

Solution of the Serine pathway in *Methylobacterium extorquens* (50 year project)

Situation in 1963 There must be a route for oxidation of acetylCoA to glyoxylate. An obvious route is to use the glyoxylate cycle but the key enzyme isocitrate is absent during growth on methanol (Large and Quayle 1963).

1970 Pat Dunstan (now Pat Goodwin). Showed that ICL is absent during growth on ethanol

Isolation of unusual mutants: A project to isolate MDH mutants. Penicillin enrichment procedure isolated mutants unable to grow on C1 or C2 but able to grow on succinate. These should be unable to oxidise methanol and ethanol. No mutants in assimilation pathways should be selected as these pathways are different.

Three types of mutant were isolated: MDH mutants. Cytochrome c mutants [unique, indicating the special role of cytochrome c in energy transduction from MDH]. A mutants with alteration in carbon assimilation pathways (PCT48) on C1 and C2 compounds showing there must be a common step in the pathway.

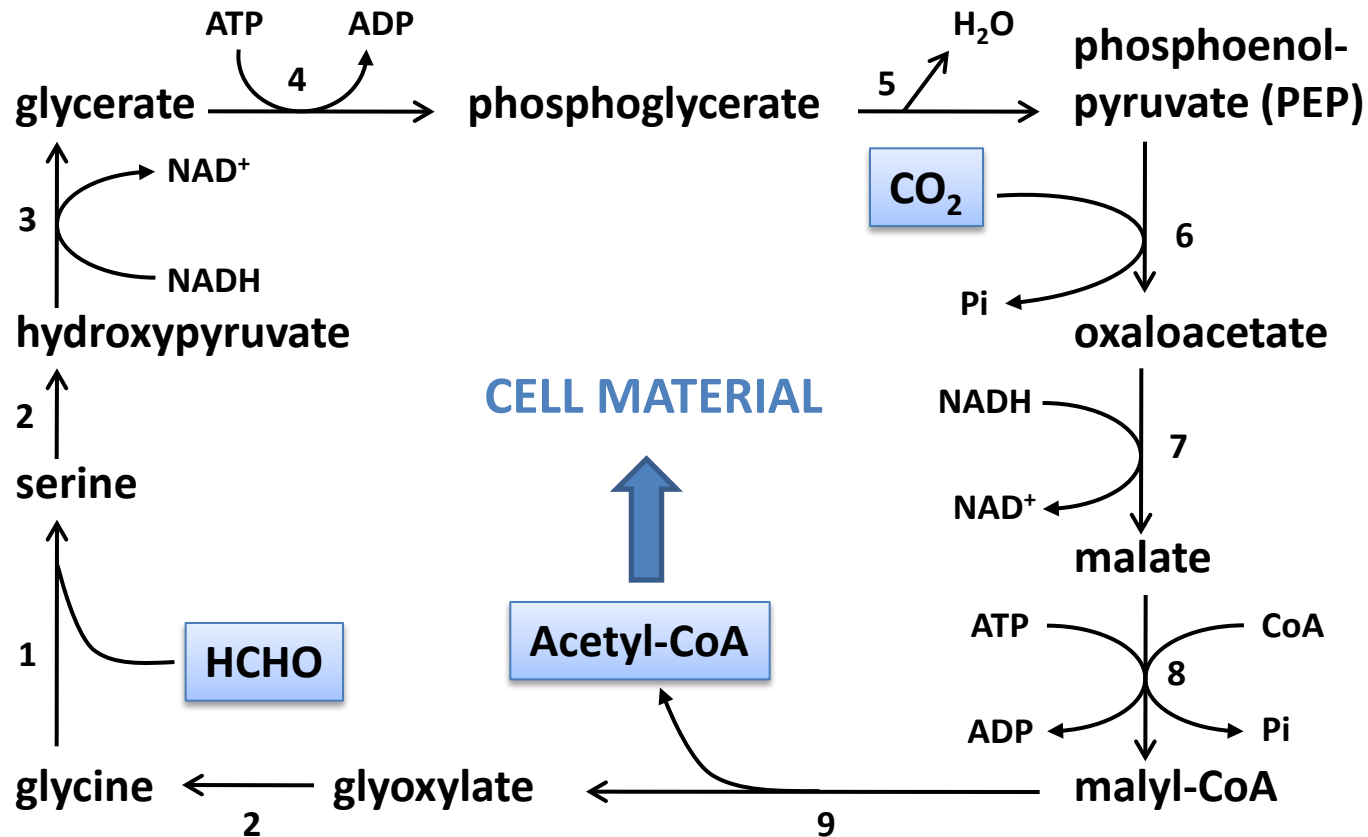


Figure 3. The serine cycle as proposed by Peel, Large, Salem and Quayle⁹⁻¹¹. The enzymes are 1, serine transhydroxymethylase; 2, serine-glyoxylate aminotransferase; 3, hydroxypyruvate reductase; 4, glycerate kinase; 5, enolase; 6, PEP carboxylase; 7, malate dehydrogenase; 8, malate thiokinase; 9, malyl-CoA lyase. After the initial proposal much further enzymological and mutant evidence was subsequently accumulated to confirm this pathway³. Note that during biosynthesis of fatty acids and poly 3-hydroxybutyrate which use acetyl-CoA as their biosynthetic starting point, this pathway is sufficient for production of acetyl-CoA from formaldehyde plus carbon dioxide.

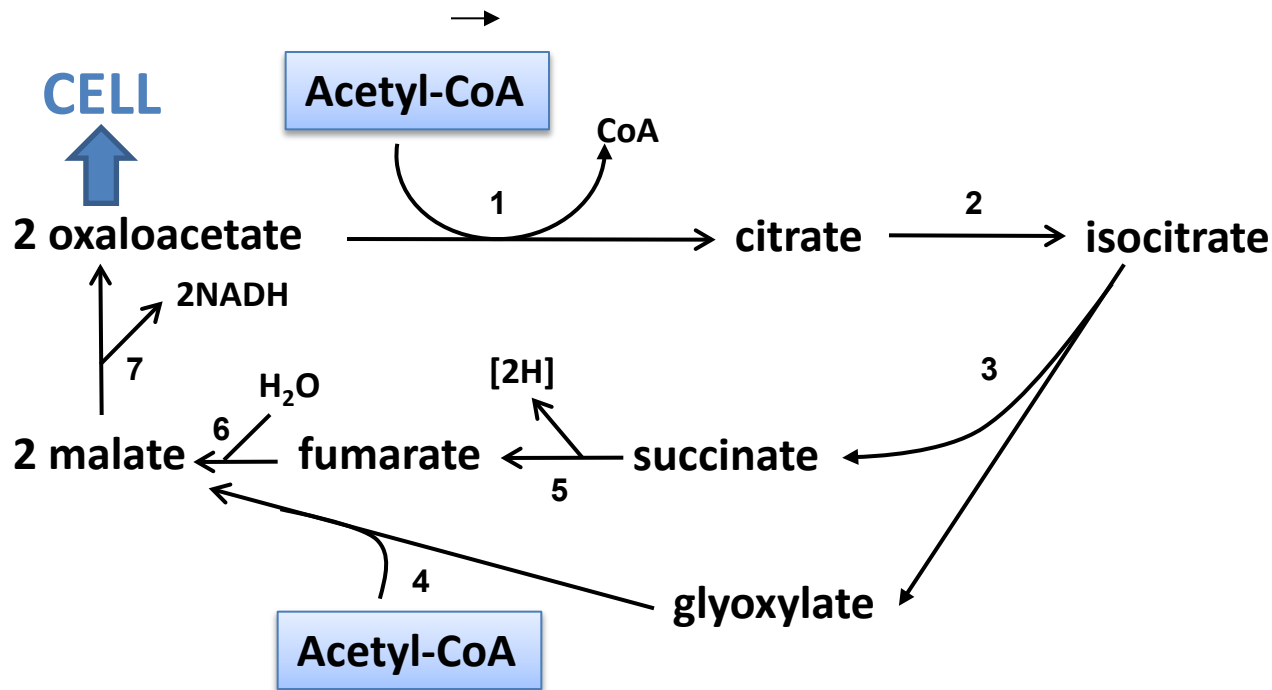


Fig. 1. Kornberg's glyoxylate cycle⁴. This achieves the condensation of 2 molecules of acetyl-CoA to malate. The enzymes are: 1, citrate synthase; 2, aconitase; 3, isocitrate lyase; 4, malate synthase; 5, succinate dehydrogenase; 6, fumarase; 7, malate dehydrogenase. In effect this cycle can be summarized as $2 \text{ acetyl-CoA} \rightarrow \text{malate}$.

