Cellular and Molecular Life Sciences

REVIEW

Structure and function of biotin-dependent carboxylases

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Abstract Biotin-dependent carboxylases include acetyl-CoA carboxylase (ACC), propionyl-CoA carboxylase (PCC), 3-methylcrotonyl-CoA carboxylase (MCC), geranyl-CoA carboxylase, pyruvate carboxylase (PC), and urea carboxylase (UC). They contain biotin carboxylase (BC), carboxyltransferase (CT), and biotin-carboxyl carrier protein components. These enzymes are widely distributed in nature and have important functions in fatty acid metabolism, amino acid metabolism, carbohydrate metabolism, polyketide biosynthesis, urea utilization, and other cellular processes. ACCs are also attractive targets for drug discovery against type 2 diabetes, obesity, cancer, microbial infections, and other diseases, and the plastid ACC of grasses is the target of action of three classes of commercial herbicides. Deficiencies in the activities of PCC, MCC, or PC are linked to serious diseases in humans. Our understanding of these enzymes has been greatly enhanced over the past few years by the crystal structures of the holoenzymes of PCC, MCC, PC, and UC. The structures reveal unanticipated features in the architectures of the holoenzymes, including the presence of previously unrecognized domains, and provide a molecular basis for understanding their catalytic mechanism as well as the large collection of disease-causing mutations in PCC, MCC, and PC. This review will summarize the recent advances in our knowledge on the structure and function of these important metabolic enzymes.

Keywords Fatty acid metabolism · Carbohydrate metabolism · Amino acid metabolism ·

Metabolic syndrome · Obesity · Diabetes · Cancer · Drug discovery · Antibiotics · Propionic acidemia · 3-methylcrotonylglycinuria · Lactic acidemia

Abbreviations

ACC	Acetyl-CoA carboxylase
BC	Biotin carboxylase
BCCP	Biotin carboxyl carrier protein
BT	BC-CT interaction
CT	Carboxyltransferase
GCC	Geranyl-CoA carboxylase
GCD	Glutaconyl-CoA decarboxylase
HCS	Holocarboxylase synthase
MCC	3-methylcrotonyl-CoA carboxylase
MCG	3-methylcrotonylglycinuria
PA	Propionic acidemia
PC	Pyruvate carboxylase
PCC	Propionyl-CoA carboxylase
PT	PC tetramerization
UA	Urea amidolyase
UC	Urea carboxylase
YCC	Acyl-CoA carboxylase (generic name)

Introduction

Biotin-dependent carboxylases are widely distributed in nature and can be found in bacteria, archaea, fungi, algae, plants, and animals. They have crucial roles in the metabolism of fatty acids, amino acids, and carbohydrates [1–4]. In some microorganisms, these enzymes also have important functions in CO_2 fixation [5, 6], methanol assimilation [7], acetyl-CoA assimilation [8–10], 3-hydroxypropionate assimilation [11], mycolic acid and methyl-branched fatty

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acid biosynthesis [12], polyketide biosynthesis [13], metabolism of terpenoids [14], and the utilization of urea as a nitrogen source [15, 16].

Biotin-dependent carboxylases have two distinct enzymatic activities and catalyze their reactions in two steps [17, 18]. First, a biotin carboxylase (BC) component catalyzes the MgATP-dependent carboxylation of the N1' atom of the biotin cofactor, using bicarbonate as the CO_2 donor (Fig. 1a). Biotin is covalently linked through an amide bond to a lysine side chain in the biotin carboxyl carrier protein (BCCP) component. In the second step of the reaction, a carboxyltransferase (CT) component catalyzes the CO_2 transfer from carboxybiotin to the acceptor of the carboxyl group (referred to as the substrate here). Most of the substrates are coenzyme A (CoA) esters of organic acids, and the site of carboxylation is on the α carbon of a saturated acid (acetyl-CoA, propionyl-CoA) or the γ carbon of an α-β unsaturated acid (3-methylcrotonyl-CoA, geranyl-CoA) (Fig. 1b). In addition, small compounds can also serve directly as the substrate, such as pyruvate and urea (Fig. 1b). Especially, the urea substrate is unique in that carboxylation occurs on a nitrogen atom.

Depending on the organism and the enzyme, the BC, BCCP, and CT components can be separate subunits or part of a multi-domain protein (Fig. 2). In addition, while the BC and BCCP components are conserved among these enzymes, the sequence and structure of the CT component depend on the chemical nature of the substrate. The CT components of enzymes that carboxylate CoA esters share recognizable sequence conservation because they recognize the CoA segment (Fig. 2). On the other hand, the CT component that carboxylates pyruvate or urea is entirely different.

Biotin must visit both the BC and CT active sites during catalysis by biotin-dependent carboxylases. A swinging-arm model had been the accepted mechanism for this translocation. The connection between biotin and BCCP contains eight methylene groups and ten rotatable single bonds, and is likely to be rather flexible (Fig. 1a). When fully extended, this flexible arm can approach a length of $\sim$16 Å (the distance from the N1' atom of biotin to the Cα atom of the lysine). Therefore, it may be expected that biotin can translocate by up to $\sim$30 Å on this swinging arm [19]. However, recent structures on the holoenzymes of pyruvate carboxylase [20, 21], propionyl-CoA carboxylase [22], 3-methylcrotonyl-CoA carboxylase [23], and urea carboxylase [24] showed that the distance between the BC and CT active sites ranges between 55 and 85 Å (Fig. 1a). Therefore, the swinging-arm model is not sufficient for biotin to reach both active sites, and hence the BCCP domain must also translocate during catalysis. This is referred to as the "swinging-domain" model (Fig. 1a).
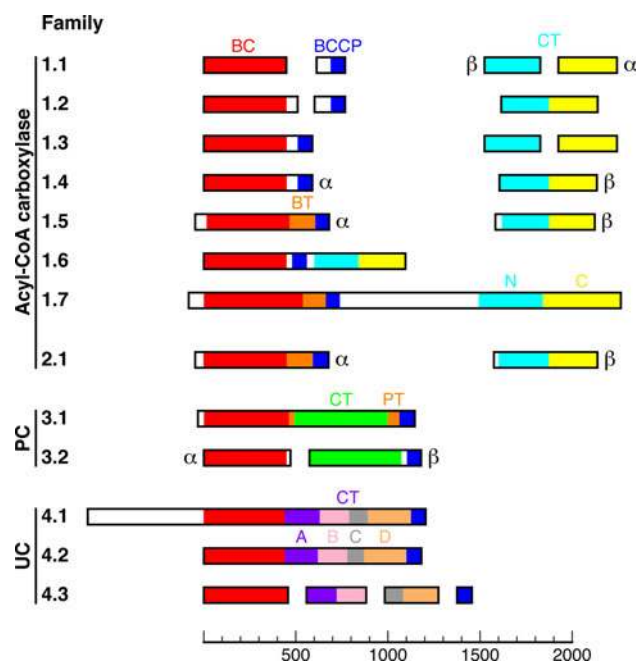
Besides biotin-dependent carboxylases, two other classes of biotin-dependent enzymes exist in nature. Biotin-
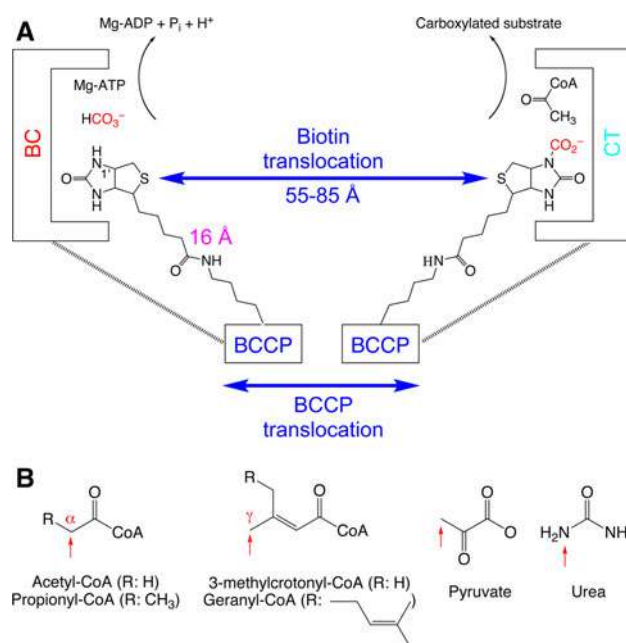
Fig. 1 The biochemical activity of biotin-dependent carboxylases. **a** Biotin is carboxylated in the active site of the biotin carboxylase (BC) component, using bicarbonate as the CO_2 donor with concomitant ATP hydrolysis. Biotin then translocates to the carboxyltransferase (CT) active site, where the CO_2 is transferred to the acceptor (substrate, acetyl-CoA is shown as an example). In the swinging-arm model, biotin itself translocates between the BC and CT active sites, while the biotin-carboxyl carrier protein (BCCP) component remains stationary. The longest distance between the N1' of biotin and the Cα atom of the covalently linked lysine residue is $\sim$16 Å, giving the swinging arm a maximal reach of $\sim$30 Å. This is significantly shorter than the distances observed in the holoenzymes so far, between 55 and 80 Å. Therefore, the BCCP domain must also translocate during catalysis, and this is known as the swinging-domain model. **b** The substrates of biotin-dependent carboxylases. The sites of carboxylation are indicated with the *red arrows*

dependent decarboxylases couple the decarboxylation of organic acids (possibly as CoA esters) to sodium ion transport in anaerobes [25–30], while the biotin-dependent transcarboxylase of *Propionibacterium shermanii* transfers the carboxyl group from methylmalonyl-CoA to pyruvate [31–33]. These enzymes are distinct from the biotin-dependent carboxylases in that they lack the BC component. They will not be specifically described further here.

Biotin-dependent carboxylases were first discovered more than 50 years ago. They have been studied intensively due to their important metabolic functions, and also feature prominently in most biochemistry textbooks. Over the past few years, there have been significant advances in our understanding of these enzymes, especially from the first structural information on several of the holoenzymes [20–24]. This review summarizes our current knowledge on the structure and function of biotin-dependent carboxylases, with emphasis on recent studies (over the past 5 years). Space limitations unfortunately prevent detailed

Fig. 2 Classification of biotin-dependent carboxylases. These enzymes are classified into four major collections and 13 different families. Domains with sequence homology to each other are shown with the *same color*. More detailed descriptions of the families can be found in the text, and common examples of acyl-CoA carboxylase family members include *E. coli* ACC (family 1.1), eukaryotic ACC (family 1.7), PCC (family 1.5), and MCC (family 2.1). The proteins are drawn to size, which is indicated with the *scale bar* at the bottom (in number of residues)

descriptions of results from earlier studies or the citation of those primary publications. These results are summarized in the many reviews that have been published in the previous years, which are cited throughout this manuscript.

Classification of biotin-dependent carboxylases

Biotin-dependent carboxylases can be classified first based on the identity of the substrate that becomes carboxylated. This is dictated by the CT component, which can be highly distinct among these enzymes (Fig. 2). These enzymes can then be further classified by how the BC, BCCP, and CT components are organized in them (Fig. 2). The different components may exist as separate subunits, often in bacteria, or they can be fused together into a large, multidomain enzyme in eukaryotes (Fig. 2). Various intermediates between these two extremes have also been observed in nature (Fig. 2).

The largest collection of biotin-dependent carboxylases uses CoA esters of (small) organic acids as the substrate; hence they are ac<u>y</u>l-<u>C</u>oA <u>c</u>arboxylases (YCCs) in general. These enzymes can have distinct substrate preferences, such as acetyl-CoA carboxylase (ACC), propionyl-CoA

carboxylase (PCC), 3-methylcrotonyl-CoA carboxylase (MCC), and geranyl-CoA carboxylase (GCC), although some of them may have a wider collection of substrates, for example enzymes that are active toward both acetyl- and propionyl-CoA (ACC/PCC). In addition, some of these enzymes can be identified based on genome sequences but have not been studied in detail biochemically, and their substrate preference is currently not known. They are referred to generically as YCCs here. Acyl-CoA carboxylases have also been referred to as ACCases [12], although ACCs are sometimes called ACCases as well.

