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MINI-REVIEW

Biotechnological potential of the ethylmalonyl-CoA pathway

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Abstract The ethylmalonyl-CoA pathway is central to the carbon metabolism of many α-proteobacteria, like *Rhodobacter sphaeroides* and *Methylobacterium extorquens* as well as actinomycetes, like *Streptomyces* spp. Its function is to convert acetyl-CoA, a central carbon intermediate, to other precursor metabolites for cell carbon biosynthesis. In contrast to the glyoxylate cycle—another widely distributed acetyl-CoA assimilation strategy—the ethylmalonyl-CoA pathway contains many unique CoA-ester intermediates, such as (2*R*)- and (2*S*)-ethylmalonyl-CoA, (2*S*)-methylsuccinyl-CoA, mesaconyl-(C1)-CoA, and (2*R*, 3*S*)-methylmalyl-CoA. With this come novel catalysts that interconvert these compounds. Among these unique enzymes is a novel carboxylase that reductively carboxylates crotonyl-CoA, crotonyl-CoA carboxylase/reductase, and (3*S*)-malyl-CoA thioesterase. The latter represents the first example of a non-Claisen condensation enzyme of the malate synthase superfamily and defines a new class of thioesterases apart from the hotdog-fold and α/β-fold thioesterases. The biotechnological implications of the ethylmalonyl-CoA pathway are tremendous as one looks to tap into the potential of using these new intermediates and catalysts to produce value-added products.

Keywords Ethylmalonyl-CoA pathway · Glyoxylate cycle · Claisen condensation · Thioesterase · Crotonyl-CoA carboxylase/reductase · 2-Methylfumaryl-CoA

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The ethylmalonyl-CoA pathway and its role in cell carbon biosynthesis

The biosynthesis of cell constituents in all living cell starts from a few so-called precursor metabolites that include the C_2 compound acetyl-CoA, the C_3 compound pyruvate (or phosphoenolpyruvate), the C_4 compound oxaloacetate, and the C_5 compound α-ketoglutarate (Neidhardt et al. 1990; Fuchs 1999). In bacterial cells, most cell carbon (about 50%) is derived from pyruvate/phosphoenolpyruvate as it is the gluconeogenic substrate leading to cell wall components and nucleotides. This is followed by acetyl-CoA (about 30%), oxaloacetate (about 13%), and alpha-ketoglutarate (about 7%). Different carbon sources enter the central part of metabolism at one or more of these precursor metabolites through peripheral and intermediary pathways. C_1 and C_2 compounds (e.g., methanol and acetate), fatty acids, waxes, (poly)hydroxyalkanoates, or alkenes enter central carbon metabolism on the level of acetyl-CoA The conversion of acetyl-CoA to cell carbon constituents is referred to as acetyl-CoA assimilation. How acetyl-CoA is converted to pyruvate/phosphoenolpyruvate, oxaloacetate, and alpha-ketoglutarate is sufficient to understand this anabolic process. Two different strategies for acetyl-CoA assimilation are known: the glyoxylate cycle (Kornberg and Krebs 1957; Kornberg et al. 1958) and the ethylmalonyl-CoA pathway (Erb et al. 2007). Both acetyl-CoA assimilation pathways convert acetyl-CoA to the citric acid cycle intermediate malate, and from there pyruvate, oxaloacetate, and alpha-ketoglutarate are made by established steps in carbon metabolism. However, the ethylmalonyl-CoA pathway is distinct from the glyoxylate cycle as one molecule of CO_2 and one molecule HCO_3^- per three molecules of acetyl-CoA are co-assimilated to

form two malate molecules. The path for malate synthesis during acetyl-CoA assimilation for either pathway is given below.

Glyoxylate cycle:

$$4\,\text{acetyl}-\text{CoA} + 2\,\text{NAD}^+ + 6\,\text{H}_2\text{O} \rightarrow 2\,\text{malate}^{2-} + 4\,\text{HSCoA} + 4\,[\text{H}] + 2\,\text{NADH} + 6\,\text{H}^+$$

(reducing equivalents generated at the succinate dehydrogenase step are most likely transferred to ubiquinone).

Ethylmalonyl-CoA pathway:

$$3\,\text{acetyl}-\text{CoA} + \text{CO}_2 + \text{HCO}_3^- + 2\,\text{NADPH} + \text{H}_2\text{O} + \text{OH}^- \rightarrow 2\,\text{malate}^{2-} + 3\,\text{HSCoA} + 2\,\text{NADP}^+ + 4\,[\text{H}]$$

(reducing equivalents generated at the succinate dehydrogenase and methylsuccinyl-CoA dehydrogenase steps are most likely transferred to ubiquinone; it is assumed that NTP generated by the succinyl-CoA synthetase is used for propionyl-CoA carboxylation).

