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Short Conceptual Overview

# The role of symmetry in the regulation of bacterial carboxyltransferase

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## Abstract

Carboxyltransferase is one component of the multifunctional enzyme acetyl-CoA carboxylase which catalyzes the first committed step in fatty acid biosynthesis. Carboxyltransferase is an $\alpha_2\beta_2$ heterotetramer and possesses two distinct but integrated functions. One function catalyzes the transfer of carbon dioxide from biotin to acetyl-CoA, whereas the other involves binding to the mRNA encoding both subunits. When carboxyltransferase binds to the mRNA both enzymatic activity and translation of the mRNA are inhibited. However, the substrate acetyl-CoA competes with mRNA for binding. Thus, mRNA binding by carboxyltransferase provides an effective mechanism for regulating enzymatic activity and gene expression. This conceptual review takes the position that regulation of enzymatic activity and gene expression of carboxyltransferase by binding to its own mRNA is at its most fundamental level the result of the symmetry in the chemical reaction catalyzed by the enzyme. The chemical reaction is symmetrical in that both substrates generate enolate anions during the course of catalysis. The chemical symmetry led to a structural symmetry in the enzyme where both the α and β subunits contain oxyanion holes that stabilize the enolate anions. Then the region of the mRNA that codes for the oxyanion holes provided the binding sites for carboxyltransferase. Thus, the symmetry of the chemical reaction formed the foundation for the evolution of the mechanism for regulation of carboxyltransferase.

**Keywords:** acetyl-CoA carboxylase; carboxyltransferase; metabolic regulation; symmetry; zinc finger.

## Introduction

> ''*The only time when science gets to its ground level is when it can interpret something in terms of symmetry*.''
>
> Jacques Monod

The quote from Jacques Monod expresses the main theme of this concept review, which is that symmetry can be the driving force for the evolution of biochemical regulation. Symmetrical relationships can be found throughout nature, from quantum mechanics to human anatomy. In the case of the enzyme carboxyltransferase, symmetry appears to have been the foundation for the regulation of enzymatic activity and expression of the genes coding for the enzyme. Because this enzyme catalyzes one of the earliest steps in fatty acid biosynthesis, this symmetry based mechanism has implications for the overall regulation of lipid metabolism in bacteria.

Carboxyltransferase is one component of the multifunctional enzyme acetyl-CoA carboxylase (ACC) which catalyzes the first committed step in fatty acid biosynthesis in all animals, plants and bacteria. The two-step reaction catalyzed by ACC is shown in Figure 1. In *Escherichia coli*, acetyl-CoA carboxylase consists of three different proteins: biotin carboxylase, biotin carboxyl carrier protein, and carboxyltransferase (1). Both biotin carboxylase and carboxyltransferase retain their activity in the absence of the other components. Biotin carboxylase catalyzes the first-half reaction in Figure 1, the ATP-dependent phosphorylation of bicarbonate to form a reactive carboxyphosphate intermediate followed by transfer of the carboxyl group to the vitamin biotin. *In vivo*, biotin is covalently attached to the biotin carboxyl carrier protein (BCCP). The second-half reaction, catalyzed by carboxyltransferase, transfers the carboxyl group from carboxybiotin to acetyl-CoA to make malonyl-CoA. In contrast to the bacterial enzyme, eukaryotic acetyl-CoA carboxylase incorporates all three functions on a single polypeptide chain with domains that correspond to each of the *E. coli* proteins (2).

## Symmetrical chemistry of carboxyltransferase

The symmetry based mechanism for the regulation of *E. coli* carboxyltransferase starts with the chemical mechanism shown in Figure 2. To transfer the carboxyl group from carboxybiotin to acetyl-CoA, a proton must first be removed from the methyl group of acetyl-CoA. The active site amino acid that acts as a base has yet to be identified. Nonetheless, the result of removing a proton from acetyl-CoA is an enolate anion. As for the other substrate carboxybiotin, the carboxyl group first dissociates from biotin to form the more electrophilic $CO_2$ which reacts with the enolate of acetyl-CoA to form malonyl-CoA (3, 4). The decarboxylation of carboxybiotin results in the formation of an 'enolate-like' form of biotin. Thus, the reaction catalyzed by carboxyltrans-

