Supplemental Material

Clark *et al.*

Supplemental Material: Clark, Helikar, Dauer. Simulating a computational biological model, rather than reading, elicits changes in brain activity

SUPPLEMENTARY MATERIALS

Table of contents

Supplemental Material: Clark, Helikar, Dauer. Simulating a computational biological model, rather than reading, elicits changes in brain activity

Supplementary tables

Table S1: Relation of students' mean behavioral accuracy with their brain activity during the system-specific task.

Contrast	Brain region	MNI coordinates			N Voxels	Max Z
		x	*y*	*z*		
Model-based > baseline	*n.s.*					
Model-based > control trials	R. Middle Frontal gyrus (BA 8)	42	26	44	213	4.04
	L. Middle Frontal gyrus (BA 6)	-26	22	56	80	4.12

Note. Student's instructional group and gender are also included in this model; n.s.: not significant
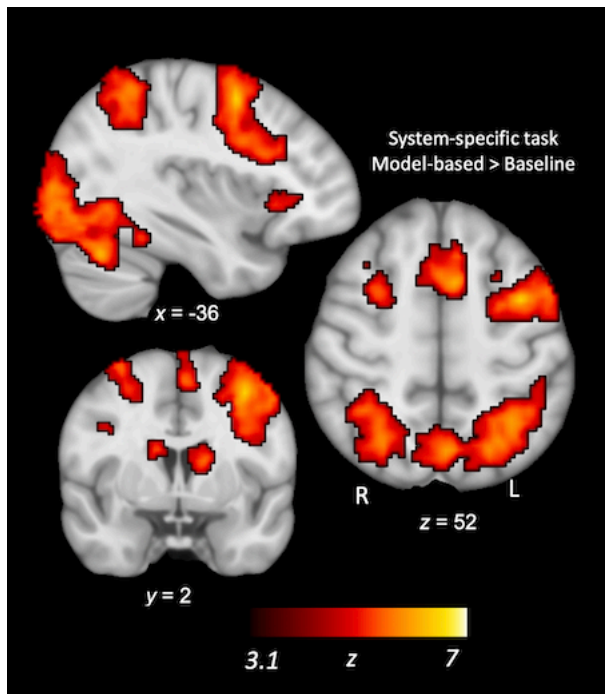
Supplementary figures

Figure S1: Mean BOLD response for model-based > baseline contrast in the whole sample for the system-specific task.
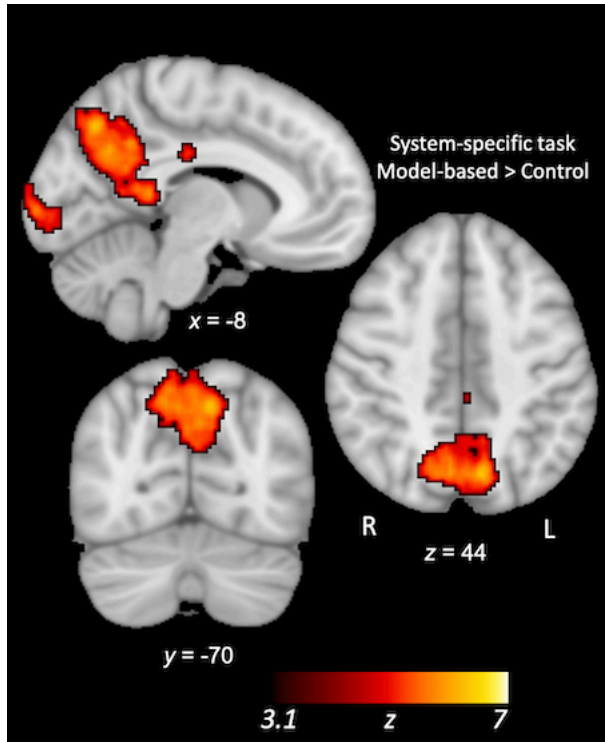
Figure S2: Mean BOLD response for model-based > control contrast in the whole sample for the system-specific task.
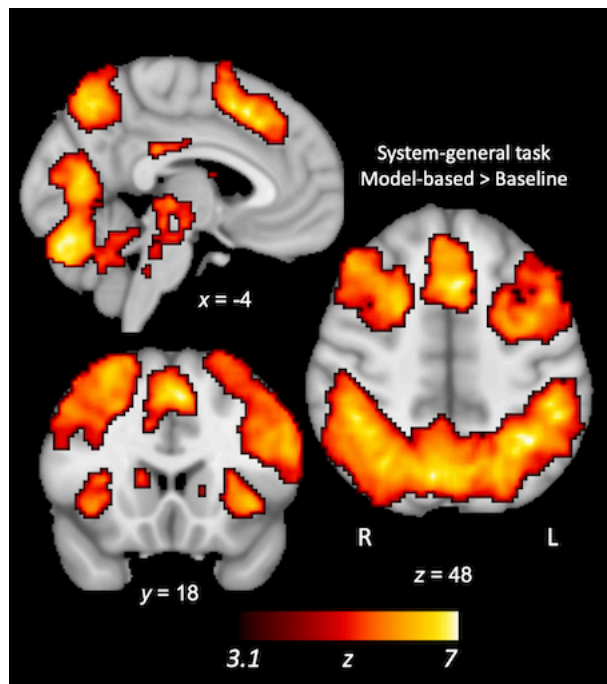
Figure S3: Mean BOLD response for model-based > baseline contrast in the whole sample for the system-general task
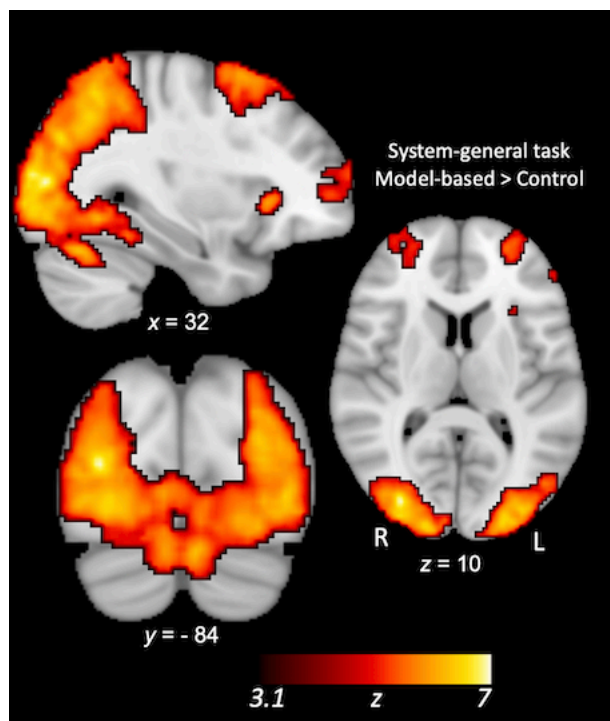
Figure S4: Mean BOLD response for model-based > control contrast in the whole sample for the system-general task

Meta-analytic cognitive decoding analysis

Group maps for these analyses are available in "Neurovault" collection: https://identifiers.org/neurovault.collection:6930

We used the Neurosynth (https://neurosynth.org/) meta-analytic decoder tool to compare unthresholded Z statistic maps obtained for all analyses where we report significant effects to other studies in the Neurosynth database. The decoder provides an indication of which terms in the Neurosynth database are associated with maps that are similar to those from our analyses, as well as providing a Pearson correlation coefficient for the association between these maps and our own. For each map where we report significant group effects, we report the top 10 associated words obtained from the database.

<u>System specific task</u>
Model-based > baseline
All participants – Results in Table 3a (https://identifiers.org/neurovault.image:373204)

Term	Correlation
Visual	.32
Reading	.30
Tasks	.30
Task	.28
Parietal	.27
Intraparietal	.27
Word	.27
Occipital	.26
Intrapietal sulcus	.26
Working memory	.25

Simulate > Read group Results in Table 3b (https://identifiers.org/neurovault.image:373205)

Term	Correlation
Pain	.14
Insula	.13
Retrosplenial	.13
Painful	.12
Navigation	.12
Inferior parietal	.12
Somatosensory cortices	.11
Somatosensory	.11
Parahippocampal	.10
Lingual	.10

System specific modeling > control trials

All participants – Results in Table 3c (https://identifiers.org/neurovault.image:373206)

Term	Correlation
Precuneus	.32
Posterior cingulate	.27
Cortex precuneus	.20
Default mode	.18
Default	.18
PCC	.18
Resting state	.18
Episodic	.18
Resting	.17
Precuneus posterior	.17

Effect of accuracy - Results in Supplemental Table 1 (https://identifiers.org/neurovault.image:373207)

Term	Correlation
Prefrontal	.14
Memory	.14
Fusiform	.13
Word	.13
Dorsolateral	.12
Task	.12
Frontal	.12
Working memory	.12
Working	.12
Demands	.11

System general task

Model-based > baseline

All participants – Results in Table 4a (https://identifiers.org/neurovault.image:382985)

Term	Correlation
Parietal	.38
Task	.34
Tasks	.33
Intraparietal	.33
Visual	.32
Intraparietal sulcus	.32
Parietal cortex	.31
Working memory	.31
Working	.30
Posterior parietal	.30

Supplemental Material: Clark, Helikar, Dauer. Simulating a computational biological model, rather than reading, elicits changes in brain activity

Model-based > control trials

All participants - Results in Table 4c (https://identifiers.org/neurovault.image:373209)

Term	Correlation
Visual	.44
Parietal	.41
Intraparietal	.39
Intraparietal sulcus	.38
Occipital	.36
Parietal cortex	.35
Task	.32
Tasks	.32
Working memory	.30
Working	.30

Simulate > Read group – Results in Table 4d (https://identifiers.org/neurovault.image:382986)
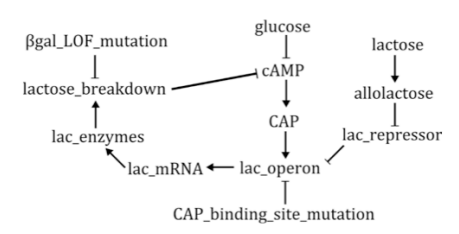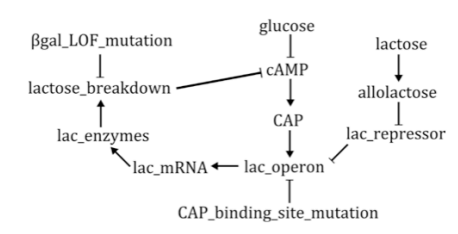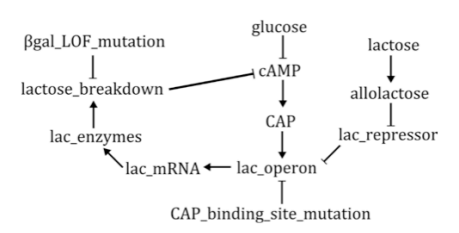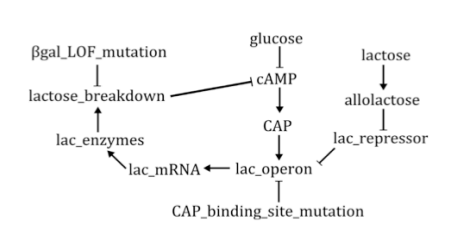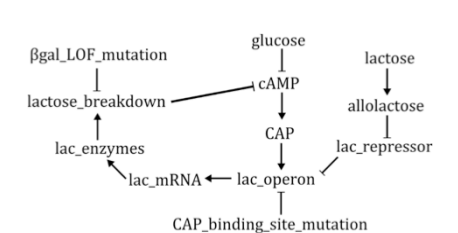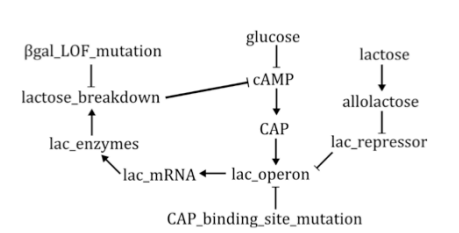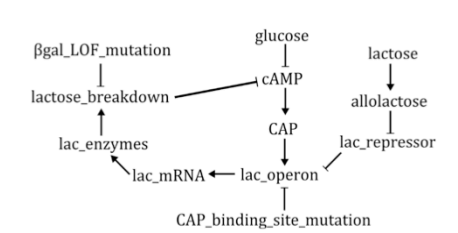
Term	Correlation
Primary motor	.21
Supplementary motor	.21
Somatosensory	.21
Motor	.21
Motor cortex	.21
Primary	.21
Supplementary	.20
Pain	.20
Sensorimotor	.19
Insula	.19

Questions included in fMRI tasks

Model-based questions included in system-specific fMRI task

Lactose is present and glucose is absent.
The target is only active if the inhibitor is inactive and the activator is active.
If both are active, then the inhibitor dominates and the target is inactive.

