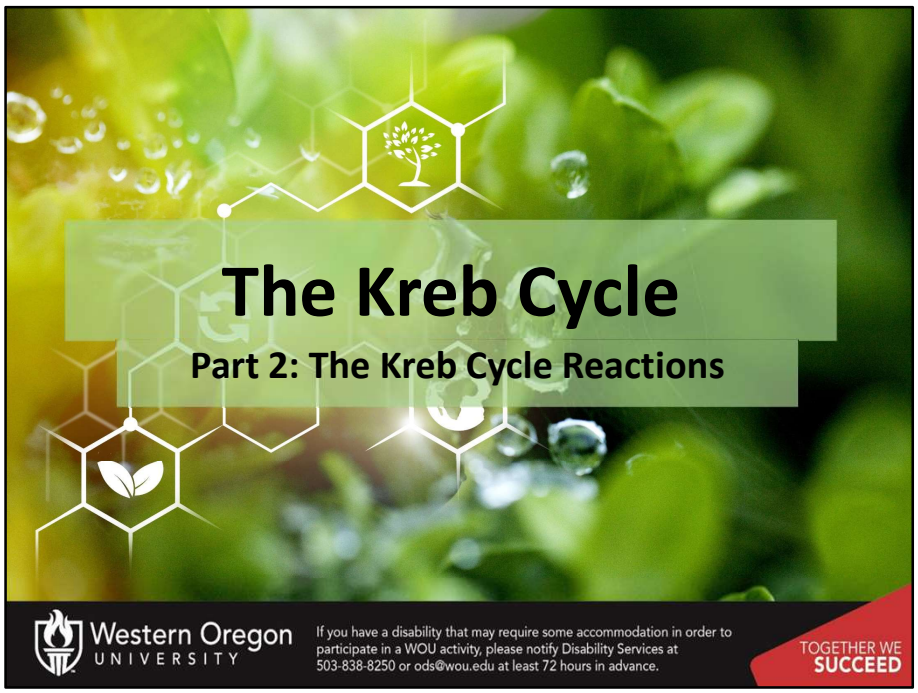
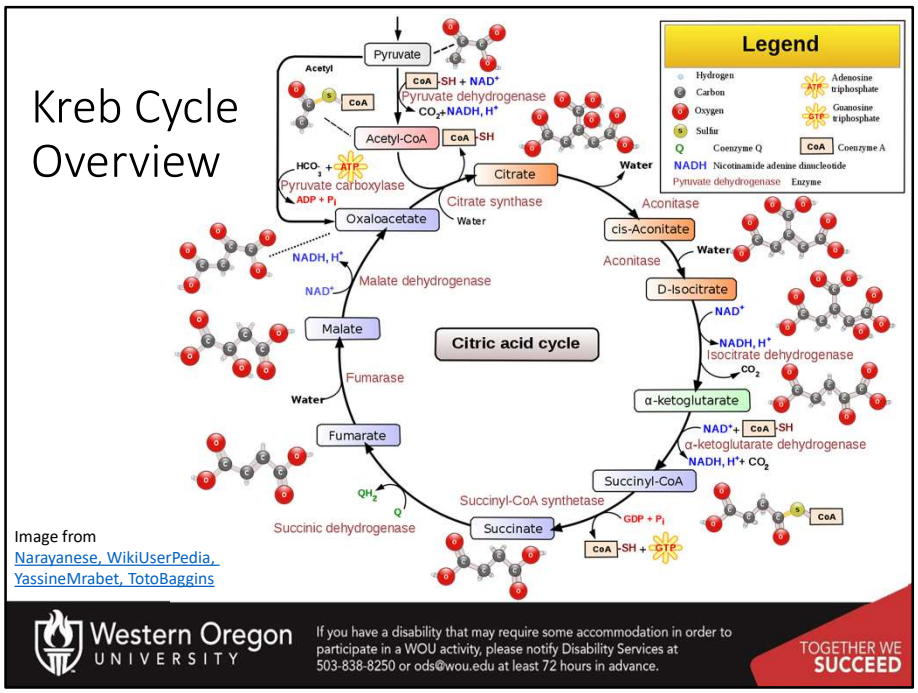
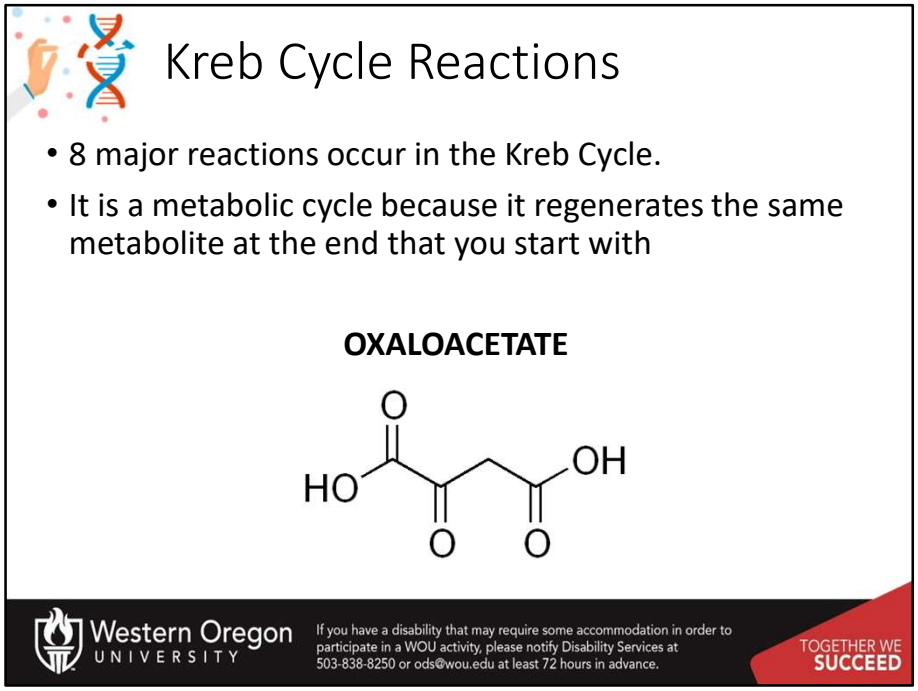


