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Discovering Cellular Respiration

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Learning Objectives

1. Describe how changes in cellular homeostasis affect metabolic intermediates

2. Perturb and interpret a simulation of celular respiration

3. Describe cellular mechanisms regulating cellular respiration

4. Describe how glucose and oxygen affect cellular respiration

5. Describe the interconnectedness of cellular respiration

6. Identify and describe the inputs and outputs of cellular respiration, glycolysis, pyruvate processing, citric acid cycle, and electron transport chain

7. Describe how different energy sources are used in cellular respiration

8. Trace carbon molecules through cellular respiration from glucose to carbon dioxide

Review of Aerobic Cellular Respiration

Cellular respiration consists of several processes that function together to convert nutrient molecules (macromolecules) into usable energy (ATP, adenosine triphosphate) to fuel cellular functions. The process of cellular respiration is analogous to a car assembly line running in reverse. Energy substrates like glucose, fatty acids and amino acids are sequentially broken down (disassembled). Energy is released during the break down and captured by converting ADP (adenosine diphosphate) into ATP. For simplicity, cellular respiration is often taught in the context of glucose break down because all four of the major processes that make up cellular respiration are utilized (Figure 1). Further, the outputs (products) of one process are often the inputs (substrates) for another process. For example, the acetyl-CoA produced during pyruvate processing is a major input for the citric acid cycle. Below is a brief description of the four processes involved in the breakdown of glucose (refer to Figure 1).

1. ***Glycolysis*** – The purpose of this ten-step process is to rearrange and convert glucose (6 carbons) into two separate molecules of pyruvate (3 carbons). Initially, energy (ATP) is invested into this process. Later, energy is released and captured by 1) converting ADP to ATP using substrate-level phosphorylation and 2) in the form of high-energy electrons that reduce the coenzyme NAD^+ to NADH.
2. ***Pyruvate Processing*** – The purpose of this process is to convert pyruvate into an acetyl group and link it to Coenzyme A (CoA). Also, high-energy electrons are harvested by NAD^+ which is converted to NADH.
3. ***Citric Acid Cycle* (CAC)**– The purpose of this process is to harvest the remaining energy stored in the two-carbon acetyl group (remember it started as 6 carbons). The CAC produces one ATP and captures 8 high-energy electrons with NAD^+ and FAD^+, which converts them to NADH and $FADH_2$, per acetyl-CoA.
4. ***Electron Transport Chain* (ETC)**– The purpose of this process is to transfer the energy stored in high-energy electrons into usable energy by producing ATP. This step utilizes O_2 as the terminal electron acceptor and chemiosmosis is used to generate ATP by oxidative phosphorylation. In addition, this step is critical for regenerating NAD^+ and FAD^+.

