


# It's *lactacular!*

## Regulation of Gene Expression in the *lac* Operon of *E. coli*



Jennifer Gillis, Elizabeth Hall,  
Lynley Holt, and John Petrie

Department of Biological Sciences, The  
University of Georgia



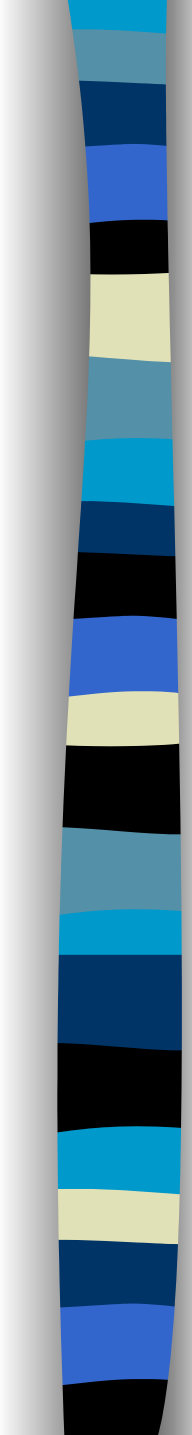
# ABSTRACT

The *lac* operon has been studied extensively as a model to understand regulation of genes and cell functions. These experiments have produced large amounts of data concerning the utilization of lactose and the expression of genes in an operon. The *lac* operon demonstrates positive and negative controls in gene regulation and shows how genes can be expressed together. It has also provided a connection that brought biochemistry and genetics together when studying *Escherichia coli* and its ability to utilize lactose (Griffiths et al, 1996).



# INTRODUCTION

Every living organism contains a metabolic circuit to obtain carbon, a fundamental function of life. Bacteria have many sources of carbon, including lactose, glucose, maltose, and galactose. Much energy would be lost if the cell simultaneously and continually synthesized enzymes to breakdown all of these sugars (Adhya, 1996). Therefore, the bacterial cell must be able to regulate the expression of its genes, thereby regulating enzyme production and in turn metabolism. The first scientists to demonstrate a transcriptionally regulated system, by working on lactose metabolism in *E. coli*, were the 1965 Nobel Prize laureates, François Jacob and Jacques Monod ([www.nobel.se](http://www.nobel.se)). They proposed a theory of double-negative control in which transcription is inhibited by a repressor protein bound to a promoter region controlling a set of genes.



Lactose, the inducer, may bind to the repressor, allosterically modifying and removing the repressor from the operator, and initiating transcription of the downstream gene set (Griffiths et al., 1996). Since the proposal of the *lac* operon, much research has been done on gene regulation and expression of this biochemical pathway, and today much more is known. This study presents a current *lac* operon system in gram-negative *E. coli* with a biochemical transport diagram. The DNA, RNA, and protein profiles of the diagram are also transformed into computer models with mathematical estimations. The computer model allowed evaluation and prediction of regulatory behaviors within the cell, and further comparisons to existing behavioral data.