The different path of malate synthesis may be one reason for the difference in distribution of these pathways in nature. A survey of all complete bacterial genomes for the presence of genes encoding the key enzymes for either pathway revealed the following: about one third of the 413 genera represented have the genes for the glyoxylate cycle enzymes (isocitrate lyase and malate synthase), about 7% for the ethylmalonyl-CoA enzymes (crotonyl-CoA carboxylase/reductase, ethylmalonyl-CoA mutase, methylsuccinyl-CoA dehydrogenase), and 1% appear to be able to use both acetyl-CoA assimilation strategies (Erb 2009a). The remaining 60% either do not require an acetyl-CoA assimilation pathway due to their substrate spectrum, utilize a so far unknown strategy, or are anaerobic bacteria. For anaerobic bacteria, acetyl-CoA can be directly converted to pyruvate by reductive carboxylation (and from there further to oxaloacetate and alpha-ketoglutarate). The enzyme pyruvate:acceptor oxidoreductase that catalyzes the reductive carboxylation step uses a low-potential electron acceptor, such as ferredoxin, that can be generated and maintained under low oxygen conditions.

Possible biotechnologically relevant products derived from the ethylmalonyl-CoA pathway

The reactions and intermediates of the ethylmalonyl-CoA pathway are shown in Figs. 1 and 2. The ethylmalonyl-CoA pathway is a non-cyclic pathway and there is no requirement to metabolically regenerate an acceptor molecule. Instead, the starting molecule acetyl-CoA and inorganic carbon may be derived from a variety of renewable carbon substrates that converge at this point of metabolism. It follows that any unique intermediate of the pathway can in principle be used as a starting point to construct a "dream pathway" leading to a value-added product. In the context of an organism, though, the requirement for energy and reducing equivalents has to be taken into account. Other considerations include secretion of products, possible toxicity, yields, and downstream processing of the desired product. In the following paragraphs, a few examples are envisioned (and remain untested) how the introduction of just a few additional enzymes may facilitate the production of interesting products. However, depending on the type and oxidation state of the starting carbon source(s), the metabolic network as a whole has to be considered to enable the withdrawal of a given intermediate from the ethylmalonyl-CoA pathway.

The initial steps of the ethylmalonyl-CoA pathway overlap with the synthesis of polyhydroxybutyrate (Korotkova and Lidstrom 2001; Alber et al. 2006; Fig. 1). Two molecules of acetyl-CoA are condensed by β-ketothiolase to form acetoacetyl-CoA that is subsequently reduced to 3-hydroxybutyryl-CoA. Acetoacetyl-CoA reductase uses NADPH as the hydride donor and specifically forms (*R*)-3-hydroxybutyryl-CoA, the substrate for polyhydroxybutyrate synthase. For assimilation purposes, however, an (*R*)-specific hydratase is proposed to catalyze the subsequent dehydration of 3-hydroxybutyryl-CoA to crotonyl-CoA (Korotkova et al. 2002; Erb 2009a). The partitioning of carbon leading to polyhydroxybutyrate versus cell carbon is determined by the competing activities of polyhydroxybutyrate synthase that polymerizes (*R*)-3-hydroxybutyryl-CoA units and reactions of the ethylmalonyl-CoA pathway. Controlling the flow through the ethylmalonyl-CoA pathway, favoring the formation of polyhydroxybutyrate synthesis, would be a desirable approach to channel different substrates that enter the central carbon metabolism at the level of acetyl-CoA to a chemically defined product. This is attractive because advances have been made in the last years to lower the cost of downstream processing for this polymer (de Koning et al. 1997; Kapritchkoff et al. 2006).

The unique reductive carboxylation of crotonyl-CoA by the enzyme crotonyl-CoA carboxylase/reductase can be considered the committed step for acetyl-CoA assimilation by the ethylmalonyl-CoA pathway. Although the reaction is reversible, the activity of crotonyl-CoA carboxylase/reductase is up-regulated at least 60-fold for *Rhodobacter sphaeroides* during growth with acetate versus succinate (Erb et al. 2007). The molecular basis for the observed regulation, however, is so far unknown. The reductive carboxylation of crotonyl-CoA is the entry point into the C_5 portion of the pathway (Fig. 1). This central part of the ethylmalonyl-CoA pathway involves C_5 intermediates very rarely seen in metabolism: (2*S*)-ethylmalonyl-CoA, (2*R*)-ethylmalonyl-CoA, (2*S*)-methylsuccinyl-CoA, mesaconyl-

