THE MCNAIR SCHOLARS JOURNAL

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Ronald E. McNair Postbaccalaureate Achievement Program
University of Washington
Office of Minority Affairs

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(fabio@astro.washington.edu)

Volume IV

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University Of Washington

McNair Program Staff

Program Director
Gabriel Gallardo, Ph.D.

Assistant Director
Steven Woodard, Ph.D.

Graduate Student Advisors
Sarah Augustine
Rahel Bisetegne
Alyson Brooks
Greg Yamashiro

McNair Program
Office of Minority Affairs
University of Washington
375 Schmitz Hall
Box 355845
Seattle, WA 98195-5845
uwmcnair@u.washington.edu
http://depts.washington.edu/uwmcnair

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In Memoriam

This fourth edition of the McNair Scholars Journal is dedicated to the memory of Marsha L. Landolt, Dean of the Graduate School. Dean Landolt, along with her husband, Robert Busch, died tragically on January 2, 2004 in an avalanche that struck their family's cabin near Soldier Mountain Ski Resort in Idaho. Dean Landolt was a passionate advocate for diversity in graduate education and a strong supporter of the McNair program and mission at the University of Washington. She was very proud of the many McNair scholars who became involved in research as undergraduates because she understood the competitive advantage that it would bring them when they applied to graduate school. She believed that diversity would enrich graduate education and the academy, and that our McNair scholars were well positioned to accomplish this goal. Dean Landolt's passion for graduate education and her commitment to diversity will be greatly missed. We hope that through our continuing work of preparing students from low income, first generation, and underrepresented backgrounds to enter graduate education, we will honor her memory as an outstanding educator and advocate for equity and diversity in higher education.

Sincerely,

Gabriel E. Gallardo, Ph.D.
McNair Director

From the
Dean of the Graduate School

It is my pleasure and honor to present the fourth issue of *The McNair Scholars Journal*. The papers contained in this volume represent a remarkable breadth of scholarship. They also represent a depth of scholarship that encompasses the best of what the University of Washington has to offer. The Scholars, their faculty mentors, the staff of the McNair Program, and all of us at this institution are justifiably proud of this work.

The McNair Scholars' Program honors the memory and achievement of the late Dr. Ronald E. McNair, a physicist and NASA astronaut. Its goal is to encourage young men and women to emulate the academic and professional accomplishments of Dr. McNair. One of the goals of the McNair Program is to encourage students who have been disadvantaged in their pursuit of academic excellence to attain not only a baccalaureate degree, but to continue a career in graduate education culminating in a doctoral degree. It is because of this goal that The Graduate School is proud to be a partner in this program. The outstanding undergraduate students who are selected to be McNair Scholars are actively recruited by our own and other graduate schools nationwide. They represent a coveted source of talent that will enhance the professoriate of the future and other leadership roles in our society.

The scholars whose work is presented in this volume will doubtless have many opportunities to pursue their post baccalaureate studies at outstanding institutions worldwide; however, I sincerely hope that the University of Washington will be fortunate enough to welcome some of these fine students into our own graduate programs. While we have benefited greatly from their presence as undergraduates, we will benefit even more by having them become our graduate student colleagues and possibly our future faculty.

Please accept my congratulations on this excellent publication.

Elizabeth L. Feetham, Ph.D.
Acting Dean of the Graduate School and Vice Provost

From the
Vice President and Vice Provost for Diversity

The McNair Scholars Journal is testimony to the fact that the McNair Program is providing an important opportunity for students to explore the wonders of research. Many of these students would never have been afforded this type of opportunity without such a program. A committed faculty mentor ensures an outstanding experience for students who are dedicated to the rigors of scholarship. Both students and faculty speak of the special working relationships that evolve as student and mentor come together as researchers. Former McNair students are making important contributions through their research in the academy and research centers that address societal issues and scientific questions of our time throughout the nation. Many of these students attribute their success as research and scholars to the McNair Program. Finally, these essays in the fourth edition of the McNair Scholars Journal are evidence of the University of Washington's goal to insure our diverse student body a quality academic experience.

Please join me in thanking the faculty, staff and students who came together through the McNair Scholars Program and made this journal possible. It is proof of the amazing things that can be accomplished through collaboration.

Nancy 'Rusty' Barceló, Ph.D.
Vice President and Vice Provost for Diversity

From the
Director

I am delighted to present the fourth edition of the McNair Scholars Journal to our reading audience. The essays and abstracts included in this volume are the culmination of work carried out by our scholars with a faculty mentor in their field of study during the 2003-2004 academic year and in the summer of 2003. The academic year and summer research component for McNair Scholars at the University of Washington has two specific goals: First, engage students in the research enterprise at the undergraduate level, so they develop the analytical and methodological skills, academic sophistication, and confidence that will make them successful students in graduate school. Second, provide students a unique opportunity to publish their undergraduate research, so that the scholars gain an early understanding of the critical role that publishing will play in their academic careers. In this respect, the McNair Journal is a key component in the preparation of our scholars for careers in research and teaching.

The excellent research contained in this volume would not be possible without the involvement of dedicated faculty on the UW campus and elsewhere who guided our scholars during their research experience. As always, I want to extend my gratitude to the faculty for their support of our students and for encouraging them to pursue a path towards graduate education. Their guidance and support has allowed our students to grow in meaningful and significant ways, while giving our scholars the foundation to enter graduate school with confidence and solid research experience.

Our journal involves a number of people who work behind the scenes to prepare the final draft for publication. I would like to extend my appreciation to the UW McNair staff, Assistant Director Steve Woodard, and our graduate student staff, Alyson, Rahel, Sarah, and Greg, for their commitment and dedication to the McNair mission and for bringing this project to completion. They are an asset to the program and have been instrumental in preparing such a high quality journal.

On behalf of the entire McNair Staff, I sincerely hope that you enjoy reading the fourth edition of the McNair Scholars Journal.

Dr. Gabriel E. Gallardo
Director, McNair Program

Journal Disclaimer

While the McNair Program Staff has made every effort to assure a high degree of accuracy, rigor and quality in the content of this journal, the interpretations and conclusions found within each essay are those of the authors alone and not the McNair Program. Any errors or omission are strictly the responsibility of each author.

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Analysis of Apicoplast targeting protein motifs in *Toxoplasma gondii* for use with homing endonuclease proteins CpaI and DpaI

Ursula Lang and Raymond J. Monnat, Jr. M.D.

Abstract

The apicoplast is an essential organelle of parasites in the phylum Apicomplexa, and has been the target for several clinically useful drugs to combat species such as Plasmodium, Toxoplasma and Cryptosporidium. Unfortunately, due to the increasing prevalence of drug resistance many of these treatments are being compromised. This research may provide a novel approach to target and degrade the genome of this organelle and in doing so kill the parasite. Homing endonucleases (HE), highly site-specific proteins with the ability to cleave DNA, with target sites in the apicoplast DNA will be used. These HE are used in conjunction with recently identified protein motifs that direct the trafficking of nuclear protein to the apicoplast. By combining these two components in a green fluorescent protein (GFP) tagged vector, one is able to track the movement of the HE into the nucleus. Due to limitations in time and experimental setbacks, the targeting vector was not successfully cloned. However, collaborations have been established to help with the Toxoplasma culture and materials are in place to continue with experimentation.

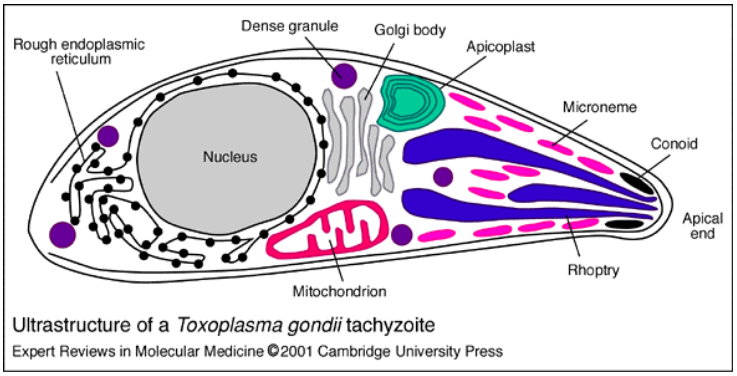
Introduction

Plasmodium falciparum, Toxoplasma gondii, and Cryptosporidium collectively sicken several 100 million people and are responsible for over 1 million deaths each year (Greenwood). Although there are drugs on the market that are effective in killing these parasites, resistance to these drugs has become increasingly prevalent (Rosenthal). Discovery of the apicomplexan plastid has proposed questions of its function, evolutionary origin, and what opportunities its bacterial-like systems offer for combating the important diseases caused by these parasites (McFadden). Macrolide antibiotics are an anti-protozoal therapy that targets metabolism in the apicoplast and has shown to be effective, thus it is anticipated that the cleavage and elimination of the apicoplast genome will markedly affect parasite viability, infectivity and pathogenesis. This research will use the model system, *Toxoplasma gondii,* to test this novel approach of apicoplast targeting.

Toxoplasma gondii:

T. gondii is an obligate intracellular parasite that is capable of infecting all mammalian cell types and establishing a latent infection in the host organism. Members of the cat family are the only known definitive hosts for the sexual stages of *T. gondii.* After tissue cysts or oocysts are ingested by the cat, viable organisms are released and invade epithelial cells of the small intestine where they undergo an asexual followed by a sexual cycle and then form tachyoites. These are excreted and can become infectious to humans through ingestion. The parasites form tissue cysts, most commonly in skeletal muscle, myocardium, and brain. These cysts may remain throughout the life of the host. The infection is suppressed by the immune system in healthy individuals, but *T. gondii* causes significant morbidity and mortality in immunocompromised individuals, including AIDS and chemotherapy patients. *T. gondii* is also a leading cause of birth defects. It is used as a model system for other apicomplexan parasites including *Plasmodium* sp., the causative agent of malaria (DeRocher).

Figure 1. Ultrastructure of a Toxoplasma gondii tachyzoite



Apicoplast:

The apicoplast during an estimated 500 million years of intracellular survival within their eukaryotic hosts is still described as being fundamentally bacterial in nature. It is thought to have arisen by two rounds of endosymbiosis of a cyanobacterial-like prokaryotic cell due to the presence of four membranes and the distinctive mechanisms of protein trafficking (McFadden). The main role of the apicoplast appears to be in fatty acid isoprenoid and heme biosynthesis (DeRocher). Encased in its four membrane structure is a 35kb circular genome that encodes a translational apparatus (rRNA, tRNA, and ribosomal protein genes) and a small number of additional open reading frames, perhaps 30 in total, that encode proteins involved in protein transport, processing or in transcription (Foth). Ten percent of the protein essential apicoplast function are encoded for by the nucleus of the cell, and are then transported to and internalized by the apicoplast. This is mediated by specialized bipartite targeting sequence consist of a secretory peptide and transit peptide (Yung). The symbiotic relationship between the apicoplast and the parasite along with knowledge of the apicoplast targeting sequence has made it a promising target for drug development.

Homing Endonucleases

Homing endonucleases are a diverse collection of proteins that are encoded by genes with mobile, self-splicing introns. These enzymes promote the movement of the DNA sequences that encode them from

one chromosome location to another; they do this by making a site-specific double-strand break at a target site (typically 14-40 bp) in an allele that lacks the corresponding mobile intron (Flick). Cellular mechanisms are then activated to repair the break and insert a copy of the HE DNA by double-strand repair. Their presence does not decrease the fitness of the host organism because they are associated with introns, which excise the element at the RNA level following transcription, or with inteins, which remove the element from within a host protein by a protein splicing reaction after translation. These DNAs have evolved to propagate between species through horizontal transmission and between individuals within a population by a process termed "homing (Gimble).

Materials and Procedures

Construction of targeting vectors encoding two different HEs along with the Acyl Carrier Protein (ACP) upstream signal/transit peptide sequence, to the apicoplast was attempted. The vectors also have a Green Florescent Protein (GFP) coding sequence downstream of the HE for the purpose of tracking the protein to the apicoplast. The two HE used were I-CpaI and I-DpaI, both members of the LAGLIDADG motif family. Recently the Monnat lab identified these two homing endonucleases to have perfect 18 bp and 21 bp target sites in the *Apicomplexan* plastid genome. These matches are not found in the human genome, and thus were believed to be highly specific for the apicoplast DNA.

The vector was a gift from Amy DeRocher, a collaborator from the Seattle Biomedical Research Institute, and was in the form of a miniprep along with agar plates of colonies. The DpaI and CpaI open reading frame PCR products, flanked with AvrII restriction sites, were a gift from Monique Turmel of the Universite Laval. The vector was digested with the AvrII and Bg1II restriction enzymes (RE) for one hour followed by a phenol extraction for enzyme inactivation. Both of these REs cut once in the vector and should give a linearized product. The vector was run on a 1% agarose gel to note size change and ensure proper cleavage (Fig. 6A). To test if the sticky ends of the DpaI and CpaI inserts could relegate, ligase was added. A series of different band sizes should form if the experiment worked (Fig. 6B). There was need to produce more vector than was originally given to us, so colonies were inoculated overnight from the original agar plates and a mini-prep of 4 mL was done.

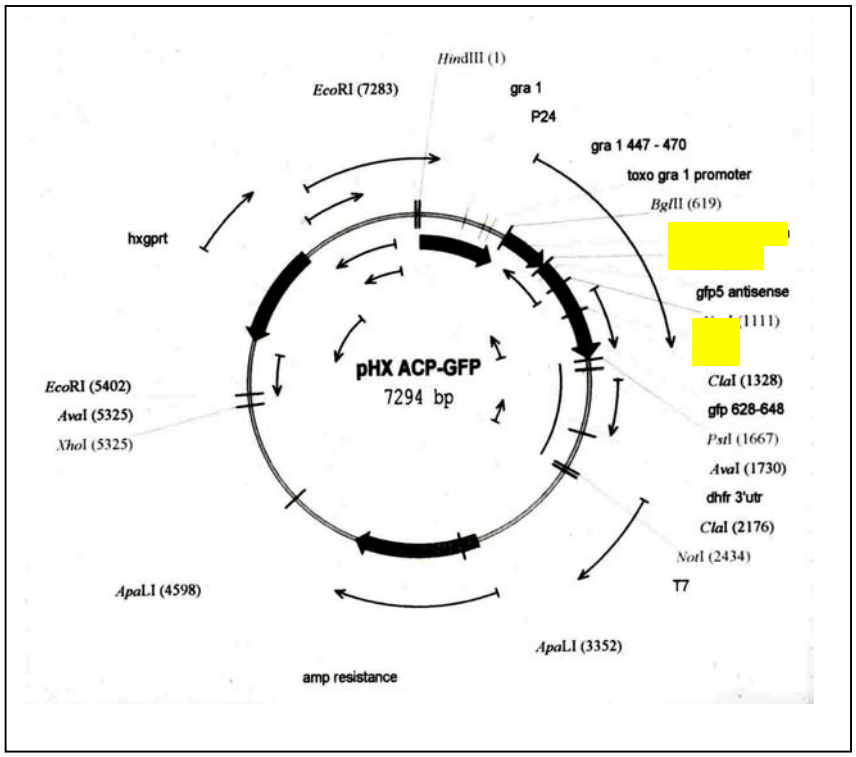


Figure 2. Schematic of the targeting Vector (pHX ACP-GFP) containing the ACP transit/signal sequence, AvrII restriction site, and GFP reporter gene. (DeRocher)

Three separate ligation reaction were done with 4 molar excess of insert to vector (60ng vector) in 20µl reaction tubes. One ligation reaction each of CpaI and DpaI insert and another reaction of vector alone without ligation enzyme to act as the negative control. *E.coli* (XL-blue chemically competent cells) was then transformed by heat-chock at 42ºC for 30 seconds with 3µl of the ligation product. This was incubated on ice for 1 hour at 37ºC, plated on ampacilin selective media, and incubated overnight at 37ºC.

The following day, twelve colonies were picked from each of the CpaI and DpaI agar plates and inoculated in 3mL of LB media with ampicillin. These were grown overnight at 37º and the following day mini-preps were done to extract the vector.

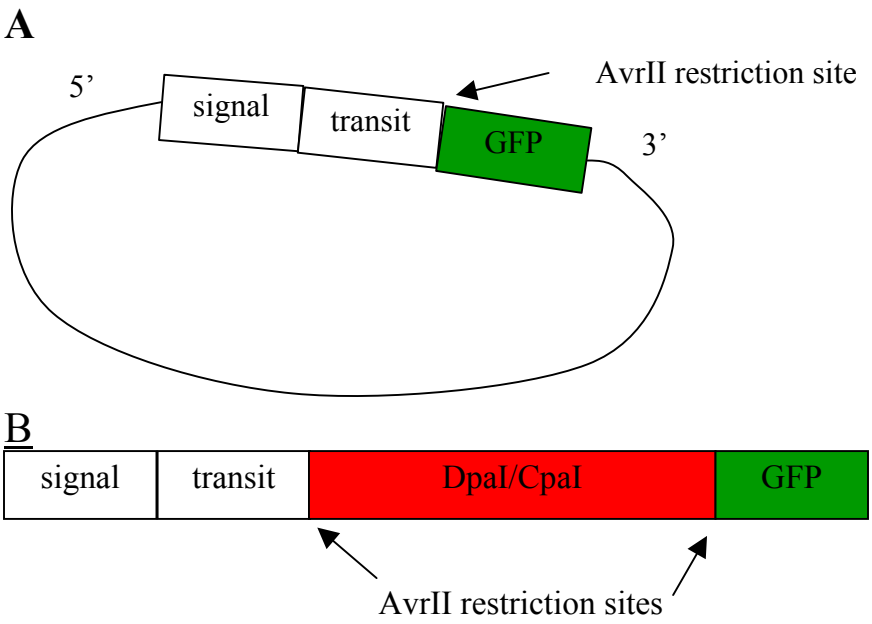


Figure 3. (A) This is a simplified schematic of the vector used in these experiments. The AvrII restriction site is used to insert the HE. (B) The region of the vector including the HE.

Restriction digest analysis was done on the 24 mini-preped vectors in order to detect the presence of the insert. The CpaI vector were cut with restriction enzyme EcoRI and the DpaI vector were cut with HindIII. Reactions of 20µl with 2µl mini-prep product were done and incubated at 37ºC for 1 hour. A 1% agarose gel in 1X TBE was used to run 10µl of digest product for gel electrophoreses (fig. 7).

Descriptions of the HE, DpaI and CpaI:

DpaI

Two Open Reading Frames (ORF):

1. first green arrow: bp 3610-4461 (including stop codon)
2. second green arrow: bp 4840-5529 (including stop codon)

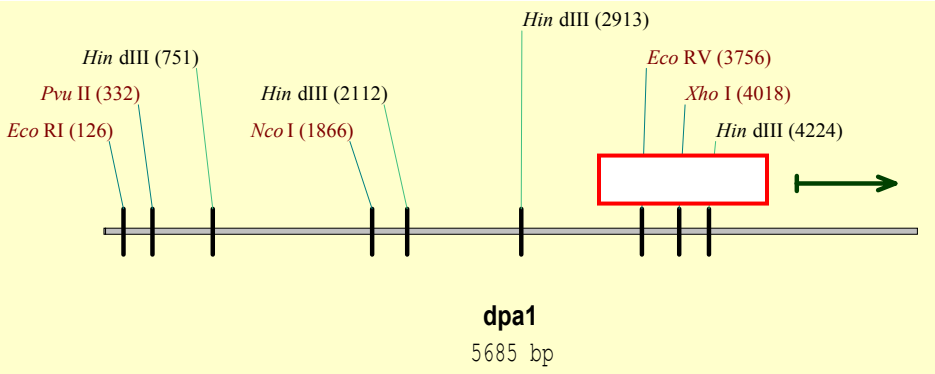


Figure 4. Restriction site map of DpaI HE. Only first ORF with three restriction sites was used in these experiments (857bp).