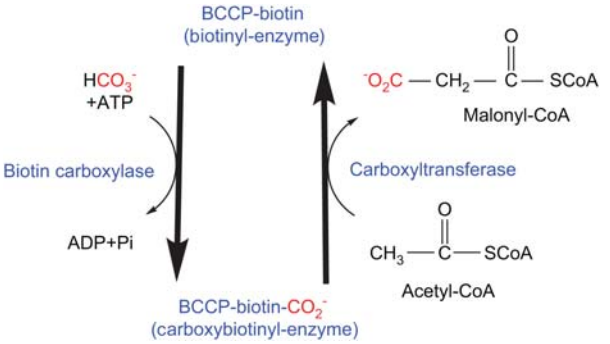


**Figure 1** Reaction catalyzed by acetyl-CoA carboxylase. BCCP denotes biotin carboxyl carrier protein.

ferase is symmetrical in that both substrates form enolate species during catalysis and the function of the enzyme is to stabilize both enolate anions.

## Structural symmetry of carboxyltransferase

The chemical symmetry of the reaction catalyzed by carboxyltransferase has also driven the evolution of carboxyltransferase to have structural symmetry. Carboxyltransferase is an $\alpha_2\beta_2$ heterotetramer (Figure 3) (5). The α subunit binds carboxybiotin-BCCP, whereas the β subunit binds acetyl-CoA. Thus, each carboxyltransferase molecule contains two active sites that lie at the interface between an α/β pair. Both the α and β subunits have a similar fold in that both have a core domain composed of repeated ββα motifs (Figure 4). The similar fold of the α and β subunits is indicative of gene duplication and suggests a common function for the two subunits.

The common function of the two subunits can be ascertained from the overall fold of the α and β subunits. The tertiary structure of the two subunits places carboxyltransferase in the crotonase superfamily of enzymes (6, 7). Although the members of this superfamily of enzymes catalyze reactions in diverse metabolic functions, the one common feature is they all catalyze reactions where an enolate anion is generated. Enzymes from the crotonase superfamily of enzymes (including carboxyltransferase) stabilize enolate anions with an oxyanion hole formed by the peptidic NH groups from adjacent glycine residues (Figure 5). Thus, because both substrates generate enolate anions during catalysis, the two subunits in carboxyltransferase that bind those two substrates evolved symmetrical folds for enolate stabilization (8).

## Genomic asymmetry of carboxyltransferase

The inherent $\alpha_2\beta_2$ symmetry in the structure of carboxyltransferase means the bacterium must produce stoichiometric amounts of both subunits. This would usually be accomplished by having the genes coding for the α (*accA*) and β (*accD*) subunits in an operon under the same transcriptional control mechanism. However, the genes coding for the α and β subunits of carboxyltransferase are not located in an operon. Instead, *accA* is located at 4.3 min of the *E. coli* chromosome, whereas *accD* is at 50 min (9). Both *accA* and *accD* are located in different gene clusters with each group producing proteins involved in a variety of metabolic functions. For instance, *accA* is located downstream of *polC* which encodes the catalytic subunit of DNA polymerase III (9), where the promoter region for *accA* is actually located in the coding region of *polC* (10). By contrast, *accD* is in a cluster between *dedA* and the *folC* gene which codes for folylpolyglutamate synthetase-dihydrofolate synthetase (11, 12). Most importantly, analysis of the upstream regulatory regions of both *accA* and *accD* has not provided any insight as to

