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Review

Acetyl-coenzyme A carboxylase: crucial metabolic enzyme and attractive target for drug discovery

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Abstract. Acetyl-coenzyme A carboxylases (ACCs) have crucial roles in fatty acid metabolism in most living organisms. Mice deficient in ACC2 have continuous fatty acid oxidation and reduced body fat and body weight, validating this enzyme as a target for drug development against obesity, diabetes and other symptoms of the metabolic syndrome. ACC is a biotin-dependent enzyme and catalyzes the carboxylation of acetyl-CoA to produce malonyl-CoA through its two catalytic activities, biotin carboxylase (BC) and carboxyltransferase (CT). ACC is a multi-subunit enzyme in most prokaryotes, whereas it is a large, multi-domain enzyme in most eukaryotes. The activity of the enzyme can be controlled at the transcriptional level as well as by small molecule modulators and covalent modification. This review will summarize the structural information that is now available for both the BC and CT enzymes, as well as the molecular mechanism of action of potent ACC inhibitors. The current intense research on these enzymes could lead to the development of novel therapies against metabolic syndrome and other diseases.

Key words. Metabolic syndrome; obesity; diabetes; structure-based drug design; fatty acid metabolism; protein structure and function; biotin-dependent carboxylases; enzyme catalysis and mechanism.

Introduction

Acetyl-coenzyme A carboxylase (ACC) (EC 6.4.1.2) catalyzes the carboxylation of acetyl-CoA to produce malonyl-CoA. This enzyme was first discovered nearly 50 years ago and has been studied extensively over the years [1]. The recent emergence of the obesity epidemic and associated clinical manifestations such as type 2 diabetes, cardiovascular diseases and atherosclerosis [2–4], which are collectively known as the metabolic syndrome, insulin resistance syndrome or syndrome X [5], has generated significant renewed interest in ACC. More than half of the population in the US is either overweight or obese [6]. While life-style changes (less food intake and more physical activity) are an important component in combating the obesity epidemic, there is also an urgent need for new therapeutic agents. ACC has critical roles in fatty acid metabolism, and represents an attractive target for therapeutic intervention in the control of obesity and the treatment of metabolic syndrome.

In humans and other mammals, there are two isoforms of the ACC enzyme, ACC1 and ACC2. ACC1 is expressed mostly in lipogenic tissues (liver, adipose, lactating mammary gland and others) and catalyzes the committed and rate-determining step in the biosynthesis of long-chain fatty acids (fig. 1A) [1, 7, 8]. The malonyl-CoA product of ACC in these tissues is used as a building block to extend the chain length of fatty acids in two carbon increments, a process catalyzed by fatty acid synthase (FAS) [1]. The long-chain fatty acids can then be incorporated into triacylglycerides and phospholipids.

In contrast, ACC2 is expressed mostly in the heart and skeletal muscle, and its malonyl-CoA product is a potent inhibitor of fatty acid oxidation in these tissues (fig. 1A) [7]. Long-chain acyl-CoAs cannot cross the mitochondrial membranes, and must be converted to acylcarnitines

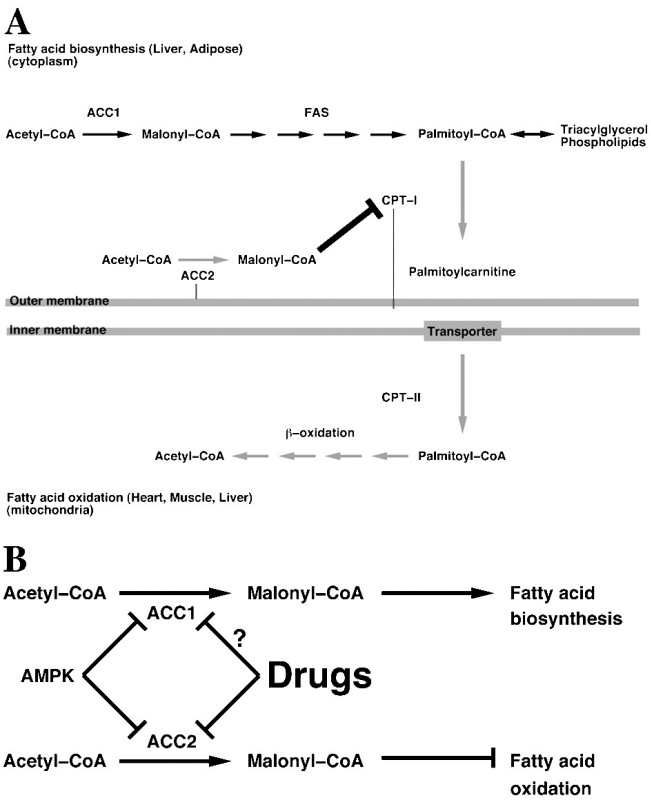

Figure 1. Acetyl coenzyme-A carboxylase (ACC) has critical roles in fatty acid metabolism. (*A*) In mammals, ACC1 controls fatty acid biosynthesis, while ACC2 controls fatty acid oxidation. (*B*) ACC is an attractive target for drug discovery. AMPK is a natural dual inhibitor of both isoforms of animal ACCs. A clinically efficacious drug should potently inhibit ACC2, but could also inhibit ACC1 to a certain extent (indicated by the question mark).

for transport into the mitochondria for β-oxidation [9–12]. The enzyme catalyzing this conversion is carnitine palmitoyltransferase I (CPT-I). ACC2 regulates fatty acid oxidation as its malonyl-CoA product is a potent (nanomolar) inhibitor of CPT-I (fig. 1A).

The importance of ACCs as drug discovery targets is validated from observations on ACC2-deficient mice [13]. These animals have drastically reduced levels of malonyl-CoA in their heart and skeletal muscle, and other studies also confirm that the level of malonyl-CoA in the heart is mostly determined by the activity of ACC [14]. As a result of the depletion of malonyl-CoA, ACC2$^{-/-}$ mice have continuous fatty acid oxidation, reduced body fat mass and body weight, despite consuming more food (hyperphagia). They are also protected against diabetes and obesity induced by high fat/high carbohydrate diets [15]. These observations suggest that inhibitors of ACC2 may be novel therapeutic agents against obesity, diabetes and metabolic syndrome in general (fig. 1B) [16, 17]. These compounds may also be active against ACC1, although currently it is not known whether selectivity between the two isoforms is necessary (see section on ACC inhibitors) (fig. 1B).

This review will attempt to summarize our current knowledge on ACCs from animals, plants, bacteria and archaea, with emphasis on recent advances. Space limitations unfortunately preclude the citation of most of the original publications, especially those before the year 2000. These can be found in the many excellent reviews that have appeared over the years on these enzymes [1, 7, 8, 16–26].

Biochemistry of ACC

ACC catalyzes the carboxylation of acetyl-CoA to produce malonyl-CoA [1, 18]. The reaction generally proceeds in two steps and is dependent on the prosthetic group biotin (fig. 2A). The first step involves the ATP-dependent carboxylation of the N1 atom in the ureido ring of biotin, catalyzed by the biotin carboxylase (BC) activity, and bicarbonate is the donor of the carboxyl group for this conversion. Divalent cations (Mg^{2+} or Mn^{2+}) are required for coordinating the ATP phosphates for catalysis. The energy required for the carboxylation reaction is derived from the hydrolysis of ATP, by the formation of an activated carboxyphosphate intermediate with the bicarbonate substrate [19, 22].

The second step of the ACC reaction involves transfer of the activated carboxyl group from the N1 atom of biotin to the methyl group of acetyl-CoA, thereby generating the product malonyl-CoA (fig. 2A). This step is catalyzed by carboxyltransferase (CT) activity. No additional energy input is required for this reaction.

Biotin is an essential vitamin (vitamin H or B_8) in higher organisms that can only be acquired from foods [20, 21]. The biotin prosthetic group is covalently linked to the biotin carboxyl carrier protein (BCCP) through an amide bond between the valeryl carboxyl of biotin and the ammonium group in the side chain of a lysine residue in BCCP (fig. 2B). This places a total of 8 methylene groups, and 10 rotatable single bonds, between the bicyclic ring of biotin and the backbone of BCCP. Biotin therefore is situated at the end of a 'swinging arm', which could facilitate its translocation between the active sites of BC and CT (fig. 2B). The distance between the active sites of BC and CT in transcarboxylase is about 7 Å based on nuclear magnetic resonance and electron spin resonance studies [1].

Multi-subunit and multi-domain ACCs

ACCs have been found in most living organisms, including archaea, bacteria, yeast, fungi, plants, animals and humans. In *Escherichia coli* and many other bacteria, as

