Volume 19 Spring 2010

MURJ

Massachusetts Institute of Technology
Undergraduate Research Journal

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Massachusetts Institute of Technology

Andrea, Field Engineer

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MURJ

Massachusetts Institute of Technology
Undergraduate Research Journal

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April 2010

Dear MIT Community,

I am honored to introduce this very special issue of MURJ celebrating our renowned Undergraduate Research Opportunities Program.

UROP's beginnings can be traced back to 1968, when MIT received a large gift from Edwin H. Land, the inventor of instant photography. Dr. Land passionately believed that opportunities for undergraduates to learn should not be limited to classroom lectures and examinations. He saw great value in students learning through practical education, or "learning by doing."

Margaret MacVicar, then a new physics instructor, believed this also. As associate provost, I asked Dr. MacVicar to use proceeds from this gift to develop a program to promote collaborative research relationships between MIT undergraduates and our faculty. Margaret approached this assignment with sound judgment, typical zeal and limitless drive, initially contending with some skeptical department heads. In fall 1969, the program was officially launched with approximately one hundred twenty-five students participating. Margaret named it the Undergraduate Research Opportunities Program, quickly to become known as "UROP."

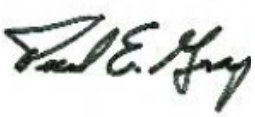
Margaret would go on to become MIT's first dean for undergraduate education, and remained ardently committed to UROP for the rest of her life. Before her untimely death in 1991, she would see UROP grow from humble beginnings to being thoroughly embedded in the MIT culture and widely regarded as the Institute's flagship academic program.

Today, UROP students are at the forefront of innovation and discovery. They play critical roles in a vast range of experimental, theoretical, clinical, and applied research projects. In the past year alone, UROP students have conducted research on Parkinson's Disease, biofuels, micro-finance, and nanotechnology. They worked on novel vaccines and cancer therapies, studied language acquisition and learning, developed educational video games, and constructed HIV monitoring devices. Last year more than two thousand students conducted research projects across all MIT disciplines, under the guidance of more than half our faculty.

Regardless of discipline, UROP students all share the common goal of making our world a better place through ingenuity, innovation, and an almost limitless enthusiasm. MURJ contributors and editors do a tremendous service in sharing the excitement of UROP with the MIT Community.

I welcome you to this issue of MURJ, and hope you will join me in honoring both this grand program and Margaret MacVicar's lasting creativity.

Sincerely,



Paul E. Gray, '54
Professor and President Emeritus

Massachusetts Institute of Technology

UNDERGRADUATE RESEARCH JOURNAL
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MURJ Staff
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murj.mit.edu

April 2010,

Dear MIT Community,

We are honored to present you Issue 19 of the MIT Undergraduate Research Journal (MURJ), a biannual publication featuring the stunning undergraduate research work at the Institute. We at MURJ maintain a high standard for the research we publish and are amazed by the quality and uniqueness of the reports in this issue. Every issue highlights the importance of undergraduates in research and the critical role they can play in science. Spanning an impressive range of fields, the research presented here involves such fascinating topics as the detection of supernovas using X-ray telescopes and the development of a better screen for prostate cancer. As this issue coincides with the 40th anniversary of the UROP program, we present a series of features, including timelines and interviews, that provide a look into the past forty years of the program. The UROP program is certainly a hallmark of MIT and we are proud to celebrate its fruitful path at the Institute with these special articles. Also showcased are numerous other features, including an interview with Professor Joanne Stubbe, and various articles we hope you find enlightening. Our writers and student researchers have made MURJ a success and allow us to fulfill our commitment to sharing science with the MIT community.

Over the past year, MURJ has changed significantly with a growing staff and the development of ties with various departments, especially with the UROP office. Now, we are proud to introduce two exciting new changes that we hope will enhance your reading experience and allow us to reach more of the community. The first change, which you will immediately notice, is the new and refreshing look of the journal featuring color and various design elements that frame the research in an aesthetic manner. The second change is the launch of our new website (murj.mit.edu) that now houses our current issue in addition to an archive of all previous issues. The renewal of our website allows us to introduce an additional feature we call web abstracts that are located at the end of this issue. These abstracts refer to online articles so be sure to visit these articles on our new website!

Our hope is that this journal is a representation of the intellectuality and spirit that the undergraduates fill the Institute with. This issue has been made possible because of the hard work of many students, departments, and faculty at MIT. We would like to thank all our executive members and associate editors for their tireless work in reviewing submissions and ensuring the upmost quality of work. We next thank Associate Dean of Academic and Research Initiatives Michael Bergren and Melissa Martin-Greene of the Office of Undergraduate Advising and Academic Programming (UAAP) for their invaluable assistance in probing the history of UROP at MIT for our feature on the past 40 years of the program. Surely, this issue would not be possible without the support of the MIT Student Activities Office, the UAAP office, MIT Publishing Service Bureau, and MIT. And lastly, a special thanks to the undergraduate authors for sharing their research with us and the larger community. We hope you enjoy the issue!

Sincerely,

Omar Abudayyeh
Co-Editor-in-Chief

Evelyn Wang
Co-Editor-in-Chief

MIT Science News In Review

[Neuroscience]

MIT's McGovern Institute Finds Cells That Keep Time

Neuroscientists and cognitive scientists have been grappling with the question of how individuals keep track of time and are able to place a series of events in chronological order. The leading theory held by scientists is that certain cells in the brain attach "time stamps" to specific events thereby associating each occurrence with a specific moment in time. When an individual recalls when some event took place, he or she simply filters through those time stamps to find the appropriate one.

Graphic of time-keeping brain cells.
Credit: Christine Daniloff, MIT News

Unfortunately, no biological evidence of such "time stamps" were to be found until the group of Ann Gaybriel, one of MIT's Institute Professors, did so in mid-October.

Her group taught two macaques (a type of Old World monkey) how to make a certain eye movement. The scientists then gave the monkeys the "go" signal for the monkeys to repeat the eye movement at their own pace. Using novel technology and mathematics developed by Naotaka Fujii (RIKEN Brain Institute, Japan) and Dezhe Jin (Penn State), the researchers were then able to track and analyze signals released by hundreds of neurons in the brains of the two monkeys.

What the researchers found was that after various time intervals (i.e. 100 milliseconds), certain groups of neurons found in the striatum and the prefrontal cortex were routinely stimulated. Gaybriel explains: "Soon enough we realized we had cells keeping time, which everyone has wanted to find, but nobody has found them before."

Scientists hope that, in the future, they can study these neurons in more depth so that they will be able to design drugs that target these cells. This may be a potentially useful therapy for diseases like Parkinson's, whose victims struggle in pursuing activities that necessitate accurate timing.

—A. Kashyap

Source: "A head of time"

– Anne Trafton, MIT News Office

http://web.mit.edu/newsoffice/2009/brain-stopwatch.html

[Biology]

Human Y Chromosome Exhibits Significant Divergence from Chimp Counterpart

The Y chromosome, responsible for what separates males from females, is often excluded from genome sequencing data. This fact is partially due to the "massive palindromic sequences," stretches of repeating DNA, that make the chromosome tricky to sequence, but is also partially due to the theory that the Y chromosome did not significantly change throughout time after diverging from the X chromosome millions of years ago. Recently, the latter idea is being challenged by

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new data found by a team of MIT researchers.

Led by biology professor and director of the Whitehead Institute David Page, scientists have now sequenced the chimpanzee Y chromosome, allowing for side-by-side comparison with the human Y chromosome sequenced in 2003.

The researchers have found that though humans and chimpanzees share 98.8 percent of their genome, the chimpanzee Y chromosome is only about one-half to two-thirds the size of the human Y chromosome. Cornell University professor of genetics Andrew Clark notes that the genomic changes are significant: "There's a dramatic amount of turnover, and it's not just degeneration – it's gain and loss of genes that do something on the Y chromosome."

Scientists hypothesize that the observed differences may be related to differing sperm production needs in each species. Since female chimpanzees mate with several males during one season, the male with the most effective sperm production is favored.

In the future, the David Page laboratory, in collaboration with Washington University's Genome Center, hopes to sequence the Y chromosomes of other mammals to study the potential divergence in those species as well.

—A. Kashyap

Source: Source: "Y chromosomes evolving rapidly" – Anne Trafton, MIT News Office

http://web.mit.edu/newsoffice/2010/y-chromosome-0114.html

[Biological Engineering]

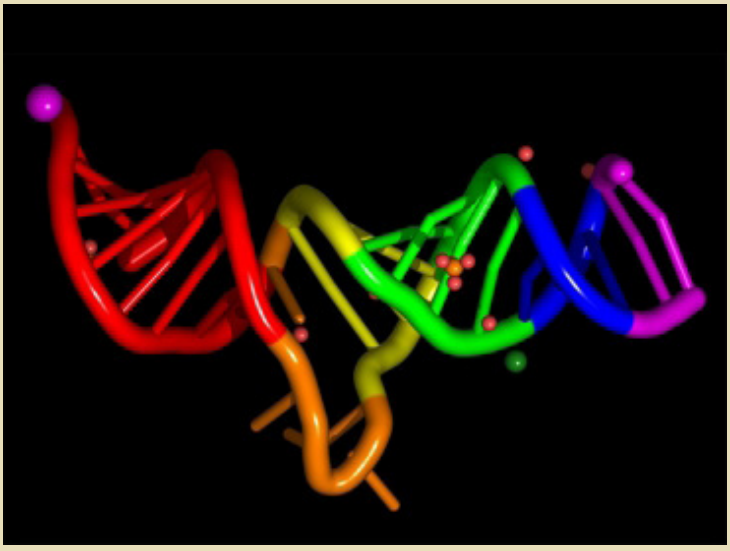
Simultaneous Silencing of Multiple Genes Paves Way for Novel Therapeutics

RNA interference (RNAi), the process by which small interfering RNAs (siRNAs) can prevent mRNAs from being translated into proteins, was first discovered by Fire and Mello in 1998. Since then, this process has been the target of scientific research as a potential avenue for new treatments of gene-related disorders.

In a collaboration between MIT and Alnylam Pharmaceuticals, researchers led by Daniel Anderson in Robert Langer's lab have been able to silence multiple genes in the hepatic cells of a mouse model. The researchers embedded siRNAs in lipid-like molecules that can pass through cells' plasma membranes. After synthesizing more than a thousand of these lipid-RNA complexes, termed "lipidoids," the scientists screened for the most effective one: C12-200.

Using the C12-200 complexes, the researchers were able to silence several genes involved in murine cholesterol metabolism with as small a dose as 0.01 milligrams of siRNA for every kilogram of solution. According to Anderson, the human equivalent would be a dose of less than one millimeter of solution, far less than what was necessitated by previous delivery models.

Because the lipidoids tend to accumulate in the spleen and liver, initial research in their use as a therapeutic is being targeted toward diseases of the liver such as hepatitis. In the future, scientists are hoping to not only broaden the scope of disorders that can be treated via RNAi but also to eventually be able to silence up to twenty genes at the same time. This would be particularly beneficial for diseases like cancer, which involves a host of different genetic malfunctions.



RNA interference.
Credit: MIT News

—A. Kashyap

Source: "New and improved RNA interference" – Anne Trafton, MIT News Office

http://web.mit.edu/newsoffice/2010/rna-int-0104.html

[Biology]

Proteins Known As Sirtuins Linked to Regulation of Metabolism

Ribbon diagram of SIR2 protein bound to nicotinamide.
Credit: Wistar Institute

Diets that involve thirty to forty percent less calorie consumption than normal have been shown to increase the lifespan and to better the overall health of a mouse model. Specifically, calorie-restricted diets have been shown to have an effect on the somatotropic signaling axis, a neural system. As less calories are consumed, growth hormone synthesis by the hypothalamus is reduced—an effect linked to extended lifespans. Now, scientists have shown that special proteins called sirtuins play a critical role in this process.

Led by biology professor Leonard Guarente, researchers wanted to investigate the role of sirtuins in growth hormone synthesis. They fed a calorie-restricted diet to both wild-type mice and mice engineered to have extremely low SIRT1 (a type of mammalian sirtuin) production in the brain. The scientists found that the wild-type mice had significantly lower levels of growth hormone when compared to the mice that had little SIRT1 production in the brain, implying that the presence of SIRT1 promotes the ability for calorie-restricted diets to lower metabolic activity.

Sirtuin's involvement in metabolic activity regulation was established earlier when previous research done in the Guarente lab showed that a calorie-restricted diet increases levels of nicotinamide adenine dinucleotide (NAD), a coenzyme that activates SIRT1 when bound to the protein.

Scientists now hope to synthesize compounds that stimulate sirtuin synthesis in order to lessen the effects of aging-related disorders like Alzheimer's and also to possibly increase the human lifespan. Such drugs are currently being produced and tested by Sirtris, a spinoff company now part of GlaxoSmithKline.

—A. Kashyap

Source: "New evidence links sirtuins and life extension" – Anne Trafton, MIT News Office

http://web.mit.edu/newsoffice/2009/guarente-sirtuins.html

[Computer Science]

New Screenshot-Based Computer Programming Makes Programming More Efficient

Before the invention of the graphical user interface (GUI), people had to memorize the text of various commands in order to use a program. The GUI made program usage much more intuitive by using icons or windows to visually represent certain operations. A GUI analogue for programming has recently been developed by MIT's Computer Science and Artificial Intelligence Lab (CSAIL).

Associate professor Rob Miller has led a group of scientists to develop Sikuli, an application that can make programming manipulations a more intuitive, visual experience. For example, without Sikuli, if a programmer wished to manipulate a certain program, he or she would have to dig through the coding of the program and either add or delete code. Such an endeavor requires deep knowledge of whatever language the individual wishes to work with. But with Sikuli, a programmer who wishes to manipulate some GUI can simply take a screenshot of the program and paste the picture into the coding.

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"New Screenshot" continued from p. 7

Sikuli, named after the Huichol Indian word for "God's eye," can also be used to enhance search engines. An individual looking for information about a certain command can simply select a picture of the icon or image corresponding to the command and then use it as a visual search term on the Web. The researchers found that such a search engine reduced the time required for people to find information by half.

The secret behind Sikuli is its pixel-based algorithms. Sikuli has no knowledge of what coding makes up a given program; it merely analyzes the appearance of the program on the monitor. Miller explains: "It's a software agent that looks at the screen the way humans do." This appearance-based functionality also means that Sikuli is a very versatile application that can work with any program that has some sort of visual interface.

People who want to use Sikuli must know some basics of the Python programming language. However, even this is becoming less true as researchers assign stretches of code to the corresponding visual operations one sees on the monitor. Now all that programmers need to do to code for some operation is to paste a "recording" of the execution of the operation into the coding.

Ultimately, this development will pave the way for more lay-friendly programming as well as more effective and efficient coding for computer science experts.

—A. Kashyap

Source: "Picture-driven computing"

– Larry Hardesty, MIT News Office

http://web.mit.edu/newsoffice/2010/screenshots-0120.html

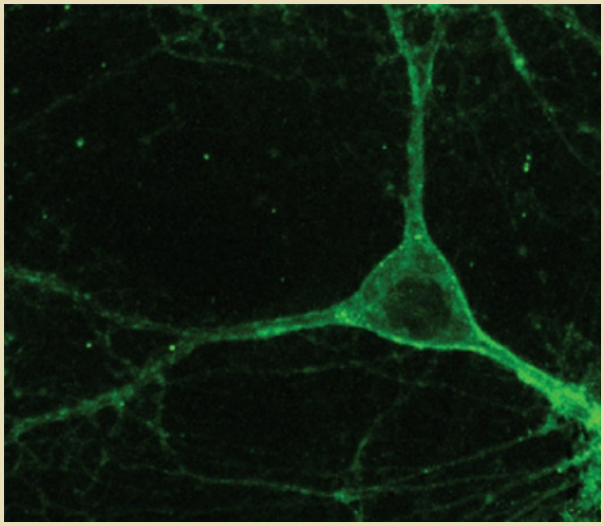
[Neuroscience]

Certain Frequencies of Light Can Halt Neuronal Activity

Around 2.5 million Americans suffer from epileptic seizures, which occur when their neurons go haywire and are stimulated excessively. Recent research done by MIT Media Lab professor Ed Boyden's group has shed light on a potential therapeutic for the disorder.

The lab has found a protein, known as Arch, which acts as a proton pump that exports protons through the plasma membrane and into the extracellular environment. The protein can be stimulated by visible light in the yellow-green region, thereby reducing the voltage across the cell membrane. This lower voltage decreases the chance for the neuron to experience an action potential, thereby temporarily halting neuronal activity.

Isolated from the Halorubrum sodomense bacterium, a type of Archaean found in the saline Dead Sea, the Arch protein was found to be more controllable and accurate than the previous protein the Boyden group attempted to use, halorhodopsin. A light-sensitive chloride pump that hyperpolarizes neurons when it imports chloride ions into the cell's intracellular environment, the halorhodopsin was not satisfactorily precise.



Arch gene expressed in a mouse neuron.
Credit: Brian Chow, Xue Han and Ed Boyden/MIT

Another advantage of the Arch protein over halorhodopsin is its extremely short refractory period. The Arch protein can be re-stimulated by light a mere few seconds after it is activated once, whereas it took minutes for halorhodopsin to be prepared for re-stimulation. Edward Callaway, a professor at the Salk Institute who was not affiliated with the study, explains the advantage: "If you have to wait a long time to get recovery, you just can't compare different conditions quickly."

The group has also found other light-sensitive proton pumps that are activated by other regions of the visible spectrum. This allows scientists to selectively activate multiple regions simultaneously depending on the protein found in each cell.

In the future, the researchers want to study this "optogenetic" process in primates to hopefully prepare it for use as a therapy in humans for stress disorders, epilepsy, or chronic pain.

—A. Kashyap

Source:"Silencing the brain with light"

– Anne Trafton, MIT News Office

http://web.mit.edu/newsoffice/2010/brain-control-0107.html

[Materials Science and Engineering]

Biomimetic Microfluidic System Uses "Micro-Ants" To Propel Particles of Much Greater Size

Cilia, minute tail-like projections, line the internal surfaces of many organs. For example, cilia which line the inside surface of the trachea beat simultaneously to propel particles and cells through the space in the trachea. Scientists at MIT have drawn inspiration from this natural process to design the next novel microfluidic system.

Led by Assistant Professor Alfredo Alexander-Katz, scientists have decided to use magnetism to replace physical micro-sized channels. Researchers have taken micro-sized plastic beads that are superparamagnetic and placed them in a liquid. The liquid system is flanked by two coils that can exert a rotating magnetic field onto the system. This field causes the beads, which have sunk to the bottom of the liquid, to self-assemble into linear chains and then rotate. Much like the beating of cilia, the rotation of the chains causes a current to build in the liquid system which propels particles through the fluid.

Because these beads, which Alexander-Katz has termed "micro-ants" because of their remarkable ability to propel objects almost one-hundred times their size, can be controlled by the magnetic field in the system, the microfluidic system is more precise and manageable than previously designed systems.

Ultimately, this microfluidic system may be used in inexpensive diagnostic chips that analyze trace amounts of bodily fluids or chemical sensors that can detect pollution in the environment. The study also sheds light on cilia function. Specifically, Alexander-Katz explains that the study may provide information as to how ciliated systems are "hydrodynamic[ally] synchronized."

