SAS Honors Program
Capstone Report
Option C
Molecular Biology of Cells, Fall 2015
Biological, Biomedical and Social Aspects of Aging, Spring 2016
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May 4, 2016

Introduction

My journey on the path to graduating with a major in Cell Biology and Neuroscience began with a mere interest in the brain and nervous system and led me to a point where I now have a panoramic view of the entire field. More importantly, it revealed an abyss of concepts still not understood, pointing me in the direction of prospective exploration and discovery. This scholarly analytic report examines my experience in two graduate level courses not only to complete Capstone Option C, but to top off my journey with Cell Biology and Neuroscience (CBN). Although I did not conduct my own specific research study, I expanded my knowledge of experimental techniques, gained exposure to the results of numerous studies, and learned to analyze these findings for biological significance. Thus, I found it appropriate to structure my review of the experience parallel to the format of an actual research-based honors thesis. In the fall of my senior year I took the graduate course entitled Molecular Biology of Cells, followed by Biological, Biomedical and Social Aspects of Aging in the spring. My personal focus during this time was, by allowing the material from both courses to complement one another, to grow better accustomed to a more integrative style of learning before I am faced with more challenging concepts in future professional settings. These graduate-level courses gave me the opportunity to get a stronger grasp on topics that had been introduced throughout the CBN track, including, but not limited to, signal transduction, DNA replication and repair, stem cells, the cell cycle, and cell motility. Without a doubt, the Capstone experience has strengthened my capacity to apply these classroom concepts to the investigation of relevant modern day issues.

Objectives

My choice to fulfill the Capstone through completion of Option C rather than an honors thesis was the combined result of my research experience and learning objectives. In my junior year, as I anticipated the Capstone slowly approaching, I started volunteering as a research assistant in an Immunohistochemistry lab, where a study on Schizophrenia attracted my interest. Although I valued the experience, I found that even after months of attending lab meetings and learning the various techniques and procedures, I did not feel competent or inspired enough to take on my own project. I attributed this lack of motivation to the realization of how time-consuming and tedious it could be to study just one pathological mechanism. What I really wanted was to bring my physiological proficiency up to par with that of the graduate students I was working with, so that I could better understand and discuss all of the different projects being worked on. I recognize that a thesis project would have been a step in this direction, but I wanted to devote more time to mastering a wide range of concepts before settling down with just one. One day my research professor asked why I wanted to go to medical school. After some discussion we concluded that since my passion was to help people in need rather than conduct scientific research, then a research-based honors thesis may not be of use to me as long as I could otherwise prove my critical thinking skills. This conversation is what first caused me to consider the possibility of completing Option C.

Before finalizing my decision, I wanted to assess whether taking a different route with the Capstone was in the interest of my learning goals. Since I had not declared my major until the end of sophomore year, my time exploring CBN courses had been thrust

into the narrow slot of two years. Naturally, I liked the idea that Option C presented the opportunity of putting extra time and attention towards consolidating more CBN knowledge. Next, I evaluated which skills I wanted to improve before medical school. When it comes to studying, my strength is detailed memorization. Having already taken a few upper-level science courses, I knew they tend to stress the idea of focusing on main concepts rather than specifics. I decided that I would benefit from taking graduate courses, which would most likely be similar in this aspect, by being able to practice this method of studying. I also realized that one of my biggest fears was having to read and analyze scientific journal articles. While completion of a Departmental Honors Thesis may have prompted me to read these types of papers for reference, graduate courses would allow me to focus more so on my analytical techniques regarding them. Ultimately, my reason for wanting to pursue higher-level coursework rather than a specific thesis topic was to broaden my horizons of CBN knowledge as much as possible before graduating.