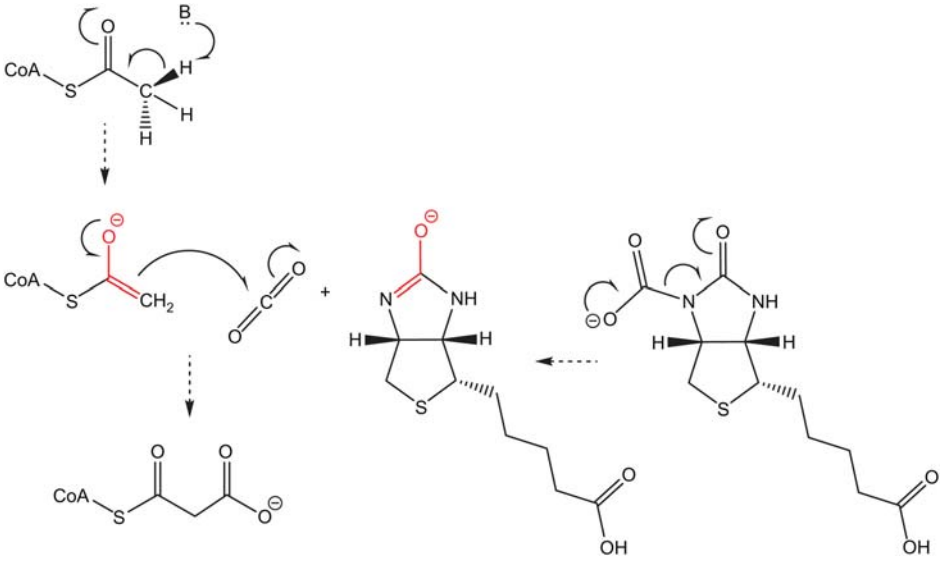


**Figure 2** Symmetrical chemistry of carboxyltransferase. During the course of the chemical reaction catalyzed by carboxyltransferase both substrates form enolate anions (highlighted in red).

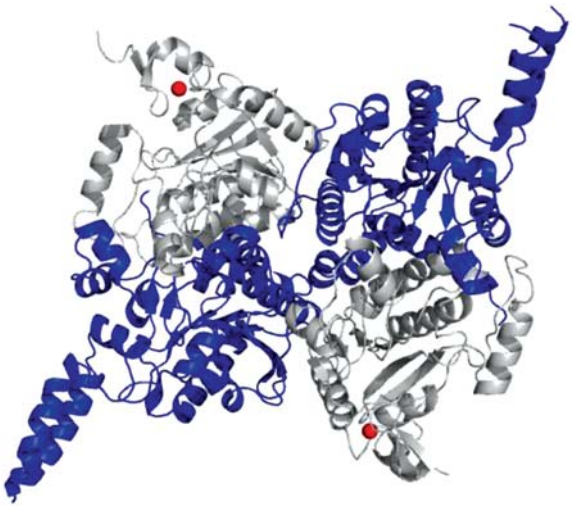

**Figure 3** Ribbon drawing of the $\alpha_2\beta_2$ heterotetramer of carboxyltransferase.
The α subunits are blue and the β subunits are white.

how the cell regulates expression of the two genes to produce stoichiometric amounts of the α and β subunits (10). Thus, if *accA* and *accD* are not expressed in a polycistronic mRNA, then how does the cell coordinate gene expression to maintain equal amounts of the α and β subunits? A possible answer was provided by solution of the crystal structure of carboxyltransferase.

## Zinc finger domain of carboxyltransferase

When the gene for the β subunit of carboxyltransferase from *E. coli* was cloned and sequenced, the authors noted that at the amino terminus there were tandem C-X-X-C sequences separated by 15 residues and speculated the protein could bind a metal ion (12). Solution of the crystal structure of carboxyltransferase from *Staphylococcus aureus* and *E. coli* along with X-ray fluorescence studies confirmed the earlier prediction of a metal ion in the enzyme (5). The metal atom is zinc, which forms part of a Cys4 zinc finger domain that is unique to the bacterial carboxyltransferase. A ribbon drawing of the zinc domain is shown in Figure 6.

## Zinc finger domain links nucleic acid binding with catalysis

Zinc finger domains are commonly associated with proteins that bind nucleic acids and analysis of the electrostatic surface potential of carboxyltransferase revealed a patch of positive charge surrounding the zinc finger domain. Thus, it was not surprising when carboxyltransferase was found to bind DNA as well as the DNA analog heparin (13). Unfortunately, DNA bound to carboxyltransferase in a cooperative manner with a half-maximal saturation of 1 μM, suggesting binding was non-specific (13). However, rather unexpectedly, DNA was found to inhibit carboxyltransferase enzymatic activity, whereas the substrate acetyl-CoA inhibited DNA binding indicating that nucleic acid binding and enzymatic catalysis were reciprocally linked (13). It is important to emphasize that this finding is in direct contrast to most dual function enzymes that bind nucleic acids. For those enzymes catalysis and nucleic acid binding are separate functions and inactivation of one does not affect the other (14, 15). By contrast, for carboxyltransferase, mutation of the cysteine residues in the zinc finger domain of carboxyltransferase abolished both nucleic acid binding and catalysis confirming the zinc finger domain is the structural motif that links nucleic acid binding and catalysis (16). Thus, the zinc finger domain is clearly important for the function of the carboxyltransferase. How-

