Name:

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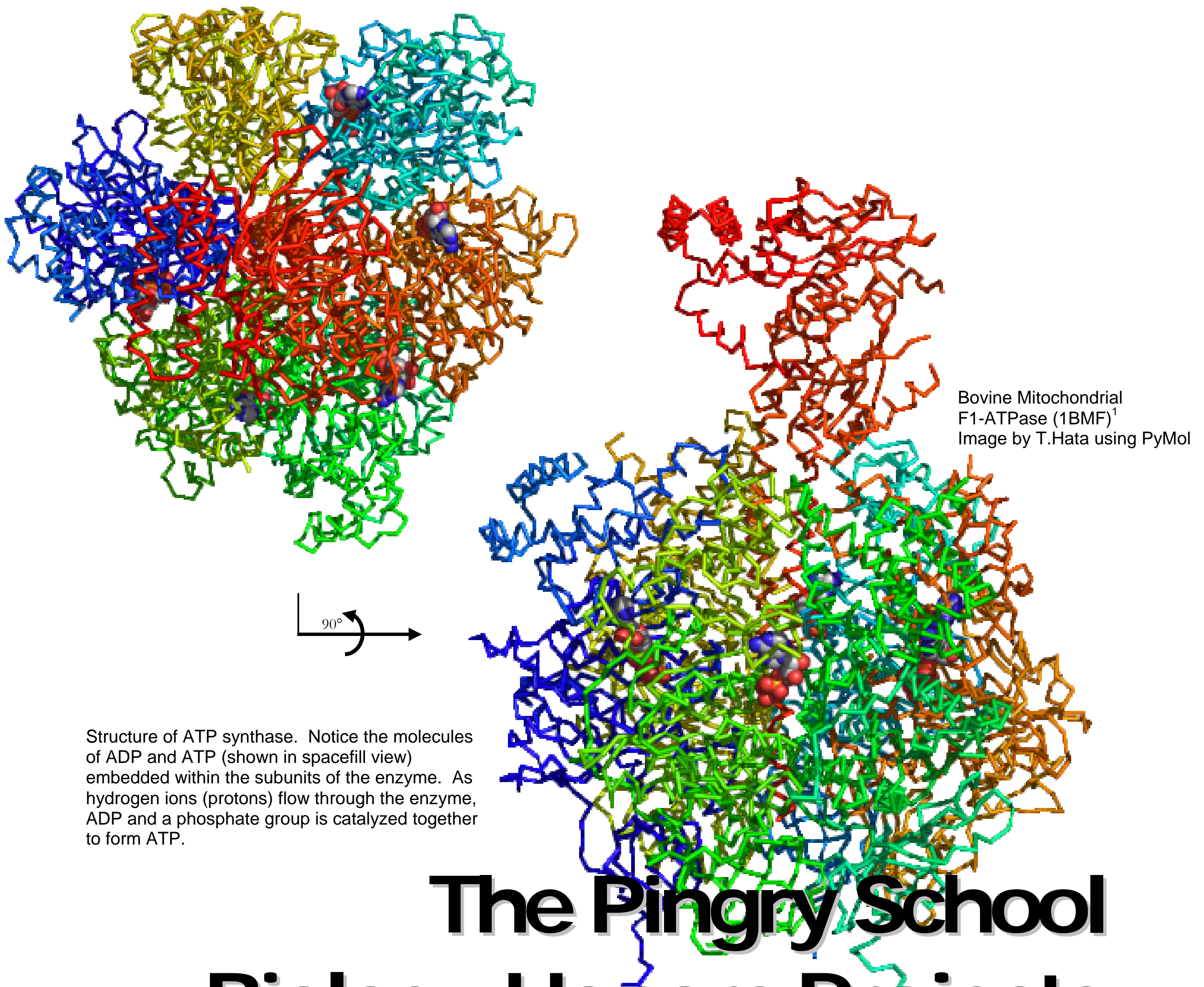
Project
3

ENERGETICS AND GENETICS

Cellular Respiration, Photosynthesis, & Genetics



Bovine Mitochondrial F1-ATPase (1BMF)[1]
Image by T.Hata using PyMol

Structure of ATP synthase. Notice the molecules of ADP and ATP (shown in spacefill view) embedded within the subunits of the enzyme. As hydrogen ions (protons) flow through the enzyme, ADP and a phosphate group is catalyzed together to form ATP.