Materials and Methods

In this section I address the way in which material was presented in these graduate courses and some of the experimental methods of particular importance in the curriculum. Both courses presented an extensive array of concepts, inducing me to exercise my ability to summarize, organize, and integrate new information. The aim of Molecular Biology of Cells was for students to master factual and conceptual knowledge in concepts of cell biology, which was tested by three exams and a cumulative final. A

term paper was due at the end of the semester, which was to critique a scientific journal article of our choice. This allowed me to begin using scientific reasoning to evaluate current research, a practice which was of even greater importance in Aspects of Aging. The transition between these two courses could not have worked better, as it allowed for the wide range of biological concepts initially mastered to then be applied to understanding one topic relevant to current health and society. Aspects of Aging was divided into four major themes, which included the impact of aging on modern societies, physiology of senescence, molecular mechanisms of aging, and the aging brain and neurodegeneration. While Molecular Biology of Cells was taught by four professors, all in the departments of CBN or Molecular Biology, each lecture in Aspects of Aging was taught by a different faculty member, and they came from a multitude of educational departments. Exposure to a variety of fields other than CBN provided a better understanding of the real-life application of biological concepts. Class sessions consisted of a formal lecture followed by questions and discussion. Each block of lectures was followed by a "journal club" day, during which we discussed our critiques of assigned scientific journal articles. This course was critical in my growth as a pre-medical student because not only was I able to practice analyzing scientific papers, but I was able to become more comfortable discussing them with my peers. The combined learning methods of the two courses effectively provided a solid foundation for future study and professional careers.

In both courses, new concepts were usually supported with evidence from the results of scientific studies, which allowed me to become more familiar with some

experimental methods. These included, but were not limited to, genetic analysis of mutants, fluorescence microscopy, and Western blots. I mention these techniques because they are relevant to my discussion in this report. The use of mutants to identify and understand new genes can either take the form of "forward" or "reverse" genetics, but a combination of both techniques is usually advantageous. Forward genetics starts with the phenotype of a mutation and traces it back to the genotype, while reverse genetics starts with a gene sequence and works towards identifying the function (Hart, 2015b). Fluorescence microscopy makes use of antibody specificity to target fluorescent dyes to a certain molecule, allowing visualization of the distribution of this molecule throughout the sample. Western blots are used often to display the detection of specific proteins in a sample. Each of these techniques serves a different purpose, but they all contributed to the knowledge gained from my work in the two graduate courses.

Results

The outcome of this Capstone experience is an even wider view and deeper understanding of CBN concepts than I had expected. I learned about too many molecular pathways and mechanisms to remember, but recognized that certain aspects of cellular function serve as overall themes to take away from the coursework. Using these themes, I was able to realize my goal of forming connections between both courses. Rather than ending my journey with a random jumble of new facts, I have mentally organized the information into a network based on overall concepts, into which I can now integrate old and new knowledge. For the purposes of this report I have drawn parallels between the

two courses using the concepts of signal transduction pathways, motor proteins, and stem cells and cell division, although there were numerous possibilities to consider.

Signal Transduction Pathways

In Molecular Biology of Cells, I extensively studied the different types of signaling pathways and their mechanisms, including growth-controlling pathways which are implicated in the metabolism of aging. This background knowledge was useful in the lecture on the IGF-like pathway in Aspects of Aging, where the focus was more on the biological effects rather than the pathway itself. Discovery of this pathway resulted from the search for genes controlling aging to determine whether lifespan could be increased. In 1993, Cynthia Kenyon isolated *C. elegans* mutants with altered rates of aging, and used forward genetic analysis to identify the genes and proteins concerned with life-span determination (Runnels, 2016). These nematodes have the ability to enter a state called Dauer, which is German for "enduring," in which growth and development is arrested at a specific larval stage if they encounter low levels of food or crowding (Runnels, 2016). This formed the basis for Kenyon's strategy, which was to use a "dauer-constitutive" mutation. She found that mutations in the *daf-2* gene greatly increase lifespan, as DAF-2 mutants lived longer than normal adults. It appeared that the DAF-2 protein controls entry into the Dauer state, increasing longevity in the absence of any effect on reproduction. The genes involved in this pathway are *daf*-2, which encodes the Insulin or IGF-1 receptor, *age-1*, which encodes the catalytic subunit of phosphatidyl inositol 3-kinase (PI3K), and *daf*-16, which encodes a FOXO-class forkhead transcription factor

(Runnels, 2016). What's more, these *C. elegans* genes in the *daf-2* pathway were found to have human homologues. The combination of *age-1* and *daf-2* is the equivalent of Daf-c in humans, and the human equivalent of *daf-16* is Daf-d (Runnels, 2016).

