

## An Investigation of Two Models of Signal Transduction During Catabolite Repression

**Catabolite repression** describes the phenomenon where certain carbon sources in bacterial growth media lead to a reduction of transcription of sensitive operons. In *Escherichia coli*, the best understood example of catabolite repression involves the lac operon. *E. coli* will not express the lac operon if glucose is present in the growth media. This is true whether or not lactose is present in the media. Other operons in *E. coli* are also repressed by glucose including the arabinose operon and the maltose operon. Although glucose is the most studied carbon source capable of catabolite repression in *E. coli*, it is not the only one capable of triggering repression of these operons. Sucrose, Fructose and other related carbon molecules can also trigger catabolite repression to varying levels.

Klug and Cummings includes a discussion of the "classic cAMP" model for catabolite repression. In this model glucose acts by inhibiting the activity of adenylate cyclase. This leads to a drop in cAMP concentrations. At lower concentrations, the cAMP is not available to bind to the catabolite activator protein (CAP). CAP in the absence of cAMP loses affinity for the Cap Binding Site doesn't bind the lac operon. In the absence of CAP binding, the promoter is unable to recruit RNA polymerase to initiate transcription. Therefore, the lac operon is not transcribed in the presence of Glucose. Alternatively, in the absence of glucose, the adenylate cyclase is not inhibited and actively produces cAMP. The cAMP binds to CAP causing it to shift to a conformation with affinity to the Cap Binding Site. CAP binds the Cap Binding Site and recruits the RNA polymerase to the promoter and initiates transcription of the lac operon.

The "classic cAMP" model was proposed in the 1970's and its general acceptance by the scientific community is evidenced by its inclusion in all the genetics textbooks. However, in the last 5 years there has been a series of papers which challenge every aspect of the classic model. Several new models for catabolite repression have been proposed in these papers. Scientists in laboratories around the world are conducting new experiments to test each model.

In this laboratory project, you will conduct an experiment to test an element of those models, the mechanism by which glucose inhibits adenylate cyclase. There are basically two models: the glucose transporter model and the glycolytic flux model.

In the glucose transporter model, it is the movement of glucose through its membrane transport protein that triggers inhibition of adenylate cyclase (See Fig 1). In this model as glucose moves through the glucose transporter it is phosphorylated to form glucose - 6 - phosphate. The phosphate that is attached to glucose comes from a protein called EIIA<sup>glc</sup>. So that when glucose is moving through the transporter, the non-phosphorylated form of EIIA<sup>glc</sup> is more abundant. The non-phosphorylated form of EIIA<sup>glc</sup> does not interact with adenylate cyclase. In the absence of this interaction adenylate cyclase is inactive and the levels of cAMP decrease.

In the glucose transporter model, when glucose is not moving through the glucose transporter, there is nothing to remove the phosphate from EIIA<sup>glc</sup>. The phosphorylated

form of this protein accumulates in the cell. The phosphorylated form of EIIA<sup>glc</sup> interacts with adenylate cyclase activating it. The active adenylate cyclase makes cAMP and the levels of cAMP increase.

In the glycolytic flux model, movement of glucose through the glucose transporter is not essential for catabolite repression. Instead, what is important in the flux of carbon through glycolysis (Fig 2.) Glycolysis is the metabolic pathway that catabolizes glucose to pyruvate. When glucose is being actively degraded by glycolysis, the concentration of phosphorylated intermediates of glycolysis such as glycerol-3-phosphate increase in the cell. In the glycolytic flux model, it is these phosphorylated intermediates that interact with EIIA<sup>glc</sup> and prevent EIIA<sup>glc</sup> from activating adenylate cyclase. Basically, the model says that abundant glucose leads to increase concentration of phosphorylated intermediates. These intermediates prevent EIIA<sup>glc</sup> from binding to adenylate cyclase and thereby prevent the activation of the enzyme. In the absence of activated adenylate cyclase, cAMP concentrations decrease.

In the glycolytic flux model, when the cells are starved for glucose, the concentrations of glycolytic intermediates decrease. As their concentrations decrease, they are not available to interact with EIIA<sup>glc</sup>. This allows EIIA<sup>glc</sup> to activate the adenylate cyclase. Adenylate cyclase produces cAMP and the concentration of cAMP increase.

For this laboratory, you will conduct an experiment to distinguish between these two models. The basic experimental strategy will be to test whether two carbon sources, galactose and glycerol, can trigger catabolite repression,. What is important about these carbon sources is that they both increase glycolytic flux, but neither of them is transported through the glucose transporter.

If the glucose transporter model were correct, then we would predict that neither of these compounds would effectively trigger catabolite repression. Since neither compound moves through the transporter, EIIA<sup>glc</sup> should remain in the phosphorylated state. And according to the glucose transporter model, this the phosphorylated EIIA<sup>glc</sup> will activate adenylate cyclase and cAMP levels will rise.