The Pingry School Biology Honors Projects

http://www.mybiology.com/honors_projects.htm

Guidelines for responses:

Read and follow these guidelines to complete this and future Honors Projects. Failure to do so may require resubmitting the project.

1. Responses need to be typed and submitted as a hardcopy to your teacher. Follow these guidelines:
    a. Use normal, 1 or 1.5 inch margins. Use 1.5 spacing throughout your project. Your name, class period, and teacher name should be on the top right corner of each page (use the Header function if using WORD. "View/Header and Footer").
    b. Each question (not sub-question, each question numbered by Roman numeral) should be on a separate page. Type the question out first, leave two spaces, and then type your response. This is a great help for us when we are grading the projects.
    c. Diagrams, if appropriate, can be drawn directly onto your project or on a separate attached page.
    d. Your project should be stapled together.
2. Ensure that you answer each question fully. Once you have composed your response, reread each question and compare it to your response. Cross off verbs which instruct you to do something when you are sure you have done them.
3. Answers should be in complete sentences and not in bulleted lists.
4. Honors Projects are to be individual work. While students may explore modules together, answers are to be individual work. You cannot explain answers to other students nor should you share answers. Many questions assess your ability to apply multiple concepts to novel questions or problems. Since your logic and reasoning is just as important as your final response, sharing answers takes away from the purpose of the Honors Projects. Be aware that lifting sentences and statements from references is plagiarism.

Introduction

Reflection of From Project 1 and 2

Through Project 1, you explored in detail the structural and functional significance of a number of well-studied proteins. You were introduced to the Protein Data Bank, the database used by scientists around the world to deposit and study known protein structures. You built your skills to utilize this database along with using MDL Chime to manipulate the coordinate files from the PDB website. I am sure that you are also familiar with their great educational resource, the Molecule of the Month features.

In Project 2, you were asked to apply your understanding of protein structure to examine how they are manufactured and processed in the cell. You learned of the significance of the ***signal peptide*, acting as the "ZIP code"**, determining the final destination of a protein. As you explored the reactions of photosynthesis and cellular respiration in class a few weeks ago, you should have realized and kept in mind that the localization of certain proteins and enzymes into specific organelles is crucial to controlling metabolic reactions in the cell. Imagine what could happen if an enzyme necessary for a reaction in the Krebs Cycle was incorrectly secreted out of the cell instead of transported to the mitochondria!
As we continue to discuss genetics and DNA technology in class, you will also learn how scientists engineer proteins by artificially manipulating these "ZIP codes."

Your last task was to hypothesize on the structure of a transmembrane protein. We will begin Project 3 with a reflection and discussion of that question.

The Topic for Project 3

The topics of Project 3 are extensions to concepts of energetics and an introduction to some advanced concepts in genetics. In addition to exploring in detail some of the enzymes that facilitate metabolic reactions, we will also discuss the fate and production of additional molecules through these reactions. The modules can roughly be divided into those dealing with energetics and genetics. It is strongly suggested that you review Chapters 6 and 7 and pay careful attention to the diagrams on page 102-103 in your text.

Module 3.1

Structure of transmembrane proteins

Recall that the function of a protein is dependent on its structure. You were asked to hypothesize on the structure of a transmembrane protein. One of the first tasks was to identify 10 possible amino acids that may make up the region of the protein spanning the internal region of the membrane. Analyze the following diagram to confirm some of your predictions. Figure 1 is a simplified diagram of glycophorin A, a transmembrane protein found on red blood cells. They play a number of biological roles including the binding of MN blood groups (similar to the ABO blood groups).