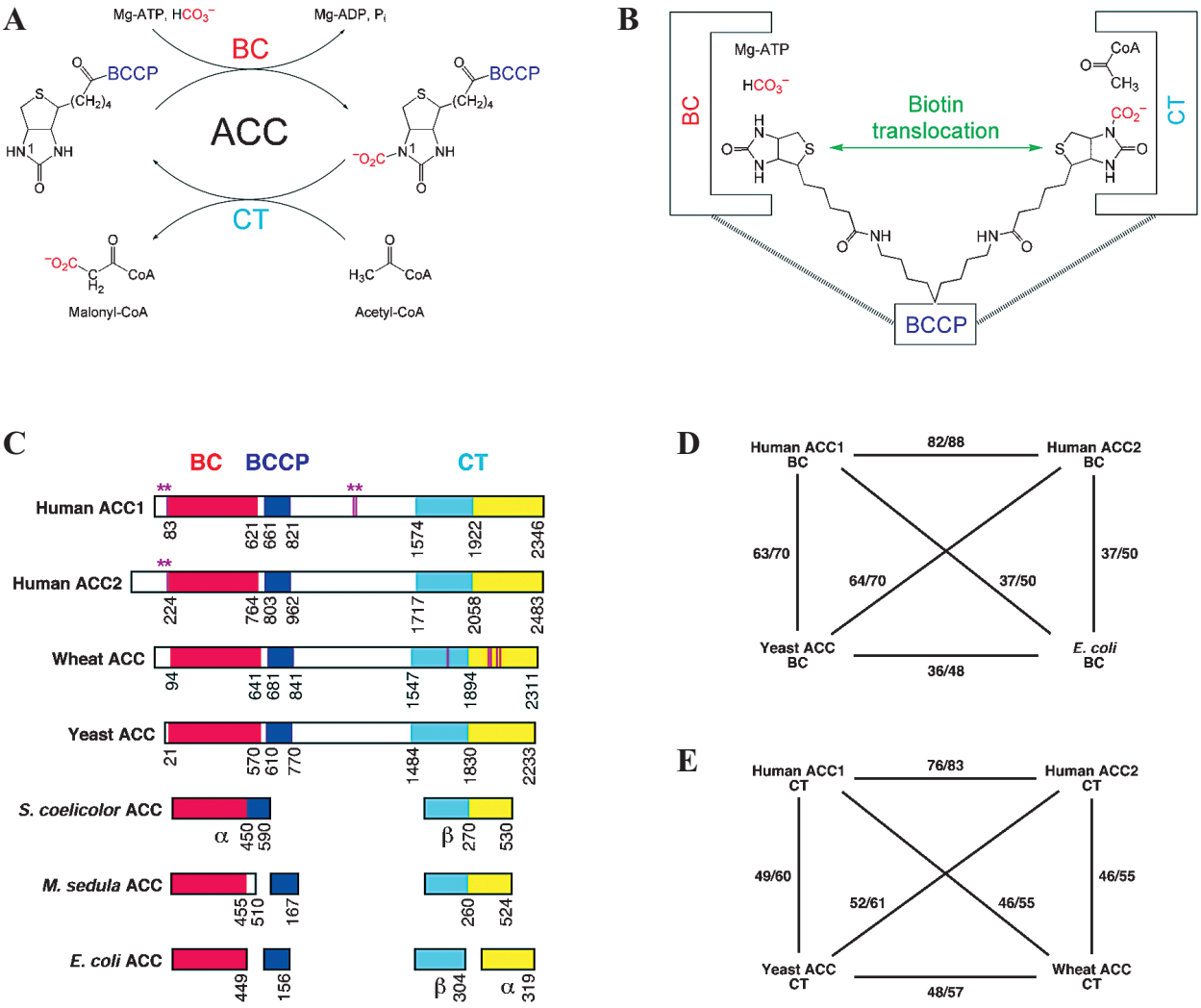

Figure 2. The biochemistry of ACC. (*A*) The reaction catalyzed by ACC proceeds in two steps, with biotin carboxylase (BC) and carboxyltransferase (CT) activity, respectively. (*B*) Biotin is situated at the end of a 'swinging arm', enabling it to translocate between the active sites of BC and CT. Connections between BC and BCCP, BCCP and CT only exist in multi-domain ACCs and are indicated by dashed lines. (*C*) Domain organization of human ACC1, human ACC2, yeast ACC, wheat ACC, *Streptomyces coelicolor* ACC, and the subunits of archael (*Metallosphaera sedula*) and *E. coli* ACC. Sites of phosphorylation in the human enzymes are indicated with the bars and stars in magenta. Sites of herbicide resistance mutations in plant ACCs are indicated with the bars in magenta in wheat ACC. (*D*) Amino acid sequence conservation among the BC domains of representative multi-domain ACCs and the BC subunit of *E. coli* ACC. For each comparison, the first number is the percent sequence identity, and the second is percent sequence similarity. (*E*) Amino acid sequence conservation among the CT domains of representative multi-domain ACCs.

well as in the chloroplasts of many plants, ACCs are multi-subunit enzymes (fig. 2C) [1, 18, 21, 22, 24, 25]. The BC subunit, with 449 amino acid residues (50 kD) for the *E. coli* enzyme, carries the biotin carboxylase activity, and biotin is linked to the BCCP subunit (156 residues, 17 kD). The CT activity is carried out by two subunits, α and β, with 319 and 304 residues (35 and 33 kD), respectively, for the *E. coli* enzyme (fig. 2C). The different subunits associate to form the holoenzyme of ACC. However, this holocomplex is unstable, and can readily dissociate under a variety of conditions [18, 22]. The BC subunit itself is a dimer in solution, and BCCPs may behave as dimers or higher oligomers. The two subunits of CT form a stable $\alpha_2\beta_2$ complex. The stoichiometry of the bacterial holoenzyme may be $(BC)_2(BCCP)_4$-$(CT\alpha,CT\beta)_2$ [27]. A BC-BCCP complex can also be isolated from *E. coli* [18, 27] and some plants [25].

In comparison, in humans and most other eukaryotes, ACC is a large (>200 kD), multi-domain enzyme that carries the BC, CT and BCCP activities in a single polypeptide (fig. 2C) [1, 18, 24]. Human ACC1 contains 2346 amino acid residues, with a molecular weight of 265 kD. Human ACC2 contains 2483 amino acid residues, with MW of 280 kD (fig. 2C). ACC2 contains a unique N-ter-

minal extension of about 140 residues, which helps to anchor this enzyme to the outer membrane of the mitochondria (fig. 1A) [7, 28]. The primary structures of the multi-domain ACCs can be divided into three parts. The N-terminal one-third of the proteins contains the BC and the BCCP domains, and the C-terminal one-third contains the CT domains (fig. 2C). The middle one-third of the proteins is unique to multi-domain ACCs, and the function of this region is currently not known.

More recently, a third form of ACC was identified in the actinomycete *Streptomyces coelicolor* (fig. 2C) [29]. This ACC enzyme has an α subunit (590 residues) that contains both the BC and the BCCP domains, and a β subunit (530 residues) that contains the CT domains. Interestingly, this subunit organization of ACC is the same as that for propionyl-CoA carboxylases (PCCs). In fact, the α subunit is shared by ACC and PCC in this organism [29].

A fourth form of ACC may exist in the archaea, where ACC is a multi-subunit enzyme, with BC, BCCP and CT subunits (fig. 2C) [30]. In contrast to *E. coli* and other bacteria, the archael CT subunit is a single protein of about 520 residues, rather than two separate subunits. This is similar to the β subunit of the ACC from *Streptomyces* [29], as well as the β subunit of PCC. The holoenzyme of archael ACC may have the stoichiometry $(BC)_4(BCCP)_4(CT)_4$ [30].

The ACCs are highly conserved among living organisms, underscoring the importance of their biological functions. For example, the BC domain of human ACC1 shares 37% amino acid sequence identity with the BC subunit of *E. coli* ACC, whereas the BC domains of human and yeast ACC share 63% sequence identity (fig. 2D). The BC subunits of *E. coli* and archael (*Metallosphaera sedula*) ACC share 47% amino acid sequence identity. The CT domains of eukaryotic ACCs share at least 45% amino acid sequence identity, and those of human ACC1 and ACC2 have 76% sequence identity (fig. 2E). In contrast to the BC domain, the sequence conservation between the CT subunits of *E. coli* and the CT domains of eukaryotic ACCs or the CT subunit of archael ACC is much lower. This may reflect different evolutionary pressure on the BC and the CT enzymes. It could also be possible that the CT structure is less sensitive to variations of its amino acid sequences.

The sequence conservation of the BCCP proteins is mostly limited to the C-terminal biotinoyl domain, which has the biotinylation site in a conserved (Ala/Val)-Met-Lys-(Met/Leu) motif [22, 24]. The *E. coli* BCCP subunit has MW of 17 kD, and the biotinoyl domain covers the C-terminal 10 kD of the protein. The N-terminal half of BCCP is extremely flexible on its own, and can be easily proteolyzed during purification, or by treatment with subtilisin, to give truncated forms of BCCP ($BCCP_S$) [18]. However, it is the full-length form of BCCP ($BCCP_L$) that exists in the holoenzyme of *E. coli* ACC, and $BCCP_L$ is much more active in the ACC reaction than $BCCP_S$. $BCCP_L$ has a K_m of about 0.3 μM as a substrate of both the BC and CT subunits, whereas $BCCP_S$ has a K_m of 25 μM.

ACCs belong to the small family of biotin-dependent carboxylases. BCCP is the only biotinylated protein and ACC is the only biotin-dependent enzyme in *E. coli* [21, 22]. Five biotin-containing proteins have so far been identified in plants [24]. In humans, there are only three other members of this family besides the ACCs, PCC, 3-methylcrotonyl-CoA carboxylase (MCC), and pyruvate carboxylase (PC) [20]. PCC catalyzes the production of methylmalonyl-CoA from propionyl-CoA, and has a critical role in the metabolism of fatty acids with odd number of carbons as well as amino acids Val, Thr, Ile and Met. Patients with deficiencies in PCC activity suffer from the metabolic disorder propionic acidemia [31]. MCC catalyzes the production of 3-methylglutaconyl-CoA, and has a crucial role in the metabolism of leucine. MCC deficiency is a common cause of organic aciduria in patients [20]. PC catalyzes the production of oxaloacetate, a crucial intermediate in gluconeogenesis [20]. Patients with PC deficiency show lactic acidemia, mental retardation, developmental delay and other symptoms.

Biological roles of ACC

ACCs catalyze the generation of malonyl-CoA, and this malonyl-CoA product can be used in diverse biological processes. The most important role of ACC is in the biosynthesis of long-chain fatty acids. In fact, ACC catalyzes the committed and rate-limiting step in fatty acid biosynthesis, and is an essential enzyme in many organisms [1, 18, 20-22, 25]. The critical roles of ACCs in regulating fatty acid metabolism in mammals have been discussed in the Introduction and will not be repeated here. ACC may also have an important role in myelin [32, 33] and the brain [34]. The malonyl-CoA product may be a satiety signal, partly explaining the hyperphagic behavior of $ACC2^{-/-}$ mice.

In plants, ACC in the chloroplast is critical for the biosynthesis of long-chain fatty acids [24, 25, 35]. Plants cannot transport fatty acids over long distance, so these compounds must be synthesized locally [25]. The plastid ACC in most plants is composed of four distinct subunits, which share significant amino acid sequence homology to those of the bacterial ACCs [21, 22, 24, 25, 35]. Interestingly, the β subunit of CT is encoded by the plastid genome, whereas the other three subunits of ACC are encoded by the nuclear genome.

In sharp contrast, the plastids of the grasses contain a multi-domain ACC, similar to the animal ACCs (fig. 2C). This unique plastid ACC of the grasses is a crucial determinant of the sensitivity of these plants to herbicides (see section on ACC inhibitors) [21, 24, 25, 36–38].