If the glycolytic flux model were correct, then we would predict that both of these compounds would trigger catabolite repression. The fact that they do not move through the glucose transporter is insignificant. What is important is that they both contribute to glycolysis (glycerol directly and galactose indirectly). Bacteria growing on these compounds should accumulate phosphorylated glycolytic intermediates. And according to the glycolytic flux model, these intermediates will prevent EIIA<sup>glc</sup> from activating adenylate cyclase and cAMP levels will decrease.

In this experiment, we will not be measuring cAMP levels directly. Instead we will assay for it indirectly, by detecting catabolite repression. To assay catabolite repression we will use *E. coli* which contain an engineered plasmids called pGLO (Fig 3). The pGLO plasmid contains a chimeric gene fusing the promoter elements from the arabinose operon to the jelly fish green fluorescent protein (gfp) open reading frame. The arabinose promoter contains a CAP Binding Site and shows the same type of catabolite repression as the lac operon promoter. Glucose prevents transcriptional initiation from this

promoter even in the presence of arabinose. The *gfp* open reading frame is serving as a reporter gene. The expression of this protein is easy to detect because it fluoresces a bright green color under UV light. Bacteria lack the equivalent of this protein and typically don't fluoresce green under UV light. This means if the arabinose promoter of pGLO is active, the cells will fluoresce green. However, if the arabinose promoter is repressed the cells will not fluoresce.

### Experiment

1. Obtain overnight cultures of *E. coli* containing the pGLO plasmids from the teaching assistant.
2. Streak out the bacteria on LB media supplemented with the following carbon sources.

LB + no supplements  
LB + 1% arabinose  
LB + 1% arabinose + 1% glucose  
LB + 1% arabinose + 1% glycerol  
LB + 1% arabinose + 1% galactose

3. Grow the bacteria overnight at 37°.
4. Store bacterial cultures at 4° until next laboratory period.
5. Visualize *gfp* expression under UV light.

### Short Report

1. Prepare a table reporting your results
2. Based on your results, write a brief essay explaining what conclusions can you draw concerning whether the glucose transporter or glycolytic flux is involved in inhibiting adenylate cyclase.
3. Suppose that you had made a second set of plates with the same compounds as before, but that you had added 1% cAMP to the media to each. How would the results have changed? (Hint draw a new table including the results you believe you would get from the five new plates.

### References

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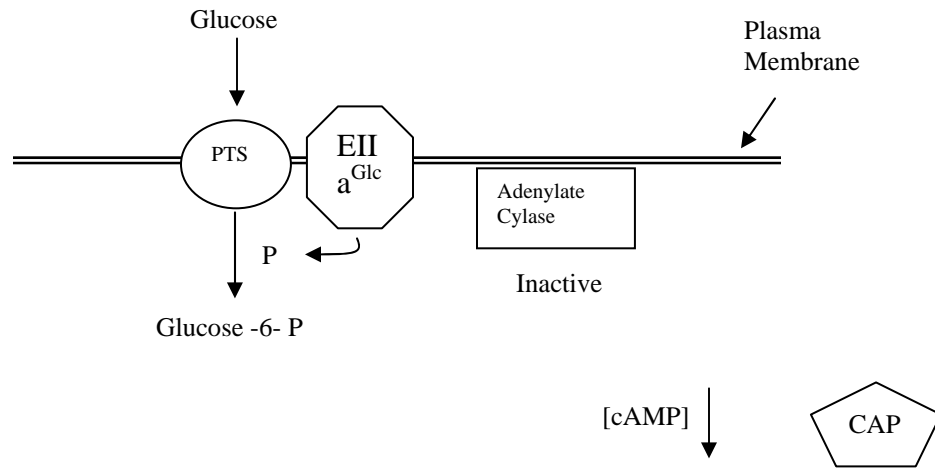
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Figure 1: Glucose transporter model

Proteins Involved

- Glucose phosphotransferase system (PTS)
- $EIIa^{Glc}$
- Adenylate Cyclase
- Catabolite Activator Protein (CAP)