βgal_LOF_mutation
glucose
lactose
cAMP
lactose_breakdown
allolactose
CAP
lac_repressor
lac_enzymes
lac_mRNA ← lac_operon
CAP_binding_site_mutation

CAP and *lac* repressor are active. What is lac_operon?

Active   Inactive
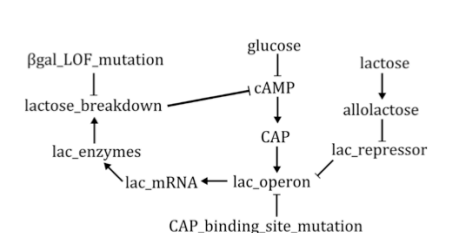

Lactose is present and glucose is absent.
The target is only active if the inhibitor is inactive and the activator is active.
If both are active, then the inhibitor dominates and the target is inactive.

βgal_LOF_mutation
glucose
lactose
cAMP
lactose_breakdown
allolactose
CAP
lac_repressor
lac_enzymes
lac_mRNA ← lac_operon
CAP_binding_site_mutation

CAP is active and *lac* repressor is inactive. What is lac_operon?

Active   Inactive

Lactose is present and glucose is absent.
E. coli mutant #5 has a non-functional lac_repressor and a constitutively active CAP.

βgal_LOF_mutation
glucose
lactose
cAMP
lactose_breakdown
allolactose
CAP
lac_repressor
lac_enzymes
lac_mRNA ← lac_operon
CAP_binding_site_mutation

Lac_operon is still expressed.

Correct   Incorrect

Lactose is present and glucose is absent. *E. coli* mutant #1 with both constitutively active CAP and constitutively active lac_repressor produces allolactose…

βgal_LOF_mutation
glucose
lactose
cAMP
lactose_breakdown
allolactose
CAP
lac_repressor
lac_enzymes
lac_mRNA ← lac_operon
CAP_binding_site_mutation

because allolactose production is independent of lac_operon expression.

Correct   Incorrect

Lactose is present and glucose is absent. *E. coli* mutant #2 has mutated CAP: CAP cannot bind cAMP. Lac_operon is not expressed…

βgal_LOF_mutation
glucose
lactose
cAMP
lactose_breakdown
allolactose
CAP
lac_repressor
lac_enzymes
lac_mRNA ← lac_operon
CAP_binding_site_mutation

because without cAMP binding, CAP remains in its inactive form.

Correct   Incorrect

Lactose is present and glucose is absent. *E. coli* mutant #4 has a non-functional cAMP. These mutants fail to break down lactose…

βgal_LOF_mutation
glucose
lactose
cAMP
lactose_breakdown
allolactose
CAP
lac_repressor
lac_enzymes
lac_mRNA ← lac_operon
CAP_binding_site_mutation

because allolactose binding lac_repressor is insufficient to activate lac_operon expression.

Correct   Incorrect

Lactose is present and glucose is absent. E. coli mutant #6 has non-functional cAMP and constitutively active CAP. This mutant can still break down lactose…

βgal_LOF_mutation
glucose
lactose
cAMP
lactose_breakdown
allolactose
CAP
lac_repressor
lac_enzymes
lac_mRNA ← lac_operon
CAP_binding_site_mutation

because constitutively active CAP doesn't need cAMP to bind the *lac* promoter to facilitate transcription.

Correct   Incorrect

Lactose is present and glucose is absent. *E. coli* mutant #1 has both constitutively active CAP and constitutively active lac_repressor. The lac_operon is never expressed…

βgal_LOF_mutation
glucose
lactose
cAMP
lactose_breakdown
allolactose
CAP
lac_repressor
lac_enzymes
lac_mRNA ← lac_operon
CAP_binding_site_mutation

because active CAP is unable to override lac_repressor's inhibition.

Correct   Incorrect

Control questions included in system-specific fMRI task

A thing is present only if is not absent.
An activator makes something active but it does not make something inactive.

What does an activator make something?

Active   Inactive

A thing is present only if is not absent.
An activator makes something active but it does not make something inactive.

Something is not active. What is it?

Active   Inactive

Letter #5 in non-functional lac repressor is f. In constitutively, the fifth letter is a T.

If I keep silent, my thoughts are not expressed.

Correct   Incorrect

The letter A is present in allolactose. It is absent in E. coli. The letter A is not absent in lactose…

because the letter A is present in the word lactose.

Correct   Incorrect

The word non-functional breaks down into non and _functional.

because it is constituted of the words note and functional

Correct   Incorrect

The word non-functional breaks down into non and _functional.

because it is constituted of the words note and functional

Correct   Incorrect

The #1 letter in the word transcription is a t. If we bind together the words lac and operon…

We can express the result as lac_operon

Correct   Incorrect

The word AMPc can be mutated into cAMP. We can mutate the word inactive: it could become activein but activein is not often expressed…

because activein is not a word

Correct   Incorrect

11

Supplemental Material: Clark, Helikar, Dauer. Simulating a computational biological model, rather than reading, elicits changes in brain activity

Model-based questions included in system-general fMRI task

Starting state is color-coded: "Active" or "Inactive"

A — B → C — D

Is "A" Active or Inactive?
Active   Inactive

Starting state is color-coded: "Active" or "Inactive"

A — B → C — D

Is "D" Active or Inactive?
Active   Inactive

Starting state is color-coded: "Active" or "Inactive"

A — B → C — D

Is "C" Active or Inactive?
Active   Inactive

Starting state is color-coded: "Active" or "Inactive"

A — B → C — D

Is "B" Active or Inactive?
Active   Inactive

Starting state is color-coded: "Active" or "Inactive"

A → B — C — D → E

Is "E" Active or Inactive?
Active   Inactive

Starting state is color-coded: "Active" or "Inactive"

A → B → D
   ↘ C → E
        ↘ F

Is "D" Active or Inactive?
Active   Inactive

Starting state is color-coded: "Active" or "Inactive"

A → B → C → D, F → E

Is "C" Active or Inactive?
Active   Inactive

Starting state is color-coded: "Active" or "Inactive"

A → B → C → D, F → E

Is "C" Active or Inactive?
Active   Inactive

12

Control questions included in system-general fMRI task

Starting state is color-coded: "Active" or "Inactive" D Is "D" Active or Inactive? Active Inactive	Starting state is color-coded: "Active" or "Inactive" A Is "A" Active or Inactive? Active Inactive
Starting state is color-coded: "Active" or "Inactive" D Is "D" Active or Inactive? Active Inactive	Starting state is color-coded: "Active" or "Inactive" E Is "E" Active or Inactive? Active Inactive
Starting state is color-coded: "Active" or "Inactive" E Is "E" Active or Inactive? Active Inactive	Starting state is color-coded: "Active" or "Inactive" E Is "E" Active or Inactive? Active Inactive
Starting state is color-coded: "Active" or "Inactive" B ( ) Is "B" Active or Inactive? Active Inactive	Starting state is color-coded: "Active" or "Inactive" E Is "E" Active or Inactive? Active Inactive

Pre-Scan Lesson Materials
<u>Background Reading</u>

Regulation of Gene Expression in Prokaryotes with Feedback Loop

Nicholas Galt, Heather Bergan-Roller, Lisa Briona, Joseph Dauer, and Tomáš Helikar
University of Nebraska – Lincoln

The E. coli genome contains approximately 4,300 genes encoding metabolic enzymes needed for cellular respiration, transport proteins essential for acquiring nutrients, regulatory proteins needed to control the production of other proteins, and many others. Because protein synthesis requires a tremendous expenditure of energy (ATP), only a subset of the available genes are actively being expressed (turned "ON") at any given time (Figure 1). The expression of many of these genes are influenced by external and internal conditions. Natural selection has favored E. coli and other prokaryotes that are able to regulate the expression of genes so that they are only expressed when they need to be expressed. In the 5 investigations of this module, you will explore the genetic control mechanisms that regulate breakdown of the disaccharide lactose in prokaryotes to exemplify gene expression and regulation present in all organisms.

Figure 1: Patterns of gene expression in prokaryotes
Only a subset of genes in the genome are actively being expressed (turned "ON") at any given time. This allows the cell to conserve energy when certain gene products (proteins) are not necessary.

The Operon Model
In prokaryotes, genes that share a similar function are often clustered together on the chromosome and their expression is coordinately controlled (i.e., if one gene is going to be expressed, all of the genes in the cluster will be expressed) by a single **promoter** and **operator**. Collectively, the promoter, operator and functionally related protein-encoding genes are called an **operon** (Figure 2). This form of gene regulation differs from eukaryotes, as eukaryotic genes are regulated individually.

The "control" region of the operon consists of the promoter and operator. The promoter is a sequence of DNA that RNA polymerase ("RNAP") recognizes and binds to initiate transcription. Promoters can also contain binding sites for regulatory proteins (transcription factors) called **activators** that function to activate transcription.

The operator is a short sequence of DNA that transcriptional regulators recognize and is analogous to an "ON/OFF" switch. Operators contain unique binding sites that repressor proteins are able to recognize: the repressor protein that is able to turn "OFF" transcription for a particular operon will not be able to turn "OFF" another. Repressor proteins prevent transcription by blocking the
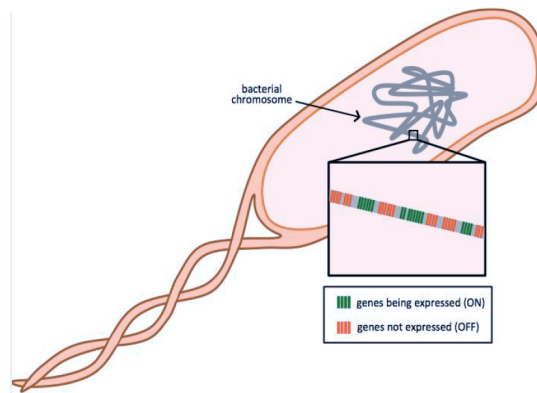
binding site in the promoter that RNAP recognizes. Activator and repressor proteins, along with corepressors and inducers, will be described in more detail in the following activities.
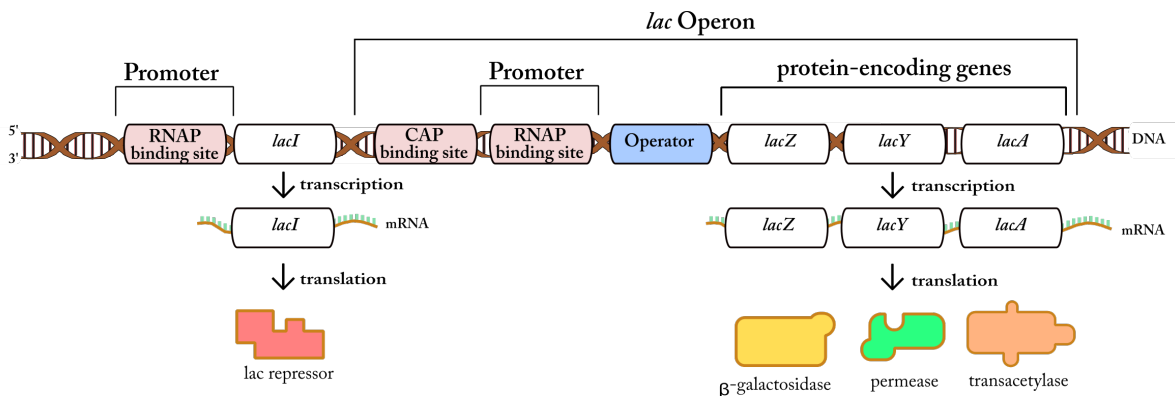

Figure 2: *lac* **Operon.**

When the operon is turned "ON," the genes within the operon are transcribed by RNAP to produce a single mRNA. mRNA containing information for more than one polypeptide is considered polycistronic mRNA ("cistron" is an old term for "coding sequence"). This polycistronic mRNA is then translated into individual polypeptides (proteins).