The cytosol of plants contains a multi-domain ACC, which is required for the extension of fatty acids to very long-chain fatty acids and the synthesis of secondary metabolites such as flavonoids [24, 25]. In *Arabidopsis*, the cytosolic ACC gene is required for proper embryonic patterning during development [39–41]. Several distinct point mutations in this gene can affect the development process. A missense mutation in the BC domain is located in the active site, and is expected to disrupt the catalysis. Three missense mutations in the CT domain are far from the active site, and their mechanism of action is unknown. A mitochondrial form of multi-domain ACC was recently characterized in barley [42] and rice [43].

In yeast, deletion of the ACC gene is lethal. Interestingly, yeast mutants lacking FAS can survive if long-chain fatty acids are provided in the medium [25]. In contrast, mutants lacking ACC arrest at the G_2/M phase of the cell cycle even in the presence of long-chain fatty acids [44]. This suggests that ACC has an essential role besides the synthesis of long-chain fatty acids. This may be the synthesis of very long-chain fatty acids, which are components of sphingolipids and ceramides and are essential for survival. ACC may also function together with nucleoporins to control the nuclear accumulation of a small GTPase [45]. Yeast contains a second isoform of ACC, known as HFA1. It is a mitochondrial enzyme, and is crucial for fatty acid and especially lipoid acid biosynthesis in that organelle [46].

In *Streptomyces coelicolor*, the malonyl-CoA product is used for the biosynthesis of both fatty acids and polyketides [29]. Many polyketide natural products are pharmaceutically important antibiotics, anticancer agents and other drugs [47]. Archael organisms do not use fatty acids in their lipids, and their ACC activity may be required for CO_2 fixation instead [30].

Kinetic studies of ACC

The catalysis of ACC starts with the formation of the carboxyphosphate intermediate in the active site of BC (fig. 3) [19, 22]. A general base of the BC enzyme may facilitate the extraction of the proton on the bicarbonate substrate, which in turn attacks the γ-phosphate of ATP to produce carboxyphosphate and ADP. Decomposition of this intermediate then produces inorganic phosphate and CO_2. One of the phosphate oxygens acts as a general base to deprotonate the N1 atom of biotin, which can then attack the CO_2 molecule to produce carboxybiotin. After translocation to the active site of CT, carboxybiotin decomposes to generate CO_2, and the N1 atom of biotin acts as the general base to extract a proton from the methyl group of acetyl-CoA (fig. 3). Mutation of residues in the active site of BC or CT failed to identify an amino acid residue that functions as the general base in their catalysis [22, 48], supporting the proposed mechanism (fig. 3). Therefore, ACC represents an example of substrate-assisted catalysis [22].

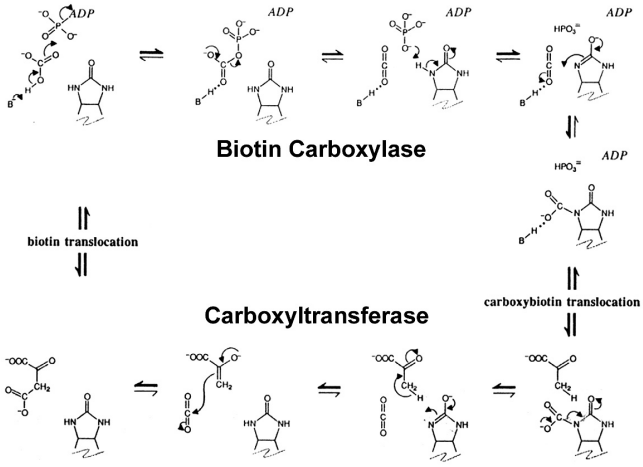

Figure 3. Chemical mechanism of ACC. Modified from [19].

BC can also catalyze the bicarbonate-dependent hydrolysis of ATP, but at a very slow rate. In the presence of biotin or BCCP, the rate of ATP hydrolysis increases up to 10,000-fold, a phenomenon known as substrate-induced synergism [22]. Biotin binding may cause a conformational change in BC that facilitates optimal substrate alignment and ATP hydrolysis. This regulatory mechanism is important, as it prevents BC from carrying out futile ATP hydrolysis in the absence of biotin. Mutations of residues in the active site of BC can easily disrupt this synergism, suggesting these residues are conserved to ensure optimal substrate alignment for catalysis [22].

The overall reaction of ACC in the forward or reverse direction, as well as the reactions catalyzed by BC or CT, can be assayed using spectrometric methods, by coupling product formation to the oxidation or reduction of the nicotinamide adenine dinucleotide (reduced) (NADH) or NAD phosphate [NAD(P)$^+$] cofactor, which leads to a change in the absorbance at 340 nm [49]. Free biotin can be used as the carboxyl acceptor for the BC reaction, and biotin methyl ester (BME) is a good carboxyl acceptor for the CT reaction, assayed in the reverse direction [48, 50]. The overall reaction of ACC in the forward direction can also be assayed using a radioactive ^{14}C-bicarbonate fixation method [51]. This radioactive assay was the basis of a screening effort that identified potent inhibitors of mammalian ACCs [52].

Kinetic studies of the multi-domain ACC from rat liver showed that the substrate ATP binds first for the forward reaction, followed by bicarbonate The ADP and P_i products of the BC reaction are released before the binding of the acetyl-CoA substrate for the CT reaction. The K_m values for the ATP, bicarbonate and acetyl-CoA substrates are 15 μM, 2.5 mM and 25 μM, respectively.

Although free biotin can be used as a carboxyl acceptor, its K_m values are very high, about 100 and 3 mM for *E. coli* BC and CT, respectively [18]. As a comparison, full-length BCCP ($BCCP_L$) has a K_m of about 0.3 μM as a substrate for both the BC and the CT subunits.

E. coli CT can also catalyze the decarboxylation of carboxylated BCCP in the absence of the acetyl-CoA substrate. As a consequence, carboxy-biotin is rather unstable when bound to CT, with a $t_{1/2}$ of 6 min [18]. In comparison, free carboxy-biotin is more stable, and this stability is enhanced in basic solutions, with $t_{1/2}$ of up to 2 h [18, 53]. The isolated CT domains of eukaryotic ACCs can catalyze the decarboxylation of malonyl-CoA in the absence of the biotin substrate [unpublished results].

Regulation of ACC gene expression

In many bacteria, the genes for BC and BCCP are located in an operon, with the BCCP gene at the 5′ end [22]. In *E. coli*, the expression of this operon is autoregulated by BCCP [54]. The regulation of gene expression for multisubunit ACCs in bacteria and plants has an additional layer of complexity. The expression of the four subunits may need to be coordinated to reflect the stoichiometry of the holoenzyme, although the mechanism of this regulation is not known [22]. The situation is even more complex in plants, where the β subunit of CT is encoded by the plastid genome. Nonetheless, the messenger RNA (mRNA) levels of the four subunits appear to be maintained at a constant ratio [24].

The expression of the yeast ACC gene is stimulated by lipid precursors such as inositol and choline, consistent with the role of this enzyme in the synthesis of very long chain fatty acids and sphingolipids [44]. In wheat, tissue-specific expression of the plastid and the cytosolic ACC genes is driven by two nested promoters [55], which give rise to alternative splicing near the 5′ end of these genes [56].

Human ACC1 gene is located in chromosome 17q12, and ACC2 gene is in chromosome 12q23. Multiple promoters have been identified for mammalian ACC1 and ACC2 genes [8, 57–59]. Use of the different promoters can lead to alternative splicing at the 5′ end [57], some of which modifies the N-terminal sequences of the encoded ACC proteins [8]. Especially, transcripts derived from the most downstream promoter (known as PIII) can encode an isozyme of ACC1 with a much shorter N-terminal segment [57, 60], which lacks the sites of phosphorylation by AMP-activated protein kinase (AMPK, see next section). This ACC1 isozyme may not be regulated by AMPK and may be constitutively active [8]. Similarly, human ACC2 also has an alternate, shorter transcript, and the encoded protein lacks the N-terminal unique segment that is believed to target this enzyme to the mitochondrial membrane [8]. The existence of isozymes of ACC1 and ACC2 offers additional flexibility in the regulation of these enzymes in different tissues, and in response to different cellular conditions.

Expression of the ACC genes is controlled by several transcriptional factors, including sterol regulatory element binding proteins (SREBP1a and SREBP1c) and carbohydrate response element binding protein (ChREBP) [61–63]. SREBP1c is regulated by insulin at the transcriptional level, and a high-carbohydrate diet can induce the expression of SREBP1c through the activation of insulin signaling. Polyunsaturated fatty acids can decrease the expression of ACC and SREBP1 [64]. ChREBP can be induced by a high-carbohydrate diet, and activates the expression of lipogenic enzymes such as ACC and FAS independent of the action of insulin [65]. The hexosamine biosynthesis pathway may also play a role in the activation of ACC and FAS expression by glucose [66].

Peroxisome proliferator-activated receptor γ coactivator-1, PGC-1α and PGC-1β, are transcriptional coactivators that can potentiate the effects of transcriptional factors and nuclear receptors [67]. PGC-1β can coactivate the expression of SREBP-1a and SREBP-1c, and has crucial roles in lipogenesis induced by high-fat diets [68].

Regulation of ACC enzymatic activity

Animal ACCs are controlled by both feedforward and feedback regulation. Citrate, a precursor of the acetyl-CoA substrate of ACC, is a feedforward allosteric activator of mammalian ACCs. On the other hand, long-chain acyl-CoA, the ultimate product of the fatty acid biosynthesis pathway, is a potent feedback inhibitor of mammalian ACCs [1, 7, 16, 18]. An important source of cytoplasmic citrate is pyruvate, produced from glycolysis and other reactions. Pyruvate is converted to acetyl-CoA in the mitochondria, which then reacts with oxaloacetate to form citrate, catalyzed by the enzyme citrate synthase of the tricarboxylic acid cycle. In the cytoplasm, citrate is converted back to acetyl-CoA by the enzyme ATP-citrate lyase. Therefore, citrate can also be considered as a transport form of acetyl-CoA from the mitochondria to the cytoplasm.