The CT components of the acyl-CoA carboxylases share readily detectable amino acid sequence conservation, because they all recognize CoA esters. It was generally believed that these enzymes have the same organization of their components. However, the recent structure of the MCC holoenzyme indicates that there may be two distinct lineages of these carboxylases [23]. Therefore, the acyl-CoA carboxylases have been divided into two separate collections, one including ACC, PCC, ACC/PCC, and most of the other YCCs (families 1.1 through 1.7), while the other consists of MCC and GCC (family 2.1) (Fig. 2). The different families of biotin-dependent carboxylases are described briefly next and in more detail in the later sections.

Family 1.1 includes the bacterial ACC enzymes (Fig. 2). They contain four subunits, with a BC subunit (~ 50 kD), a BCCP subunit (~ 17 kD), and two subunits (α and β, ~ 33 kD each) for the CT activity. These enzymes are also referred to as the multi-subunit ACCs [2]. The holoenzyme has the stoichiometry $(BC)_2(BCCP)_4(CT\alpha, CT\beta)_2$, but it is generally unstable and readily dissociates during purification. These enzymes are also found in the chloroplasts of many plants, reflecting the evolutionary origin of this organelle.

Family 1.2 includes the ACC/PCC enzymes from *M. sedula* [34], *A. fulgidus*, and other archaeal organisms, but it appears to be absent in *M. jannaschii*. Compared to the multi-subunit bacterial ACCs, the two CT subunits are fused into a single protein (~ 60 kD), while the BC and CT components remain as separate subunits. The holoenzyme has the stoichiometry $(BC)_4(BCCP)_4(CT)_4$.

Family 1.3 represents another possible way of fusing the different components of the multi-subunit bacterial ACCs, where the BC and BCCP subunits are fused together but the CT subunits remain separate. An actual example of such an enzyme has not been identified as yet, but its existence may be expected.

Family 1.4 includes the ACC/PCC/acyl-CoA carboxylases (YCCs) in *S. coelicolor*, *M. tuberculosis*, and other organisms [12]. The BC and BCCP components are fused into a single protein (α subunit, ~ 65 kD), and the two CT subunits are also fused (β subunit, ~ 60 kD). The

holoenzyme has the stoichiometry $\alpha_6\beta_6$ or $\alpha_2\beta_2$ [12]. Another subunit (ε) is required for some of these enzymes to achieve maximal activity [12].

Family 1.5 includes the PCCs from various organisms. The difference to family 1.4 is that the BC-BCCP fusion (the α subunit, $\sim$73 kD) also contains a BT domain, which mediates the interactions between the BC (in the α subunit) and CT (the β subunit, $\sim$55 kD) domains [22]. This domain is likely absent in family 1.4, as the linker between BC and BCCP is too short to accommodate the residues of the BT domain in that family. However, it is possible that family 1.4 contains a modified version of the BT domain, for example a structure similar to that of the PT domain in pyruvate carboxylase (family 3.1) [21]. PCC holoenzyme is a 750 kD $\alpha_6\beta_6$ dodecamer.

Family 1.6 includes the acyl-CoA carboxylases from *P. aeruginosa* and some other bacterial organisms. These enzymes can be readily identified based on sequence searches, but they have not been characterized biochemically (hence they are called YCCs here). All four subunits of the multi-subunit bacterial ACCs are fused together in these enzymes, giving rise to a multi-domain protein of $\sim$120-kD molecular weight.

Family 1.7 includes the ACCs from most eukaryotic organisms. These proteins can be thought of as being made of three parts of equal lengths. The N-terminal one-third of the proteins contains the BC, BCCP, and possibly a BT domain, and the C-terminal one-third contains the CT activity. The middle one-third is unique to eukaryotic ACCs and has no other close sequence homologs in the database. The structure and function of this part of eukaryotic ACCs is currently not known. These enzymes are generally referred to as the multi-domain ACCs, with $\sim$250-kD molecular weight, and they function as 500-kD homodimers and possibly higher oligomers. In light of the multi-domain bacterial YCCs of family 1.6, it is probably more appropriate to refer to family 1.7 as the multi-domain eukaryotic ACCs. The multi-domain bacterial YCCs lack the middle one-third of the multi-domain eukaryotic ACCs, and they may lack the BT domain as well (Fig. 2).

Family 2.1 includes the MCCs and GCCs from various organisms. The overall domain organization of these enzymes appears quite similar to that of PCCs (family 1.5). However, the crystal structure of MCC indicates a large difference in the architectures of the β subunit and the holoenzyme [23], and therefore it has been placed into a separate family. GCC is assigned to this family based on sequence conservation and the similarity of its substrate to that of MCC (Fig. 1b). These holoenzymes are also 750-kD $\alpha_6\beta_6$ dodecamers.

Besides the acyl-CoA carboxylases, two other major collections of biotin-dependent carboxylases use small organic compounds as substrates, specifically pyruvate and urea (Fig. 1b). Family 3.1 includes the pyruvate carboxylases (PCs) that are present in most organisms, from bacteria to humans. It is a single-chain, multi-domain enzyme of $\sim$130 kD and functions only as a 500-kD tetramer. Besides the BC, CT, and BCCP domains, structural studies have revealed the presence of another domain, known as the PT (PC tetramerization) or the allosteric domain, in these enzymes [20, 21].

Family 3.2 includes the two-subunit form of PCs. They are found in some bacteria (such as *P. aeruginosa*) as well as archaea (*M. jannaschii*). The α subunit contains the BC component ($\sim$52 kD), and the β subunit contains CT and BCCP ($\sim$65 kD). The stoichiometry of the holoenzyme is $\alpha_4\beta_4$. Whether the PT domain also exists in these enzymes is currently not known.

Family 4.1 includes the urea carboxylase (UC) found in yeast and some other fungal organisms. It is a single-chain, multi-domain enzyme of $\sim$200 kD, with an allophanate hydrolase (also known as the amidase) domain fused at the N-terminus. The entire enzyme is known as the urea amidolyase (UA). The CT component consists of four subdomains (A, B, C, D) and is distinct from that in acyl-CoA carboxylase and PC [24]. UC functions as a monomer.

Family 4.2 includes the UCs found in many bacterial organisms, some fungal species, and green algae. They are different from family 4.1 in that they lack the allophanate hydrolase domain, and hence they are somewhat smaller, $\sim$130 kD.

Family 4.3 includes the UC found in *P. aeruginosa* and possibly other bacterial organisms. It is a multi-subunit form of the enzyme, with a BC subunit ($\sim$50 kD), BCCP subunit ($\sim$10 kD), and two subunits for the CT activity ($\sim$35 kD, each containing two domains of the multi-domain form of the enzyme).

Overall, four major collections of biotin-dependent carboxylases can be identified based on current biochemical, sequence, and structural information, which can be further divided into 13 families. The availability of genome sequences has enabled the identification of all biotin-dependent enzymes in many organisms, which also provides insight into the evolution of these enzymes [35, 36]. An inventory of such enzymes can now be compiled for these organisms (Table 1). *E. coli* has only one biotin-dependent enzyme, a multi-subunit bacterial ACC (family 1.1, Fig. 2). The yeast *S. cerevisiae* has two multi-domain eukaryotic ACCs (family 1.7), two PCs (family 3.1), and one urea amidolyase (family 4.1), while most other fungal species has only one ACC, one PC, and one UA. There are five biotin-dependent carboxylases in humans, ACC1, ACC2, PCC, MCC, and PC. The structure and function of representative enzymes in these different families will be described in more detail below.

Table 1 Inventory of biotin-dependent carboxylases in some common organisms

Organism	Biotin-dependent carboxylase (family number)
Bacteria	
Escherichia coli K-12	Multi-subunit ACC (1.1)
Pseudomonas aeruginosa PAO1	Multi-subunit ACC (1.1), multi-domain YCC (1.6), MCC (2.1), GCC (2.1), two-subunit PC (3.2), multi-subunit UC (4.3)
Ruegeria pomeroyi DSS-3	Multi-subunit ACC (1.1), PCC (1.5), MCC (2.1), GCC (2.1), PC (3.1)
Cupriavidus metallidurans CH34	Multi-subunit ACC (1.1), multi-domain YCC (1.6, 2 copies), MCC (2.1), GCC (2.1), PC (3.1)
Deinococcus radiodurans R1	Multi-subunit ACC (1.1), multi-domain YCC (1.6), PCC (1.5), MCC (2.1)
Streptomyces coelicolor A3(2)	Two-subunit YCC (1.4), PCC (1.5, 2 copies), MCC (2.1, 2 copies), PC (3.1)
Staphylococcus aureus	Multi-subunit ACC (1.1), PC (3.1), multi-subunit UC (4.3)
Bacillus subtilis	Multi-subunit ACC (1.1), three-subunit YCC (1.4), PC (3.1)
Archaea	
Methanocauldococcus jannaschii	Two-subunit PC (3.2)
Metallosphaera sedula	Two subunit YCC (1.4)
Fungi	
Saccharomyces cerevisiae	Eukaryotic ACC (1.7, 2 copies), PC (3.1, 2 copies), UC (4.1)
Kluyveromyces lactis	Eukaryotic ACC (1.7), PC (3.1), UC (4.1)
Schizosaccharomyces pombe	Eukaryotic ACC (1.7), PC (3.1)
Animals	
Xenopus laevis	Eukaryotic ACC (1.7), PCC (1.5), MCC (2.1), PC (3.1)
Danio rerio	Eukaryotic ACC (1.7), PCC (1.5), MCC (2.1), PC (3.1)
Mus musculus	Eukaryotic ACC (1.7, 2 copies), PCC (1.5), MCC (2.1), PC (3.1)
Homo sapiens	Eukaryotic ACC (1.7, 2 copies), PCC (1.5), MCC (2.1), PC (3.1)

Acetyl-CoA carboxylase (ACC)

Biological functions of ACC

ACC catalyzes the conversion of acetyl-CoA to malonyl-CoA, which serves many functions [37]. It provides the two-carbon building block for fatty acid biosynthesis in most living organisms, and is also used for polyketide biosynthesis in some organisms (Fig. 3). In mammals, ACC1 (also known as ACCα) is cytoplasmic and catalyzes the rate-limiting and committed step of long-chain fatty acid biosynthesis in liver, adipose, and other lipogenic tissues. In the yeast *S. cerevisiae*, the cytosolic ACC1 is essential for viability. There is also a mitochondrial form of the enzyme (known as HFA1), which is important for growth on lactate or glycerol as carbon source and for fatty acid (especially lipoic acid) biosynthesis in this organelle [38–40]. However, this isoform is unique to *S. cerevisiae* and is not present in other fungal organisms, although ACC activity has been reported in the mitochondria of some plants. Malonyl-CoA for fatty acid biosynthesis in the mitochondria of other organisms may be produced by malonyl-CoA synthetase [41, 42] and/or PCC working on acetyl-CoA as the substrate.

Mammals carry a second isoform of ACC, ACC2 (also known as ACCβ), which is highly conserved with ACC1, with 73 % amino acid sequence identity between human ACC1 and ACC2. ACC2 is associated with the outer mitochondrial membrane through a 140-residue segment at the N-terminus that is absent in ACC1, the first 20 residues of which are highly hydrophobic [43]. However, most of the ACC2 protein faces the cytosol (Fig. 3). This isoform is primarily expressed in heart and muscle tissues, as well as liver. The malonyl-CoA product is a potent inhibitor of carnitine palmitoyltransferase I (CPT-I), the crucial enzyme for the transport of long-chain fatty acyl-CoAs into the mitochondria for β-oxidation (Fig. 3) [44, 45]. There are differences in the expression patterns of human and rodent ACCs [46]. In addition, a form of ACC2 lacking the N-terminal mitochondrial targeting segment is expressed at high levels in white adipose tissue in humans and can participate in de novo lipogenesis [47].

Single-nucleotide polymorphisms (SNPs) in ACC1 and ACC2 are associated with hypertriglyceridemia and hypercholesterolemia, respectively, in patients taking antipsychotic drugs [48]. Polymorphisms in the promoter or the coding region of the *ACC1* gene is linked to fatty acid composition in porcine meat [49], fatness traits in

Fig. 3 Functions of biotin-dependent carboxylases in mammals. Reactions involving the five biotin-dependent carboxylases are shown in *blue*. Selected intermediates of the TCA cycle (*green*) are shown. Glutamine can also be used for anaplerosis, especially in some cancer cells. *Dashed arrows* indicate pathways with more than one step. In other organisms, the biotin-dependent carboxylases have similar functions (with the exception of ACC2), and they are also involved in additional cellular processes

chickens [50], fatty acid composition in beef [51], and milk production traits in goats [52].