Key Concepts and Learning Objectives
Key Concepts of Gene Regulation and Expression (*adapted from Khodor et al., 2004*)
These are major ideas about gene expression and regulation that are exemplified by prokaryotic operons but apply to all gene expression and regulation, regardless of organism.

The expression of genes is regulated.
- *Not all genes need to be expressed at all times.*
  - Gene products are regulated in their timing and abundance.
  - Expression of some gene products needs to be regulated in response to external stimuli (e.g., change in available nutrients) or internal processes (e.g., cell cycle progression).
- *Components of processes that work together are often regulated together.*
  - It is energetically favorable to co-regulate the expression of genes that encode proteins involved in a pathway or process.
  - Co-regulated genes can be organized into operons or share common transcription factors and their consensus binding sites.
- *RNAP and regulator proteins (trans-acting elements) interact with regulatory regions (cis-acting elements) by binding to either promote or prevent transcription.*
  - Cis-acting elements (e.g., operator, promoter) are sequences of DNA.
  - Trans-acting elements (e.g., repressor, transcription factors) are gene products (functioning proteins).
- *DNA mutations can impact gene regulation.*
  - Mutations in cis-acting elements will affect only the protein encoded by the gene where the cis-acting element is located.

> Mutations in trans-acting elements will affect the expression of all genes whose transcription is regulated by the trans-acting element.
- *Gene expression can be controlled via feedback mechanisms.*
  - Both substrates (starting materials) and pathway end-products can influence ongoing gene expression through both positive and negative feedback loops.

Learning Objectives
These are actions that the learner will have the opportunity to accomplish during the following activities to demonstrate understanding of gene expression and regulation.
   A. Interpret visual representations of dynamic cellular events.
   B. Describe mechanistically how prokaryotic genes are regulated.
   C. Relate specific *lac* operon and *trp* operon examples to general "Key Concepts of Gene Expression and Regulation."
   D. Predict the impact of mutations on gene expression and regulation.
   E. Investigate the computational model through simulations.
   F. Interpret simulation results.
   G. Relate simulation results to cellular events.
   H. Explain mechanistically the influence of nutrition on the *lac* and *trp* operons.
   I. Describe the composition (i.e., macromolecular composition) of elements related to operons.
   J. Describe the mechanisms by which *E. coli* ensures glucose is preferentially used as an energy source.

The *lac* Operon
The lac operon (Figure 2) is a cluster of three genes that function together to import and metabolize the disaccharide lactose (lac) into glucose and galactose. For most prokaryotes, glucose is the preferred carbohydrate (sugar) because it can directly enter glycolysis. For example: if you drank a glass of milk, lactose would be readily available to the *E. coli* living in your intestines but glucose would be in short supply. In such a scenario, the lac operon would be switched "ON" to produce the enzymes required for the *E. coli* to utilize the lactose. Regulation of the lac operon is therefore an example of gene regulation responsive to changes in environmental conditions.

Regulation of the Lac Operon
The activity of the lac operon is controlled by two different regulatory proteins. The first is the ***lac* repressor** which is produced by the regulatory gene *lacI*. When active, the lac repressor binds the ***lac* operator** and blocks transcription of *lac* operon (Figure 3). Repressors exist in two forms: active (able to bind the operator) and inactive (unable to bind the operator). The lac repressor is synthesized in its active form.

A co-factor (regulatory molecule) called an **inducer** (acts to *induce* transcription) is required to deactivate the lac repressor. Binding of the inducer to the repressor induces an allosteric conformational change that converts the lac repressor to its inactive form, and makes the lac repressor unable to bind the *lac* operator. As lactose enters the cell, some of it is isomerized into the inducer **allolactose**. Allolactose's binding to the lac repressor causes the lac repressor to change shape, inactivating it (Figure 3). This form of regulation saves the cell energy by only producing the enzymes required for breaking down lactose when lactose is present. The lac operon is considered an inducible operon because its default state is "OFF" and transcription is only turned "ON" in the presence of an inducer (allolactose).

The second regulatory protein is an **activator** (*activates* transcription) called **Catabolite Activator Protein** (**CAP**; also called Catabolite Receptor Protein, CRP). CAP is also an **allosteric** protein existing in two forms (active and inactive). Unlike lac repressor, CAP is synthesized in its inactive form. CAP changes to its active form when bound to **cyclic AMP** (**cAMP**). Activated CAP binds the CAP-binding-site immediately upstream of the RNAP binding site in the promoter, and activates transcription by recruiting RNAP to the lac promoter (Figure 3).
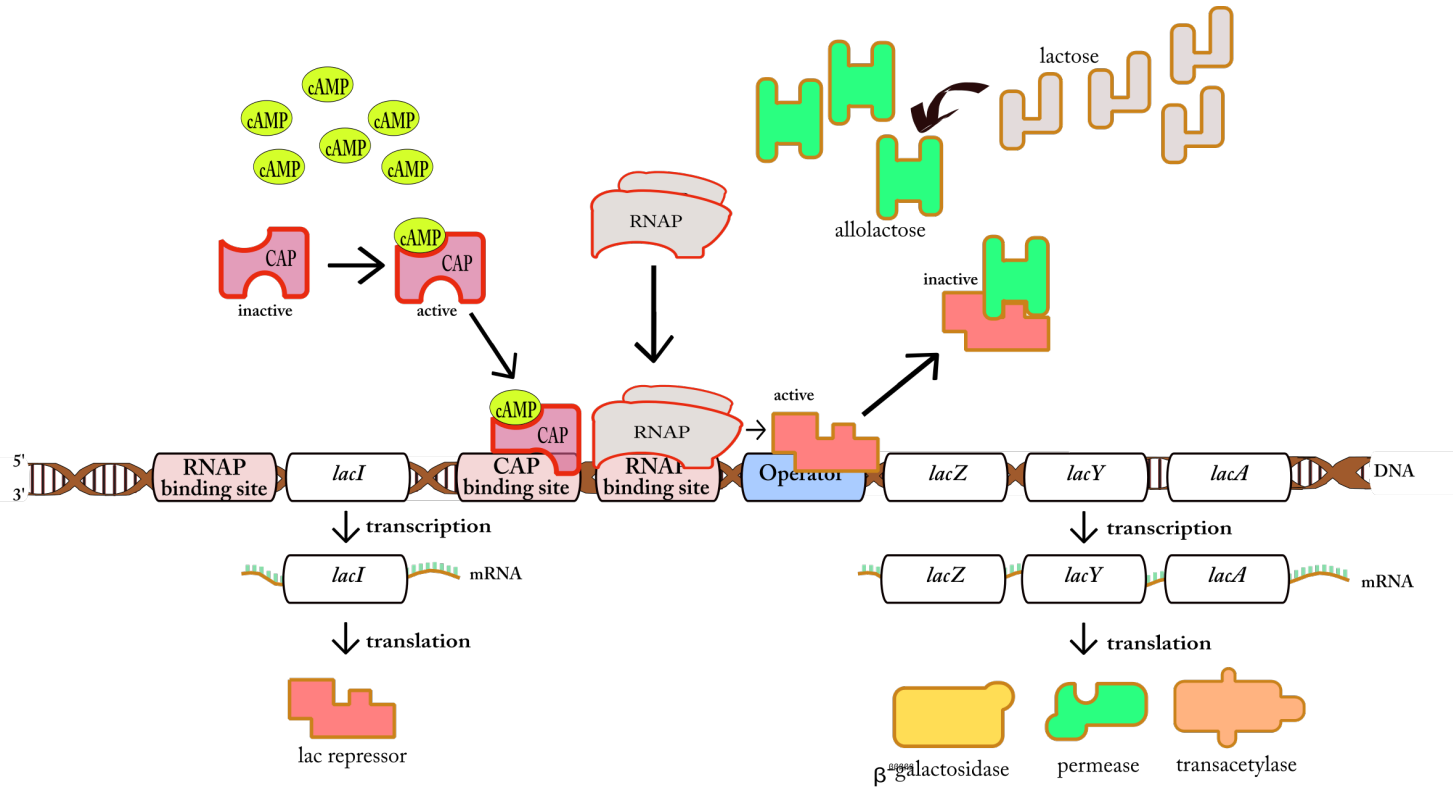

Figure 3: Regulation of the *lac* operon

As previously mentioned, glucose is the preferred energy source for cells because glucose doesn't require additional pre-processing to enter the glycolytic pathway, and because glycolysis enzymes are **constitutively** synthesized (made continuously at a steady rate). Glucose normally inhibits the production of cAMP; however, low levels of glucose allow the cell to produce cAMP, which is then able to bind to CAP, activating it. This enables the cell to metabolize lactose as an alternative energy source.

The two different regulatory proteins, the lac repressor and CAP, allow the lac operon to integrate two different signals: cellular lactose and cellular glucose levels. Further, the *lac* operon is only "ON" when two conditions have been met. The purpose of this module is to determine under what conditions the *lac* operon is "ON" (being transcribed).

<u>Table of Positive and Negative Regulators</u>

Regulation of Gene Expression in Prokaryotes with Feedback Loop

Nicholas Galt, Heather Bergan-Roller, Lisa Briona, Joseph Dauer, and Tomáš Helikar
University of Nebraska – Lincoln

Activity 1: Identify the components of the lac operon, and their regulators

The first step in building a computational model of the lac operon is to determine what you want the model to do. **The goal of your model is to demonstrate the conditions required for the *lac operon* to be active.** Specifically, the activity of the operon in response to environmental glucose and lactose. The components of the *lac* operon, the cell, and extracellular environment that you will need to include in your model are listed in the table below. Based on the background reading, identify the positive and/or negative regulators for each of the components and complete the table.

Component	Positive Regulators	Negative Regulators	Relationship
glucose	N/A	N/A	external component
lactose	N/A	N/A	external component
lac mRNA			
allolactose			
cAMP			
lac repressor			
CAP	cAMP		cAMP binds to and activates CAP
lac operon			
lactose breakdown			
lac enzymes			

Table 1: summary of *lac* operon components and regulators.

Supplemental Material: Clark, Helikar, Dauer. Simulating a computational biological model, rather than reading, elicits changes in brain activity

Regulation of Gene Expression in Prokaryotes with Feedback Loop

Nicholas Galt, Heather Bergan-Roller, Lisa Briona, Joseph Dauer, and Tomáš Helikar
University of Nebraska – Lincoln

Investigating the *lac* Operon System

The purpose of this section is to investigate how the external environment and intracellular (inside the cell) conditions influence the lac operon system. You will be using a published model of the lac operon system to conduct simulations to verify your own predictions about the dynamic interactions between the components of the model. The instructions below will get you set up to investigate the lac operon system.