The active form of animal ACCs is a large, linear polymer, with a molecular weight of about 8 million Da for the complex [1, 18]. This polymer is made up of 10–20 protomers, which are dimers of the ACC enzyme. Citrate and long-chain acyl-CoA regulate the enzymatic activity of ACC partly by perturbing the equilibrium of this polymerization process. Citrate promotes the polymerization of ACC, and thereby leads to enzyme activation. The K_a of citrate for ACC1 and ACC2 is about 2 mM [7], although this activation may precede the polymerization process, indicating that citrate may cause an activating

conformational change in the protomer [69]. Besides citrate, other anions can also promote polymerization, including isocitrate, malonate, sulfate and phosphate.

In contrast to citrate, long-chain acyl-CoA is bound tightly by ACC, with K_i in the nanomolar range, and promotes the dissociation of the polymer into protomers. This inhibition may partly explain the lean phenotype observed in mice deficient in stearoyl-CoA desaturase 1 (SCD1), which is the crucial enzyme for producing unsaturated fatty acids [70]. The lack of SCD1 activity may produce higher than normal levels of saturated long-chain acyl-CoAs, which leads to the inhibition of ACCs and consequently elevated fatty acid oxidation [11]. Stimulation of AMPK may also play a role in metabolism in SCD1-deficient mice [71].

Besides acute regulation by citrate and long-chain acyl-CoAs, animal ACCs are also regulated by covalent modification, specifically phosphorylation of several critical Ser residues [7]. AMPK is the critical kinase that can phosphorylate both isoforms of ACC under physiological conditions [72], which significantly inhibits the activity of these enzymes (fig. 1B) through a reduction in the V_{max} and desensitizes them towards citrate activation. Phosphorylation by AMPK also potentiates the inhibitory effects of palmitoyl-CoA [73]. The sites of phosphorylation by AMPK include Ser79, Ser1200, Ser1215 in ACC1 and Ser218 in ACC2 (fig. 2C). Ser79 in ACC1, and equivalently Ser218 in ACC2, are located just prior to the BC domain (fig. 2C), and their phosphorylation may be sufficient to inhibit the activity of ACCs, as ACC2 lacks the phosphorylation sites equivalent to Ser1200 and Ser1215 of ACC1 (fig. 2C). Besides AMPK, protein kinase A (PKA) can also phosphorylate ACC1 and ACC2, at Ser77 and Ser1200 in ACC1, although the physiological relevance of this covalent modification is not yet established [7, 8].

AMPK is a master metabolic switch that becomes activated in response to a variety of stress signals and exercise [74–78]. AMPK can also be activated by various adipokines, such as leptin and adiponectin [3, 78–82]. Activated AMPK in turn regulates the catalytic activity of a large number of enzymes and cellular processes through phosphorylation, ultimately leading to inhibition of ATP-consuming pathways (such as fatty acid biosynthesis) and activation of ATP-producing pathways (such as fatty acid oxidation). Therefore, AMPK is a natural dual inhibitor of both isoforms of mammalian ACC (fig. 1B).

ACCs from *E. coli* and the yeast *Schizosaccharomyces pombe* are not regulated by citrate or long-chain acyl-CoAs [18]. ACC from the yeast *Saccharomyces cerevisiae* is activated by citrate, although citrate does not affect the homotetramer oligomerization state of this enzyme [1]. *E. coli* ACC is inhibited about 70% by 40 μM medium- to long-chain acyl carrier proteins, which are the direct products of FAS [22].

ACCs as targets for drug action and drug discovery

In humans, ACC1 and ACC2 have central roles in fatty acid biosynthesis and fatty acid oxidation (fig. 1A), making these enzymes attractive targets for the discovery of novel therapeutics against obesity, diabetes and other manifestations of the metabolic syndrome (fig. 1B) [16, 17].

Very few potent, small molecule inhibitors of mammalian ACCs are currently known. A series of such compounds that inhibit both isoforms of mammalian ACCs have recently been reported [52]. Compound CP-640186 (fig. 4A) has IC_{50} (inhibitory concentration 50%) values of about 50 nM against ACC1 and ACC2 from rat, mouse and monkey. In test animals, this compound reduced tissue malonyl-CoA levels, inhibited fatty acid biosynthesis and stimulated fatty acid oxidation, and most importantly reduced body fat mass and body weight and improved insulin sensitivity [17]. These observations demonstrate the promise of ACC inhibitors in the treatment of metabolic syndrome.

Stimulation of fatty acid oxidation is likely the principal route for achieving a favorable clinical outcome for ACC inhibitors. Therefore, any clinical candidate should potently inhibit ACC2. It is currently not known whether simultaneous inhibition of ACC1 is required, or tolerated, for a successful drug (fig. 1B) [17]. The CP-640186 compound is non-selective against the two isoforms, and appears to have performed well in animal models, lending support to the idea that isoform non-selective inhibitors may be adequate, or possibly even superior [17]. Complete specificity, inhibiting ACC2 without having any effect on ACC1, is probably not a prerequisite. Consider-

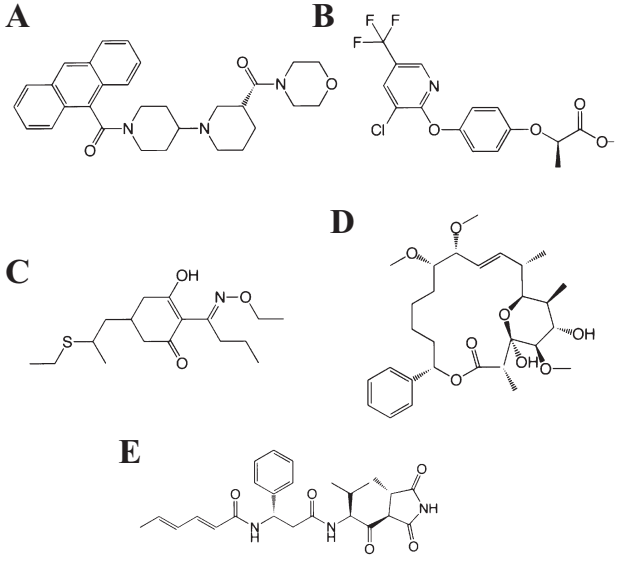

Figure 4. Chemical structures of representative ACC inhibitors. (*A*) CP-640186 [52]. (*B*) Haloxyfop. (*C*) Sethoxydim. (*D*) Soraphen A [104]. (*E*) Moiramide B [111].

ing the high degree of conservation between the two isoforms (see section on CT structure), achieving such a degree of absolute specificity is likely to be difficult in practice as well.

A benzoic acid compound, after being converted to its CoA ester in the liver, is an inhibitor of both 5-hydroxy-3-methylglutaryl (HMG)-CoA reductase (K_i of 18 μM) and ACC (K_i of 70 μM) [83]. The compound can lower blood cholesterol and triglyceride levels in normal and Zucker fatty rats. The CoA ester of an ω-hydroxy-alkanedicarboxylic acid compound is an inhibitor of ACC [84]. It reduced serum triglyceride and free fatty acid levels, fasting glucose and insulin levels, as well as body weight in animal models.

Analogs of long-chain fatty acids have been developed as inhibitors of ACCs [17]. These compounds are converted to their CoA esters and inhibit the enzymes by mimicking palmitoyl-CoA. Linking the biotin and CoA substrates together produced a bisubstrate analog inhibitor of ACC [85]. A chloroacetylated biotin derivative can inhibit animal ACCs and reduce lipid accumulation [86].

In plants, ACC is a proven target for drug action. Two classes of compounds kill sensitive plants by inhibiting their fatty acid biosynthesis, and they have been used commercially as herbicides for more than 20 years. These aryloxyphenoxypropionate (FOP, also known as APP, fig. 4B) and cyclohexanedione (DIM, also known as CHD, fig. 4C) compounds are reversible inhibitors of grass plastid ACC, and kinetic studies suggest they may interfere with the CT activity of the ACC enzyme, with K_i in the nanomolar range [21]. The two classes of compounds are mutually exclusive inhibitors, suggesting that they may share a common binding site. The CoA esters of the FOPs are more potent inhibitors of ACC than the parent compounds, although it is not known whether this mode of action actually occurs in vivo [87, 88].

The wide use of these herbicides has led to the emergence of resistant weeds. It has also exerted selection pressure on the nucleotide diversity in the black-grass ACC gene [89]. Genetic analyses identified several single sites of mutation in the CT domain that determine resistance (fig. 2C), confirming the kinetic results that these herbicides inhibit CT activity [24]. A single-site Ile-to-Leu mutation (at the position equivalent to residue 1705 in yeast ACC), which represents an extremely subtle change in the side chain of the amino acid, is sufficient to confer resistance to both the FOPs and the DIMs by the ACC enzyme from many grasses, while those that are insensitive to the herbicides have a Leu residue at this position [90–94]. The mutation does not appear to affect the catalytic properties of the enzyme. Cooperativity was observed in the binding of herbicides to the mutated, resistant or the naturally insensitive ACC enzyme homodimer, whereas this phenomenon is not observed with the sensitive enzyme [95].

The second single-site mutation that confers resistance to herbicides is an Ile-to-Asn change (equivalent to residue 1967 in yeast ACC), again in the CT domain of ACC [96, 97]. Interestingly, this mutation only affects sensitivity to FOP compounds, but has minimal impact on the potency of DIM compounds. The binding sites of these two classes of compounds may not entirely overlap. Mutations at three additional sites in the CT domain have been identified from resistant weeds [98]. An Asp→Gly change (equivalent to residue 2004 in yeast ACC) confers resistance to both the FOPs and DIMs, whereas a Trp→Cys and a Gly→Ala change (1953 and 2022 in yeast ACC) confer resistance to the FOPs only. Efforts are under way to develop new inhibitors that have activity against the plastid ACC from resistant plants [99].

Cereal crops such as wheat are resistant to the herbicides even though their plastid ACC enzymes are sensitive. These plants can rapidly metabolize the herbicides to inactivate them, offering another mechanism for achieving herbicide resistance at the whole plant level [37, 100]. A new DIM compound was found to inhibit the plastid ACC enzyme from rice, but is not harmful to the rice plant [101].

These herbicides are weak inhibitors against mammalian and yeast ACCs [48, 102]. These enzymes contain a Leu residue at the first position (1705 of yeast ACC), and a Val residue at the second position (1967 of yeast ACC). However, these differences are not the sole determinant of herbicide sensitivity, as the L1705I/V1967I double mutant of yeast CT domain is not more sensitive to the herbicides [103]. There must be additional structural differences between these enzymes and the plastid ACCs from grasses (see section on CT structure).