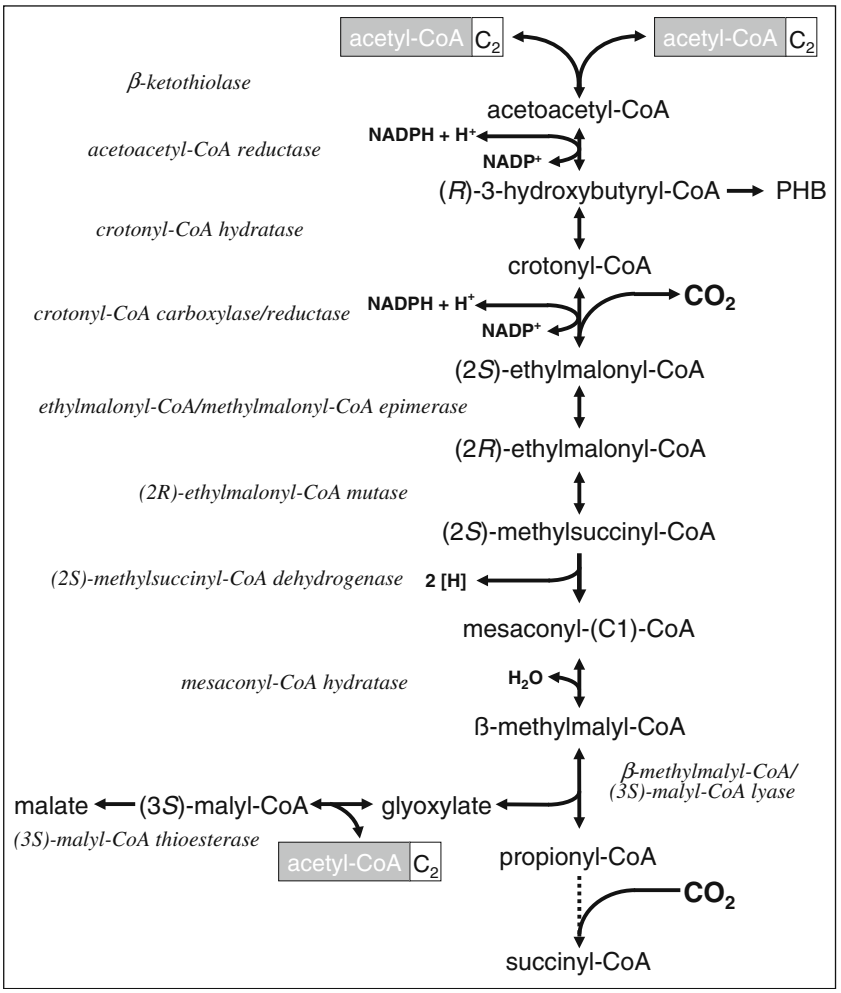

Fig. 1 Ethylmalonyl-CoA pathway for acetyl-CoA assimilation. The ethylmalonyl-CoA pathway and the participating enzymes are shown in detail. An (*R*)-specific crotonyl-CoA hydratase is probably involved in the conversion of 3-hydroxybutyryl-CoA to crotonyl-CoA. (*R*)-3-Hydroxybutyryl-CoA is also the precursor for polyhydroxybutyrate (*PHB*) synthesis. The electron acceptor for (2*S*)-methylsuccinyl-CoA dehydrogenase has so far not been determined. The conversion of propionyl-CoA to succinyl-CoA includes the carboxylation of propionyl-CoA by an ATP- and bicarbonate-dependent propionyl-CoA carboxylase, conversion of the product (2*S*)-methylmalonyl-CoA to (2*R*)-methylmalonyl-CoA by the promiscuous enzyme methylmalonyl-CoA/ethylmalonyl-CoA epimerase, and a carbon skeleton rearrangement catalyzed by a specific methylmalonyl-CoA mutase that is distinct from ethylmalonyl-CoA mutase. The structures of the intermediates are shown in Fig. 2