Part I: Accessing the *lac* Operon Model

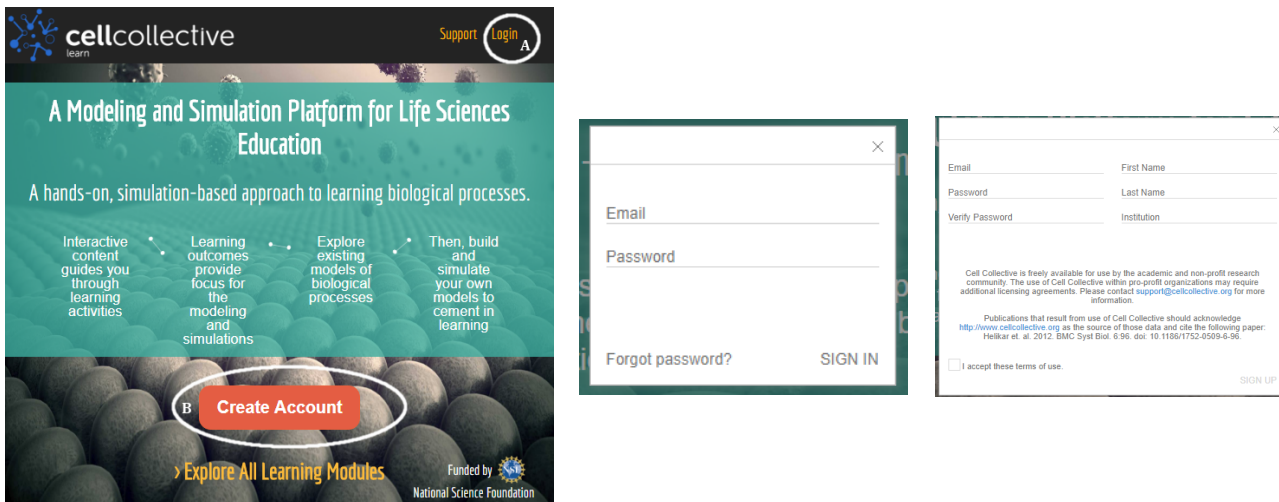### Step 1: Navigate to learn.cellcollective.org and login/signup

Figure 1: accessing Cell Collective Learn and logging in/signing up. Left: Login with an existing account (A) or create an account (B). Center: the login screen. Right: the signup screen.

In your web browser (Google Chrome, Mozilla Firefox or Apple Safari), go to the URL learn.cellcollective.org. If you already have an account with Cell Collective, select Login (Figure 1, Left, A). If you need to sign up for a new account, select "Create Account" (Figure 1, Left, B).

Step 2: Enter module "Regulation of the lac Operon with feedback"

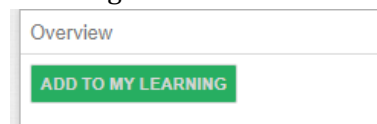Open the module "Regulation of the lac operon with feedback." Click on the tab, "Add to My Learning"

Figure 2: The "Add to My Learning" Button. Once you have added the module to "My Learning" and hover over it, it will be green.

Next, navigate home by pressing "Cell Collective Learn" words in orange at the top right.

Step 3: Enter module "Regulation of the lac Operon with feedback" through the My Learning Tab

Click on "My Learning" and click on the "Regulation of the lac operon with feedback" module to open it. Once you are in the Module, click on "Model" to leave the Overview tab.
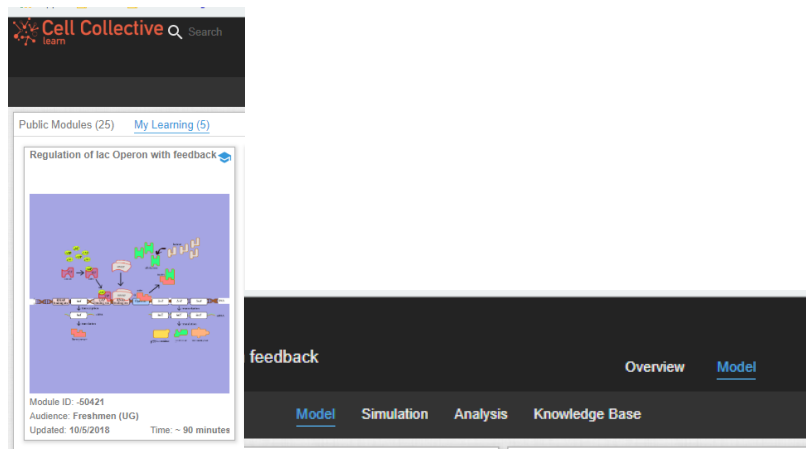
Figure 3: Left:The "Regulation of lac Operon with feedback" under the My Learning tab. If you are under My Learning, it will be underlined and highlighted in blue. Right: Click on 'Model' (highlighted and underlined) to leave the Overview section and access the lesson activities.

Step 4: Choosing the Simulation Tab
These analyses will be done through simulation. Choose the simulation tab (Figure 4, below).
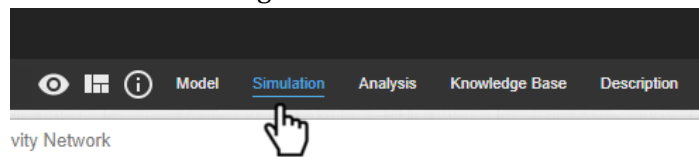
Figure 4: choosing the Simulation tab.

Step 5: Simulation Setup
1. In the SIMULATION CONTROL panel, set the Sliding Window size to 50 (Figure 5).

Figure 5: SIMULATION CONTROL panel settings.

2. In the EXTERNAL COMPONENTS panel, all components should be "off" (activity level = 0%). These will be changed in the following investigations to simulate various scenarios.
3. In the INTERNAL COMPONENTS panel, ensure that all components are visible (Figure 6).
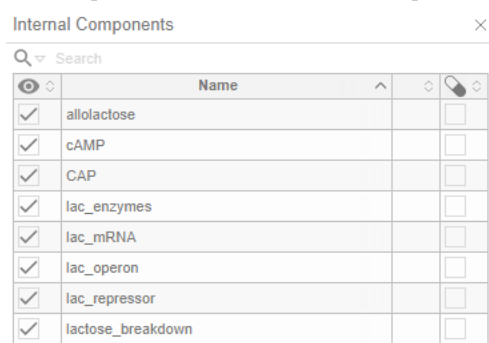
Figure 6: INTERNAL COMPONENTS panel settings.
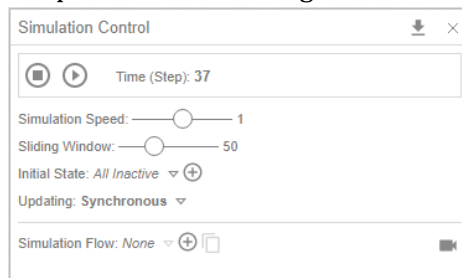
Part 2: Investigations

Investigation 1: Only lactose

One morning you're running late for class and only have time for a glass of milk for breakfast. As a result, the *E. coli* in your gut only have lactose (the sugar in milk) as an energy source. You will investigate how the presence of only lactose affects the lac operon system.

1. **Make a prediction:** When only lactose is available, the lac operon is _____. (circle one)
   a. On
   b. Off

2. **Support your prediction with a mechanism**: Predict HOW the presence of lactose affects the lac operon, making specific reference to components and their interactions. Refer to the Cell Collective model as necessary (1pt for a mechanistic answer. It doesn't have to be correct).

3. **Simulate the model**:
   a. In the window titled "EXTERNAL COMPONENTS:"
      i. Set "lactose" slider to 100.
      ii. Set "glucose" slider to 0.
      iii. Keep betaGal_LOF_mutation at 0.

21

        iv.   Keep CAP_binding_site_mutation at 0.
- b. Start the simulation by clicking on the play button under Simulation Control. Click the pause button after ~75 steps (shown on the x-axis).
- c. The activity of each component in the model can be observed in the SIMULATION GRAPH. To see specific components in the graph, hover the cursor over the component name in the key.

4. **Record the results**:

A.   Allolactose is ___ when only lactose is present.
- a. Present (activity level = 100)
- b. Absent (activity level = 0)

B.   The lac repressor is ___ when only lactose is present.
- a. Active
- b. Inactive

C.   The lac operon is ___ when only lactose is present.
- a. Expressed
- b. Not expressed

5. **Do your simulation results match your prediction?** (circle one)   Yes   No
If your prediction was not correct, continue to play with the simulation to understand the following:
- a) how simulation results translate to events inside the cell
- b) how the presence of only lactose affects the regulation of the lac operon

6. **Describe the mechanism correctly**: If your predicted mechanism is incorrect, fix it below and describe the mechanism correctly. Describe HOW the presence of lactose affects the *lac* operon. Be sure to describe all of the components and their interactions based on the Cell Collective model.

7. **What is the evolutionary significance of this mechanism?**

8. **WHY is the lac operon ON when lactose is present but turned OFF when lactose is absent?**

Investigation 2: Lactose and glucose
Another morning, you have plenty of time before class so you enjoy a bowl of cereal with marshmallows. As a result, the *E. coli* in your gut has both lactose from the milk and glucose from the marshmallows available in its environment. Investigate how the present of both lactose and glucose affects the lac operon system.
1. **Make a prediction**: When both glucose and lactose are available, the lac operon is _____. (circle one)
- a. On
- b. Off

2. **Support your prediction with a mechanism**: Predict HOW the presence of glucose and lactose affects the lac operon, making specific reference to components and their interactions. Refer to the Cell Collective model as necessary (1pt for a mechanistic answer; it doesn't have to be correct).

3. **Simulate the model**:
    d. In the window titled "EXTERNAL COMPONENTS:"
        i. Set "lactose" slider to 100.
        ii. Set "glucose" slider to 100.
        iii. Keep betaGal_LOF_mutation at 0.
        iv. Keep CAP_binding_site_mutation at 0.
    e. Start the simulation by clicking on the play button under Simulation Control. Click the pause button after ~75 steps (shown on the x-axis).
    f. The activity of each component in the model can be observed in the SIMULATION GRAPH. To see specific components in the graph, hover the cursor over the component name in the key.

4. **Record the results**:
    A. Allolactose is ____ in the cell when both glucose and lactose are present.
        a. Present (activity level = 100)
        b. Absent (activity level = 0)

    B. cAMP is ____ in the cell when both glucose and lactose are present.
        a. Present (activity level = 100)
        b. Absent (activity level = 0)

    C. CAP is ____ in the cell when both glucose and lactose are present.
        a. Active
        b. Inactive

    D. The lac repressor is ____ in the cell when both glucose and lactose are present.
        a. Active
        b. Inactive
    E. The lac operon is ____ in the cell when both glucose and lactose are present.
        a. "ON"
        b. "OFF"

5. **Do your simulation results match your prediction?** (circle one)   Yes   No
If your prediction was not correct, continue to play with the simulation to understand the following:
    a) how simulation results translate to events inside the cell
    b) how the presence of glucose and lactose affect the regulation of the lac operon

6. **Describe the mechanism correctly:** If your predicted mechanism is incorrect, fix it below and describe the mechanism correctly. Describe HOW the presence of glucose and lactose affects the *lac* operon. Be sure to describe all of the components and their interactions based on the Cell Collective model.

7. **What is the evolutionary significance of this mechanism?**

8. **WHY does the lac operon shut off when glucose is present, even if lactose is present?**

Investigation 3: CAP

For this investigation you will investigate the role of the catabolite activator protein (CAP). When active, CAP binds to the CAP binding site in the promoter of the *lac* operon.

1. **Make a prediction**: In a situation where the lac operon would normally be ON (lactose present, glucose absent), predict how the activity of the lac operon and lactose breakdown would be affected if a cell acquired a deleterious mutation in the CAP binding site so that active CAP can no longer bind the operator in the lac operon.

    A. How would this mutation affect the lac operon? Lactose is present and glucose is absent but the lac operator is mutated so CAP cannot bind the operator. (circle one)
        a. The lac operon would always be "ON"
        b. The lac operon would always be "OFF"

    B. How would this mutation affect lactose breakdown? Lactose is present and glucose is absent but the lac operator is mutated so CAP cannot bind the operator. (circle one)
        a. Lactose breakdown would be more active with the mutation
        b. Lactose breakdown would not change with the mutation
        c. Lactose breakdown would not occur with the mutation

2. Support your prediction with a mechanism: Predict HOW a mutated CAP that cannot bind the operator affects lac operon activity and lactose breakdown when lactose is present and glucose is absent, making specific reference to components and their interactions. Refer to the Cell Collective model as necessary (1pt for a mechanistic answer; it doesn't have to be correct).