Soraphen A is a macrocyclic polyketide natural product (fig. 4D) that is secreted by the soil-dwelling myxobacterium *Sorangium cellulosum* as a fungicide [104, 105]. It is a potent inhibitor of the BC domain of eukaryotic ACCs, with K_d of about 1 nM, but it has no effect on bacterial BC subunits [17, 106, 107]. The compound has been tested extensively as a broad-spectrum fungicide for agricultural applications [105]. It is also being developed for use as a dominant selection marker for fungal transformation [108].

In bacteria, inhibiting fatty acid biosynthesis is an attractive approach for developing novel antibiotics [109, 110]. Most of the inhibitors are directed against the FAS enzyme complex, while very few inhibitors of bacterial ACCs are known. Recently, a potent inhibitor of the CT activity of bacterial ACC was reported [111–113]. Moiramide B (fig. 4E), a natural product, is competitive with the acetyl-CoA substrate and has a K_i value of 5 nM against the CT subunits of *E. coli* and other bacteria, while having little inhibitory activity against the eukaryotic ACCs.

Inhibition of ACC may also be efficacious for the treatment of infections by apicoplexan parasites, which in-

clude *Plasmodium falciparum* (the causative agent for malaria) and *Toxoplasma gondii* [26, 114]. De novo fatty acid biosynthesis in these organisms is localized in the apicoplast, which contains a multi-domain ACC. Interestingly, *T. gondii* ACC is inhibited with moderate potency (IC_{50} of 5–20 μM) by FOPs, while the enzyme is insensitive to DIMs [115]. This apicoplast enzyme has a Leu residue at the position (equivalent to 1705 in yeast ACC) where the Ile →Leu mutation confers resistance to the herbicides in plant ACCs. The Leu →Ile mutant is surprisingly resistant to FOPs, suggesting the possible presence of structural differences in this region of the *T. gondii* enzyme compared with the plant enzymes.

ACC contains two catalytic activities, BC and CT. Shutting down either activity should be sufficient to inhibit the overall reaction of the enzyme. Almost all the ACC inhibitors are targeted against the CT, while soraphen A is the only potent inhibitor of BC that is currently known. In animals, it is conceivable that inhibitors could be found that disrupt the polymerization process of the enzyme.

Structure of ACC

The holoenzyme of bacterial ACC is unstable and readily dissociates into the individual subunits. Therefore, the different subunits can be studied separately, and the first structural information on the ACCs was obtained for the BC subunit of *E. coli* ACC [116]. This was followed by the crystal structure of the biotinoyl domain of *E. coli* BCCP [117]. Crystals of the CT subunits of *E. coli* ACC have also been obtained, but they do not diffract to sufficient resolution for structural studies [22].

The exceptional size of eukaryotic multi-domain ACCs (>200 kD) probably precludes any detailed structural studies of these enzymes in their entirety. However, it has been possible to study the domains in these enzymes separately. The first structural information on the CT activity of ACC was obtained for the yeast enzyme [48], and the first structure of an eukaryotic (yeast) BC domain was also determined recently [107].

BC

Crystal structures the free enzyme of *E. coli* BC as well as the complex of an active site mutant (Glu288Lys) with ATP have been determined [22, 116, 118]. The crystal structure of the BC subunit of *Aquifex aeolicus* PC is highly homologous to that of the *E. coli* enzyme [119]. The only structures of a eukaryotic BC domain are for the yeast ACC, both the free enzyme and the complex with the potent natural product inhibitor soraphen A [107].

The structure of BC has the ATP-grasp fold, which is shared by several enzymes with related biochemical functions – the ATP-dependent acylation of an amine or amide nitrogen [120, 121]. The catalysis by these enzymes generally proceeds through the formation of an acylphosphate intermediate. The structure of BC contains three domains, A, B and C domains, as well as the AB linker that connects the A and B domains (fig. 5A). The A and C domains and the AB linker form a cylindrical structure (fig. 5A). The active site of the enzyme is located at the top of this cylinder, where ATP is located. The B domain is a lid over the active site. The adenine base of ATP is recognized specifically by the enzyme, through hydrogen bonding to its N1 and N6 atoms. The phosphates of ATP interact with a glycine-rich loop in the B domain and several Arg, Lys and His side chains from the B and C domains, as well as a Lys residue from the AB linker.

The B domain may undergo a large conformational change during catalysis. In the structure of BC in complex with ATP, the B domain is in intimate contact with the ATP molecule and closes the active site (fig. 5A). In contrast, in the structures of the free enzyme of bacterial BC subunits [22, 119], the B domain assumes an open conformation, where it is positioned away from the A and C domains and can be more disordered (fig. 5B). Interestingly, the B domain of yeast BC assumes a closed conformation even in the absence of ATP in the active site (fig. 5A).

The bacterial BC subunits are dimers in solution [22]. The crystal structures show that the two monomers are associated through their C domains (fig. 5C). The active sites of the two monomers are located far from the dimer interface, and it may therefore appear that each monomer contains a complete active site (fig. 5C). The BC dimer does not exhibit cooperativity in catalysis [22]. However, dimerization is required for the activity of the BC subunits. Moreover, a heterodimer of *E. coli* BC with one wild-type active site and an inactivated mutant active site has essentially no activity [22]. The exact molecular mechanism for this dimerization requirement is currently not known. Eukaryotic BC domains are monomeric in solution and are catalytically inactive [106, 107], suggesting that dimerization may also be required for the activity of these BC enzymes.

Soraphen A is bound in an allosteric site, about 25 Å from the active site of the BC domain, at the interface between the A and C domains (fig. 5A) [107]. While the active site of BC is located at the top of the cylinder formed by the A and C domains, soraphen A is located at the bottom of this cylinder, therefore at the opposite face of the BC domain from the active site (fig. 5A).

Soraphen A has extensive interactions with the BC domain (fig. 6A), and most of the residues that are in contact with this natural product are highly conserved among the eukaryotic BC domains. This provides a molecular basis for the potent activity of this polyketide against eukaryotic ACCs. The structure also explans the resistance mutations against soraphen A, for example Ser77Tyr and Lys73Arg,

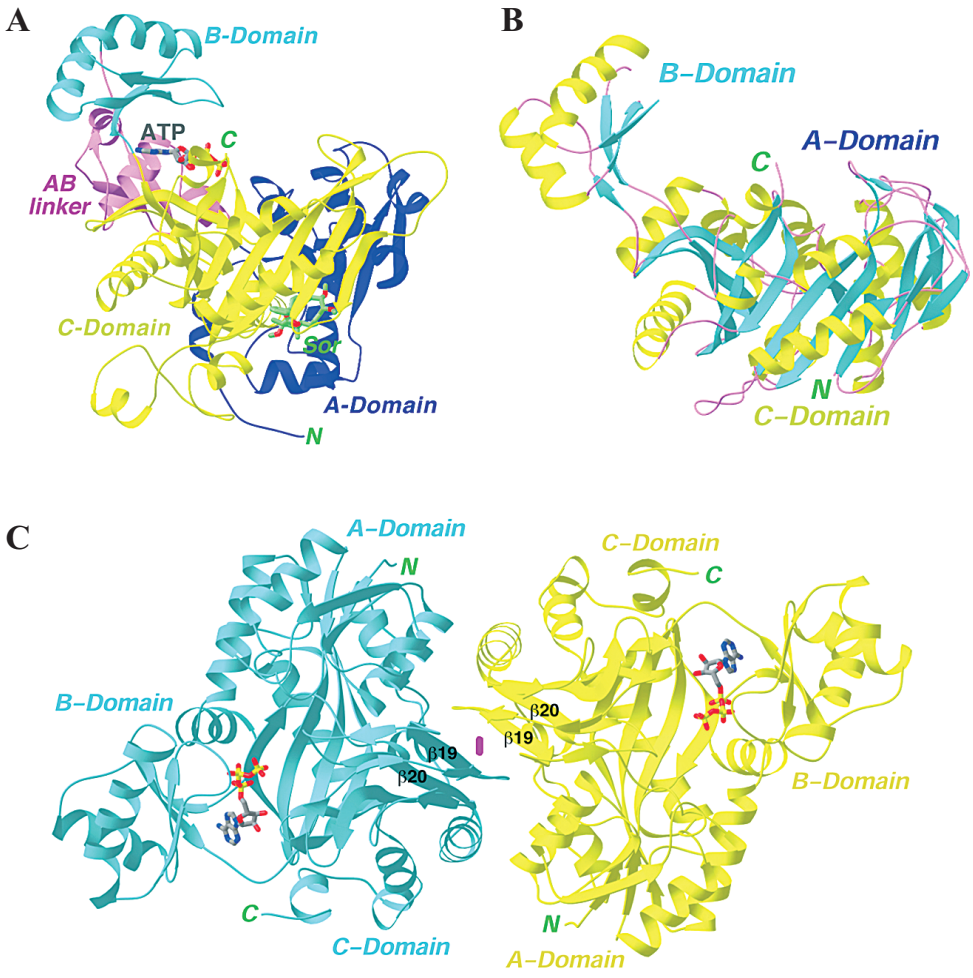

Figure 5. Structures of biotin carboxylase (BC). (*A*) Structure of yeast BC domain in complex with soraphen A [107]. The A, B and C domains are given different colors. Soraphen A is shown as a stick model in green for carbon atoms, labeled Sor. The expected position of ATP, as observed in the *E. coli* BC subunit [118], is shown in gray. (*B*). The open form of *E. coli* BC subunit free enzyme [116]. The B domain is located far from the rest of the structure. (*C*) Dimer of *E. coli* BC subunit. The bound position of ATP is shown [118]. The dimer axis is indicated with the magenta oval.

as they have important roles in binding soraphen A (fig. 6A).

The compound is located on the surface of the BC domain, and half of its surface is exposed to the solvent (fig. 6B). The binding of soraphen A leads to the burial of the side chain of Trp487 (fig. 6A), which can be used as the reporter for a fluorescence-based binding assay [107]. This allowed the characterization of the impact of mutations in the binding site, and the observed effects provide further support for the structure of the complex.