The activity of both ACC1 and ACC2 in mammals are stimulated by citrate, inhibited by long-chain saturated acyl-CoA, and inactivated by phosphorylation [53], especially AMP-activated protein kinase (AMPK, at Ser80 in ACC1, Ser222 in ACC2) and cAMP-dependent protein kinase (protein kinase A, at Ser1201 in ACC1). Steady-state kinetic studies show that citrate increases the k_{cat} (threefold) and k_{cat}/K_m (tenfold) of the ATP and acetyl-CoA substrates for human ACC2, with a K_a of ~ 0.5 mM [54]. In comparison, another report shows that human ACC2 is activated 1,000-fold by citrate, while ACC1 is activated only fourfold [55]. The data also suggest two binding sites for citrate, a higher-affinity ($K_d \sim 1$ mM) activating site and a lower-affinity ($K_i \sim 30$ mM) inhibitory site. A cytosolic protein MIG12 (~ 20 kD) promotes the polymerization and stimulation of ACC1 by citrate, and is incorporated into the ACC1 polymer [56]. The activity of MIG12 is negatively regulated by forming a complex with another protein, Spot 14 (~ 17 kD) [57].

Human ACC1 interacts with BRCA1 in a phosphor-ylation-dependent manner, which reduces the activity of ACC1 [58, 59]. The tandem BRCT domains of BRCA1 recognize the phosphorylated Ser1263 residue of ACC1, in a (pS)PTF motif, and the structure of this complex has been reported [60]. The phosphorylation of Ser1263 is regulated by the cell cycle, possibly through a proline-directed protein kinase such as the cyclin-dependent kinase (CDK).

In plants, plastid ACC (ACC1) produces malonyl-CoA for the biosynthesis of long-chain fatty acids, while cyto-plasmic ACC (ACC2) is important for secondary metabolism, including the synthesis of very long-chain fatty acids, flavonoids, cuticular waxes, and other com-pounds, and for proper embryonic development [61–63]. Plastid ACC in dicots is a multi-subunit enzyme, similar to the multi-subunit bacterial ACCs [64, 65]. On the other hand, the plastid ACC in grasses is a single-chain, multi-domain enzyme, similar to the multi-domain eukaryotic ACCs and arising through gene duplication of the cytosolic ACC. This multi-domain plastid ACC is the target of three classes of herbicides (see below). The BCCP subunit of the

multi-subunit plastid ACC of *Arabidopsis thaliana* forms a complex with the signaling protein PII, which reduces the V_{max} of ACC but does not affect its K_m for acetyl-CoA [66]. 2-oxoglutarate, pyruvate or oxaloacetate can disrupt this inhibition. A second isoform of BCCP (BCCP2) in *A. thaliana* plastids is expressed at much lower levels and cannot rescue the lethal phenotype of BCCP1 null mutants [67]. Two additional BCCP-like proteins lack the biotinylation motif (MKM) but co-purify with the 1–2 MDa ACC complex from *A. thaliana* chloroplasts [68].

ACC has important functions in other organisms as well. In the apicomplexan pathogen *Toxoplasma gondii*, ACC1 in the apicoplast is required for fatty acid biosynthesis [69]. In *Trypanosoma brucei*, RNAi knockdown of ACC results in reduced virulence of the pathogen, and lower fatty acid elongation in procyclic forms [70]. *Aedes aegypti* mosquitos with deficient ACC activity have reduced lipogenesis and produce significantly fewer eggs [71]. Human cytomegalovirus (HCMV) upregulates the expression and activity of ACC1 in infected cells, and inhibition of ACC1 attenuates viral replication [72]. In archaea and some bacteria, ACC (and PCC) activity is important for CO_2 fixation (possibly coupled with ammonia oxidation) [5, 6].

ACCs as drug discovery targets

The crucial roles of ACCs in fatty acid metabolism make them attractive targets for drug discovery against a variety of human diseases, including bacterial infections, fungal infections, type 2 diabetes, cancer, artherosclerosis, and others [2, 3, 73–77]. Besides microbial infections, many of the other diseases are also manifestations of the metabolic syndrome, which is linked to the current obesity epidemic [78–80]. It has been projected that $\sim 50\%$ of the adult population in the US will be obese by the year 2030 [81], indicating a pressing need for new therapeutic agents and modalities in this area.

The importance of ACCs as targets for drug discovery against the metabolic syndrome was first validated by observations on the ACC2 knockout mice [82–86]. These mice have elevated fatty acid oxidation, increased energy expenditure, reduced body fat and body weight, improved insulin sensitivity, smaller heart size but with normal function, and normal life span and fertility. The animals have increased food intake (hyperphagia), due to reduced malonyl-CoA levels in the brain [87, 88]. In comparison, a null mutation in the *ACC1* gene causes embryonic lethality in mice [89]. ACC1 knockout in the liver in mice reduced hepatic lipid accumulation but did not disturb glucose homeostasis [90]. ACC1 knockout in the adipose tissues in mice reduced lipid accumulation in these tissues, but also caused prenatal growth retardation and impaired bone development [91].

Knockdown of ACC1 and ACC2 expression with antisense oligonucleotides confirms the beneficial effects of inhibiting these enzymes [92]. Mice on a high-fat diet show improved peripheral insulin sensitivity after treatment with the potent ACC inhibitor soraphen A [93]. Downregulation of ACC2 activity by the adipokine CTRP1, through AMPK-mediated phosphorylation, leads to elevated fatty acid oxidation and reduced adiposity in mice [94]. On the other hand, overexpression of ACC2 is associated with increased production of proinflammatory cytokines in a human renal cell, which can be reversed by inhibition of p38 MAP kinase [95]. This may be a mechanism for diabetic nephropathy development. An SNP in ACC2 is associated with type 2 diabetic nephropathy and proteinuria [96–98], while another SNP in ACC2 is linked to increased risk for metabolic syndrome [99].

Two recent reports have failed to observe the beneficial effects of ACC2 knock out or down regulation, as elevated fatty acid oxidation did not change energy expenditure or adiposity [100, 101]. Another study failed to demonstrate a correlation between ACC2 phosphorylation (down regulation) and fatty acid oxidation in skeletal muscle [102]. Some of these differences could be due to variations in the genetic background of the mice or the strategies that were used to create the knockout mice [86, 103, 104].

A large number of potent (nanomolar) inhibitors have been reported against human ACCs [105–116]. Some of these compounds have nearly equal activity against both isoforms (isoform nonselective), while others are more selective toward ACC2. They can reduce tissue malonyl-CoA levels, inhibit fatty acid biosynthesis, enhance fatty acid oxidation, reduce plasma triglyceride levels, improve insulin sensitivity in cells or in rodent models. However, long-term treatment with an inhibitor of both ACC1 and ACC2 did not lead to sustained reduction in hepatic triglyceride levels or body weight in rodent models, although the compound was able to stimulate fatty acid oxidation [117]. Long-term down-regulation of ACC1 activity in β-cells leads to reduced glucose-stimulated insulin secretion [118], indicating that ACC2 inhibition may be the more desirable approach for diabetes therapy.

Recent studies suggest that ACCs are also attractive targets for anti-cancer agents. ACC is over-expressed in liver, prostate, breast, and other cancers [119–122], and RNAi knockdown or chemical inhibition of ACC1 leads to growth inhibition and apoptosis [111, 123–125] as well as reduced tumor cell invasion [126]. AKR1B10 (aldo–keto reductase family 1 B10) is overexpressed in some cancer cells and can form a complex with ACC1 and stabilize its cellular levels [127], consistent with the importance of ACC1 in cancer. As discussed earlier, ACC1 activity is downregulated through a phosphorylation-dependent interaction with BRCA1 [58, 59].

Plastid multi-domain eukaryotic ACCs from grasses are the targets of three classes of commercial herbicides, aryloxyphenoxypropionates (APPs or FOPs), cyclohexanediones (CHDs or DIMs), and pinoxaden [128–131]. FOP and DIM inhibitors have been used in the field for more than 30 years, and resistance mutations have been reported against them [132–136]. Pinoxaden was introduced in 2006, but resistance grasses were already present, likely due to cross resistance to the FOPs and/or DIMs [137, 138]. Additional pinoxaden resistance mutations have also been reported [136, 139]. A herbicide-resistant green foxtail plant has higher fitness than the wild-type [140]. Dimeric cyclohexanedione compounds also have activity against the malaria parasite *Plasmodium falciparum* [141].

Potent inhibitors that target the BC activity of bacterial ACCs have been developed, with potential use as antibiotics [142–146]. These compounds bind selectively to the ATP binding site of BC, and the initial leads were identified by directed high-throughput screening, virtual screening, and fragment-based approaches. For the CT activity, the natural products moiramide and andrimid are known inhibitors [146]. A single-site mutation (M203L) in the β subunit of *E. coli* CT produces a fivefold resistance to andrimid [147]. Components of cinnamon oil are also inhibitors of CT activity, partly explaining the antibacterial effect of cinnamon bark [148].

Structures of ACC BC component

The ACC holoenzymes have been difficult to study at the structural level. The multi-subunit bacterial ACCs dissociate readily during purification, while the multi-domain eukaryotic ACCs are exceptionally large ($\sim$250-kD monomers). On the other hand, using the divide-and-conquer approach, structural information has been obtained for the BC, BCCP, and CT components of both bacterial and eukaryotic ACCs. The structure of a bacterial BC subunit (from *E. coli* ACC) was first reported in 1994 [149], and the structure of a eukaryotic BC domain (from yeast ACC) was first reported in 2004 [150]. Currently, a large number of structures are available for bacterial and eukaryotic BC components [143, 151–157].

The BC structure contains three domains, A, B, and C domains (Fig. 4a). Residues in domains A and C form the active site. Domain B undergoes a large conformational change to close over the active site during catalysis. Eukaryotic BC domain contains several inserted segments, especially at the N-terminus and between domains A and B (the AB linker), which explains its larger size ($\sim$550 residues) compared to bacterial BC subunits ($\sim$450 residues) (Fig. 4b).

The structure of *E. coli* BC in a pentary complex with the substrates MgADP, bicarbonate, and biotin provides detailed insights in the catalysis by this enzyme (Fig. 4a) [154]. The substrates are recognized by an intricate network of ionic, hydrogen-bonding and van der Waals interactions (Fig. 4c). One of the oxygen atoms of bicarbonate is poised to initiate the nucleophilic attack on ATP to form the carboxyphosphate intermediate, and Glu296 is the general base that extracts the proton from bicarbonate. The orthophosphate (PO_4^{3-}) derived from the decomposition of carboxyphosphate is the general base that extracts the proton on the N1$'$ atom of biotin [17], and Arg338 stabilizes the resulting enolate biotin intermediate for the carboxylation reaction.

Mutation of residues in the BC active site (including Glu296 and Arg338) confirms their importance for catalysis. Mutation of Gly165 and/or Gly166 in the glycine-rich loop in domain B and near the phosphates of ATP has no effect on the k_{cat} but leads to a 40-fold increase in K_m for ATP [158]. The catalytic activity of *E. coli* BC can be inhibited by ATP at high concentrations (substrate inhibition) and other nucleotides [157]. A second ADP molecule can be bound in the BC active site, with its phosphate groups occupying the binding sites of bicarbonate and biotin.

Escherichia coli BC is a dimer (Fig. 4a), and studies suggest that catalysis at the two active sites of the dimer may be coupled [159]. A chimeric mutant where one of the active sites of the dimer is knocked out is essentially inactive. However, each active site of the dimer is located $\sim$25 Å away from the dimer interface, with no contributions from residues in the other monomer (Fig. 4a). It is postulated that there may be long-range communications between the two active sites, through the dimer interface. The two monomers may undergo alternating catalysis, with one monomer binding substrates and catalyzing turnover and the other releasing products. Half-site reactivity of the BC subunit has also been proposed [152, 160], although no cooperative behavior is observed from kinetic studies.

Mutations in the dimer interface of *E. coli* BC can produce mutants that are monomeric in solution [161]. These mutants are still catalytically active, indicating that dimerization is not absolutely required for BC catalysis in vitro. On the other hand, dimerization of BC is required in vivo, possibly for the assembly of the ACC holoenzyme [162].