Alexander-Katz is confident that he can apply the technology to microfluidic chips "within a year or so."

—A. Kashyap

Source: "'Micro-ants': Tiny conveyor belts for the 21st century" – David L. Chandler, MIT News Office
http://web.mit.edu/newsoffice/2009/micro-ants.html

"Micro-ants".
Credit: Christine Daniloff, MIT News

Chanel bag.
Credit: U.S. Marshals Service via Washington Post

[Economics]

Value of Luxury Goods Dependent on the Status of Consumer

Ever since the 1899 publication of Thorstein Veblen's seminal work *The Theory of the Leisure Class*, most economists have agreed that much of the value of luxury goods is in their use as a status symbol—as opposed to the inherent quality of the good. Recent research on counterfeit luxury goods at MIT is expanding on this theory by showing that individuals can only assess the quality of a good when given some context.

Sloan School of Management assistant professor Renee

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Richardson Gosline surveyed consumers' ability to discern counterfeits from authentic luxury goods by asking consumers to identify authentic goods from over twenty photos of both fake and real luxury handbags. Before the assessment, the consumers believed their ability to differentiate real goods from fake ones would rank a 6.2 on a 7-point scale on average. The consumers also noted that they would, on average, pay almost eight-hundred dollars for an authentic bag.

But after Gosline only showed pictures of handbags placed on shelves, thus depriving the good from any visual context, the consumers lost confidence in their ability to discern real from fake. On average, their self-assessed ability to determine real goods from fake goods fell to 3.1; they also reported that, on average, they would only pay around four-hundred dollars for the bags depicted in the photos.

However, after Gosline showed the participants photos of people wearing or using the same handbags, thereby contextualizing the goods, consumer confidence and willingness to pay large amounts of money rose up to original levels. Gosline explains: "Basically these consumers look at the person, the setting, and determine the authenticity by seeing if the person's image corresponds with the image they have of the brand."

This study is not promising for the luxury good industry, which has, in recent years, been plagued by massive counterfeiting. The International Chamber of Commerce estimates that the counterfeiting industry makes up five to seven percent of the global market.

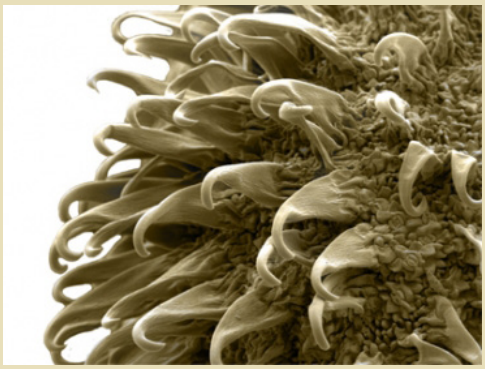
—A. Kashyap

Source:"The real thing?"– Peter Dizikes

http://web.mit.edu/newsoffice/2009/real-fake-1201.html

[Chemical Engineering]

New Nanotherapeutic Developed to Repair Vascular Damage



Scanning electron microscope (SEM) image of nanoburr particle.
Credit: MIT News

When arteries are damaged, scar tissue forms in the vicinity of the vessel damage. However, often times, the developing scar tissue can clog the arteries. Researchers in Robert Langer's lab, in collaboration with Harvard Medical School's Omid Farokhzad, have recently been able to apply the burgeoning field of nanotechnology to solve this problem.

Researchers developed nanoparticles called "nanoburrs" that selectively adhere to the damaged walls of arteries. Scientists coated the nanoparticles with the C11 amino acid sequence, which strongly binds to the basement membrane, a layer found in arterial walls that is only exposed when arteries are damaged. Cell division-inhibiting drugs, like paclitaxel, are bound to a biopolymer chain consisting of polylactic acid (PLA), which are then enclosed in the nanoparticles. When released at the designated areas, the drug can inhibit scar tissue replication. Advantageously, because the drug cannot be released until the PLA chain is degraded via hydrolysis, drug delivery time can be controlled by varying the length of the PLA chain that binds the drug. An additional useful feature of the nanoparticles is that they can also home to their intended targets. For example, nanoburrs systemically injected into mice tail veins were able to localize to damaged regions of the murine carotid artery.

The nanoburrs may either supplant or be used in conjunction with the current treatment for arterial damage which involves inserting a vascular stent into the damaged region which then stabilizes the artery while simultaneously delivering pharmaceuticals. The nanoburrs are especially useful for treating places where arteries fork, regions which cannot be treated by stents. Farokhzad notes that the new technology has broad applications and can be used to treat any disease "where vascular permeability or vascular damage is commonly observed."

—A. Kashyap

Source: "New 'nanoburrs' could help fight heart disease" – Anne Trafton

http://web.mit.edu/newsoffice/2010/nanoburrs.html

World Science News In Review

[Ecology]

Fig Trees Punish Non-Pollinating Wasps

In a classic example of mutualistic symbiosis, wasps lay their eggs in the fruit of a fig tree; the wasp offspring derives shelter and nourishment from the fruit. In exchange, the wasp pollinates the fig tree allowing for the tree's spread. Depending on the wasp species and fig tree species, wasps either actively pollinate the tree via a pouch-like organ or they passively collect pollen when they interact with the tree.

Charlotte Jandér of Cornell University's Neurobiology and Behavior department wanted to know "what forces maintain this 80-million-year-old arrangement between figs and their wasp pollinators [...] What prevents the wasps from reaping the benefits of the relationship without paying the costs?"

For six unique fig wasp-fig tree species combinations, Jandér sequestered a pollen-bearing wasp and a non-pollen-bearing wasp with the fruit of a fig tree by wrapping a mesh bag containing the insect around the fruit. The wasps then laid eggs in the fig fruits. Those wasps that did not bear any pollen when they laid their eggs in the figs were then punished by fig trees, which dropped their fig fruits thereby killing the wasp offspring that lived inside.

Interestingly, only the species of fig trees that necessitated active pollination resorted to this punishment. Fig trees that functioned via passive pollination did not proactively punish any non-pollen-bearing wasps.

Jandér notes that this study has interesting implications in terms of what drives mutualistic interactions between different organisms: "Sanctions seem to be a necessary force in keeping this, and other, mutually-beneficial relationships on track when being part of a mutualism is costly."

Jandér wraps bag that contains either a pollen-bearing or non-pollen-bearing wasp around the fruit of a fig tree.
Credit: Marcos Guerra, ScienceDaily

—A. Kashyap

Source: ""Punishment Important in Plant-Pollinator Relationship" – ScienceDaily
http://www.sciencedaily.com/releases/2010/01/100114143513.htm

[Neuroscience]

New Gene Variant Found That Lowers Risk for Dementia and Alzheimer's Disease

Many biomedical researchers are attempting to find novel therapeutics for neurodegenerative disorders like Alzheimer's disease, which alone afflicts 2.4 to 4.5 million Americans. Led by Richard B. Lipton, M.D., scientists at the Albert Einstein College of Medicine at Yeshiva University have recently identified what they term a "longevity gene" that slows down the rate of natural cognitive decline in older individuals.

The gene of interest is a variant of the CETP gene, which is responsible for the synthesis of the cholesteryl ester transfer protein. Individuals with the gene variant have higher levels of high-density lipoproteins (HDLs) or "good cholesterol."

The researchers wanted to learn if there was any correlation between the presence of the CETP variant and the rate of cognitive decline in the elderly. They selected 523 healthy individuals of at least seventy years of age from the Einstein Aging Study, a group of racially diverse Bronx residents. After blood samples were taken to determine each individual's genotype for the CETP gene, the participants would be tested to determine any decrease

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"New Gene Variant" continued from p. 10

in cognitive ability or any onset of neurodegenerative disorders over the course of four years.

The researchers found that those individuals homozygous for the beneficial CETP gene variant had a lesser decline in cognitive ability and a smaller risk for cognitive disorders. Amy Sanders, M.D., elaborates: "More specifically, those participants who carried two copies of the favorable CETP variant had a 70 percent reduction in their risk for developing Alzheimer's disease compared with participants who carried no copies of this gene variant."

Research in the future is focusing on synthesizing compounds that mimics the performance of the protein product of the CETP gene variant with the hope that these drugs will lower the risk of cognitive disorders in the elderly.

—A. Kashyap

Source: "'Longevity Gene' Helps Prevent Memory Decline and Dementia" - ScienceDaily

http://www.sciencedaily.com/releases/2010/01/100112165234.htm=

[Computational Biology]

Bacterial Behavior Sheds Light on Game Theory

The study of game theory, the science of how and why individuals make certain choices during competitive situations, has recently taken an interesting turn. Researchers have begun investigating the behavior of single-celled organisms in the hope of applying any newfound knowledge to human interactions.

Scientists at the University of California, San Diego's Center for Theoretical Biological Physics have been using theoretical mathematics, chemistry, and physics to model the genomic and proteomic interactions among colonies of Bacillus subtilis under conditions of environmental stress.

Under stress, a bacterium faces two options. It can undergo sporulation, a process that involves more than 500 genes, in which the bacterium places a copy of its genome in a durable capsule known as a spore. These spores, which are extremely durable even during harsh conditions, can then germinate into new bacteria. The mother cell that sacrifices its genome to form the spore then bursts open releasing its intracellular contents to the environment.

The other option for the bacterium is to enter the "competence intermediate state" in which it makes its membrane more permeable to its extracellular environment so that it can assimilate the cellular contents released by other bacteria that have sporulated. Though this option may allow for survival, the bacterium risks death.

Bacillus subtilis colonies.
Credit: Eshel Ben Jacob, ScienceDaily

UCSD physics professor José Onuchic explains how these two options present a Prisoner Dilemma-like predicament: "It pays for the individual cell to take the risk and escape into competence only if it notices that the majority of the cells decide to sporulate. [...] But if this is the case, it should not take this chance because most of the other cells might reach the same conclusion and escape from sporulation."

The conclusion of the "game" is that only ten percent of the bacteria enter the "competence intermediate state." The scientists have found that the decision a bacterium makes is dependent upon the chemical signals being released by its neighboring bacteria. Moreover, though the researchers keep refining their studies, they conclude that stochastic processes still pervade the decisions the bacteria make. Onuchic explains: "Another interesting fact is that the same cells in the same environment, in this case, bacteria in the colony, can actually in a statistical matter choose two different outcomes: sporulation or competence."

The researchers hope their studies will have applications in sociology, economics, and even cancer biology.

—A. Kashyap

Source: "Bacteria Provide New Insights Into Human Decision Making" - ScienceDaily

http://www.sciencedaily.com/releases/2009/12/091211200341.htm

[Earth Science]

Scientists Say Humans, Not Climate Change, Brought About Extinction of Prehistoric Australian Megafauna

Megafauna, including giant reptiles, large marsupials like the depicted *Diprotodon*, and huge flightless birds, roamed Australia during the Pleistocene Epoch until they went extinct around 40,000 years ago. New evidence presented by Australian scientists seems to point to humans as the cause for the towering giants' fate—as opposed to climate change, the current leading theory.

Diprotodon.
Credit: Peter Trusler, Melbourne Museum

Richard Roberts, a professor at the University of Wollongong, and Barry Brook, a professor at the University of Adelaide, used novel methods to directly date the bones and teeth of various megafauna species found at Cuddie Springs, a site in New South Wales. They then proceeded to directly date the Aboriginal artifacts also at the site.

The researchers found that the megafauna and humans only coexisted for approximately 5,000 to 15,000 years. Roberts explains: "These results provide no evidence for the late survival of megafauna at this site. [...]Given that people arrived in Australia between 60,000 and 45,000 years ago, human impact was the likely extinction driver, either through hunting or habitat disturbance."

This direct dating method contradicts previous research at Cuddie Springs, which found that the fossils of the extinct animals were located in the same strata of earth as the Aboriginal tools, implying that the megafauna and humans lived concurrently. Roberts believes that, over the years, the layers containing the human artifacts and the fossils must have mixed.

In the future, Professor Barry Brook wants these new methods to be applied to other sites of interest such as those in Kangaroo Island, the province of Victoria, and Papua New Guinea.

—A. Kashyap

Source: "Humans Caused Demise of Australia's Megafauna, Evidence Shows" - ScienceDaily

http://www.sciencedaily.com/releases/2010/01/100121141109.htm

[Biology]

Stem Cell-Derived Nerve Cells Successfully Form Neural Connections After Transplant

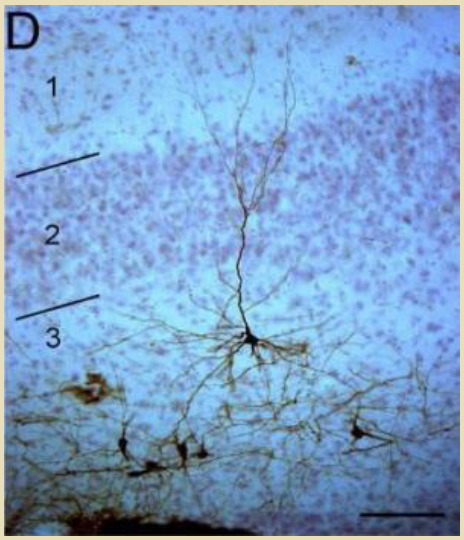
Though previous studies have confirmed that stem cells can be differentiated into the neural lineage, recent work has shown for the first time that these differentiated stem cells can also form the correct neural connections with neighboring cells.

Using two different protocols, scientists led by James Weimann of Stanford Medical School attempted to differentiate embryonic neural stem cells into cortical neurons. When deemed ready, the cells were then implanted into the cortexes of infant mice, specifically the regions of the cortex responsible for sight, motor skills, and tactility.

Weimann found that the implanted cells were able to develop neural fibers that connected to the cerebral cortex and spinal cord—as they should. Somehow, the cells "knew" where it ought and where it ought not to form connections. Cells implanted into the region of the cortex responsible for mobility formed connections with the spinal cord but not the superior colliculus; cells implanted into the cortex region

continued on p. 14

"Stem Cell" continued from p. 15



A single stem cell-derived neuron.
Credit: Weimann et al. The Journal of Neuroscience 2010, ScienceDaily.com

responsible for sight connected to the pons and the superior colliculus but not the spinal cord.

Moreover, the ability for the implanted cells to form the correct connections was protocol-dependent as only the cells cultured via one of the two protocols were able to do so.

Scientists' next step is to test if they can achieve similar results in adult mouse models. Researchers also wish to gain insight into how exactly these implanted cells were able to know how to develop. Hopefully, this line of research can provide a new therapy for people suffering from brain-related disorders like Lou Gehrig's disease (amyotrophic lateral sclerosis).

—A. Kashyap

Source: "Neurons Developed from Stem Cells Successfully Wired With Other Brain Regions in Animals" - ScienceDaily

http://www.sciencedaily.com/releases/2010/01/100119172751.htm

[Chemistry]

Scientists in France and Sweden Measure Energy Required for Viral Infection

Current therapeutics to counter viral infections are rendered ineffective in the long run by a virus strain's ability to rapidly mutate. The problem is plaguing vaccine and drug manufacturers who must constantly synthesize novel pharmaceuticals in this biological arm race against the virus. So scientists at Lund University in Sweden and the University of Lyon in France are attempting to develop a physics-based approach to stopping viruses.

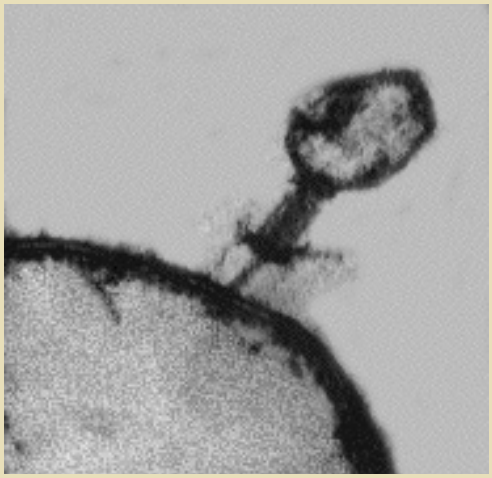
Led by Alex Evilevitch (Lund), the scientists have calculated the amount of energy required for a virus to inject its nucleic acid into E. coli bacteria using "isothermal titration calorimetry." The scientists believe that this internal energy in the virus is responsible for the virus's extremely high internal pressure, on par with the pressure at 500 meters below sea level. The high internal pressure, in turn, allows the virus to eject its genome at a high speed. Researchers hope that they can lower the internal energy of the virus thereby rendering them incapable of infecting target cells.

The researchers have also determined that the amount of internal energy in the virus seems to positively correlate to the water content of the virus's protein-based capsid. Thus, they are now focusing on finding novel ways to regulate the capsid water content of viruses.

—A. Kashyap

Source: "Energy of Attacking Virus Revealed" - ScienceDaily

http://www.sciencedaily.com/releases/2010/01/100120085346.htm



Virus infecting cell by injecting genome into target cell.
Credit: www.dform.com

[Biology]

Austrian Scientists Use Insect Cells to Develop Faster Method to Generate Vaccines

The 2009 H1N1 pandemic gripped many people around the world in fear. The demand for large supplies of vaccines against the virus was immediate. However, according to Florian Krammer, a scientist at the University of Natural Resources and Applied Life Science in Vienna, "classical manufacturing methods for vaccines fail to satisfy this demand."

The "classical manufacturing method" utilizes embryonated chicken eggs, a process that may take several months. Moreover, the manufacturing of traditional vaccines can also suffer from a lack of egg supply. Krammer and others, thus, attempted to find a more efficient way to synthesize vaccines using insect cells.

From these insect cells, the researchers were able to generate what they termed VLPs, short for recombinant influenza virus-like particles. Because these particles lack genomes, they are not infectious.

Such particles exhibit many advantages over traditional egg-derived vaccines. For example, researchers were able to generate these VLPs in a mere ten weeks, thereby showing that their method could prove to be a faster and more cost-effective one—especially in the case of a virus that rapidly mutates. Furthermore, because the VLPs are not derived from eggs, people that are allergic to eggs can still have the vaccine.

Editor of Biotechnology Journal Alois Jungbauer concludes: "Virus-like particles will be one solution to tackle the biological variability of influenza pandemics. Mutated strains can be quickly engineered. So in this respect the teams' work is an extremely valuable contribution to modern vaccine production."

—A. Kashyap

Source: "Insect Cells Provide the Key to Alternative Swine Flu" - ScienceDaily

http://www.sciencedaily.com/releases/2010/01/100104191928.htm

MURJ Features

40 Years of UROP

A Special Look into the Past Forty Years of MIT's Undergraduate Research Opportunities Program

The Growth of UROP at MIT

Timeline Compiled by MIT's UROP Program

Today: Eighty-five percent of graduating seniors will have participated in UROP at least once during their undergraduate years. Six of MIT's current faculty Nobel Laureates have been/are active UROP Supervisors. In UROP's forty years, well over $100M has been allocated to support UROP students and their projects.

2009: UROP alum and current HST graduate student, Geoffrey Von Maltzahn, '03, is awarded the $30,000 Lemelson-MIT Student Prize for innovations in the fight against cancer. UROP celebrates its 40th anniversary with a day-long research symposium.

2007: The International Research Opportunities Program (IROP) is formally launched to support UROP research taking place overseas.