It would have been difficult to follow the material presented in this discussion without first understanding the structure of a simple signal transduction pathway. As discussed in Molecular Biology of Cells, a simple signaling pathway begins with the release of a ligand, or signal molecule, and the transport of this ligand to its target (D'Arcangelo, 2015). In the IGF-1 pathway in humans, Growth hormone is the first signal released, traveling from the anterior pituitary gland in the brain to the liver, where it stimulates the release of IGF-1. IGF-1 is a ligand known to modulate cell survival, growth, puberty, and gonadal function, which may all contribute to the aging process (Runnels, 2016). When the ligand binds to its receptor on its target cell, it then triggers a conformational change in this receptor which activates it. (D'Arcangelo, 2015). It is in this way that insulin-like ligands activate the DAF-2 receptor in *C. elegans* or the insulin/ IGF-1 receptor in humans. The next step in basic signal transduction is the activation of second messengers such as AGE-1, which is the catalytic subunit of PI3K, a second messenger common in growth-controlling signaling pathways in humans. These second messengers then exert their effects on Effector proteins, which mediate the biological functions of the pathway. DAF-2 and AGE-1 inhibit the effector DAF-16/FOXO, whose downstream gene targets control antioxidants, chaperones, antimicrobials, metabolic genes, and novel genes—all factors contributing to a long life span (Runnels, 2016). Another common effector involved in this pathway is Akt, a protein kinase which also

inhibits DAF-16 and promotes growth. Therefore, *daf-2* and *age-1* mutants allow for constant expression of *daf-16*, meaning an extended lifespan for these animals.

Later studies found that the role of insulin/IGF-1 signaling in controlling aging is evolutionarily conserved in organisms such as Drosophila, mice and mammals. Evidence supporting the role of IGF-signaling in human aging includes the fact that DNA variants in the insulin receptor gene are linked to longevity in a Japanese cohort. Also, mutations known to impair IGF-1 receptor function are overrepresented in an Ashkenazi Jewish cohort of people aged 100 or older (Runnels, 2016). Using the concepts of signal transduction from Molecular Biology of Cells in conjunction with data from Aspects of Aging allowed me to fully understand and appreciate the role of cell signaling in aging.

Motor Proteins

My graduate coursework led to a proficiency in the properties of the different filaments involved in cell motility and their role in maintaining normal cellular function. In Molecular Biology of Cells, I gained more insight into the functions of microtubules, which are highly dynamic filaments responsible for the organization and long-range transport of organelles throughout the cell (Kwan, 2015b). They are made from α and ß tubulin dimers, and interaction of tubulin with Microtubule Associated Proteins (MAPs) can either stabilize or destabilize these structures. While some MAPs enhance elongation and stability of microtubules, others such as kinesin 13 and stathmin destabilize the ends of microtubules and enhance shortening. As seen in the Aspects of Aging course, the changes seen in memory and behavior during aging depend on the activity of

microtubules in mature neurons (Shumyatsky, 2016). Specifically, the process of learning induces changes in the stathmin-dependent dynamics of microtubules which are essential for memory formation. As touched upon in Molecular Biology of Cells, stathmin is a negative regulator of microtubule formation. This means that unphosphorylated stathmin binds to tubulin dimers and leads to microtubule disassembly, while stathmin phosphorylation leads to its release of tubulin and allows for microtubule assembly (Shumyatsky, 2016). A learned fear experiment conducted by Uchida et al. (2014) tested for memory formation in mice. They found that in the dentate gyrus, an area of the hippocampus responsible for the formation of new episodic memories, learning induces biphasic shifts in stathmin phosphorylation and the stability of microtubules. This biphasic shift means that stathmin is first destabilized, then stabilized. It consists of an early phase at about one hour after learning and a late phase about eight hours after learning, after which the formation of long term memories occurs. In the early phase stathmin activity is high and thus microtubule stability is low. Then in the late phase, stathmin activity is low and microtubule stability is high (Shumyatsky, 2016).

