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Understanding Gene Regulation- Operons and their key importance to cellular functionality

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For many years, scientists were puzzled as to how cells regulate gene expression. How could a cell produce different amounts of genetic product at different times? How could a cell start and stop production of specific genetic products? Scientists discovered operons using a wide variety of experiments involving polymerase chain reaction and various blotting and recombinant techniques. While the area is still open to much study, a lot has been learned about operons thus far. A few examples discussed below include how operons can be influenced by antibiotics, how operons can be modified to completely repress expression, and how gene expression is influenced by the distance from the gene to the end of the operon. The results found from these experiments are exciting, and could one day potentially aid in curing diseases and disorders, as well as answering many questions about genetic expression.

Introduction.
Cells possess an extremely large genome with a wide variety of information. While much of it is expressed at some point throughout a cell's life, different amounts of genetic product are needed at different times. This is the fundamental concept between operons. Operons allow genes to be expressed and repressed. Essentially, the operon is a switch, made up of the promoter, operator, the genes themselves, and the terminator. The promoter is the sequence that initiates transcription of the genes. The operator is the sequence to which a transcription factor binds and, after transcription of the genes, the terminator signals the end of transcription. Repressors are additional parts of operons, proteins that bind to the operator sequence to prevent transcription (Figure 1.a) (1). Operons also have activator proteins that bind to the operator sequence to enable transcription. Different operons vary in their use of activator and repressor proteins (Figure 1.b)

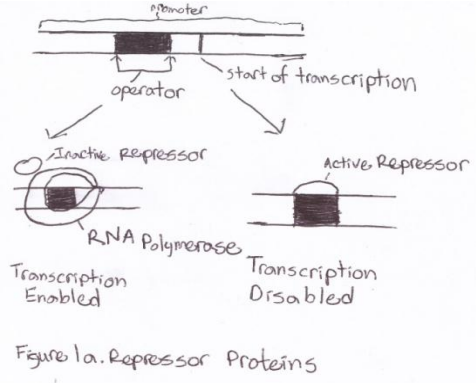

Figure 1a. Repressor Proteins

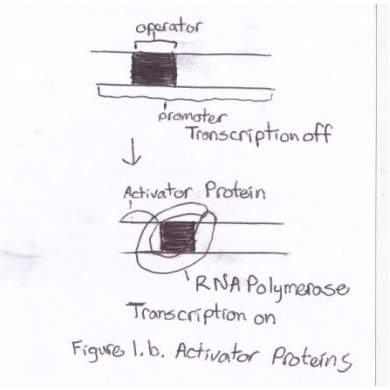

Figure 1.b. Activator Proteins

Recent Progress

Recently, as stated above, several exciting discoveries about operons have made. To start off, it has recently been discovered that antibiotics can be used to affect operons. By doing this, an element of control over operons can be exerted by the experimenter. This was conducted by fusing the tetracycline resistance repressor to the activating domain of virion protein 16 of the herpes simplex virus, creating a tetracycline controlled transactivator (tTA). This transactivator stimulates transcription from a minimal promoter sequence (1). This is made possible due to the fact that the tTA binds to the tetracycline operator sequence (tetO), which is prevented by tetracycline. The transactivator was then integrated to a Luciferase gene controlled by a tTA dependent promoter. Following this, luciferase activity was monitored in the presence or absence of tetracycline (2). The results found were as expected; luciferase activity was much lower in the presence of tetracycline than without it. As seen in (figure 2), small increases in tetracycline affected the luciferase activity negatively by several orders of magnitude.

The second study conducted also dealt with controlling operons. They created a plasmid vector containing the pBAD promoter of the arabinose operon and the positive and negative regulator of the promoter, araC. Essentially, in the presence of arabinose, transcription was activated by the pBAD promoter. The phoA gene was used to monitor expression and it was found that the ratio of induction to repression can be 1,200 fold, compared to other less efficient methods (3). Thus, expression of the gene can be modified greatly, and repressed to very low levels in the presence of glucose, which represses transcription. Additionally, it was found that due to the kinematics of this method of expression, it affects the temperature of the cell less than that of others. Following the creation of the pBAD vector, cells were grown in arabinose positive and arabinose negative cultures, and alkaline phosphatase (AP) activity, a protein closely linked to phoA production was measured. This was conducted first by shifting cells with the new vector (ara) and wild type cells (ara^+) from an arabinose medium to a glycerol medium, and then shifting the two types of cells from a glycerol medium to an arabinose medium. As expected and stated in (figure 3), amounts of AP activity were far greater in the arabinose culture.

The third study conducted dealt with the effect of the distance from the end of an operon to a specific gene on the magnitude of expression. This was found to be a result of increased transcription of the genes further from the end. The study was conducted by use of the well-studied Lac operon, inserting genetic sequences for cyan, yellow and cherry fluorescent proteins. These genetic sequences were inserted at various positions from the start codon to the end of the operon. As seen in (figure 4), average expression of the proteins when plotted as a function of distance from the end of the operon had a strong, positive, linear correlation. The study also found that gene expression increases with distance due to increased protein production and not decreased protein degradation or delay in expression by downstream genes by comparing the concentration of CFP and YFP at the first and third position in an operon at varying times. A final noteworthy discovery was that after conducting Northern blots to measure mRNA concentrations and their decay, it was determined that protein production is six times greater during mRNA transcription than during its release (4).