The polyketide natural product soraphen A is an allosteric inhibitor of eukaryotic ACC, binding to the BC domain using the equivalent surface area as that for dimerization of *E. coli* BC (Fig. 4b) [150]. Conformational differences in this region of eukaryotic BC allow soraphen A binding but disallow dimerization. The BC domain of eukaryotic ACC is monomeric in solution and is catalytically inactive [163]. A BODIPY-labeled soraphen analog has been developed and can be useful for screening for new

Fig. 4 Structural information on the BC component.
a Structure of the BC subunit dimer (*cyan* and *yellow*) of *E. coli* ACC in complex with MgADP, bicarbonate, and biotin [154]. The twofold axis of the dimer is indicated with the *black oval*. b Structure of the BC domain of yeast ACC in complex with the inhibitor soraphen A (*green*) [161]. The sub-domains of BC are given *different colors*. The bound position of ATP is also shown to indicate the location of the active site. The view is similar to that for the top monomer in a. c *Stereo* figure showing detailed interactions between MgADP, bicarbonate, and biotin with the active site of the *E. coli* BC subunit. Several segments of the protein, including the glycine-rich loop, are omitted for clarity. The structure figures were produced with the program PyMOL (www.pymol.org)
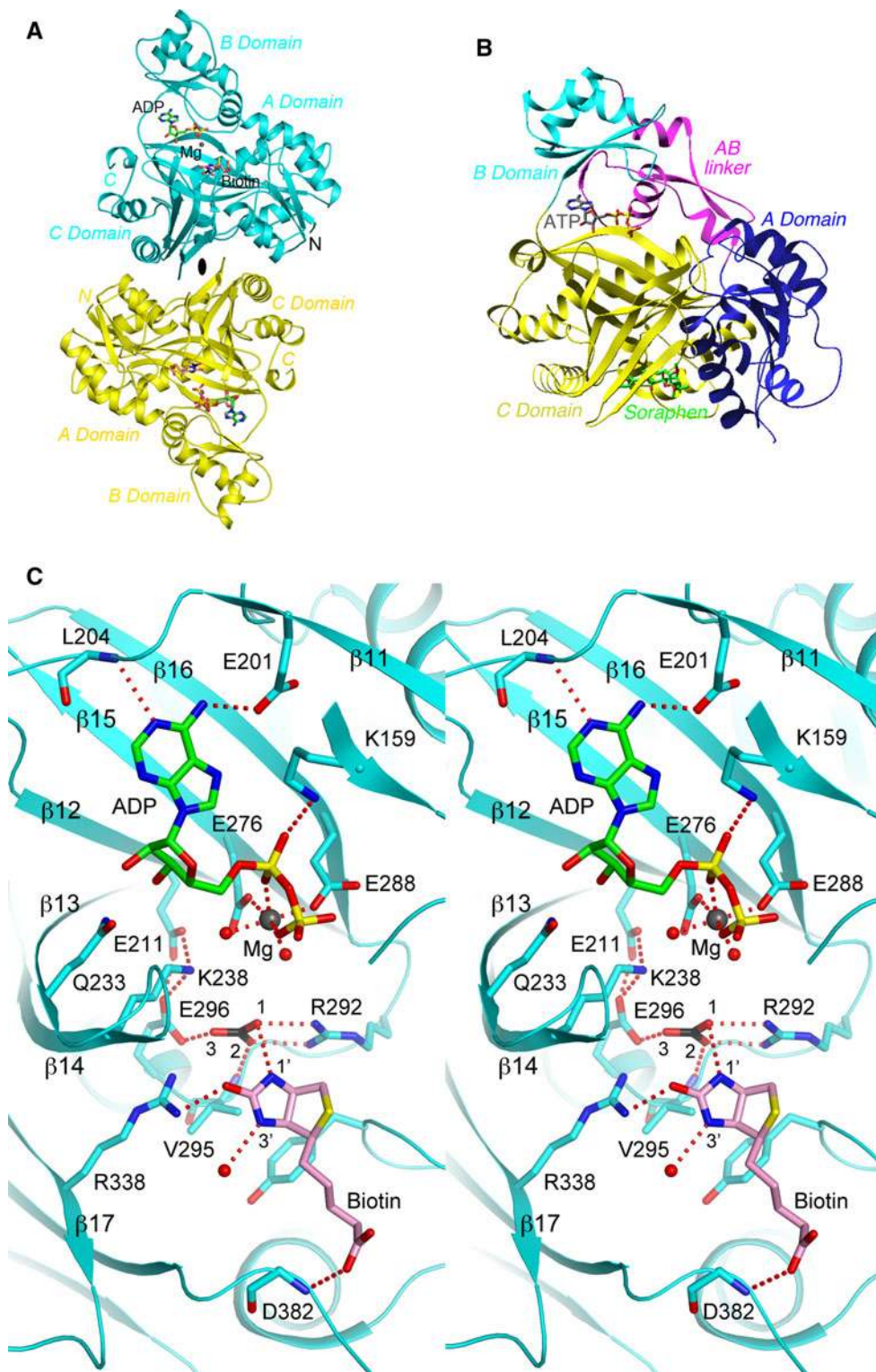
compounds and characterizing inhibitor binding to the BC domain [155].

The AMPK phosphorylation sites in mammalian ACCs are located in the N-terminal extension of the BC domain. The structure of human ACC2 BC domain phosphorylated by AMPK shows that the segment enclosing the phosphorylated Ser222 residue is located in the soraphen-binding site [156]. Therefore, structural information on soraphen A binding and the phosphorylated BC domain supports the hypothesis that this region of BC can

allosterically regulate catalysis, although the exact molecular mechanism for this long-range communication is currently not known.

Structures of ACC BCCP component

The BCCP domain of *E. coli* ACC, including the "thumb" feature, is more flexible in the apo protein [164]. Upon biotinylation, the thumb interacts with biotin [165], leading to its stabilization as well as that of BCCP domain overall [164, 166]. Solution structure of the BCCP domain of human ACC2 is similar to that of BCCP subunit of *E. coli* ACC [165], except that the human protein does not have the thumb feature [167]. As a result, the covalently attached biotin is flexible in human BCCP, while it interacts with the thumb in *E. coli* BCCP. This thumb structure interferes with biotinylation by the human holocarboxylase synthase (HCS), though it has no effect on biotinylation by the *E. coli* BirA enzyme [168]. In comparison, an engineered *E. coli* BCCP lacking the thumb feature, and the BCCP domain of human PCC (which also lacks the thumb feature) can be readily biotinylated by HCS [168].

Structures of ACC CT component

The structure of a eukaryotic CT domain (from yeast ACC) was first reported in 2003 [169], and the structure of bacterial CT subunit was first reported in 2006 [170]. The structure of the CT domain contains two sub-domains, N and C domains (Fig. 5a), which are equivalent to the β and α subunits of bacterial CT (Figs. 2, 5b). Each domain/subunit has the crotonase fold (a β–β–α superhelix). The active site of CT is located at the bottom of a "canyon" in the interface of a dimer of the CT domains (Fig. 5c) or an $\alpha_2\beta_2$ heterotetramer of the bacterial CT subunits. Therefore, CT must dimerize to be catalytically active.

Using the yeast CT domain as a surrogate since crystals of the CT domain of grass plastid ACCs are not yet available, the binding modes of all three classes of herbicides, haloxyfop, tepraloxydim, and pinoxaden, have been determined [171–173]. The compounds inhibit ACC by competing against the binding of the acetyl-CoA substrate. Despite their chemical diversity, the three compounds share two common anchoring points of interactions with the CT domain—a negatively charged oxygen atom that likely mimics the oxyanion in the substrate during catalysis, and a small hydrophobic group (methyl or ethyl) that is probably located in the binding site for the acetyl group. Each compound also establishes unique interactions with the CT domain. Haloxyfop binding requires a large conformational change in the active site, in the dimer interface of the CT domain, while tepraloxydim and especially pinoxaden require much smaller changes. Most of the herbicide resistance mutations are located in or near the binding sites, consistent with their effects on herbicide binding although the exact molecular mechanism is still not fully understood.

Studies using the yeast CT domain also reveal that a class of inhibitors of mammalian ACCs, as exemplified by the CP-640186 compound, block the binding of (carboxy)biotin to the CT-active site (Fig. 5a) [108, 174]. Structures of the human and bovine ACC2 CT domain have been reported at 3.2 Å and 2.4 Å resolution, respectively [112, 175]. In a different approach, nine mutations are introduced into the active site of yeast ACC CT domain to "humanize" this protein [176]. The resulting mutant binds human ACC inhibitors with similar potency as the human CT domain and produces crystals that diffract up to 2.4 Å resolution.

A unique feature of bacterial CT is the presence of a zinc finger in the β subunit (Fig. 5b) [170], which binds the CT mRNA and inhibits its translation, and this inhibition can be reversed by the substrate acetyl-CoA [177]. *E. coli* CT also binds and is inhibited by single-stranded, double-stranded, and hairpin DNA [178].

Propionyl-CoA carboxylase (PCC)

Biological functions of PCC

PCC catalyzes the conversion of propionyl-CoA to *D*-methylmalonyl-CoA. In most organisms, it is crucial for the catabolism of β-branched amino acids (Thr, Val, Ile) and Met, cholesterol side chain, and fatty acids with an odd number of carbon atoms (Fig. 3). Mammalian PCC is localized in the mitochondrial matrix, and is activated by monovalent cations (K^+, NH_4^+, Cs^+) [179–181]. PCC activity is also important for CO_2 fixation in some archaeal organisms [5, 6], methanol assimilation in *Methylobacterium extorquens* [7], acetyl-CoA assimilation in α-proteobacteria [10], 3-hydroxypropionate assimilation in *Rhodobacter sphaeroides* [11], mycolic acid and methyl-branched long-chain fatty acid biosynthesis in *M. tuberculosis* [12], and polyketide biosynthesis in *Streptomyces* and other organisms [13].

Inherited deficiencies in PCC activity in humans are linked to propionic acidemia (PA), with symptoms usually first appearing during the neonatal period, including vomiting, lethargy, ketoacidosis, delayed growth, cardiomyopathy, mental retardation, and death in severe cases. A large number of autosomal recessive mutations in both subunits of PCC have been identified, including missense mutations, a few nonsense mutations, insertions/deletions, and splicing mutations [182–189]. PCC is expressed in the brain and may be important for neurodevelopment, which may be related to the
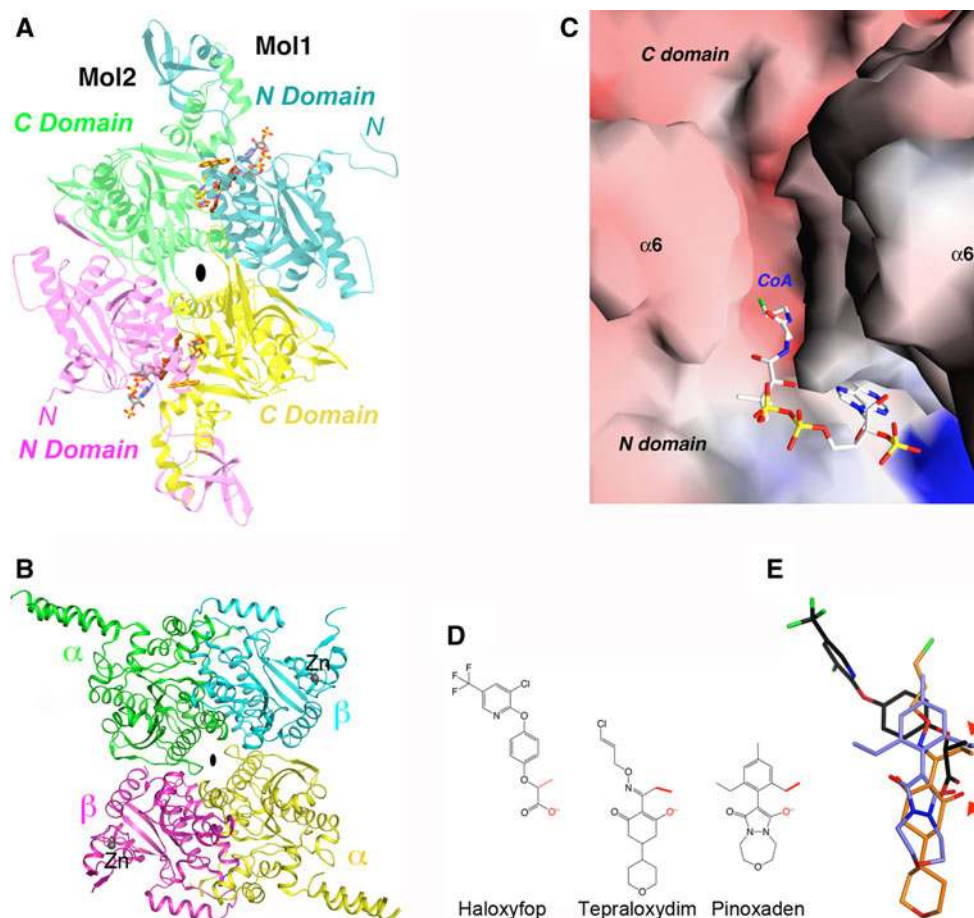
Fig. 5 Structural information on the CT component of acyl-CoA carboxylases. **a** Structure of the CT domain dimer of yeast ACC in complex with tepraloxydim (*brown*) [172]. The N and C domains for monomer 1 are colored in *cyan* and *yellow*, respectively, and those for monomer 2 in *magenta* and *green*. The bound positions of CoA (*gray*) [169] and CP-640186 (*gold*) [174] are also shown. **b** Structure of the CT subunit of *S. aureus* ACC [170]. The zinc ions are shown as spheres (*dark gray*). The view is similar to that for **a**. **c** Molecular surface showing the canyon in the active site region of the CT dimer.