2000: In April, John M. Grunsfeld '80, a former UROP student, returns to MIT to discuss his space shuttle mission to repair the Hubble telescope and presents UROP memorabilia that accompanied him into space.

1997-1998: UROP launches an expanded website to compliment its print UROP Directory.

1993: UROP introduces a mentoring program to link students who have never undertaken a UROP with upperclassmen experienced in research.

1987: Jennifer Wiseman discovers the "Comet Wiseman-Skiff" as a UROP student, under the mentorship of Prof. Jim Elliot in EAPS, who remains an active UROP mentor.

1979: UROP celebrates its 10th Anniversary with a birthday party in Lobby 10.

1975-1976: Twenty-eight percent of the student body participates in UROP during the academic year. Today, approximately half of the student body participates during the academic year.

1973-1974: Direct UROP funding introduced, enabling students to seek centrally administered funds to support their UROP research. The UROP hourly rate is $2.50/hour.

1971: UROP's first service-learning oriented projects take place.

Fall 1969: Prof. Margaret MacVicar launches UROP with fewer than 200 student participants. Forty years later, 85% of MIT graduates will participate in UROP at least once.

2009: On October 29, UROP marks its 40th Anniversary with an all-day research symposium, introduced by President Susan Hockfield.

2008: The PBS series Design Squad wins the prestigious George Foster Peabody Award. Members of the MIT Community (including several UROP students) play important roles in developing this series aimed at increasing young peoples' understanding of engineering and the design process.

2002-2003: Over 2000 students conduct UROP projects.

1999: UROP student Mike Tarkanian '00 plays a key role in the discovery that ancient Mesoamericans were processing latex to produce rubber as early as 1,600 BC.

1995: More than half of the MIT faculty are actively mentoring UROP students.

1989: UROP celebrates 20 years at MIT with a celebration in Lobby 13. Over 2000 projects are conducted this year and about 75% of the student body is reported to have participated in UROP at least once before graduation.

1986: UROP's founder, Dr. MacVicar, receives a Commendation in Higher Education Award from the Charles A. Dana Foundation for her work in establishing UROP.

1978: New York Times Magazine features an article about MIT UROP, citing the program as an innovation in higher education and an inspiration for a number of other universities that begin undergraduate research programs.

1974-75: Sixteen percent of UROP participants are female. By 2003-04 female students represented 45% of all UROPs.

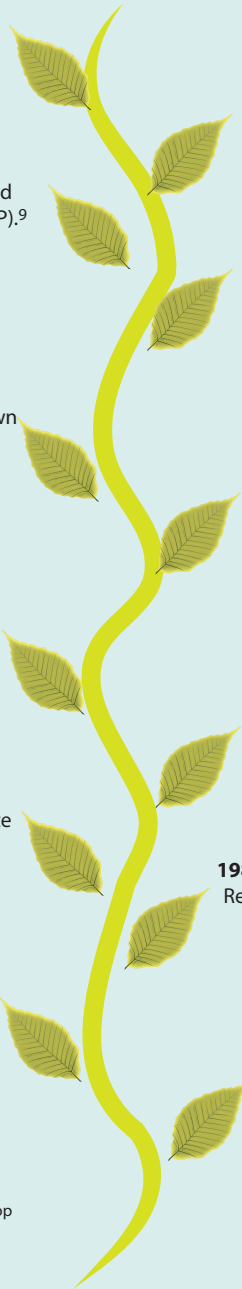
1972: Nearly 1000 students are reported as participating in UROP.

1970: Twenty-seven departments, labs, and programs are open to UROPs. Today, over 70 different academic departments, laboratories, and centers actively participate in UROP.

The Growth of UROP at other Universities

Timeline Compiled by MIT's UROP Program

Since UROP's founding in 1969, a number of other universities both nationwide and abroad have established their own "UROP" programs.



~2007: Cambridge University, UK establishes the Undergraduate Research Opportunities Programme (UROP).[11]

~2007/2008: RWTH Aachen University establishes UROP as a new Undergraduate Research Opportunities Programme.[10]

~2006: University of Reading, UK begins a summer focused Undergraduate Research Opportunities Programme (UROP).[9]

1997-1998: Boston University introduces their Undergraduate Research Opportunities Program (UROP).[8]

1995: The University of California, Irvine launches their own Undergraduate Research Opportunities Program (UROP).[7]

1994: The University of Colorado, Denver establishes an Undergraduate Research Opportunities Program (UROP).[6]

1992: Louisiana Sea Grant College Program starts an Undergraduate Research Opportunities Program (UROP).

1990: Carnegie Mellon introduces the campus-wide Undergraduate Research Initiative (URI) program.[5]

1989: University of Michigan launches their Undergraduate Research Opportunity Program (UROP).[4]

1987: Georgia Institute of Technology begins an Undergraduate Research Opportunities Program (UROP) program in the School of Electrical and Computer Engineering. The program was modeled after MIT's UROP. [3]

1987: The University of New Hampshire establishes the Center for Undergraduate Research. [2]

1981: Imperial College, London starts its Undergraduate Research Opportunities Programme (UROP), which over its history "has hosted over 2000 students, the core of whom have gone on to establish careers in research and development both in the academic and commercial environments."[1]

[1] http://www3.imperial.ac.uk/urop/abouturop/uropforstudents/whatisurop
[2] http://www.unh.edu/undergrad-research/urop.html
[3] http://www.ece.gatech.edu/orgs/urop/background.html
[4] http://www.lsa.umich.edu/urop/about/
[5] http://www.cur.org/publications/aire_raire/cmu.asp
[6] http://www.cudenver.edu/Who%20Am%20I/Current%20Students/UROP/Pages/default.aspx
[7] http://www.urop.uci.edu/ (under About UROP)
[8] http://www.bu.edu/urop/
[9] http://www.reading.ac.uk/internal/UROP/UROP_Home.asp
[10] http://www.exzellenz.rwth-aachen.de/ca/k/sav/lang/en/
[11] http://www.eng.cam.ac.uk/teaching/urops/index.html

Interview with Kim Vandiver

Dean of Undergraduate Research

By: Omar Abudayyeh and Paul Baranay

MURJ: Can you tell us a little about what your roles are as the Dean of Undergraduate Research?

I took this role on about 10 years ago. At the time, Margaret MacVicar was essentially the first faculty member who basically built the UROP program and was essentially the first Dean for Undergraduate research by 1991. Between 1991 and 1998, there had not been a faculty director of UROP and so I got recruited by the chancellor. We needed some faculty involvement in certain policy and things like that in the office and so I said I'd like to do that. It also happened that I created the Edgerton Center in 1992 and I'm a big believer of hands-on experience-based education. I had long thought about the combination of the two things, as the Edgerton involved hands-on research and the UROP was a great program that students already knew about and that allowed them to get out there and build things. It was a great combination for me to have both of those resources that I could coordinate together for the benefit of the students. That's how I got to be where I am. My real role in it these days is I'm basically the faculty director of UROP. The day-to-day operations are run fantastically by Micheal Bergren and Melissa Martin-Greene and the people of the UAAP.

More recent things that I'm interested in are what I call IROPs because the Edgerton center is home of D-Lab. D-Lab gets students involved in international development projects and UROP is a nice mechanism for students being able to continue to work on projects that they might start in D-Lab. If students wanted to spend their summer in Ghana pursuing a project that was started on alternative fuels that they learned about in D-Lab, IROP is a way for students to apply for funding and get involved and do things that were international. In the last few years now there has been a lot more interest in giving MIT students more international experiences. The creation of this IROP concept was something I was hand-in-hand with Julie Norman in developing.

> ***"What he got from doing that UROP and having a faculty member saying, 'No big deal, you can learn it in a couple weeks,' is that it built his confidence in himself that he could do just about anything if he put his mind to it."***

MURJ: So what has been your influence on the program as the Dean?

Early on when I took on this role 10 years ago the question came up regarding just what are students' IP rights. It's pretty clear if you are a UROP student working on a sponsored research project for a faculty member, then the Office of Sponsored Programs rules apply and basically that means for most sponsors the IP belongs to MIT and the sponsors have royalty free license to use any intellectual property that comes from the project. If they want to have exclusive rights to it, they have the right to negotiate with MIT to make it happen. What happens though when you apply to the UROP office for funding to work on something that's maybe even your own idea that you want to pursue? It's not sponsored research money but actually MIT money and we're going to give it to you to work on a new energy invention or something. Who owns the IP? MIT has clauses about things like this. It's process and procedures that mostly applies to employees and it says that if you make substantial use of MIT resources then MIT has an ownership interest in the IP. We came to the conclusion that in the case of UROP students even if you got funding from the UROP office, UROP students should own the IP. That's the sort of thing that comes up. If you invent something, even though you were paid by the UROP office, we still want you to have the IP. The why about that is actually interesting. I think in the long run that if you invent this new energy

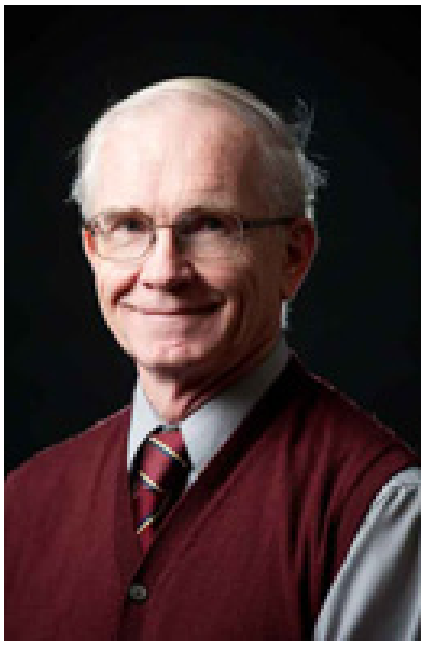
device and you go off and build an industry around it, MIT will benefit more from your interest in paying back and supporting the next generation of UROP students then we would if we exacted our licensing rights. That's why you need a faculty hand in the guidance of a program like UROP.

MURJ: So you addressed this a little already, but can you discuss the impact of UROP on MIT students?

Well, I had a phone call many years ago. I think it came into the office as an email and not right to me. It was a past student and at the time a relatively recent student, only five or six years out. He basically contacted the office and said I wouldn't be where I am today without my UROP experience and my company was just bought out for 100 million dollars and I want to do something to ensure that UROP is safe for the next generation. He gave us a half a million dollars and we had conversations about what made him feel this way. I think his story is an interesting one and it's not just interesting, but also very typical. This was in the software boom of the mid-90s and he got out and done something that made him a big chunk of money. In my conversations with him afterward, I asked why and what it had to do with his first UROP.

He went in and knocked on the door looking for that first UROP, which is hard to do. This guy asked him if he could program in C and he said no. To his great surprise, the response of the faculty member was that it didn't matter and he could learn it in a couple weeks. It wasn't that he did a particular UROP and learned exactly how to do A-B-C and went out to build his company on A-B-C. What he got from doing that UROP and having a faculty member saying, "No big deal, you can learn it in a couple weeks," is that it built his confidence in himself that he could do just about anything if he put his mind to it and that a faculty member at MIT basically endorsed and gave him permission to go do those kinds of things. He had a sequence of two to three different UROPs in the course of being an undergrad here and they just set him up in the right frame of mind that he could go out and do things and so he went out and built a company. The guy's name is Niel.

That's a story way of telling what UROP does for students. I think it gives you a place where you can actually solve unstructured problems and do hands-on stuff. You find out that it isn't just book learning and there's no right answers necessarily.



"MIT students are really smart and can do things that you can't get a grad student to do. "

You get what I call experiential learning. Maybe all you learn from it is you take on your first UROP and work in a biology lab and discover you don't really like working in a biology lab. That's a heck of an important lesson to learn at a relatively small cost and you go on and try something else so that when you leave here you make the right decision about where to go for grad school and the right decision about what career to pursue. You really have to do something to know whether or not you like it or if it excites you.

MURJ: How has the program evolved over the years?

My knowledge of the early years is non-existent. I knew Margaret MacVicar for a long time. She was a close colleague. So I will have to do it a little bit by assumption. I have been called upon by other schools that don't have UROP programs and have talked with their faculty about starting UROP programs. I did this at a very well known university in the UK that had a bunch of faculty in the room for a couple of hours and gave a couple presentations on UROP. On the first visit there for most of the two hours, I was told all the reasons it couldn't possibly work there. It has to do with undergraduates don't know enough to make contributions. Undergraduates will break stuff in the lab. You can't trust them. Undergraduates don't have time. Undergraduates can't this, don't that. So that's what I got for two hours. There were a couple of students in the room that had been to MIT and had done UROPs here. Finally, one of them burst after about the third time a faculty said, "Our students just don't have time to do that. They're too busy doing other things." That student was just about ready about to explode and finally just

couldn't contain himself any longer and put the faculty member straight. He said, "You know we have time to go down to the pub and drink beers. We have time to play football. We have time to do this. Some of us have time to do UROP and that we ought to be doing it here." I think what happened early on at MIT is that, like this, faculty members were skeptical and it took time to convince them that it's actually valuable. Over the years, UROP got its roots down and started to flourish, and now if you told faculty they couldn't have UROP students, you'd have war on your hands. MIT students are really smart and can do things that you can't get a grad student to do. It's not just smarts. It has to do with students come here for Master's Degrees and Ph.D.s and you have to assign them a problem that's big enough to be adequate for a thesis. That might take a typical Master's student a year or so and Ph.D. students three to five years. However, shorter projects and problems come along in the course of research that really need to get done, but you don't want to divert your grad student from making progress on a big problem to writing up a piece of code that you really need to get done.

I'll give you a quick story. I had a grad student who wasn't particularly gifted in making things, but we needed to do an experiment in a wind tunnel. I had another student who walked into my office looking for a UROP and I interviewed him. I doubt they still do this with TEAL, but in 8.02 years ago they would give kids a package of parts and say that there was a contest to go make an electrical motor. Later in the semester, they would bring them to class and have the RPM measured and the one who could crank it out the fastest wins. So this kid as a freshman had won that. This was the kind of kid I needed because I needed someone that could build the model for the wind tunnel experiment and make it work. And he did and he actually stayed on for his senior year and the next year as a Master's student and the second year we really did the experiment right, not that the first year he failed, just that the first time you do a complicated experiment you always fail. You just have to figure out what you did wrong and get it right. I needed both students. I needed the student who could build stuff in the shop and combine it with the grad student who was good at analysis and data crunching and without both of them we could have never succeeded in the project. The fact that you have to realize is that there are all sorts of problems that are of the size and scale that are appropriate for undergraduates that aren't necessarily the size and scale appropriate for the graduate students and you need both.

"What happens though when you apply to the UROP office for funding to work on something that's maybe even your own idea that you want to pursue? It's not sponsored research money but actually MIT money and we're going to give it to you to work on a new energy invention or something."

MURJ: How do you think the UROP program will change and hopefully improve over the next 10, 20, and maybe even 40 years?

At least certainly in the next few years, I think you'll see a lot more of the international UROPS. I think the U.S. in order to be competitive internationally will need students to be able to pick up their passport and head out and go do things in different places, and students will need those experiences as undergraduates to have the confidence to work in Germany or in Asia or wherever. So I think we will continue to sponsor those kinds of experiences and so they're going to grow. Universities like MIT are going to have to change and adapt in order to be able to survive in a tight resources climate. Tuition is getting close to $40,000 and the cost of a year at MIT when you throw everything in is over $50,000. Around the country tuition rates have been going up so fast and that cannot be sustained. Universities in order to survive are going to have to figure out different models of working and the value propositions for why should you and your parents pay to have you come here rather than getting your degree from the University of Phoenix. So what do you get out of being here that makes it worth it? We can say what that is today, but what about 10 or 20 years from now? I think we are going to have to get both better at making it cost less, but also getting very clear on the value of what it is you did here that you can't get if you are not here. I think UROP actually is a key part to the answer of that question. You cannot get the UROP experience at the University of Phoenix.

Interview with Melissa Martin-Greene

UROP Program Coordinator

By: Omar Abudayyeh and Paul Baranay

MURJ: Tell us about yourself; where you're from, how long you've been at MIT.

MM: Well, I was born and raised in Massachusetts and I now reside in New Hampshire. I have been at MIT working with UROP since August 1997.

MURJ: What were some of the reasons for starting the UROP program 40 years ago?

MM: UROP's founder Prof. Margaret MacVicar was inspired by a number of innovations in education that were occurring at the time. In particular, she drew inspiration from a few experimental introductory research classes and a speech called Generation of Greatness that Dr. Edwin Land, founder of the Polaroid Corporation gave at MIT in 1957. The speech stressed the importance of learning by doing and the need to get undergraduates involved in hands-on learning projects and research as early as possible in their careers. Before the start of UROP, students were involved with research through theses and lab classes, but there was no centralized mechanism through which students could engage in research.

Professor MacVicar was inspired by Dr. Land's belief in the importance of hands on learning. She agreed with him - as did the other faculty instrumental in the early days of UROP - that allowing undergraduates to engage in research as junior colleagues of faculty would greatly benefit not only the students themselves, but also faculty, departments, and the overall research enterprise. Without a formal means to engage in research, students did not have much opportunity to really learn about the research environment at the undergraduate level. Before UROP, many students had to wait until graduate school to be exposed to research. Therefore, UROP provided a means to get students involved in research earlier in their academic careers and afforded the opportunity to apply classroom knowledge to real-world problems.

MURJ: What are the visible results you see from the UROP program over the 40 years that have been invested in it?

MM: Students are making a difference, giving back to communities, and advancing spheres of academic discovery. They are discovering their passions, honing their skills, learning to solve real research problems and how to think abstractly about issues. They are making real connections in the research arena, not only with faculty and other mentors here at MIT, but also through the connections they make at conferences, professional society meetings, or by being published in journals.

Through UROP, students have the freedom to explore their interests at any stage of their undergraduate career beyond what is required of them in the classroom. UROP provides an added benefit for our students, such as the ability to engage in research across all disciplines and at anytime from freshman through senior year. Undergraduate research programs at other schools might limit participation to only those students from a given class year or major.

Some students choose their majors because of their UROP experiences and others change their chosen direction because of the exposure that UROP provides. Students explore new areas of interest and subjects

"A number of colleges and universities worldwide have begun their own undergraduate research programs since MIT's UROP was founded, several of which credit MIT's UROP as an inspiration."

that they previously had not studied. I have also observed students having the opportunity to publish for the first time. Each year UROP students co-author papers in professional journals, society publications, and MIT journals like MURJ.

UROP is also one of the more powerful means through which students connect with faculty outside of the classroom.

MURJ: So how has the UROP program evolved over the years?

MM: The basic tenets of the UROP program have remained intact since UROP's inception- all UROP work is worthy of academic credit, conducted under the supervision and mentorship of an MIT faculty member, and proposed through our office. At the foundation of the UROP program is a strong belief in the transformative power of research engagement for students. While the philosophy behind UROP has essentially remained unchanged, many of the practical and administrative aspects of the program have evolved over the past forty years. I think introducing a pay option was probably instrumental in ensuring that UROP continues to be successful in the present day. Certainly, students need to finance their MIT education and UROP is one of the ways to do that. From surveys and various studies conducted over the years, it is clear that students would engage in UROP whether or not pay was available, but pay is a strong motivator. Without the paid UROP option, some students would not be able to participate because they would need to focus on more traditional campus jobs.