3. **Simulate the model**:
    a. In the window titled "EXTERNAL COMPONENTS:"
        i. Set "lactose" slider to 100.
        ii. Set "glucose" slider to 0.
        iii. Keep betaGal_LOF_mutation at 0.
        iv. Set CAP_binding_site_mutation to 100.
    b. Start the simulation by clicking on the play button under Simulation Control. Click the pause button after ~75 steps (shown on the x-axis).
    c. The activity of each component in the model can be observed in the SIMULATION GRAPH. To see specific components in the graph, hover the cursor over the component name in the key.

4. **Record the results**:
    A. CAP is ___ in the cell when lactose is present, glucose is absent, and the lac operator is mutated so CAP cannot bind the operator.
        a. In its active configuration

24

   b. In its inactive form

   B. The lac operon is ___ in the cell when lactose is present, glucose is absent, and the lac operator is mutated so CAP cannot bind the operator.
      a. "ON"
      b. "OFF"

   C. The lac mRNA is ___ in the cell when lactose is present, glucose is absent, and the lac operator is mutated so CAP cannot bind the operator.
      a. Present (activity level = 100)
      b. Absent (activity level = 0)

   D. The lac enzymes are ___ in the cell when lactose is present, glucose is absent, and the lac operator is mutated so CAP cannot bind the operator.
      a. Present (activity level = 100)
      b. Absent (activity level = 0)

   E. Lactose breakdown is ___ in the cell when lactose is present, glucose is absent, and the lac operator is mutated so CAP cannot bind the operator.
      a. Occurs
      b. Does not occur

5. **Do your simulation results match your prediction?** (circle one)   Yes   No
   If your prediction was not correct, continue to play with the simulation to understand the following:
      a) how simulation results translate to events inside the cell
      b) what role CAP plays in regulating the lac operon

6. **Describe the mechanism correctly**: If your predicted mechanism is incorrect, fix it below and describe the mechanism correctly. Describe HOW a mutated CAP that cannot bind the operator affects lac operon activity and lactose breakdown when lactose is present and glucose is absent. Be sure to describe all of the components and their interactions based on the Cell Collective model.

7. **How would a colony of *E. coli* with this mutation survive in a dairy barn?**

8.   **What role does CAP play in regulating the *lac* operon?**

Investigation 4: Lac Z
For this investigation, you will be investigating the lacZ gene product. The lacZ gene is part of the *lac* operon and codes for the enzyme β-galactosidase, the enzyme responsible for breaking down lactose into glucose and galactose.

1. **Make a prediction**: In a situation where the lac operon would normally be ON (lactose present, glucose absent), predict how the activity of the lac operon and lactose breakdown would be affected if a cell acquires a loss-of-function ("LOF") mutation that makes the lacZ gene product β-galactosidase nonfunctional.

   A. How would this mutation affect the lac operon? Lactose is present, glucose is absent and the lacZ gene is mutated so that β-galactosidase is nonfunctional. (circle one)
      a. The lac operon would be ON
      b. The lac operon would be OFF

   B. How would this mutation affect expression of the lacZ gene? Lactose is present, glucose is absent and the lacZ gene is mutated so that β-galactosidase is nonfunctional. (circle one)
      a. The lacZ gene would be expressed into its enzyme
      b. The lacZ gene would NOT be expressed into its enzyme

   C. How would this mutation affect lactose breakdown? Lactose is present, glucose is absent and the lacZ gene is mutated so that β-galactosidase is nonfunctional. (circle one)
      a. Lactose breakdown would be more active with the mutation
      b. Lactose breakdown would not change with the mutation
      c. Lactose breakdown would not occur with the mutation

2. **Support your prediction with a mechanism**: Predict HOW a nonfunctional lacZ gene product (β-galactosidase) affects *lac* operon activity and lactose breakdown when lactose is present and glucose is absent, making specific reference to components and their interactions. Refer to the Cell Collective model as necessary (1pt for a mechanistic answer; it doesn't have to be correct).

3. **Simulate the model**:
   a. In the window titled "EXTERNAL COMPONENTS:"
      i. Set "lactose" slider to 100.
      ii. Set "glucose" slider to 0.
      iii. Set betaGal_LOF_mutation to 100.
      iv. Set CAP_binding_site_mutation to 0.
   b. Start the simulation by clicking on the play button under Simulation Control. Click the pause button after ~75 steps (shown on the x-axis).
   c. The activity of each component in the model can be observed in the SIMULATION GRAPH. To see specific components in the graph, hover the cursor over the component name in the key.

4. **Record the results**: Interpret the simulation results to determine the state of the model components when lactose is present, glucose is absent, and lacZ gene is mutated so that its gene product β-galactosidase is nonfunctional.
   A. The lac operon is ___ in the cell when lactose is present, glucose is absent, and lacZ gene is mutated so that its gene product β-galactosidase is nonfunctional.
      a. "ON"
      b. "OFF"

B. The lac mRNA is ___ in the cell when lactose is present, glucose is absent, and lacZ gene is mutated so that its gene product β-galactosidase is nonfunctional.
   a. Present (activity level = 100)
   b. Absent (activity level = 0)

C. The lac enzymes are ___ in the cell when lactose is present, glucose is absent, and lacZ gene is mutated so that its gene product β-galactosidase is nonfunctional.
   a. Present (activity level = 100)
   b. Absent (activity level = 0)

D. Lactose breakdown is ___ in the cell when lactose is present, glucose is absent, and lacZ gene is mutated so that its gene product β-galactosidase is nonfunctional.
   a. Occurs
   b. Does not occur

5. **Do your simulation results match your prediction?** (circle one)   Yes   No
   If your prediction was not correct, continue to play with the simulation to understand the
   Following:
   a) how simulation results translate to events inside the cell
   b) the role lacZ plays in lactose breakdown

6. **Describe the mechanism correctly**: If your predicted mechanism is incorrect, fix it below and describe the mechanism correctly. Describe HOW a nonfunctional lacZ gene product (β-galactosidase) affects *lac* operon activity and lactose breakdown when lactose is present and glucose is absent. Be sure to describe all of the components and their interactions based on the Cell Collective model.

7.   In the presence of lactose and the absence of glucose, a mutation in the CAP binding site described in Investigation 3 has the same phenotype as the mutation in the lacZ gene described here. How are the mechanisms different?

Investigation 5: Feedback loop
Regulation of the lac operon is a little more complicated than shown thus far; there are also positive and negative feedback loops that allow for more tightly regulated lac operon gene expression. We will investigate one mode of feedback using the model and simulation "fMRI - Regulation of the Lac Operon".

To navigate to this module, go to cellcollective.org. The top left corner will say "Cell Collective Research." Search for fMRI. This will bring up a panel that looks like Fig. 7.

Supplemental Material: Clark, Helikar, Dauer. Simulating a computational biological model, rather than reading, elicits changes in brain activity
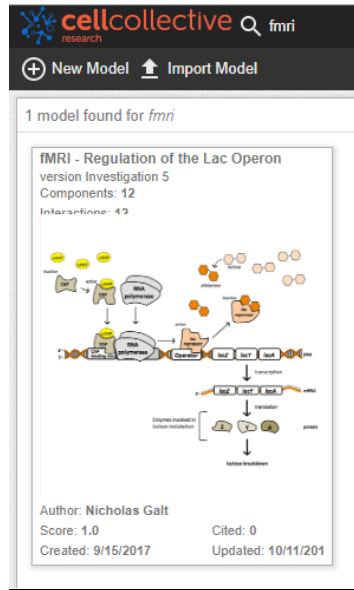

Figure 7. Screenshot of module used in Investigation 5.

Click on the title, "fMRI - Regulation of the Lac Operon" to open the module. Then click on the simulation tab (Fig 8). This will allow you to begin with the investigation.
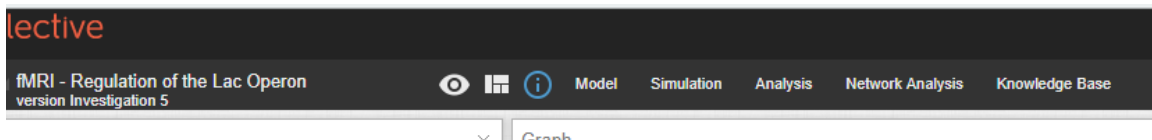

Figure 8. Screenshot of headings under the fMRI - Regulation of the Lac Operon module. Click on the 'Simulation' tab to access the simulations.

1. Make a prediction: Recall that high levels of intracellular glucose inhibits cAMP production. Also, in the presence of lactose and the absence of glucose, the lac operon would normally be "ON."
    A. In this situation (lactose is present and glucose is absent), how would lactose breakdown affect cAMP levels in the cell? (circle one)
        a. cAMP levels will remain steady in response to lactose breakdown
        b. cAMP levels will increase in response to lactose breakdown
        c. cAMP levels will decrease in response to lactose breakdown
        d. cAMP levels will fluctuate in response to lactose breakdown

    B. In this situation, how would lactose breakdown affect CAP activity in the cell? (circle one)
        a. CAP will remain active
        b. CAP will become inactive
        c. CAP activity will fluctuate

    C. In this situation, how would lactose breakdown affect lac operon mRNA levels? (circle one)
        a. mRNA levels will remain steady in response to lactose breakdown
        b. mRNA levels will increase in response to lactose breakdown

      c.   mRNA levels will decrease in response to lactose breakdown

      d.   mRNA levels will fluctuate in response to lactose breakdown

2. Support your prediction with a mechanism: Predict HOW lactose breakdown affects cAMP levels, CAP activity, and *lac* operon mRNA levels. Be sure to describe all their components and their interactions; refer to the v1.1 simulation model if necessary. (1pt for a mechanistic answer; it doesn't have to be correct).

3. **Simulate the model:**
   a. In the window titled "Eχτεrnal Components:"
      i. Set "lactose" slider to 100.
      ii. Set "glucose" slider to 0.
      iii. Set betaGal_LOF_mutation to 0.
      iv. Set CAP_binding_site_mutation to 0.
   b. Start the simulation by clicking on the play button under Simulation Control. Click the pause button after ~75 steps (shown on the x-axis).
   c. The activity of each component in the model can be observed in the Simulation Graph. To see specific components in the graph, hover the cursor over the component name in the key.

4. **Record the results**. When lactose is present and glucose is absent, and we include in our model the additional complexity that glucose inhibits cAMP production:
   A. Allolactose levels _____
      a. Rise then level out, remaining steady
      b. Fluctuate regularly
      c. Initially start out high, but fall rapidly to 0

   B. cAMP levels _____
      a. Rise then level out, remaining steady
      b. Fluctuate at the same regular rate (peaks and valleys are consistent)
      c. Fluctuate at reducing levels, eventually reaching a regular rate of fluctuation (attenuation)

   C. CAP activity _____
      a. Fluctuates at the same regular rate (peaks and valleys are consistent)
      b. Fluctuates at reducing levels, eventually reaching a regular rate of fluctuation (attenuation)
      c. Remains unchanged even with this additional complexity included

   D. The lac repressor activity _____
      a. Decreases to 0 (becomes inactive) in response to allolactose levels
      b. Fluctuates at a regular rate (binds the operator then falls off, binds the operator then falls off, ...)
      c. Remains active but at a low activity level

   E. mRNA levels _____
      a. Fluctuates at the same regular rate (peaks and valleys are consistent)
      b. Fluctuate at reducing levels, eventually reaching a regular rate of fluctuation (attenuation)

        c.   Remains unchanged even with this additional complexity included

5. **Does your simulation results match your prediction?** (circle one) Yes   No
   If your prediction was incorrect, continue to play with the simulation to understand the following:
   a) How simulation results translate to events inside the cell
   b) What role lactose breakdown has on regulation of the lac operon

6. Describe the mechanism correctly: If your predicted mechanism is incorrect, fix it below and describe the mechanism correctly. HOW does lactose breakdown affect the various components of the *lac* operon and its regulatory elements? Be sure to describe all of the components and their interactions based on the Cell Collective model.