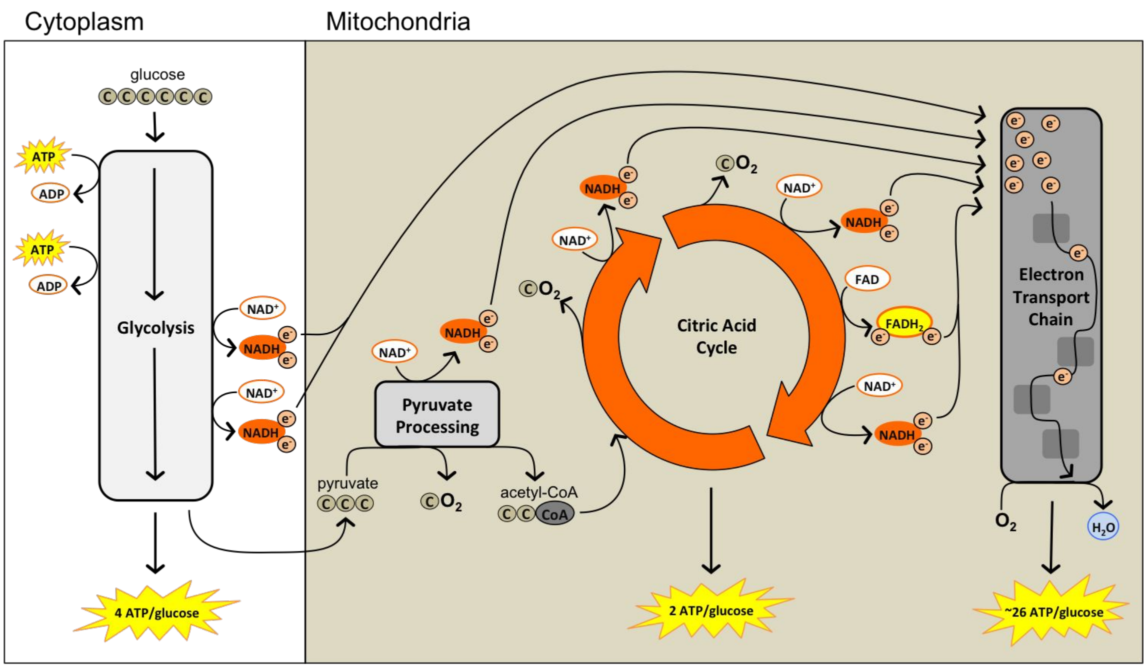

Figure 1 Overview of Cellular Respiration in Eukaryotes
This diagram allows you to trace the carbon atoms that were part of glucose being released as CO2, and the electrons that originate in glucose being used to generate ATP in the electron transport chain.

Fermentation

In the absence of oxygen (anaerobic conditions) or in an oxygen-deprived state (during an intense workout), the pyruvate produced by glycolysis remains in the cytoplasm where it is reduced (gains electrons**).** In animals, an enzyme called lactate dehydrogenase transfers electrons from NADH to pyruvate to produce lactate (lactic acid) and NAD^+ (Figure 2). The purpose of this process is to regenerate NAD^+ to be used again during glycolysis. Without fermentation, glycolysis would stop because all of the NAD^+ would remain reduced as NADH.

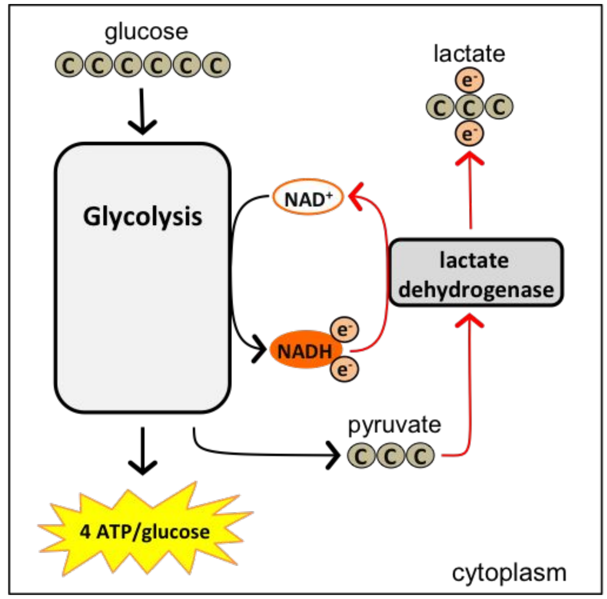

Figure 2 Lactic Acid Fermentation

Regulation of Cellular Respiration

The mechanisms regulating cellular respiration are elaborate. They ensure a sufficient supply of ATP and avoid the wasteful overproduction of ATP when supplies are high through *feedback inhibition* (negative feedback loops). Feedback inhibition occurs when a particular intermediate (or output) inhibits or slows down its own production when its concentration is at a sufficient level (Figure 3). Many of the metabolic intermediates of cellular respiration regulate their own production in this manner.