More recently, the introduction of a more formalized program (IROP) for promotion and recognition of international research is another way in which UROP has evolved. Several

"I hope that MIT students will continue to recognize the importance of engaging in research at the undergraduate level, especially as a post-baccalaureate education becomes increasingly important in society."

other schools have their own brand of international research programs, but I think that formalizing such a program here at MIT opens even more doors of exploration for our students.

MURJ: Can you discuss some success stories that UROP has had?

MM: There are more UROP successes than could possibly be captured in one article, but I will mention some standouts. One example would be Mike Tarkanian,'00 who was a UROP in the Center for Materials Research in Archaeology and Ethnology (CMRAE). Mike worked with Prof. Dorothy Hosler on a project studying materials archaeology and through his research discovered that ancient Meso-Americans were fabricating rubber and using it to create various items as early as 1600 B.C. This was definitely a first, not only for the UROP student, but also for modern archaeology.

Another example is Ms. Jennifer Wiseman, '87, who discovered the comet "Wiseman-Skiff" as a UROP student under the mentorship of Prof. Jim Elliot in EAPS.

Yet another example is Daedalus, an endeavor on which a number of UROP students, faculty, and others collaborated. Daedalus was a lightweight airplane that was flown across the Mediterranean Sea from Crete to just offshore of the island of Santorini, completely under human power. The flight set aviation records that stand to this day.

More recently, Mr. Geoffrey Von Maltzahn, class of 2003 and HST graduate student, was awarded the 2009 $30,000 Lemelson-MIT Student Prize for innovations in the fight against cancer. Geoff participated in UROP with Dr. Shuguang Zhang designing and developing a new class of self-assembling surfactant peptides, and was awarded the 2003 MIT Randolph G. Wei Award for those research efforts. Geoff was also the MIT student delegate to the 2003 Coalition for NSF Funding in Washington, DC, and had the opportunity to present his research and represent MIT's NSF-funded undergraduate students to members of the U.S. Congress.

UROP students not only contribute to research related to curing diseases like cancer research, HIV, and Parkinson's disease research, but also to issues of energy and the environment, including

"Students are making a difference, giving back to communities, and advancing spheres of academic discovery. They are discovering their passions, honing their skills, learning to solve real research problems and how to think abstractly about issues."

campus sustainability. The MIT Energy Initiative is actively engaged in recruiting UROP students and faculty members to work on research related to campus energy resources and more global energy problems.

UROP students have gone on to patent inventions and start companies based on UROP research done at MIT in collaboration with MIT faculty. We also have NASA astronauts who were UROP students during their undergraduate years. Furthermore, forty active faculty members started their research careers as UROP students here at the Institute.

MIT research has its way of reaching out to the various facets of society. UROP students are engaged in service learning projects related to D-Lab and other initiatives enabling them to apply their engineering expertise to real problems in the developing world. We also support IROP projects that take students to various countries in order to collaborate with MIT colleagues abroad. It is great to see MIT's mission fulfilled by undergraduates who are engaged in hands on learning and using expertise developed in the classroom to help address real world issues.

MURJ: How does UROP's program compare to programs at other schools? Has it been a source of inspiration for other programs?

MM: MIT's UROP program was at the forefront of formalizing the undergraduate research relationship between students, faculty and academic departments and the program has definitely served as inspiration for the myriad of undergraduate research programs that have developed at other schools. A number of colleges and universities worldwide have begun their own undergraduate research programs since MIT's UROP was founded, several of which credit MIT's UROP as an inspiration. Georgia Tech is one such university and the Imperial College in London is another. All you need to do is "Google" UROP and you will see how many variations of the UROP schema there are worldwide.

MURJ: Where do you see the UROP program going in 40 years?

MM: In the years ahead, I would like to see UROP going strong with an active and vibrant undergraduate participation level. Currently, 85% of graduating seniors will have done a UROP at least once. I hope that MIT students will continue to recognize the importance of engaging in research at the undergraduate level, especially as a post-baccalaureate education becomes increasingly important in society. I hope that more of our undergraduate population will recognize the benefits of undergraduate research and continue their active engagement in UROP during the years to come.

In the future, I think that UROP will be more engaged in international research. We have already seen a great deal of interest in global education at MIT and at the national level. So, I think that people recognize how important it is to have a variety of international experiences and I believe UROP will continue to grow and evolve in this area, reaching out to research sectors beyond campus borders.

I believe UROP empowers students to develop tools that will help aid the nation and the world, as we deal with the current energy crisis and beyond. I believe that our students will be on the front lines of the development of new vaccines and the cures for various diseases that have plagued mankind for generations. I hope that UROP experiences will continue to inspire MIT students to serve others, not only here at MIT, but also as they continue on to graduate school and careers.

Thanks to UROP, MIT undergraduates have the unique opportunity to apply the collaborative research skills developed here at the Institute to help solve some of the greatest scientific problems and social issues of our time.

MicroRNAs:

Potent Gene Regulators of Consequence in Immune System Development

By: Andrew T. Shie
Department of Biology

Hematopoiesis, or the formation of blood cells, is one of the most important processes to have evolved in higher organisms. Suitably, this complex process utilizes an equally intricate regimen of genetic regulation to ensure the proper execution of all hematopoietic tasks. One such task is monitoring the development of cells that, when mature, serve as the body's most critical line of defense against foreign pathogens. Abnormal regulation of this hematopoietic process results in classic immune-related disorders such as leukemia or immunodeficiency.

Though a wealth of information exists on pre-transcriptional regulation of immune cell development – an event largely controlled by cytokines and transcription factors [1] – less is known about the corresponding post-transcriptional regulation. However, the discovery of microRNAs (miRNAs) heralded the arrival of such a regulatory mechanism: one that operates post-transcriptionally to inhibit mRNA function, plays a key role in many aspects of development [2], and is naturally abundant in animals [3]. This review focuses on the mechanisms of miRNA-mediated regulation of gene expression and provides diverse examples of individual miRNAs already known to have significant, established roles in controlling development of the mammalian immune system.

MiRNA biogenesis and mechanisms of action

Functional miRNAs are produced from longer primary RNA transcripts, which are processed in a series of maturation steps [4] (Figure 1). Following transcription of the miRNA gene, the resulting primary transcript folds and self-base-pairs to produce a "pri-miR" bearing hairpin structures. These are then processed within the nucleus to give rise to ~60-nucleotide-long pre-miR step-loops; this modification is achieved through the action of a nuclear microprocessor complex composed of the RNase III enzyme Drosha and other associated proteins [5, 6]. The pre-miR is then transported out of the nucleus by Exportin-5 in a process dependent on Ran-GTP (and the Ran cycle, which directs transport of materials in and out of the cell nucleus) [7-9]. Once in the cytoplasm, the pre-miR is further processed into a functional miRNA duplex ~22 nucleotides in length through the combined action of the cytoplasmic RNase III enzyme, Dicer, and its partners [10, 11]. The guide strand of the mature miRNA is selectively loaded into the RNA-induced silencing complex (RISC), which contains the catalytically active Argonaute2 (AGO2) RNase [12]. This loading creates the functional unit that directs post-transcriptional repression of mRNAs, and the complementary "passenger" strand is subsequently degraded [13]. Information regarding processing and loading of the guide strand into the RISC is believed to be contained in the nucleotide sequences flanking the catalytic region of the pri- and pre-miRs [13-15].

Though the aim of gene regulation by miRNAs in mammals is clearly to reduce the production of targeted proteins, the mechanism by which miRNAs prevent protein-coding mRNA from being translated has not been completely resolved. Some of the evidence suggests miRNAs can perform their inhibitory functions by inducing mRNA instability and eventual degradation (Figure 1). For instance, studies utilizing DNA microarrays have shown that, by artificially introducing miRNAs into cells, the amount of mRNAs containing putative miRNA binding sites decreases [16]. While this clarifies the ability of miRNAs to interfere with mRNA stability, the manner by which miRNAs mediate such an effect remains unclear.

Situations have also been discovered in which miRNAs function by inhibiting translation (Figure 1). In particular, miRNAs

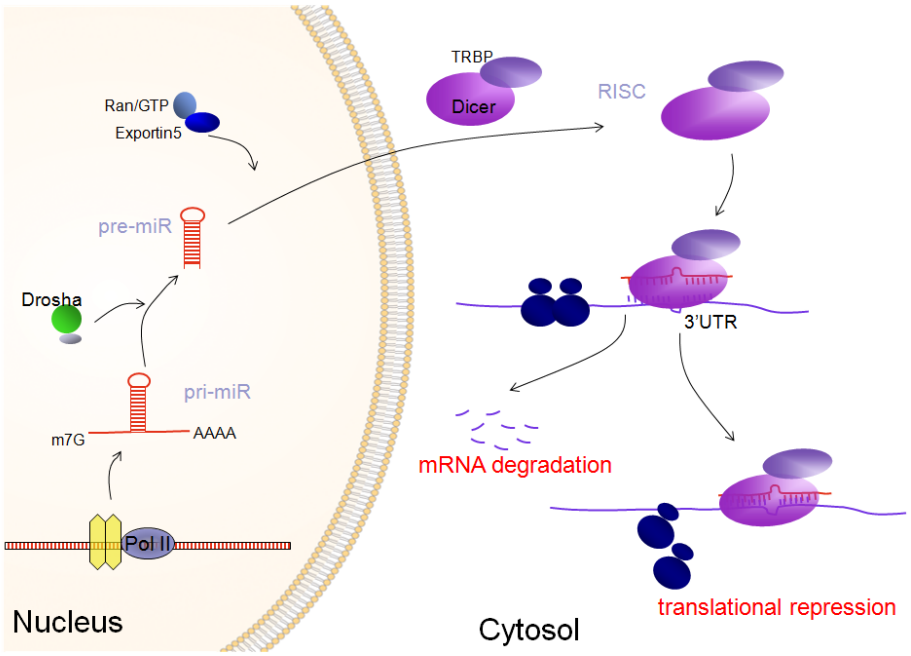


Figure 1. Biogenesis and function of animal microRNAs (miRNAs)

naturally expressed by the cell have been shown to be associated with polysomes, which are clusters of ribosomes that attach along the length of a single mRNA to simultaneously synthesize protein. Based on analyses of polysomal distribution on mRNA, it has been suggested that miRNAs either inhibit movement of ribosomes along the mRNA transcript or prevent the initiation of translation altogether [17, 18]. However, much of the evidence points toward the latter. Specifically, miRNAs have been observed to interfere with the ability of cap-binding proteins to recognize the 7-methylguanosine (m7G) mRNA 5' cap [19]. Further research identified AGO2 as containing a cap-binding-protein-like motif; this suggests that, by binding to the m7G cap itself, AGO2 impedes recognition of the m7G cap by the translation-initiating factor e1F4E [20].

One final issue of extreme relevance is determining how miRNAs target genes. Early research into miRNA/target gene interactions yielded a fundamental principle: miRNAs regulate gene expression by forming base-pair interactions with evolutionarily conserved sites in the 3' untranslated regions (UTRs) of target gene mRNAs [21, 22]. More recently, it has been postulated that miRNA targeting revolves mostly around the perfect, sequential pairing of 7 or 8 "seed nucleotides" in the mRNA with the 5' end of an miRNA [23]. Computational programs adhering to these basic principles have since been developed to aid in the identification of miRNA target genes. These programs have in fact revealed at least two additional principles: 1) that a single miRNA can target multiple genes, and 2) that a single gene may be acted upon by multiple miRNAs [24]. Though these programs have vastly improved target gene prediction, they are not fail-proof, due to the constant emergence of miRNAs with unexpected behaviors. For example, some mRNAs can be repressed via 5' UTRs [25], and situations exist in which perfect pairing of seed nucleotides is not necessary for gene regulation [26]. These exceptions suggest a complexity of miRNA/mRNA interactions that we are only beginning to appreciate.

MiRNAs and hematopoietic lineage development

Dynamic processes such as immune system development require frequent micro-adjustments to gene expression, which post-transcriptional regulatory mechanisms can provide. Since miRNAs occupy a crucial niche in the area of post-transcriptional regulation, it is not surprising that they have already been widely implicated in immune cell differentiation. Studies show that mice with knockouts of Dicer in T cell precursors exhibit severe defects in mature T cell production [27], while targeted deletions of individual miRNA genes result in mice with various immune deficiencies [28, 29]. In many cases, altered levels of particular miRNA expression have been observed in various leukemias and lymphomas [30-32]. Follow-up studies also show that certain leukemias are in fact characterized by distinct signatures of miRNA expression, further suggesting the functional significance of miRNAs in leukemogenesis [31, 32].

A variety of studies have already elucidated several miRNA functions pertinent to immune system development. One of the earliest revelations of miRNA function in vertebrate cells was the discovery of miR-181a's ability to modulate B and T cell differentiation [33]. Another miRNA, miR-155, plays a regulatory role in T cell differentiation and function [28, 29, 34]. Likewise, miR-223 seems to be critical in regulating the development of granulocytes [35, 36]. It is worth noting that these particular miRNAs were investigated because they were observed to be differentially expressed in specific hematopoietic tissues; indeed,

miR-181a, miR-155, and miR-223 have all been shown to be either hematopoietic- or hematopoietic cell lineage-specific [33, 37].

An additional paradigm of particular interest comes from recent studies showing that the lymphocyte-specific miRNA, miR-150, regulates B cell differentiation. When forcibly overexpressed in hematopoietic stem and progenitor cells, miR-150 triggers a specific and severe defect in the production of mature B cells; this was shown to be a result of miR-150 blocking the progression of premature B cells from the pre-B stage to the pro-B stage, an intermediate step in the pathway of B cell development [38]. Additional studies identified the transcription factor Myb as the potential primary target of miR-150 [39]. However, Myb is known to be involved in a multitude of hematopoietic processes, including T cell, myeloid, and erythrocyte development [40-43]. As a result, there is some unlikelihood of Myb downregulation being the sole contributor to the observed phenotype of B cell-specific impaired development. The broad effects of Myb on hematopoiesis have therefore motivated additional research to uncover the genetic targets of miR-150 that are as crucial but more B cell-specific than Myb.

MiR-150 is of extra interest because deletion of miR-150 in mice results in a significant increase in the production of "natural" antibodies (antibodies present in the serum of an individual without known prior exposure to the corresponding antigen). In addition, miR-150 expression was observed to be downregulated immediately following activation of B cells by antigen stimulation [39]. These discoveries indicate that miR-150 (and miRNAs in general) may regulate not only immune system development but also the immune response. Indeed, this area of research is now one of active exploration. In any case, while it is obvious that miRNAs greatly influence immune system development, there remains plenty of room for additional research into characterizing the specific roles they assume.

Connecting the dots, future directions

Homeostasis of the immune system is a finely tuned process; consequently, its extensive regulation by a quantitative gene regulatory mechanism like miRNAs comes as no surprise. As miRNAs act right before the step of protein synthesis, they are well-suited for micromanagement of gene expression [44]. In effect, miRNAs can be seen as an additional layer to the adjustable modulation of protein synthesis already conferred by other regulatory mechanisms, such as chromatin modification and transcriptional control. However, our understanding of miRNA-controlled, post-transcriptional gene regulation is not yet integrated with the established role of pre-transcriptional elements (cytokines, growth factors, transcription factors, etc.) in immune system homeostasis. Determining the interplay between the two will not only serve to bolster existing knowledge but also potentially reveal new notions behind the various facets of immune system regulation.

Finally, since it is known that abnormal miRNA regulation is tightly correlated with leukemia and other hematopoietic disorders, unraveling the functions and mechanisms of miRNAs in hematopoietic-cell lineage differentiation will provide added insight into leukemogenesis. A multitude of miRNAs are observed to have either oncogenic or tumor suppressor effects [45-48] (the latter of which miR-150 is a possible example); for that reason, there is enormous promise for novel therapeutics targeting miRNAs to be developed as a means to modulate aberrant immune system development.

MiRNAs are encoded in the animal genome by specific genes and transcribed primarily by RNA polymerase II into long primary transcripts (pri-miRs); these exhibit characteristic hairpin structures and are modified with both a 7-methylguanosine (m7G) 5' cap and a 3' poly(A) tail. A nuclear "microprocessor" consisting of the RNase III enzyme Drosha and other associated proteins cleave the pri-miR to form stem-loop pre-miRs ~60 nucleotides in length. Pre-miRs are then transported out of the nucleus with the aid of an Exportin-5/Ran-GTP complex. The cytosolic RNase III enzyme Dicer further processes the exported pre-miRs into ~22-nucleotide-long mature miRNA duplexes; the RNA-binding protein TRBP facilitates cleavage by Dicer. The final step of miRNA biogenesis involves the selective loading of the catalytic strand into an RNA-induced silencing complex (RISC). The resulting functional miRNA/RISC unit is then able to repress gene expression by base-pairing to the 3' untranslated regions (3' UTRs) of target mRNAs, leading to either increased mRNA instability/degradation or inhibition of translation.

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Diagnostics and the Developing World:

Vital Technology that Could Help Level the Playing Field of Global Healthcare

By: Sonya Makhni

Twenty years ago, the developed world was confident that the then-recent advancements in health care technology and practices could be successfully shared with the developing world by the year 2000 [6]. In a historic conference headed by the WHO and UNICEF, the project "Health Care for All by 2000" was proposed; its strategies would finally bring equality of health care to all corners of the developing world using new technologies that were both effective and economical [1, 8]. The advent of vaccinations, oral rehydration solutions, antibiotics, pesticides, and water pumps would significantly improve conditions for those in the developing world—at least theoretically [6].

This, however, was not the case at all. Effective vaccinations were rendered useless without the proper means of storing them. Broken water pumps sat idle while residents lacked the means and knowledge to repair them. Sporadic use of pesticides only sustained diseases, such as malaria [6]. As the developing world's struggle for adequate healthcare becomes more evident, the developed world reaches for more novel solutions based on technology [3].

This review will offer a description of a previously held approach, why it ultimately failed, a new focus on different technologies, such as microfluidic diagnostics, and why they offer promise in rectifying the global healthcare situation.

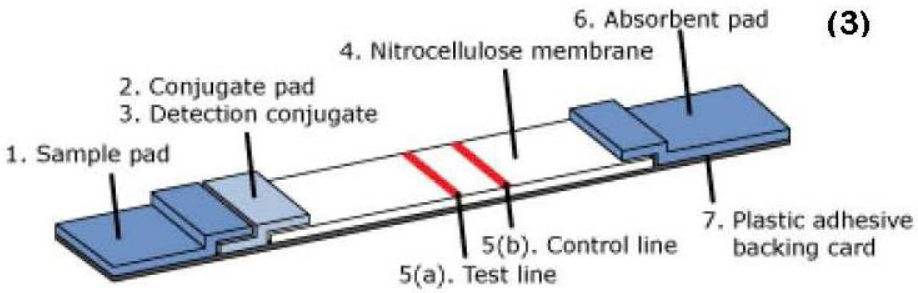
> ***"A new focus on different technologies, such as microfluidic diagnostics, and why they offer promise in rectifying the global healthcare situation."***

Medical Device Distribution: A Failed Approach

One very obvious disparity between the developing and developed worlds is the availability of medical technology. In recent years, as a means to provide these regions with more technology, the developed world has channeled to hospitals thousands of medical devices; in fact, over 95% of all medical equipment in these hospitals comes from outside sources [6]. An issue with donated equipment is that when devices break or need maintenance, as they inevitably do, hospital personnel and physicians are often times unable to make necessary repairs. A study conducted by Engineering World Health (EWH) of medical equipment in 33 developing hospitals revealed that almost 1000 pieces of equipment were labeled as broken by hospitals and were no longer in use [6]. Moreover, 39% of all equipment never functions to begin with [6].

