# Questions on cAMP Model

1. Adjust the amount of CO2 in the water and hit the setup button.
   1. What changes in the world?
   2. What changes in the gene enrichment graph?

a. The amount of CO2 in the ocean increases if the slider is set to 800ppm.

b. The graph bars increase or decrease pretty dramatically if the slider is set to 800ppm. At 400ppm all the graph bars are basically at zero. At 800ppm, ribosomes have a positive gene enrichment while all the other processes have a negative gene enrichment.

1. Set the CO2 at 400ppm, which is the current concentration of carbon dioxide in the atmosphere. Hit the “go” button. Let the simulation run until it appears to have reached a steady state, then click on the “go” button again to toggle it off.
   1. Describe what you see in the “world”, and also on the graph called “CO2 Distribution”.

In the world, carbon dioxide molecules move from the ocean, into the cell, into the chloroplast, and then into the pyrenoid. In the graph, the CO2 concentration in the ocean decreases slightly (black) while the CO2 concentration in the pyrenoid increases drastically and never drops. At steady state, the concentrations in the ocean, chloroplast, and cell are roughly identical, while the CO2 concentration in the pyrenoid is approximately a factor of four times larger.

* 1. The world depicts one regulatory pathway for a “Carbon-Concentration Mechanism”, or “CCM”. Based on your answer to question a., why is this an appropriate name?

The pathway makes it so that the concentration inside the pyrenoid is about four times as large as the concentration in the ocean.

* 1. Record the ending concentrations of CO2 in the pyrenoid, and in the ocean. You may need to estimate these numbers from the graph “CO2 Distribution”.

|  |  |  |
| --- | --- | --- |
| Atmospheric CO2 Concentration | Steady state Concentration of CO2 in Pyrenoid | Steady state Concentration of CO2 in Ocean |
| 400 ppm | ~0.60 | 0.13 |

* 1. The CCM consists of several “transporters” of two types. Pores allow CO2 to pass in both directions, and a “pump” acts as a one-way valve. Where is the pump located? Why do you think it is located there?

The pump is located between the chloroplast and the pyrenoid. It makes sense to be there because it’s the only active component and should pump only into the volume where the CO2 needs to be concentrated.

* 1. The “ATP Used” graph shows that some energy must be used by the diatom in the CCM process being depicted. Why do you think this is true?

The pump requires energy in order to shuttle CO2 “up” a concentration gradient. In addition (and this is not so clear from the model), holding the transporters “open” requires energy, and the wider the openings, the more energy is required.

* 1. Record the amount of ATP required to reach steady state, and the amount of ticks required. This can be done by placing your mouse over the appropriate point on the “ATP Used” graph, and reading off the x and y values.

|  |  |  |
| --- | --- | --- |
| CO2 Concentration | Ticks to reach Steady State | ATP Used to reach Steady State |
| 400 ppm | ~1000 | ~40 |

Note that the model continues to require energy in order to keep the transporters open even after the concentration of CO2 in the pyrenoid saturates. In a better model (and I would guess in the diatoms, although this is purely a guess), the transporter size would vary in feedback with the concentration of CO2 in the pyrenoid.

1. Set the CO2 at 800ppm, which is the projected concentration of carbon dioxide in the atmosphere near the end of this century. Hit the “go” button. Let the simulation run until it appears to have reached an steady state, then click on the “go” button again to toggle it off.
   1. What happens to the size of the transporters at this higher concentration of CO2?

The transporters decrease in width.

* 1. Compare the data you collected in the previous question by completing the tables show below:

|  |  |  |
| --- | --- | --- |
| CO2 Concentration | Ticks to reach Steady State | ATP Used to reach Steady State |
| 400 ppm | ~1000 | ~40 |
| 800 ppm | ~1000 | ~33 |

|  |  |  |
| --- | --- | --- |
| Atmospheric CO2 Concentration | Steady State Concentration of CO2 in Pyrenoid | Steady State Concentration of CO2 in Ocean |
| 400 ppm | ~0.60 | 0.13 |
| 800 ppm | ~0.60 | 0.26 |

1. Summarize the significance of the tables you just filled in by answering the following questions:
   1. What changed as the concentration of CO2 in the ocean increased?

The diatom used less energy to reach steady state, and the concentration of the ocean at steady state was twice as high (consistent with the atmospheric CO2 concentration).

* 1. What did not change as the concentration of CO2 in the ocean increased?

The saturated steady state concentration in the pyrenoid was the same, and it took approximately the same amount of time to reach that concentration.

* 1. What is the overall effect of the increased concentration of CO2 in the ocean for the diatom?