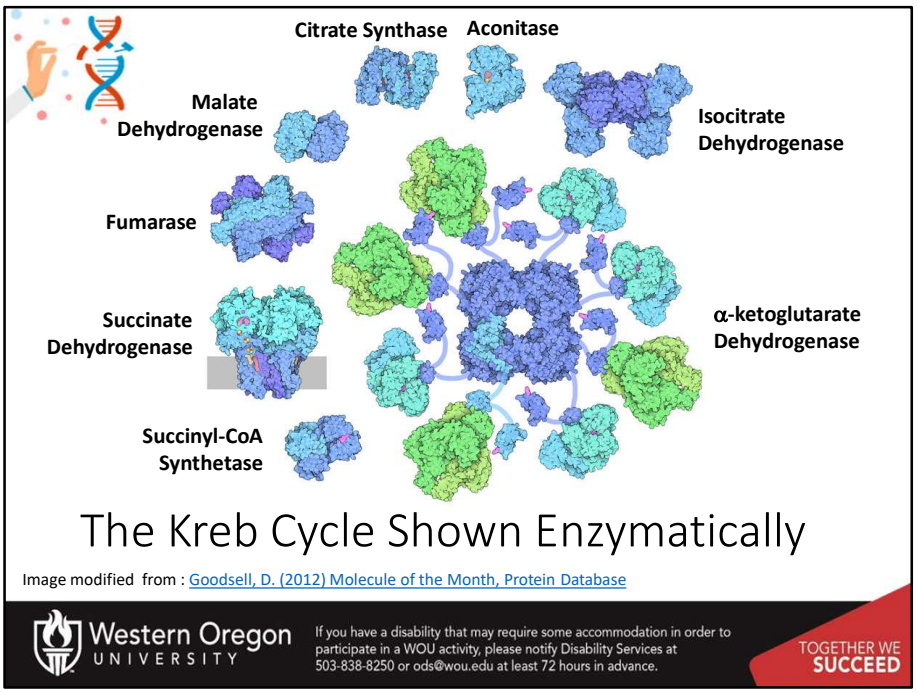
Welcome to part two of our lecture series on the Kreb Cycle. This tutorial will go through the metabolic reactions of the Kreb Cycle in more detail.



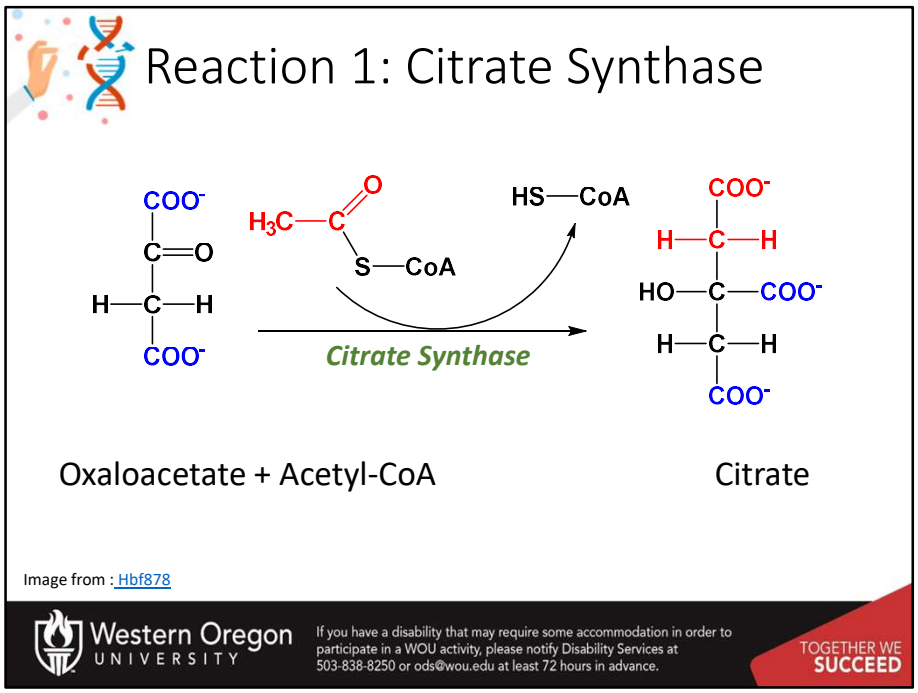
The Kreb Cycle has a total of 8 metabolic reactions involved in the full oxidation of our food molecules into carbon dioxide.



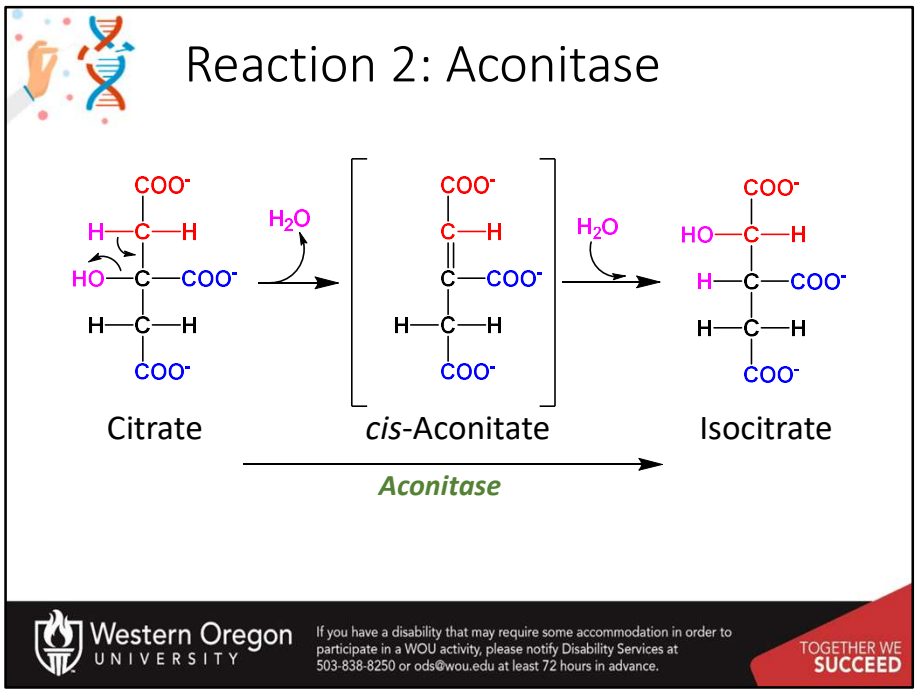
The eight reactions of the citric acid cycle use a small molecule--oxaloacetate--as the starting place for the reaction cycle. The cycle starts by addition of an acetyl group to oxaloacetate, then, in eight steps, the acetyl group is completely broken apart, restoring the oxaloacetate molecule for another round. In a typically biological twist, it's not quite this simple. You might imagine that the enzymes could just pop off the two carbon atoms of the acetyl group, using the oxaloacetate as a convenient carrier. However, by carefully labeling particular carbon atoms in these molecules, scientists have found that things get shuffled around a bit, and two carbon atoms in the original oxaloacetate are the parts that are actually released as carbon dioxide. Then, at the end of the cycle, the original acetate atoms are shuffled around to recreate the oxaloacetate.