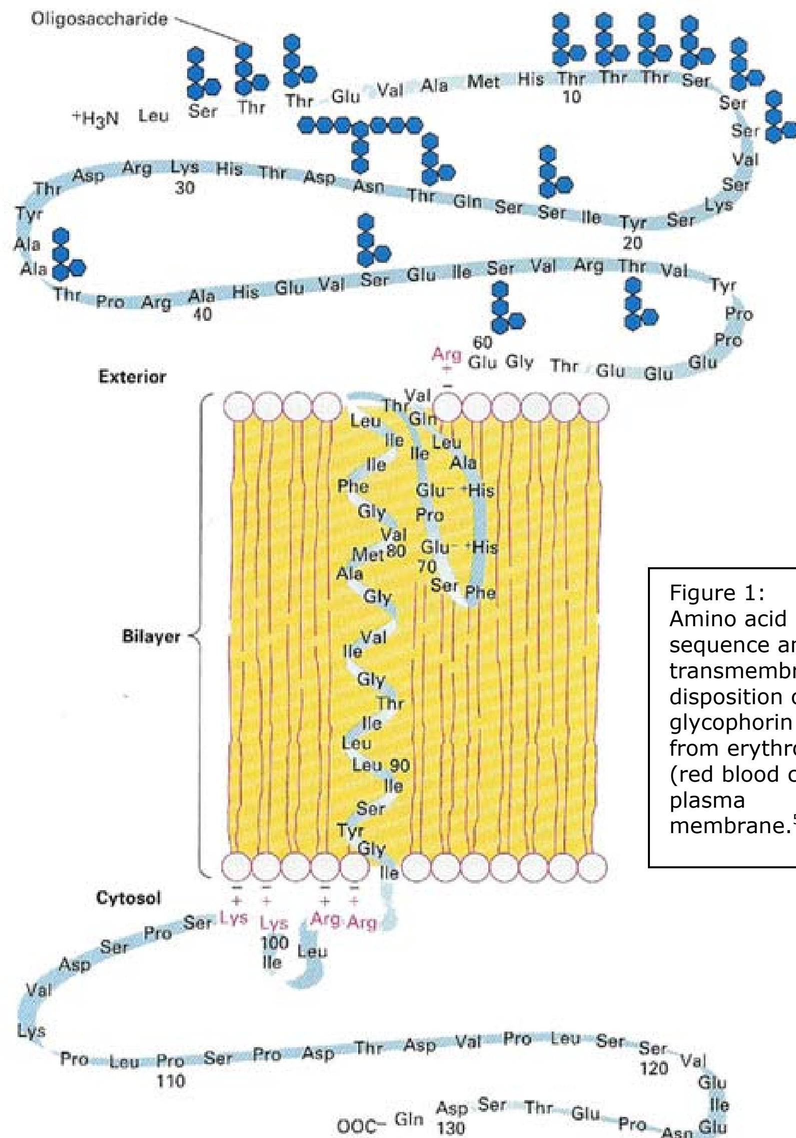


Figure 1: Amino acid sequence and transmembrane disposition of glycophorin A from erythrocyte (red blood cell) plasma membrane.[5]

Glycophorin A is actually found as a homodimer. (A dimer is a protein made of two separate polypeptides; a homodimer is made of two identical polypeptides) This diagram shows just one of these two polypeptides. Notice that the amino acids are numbered from the amino end (the NH_3^+ end). Thus, we see that each polypeptide is constructed of 131 amino acids. Let us take a closer look at the region spanning the membrane. Residues 62-95 (Val-Ile) are buried in the membrane, with the sequence from position 74

through 94 (Thr-Gly) forming an α-helix. We see that many of these amino acids are non-polar, which should be consistent with your predictions for the question in Project 2. You should also notice two ionic interactions between Glu and His forming distinct tertiary structures within the membrane. The author of this diagram also suggests additional interactions that stabilize this protein in place; the positively charged Arg and Lys residues interact with the negatively charged phospholipid head groups at both surfaces of the membrane. Finally, note the numerous carbohydrate residues attached to amino acids in the extracellular domain. These oligosaccharides (short chains of carbs… recall the terms disaccharide, polysaccharide) play a number of roles in the body including cell-cell recognition.

I. With the understanding that the membrane-spanning region of the protein would contain nonpolar amino acids, evaluate the chemical properties of the amino acids that make up the regions extending out of the membrane. We expect many of these to be either charged or polar (recall that both of these would interact with water). Evaluate this expectation by doing the following:

   For the region 62-95, what percentage of these amino acids is non-polar? Calculate the percentage of the residues in this region that are charged or polar. Also evaluate residues 1 through 60 and 102 through 131. What percentage of these amino acids is non-polar? Show your calculations along with your answers.