Yeast BC domain shares 37% amino acid sequence identity with the *E. coli* BC subunit (fig. 2D). While their overall structures are similar, there are significant differences between them, especially in the soraphen A binding site [107]. The largest difference in this region is in the positioning of strand β19 in the central β sheet of the C domain, which is shifted by about 3 Å in the *E. coli* BC subunit towards the soraphen A binding site (fig. 6C). As a consequence, the binding site is blocked by this β strand in the *E. coli* enzyme (fig. 6D). Therefore, soraphen A does not inhibit the bacterial BC subunits due to structural differences between the bacterial and eukaryotic BC in its binding site.

Located in an allosteric site, soraphen A may indirectly affect substrate binding in the active site or it may function through a novel mechanism. There is little conformational change in the BC domain upon soraphen A binding, so it is unlikely for soraphen A to affect substrate binding [107]. Structural comparison with the bacterial BC dimer suggests that soraphen A may be located in the interface of the putative dimer of eukaryotic BC domains (fig. 6E). Therefore, soraphen A binding may disrupt the dimerization of eukaryotic BC domains. Native gel electrophoresis experiments with the BC domain provide evidence in support of this structural prediction. Soraphen A promotes the appearance of a fast migrating band in this gel, whereas it

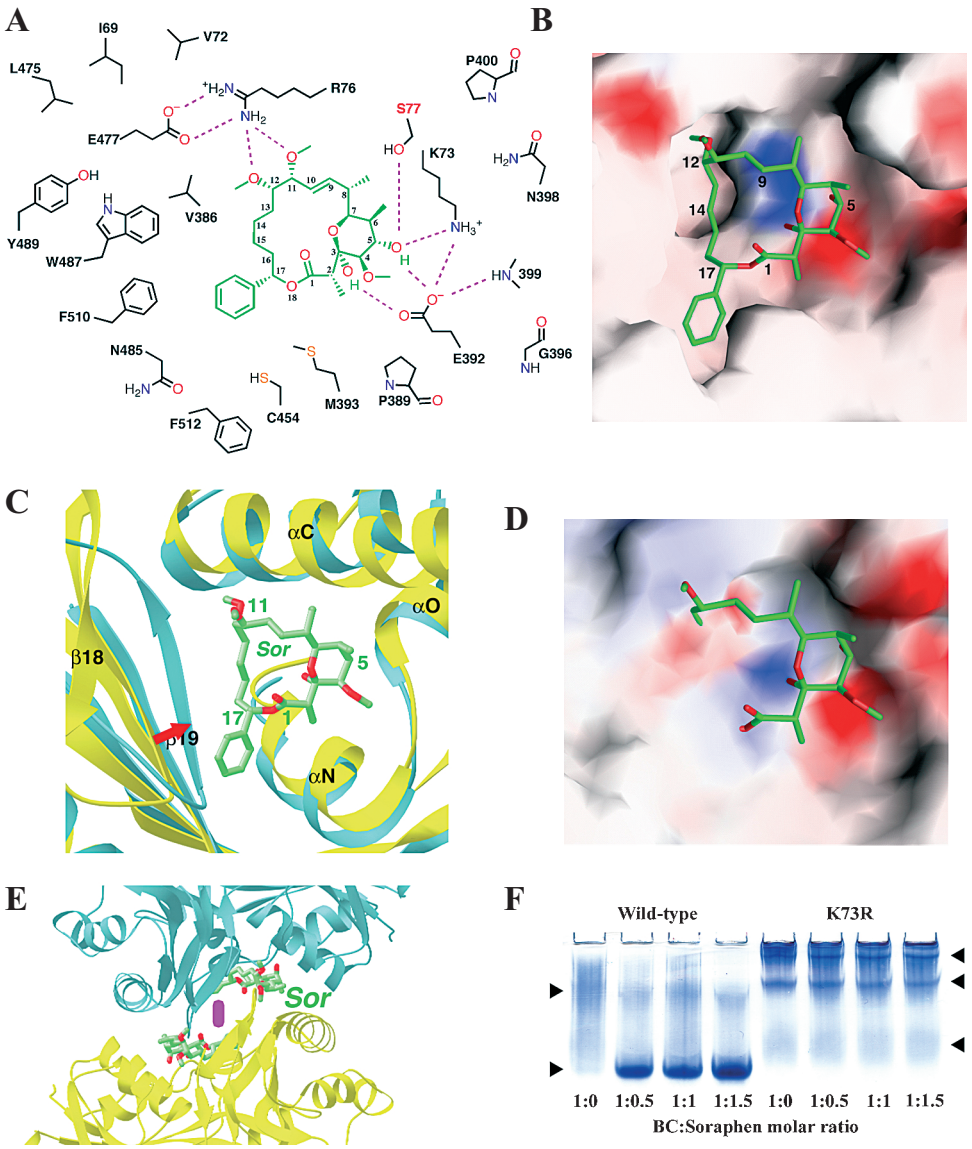

Figure 6. The binding mode of soraphen A to the yeast BC domain. (*A*) Schematic drawing of the interactions between soraphen A and the yeast BC domain [107]. (*B*) Molecular surface of the BC domain in the soraphen A binding site. (*C*) Structural comparison between yeast (in yellow) and *E. coli* (cyan) BC in the soraphen A binding site. The shift in the position of strand β19 is indicated with the red arrow. (*D*) Molecular surface of the *E. coli* BC subunit in the soraphen A binding site. The soraphen A molecule is shown for reference, and has extensive steric clash with the bacterial BC subunit. (*E*) The dimer interface of the *E. coli* BC subunit [118]. The position of soraphen as observed in the yeast BC domain structure is shown for reference, in green for carbon atoms. (*F*) Native gel showing the electrophoretic mobility of wild-type and Lys73Arg mutant of yeast BC domain in the absence or presence of soraphen. Possible bands in the gel are marked with the arrowheads.

has no effect on the migration behavior of the soraphen-insensitive Lys73Arg mutant (fig. 6F). Soraphen A may inhibit the BC activity via a novel mechanism, by functioning as a protein-protein interaction inhibitor to disrupt the dimerization (or oligomerization) of the enzyme.

CT

The yeast CT domain is the only CT enzyme from ACC for which structural information is currently available, as a free enzyme or in complex with CoA [48], the herbicides haloxyfop or diclofop [103], or the inhibitor CP-640186 [50]. Crystal structures of CT from other enzymes have also been reported, including the CT subunit of glutaconyl-CoA decarboxylase (Gcdα) from *Acidaminococcus fermentans* [122], the transcarboxylase 12S core from *Propionibacterium shermanii* [123], and the β subunit of PCC from *Streptomyces coelicolor* [124].

The structure of yeast CT domain contains two subdomains (fig. 7A) [48], N and C domains, which are equiv-

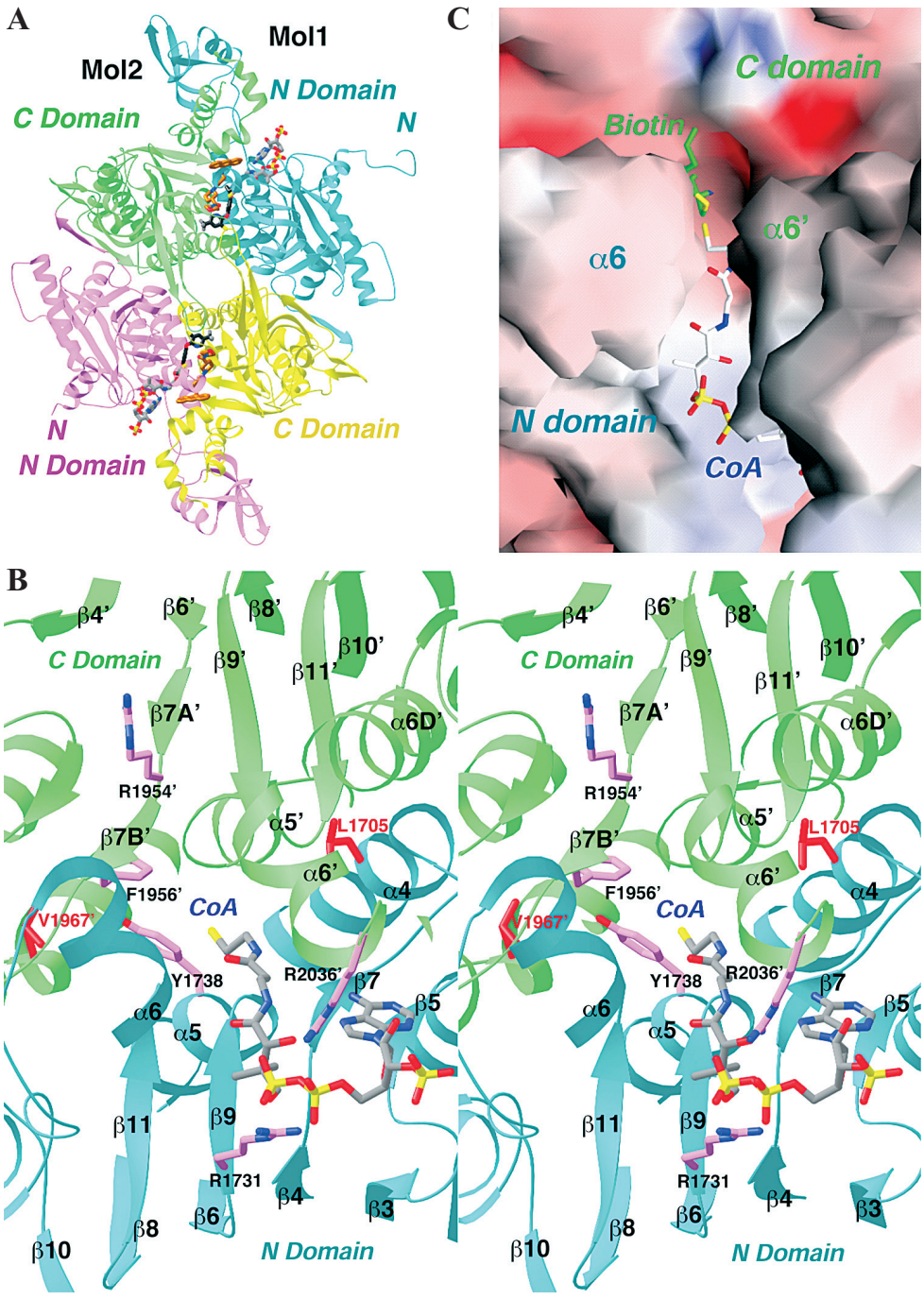

Figure 7. Crystal structure of the CT domain of yeast ACC. (*A*) Schematic drawing of the structure of yeast CT domain dimer [48, 50, 103]. The N domains of the two monomers are colored in cyan and magenta, and the C domains are colored in yellow and green. The positions of CoA (gray), haloxyfop (black) and CP-640186 (gold) are shown. (*B*) The active site of yeast CT domain. The N domain is shown in cyan, and the C domain of the other monomer in green. The side chains of residues in the active site are shown in purple. The prime (′) in the labels indicates the C domain of the other monomer of the dimer. (*C*) Molecular surface of the active site region of yeast CT domain. The side chain of Lys1764 (in helix α6, 15 Å from the active site) has been removed to facilitate the viewing of the active site. The bound position of biotin in the β subunit of PCC is shown for reference in green [124].

alent to the β and α subunits of *E. coli* CT (fig. 2C). Both domains have the β-β-α superhelix fold that is found in crotonase and other proteins [22, 125]. Crotonase and several other enzymes of this superfamily have important roles in the β-oxidation of fatty acids, indicating the intriguing possibility that ACC and these enzymes might share a common evolutionary origin. The N and C domains of CT also contain many unique features, such as significant surface insertions (fig. 7A). In addition, the binding site for the adenine base of acyl-CoA exists only in the N domain and is absent in the C domain.