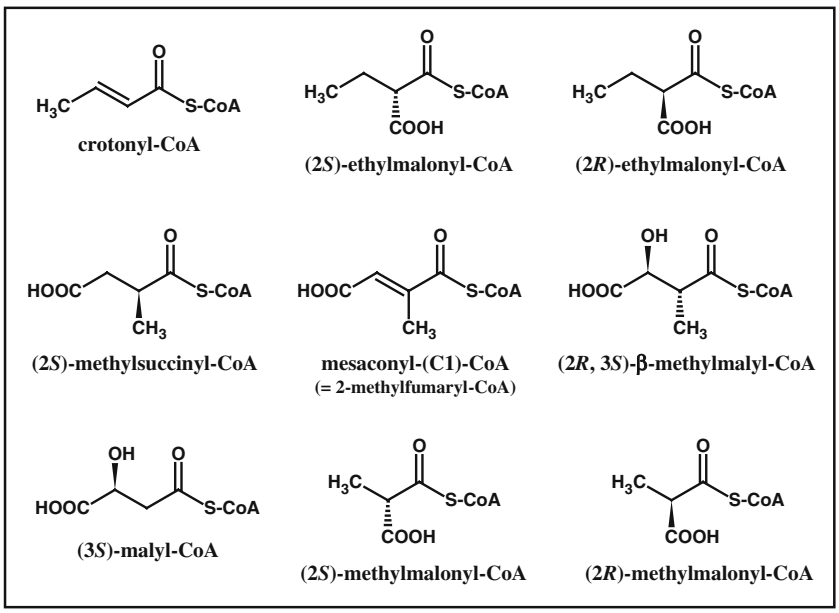

Fig. 2 Unusual intermediates of the ethylmalonyl-CoA pathway

(C1)-CoA, and (2*R*, 3*S*)-methylmalyl-CoA (for the structures of these compounds, see Fig. 2).

Removal of the CoA moiety by the introduction of a specific thioesterase could lead to the production of stereochemically defined acids, most of which are currently commercially unavailable: (2*S*)-ethylmalonate, (2*R*)-ethylmalonate, (2*S*)-methylsuccinate, mesaconate, and (2*R*, 3*S*)-methylmalate.

The general reaction scheme for thioesterases hydrolyzing thioester bonds of CoA esters is shown below:

$$R - COSCoA + H_2O \rightarrow R - COO^- + HSCoA + H^+.$$

The prospect for identifying specific thioesterases that would act on these intermediates seems favorable as many thioesterases have been characterized. Thioesterases are either domains of protein complexes such as non-ribosomal peptide transferases, polyketide synthases, and fatty acid synthases or constitute separate enzymes (Barnes and Wakil 1968; Lin and Smith 1978; Cho and Cronan 1993; Benning et al. 1998; Li et al. 2000; Kim et al. 2002; Schwarzer et al. 2002; Koglin et al. 2008). Most characterized thioesterases belong to the class of alpha/beta-fold hydrolases or the hotdog-fold protein family and both classes contain thioesterases that act on CoA esters (Table 1). Therefore, there may be numerous candidates for mutagenesis to altered substrate range. Apart from these two major families, a new class of thioesterases has been discovered as part of the ethylmalonyl-CoA pathway. The characterized member of this new class of thioesterases specifically hydrolyzes (3*S*)-malyl-CoA and the enzyme belongs to the superfamily of malate synthases (Erb et al. 2010). Members of this superfamily adopt a β_8/α_8 barrel-fold and up to now were only known to catalyze Claisen condensation reactions (Howard et al. 2000; Goulding et al. 2007). So far, it is not possible to identify members of this large family specifically as thioesterases based on primary amino acid sequence analyses. Eventually, however, it may be possible to extend the number of enzymes as starting points for mutagenesis studies to include members of this family.

Another possibility to release free acids from CoA intermediates would be the use of CoA transferases that transfer the CoA residue directly from the intermediate to the substrate, which is used in the process to produce a specific acid. At least three different classes of CoA transferases have been described (Pickart and Jencks 1979; Buckel et al. 1981; Heider 2001; Berthold et al. 2008; Fleck and Brock 2008).

Specific thioesterases that exclusively hydrolyze (2*S*)-ethylmalonyl-CoA, (2*R*)-ethylmalonyl-CoA, (2*S*)-methylsuccinyl-CoA, mesaconyl-(C1)-CoA, and (2*R*, 3*S*)-methylmalyl-CoA or specific CoA transferases that transfer the CoA moiety from these compounds to other carboxylic acids have, to my knowledge, not been described. Characterized CoA thioesterase and CoA transferases, however, provide a solid basis for protein engineering with the goal in mind of altering substrate specificities of these enzymes. Success for this approach requires not only an increase in specificity for the desired substrate but also at the same time a decrease of unspecific activities possibly targeting other metabolic CoA intermediates.