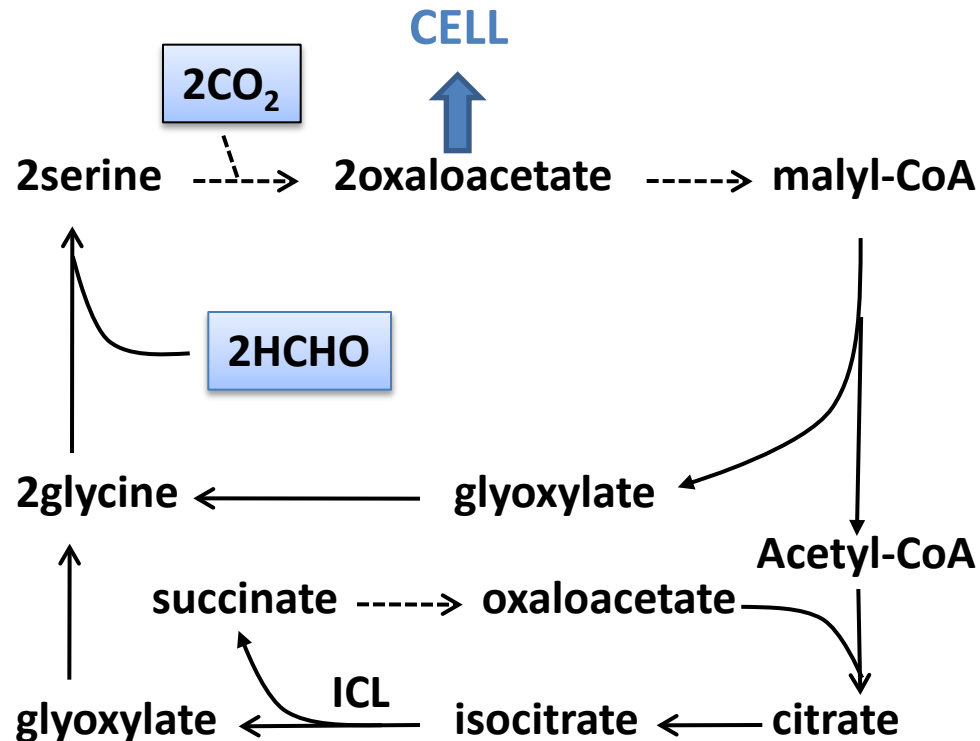


Fig. 4. The serine cycle in methylotrophic bacteria having isocitrate lyase [ICL]³. The upper part of the Figure shows the serine cycle as shown on Fig 3. The lower part shows the oxidation of acetyl-CoA to glyoxylate by isocitrate lyase together with the non-decarboxylating enzymes of the TCA cycle.

Solution of the Serine pathway in *Methylobacterium extorquens* (50 year project)

Situation in 1963 There must be a route for oxidation of acetylCoA to glyoxylate. An obvious route is to use isocitrate lyase but this enzyme is absent during growth on methanol (Large and Quayle 1963).

1970 Pat Dunstan (now Pat Goodwin). Showed that ICL is absent during growth on ethanol

Isolation of unusual mutants: A project to isolate MDH mutants. Penicillin enrichment procedure isolated mutants unable to grow on C1 or C2 but able to grow on succinate. These should be unable to oxidise methanol and ethanol. No mutants in assimilation pathways should be selected as these pathways are different.

Three types of mutant were isolated: MDH mutants. Cytochrome c mutants [unique, indicating the special role of cytochrome c in energy transduction from MDH]. A mutants with alteration in carbon assimilation pathways (PCT48) on C1 and C2 compounds showing there must be a common step in the pathway.



Yuri Me, Pat Dunstan (now Goodwin) and Sasha Netrusov in Kiev 12 days after Chernobyl

The assimilation of ethanol in *M. extorquens* by study of ^{14}C -acetate assimilation

After growth on Methanol early label was in glycollate (reflects early glyoxylate label) & citrate

After growth on Ethanol early label was in glycine (reflects early glyoxylate label) & citrate

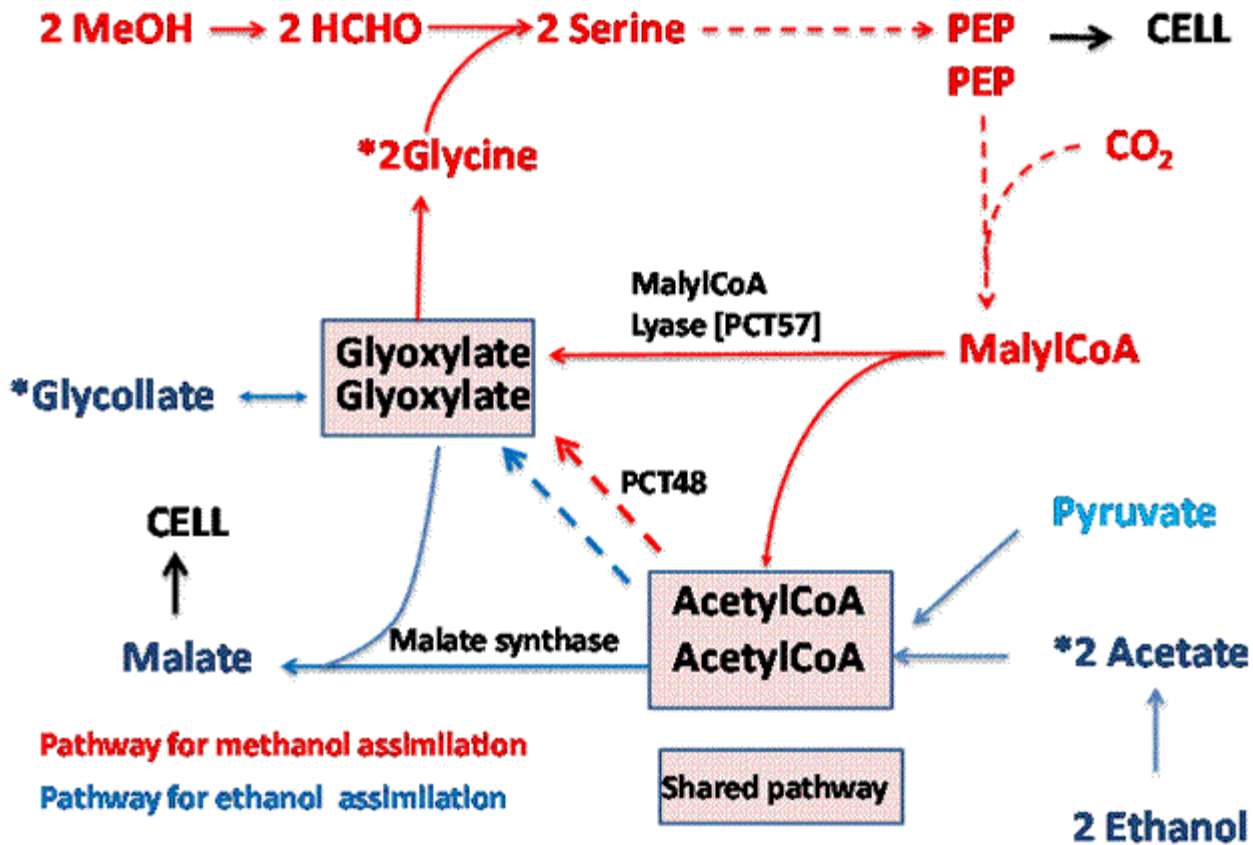
SO; there is an unknown common route for rapid metabolism of acetylCoA to glyoxylate during growth on C1 and C2 substrates.