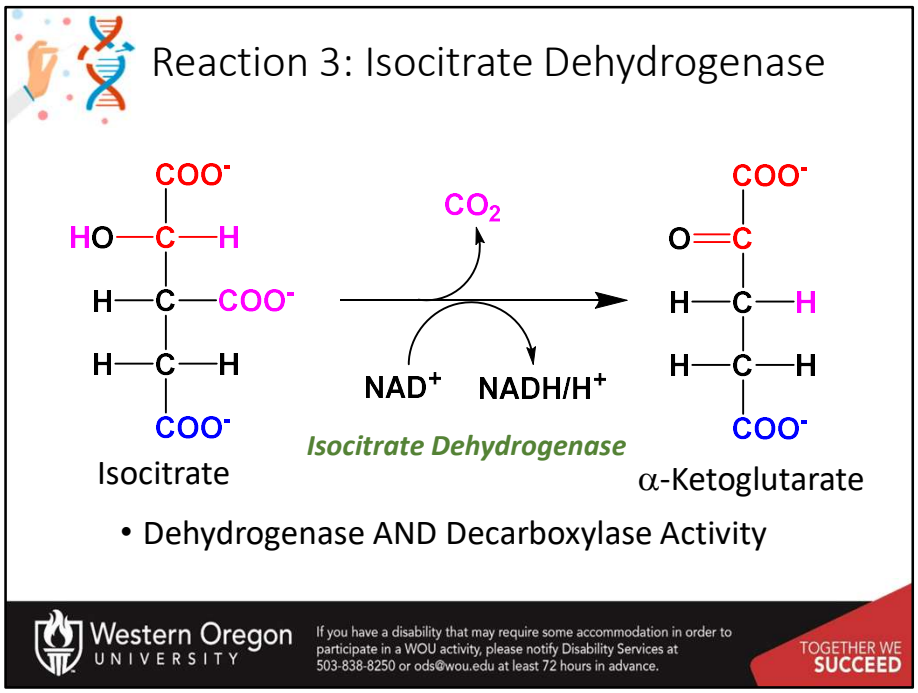
The citric acid cycle, also known as the Krebs cycle or the tricarboxylic acid cycle, is at the center of cellular metabolism, playing a starring role in both the process of energy production and biosynthesis. It finishes the sugar-breaking job started in glycolysis and fuels the production of ATP in the process. It is also a central hub in biosynthetic reactions, providing intermediates that are used to build amino acids and other molecules. The citric acid cycle enzymes, shown here, are found in all cells that use oxygen, and even in some cells that don't. In addition, all of these enzymatic reactions take place in the matrix of the mitochondria, where they are tethered closely to the innermembrane, and in fact, the succinate dehydrogenase is actually a membrane bound protein and participates in the next stage of the process, the electron transport chain.



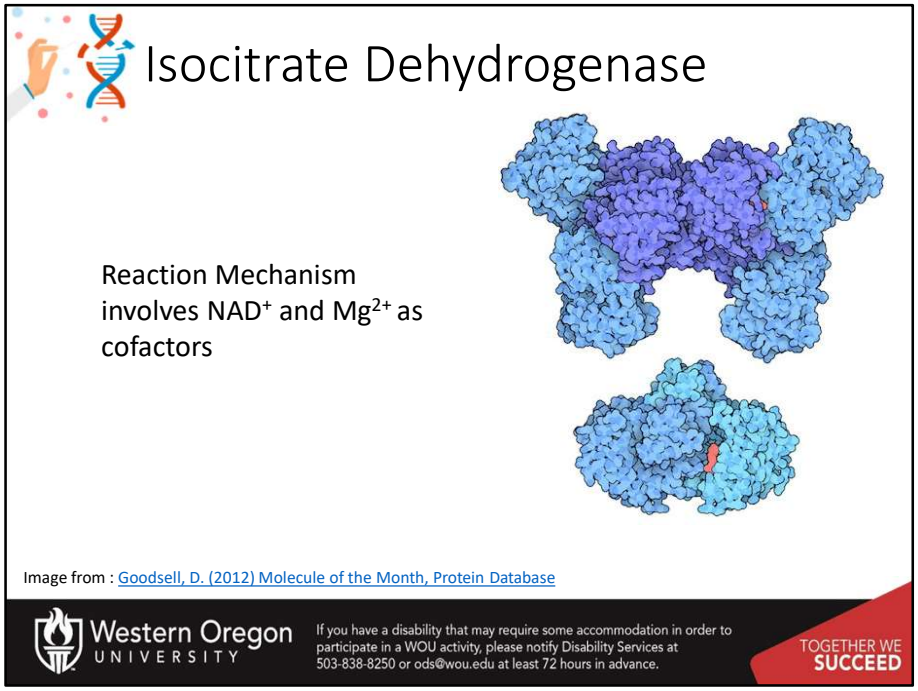
The first reaction in the citric acid cycle is the formation of citric acid (or citrate) from acetal-coA and oxaloacetate. CoASH is released in the process. Note that the two carboxylic acid groups shown in blue originate from oxaloacetate, while the acetate-derived carboxylic acid group and methyl carbon are shown in red. This will allow you to visualize what happens to these positions throughout the Kreb cycle reactions.



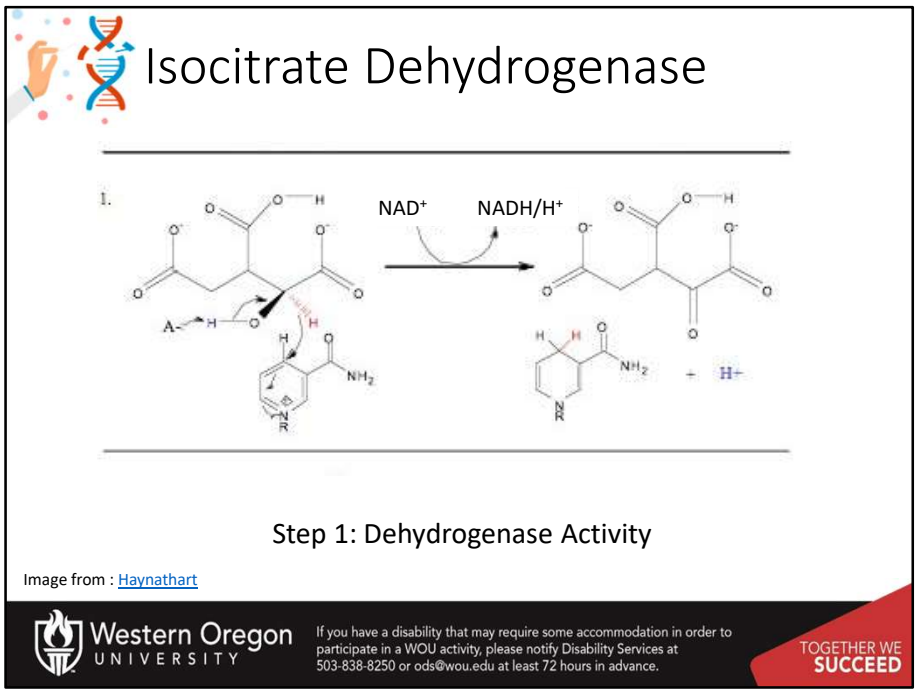
Reaction 2, is the isomerization of citrate to isocitrate, and it is mediated by an enzyme named Aconitase. This is named for the cis-aconitate intermediate that forms during the reaction. In fact, on many Kreb Cycle diagrams, the cis-aconitate is also shown on the diagram. Thus, you must be careful when you are labeling a Kreb cycle diagram that this intermediate may be shown or it may not be shown.