Basically the diatom is able to do what it did before in the same amount of time using less energy.

1. Describe the gene regulation process which is occurring. Which of the following are up-regulated by increased carbon dioxide? Which are down regulated?

|  |  |  |
| --- | --- | --- |
| Item | Up-regulated by CO2 | Down-regulated by CO2 |
| CYCc | X |  |
| cAMP | X |  |
| Transcription Factor | X |  |
| CCM Transporters |  | X |

1. The graph called Gene Enrichment shows the expression of various processes or classes of proteins in the cell, compared to their expression at a CO2 concentration of 400ppm.
   1. Which processes are happening less as the amount of CO2 increases?

* Ion Transport
* Transcription
* Kinases
* Photosynthesis
* TCA Cycle
  1. Based on what you know about the processes you just listed, do you believe that the cell is “going faster” or “going slower” as the concentration of CO2 increases?

The question is poorly worded, but I am trying to get at the idea that the diatom can slow down processes which ultimately lead to energy production (eg oxidative phosphorylation, kinases, TCA and photosynthesis) because they are saving energy by reducing CCM.

1. Ribosomes are the only process/class of proteins which are being expressed MORE as the concentration of CO2 increases. Based on what you know about the role of ribosomes (you may need to remind yourself about their role before proceeding), why do you think ribosomes are being expressed MORE? (\*\*\*)

Multiple answers are possible here. Ribosomes ultimately function to translate RNA into proteins. Hence they might be needed in cases where lots of proteins or new proteins need to be formed. The paper from which this model was derived suggests that diatoms might need to make new proteins in order to adjust to the higher levels of CO2.

(\*\*\*) Might want to omit this question because in talking to Justin it’s not clear that there is any relationship between ribosomal expression and function.

Auxiliary Features of the Model

1. Hennon, et. al. hypothesized the gene network model shown in the simulation. They believed, specifically, that various CCM related genes were controlled by a molecule which affects transcription factors which have a cis-regulatory sequence downstream of genes in the CCM pathways. (wording?)

COULD THIS BE SHOWN IN THE NUCLEUS OF THE MODEL SOMEHOW?

This molecule is called cAMP. To test their theory, they looked at the expression of several proteins in the CCM pathway when cAMP was degraded by hydrolysis (water addition to split into two or more pieces) by IBMX.

Run the simulation, then click on the button which says Knockout cAMP with IBMX. What happens in the simulation? If the simulation provided direct experimental evidence, would this evidence support their hypothesis?

In the simulation, when IBMX knocks out cAMP, the CCM transporters turn “OFF”, and the cell no longer moves CO2 into the pyrenoid at all. The model depicts this by “closing” the transporters.

Their results are consistent with the hypothesis. If IBMX is known to knockout cAMP, and concurrently the CCM mechanisms shut down, then it could be that cAMP is controlling the CCM mechanisms.

1. Run the simulation, then click on the button which says “Calvin Cycle On”. What happens in the Calvin Cycle? How does the simulation show this process?

The Calvin cycle produces three carbon molecules by “fixing” three CO2 molecules together with five water molecules. Energy is used in the process. The simulation shows this explicitly because three CO2 molecules move around the loop, and then a G3P molecule replaces them. The G3P molecules move off the screen cause there used to be a graph which counted them on the left hand side of the world. The graph was removed because it seems to draw focus around from the CCM mechanisms.

1. All simulations or models have aspects which are consistent with the real world, and aspects which are flawed. What are some of the flaws with this model?

There are lots of answers to this question. From a diatom researcher’s point of view, the actual CCM mechanism involves bicarbonate (HCO3-) which is also pumped into the cell. In addition, several proteins called carbonic anhydrases are involved in converting CO2 into bicarbonate in the cell, and then bicarbonate back to CO2 in the pyrenoid. See for example Hopkinson at the University of Georgia, “Efficiency of the CO2-concentrating mechanism of diatoms”, PNAS, 2011.

In addition, the actual mechanism whereby the transporters control the flow of CO2 is not related to the opening size of the transporters, nor are the energetic costs of keeping the CCM transporters open or of the pump in the pyrenoid membrane accurate. These were conveniences choses for the sake of making the model “work”.

From a student’s point of view, possible simplifications include:

* CO2 is not “regenerated” in the ocean space, while in the real world we don’t know if the diatoms will dramatically decrease the amount of CO2 in the ocean.
* CO2 would “move” all around the cell, not just in the space it is confined to in the model.
* The G3P shown disappears rather than going on to be incorporated into six-carbon sugars or metabolized.
* There is no mechanism that shows how IBMX knocks out cAMP.