Figure 4 represents a simplified view of where feedback inhibition occurs in cellular respiration and shows that the outputs of each process (i.e., pyruvate, acetyl-CoA, NADH and $FADH_2$) can inhibit or slow down their own production. For example, if the electron transport chain was damaged or inhibited NAD^+ and FAD^+ could no longer be regenerated resulting in the build-up of NADH and $FADH_2$. As the levels of NADH and $FADH_2$ increase in the mitochondria, they will begin to inhibit both pyruvate processing and citric acid cycle. As the citric acid cycle slows down, acetyl-CoA levels will rise which will further inhibit pyruvate processing. Interestingly, glycolysis could continue in this situation because an alternative pathway, lactic acid fermentation, allows for pyruvate and NADH to be processed within the cytoplasm preventing them from building up and inhibiting their own production.

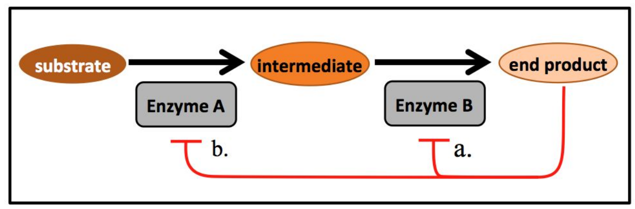

Figure 3 Feedback Inhibition
A simple metabolic pathway that requires two enzymes and contains one intermediate metabolite. (a) The end product inhibits its own production directly. (b) The end product inhibits its own production indirectly by inhibiting an upstream enzyme.

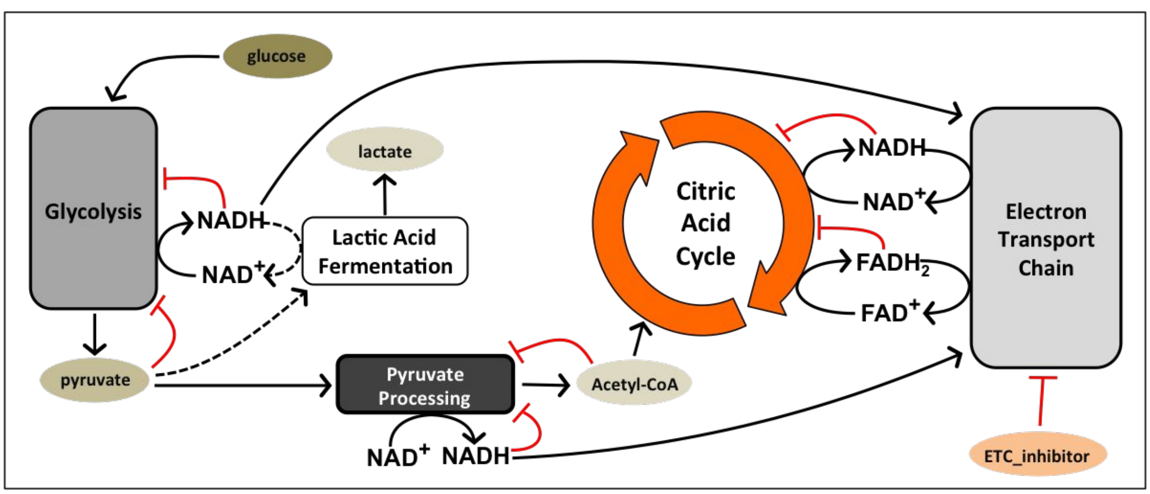

Figure 4 Regulation of Cellular Respiration

Activity: Cellular Respiration Simulation

The purpose of this learning module is to explore the fundamental properties and components of cellular respiration. To do so, you will be using a computer simulation to become familiar with the components of cellular respiration and the mechanisms regulating this complex process.