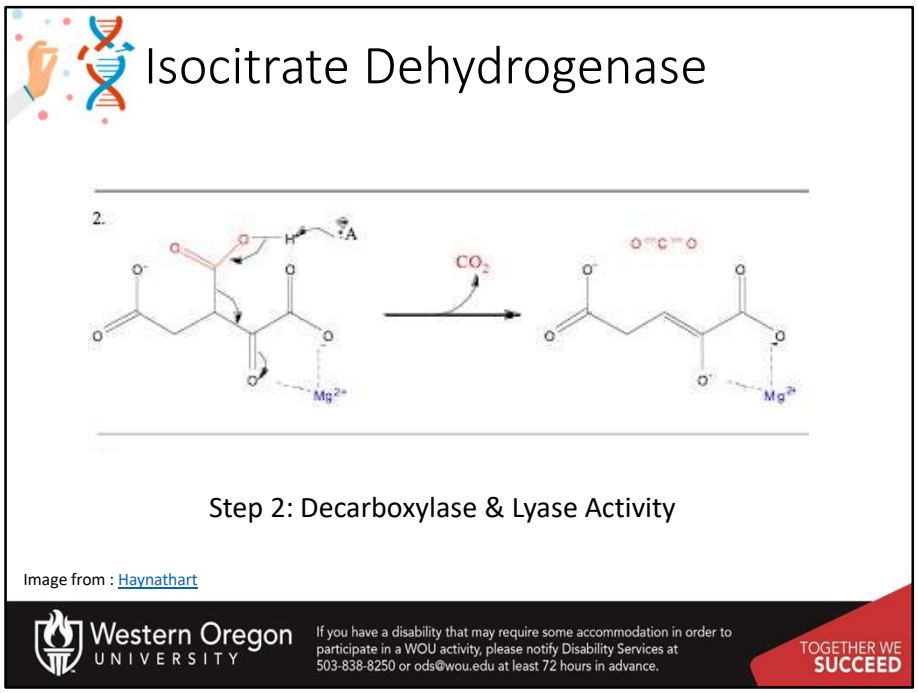
Within the citric acid cycle, isocitrate, produced from the isomerization of citrate, undergoes both oxidation and decarboxylation to form αλπηα-Ketoglutarate. CO2 is released and a molecule of NADH is formed in the process.



This is accomplished by the isocitrate dehydrogenase enzyme. Note that the enzyme is named for its oxidation role, not the decarboxylation step. Isocitrate dehydrogenase uses NAD+ and Mg2+ as cofactors within the reaction mechanism that is shown on the next few slides.

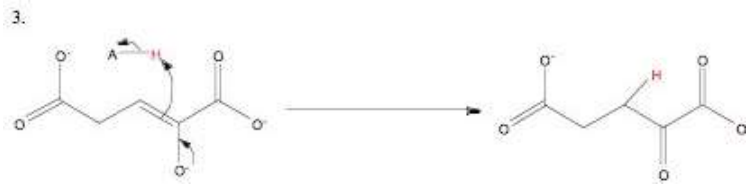


Step 1 details the dehydrogenase activity of the enzyme. First, an acidic residue in the active site deprotonates the alcohol position of isocitrate. The electrons flow into the molecule generating the carbonyl functional group. This enables the extraction of the electrons and hydrogen from the C2 carbon position by the NAD+ cofactor. The proton is released into the environment by the acidic residue in the active site when NADH exits the active site.



In step 2, the decarboxylation occurs. In this reaction mechanism, the Mg2+ cofactor stabilizes the carbonyl function group and the C1 carboxylic acid functional group. An acidic base within the active site drives the decarboxylation which forms an alkene intermediate. Again, the carbonyl oxygen is charge stabilized by interaction with the metal cofactor.