This study shows that hospitals in the developing world have numerous barriers to effectively using donated technologies. First, locals lack spare parts and other consumables necessary for equipment. These must be purchased internationally and hospitals usually cannot afford these products. Second, many locations do not function under reliable power and water sources. [1]. Such a predicament makes storage of vaccinations and patient samples difficult or impossible. Lastly, local personnel do not have the technical expertise to troubleshoot or repair equipment. [1].

It is clear that current technologies are needed in the developing world,



Lateral flow test (left). Lateral flow test strip (right).

but past experience shows that simply providing hospitals with medical devices is not effective. For this reason biomedical engineers have begun focusing on bringing these regions technologies specific to both their resources and needs.

Microfluidic Diagnostics

Focus has since been directed upon designing technologies that provide results similar to devices used in the developed world but do not require as much power and resources to maintain [5]. Microfluidic diagnostics is one such technology. It is designed to replace expensive and lengthy diagnostic procedures but still provide comparable results—only on a single, simple test strip [7]. Infectious diseases, drug use, high cholesterol, and other states can be easily diagnosed without complex equipment or highly trained personnel. The diagnostics require only a small fluid sample and consist of a solid surface that catches analytes (antibodies or antigens). Biomarkers within the slide bind to the analytes, permitting detection by the naked eye. [3] Additionally, the diagnostics deliver test results quickly and clearly [2]. There are several types of diagnostic technologies, one of which is called the Lateral-flow test. [3].

Lateral-Flow Tests: How They Work

Also called immunochromatographic strip (ICS) tests, lateral-flow tests are designed to detect analytes, such as antigens or antibodies, and the diagnostics can test multiple analytes at once. Specimen samples can include blood, urine, saliva, feces, serum, or plasma [3].

Step 1

The sample is first placed on the sample pad, which is composed of cellulose or glass fiber. A signal reagent is used for detection purposes (usually made with gold, colored latex, or fluorescent components) [3].

Step 2

Detector molecules are solubilzed and are able to bind to the analyte that is present in the sample [3].

Step 3

A Nitrocellulose (NC) membrane pulls the fluids away from the sample pad via capillary action. The NC membrane is a useful material composed of a thin Mylar sheet and NC coating [3].

The fluid (containing analyte and marker) continues to move onto the membrane. A capture binding protein immobilizes the liquid, forming a distinct red line signal called the test line. A visible test line indicates a positive test result for the target analyte. Test completion is revealed by a second control line in which excess marker-molecule is trapped. This line should always be visible; the test is invalid if it is not [3].

Step 4

An absorbent wicking pad, composed of cellulose fiber sheets, uses capillary action to maintain flow and prevent backflow into the membrane. Excess fluid and reagents move towards this pad [3].

Benefits

The benefits of diagnostics are numerous. As previously mentioned, results are rapid and easy to read. This is crucial in developing areas, for patients do not often return to clinics to receive test results. Highly trained technical personnel are rare in these areas, so easy-to-read results are necessary [6]. Diagnostics

will undoubtedly work to increase the efficacy of already developed treatments. For example, drugs for several treatable infectious diseases are known, but there does not yet exist an efficient system of identifying affected patients [5]. They will also be useful in controlling infection outbreaks, for widespread treatment requires prompt diagnoses and localization [7]. Additionally, the diagnostics will provide useful data in tracking an individual's treatment progress in a cost-effective and practical manner [7]. Finally, risk of contamination is decreased because the sample is isolated within the diagnostic's compact design; wastes are segregated and equipment components need not be cleaned or maintained as stringently [7].

Barriers

Diagnostics still need to be able to withstand the varying conditions typical of developing countries. Typical lab conditions do not always guarantee a steady supply of running water and electricity, so storage and use of diagnostics cannot be dependent on these factors [7]. They must be lightweight for shipping purposes and able to withstand a wide range of temperatures, ranging from 10-40 °C [7]. In order to minimize shipping weight, diagnostics need to be packaged in a dry form so that fluids need only be added locally [7]. Versatility of function is also important; ideally, the diagnostics would be able to test for a wide range of conditions without needing much more than a simple change in reagents [6]. Finally and most importantly, they must be inexpensive. Cost effective materials must be engineered in order maximize function and minimize costs. [6,7].

MIT's Innovations in International Health Lab

An organization involved in producing such technologies is MIT's Innovations in International Health (IIH) Lab. Dedicated to accelerating global health technologies by empowering local healthcare workers and engineers, IIH is a collaborative effort. It has partners in 12 different countries worldwide, including Nicaragua, India, Ghana, and Ethiopia. Working in conjunction with these researchers, IIH produces medical technologies that can be distributed throughout the developing world.

"Infectious diseases, drug use, high cholesterol, and other states can be easily diagnosed without complex equipment or highly trained personnel."

Of the many projects conducted in IIH labs, one—Medical Education Design Invention Kits (MEDIK)— aims to demystify useful technologies so that local healthcare workers and residents may learn to design and trouble-shoot useful technologies, such as the diagnostics. The ability to create their own technologies is of utmost importance so that they can engineer devices for their own circumstances. Teaching local residents to innovate, design, and develop offers a dependable solution that frees them from dependence upon inaccessible materials and aid. IIH is also developing drug delivery, vital signs, and prosthetic kits (and others) with the same purpose. An ability to create one's own medical devices provides a hopeful step forwards to decreasing the global healthcare gap.

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National Medal of Science Winner Dr. Joanne Stubbe

Abridged Interview

By: Akansh Murthy

Dr. Joanne Stubbe is a Novartis Professor of Biology and Chemistry at the Massachusetts Institute of Technology. She received her Bachelors degree in Chemistry at the University of Pennsylvania and her PhD in Chemistry at the University of California, Berkeley. Her recent awarding of the National Medal of Science prompted me to have a small chat with her.

AM: Both your Bachelor's degree and your PhD were in fields of Chemistry. What first drew you towards Chemistry?

JS: It was high school. I had a fantastic high school teacher. And I loved colors and I loved crystals. In contrast to most everyone else who liked explosions. And I would say that's what drew me to chemistry. I was also fortunate enough to work in biochemistry labs. So I sort of learned what it was like to work in actual laboratories. I worked through my undergraduate years with NSF funded research dealing with chemistry also. I just loved walking around labs and watching things change. Also, back when I was a student, biology was not molecular based at all and I just wasn't interested in it. I was interested in mechanism-detailed chemical transformations and how things worked.

AM: You recently won the National Medal of Science, the nation's top science honor [presented by President Obama], for your work on ribonucleotide reductases, polyester synthases, and natural product DNA cleavers. Could you go into a little detail regarding these terms but still paint an understandable picture for the general audience?

JS: Well, most people think that free radicals are bad right? They believe that free radicals generate highly reactive species inside the body that are uncontrollable and harmfully react with genetic material, leading to mutations in the DNA. However, general textbooks ignore the fact that there are many, many reactions inside our bodies that use the chemical reactivity of free radicals with exquisite specificity to do incredibly important reactions. So, my lab has spent a lot of time trying to understand free radical chemistry and primary metabolic pathways. One of the primary pathways is how one can make the building blocks of DNA. Ribonucleotide reductase, an enzyme, is the only way that one can make deoxy- nucleotides. And if one look at the chemistry of this, it involves very complex free radical chemistry. Free radicals are thus incredibly important in these processes and very underappreciated. Even all of our vitamins (B6) use free radical chemistry.

"Science changes and the way science works changes with time."

AM: Do you feel that discoveries such as those in your work (ribonucleotide reductases, bleomycin, etc.) will be

good cancer fighters compared to the status quo?

JS: Well, I'm all about going against the status quo (laugh). One of the tools we use is mechanism-based inhibition. We design molecules that are chemically really unreactive but they look like the normal substrate for the catalyst. So, they can bind in that active site, where that chemistry takes place. But, we add a modification to the molecules so that they can go through the same first step but then do something else such as inactivation of enzymes. As it turns out, by studying ribonucleotide reductases, we found a lot of mechanism-based inhibitors. A variation of one is currently being used clinically to treat pancreatic cancer. So with mechanism-based inhibition, we can make the molecules modify steps in nucleotide metabolism, inhibit rapidly dividing cells or even induce apoptosis, so as to kill those cells that are metastasizing. Most drugs today don't follow this approach. Current methods just randomly screen without a rational targeting idea behind the model and not surprisingly are having problems.

AM: Has your family had any influence on your work?

JS: My family doesn't understand anything I do (laugh). I'm a black sheep. I think they just thought I was weird until I won the National Medal of Science. Then, they all got really excited, simply because they got to go to the White House. My father was a mathematician and my mother was a teacher. I was always taught that I could do anything I wanted to do. One of my brothers went to MIT and he was a Chemistry major. What I work on is complicated so it's hard to keep them updated. So my family is science-oriented but my work is still something that they can't easily understand so…

"With mechanism-based inhibition, we can make the molecules modify steps in nucleotide metabolism, inhibit rapidly dividing cells or even induce apoptosis, so as to kill those cells that are metastasizing."

AM: What are your research and personal plans for the future?

JS: I plan to retire (laugh).

AM: Short and sweet huh? (laugh).

JS: Yes, I think one needs to know when it's time to head out. I see way too many people hanging around for too long. Science change and the way science works changes with time. What I learned during my PhD has nothing to do with what I do now. That's what I like about academics- learning new stuff. But, one finally comes to a stage where maybe it's time to stop. People are collecting and processing hundreds of pieces of information at once. It's gotten to a point where there's so much information that it can't be sorted in a way that one can think about it readily and I'm not sure I want to keep going in that direction. I like to understand things in detail and I appreciate the big picture of things but I value the specifics. This issue is for the next generation to figure out and I'm not sure I want to be involved in that (laugh).

AM: Fair enough. My last question is regarding your advice for growing and passionate undergraduates who are seeking to go above and beyond, in terms of research?

"I'm all about going against the status quo."

JS: I don't know- for me, it was passion. There are so many exciting things to choose from- I don't know how one can choose. Probably, as undergraduates start doing UROPs, they'll start to get more excited. I think I've been extremely fortunate to be paid to do what I love. I mean- how many people can do that and can't wait to get to work in the morning? I think a lot of students of your age are more focused on immediate results rather than the practical outcome of a project. But that shouldn't be the main idea. Labs, at least for me, are very exciting places and if undergrads at MIT are fortunate enough to get into one, they should take full advantage of the opportunity.

It turns out that Dr. Joanne Stubbe is a very personable and friendly professor who is willing to share some of her glory with the students of Massachusetts Institute of Technology. Not many high level professors have enough time to really connect with undergraduate community. Many thanks to Dr. Stubbe.

MURJ UROP Summaries

PEG-based drug delivery platform for vocal fold regeneration[1]

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The vocal folds, a twin pair of mucus membranes stretched across the larynx, are primarily responsible for human phonation such as speech. In the presence of a tracheal air stream, the vocal folds are stimulated to vibrate up to a 60% strain at frequencies of 75-1000 Hz1. Vocal fold scarring, caused by mechanical stresses and environmental factors, can diminish their pliability and is the cause for most human hoarseness. Consequently, there has been a focus on developing biomaterials that can mimic the viscoelastic properties of the vocal fold and can be directly injected to treat vocal fold disease [2,3].

Our research group has been developing an injectible hydrogel made from UV-crosslinked Polyethylene glycol (PEG) and Polyethylene glycol diacrylate (PEGDA) polymers. After incubation and shearing, gels composed of 70% PEG and 30% PEGDA showed desirable viscoelastic and vibrational properties during mechanical tests and animal studies. However, recently completed animal studies have indicated the possibility of an immune response against PEG gels injected into the vocal folds. Therefore, it was necessary to modify the basic PEG hydrogel into a multi-functional implant capable of improving the biocompatibility and in vivo residence time of the gels. The goal of this current study is to prepare polymeric nanoparticles encapsulating the anti-inflammatory glucocorticoid Dexamethasone and the anti-fibrotic drug Rapamycin, in order to prevent an immune response. [4,5] Such loaded nanoparticles can be incorporated within the hydrogels, which can provide an effective, local, and controlled drug release.

Nanoparticles are made from the biocompatible copolymer poly(lactic-co-glycolic acid) (PLGA), which is adjustably biodegradable depending on the molecular weights of the monomers used6. Using an optimized single emulsion synthesis method, we are able to create drug-loaded nanoparticles approximately 200 nm in size, with an approximate 30% encapsulation efficiency for Rapamycin and 70% encapsulation efficiency for Dexamethasone. Subsequent drug release studies of these nanoparticles indicate that, when incubated in PBS for one week these nanoparticles can release up to 3 µg/mL of Rapamycin and up to 12 µg/mL of Dexamethasone. These concentrations were determined via high-performance liquid chromatography (HPLC). We have also demonstrated that nanoparticles created with higher molecular weight PLGA polymers exhibit slower drug release rates, which can be desirable in order to prolong its therapeutic efficacy in vivo. Thus, these synthesized nanoparticles have the capability of releasing drug into its immediate medium in a time-controlled manner.

Next, these nanoparticles were incorporated into the PEG hydrogels by mixing nanoparticles into the gel precursor solution at a concentration that we demonstrated would not significantly affect the mechanical properties of the gel. To measure the amount of drug released by the nanoparticle-hydrogel system, the drug concentrations of the gels were measured at different times upon dissolution after incubation in PBS. According to preliminary results, each PEG-nanoparticle gel released a maximum of approximately 150 µg of Dexamethasone after 3 days, with 100 µg leaking out of the gel and 50 µg remaining in the gel. From these results, we can conclude that drug-releasing nanoparticles incorporated into our PEG hydrogels are able to be released by the hydrogel system. Overall, these initial results provide evidence that with further modifications and in vivo testing, a PEG-hydrogel platform for controlled release of anti-scarring and anti-inflammatory therapeutics can be created for vocal fold disease, mitigating the potential problems encountered during animal testing.

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Micro-X[1]

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Micro-X is an X-ray telescope slated for launch in January of 2011 as part of NASA's sounding rocket initiative. The purpose of the mission is to observe the Puppis A supernova remnant using an array of transitions edge sensors (TESs), thermometers based on superconductors in their transition region. In conjunction with microcalorimeters, TESs may be used as high energy-resolution photon detectors. TESs are being developed for use on future NASA missions such as IXO. Micro-X will be the first space mission to utilize this technology. It promises to provide valuable insights into the origins of the universe.

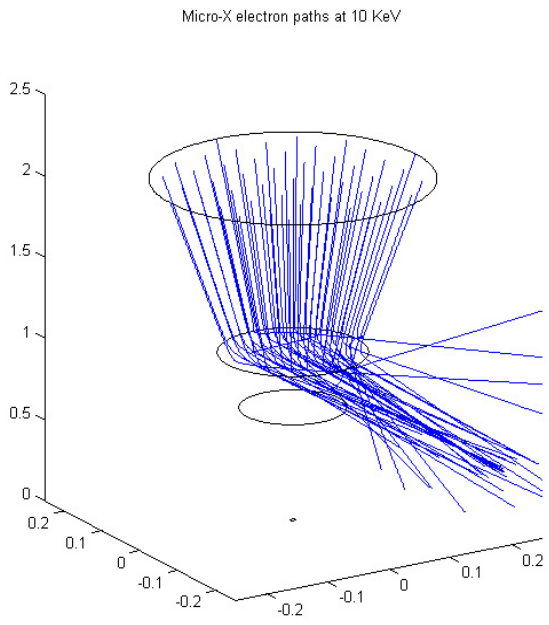
One possible issue facing X-ray telescopes is electron noise. The Chandra X-ray Observatory includes an array of permanent magnets called a "magnetic broom" to eliminate the background noise from incident electrons by deflecting them away from its detector. While electrons should present a less significant problem for Micro-X than for Chandra due to its lower orbit and smaller detection area, we determined from the best data available that it would be worthwhile to add a magnetic broom to the Micro-X design. Our simulation and design work on a magnetic broom for Micro-X was incorporated into the new design for the telescope's optics bench.

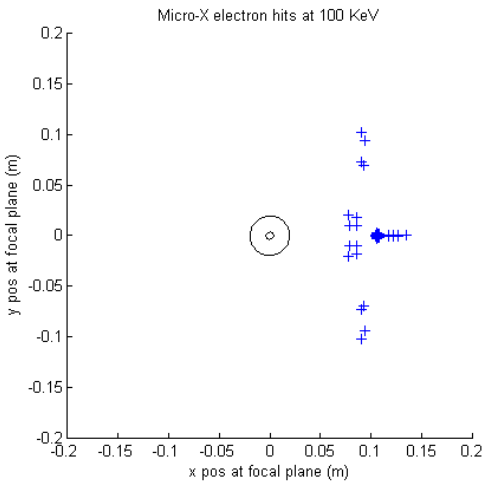
We wrote a MATLAB simulation based on a Runge-Kutta solver to calculate ion paths in magnetic fields. It can generate paths from an arrangement of dipoles and starting positions output plots of paths and ending positions. To ensure that the simulation was accurate, we used it to recreate simulations done by the Chandra team and checked our results against their data, which was available on the Internet.

After plotting field strengths and doing some test plots in the Chandra configuration, we were satisfied that the simulation worked. Chandra used an arrangement of permanent magnets that provided a reasonably constant magnetic field within the aperture, so we scaled the same geometry down for Micro-X. We tested a number of positions and strengths for the magnets and finally settled on one that would allow us to bolt small, commercially available neodymium-iron-boron magnets to an existing aperture on the optics bench.

The final magnet strength was chosen based on availability. The Micro-X magnetic broom uses eight magnets, each with a dipole moment of about 1 A-m^2 (the magnets in Chandra had a dipole strength of 45 A-m^2). The expected strength of the field at the center of the magnetic broom is 3.2 x 10^{-4} Tesla.

We purchased the magnets and assembled the broom. The field strength measured experimentally with a Gauss meter agreed very well with my simulations, so we can be confident that the broom will be effective for Micro-X and that electron noise won't be a significant barrier as we seek to demonstrate and test the capabilities of TES technology.





The diagrams above show some examples of the outputs of the simulator; on the left is a display of paths of 10KeV electrons, while the right depicts where the electrons would hit the focal plane. Both are set for the Micro-X optical bench geometry; the top circle on the right graph represents the optics, and the bottom circle is the filters and detectors on the focal plane. The magnets are arranged around the circle below the optics, about a meter away from the focal plane.

Perceptual sound synthesizer:

A study of auditory perception through language[1]

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A sound can be described by its amplitude (volume) and its frequency (pitch). However, two sounds with the same frequency and amplitude can sound very different because of a third parameter known as timbre which encompasses all characteristics of the sound not associated with its amplitude or frequency. These characteristics include the envelope – an analysis of the sound, particularly its amplitude, in the time domain – and the spectrum – an analysis of the frequencies and harmonics, and of the sound in the frequency domain. Synthesizers can then generate different sounds through algorithms that recreate the ADSR envelope and perform spectral analysis. However, in order to create or modify particular sounds for a composition, a musician will generally have to experiment with numerous technical parameters. This experience can be very unintuitive for novice electronic composers.

