Investigating the ‘Apobec’ Protein Family for better Immune Health

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Background

Nancy Li is an undergraduate student at USC who is pursuing her bachelor’s degree in Biological Sciences, with a minor in business. A native of Palo Alto, the heart of Silicon Valley, Li has always been fascinated and immersed in science and technology. Her parents are both scientists, which made her engrossed in research at a very young age. As Li says, “ I remember the first time I set foot in to a lab when I was six years old. Since then, I have been exploring and exposing myself to different subjects of research.” Specifically, Li is interested in research focusing on understanding diseases and disorders. She hopes that developing such an understanding of this comprehensive field will help her become a successful pediatrician.

Nancy Li has been a research intern in Dr. Myron Goodman’s lab for almost two years. Dr. Goodman is a biochemistry professor is currently focused on three major projects researching the molecular basis of mutagenesis. The first is centered on investigating biochemical and physical-chemical mechanisms that control DNA replication fidelity. The second focuses on examining DNA repair systems, such as the SOS-induced error prone repair in *E. coli*. Goodman’s third project is concentrated on identifying and studying mechanisms of different DNA processes, such as replication, repair and nucleotide metabolism.

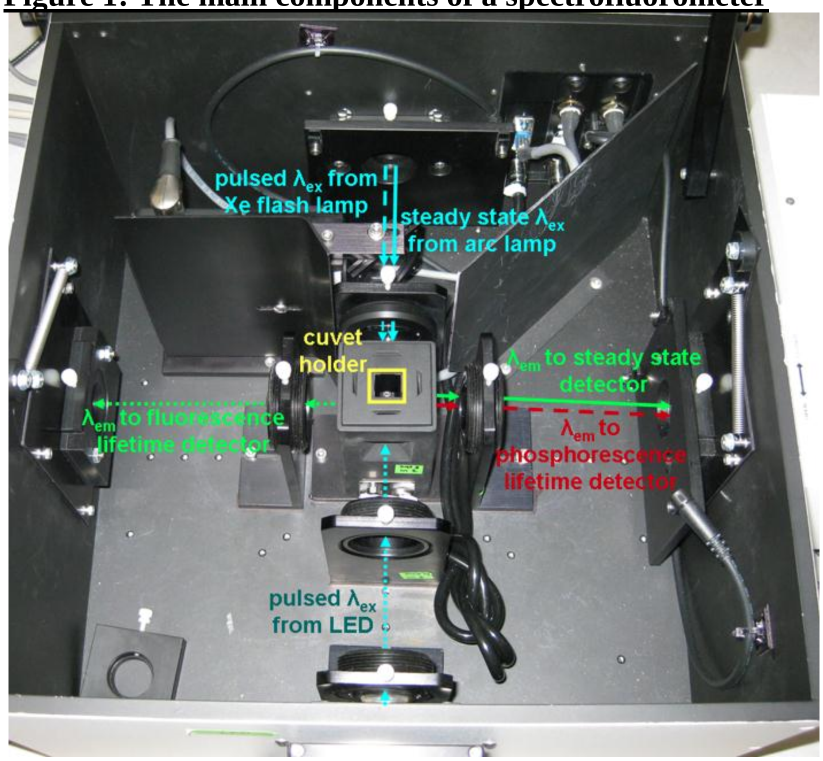
The main purpose of Li’s research is to investigate biochemical and physical mechanisms that govern DNA replication through the effects of different protein components. The lab’s research concentrates on the Apobec (Apolipprotein B mRNA editing enzyme, catalytic polypeptide-like) protein family. Li decided to intern for Dr.

Goodman because she did not have much prior experience in biochemistry and wanted to learn more about the subject through research. “Little did I know, this would be one of my most fascinating and rewarding research experiences”, says Li.

Methods and Materials

The main machinery used in the research is a fluorescence and phosphorescence spectrofluorometer. Specifically, Li measures the DNA and protein binding through the spectrofluorometer. This measures wavelengths and intensity of wavelength emissions at different chemical concentrations of a protein sample. Figure 1 shows an image of the inside of a spectrofluorometer. Each protein sample with different concentrations is pipetted in a cuvette, a small, square cross-section tube that is used to hold samples for spectroscopic experiments. The spectrofluorometer uses light with high intensity, in order to provide the sample (in this case, DNA and protein), with as many photons as possible, which maximizes the number of molecules in the excited state.