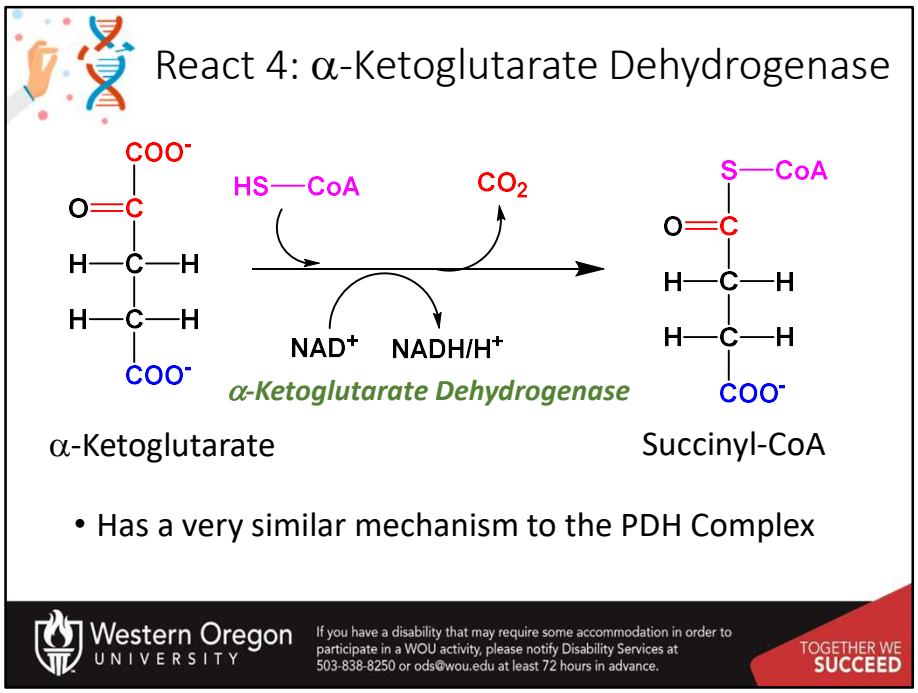
Isocitrate Dehydrogenase



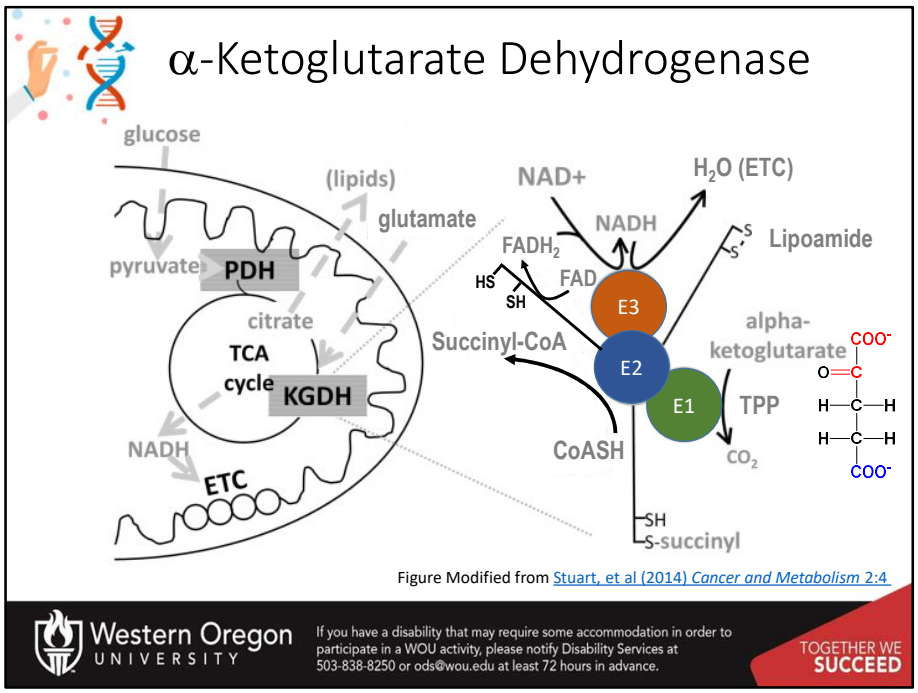
Step 3: Ketone Formation

Image from : Haynathart

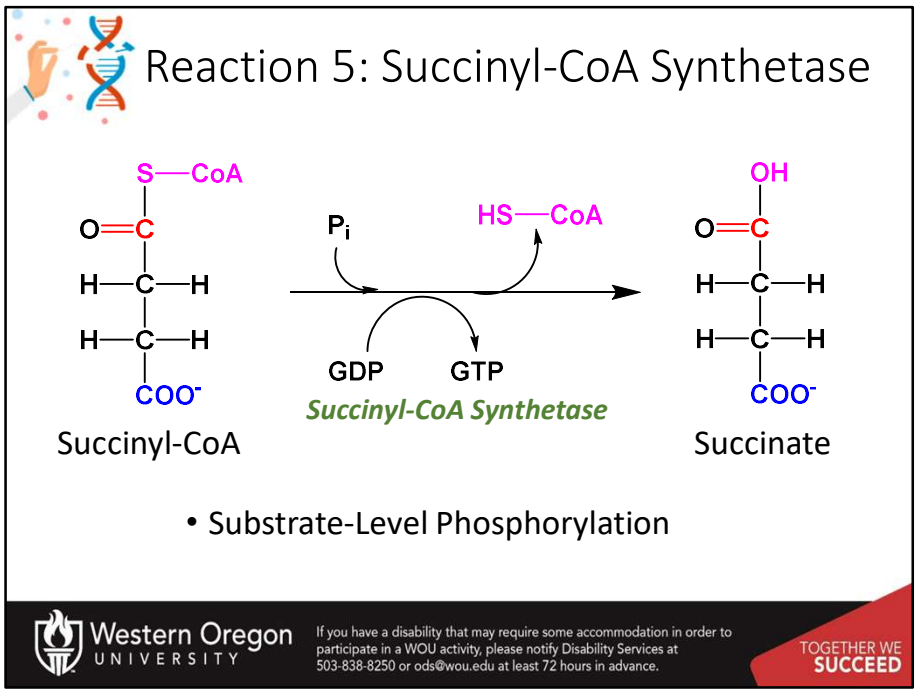
As the carbonyl electrons fall back into the molecule forming the ketone functional group, tthe pi-bond between the C2 and C3 positions, abstracts a proton from an acidic residue in the active site to form alpha-ketoglutarate. Take a moment to compare this mechanism with that found in the Pyruvate Dehydrogenase Complex, and that of the Glyceraldehyde 3-Phosphate Dehydrogenase from the Glycolytic pathway. This exemplifies the many different ways dehydrogenase enzymes can work to oxidize biological molecules.



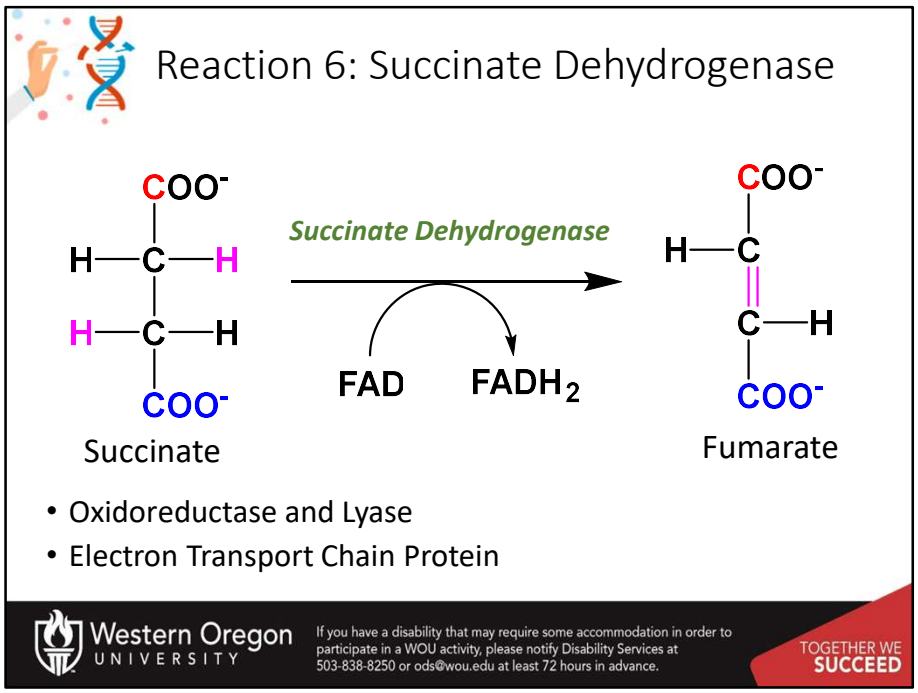
The alpha-ketoglutarate dehydrogenase (KGDH) has a reaction mechanism that is very similar with pyruvate dehydrogenase (PDH) complex. It also liberates CO2 and a molecule of NADH and a proton, in the process of forming Succinyl-CoA.