Module 3.2

Enzymes in the electron transport chain

We will now take a closer look at two proteins that play a role in ATP synthesis in the mitochondria. Much of the ATP in our cells is produced through the action of ATP Synthase, the enzyme in the final step of electron transport phosphorylation. Review the diagram below:

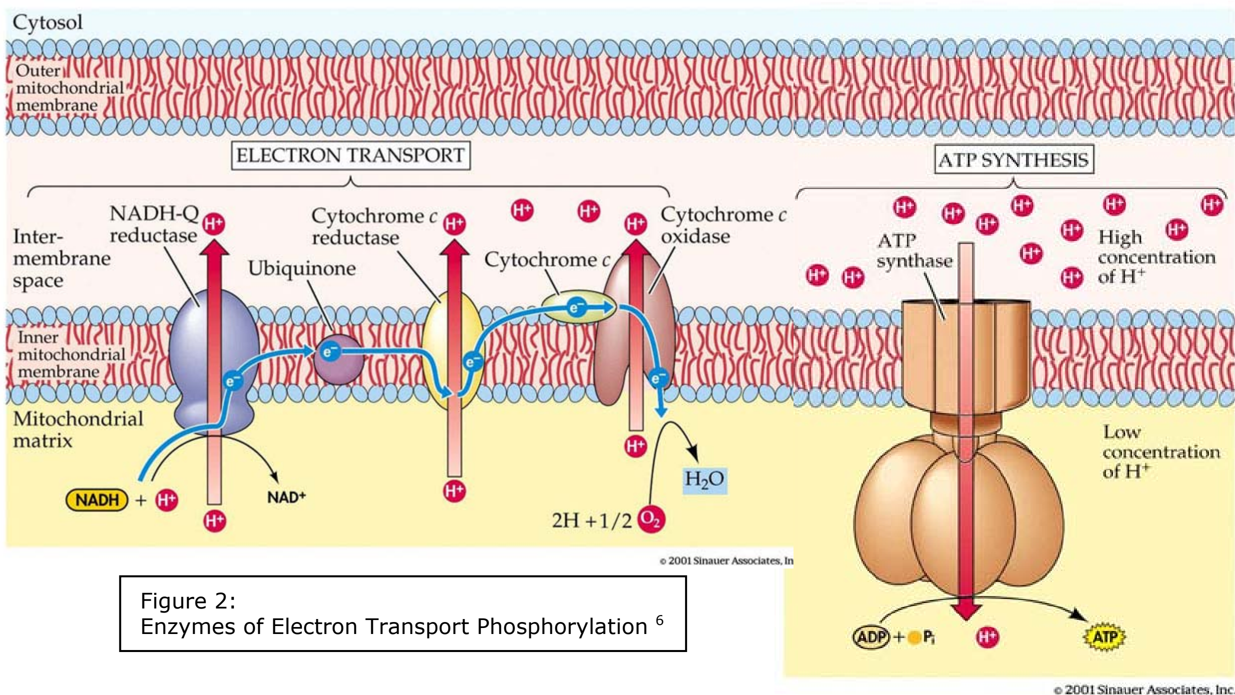


Figure 2:
Enzymes of Electron Transport Phosphorylation [6]

You should already be familiar with the roles of most of these proteins. Notice that the name of each enzyme is identified here. Pay careful attention to **ATP synthase** and **cytochrome C**. If you do not recall the mechanisms of ATP synthesis, I suggest you review your notes and diagrams in your text before you continue.

ATP Synthase

The structure and mechanisms of ATP synthase was first identified by a number of scientists in the mid-90's. Three of the scientists credited for their work with ATP synthase received the Nobel Prize in Chemistry in 1997.

Visit the Nobel e-museum at www.nobel.se and go to the "Chemistry" section. Continue to "Laureates" and to "1997." Here, along with pictures of Dr. Boyer, Dr. Walker, and Dr. Skou, you should see a link titled "Illustrated Presentation" towards the top right. Continue through this module by using the arrow buttons. Pay particular attention to the diagram titled "Four stages in ATP synthesis" on the third page.

Additional resources:
Dr. Hongyun Wang's molecular models web page is a good source for how ATP Synthase works. http://www.cse.ucsc.edu/~hongwang/ATP_synthase.html
Students unfamiliar with how motors work may find it useful to visit the Old Sturbridge Village website which has a good tutorial on how simple water wheels work.
http://www.osv.org/education/WaterPower/index.html. Another helpful animated image can be found on http://people.howstuffworks.com/hydropower-plant2.htm.
Finally, also visit the PDB website and read the Molecule of the Month feature on ATP Synthase (www.pdb.org, recall that you can access past features from the "Previous Features" link).

