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PUBLICATION
Hailong Zhang, Zhiru Yang, Yang Shen, Yang and Liang Tong, "Crystal Structure of the Carboxyltransferase Domain of Acetyl-coenzyme A Carboxylase," *Science*, **299**, 2064-2067,

FUNDING
Columbia University

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Crystal Structure of the Carboxyltransferase Domain of Acetyl Coenzyme A Carboxylase

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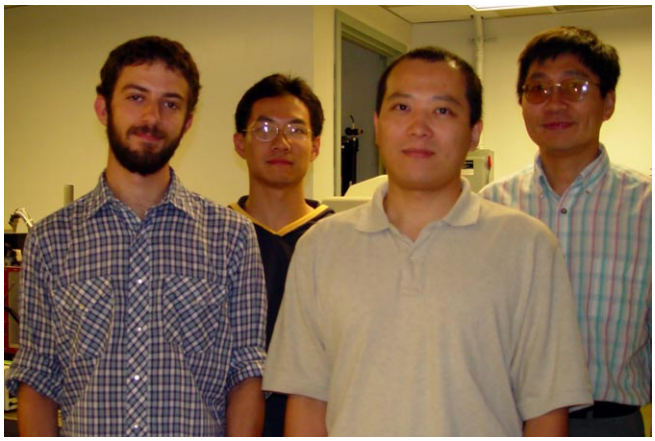
Acetyl-CoA carboxylases are crucial for the biosynthesis and oxidation of long-chain fatty acids. They are targets for drug development against obesity and diabetes, and several herbicides function by inhibiting the carboxyltransferase (CT) domain of these enzymes in plants. We have determined the crystal structures of yeast CT free enzyme and the CoA complex at 2.7 Å resolution. The structure of CT comprises two domains, both belonging to the crotonase/ClpP superfamily. The active site is at the interface of a dimer. The herbicides target the active site of CT, providing a lead for inhibitor development against human ACCs.



Left to right: Bengamin Tweel, Yang Shen, Hailong Zhang, Liang Tong

Mammalian, yeast, and most other eukaryotic ACCs are large, multi-functional enzymes, containing the biotin carboxylase (BC) domain, the biotin carboxyl carrier protein (BCCP) domain, and the carboxyltransferase (CT) domain (**Figure 1a**). BC catalyzes the ATP-dependent carboxylation of a biotin group covalently linked to a lysine residue in BCCP, and then CT catalyzes the transfer of the carboxyl group from biotin to acetyl-CoA to produce malonyl-CoA (**Figure 1b**). In *E. coli* and other bacteria, ACCs are multi-subunit enzymes, with BC, BCCP, and two subunits for the CT (**Figure 1a**).

We have determined the crystal structures of the CT domain of yeast ACC, which constitutes the 90 kD fragment at the C-terminus of the protein (**Figure 1a**). Each CT domain molecule is made up of two sub-domains, N and C domains, that are intimately associated with each other (**Figure 1c**). The two domains share similar polypeptide backbone folds, with a central β-β-α super-helix. However, the amino acid sequence identity between the structurally equivalent residues of the two domains is only 12 %, underscoring the lack of sequence conservation between them. The backbone fold is similar to that of the crotonase/ClpP superfamily, even though the amino acid sequence identity between CT domains and these other proteins is less than 14 %.

A dimer of the CT domain is observed in the crystal as well as in solution. About 5300 Å^2 of the surface area of each monomer is buried in the dimer interface, involving mostly residues that are highly conserved among the ACCs. The dimer is formed by the side-to-side arrangement of the two monomers, such that the N domain of one molecule is placed next to the C domain of the other (**Figure 1c**).

The structure of the CoA complex shows that the active site of the enzyme is at the interface of the dimer (**Figures 1c, 2a**). Residues in this active site are well conserved among the various CT domains.
Herbicides that target the CT domain are powerful inhibitors of plastid ACC and can kill sensitive plants by shutting down fatty acid biosynthesis. Mutagenesis and biochemical studies showed that an Ile residue in the CT domain plays an important role in determining the sensitivity of the wheat enzyme to the commercial herbicides. An Ile→Leu mutation renders the wheat enzyme resistant to the herbicides, and plant ACCs that are

insensitive to the herbicides have a Leu residue at this position. This residue is equivalent to Leu1705 in yeast ACC, which is located deep at the bottom of the active site cavity and also in the dimer interface. Thus, mutagenesis and structural information suggest that the herbicides target the active site of CT.

Our kinetic experiments confirm that the herbicide haloxyfop is a competitive inhibitor of yeast CT with respect to the substrate malonyl-CoA (**Figure 2b**).