Another possibility to synthesize value-added chemicals starting from intermediates of the central part of the ethylmalonyl-CoA pathway is the two-step reduction of ethylmalonyl-CoA to 2-ethyl-3-hydroxypropionate. A similar reaction sequence has been proposed for the formation of 3-hydroxyisobutyrate from methylmalonyl-CoA (Marx et al. 2007), which is an intermediate later in the ethylmalonyl-CoA pathway. Both 3-hydroxyalkonates, 2-ethyl-3-hydroxypropionate, and 3-hydroxyisobutyrate could be extremely interesting platform chemicals, as has been recently reviewed for 3-hydroxyisobutyrate (Rohwerder and Müller 2010).

The prospect of engineering organisms to take advantages of these approaches is good as at least two organisms already considered for biotechnological applications contain the ethylmalonyl-CoA pathway: *R. sphaeroides* and *Methylobacterium extorquens*.

Table 1 Three recognized classes of thioesterases based on their three-dimensional structure

Class	Enzyme superfamily	Prototype	References
Hotdog-fold thioesterase	Hotdog-fold superfamily	4-Hydroxybenzoyl-CoA thioesterase from *Pseudomonas* sp. strain CBS-3	Benning et al. (1998)
		4-Hydroxybenzoyl-CoA thioesterase from *Arthrobacter* sp. strain SU[a]	Thoden et al. (2003); Zhuang et al. (2003)
α/β-Hydrolase-fold thioesterase	Esterase/lipase superfamily	acyl-CoA thioesterase I (TseA) from *Escherichia coli*	Barnes and Wakil (1968); Cho and Cronan (1993) Lo et al. (2003)
β_8/α_8 Barrel-fold thioesterase	Malate synthase superfamily	(3*S*)-Malyl-CoA thioesterase	Erb et al. (2010)

[a] The two 4-hydroxybenzoyl-CoA thioesterases form distinct clades within the class of hotdog-fold thioesterase and the alignment of their amino acid sequences is not straightforward (Thoden et al. 2002). They also differ in their arrangement of subunits (Cao et al. 2009)

The ethylmalonyl-CoA pathway is central to the carbon metabolism of at least two biotechnologically relevant organisms, *R. sphaeroides* and *M. extorquens*

The ethylmalonyl-CoA pathway is used as the acetyl-CoA assimilation strategy in the metabolically versatile purple non-sulfur bacterium *R. sphaeroides* (Fig. 3a). *R. sphaeroides* has many desirable features for use in biotechnological applications. It can grow phototrophically in the light under anaerobic conditions, but can easily transition to chemoheterotrophic growth (aerobically in the dark). The clear advantage is that it can "turn itself anaerobic," a fact that has been used by many researcher working with the organism in the past: Aerobic medium is inoculated and then the culture vessel is sealed and placed in the dark. After several hours, the culture (now anaerobic because the oxygen has been used up through respiration) is placed in the light and growth continues. During phototrophic growth, *R. sphaeroides* uses a wide variety of carbon sources exclusively for synthesis of cell mass, and it does so with amazing efficiency. The reductant that is generated during assimilation of a substrate that is more reduced than cell carbon is transferred to exogenous CO_2 and converted to additional cell mass through the Calvin–Benson–Bassham cycle (Wang et al. 1993). CO_2 fixation through the Calvin–Benson–Bassham cycle can also occur with substrates that are more oxidized than cell mass, for example malate, because more CO_2 and reductant are transiently generated during the initial assimilation-steps than is released at the end (Laguna et al., submitted). This extra carbon is therefore not lost but can be converted to additional cell mass by the Calvin–Benson–Bassham cycle; at the same time, this allows the cell to maintain redox balance. In the case of growth with substrates that enter the central carbon metabolism on the level of acetyl-CoA, CO_2 generated from decarboxylation of the product of the ethylmalonyl-CoA pathway malate (to form C_3 precursor metabolites) is directly re-assimilated by the ethylmalonyl-CoA obviating the requirement of the Calvin–Benson–Bassham cycle (Laguna et al., submitted). The ability of *R. sphaeroides* to make total use of the carbon source for cell mass biosynthesis (of course, CO_2 will still be generated during growth on an oxidized substrate) may be exploited by channeling a given substrate to a desired product with maximum yield. Also, the withdrawal of intermediates of the ethylmalonyl-CoA pathway in order to produce specific products may be possible without effecting growth of the organism. For *R. sphaeroides*, a mutational block in the ethylmalonyl-CoA pathway still allows the growth on substrates not requiring the pathway, such as succinate (Erb et al. 2008, 2009b, 2010). Because *R. sphaeroides* utilizes many carbon substrates, a possibility would also be to streamline various substrates toward the desired product by manipulating central carbon metabolism, eliminating the need to start with a pure carbon source. Apart from the ability to fix CO_2, *R. sphaeroides* can also fix molecular nitrogen, a fact advantageous for biotechnological considerations. The genomes of four different strains of *R. sphaeroides* are fully sequenced (Choudhary et al. 2007; Lim et al. 2009). Transcriptome analysis by microarrays has been established