Musicians often use common adjectives such as "cool," "smooth," and "dry" to describe sounds. One might hypothesize at first that people of different musical backgrounds or different cultures might use different descriptive words to describe certain sounds. A study was conducted by Sarkar et. al. in order to investigate the relationship between auditory perception and language. A survey was given to 844 individuals of different musical backgrounds. Each subject was allowed to describe various sound samples with given words. Through a statistical analysis of the results, it was determined that musical background and training are weak factors in the description of sounds. Subjects tended to assign certain words to specific sounds regardless of musical or cultural background. These results suggest that a functional relationship exists between words and timbre, and these words correlate with features of the sound in both the time domain and the frequency domain. By creating a generalization of this mapping, one can then create a synthesizer that can create or modify sound inputs through the use of these adjectives – "make the sound sharper," for example – rather than with technical parameters, thereby making the process more intuitive for the user.

This research project deals with analysis of data from previous studies in order to find sound and word correlations. Using the data from the surveys to analyze the profiles of the users, it is possible to build a clear profile of how cultural and musical factors affect the perception of timbre. Once certain sounds are matched to words, features of the sound such as the envelope and spectrum, can be extracted, and these features can then be matched with the words. This data can then be used to create an ordered database of features tagged with the descriptive words, and further correlations can then be used to create the synthesizer engine that processes audio based on verbal descriptors.

The goal of this project is to continue this data analysis so that correlations between the user's background, the descriptors, and the sounds can be refined and analyzed in greater detail. Preliminary results from the first survey showed that people tended to agree on the usage of certain words, like "bright", "resonant", and "full" to describe certain sounds. Subjects also tended to agree on what words not to use to describe the sounds. However, there were also certain words that people tended to disagree on, such as "open," "hard," and "thin." Further analysis of the data as well as subsequent surveys targeted to groups of users of specific demographic categories can reveal more information about word agreements or word disagreements. The second goal is to create a synthesizer that will be able to extract sound data based on descriptive words and easily modify them.

Work towards the second goal was done during the summer of 2009. A framework for the synthesizer was built using Python, SQL, and the sound analysis tool known as VAMP. The two parts of this synthesizer are sound retrieval and sound modification. For sound retrieval, VAMP was used to extract key features from sounds in both the spectral and time domain. These features were used to build feature vectors corresponding to sounds and words to create a space where descriptive words could be mapped to particular regions. This data was stored in a database in order to train the system. A second database contained information about sounds to be retrieved by the system and their corresponding feature vectors also generated by VAMP. The k-Nearest Neighbors algorithm was implemented for this phase. A user can enter words into the system, which will then search through the training data for the words. If the words are not in the training database, the system uses WordNet through the Natural Language Toolkit to recursively search for synonyms and see if those synonyms are in the training data, which is then displayed for the user to select. Once the final words are selected, the corresponding feature vectors are returned and are then compared with the feature vectors in the retrieval database using the k-NN algorithm. The nearest vectors are then selected, and the corresponding sounds are retrieved by the system. Several other features of the system, such as the ability for users to record and upload their own sounds for comparison searches, were also implemented.

This project was continued through the fall of 2009. Feature extraction and analysis was improved and a synthesis engine for sound modification was also implemented. The feature extraction is linked to VAMP, an external program, which is prone to many errors during processing. Work was done to utilize the VAMP functions directly in Python so that the system can run much faster. The synthesizer also had to be able to modify the sound from descriptive words, which requires the application of certain auditory filters to achieve the desired effect. An algorithm was implemented to select the correct filters based on the modifiers that a user inputs into the system. Sound modification is essentially the process of transposing one feature vector to another, and each filter affects each element of a feature vector differently by increasing or decreasing the value. The problem now is essentially to find the right combination of filters that, when cascaded, will transpose the current feature vector into a new feature vector. This is essentially an n-dimensional vector-packing problem, with each filter represented by a vector and the algorithm finding the right combination of vectors that sum to a given vector. This modification system is currently being implemented.

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Giant multishell CdSe quantum dots in quantum dot light emitting diode devices[1]

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Quantum dots (QD), semiconductor nanocrystals that confine an electron and its hole in all three dimensions, possess a unique size dependent property of a tunable band gap. This tunable band gap, which results in the ability to emit any wavelength of light, along with a broadband absorption and a narrowband emission, causes the dots to be desirable material for optoelectronic devices. Current research has shown that quantum dot light emitting diodes (QD-LED), when combined with organic materials, possess a twenty five fold improvement in luminescent efficiency, making these devices novel approaches for a new display technology capable of exhibiting colors far beyond the current color gamut [1]. However, the external quantum efficiency, or the number of photons emitted per electron injected in the device, varies depending on the size of the QDs and the amount of current passed through them, suggesting that the mechanism of operation for these QD-LEDs is not fully understood. One proposed explanation for the phenomenon is Auger recombination, where the energy from an excited electron recombining with a hole is transferred to another electron, which then relaxes to its ground state without emitting a photon [2]. This Auger recombination is common among QDs and leads to luminescent quenching, which in turn decreases the external quantum efficiency.

However, recent studies have discussed the development of a new class of quantum dots that significantly reduce the amount of Auger recombination. These giant nanocrystal quantum dots (G-NQDs), which are synthesized by growing fifteen layers of an inorganic shell with a higher band gap onto the dots, have been reported to possess greater chemical stability after purification techniques and a higher quantum yield, a measure of the intensity of photoluminescence, than regular dots [3]. Both properties are of crucial importance for optoelectronic devices.

In conjunction with the Bulovic Group, our current project involves synthesizing these G-NQDs and inserting them into QD-LED devices. With these devices, we aim to test the Auger recombination hypothesis and to study the effects of dot size on device performance. The results of this study will hopefully lead to greater insight on the device mechanism of QD-LEDs and further the process of developing a new class of display screen technology.

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Independent sets and graph homomorphisms[1]

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Given a graph, consisting of vertices and edges, the term independent set denotes a subset of its vertices with no pair connected by an edge. As an example, Figure 1 shows all seven independent sets of a 4-cycle graph. For a given family of graphs, it is reasonable to wonder which graphs maximize the number of independent sets. This question has important applications within mathematics—for example, in combinatorial number theory—as well as in fields such as statistical mechanics.

Specifically, we are interested in the family of d-regular graphs, which are graphs in which every vertex has d edges attached. These graphs arise naturally from systems with structural regularities, e.g., the interactions between neighboring molecules in a lattice. In 1991, Noga Alon [1] proposed an answer to the question of which graphs maximize the number of independent sets. He conjectured that, among d-regular graphs with the same number of vertices, the number of independent sets is maximized for a disjoint union of complete bipartite graphs. However, no proof was known at the time. In 2001, Jeff Kahn [3] partially resolved Alon's conjecture by limiting to the case when the graph is already bipartite. Last summer, we fully resolved Alon's conjecture [4].

It is known that independent sets form a special case of a more general construction known as graph homomorphisms [2]. There exist generalizations of Alon's conjecture for graph homomorphisms, but they have only been proven in bipartite cases, so the aim is to find a proof that works in general. Unfortunately, our proof for the independent sets problem does not seem to readily extend to graph homomorphisms, so we need to search for other ideas. Our current results include a proof in a special case of the graph homomorphism problem. This special case is interesting because it implies results about lattice points inside the stable set polytope, which generalize my previous results on independent sets.

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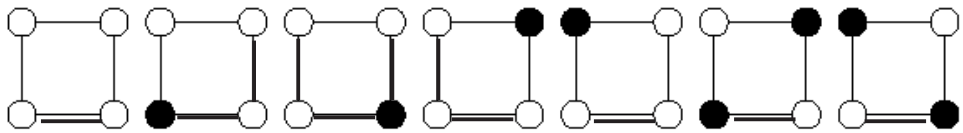

Figure 1: The 4-cycle graph has 7 independent sets

The chemical biology of phosphorothioate modifications of DNA in prokaryotes:

Development of affinity purification methods[1]

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The study of DNA modifications is an important frontier in biological research that has advanced the understanding of many cellular processes. In particular, these types of modifications often play a critical role in DNA replication, transcription, and translation in all organisms. Over the past twenty years, synthetic phosphorothioates (PT), in which a non-bonding oxygen in the DNA phosphate backbone is replaced with sulfur, have generated interest due to their ability to confer nuclease resistance to oligonucleotides bearing the modification [1]. For example, in well-documented forms of gene therapy, antisense DNA strands used as transcriptional inhibitors can be stabilized by PT [2]. In addition to blocking gene expression, oligonucleotides bearing PTs have been found to bind to anti-DNA antibodies, which has implications for the medicinal use of PTs in the diagnosis and treatment of autoimmune diseases [3].

Recently, the Dedon lab discovered that PTs occur naturally as sequence-selective and stereo-specific DNA backbone modifications in bacteria that harbor a five-gene cluster termed the dnd locus [4]. Much remains unknown about the biochemical and evolutionary significance of natural phosphorothioation, however, so new tools are needed to discover, quantify and map PT sites in DNA. By developing tools that can purify PT-containing DNA fragments for subsequent DNA sequencing, we can determine the genomic location of PTs and identify consensus sequences that will then allow us to elucidate the function of naturally occurring PTs. For example, PTs may be found to act as a type of regulatory mechanism if DNA sequencing reveals that specific gene families are preferentially phosphorothioated. Here we focus on the development of two different affinity purification methods to efficiently isolate DNA fragments containing the modification. Affinity purification methods exploit a physicochemical property of the target molecule to selectively bind it for removal from a mixture. Both methods described here utilize the intrinsic nucleophilicity of the PT sulfur to react with electrophilic receptors.

The first method is conceptually based on previous studies that have demonstrated the ability of thiolated DNA oligonucleotides to bind to gold nanoparticles (AuNPs) and assemble complex nanostructures [5,6]. To assess the feasibility of using AuNPs to isolate DNA fragments containing PT modifications, we used a gel mobility shift assay to assess binding of PT-modified oligonucleotides to AuNP. 30-mer oligonucleotides designed with a PT linkage located at the 3' end were incubated with sodium citrate-coated AuNPs. The mixtures were then subjected to the mobility shift assay, in which AuNPs combined with PT-containing DNA exhibited slight band retardation on the gel as compared to AuNPs mixed with PT-free DNA. Preliminary results also indicate that a higher ratio of DNA to AuNPs may help to emphasize the shift.

The second method involves a protocol originally designed for protein purification, in which the sulfur in the amino acid cysteine is alkylated with a biotinylated iodoacetyl-PEG molecule and purified by employing biotin's affinity for streptavidin linked to a solid matrix. We assessed the utility of the iodo-PEG-biotin probe for affinity purification of PT-modified DNA by reacting the probe with PT-containing dinucleotides and oligonucleotides. Analysis by HPLC and mass spectrometry revealed complete reaction of the probe with the PT-containing dinucleotides, with probe release achieved by base-mediated desulfuration. Additionally, a blotting assay used with an avidin-conjugated horseradish peroxidase was performed to confirm selective biotinylation of plasmid DNA containing PTs, demonstrating the feasibility of labeling large PT-containing DNA fragments. Our current work is now centered on isolating this entire probe-PT-DNA complex with a user-friendly column method and evaluating the binding and elution process with 32P radiolabeled DNA.

Overall, both approaches provide insight as to how we may be able to efficiently purify and study PT-modified DNA in bacterial genomes. This will allow for the identification of the sequence context, which can then be used to probe available genome sequences of organisms that have been proven or suspected to utilize PT modifications. PTs have recently been identified in numerous bacterial species and marine organisms, and optimizing affinity purification

methods will lead to the development of rapid genomic screening tools that will better define location as an insight into function.

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Scalability of renewable energy sources[1]

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During the course of this project, the comparative merit of several alternate energy processes on the national and global stage was analyzed. The purpose of this study was to investigate the following five energy sources: geothermal, wind power, solar power, bioenergy, and nuclear power. For each option, we examined the technical parameters of current systems, the amount of power produced by each unit, current usage within the United States and internationally, projected limitations to the system, research and development needed to improve the technology, and the overall scalability of the process. By investigating each alternative energy source in turn, this project aims to comparatively determine the most comprehensive candidate for large-scale construction and use in the United States. After conducting research on the logistics, economic considerations, projected power output, emissions, and existing operational statistics of each prospective energy source, report detailing the results has been compiled.

The three criteria we have defined for a potential large-scale energy system are: renewability of the process, efficiency of the technology (the power produced significantly outweighs the amount required to conduct the process), and scalability of the system (the number of units needed to produce a given amount of power can realistically be implemented). Once these criteria have been satisfied, researchers and policymakers will be able to determine which alternative technology is most appropriate for widespread implementation, and how other options may be integrated into the energy structure of the United States. Additional recommendations and incentives toward public support and involvement in renewable energy were discussed as well. A supplementary goal of this study was to examine means through which researchers, policymakers, and civilians can act together to encourage American energy independence and diversify the national energy portfolio.

After consideration of economic, environmental and governmental factors, this investigation has concluded that geothermal technology is the most scalable of the five surveyed. Effective systems may be implemented across the country with additional research and development. Bioenergy and nuclear power are ranked below geothermal because significant development in a variety of fields (i.e. infrastructure, waste management) is necessary for the technologies to assume a more efficient, cost-effective, and scalable place in the national energy portfolio. Wind power assumes the lowest rating among the five due to the high costs involved with installation of turbines, the necessary construction of transmission lines, and the unwieldy amount of turbine systems that must be installed to have an appreciable effect on American electricity generation.

Aside from proceeding with advanced research, development, and governmental regulation in the geothermal, bioenergy, and nuclear fields, a combined solution based upon more than one renewable technology is most advisable. In such a scenario, natural land structures, chemical characteristics, and regional demand would play an important role in determining which method would be applied to a certain area. Furthermore, this study recommends the drafting of governmental tax incentives, a carbon tax, and/or a cap and trade plan in order to shift energy generation in favor of renewable sources. These options may have the additional effect of heightening public awareness of the benefits of sustainable technology, increasing morale by offering monetary motivations to use it, and restructuring the American energy program with room for expansion throughout the twenty-first century.

Properties of elastic materials and design of flexible MEMS structures[1]

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Microelectromechanical systems (MEMS) are tiny micron-scale machines with diverse applications in sensors, actuators, and integrated systems. Common examples include accelerometers, inkjet printing heads, and lab-on-chip chemical reactors and bio-sensors. They are traditionally fabricated with the same basic processes that are used for transistors in the semiconductor industry, which are made up of successive layers of materials deposited and patterned atop a silicon substrate.

Ultimately, the bulk properties of the device are limited to that of the silicon substrate used. However, there are applications where such a rigid, heavy substrate is undesirable. To circumvent this barrier, we are currently researching new materials, fabrication techniques, and designs for devices requiring greater elasticity, ruggedness, lower cost, and lesser weight. After fabricating several prototypes and running an array of mechanical experiments, we determined that the woven ABS plastic (acrylonitrile butadiene styrene) deposited by rapid-prototyping 3D-printers is actually a viable material for creating MEMS structures.

The cantilever beam is one such structure ubiquitous to MEMS. Essentially, it is a long and thin 'diving board' with one end rigidly attached to a support and the other end free to deflect up and down. If a point mass is placed on the free end, the entire beam functions as a spring, much like a swimmer standing on the edge of the diving board. The entire system can be thought of as a second order spring-mass-dashpot system with a resonant frequency and damping loss. The spring constant of this structure is given by Equation 1.

$$K = \frac{EWH^3}{4L^3}$$

Equation 1: The spring constant is related to the cantilever's length L, thickness H, width W, and material's tensile modulus E. Note the strong dependence on thickness and length. [1]

The performance of many useful MEMS structures depends on the tensile modulus, so an experiment was designed to determine this parameter. A structure with eight cantilevers of varying length and thickness extending outwards from a rigid base was fabricated (Picture 1). The frequency response of each cantilever was obtained by driving the base with sinusoidal vibrations. The damping ratio and tensile modulus (2 gigapascals) were obtained from the resonant frequency and bandwidth of the frequency response. At resonance, the amplitude of the cantilever oscillation was 60 times the drive amplitude. The low dashpot coefficient suggests that this material is well-suited for use in a mechanical amplifier due to low mechanical loss.

Mechanical resonators often find application in energy harvesting devices. These devices convert mechanical vibrations from the ambient environment to electrical power, typically for remote sensor nodes [2]. Because of the favorable mechanical results, we decided to use this material to create an energy harvesting device for the DARPA Hybrid Insect-MEMS project [3]. This project's goal is to embed electronics on-board a live moth so that its flight can be remote-controlled. The energy harvester uses magnetic induction to convert vibrations produced by the moth during flight to electrical power for the on-board electronics [4]. Because the moth must be able to fly with the energy harvester, the device must be lightweight and compact.

The structure shown in Picture 2 is a modified cantilever structure designed to provide a vertical travel path for the proof mass, unlike a regular cantilever. The motion amplification can be seen by comparing the blurred region at the base and at the free end of the beam. The proof mass is composed of a set of magnets which produce electrical power when swung past a coil (not shown). The amplified vibration amplitude increases the magnet velocity, increasing overall power output. The entire system weighs 1 gram, fits into a volume of about 3 milliliters, and is capable of producing 1 milliwatt of power from the moth's narrowband 25 Hz vibrations, which have an amplitude of about 1 millimeter [4]. This amount of power meets the requirements posed by the on-board electronics.

Overall, the 3D-printed ABS plastic allowed us to iterate resonator designs rapidly. The material itself allowed us to create a lightweight, low-frequency, narrowband resonator with a light proof mass. Normally, stiffer materials are used in energy harvest-

ers, so a heavy proof mass is required to bring down the resonant frequency. However, since mass was a limiting design constraint, the use of the supple ABS plastic allowed us to circumvent this constraint. Finally, the low mechanical loss of this material enabled it to greatly amplify the tiny vibrations and improve the power output of the harvester.

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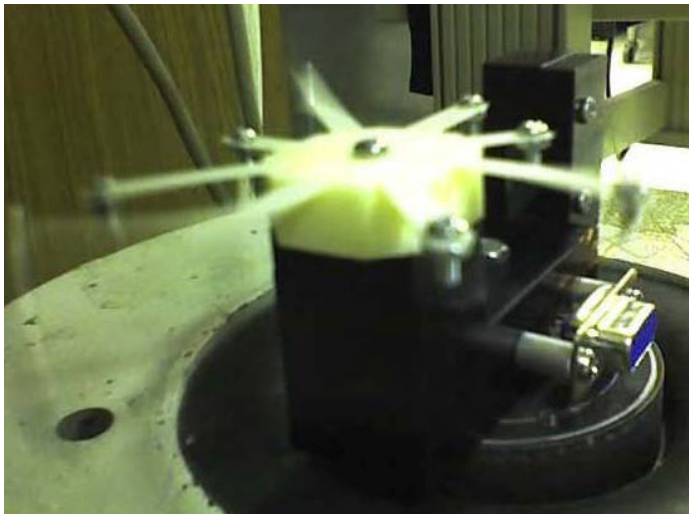

Figure 1. An eight-armed structure was fabricated to extract the material properties of the 3D-printed ABS plastic from the frequency response of the cantilever beams. The picture shows several of the arms resonating in response to the base being driven by vibrations produced by a shaker table.