7. Is this an example of positive or negative feedback? Justify your answer.

8. Why does this feedback occur – what is the evolutionary advantage to the effects lactose breakdown causes in the cell?

Supplemental Material: Clark, Helikar, Dauer. Simulating a computational biological model, rather than reading, elicits changes in brain activity

<u>Read Version</u>

Regulation of Gene Expression in Prokaryotes with Feedback Loop

Nicholas Galt, Heather Bergan-Roller, Lisa Briona, Joseph Dauer, and Tomáš Helikar
University of Nebraska – Lincoln

Investigating the *lac* Operon System

The purpose of this section is to investigate how the external environment and intracellular (inside the cell) conditions influence the lac operon system. You will be using a published model of the lac operon system to conduct simulations to verify your own predictions about the dynamic interactions between the components of the model. The instructions below will get you set up to investigate the lac operon system.

Investigation 1: Only lactose

One morning you're running late for class and only have time for a glass of milk for breakfast. As a result, the *E. coli* in your gut only have lactose (the sugar in milk) as an energy source. You will investigate how the presence of only lactose affects the lac operon system.

1. **Make a prediction:** When only lactose is available, the lac operon is _____. (circle one)
   a. **On**
   b. Off

2. **Support your prediction with a mechanism**: Predict HOW the presence of lactose affects the lac operon, making specific reference to components and their interactions. Refer to the Cell Collective model as necessary (1pt for a mechanistic answer. It doesn't have to be correct).

   Lactose →allolactose ⊣ lac repressor ⊣ *lac* operon

   When lactose enters the E. coli cell, some of it is isomerized into the inducer allolactose. Allolactose binds lac repressor, triggering a conformational change that inactivates lac repressor. Inactive lac repressor can no longer repress expression of the *lac* operon, so you get *lac* mRNA, *lac* enzymes, and ultimately lactose breakdown.
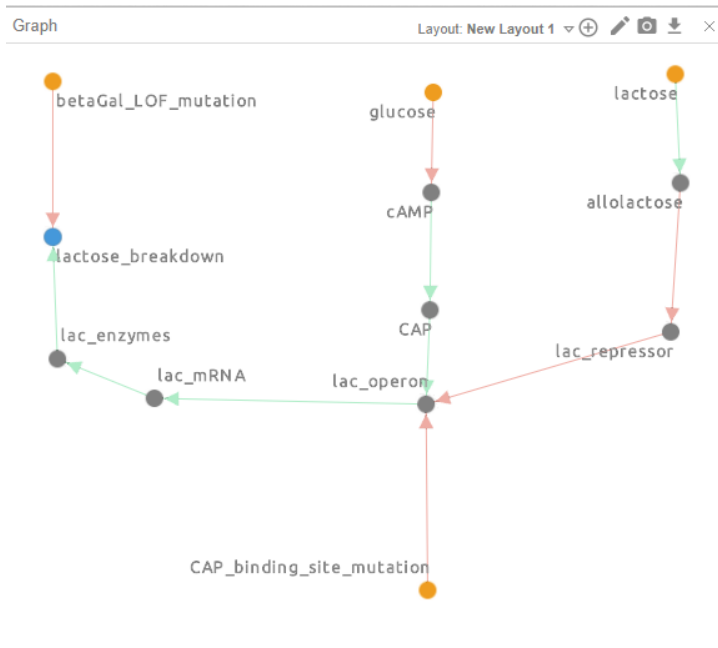
3. Simulate the model

Figure 1: lac operon model for Investigations 1 - 4.
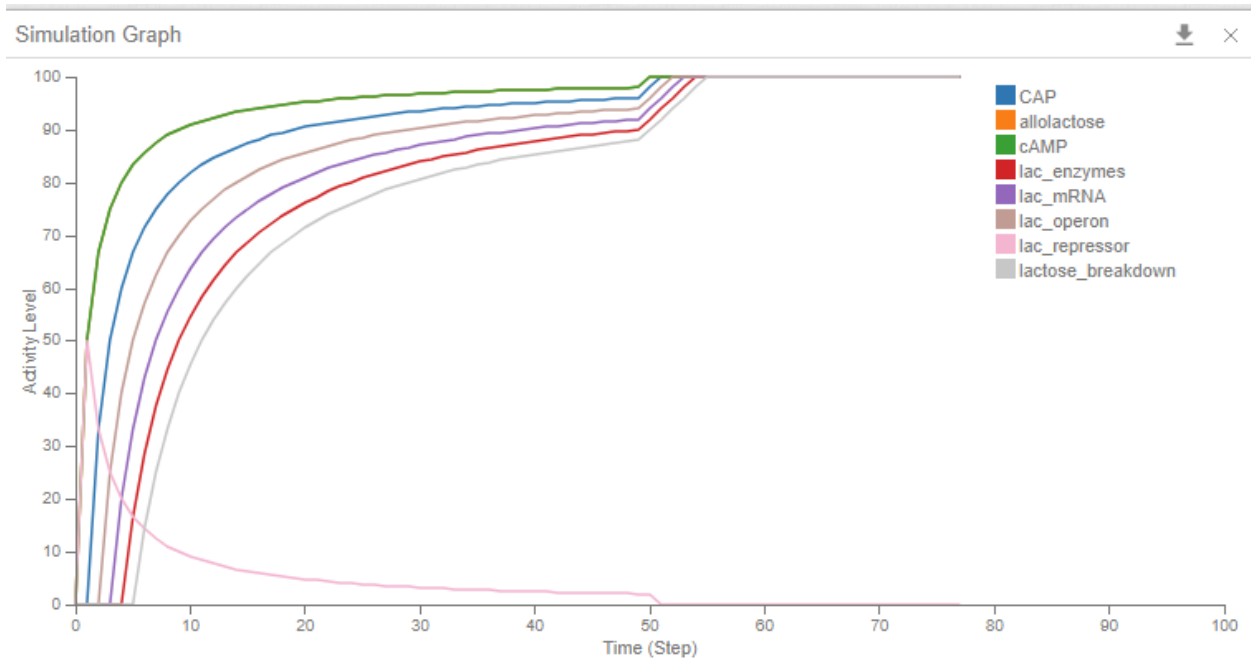

Figure 2: "Only lactose" simulation results.

4. **Record the results**:

    A.   Allolactose is ____ when only lactose is present.

            a.   Present (activity level = 100)

            b.   Absent (activity level = 0)

      B.   The lac repressor is ___ when only lactose is present.
- a. Active
- **b. Inactive**

      C.   The lac operon is ___ when only lactose is present.
- **a. Expressed**
- b. Not expressed

5. **Do your simulation results match your prediction?** (circle one)   **Yes**   No

If your prediction was not correct, continue to play with the simulation to understand the following:
- a) how simulation results translate to events inside the cell
- b) how the presence of only lactose affects the regulation of the lac operon

6. **Describe the mechanism correctly**: If your predicted mechanism is incorrect, fix it below and describe the mechanism correctly. Describe HOW the presence of lactose affects the *lac* operon. Be sure to describe all of the components and their interactions based on the Cell Collective model.

Refer to #2

7. **What is the evolutionary significance of this mechanism?**

Making protein is energetically expensive. By making the enzymes capable of metabolizing lactose only when lactose is present allows the cell to only make proteins when they are required.

8. **WHY is the lac operon ON when lactose is present but turned OFF when lactose is absent?**

Without lactose to make allolactose, lac repressor is active. Active lac repressor binds the operator preventing transcription: the default state of lac operon expression is "off."

Investigation 2: Lactose and glucose

Another morning, you have plenty of time before class so you enjoy a bowl of cereal with marshmallows. As a result, the *E. coli* in your gut has both lactose from the milk and glucose from the marshmallows available in its environment. Investigate how the present of both lactose and glucose affects the lac operon system.

    1. **Make a prediction**: When both glucose and lactose are available, the lac operon is _____. (circle one)
- a. On
- **b. Off**

    2. **Support your prediction with a mechanism**: Predict HOW the presence of glucose and lactose affects the lac operon, making specific reference to components and their interactions. Refer to the Cell Collective model as necessary (1pt for a mechanistic answer; it doesn't have to be correct).

            Lactose →allolactose ⊣ lac repressor ⊣ *lac* operon

Glucose ⊣ cAMP → CAP → *lac* operon

As observed in the "lactose only" scenario, some lactose is isomerized into the inducer allolactose. Allolactose is able to bind lac repressor, inactivating it. Inactivating lac repressor prevents it from binding to the operator: if this was the only level of regulation, then transcription could now proceed. But the *lac* operon has two levels of transcriptional control, not just one. In addition to needing the repressor removed from the operator, it also requires the activator CAP to bind to the CAP binding site before *lac* operon transcription can occur. Only activated CAP can bind to the CAP binding site. CAP is activated by forming a complex with cAMP. cAMP production is inhibited by glucose. Therefore, even with lactose available, if glucose is present the *lac* operon will not be transcribed because CAP cannot be activated.
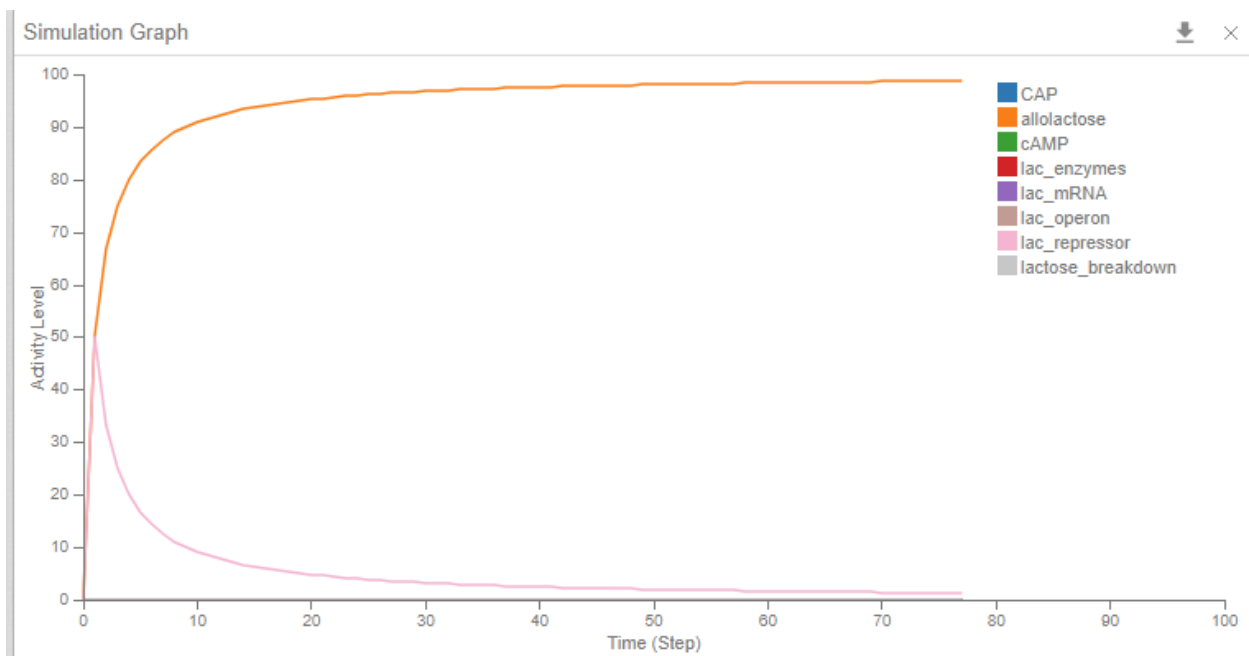
3. **Simulate the model**:

Figure 3: "lactose and glucose" simulation results.