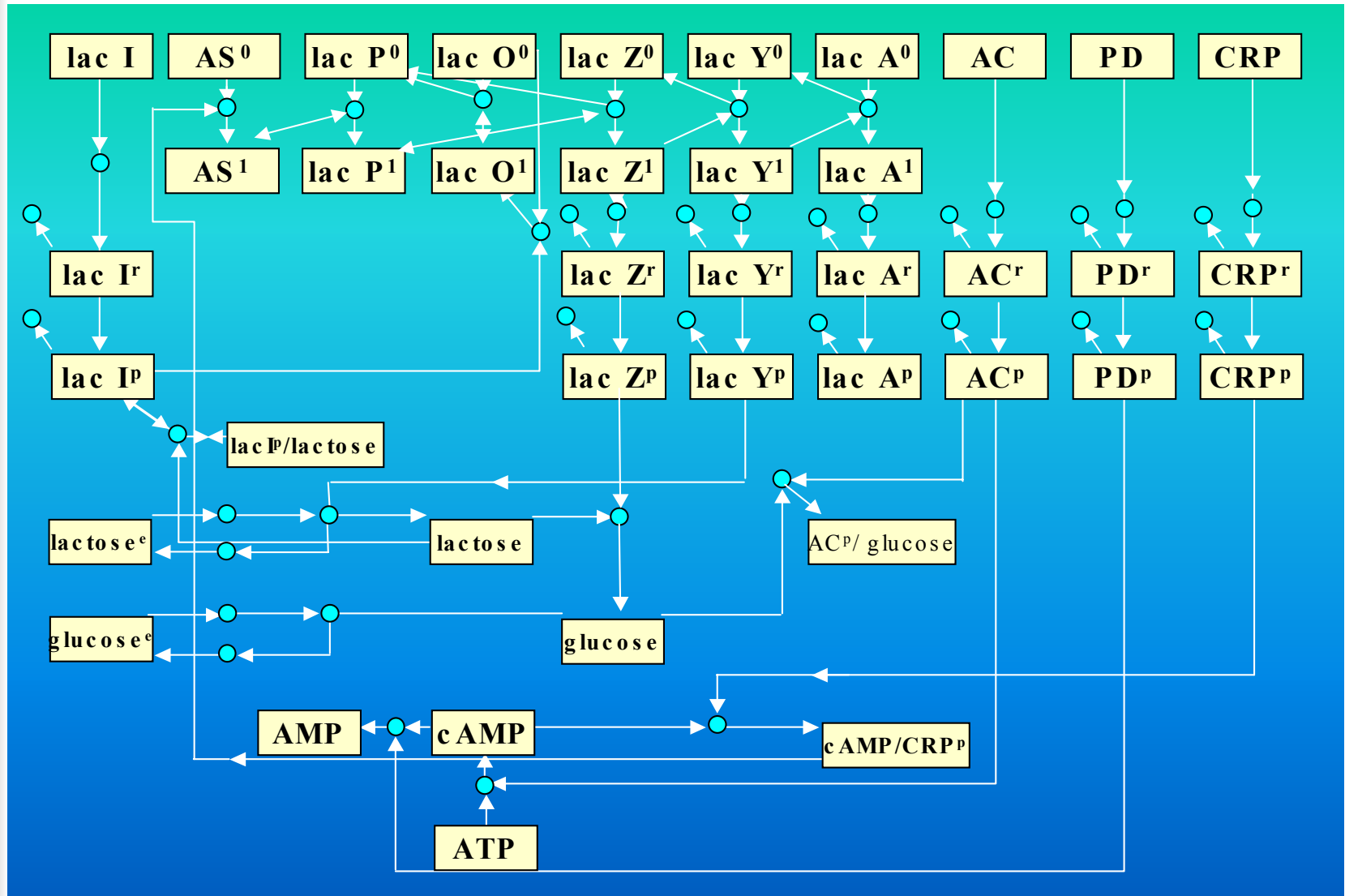


# MATERIALS AND METHODS

A diagram of the lactose metabolism circuit was compiled detailing the genes, regulators, enzymes, and substrates involved (figure 1). To compile the circuit, illustrations of the lac operon (figure 2-6) were used ([www.blc.arizona.edu](http://www.blc.arizona.edu)). Literature from a medley of general genetics texts and papers aided in determining the genes and enzymes involved. Once the biochemical pathway was resolved, chemical equations of the forward and backward reactions were formulated with estimated reactions rates. A file with the initial concentrations and biochemical reactions of all circuit components was input and run on the Origin 2000 UNIX computer using kin1.13 by Berndt Schuttler, Department of Physics at the University of Georgia ([genegenetic.uga.edu](http://genegenetic.uga.edu)). Certain parameters such as concentration levels

and the presence of genes and proteins are manipulated to determine their affects on the substrates of the reactions.