CoA is recognized by the N domain of one monomer, in the bottom half of the canyon. Biotin is recognized by the C domain of the other monomer, in the top half of the canyon. The side chain of Lys1764 has been omitted in this figure. **d** Chemical structures of the herbicides haloxyfop (FOP), tepraloxydim (DIM), and pinoxaden. The two anchoring points of interaction with the CT domain are highlighted by the *red arrows*. **e** Overlay of the binding modes of haloxyfop (*black*), tepraloxydim (*brown*), and pinoxaden (*light blue*)

neurological effects of PCC deficiency [190], including epilepsy [191] and seizure [192].

PCCα$^{-/-}$ mice die of propionic acidemia within 2 days after birth, but they can be rescued by a liver-specific transgene [193]. Anti-sense morpholino oligonucleotides have been used to correct splicing defects in PCC, which restored normal PCC activity in patients' fibroblasts [194]. In another study, a modified U1 snRNA was used to correct a splicing defect, but it did not restore PCC enzymatic activity [195]. Successful rescue of PCC deficiency in mouse models by gene therapy has been demonstrated [196, 197], indicating a potential treatment strategy for PCC deficiency in humans.

Structure of PCC

The crystal structure of the 750-kD $\alpha_6\beta_6$ holoenzyme of a bacterial PCC was reported recently, and a similar structure was observed for human PCC based on a cryo-electron microscopy reconstruction [22]. The holoenzyme obeys 32-point group symmetry, with a central β_6 hexamer core (Fig. 6a) and three α subunits at each end of the β_6 core (Fig. 6b). Therefore, the structure of the PCC holoenzyme consists of four layers, with three subunits in each layer, $\alpha_3-\beta_3-\beta_3-\alpha_3$.

The β_6 hexamer core is in the shape of a short cylinder and there are intimate contacts among the six monomers. The overall structure of this hexamer core is similar to the

Fig. 6 Striking differences in the overall architecture of the holoenzymes of PCC and MCC. **a** Crystal structure of the bacterial PCC holoenzyme [22], viewed down the twofold symmetry axis within a β_2 dimer. The domains are colored as in Fig. 2. The four layers of the structure are indicated. **b** Structure of the PCC holoenzyme, viewed down the threefold symmetry axis. **c** Crystal structure of the *P. aeruginosa* MCC holoenzyme [23], viewed down the twofold axis within a β_2 dimer. **d** Structure of the MCC holoenzyme, viewed down the threefold axis. **e** Structure of the MCC holoenzyme, after a $\sim 60°$ rotation around the vertical axis from **c**. The view is down the twofold axis relating two β_2 dimers. **f** Structure of the MCC holoenzyme, after a $\sim 60°$ counterclockwise rotation from panel D
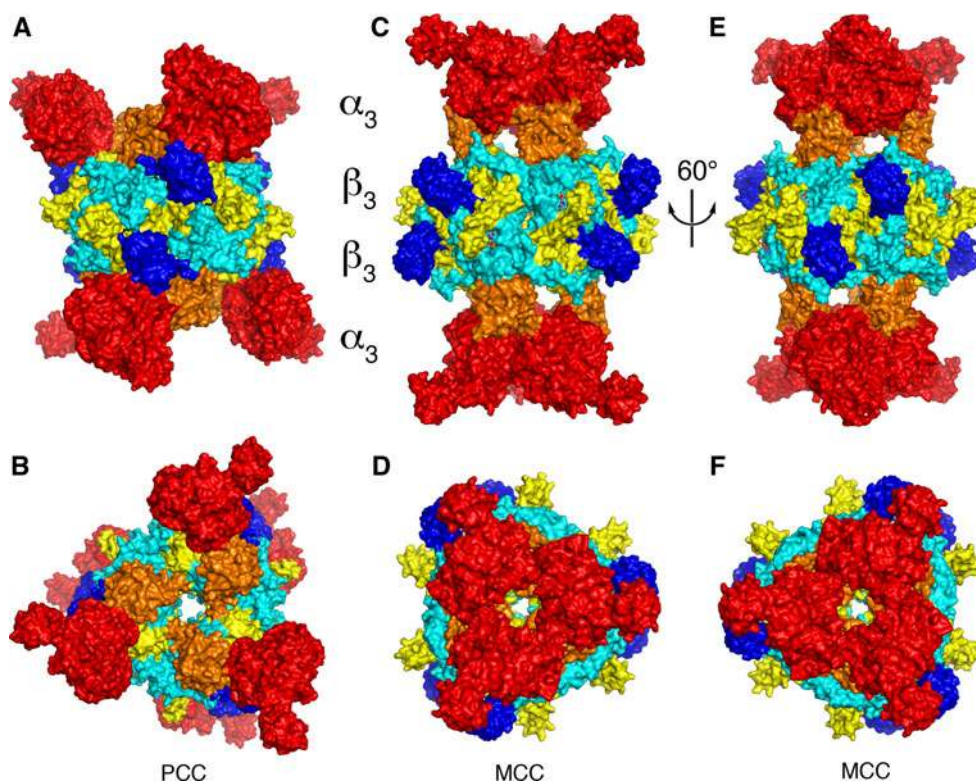
12S subunit of transcarboxylase [31] as well as the β subunit of *S. coelicolor* and *M. tuberculosis* acyl-CoA carboxylases [198–200]. In addition, the β_2 dimer of PCC, with one monomer from each layer of the β_6 hexamer (Fig. 6a), is similar to the dimer of the CT component of ACC (Fig. 5a). The active site is located at the interface of this dimer (Fig. 7a), at the bottom of a deep canyon (Fig. 7b). In the PCC crystal, BCCP-biotin (from the α subunit) is bound in the CT active site, interacting with residues in the C domain of one β subunit (Fig. 7b). The propionyl-CoA substrate is recognized by the N domain of the other subunit (Fig. 7b).

In comparison, the α subunits are arranged as monomers, splayed far apart from each other, and there are essentially no contacts among them in the PCC holoenzyme (Fig. 6b). This is in sharp contrast to the dimeric organization of the BC subunit of bacterial ACCs (Fig. 4a) and the BC domain of pyruvate carboxylase (see below). While the overall structure of the BC domain of the PCC α subunit is similar to that of the bacterial BC subunit, sequence, and conformational differences for those residues in the putative dimer interface in PCC precludes the formation of a similar dimer.

There are few contacts between the BC domain in the α subunit and the β subunit (CT component) (Fig. 6a). Instead, interactions between the α and β subunits in PCC is mediated by a previously unrecognized domain, located between the BC and BCCP domains in the primary sequence of the α subunit (Fig. 2). It has been named the BT domain [22], for its role in mediating BC-CT interactions. The structure of this domain has a novel backbone fold, with a long helix surrounded by an eight-stranded anti-parallel β-barrel (Fig. 7c). A "hook", comprising the C-terminal part of the helix and the loop connecting it to the first strand of the β-barrel, has a central role in the interactions between the α and β subunits in the PCC holoenzyme.

The distance between the BC and CT active sites in the PCC holoenzyme is ~ 55 Å (Fig. 7a). This cannot be reached by biotin in the swinging-arm model. Therefore, the BCCP domain (in addition to its attached biotin) must translocate during PCC catalysis, hence the swinging-domain model (Fig. 1a).

The structure of the PCC holoenzyme also provides a framework for understanding the large collection of disease-causing mutations. The missense mutations are distributed throughout the entire enzyme (Fig. 7a). Some of them affect substrate binding and/or catalysis, while others affect protein stability [201–207]. At the same time, few of the mutations are located directly in the interface between the subunits of the holoenzyme, consistent with the extensive nature of the interface. Single-site mutations in this interface may not be sufficient to disrupt the holoenzyme. In fact, five mutations in the hook region are needed to disrupt the interactions between the α and β subunits [22].
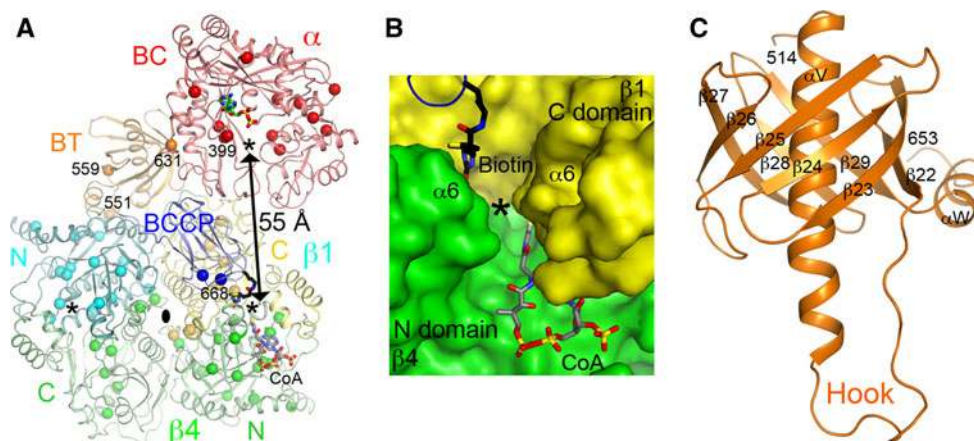
Fig. 7 The active sites of PCC. **a** Relationship between the BC and CT active sites (indicated with the *asterisks*) in the PCC holoenzyme. The CT active site is located at the interface of a β_2 dimer, with the β subunit from the bottom layer colored in *green*. Sites of disease-causing missense mutations are indicated with the *spheres*. The *third asterisk* indicates the other active site of the CT dimer. **b** Molecular surface of the active site region of CT. The observed position of biotin is shown (stick model in *black*). The position of CoA (*gray*) is modeled based on that in the structure of the yeast ACC CT domain [169]. **c** Structure of the BT domain of the bacterial PCC α subunit. The hook region is labeled

3-Methylcrotonyl-CoA carboxylase (MCC)

Biological functions of MCC

MCC catalyzes the conversion of 3-methylcrotonyl-CoA to 3-methylglutaconyl-CoA (Fig. 1b). It is essential for the catabolism of leucine and isovalerate in most living systems (Fig. 3) [61]. In *Pseudomonas* organisms, MCC is required for the metabolism of acyclic terpenoids [14, 208–212]. In *Arabidopsis*, MCC activity is required for seed development and germination [213]. MCC expression level is higher in male zebra finches, which may be important for the development of their singing behavior [214].

MCC shares good sequence conservation with PCC and its holoenzyme is also a 750-kD $\alpha_6\beta_6$ dodecamer. On the other hand, while PCC carboxylates the α carbon of the substrate, MCC carboxylates the γ carbon of an α–β unsaturated acid (Fig. 1b). MCC is located in the mitochondrial matrix in eukaryotes, and mutations in the N-terminal mitochondrial targeting sequence of both subunits can affect their localization [215]. Human MCC has been expressed and purified from the baculovirus system in the active form [216]. It demonstrated hyperbolic kinetics with ATP and 3-methylcrotonyl-CoA, while the *Pseudomonas* MCC showed sigmoidal kinetics toward ATP [212].