Scientists have successfully observed parts of ATP synthase in action. Dr. Yoshida succeeded in attaching a fluorescently tagged protein filament to part of ATP synthase that was secured on a microslope slide. His lab was able to record the movement of this filament while viewing under a microscope.

Visit http://www.res.titech.ac.jp/~seibutu/projects/f1_e.html for a brief description on how this was done along with video clips you can download to view. If you are having trouble accessing this site, the information and videos are also available on the Honors Bio website.

Cytochrome C

Cytochrome C is an electron carrier protein found in the inner membrane of the mitochondria. It is responsible for transporting electrons between two larger protein complexes in the electron transport chain (see Figure 4).

Visit the Protein Data Bank's "Molecule of the Month" feature at www.pdb.org. Go to "Cytochrome C". A note of caution; the protein referred to as *cytochrome C reductase* in Figure 4 is also called *cytochrome bc1*. You will see the later name used on the PDB website.

II. Answer the following questions.

a. The activity of ATP synthase is often compared to a molecular "motor", "rotor", or "turbine". Why do you think this is? What characteristic of ATP synthase function resembles a rotor used to generate electricity? In a few sentences, summarize how ATP synthase phosphorylates ADP to synthesize ATP by describing how hydrogen ions flow through ATP synthase to produce ATP.

b. Explain the molecular basis of how cytochorome C transports electrons. How are electrons bound to the protein? How are the electrons transferred to one protein to anther? What characteristic of the lipid bilayer are we assuming in this model of electron transport?

Module 3.3

Photosynthesis

Fate of sugars produced through the light-independent reactions

From your reading and your discussions in class, you should now be familiar with the production of PGAL through the light-independent reactions (The Calvin Cycle) of photosynthesis. PGAL, or phosphoglyceraldehyde, is also referred to by a few other names. The same molecule is sometimes referred to as G3P (for glyceraldehyde 3-phosphate) or as a triose phosphate, as we will see in the following diagram. Most importantly, the product of the light-independent reactions is a three-carbon sugar with a single phosphate group. Two of these can be synthesized into glucose through additional enzymatic reactions.

In the following diagram, you see the number of reactions involved in facilitating the production of starch and sucrose in a plant cell from PGAL. You should also see that these reactions are localized: starch production in the chloroplast and sucrose production in the cytosol (cytoplasm). Sucrose is the form by which carbohydrates are transported through the rest of the plant. It is carried through the vascular system of the plant (like the blood vessels in an animal) from photosynthetic cells to the rest of the plant; e.g. to the root cells that are not producing their own sugars.

Figure 3:
Reactions and localization of starch and sucrose synthesis. [8]

The syntheses of starch and sucrose are competing processes that occur in different cellular compartments. Starch synthesis and deposition is localized in the chloroplast. Sucrose is synthesized in the cytosol. The partitioning of PGAL (labeled as Triose Phosphate) between starch synthesis in the chloroplast and sucrose synthesis in the cytosol is determined in part by the concentration of phosphate groups (labled as P_i). Keep in mind that these will accumulate as ATP is hydrolyzed into ADP. When the cytosolic P_i concentration is high, chloroplast PGAL is exported to the cytosol in exchange for P_i and PGAL can be metabolized for ATP production or used in sucrose synthesis. When the cytosolic P_i concentration is low (for example, when ATP consumption is low), PGAL is retained within the chloroplast, and starch is synthesized. Note that the reactions on this diagram can be reversed.[8] The two digit numbers on the diagram (e.g. 5-5) refer to the names of enzymes and structure of intermediates listed on a handout that should be downloaded at www.mybiology.com/honors_projects.htm.

What was described above was studied by scientists using cell fractionation, in which organelles are isolated from cells and separated. Scientists are then able to isolate and study enzymes within the compartments.