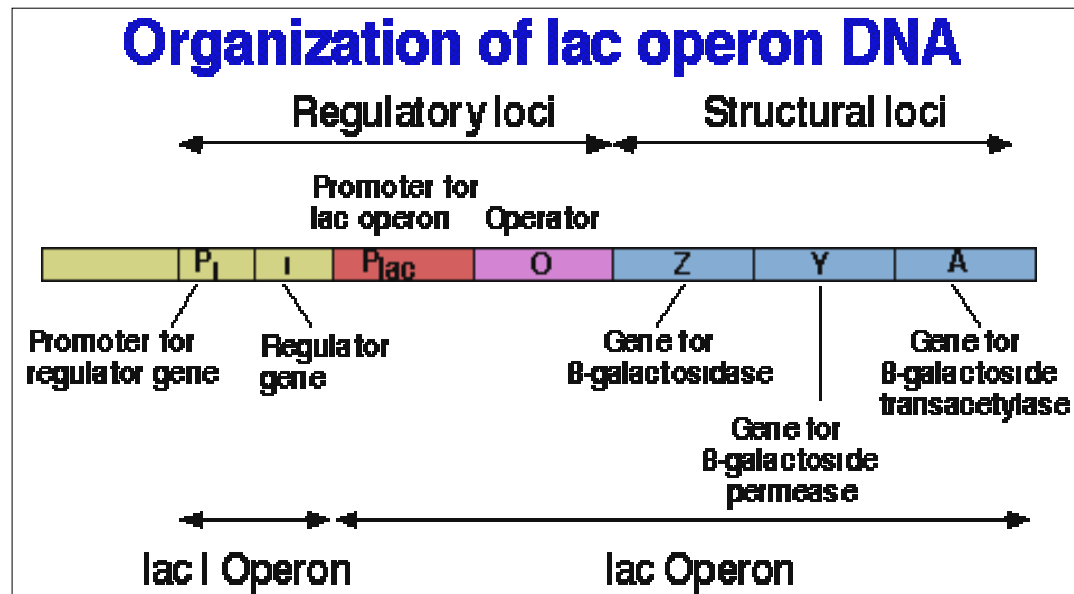
Figure 1



# RESULTS AND DISCUSSION

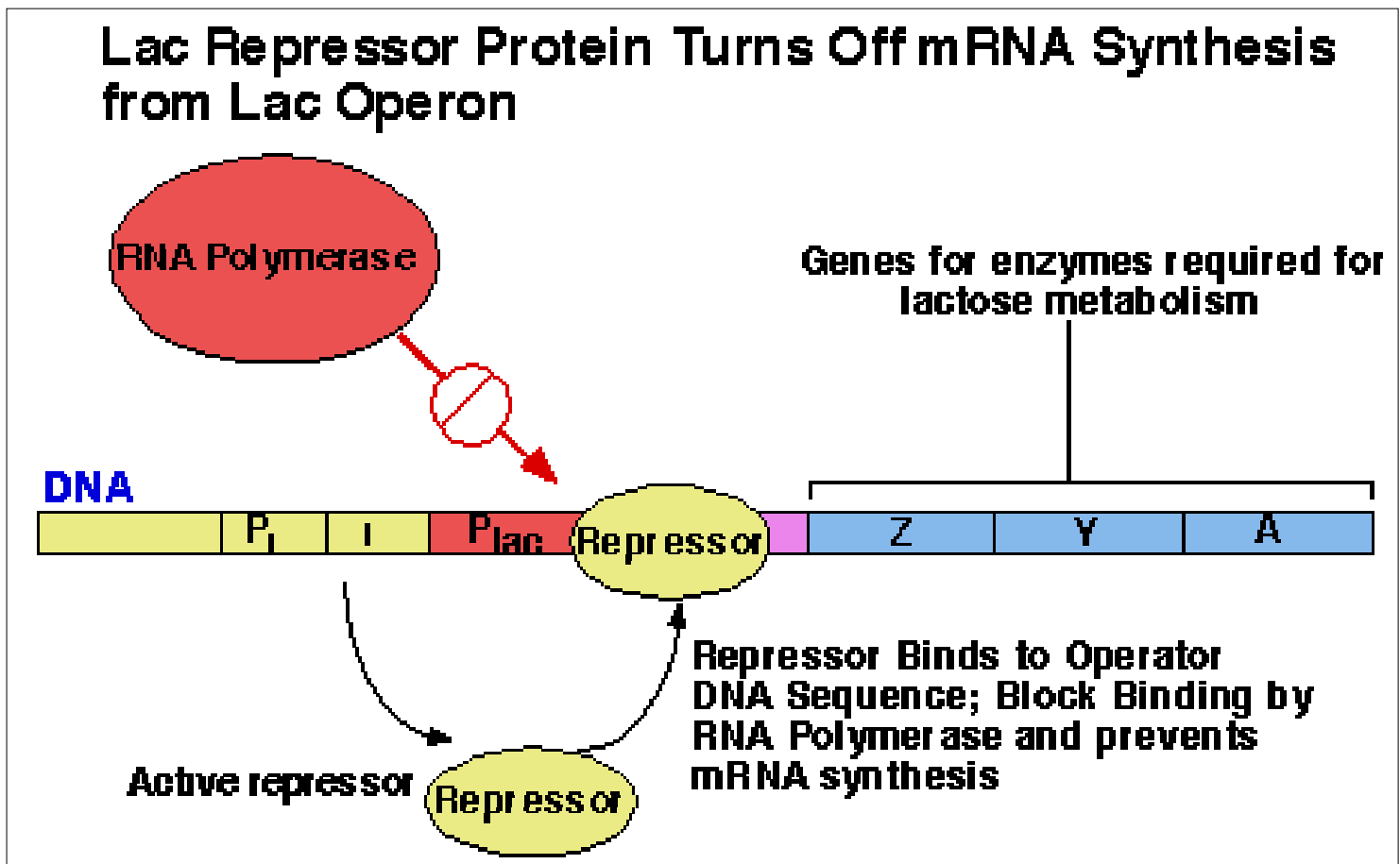
- An operon, which appears to be unique to bacteria, is a contiguous set of genes transcribed from a single promoter into one mRNA molecule. The mRNA is translated into different proteins corresponding to the separate genes (Dale, 1994). The *lac* operon in *E. coli* consists of three genes: *lacZ*, *lacY*, and *lacA* (figure 2).

Figure 2



- Three operators are now known exist In the *lac* operon. In negative control, the absence of lactose results in a bound repressor protein (*lacI*) to the operators (O), preventing transcription (figure 3) (Adhya, 1996).