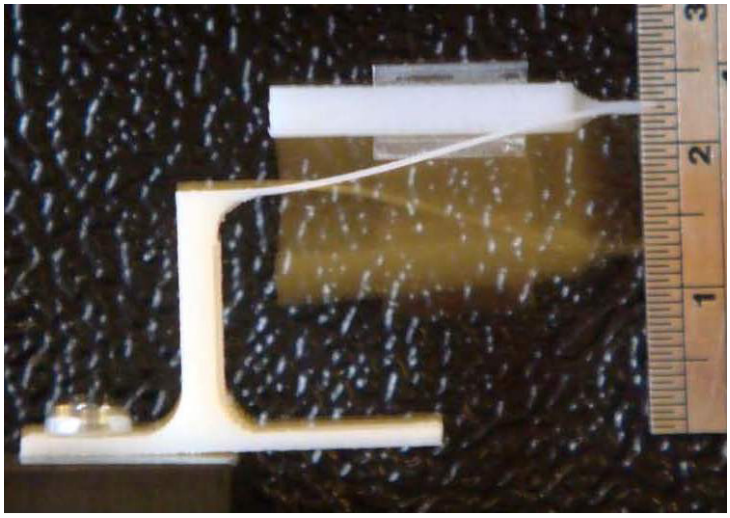

Figure 2. The mechanical resonator made from the ABS plastic is a modified cantilever structure designed to provide vertical motion for the proof-mass. The motion amplification can be seen by comparing the blurred region at the base and at the free end of the beam. The ruler shows centimeters.

Effects of different drugs on the cognitive functioning of schizophrenic patients[1]

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Schizophrenia is a chronic, severe, and debilitating mental disorder characterized by hallucinations, delusions, and other unusual thought content and social behavior. Previous studies done by Dr. Evins and her colleagues in the Center for Addiction Medicine (CAM) at the Massachusetts General Hospital (MGH) showed that a single dose of transdermal nicotine could improve the episodic memory of non-smoking schizophrenics [3]. However, this effect was less strong in the healthy controls. The placebo did not have an effect on the recognition of novel items. Furthermore, nicotine only affected the episodic memory of novel items and not previously-viewed items. In addition to improving episodic memory, nicotine could enhance the reward responsiveness of non-smokers[2]. Other studies done by Dr. Evins' group at CAM showed that the nicotinic acetylcholine receptor was involved in attentional processes and was impaired in schizophrenics [1]. Thus, nicotinic agonists may treat cognitive impairment in schizophrenics.

This past year, we investigated the other effects of transdermal nicotine on schizophrenic patients as well as the effects of nicotinic agonists and antagonists. One study we worked on involved understanding the effect of nicotine on attention in schizophrenics as compared with normal matched controls. Previous studies showed that nicotine improved the episodic memory and the reward responsiveness of schizophrenic patients, but the effect on attention was still unclear.

Another study we worked on sought to understand the effects of a nicotinic agonist and antagonist on cognitive functions in schizophrenics as compared with healthy controls. The nicotinic agonist used in the study was varenicline and the nicotinic antagonist was mecamylamine. Since nicotinic agonists enhanced the action at the nicotinic acetylcholine receptor, the hypothesis was that varenicline would improve the cognitive functioning of schizophrenics. Similarly, mecamylamine should decrease the mental functioning of these patients. Since the nicotinic acetylcholine receptor was not impaired in healthy people, these drugs should not have a major effect on the cognitive functioning of the healthy controls.

Lastly, we looked at the effects of a dietary supplement on the symptoms, cognitive functioning, and smoking cessation of schizophrenics. Sarcosine is an N-methyl derivative of glycine and may help negative and positive symptoms of schizophrenia by increasing the brain glycine concentration and thus increasing NMDA receptor activation. Brain glycine concentration was measured by MR scans at McLean Hospital.

All of the above three studies were randomized and double-blind. With the exception of the sarcosine study, all subjects in the other two studies had to be non-smokers to avoid the confounding effects of nicotine withdrawal and reinstatement.

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MURJ Reports

Novel regulators controlling biofilm formation through the GacS/GacA two-component system in *Pseudomonas aeruginosa*[1]

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The opportunistic pathogen Pseudomonas aeruginosa is the primary cause of death in chronic bacterial lung infections of cystic fibrosis patients. The development of antibiotic resistance in P. aeruginosa is a major cause of the pathogen's morbidity and is strongly correlated with the formation of biofilms. Biofilm formation requires highly coordinated activation and repression of involved genes in response to environmental cues. Two component regulatory systems, comprised of a pair of proteins including a sensor kinase and a response regulator, are one of the main ways P. aeruginosa adapts to diverse environments such as the lung. One such two-component pathway, GacS/GacA, regulates a variety of virulence and stress-responsive genes, and is involved in the formation of biofilms. To identify genes involved in regulation of biofilms, a negative GacS/GacA signaling reporter strain, PAK exoS-lacZ, was screened for overexpression and transposon mutants. Approximately 3,500 overexpression mutants were screened and 36 were selected for up or down regulation of exoS expression, sequenced, analyzed by epistatic analysis, and assayed for biofilm phenotype. In addition, about 1,500 transposon mutants were screened and 15 were selected for up or down-regulation of exoS, sequenced by arbitrary PCR, and assayed for biofilm phenotype. Six genes were identified as potential regulators of the GacS/GacA pathway: lpdV, spoT, panC, recO, rimI and ilvI. Additionally identified were candidate effector molecules, which may play an important role in the regulation of the GacS/GacA pathway and in the regulation of biofilm formation.

Introduction

The formation of bacterial biofilms is strongly correlated with the development of antibiotic resistance and therefore commonly with a pathogen's virulence. Biofilm formation requires highly coordinated activation and repression of involved genes, as well as complex chemical communication among bacterial cells. Two-component regulatory systems, comprised of a pair of proteins that include a sensor kinase and a cognate response regulator, are one of the main ways bacteria adapt to diverse environments. An input signal received by a histidine kinase stimulates autophosphorylation of a conserved histidine residue by ATP. This autophosphorylated kinase donates its phosphoryl group to its cognate response regulator, which readily transfers the group to its conserved aspartic acid residue. Phosphorylation of a response regulator typically activates an effector domain which in turn typically binds to DNA to regulate gene expression [7]. One such two-component pathway, GacS/GacA, regulates a variety of virulence and stress-responsive genes, and is conserved in a wide range of pathogens [6].

In the opportunistic pathogen *Pseudomonas aeruginosa*, GacS/GacA signaling has been shown to promote the expression of biofilms. GacS is a histidine kinase, which when autophosphorylated, initiates a phosphorelay which ultimately results in the activation of its cognate response regulator, GacA (Figure 1). While much about the GacS/GacA two-component signaling pathway is still unknown, recent work has examined the function of small regulatory RNAs, *rsmY* and *rsmZ,* in the regulation of gene expression in response to GacS/GacA signaling [1]. Researchers have shown that the sole output of the GacS/GacA pathway is GacA's direct regulation of transcription of *rsmY* and *rsmZ*. These small regulatory RNAs in turn modulate other genes' expression by inhibiting the RNA binding protein RsmA (or CsrA). RsmA promotes or represses protein expression of hundreds of target mRNAs by affecting their stability. Therefore, inhibition of RsmA activity by *rsmY* and *rsmZ*, reverses these post translational regulatory effects [5]. Of the hundreds of gene expressional changes induced by *rsmY* and *rsmZ* inactivation of RsmA, some are modulated directly through the stability of their precursor mRNAs, while others are modulated by mRNA stability of a transcriptional regulator, resulting in a broadening of gene expressional changes at the level of transcription.

One such gene, *exoS*, encodes a cytotoxin of the type III secretion (T3S) system and is strongly down-regulated in the presence of *rsmY* and *rsmZ* -- likely as a result of the destabilization of an mRNA precursor for an *exoS* transcriptional regulator [1]. In general, high

levels of *exoS* expression are present in an acute infection state and reciprocally, *exoS* expression is strongly inhibited in the chronic biofilm state. Since *exoS* expression dramatically modulates between high and low levels during acute and chronic infection respectively, *exoS* is a superb candidate to be a negative reporter for the RetS/GacS/GacA and *rsmY/rsmZ*/RsmA pathway, and as a negative reporter of the *P. aeruginosa* biofilm state [1].

Although outputs of the GacS/GacA pathway and the pathway's importance to bacterial phenotypes are understood, little is known about the regulation of the GacS/GacA two-component pathway itself. It has been shown that sensor kinase RetS inhibits histidine kinase GacS via formation of inactive heterodimers, thereby preventing phosphorylation of response regulator GacA [4]. Another sensor kinase, LadS, promotes GacS activity although its mechanism is unknown [3]. It is thought that other unknown factors help to regulate GacS activity in response to environmental changes. Similarly, multiple other players likely take part in the phosphorylation of response regulator GacA, in response to activated GacS, perhaps by means of a phosphorelay.

To identify possible novel regulators of the GacS/GacA pathway, we received strains and constructs to use an *exoS-lacZ* reporter gene to visualize the upregulation and downregulation of GacS/GacA signaling and *P. aeruginosa* biofilm phenotype. Suicide vector pBTK30 was used to introduce a mini-transposon into *exoS-lacZ* reporter strain. Mutants which displayed increased or decreased *ExoS* expression were isolated and sequenced through arbitrary PCR. In parallel, we also performed overexpression, using a PA14 genomic library, and isolated genes whose overexpression affects *ExoS* expression. In this way, we isolated a variety of novel GacS/GacA pathway regulators and performed assays to determine the functions of several of these regulators and their possible place in the pathway. These novel regulatory elements may be involved in the regulation of the GacS/GacA pathway and the coordination of biofilm formation.

Materials and Methods

Plasmids, strains and culture conditions

Nearly all strains studied were derived from *P. aeruginosa* strain PAK with the exceptions of PA14 for the creation of a genomic library and *E. Coli* strains used as donors or vector carriers. Strains and plasmids are listed in Table A. Strains were maintained on Luria-Bertani (LB) broth with antibiotics when required (150 µg ml^{-1} carbenicillin, 75 µg ml^{-1} gentamicin, 25 µg ml^{-1} Irgasan). Growth incubations were carried out at 37°C at 300 rpm.

Creation of a PA14 genomic library

PA14 genomic libraries of 2-4 kb and 4-8kb were created in high-copy plasmid pMQ64. PA14 genomic DNA was isolated using Genta® PureGene® Genomic Prep Solutions, then partially digested with restriction enzyme BfuCI. Resulting genomic DNA segments were electrophoresed on a 1% agarose gel and segments of 2-4kb and 4-8kb were excised and gel purified with QIAXII [10]. pMQ64 was purified from *E. coli* + pMQ64 using HiSpeed Plasmid Maxi Kit [10], linearized with restriction enzyme BamHI, and dephosphorylated with Shrimp Alkaline Phosphatase (SAP). Genomic segments were introduced into linearized pMQ64 using Quick Ligation Kit (New England BioLabs, Inc) and ligations were used to transform DH5α -$T1^R$ chemically competent cells. 2-4kb and 4-8kb PA14 genomic libraries were purified from the transformed cells using QIAprep Spin MiniPrep Kit [10].

Overexpression screen

The PA14 genomic libraries were transformed into reporter strain PAK*exoS*::GL3 through a standard *Pseudomonas* electroporation protocol [2]. Mutants exhibiting promotion and inhibition of *exoS* expression were selected through blue white screening on gentamicin plates supplemented with 50 µg ml^{-1} X-gal. Putative mutants were reaffirmed by restreaking for single colonies, onto gentamicin

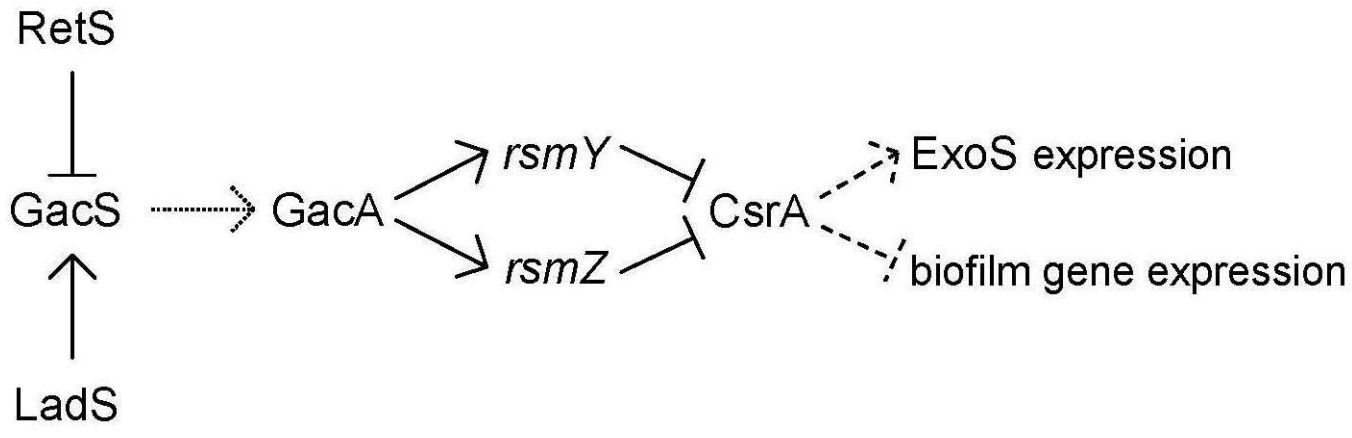

Figure 1. Summary of the GacS/GacAtwo-component signaling pathway reciprocally controlling expression of *exoS* and a variety of genes involved in biofilm formation. Protein kinases, RetS and LadS, repress and activate histidine kinase GacS, which activates response regulator GacA through a phosphorelay, indicated by a dotted. GacA promotes transcription of two small regulatory RNAs *rsmY* and *rsmZ*, which bind to and inhibit CsrA, an RNA-binding protein involved in post-transcriptional regulation of target genes. Active CsrA indirectly, as indicated by dashed arrows, promotes the expression of ExoS and inhibits biofilm gene expression.

Table A. Bacterial strains and plasmids used in this study.

Strains/plasmids	Characteristics	Source and reference
P. aeruginosa		
PAK	wild-type	J. Skerker, Broad Institute
PAK*exoS*::GL3	*PAK with exoS* promoter-*lacZ* fusion insert	S. Lory, Harvard Medical School
PAK Δ*retS exoS*:: GL3	PAK with deletion of *retS* and exoS-lacZ fusion insert	S. Lory, Harvard Medical School
PAK Δ*gacA* exoS::GL3	PAK with deletion of *gacA* and exoS-lacZ fusion insert	S. Lory, Harvard Medical School
PA14	wild-type	D. Newman, MIT
E. Coli		
DH5α-T1^R	F-φ80lacZΔM15 Δ(lacZYA-argF)U169 recA1 endA1 hsdR17(rk-, mk+) phoA supE44 thi-1 gyrA96 relA1 tonA	Invitrogen
SM10 λpir pBTK30	Donor strain for conjugation	S. Lory, Harvard Medical School
Plasmids		
pMQ64	6 kb mobile gentamicin resistance plasmid; M13 cloning site, *lacZ* with P lac promoter	L. Croal, MIT
pBTK30	mini-transposon suicide delivery vector (ori R6K), mariner C9 transposase, *oriT* RK2, *bla* ampicillin resistance	S. Lory, Harvard Medical School

and X-gal supplemented plates, and selecting for the retention of suppressed or enhanced β-Galactosidase activity. Plasmids from these mutants were puri- fied with QIAprep Spin Miniprep Kits [10] and were sequenced by the MIT Biopolymers Laboratory. Sequence analysis was carried out using ncbi BLAST and microbes online.

Epistasis Analysis

Plasmids purified from over expression mutants which exhibited downregulated *exoS* expression were electroporated into PAKΔ*gacA exoS*::GL3. Likewise, plasmids purified from over expression mutants that exhibited upregulated *exoS* expression were electroporated into PAKΔ*retS exoS*::GL3. Retention of loss of mutant phenotype was observed.

Transposon Mutagenesis Screen

The mini-transposon pBTK30 was introduced into PAK*exoS*::GL3 via conjugation. Liquid cultures of donor strain SM10 λpir pBTK30 (in LB/carbenicillin) and PAK*exoS*::GL3 (in LB) were combined (total volume 1 mL) in a 1:1 donor to recipient ratio, using cell density calculations determined by OD_{600} readings. The combined cultures were immediately centrifuged and washed once with 1 mL of LB. Next, cells were resuspended in 1.2 mL LB, spotted in 75µL aliquots onto LB plates, and incubated at 37˚ for 3 hours. Spots were scraped from the plate, and harvested cells were resuspended in 100µL LB and plated in 10µL dilutions onto LB supplemented with gentamicin, Irgasan and 50 µg ml^{-1} X-gal. Mutants exhibiting either up or down regulation of *exoS-lacZ* expression were selected through blue white screening on gentamicin plates supplemented with 50 µg ml^{-1} X-gal. Putative mutants were reaffirmed by restreaking on gentamicin and X-gal supplemented plates for single colonies and selecting for the retention of suppressed or enhanced β-Galactosidase activity.

Arbitrary PCR and Sequencing of Transposon Mutants

Suppressed gene mutants resulting from transposon mutagenesis were amplified using arbitrary primed PCR [9]. The following primers were used: Rnd1-Pa(1) = GGCCACGCGTCGACTAGTACNNNNNNNNNNGATAT; Rnd1-BTK30 = CACCGCTGCGTTCGGTCAAGGTTC; Rnd2-Pa = GGCCACGCGTCGACTAGTAC Rnd2-BTK30 = CGAACCGAACAGGCTTATGTCAATTC; BTK30-Seq = TGGTGCTGACCCCGGATGAAG. Primary PCR reactions (51µL) were set up with 45µL Platinum Supermix (Invitrogen), 1 µL DMSO, 3µL template (prepared by boiling) and 1µL of each primer. Secondary PCR reaction (50µL) were ser up with 42µL Platinum Supermix (Invitrogen), 1µL DMSO, 5µL of the primary PCR and 1µL of each primer. Cycling conditions for primary and secondary reactions were: 1. 95˚C, 5 minutes; 2. 94˚C, 30 seconds; 3. 30˚C, 30 seconds; 4. 72˚C, 60 seconds (return to step #2 5 times); 5. 94˚C, 30 seconds; 6. 45˚C, 30 seconds, 7. 72˚C, 60 seconds (return to step #5 29 times); 8. 72˚C, 5 minutes; 9. 4˚C, ∞

Biolfim Assays

Biofilm assays were carried out on overexpression mutants as well as transposon mutants of interest. In modification of the biofilm ring assay [9], 150µL of LB was inoculated with 3 µL of overnight-grown culture in a well of a 96-well U-bottom Falcon plate (Falcon #3911). Following incubation at 37˚C for 20 hours, media was removed and wells were washed with 200µL of distilled water. Wells were stained for 15 minutes in a solution of .05% crystal violet and then washed thoroughly with distilled water.