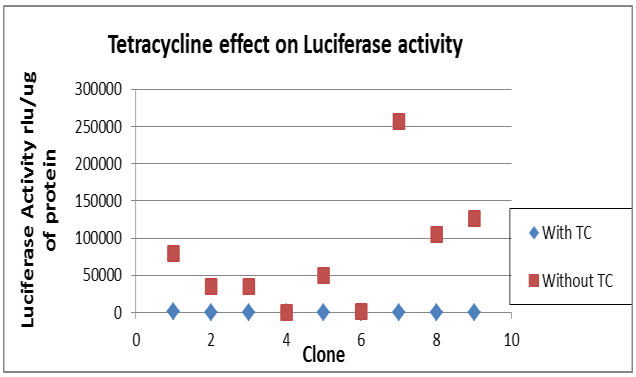

Figure 2: Tetracycline effect on Luciferase Activity

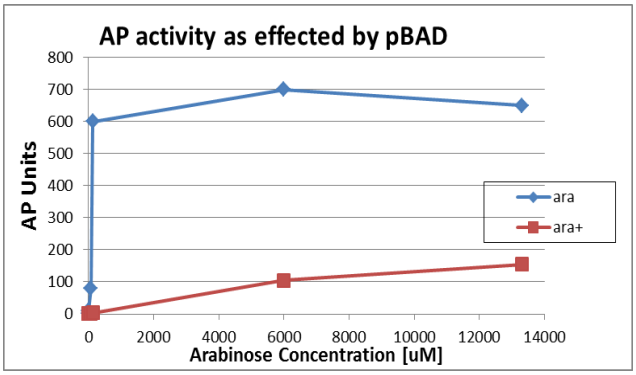

Figure 3: AP activity as affected by pBAD vector

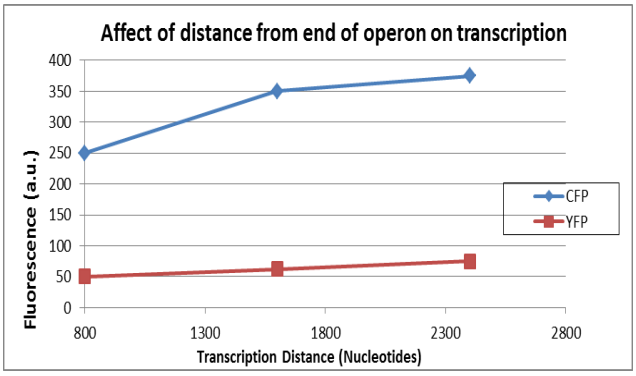

Figure 4: Effect of distance from end of operon on transcription

Discussion

The studies mentioned all deal with key concepts regarding operons. The first study addresses how efficiently gene expression can be modified by the presence of an antibiotic that prevents transcription of an operon. The real significance of this, however, comes from the pairing of a repressor with an activator. This proved to be much more efficient at inactivating the gene. Results from the study show that AP activity decreases greatly in the presence of tetracycline. This is due to the lower concentration of activators needed to carry out this process than repressors, which, by contrast, compete with transcription factors for binding in a promoter region. This is extremely important because it shows that genetic modification can take place with relatively small, nontoxic amounts of activator (which in this case is the antibiotic, tetracycline). The arabinose study demonstrates the fact that genes can potentially turned completely on or off. The fact that the pBAD vector containing the pBAD promoter turned on transcription in the presence of arabinose was proved by higher levels of AP activity. Furthermore, this method of gene regulation has a very low impact on cellular temperature, which means it interferes less with other cellular reactions affected by temperature. This is an exciting find because by using this method, scientists can conduct a wide variety of genetic experiments in the future requiring them to completely turn off certain genetic sequences while not affecting the temperature of the cell. This will allow for the study of a wide variety of phenotypes of cells. It is very likely that this methodology will lead to many exciting new finds in the future. The third study found that genetic expression of a specific gene varies based on its distance from the end of the operon. Specifically, the further away from the end it is, the greater expression will be. Again, this has many exciting implications for further research. In future experiments using this concept, scientist will be able to vary expression of certain genes, causing changes in phenotypes, as well as possible changes in productivity of cellular functions. While it may seem that these are very small advances, when put together it is possible that in the future, cures for diseases and disorders will be discovered thanks to these discoveries. It should also be noted that while many advances have been made in this field thanks to the intense study and modification of operons, there is still progress to be made. Specifically, many modifications to genomic sequences still have unclear effects on phenotypes. Although operons play a significant role in this, it is a much more complex system. However, with the increases in the understanding of operons and other aspects of genetic regulation, advances that could save lives one day are ever present.

References

1 Alberts, B., Bray, D., Hopkin, K., Johnson, A., Lewis, J., Raff, M., Roberts, K., & Walter, P. (2010). *Essential cell biology*. (3rd ed., pp. 25-277). New York: Garland Science, Taylor and Francis Group, LLC. DOI: www.garlandscience.com

2 Gossen, M., & Bujard, H. (1992). Tight control of gene expression in mammalian cells by tetracycline-responsive promoters. *Proceedings of the National Academy of Sciences of the United States of America*, *89*(12), 5547-5551.

3 Guzman, L., Belin, D., Carson, M., & Beckwith, J. (1995). Tight regulation, modulation, and high-level expression by vectors containing the arabinose p-bad promoter. *Journal of Bacteriology*, *177*(14), 4121-4130.

4 Lim, H., Lee, Y., & Hussein, R. (2011). Fundamental relationship between operon organization and gene expression . *PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA*, *108*(26), 10626-10631.