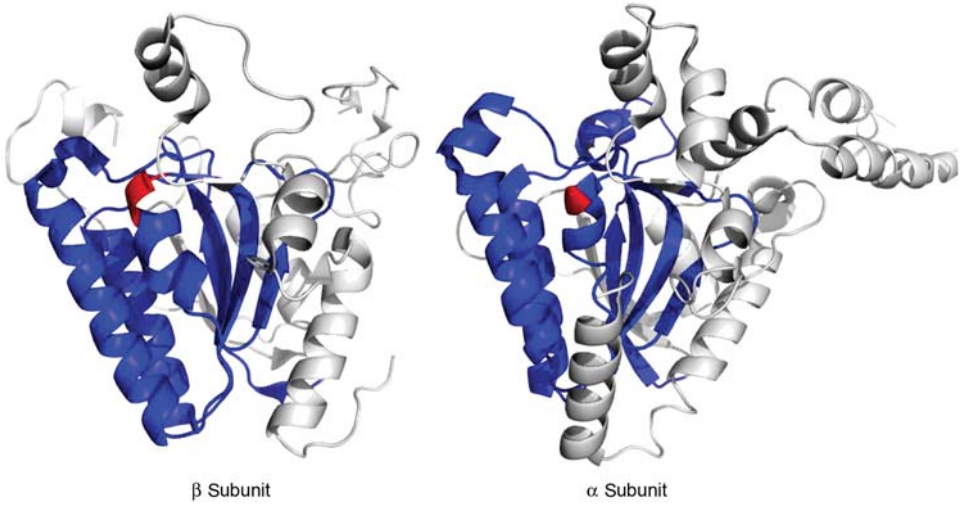


**Figure 4** Ribbon drawing of the α and β subunits of carboxyltransferase showing the similarity in tertiary structure.
The part of the α and β subunits that is similar is highlighted in blue. The red denotes the glycine residues located at the amino terminus of an α helix that form the oxyanion hole.

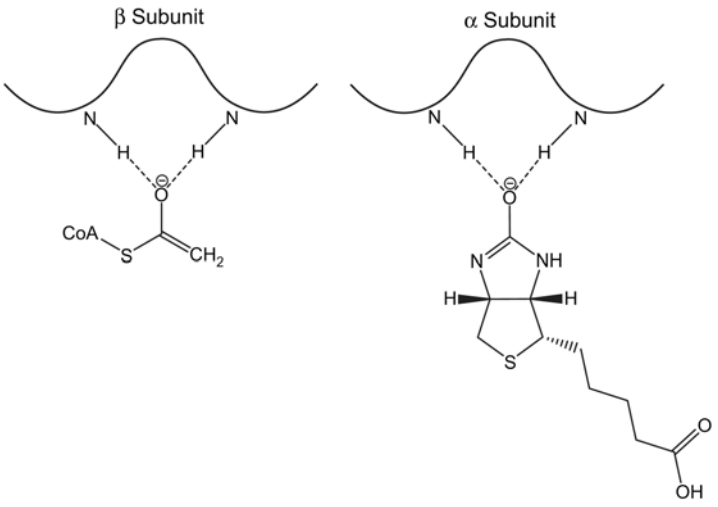


**Figure 5** Schematic showing the oxyanion holes in the α and β subunits and how they interact with the enolate anion intermediates in acetyl-CoA and biotin.

ever, if DNA binding is not the physiological role of the zinc finger domain then what is?

