**Citric Acid Cycle**

**13.1 The PDH Reaction**

The PDH Complex: E1, E2, E3

* Located in mitochondrial matrix; pyruvate must be transported into mitochondria from cytosol
* E1, E2, E3 make up PDH complex
* Catalyze oxidative decarboxylation of pyruvate and transfer of acetyl unit to CoA

Step 1: Decarboxylation of Pyruvate (E1)

* E1 = pyruvate dehydrogenase
* Thiamine = Vitamin B1
* Cofactor for several decarboxylation reactions
* Thiazolium ring N+ stabilizes the carbanion attached to TPP

Step 2: Transfer from E1 to E2

* Disulfide bond = active portion
* Accepts hydroxyethyl group
* Transfer regenerates E1 and oxidizes hydroxyethyl to acetyl group

Step 3: Acetyl Transfer to CoA (E2)

* E2 transfers acetyl group to CoA, forming acetyl CoA
* Lipoamide is reduced
* Acetyl CoA = thioester (high energy); retains energy from oxidation of hydroxyethyl to acetyl group

Step 4: Oxidation of E2 (E3)

* E3 re-oxidizes E2’s lipoamide group (restoring disulfide bond) by reducing a disulfide bond on itself

Step 5: Oxidation of E3

* E3 transfers electrons to NAD+ via FAD
* Electrons move to FAD to form FADH2.
* Electrons move from FADH2 to NAD+ to form NADH and H+.
* E3 is restored to its oxidized state.

**14.2: The Eight Reactions of the Citric Acid Cycle**

Citrate Synthase

* Condensation reaction between oxaloacetate and acetyl CoA
* Citrate synthase changes conformation upon substrate binding; metal ion cofactor is not required for C-C bond
* Low barrier hydrogen bonds stabilize transition state intermediate
* CoA is reused by PDH or succinyl CoA synthetase
* Exergonic reaction:
* Energy fueled by hydrolysis of thioester bond in acetyl CoA

Aconitase

* Reversible isomerization of citrate to isocitrate
* Enzyme is asymmetrical, thus, only one carboxymethyl arm participates in reaction

Isocitrate Dehydrogenase

* Oxidative decarboxylation of isocitrate to a-ketoglutarate
* Oxidation requires NAD+
* Carboxylate group eliminated as CO2
* Reaction intermediate stabilized by Mn2+ ion
* CO2 molecules diffuse out of cell; O2 is uninvolved

α-Ketoglutarate Dehydrogenase

* Oxidative decarboxylation
* Transfer of remaining 4C fragment to CoA
* Free energy conserved in thioester bond
* Complex resembles PDH in structure and mechanism
* E3 is a member of both complexes
* Release different carbons than those that entered as acetyl CoA

Succinyl-CoA Synthetase

* Exergonic reaction:
* Drives synthesis of GTP from GDP and Pi
* Reversible reaction, because overall near 0
* Coupling of exergonic reaction to phosphoryl group transfer to NDP = direct phosphorylation
* Active site His transfers phosphoryl group from substrate to GDP
* Requires movement of protein loop containing phospho-His side chain must travel to distant ADP (35 A away)

Succinate Dehydrogenase

* SDH is located in mitochondrial membrane; lipid soluble e- carrier: Q accepts electrons from FADH2 to re-oxidize

Fumarase

* Hydration/reduction

Malate Dehydrogenase

* Oxaloacetate is the substrate for the subsequent reactions, thus, citrate synthase activity pulls malate dehydrogenase forward

**14.3: Thermodynamics of the Citric Acid Cycle**

Regulation of CAC

* Citrate synthase: sensitive to concentrations of substrate; inhibited by products of 1 and 4 (competitive), and NADH
* Isocitrate dehydrogenase: inhibited by NADH (product)
* α-ketoglutarate dehydrogenase: inhibited by product and NADH
* Ca2+ activates dehydrogenases (signals need for energy)
* ADP activates 3, signaling low ATP

**14.4: Anabolic and Catabolic Functions of the Citric Acid Cycle**

Anaplerotic Reactions

* Pyruvate Carboxylase: activated by acetyl-CoA (indicating low CAC activity) to increase oxaloacetate concentration
* Replenishment of CAC intermediates to increase flux; CAC = energy generating catalytic cycle

Citrate Transport System

* ATP-citrate lyase undoes exergonic citrate synthase reaction
* Provides Acetyl-CoA for fatty acid synthesis (cytosolic)
* Pyruvate regenerated by cytosolic malic enzyme
* Citrate and pyruvate can cross the mitochondrial membrane via specific transport proteins.
* Provides acetyl-CoA for lipid synthesis = “acetyl-CoA shuttle”
* Pyruvate is regenerated