In mutant 48 there was no rapid assimilation of acetate into glyoxylate (only citrate).

This same route was shown to operate on propanediol, 3-hydroxybutyrate and lactate

Problem: need to identify enzymes involved.

From Dunstan, Drabble, Taylor & Anthony (1972/76)



The shared pathway for methanol and ethanol assimilation

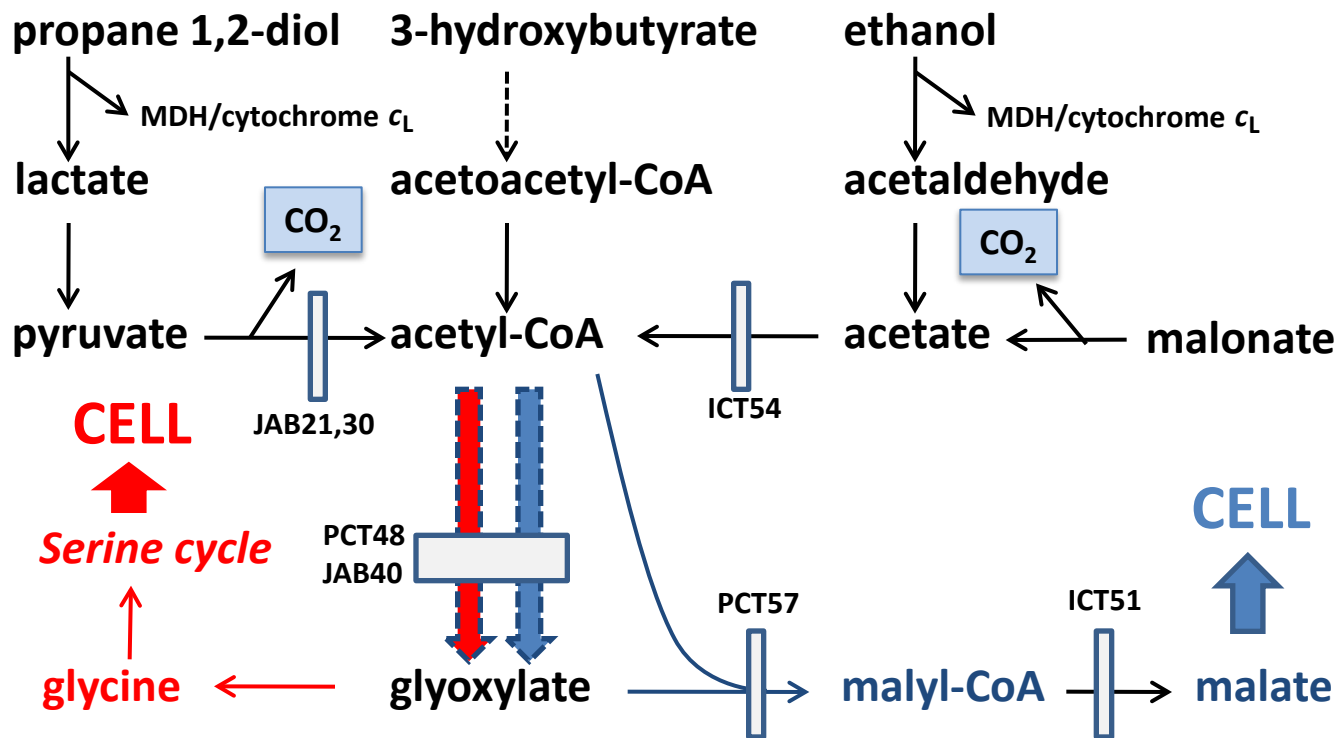


Fig. 5. Pathways for growth of *M. extorquens* on substrates metabolized by way of acetyl-CoA, based on the work of Pat Dunstan, John Bolbot and Ian Taylor ^{12, 17-19, 21, 22}. NB: only the carbon balance is illustrated. Red indicates pathway on C₁ compounds; blue indicates pathway on C₂ and related compounds. In short-term labeling experiments glyoxylate would arise by equilibration with glyoxylate. The growth substrates include ethanol, acetate (a poor substrate), 3-hydroxybutyrate, malonate, propanediol, lactate and pyruvate. Propanediol and ethanol are oxidized by methanol dehydrogenase (MDH) whose electron acceptor is cytochrome c_L¹⁶; there is no growth of mutants lacking these proteins. For oxidation of propanediol by MDH an additional modifier protein is required to alter its substrate specificity²². Note that condensation of glyoxylate and acetyl-CoA to malate requires two enzymes: malyl-CoA lyase and malyl-CoA hydrolase.

Glyoxylate Regeneration Cycles

Mila Chistaserdova and Mary Lidstrom as a result of their work using mutants and some biochemistry produced many complex pathways, called Glyoxylate Regeneration cycles'

The solution was finally obtained in the lab of Georg Fuchs in Fribourg by very thorough enzymology and complex labelling techniques. Erb, Berg, Alber, Spanheimer, Ebenau-Jehle and Fuchs.

The EthylmalonylCoA pathway (EMC pathway)