The chloroplast is one of a group of organelles known as plastids. Plastids have their own DNA, and make many of their own proteins and enzymes. The different types of plastids include amyloplasts (store starch), etioplasts, elaioplasts (store fat), chromoplasts (pigments, especially in colored portions of plants such as petals and ripe fruits), and leucoplasts in addition to the chloroplast. Each type of plastid is a differentiated form which derives from a proplastid. Just as each different type of cell in your body has the same genes, but expresses them differently, similarly each different type of plastid has the same genes, but the way in which the genes are expressed is different. As a result, each enzyme can be turned off, turned up, or turned down.[9]

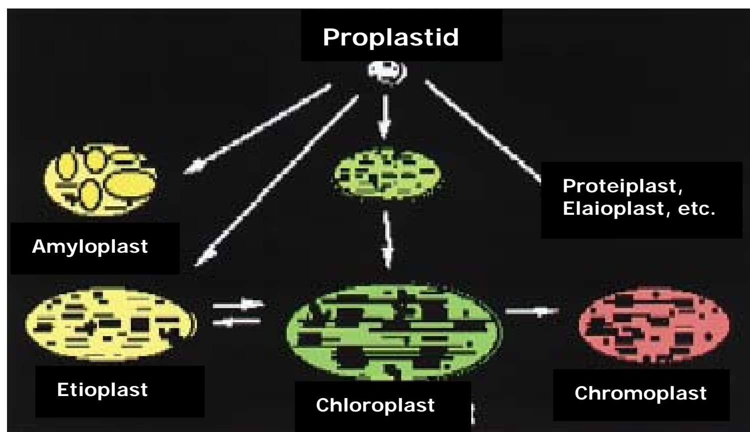


Figure 4: Development of plastids.

Now let's apply our understanding of biochemistry to understand different types of corn (*Zea mays*). Corn comes in a large variety of different types which include flour corn and sweet corn. In flour corn, the carbohydrates in the amyloplasts are almost entirely composed of starch. In sweet corn, significant amounts of sugars remain. In other varieties of corn, the number and activity of elaioplasts affects fat content. All of this is controlled by the genetics of the plant. Just like some people produce more melanin resulting in darker skin, enzyme levels in different types of corn can differ significantly. This leads to the many variety of corn; the same concept applies to the variety we see in other plants.

Imagine you had the ability to increase or decrease the rate of individual reactions in Figure 5. How might you engineer sweeter corn? How about engineering plants that store more of its sugars as starch? Recall that advances in genetic engineering allows us to manipulate individual genes in plants.

Based on the prior information provided to you, it seemed that this could be easily accomplished by manipulating the relative levels of free phosphates (P_i) in the chloroplast and cytosol. This idea, although it may seem logical, is not biologically possible; you can't "inject" phosphates into each cell of a plant. **All biological systems are controlled by mechanisms involving different enzymes**. For example, although you cannot directly change the amount of P_i in the chloroplast, you could decrease the levels of enzymes that facilitate reactions (5-2) or (5-5).

In order to produce higher levels of sucrose in corn cells to make them sweeter, one might decrease levels of enzymes facilitating (5-5) and (5-7) while increasing levels of enzymes facilitating (6-8) and (6-9). Notice that pathways in both the chloroplast and the cytosol are identical until the formation of Glucose-1-phosphate. Thus, the ultimate fate of the carbon fixed through photosynthesis depends on what enzyme further modifies Glucose-1-phosphate.

At the time you were asked to think about these questions, you were not yet familiar with the role of DNA on determining amino acid sequence and protein structure; I simply told you to "imagine that you had the ability to manipulate the sequence of amino acids on a particular protein." You should now know exactly how this could be accomplished.

Using the tools of biotechnology, including restriction enzymes and DNA ligase, scientists can, with relative easy, "cut & paste" strand of DNA together. The gene coding for a particular enzyme can be modified to contain the signal peptide sequence for a different target location.

Visit the Protein Data Bank's "Molecule of the Month" feature at www.pdb.org. Read the module under "Restriction Enzymes" in 2000 and "DNA Ligase" in 2004.