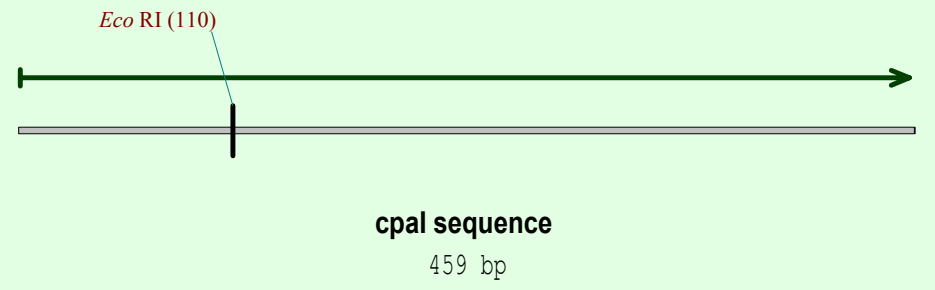
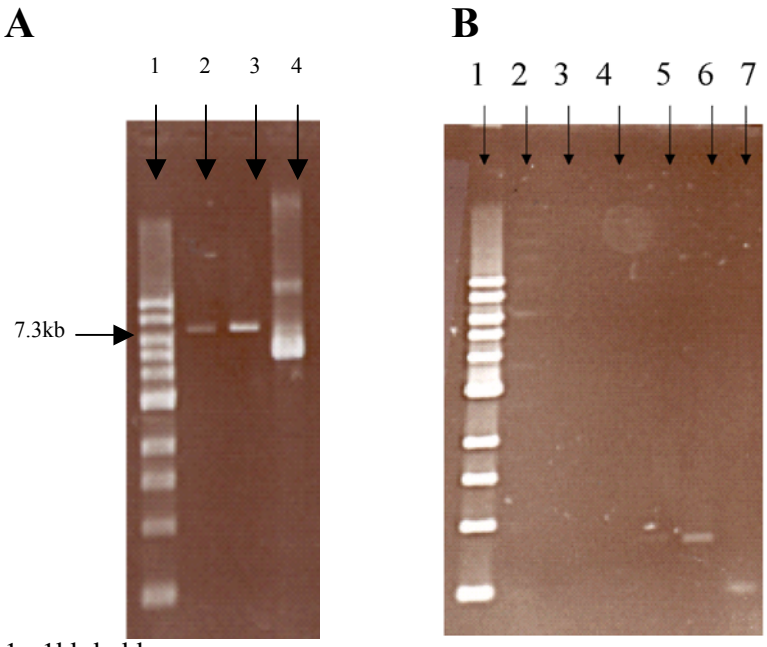


Figure 5. Restriction site map of CpaI HE (only the ORF). The EcoRI site was used to do determine the orientation of the insert once it was ligated into the vector (459pb).

Results

The initial analysis of the vector and inserts were done by cutting them with the AvrII restriction enzyme. The vector was found to have linearized as predicted (fig. 6A) at 7.3kb and on subsequent ligation, was found to re-ligate efficiently. This demonstrated that the 4 bp overhangs were still intact. Since the inserts had AvrII 4 bp overhangs on both the 5' and 3' ends, ligase was added to check that they could re-ligate. The gel of the CpaI insert after ligation ran a ladder of different DNA sizes, which affirmed the integrity of the ends. The DpaI results were not visible on the gel, although a 857bp band also was not detected in the ligase positive lane (fig. 6B). This indirectly supports the conclusion that the DpaI overhang ends are also intact.

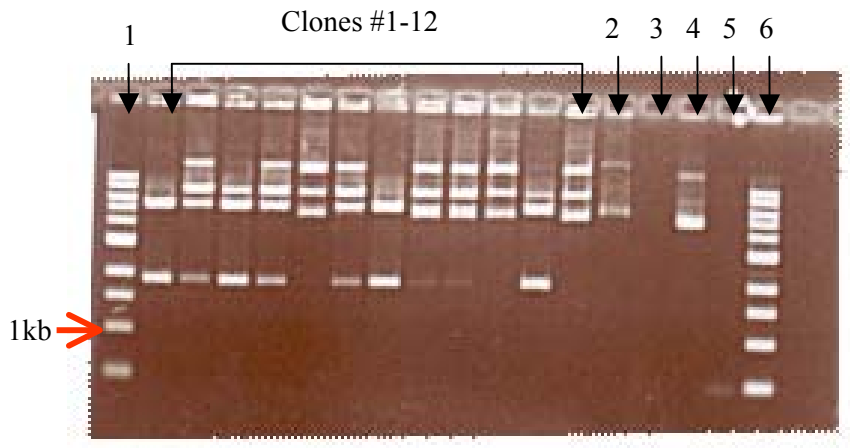


1: 1kb ladder
2: Vector with AvrII digest
3: Vector with BglII digest
4: Vector uncut

1: 1kb ladder
2: CpaI with ligase
3: CpaI without ligase
4: DpaI with ligase
5: DpaI without ligase
6: DpaI uncut
7: CpaI uncut

Figure 6. Preparation of the vector involved cleaving it with AvrII restriction enzyme. (A) The 7.3kb band represent the linearized vector which ensures that there is only one cleavage site. (B) The CpaI and DpaI HE were previously digested with AvrII, so adding ligase will create a sequence of different band sizes if the sticky-ends are intact.

The CpaI and DpaI inserts contain EcoRI and HindIII sites respectfully, and the vector also contains two EcoRI sites and one HindIII site. With this information, the sizes of fragments with a correctly ligated insert were predicted to be: 1.5kb and 6.6kb for the DpaI fragment and 1061bp (~1kb), 4.8kb, and 1881bp for CpaI (Fig. 7).



1: 1kb ladder
2: Vector cut with EcoRI
3: CpaI cut with EcoRI
4: Vector uncut
5: CpaI uncut
6: 1kb ladder

Figure 7. Several colonies of clones were inoculated overnight, mini-preped, and cut with EcoRI for CpaI HE and with HindIII for DpaI HE. With the CpaI insert correctly ligated into the vector, there should be a 1kb fragment visible.

All of the clones that were picked were negative for the correctly ligated insert. Both the DpaI and CpaI ligations were attempted several times with poor results. Some of the experimental parameters that were varied were digest time from 1-24 hours, insert to vector DNA ratio during ligation, and number of clones picked. All of these variations gave similar negative results. Further recommendations would be to gel purify the vector and inserts after digestion with AvrII and gel electrophoresis. The four base pairs that are cleaved off of the insert ends may interfere with the sequential ligation. The AvrII restriction enzyme was not very efficient at digesting the vector, so the background of undigested vector was high in the transformations. A different enzyme may be more effective for this experiment. As a result of these difficulties, the targeting vector was never created and the research was not able to proceed.

Conclusion

This research unites knowledge of apicoplast targeting mechanisms with homing endonuclease function through a novel approach to *Toxoplasma gondii* drug therapy. The apicoplast genome was to serve as the substrate for the homing endonucleases used in these experiments. The experiments in this paper attempted to construct targeting/signaling vectors containing the homing endonucleases, CpaI and DpaI, that would in effect degrade the apicoplast genome and kill the parasite.

The targeting/signaling vector containing the homing endonucleases to the apicoplast was not successfully constructed, so nothing can be concluded about the ability of the HE to be expressed and cleave the DNA. However, through the collaborations we established, much of the preparation for future work has been done. Some of the components of the experiments need to be rethought such as what restriction enzymes to use in cleaving the DNA. Other, more efficient, methods of screening colonies for the desired vector can be done such as PCR analysis.

The apicoplast has been the focus for research because of its unique bacterial-like properties, and antibiotics inhibiting its molecular processes are already in chemotherapeutic use. Although the experiments did not proceed as expected, with continued protocol modifications, we are hopeful that the results will contribute to drug development.

Acknowledgements

This work would not have been made possible without the advice and financial support of the McNair program throughout the academic year, and from the University of Washington GenOM Project (NIH HG02360-03S1) during the summer months. Lisa Peterson was a great support throughout the whole process. I thank all the members of the Monnat lab for their friendship, technical support, and especially Dr. Raymond Monnat for his careful guidance and creative mind.

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Analysis of Association between Abasic Endonuclease Activity and Abasic Site Abundance in Glioma Genomic DNA

Phoebe Lee and John Silber
Department of Neurological Surgery, University of Washington

ABSTRACT

Abasic sites are the most common type of oxidative free radical induced DNA damage. Base excision repair (BER) pathway is the main process for repairing oxidative damages including abasic sites. BER is initiated by the endonuclease activity of the Ape1/Ref-1, an abundant enzyme ubiquitously expressed in human cells. Emerging evidence indicates that Ape1/Ref-1 level reflects the level of oxidative stress experienced by cells. We have discovered that Ape1/Ref-1 activity is elevated in gliomas compared to adjacent histologically normal brain for more than 90% of the glioma/normal tissue pairs studied. Therefore, we hypothesize that there is a direct association between the level of abasic sites and Ape1/Ref-1 activity in human gliomas, reflecting a response to elevated oxidative stress. To examine our hypothesis, we quantitated abasic site abundance in genomic DNA of cultured glioma cells and developing brains using an aldehyde reactive probe (ARP). Our results suggest that DNA isolated from developing brain contain a co-purified ontaminant that is ARP reactive. I discuss an experimental test of this sub-hypothesis, as well as possible explanations for observed data.

INTRODUCTION

Base excision repair pathway (BER) is the main defense against the deleterious effects of oxidation or alkylation induced DNA base modification, spontaneous base loss, and free radical induced strand breaks (Evans et al., 2000; Krokan et al., 2000). Many of these DNA lesions are generated by anti-cancer agents and environmental mutagens that produce free radicals. The first step of the BER pathway involves a specific glycosylase that hydrolyzes the *N*-glycosylic bond between altered base and deoxyribose, producing a baseless (i.e. abasic) site. Ape1/Ref-1, the major mammalian apurinic/apyrimidinic endonuclease, incises the phosphodiester backbone 5' to the abasic site. Following the nick, the baseless deoxyribose is excised by the 5' phosphodiesterase activity of DNA polymerase β and the resulting single nucleotide gap is then filled by polymerase β using the complementary strand as a

template, concluding with DNA ligase sealing the repaired strand (Wilson et al., 2001). Box 1 illustrates this "short path" mechanism of BER as well as an alternative "long path" pathway that utilizes many of the same enzymes but applies to different types of damage. In addition to its endonuclease activity, Ape1/Ref-1 is also a redox protein that activates a number of the DNA binding protein such as FOS, JUN, as well as transcriptional factors including NF-K β and p53. Box 2 illustrates the multifunctional nature of the enzyme (reviewed in Evans et al., 2000).

Ape1/Ref-1 is an abundant enzyme ubiquitously expressed in human cells. Notably, Ape1/Ref-1activity is heterogeneous within normal human tissues with the greatest activity observed in proliferating cells. A dependence on proliferation is evidenced by the reduction of Ape1/Ref-1 protein and mRNA content that accompanied cessation of proliferation in maturing rodent brain (Grosch et al., 1998). Also, tumorgenesis is associated with elevated Ape1/Ref-1 protein content and activity (reviewed in Evans et al., 2000), indicating an association between Ape1/Ref-1 and proliferation. Our analysis of Ape1/Ref-1 activity in 58 pairs of adult glial tumors/and adjacent histologically quiescent normal brain revealed that activity was an average of 12-fold greater in tumors than flanking normal brain cells in greater than 90% of the pairs studied (Bobola et al., 2001). Moreover, regression analysis revealed a strong position correlation between Ape1/Ref-1 activity and glioma proliferation rate (i.e. fraction of cells in S-phase as assessed by flow cytometry).

The mechanisms underlying the apparent association of Ape1/Ref-1with proliferation are not well understood. Several laboratories including ours (Silber et al., 2002), have demonstrated a transient elevation of Ape1/Ref-1protein content and activity in response to low-level oxidative free radicals (Ramana et al., 1998). Proliferation elevates the abundance of endogenous oxidative free radicals as a consequence of greater oxygen utilization. Our hypothesis is that glioma Ape1/Ref-1 activity reflects endogenous generation of oxidative free radicals. A test of our hypothesis is to seek associations between oxidative free radical DNA damage and Ape1/Ref-1 activity. The most abundant oxidative free radical modifications in DNA are abasic sites and fragmented deoxyribose at the ends of strand breaks (Demple and Harrison, 1994). To quantitate this damage, we used N-aminoxymethylcarbonylhydrazino D-biotin, or aldehyde reactive probe (ARP), a biotinylated hydroxylamine derivative that covalently links to the aldehyde group of the ring-open form of deoxyribose found in abasic sites and fragmented deoxyribose moieties.

Experimental design

Our plan is to measured the abundance of abasic sites in glioma/normal brain pairs, and seek correlation between abasic site levels and Ape1/Ref-1 activity. To verify our experimental approach, we assayed cultured glioma cells (SNB19) and developing brain tissue samples for Ape1/Ref-1 activity and abasic site abundance. Cell line samples were used as reference to the tissue samples, since we observed that MX treatment ablated all ARP signal in previous studies. Developing brain tissues were used because similar to cancerous cells, developing brain are continuously proliferating, thus maintaining a state of elevated oxidative stress. DNA extracted from developing brain of different gestational age was treated with a molar excess of ARP. To demonstrate specificity of ARP binding, a portion of DNA was treated with methoxyamine (MX), which also covalently bind aldehyde groups, prior to reaction with ARP. The biotinylated DNA samples were bound to a nitrocellulose membrane using a vacuum blot apparatus. The amount of biotinylated abasic sites was quantified with an ELISA-like assay via biotin-avidin horseradish peroxidase conjugated with a fluorescent tag. Sample band intensities were determined by using NIH Image Program, and the number of abasic sites was quantitated by simultaneously assaying calf thymus DNA containing known levels of abasic sites (abasic sites/10^6 nucleotides) to provide a standard curve.

MATERIALS AND METHODS

DNA extraction from cultured glioma cells and developing brain was performed by detergent lysis followed by RNase digestion and differential salt precipitation of proteins. DNA was precipitated by isopropanol precipitation and excess salt removed by repeated washes with 70% ethanol. This protocol rather than the customary phenol extraction technique was employed to avoid induction of addition abasic sites during extraction due to phenol's property as a weak oxidant that in the presence of metal ions can generate oxidative free radicals.

Part of the glioma tumor/and normal pairs was treated with MX prior to ARP treatment. 10µg of DNA were incubated with 5mM final concentration of MX (Sigma) at 37^o C for 2 hrs in 100µl reaction volume. DNA was kept for at least1 hr in 95% ethanol and 0.3M final concentration sodium acetate at -20^o C, followed by centrifugation to pellet DNA at 16,800 rpm for 20 min via SS34 rotor, and the pellets were washed with 70% ethanol in 4 ^{o} C. DNA was resuspended in 10mM Tris, pH 7.4, and absorbance of samples was measured at 260 and 280 nm to

calculate yield and purity. All A_{260}/A_{280} were ≥ 1.8, indicating minimal protein contamination.

Different samples of 10 µg DNA was incubated in 2mM final concentration ARP (Dojindo Molecular Technologies, Inc., Gaithersburg, MD) at 37 °C for 20 min in 100µl reaction volume. DNA was spun to pellet at 16,800 via SS34 rotor and washed 2-30 min intervals with 70% ethanol in 4°C. DNA was resuspended in 10mM Tris, pH 7.4 and absorbance at 260 and 280nm was again measured to determine yield and purity; all A_{260}/A_{280} ratio were ≥ 1.8. (Liu et al., 2002)

DNA were heated at 100°C for 10 min followed by cooling in ice water, 0.01–0.3 µg of cellular DNA was brought to a total of 0.3 µg by addition of carrier calf thymus DNA pretreated with MX to eliminate abasic sites, as described below; at least two different amounts of cellular DNA were analyzed in every experiment. DNA samples were mixed with an equal volume of 2 M NH_4 acetate and loaded onto nitrocellulose filters saturated with 1 M NH_4 acetate by using a vacuum manifold. The nitrocellulose filters were then soaked in 5x SSC (0.75 M NaCl and 0.075 M trisodium citrate) for 15 min at 37°C, air dried, and baked for 2 hrs at 80°C in a vacuum oven.

After soaking for 1 hr at room temperature in 20 mM Tris-HCl (pH 7.5), 0.1 M NaCl, 1 mM EDTA, 0.5% casein, 0.25% BSA, and 0.1% Tween 20, the membrane was incubated with streptavidin-conjugated horseradish peroxidase at room temperature for 45 min. The membrane was rinsed for 35 min in 20 mM Tris-HCl (pH 7.5), 0.26 M NaCl, 1 mM EDTA, and 0.1% Tween 20, and the bound peroxidase activity visualized by enhanced chemiluminescence. Images on X-ray film were photographed with a digital camera, and band (pixel) density was quantitated by using NIH Image version 1.68. (Silber et al., 2002)

Abasic site abundance in cellular DNA was determined by comparison with a standard curve constructed with reference DNA containing 1080 abasic sites/10^6 nucleotides. Standards were incubated with ARP and processed simultaneously on the same nitrocellulose filter with cellular DNA samples. In our experience, band density of standards is linear within the range of 0.8 to 35 (or up to 200 with shorter exposure times) abasic sites/10^6 nucleotides when 0.3 µg of DNA is blotted. (Silber et al., 2002) Previously, we extracted DNA samples from three developing brain tissues to assay for Ape1/Ref-1 activity and measured abundance of abasic sites.

RESULTS AND DISUSSION

We had previously used ARP to quantify abasic site abundance in cultured human glioma cells (Silber et al., 2002). Importantly, reducing or elevating Ape1/Ref-1-mediated abasic endonuclease activity was accompanied by increased or diminished levels of abasic sites. Notably, treatment of the DNA with methoxyamine (MX), a primary amine that reacts with aldehydes, blocked binding of ARP to the DNA. These results validated the assay for abasic sites. In subsequent experiments, we encountered two unexpected problems: one, ARP signal intensity failed to correspond to amount of DNA analyzed and two, pre-treatment with MX of DNA extracted from brain tissue did not abolish reaction with ARP.

As illustrated in Fig. 1, the fluorescence signal generated by ARP binding was not proportional to the amount of DNA analyzed; for example, ARP signal intensity for 0.05μg DNA was greater than 0.2μg DNA. We suspected the integrity of the ARP that had been stored in solution in PBS at –20 °C for approximately 6 months. Preparing a fresh stock solution of ARP eliminated this problem as illustrated in Fig. 2. ARP reactivity of SNB19 DNA treated with MMS (an alkylating agent that produces abasic sites) in the absence or presence of lucanthone (a drug that inhibits Ape1/Ref-1 activity) was measured. The result of the test showed proportionality of ARP reactivity with the amount of DNA analyzed and larger abundance of abasic sites in the lucanthon treated cells. These data suggest that ARP in solution is not stable to repeated freezing and thawing. It is likely that the ARP or its biotin moiety was degraded so that it could not bind the strepavidin conjugated horseradish peroxidase stoichiometrically.

Figure 3 shows ARP reactivity of DNA isolated from developing brain 26, 84, and 88 at gestational age 78, 137, and 111 days, respectively. The abasic site endonuclease activity had been assayed; the abundance of abasic sites was measured after MX treatment, and resulted data showed measurable ARP signal for all three samples. This shows that MX treatment did not ablate all of the ARP signal. We suspect the residual ARP signal of the MX-treated DNA resulted from co-purified contaminants that could react with ARP but not MX. Subtracting the MX-treated ARP signal densities (i.e. background) provided an estimate of relative abasic site levels. Fig. 3 shows that the background signal density varied widely among the tissues, ranging from about 10 to 15% to 80% of the total ARP-signal density. The corrected densities show

that sample 26 had the largest and 88 the smallest abundance of abasic sites.

These limited data reveal no clear relationship between Ape1/Ref-1 activity and abasic site abundance. Interestingly, the Ape1/Ref-1 activity of samples 26 and 84 was inversely proportional to abasic site abundance, suggesting that abasic site levels reflected the ability to repair this lesion. In contrast, sample 88 displayed the smallest number of abasic sites and was accompanied by an activity that was an intermediate to that of samples 24 and 86. Conceivably, the determinants of Ape1/Ref-1 levels in developing brain may be multi-factorial, reflecting the requirement not only for the DNA repair activity of Ape1/Ref-1 but also its re-dox function. In addition, the samples may differ in their tolerance of unrepaired abasic sites and sample 88's physiological behavior might innately deviate from the norm, as the tissue type, DNA extraction protocol and other experimental treatments were identical for all tissue samples. The recent characterization of mammalian DNA polymerases capable of synthesizing past a baseless nucleotide (Zhang et al., 2002) is one possible mechanism to circumvent the lethality of abasic sites. Analysis of a greater number of samples should provide additional insights into these possibilities.

The failure of MX to ablate ARP binding to DNA isolated from developing brain was completely unanticipated and was contrary to our observations for DNA isolated from cultured glioma cells. It is likely that DNA isolated from tissue contained a contaminant that bound ARP. Moreover, it is likely that binding of ARP was not via reaction with an aldehyde group since MX could not block the binding. This finding makes it unlikely that high molecular weight polysaccharide moieties such as those commonly found on membrane glycoproteins as a possible contaminant. Therefore, we hypothesize the source of contaminants to be extracellular matrix macromolecules, as tissue cells are surrounded with an extensive extracellular maxtrix while cultured cells lack such environment. In support of our hypothesis, MX treatment of the human glioma cell line SNB19 DNA either completely ablated or greatly diminished the intensity of the ARP signal. Since background signal make up a significant portion of the total signal intensity, there is a need to minimize the contaminants to ensure the data accurately reflects the abundance of abasic site.