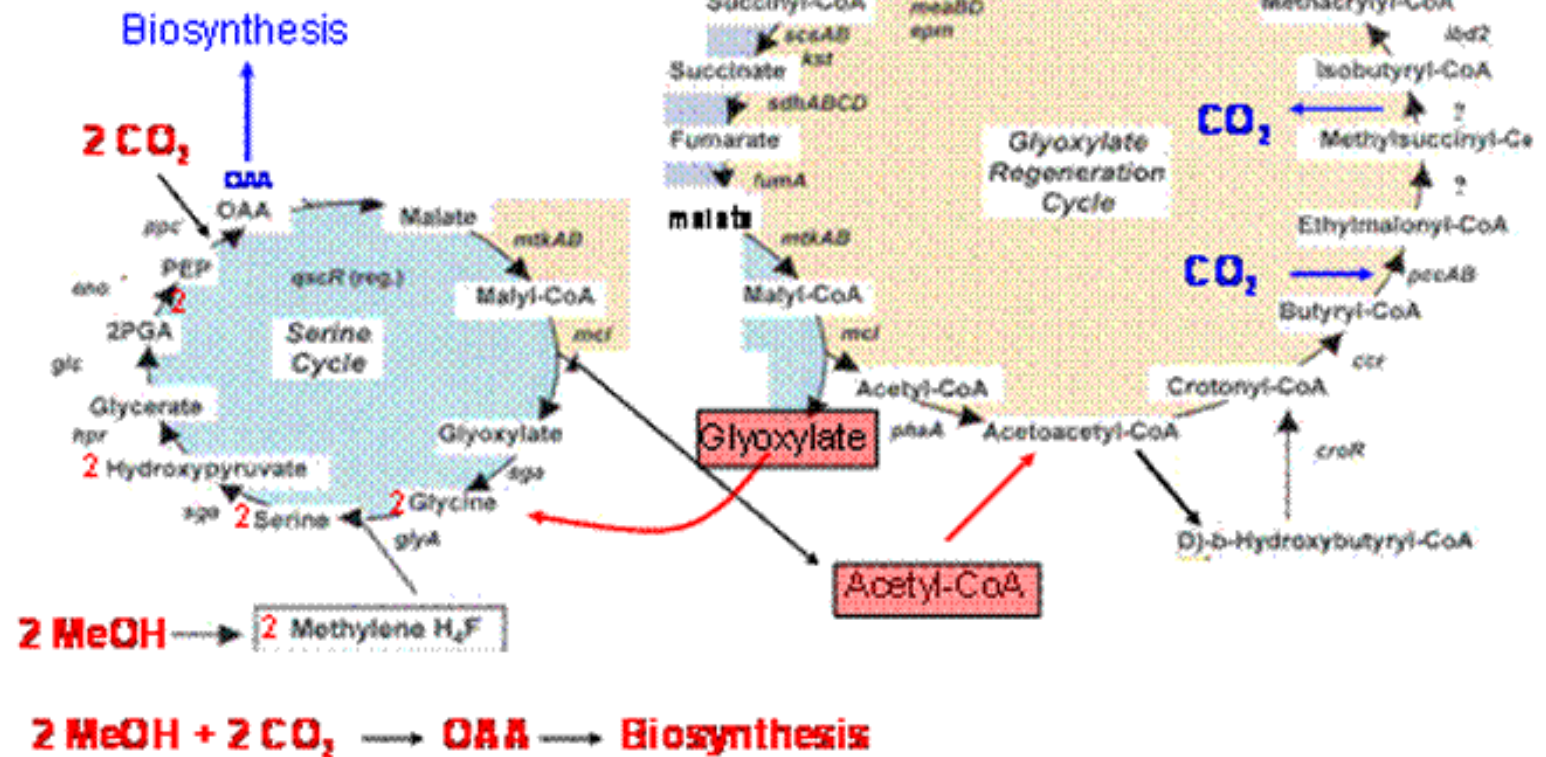
This was done for acetate assimilation in *Rhodobacter sphaeroides* but was soon shown to be the common pathway also involved in methanol and ethanol assimilation in *M. extorquens*.

Most of the following slides are the Figures from my review:

How half a century of research was required to understand bacterial growth on C1 and C2 compounds: the story of the Serine Cycle and the Ethylmalonyl-CoA pathway. Science Progress 94, 109-138, 2011

Pathway based on Chistoserdova,
Lidstrom and colleagues

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



The Glyoxylate Regeneration Cycle Mila Chistoserdova and Mary
Lidstrom



Mary Lidstrom (right)



Mila Chistaserdova (right)

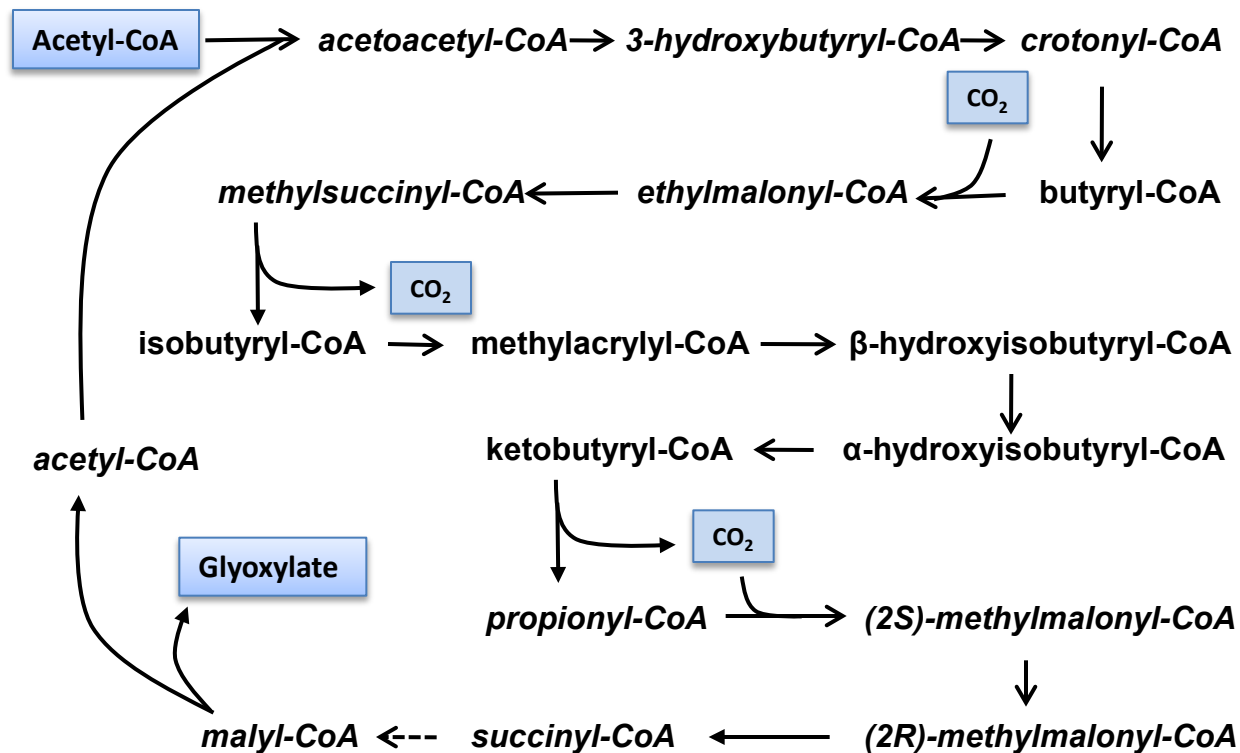


Fig. 7. The glyoxylate regeneration cycle (GRC) for oxidation of acetyl-CoA in *M. extorquens* as proposed by Lidstrom, Chistoserdova and colleagues^{27, 31-33}. Their papers should be consulted for details of the extensive experimental work, mainly using mutants and radioactive substrates that led to this [necessarily] speculative proposal. The compounds in italics were later shown to be intermediates in the ethylmalonyl-CoA (EMC) pathway. The solid arrows merely indicate proposed reactions (or series of reactions); they do not necessarily indicate that such reactions are known reactions.