## Carboxyltransferase binds the mRNA encoding the α and β subunits

Because RNA binding proteins are known to bind DNA nonspecifically and considering that the closest structural homolog of the carboxyltransferase zinc finger domain was from the 50S ribosomal protein L37Ae (5), the ability of carboxyltransferase to bind RNA was investigated. Carboxyltransferase was found to bind the mRNA coding for both the α and β subunits (16). However, unlike DNA binding to carboxyltransferase, the binding of mRNA was hyperbolic with a $K_d$ value of approximately 150 nM. Moreover, the fact that carboxyltransferase exhibited little to no affinity for the mRNA coding for EF-Ts, which is comparable in size to the mRNA coding for both the α and β subunits, indicates specific binding of carboxyltransferase to the mRNA coding for both the α and β subunits.

As for a physiological function, the binding of carboxyltransferase to the mRNA coding for both the α and β subunits was found to inhibit translation and the substrate acetyl-CoA relieved the inhibition (16). In a reciprocal manner, mRNA inhibited catalysis by carboxyltransferase, whereas acetyl-CoA relieved the inhibition. All of these observations taken together suggested that the role of the zinc finger domain on carboxyltransferase was to bind to the mRNA coding for the α and β subunits in order to regulate carboxyltransferase activity and gene expression.

A model for the regulation of carboxyltransferase activity and gene expression is shown in Figure 7 and is based on the fact that fatty acids in bacteria are only used for membrane biogenesis. Thus, in stationary phase when acetyl-CoA levels are low carboxyltransferase acts as a 'dimmer switch' by binding to the mRNA coding for the α and β subunits and inhibiting translation as well as enzymatic

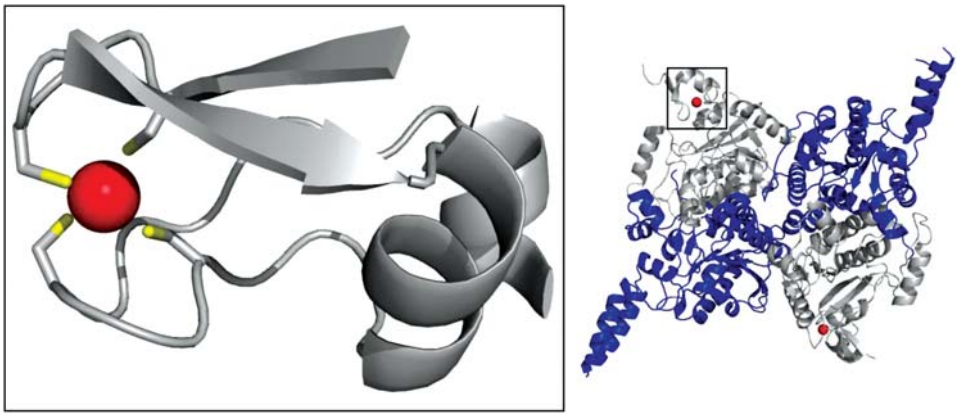

**Figure 6** Ribbon drawing of the zinc finger domain.
The location of the zinc finger domain in the $\alpha_2\beta_2$ heterotetramer of carboxyltransferase is shown in the box. The zinc atom is represented as a red sphere in this Figure and in Figures 3 and 4 as well.