A test for our hypothesis for the source of contaminant is to do a complete digestion of tissue extracted DNA with DNase before treatment with ARP. If ARP signal remains in the absence of DNA, it would confirm the presence of co-purified contaminates. To attemp to reduce contamination, we will use alternative purification procedures utilizing

chaotroptic agents other than SDS (e.g. NaI) to dissolve the tissue and lyse cells.

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Presence and Effects of *Tritonia* Peptides on Velar Ciliary Activity in *Tritonia Diomedea* Larvae

Hoang Nhan

Abstract

Ciliated cells are an important component of locomotion during both adult and larval stages of several marine gastropods. For example, the adult sea slug, Tritonia diomedea, uses a cilia-covered foot to crawl whereas the veliger larvae swim in the water column using cilia of the larval velar lobes. The cilia providing the propulsive force in both adults and larvae are thought to be under direct nervous control. In adult T. diomedea, TPep has been shown to control ciliary locomotion; however, it is not known if TPep regulates ciliary activity in larval T. diomedea. Here I used immunohistochemistry to investigate whether TPep is present in T. diomedea larvae. My results indicate that TPeptidergic cells and cell processes are present at the base of ciliated cells within the velar lobes of T. diomedea larvae immediately after hatching. These neurons, however, did not innervate the presumptive foot tissue, which bears functional cilia that will serve as the primary effector cells during adult crawling. This distribution of TPep persists at least until metamorphic competence. To determine if this neurotransmitter directly control larval locomotion, I tested whether application of TPep alters the ciliary beat frequency (CBF) of velar cilia. My results indicate that TPep (in concentrations from 10^{-8} to 10^{-3}M) did not alter the CBF of velar cilia. Therefore, although TPep was shown to exist in the nervous system of larval T. diomedea, it does not seem to participate in the neural circuitry controlling larval or presumptive foot ciliary activity. In addition, the results suggest that the neural circuitry controlling adult ciliary crawling develops de novo during metamorphosis from larval to juvenile form.

Introduction

Ciliated cells exist in almost all animals and serve many diverse functions. Cilia are commonly used for transportation of food, gametes, excretory products, and for locomotion (Sleigh 1974). Cilia help many animals, such as veliger larvae of marine gastropods (Koshtoyants *et al.* 1961; Fretter 1967), and larvae of other marine forms (Mackie *et al.* 1969) to move through fluid medium. The current study is designed to investigate the presence and function of the neurotransmitter TPep in the control of *T. diomedea* larval ciliary activity.

The neural mechanisms underlying ciliary activity has been studied in free-swimming larvae of some marine invertebrates because of their simple nervous systems and their obvious, accessible cilia (Mackie *et al.* 1976). For instance, dopamine (DA) and serotonin (5-HT) were found to control the stability and direction of ciliary beating in sea urchin larvae (Wada *et al.* 1997); laser ablation revealed regulation of ciliary activity by serotonergic neurons in pond snails (Kuang and Goldberg 2001); and larvae of nudibranch molluscs have serotonergic components in the apical sensory organ and its associated axons that affect their velar cilia function (Kempf *et al.* 1997). However, the role of the cilio-regulatory neurotransmitters in controlling CBF of larval gastropods is not known.

In contrast, previous studies indicate that certain aspects of ciliary activity in many adult gastropods are under nervous control via innervation by central nervous system neurons (Audesirk 1978a; Audesirk 1978b; Willows *et al.* 1997). In adult gastropods, serotonin (5-HT) was found to affect ciliary beat frequency, both in intact epithelia and isolated cells (Buznikov and Manukhin 1962; Audesirk *et al.* 1979; Goldberg *et al.* 1994). A group of pedal peptides isolated from *T. diomedea* central neurons, known as TPep, was also found to increase ciliary beating in the foot epithelium (Willows *et al.* 1997).

The larvae of *T. diomedea*, however, use velar cilia rather than pedal cilia to swim during early larval stage; approximately the first 30 days after hatching (Hadfield 1977; Kempf 1977). The foot structure does exist during the larval stage but is not used for locomotion until the animal settles or achieves metamorphic competence. It is not known whether the mechanisms and the neurotransmitters controlling pedal ciliary activity in adults similarly control velar ciliary activity in larvae, even though the velar lobes in larvae and the foot in adult both function in locomotion. The effect of neurotransmitters on the larvae's central nervous system at both the cellular and behavioral levels is accessible to analysis because (1) the larvae can be cultured from embryos through

metamorphosis to reproductively mature slugs in the laboratory; (2) their nervous system is simple; and (3) their cilia are readily accessible. In addition, the direct innervation of velar lobes by neuronal cells provides an excellent opportunity for studying the direct relationship between nervous controls and behavioral outputs.

In this experiment, I investigated the distribution of TPep in *T. diomedea* veliger larvae during development from hatchling to metamorphic competence. In addition, I examined whether TPep has the same effect on velar ciliary beating in veliger larvae as it does on pedal ciliary beating in adults. Immunohistochemical results showed that TPep exists at the base of the ciliated cells in each velar lobe during all larval stages, from hatching until metamorphic competence. However, tests of the direct effects of TPep on CBF were inconclusive. Therefore, the role of TPep in *T. diomedea* larval ciliary activity remains unknown.

Methods

Larvae sampling. Egg masses laid by SCUBA- or trawl-collected *Tritonia diomedea*, were collected and maintained in aerated seawater at approximately 10°C. Culture water was changed regularly until larvae were released from embryos. Larvae cultures were maintained in filtered seawater (FSW) with antibiotics (0.006 g/ml of Penicillin G and 0.005 g/ml of Streptomycin in FSW). Cultures were incubated at 19°C and water was changed every two days. The developmental period of *T. diomedea* is approximately a month from the time of hatching until reaching metamorphic competence (~330μm shell length) (Hadfield 1977; Kempf 1977). In nature, *T. diomedea* larvae are obligate planktotrophs; however, in laboratory culture, they were fed with unicellular algae *Isochrysis* and *Pavlova* in 1:1 ratio, according to the method established by Kempf and Willows (1977).

Larval TPep immunohistochemistry

Approximately 100 larvae were sampled every two days and immersed in fixative for TPep immunohistochemistry (as described below). Nearly half of each sample was lost during different steps of the following procedure. First, larvae were anesthetized in a solution consisting of 7.5% MgCl and FSW in a 1:1 ratio at 4ºC for 15 minutes. Samples were then fixed in 4% paraformaldehyde in FSW with 50 mM Tris at 4ºC for 2-3 hours. After decalcifying larvae's shells with 10% ethylenediaminetetraacetic acid, disodium salt (EDTA) in phosphate buffer (PB, pH ~7) for 30 minutes, samples were rinsed three times in

Millonigs PB rinse over a period of an hour at room temperature (RT), followed by permeabilization in phosphate buffered saline (PBS) with 4% Triton X-100, and 0.1% sodium azide (PTA). Permeabilized specimens were then incubated in blocking solution A (PTA / 4% Triton X-100 / 6% normal donkey serum) at 4ºC for 12 hours, then in a 1:500 primary rabbit anti-TPep antibody and PTA (0.1% Triton X-100) solution at 4ºC for 48 hours. The samples were rinsed in blocking solution B (PTA / 0.1% Triton X-100 / 6% normal donkey serum) three times for 30 minutes each at RT, and then were incubated in a secondary donkey-anti-rabbit antibody (1:800 conjugated to Alexafluor 594 nm (Molecule Probes) in blocking solution B at 4ºC for 12 hours. Finally, the specimens were rinsed in Millonigs PB rinse over two hours at RT and mounted in VectaShield mounting medium (Vector Labs Inc.). Larvae were examined using a Bio-Rad MRC-600 confocal laser-scanning microscopy with a far-red filler-set 633/655.

The control larval samples for immunohistochemistry labeling were processed using the exact described procedure, but omitting the addition of primary antibody in the first antibody incubation step.

Effects of TPep on larval velar cilia

Veliger larvae were obtained from the same cultures used for immunohistochemistry as described previously. Individual larva in FSW was transferred to a 24x60mm glass cover slip (Corning Instruments). The cover slip was then placed on a Peltier plate mounted to the stage of a Nikon TMS inverted microscope. The Peltier plate was used to keep the specimen at a constant temperature of approximately 2°C.

To position the larva, I used a micropipette with an internal diameter of approximately 40µm to suction the larval shell. The micropipette was mounted on a micromanipulator to maneuver of the specimen. A similar micropipette, positioned at the opposite side of the specimen, was used to apply various concentrations of TPep to the larva.

I applied increasing concentrations of TPep, from 10^{-8}M to 10^{-3}M, with FSW rinses between applications. Each time, I increased the TPep concentration 10-fold and each FSW rinse lasted 30 minutes to an hour. Measurements of ciliary beat frequency (CBF) were preceded approximately 10 seconds by an application of transmitters so that the specimen would be freshly immersed in the desired TPep concentration. The solutions were all kept at ~2°C so that temperature differences between the specimen and the solution were minimized and would be less likely to affect CBF. The entire experiment lasted approximately 4 hours.

To measure the CBF, I mounted a Panasonic Color CCTV camera (WV-CP412) to the microscope. The camera was connected to a Panasonic Color Video Monitor (CT-2086Y) that displayed the image of the specimen. With a 40x objective, the pedal cilia and long pre-oral cilia, as seen on the screen, were between 3 cm and 9 cm long, corresponding to approximate actual lengths of 10μm and 30μm, respectively. I fixed a Fotonic Sensor from MTI Instruments (KD-38) to the screen such that it would transect the stroke path of a cilium parallel to the plane of view. The analog signal from the sensor went through a Krohn-Hite electric frequency filter (Model 3750) with a high-pass of 5 Hz and a low-pass of 30Hz. The signal was then fed through a custom-built amplifier and into an Astro-Med DASH-4U digital data recorder. The recorder collected 26.11 seconds of data with a sampling rate of 5 KHz. The data were analyzed using the fast Fourier transform (FFT) function on the recorder unit. This method is similar to that used by Braga et al. (1986). Three measurements were taken for each solution change. Besides measuring the CBF of long velar cilia, I also measured the CBF of short velar cilia and pedal cilia whenever possible. The most common frequency in each recording was determined, and the effects of the concentrations of transmitter were examined using a one-way ANOVA.

A second experiment was designed to determine if the effect of sheer mechanical force exerted upon the larvae could affect CBF. Instead of alternating between TPep and FSW application, the larva was spritzed with only FSW during the entire experiment.

Results

1. Presence and location of TPep in Tritonia diomedea larvae

Tritonia diomedea hatch 10 days after oviposition and spend their planktotrophic larval stage for approximately a month before achieving metamorphic competence (Hadfield 1977; Kempf 1977). Figure 1 shows the basic anatomy of a nudibranch veliger larva. Twenty-five samples of larvae were examined ranging from 0 to 26 days after hatching. Immunohistochemistry data showed that TPep labeling was consistently present in all larval samples. TPep was primarily distributed in the larvae's central nervous system and at the base of the ciliated cells at each velar lobe (Figure 2a). This pattern of TPep distribution was seen in both the newly hatched and the metaphorically competent larvae. However, no labeling was seen on the ciliated foot structure of any of the larvae.

Negative controls samples labeled with only secondary antibody failed to show the fluorescent labeling patterns as was seen in the experimental samples (Figure 2b). Both the control and experimental samples showed non-specific labeling around the stomach area of the larvae.

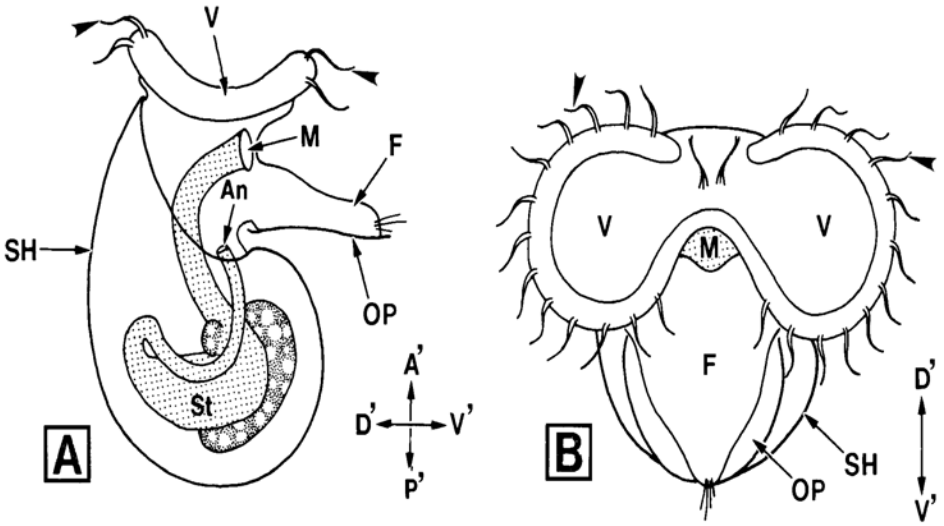

Figure 1. Sketches that illustrate major body components and axes of a generalized nudibranch veliger in two orientations. Digestive tract and large left and small right digestive diverticula are stippled. **A**: Right sagittal view. **B**: Anterior transverse view. Arrowheads indicate velar cilia. **An**, anus; **F**, foot; **M**, mouth; **OP**, operculum; **SH**, larval shell; **St**, larval stomach; **V**, velar lobes. Orientation axes: **A'**, anterior; **P'**, posterior; **D'**, dorsal; **V'**, ventral. (Kempf, 1997)

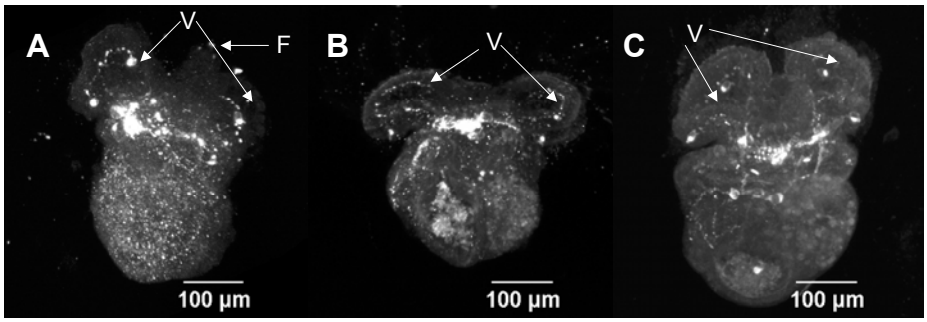

Figure 2a. Experimental larvae show consistent immunolabeling patterns for TPep. Larvae of 0-day (A), 8-day (B), and 26-day post hatched (C) labeled for TPep. Fluorescent labeling is seen at the base of the ciliated cells and within the larvae's CNS.

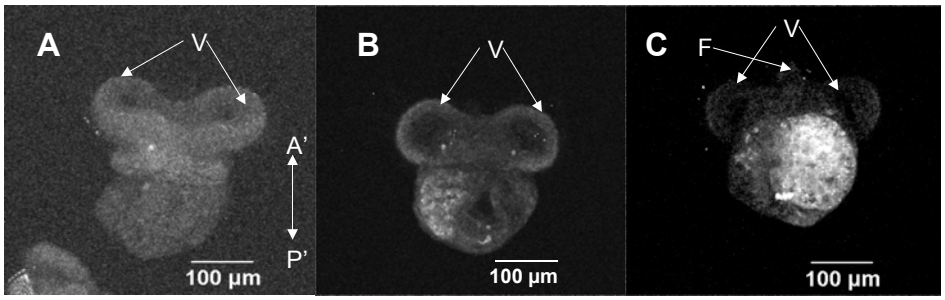

Figure 2b. Control larvae show no immunolabeling of TPep. Larvae of 0-day (A), 4-day (B), and 6-day post hatched (C) labeled for TPep. No specific labeling is seen at the CNS, the velar lobes, or the foot. A':anterior; P':posterior.

2. Direct effect of TPep on larval ciliary activity

In the controls, application of FSW alone did not show a significant effect on long velar cilia's CBF ($p<0.14$), with the CBF varied between 6 and 14 Hz (Figure 3). Temperature remained quite constant during each trial, ranging from 0.5 to 2.0ºC, and did not exert any influence on normal CBF (Figure 4).

TPep exerted different effects on different types of cilia. For long cilia on the velar lobes, TPep had a significant influence on larval CBF ($p<0.03$), but overall, an increase in TPep concentration did not result in either an increase or decrease in CBF. For short cilia and pedal cilia, an increase in TPep concentration did not show any significant change in CBF.

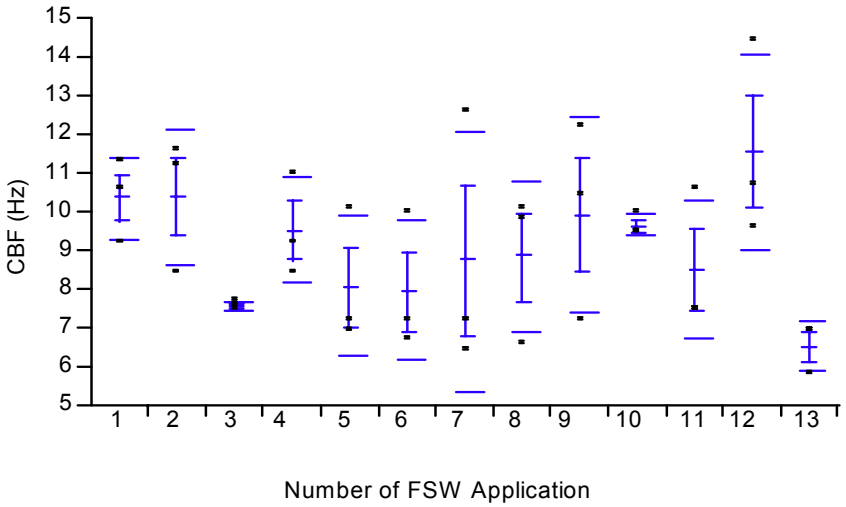

Figure 3. Effect of FSW application on long velar cilia's CBF. Application of FSW alone over time did not show a significant influence on CBF ($p<0.14$).

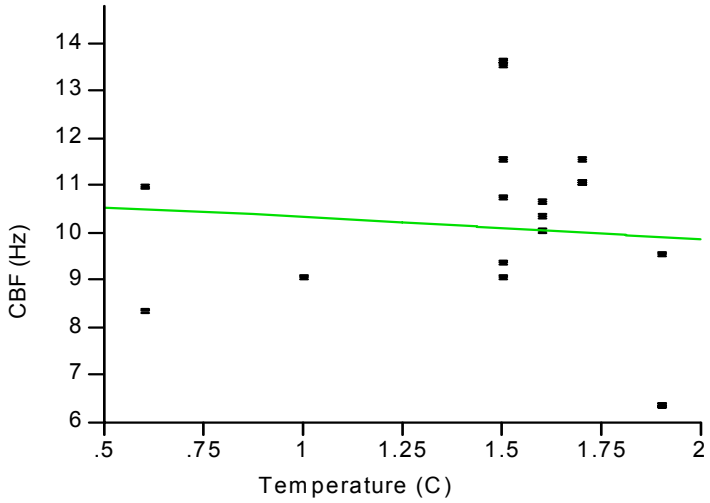

Figure 4. Effect of temperature on long velar cilia's CBF. Changes of temperature occurred during the experiment was minute and did not affect the CBF of larvae ($p<0.73$).

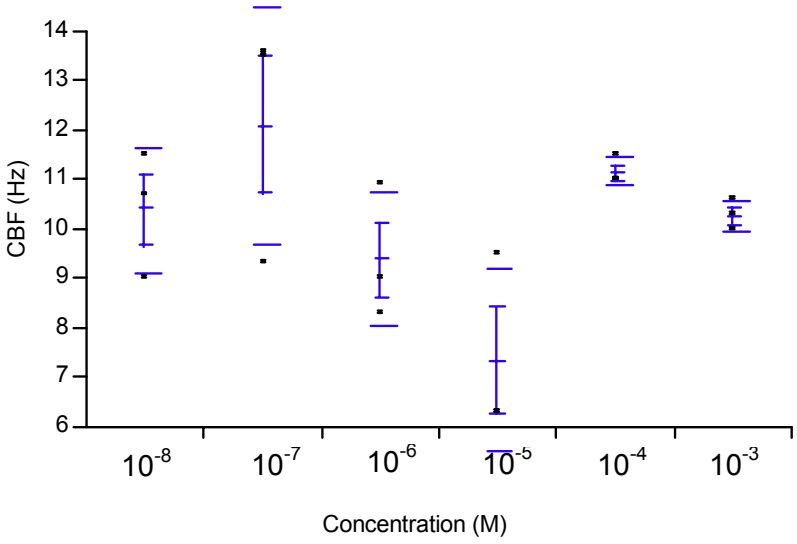

Figure 5. Effect of TPep on long velar cilia's CBF. Changes in concentration of TPep did exert a significant effect on the long velar cilia's CBF ($p<0.03$). However, data did not show a consistent trend of either increasing or decreasing CBF along with increasing TPep concentration.