Glucose Present



Glucose Absent

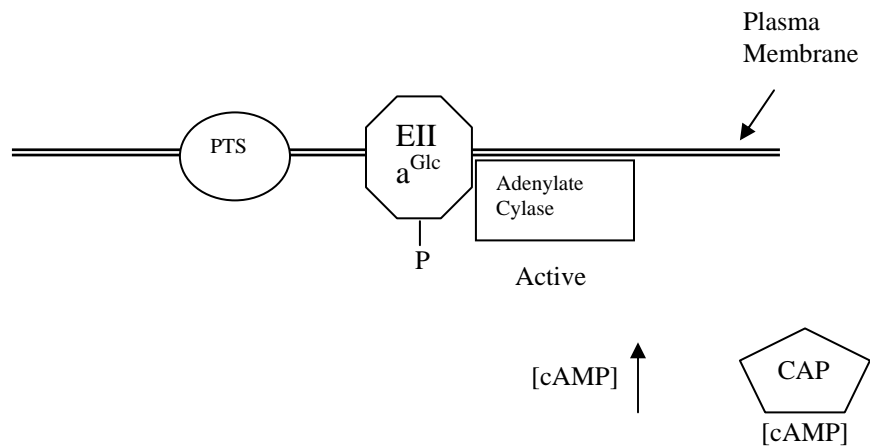
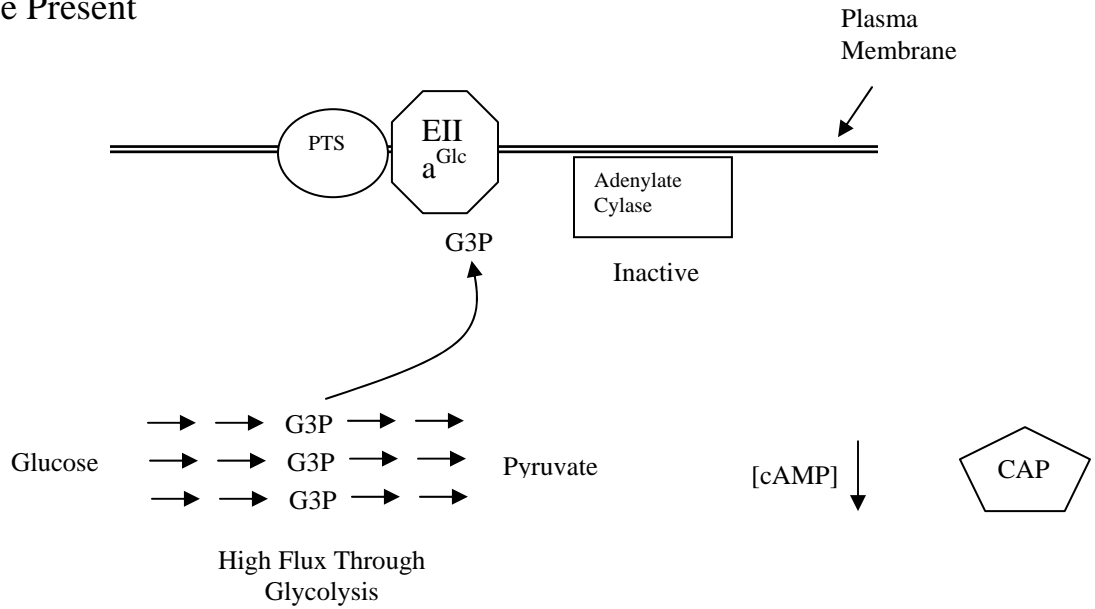


Figure 2: Glycolytic Flux Model

Proteins Involved

- Glycolytic Enzymes
- EII<sup>a</sup><sub>Glc</sub>
- Adenylate Cyclase
- Catabolite Activator Protein (CAP)

Glucose Present



Glucose Absent

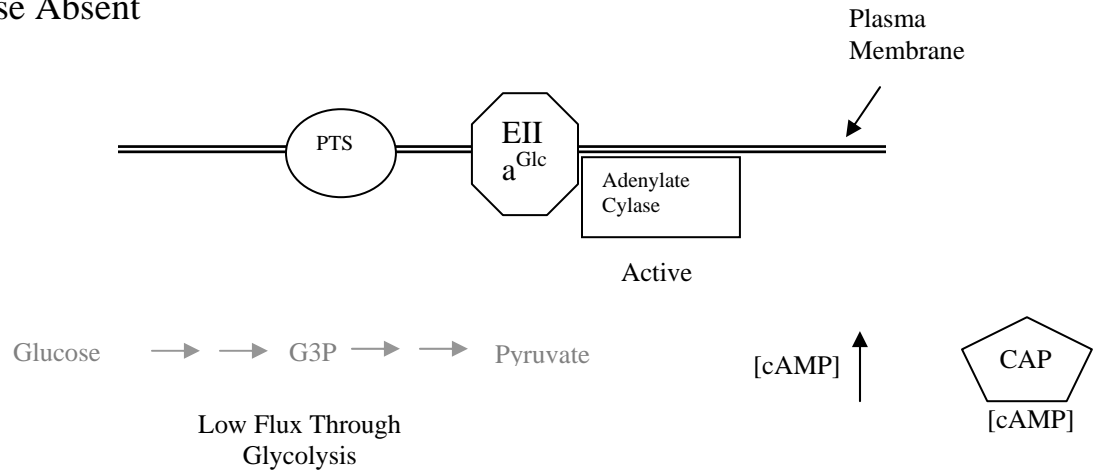


Figure 3: Map of pGLO plasmid