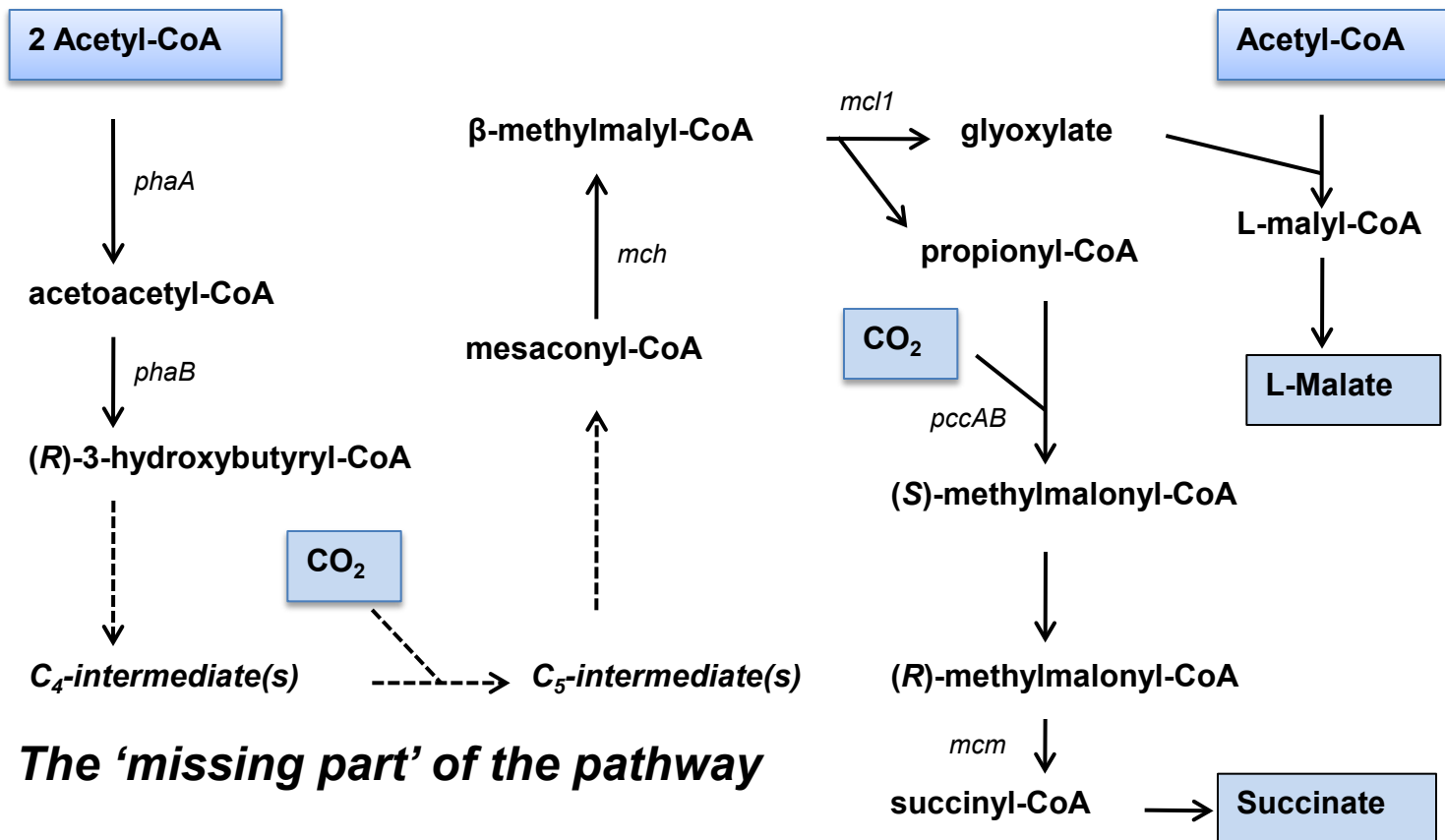


Fig. 9. Proposed pathway for acetyl-CoA assimilation by *Rhodospirillum rubrum*. This Figure is re-drawn from the 2006 paper by Alber, Spanheimer, Ebenau-Jehle and Fuchs⁴³. The gene *phaA* encodes β -ketothiolase; *phaB*, acetoacetyl-CoA reductase; *mch*, mesaconyl-CoA hydratase; *mcl1*, L-malyl-CoA/ β -methylmalyl-CoA lyase; *pccAB*, propionyl-CoA carboxylase and *mcm* encodes (R)-methylmalonyl-CoA mutase. Although the enzymes catalyzing the conversion of the C₄ compound 3-hydroxybutyryl-CoA to the C₅ intermediate mesaconyl-CoA were not known at the time, it was suggested that this process probably involves a carboxylation step, as was subsequently demonstrated when the ethylmalonyl-CoA pathway was finally elucidated (Figs. 10-12).

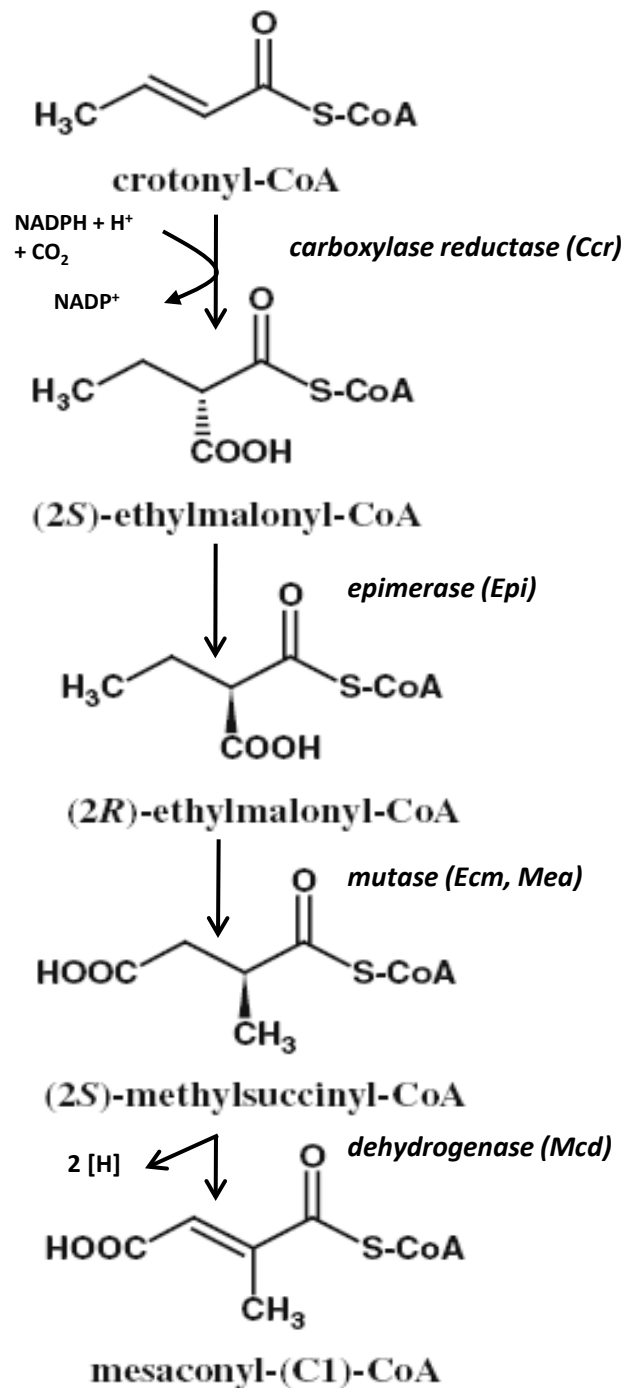
Fig. 10. The ‘missing’ part of the ethylmalonyl-CoA (EMC) pathway. The conversion of crotonyl-CoA to to mesaconyl-CoA depends on three novel enzymes:

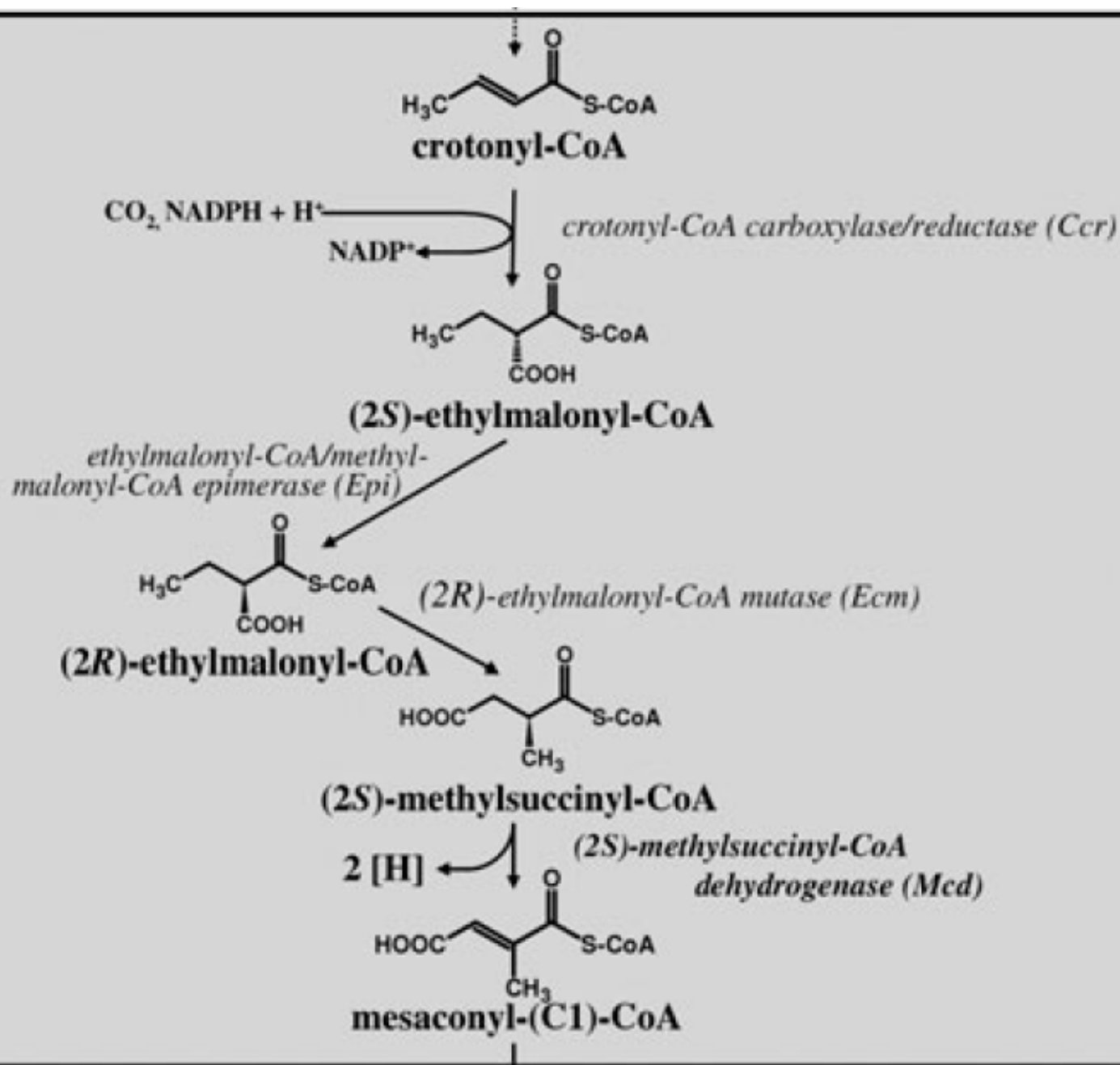
crotonyl-CoA carboxylase/reductase⁴⁴,

(2R)-ethylmalonyl-CoA mutase⁴⁶ and

(2)-methylsuccinyl-CoA dehydrogenase⁴⁷.

The two forms of ethylmalonyl-CoA are interconverted by ethylmalonyl-CoA/methylmalonyl-CoA epimerase.





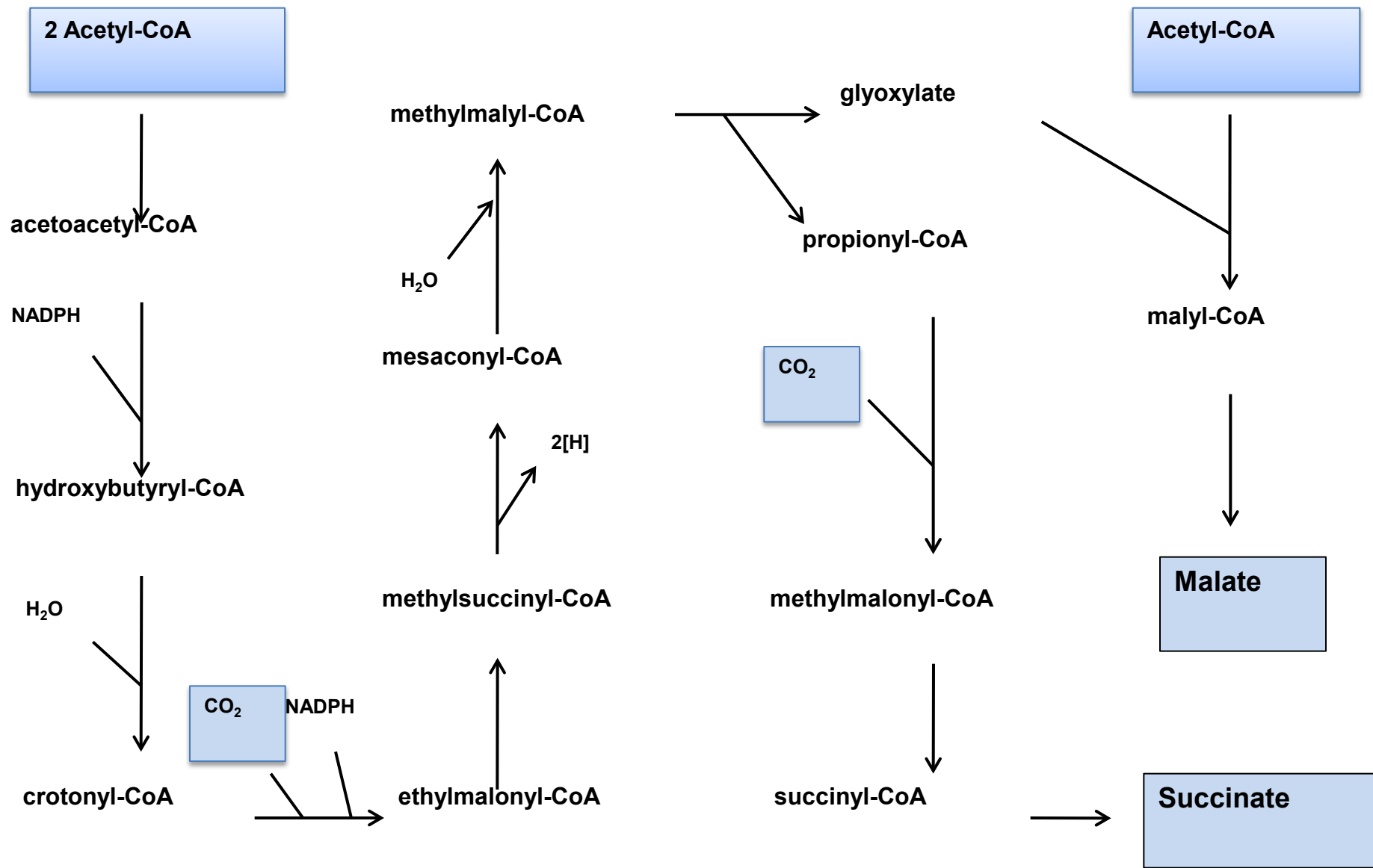


Fig. 12. The ethylmalonyl-CoA (EMC) pathway for acetyl-CoA assimilation in *Rhodobacter sphaeroides*, Note that there are two forms (R and S) of ethylmalonyl-CoA and two forms (R and S) of methylmalonyl-CoA (see Fig. 11) which are interconverted by the same epimerase.