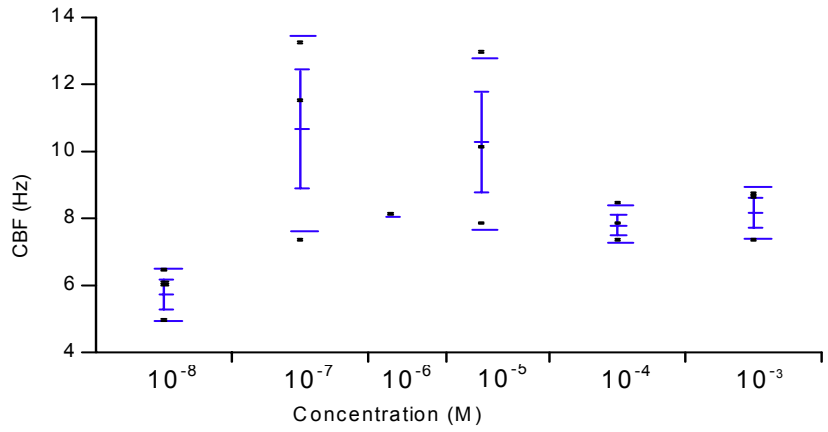

Figure 6. Effect of TPep on short velar cilia's CBF. Changes in concentration did not show an effect on short velar cilia's CBF ($p<0.08$).

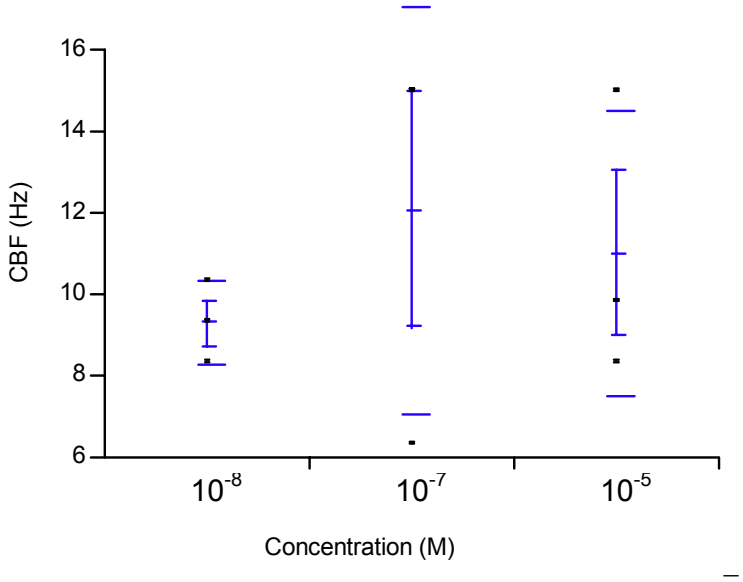

Figure 7. Effect of TPep on larval foot cilia's CBF. Changes in concentration did not show an effect on foot cilia's CBF ($p<0.65$). Measurements could not be taken at the higher concentrations due to the position of the foot seen on the TV monitor.

Discussion

Previous experiments showed that TPep is present in the foot of adult *Tritonia diomedea* (Cain, 2001). Immunohistochemical images from this experiment showed that TPep was also present in *T. diomedea* larvae from the day they were hatched through metamorphic competence. TPep was located mainly in the central nervous system of larvae and along the base of the ciliated cells on velar lobes. This pattern of TPep distribution was seen in the newly hatched as well as the nearly settled ones. This evidence suggests that TPep is involved in specific neural circuits that control certain behaviors (perhaps ciliary activity), which are critical during development. Control samples with no primary antibody added showed no fluorescent cells, thus confirming that TPep labeling is specific to TPep only.

TPep exists in the foot of the adult *T. diomedea* and controls its pedal ciliary activity (Willows *et al.* 1997). However, no TPep labeling was seen at the ciliated foot structure of *T. diomedea* larvae. This observation is consistent with prior knowledge. Because the foot of the larva is not used for locomotion like it is in the adult, TPep is not needed at that location.

However, direct application of TPep to larval cilia produced different effects on different types of cilia. First, TPep did not show any influence on pedal cilia. Immunohistochemistry data indicated that TPep is absent from the foot, which might explain the failure of externally applied TPep in affecting larval pedal ciliary beat frequency. However, pedal cilia beat constantly, and this suggests that perhaps pedal cilia of *T. diomedea* larvae are under the control of other neurotransmitters. Second, TPep is present at the base of the ciliated cells of the velar lobes, which included both short and long velar cilia. Interestingly, TPep affected the CBF of long velar cilia but not short velar cilia. Similarly to pedal cilia, short velar cilia appear to beat constantly; therefore, the data also suggests that short velar cilia are under nervous control, operated through different neurotransmitters than TPep.

In contrast, TPep had significant effect on long velar cilia's beat frequency ($p<0.03$), although the observed responses were not what I predicted. I expected to see a positive correlation between TPep concentration and CBF. Behavioral data, however, showed an initial decrease in CBF between the concentrations of 10^{-8}M and 10^{-5}M, and then an increase at the concentrations of 10^{-4} and 10^{-3}M. One possible explanation for this behavior is the effect of chemical overdose. The concentrations of TPep from 10^{-8}M to 10^{-5}M have been shown to be effective in controlling pedal ciliary beating (Willows *et al.* 1997).

When exposed to TPep at those concentrations, the larva showed a consistent trend of decreasing CBF. Yet, as TPep concentration exceeded the normal range, the larva increased its CBF. High concentration of TPep might have damaged the animal, and thus its reaction to TPep at that time was non-specific, which made the overall data set inconclusive. Some other possible complications might be due to the inability of TPep to penetrate the tissue or that the TPep solution was diluted when it was squirted onto the preexisting FSW. In addition, the current method did not allow the complete removal of preceding FSW solution prior to administering a new dose of TPep.

In summary, future experiments need to address the following issues. First, researchers need to conduct more trials and take more measurements during each trial in order to increase the results' statistical power. Second, current data suggested that more observations should focus on the interaction of cilia and TPep at concentrations between 10^{-8}M and 10^{-5}M. Therefore, diluting TPep in smaller increments within that range might help to elucidate the true effect of TPep on long velar cilia. Third, an improved method is needed to minimize dilution of TPep due to the presence of preexisting FSW.

Despite of the inconclusiveness of the behavioral data, my immunohistochemistry data confirmed the presence of TPep at the CNS and at the base of the ciliated cells of velar lobes in *T. diomedea* larvae. It provides further insights into the neural development of *T. diomedea* and a foundation for future research on the role of TPep in nudibranch veliger larvae.

Acknowledgements

I thank Dr. Shaun Cain and soon-to-be Dr. Michael Baltzley for their everlasting guidance, inspiration, encouragement, and sarcasm; my accomplice Marcel Tam for his priceless support/entertainment, and his meticulous *Tritonia* babysitting skill; Owen Woodward for his assistance in experimental set-up; Dr. A.O.D. Willows, Dr. Pedro Verdugo, and Russell Wyeth for their insightful suggestions. This project was funded by the Mary Gates Endowment and McNair Research Stipend.

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Hoang Nhan
Friday Harbor Laboratories
University of Washington, Friday Harbor, Washington 98250, USA

Characterization of Plasma Potential near Ion Thruster Discharge Cathode

Sonca Nguyen

Abstract

Kameyama and Wilbur[1] have proposed a potential hill model to explain ion energy spectra near the cathode inside a discharge chamber of an ion thruster. The plasma potential hill could be responsible for keeper erosion, a concerning issue which can possibly affect the thruster's operating capability. The focus of this work is to investigate the ion energy spectra near the cathode and to determine if such a hill-like structure actually exists. Using electrostatic probes, we have characterized the plasma potential near the cathode. Experimental results indicate the presence of a small potential hill. However, these results do not provide strong evidence to support Kameyama and Wilbur's model with confidence.

Nomenclature

V_p = plasma potential

V_f = floating potential

T_e = electron temperature

e = electron charge

I_e = electron current

I_i = ion current

Introduction

The ion thrusters on the Deep Space 1 (DS1) spacecraft accumulated 16, 265 hours of operation in space before the mission was terminated. This mission successfully demonstrated that ion thrusters can serve as a reliable primary propulsion system.[2] This success has led to efforts to develop more powerful thrusters. NASA is planning a future DAWN mission to orbit Vesta and Ceres, two asteroids located in the asteroid belt between Mars and Jupiter.[3] NASA plans to use NSTAR-type ion thrusters tested on the DS1 spacecraft for this mission.[4] The thrusters to be employed require over 30,000 hours of operation and will process over 400 kg of xenon fuel.

The Extend Life Test (ELT) performed at the Jet Propulsion Laboratory (JPL) has shown that by the end of 30,325 hours of operation, the discharge cathode keeper face totally eroded and exposed the cathode orifice plate to the plasma.[5] Although the ELT has verified that keeper erosion did not affect the thruster's operating capability, the detrimental erosion detected in the keeper face is, nonetheless, an issue that needs to be addressed. While several groups in the electric propulsion community have offered models to elucidate the cause of the erosion, very few experimental works have been performed to explain this phenomenon.

Kameyama and Wilbur from Colorado State University (CSU) proposed that there is a plasma potential hill near the cathode.[1] Many previous works in this area of research have often discussed this potential hill model in their work, especially to explicate the cause of keeper erosion. In particular, Williams *et al.* in their laser induced fluorescent (LIF) work detected back-flowing ions near the region of erosion via ion velocity mapping at an energy level of a few volts.[6] Williams *et al.* measured velocity vectors emanating from a common region, and the energy distributions they obtained were consistent with the potential hill model.[7]

In a different study, Domonkos *et al.* offered explanation for the probable causes of keeper erosion. They asserted that two possible erosion processes occur inside the discharge chamber: electron backstreaming and sputtering by xenon ions of the ion collecting surfaces of the keeper.[8] Domonkos *et al.* predicted that electron backstreaming is most pronounced on the centerline. Furthermore, a large fraction of the backstreaming electron current is collected by the discharge cathode and keeper. In the same report, they asserted that with sufficient current density and acceleration potential, the backstreaming electron beam can vaporize components. This suggests that erosion of the keeper face can be a result of vaporization caused by a backstreaming electron beam. They further proposed that ion current distribution on the keeper causes increased wear in the orifice, and sputtering caused by doubly charged ions is the most significant factor in keeper erosion.

Foster and Patterson's different approach in their recent study of the downstream ion energy distributions proposed that ions inside the cathode gain energy via charge exchange in collisions with the cathode orifice wall as they exit toward the discharge chamber.[9] Their results show that the ions falling out of the discharge plasma result in a dominant ion signal and energetic ions were detected in the tail of the distribution function.[10]

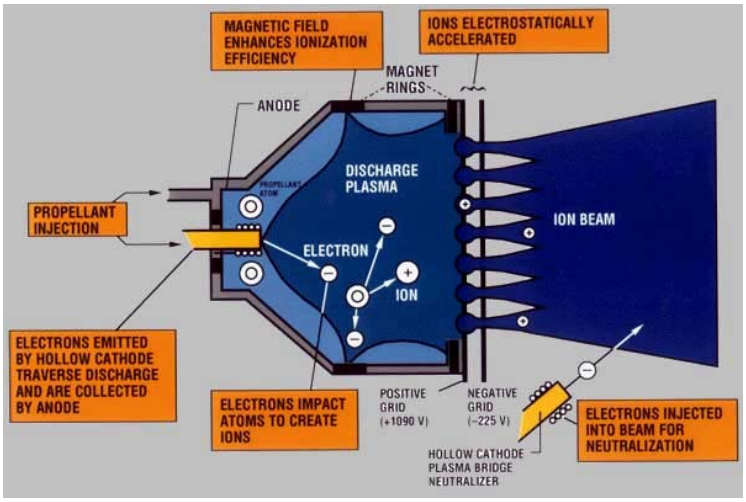
Several people, including Katz *et al*.,[11] have also addressed the need to understand plasma behavior through 1-D and 2-D models. While the model Katz *et al*. presented was simple, it nevertheless shows evidence of a potential hill. Katz *et al*.'s 1-D model suggests that a double ion concentration, believed to be responsible for most of the ion sputtering, is only a small fraction of the total ions.

All of the previous works indicate a strong need for experimental characterization of the plasma potential near the cathode to validate the potential hill model. The work presented here is part of the on-going effort to understand the ion energy spectra inside the discharge chamber, especially near the cathode. The plasma potential is characterized for two different flow rates and different discharge currents. The results show a trend that indicates the presence of a small potential hill. The magnitude of the hill is discovered to be dependent on the operating conditions. This experiment was performed in the Electric Propulsion Research Building at NASA Glenn Research Center under the guidance of Dr. John E. Foster.

Description of an Ion Engine

Figure 1 shows a side view of a typical engine. Important features to note are the hollow cathode, discharge chamber, magnetic field, and positive and negative grids.

Figure 1. A side view of a typical ion engine. The hollow cathode emits electrons, which interact with the neutral gas to form plasma inside the discharge chamber. An ion beam is generated to provide thrust. Courtesy of NASA Glenn Research Center.



Propellant, usually xenon, is injected into both the hollow cathode and the discharge chamber. The hollow cathode's primary function is to emit electrons into the discharge chamber. Neutral gas in the discharge chamber is ionized through electron bombardments and plasma is generated. The magnetic rings along the plasma chamber confine the electrons to remain in the core of the discharge chamber and to prevent them from accelerating to the chamber wall, the anode. The ions in the plasma are attracted to the negative grid, which is also known as the accelerator grid. As the result, an ion beam is created, which produces thrust.

Theory

Kameyama and Wilbur presented a report in 1998 at the International Symposium on Space Technology and Science in Japan

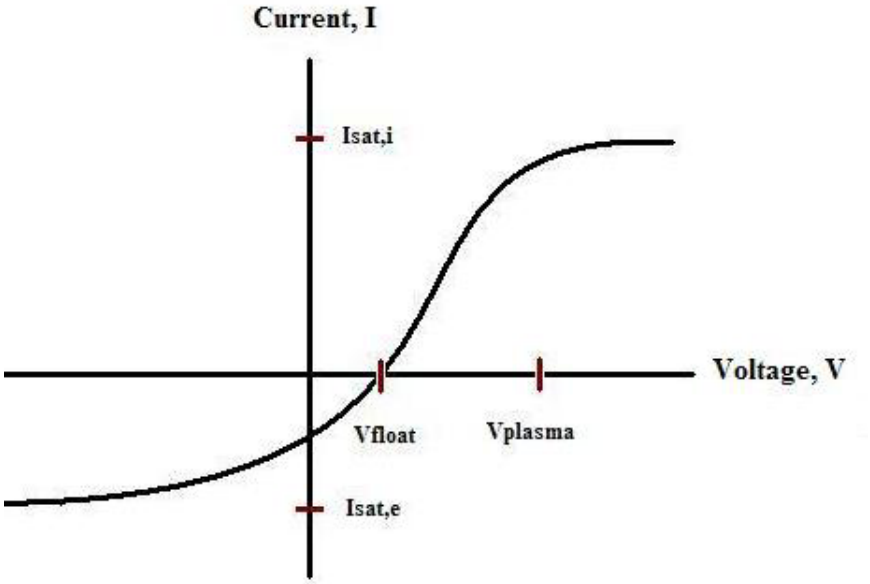
proposing that a plasma potential hill exists in a small region immediately downstream of the discharge cathode.[1] Their model applies conservation equations to account for the primary electrons from the cathode, ions due to impact collisions, and secondary electrons. In the region between 1 and 5 mm downstream of the cathode, their model suggested that there is a peak in potential. They asserted that when the electrons ejected from the cathode ionized the neutral gas atoms, the secondary electrons which have very low mass can easily escape this region of ionization. However, the massive ions tend to accumulate and thus induced an electric field there. The potential hill is formed from the accumulation of the ions and its height increases until stability is achieved.

The existence of the potential hill results in the ions upstream of the hill to accelerate back towards the cathode and the ions downstream of the hill to accelerate toward the accelerator grids. If the model is accurate, it has been suggested that the ions that accelerate towards the cathode collide into the keeper and thus erode the keeper surface. Although not stated in their paper, the energy of some fraction of the ions impacting the keeper is postulated to be high enough to exceed the sputtering threshold, which results in the erosion of the keeper surface.

Results presented in this paper were taken from single Langmuir probes. In the early 1920's when the study of plasma physics was still at its early stage, Irving Langmuir proposed a technique to measure plasma parameters, which is now known as the single Langmuir probe. This technique involves inserting a single probe into the plasma and measuring the current drawn to the probe as a function of applied bias voltage.

Measurements obtained from the single Langmuir probe are usually displayed in a I-V (current-voltage) characteristic curve shown in Fig. 2. The I-V curve provides the following information about the plasma: floating potential, plasma potential, ion saturation current, and electron saturation current.

Figure 2. Current-voltage characteristic curve obtained from a single Langmuir probe. Important plasma parameters are labeled.



In regions where the I-V characteristic curves do not exhibit clear electron saturation, the plasma potential could not be determined from I-V curves. However, the Langmuir probe can accurately measure the electron temperature and floating potential. The plasma potential is then calculated from the measured electron temperature and floating potential using Equation 1.[12]

$$V_p = V_f + \frac{kT_e}{e}\ln\left(\frac{I_e}{I_i}\right) \tag{1}$$

By assuming the flux condition for the electron and ion current, the plasma potential is reduced to the following equation.

$$V_p = V_f + \frac{kT_e}{e}\ln\left(\frac{M/Z}{(2m\pi)^{1/2}}\right) \tag{2}$$

where Z is the charge of the ion, m is the electron mass, and M is the mass of the ion.

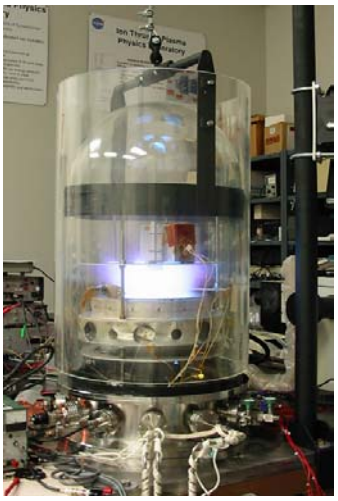
Replacing the values for xenon, Equation 2 may be simplified.

$$V_p = V_f + 5.27T_e \tag{3}$$

Experiment Setup

A bell jar located in Vacuum Facility 65 in the Electric Propulsion Research Building at the NASA Glenn Research Center, shown in Fig. 3, was used to operate the plasma discharge under vacuum condition. The base pressure of the bell jar was between 10^{-7} and 10^{-8} Torr and the pressure was at 1.3×10^{-4} Torr when the discharge was under operation with xenon as the propellant.

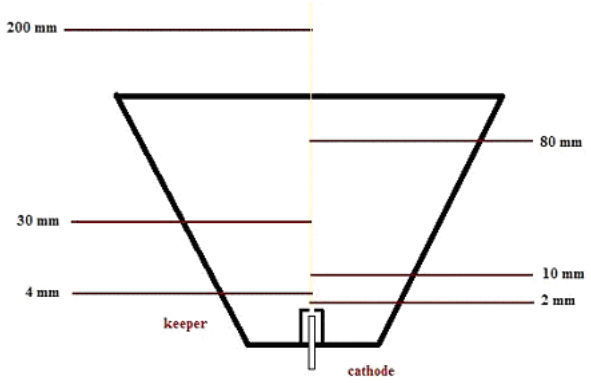
Figure 3. Bell jar in experimental set-up. Discharge plasma is glowing.



A 30-cm discharge chamber with the same geometry as that of a NASA Solar Electric Propulsion Technology Applications Readiness (NSTAR) engine was tested. A NASA Evolutionary Xenon Thruster (NEXT) cathode was used to generate the discharge. The keeper electrode assembly was included in the cathode assembly and it floated nominally to approximately 5 V with respect to the cathode. A flow meter was used to measure the flow rates.

Langmuir probes were placed along the following locations downstream of the keeper orifice: 2, 4, 10, 30, 80, and 200 mm. Figure 4 illustrates the probe locations along the discharge chamber's axial direction. The data was taken at 4.33 sccm and at 3.55 sccm flow rate.

Figure 4. Probe locations along discharge chamber's axial direction.