Deficiencies in MCC activity in humans are linked to 3-methylcrotonylglycinuria (MCG), which constitute one of the most frequently observed inborn errors of metabolism [217–226]. The clinical manifestations of MCG are highly variable, from asymptomatic individuals to neonatal onset, severe cases that can result in death. Neurological symptoms such as psychomotor retardation, seizure and coma have also been observed. Like PCC, a large number of autosomal recessive mutations in both subunits of MCC have been identified.
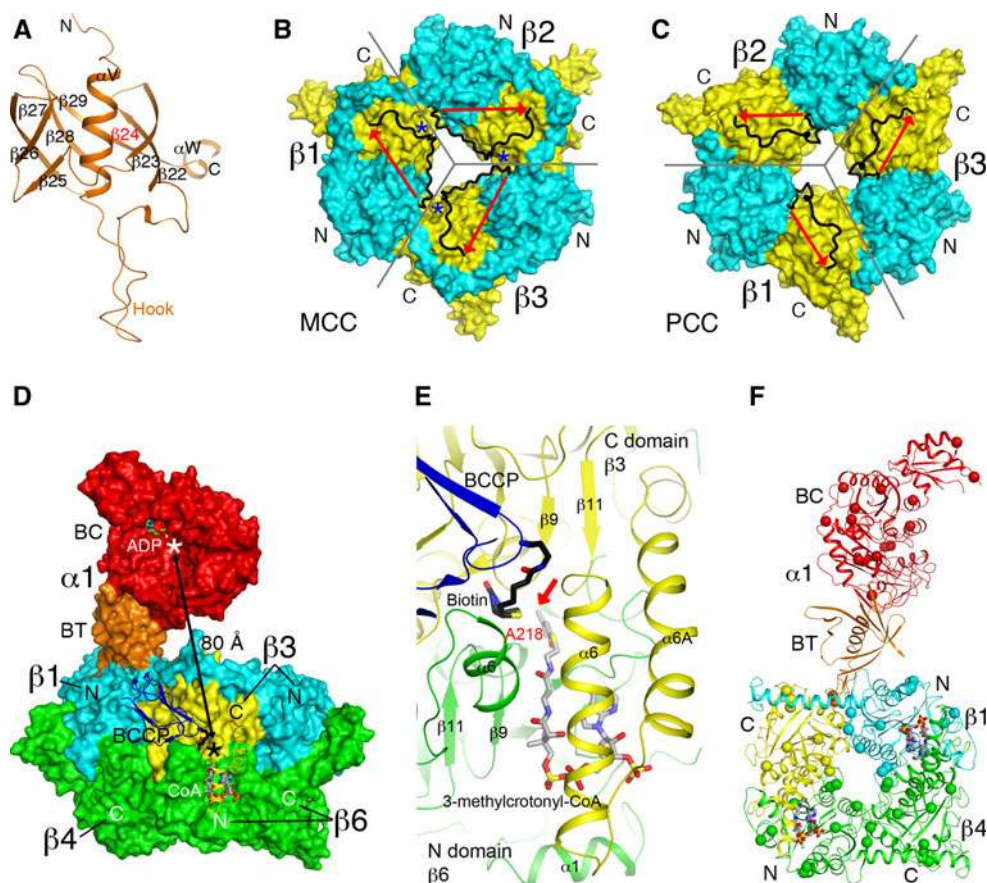
Structure of MCC

The structure of the *P. aeruginosa* MCC holoenzyme also obeys 32-point group symmetry, with three subunits in each of four layers (Fig. 6c) [23]. However, despite its sequence conservation with PCC, the overall architecture of the MCC holoenzyme is strikingly different from that of PCC. This is especially apparent for the locations of the α subunit relative to the β subunit, the organization of the N and C domains in the β subunit, and the structure and placement of the BT domain. As a result of these differences, the overall shape of the MCC holoenzyme is highly distinct from that of PCC (Fig. 6a, c).

Rather than being splayed away from each other as in PCC, the three α subunits at each end of the central β_6 core of MCC are located close to each other, in fact showing trimeric association (Fig. 6d). In addition, the BC domain in the α subunit is located ~20 Å above the β subunit, and there is no contact between them. The BT domain bridges the BC domain and the β subunit. While the overall structure of this domain is similar to that in PCC, there are also important differences. Especially, the hook in the MCC BT domain has a different conformation (Fig. 8a), contributing to the different architecture of the MCC holoenzyme compared to PCC. The *Pseudomonas* MCC BT domain lacks the third strand of the eight-stranded β-barrel (Fig. 8a), although this strand is likely present in human and most other MCCs [23].

In the β subunit, the positions of its N and C domains in MCC (Fig. 8b) are swapped relative to those in PCC (Fig. 8c).

Fig. 8 Structure of *P. aeruginosa* MCC. **a** Structure of the BT domain of the *P. aeruginosa* MCC α subunit. The missing third strand is indicated in *red*. **b** Structure of the β_6 hexamer of MCC. The subunit boundaries are indicated with the *gray lines*. The N (*cyan*) and C (*yellow*) domains are labeled. The linker between the two domains is shown in *black*, with the direction given by the *red arrow*. The linkers from neighboring subunits come very close to each other at one point (*blue asterisk*), and therefore only a small change is needed to switch to the connectivity seen in the PCC β subunit. **c** Structure of the β_6 hexamer of PCC, shown in the same scheme as panel B. The linker in PCC β runs in the opposite direction compared to MCC β.

d Relationship between the BC and CT active sites (indicated with the *asterisks*) in the MCC holoenzyme. While the BT domain of the α subunit contacts one β_2 dimer (β1 and β4), the BCCP domain of that α subunit is actually located in the active site of a different β_2 dimer (β3 and β6). **e** Binding modes of biotin (*black*) and 3-methylcrotonyl-CoA (*gray*) to the CT active site of MCC. The position of 3-methylcrotonyl-CoA is modeled based on that of CoA in MCC [23] and crotonyl-CoA in GCDα [30]. The carbon atom to be carboxylated is indicated with the *red arrow*. **f** Locations of disease-causing missense mutations in the structure of MCC (indicated with the *spheres*). Only the β1–β4 dimer is shown

This difference is not due to a swap of the two domains in the primary sequences of the two β subunits. Instead, it is due to a different connectivity between the N and C domains. In essence, each layer of the β_6 hexamer contains alternating N and C domains of the three subunits. While PCC uses one way of connecting neighboring N and C domains to make a β subunit (Fig. 8c), MCC uses the alternative way, such that the linker between the two domains runs in the opposite direction as compared to PCC (Fig. 8b).

The N and C domains of the β subunits in PCC and MCC have the same backbone fold. The pseudo symmetry operation relating the two domains is a rotation of ∼60° along the threefold axis of the β_6 hexamer. Therefore, the β_6 hexamers of MCC and PCC have pseudo sixfold symmetry. The swapping of the N and C domains in MCC

follows this pseudo symmetry, equivalent to a 60° rotation around the threefold axis of the hexamer (Fig. 8b, c), and therefore does not lead to a large change in the overall shape of the hexamer. Such a rotation also places the BCCP domain in the same position in the MCC holoenzyme compared to PCC (Fig. 6e, f).

The connectivity between the N and C domains observed in MCC β subunit is the same as that in the α subunit of the Na$^+$-transporting glutaconyl-CoA decarboxylase (GCDα) [27, 30]. However, GCDα is a dimer only, and therefore the different connectivity leads to a distinct organization of the active site in GCDα dimer compared to the CT dimer of ACC (Fig. 5a). Like MCC and GCC, GCDα is also active on the γ carbon of an α-β unsaturated acid. It is possible that these three enzymes

share a common evolutionary origin, representing a second lineage of biotin-dependent carboxylases that act on CoA esters. Another common feature among MCC, GCDα and likely GCC is the extended N-terminal segment of the β subunit, which has extensive interactions with the C domain of the same subunit (Fig. 8b). In comparison, the N-terminal segment of PCC β subunit is much shorter (Fig. 8c). These additional interactions may be the molecular basis for the swapping of the positions of the N and C domains [23].

The distance between the BC and CT active sites in the MCC holoenzyme is ~ 80 Å (Fig. 8d), indicating that the BCCP domain must translocate during catalysis (the swinging-domain model). BCCP-biotin is located in the CT active site in the MCC structures (Fig. 8d). Compared to the structure of PCC, biotin is bound deeper into the CT active site, consistent with its carboxylation of the γ carbon of the substrate. The N1′ atom of biotin is ~ 6 Å from the γ carbon of the 3-methylcrotonyl-CoA substrate (Fig. 8e), modeled based on the observed positions of CoA bound to MCC [23] and crotonyl-CoA bound to GCDα [30]. A conformational change for two helices in the CT active site (α6 and α6A, Fig. 8e) is observed upon CoA binding in MCC [23]. A similar conformational change is also observed in GCDα [30].

The disease-causing mutations are distributed over the entire structure of the holoenzyme (Fig. 8f). Some of them are located in the BC or CT active site. For example, the R385S mutation in the α subunit abolishes the stabilization of the biotin enolate during BC catalysis. The A214T mutation in the β subunit is centrally located between biotin and 3-methylcrotonyl-CoA in the CT active site (Fig. 8e) and may block binding of either or both substrates. Some of the mutations may also disturb the interaction between the BCCP or the BT domain and the β subunit [23].

Geranyl-CoA carboxylase (GCC)

GCC is classified into the same family as MCC since its site of carboxylation in the substrate is also on the γ carbon of an α-β unsaturated acid (Fig. 1b). The amino acid sequences of bacterial GCC are highly conserved with those of MCC, and the holoenzyme of GCC is likely also an $\alpha_6\beta_6$ dodecamer. At the same time, the geranyl group is much larger than the 3-methylcrotonyl group, and therefore the CT component (β subunit) of GCC is expected to have a larger pocket in the active site to accommodate this group. In fact, *P. aeruginosa* MCC cannot carboxylate geranyl-CoA, while GCC has activity toward both geranyl-CoA and 3-methylcrotonyl-CoA [212].

GCC is found in *Pseudomonas* organisms and several other bacteria (Table 1) [14, 208–212, 227]. It is important for the metabolism of the geranyl group and other acyclic terpenes. In fact, some *Pseudomonas* organisms can use these compounds as the sole carbon source. Through reactions that are similar to β-oxidation, γ-carboxygeranyl-CoA is converted to 3-methylcrotonyl-CoA (releasing two acetyl-CoAs and one acetate), and MCC facilitates the further degradation of this compound.

GCC activity has been purified from maize leaves, and it is probably also important for the metabolism of acyclic terpenes in plants [61, 228]. The biotin-containing component of this enzyme was found to have a molecular weight of ~ 122 kD, which is significantly larger than the ~ 75-kD α subunit of bacterial GCC (and MCC). Plant GCCs may constitute another family of biotin-dependent carboxylases, although a sequence for this protein is currently not available.

Other acyl-CoA carboxylases

Streptomyces, *Mycobacterium*, and *Corynebacterium* organisms have a collection of $\alpha_6\beta_6$ or $\alpha_2\beta_2$ acyl-CoA carboxylases (family 1.4, Fig. 2) [12, 229]. These enzymes are different from PCC (family 1.5) in that they probably lack the BT domain in the α subunit. In addition, they have more diverse substrate preferences, being active toward acetyl-, propionyl-, and/or butyryl-CoA [230, 231]. Moreover, some of these enzymes require the presence of a third subunit, ε subunit (~ 7 kD in *S. coelicolor* and ~ 25 kD in *M. tuberculosis*), for maximal activity, although the exact stoichiometry of this subunit is currently not known [12, 232, 233]. These acyl-CoA carboxylases are important for the biosynthesis of fatty acids, branched-chain fatty acids and other compounds, such as mycolic acid [234, 235]. An acyl-CoA carboxylase in *Mycobacterium tuberculosis* is crucial for mycolic acid biosynthesis and pathogenesis [236].

The structures of the β_6 hexamer of these enzymes have 32 symmetry [198–200, 237], and are similar to that of PCC [22] and the 12S subunit of transcarboxylase [31]. An Asp residue near the CT active site may be important for substrate preference for propionyl-CoA, while mutating this residue to Ile changes the preference to acetyl-CoA [198, 200]. On the other hand, an equivalent mutation in PCC is not able to change the substrate preference of that enzyme [22].