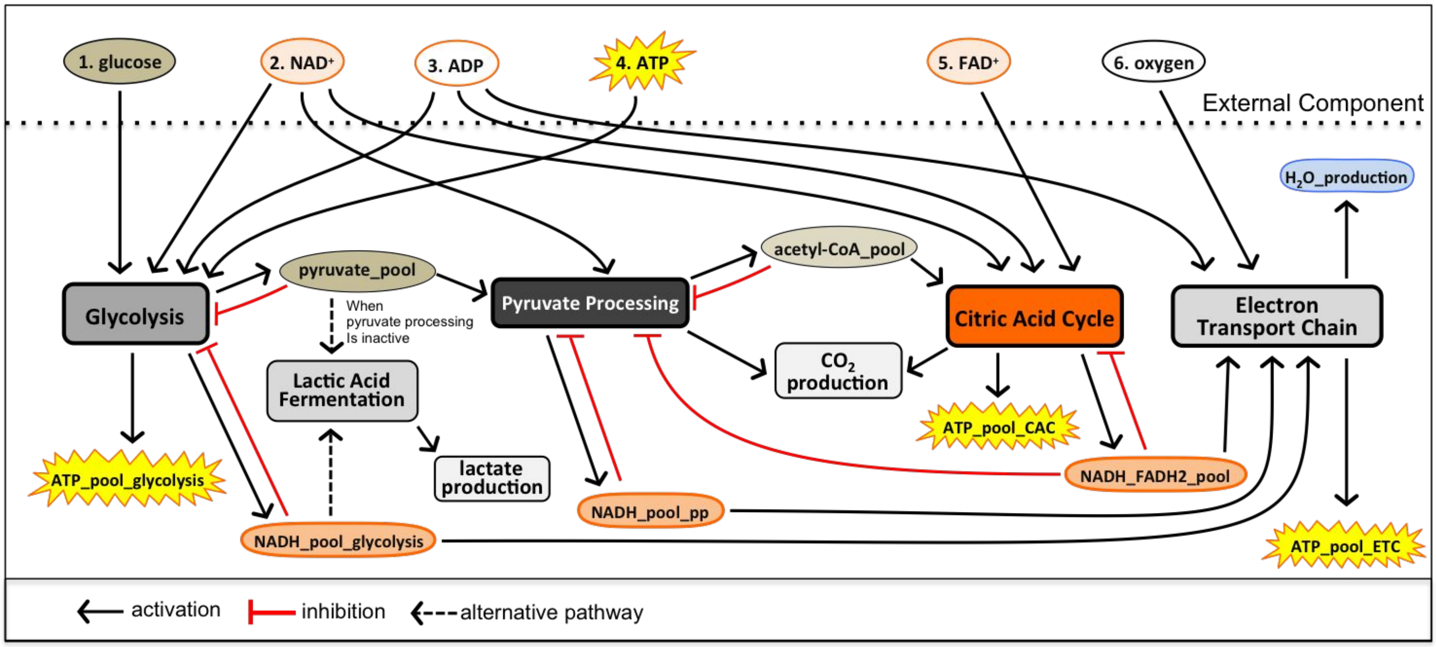

Figure 5 Cellular Respiration Dynamic Model

Computational Model of Cellular Respiration

In this activity you will be using a computational modeling and simulation software called the Cell Collective to explore the components and dynamics of cellular respiration. Figure 5 represents a simplified computational model of cellular respiration that was built using the Cell Collective. The components of the model represent important molecules (e.g., glucose and ADP) and processes (e.g., glycolysis and pyruvate processing). The components are connected using arrows to represent positive regulation (activation) and blunted lines to represent negative regulation (inhibition) which are collectively called *interactions*. Simulating the model allows you to observe all of the components interacting together and mimics the activities occurring in a cell. To interpret simulation data, it is critical that you understand the model components and their functions. **To begin, complete the task below.**

Exercise 1: Use arrows to connect each input to its function. (note: some inputs may share a similar function)

Inputs	**Function**
glucose	electron carrier
NAD^+ and FAD^+	final electron acceptor
ADP	energy acceptor
ATP	carbon, electron, and energy source
oxygen	immediate energy source

Exercise 2. Simulation of Cellular Respiration Using Cell Collective

Part 1: Access to the Dynamic Model of Cellular Respiration

a. Go to learn.cellcollective.org
b. Sign Up and Login to the Cell Collective.
c. From the Home page, select “Discovering Cellular Respiration with Simulations” (red box, Figure 6) then click the “Start Activity” button (blue box, Figure 6).
d. Next, click “Simulation” on the top menu bar to enter the Simulation workspace.

