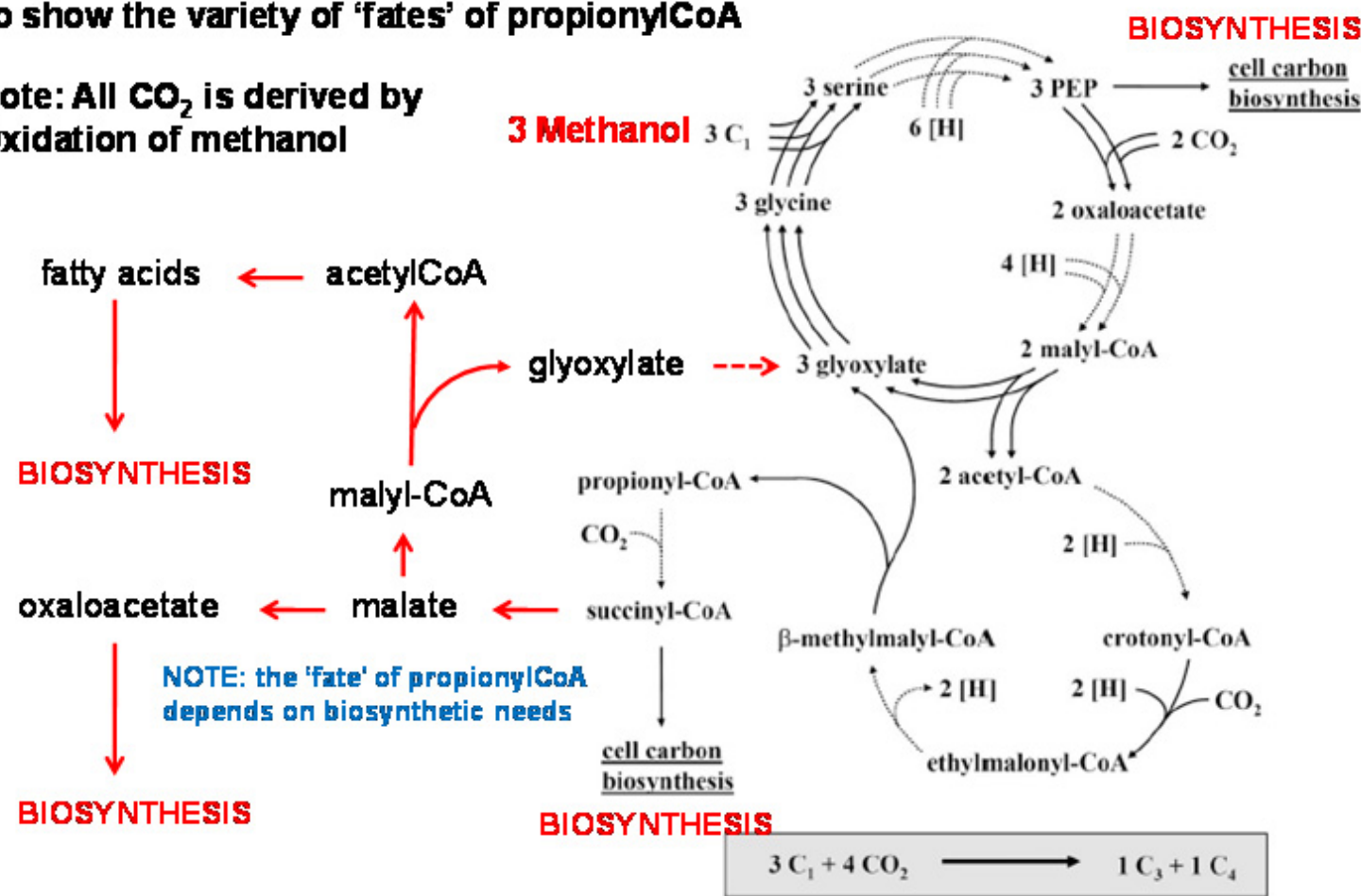
The ethylmalonyl-CoA pathway

For assimilation of C1 compounds

I have modified this slightly from original to show the variety of 'fates' of propionylCoA

Note: All CO₂ is derived by Oxidation of methanol

3 Methanol



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HOME

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THANKS for help to [Hugh Anthony](#) and [Carl Gore](#)