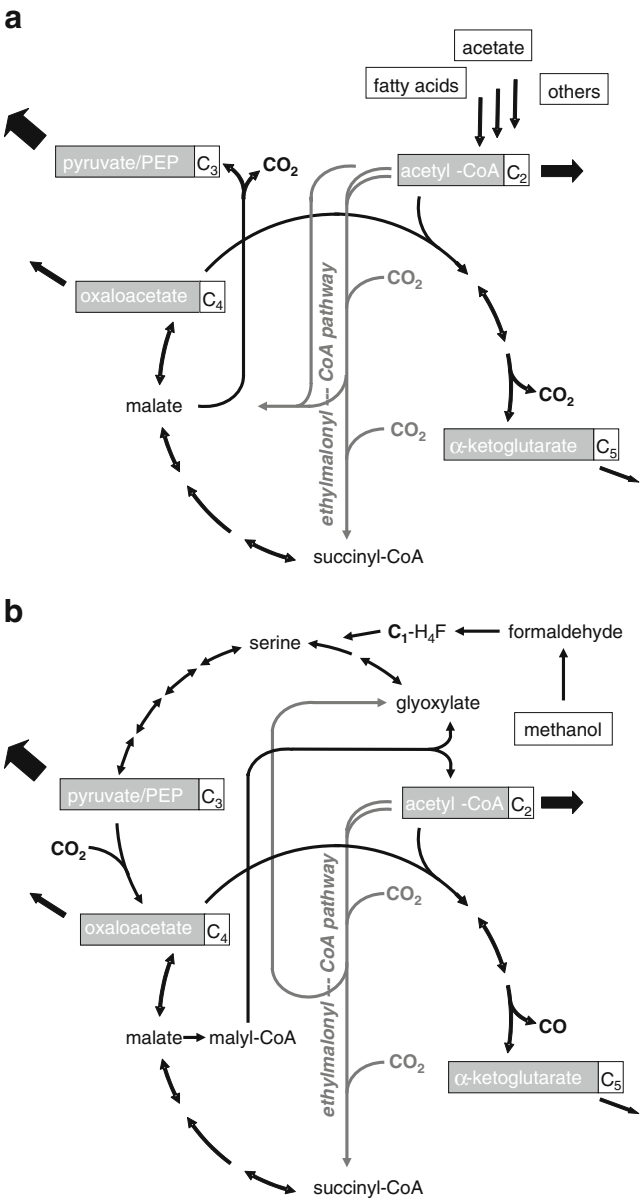

Fig. 3 Central role of the ethylmalonyl-CoA pathway in carbon metabolism. **a** Acetyl-CoA assimilation by *R. sphaeroides*. The assimilation of acetyl-CoA leads to the production of precursor metabolites (acetyl-CoA, pyruvate, oxaloacetate, and α-ketoglutarate) that are used for the biosynthesis of cell constituents. The estimated relative amounts withdrawn in each case are indicated by the *thickness of the arrow bar*. **b** Methanol assimilation by *M. extorquens*. C_1 units are assimilated by the serine cycle (Large et al. 1961). Precursor metabolites are taken out of the serine cycle for cell carbon biosynthesis and glyoxylate is being replenished by the ethylmalonyl-CoA pathway

for *R. sphaeroides* (Roh et al. 2004; Arai et al. 2008), and several global regulators in carbon metabolism, such as CbbR (Gibson and Tabita 1993), RegAB, or PrrAB (Sganga and Bauer 1992; Bauer and Bird 1996; Eraso and Kaplan 1994; Joshi and Tabita 1996), have been identified. Genetic tools to alter the metabolism of *R. sphaeroides* are available (Kovach et al. 1995; Schäfer et al. 1994; Quandt and Hynes 1993) and random mutagenesis is also possible (Larsen et al. 2002). An expression vector for high-level protein production was also recently developed for *R. sphaeroides* (Ind et al. 2009).