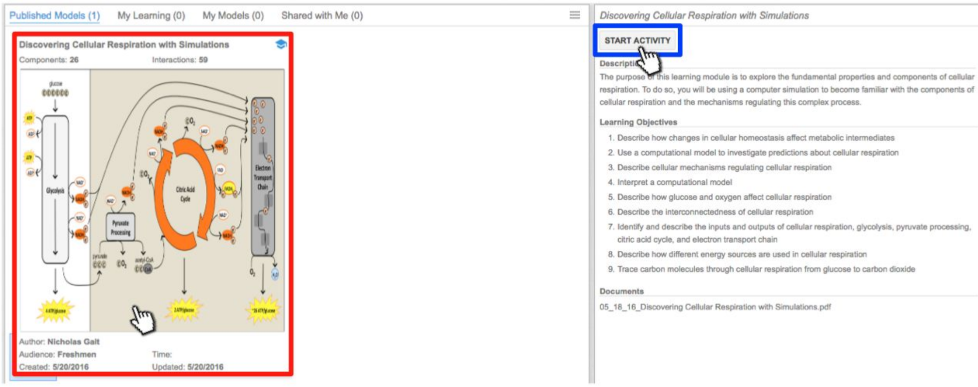

Figure 6

Part 2: Using the Cell Collective to Monitor Cellular Respiration

The purpose of this simulation is to explore both the inputs and outputs associated with aerobic cellular respiration and fermentation (see Figure 5). When the necessary inputs are selected, you can observe the activity of each process increase (dotted line, Figure 7).

a. To begin, adjust the Sliding Window size to 100 (Red arrow, Figure 7). Next, provide the cell with the six major inputs of cellular respiration. The inputs are set as “External Components” so you can control which inputs your cell will have available for cellular respiration. Adjust the activity of the following sliders to 95 (red box, Figure 7).

   i. 1.glucose
   ii. 2.NAD^+
   iii. 3.ADP
   iv. 4.ATP
   v. 5.FAD^+
   vi. 6.oxygen

b. Next, you will need to select the components of cellular respiration that you want to observe. Under “Internal Components” (blue box, Figure 7), check the following:

   i. glycolysis
   ii. pyruvate_processing
   iii. citric_acid_cycle
   iv. electron_transport_chain
   v. lactic_acid_fermentaiton

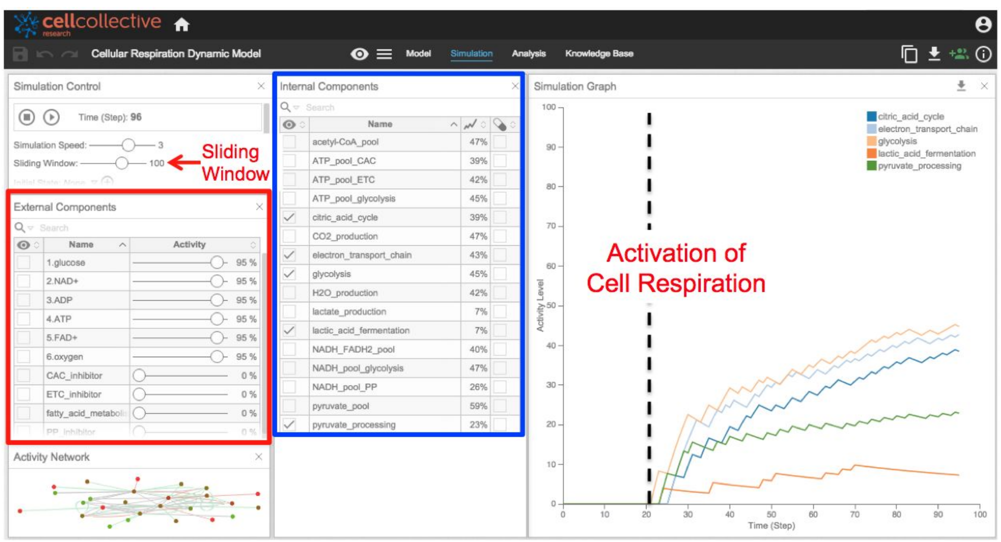

Figure 7 Simulation Setup

c. Start the simulation by clicking on the *play* button in the “Simulation Control” panel (top left of the screen). To evaluate the activity of a process it may be useful to click the “Pause” button. To clear the graph and restart the simulation, click the *stop* button.