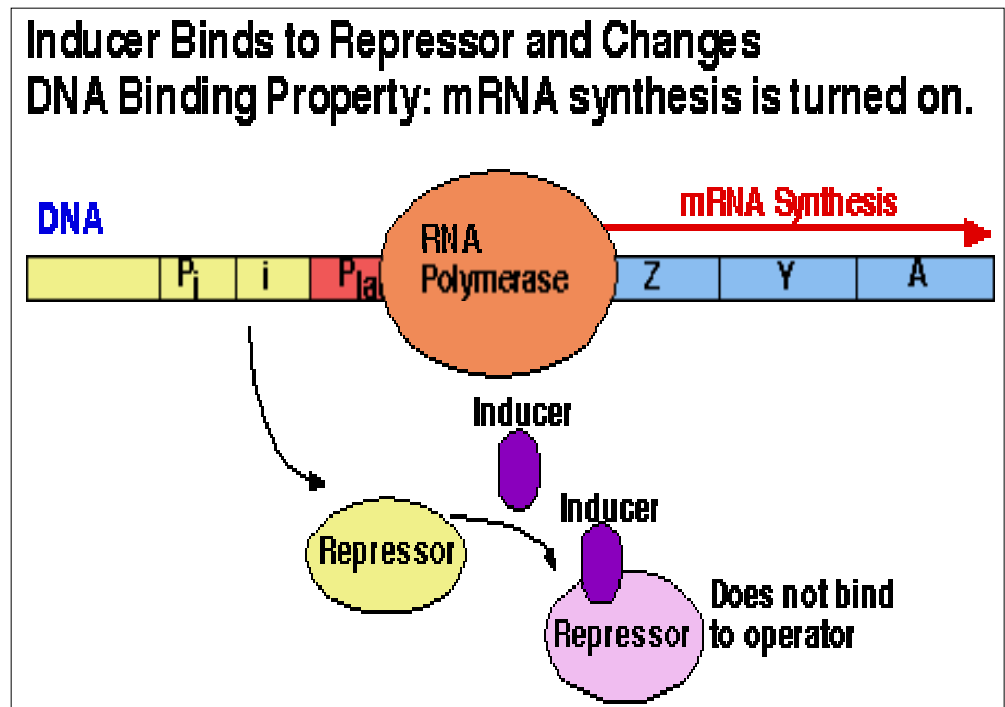
Figure 3

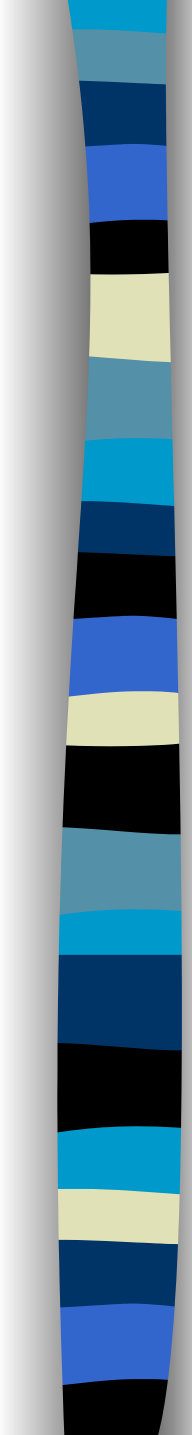


- When present, the lactose inducer binds and allosterically changes the repressor protein, removing it from the operators (figure 4). The resulting transcription leads to the gene products, B-galactosidase (*lacZ*), permease (*lacY*), and transacetylase (*lacA*).

- Permease is responsible for bringing extracellular lactose into the cell, B-galactosidase breaks down lactose into glucose and galactose, and the function of transacetylase remains unknown (Griffiths et al., 1996).

Figure 4



- 
- A mode of positive control is also active in the *lac* operon. Cyclic AMP, encoded by the *cyp* gene and the product of adenylate cyclase, binds to the cAMP receptor protein (CRP), contributing to transcription initiation. The cAMP/CRP complex binds to a region known as the activation site (AS). The positive control of this cAMP/CRP complex explains the phenomenon of catabolite repression, also called the “glucose effect”. When glucose is present in the cell, a smaller amount of cAMP is formed, eventually causing less lactose metabolism (Adhya, 1996).
  - The *E.coli* cell prefers glucose as its carbon source, and won't metabolize lactose until all glucose is used up (Lin et al., 1984). When glucose is broken down, the levels of ATP increase, and cAMP levels fall.

- Transcription is dependent on the cAMP/CRP complex, therefore, the operon is repressed when glucose levels are high. As the glucose is used up, levels of ATP fall, cAMP levels increase, the *lac* operon is induced, and lactose is then metabolized (figures 5-6) (Dale, 1994).

