Activity 33 Exploring the Citric Acid Cycle

PRE-ACTIVITY ASSIGNMENT

- Which intermediate from the cycle is removed to make fat?
  Citrate transported to cyotosol, made into acetyl Co-A
- Which intermediate is removed to make glucose?
  Malate transported out of mitochondria, converted to oxaloacetate for gluconeogenesis
- Which intermediate is removed to make glutamate?
  α-ketoglutarate
- Where do fats enter the cycle?
  At acetyl-CoA, succinyl-CoA (fats oxidized in mitochondria, so no transport needed)
- Write out the overall reaction of the TCA cycle.
  $3NAD^+ + FAD + GDP + P^i + Acetyl\text{-}CoA \rightarrow 3NADH + FADH_2 + GTP + CoA + 2\ CO_2$

IN-CLASS ACTIVITY

Critical Thinking Questions

2. FFE: citrate synthase, isocitrate dehydrognease, α-ketoglutarate dehydrogenase
3. FFE reactions
4. Branch points:
   - Acetyl-CoA entering from pyruvate/glycolysis and fatty acids
   - Brach to amino synthesis from α-ketoglutarate
   - Branch to fatty acid synthesis from citrate
   - Branch to glucose synthesis from malate

   The points of regulation match pretty well with branch points. Malate levels likely concentration controlled as opposed to enzyme regulated.
5. This enzyme is inhibited by all factors listed in question:

   NADH makes sense because it is a product of TCA

   ATP is also ultimately a product (of oxidative phosphorylation, not TCA directly)

   Succinyl CoA makes sense because if its levels are high, indicates that TCA is not moving forward.
6. ADP is allosteric activator; Reaction is controlled by the simple mechanisms of substrate availability (isocitrate, NAD^+ or $NADP^+$, Mg^{2+} / Mn^{2+}), product inhibition (by NADH and α-ketoglutarate), and competitive feedback inhibition (by ATP); Ca^{2+} is an activator so that ion that stimulates muscle contraction also stimulates ATP production.
7. OAA is regenerated so the cycle has unlimited capacity for oxidizing incoming acetyl-CoA.
8. Anapleurotic reaction are those replenish cycle intermediates. Pyruvate is converted to OAA as part of gluconeogenesis. Activation by acetyl CoA makes sense because if there is a lot of it present, more OAA would be needed to run the cycle.

9. Because the enzyme IS chiral it will interact with the substrate in a completely chiral manner. Most books have a good picture of this for discussion.
10. Oxidative reactions: isocitrate dehydrogenase, α-ketoglutarate dehydrogenase, succinate dehydrogenase

    FAD is stronger oxidizing agent and can create double bonds. Consequence is that you don't get as much ATP in oxidative phosphorylation.
11. GTP (which is readily interconvertable with ATP) is made at the succinyl-CoA synthetase step. Cleavage of succinyl-CoA is coupled to GTP production.

POST-ACTIVITY SKILL EXERCISES

1. Fluoroacetyl CoA can substitute for acetyl CoA in TCA. This compound reacts with citrate synthase to produce fluorocitrate, a metabolite of which then binds very tightly to aconitase, thereby halting the cycle. This mechanism is consistent with the observed accumulation of citrate. Toxicity results from a general inhibition of oxidative metabolism.
2. Two ^{14}C-pyruvates are used. One proceeds through pyruvate carboxylase to make OAA. The other proceeds through pyruvtae dehydrogenase to make acetyl CoA. In order to not deplete cycle intermediates, these two would have to be added to an exisiting OAA and acetyl-CoA. These two combine to form citrate and then on to α-ketoglutarate. If the α-ketoglutarate is removed, no TCA intermediates are removed since OAA made was not originally part of the TCA intermediates routinely present but was an anapleurotic molecule made from pyruvate.

   α-ketoglutarate is $^{-}OOC\text{-}*CH_2\text{-}CH\text{-}*C(=O)\text{-}COO^{-}$ with labels from the two pyruvates as shown by stars.

   Sequence of events: pyr (to make OAA) + CO_2 (to make OAA) + 2pyr (to make acetyl-CoA) + OAA (in cycle) → 2 citrate + 2 CO_2 → 2 α-ketoglutarate + 2 CO_2