d. To see the activity of other components in the model just check the box next to its name. For example, you can observe the process specific levels of NADH and FADH2 by selecting “NADH_FADH2_pool” for the citric acid cycle, NADH_pool_glycolysis” and “NADH_pool_PP” for pyruvate processing.

e. Continue this activity by completing the questions on the next page.

Exercise 3. The purpose of this section is to learn how the processes of cellular respiration are interconnected. You will be conducting simulations to verify your own predictions about the dynamic interactions between the components of cellular respiration. Complete each statement thoroughly.

Investigation 1: Interconnectedness of Cellular Respiration
The processes of cellular respiration are interconnected in that the outputs (products) of one process may be the inputs (substrates) of another process. Most cell types can harvest energy from a variety of substrates. However, brain cells primarily rely on glucose as an energy source.

A. **Prediction**. Predict how the activity of the following processes (below) would be affected if glucose was no longer available in brain cells.

1. Pyruvate processing would (circle one):	Increase	Decrease	Remain the same
2. Citric acid cycle would (circle one):	Increase	Decrease	Remain the same
3. Electron transport chain would (circle one):	Increase	Decrease	Remain the same

B. **Defend your prediction.** Describe HOW the components involved would interact to affect the processes of cellular respiration if glucose was not available in brain cells. (Hint: Use Figure 5)
For the first one, we have provided an example of a mechanism for you. You just have to fill in the blanks or circle

If glucose was not available, glycolysis would be **active/inactive** and not produce _________ to fuel pyruvate processing. Therefore, pyruvate processing would be **active/inactive** and not produce _________ to fuel the citric acid cycle which would then be **active/inactive**. The electron transport chain would be **active/inactive** because _________ and _________ would not be available to supply electrons for oxidative phosphorylation.

C. **Test your prediction with Cell Collective simulation.** Simulate normal cellular respiration as you did in Task 2
1. Monitor the activity levels of each process (glycolysis, pyruvate_processing, citric_acid_cycle and electron_transport_chain) and the inputs/outs of each process (e.g., pyruvate_pool, NADH_pool_glycolysis, acetylCoA_ pool, etc.).
2. Once the activity levels of the "Internal Components" have normalized (i.e., leveled off)
3. Adjust the "1.glucose" slider to 0 (See red box, Fig. 7).

D. **Record the results.**

1. Glycolysis (circle one):	Increases	Decreases	Remains the same
2. Puruvate_pool (circle one):	Increases	Decreases	Remains the same
3. Pyruvate_processing (circle one):	Increases	Decreases	Remains the same
4. Acetyl-CoA_pool (circle one):	Increases	Decreases	Remains the same
5. Citric_acid_cycle (circle one):	Increases	Decreases	Remains the same
6. NADH_pool_glycolysis (circle one):	Increases	Decreases	Remains the same
7. NADH_pool_PP (circle one):	Increases	Decreases	Remains the same
8. NADH_FADH2_pool (circle one):	Increases	Decreases	Remains the same
9. Electron_transport_chain (circle one):	Increases	Decreases	Remains the same

E. **Evaluate your prediction.** Do your simulation results match your predictions? (circle one): Yes No

If your prediction was not correct, continue to play with the simulation to understand the following:
1) how simulation results translate to events inside the cell
2) how the glucose affects the processes of cellular respiration

F. **Describe the mechanism correctly.** Describe **HOW** the components of cell respiration interact when glucose is removed.