A *Rhizobium etli* acyl-CoA carboxylase has ~ 15-fold higher activity toward propionyl-CoA than acetyl-CoA, and it is activated substantially by mono-valent cations, K^+, NH_4^+ and Cs^+ [238].

Pyruvate carboxylase (PC)

Biological functions of PC

PC catalyzes the conversion of pyruvate to oxaloacetate (Fig. 1b) and it is an important enzyme in intermediary metabolism [4, 239–243]. In mammals, PC is localized in the mitochondrial matrix and has crucial roles in gluconeogenesis in liver and kidney (being the first enzyme in the gluconeogenesis pathway), lipogenesis and glyceroneogenesis in adipocytes, and biosynthesis of the excitatory neurotransmitter glutamate in astrocytes (Fig. 3). PC has an important anaplerotic role, replenishing the intermediates of the tricarboxylic acid (TCA) cycle that have been withdrawn for the biosynthesis of glucose, fatty acids, amino acids and other molecules [244]. Tumor cells depend on PC for anaplerosis in the absence of glutamine (Fig. 3), and cells with high PC activity may be resistant to inhibition of glutamine metabolism [245].

PC is important for glucose-induced insulin secretion by pancreatic β-cells in rats [246], and knock-down of PC activity by shRNA in rat insulinoma cells leads to impaired insulin secretion [247]. However, PC protein level and enzymatic activity are low in human pancreatic β-cells, and these cells may use a different pathway for stimulating insulin secretion [248].

Fungal PC is localized in the cytoplasm, in contrast to other eukaryotes. *S. cerevisiae* is unique in that it carries two PC enzymes, and the expression levels of the two enzymes are regulated independently in response to different nutrients [4]. PC is required for the assembly, import, and/or activation of some peroxisomal enzymes in several fungi, including alcohol oxidase in *Hansenula polymorpha* [249] and D-amino acid oxidase in *Pichia pastoris* [250]. This function is mediated by the CT domain of PC [251], but involves residues on the opposite face of the CT domain from the active site and is independent of its catalytic activity [252]. PC activity is important for carbon metabolism and virulence of the human pathogen *Listeria monocytogenes* [253].

PC is a single-chain, multi-domain enzyme in most organisms (family 3.1, Fig. 2), and is active only as a 500-kD tetramer. In archaea and some bacteria, such as *P. aeruginosa* [254], PC is in the two-chain form (family 3.2, Fig. 2), but it is also active as a tetramer of the two chains. Acetyl-CoA stimulates single-chain form of PC, by activating the BC activity, but it has no effect on the two-chain form of PC [255, 256]. An ATP analog, MgTNP-ATP, also activates PC activity and can allosterically compete with acetyl-CoA [257]. On the other hand, aspartate, glutamate and α-ketogluratate are allosteric, feedback inhibitors of the CT activity of PC [258].

Deficiency in PC activity is a rare, autosomal recessive metabolic disorder in humans, and has been associated with three forms of clinical manifestations [259–264]. Patients with form A PC deficiency have chronic, mild to moderate lactic acidemia, psychomotor retardation and hypotonia, and many patients die within a few years of birth. Form B deficiency is associated with the most serious symptoms, with severe lactic acidemia, hypoglycemia, hyperammonemia, anorexia, convulsions, and the patients generally die in the first months of life. Form C deficiency shows occasional mild lactic acidemia and may also show mild neurological symptoms. A collection of missense and insertion/deletion mutations has been identified in patients suffering from PC deficiency.

Structure of PC

Crystal structures of the full-length *Rhizobium etli* PC (RePC) [20, 265], *Staphyloccocus aures* PC (SaPC) [21, 266], and human PC (HsPC) lacking only the BC domain [21] are currently available. The overall structure of the tetramer is in the shape of a square (or a diamond), with approximate 222 symmetry for SaPC (Fig. 9a). The structure consists of two layers, with two monomers in each layer (Fig. 9b). BC and CT dimers are located at alternate corners of the square, creating an extensive interface between the two layers. On the other hand, there are few contacts between the two monomers in the same layer (Fig. 9a). The overall structures of the four monomers in the tetramer are similar to each other, but there are differences in the relative positions of their BC and CT domains. This is especially true for RePC, where the two monomers in one layer show large differences to those in the other layer. As a result, the RePC tetramer shows significant asymmetry between the two layers (Fig. 9c, d) [20, 265].

Structural information on these PC enzymes reveals the presence another domain, named as the PT (PC tetramerization) [21] or allosteric [20] domain. The structure of this domain contains a helix (formed by 30 residues between the BC and CT domains, Fig. 2) surrounded by a highly twisted, four-stranded anti-parallel β-sheet (formed by 60 residues between the CT and BCCP domains) (Fig. 9e). In HsPC and SaPC, the PT domain is important for tetramerization, hence its name [21]. A PT domain in one layer interacts with a PT domain in the other layer, and mutations in this PT–PT interface can disrupt PC tetramerization and catalytic activity. In contrast, the equivalent allosteric domain in RePC does not appear to be important for tetramerization (Fig. 9c) [20]. RePC lacking the BC domain is a dimer in solution [265], while the same construct for HsPC is a tetramer [21].
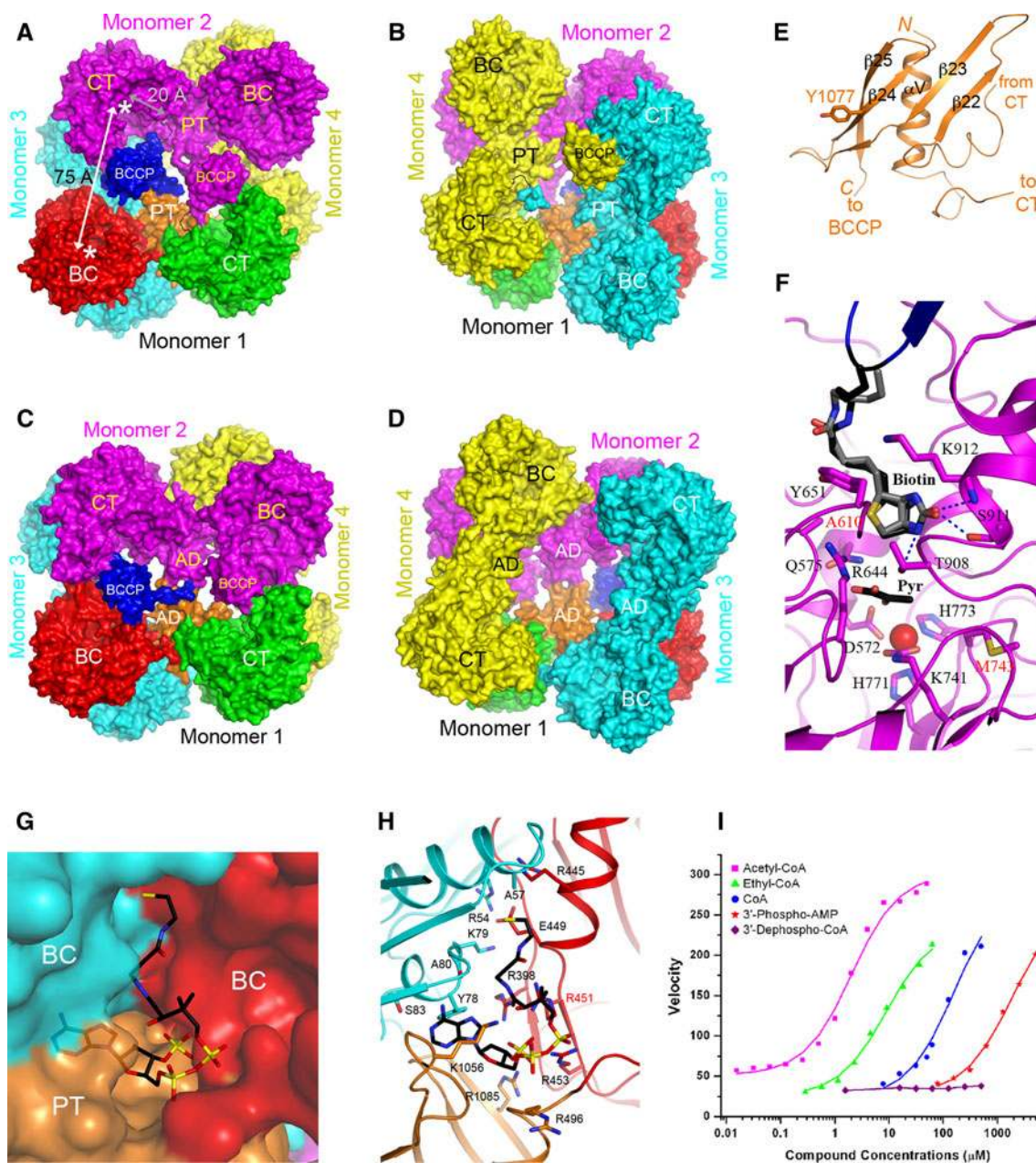
Fig. 9 Structure of PC. **a** Crystal structure of SaPC tetramer [21]. The domains of monomer 1 are colored as in Fig. 2, and the other three monomers are in *magenta*, *cyan* and *yellow*. The BC and CT active sites are indicated with the *asterisks*. The distance between the exo site and the CT active site is also labeled (*gray*). **b** Structure of SaPC tetramer, viewed from the bottom layer. **c** Crystal structure of RePC tetramer [20]. **d** Structure of RePC tetramer, viewed from the bottom layer. **e** Structure of the PT domain of SaPC [21]. **f** Structure of the active site region of the CT domain, in complex with BCCP (*blue*). The position of biotin in the SaPC structure is shown in *black*, and that in the HsPC structure in *gray* [21]. **g** Molecular surface of the binding site of CoA in SaPC [266]. **h** Detailed interactions between CoA and the binding site in SaPC. **i** The activity of various acetyl-CoA analogs in stimulating the catalysis by SaPC [266]

The structure of the PT domain shares remote similarity to that of the BT domain in PCC and MCC. The eight-stranded β-barrel in the BT domain (Fig. 7c) is replaced by a four-stranded anti-parallel β-sheet in the PT domain (Fig. 9e). However, while the helix is connected directly to the β-barrel in the BT domain, the CT domain of PC is inserted between the helix and the β-sheet in the PT domain.

The structure of the CT domain of PC contains a tri-osephosphate isomerase (TIM) barrel with a long C-terminal extension, and has similarity to that of the 5S (CT) subunit of transcarboxylase [32] and the CT domain of oxaloacetate decarboxylase [29, 267]. In SaPC and HsPC structures [21], one BCCP and its covalently attached biotin is bound in the CT active site of the other monomer in the same layer (Fig. 9b), providing direct

insight into the molecular mechanism of the CT reaction. In contrast, the BCCP domains are in a non-productive location and their attached biotins are disordered in the RePC structure (Fig. 9c) [20].

In the CT active site, Thr908 is hydrogen-bonded to the N1′ atom of biotin (Fig. 9f), indicating that it may play an important role in catalysis. The structure of the T908A mutant of SaPC is essentially identical to that of the wild-type enzyme, but the mutant is inactive [266]. Kinetic studies with the equivalent mutant of RePC also confirm the importance of this residue in the CT reaction [268]. The disease-causing A610T and M743I mutations are located in the active site of the CT domain, and are expected to disrupt catalysis by interfering with the binding of biotin (A610T mutation) [21, 266] or pyruvate (M743I mutation) (Fig. 9f) [21, 32]. The importance of other residues in the CT active site has also been assessed by mutagenesis studies in SaPC [266] and RePC [269].

The structural information defines the molecular basis for why PC is only active in the tetrameric form [20, 21]. BCCP-biotin is carboxylated in the BC domain of its own monomer, but it then transfers the CO_2 to pyruvate in the CT domain of the other monomer in the same layer (Fig. 9a). Therefore, both monomers in each layer are required for catalysis, but their conformation is stabilized only in the context of the entire tetramer because there are few interactions between two monomers of the same layer.

The distance between the BC and CT active sites in PC is ∼75 Å (Fig. 9a), supporting the swinging-domain model for catalysis (Fig. 1a). Binding of BCCP-biotin in the CT active site is observed in SaPC and HsPC [21]. Recently, BCCP-biotin in a nonproductive binding mode in the BC domain of RePC was reported [265].