The successful development of inhibitors against the active site of the CT domain of plant ACCs holds promise for the development of inhibitors against other CT domains, especially those of human ACCs. Our structural information on the CT domain provides a starting point for understanding the catalysis by this enzyme as well as for designing and optimizing its inhibitors.

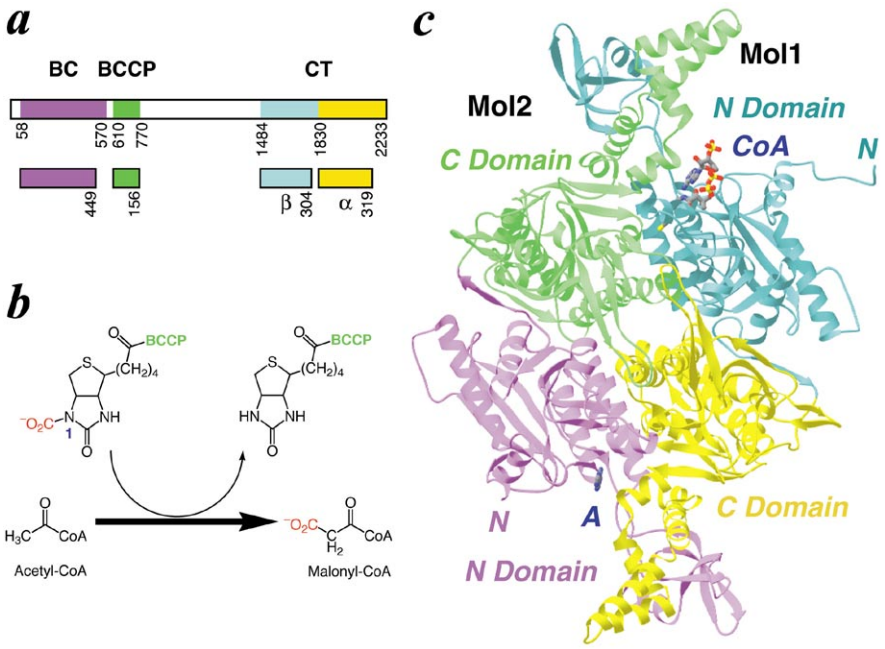


Figure 1. *Structures of ACCs.* **(a)**. *Schematic drawing of the primary structures of eukaryotic, multi-domain ACC and bacterial, multi-subunit ACC.* **(b)**. *The chemical reaction catalyzed by CT.* **(c)**. *Schematic drawing of the structure of the CT domain dimer of yeast ACC. The CoA molecule bound to one monomer is shown as a stick model.*

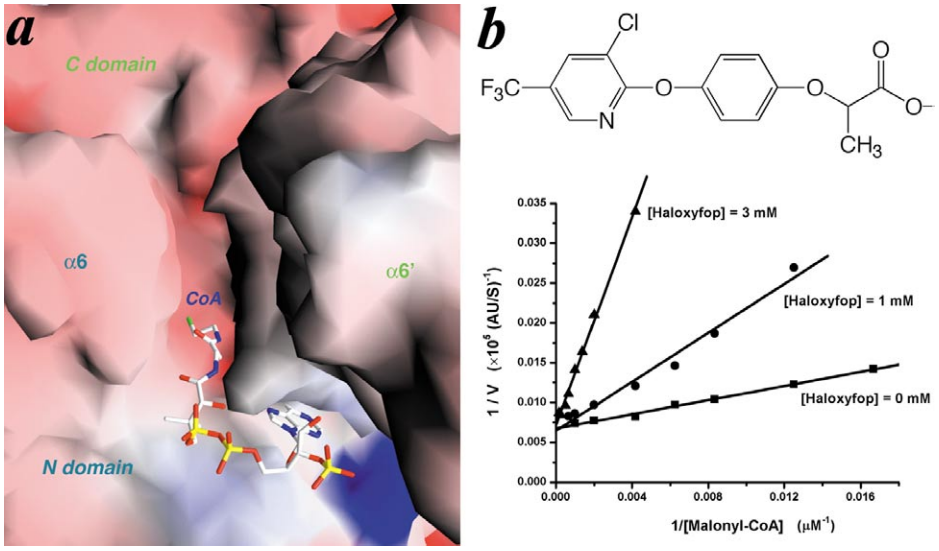


Figure 2. *The active site of CT.* **(a)**. *Molecular surface of the active site region of CT.* **(b)**. *Double-reciprocal plot showing the competitive inhibition of the yeast CT domain by the herbicide haloxyfop.*