Investigation 2: Other Sources of Energy

Glucose is not the only energy source available to a cell. After eating a large ribeye steak, for example, your cells would be provided with amino acids (from muscle) and fatty acids (from fat) but very little glucose to fuel cellular respiration. Specifically, some amino acids can be used as energy substrates in glycolysis and the citric acid cycle. For this investigation you will identify which process of cell respiration utilizes fatty acids.

A. **Prediction**. What process(es) become significantly more active when fatty acids are being metabolized (broken down)?

(circle all that apply): Glycolysis Pyruvate Processing Citric Acid Cycle Electron Transport Chain

B. **Test your prediction with Cell Collective simulation.**

1. Adjust the NAD+, ADP, ATP, FAD+ and oxygen sliders to 95 and adjust the glucose slider to 10 to see the effects of fatty acid metabolism more clearly.
2. Monitor the activity levels of each process (glycolysis, pyruvate_processing, citric_acid_cycle and electron_transport_chain).
3. Wait for the activity levels of the "Internal Components" to normalize (i.e., leveled off)
4. Adjust the "fatty_acid_metabolism" slider to 100 (See red box, Fig. 7).

C. **Record the results.**

1. Glycolysis (circle one): increases decreases remains the same

2. Pyruvate_processing(circle one): increases decreases remains the same

3. Citric_acid_cycle (circle one): increases decreases remains the same

4. Electron_transport_chain (circle one): increases decreases remains the same

D. **Evaluate your prediction.** Do your simulation results match your predictions? (circle one): Yes No

If your prediction was not correct, continue to play with the simulation to understand the following:

1) how simulation results translate to events inside the cell
2) where fatty acids are DIRECTLY utilized by cellular respiration

E. Which process does fatty acids feed directly into? (circle one):

Glycolysis Pyruvate Processing Citric Acid Cycle Electron Transport Chain

F. Defend why you think fatty acids feed directly into the process you've chosen above by 1) describing what's occurring in the cell and 2) using your simulation results to support your answer. (Hint: it may be useful to compare the inputs and outputs of each process that was active)

Investigation 3: Feedback Inhibition

Carbon dioxide (CO_2) is a major output of cellular respiration. It is produced during both pyruvate processing and the citric acid cycle.

A. **Prediction**. If a cell was treated with a drug that completely inhibited the citric acid cycle, would the production of carbon dioxide (CO_2) (circle one):

increase decrease remains the same stop completely

B. **Defend your prediction.** Provide a mechanistic explanation to describe HOW the components involved would interact to affect the production of carbon dioxide if the citric acid cycle was inhibited. (Hint: remember feedback inhibition and Figure 5)

C. **Test your prediction with Cell Collective simulation.** Simulate normal cellular respiration as you did in Task 2. (Be sure the glucose slider is back up to 95)

1. Monitor citric_acid_cycle, CO2_production, acetyl-CoA_pool and pyruvate_processing.
2. Once the activity levels of the "Internal Components" have normalized (i.e., leveled off),
3. Adjust the "CAC_inhibitor" slider to 100 (See red box, Fig. 7).

D. **Record the results.**

1. Acetyl-CoA_pool (circle one):	increased	decreased	remains the same	stop completely
2. CO_2 production (circle one):	increased	decreased	remains the same	stop completely
3. Pyruvate processing (circle one):	increased	decreased	remains the same	stop completely
4. Citric acid cycle (circle one):	increased	decreased	remains the same	stop completely

E. **Evaluate your prediction.** Do your simulation results match your predictions? (circle one): Yes No

If your prediction was not correct, continue to play with the simulation to understand the following:
1) how simulation results translate to events inside the cell
2) how the citric acid cycle and carbon dioxide production are related in cellular respiration

F. **Describe the mechanism correctly.** Describe **HOW** carbon dioxide production is affected when the citric acid cycle is inhibited. Be sure to include all of the components involved and describe how they interact.

Investigation 4: Feedback Inhibition and an Alternative Pathway
A new drug has been developed to combat obesity. One of the effects of the drug is that it completely inhibits pyruvate processing.