Procedure

The plasma potential, electron temperature, and floating potential were measured using the SmartSoft program at the locations listed in the experimental set-up at two different flow rates: 4.33 and 3.55 sccm. The discharge currents were varied between 11.0 and 15.0 Amps in 0.5 Amp increments. The plasma potential was determined using Equation 3.

Results

In this experiment, the cathode potential is defined as ground. Figure 5 shows plots of plasma potential with respect to ground versus distance of the probe measured from the keeper surface for 3.55 sccm flow rate. The x-axis scale extends from the keeper (x=0) to the location of probe 4 (x=30 mm). In reducing the scale of the x-axis, details of the plasma potential near the cathode are shown. Figure 6 shows identical plots but with the x-scale extended to include all six probes (x=200 mm). This figure fully characterizes the plasma potential from the keeper to the exit plane of the engine in 1-D.

The plots in these figures are varied by discharge currents from 11 Amps to 14 Amps and the voltage was varied between 26 and 30 volts. The most appreciable plasma potential difference between the plots is the data obtained from Probe 3, as shown in Figure 5. In other locations, the

plasma potential varies with discharge current but the variation from probe 3 is greatest.

The general trend illustrated in Fig. 5 indicates the plasma potential is highest at 2 mm and then continuously decreases to 30 mm. Figure 6 shows that the plasma potential at 80 mm is higher than that at 30 mm. Somewhere between 10 and 80 mm, the plasma potential is at a minimum. From 80 mm, the plasma potential continues to increase passing the exit plane. The height of the plasma potential hill discovered near the cathode varies between 5 volts and 7 volts by inspection.

A similar phenomenon is detected for 4.33 sccm flow rate. Figure 7 shows plots of plasma potential versus probe position for 4.33 sccm flow rate with the scale of the x-axis set at 30mm. Evidence of a plasma potential hill is exhibited through these figures. The hills shown by these figures are a little more pronounced than those of the lower flow rate discussed previously. The height of the hill ranges from 7 to 10 volts by inspection.

Similar to the lower flow rate, the plasma potential at 4.33 sccm flow rate varies appreciably at 10 mm (probe 3) with discharge currents. Figure 8 shows similar plots with an extended axial distance. The results obtained at this flow rate have similar trends as those obtained at 3.55 sccm. The heights of the potential hills are slightly higher than the height of the hills obtained at 3.55 sccm.

Measurements taken during this experiment were at discrete locations along the axial direction of the engine. Due to the limited number of probes used, the actual maximum and minimum could be between data points. The plasma potential was supposed to be characterized in the axial direction along the center line of the cathode from the orifice to the exit plane of the engine. The probe tips were not precisely placed along the center line due to some physical limitations. This imprecision might contribute to some small error in the analysis, especially if the potential varies radially.

Conclusions

Plasma potential was measured at 6 locations along the axial direction from the cathode keeper. The results indicate evidence of a plasma potential hill for different flow rates and operating conditions. While the results indicate the presence of a potential hill, the heights of the hill at both 4.33 sccm and 3.55 sccm did not match the required height of the theoretical hill to support Kameyama and Wilbur's potential hill model, which may contribute to the erosion of the cathode keeper.

Future Work

The need to understand the plasma behavior inside the discharge chamber, particularly near the cathode, is immense. For this experiment, probes were placed at discrete locations. A finer resolution of data points will provide more conclusive results and thus a deeper understanding of the plasma behavior and is highly recommended for future work. In addition, non-intrusive techniques, such as an electron beam, are also highly recommended and should be examined as an alternative measuring device.

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Figure 5. Near-field V_p measurements of all 6 probes at 3.55 sccm.

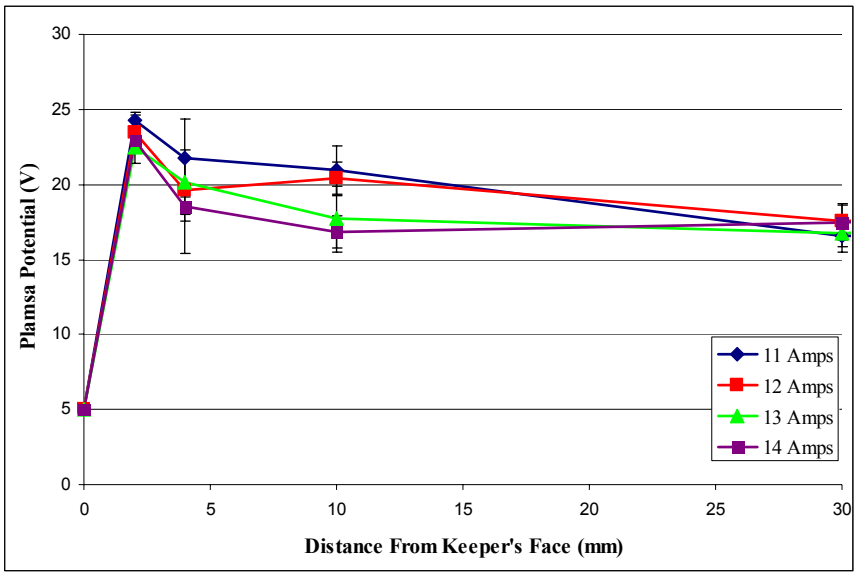


Figure 6. Near-field V_p measurements at 3.55 sccm.

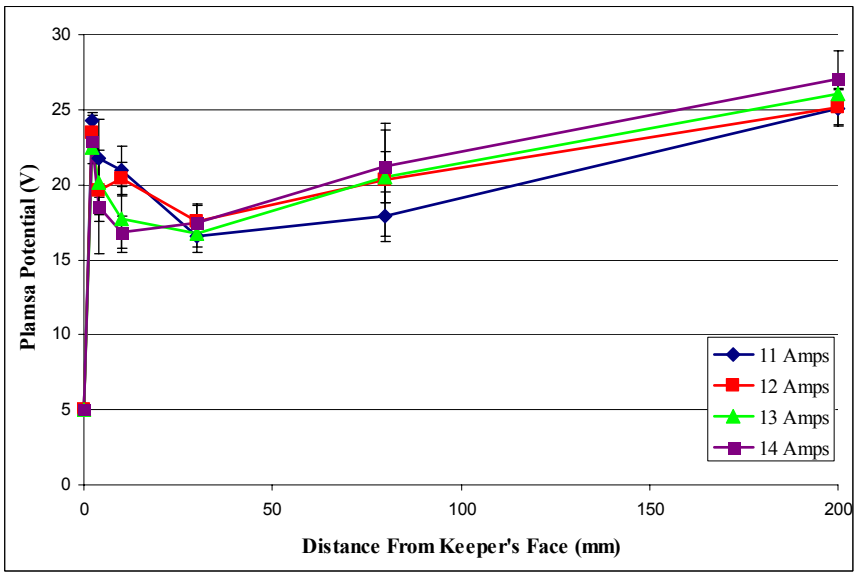


Figure 7. Near-field V_p measurements of all 6 probes at 4.33 sccm.

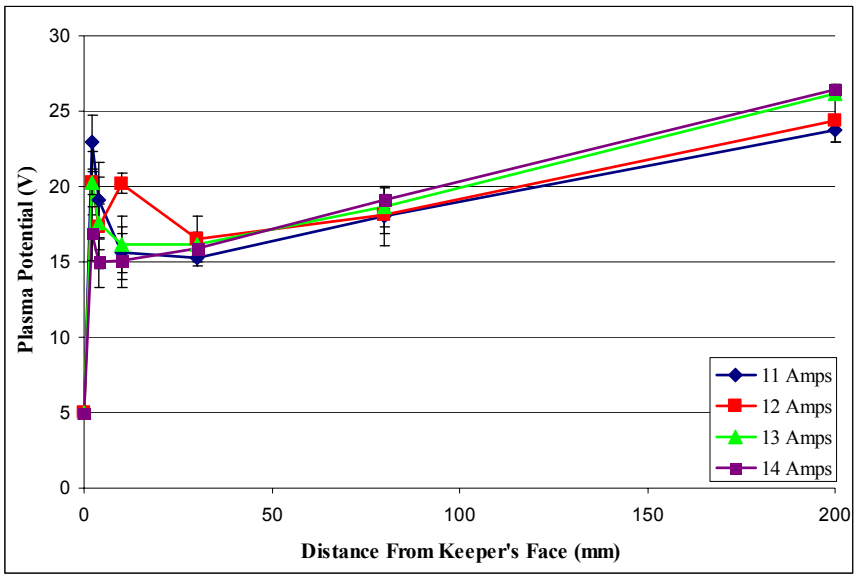
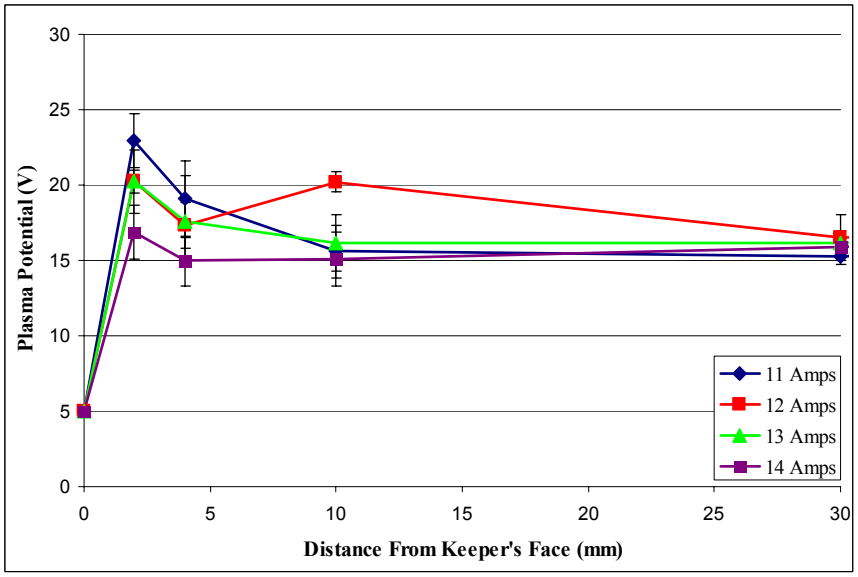


Figure 8. Near-field V_p measurements at 4.33 sccm.



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Sonca Nguyen
Department of Aeronautics and Astronautics
sonca@u.washington.edu

My research interest is in electric propulsion.

My intended Ph.D. program is Aerospace Engineering.

Recruiting Rounds:
Recruiting Minority Middle School and High School Students into Nursing

Janelle Sagmiller

ABSTRACT

Currently our nation finds itself amidst a nursing shortage. There is little research regarding recruitment of untapped populations such as minority middle and high school students. ***METHODS:*** *A pretest-posttest quasi-experimental design utilizing the Perception of Nursing Survey was used to identify the impact of the Recruiting Rounds PowerPoint Presentation (RRPP), on middle and high school students' perceptions of, and interests in, the nursing profession. A convenience sample of 450 students residing within a Northwester U.S. school district with greater than 60% minority students was selected. Questions included: Was there a difference in student interest level and perception about nursing from pretest to posttest following the RRPP? If so, do the changes reflect a more positive or negative interest level and opinion of the nursing profession?* ***RESULTS:*** *Of the 450 eligible students, only 174 questionnaires were adequate for analysis due to availability of students from scheduled classes and properly completed forms. After approximately 40 minutes of time lapsing from pretest to posttest, results indicate ($p \leq 0.01$) student interest in and perception of nursing changed from a slightly positive view to a more positive view. Findings of total opinion ($p \leq 0.049$) and total interest ($p \leq 0.01$) scores suggest gender has slight correlation with scores. Findings of demographic variables and student scores are also discussed.* ***CONCLUSION:*** *These results indicate the possibility for recruitment strategies to be developed when recruiting this population. Identification of key characteristics that appeal to this population is warranted, and could help shape future tools for nursing recruitment.*

Introduction/ Specific Aims

Currently, our nation finds itself amidst a nursing shortage. In 2002, the U.S. Department of Health and Human Services reported that 90 percent of nursing homes were experiencing staff shortages (Barkley & Kohler, 1992). Today, more than 75% of our nation's hospitals advertise vacant nursing positions, and for tomorrow's 2010 projection by the US Bureau of Labor Statistics, more than one million nurses will be needed to serve our population (Barkley & Kohler, 1992).

To further complicate our nursing shortage problem, presently we experience a health service disparity; wherein, racial and ethnic minority groups represent only 14% of the registered nurse population but comprise over 28% of the nation's population (Villarreul, Canales, & Torres, 2001; Barbee & Gibson, 2001). This figure indicates the percentage of practicing minority registered nurses is not representative of the population. Since representation of ethnic minority nurses is critical for quality care in serving diverse populations, there is a need to recruit minorities into nursing (Dowell, 1996; Sherrod, 1995). Both the Institute of Medicine and the federal government recognize this need, and consequently, one primary objective for Healthy People 2010 calls for an increase in the number of all health profession degrees awarded to under-represented racial and ethnic minority groups (DHHS, 2000).

One suggestion for alleviating our nationwide nursing shortage is to recruit middle school and high school students into the field. This study proposes to focus on the importance of recruiting minority high school and middle school students into nursing. A potential positive impact of recruiting this population into nursing is nursing school enrollment may increase in the future. Another positive impact of recruiting minority students into nursing is the capacity to increase the quality of care for diverse populations (Hodgeman, 1999).

Recruiting Rounds Power-Point Presentation (RRPP), an oral classroom presentation, was the nursing profession recruitment strategy tool, developed by the investigator, which targeted minority middle school and high school students. This study aimed to identify the impact of the RRPP on middle school and high school student's perceptions of and interest in the nursing profession. Questions asked throughout this study included: Was there a difference in student's interest level and opinion about nursing as a potential career choice from pretest to posttest, following the RRPP? If so, do the changes reflect a more positive or negative interest level and opinion of the nursing profession?

By using the Perception of Nursing Survey (PNS) developed by Grossman, Arnold, Sullivan, Cameron, and Munro (1989) for school age children, demographic variables such as gender, cultural background, and school age where compared to opinion scores about nursing generated from a 14-item questionnaire. Each student's opinion score was then compared to student's interest level in pursuing nursing. In addition to the 14-item questionnaire, there was a spot for students to rate their current interest in nursing on a Lickert scale of 1-10, with one equivalent to the least amount of interest and ten corresponding to the most amount of interest they currently have in nursing.

Background

Recruiting younger people

The viability of the nursing profession depends upon successful recruitment efforts and successful recruitment targeting middle school and high school populations. After identifying nursing perceptions held by minority high school students, Rossiter and Yam (1998) suggest that, "the nursing profession should be promoted to students at various critical stages during their school years," which would help to, "reinforce and refocus their attention on the nursing profession, thus enabling them to make a more informed and balanced decision in their career choice." Another study conducted in 2000 concurred with the previous findings, and stressed that recruiting should begin earlier than high school age, since career choice is a process that begins early in life (Leonard & Iannone). The critical stage in development which Rossiter and Yam eluded to in their study, has been defined as the age period between 10 and 14; wherein, programs have the most impact helping the student transition from middle school to high school and they can make course decisions accordingly (U.S. Department of Education, 1998). Finally, the U.S. Department of Education also recognizes the need to begin career awareness programs early in academic programs. The significance to early awareness programs is that during the critical middle school stage, students are able to make decisions regarding high school course selections, while at the same time gaining experience that can broaden their knowledge for future career choices (Gonzalez, Kearns, Lafferty, Lampignano & Papps, 2000). Recruitment of minority middle school and high school students then must be addressed, and successful recruitment strategies must be sought after and employed.

Changing perceptions of nursing

However, before embarking on recruitment strategies, Reiskin and Haussler (1994) claim that, "if colleges and universities are to recruit and retain culturally diverse students, they must first know how high school students perceive nursing." The public's perception of nursing has changed from a 1940's view of competent, intelligent, and courageous nurses undertaking independent, science-based actions in their practice, to the recent image of nurses characterized as: caring, useful to society, educated- although not linked with being "scientifically knowledgeable" or perceived as not having enough knowledge for autonomous practice like doctors (Droes, Hatton & Kramer, 1993). Despite the studies conducted on the general public's view of nurses, what about high school and middle school students' perceptions of the nursing profession?

Such negative public images could influence high school and middle school students when they decide to choose nursing as a future career, thus it is important to identify what current perceptions high school and middle school students have regarding nursing. A number of studies have looked upon high school students' perceptions of nursing; however, there is scant research on middle school students' perceptions of nursing.

To begin with, the literature review found high school students' perceptions to be similar to those views held by the general public. Despite most high school students being aware of the nursing profession as caring and helping, they perceive nursing as a low status career with unpleasant tasks such as manual labor, working inflexible working hours around a busy work environment, with little income and job security (Grossman et al., 1989; Grossman & Northrop, 1993; Helmsley-Brown & Foskett, 1999; Marriner-Tomey, Schwier, Marticke & Austin, 1990; Marriner-Tomey, Schwier, Marticke & May, 1996). In addition to theses views, studies have identified high school students lacking knowledge about diverse nursing career opportunities in teaching, research, management and administration (Grossman et al. 1989; Grossman & Northrop, 1993).

Comparable negative attitudes towards nursing have also been found in studies centered on minority high school students' perceptions of nursing. Although, ethnic students did perceive nursing as caring, they viewed nursing to be passive, weak, powerless, and lacking knowledge and independence. For example, in a 1997 study wherein 157 high school students were interviewed by questionnaire collecting data on their perception and knowledge of nursing. Most students thought knowledge was not needed to provide care. Students also considered

nursing not to incorporate science and technology into their practice; however more intriguing was the thought that nurses obey all doctors orders without reasoning (Rossiter, Bidwell & Chan, 1998). Later another study conducted by Rossiter and Yam (1998) found there was a recruitment problem when attracting minority students into nursing, because student perceptions still view nurses lacking status, power, autonomy and control.

Despite the glum appearance of unchangeable nursing perceptions, a few studies have shown that high school students' perceptions of nursing can be positively changed. In a nursing summer recruitment institute targeting minority high school students, Sherrod found pretest-posttest Perception of Nursing Survey (PNS) scores significantly differed upon completion of the institute (1995). Upon completion of the institute, significant student scores indicated students regarded nursing as a career in which one could manage large groups of people, have impact on international healthcare, and teach in college or university (Sherrod, 1995). In another pretest/posttest design, conducted on 451 high school biology students using the Nursing Image Questionnaire, the study found the image building process successful in positively increasing students' valuation of the nursing career (Droes et al., 1993). Lastly, in an attempt to capture middle school students' perceptions of nursing and how they can change with an intervention, another study employing the PNS found significant differences in perceptions pre nursing camp opinion scores compared to post camp opinion scores in areas such as nursing, "provides the opportunity to manage many people, teach in a college or university, be in demand and sought after, be an executive, design and direct health care programs, and be on the cutting edge of science," (Drenkard et al., 2002).

Successful Recruitment Strategies

The above three intervention based practices to positively influence student perceptions have proved successful in that the majority of students' perceptions were changed.

In a review of past high school and minority recruitment, again, more studies and attempts have been made to capture high schools students into careers like nursing. For example, university and college based recruitment programs employ a variety of recruitment strategies including, nursing brochures, high school scholarship programs, and offer didactic and hands-on learning experiences along with nursing mentorship programs (Hodgeman, 1999). Hospital based programs

range from 8-day nursing camps to comprehensive 2-year programs that prepare students for college entry (Sherrod, 1995).

However, another form of recruitment that takes nursing to the student is classroom recruiting. The state of Indiana utilized a collaboration of nursing service and nursing education that culminated in a project entitled Nursing 2000 which targeted high school and middle school students into nursing careers. In a study that reviewed Nursing 2000 recruitment strategies, classroom recruiting was found to be, "a powerful influencing factor in the promotion of nursing as a viable career," with an estimated perceived influence on career choice of 37% (Wilson & Mitchell, 1999).

Classroom recruitment continues to be a successful recruiting tool that brings students information in ways that can be made creative by the presenter. In addition to the malleable organization of classroom presentations, with finesse and determination, the target population to be recruited can compose the audience; thus giving classroom presentations an upper hand when choosing whom to recruit.

METHODS

Sample

Sampling criteria for the school age students included public middle and high school children. Since minority high school and middle school students are the target population for this study, one school district in the Northwest with greater than 60% minority composition was conveniently selected for the intervention. Within that school district all public middle schools and high schools were selected to receive the presentation intervention. A random sample of students was not possible due to each individual school's curriculum and scheduling conflicts. For that matter, each public school principal selected the sample of students based upon accessibility within the school's curriculum. The target sample size of 450 students was utilized based upon the original study sample size Grossman used in 1989 with the advent of the Perception of Nursing Survey (PNS). Ideally, 225 middle school students and 225 high school students would have composed the 450-student sample size. However, due to availability of students and classroom scheduling, only 155 middle school students and 98 high school students were available for the presentation, making a total of 253 students available.