Yeast CT domain is a dimer in solution. The two monomers of the dimer are arranged in a head-to-tail fashion, such that the N domain of one monomer is in contact with the C domain of the other monomer (fig. 7A). Most of the residues in this interface are highly conserved among eukaryotic multi-domain ACCs, and the dimeric nature of the CT domains is also consistent with the $\alpha_2\beta_2$ stoichiometry of the bacterial CT subunits. The CT domain of human ACC1 is also dimeric in solution [50], and the structure of the yeast CT domain dimer is likely a good model for the human enzyme as well.

The active site of the CT domain is located at the dimer interface (fig. 7A) [48]. While most of the CoA molecule is associated with the N domain, the thiol group of CoA is situated right at the dimer interface (fig. 7B). The α6 helix from the N domain of one monomer and the α6′ helix from the C domain of the other monomer form two walls of a deep canyon in the active site of the CT domain (fig. 7C), and the small β sheets of the β-β-α superhelix of the two domains form the floor of the canyon (fig. 7B). It is expected that the biotin substrate will approach the active site from the C domain of the other monomer, and this has been confirmed in the structure of the β subunit of PCC in complex with biotin and propionyl-CoA (fig. 7C) [124].

There is essentially no conformational change in the CT domain upon CoA binding. The adenine base of CoA is recognized by the CT domain through specific hydrogen bonding to its N1 and N6 atoms [48]. The phosphate groups of CoA are located close to several Lys and Arg residues (fig. 7B). Arg1731 interacts with both the α and β phosphate of CoA, and mutation of this residue leads to a 14-fold increase the K_m of the malonyl-CoA substrate [48]. Mutation of another residue that contact the phosphates, Arg2036′ (with prime indicating the second monomer), has no effect on enzyme activity, suggesting it has little contribution to CoA binding. Mutation of the Arg1954′ residue (fig. 7B) has the largest impact on the catalysis by the CT domain, producing a 75-fold increase in the K_m for malonyl-CoA and 300-fold decrease in the k_{cat}/K_m. This residue is strictly conserved among the multi-domain ACCs and may be involved in recognizing the carboxyl group of the malonyl-CoA or carboxybiotin substrate.

The FOP compounds are bound at the dimer interface (fig. 7A), in a novel pocket in the active site region of the CT domain [103]. The pyridyl ring of haloxyfop shows π-stacking interactions with the side chains of Tyr1738 and Phe1956′, and the phenyl ring is sandwiched between two peptide planes (fig. 8A). Even though there is no spatial overlap between haloxyfop and CoA (fig. 7A), steric clash between the herbicide and the acetyl or malonyl group of the substrate is expected. This is supported by kinetic studies showing that haloxyfop is a competitive inhibitor versus the malonyl-CoA substrate for the yeast CT domain [48], although the K_i against the yeast CT is rather weak, about 250 μM.

A large conformational change in the active site region of the CT domain is observed in the haloxyfop complex (fig. 8B), and this change is required for the binding of this herbicide. The side chains of Tyr1738 and Phe1956′ assume new conformers, and the main chain of Phe1956′ shifts by about 2 Å (fig. 8B). In the free enzyme, these two residues contact each other and help cover the hydrophobic core of the dimer (fig. 7B). Their positions in the free enzyme structure actually clash with the bound position of haloxyfop (fig. 8B). The conformational changes of these and other residues, together with a change in the organization of the dimer (fig. 8B), create a binding pocket that opens up into the core of the dimer. This conformation of the CT domain is incompatible with the binding of the acetyl- or malonyl-CoA substrate, and therefore it may not have a role in the catalysis by this enzyme.

Two residues that confer resistance to the herbicides when mutated, which are equivalent to Leu1705 and Val1967 of yeast CT, are located at opposite ends of the haloxyfop binding site (fig. 7A, B) [103]. These are the only two residues in the binding site that show variations among the multi-domain ACCs (fig. 9 and see below). However, the exact molecular mechanism of the resistance mutations is currently still not clear, as neither of the two residues show crucial contacts with the herbicide (fig. 8A). A double mutant of yeast CT, changing both residues to Ile, is not more sensitive to the herbicides [103].

The three additional mutations that confer resistance to the herbicides in black-grass [98] are not in direct contact with haloxyfop, but are located close to the binding site. The Trp →Cys mutation is equivalent to residue Trp1953′ in yeast CT. This residue has π-stacking interactions with the side chain of Trp1924′, which is located next to the trifluoromethyl group of haloxyfop (fig. 8A). Like Val1967′ in this region of the binding site, the Trp →Cys mutation confers resistance only to the FOPs [98]. The Asp →Gly mutation is equivalent to residue Asp2004′ in yeast CT. This residue is hydrogen-bonded to the side chain of the strictly conserved Trp2000′ at the C-terminal end of the α5′ helix, while the N-terminal end of this helix interacts with haloxyfop (fig. 8A). The mutation may have dis-

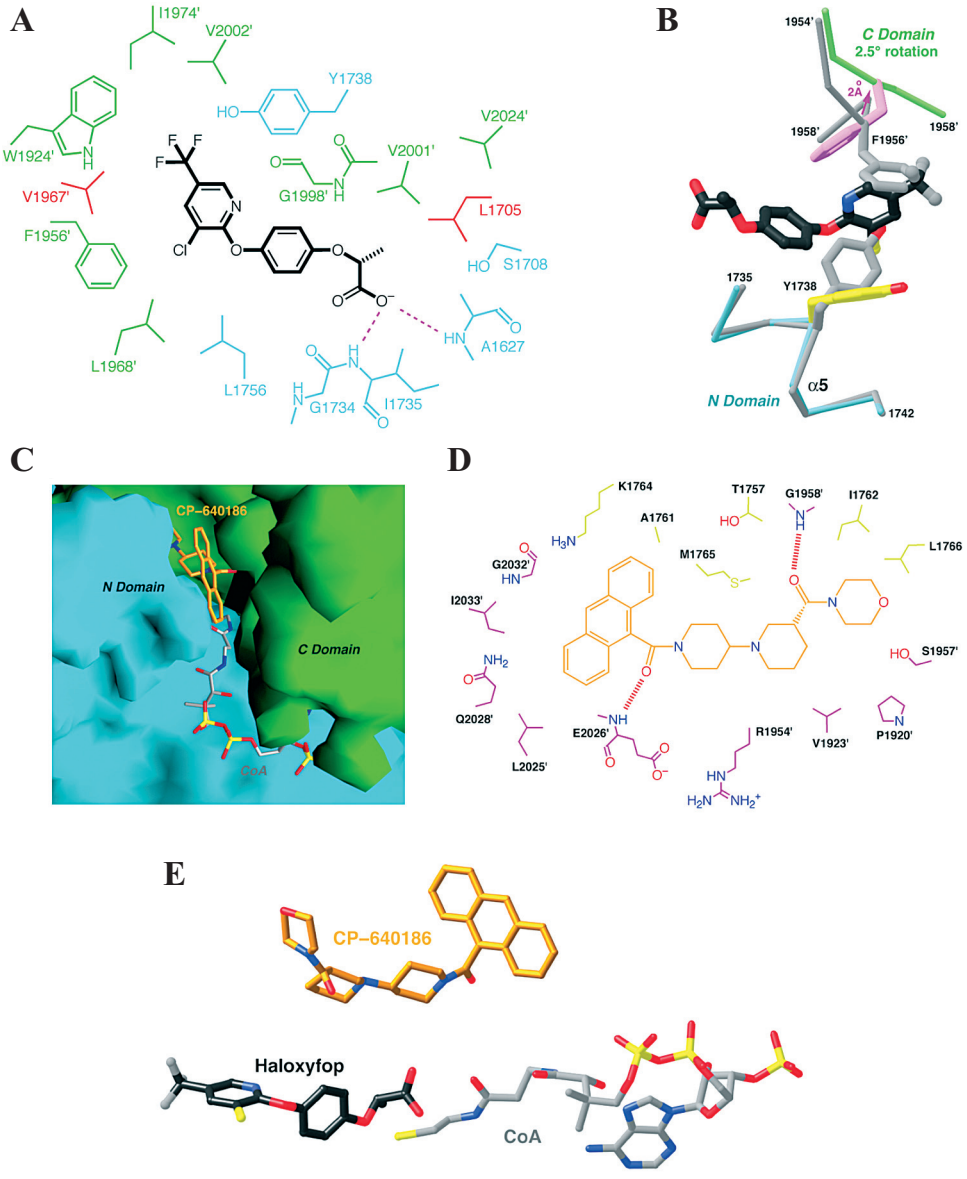

Figure 8. The binding mode of inhibitors to yeast CT domain. (*A*) Schematic drawing of the interactions between haloxyfop and the yeast CT domain [103]. (*B*) Structural overlay of the CT domain free enzyme (in gray) and the haloxyfop complex (in cyan and green for the N and C domains) near the inhibitor binding site. (*C*) Molecular surface of the active site region of yeast CT. The CoA and CP-640186 molecules are shown in gray and gold, respectively. (*D*) Schematic drawing of the interactions between CP-640186 and the yeast CT domain. (*E*) Three distinct binding regions in the active site of CT. Comparison of the binding modes of CP-640186 (in gold), haloxyfop (black) and CoA (gray).

rupted the conformation of this helix, and hence interfered with herbicide binding. The Gly→Ala mutation is equivalent to residue 2022′ in yeast CT. Interestingly, yeast ACC has an Ala residue at this position, which is located in a hydrophobic environment that contains Leu1705 (fig. 7B).