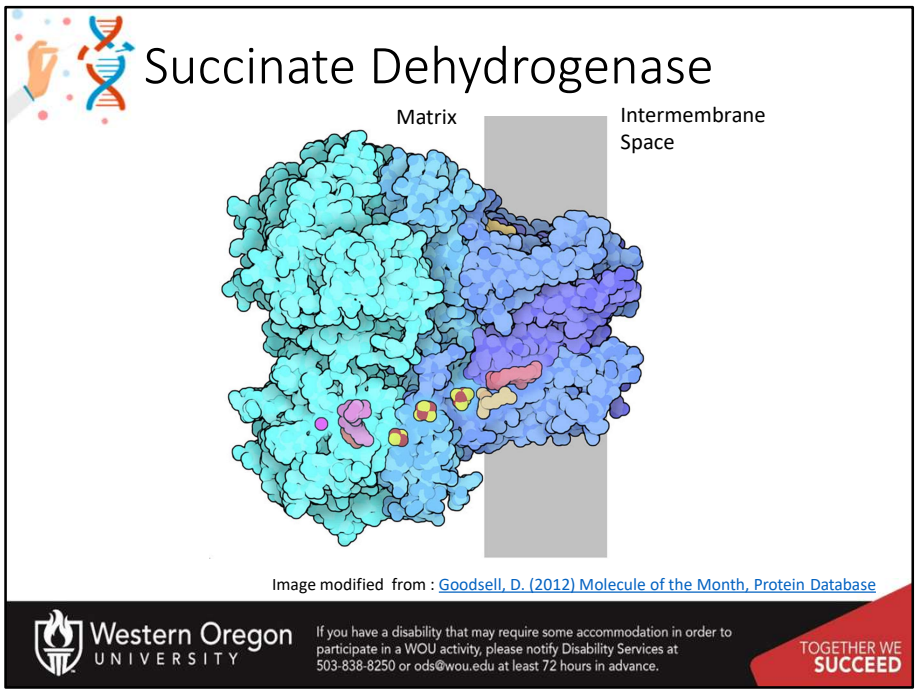
Similar to the pyruvate dehydrogenase (PDH), the alpha-ketoglutarate dehydrogenase (KGDH) also uses CoASH as a substate, and TPP, Lipoamide, FAD, and NAD+ as cofactors. In this case TPP attacks the alpha ketoglutarate C2 carbonyl carbon, mediating the release of the CO2 that was originally from the acetate molecule. The remaining succinyl group is then transferred to the lipoamide cofactor. Partial reduction of the lipoamide from the alpha-ketoglutarate has also occurred at this step. On the E2 complex, the succinyl group is transferred to Coenzyme-A as a thioester and released from the enzyme. The lipoamide cofactor is fully reduced in the process. The lipoamide cofactor releases the electrons the bound FAD housed on the E3 subunit. This restores the oxidized state of lipoamide for a second round of enzymatic function. Finally, the electrons are moved from the FADH2 to the labile NAD+ cofactor where they can be transported to the electron transport chain.



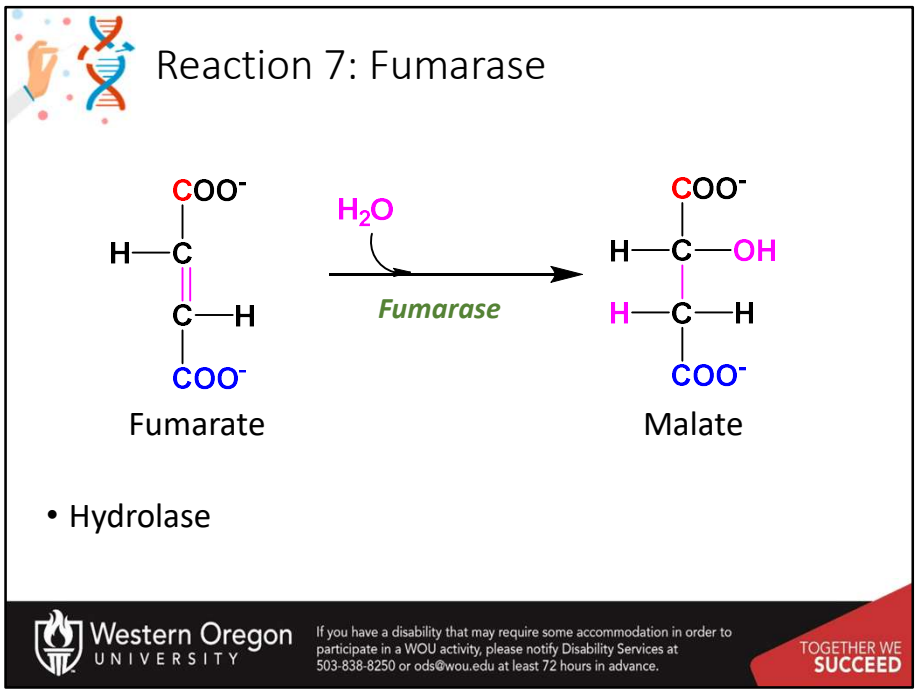
The Succinyl-CoA synthetase is an enzyme that creates a molecule of GTP (ATP equivalent) through the phosphorylation of GDP. This process releases the Coenzyme A and forms a molecule of succinate.



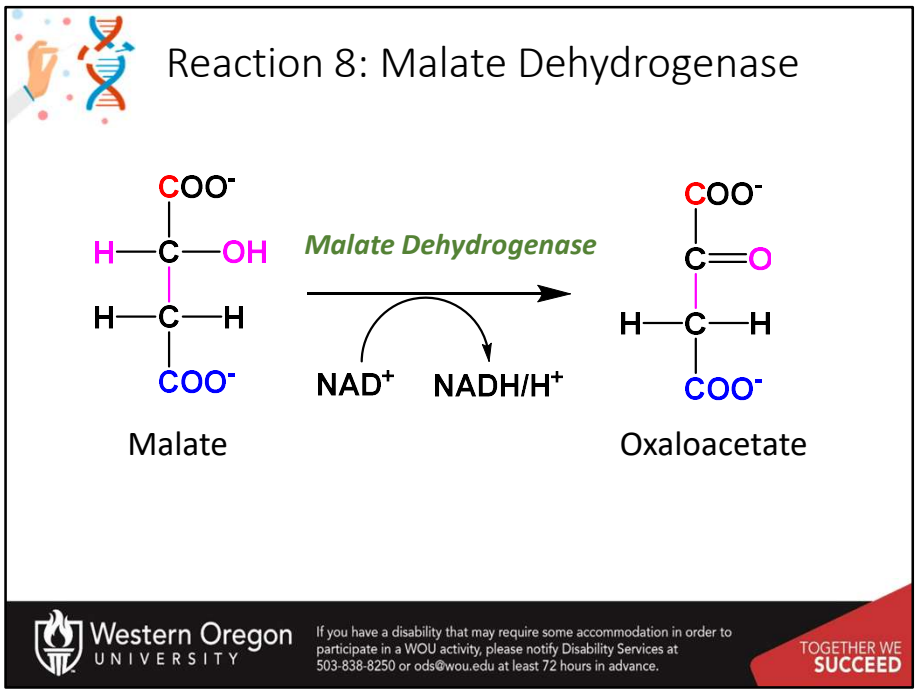
Succinate dehydrogenase then mediates the oxidation of succinate to form fumarate through another novel dehydrogenase mechanism.