Figure 5

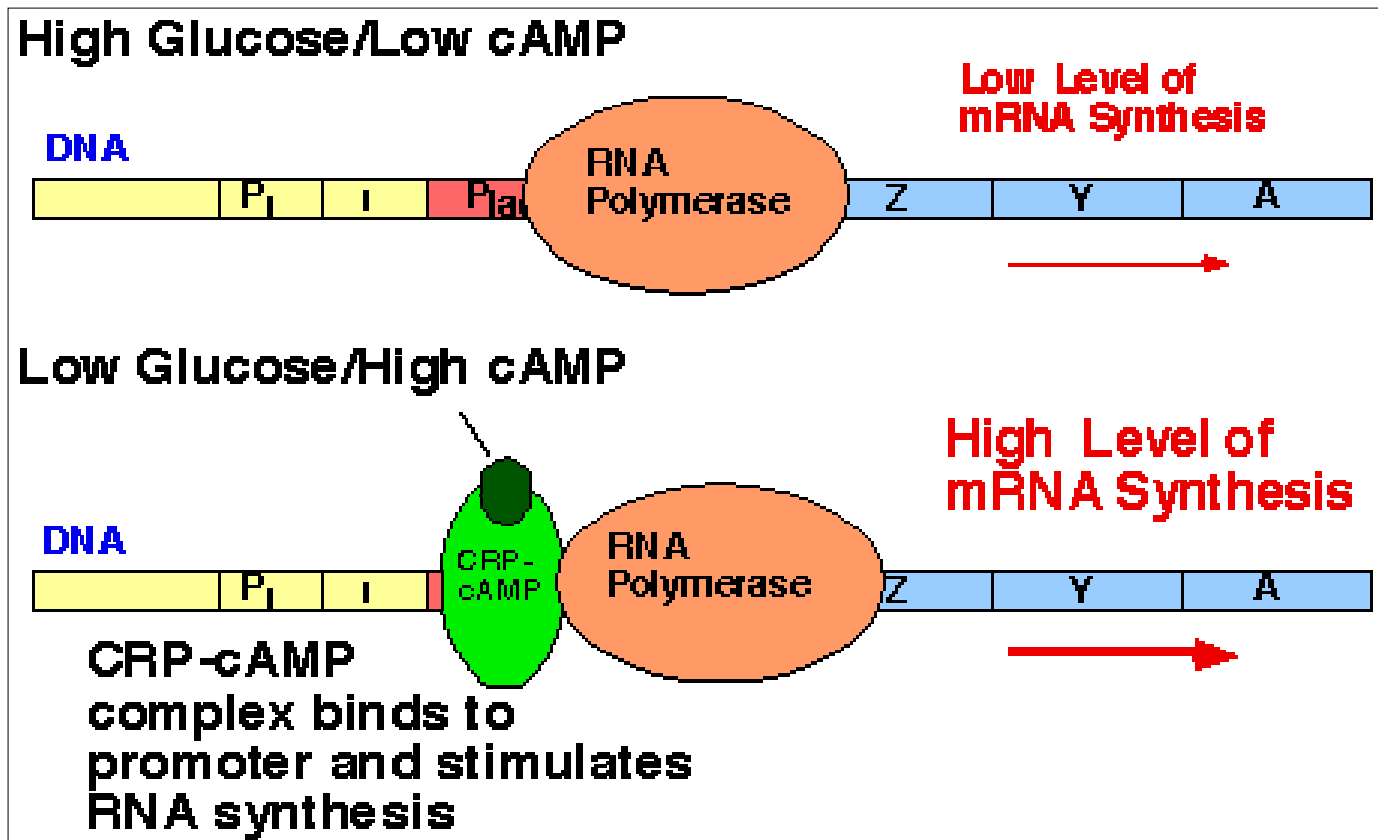


Figure 6

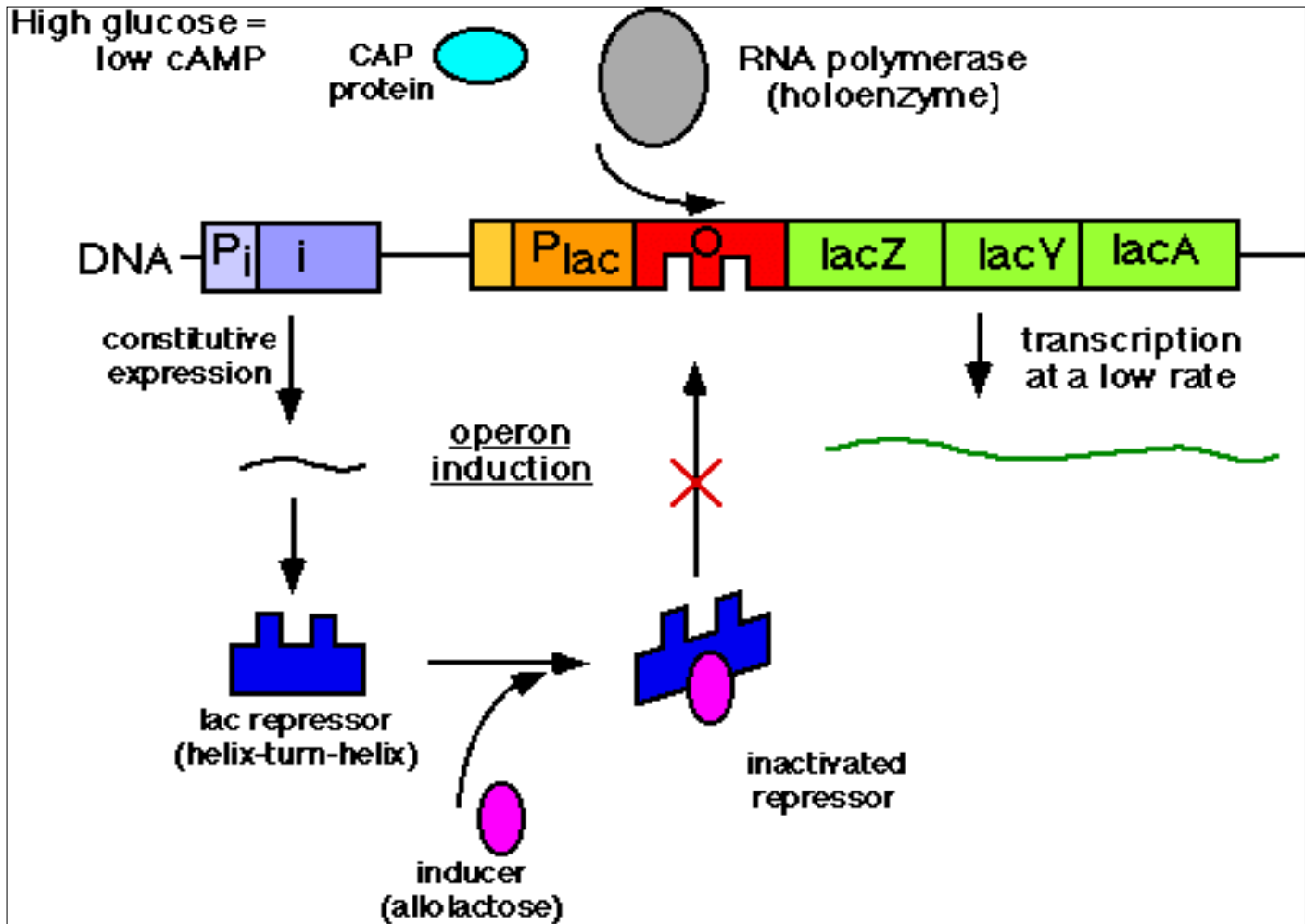
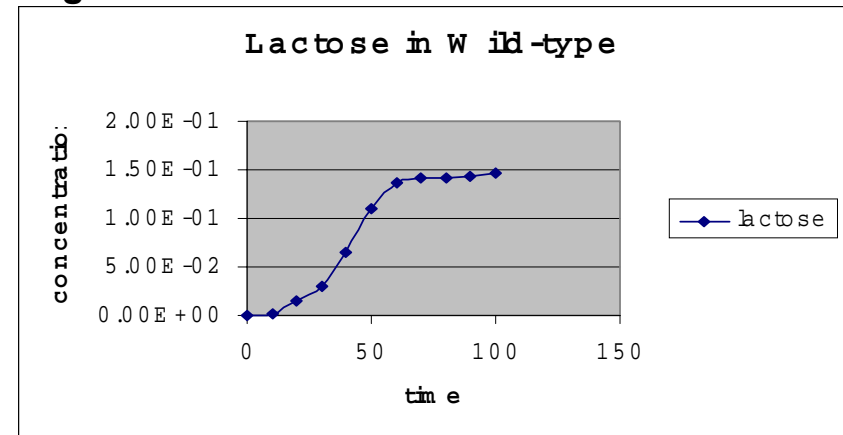


Figure from [www.blc.arizona.edu](http://www.blc.arizona.edu)

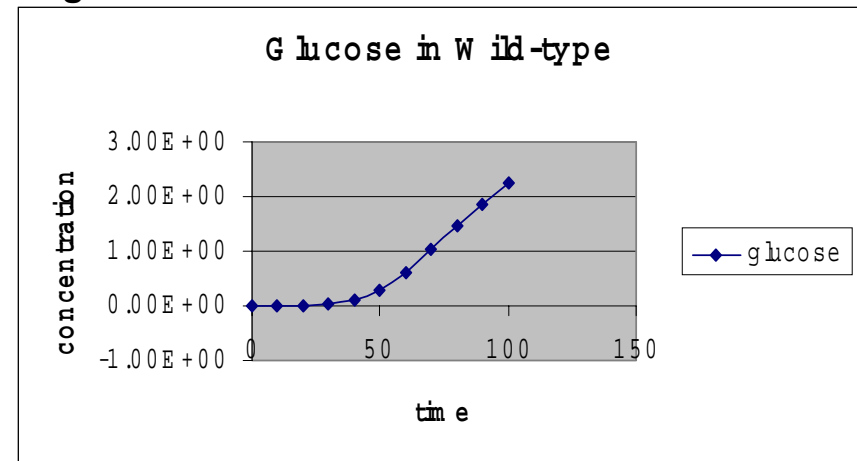
■ Lactose was available for the cell to utilize in the computer program. Glucose concentrations in wild-type show a steady increase as time progresses. This indicates the cell metabolizes lactose yielding glucose (figure 7) .

■ Glucose was not introduced in the extracellular level, so its concentration began very low and increased steadily as lactose was metabolized into glucose and galactose.(figure 8).