A. **Prediction**. How would the activity of glycolysis and lactic acid fermentation be affected if a cell was treated with a drug that completely inhibits pyruvate processing?

1. Glycolysis would (circle one):	increase	decrease	remains the same	stop completely
2. Lactic acid fermentation would (circle one):	increase	decrease	remains the same	stop completely

B. **Defend your prediction.** Provide a mechanistic explanation to describe **HOW** the components involved would interact to affect glycolysis and lactic acid fermentation if pyruvate processing was inhibited. (Hint: use Figure 5)

C. **Test your prediction with Cell Collective simulation.** Simulate normal cellular respiration as you did in Task 2. (Be sure the CAC_inhibitor slider is back down to 0)
1. Monitor pyruvate_processing, pyruvate_pool, glycolysis, lactic_acid_fermentation and NADH_pool_glycolysis
2. Wait for the activity levels of the "Internal Components" to normalize (i.e., leveled off)
3. Adjust the "PP_inhibitor" slider to 100 (See red box, Fig. 7).

D. **Record the results.**

1. Pyruvate_processing (circle one):	increases	decreases	remains the same	stops completely
2. Glycolysis (circle one):	increases	decreases	remains the same	stops completely
3. Lactic_acid_fermentation (circle one):	increases	decreases	remains the same	stops completely
4. NADH_pool_glycolysis is (circle one):	increases	decreases	remains the same	stops completely

E. **Evaluate your prediction.** Do your simulation results match your predictions? (circle one): Yes No

If your prediction was not correct, continue to play with the simulation to understand the following:
1) how simulation results translate to events inside the cell
2) how the components of cellular respiration affect the processes of cellular respiration

F. **Describe the mechanism correctly.** Describe the mechanism of **HOW glycolysis and lactic acid fermentation** are affected when pyruvate processing is inhibited. Be sure to include all of the components involved and describe how they interact.

Investigation 5: Feedback Inhibition. Oxygen is a key component of cellular respiration.

A. **Prediction**. What would happen to the behavior (i.e., activity) of lactic acid fermentation if oxygen was removed from the cellular environment? (circle one)

increase   decrease   remain the same

B. **Defend your prediction.** Provide a mechanistic explanation to describe **HOW** the components involved would interact to affect lactic acid fermentation if oxygen was not present in the cellular environment. (Hint: remember feedback inhibition)

C. **Test your prediction with Cell Collective simulation.** Simulate normal cellular respiration as you did in Task 2. (Be sure the PP_inhibitor slider is back down to 0)

1. Monitor the activity of all components that connect lactic_acid_fermentation to the electron_transport_chain
2. Wait for the activity levels of the "Internal Components" to normalize (i.e., leveled off)
3. Remove oxygen from the environment by adjusting the "6.oxygen" slider to 0 (See red box, Fig. 7).

D. **Record the results.**

1. Lactic_acid_fermentation (circle one):	Increases	Decreases	Remains the same
2. Pyruvate_pool (circle one):	Increases	Decreases	Remains the same
3. Pyruvate_processing (circle one):	Increases	Decreases	Remains the same
4. Acetyl-CoA_pool (circle one):	Increases	Decreases	Remains the same
5. Citric_acid_cycle (circle one):	Increases	Decreases	Remains the same
6. NADH_pool_glycolysis (circle one):	Increases	Decreases	Remains the same
7. NADH_pool_PP (circle one):	Increases	Decreases	Remains the same
8. NADH_FADH2_pool (circle one):	Increases	Decreases	Remains the same
9. Electron_transport_chain (circle one):	Increases	Decreases	Remains the same

E. **Evaluate your prediction.** Do your simulation results match your predictions? (circle one):   Yes   No

If no, continue to play with the simulation to understand the following:
1) how simulation results translate to events inside the cell
2) how oxygen affects cellular respiration

F. **Describe the mechanism correctly.** Describe the mechanism of how lactic acid fermentation is affected when oxygen is removed. Be sure to include all of the components involved and describe how they interact.