4. **Record the results**:
   A. Allolactose is ____ in the cell when both glucose and lactose are present.
      a. **Present (activity level = 100)**
      b. Absent (activity level = 0)

   B. cAMP is ____ in the cell when both glucose and lactose are present.
      a. Present (activity level = 100)
      b. **Absent (activity level = 0)**

   C. CAP is ____ in the cell when both glucose and lactose are present.
      a. Active
      b. **Inactive**

   D. The lac repressor is ___ in the cell when both glucose and lactose are present.
     a. Active
     b. **Inactive**

   E. The lac operon is ___ in the cell when both glucose and lactose are present.
     a. "ON"
     b. **"OFF"**

5. **Do your simulation results match your prediction?** (circle one)   **Yes** No
If your prediction was not correct, continue to play with the simulation to understand the following:
 a) how simulation results translate to events inside the cell
 b) how the presence of glucose and lactose affect the regulation of the lac operon

6. **Describe the mechanism correctly:** If your predicted mechanism is incorrect, fix it below and describe the mechanism correctly. Describe HOW the presence of glucose and lactose affects the *lac* operon. Be sure to describe all of the components and their interactions based on the Cell Collective model.

<div align="center">**See #2**</div>

7. **What is the evolutionary significance of this mechanism?**

Glucose doesn't require pre-processing before entering glycolysis the way lactose does. If glucose is available, the cell will use glucose to the exclusion of other food sources to conserve energy.

8. **WHY does the lac operon shut off when glucose is present, even if lactose is present?**

E. coli preferentially uses glucose as an energy source, so it will use lactose (and express the lac operon to process lactose) only if glucose is not available.

Investigation 3: CAP

For this investigation you will investigate the role of the catabolite activator protein (CAP). When active, CAP binds to the CAP binding site in the promoter of the *lac* operon.

1. **Make a prediction**: In a situation where the lac operon would normally be ON (lactose present, glucose absent), predict how the activity of the lac operon and lactose breakdown would be affected if a cell acquired a deleterious mutation in the CAP binding site so that active CAP can no longer bind the operator in the lac operon.

   A. How would this mutation affect the lac operon? Lactose is present and glucose is absent but the lac operator is mutated so CAP cannot bind the operator. (circle one)
      a. The lac operon would always be "ON"
      b. **The lac operon would always be "OFF"**

   B. How would this mutation affect lactose breakdown? Lactose is present and glucose is absent but the lac operator is mutated so CAP cannot bind the operator. (circle one)
      a. Lactose breakdown would be more active with the mutation
      b. Lactose breakdown would not change with the mutation
      c. **Lactose breakdown would not occur with the mutation**

2. Support your prediction with a mechanism: Predict HOW a mutated CAP that cannot bind the operator affects lac operon activity and lactose breakdown when lactose is present and glucose is absent, making specific reference to components and their interactions. Refer to the Cell Collective model as necessary (1pt for a mechanistic answer; it doesn't have to be correct).

   No glucose → ↑ cAMP levels → activated mutated CAP → since CAP can't bind CAP binding site, there's no lacZ transcription → no lacZ transcription means no β-galactosidase → no β-galactosidase means no lactose breakdown
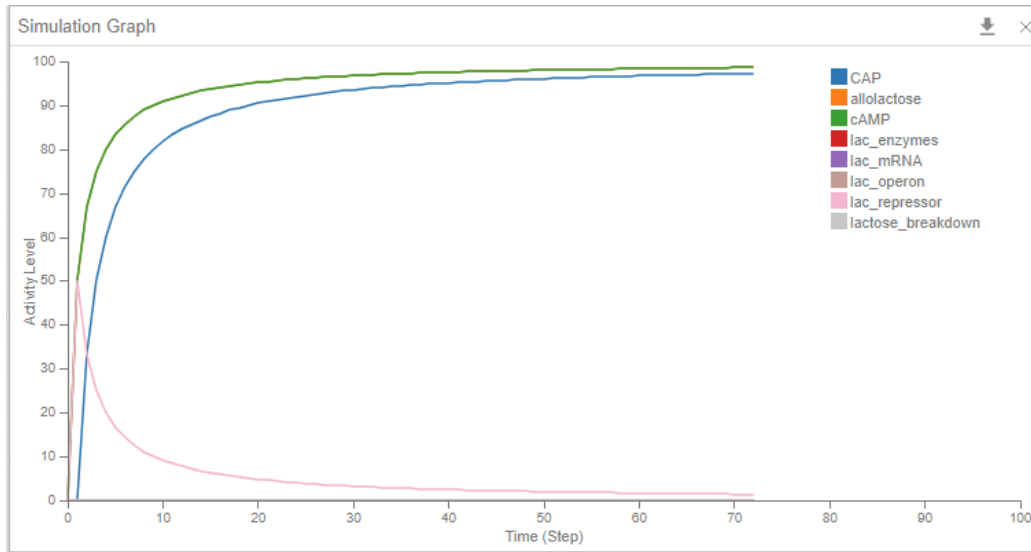
3. **Simulate the model**:

Figure 4: "lactose only with CAP_binding_site_mutation" simulation results.

3. **Record the results**:
   A. CAP is ___ in the cell when lactose is present, glucose is absent, and the lac operator is mutated so CAP cannot bind the operator.
      a. **In its active configuration**
      b. In its inactive form

   B. The lac operon is ___ in the cell when lactose is present, glucose is absent, and the lac operator is mutated so CAP cannot bind the operator.
      a. "ON"
      b. **"OFF"**

   C. The lac mRNA is ___ in the cell when lactose is present, glucose is absent, and the lac operator is mutated so CAP cannot bind the operator.
      a. Present (activity level = 100)
      b. **Absent (activity level = 0)**

   D. The lac enzymes are ___ in the cell when lactose is present, glucose is absent, and the lac operator is mutated so CAP cannot bind the operator.
      a. Present (activity level = 100)
      b. **Absent (activity level = 0)**

   E. Lactose breakdown is ___ in the cell when lactose is present, glucose is absent, and the lac operator is mutated so CAP cannot bind the operator.
      a. Occurs
      b. **Does not occur**

5. **Do your simulation results match your prediction?** (circle one)   **Yes**   No

37

If your prediction was not correct, continue to play with the simulation to understand the following:
   a) how simulation results translate to events inside the cell
   b) what role CAP plays in regulating the lac operon

6. **Describe the mechanism correctly**: If your predicted mechanism is incorrect, fix it below and describe the mechanism correctly. Describe HOW a mutated CAP that cannot bind the operator affects lac operon activity and lactose breakdown when lactose is present and glucose is absent. Be sure to describe all of the components and their interactions based on the Cell Collective model.

<mark>See #2</mark>

7. **How would a colony of *E. coli* with this mutation survive in a dairy barn?**

<mark>While lactose is clearly the most common sugar in a dairy barn, it is not the only sugar: sucrose can be found in spilled coffee drops from a dairy worker's morning cup o' joe; glucose can be found in the cud or sputum from a cow's meal; other sugars are available in the bodily excretions from other dairy barn residents such as cats and mice. A colony of E. coli unable to process lactose would still survive in the dairy barn environment.</mark>

8. **What role does CAP play in regulating the *lac* operon?**

<mark>Activated CAP is the *lac* operon activator: it recruits RNAP to the lac promoter, facilitating transcription. RNAP by itself has very low affinity for the lac operon promoter; activated CAP increases RNAP's affinity.</mark>

Investigation 4: Lac Z
For this investigation, you will be investigating the lacZ gene product. The lacZ gene is part of the *lac* operon and codes for the enzyme β-galactosidase, the enzyme responsible for breaking down lactose into glucose and galactose.
1. **Make a prediction**: In a situation where the lac operon would normally be ON (lactose present, glucose absent), predict how the activity of the lac operon and lactose breakdown would be affected if a cell acquires a loss-of-function ("LOF") mutation that makes the lacZ gene product β-galactosidase nonfunctional.
   A. How would this mutation affect the lac operon? Lactose is present, glucose is absent and the lacZ gene is mutated so that β-galactosidase is nonfunctional. (circle one)
      a. <mark>**The lac operon would be ON**</mark>
      b. The lac operon would be OFF

   B. How would this mutation affect expression of the lacZ gene? Lactose is present, glucose is absent and the lacZ gene is mutated so that β-galactosidase is nonfunctional. (circle one)
      a. <mark>**The lacZ gene would be expressed into its enzyme**</mark>
      b. The lacZ gene would NOT be expressed into its enzyme

    C. How would this mutation affect lactose breakdown? Lactose is present, glucose is absent and the lacZ gene is mutated so that β-galactosidase is nonfunctional. (circle one)
   - a. Lactose breakdown would be more active with the mutation
   - b. Lactose breakdown would not change with the mutation
   - c. **Lactose breakdown would not occur with the mutation**

2. **Support your prediction with a mechanism**: Predict HOW a nonfunctional lacZ gene product (β-galactosidase) affects *lac* operon activity and lactose breakdown when lactose is present and glucose is absent, making specific reference to components and their interactions. Refer to the Cell Collective model as necessary (1pt for a mechanistic answer; it doesn't have to be correct).

    Lactose still enters cell → allolactose still formed → lac repressor still inhibited → *lac* operon still expressed → nonfunctional β-galactosidase synthesized → lactose is not broken down
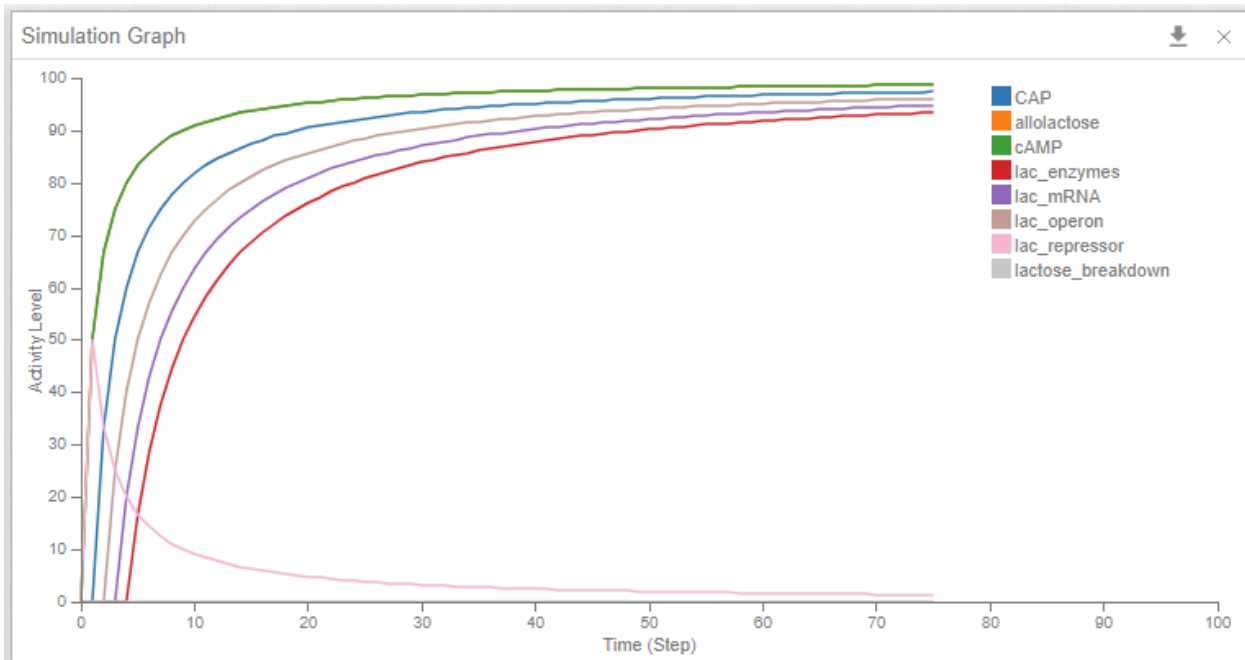
3. **Simulate the model**:

Figure 5: "lactose-only and betaGal_LOF_mutation" simulation results

4. **Record the results**: Interpret the simulation results to determine the state of the model components when lactose is present, glucose is absent, and lacZ gene is mutated so that its gene product β-galactosidase is nonfunctional.
   - A. The lac operon is ____ in the cell when lactose is present, glucose is absent, and lacZ gene is mutated so that its gene product β-galactosidase is nonfunctional.
      - a. **"ON"**
      - b. "OFF"

    B. The lac mRNA is ___ in the cell when lactose is present, glucose is absent, and lacZ gene is mutated so that its gene product β-galactosidase is nonfunctional.
- a. **Present (activity level = 100)**
- b. Absent (activity level = 0)

    C. The lac enzymes are ___ in the cell when lactose is present, glucose is absent, and lacZ gene is mutated so that its gene product β-galactosidase is nonfunctional.
- a. **Present (activity level = 100)**
- b. Absent (activity level = 0)

    D. Lactose breakdown is ___ in the cell when lactose is present, glucose is absent, and lacZ gene is mutated so that its gene product β-galactosidase is nonfunctional.
- a. Occurs
- b. **Does not occur**

5. **Do your simulation results match your prediction?** (circle one)   **Yes**   No
   If your prediction was not correct, continue to play with the simulation to understand the
   Following:
   a) how simulation results translate to events inside the cell
   b) the role lacZ plays in lactose breakdown

6. **Describe the mechanism correctly**: If your predicted mechanism is incorrect, fix it below and describe the mechanism correctly. Describe HOW a nonfunctional lacZ gene product (β-galactosidase) affects *lac* operon activity and lactose breakdown when lactose is present and glucose is absent. Be sure to describe all of the components and their interactions based on the Cell Collective model.

<div align="center">**See #2**</div>

7. **In the presence of lactose and the absence of glucose, a mutation in the CAP binding site described in Investigation 3 has the same phenotype as the mutation in the lacZ gene described here. How are the mechanisms different?**

   Both mutations have an end phenotype of "unable to metabolize lactose." The mutation in the CAP binding site prevents the activator from binding, meaning that lac operon transcription cannot occur in the first place. The lacZ mutation is further downstream: the lac operon is transcribed, but the lacZ gene product is non functional.

Investigation 5: Feedback loop
Regulation of the lac operon is a little more complicated than shown thus far; there are also positive and negative feedback loops that allow for more tightly regulated lac operon gene expression. We will investigate one mode of feedback using the model and simulation "Regulation of the Lac Operon version 1.1 Investigation 5."
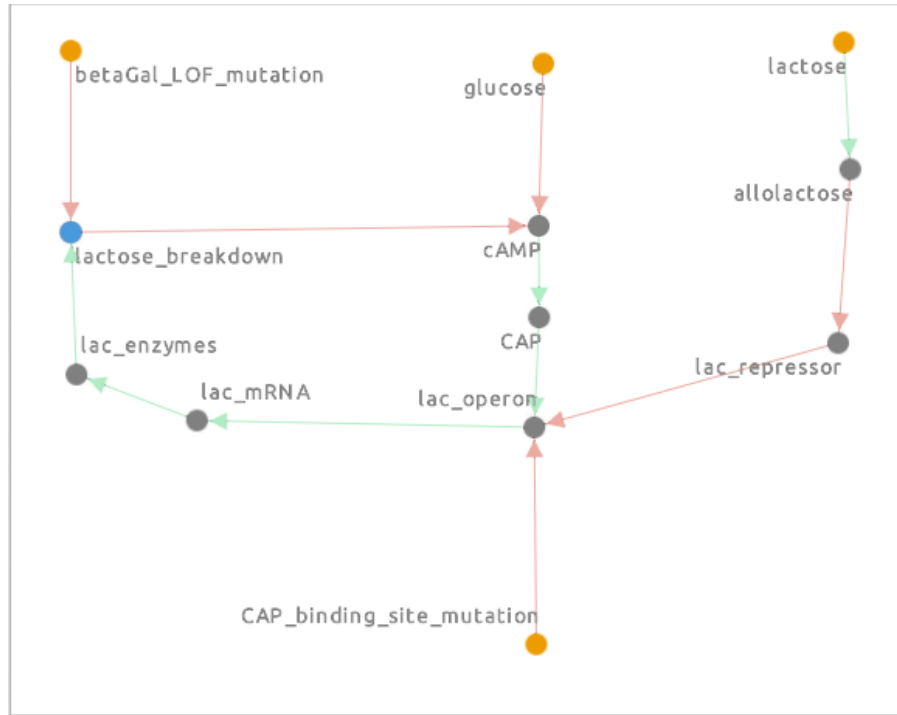
Figure 6: lac Operon model with feedback loop

1. Make a prediction: Recall that high levels of intracellular glucose inhibits cAMP production. Also, in the presence of lactose and the absence of glucose, the lac operon would normally be "ON."
   A. In this situation (lactose is present and glucose is absent), how would lactose breakdown affect cAMP levels in the cell? (circle one)
      a. cAMP levels will remain steady in response to lactose breakdown
      b. cAMP levels will increase in response to lactose breakdown
      c. cAMP levels will decrease in response to lactose breakdown
      d. **cAMP levels will fluctuate in response to lactose breakdown**

   B. In this situation, how would lactose breakdown affect CAP activity in the cell? (circle one)
      a. CAP will remain active
      b. CAP will become inactive
      c. **CAP activity will fluctuate**

   C. In this situation, how would lactose breakdown affect lac operon mRNA levels? (circle one)
      a. mRNA levels will remain steady in response to lactose breakdown
      b. mRNA levels will increase in response to lactose breakdown
      c. mRNA levels will decrease in response to lactose breakdown
      d. **mRNA levels will fluctuate in response to lactose breakdown**

2. Support your prediction with a mechanism: Predict HOW lactose breakdown affects cAMP levels, CAP activity, and *lac* operon mRNA levels. Be sure to describe all their components and their interactions; refer

to the v1.1 simulation model if necessary. (1pt for a mechanistic answer; it doesn't have to be correct).

==Lactose is broken down into glucose and galactose by β-galactosidase. This lactose-derived glucose increases intracellular levels of glucose. High levels of glucose inhibit cAMP production; CAP will not be able to bind to the CAP binding site so mRNA production will decrease. The cell preferentially uses glucose however, so as the glucose is processed intracellular glucose levels fall, allowing cAMP production and CAP activation. This in turn increases lactose import and processing, continuing the cyclic fluctuations as long as lactose is the dominant energy source.==
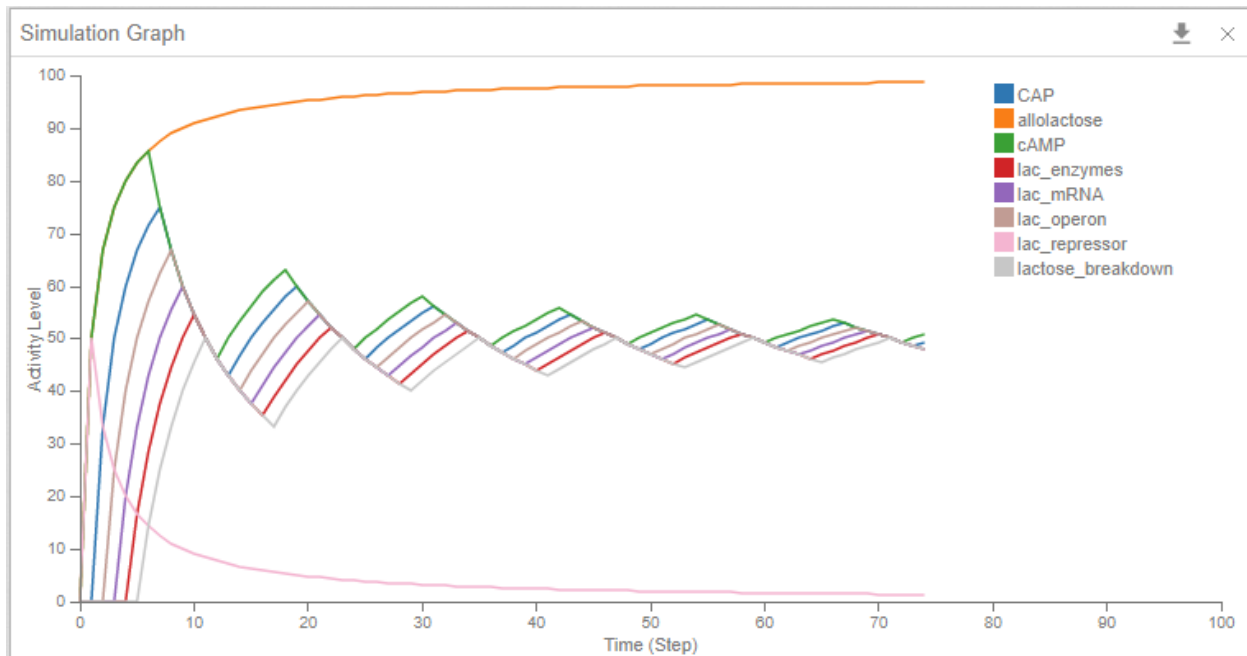
3. **Simulate the model:**

Figure 7: "lac operon with feedback loop" simulation results

4. **Record the results**. When lactose is present and glucose is absent, and we include in our model the additional complexity that glucose inhibits cAMP production:
    A. Allolactose levels _____
        a. **Rise then level out, remaining steady**
        b. Fluctuate regularly
        c. Initially start out high, but fall rapidly to 0

    B. cAMP levels _____
        a. Rise then level out, remaining steady
        b. Fluctuate at the same regular rate (peaks and valleys are consistent)
        c. **Fluctuate at reducing levels, eventually reaching a regular rate of fluctuation (attenuation)**

    C. CAP activity _____
        a. Fluctuates at the same regular rate (peaks and valleys are consistent)

        b. **Fluctuates at reducing levels, eventually reaching a regular rate of fluctuation (attenuation)**

        c. Remains unchanged even with this additional complexity included

  D. The lac repressor activity ____

        a. **Decreases to 0 (becomes inactive) in response to allolactose levels**

        b. Fluctuates at a regular rate (binds the operator then falls off, binds the operator then falls off, …)

        c. Remains active but at a low activity level

  E. mRNA levels ____

        a. Fluctuates at the same regular rate (peaks and valleys are consistent)

        b. **Fluctuate at reducing levels, eventually reaching a regular rate of fluctuation (attenuation)**

        c. Remains unchanged even with this additional complexity included

5. **Does your simulation results match your prediction?** (circle one) **Yes**　　No

   If your prediction was incorrect, continue to play with the simulation to understand the following:

    a) How simulation results translate to events inside the cell

    b) What role lactose breakdown has on regulation of the lac operon

6. Describe the mechanism correctly: If your predicted mechanism is incorrect, fix it below and describe the mechanism correctly. HOW does lactose breakdown affect the various components of the *lac* operon and its regulatory elements? Be sure to describe all of the components and their interactions based on the Cell Collective model.

<div align="center">

See #2

</div>

7. **Is this an example of positive or negative feedback?** Justify your answer.

  Negative feedback because observed component activity levels eventually attenuated. If it was positive feedback, activity levels would have kept increasing.

8. **Why does this feedback occur – what is the evolutionary advantage to the effects lactose breakdown causes in the cell?**

  ***E. coli* preferentially uses glucose as an energy source: if it's available, the cell will use it. Also, bacterial mRNA and proteins are short-lived, being regularly recycled into alternative products as the cell responds to an ever-changing environment. To conserve resources, the cell will only make lactose-processing proteins when lactose is in the environment and glucose is not. These fluctuations allow *E. coli* to constantly poll/adapt to their environment.**