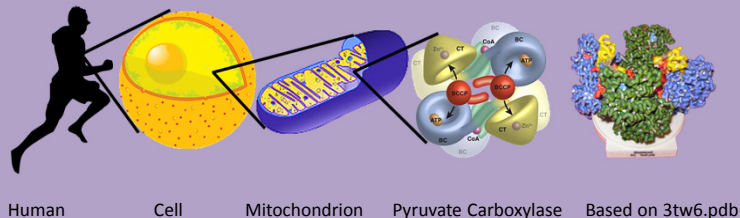


(1) Abstract

Cells exist in a state of continuous metabolic flux. The Krebs cycle, a central metabolic hub in the cell, is responsible for supplying precursors for the synthesis of amino acids, nucleotides, and compounds required for energy transfer. During periods of increased metabolic flux, metabolites in the Krebs cycle become depleted and must be replenished. Pyruvate carboxylase (PC), a multifunctional enzyme, replenishes the Krebs cycle by catalyzing the conversion of pyruvate to oxaloacetate, a Krebs cycle intermediate. The Shorewood SMART Team (Students Modeling A Research Topic) created a model of PC using 3D printing technology. PC contains four distinct domains: biotin carboxylase (BC), central allosteric, carboxyltransferase (CT), and biotin carboxyl carrier protein (BCCP). The overall reaction is initiated by BCCP-biotin carboxylation in the BC domain. BCCP-carboxybiotin physically translocates to the CT domain to transfer its carboxyl group to pyruvate. The active site of the CT domain undergoes a reconfiguration upon pyruvate binding to accommodate the docking of BCCP-carboxybiotin for pyruvate carboxylation. With the rise in antibiotic resistance, understanding how PC functions may provide a target in developing new antibiotics, whereby the new drug would eliminate critical metabolic activity, thus killing the bacteria.

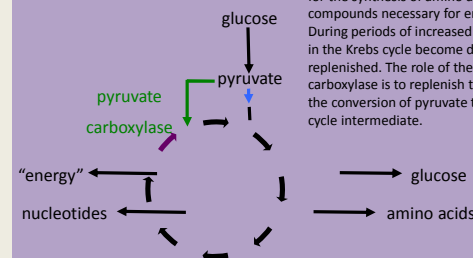
(2) Importance of Energy

Living cells perform work to stay alive. In order for the cell to maintain homeostasis, energy transduction must occur by way of chemical transformations. One method for deriving energy is the Krebs cycle, which occurs in the mitochondrion of the cell. Krebs cycle intermediates, utilized for energy transduction and biosynthetic pathways, must be replenished. Cycle replenishment is accomplished by the enzyme pyruvate carboxylase converting pyruvate to oxaloacetate, a vital Krebs cycle intermediate.



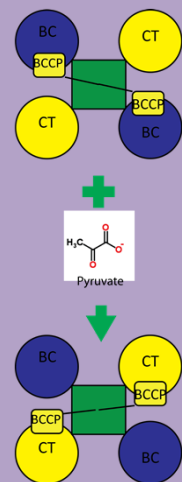
Pyruvate carboxylase is a multifunctional enzyme that is found in the mitochondrion of the cell in all eukaryotes. The function of this enzyme is to replenish the Krebs cycle intermediate oxaloacetate during periods of increased metabolic flux.

(3) The Krebs Cycle

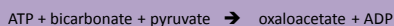


The Krebs cycle is responsible for supplying precursors for the synthesis of amino acids, nucleotides, and other compounds necessary for energy transfer in the cell. During periods of increased metabolic flux, metabolites in the Krebs cycle become depleted and must be replenished. The role of the enzyme pyruvate carboxylase is to replenish the Krebs cycle by catalyzing the conversion of pyruvate to oxaloacetate, a Krebs cycle intermediate.

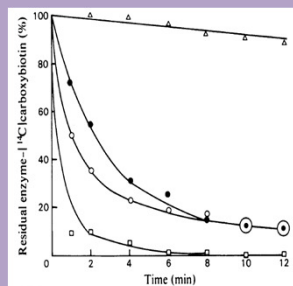
(4) BCCP Translocation



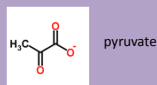
Pyruvate carboxylase is comprised of four functional domains assembled on a single polypeptide chain. These domains consist of a 1. biotin carboxylase (BC) domain, 2. carboxyl transferase (CT) domain, 3. biotin carboxyl carrier protein (BCCP) domain, and 4. allosteric domain^{1,2}. BCCP physically translocates from the BC domain to the CT domain in the presence of ATP, bicarbonate and pyruvate.



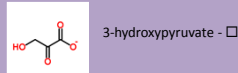
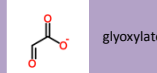
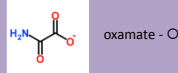
(5) BCCP Translocation in PC with Structural Analogs of Pyruvate



The above graph depicts the decarboxylation of carboxybiotin in the presence of various structural analogs of pyruvate. In the absence of a pyruvate analog, the decarboxylation of carboxybiotin is minimal. When an analog of pyruvate is added to the reaction mixture, the decarboxylation of carboxybiotin occurs rapidly over time. All depicted analogs contain both the carboxyl and oxo groups of pyruvate, suggesting a role for these functional groups in binding the ligand in the active site of the CT domain³.

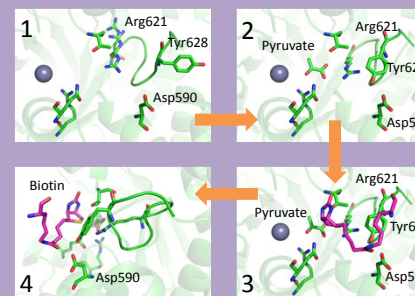


Structural analogs of pyruvate



Key: The chemical structures are analogs of pyruvate and are depicted in the graph.

(6) Active Site of the Carboxyl Transferase Domain



Upon binding of pyruvate in the active site of the carboxyl transferase domain, Tyr628 moves within hydrogen bonding distance to Asp590. The repositioning of Tyr628 assists in reconfiguring the active site to accommodate carboxybiotin docking. An important concept to note here is that structure determines function⁴.

(7) Biological Significance

The basic research question centered on pyruvate carboxylase is concerned with how multifunctional enzymes coordinate catalysis. Multifunctional enzymes combine two or more spatially distinct active sites to accomplish an overall reaction. Pyruvate carboxylase, a multifunctional enzyme, catalyzes the carboxylation of pyruvate to oxaloacetate by utilizing three spatially distinct domains. These functional domains are the BC, CT and BCCP domains. Understanding how pyruvate carboxylase coordinates catalysis between spatially distinct active sites will serve as a model for understanding the regulation of multienzyme complexes in the cell. Because pyruvate carboxylase plays a critical role in central metabolism, it may also serve as a potential target for the development of novel therapeutics against various disease states.

(8) References

1. St. Maurice, M. et al. (2007) *Science* 317, 1076-1079.
2. Lietzan, A.D. et al. (2011) *Biochemistry* 50, 9708-9723.
3. Goodall, G.J. et al. (1981) *Biochem J* 199, 603-609.
4. A.D. Lietzan and M. St. Maurice unpublished data.