Instrument

The Perception of Nursing Survey (PNS) was used to assess the impact of the Recruiting Rounds Power Point presentation (RRPP) on high school and middle school students' opinion and interest level of the nursing profession. Since the tool has been used in previous studies, content validity was established with 0.92 proportion of expert agreement of total items judged as valid ($p<.05$) and Cronbach's alpha for reliability was 0.76 (Grossman et al., 1989). The beginning of this survey included a survey list of 14 questions measuring student opinions of nursing. The survey, answerable by: yes, somewhat, unsure or no, was coded as: 4= yes, 3= somewhat, 2= unsure, and 1= no. Following the 14 item opinion questionnaire, a Lickert scale of 1-10 was employed to elicit students' interest level in nursing with one signifying least interest and a score of ten corresponding to most interest in nursing. As well as gathering data on opinion and interest in nursing, the PNS also gathered demographic data such as age, gender, school grade and cultural background.

Design

A single group, pretest-posttest, quasi-experimental design proved most useful when measuring each student's outcome. Public middle school students enrolled in grades 6-9, with typical ages of 11-13, and public high school children (enrolled in grades 9-12, with general ages ranging from 13-19) were defined as students. Demographic independent variables such as age, gender, cultural background, and school grade were identified by use of the PNS questionnaire. Dependent variables such as interest in and opinion about nursing were measured by the same opinion questionnaire.

Next Page: Perception of Nursing Survey (PNS) Questionnaire (The Perception of Nursing Survey was developed by: Grossman, D., Arnold, L., Sullivan, J., Cameron, M., & Munro, B. (1989).

Do you think a career in nursing provides the opportunity for you to:

1. Help people live healthy lives?
   Yes Somewhat Unsure No

2. Master high-technology instruments?
   Yes Somewhat Unsure No

3. Care for individuals, families, and communities during time of need?
   Yes Somewhat Unsure No

4. Manage large groups of people?
   Yes Somewhat Unsure No

5. Be a leader in directing and influencing national health policy and legislation?
   Yes Somewhat Unsure No

6. Teach in a college or university?
   Yes Somewhat Unsure No

7. Be a member of a prestigious profession?
   Yes Somewhat Unsure No

8. Be in demand and sought after?
   Yes Somewhat Unsure No

9. Be free to change your career focus during your professional life?
   Yes Somewhat Unsure No

10. Be an executive?
    Yes Somewhat Unsure No

11. Design and direct health programs for business and athletic organizations?
    Yes Somewhat Unsure No

12. Have an impact on international health care?
    Yes Somewhat Unsure No

13. Be on the "cutting edge" of scientific research?
    Yes Somewhat Unsure No

14. Achieve financial success?
    Yes Somewhat Unsure No

Please rate your interest in nursing on a scale of 1-10, with 1 being least and 10 being the most interest in nursing: 1 2 3 4 5 6 7 8 9 10
Least Most

Please circle the response that best describes you

Gender Male Female

Age 11 12 13 14 15 16 17 18 19

Grade 5th 6th 7th 8th 9th 10th 11th 12th

Cultural Background Native American African-American Asian American
Hispanic Caucasian Pacific Islander Other_____

Procedures/Protocols

To access the specific population described above, gaining permission from: the selected school district superintendent, the middle school and high school principals, the University of Washington Human Subjects Review Board, as well as parental and student permission was needed. First, the selected school district superintendent and researcher had a meeting determining the appropriateness of the study conducted with students as well as detailing the rights of parents and students' for declining participation in the study.

After superintendent permission was obtained, sample take-home permission slips were constructed, and sent to principals for proofing of appropriate content. Next, a complete summary of intentions and identification of school district and schools to be studied, with copies of permission documents, were sent to the University of Washington Human Subjects Review Board. The permission slip, with English on one side and Spanish on the other, included information regarding the study's purpose, reason why their child was selected, area to be marked to decline study participation and a clause stating that students will again have the choice to opt out of the study and participate in another activity offered at the time.

Students were screened for eligibility by the following criteria: 1) identified as an enrolled student of selected school, 2) resident within selected school district, and 3) attending regular classes appropriate for age and year in school. Upon student and parental acceptance of partaking in study, before the presentation began, students were again reminded of their right to not participate. Completion of both pretest and posttest questionnaire were explained as essential to the study, as well as participation in the RRPP intervention presentation. Before the presentation, pretest questionnaires were handed to students for completion, and then collected by instructor before the presentation began. Immediately after the presentation, students were given the posttest questionnaire and again questionnaires were collected once students had finished answering questions. No pilot test procedures were conducted.

Protection

Due to the test population being minors, and the delicate school district selection for a high minority enrollment, students' identity and enrollment of selected school district were kept anonymous. The investigator explained to students that their responses would be kept confidential and that only the investigator would have access to the

questionnaires. While students were directed not to put their name on the questionnaires at any time, demographic information such as gender, age, school grade and cultural background were self-reported by each student. All research questionnaires were color coded in either one of two colors, according to which questionnaire was used at the time. For example, a bright orange questionnaire was used as the pretest questionnaire, and bright yellow was used as the posttest questionnaire.

Each student was given the opportunity to generate their own Student Identification Fun Code to be used on the questionnaires. Sticky notes, with different numbers on each note, were randomly passed to each student by the investigator. Next, on the sticky note, students were asked to write down an animal followed by a color of their choosing. This number- animal- color code became the Student Identification Fun Code, and students utilized their unique generated code on both pretest and posttest questionnaires, so responses could be tracked and comparisons made. After students generated their code and completed the first questionnaire, the investigator began the RRPP presentation. Each presentation lasted approximately 42 minutes, after which students were given the posttest survey and asked to hand in upon completion.

Results

For analysis of the data, the students' scores were separated into two categories. First, there is the overall perception of the nursing profession score fashioned by the 14 item questionnaire answerable and coded by: yes= 4, somewhat=3, unsure=2 and no=1. The maximum score, indicating a more positive opinion of nursing for this section was 56; whereas the minimum score, reflecting a more negative opinion of nursing, was 14. Second, the students' Lickert scores were calculated by their pre and posttest interest level in nursing, with one representing least interest in nursing and ten representing most interest in nursing. Both the opinion and interest score were analyzed in context of demographic variables, as well as analyzed in context of each other.

For the purposes of this study, some cultures represented on the PNS, are not utilized when comparing scores. Based upon student self identification, there were less than 5 students from each cultural group: African American, and Asian American / Pacific Islander. As a result, these cultural groups are not included in data sets looking just at culture due to low representation; where as, Native American, Hispanic, Caucasian and Multi-cultural groups are utilized when comparing culture opinion and interest level scores.

Only completely filled out questionnaires were utilized for data analysis. Of the 253 students available to participate in this study, only 174 students with matching pretest and posttest questionnaires were utilized for data analysis. Of the 253 available students, 9 students declined participation, 10 students marked double answers on questionnaires, 52 students had incomplete questionnaires (often times missing only one question), and 8 were discounted because of low cultural background representation. It is important to note that while many questionnaires were eliminated because of double responses or missed items, the majority of students showed an interest in participating in this study.

Within the overall opinion score for all students, the average pretest score was 39.914 and average posttest opinion score was 45.754 (see Table 1)**.** Thus indicating an overall 5.84-point perception increase. In other words, students' opinion of nursing positively increased by 10%, or elevated from a good (positive) view of the nursing profession, to a better (more positive) view of nursing. Similar to the opinion score, interest scores between genders differ with girls having a higher preliminary interest than boys, as well as higher posttest interest scores (Table 1). In general, girls had a more positive perception of nursing than boys, as illustrated by their consistently higher pretest and posttest scores for both the opinion section and interest level.

Average total perception scores at pretest and posttest for school and culture are shown below in Table 2. On average, middle school students viewed nursing more positively than high school students and of the majority cultural groups (Native American, Hispanic, Caucasian and Multi-cultural) Caucasian students regarded nursing more positively than other cultural groups. However, multicultural students had the greatest average increase in perception of nursing at 15.7% perception increase compared to the lowest opinion increase (7.9%) held by Native students.

Multivariate tests indicate there was no relationship or correlation for students' total opinion score between demographic variables such as cultural background or school grade (middle school and high school). However, results of One-way ANOVA found gender to have a slight relationship to both the pretest and posttest opinion score with a pretest p value of 0.061 and posttest p=0.089 (Table 3), with general analysis of tests between subject effect showing gender's p= 0.049 (Table 4). Thus illustrating that during the pretest, girls tend to score higher than boys, but during the posttest, boys score nearly as high as girls. On both tests, girls tended to have a higher average pretest (40.929) and posttest (46.786) score than boys' pretest (38.900) and

posttest scores (44.722) (see Table 1). The slope for both boys and girls is 5.8, indicating that between the pretest to posttest (about one hour), the treatment influenced both genders on average equally in perception of nursing, with girls continually having a more positive perception of nursing than boys.

Table 1: Mean Scores Between Gender for Pretest and Posttest Opinion and Interest Scores

	Gender	Mean	Std. Deviation	N
Opinion Score 1				
	Male	38.9	8.13	90
	Female	40.9	5.73	84
	Total	39.9	7.13	174
Opinion Score 2				
	Male	44.7	8.94	90
	Female	46.8	7.15	84
	Total	45.7	8.17	174
Interest Level 1				
	Male	4.42	2.5	90
	Female	6.05	2.59	84
	Total	5.21	2.66	174
Interest Level 2				
	Male	6.28	2.76	90
	Female	7.38	2.51	84
	Total	6.81	2.69	174

Table 2: Mean Total Opinion Scores for School and Culture

	Total score for School	Mean	Std. Deviation	N
Opinion Score 1				
	Middle School	40	7.2	113
	High School	39.5	7.03	61
	Total Score for Culture			
	Native American	40.1	6.5	43
	Hispanic	39.6	7.79	92
	Caucasian	41.8	6.69	10
	Multi-cultural	39.2	6.82	21
Opinion Score 2				
	Total Score for School			
	Middle School	46.1	7.59	113
	High School	44.9	9.1	61
	Total Score for Culture			
	Native American	44.7	8.9	43
	Hispanic	45.5	8.5	92
	Caucasian	47.2	5.51	10
	Multi-cultural	48	6.85	21

Multivariate tests also indicate there is no relationship or correlation for students' total interest score between demographic variables (cultural background or school grade). However, results of One-way ANOVA find gender to have a slight relationship to both the pretest ($p=\leq 0.01$) and posttest interest score ($p=0.007$) (See Table 5). Further analysis of tests between subject effect demonstrate gender's $p=\leq 0.01$ (Table 6), suggesting a relationship between gender and interest scores.

Table 3: One-way ANOVA Between Gender and Total Opinion Scores

Total Opinion Score 1		**Sum of Squares**	**df**	**Mean Square**	**F**	**Sig.**
	Between Groups	178.794	1	178.79	3.57	0.061
	Within Groups	8613.671	173	50.079		
	Total	8792.466	174			
Total Opinion Score 2						
	Between Groups	193.058	1	193.058	2.916	0.089
	Within Groups	11385.798	173	66.197		
	Total	11578.856	174			

Table 4: Tests of Between-Subjects Effects for Gender and Total Opinion Score

Source	**Type III Sum of Squares**	**df**	**Mean Square**	**F**	**Sig.**
Intercept	637738.115	1	637738.115	6919.672	≤0.01
Gender	363.77	1	363.77	3.947	0.049
Error	15852.149	172	92.164		

With regards to pretest and posttest nursing interest scores, on average students' interest in nursing increased by 16% after the presentation (Table 1). On the Lickert scale of 1-10, Table 7 illustrates Caucasian students had the greatest average pretest interest score (5.6), while Hispanic students had the highest average posttest interest score (7.03) in nursing. On average, Multicultural students' interest in nursing increased by 19.5%, making them the cultural group with the greatest interest score increase (Table 7).

Table 5: One-way ANOVA Between Gender and Total Interest Scores

Total Interest Score 1		Sum of Squares	df	Mean Square	F	Sig.
	Between Groups	114.787	1	114.787	17.727	≤0.01
	Within Groups	1113.765	173	6.475		
	Total	1228.552	174			
Total Interest Score 2						
	Between Groups	52.876	1	52.876	7.567	0.007
	Within Groups	1201.865	173	6.988		
	Total	1254.741	174			

Table 6: Tests of Between-Subjects Effects for Gender and Total Interest Score

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	12647.532	1	12647.532	1152.373	≤0.01
Gender	161.738	1	161.738	14.37	≤0.01
Error	1887.736	172	10.957		

Both Tables 4 and 6 have time as the intercept during test analysis. In both cases, the intercept, or time, ($p \leq 0.01$) is attributed in accounting for the relationship between scores from pretest to posttest. While the time lapse, approximately 40 minutes, may suggest the intervention greatly influenced the scores, it cannot be substantiated without further testing utilizing control groups.

Table 7: Mean Total Interest Scores for School and Culture

	Total score for School	Mean	Std. Deviation	N
Interest Score 1				
	Middle School	5.31	2.48	113
	High School	5.02	2.99	61
	Total Score for Culture			
	Native American	5	2.81	43
	Hispanic	5.43	2.73	92
	Caucasian	5.6	2.91	10
	Multi-cultural	4.67	2.7	21
Interest Score 2				
	Total Score for School			
	Middle School	6.85	2.69	113
	High School	6.74	2.73	61
	Total Score for Culture			
	Native American	6.49	2.9	43
	Hispanic	7.03	2.68	92
	Caucasian	6.9	2.08	10
	Multi-cultural	6.62	2.77	21

Between the middle and high school students, middle school students on average had the highest pretest interest score (5.31) and posttest interest score (6.85). However, high school students interest scores increased on average by 17.2%, versus 15.4% for middle school students (Table 7).

Of the questions eliciting an opinion of nursing, general results indicate students regard nursing as a profession that cares for individuals, families and communities, as well as helps people live healthy lives and is a profession that leads to financial success. The top three opinion items which scored the lowest include students perceive the nursing profession not in demand and sought after, nor teaching in college and universities

as well as not linked to being a member of a prestigious profession. Discussion of these results will be covered in the following section.

Discussion

Overall, results indicate that after one hour, students changed their interest in nursing as well as their opinions in nursing. This change in opinion scores reflects the same findings as described earlier in the literature review found by Sherrod and Drenkard. In general, both interest scores and opinion scores of nursing become more positive over time. Demographic variables such as culture and school classification do not appear to have as much of an impact on student scores, as does gender.

Results illustrate that middle school students had a higher pretest and posttest interest in nursing than high school students, Caucasian students had a higher pretest interest score, although Hispanic students had the highest posttest interest score, and girls do have a preliminary interest in nursing, scoring on average a 6.05, and then increasing to an average of 7.38. Meanwhile, boys tend to have a lower pre-interest score (4.42) that does increase over time (6.28). In essence, results indicate there is a difference by gender from pretest to posttest. As well as the rate by which girls and boys increase in interest over time is significant, with boys' interest increasing at a greater rate, even though their posttest interest scores are lower than those belonging to girls. Overall the results indicate girls' interest in nursing was high before the presentation and changed much higher over time; whereas, boys' preliminary interest was fair and increased to a high interest in nursing.

Total opinion scores changed as well. Again middle school students had higher scores than high school students, Caucasian students had higher pretest opinion scores; however, Multi-cultural students had the highest posttest opinion score. When looking at opinion scores between gender, gender proves to have an affect upon opinion scores, with girls having the highest pretest and posttest scores. For opinion the rate of change between genders appears to be the same, indicating that no particular gender is having a faster rate of opinion score change.

Perhaps the most interesting finding is that time is the overall variable which appears to have consistent results when looking upon both interest level and opinion of the nursing career. Over time, scores

change which may indicate that the intervention (RRPP) is having the greatest affect upon changes in scores.

When discussing the top three highest and lowest scored opinion items, it should be noted that the mean scores are from all students from pretest to posttest. The top three scoring item illustrate that students generally perceive nursing as a caring, helping and financially stable profession. Within this category, on average there was an 8% increase of opinion concerning nursing providing a financially successful profession (from 3.28 pretest score to 3.61 posttest score, indicating that students were increased from an ambivalent opinion to a stronger belief that nursing profession leads to financial success. On the other end of the spectrum, the lowest scoring items in general (nurses in demand and sought after, nursing providing the opportunity to teach in college or at a university, and being a member of a prestigious profession) move in opinion from students feeling unsure, to students feeling more like nursing *does* provides them the prospect to accomplish the three mentioned opportunities.

Limitations

Possibly one of the greatest limitations to this study was availability of a control group from which time effect could have been tested. Sample size is very crucial to this study; however, availability of students varied greatly. Whereas the study was immensely fortunate to have the students partake in the intervention, scheduling within the school curriculum was cumbersome. The investigator raises the question that timing within the school year for data collection may have an effect on outcomes. For example, results gathered from the beginning of the school year may differ with results gathered at the end of the school year, as well as data collected around state test time versus near vacation time. Since data collection within schools can be difficult, it is appropriate to note that events like a fire drill, as happened during this study twice, may have an affect on findings as well. Any disruptions affecting student attention and investigator-student connection may be damaging to the study, and therefore careful planning as well as communication between research team and school must take place in order to guard against any potentially data gathering interruption. If there were an opportunity to have more students, then provisions for a time control group would have been warranted and added to this study. Without the time control, the data is best used for speculation rather than making definitive conclusions.

Apart from sample size and lack of control group, not having all questionnaires returned also limits the study. Incomplete and not turned

in questionnaires may be a result of confusing questionnaire phrases and vocabulary. During the time students were filling out questionnaires, there were vocal questions from students about the meaning of phrases such as, “sought after”, and “cutting edge” as well as questions about vocabulary: “prestigious”, and “high-technology”.

Conclusions and Implications

Largely, results indicate from pretest to posttest that both student interest in nursing and opinion of the nursing profession changes. For the most part, change is more positive than negative and indicates over the course of the Recruiting Rounds Power Point (RRPP) presentation, student interest and opinion of nursing increases. These implications suggest that aspects of the presentation reach students and influence them, as well as the presentation cuts across culture and school grade affecting them equally. As well as particularly shaping boys interest in nursing, more so than shaping girls interest in nursing.

A short term influence of the RRPP, is when students are deciding which classes to take in the upcoming years, they may choose science courses that will enable them to enroll in college prior to nursing school enrollment. Or perhaps high school students will think of changing their class schedule in order to incorporate classes leading them toward nursing preparation. Of course, a potential long term implication is that there may be an increase in nursing school enrollment, with the advent of these students graduating high school and enrolling in nursing school. Possibly, these recruited nursing school students also represent underserved and minority populations, thereby helping change the face of nursing into a more diverse profession.

Further study may be conducted in order to identify what aspects of the RRPP are interesting to minority students, as well as uninteresting to students. As mentioned earlier, there may be a more definitive study conducted with a larger sample size, including a control group that controls for time. A longitudinal study would prove useful as well, that tracks student career choice after they are exposed to the RRPP.

Essentially, the study concludes there are changes in both student opinion and interest in nursing for the positive. Whether or not the RRPP actually recruits students into nursing for the future, at least public opinion and interest in nursing have the potential for changing. By helping re-educate the public about the nursing profession, this study has provided another process in not only disseminating nursing career information, it raises public interest and opinion about the noble nursing profession.

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Dedication

I would like to dedicate this study to the profession of nursing and my mentors throughout the research process. Key to the profession of nursing is the new up and coming nurses, so I also dedicate this study to those students and their community whom shared their time to learn a little more about nursing. We look forward to counting you in our ranks.

Biosynthesis of Novel Analogs of Myxalamid via Feeding Experiments

Kwun Wah Wen

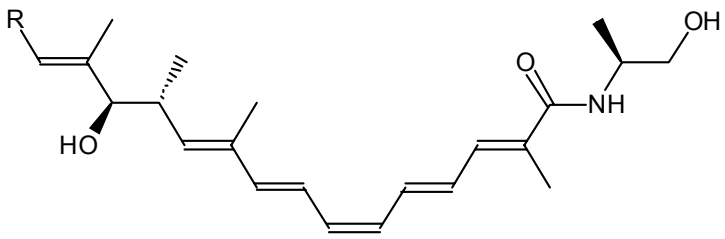
Abstract

The myxalamids belong to one of four groups of antibiotics that have been isolated from the myxobacteria Sigmatella aurantiaca Sga15 and Myxococcus xanthus Mx12. These secondary metabolites are biologically active against yeasts and molds, and are inhibitors of the electron transport in the respiratory chain. Myxalamid B has demonstrated to block the NADH oxidation in beef heart submitochondrial particles by disrupting cytochrome reduction. These natural compounds are catalyzed by two very large and complex polyfunctional enzymes, polyketide synthases (PKSs) and non-ribosomal peptide synthetases (NRPSs). The biosynthetic gene cluster of myxalamids has been reported by Müller and co-workers. There has been evidence that the loading domain of the PKSs and the subsequent enzymes responsible for the myxalamid biosynthesis are rather flexible in term of their substrate-specificity. We manipulated the biosynthetic processes in myxalamids producer using unnatural N-acetylcysteamine (SNAC) derivatives to mimic the CoA starter units which are naturally accepted by both PKSs and NRPSs of the myxalamid biosynthesis to generate novel analogs of myxalamids and study the substrate-specificity of the myxalamid PKS. We have successfully generated a number of novel natural products by feeding SNAC derivatives of free acids to the myxalamid producers, S. aurantiaca pEPS7-1. The feeding experiments with benzoyl SNAC and isovaleryl SNAC have demonstrated that the two major enzymes complexes (PKSs and NRPSs) responsible for myxalamid production, to some extent, can accept unnatural substrates. The next stage will be to assay the potential antibiotic/biological activities of the unnatural myxalamid products. This research approach could be a new tool to search for new potential pharmaceutical natural products.