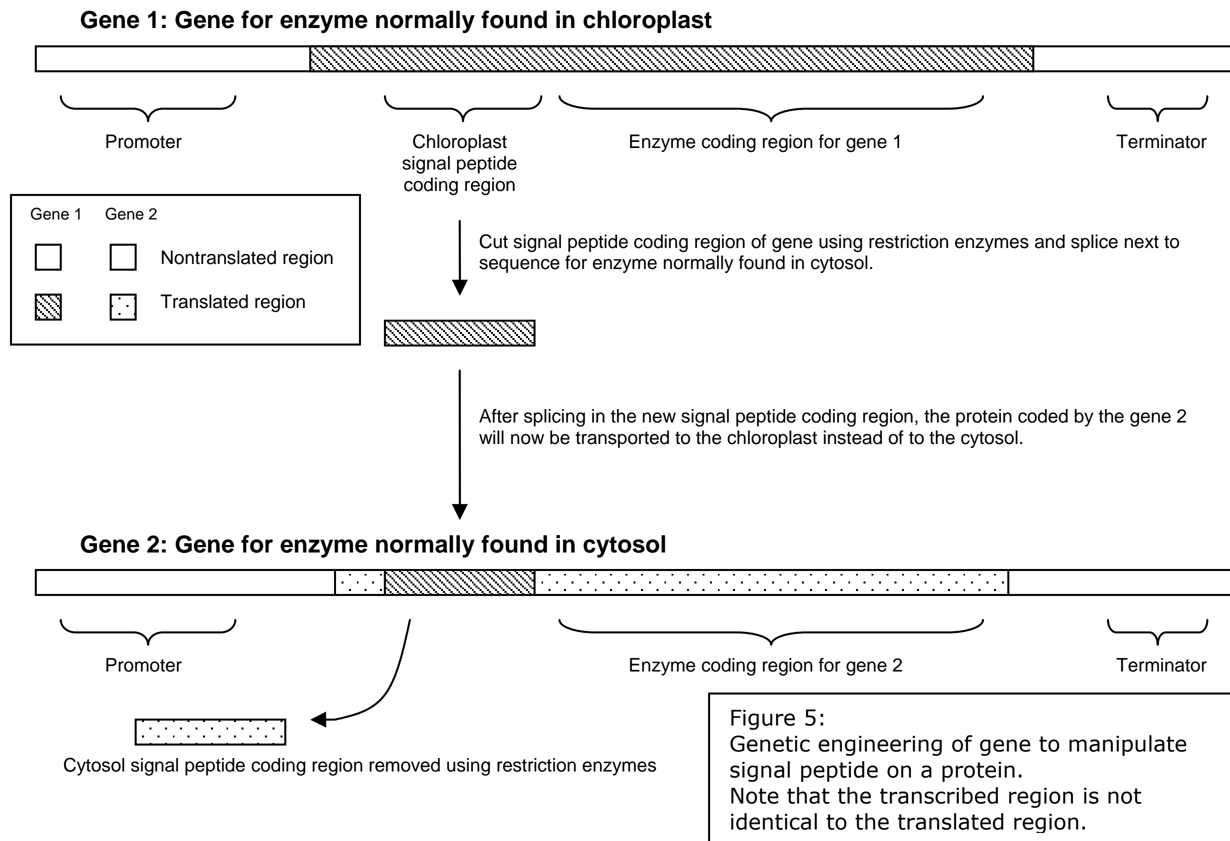


Figure 5:
Genetic engineering of gene to manipulate signal peptide on a protein.
Note that the transcribed region is not identical to the translated region.

The diagram above describes how it may be possible to engineer a plant so that a protein normally found in one organelle can be directed to a different organelle. This will ultimately affect the functions and characteristics of the cell and the entire plant.

III. In your own words, describe the function of the signal peptide. Then describe how you would use manipulate the signal peptide on various genes to engineer corn that may produce more sugars as sucrose instead of storage as starch, possibly leading to the production of sweeter corn.

Module 3.4

Genetic Engineering: Modifying enzymes and proteins through modification of DNA

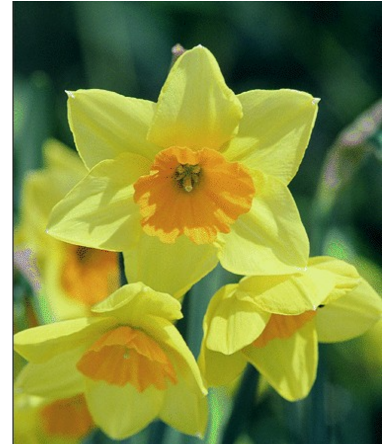


Figure 6:
The daffodil flower contains high levels of beta-carotene which also gives it its distinctive yellow color.