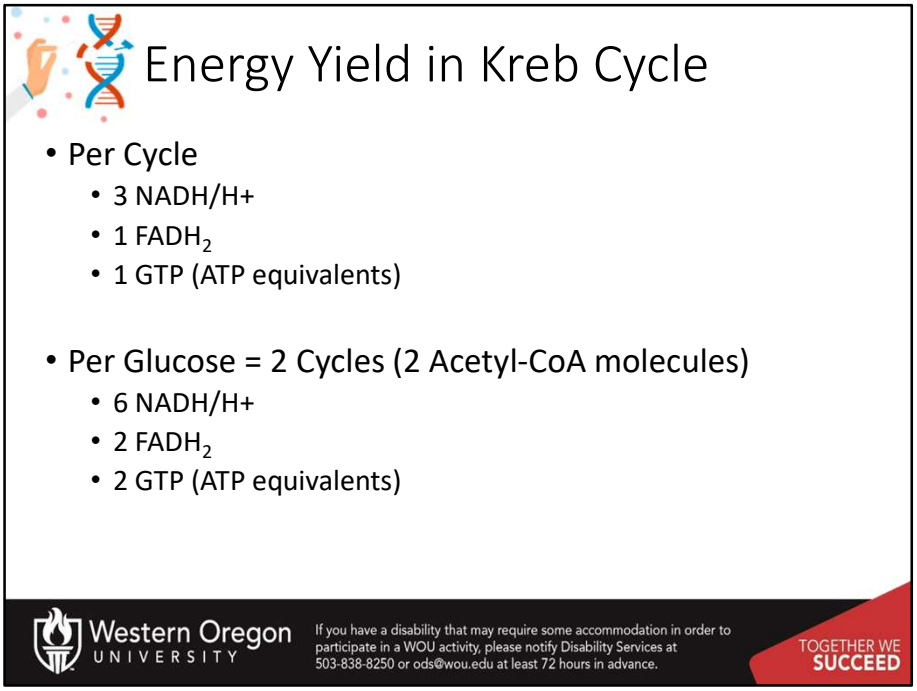
This step is performed by a protein complex that is bound in the membrane of the mitochondrion. It links this citric acid cycle task directly to the electron transport chain (ETC) and makes the transfer of electrons harvested from the food molecules streamlined. It first extracts hydrogen atoms from succinate, transferring them to the carrier FAD. The resulting product is fumarate. Interestingly, this protein is also Complex II in the electron transport chain, where it can directly transfer the electrons harvested from the succinate into the ETC. We will come back to this complex in the next section covering the ETC.



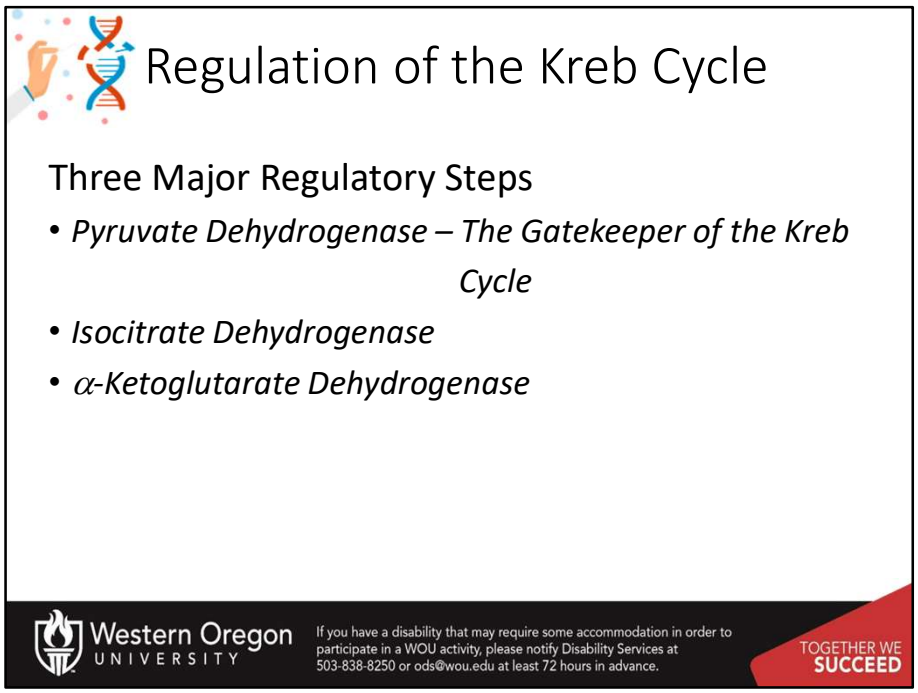
In reaction 7, the lyase, known as fumarase, converts fumarate to malate. This enzyme is also a hydrolase, as water is incorporated into the final structure (shown in pink, here).



The final reaction is one that we visited in the gluconeogenic pathway leading to the oxidation of malate to form oxaloacetate. One final energy carrier, NADH & H+, is formed.



Overall the total energy yielded in one turn of the citric acid cycle are 3 molecules of NADH & H+, 1 FADH2, and 1 GTP. This is doubled for the energy potential in 1 glucose molecule as two molecules of acetyl-CoA will enter the Kreb cycle.

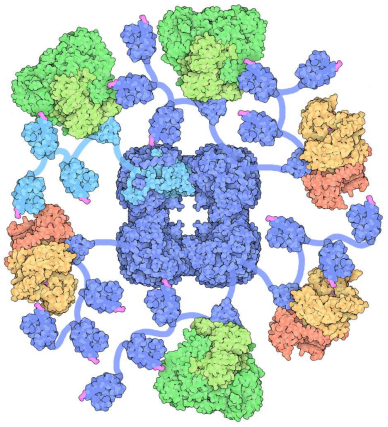


Regulation of this pathways is mediated primarily by three of the major dehydrogenase enzymes in the pathway. These include: Pyruvate dehydrogenase which serves as a gatekeeper prior to the beginning of the Kreb Cycle, followed by the next two dehydrogenase/carboxylase steps in the pathway, mediated by isocitrate dehydrogenase and alpha-ketoglutarate dehydrogenase. Essentially all of the steps were CO_2 is released. All of these enzymes are highly regulated by the energy load of the cell.

Pyruvate Dehydrogenase Complex

Regulation of the PDH Complex

- Allosteric Regulation
  - **High NADH = Inactivation**
  - **High Acetyl-CoA = Inactivation**
  - **High ATP = Inactivation**
  - **High ADP = Activation**
- Phosphorylation
  - **PDH Kinases inactivate PDH**
  - **PDH Phosphatases activate**



Gatekeeper of the Kreb Cycle

Image from : Goodsell, D. (2012) Molecule of the Month, Protein Database

For example, PDH is inactivated when high energy load is present in the cell. For example, if NADH or ATP levels are high, the enzyme will have low activity. Similarly it undergoes negative feedback inhibition with the acetyl-CoA product of the reaction. Phosphorylation also plays a role in PDH regulation. Energy-sentitive PDH kinases are active when the cell has high energy. This causes the phosphorylation and inactivation of the E1 subunit, blocking all activity of the enzyme. Alternatively, dephosphorylation activates PDH.