Figure 1: The main components of a spectrofluorometer



Members of the Apobec protein family interfere with reverse transcription by acting as activated induced deaminases that induce deoxycytidine to deoxyuridine mutations. Transcription is the process of a segment of DNA being copied into RNA, which is the first step of gene expression. Reverse transcription, however, is when DNA is made with an RNA template. Deaminases are a class of enzymes that catalyze the process of deamination, the removal of an amine group from a molecule. Figure 2 shows the steps of reverse transcription. This can lead to consequences, such as mutations in

DNA bases, as seen in Li's research. Figure 3 shows the data that is received in such experiments that Li conducts. The Y-axis of the graph represents the protein concentration, while the X-axis represents the difference in anisotropy. Fluorescence anisotropy is used to generate binding curves for DNA-binding proteins. It is the when the light emitted by a fluorophore has different intensities for different protein concentration. The difference in anisotropy is used to calculate the degree of binding of the protein to the DNA.

Figure 2. The Process of Reverse Transcription

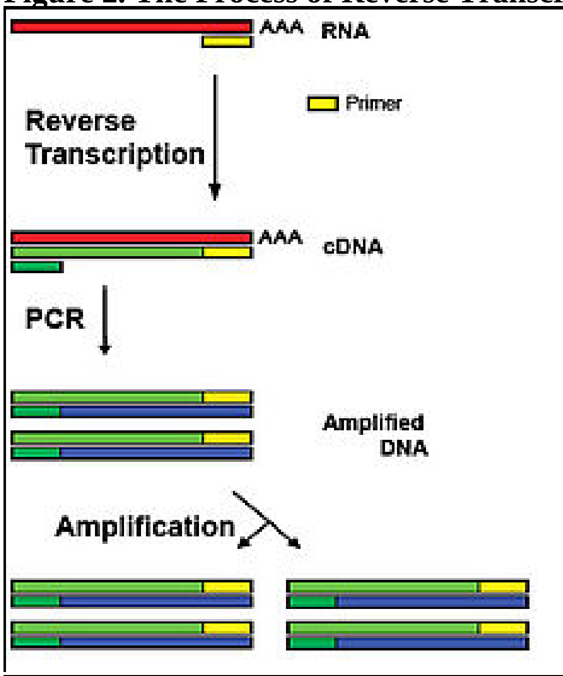
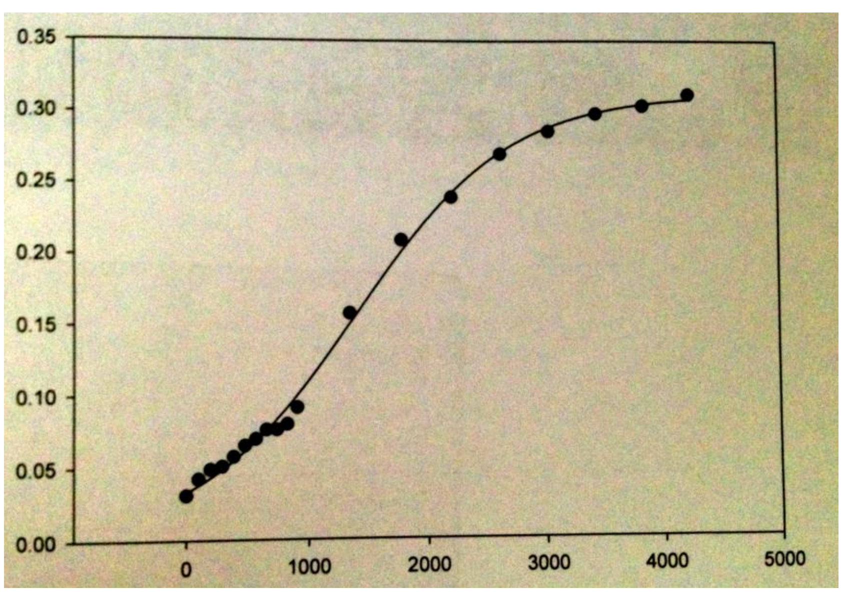


Figure 3: Graph measuring Protein concentration (Y-axis) and Anisotropy (X-axis)



Applications of the Apobec Protein Family for the Future

The Apobec family plays a role in mutating a deoxycytidine to a deoxyuridine, both of which are nucleosides. This protein family includes 11 proteins that all have one thing in common: inducing mutations (change in genetic information) for the better. Although mutations are commonly known to harm human health, this special family of proteins does the opposite. “The Apobec protein family is extremely important to research for human health because it has been found to play important roles in adaptive and innate immunity”, mentions Li. For example, Apo3, a member of this protein family, acts to restrict retroviruses. In medicine, this is important for various diseases caused by retroviruses, such as different types of cancer and AIDS. If known how to correctly incorporate these types of proteins in retrovirus-induced diseases, the human population would greatly benefit from that discovery.