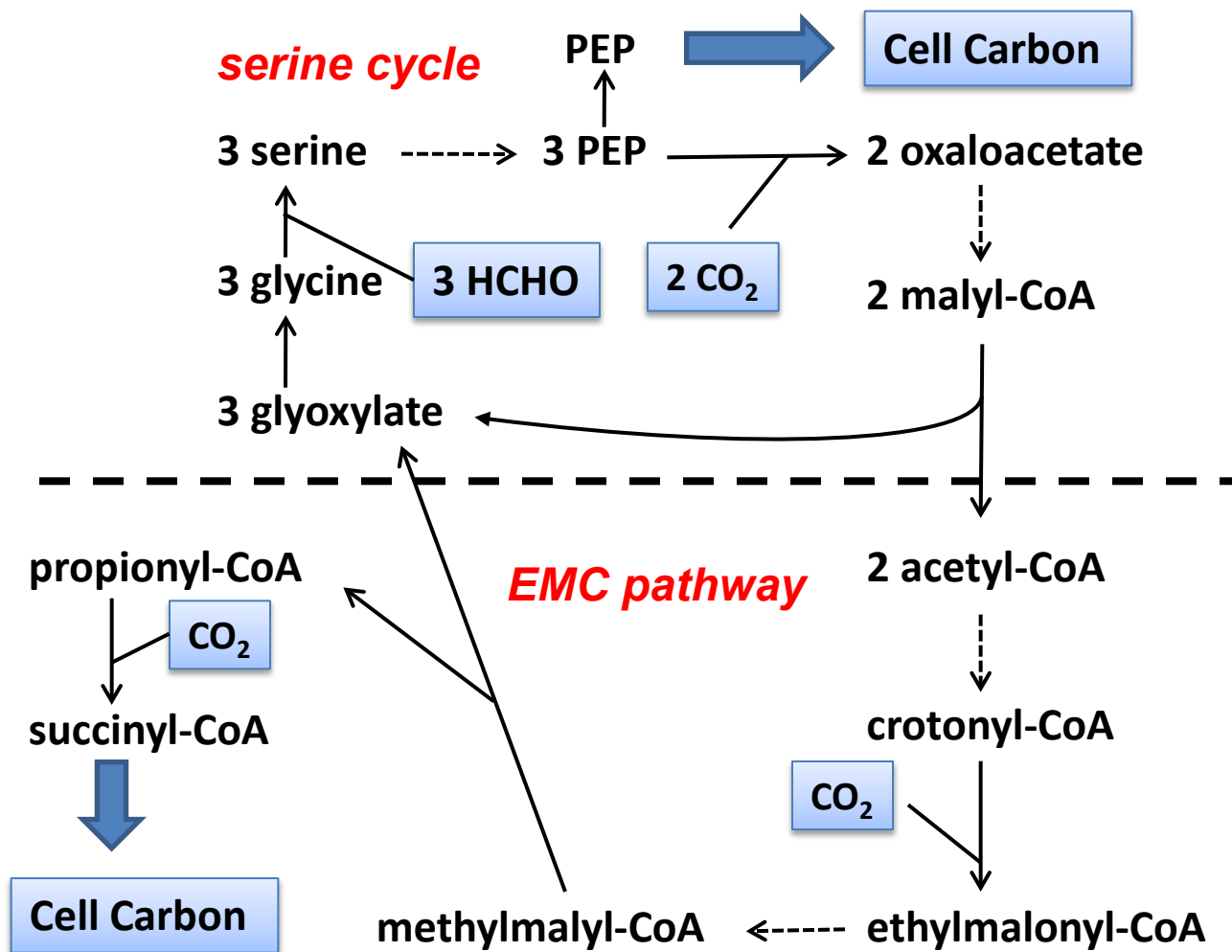


Fig. 13. The serine/EMC cycle for assimilation of C₁ compounds by methylotrophs⁴⁴. The ethylmalonyl-CoA (EMC) pathway for oxidation of acetyl-CoA to glyoxylate (lower half) (Fig. 12) is coupled to the serine cycle as shown on Fig. 3 (upper half). This is taken from the 2007 paper of Erb et al.⁴⁴ but for convenience only the carbon skeletons are shown. Dotted lines indicate that more than one reaction step is involved. Note that if acetyl-CoA is required as the biosynthetic precursor of membrane fatty acids or the storage compound poly 3-hydroxybutyrate then the EMC pathway is not required for oxidation of acetyl-CoA to glyoxylate.



Frieburg group: Georg Fuchs, Toby Erb and ? sorry



Celebrating
X, Georg Fuchs, Ivan Berg, Y, Z,
Toby

Sorry no picture of Birgit Alber

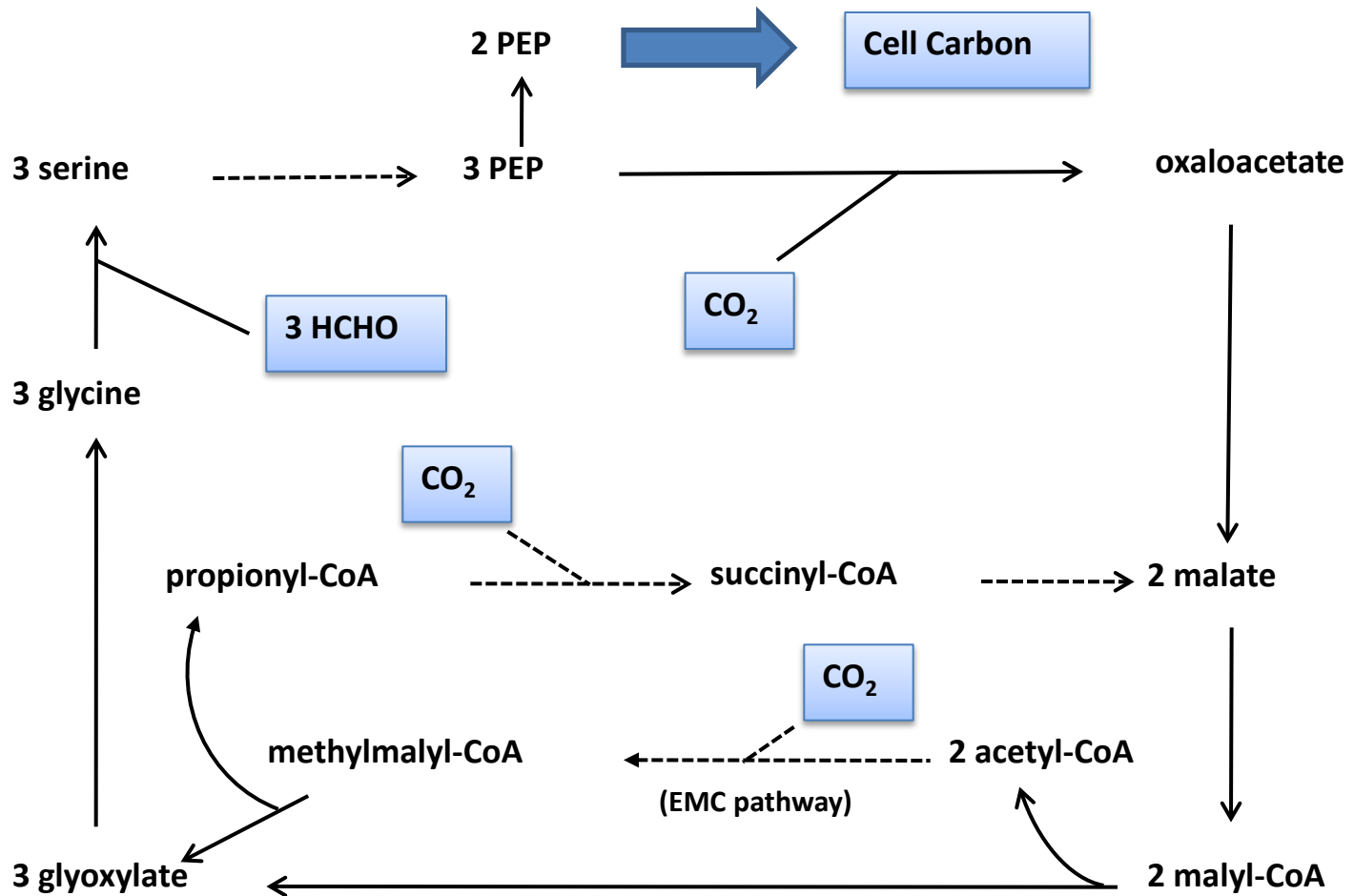


Fig. 14. **The serine/EMC cycle for assimilation of C1 compounds as it occurred during experiments described by Vorholt and colleagues⁵⁴** (re-drawn for ease of comparison with Figs. 3 and 13). This depiction of the pathway shows the succinyl-CoA, derived from propionyl-CoA, being 'recycled' to produce a third glyoxylate.