The overall structures of the BC domain monomer and dimer of PC are similar to those of the BC subunit of E. coli ACC. Mutation of residues in RePC equivalent to those important for substrate binding and catalysis in E. coli BC [154] confirms their importance for PC catalysis as well [270].

The activator CoA is bound at the interface of the BC and PT domains of one monomer and the BC domain of another monomer (Fig. 9g) [20, 266]. A change in the organization of the dimer of the two BC domains is observed upon CoA binding [266], consistent with the fact that acetyl-CoA primarily activates the BC activity. The phosphate groups of CoA interact with a collection of Arg and Lys residues (Fig. 9h). Especially, Arg541 recognizes the 3′-phosphate group of CoA, which is critical for the activating effect of CoA (Fig. 9i), and the R541C disease-causing mutation abolishes acetyl-CoA activation [21]. In SaPC, CoA binding made the four monomers of the PC tetramer more similar to each other, essentially making the tetramer more symmetric. The symmetrical structure of

SaPC is also supported by cryo-EM studies [266, 271]. In contrast, a highly asymmetric tetramer is observed in the RePC structure, and only two CoA molecules (the 3′-phospho-ADP portion) are bound to the tetramer [20]. However, this asymmetry may be an inherent property of RePC and does not depend on CoA binding, as the structure without CoA shows similar asymmetry [265].

In the SaPC structure, the other three biotin groups are located in another binding site, at the interface between the PT and CT domains of the other monomer in the same layer, ∼20 Å from the CT active site (Fig. 9a) [21]. This exo site does not exist in HsPC due to sequence and structural differences. The functional importance of this binding site is currently unknown.

Urea carboxylase (UC)

Biological functions of UC

UC catalyzes the carboxylation of urea to produce allophanate, which is then hydrolyzed to generate ammonia and CO_2 (Fig. 10a) [15, 16, 272]. Therefore, urea amidolyase (UA, family 4.1) can catalyze both steps of this conversion, while a separate allophanate hydrolase activity is needed for UC (families 4.2 and 4.3).

UC is found in some bacteria, fungi, and green algae, and its activity is important for the utilization of urea as a nitrogen source in these organisms [15, 16, 272]. A second pathway for urea utilization is catalyzed by the nickel-dependent enzyme urease, which can convert urea to ammonia in a single step. Some of the fungal organisms contain both pathways, although only the UA pathway is present in S. cerevisiae. In fact, the UA form of this enzyme is found primarily in fungal species. In Saccharomyces kluyveri, UA is part of a pyrimidine degradation pathway, converting the urea product of uracil degradation to ammonia and enabling this organism to use uracil as the sole nitrogen source [273]. This is a distinct pathway for uracil catabolism, and it may also be present in other eukaryotes.

In Candida albicans, UA activity is important for the virulence of this important human pathogen. The CO_2 product of UA is a signal for hyphal switching [274], while the ammonia product can be secreted to alkalinize the extracellular environment, which also induces the yeast-hyphal transition. The hyphal form is important for C. albicans to escape phagocytosis by macrophages, thereby evading the host immune system [275].

Structure of UC

The structure of the UC component of K. lactis urea amidolyase was reported recently (Fig. 10b) [24]. In

Fig. 10 Structure of the urea carboxylase (UC) domain of *K. lactis* urea amidolyase. **a** The chemical reaction catalyzed by UC, converting urea to allophanate, which is then hydrolyzed to ammonia and bicarbonate. **b** Crystal structure of *K. lactis* UC [24]. The domains are labeled, and the active site of BC is indicated with the *asterisk*. **c** Interactions of biotin and urea with the CT active site of UC, located at the interface between the B and D domains
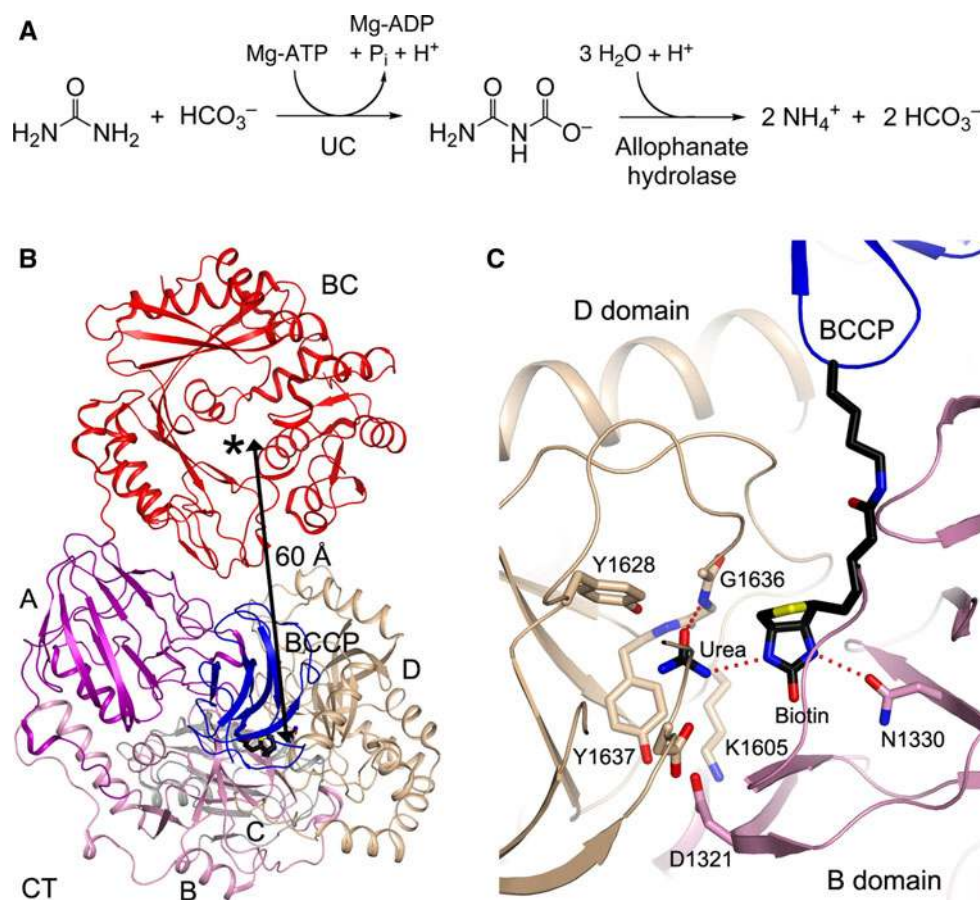
contrast to the other biotin-dependent carboxylases, the UC component is catalytically active as a monomer. The CT domain of UC consists of four sub-domains, named A, B, C and D (Fig. 10b), with many structural and sequence homologs. Especially, domains A and B together are similar to the KipA subunit, and domains C and D the KipI subunit of the *B. subtilis* KipA-KipI complex [276, 277]. KipI binds and inhibits the autophosphorylation of the histidine kinase KinA, thereby inhibiting sporulation. On the other hand, KipA can sequester KipI and prevent it from inhibiting KinA activation. CT and KipA-KipI have ~20–35 % sequence identity, but residues that are important for CT catalysis are not conserved in KipA and KipI. Therefore, the KipA-KipI complex is unlikely to have CT activity. In addition, both domains B and D have the cyclophilin fold, although they do not share any recognizable sequence homology with cyclophilin.

The active site of the CT domain is located in a cleft at the interface between the B and D domains (Fig. 10c) [24]. The biotin head group is fully extended away from the BCCP domain and interacts with highly conserved residues in the active site. The urea substrate is recognized by hydrogen-bonding interactions with the active site. Especially, one of the urea nitrogen atoms is directly hydrogen-

bonded to the N1′ atom of biotin. Mutations of residues in the active site region have detrimental effects on the catalysis by UC.

The overall structure of the BC domain of UC is similar to that of other BC components. However, the BC domain is monomeric in UC, as the interface that mediates the dimerization of the BC subunit of bacterial ACC is not conserved in UC. The BC domain interacts with domains A and D of CT in the UC holoenzyme. The distance between the BC and CT active sites in UC is ~60 Å (Fig. 10b), indicating that the BCCP domain must translocate during catalysis, consistent with the swinging-domain model (Fig. 1).

Common features in the catalysis by biotin-dependent carboxylases

The BC component has strong sequence conservation among the biotin-dependent carboxylases. Insights into the BC reaction are obtained from the structure of the *E. coli* BC subunit in complex with substrates [154], which indicates the crucial importance of two residues, Glu296 as a general base to extract the proton from bicarbonate and Arg338 to stabilize the biotin enolate oxyanion. The

hydrolysis of ATP, via the formation of a carboxyphosphate intermediate, serves at least two functions: (1) the dehydration of bicarbonate to CO_2, which is a stronger electrophile; (2) the production of orthophosphate (PO_4^{3-}), which is a strong base that is capable of extracting the proton on the N1′ atom of biotin [17]. This mechanism is likely shared among all biotin-dependent carboxylases, and Glu296 and Arg338 of *E. coli* BC are conserved among these enzymes.

The CT components of the acyl-CoA carboxylases show sequence and structural conservation. The acyl-CoA substrate is recognized by a domain/subunit with the crotonase fold, and it may be speculated that this part of the CT component evolved from a primordial crotonase. This fold is capable of binding CoA esters, and it also provides an oxyanion hole, in the form of two main-chain amides, that stabilizes the enolate oxyanion of the acyl group during catalysis. Remarkably, the main-chain carbonyl of one of the oxyanion hole residues recognizes the N6 amino group, and the main-chain amide of the following residue is hydrogen-bonded to the N1 atom of the adenine base of CoA. Biotin is recognized by a separate domain/subunit, but also with the crotonase fold. The equivalent oxyanion hole in this domain/subunit stabilizes the biotin enolate oxyanion during the CT reaction. It might be possible that the biotin-binding domain/subunit of CT arose through gene duplication of a crotonase enzyme and then evolved its specificity toward biotin.

The CT components of PC and UC are entirely different in sequence and structure from those of acyl-CoA carboxylases. However, they share the common feature of providing two oxyanion holes to stabilize the intermediates during the CT reaction. On the other hand, while acyl-CoA carboxylases use primarily (carboxy)biotin for catalysis [17, 169], side chains of conserved amino acids are important for the CT reaction in PC and UC, for example Thr908 in SaPC [21, 266, 268] and Lys1605 in KlUC [24].

Summary and perspective

Biotin-dependent carboxylases are crucial enzymes for metabolism and many other cellular processes. They produce compounds that are important for the biosynthesis of many molecules and/or help degrade potentially toxic metabolic intermediates. ACCs are drug discovery targets against type 2 diabetes, cancer, microbial infections, and other diseases in humans. Three classes of herbicides function by inhibiting the plastid ACC of grasses, demonstrating the feasibility of small-molecule ACC antagonists. On the other hand, deficiencies in PCC, MCC and PC activity are linked to serious diseases in humans, especially infants. Development of agents that can restore the activity of the deficient enzyme, possibly through gene therapy, will be of great benefits to these patients.

Structural information on the holoenzymes of biotin-dependent carboxylases demonstrates the remarkable degree of variability in these enzymes. They share conserved domains, which have similar structures on their own. However, the arrangement of these domains in the holoenzyme monomers and especially the architecture of the holoenzyme oligomers can be dramatically different, for example the comparison between PCC and MCC or between SaPC and RePC. These observations also serve as a cautionary tale for the limitations of homology modeling on such large, multi-domain systems.

The past few years have seen great progress in the studies on biotin-dependent caboxylases, especially with the first structural information on most of the holoenzymes (PCC, MCC, PC, UC). At the same time, many important questions remain to be answered. For example, structural information on the holoenzymes of multi-subunit and multi-domain ACCs is still not available. In addition, while the recognition of BCCP by the CT component has been observed in most of the holoenzyme structures, it is not clear how BCCP binds to the BC component for catalysis. Moreover, the molecular basis for substrate selectivity of the acyl-CoA carboxylases is still poorly understood, and presently one cannot readily determine the substrate preference(s) of a given acyl-CoA carboxylase based on a sequence analysis of its CT component. While substitutions of amino acid side chains are likely to play a major role in determining substrate selectivity (for example the size and/or shape of the acyl-CoA binding pocket), conformational differences in the main chain of residues in the active site may also be important and further structural information is needed to illuminate such differences. Therefore, the coming years should continue to be highly exciting in the studies of biotin-dependent carboxylases.

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