Several attempts at determining the binding mode of the DIM compounds to the yeast CT domain have not been successful. These compounds are not sensitive to the mutation of the residue equivalent to Val1967′, and therefore may not contact this part of the binding site (fig. 8A). Val1967′ becomes accessible to herbicide binding only after the conformational change in the dimer interface (fig. 7B). Therefore it will be interesting to observe whether DIM binding is dependent on a conformational change in the CT domain.

CP-640186, a potent inhibitor of mammalian ACCs [52], is also bound in the dimer interface, in the active site region of the CT domain (fig. 7A) [50]. In contrast to the FOP compounds, the binding of this compound does not cause any significant conformational changes in the CT domain. The anthracene group of the inhibitor is situated

in the canyon of the CT active site, having tight interactions with helices $\alpha 6$ and $\alpha 6'$ from the two monomers (fig. 8C). The rest of the inhibitor is situated over the C domain of the second monomer (fig. 8C), suggesting that CP-640186 may utilize the biotin binding site of the enzyme (fig. 7C). The compound is noncompetitive versus the acetyl-CoA or malonyl-CoA substrate [50, 52]. Both carbonyl oxygens of the inhibitor are involved in hydrogen-bonding interactions with main chain amides from the CT domain (fig. 8D).

The presence of the anthracene fluorophore in CP-640186 allowed the development of a fluorescence anisotropy binding assay to measure the affinity of this compound for various CT domains [50]. CP-640186 is a moderate inhibitor of the yeast CT domain, with K_d of about 7 μM. It is more potent against the CT domain of human ACC1, with a K_d of 1.2 μM. However, the IC_{50} of this compound against full-length rat ACC1 and ACC2 is 50 nM, and the morpholino group is important for high-affinity binding [52]. One face of this group is exposed to the solvent in the complex with the yeast CT domain. The BCCP domain in the full-length ACC may help cover this part of the binding site and shield the compound from the solvent. In addition, the IC_{50} values against the rat enzymes were measured in the presence of the activator citrate, which can protect the enzyme against inactivation by avidin [52]. It is possible that citrate promotes the tight association of the BCCP and CT domains, thereby making biotin inaccessible to avidin, and conferring additional potency to the CP-640186 compound against the mammalian ACCs.

Remarkably, there is little structural overlap among the bound positions of CoA, haloxyfop and CP-640186 (fig. 8E), suggesting the presence of at least three distinct regions for inhibitor binding in the active site of the CT domain. Nanomolar inhibitors against the plant ACCs have been developed using the haloxyfop binding site [36], and nanomolar inhibitors against the mammalian ACCs have been developed using the CP-640186 binding site [52]. This bodes well for the identification and development of potent inhibitors against human and other ACCs.

The active site region of the CT domain is highly conserved among the multi-domain ACCs (fig. 9). For example, in the immediate neighborhood (within 5 Å) of the CP-640186 molecule, there are only six amino acid differences between the CT domains of yeast ACC and human ACC1. This suggests that the overall binding mode of the compound to the human enzymes should be similar, and the yeast enzyme is a good surrogate for defining the molecular mechanism of action of mammalian ACC inhibitors (until the human enzymes are crystallized). There are six amino acid differences between human ACC1 and ACC2 in the active site region of CT, none of which is in contact with the inhibitors or the substrate (fig. 9). It remains to be seen whether these differences are sufficient for the development of isoform selective inhibitors of mammalian ACCs. It may also be possible that amino acid differences between ACC1 and ACC2 outside the active site region can indirectly affect the conformation of the active site, which could help enhance the selectivity of inhibitors.

The tertiary and quaternary organization of the yeast CT domain is also observed in the 12S core of the transcarboxylase from *Propionibacterium shermanii* [123] as well as the β subunit of PCC from *S. coelicolor* [124]. The structures of these two enzymes are highly homologous to each other, as they share 51% amino acid sequence identity. The sequence conservation between them and the yeast CT domain is much weaker, in the 20% range, underscoring their remarkable conservation in structure.

The structure of the CT subunit of glutaconyl-CoA decarboxylase (Gcdα) also contains two domains with the

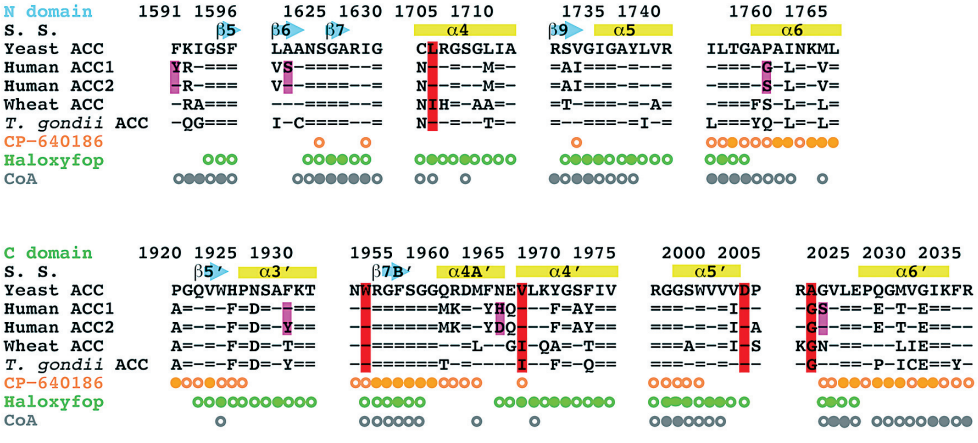

Figure 9. The active site of the CT domain is highly conserved among the eukaryotic multi-domain ACCs. Residues that are within 5 Å of CP-640186, haloxyfop and CoA are indicated by filled circles in gold, green and gray, respectively. Residues that are within 10 Å are indicated by the open circles. Residues that confer herbicide resistance when mutated are highlighted in red. Residues that are different between human ACC1 and ACC2 are highlighted in magenta.

crotonase fold, and the enzyme is a dimer in solution [122]. Surprisingly, the arrangement of the two domains in Gcdα monomer is entirely different from that seen for the CT domain of yeast ACC, and as a consequence the organization of the Gcdα dimer is different as well. The unique biological function of this enzyme, providing energy for a sodium ion pump, may be consistent with this novel structural organization [122].

BCCP

The structure of the C-terminal biotinoyl domain of BCCP contains a central β-sandwich, with pseudo-twofold symmetry (fig. 10) [22, 117]. The biotinylation motif is located in a tight β-turn of this β-sandwich (fig. 10). The ureido ring of biotin interacts with a unique inserted segment in the structure of BCCP, known as the thumb (fig. 10), and this interaction also helps to stabilize the biotinoyl domain [126]. The lipoyl domain of pyruvate dehydrogenase shares the same structural features as the biotinoyl domain, suggesting a possible evolutionary relationship between the two proteins [22].

Residues of BCCP outside the biotinoyl domain are highly flexible in structure. A Pro/Ala-rich linker segment is located just prior to the biotinoyl domain. Deletion of this linker results in an inactive mutant, although it can still be biotinylated [127]. The first 30 residues at the N terminus may be involved in the dimerization of BCCP, while the biotinoyl domain of BCCP is monomeric in solution, even at 1–2 mM concentrations [22]. These residues also mediate the interactions between BCCP and BC, forming a $(BC)_2(BCCP)_4$ complex [27]. It may be possible that each BC active site interacts with a BCCP dimer in this complex.

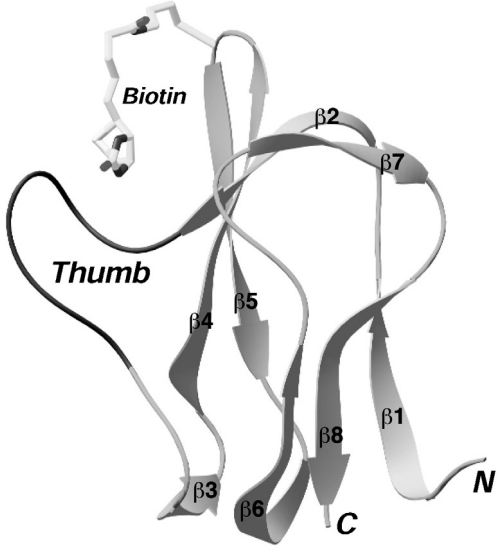

Figure 10. Structure of BCCP. Structure of the monomer of *E. coli* BCCP [117]. The biotin prosthetic group is shown, and the 'thumb' is labeled.

Summary and perspectives

ACCs are biotin-dependent carboxylases and catalyze the production of malonyl-CoA from acetyl-CoA with the hydrolysis of ATP. Multi-subunit ACCs are found in bacteria, archaea and the chloroplasts of most plants, whereas multi-domain ACCs are found in most eukaryotes. ACCs have crucial roles in the biosynthesis and metabolism of fatty acids, as well as other important secondary metabolites, and ACC activity is crucial for the viability of most living organisms. The activities of these enzymes are regulated both at the transcriptional level and at the protein level, in response to a variety of cellular and nutritional signals. Structural information on the subunits or domains of these enzymes has greatly enhanced our understanding of their catalytic mechanism as well as the molecular mechanism of their inhibition.

This is an exciting time for research on enzymes involved in fatty acid metabolism and the regulation of their expression and activity. The important roles of these enzymes in the pathogenesis of obesity, type 2 diabetes and other clinical manifestations of the metabolic syndrome have generated a significant amount of new and renewed interest in them. The current intense research activity in this area should significantly increase our basic understanding of the expression, regulation and mechanism of these enzymes. Just as important, it can be expected that the research activity could lead to the development of modulators of these enzymes that prove efficacious in the clinic. This should be of tremendous benefit to the population in general, in our fight against the obesity epidemic.

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