Introduction

Myxobacteria have been well established as a potential source for natural products with strong biological activities. These bacteria are Gram-negative gliding bacteria (Jansen 1983) that utilize organic materials from the environment as food by the action of their extracellular hydrolytic enzymes. It is characteristic that this kind of bacteria of demonstrates vegetative growth when there are adequate nutrients, but also socializes and aggregate to form multicellular structures called fruiting bodies during starvation. An immense variety of their secondary metabolites have been shown to effectively inhibit cancer cell growth (the epothilons) and the electron transport via the respiratory chain (myxalamid, soraphen, and the myxothiazol).

Four natural myxalamids (A-D, see Figure 1) have been isolated from the myxobacteria *Sigmatella aurantiaca* Sga15 and *Myxococcus xanthus* Mx12 (Amos 1994; Gerth 1983; Jansen 1983). The chemical structures of myxalamids A and B were determined based on the spectral data of the from the ozonolysis products of myxalamid A and di-O-acetylmyxalamids (Jansen 1983). The myxalamids are active against yeasts, molds, and some Gram-positive bacteria (Gerth 1983), and are inhibitors of the electron transport in the respiratory chain (Hunter 1995). Myxalamid B, for example, has demonstrated to block the NADH:ubiquinone oxidoreductase at the site of complex I in beef heart submitochondrial particles (Friedrich 1994; Gerth 1983). Despite their potent biological activities, myxalamids showed a relatively high cytotoxicity, which in turn has hampered their further development as drugs. The goal of this project is to overcome this problem by generating novel, less toxic analogs of myxalamid that may have potential anti-fungal activities through feeding experiments with unnatural starter units and genetic manipulations of the myxalamid biosynthetic genes.



Myxalamid A	R= 1-methyl-propyl
Myxalamid B	R= isopropyl
Myxalamid C	R= ethyl
Myxalamid D	R= methyl

Figure 1. Chemical structures of myxalamids A-D.

The biosynthetic gene cluster of myxalamids has been reported by Müller and co-workers (Beyer 1999; Silakowski 2001). Myxalamids are biosynthetically derived from acetate, propionate, and amino acids. The whole process of myxalamid biosynthesis is mainly catalyzed by two very large and complex polyfunctional enzymes, polyketide synthases (PKSs) and non-ribosomal peptide synthetases (NRPSs). Both PKS and NRPS systems are organized in modules, each of which is responsible for the extension and modification of the chain (Silakowski 2001; Figure 2). The affinity of the PKS enzyme system for binding its substrates is a prerequisite for the elongation of the polyketide chain in the synthetic process (Beyer 1999).

It has been known that the organization of the first open reading frame (ORF) of the myxalamid PKS gene cluster is rather unusual (Silakowski 2001). Moreover, the myxalamids also contain some other unique features: 2-methylbutyryl coenzyme A (CoA) and isobutyryl CoA derived from isoleucine and valine, respectively, are used as starter units, together with the common ones, acetyl CoA and propionyl CoA. In addition, the polyene-polyketide backbone is linear, terminating in a 2-amino-propanol structure derived from the amino acid alanine. Based on this evident, it is likely that the loading domain of the PKSs and the subsequent enzymes responsible for the myxalamid biosynthesis are rather flexible in term of their substrate-specificity.

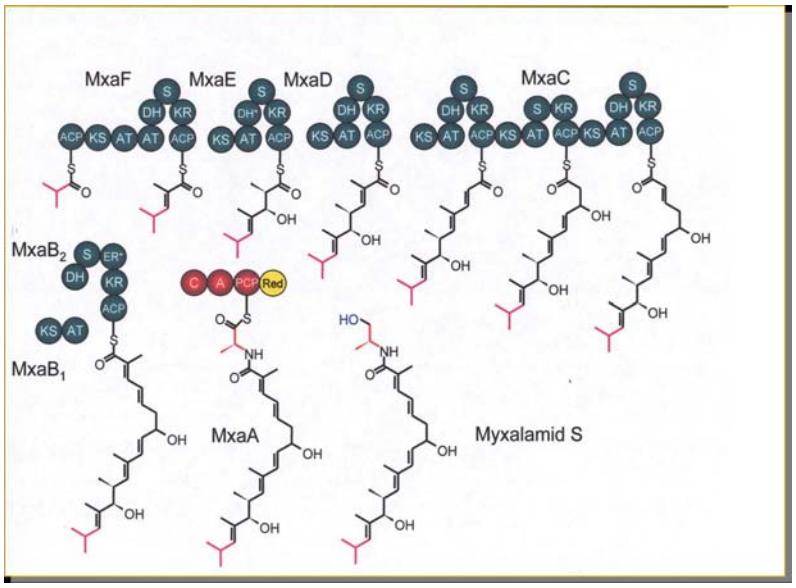


Figure 2. Processing by PKS and NRPS in myxalamid biosynthesis.

Materials and Methods

Synthesis of N-acetylcysteamine (SNAC) derivatives.

To a solution of the carboxylic acid in methylene chloride was added N-acetylcysteamine, DDC (or EDAC) and DMAP at 0 °C. The mixture was warmed up to room temperature and stirred for several hours. Thin layer chromatography (TLC) (solvent system: 50% hexane -50% ethyl acetate) was used to monitor the progress of the reaction. After the reaction was completed, the reaction mixture was transferred to a separatory funnel. Equal amount of chloroform and saturated aqueous solution of ammonium chloride was added and the SNAC derivative was extracted to the organic fraction. The organic layer was then washed with brine solution and the organic solvent was evaporated in vacuo. The sample was further purified by recrystallization or by silica gel column chromatography. TLC and mass spectroscopy were used to locate and identify the desire product. The entity of the sample was checked by proton NMR spectroscopy.

Feeding unnatural N-acetylcysteamine derivatives to mutant strain of Stigmatella aurantiaca Sga15.

The myxalamid producing strain were grown in tryptone starch medium (10 g of tryptone, 2 g of $MgSO_4 \cdot 7H_2O$, 4 g of soluble starch, 11.9 g of HEPES Pufferan buffer/liter; pH adjusted to 7.2 with KOH). The cells were precultured in 3 ml of tryptone starch medium in a shaker 160 rpm 30 °C for three days until reaching 1×10^7 cells/ml. 1 ml of the cells suspension was then transferred to a new flask containing 25 ml of the same medium after the addition of kanamycin to a final concentration of 50 μg/ml. The culture was grown for another two days under the same condition. 10 ml of the cells suspension was inoculated into a 100 ml tryptone starch medium and grown under the same condition. Feeding experiments with either the free acids or their respective SNAC derivatives (both sterilized with Millipore filter) are conducted at 12 h, 24 h, and 36 h after the inoculation. 1 g of non-ionic resin (Amberlyst XAD-1180) is added after 48 h of fermentation. The culture is grown for

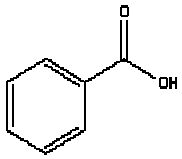
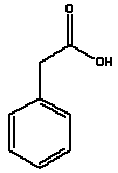
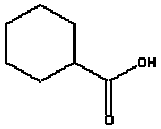
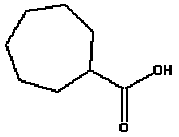
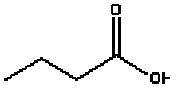
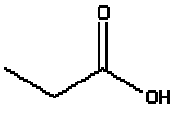
4 days and the cells and resin are harvested by centrifugation at 5000 rpm for 10 minutes. The supernatant is discarded and the cell/resin residues are extracted with 3 x 50 ml acetone and filtered. Sodium sulfate is added to remove the water in the sample and the sample is evaporated and weighed. The incorporation of the precursors into myxalamids was determined after acetone extraction of cells by HPLC-MS (Series 1100 HPLC, Hewlett-Packard, Palo Alto, CA; SCIEX API 2000 ESI-MS, PerkinElmer).

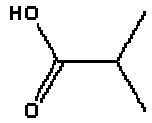
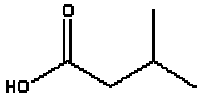
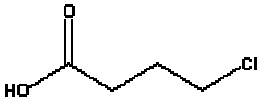
Results

Synthesis of N-acetylcysteamine (SNAC) derivatives.
SNAC derivatives, were synthesized by the general reaction shown in Figure 3. In each synthesis, carboxylic acid candidate and N-acetylcysteamine were allowed to react through esterification coupled by EDAC and DMAP. The carboxylic acid candidates containing various functional groups and their respective chemical structures are shown in Table 1. The resulting SNAC derivatives were fed to the fermentation culture of myxalamid producer and the products were analyzed by high performance liquid chromatography (HPLC) and electrospray mass spectroscopy.

Figure 3. Synthesis of SNAC derivatives.

Table 1. Carboxylic acid candidates for the chemical synthesis of SNAC derivatives.

Functional group	Chemical structure
Aromatic	 Benzoic acid
	 Phenyl acetic acid
Cyclic aliphatic	 Cyclohexanecarboxylic acid
	 Cycloheptanecarboxylic acid
Linear	 Butyric acid
	 Propionic acid

Branching	 Isobutyric acid
	 Isovaleric acid
Halogenic	 3-Bromopropionic acid
	 4-Chlorobutyric acid

Feeding unnatural N-acetylcysteamine derivatives to mutant strain of Stigmatella aurantiaca Sga15 produces novel myxalamid analogs.

In our feeding experiments using benzoyl SNAC and isovaleryl SNAC, the myxobacterial strain pEBS7-1 gave a number of new secondary metabolites as judge from their high performance liquid chromatography (HPLC) analysis (Figures 4 and 5). Mass spectroscopy and fragmentation data revealed that the new metabolites were the benzoyl and isovaleryl derivatives of the myxalamids, suggesting the incorporation of benzyol SNAC and isovaleryl SNAC into myxalamid PKS (Figures 6 and 7).

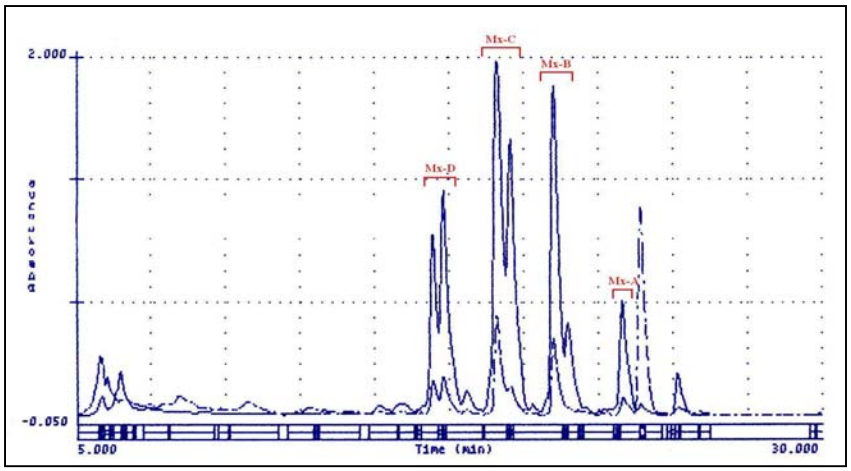
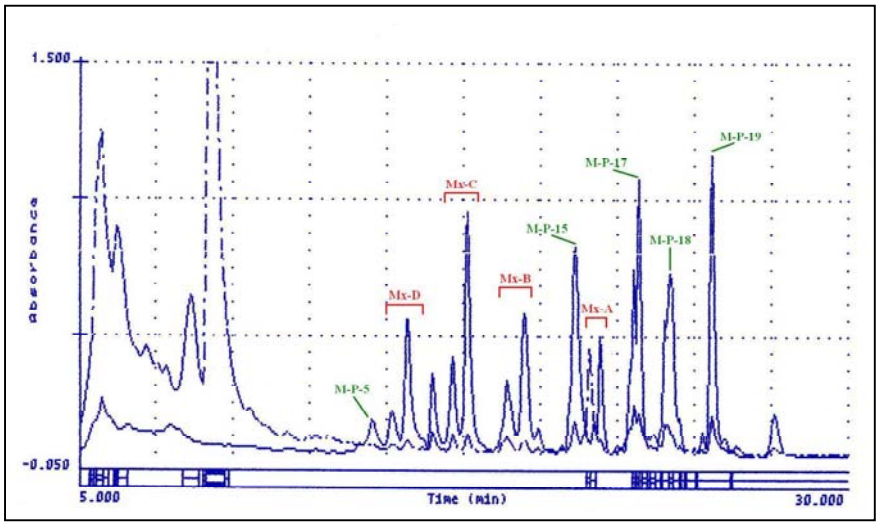
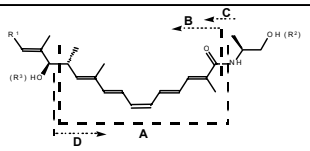
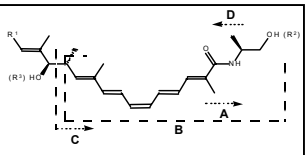


Figure 4. HPLC of no feeding of SNAC to pEBS7-1 of Stigmatella aurantiaca Sga15.

Figure 5. HPLC of benzoyl SNAC fed to pEBS7-1 of Stigmatella aurantiaca Sga15.







Compound Name	Source	M+H	Fragmentation A	B	C	D	M+Na	Fragmentation A	B	C	D
Myxalamid D	No feeding	374	215	299	356	356	396	166	296	312	378
Myxalamid C	No feeding	388	215	313	370	370	410	166	296	312	392
Myxalamid B	No feeding	402	215	327	384	384	424	166	296	312	406
Myxalamid A	No feeding	416	215	341	398	398	438	166	296	312	420
M-I-8	IVS	416	215	341	398	398	438	166	296	312	420
M-I-10	IVS	458	215	299	—	440	480	250	294	395	—
M-I-11	IVS	472	215	313	—	454	494	250	294	395	—
M-P-5	BS	396	215	321	378	378	418	166	296	312	400
M-P-15	BS	478	215	299	—	460	500	270	294	415	378
M-P-17	BS	492	215	313	—	474	514	270	294	415	392
M-P-18	BS	506	215	327	—	488	528	270	294	415	406
M-P-19	BS	492	—	313	—	460	514	270	—	415	392

Figure 6. Mass Spectroscopy Analysis of Myxalamid Analogs.

Feeding unnatural N-acetylcysteamine derivatives to mutant strain of Stigmatella aurantiaca Sga15 alters the actual and relative yields of natural myxalamids.

Feeding the myxalamid producer pEBS7-1 with isobutyryl SNAC and propionyl SNAC showed apparent amplification of myxalamids B and C peaks (Figures 8 and 9), respectively, suggesting that isobutyryl CoA and propionyl CoA are the precursors of these two myxalamids. Feeding with phenyl acetic acid showed inhibition in the production of myxalamid D, whereas 3-bromopropionyl SNAC and 4-chlorobutyryl SNAC were found to be highly toxic to the bacteria. This finding was coherent with previously reported results, where chloropropionyl CoA was found to be inhibitor of acyl CoA utilizing enzymes (Saurez 1983).

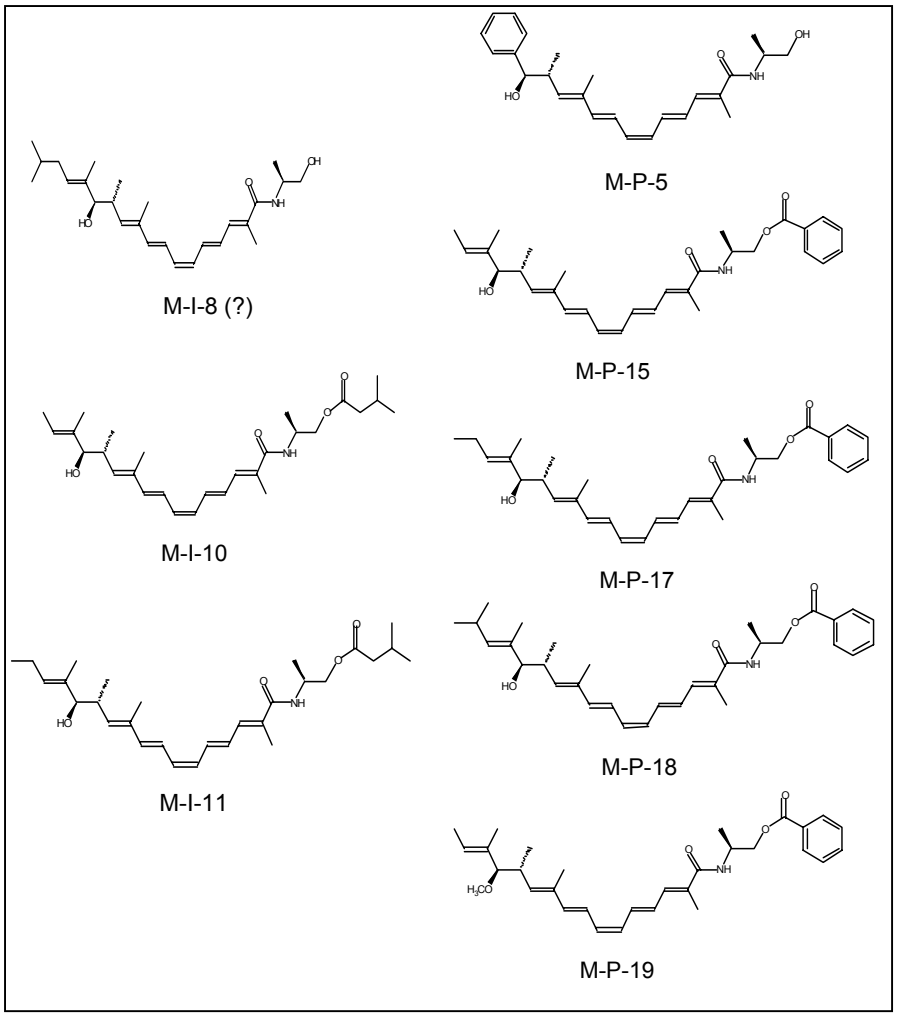


Figure 7. Novel benzoyl and isovaleryl analogs of myxalamid.

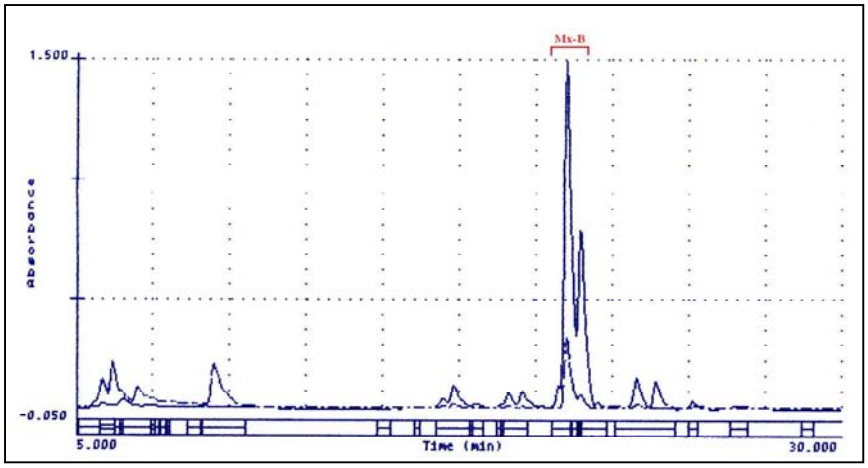


Figure 8. HPLC of propionyl SNAC fed to pEBS7-1 of Stigmatella aurantiaca Sga15.