Methylotrophic bacteria that use the serine cycle for the assimilation of methylated compound require a pathway to regenerate glyoxylate from acetyl-CoA (Large et al. 1961; Anthony 1982). Glyoxylate yields glycine, the primary acceptor molecule for C_1 units, forming serine. Depending on the species, either reactions of the glyoxylate cycle or the ethylmalonyl-CoA pathway are used. Even before the ethylmalonyl-CoA pathway was biochemically characterized in *R. sphaeroides*, the genes involved in the regeneration of glyoxylate from acetyl-CoA during methanol assimilation by the isocitrate lyase-negative bacterium *M. extorquens* were already identified (Chistoserdova and Lidstrom 1996; Smith et al. 1996; Korotkova and Lidstrom 2001, 2004; Korotkova et al. 2002, 2005; Chistoserdova et al. 2003). The operation of the ethylmalonyl-CoA pathway was directly demonstrated in *M. extorquens* by short-term ^{13}C-labeling experiments and time-dependent appearance of all predicted CoA ester intermediates (Peyraud et al. 2009; Fig. 3b). *M. extorquens* is probably the best-studied methylotroph (Large et al. 1961; Anthony 1982; Chistoserdova et al. 2003; Chisterodova et al. 2009) and is being considered for methanol-based biotechnology, as recently reviewed by Schrader et al. (2008). In comparison to methylotrophic bacteria that use the glyoxylate cycle, *M. extorquens* has a higher efficiency of carbon recovery due to the increased CO_2 fixation ability of the ethylmalonyl-CoA pathway (Peyraud et al. 2009). The elucidation of the ethylmalonyl-CoA pathway not only completes the picture for carbon utilization in this biotechnological relevant organism but also opens up possibilities to explore the unique intermediates for the production of crude and fine chemicals.

Other bacteria, including many marine α-proteobacteria, are expected to use the ethylmalonyl-CoA pathway (Erb et al. 2009b), but have been less well studied in regard to their carbon metabolism. Actinomycetes, like *Streptomyces* and *Salinospora* species, also utilize the pathway—or part of it—to supply precursors for the synthesis of secondary metabolites, including antibiotics (reviewed in Chan et al. 2009).

Novel catalysts involved in the ethylmalonyl-CoA pathway and their biotechnological potential

With the emergence of unusual metabolic intermediates as part of the ethylmalonyl-CoA pathway come novel enzymes that catalyze their interconversion. Although these enzymes will function best in the context of an organism due to their requirements of complex cofactors, such as coenzyme A, coenzyme B_{12}, and NADPH, they may also be considered as catalysts in vitro after alteration.

(2*R*)-Ethylmalonyl-CoA mutase, (2*S*)-methylsuccinyl-CoA dehydrogenase, and mesaconyl-CoA hydratase define distinct clades within large enzyme families based on their substrate specificities (Alber et al. 2006; Erb et al. 2008, 2009b; Zarzycki et al. 2008). The B_{12}-dependent (2*R*)-