**Figure 7**

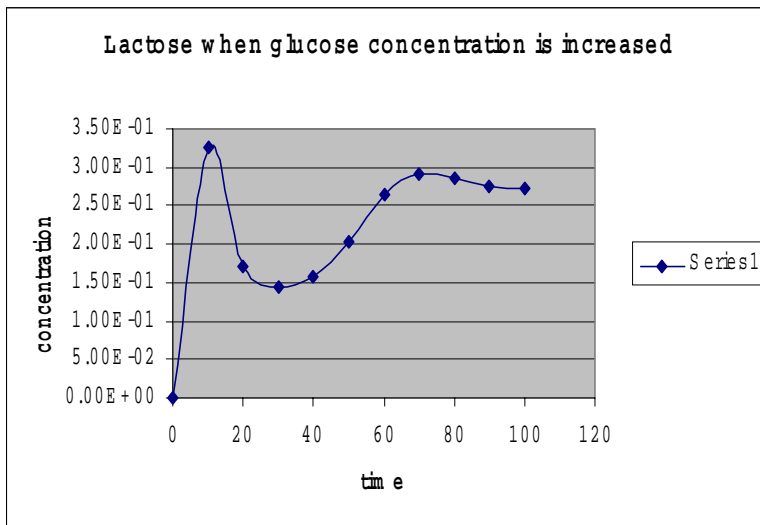


**Figure 8**

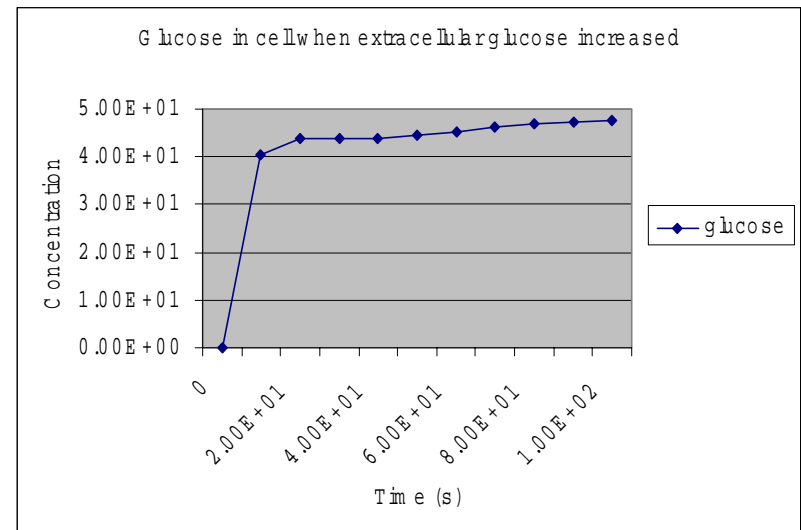


- Extracellular glucose levels increased in the computer simulated model showed the cell's use of glucose over time. Figure 9 indicates the cell immediately utilized it's glucose carbon source. The intracellular concentration levels off as all the source runs out.
- Lactose concentrations fall off quickly , indicating that the cell recognized the presence of glucose, which it prefers to bind. Levels increase again as the glucose source runs out (Figure 10).

**Figure 9**



**Figure 10**



■ When a gene is knocked out, it prevents transcription of its RNA, and hence its protein. This will therefore inhibit the operon's function as a whole. *LacZ* is translated into a protein, beta-galactosidase, which breaks down lactose into glucose and galactose.

■ Without the *lacZ* gene, the cell is unable to bring lactose into the cell, and would not be able to metabolize it into glucose and galactose.

Therefore, concentrations of lactose and glucose are at zero (Figures 11-12).

Figure 11

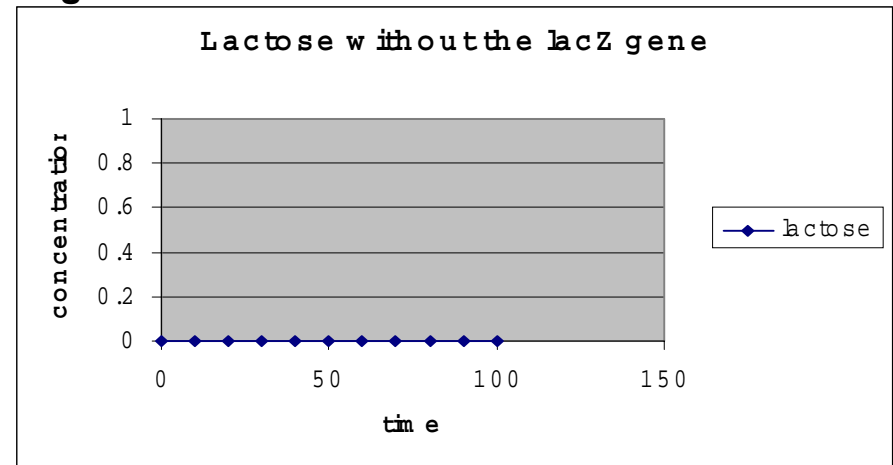
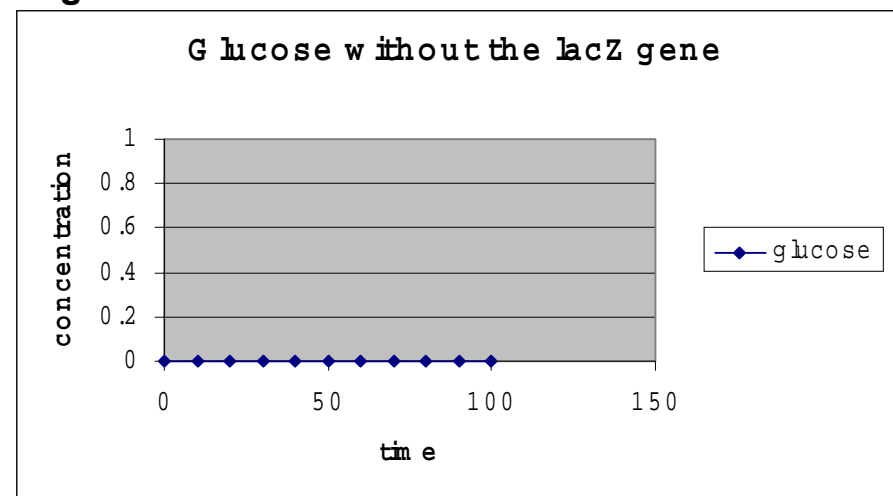
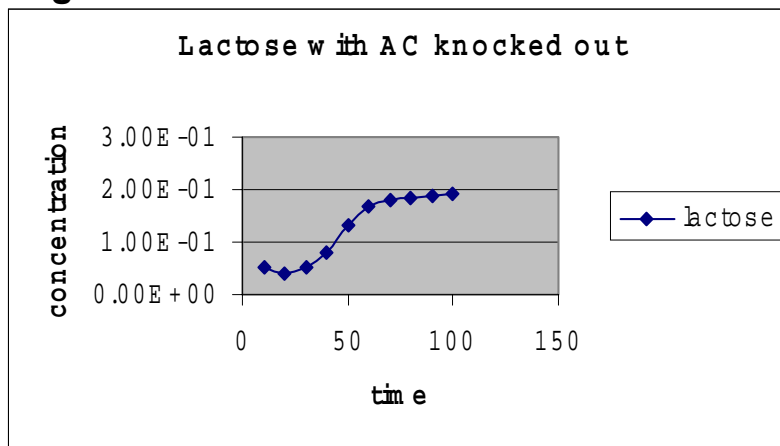