To prove that memory is indeed regulated by these microtubule dynamics, it is useful to consider drugs that interfere with microtubule dynamics. I had already been exposed to this idea in Molecular Biology of Cells, where I learned that the small molecule Taxol inhibits cell division by stabilizing microtubules, preventing them from shortening and pulling sister chromatids apart. Such drugs can be used to treat cancer; as cancer cells divide quickly, they are more sensitive to the effects of Taxol (Kwan, 2015b). In Aspects of Aging, we discussed a study showing that Taxol inhibits memory at the

early phase and enhances memory at the late phase. Therefore, when microtubules become hyper-stable at eight hours after learning, injecting Taxol into the dentate gyrus at this time helps consolidate memory formation (Shumyatsky, 2016). All of these concepts ultimately led to the conclusion that interactions between stathmin and microtubules are involved in the memory decline seen in aging. We studied western blots which showed a reduced amount of stathmin in the dentate gyrus of aged mice. Less stathmin is associated with increased stability of microtubules, causing them to be less dynamic. This results in less pronounced differences between the early and late phases of the biphasic shift, accounting for weaker memory formation. Being able to apply such detailed knowledge of mechanisms to the real-life issue of aging gave me a better understanding of the significance of microtubules than I had ever achieved in previous biology courses.

Stem Cells and Cell Division

Molecular Biology of Cells explored the features of stem cells and their potential clinical uses, an area growing more and more significant in current research. Stem cells are undifferentiated cells that have unique cellular properties of self-renewal and pluripotency (Kwan, 2015c). They are capable of dividing and renewing themselves indefinitely, unlike terminally differentiated cells such as neurons that do not divide. Pluripotency means that these although these cells are not destined to become any specific type of cell, they can give rise to specialized, or differentiated, cells that are destined for specific tissues. This happens in response to internal and external signals. These special properties mean that stem cells can have the potential to produce new cells

of the liver, heart, blood, or other types of tissue. The course also covered the potential clinical impact of stem cells. For instance, stem cells can be used for the clarification of complex events in human development, such as molecular mechanisms of self-renewal (Kwan, 2015c). I was able to see this concept unfold in Aspects of Aging, where the lecture on stem cells illustrated the importance of signals in gene expression and cell differentiation as we age. What's more, this discussion called for the knowledge of cell cycle regulation that I had also gained from Molecular Biology of Cells.

According to the Stem Cell Theory of Aging, the aging process is a result of the inability of stem cells to continue to replenish tissues with functional differentiated cells capable of maintaining that tissue's original function, which is normally achieved through asymmetric cell division (Zhang, 2016). During aging, a failure of self renewal or abnormal differentiation can result in a skewed lineage or altered fate of differentiated progeny. Stem cells maintain their "stemness" by coordinating various cellular processes including polarized trafficking, maintenance of asymmetric protein distribution, mitotic spindle alignment, and formation of diffusion barriers. Defects in any of these processes of cell division can lead to stem cell aging and age-related diseases (Zhang, 2016).

While studying the cell cycle in Molecular Biology of Cells, I discovered the role of regulatory proteins, such as the cell division cycle (Cdc) proteins, which control various activities required for entry into different stages of the cell cycle (Kwan, 2015a). In Aspects of Aging I then learned of one protein in particular, Cdc42, which is implicated in the maintenance of asymmetric division through its interaction with Partitioning (Par) proteins. Par proteins localize to specific regions of the cell membrane,

controlling polarization and thus asymmetric division (Zhang, 2016). A study conducted by Florian et al. allowed us to observe the polarity defects associated with aging. Using fluorescence microscopy, they demonstrated that activation of Cdc42 by an extracellular signal called Wnt5a induced aging-like phenotypes in young stem cells *in vivo*. These cells showed altered protein localization and skewed differentiation, illustrating the role of Cdc42 and Par proteins in these processes (Zhang, 2016). Understanding the potential clinical impact of stem cells requires an in depth knowledge of cell division regulation; however, mastering these concepts may lead to regenerative therapy to treat Parkinson's, Alzheimer's, ALS, spinal cord injury, and other conditions involving cell death.

Discussion

The Capstone experience was intellectually useful because it expanded and strengthened my knowledge of aspects of cellular function relevant to the medical field today. Also important is the fact that these courses gave me much-needed exposure to the scholarly challenge of working closely with medical journal articles, whether applying them to new concepts learned in class or analyzing articles on my own. What's more, the graded term paper in Molecular Biology of Cells provided me with feedback of my written analytical skills, and the journal club discussions in Aspects of Aging provided me with feedback of my spoken analytical skills. In this section I reflect upon three of my analyses and the progression in my ability to criticize them.