Isocitrate Dehydrogenase

Regulated Allosterically by Energy Load in the Cell

- Low energy activates the pathway
  - **High ADP levels**
- High Energy shuts off the pathway
  - **High ATP**
  - **High NADH**

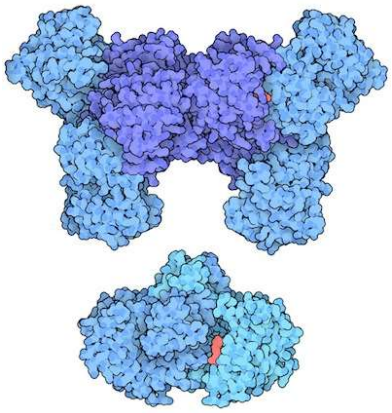


Image from : Goodsell, D. (2012) Molecule of the Month, Protein Database

Similarly, Isocitrate Dehydrogenase is activated by low energy level states (ie high ADP levels) and inhibited when energy is plentiful (high ATP or high NADH).

α-Ketoglutarate Dehydrogenase

Regulated Allosterically by Energy Load in the Cell

- Negative Feedback Inhibition
  - **Succinyl-CoA**
- High Energy shuts off the pathway
  - **High ATP**
  - **High NADH**

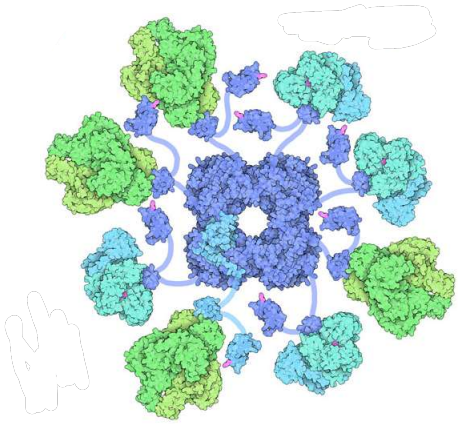
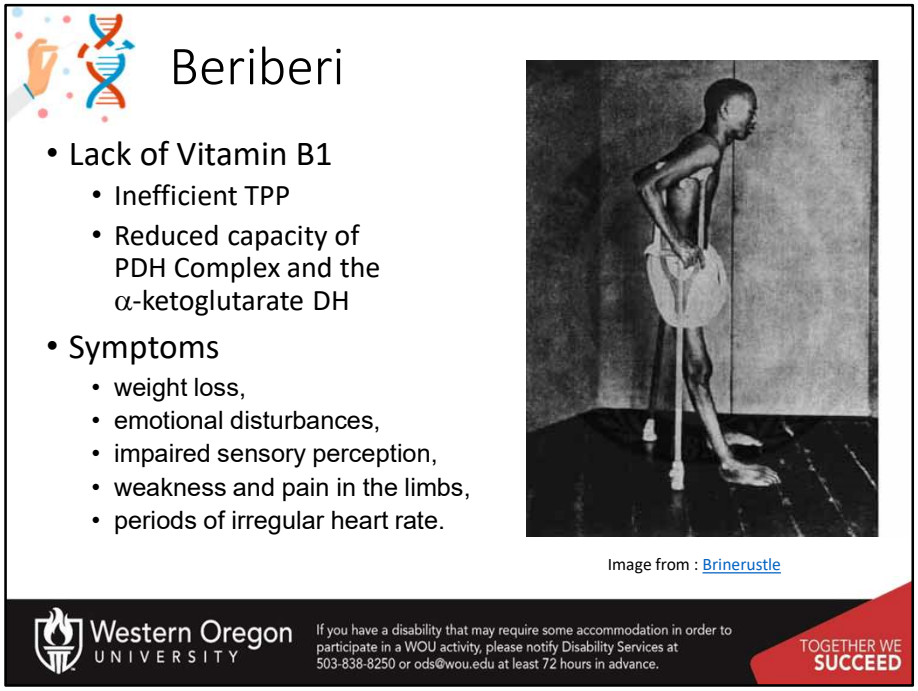


Image from : Goodsell, D. (2012) Molecule of the Month, Protein Database

Following suit, the alpha-ketoglutarate enzyme undergoes negative feedback inhibition by an accumulation of the succinyl-CoA product levels. High levels of ATP and NADH are also inhibitory.



Note that vitamin deficiencies can cause disease states, such as Beriberi. A lack of vitamin B1 will lead to inefficient production of the TPP cofactor required for the PDH and KGDH complexes. Thus, these enzymatic systems to not function well to produce ATP. Patients will experience weight loss, emotional distubances, and other neurological and muscular symptoms.

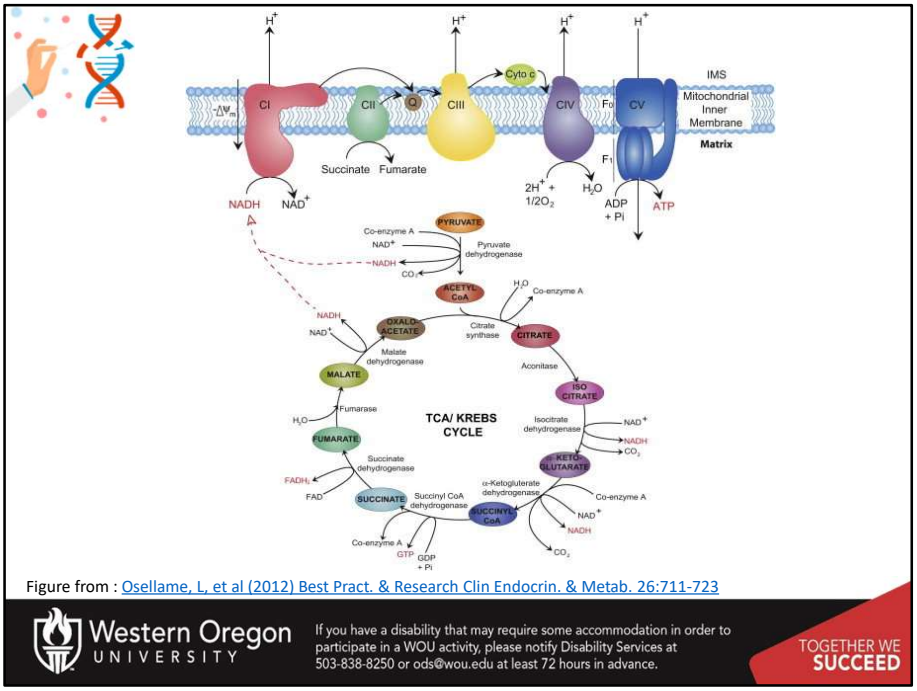


Figure from : Osellame, L, et al (2012) Best Pract. & Research Clin Endocrin. & Metab. 26:711-723

In the next lecture, we will learn how the electrons harvested in the Kreb cycle are used to generate ATP through the electron transport chain.