Julia Vorholt; confirmation of the Ethylmalonyl pathway (Zurich)

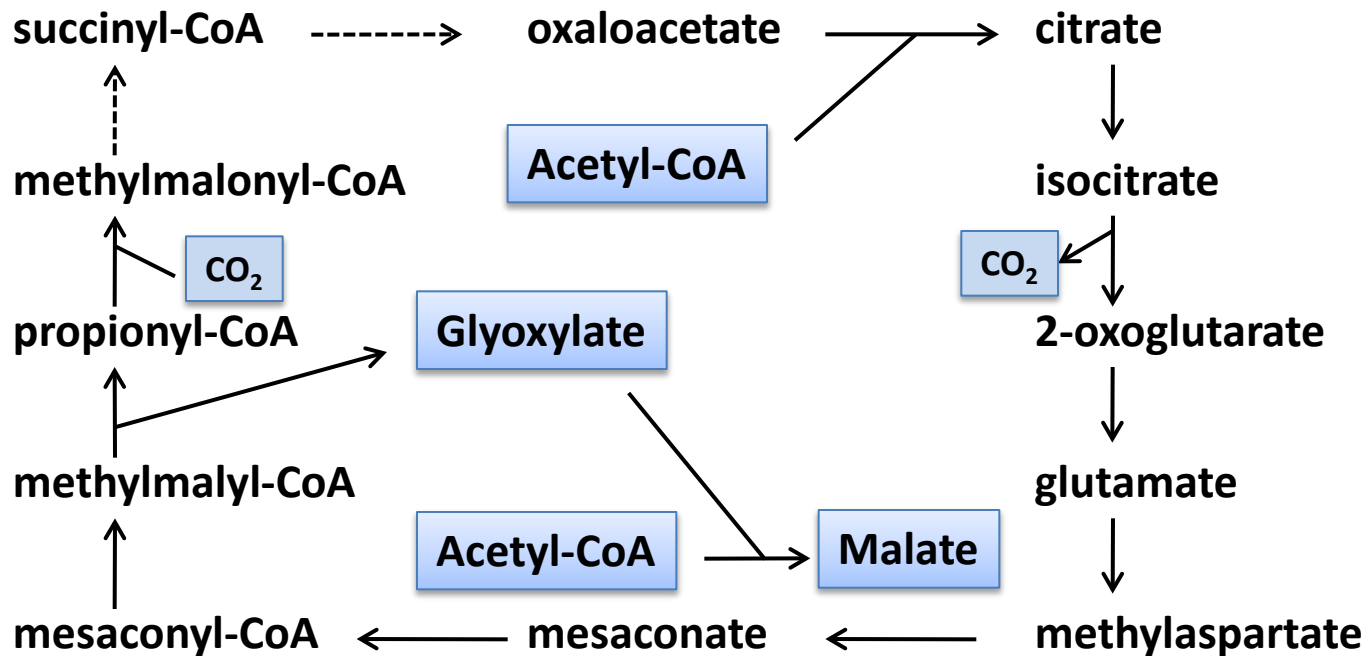


Fig. 6. The methylaspartate cycle²⁴. This pathway for oxidation of acetyl-CoA to glyoxylate in methylotrophs was proposed in 1984 by Shimizu, Ueda and Sato²³. Only the carbon skeletons have been included. The left hand side from mesaconyl-CoA to succinyl-CoA remains an essential part of the serine pathway as it is now understood. This cycle has recently been shown by Ivan Berg and colleagues to operate in haloarchaea for assimilation of C₂ compounds²⁴. In the complete methylaspartate cycle the glyoxylate condenses with a second molecule of acetyl-CoA to give malate, the overall carbon balance being the same as the glyoxylate cycle (Fig. 1).

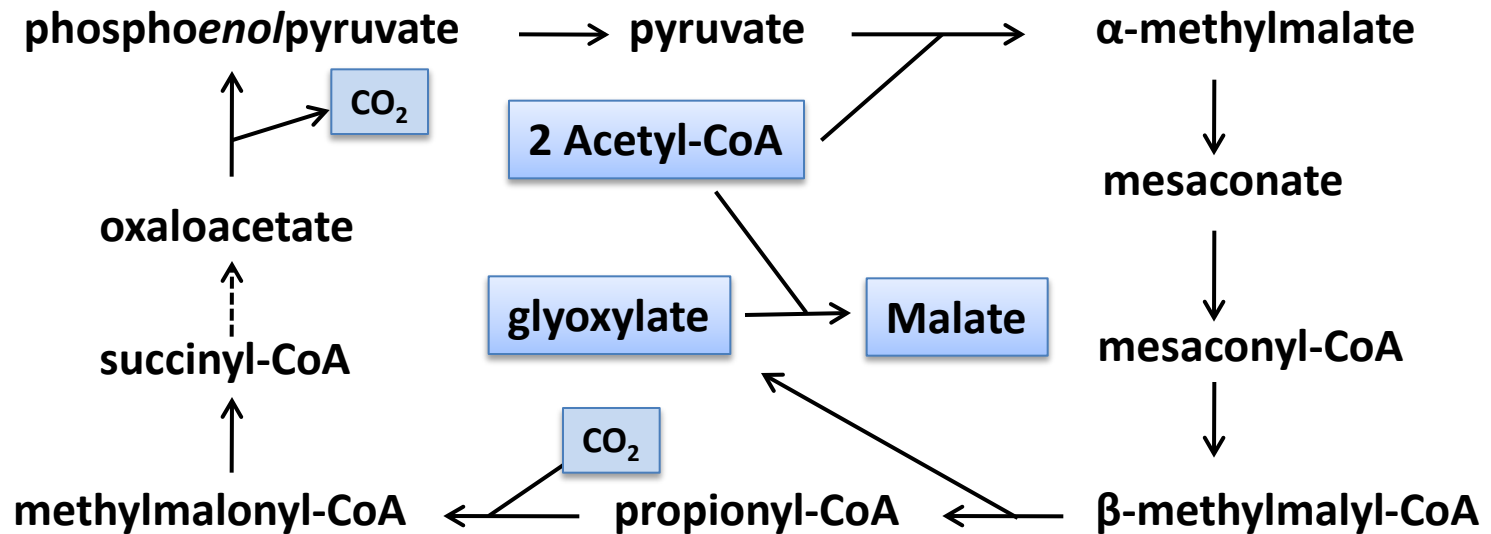


Fig. 8. The citramalate cycle proposed in 1977 for oxidation of acetyl-CoA to glyoxylate in *Rhodospirillum rubrum* by Ivanovsky's group in Moscow (note: citramalate is α-methylmalate)^{39,40}. The pathway is completed by the condensation of the glyoxylate with a second acetyl-CoA to give malate.