Figure 12

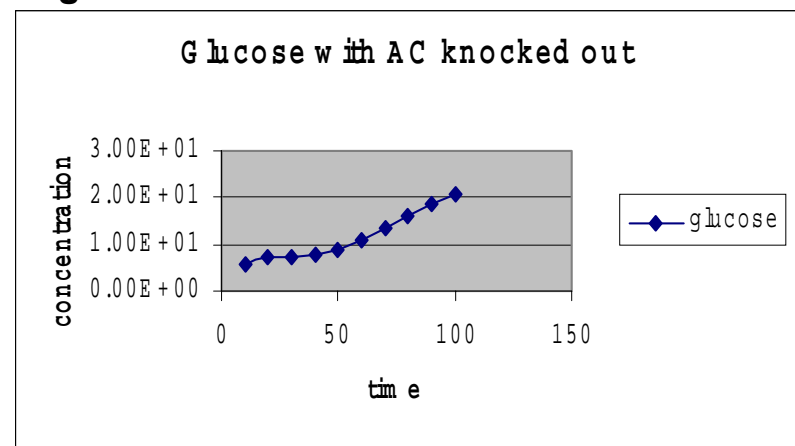


- By knocking out the AC gene, you prevent the transcription and translation of its gene and protein. Adenyl cyclase (AC), breaks down ATP into cAMP which in turn binds to CRP. The cAMP/CRP complex bind to the activator region increasing the affinity of polymerase. By knocking out the AC, less lactose is metabolized because the operon isn't as active (Figure 13).
- Glucose is left free in the cell and glucose concentrations are relatively unchanged as compared to wild-type (Figure 14).

**Figure 13**



**Figure 14**





# CONCLUSIONS

The *lac* operon is a well-studied model presenting a way to understand gene regulation and expression. It helped advance the study of genetics significantly by providing scientists the ability to test their theories experimentally and formulate tangible results. The studies of the *lac* operon provided the link to connect two sciences, biochemistry and genetics, by studying bacterial genetics and regulation using techniques considered biochemical. It was one of the first models to demonstrate the regulation of gene expression in bacteria. Even though the earlier attempts to study the operon led to a model more simplified than it actually is, gene regulation proved to be the central device for gene expression in prokaryotes (Brock, 1990).



# REFERENCES

- Adhya, Sankar. 1996. "The *Lac* and *Gal* Operons Today." In *Regulation of Gene Expression in Escherichia coli*. E.C.C. Lin and A. Simon Lynch, eds. R.G. Landes Company.
- Brock, Thomas D. 1990. "Gene Expression and Regulation." In *The Emergence of Bacterial Genetics*. Cold Spring Harbor Laboratory Press.
- Dale, Jeremy W. 1994. "Regulation of Gene Expression." In *Molecular Genetics of Bacteria*. John Wiley and Sons, Ltd.
- Griffiths, A.J.F., Miller, J.H., Suzuki, D.T., Lewontin, R.C., Gelbart, W.M. 1996. *An Introduction to Genetic Analysis*. W.H. Freeman and Company, New York.
- Lin, E.C.C., Goldstein, R., Syvanen, M. 1984. *Bacteria, Plasmids, and Phages: An Introduction to Molecular Biology*. Harvard University Press.