For my term paper assignment in Molecular Biology of Cells, I chose to review an article by Nayak et al. entitled, "Tricellulin deficiency affects tight junction

architecture and cochlear hair cells." This study considered the effects of genetic mutations causing defects in tight junction proteins such as tricellulin, which are often linked to hearing loss. Tricellulin is normally found at all tricellular tight junctions in the cochlear duct of the inner ear, and its deficiency leads to recessive nonsyndromic deafness (DFNB49). The authors of this article set out to determine tricellulin function in the inner ear, and likewise the mechanism underlying DFNB49. To carry this out, they created a knockin mouse model with a mutation orthologous to the Tric gene mutation underlying DFNB49 in humans. This mutation was predicted to result in a truncated tricellulin protein. They found that these mutants do not express tricellulin at tricellular tight junctions in the inner ear sensory epithelium, experience rapidly progressing hearing loss and grow profoundly deaf by P30, show progressive hair cell degeneration by the third week of life, and show affected ultrastructure of tricellular tight junctions in the inner ear. DFNB49 causes loss of full-length, functional tricellulin in mutant mice and disrupts the connection between bicellular and tricellular tight junctions in the inner ear, which leads to loss of mechanosensory hair cells in the organ of Corti. I decided that this study successfully provided a strong foundation for further study with its thorough examination of tricellulin function and strong factual support. An extensive analysis left me with few unanswered questions concerning the physiology of tricellulin mutation and corresponding hearing loss. Moreover, they did a good job of addressing the multiple functions of tight junctions rather than limiting their discussion to just one. This demonstrates their ability to look at the bigger picture rather than focusing on any one detail, which minimizes any doubts the reader might have towards the validity of their

arguments. I also took notice of the fact that the authors considered an impressively wide range of tricellulin function. For instance, since one of their arguments is that a lack of tricellulin disrupts the connection between bicellular and tricellular junctions, it is important that they also realized that "changes in the ultrastructure of the tricellular tight junctions seen in the absence of tricellulin might also be due to defects in the cytoskeletal architecture or organization of other tight junction proteins" (Nayak 6), another factor that could affect normal development. Yet another positive aspect I found in this article is the fact that its arguments are based on factual information rather than opinion. Specifically, in their discussion of progressive hair cell degeneration due defective tricellulin, the authors examined the morphology of the cochlear epithelium at various postnatal developmental stages. They provided a figure displaying the degeneration pattern they observed by immunofluorescence, and by observing this figure even before reading their findings, I was able to clearly see the effects they discussed. This suggests that they did not try to manipulate the reader into seeing certain effects. Overall, I failed to find discussion that seemed too opinionated to be significant. Despite detailed arguments and sufficient data, this article could have been stronger in aspects concerning organization. For example, these authors might have considered providing subtitles in their results section to make for a more clear understanding of the flow of ideas. Also, although ideas from the results and discussion sections did not necessarily contradict one another, their scattered presentation rendered confusion about the conclusions drawn. Ultimately, I found that this article provided a strong basis for further study with a wide

range of data and an extensive analysis, but the discussion section was hard to follow in relation to the results.

One of the articles assigned for journal club discussion in Aspects of Aging was entitled, "Does oxidative damage to DNA increase with age?" This paper by Hamilton et al. provides useful insight into some of the physiological effects of aging, specifically that oxidative damage to DNA increases with age. One critique I had was that the authors failed to mention the possible external factors, such as housing conditions, that could have contributed to oxidative damage. For instance, female mice were housed in groups of four while male rats and mice were housed individually; one type of housing may be more stressful than the other. Another extraneous factor that could affect the results is handling of the rodents. Including these potential factors would have made the conclusions more valid. One purpose of this study was to determine the effect of dietary restriction on age-related oxidative damage. However, caloric restriction was only carried out using the male rodents, and it might be useful to carry out another experiment in which this is done for females as well, as males and females have differences in metabolism. In comparison with my term paper in Molecular Biology of Cells, I already showed improvement in thinking critically about experimental procedures and thinking of potential areas for future study, rather than simply pointing out strengths.