Now think about this. Imagine that scientists identified the genes from the daffodil flower that codes for the enzymes necessary to make vitamin A (the enzymes produce beta-carotene, which is converted into vitamin A. Beta-carotene is also responsible for giving the daffodil its bright yellow color). The scientists then take these genes and splice them into the rice plant. The result was a more nutritious rice plant that could produce higher levels of vitamin A. This was actually accomplished by Dr. Ingo Protykus and Dr. Peter Beyer a few years ago in hopes of helping children in developing countries that go blind because of insufficient vitamin A. This engineered rice was called "Golden Rice" for the yellow tint caused by the elevated amount of beta-carotene in the grain (seed).

Figure 7:
Goldren rice received its name from the yellow tint caused by elevated amounts of beta-carotene. Vitamin A deprivation during development can lead to blindness.

Does "Golden rice" sound like a great thing to you? Although it was developed with what most would consider great intentions, it turned out to be one of the most controversial GMO's to be announced. Go to www.mybiology.com/honors_projects.htm to download an

article describing Golden rice. Also, search the internet with the keyword "golden rice" to browse through other resources describing the technology and product. It is my guess that you will come across a significant number of anti-GMO websites demonstrating how controversial GMO's continue to be.

IV. Using what you understand about how genes can be manipulated, consider the following information and answer the question.

Recall that the promoter region is responsible for attracting RNA Polymerase to initiate transcription. If you think about it, every single gene in a cell must be preceded by a promoter region; yet, not all genes are expressed in a single cell. A leaf cell is a leaf cell because it expresses the genes necessary for the structure and functions of a leaf. A root cell, in contrast, will most definitely express a different set of genes. We describe this by saying that different cells have "different patterns of gene expression." Scientists now understand that this is partially due to differences in the promoter sequence. In other words, the promoters activated in a leaf cell have a slightly different sequence than those activated in a root cell. This difference is recognized by the various transcription factors found in eukaryotic cells to control gene expression.

Consider Golden rice. Imagine that scientists found that elevated amounts of beta-carotene in the root cells disrupt its functions. Thus, it is not favorable to have this new gene expressed in the root cells.

a. Since the beta-carotene is only beneficial when digested by a person, which cells would be the NECESSARY target of the new gene? What cells would not need to express the new genes to maintain the benefits of Goldren rice?

b. Using appropriate diagrams (similar to figure 5), describe what you might do to produce a more efficient type of Goldren rice that has the beneficial nutritional values but does not produce beta-carotene where it could possibly disrupt normal cellular processes in non-target cells.

First I screened 1×10^9 plaques, recovered phage, grew that up and did a phage prep, did a partial XbaI/BsteI digest, excised a fragment from a low-melt gel, used it as a probe on a genomic library, did a BAC prep, cut with *Not* I and treated with Klenow, ligated on some adapters, cloned it into an expression vector, packaged it into a phage, and then I left it out on my bench over the weekend...

Figure 8: Genetic engineering, although simple by concept, often requires complex procedures and equipment.

Notes and Bibliography

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   Structure at 2.8 A resolution of F1-ATPase from bovine heart mitochondria.
   Nature 370 *pp.* 621 (1994)

   Image generated using PyMol

2. Research Collaboratory for Structural Bioinformatics
   H.M.Berman, J.Westbrook, Z.Feng, G.Gilliland, T.N.Bhat, H.Weissig, I.N.Shindyalov, P.E.Bourne

   The Protein Data Bank
   Nucleic Acids Research, 28 pp. 235-242 (2000)

3. MDL Chime is a registered product of MDL Information Systems, Inc. The distribution and use of the product through www.mybiology.com is limited to educational use only.

4. "Molecule of the Month" features are illustrated and written by David S. Goodsell of the Scripps Research Institute and Shuchismita Dutta at the Protein Data Bank.

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The Pingry School Biology Honors Projects have been developed and written by Tommie S. Hata. Thanks to those who have contributed by offering suggestions and providing help with content. A special thanks to Dr. Tim Herman and Dr. Mike Patrick of the Center for BioMolecular Modeling for their inspiration through the summer modeling institute and those at the Protein Data Bank for their work with the PDB website.

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