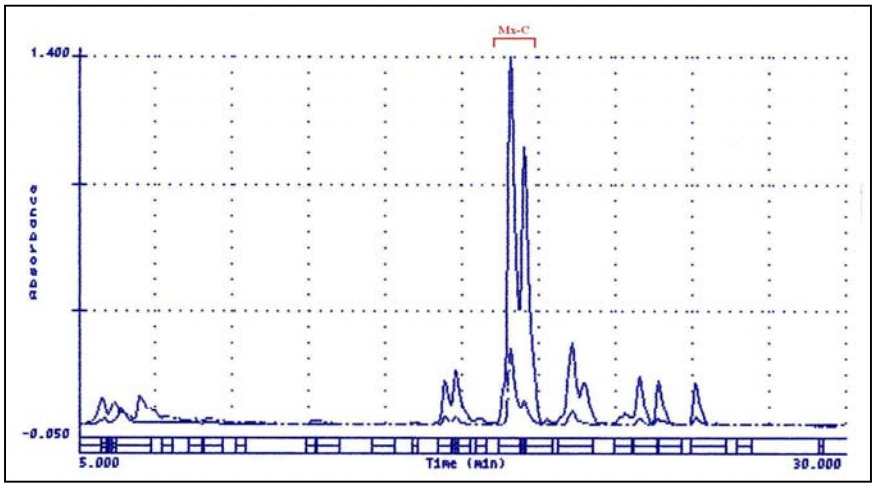


Figure 9. HPLC of isobutyryl SNAC fed to pEBS7-1 of Stigmatella aurantiaca Sga15.

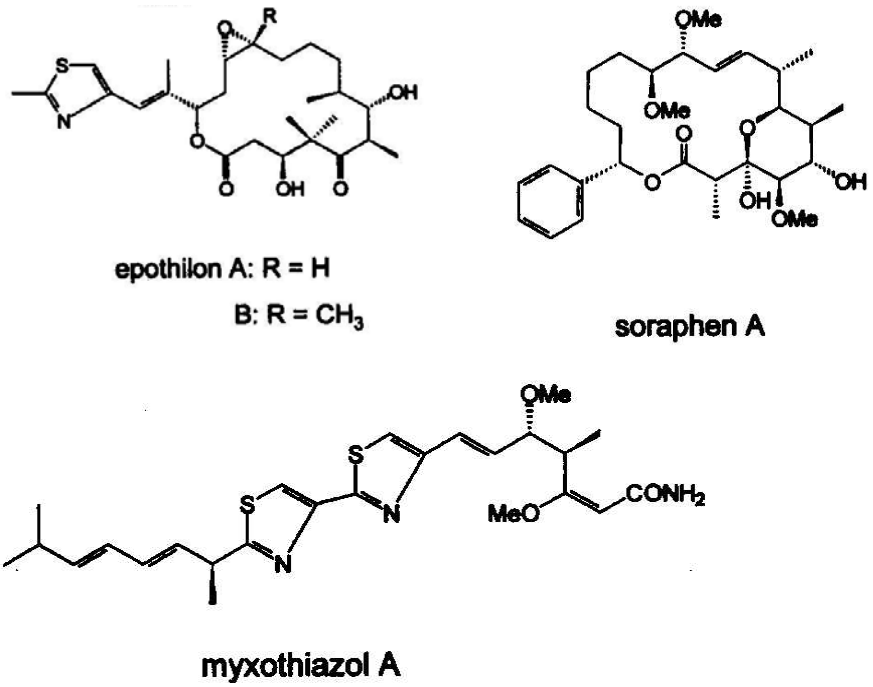


Figure10. Structures of the epothilons, soraphen A and myxothiazol A.

Discussion

Since the myxalamid producing strains were shown to utilize unnatural SNAC derivatives (e.g. benzoyl SNAC and isovaleryl SNAC) to make new analogs of myxalamid, we then will synthesize more SNAC derivatives from other plausible acid candidates to characterize the substrate-specificity of the PKS loading domain and generate more novel metabolites for drug development purposes.

All these experimental results were significant both in terms of understanding the enzyme-substrate interactions in the PKS myxalamid biosynthesis and the practical applications of manipulation techniques in generating myxalamid analogs. However, more feeding experiments have to be implemented in order to generalize the enzymatic preference

in relation to substrate conformations (spatial arrangements of atoms) and functionalities.

The significance of enhancing and suppressing the production of natural myxalamids lies in the fact that it might be possible to isolate particular natural myxalamids by feeding SNAC derivatives that amply their production and derivatives that inhibit the production of uninterested natural myxamids.

One of the compounds that we will do feeding experiments in the future is 1-methyl butyryl SNAC. It resembles the precursor molecule (1-methyl butyryl CoA) of myxalamid A, whose production by both wild-type and mutant strains the *Stigmatella aurantiaca* Sga15 has been found to be the least among natural myxamids. With the results of dramatic amplification of myxalamids B and C production by feeding with compounds that mimic their respective precursors, we anticipate consistent stimulation to myxalamid A biosynthesis when the myxalamid producer is fed with 1-methyl butyryl SNAC.

Although we have successfully produced myxalamid analogs of benzoyl SNAC and isovaleryl SNAC using biotransformation techniques, the yield of these new compounds was relatively low compared to that of the four natural myxalamids (A-D), limiting further biological evaluations of these compounds. To resolve this problem, we propose to genetically engineer a more efficient PKS loading domain for myxalamid producers to increase their PKS enzyme affinity for benzyl moiety by swapping the benzyl-specific PKS loading domain from the soraphen producer. We also propose to interchange the PKS loading domains amongst the producers of epothilon, myxothiazol and soraphen (Figure 10). It is the hope of the author that the swapped loading domains will enhance the efficiency of these producers to uptake foreign starter units of the donors, and incorporate them in the biosynthetic pathways of the antibiotics.

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Student Abstracts

Leslie Edward Byrd II

Dielectrical Sensing For Industrial Applications

The main focus for the two projects presented here is non-destructive, non-invasive testing. This is an important concept for industry because it addresses the issue of controlling the composition or state of products during their production process, as apposed to stopping the process and testing or completing the process and testing afterwards.

Paper Project. It has been brought to attention that paper-manufacturing companies would like to have a greater knowledge of the moisture, titanium dioxide, and other components content in their paper pulp during their paper making process. The sensors that measure the paper now do not perform well in the early stages of the process when there are large amounts of moisture and titanium dioxide in the paper pulp, so this is the portion of the manufacturing process that is the focus of this project. The system is developed to conduct real time capacitive spectroscopy sensing in order to determine the composition and feedback the information. The non-destructive nature of the sensor works well with the feed forward control and the dimensions for the sensor will work well in the paper machines.

Pharmaceutical Project. Pharmaceutical companies have recently become subject to strict regulations, specifically concerning the accuracy of the composition to the tablets. New regulations specify the coating thickness put on tablets. Experimentally, the coating thickness of the tablets is in the order of 50 micrometers. The overall design of the sensor is a MEMS (Micro Electrical Mechanical System) application using the dielectric spectroscopy concept coupled with a thermal expansion concept. In order to determine the coating thickness or the tablets,

readings must be taken at different distances, and because of the small size of the tablets, thermal expansion of the sensor is the route we have chosen.

Plastic Project. The automotive industry in the past few years has been leaning towards using non-metal materials for the exterior of their automobiles. Due to mass production and the way the factories are set up, a whole car is not manufactured one at a time, but rather different parts are mass-produced in different manufacturing plants in different places. These places can be anywhere from next to each other to all over the world. The problem with making the exterior of the cars at different places all over the world is the different environmental conditions the parts are exposed to, specifically the different temperatures and humidity. When these parts are painted with different moisture content, the paint dries at a different rate and caused the final color to be slightly off depending on the drying rate. Instead of these companies shipping all the parts to one warehouse where they could stay for extended periods of time at the same temperature to ensure the same moisture content (because that becomes expensive), they have shown desire for non-invasive sensors that will measure the moisture level in the plastic. The dielectric spectroscopy sensor is our approach.

Mentors: Dr. Alexander Mamishev, Kishore Sundara-Rajan (Graduate Student)

Lorena Chavez

Is the Face Inversion Effect Exclusive to Faces?

Several studies have pointed out that face recognition is disproportionately impaired by stimulus inversion when compared to recognition of other types of visual stimuli. Previous research has also shown that famous faces, when compared to houses, have a greater inversion effect. The present study sought to find out if the inversion effect is a product of familiarity with the stimuli or of "face specialness." Data will be

obtained from 24 groups of 5-8 UW students each who will be shown either right side up or inverted pictures of famous buildings and houses. Memory recognition for the two categories of stimuli will be recorded for each subject. Data collection is in progress and results are pending. Since the Face Inversion Effect has been attributed to the "specialness" of faces and not due to the familiarity factor, our study will take a closer step towards unveiling the origin of the Inversion Effect.

Faculty Mentor: Dr. Geoff Loftus, Department of Psychology

Madalene Day

Understanding Racial Biases In Pre-Adoptive Parents

Transracial adoption, which is defined as an adoption of a child who is of a different race or ethnic group from the adoptive parents, is becoming increasingly more common. With this comes a need for understanding how racial biases will impact the lives of both the adopted child and adoptive family. I assess racial biases in pre-adoptive parents through explicit and implicit measures to gain a greater understanding of how racial biases relate to the desire to transracially adopt. The participants in this study are pre-adoptive parents from Antioch Adoptions in Redmond, WA. The explicit measures will consist of asking the participants to choose statements that best fit their feelings on issues of race, ethnicity and identity. Additionally I will use the Modern Racism Scale and ask participants to rate their personal feelings about transracial adoption on a 9-point likert scale. The implicit measure will consist of three implicit attitude tests (IATs). The IATs will compare pictures of black children vs. white children, dark-skinned black children vs. light-skinned black children and light-skinned black children vs. white children. I am currently collecting data, however, from the racial attitudes literature. I expect to see that people will have a stronger preference for white children and I hypothesize that there will be a preference for light-skinned black children as well. I also expect to find that the strengths of people's

associations with white children will inversely correlate with the strength of their desire to transracially adopt. The results from this project will help Antioch Adoptions to better understand how to direct their teaching and preparation of pre-adoptive parents. Furthermore the methods of this project may be a useful tool for the agency to use in their counseling of the pre-adoptive parents who wish to transracially adopt.

Faculty Mentor: Dr. Anthony Greenwald

Jennifer Ann Devine

Poverty Discourse in the American Northwest: A Comparative Analysis in Kittitas County

My research project is part of a larger project entitled, "Reinterpreting Geographies of Poverty and Inequality in the American Northwest." The larger project "attends to the ways in which cultural and economic processes intersect to produce place-specific constructions of poverty and the poor, resulting from broader processes of uneven development." Research demonstrates that the economic sector in the rural Northwest that is most closely associated with poverty is large-scale agriculture. In Washington State in particular, this sector employs a considerable number of Hispanic as well as white workers. Therefore, to fully understand poverty in these agricultural communities, research needs to comparatively examine the lives of its white and Hispanic residents. We need to understand how labor markets, work experiences and their intersection with the cultural and political constructions of poverty structure both opportunity and exclusion for both of these groups.

This investigation examines both similarities and differences of Hispanic and white workers' experiences living in Kittitas County. Using qualitative methods, my individual project establishes Hispanic personal and community histories of livelihood and labor in the area, investigates rural and social change, and determines how Hispanics discuss poverty and the

rural poor in contrast to white populations in Kittitas County of Washington State. This paper will unpack the dynamic social forces and structures that historically and contemporarily shape Hispanic landscapes in Kittitas understood from the perspective of a community's history.

Faculty Mentors: Dr. Victoria Lawson and Dr. Lucy Jarosz, Geography Department

Oluwatope Fashola

Is there a Burden of "Acting White" Keeping African American Students from Academic Success?

The threat behind the oversimplification of the oppositional culture theory is that it puts full responsibility of the educational gap on involuntary minority groups. The purpose of this project is to test the oppositional culture theory as a method of uncovering larger questions regarding minority education. In order to test this hypothesis I will use data from the 2000 and 2002 Beyond High School Project survey data. Data collected from this project will be analyzed according to the stipulations made by the oppositional culture theory, specifically testing the effect of family, economic, and school factors that may be helping to perpetuate an educational disparity between white students and black students. Within Ogbu's hypothesis he acknowledges racial inequality, stagnate occupational mobility, and unequal treatment within education institutions, bringing together evidence that this historical injustices are having a present effect on African Americans. However, Ogbu's conclusion based on these ethnographic findings explicitly states that it is the African American creation of a "folk theory of success" which keeps African American students from succeeding. This anecdote is one of many observations that aid in the confusion and oversimplification surrounding the theory. Horvat and Lewis (2003) believe that the oppositional culture theory has been oversimplified into a culture of poverty theory that focuses on a "burden of acting white," leading policymakers

to overlook the collaboration and culmination of multiple forces on the educational outcomes of black students. Others (Foley 1991; Slaughter-Defoe 1990) have argued that Fordham and Ogbu's (1986) oppositional culture theory overlooks or minimizes within-group variation. Ainsworth-Darnell and Downey worry that the oppositional culture model has become so respected in the academic community that it threatens to divert attention from other explanations for the racial gap in school performance (1998). The reason that this theory is so controversial is that black-white achievement gap has been and continues to be one of the most important issues regarding education in America. Therefore, understanding how to improve the academic achievement of black students and how to remove roadblocks to their success are crucial.

Faculty Mentor: Dr. Charles Hirschman, Department of Sociology

Bárbara Guzmán

Balancing College Aspirations and Cultural Expectations Among Mexican Female High School Graduates in Manson, Washington

According to Census 2000, people of Mexicans descent have a lower educational success rate than any other ethnic group. Within this minority group, awareness should be given to Mexican females. In addition to racial issues that influence females not to pursue a higher education (stereotypes), traditional roles that evolve from parental or social responsibilities can further influence decisions not to pursue a college education. The probability of coming into contact with females who experienced these circumstances during my attendance at Manson High School (located in North Central Washington) was very high, and inspired this study. An informal ten-question questionnaire was administered to five female Manson high school graduates and one high school senior in order to gather information about their backgrounds and to

gain an understanding as to why they have not pursued higher education. Unfortunately, more research is required with a larger sample of Mexican females in a variety of backgrounds to truly represent Mexican females in the United States. However, this research, along with previous work, will provide materials and information needed to begin the process of changing the attitudes towards education for underrepresented students.

Faculty Mentor: Dr. Gabriel E. Gallardo, American Ethnic Studies

Hoang Nhan

The Presence of TPep and Dopamine in Tritonia diomedea Veliger Larvae and Their Effects on Larval Velar Cilia

Ciliated cells are an important component of locomotion during both adult and larval stages of several marine gastropods. For example, the adult sea slug, Tritonia diomedea, uses a cilia-covered foot to crawl whereas the veliger larvae swim in the water column using cilia of the larval velar lobes. The cilia providing the propulsive force in both adults and larvae are thought to be under direct nervous control. In adult T. diomedea, two neurotransmitters, TPep and dopamine (DA), have been shown to control ciliary locomotion; however, it is not known if either TPep or DA regulate ciliary activity in larval T. diomedea. Here we used immunohistochemistry to investigate whether either TPep or DA is present in T. diomedea larvae. Our results indicate that TPeptidergic and dopaminergic cells and cell processes are present at the base of ciliated cells within the velar lobes of T. diomedea larvae immediately after hatching. These neurons, however, did not innervate the presumptive foot tissue, which bears functional cilia that will serve as the primary effector cells during adult crawling. This distribution of TPep and DA persists at least until metamorphic competence. To determine if these neurotransmitters directly control larval locomotion, we tested whether application of TPep and DA alters the ciliary beat frequency (CBF) of velar cilia. Our results

indicate that neither transmitter (in concentrations from 10-8 to 10-3M) alters the CBF of velar cilia. Therefore, although TPep and DA were both shown to exist in the nervous system of larval T. diomedea, neither transmitter seems appears to participate in the neural circuitry controlling larval or presumptive foot ciliary activity. In addition, the results suggest that the neural circuitry controlling adult ciliary crawling develops de novo during metamorphosis from larval to juvenile form.

*Research conducted at Friday Harbor Laboratories, Univ. of Washington, Friday Harbor, WA

Faculty Mentors: Dr. Shaun Cain, Department of Biology
Contributors: Marcel Tam Department of Neurobiology, University of Washington, Michael Baltzley, Department of Biology, Univ. of North Carolina

Nhi Nguyen

Behavioral Signatures in Youth Sport Coaches

The primary purpose of this investigation is to examine the effect of game status (i.e. winning/tied/losing) on coaching behaviors in the context of youth baseball. Coaching behaviors have been identified as important predictors of children's enjoyment and experiences in youth sports. Children who perceive their coaches as being supportive, instructive, and less punitive typically report lower levels of competitive anxiety, greater levels of enjoyment and intrinsic motivation, and higher self-esteem. Participants in the current investigation were 51 male baseball coaches from the greater Seattle area. Each of the coaches was observed during competition. A total of 215 observations (games) were conducted with an average of four observations per coach. The Coaching Behavioral Assessment System (CBAS, Smith & Smoll, 1990) was employed to evaluate how frequently coaches engaged in supportive, punitive, and instructional behaviors. The game status and coaching behaviors were recorded during each inning. A one-way MANOVA was

conducted to examine game status related differences in coaching behaviors. Significant main effects were observed for supportive and punitive behaviors. Post-hoc analyses identified that coaches were more supportive and less punitive when their team was winning or tied, rather than losing. The discussion focuses upon the situational effects of game status on coaching behaviors and its implications for the enjoyment and continued involvement of young athletes.

Faculty Mentors: Dr. Sean Cumming, Dr. Ron Smith, and Dr. Frank Smoll

Sonca Nguyen

Design and Analysis of a Langmuir Probe in a Z-Pinch

Magnetically confined plasma configurations are prone to instabilities. At the University of Washington, the ZaP Flow Z-pinch experiment team is investigating the concept of using sheared flow to stabilize a Z-pinch plasma configuration. One new probe utilized in this experiment is a Langmuir triple probe. Data to be obtained from this probe include floating potential, ion saturation current, and double probe potential. From these three data points, other desired plasma parameters can be derived: plasma potential, electron saturation current, electron temperature, and ion density. This diagnostic is still in its development stage, and thus this report only covers the design and setup of the probe and results from the floating potential characterization.

Faculty Mentors: U. Shumlak

Zawanblichi Parker

Locational Marginal Pricing

Locational Marginal Pricing (LMP) is a type of pricing approach used to manage the efficient use of power transmission when congestion occurs on the power grid. Congestion occurs when there are one or more restrictions on the transmission system that prevents the least expensive supply of energy from serving the demand. LMP is used in heavily congested areas and through regional transmission organizations such as PJM (Pennsylvania, Jersey, Maryland) and New England Independent System Operator (NEISO). Price forecasting for power markets using LMP is much more complicated than methods used in zonal pricing systems thus, power prices in congested areas are higher than those in un-congested areas. LMP provides market participants a clear indication of the price of electricity at every location on the grid and in turn, assists in forecasting power market prices.

Faculty Mentors: Dr. Chen Ching Liu, Graduate Student advisor Guang Li

Maria Rodriguez

Structure, Architecture, and Characterization of the Skeleton found in the Sea Sponge *Euclectella Aspergillum* – Materials Science Aspects

Nature has perfected material processing techniques optimized for their environment. It is this specific characteristic that has enabled them to evolve for millions of years. Recently, scientists and engineers have been intrigued to study the structural elements and properties of natural biological materials found in sea animals, plants, and insects, whose constituents are essentially made up of anisotropic composites. The Euplectella Aspergillum sponge, or Venus Flower Basket, is of particular interest because of the structural and mechanical properties

incorporated into its very lightweight, yet stiff, skeleton. The purpose of our study is to characterize the structural configuration of its intricate system, its composition and fine structure, and compare it to commonly known configurations established for high-performance structural composite-fiber weaves for cylinders. An attempt will also be made to characterize the chemical nature of the joints in the structure.

Faculty Mentor: Dr. George Mayer

Kwun Wah Wen

The propensity of helper T cells to become TH2 cells (TH2 bias) correlates with higher susceptibility to infection by the protozoal parasite Leishmania major. TH2 bias was found to be regulated by Dice1, a quantitative trait locus on mouse chromosome 16 using linkage analysis. More recently, interval-specific congenic mapping was used to resolve Dice1 into Dice1.1 and Dice1.2, genetic loci that independently confer TH2 bias. Interestingly, Dice1.2 but not Dice1.1 was found to control susceptibility to infection by L. major. Interval-specific congenic mapping involves the identification and definition of a set of recombinant intervals by genotyping markers polymorphic between the parental strains. In order to narrow the minimal Dice1.1 and Dice1.2 genetic intervals, I am mapping the endpoints of the congenic intervals that define these loci. My results have diminished the physical distances of Dice loci and lay the groundwork for identifying the individual genes within Dice1.1 and Dice1.2 controlling TH2 bias and L. major susceptibility.

Faculty Mentor: Dr. Mark Bix