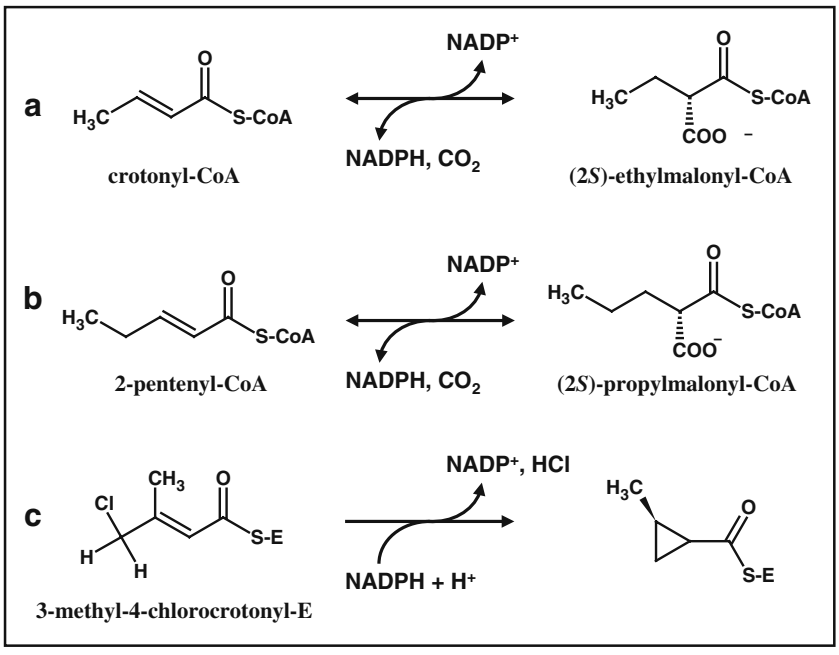

Fig. 4 Unprecedented reactions catalyzed by enzymes related to crotonyl-CoA carboxylase/reductase. **a** Reaction catalyzed by crotonyl-CoA carboxylase/reductase from *R. sphaeroides*, *M. extorquens*, and *Streptomyces coelicolor* (Erb et al. 2007, 2009a). **b** One reaction catalyzed by (chloro)crotonyl-CoA/2-pentenyl-CoA carboxylase/reductase (Sal G) from *Salinospora tropica* (Liu et al. 2009; Eustáquio et al. 2009). **c** Reaction catalyzed by the enoyl-CoA reductase domain of CurF from *Lyngbya majuscula* (Gu et al. 2009)

ethylmalonyl-CoA mutase in particular requires very specific binding of its substrate in order to avoid unwanted side reactions due to its radical-based mechanism (Rétey 1990; Vlasie and Banerjee 2004; Buckel et al. 2006); therefore, ethylmalonyl-CoA mutases can be easily distinguished from other B_{12}-dependent acyl-CoA mutases as they form a very deep branch in a neighbor-joining tree for representatives of this family (Erb et al. 2008). The characterization of new members of B_{12}-dependent acyl-CoA mutases ((2*R*)-ethylmalonyl-CoA mutase), flavin-dependent acyl-dehydrogenases ((2*S*)-methylsuccinyl-CoA dehydrogenase) and *R*-specific hydratases (mesaconyl-CoA hydratase) will allow for a better understanding of substrate recognition by these enzymes. A clear assessment of structure/function relationship will facilitate the discovery of similar enzymes with different traits, such as increased thermostability, also through the analyses of candidate genes in the continuously growing environmental databases. At the same time, it provides a basis for design or discovery of enzymes with novel substrate and cofactor specificities.

Crotonyl-CoA carboxylase/reductase and (3*S*)-malyl-CoA thioesterase on the other hand catalyze unprecedented reactions as members of enzyme superfamilies (Erb et al. 2007, 2009b, 2010). As mentioned above, (3*S*)-malyl-CoA thioesterase belongs to the superfamily of malate synthases that catalyze the Claisen condensation between acetyl-CoA and glyoxylate (Erb et al. 2010). Also part of this family are malyl-CoA lyases and the subunit of citrate lyase that catalyzes the cleavage of an enzyme-bound citryl moiety into oxaloacetate and an enzyme-bound acetyl group (Meister et al. 2005); however, (3*S*)-malyl-CoA thioesterase represents the first described thioesterase of this family (Erb et al. 2010).

Crotonyl-CoA carboxylase/reductase belongs to the superfamily of medium-chain alcohol dehydrogenases and related dehydrogenases/reductases. Members of this family either reduce C=O or C=C bonds. Very surprisingly, crotonyl-CoA carboxylase/reductase was found to catalyze the reductive carboxylation of crotonyl-CoA to (2*S*)-ethylmalonyl-CoA (Erb et al. 2007). The enzyme also catalyzes the reduction of crotonyl-CoA to butyryl-CoA in the absence of CO_2, but with only about one tenth of the maximum rate (Erb et al. 2009b). The complete stereochemical course of both the carboxylation reaction and the reduction reaction catalyzed by the enzyme was elucidated and a reaction mechanism has been proposed (Erb et al. 2009b). A catalyst for the stereoselective carboxylation of an enoyl thioester may even find an application in organic synthesis. Crotonyl-CoA carboxylase/reductase also accepts acrylyl-CoA as a substrate and therefore is able to produce two extender units for modular and iterative polyketide synthases responsible for the synthesis of chemotherapeutic chemicals: (2*S*)-ethylmalonyl-CoA and (2*S*)-methylmalonyl-CoA (Chan et al. 2009). Enzymes related to crotonyl-CoA carboxylase/reductase have been recently shown to also use substrates derivatives, such as chlorocrotonyl-CoA and 2-pentenyl-CoA, or even catalyze an unprecedented dehalogenating cyclopropanation of an enzyme-bound 3-methyl-4-chlorocrotonyl-substrate (Fig. 4; Eustáquio et al. 2009; Liu et al. 2009; Gu et al. 2009). Therefore, the enzyme superfamily of medium-chain alcohol dehydrogenases and related dehydrogenases/reductases can now be extended to include members that catalyze a α-carbon–carbon bond formation coupled to the reduction of an α,β-double bond. Among others, this finding has major implication for broadening the type of elongation units available for polyketide synthases, either by discovery or design.

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