The next paper assigned for journal club discussion was "Evoked Potentials and Memory/Cognition Tests Validate Brain Atrophy as Measured by 3T MRI (NeuroQuant) in Cognitively Impaired Patients." In this study, Braverman et. al. set out to develop predictive models of forms of cerebral atrophy, for many cognitive diseases such as

dementia are related to this form of neurodegeneration. The authors hypothesized that subjects positive for cerebral atrophy would score lower on cognitive memory tasks, and that eventually studies such as these could lead to cost-effective diagnostic methods in primary care medicine. However, the subjects used in this study were all taken from a primary care facility and most of them already had pre-existing neurological deficits. Although the NeuroQuant software compares subjects' results to a database of normal healthy brains of the same age as a sort of control, there are many extraneous factors that could affect the data for this non-random group. In addition, this claims to be the largest study evaluating the relationships between 3T MRI, P300 evoked wave potentials, and cognitive memory tests in order to validate brain atrophy. This points attention to the fact that perhaps this is too large a sample size for a study of this sort. For instance, in order to asses the relationship between lobe damage and P300 latencies and amplitudes, final models were built by reducing the full models through a mixed stepwise regression method, in which only the main effects were considered. The authors say that over 1000 combinatorial models were examined. There are potential difficulties with the stepwise regression method, especially with so many different variables. It is questionable whether the authors merely molded the results to fit some of their pre-determined ideas. In general, I found the results of this paper to be all over the place. The discussion section did not delve into physiological causation whatsoever, and even though counterintuitive results were presented, the authors did not try to account for these at all. Therefore, it may leave the reader feeling more confused about these theories. Overall, while the data did show associations between atrophy and cognition tests, I did not find the conclusions

compelling enough to be able to lead to diagnostic tools in neurodegeneration. Looking back on my analysis, I again noticed an improvement in my analytical skills, for I was able to notice weaknesses in the way the authors manipulated the data rather than just how they presented it. Ultimately, my work in these two courses stimulated growth in my ability to comprehend and critique biomedical literature, which will certainly come in handy in the future.

Significance and Future Direction

My capstone experience enhanced my undergraduate studies by allowing me to fine tune my ability to comprehend and analyze current scientific research. I gained much more exposure to working with medical journals than I otherwise would have as an undergraduate. I also feel more proficient in being able to discuss a range of physiological problems than I did when I first volunteered as a research assistant. This desire to master a variety of topics in both cell biology and neuroscience has personal significance, which makes my experience even more meaningful. To begin with, someone close to me is affected every day by a skin disease called familial benign chronic pemphigus, or better known as Hailey-Hailey disease. He had joked of high hopes that for my capstone I would conduct research and finally find the cure to his condition. Although at the time I had little faith in this possibility, I held on to the fact that the pathology of his disease concerns cell junctions and cell adhesion, a major topic in cell biology. Studying this topic in a graduate-level course as an undergraduate student was a good first step towards understanding the cause of the disease and perhaps even

finding a cure, way down the road when I have even more knowledge and experience. Furthermore, my grandma in Philippines, although not officially diagnosed, shows evident signs of Parkinson's disease. Of course, it was especially meaningful for me to learn about the pathology of this neurodegenerative disease in both courses. As mentioned earlier, my ultimate goal is to become a doctor and to help patients in need. This passion was enhanced when I went on a global medical brigade to Nicaragua this past winter. We set up a free medical clinic for three days in one community, and I was able to witness up-close a multitude of doctor-patient interactions, through which I heard countless medical complaints from patients followed by medical advice from doctors. This experience reminds me that my reason for wanting to master a wide range of concepts stems from my ambition to help a wide range of people. Each of these parts of my life give me motivation to go further in depth with my studies, and Option C seemed the best way to do so while keeping each of them in mind. Indeed, the two courses covered numerous topics I could relate to, such as cell adhesion and neurodegenerative disorders, which made learning easier and more intriguing, and reinforced my appreciation for CBN more than an Honors Thesis could have.

Overall, my proficiency in CBN topics as a result of these courses will serve as a basis for future studies. After graduating, I want to read more journal articles and continue to practice analyzing them, in order to maintain this skill. Specifically, I want to read more papers about Hailey-Hailey disease and Parkinson's, for these issues are particularly important to me. My capstone experience was not only good way to end my undergraduate career, but a good way to begin my post-graduate undertakings.

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