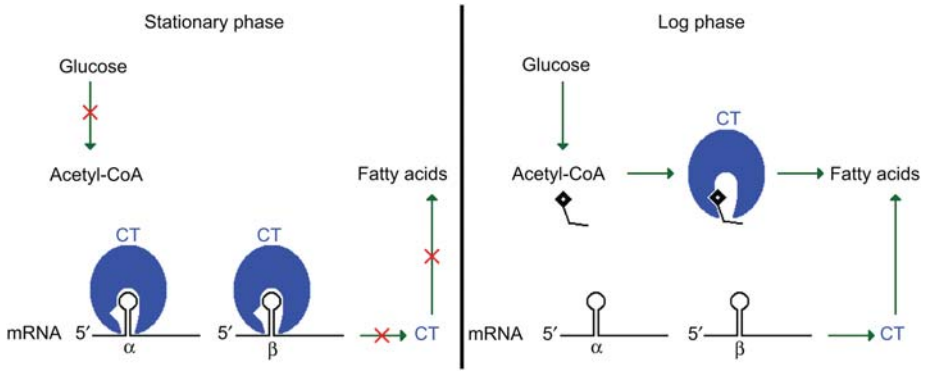


**Figure 7** Model for the regulation of carboxyltransferase (CT) activity and gene expression.

activity. By contrast, during log phase the high concentration of acetyl-CoA competes with mRNA for binding to carboxyltransferase thereby allowing catalysis to occur as well as translation of mRNA to synthesize more carboxyltransferase. A salient feature of this model is that it does not require transcription of the genes for the α and β subunits which allows for a rapid response to an increase in nutrients. Most importantly, the model accounts for how the cell overcomes the inherent asymmetry in the genomic organization of *accA* and *accD* to maintain stoichiometric amounts of the two subunits. The question now is how is the symmetry of the chemical mechanism, and the symmetry in the tertiary structure of the α and β subunits, connected to the model for regulation in Figure 7.

## Symmetry-based mRNA binding by carboxyltransferase

The symmetry in the regulatory model presented above stems from the sequence of mRNA where carboxyltransferase binds. Carboxyltransferase bound to both mRNA molecules within the coding region (16). Binding assays on progressive truncations starting at the 3′ end of the mRNA revealed carboxyltransferase bound to the 5′ end of the coding region of the α and β mRNA molecules including a region referred to as the 'symmetry box' (Figure 8). It turns out that the 'symmetry box' codes for the structural region of the α and β subunits that is the same (i.e., symmetrical) between the two subunits (the blue highlighted region in Figure 4). That is the part of the tertiary structure which contains the oxyanion

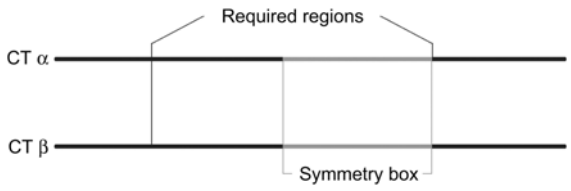


**Figure 8** Schematic showing the regions of the mRNA for the α and β subunits that are required for carboxyltransferase (CT) binding.

holes that stabilize the enolate anions generated in both substrates during catalysis (Figure 5). The section of tertiary structure that is common between the α and β subunits (the blue highlighted region in Figure 4) has a 41% amino acid sequence identity compared to only a 19% amino acid sequence identity outside this region (16). By contrast, the sequence of nucleotides in the 'symmetry box' does not show an increase in conservation compared with the sequence outside this region. However, considering that carboxyltransferase probably binds to a specific three-dimensional structure in the mRNAs coding for the α and β subunits and given that nucleotide sequence is neutral in the folding of RNA (17), it is therefore not surprising there is no increase in nucleotide sequence identity in the 'symmetry box'.

The obvious question for future studies is do the mRNA molecules coding for the α and β subunits, or at the very least the symmetry box regions, have similar tertiary folds? The three-dimensional structure for at least part of the two mRNA molecules is expected to be homologous because carboxyltransferase binds with similar affinities to the same region of the two mRNA molecules (16). Moreover, how does the zinc finger domain bind to the mRNA? Because carboxyltransferase contains two zinc finger domains does one mRNA molecule bind to only one zinc finger domain of carboxyltransferase or are both zinc finger domains involved in binding a single mRNA? These will be challenging questions to answer given that 680 nucleotides of both mRNA molecules are required for carboxyltransferase binding which make them too large for structure determination by either X-ray crystallography or NMR.

In summary, nature initially used the symmetry of the chemical reaction catalyzed by carboxyltransferase as the driving force for the evolution of the tertiary structure of the α and β subunits. Then, to achieve stoichiometric amounts of the α and β subunits the inherent symmetry in the mRNA coding for the two subunits served as a binding site for the zinc finger domain of carboxyltransferase which eventually led to coordinated gene expression and regulation of enzyme activity. This is an excellent example of the point made by Jacques Monod at the beginning of this article. Namely, that symmetry lies at the heart of natural phenomena. In this case,

symmetry led to regulation of carboxyltransferase and ultimately to play a role in the regulation of fatty acid synthesis.

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