Ca++ Phenotypic Dependency Assay

Transposon mutants suspected of displaying a Ca++ dependent phenotype were restruck onto plates of LC media (10g/L tryptone, 5g/L yeast, and 25g/L NaCl) supplemented with $CaCl_2$ in concentration of 0.025M $CaCl_2$, 0.125M $CaCl_2$, or 0.25M $CaCl_2$ and all with 50 µg ml^{-1} X-gal. The mutant's *exoS-lacZ* expression phenotype was

examined on each calcium plate, as compared to a corresponding mutant known to be acting in the GacS/GacA pathway, PAKΔ*retS exoS*::GL3.

Results

28 over-expressed PA14 genomic segments found to alter exoS expression

To identify genes involved in the regulation of *exoS*, we screened ~3,500 colonies of overexpression mutants resulting from the electroporation of PAK*exoS-lacZ* with a PA14 genomic library in the high copy plasmid pMQ64. 34 mutants were identified that displayed varying degrees of increased of decreased *exoS-lacZ* expression, relative to an empty vector control. Figure 2A outlines the process of library creation and overexpression screening while Figure 2B shows examples of up and down regulating mutants and our phenotypic classification system. Overexpression mutants were subject to two rounds of phenotypic reconfirmation, and then sequenced. The 20 distinct genomic segments conferring downregulation of *exoS-lacZ* when over expressed were categorized as follows: 11 conferred strong downregulating phenotypes (W1=white1), 6 conferred moderate downregulating phenotypes (W2), and 3 conferred only slight phenotypes (W3), and were disregarded for further analysis. Upregulation mutants were harder to isolate due to the very slight phenotypic variation between these mutants and wild-type PAK *exoS-lacZ*. Of the 8 distinct genomic segments conferring upregulation in over expression mutants, 2 conferred a strong upregulation (B1=blue1), while 2 conferred a moderate upregulating phenotype (B2), and 4 conferred a slight upregulating phenotype (B3). Those conferring slight phenotypes were disregarded for further analysis. All overexpression mutants were categorized by phenotype and sequenced. Overexpression mutant inserts returning clear sequence and not concatemers (2 found) are reported in Table 1.

Several genes were identified in open reading frames of these overexpressed genomic segments, and are therefore potential regulators of *exoS*. These genes were categorized into those involved in drug resistance, metabolism, two-component signaling, ribosomes, transcription, secretion and DNA damage. One gene which was identified, *mexR*, is involved in drug resistances. The following genes involved in metabolism were found in complete open reading frames: *lpdV, glcC, glcD, pslM, pslL, folK, panB*, and *panC*. Isolated segments containing ribosomal genes *rimJ* and

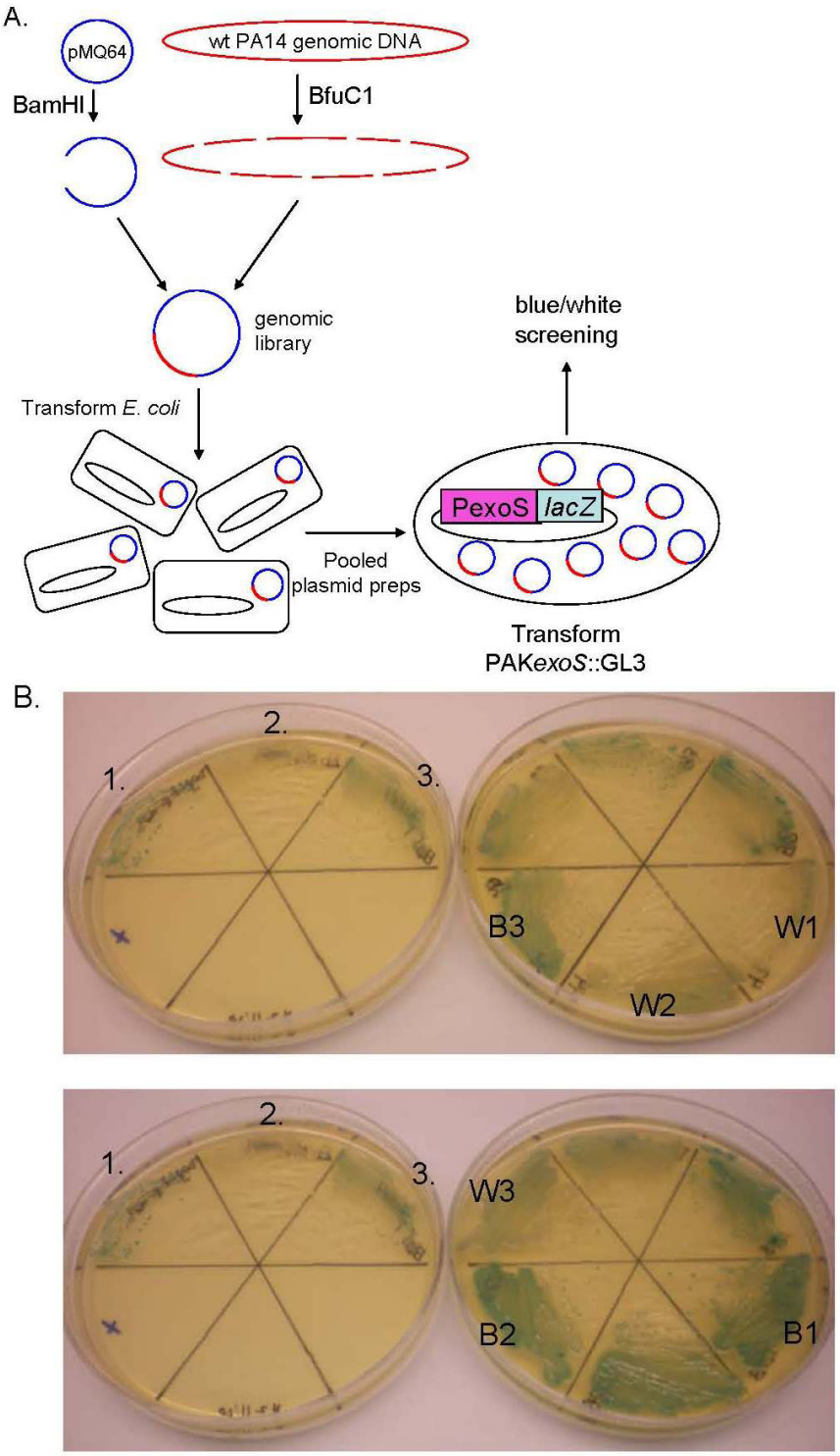

Figure 2. Overexpression mutant construction and categorization. **A.** Diagram overview of mutant construction and screening. **B.** Identification of mutants displaying up or down-regulation of *exoS-lacZ* reporter expression through blue/white screening. Control strains are numbered as follows: 1.*exoS-lacZ*ΔgacA/pMQ64; 2. *exoS-lacZ*/pMQ64; 3. *exoS-lacZΔretS*/pMQ64. W1 is the strongest white phenotype, W2 is moderate and W3 is the weakest. B1 is the strongest blue phenotype, B2 is moderate and B3 is the weakest.

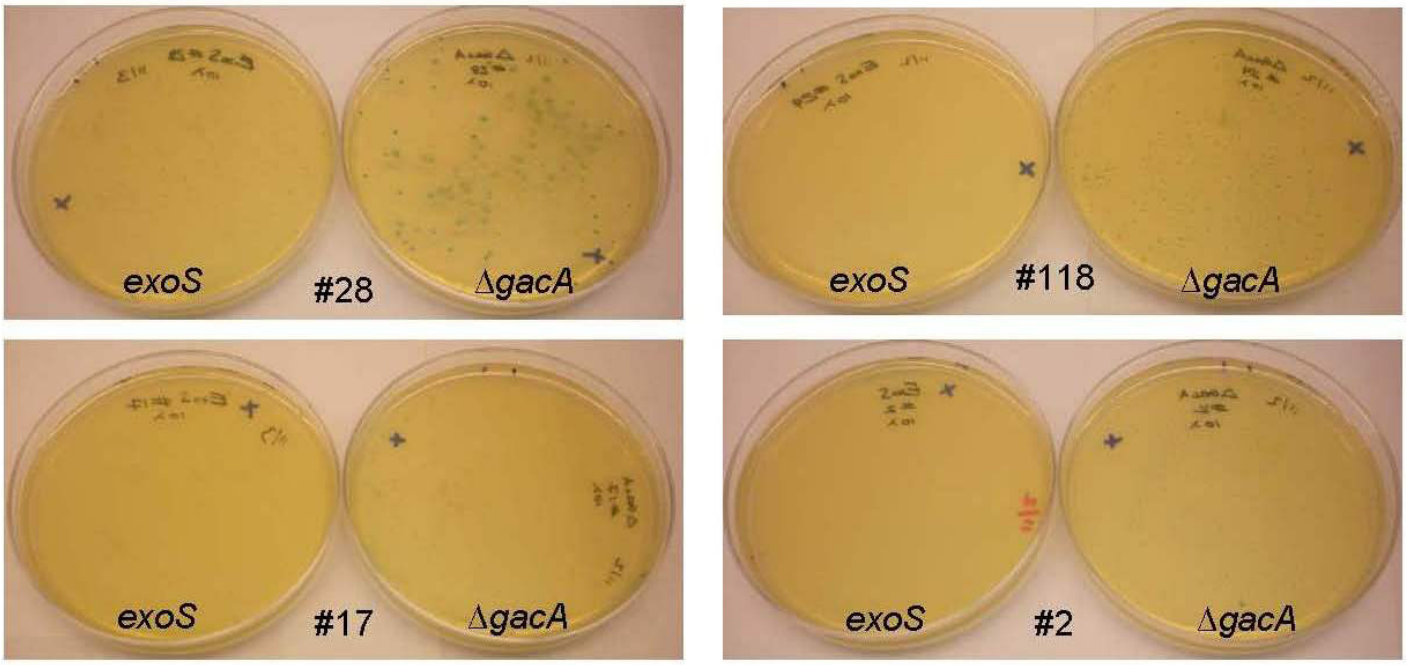

Figure 3. Epistasis analysis compares the level of gacA dependence in four *exoS* down regulating overexpression mutants. The phenotypic difference between in PAK*exoS-lacZ*/pMQ64#28 and PAKΔ*gacAexoS-lacZ*/pMQ64#28 is quite strong, suggesting the mechanism of pMQ64#28 *ExoS* downregulation is dependent upon GacA.

Table 1. Overexpressed genomic segments conferring up or down regulation of *exoS*.

Mutant #	Phenotypic Categorization*	Complete ORFs	Incomplete ORF's	gene of interest	potential function
116	W2	PA14_05520, PA14_05510, PA14_05500, PA14_05480	PA14_05530, PA14_05460	mexR	drug resistance
143,147	W1	PA14_35490	PA14_35500 , PA14_35470	lpdV	amine/metabolic
17	W2	PA14_70710, PA14_70690	PA14_70680, PA14_70710	glcC, glcD	metabolic
73	W3	PA14_35590, PA14_35600	PA14_35620, PA14_35570	pslM, pslL	metabolic
109	W1	PA14_34460, PA14_34490, PA14_34500, PA14_34510	PA14_34450, PA14_34520	COG-TauB, COG-TauA	metabolic
42,47	W1	PA14_62570, PA14_62580, PA14_62590	PA14_62600	folK, panB, panC	metabolic
28	W2	PA14_69470	PA14_69450, PA14_69480	algR	response regulator
2	W1	PA14-39570, PA14_39580	PA14_39590	rimJ	ribosomal
141	W1	PA14_06240, PA14_06250, PA14_06260, PA14_06270, PA14_06280	PA14_06230, PA14_06290	COG-RimI, COG-LysR	ribosomal
44	W1	PA14_26760, PA14_26750	PA14_26770	COG-AcrR	transcription
122	W2	PA14_03090, PA14_03080, PA14_03070, PA14_03050	PA14_03100, PA14_03040	COG-RimI	transcription
84	W1	PA14_30980, PA14_30990, PA14_31000	PA14_31010, PA14_30970	COG-LysR	transcription
145	W1	PA14_21820, PA14_24110, PA14_62510	PA14_21790	tRNA-Val, tRNA-Asp	transcription
24	W3	PA14-73390	PA14_73370, PA14_73400		
29	W2	PA14_47010, PA14_47030	PA14_47040, PA14_47000		
66	W1	PA1411160, PA14_11150	PA14_11170, PA14_11140		
118	W1		PA14_57070		
140	W2	PA14_54290, PA14_54300, PA14_54320, PA14_54330, PA14_54340, PA14_54350	PA14_54270, PA14_54370	pdxJ, recO, era, rnc, lepB	DNA damage
150	W3	PA14_59410, PA14_59430	PA14_59400		
120,142	W1	PA14_65590	PA14_65605, PA14_65580		
58, 59	B1	PA14_66580	PA14_66570, PA14_66600	COG-DamX	secretory
129	B1		PA14_58350		
127	B2	PA14_36080	PA14_36070, PA14_36090		
128	B2	PA14_32810	PA14_32790		
133	B3		PA14_71940	wzt	transporter
126	B3	PA14_55390	PA14_55400, PA14_55380		
134	B3		PA14_00700		
136	B3		PA14_55637		

*Categorization scores are W, white, for down-regulating mutants and B, blue, for up-regulating mutants. Numbering is as follows: 1=very stong phenotype, 2=mediocre phenotpye, 3=lesser, but still distinctly distinguishable from wild type.

rimI were also identified. Two other genes, *recO*, involved in DNA-damage response, and *damX*, involved in secretion, were also present as full open reading frames of these overexpressed segments. A final identified complete gene, *algR*, is the response regulator of the LytR/AlgR two-component signaling pathway. Several other hypothetical proteins and genes were identified as well as several genes present in incomplete open reading frames (Table 1).

Epistasis Analysis of Over-expression Mutants

To determine which overexpressed segments may be involved in biofilm formation, we electroporated plasmids isolated from our over expression mutants into deletion mutants of opposing *exoS-lacZ* phenotypes. In this way, downregulating plasmids were expressed in upregulating deletion mutant Δ*gacA* and upregulating plasmids were expressed in downregulating deletion mutant Δ*retS*. Thus, dependence or independence from the GacS/GacA two-component signaling pathway was established (Figure 3). Phenotype differences between mutant plasmids in wild-type *exoS-lacZ* and in either Δ*gacA exoS-lacZ* or Δ*retS exoS-lacZ* were used to score relative phenotypic dependencies on GacA or RetS (Table 2). 18 downregulating over expression plasmids and 5 upregulating over expression plasmids chosen were chosen for further investigation by epistasesis analysis based upon their phenotypic strength in *exoS-lacZ.* In all cases, the deletion of *retS* dominated over the 5 *exoS-lacZ* upregulating overexpression plasmids. Therefore, our measurement of phenotypic change does not suggest a dependence of the upregulating mutant phenotype on *retS* but instead suggests that GacS/GacA signaling is the most potent way to abolish *ExoS* expression. Of the 18 downregulating mutants, 10 were found have downregulation that was strongly (score ≥4) GacA dependent, suggesting that these mutants exert their effects on *ExoS* expression through the GacS/GacA two component pathway.

15 knocked-down genes found to alter exoS expression

In order to screen for genes whose disruption alters *exoS* expression, we carried out transposon mutagenesis on the *exoS-lacZ* reporter strain. Approximately ~1500 transposon mutants were screened and 15 mutants were identified and categorized by phenotypic strength, and they are listed and summarized in Table 3. 12 mutants displayed decreased *exoS-lacZ* expression and 3 displayed increased *exoS-lacZ* expression. 3 of the 12 mutants found displayed a perfectly white phenotype, suggesting less *exoS-lacZ* expression than our negative control strain, Δ*retS exoS-lacZ*, which displays reduced *ExoS* expression as a result of removing the inhibitor of GacS/GacA signaling. The disrupted gene in two of these particularly white mutants was found to be the *lacZ* of the *exoS-lacZ* reporter fusion, which is not native to PAK, and therefore has no PA number.

In order to identify the disrupted genes of interest, the genomic DNA downstream of the BTK30 transposon insert was amplified in each mutant using arbitrary PCR (Figure 4) and the PCR products were sequenced. Disrupted genes were identified using BLAST (Table 3). Genes which were found to suppress *exoS* when disrupted include *spoT, pvdJ, ggt,* PA14_01150, and *ilvI*. *retS*, which codes for a protein kinase known to inhibit GacS/GacA signaling, was also found to be disrupted in *exoS* suppressing transposon mutant. Transposon disruption of genes *gldG, spuG* and *proA* were found to promote *exoS-lacZ* expression.

Biofilm Assays of Transposon Mutants

Biofilm assays were performed to further characterize the 15 transposon mutants (Figure 7). As expected, results showed a negative correlation between *exoS-lacZ* expression and biofilm formation. Transposon mutants #1, 9, 16, 24, and 25 displayed particularly strong biofilm phenotypes, while mutants #31, 33, and 39 displayed particularly weak biofilm phenotypes. Mutants #2, 5, 6, 11, 13, 15, and 18, which displayed strong *exoS-lacZ* up or down regulation did not display the predicted corresponding biofilm phenotype. This was expected in mutants 5 and 15 because while these mutants display no *exoS-lacZ* expression due to reporter construct disruption, they still display normal expression of *exoS* itself and therefore would be expected to display a wild-type biofilm phenotype.

Variation of transposon mutant exoS expression in response to Calcium levels

As noted above, transposon mutant #2 corresponds to a disruption of the gene *ilvI*, the large subunit of acetolactate synthase. Since lactate chelates calcium and *exoS,* like all T3S genes, is more strongly expressed and is depleted calcium conditions, this raises the question of whether *exoS* expression is down regulated in mutant #2 simply as a result of higher calcium levels. In order to investigate more about transposon mutant #2, we tested the effects of varying levels of calcium on *exoS-lacZ* expression in this mutant and result-

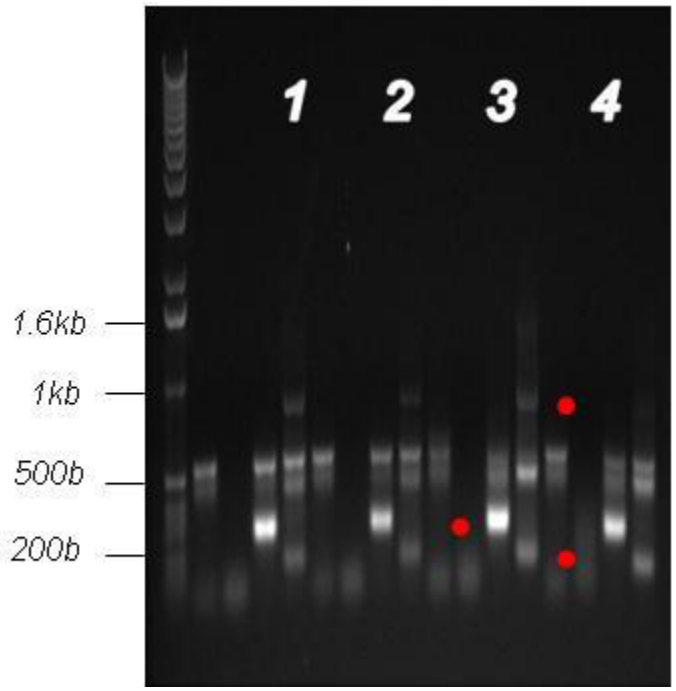

Figure 4. Second round of arbitrary PCR on 1:PAK*exoS-lacZ,* 2: H2O, 3: PAK*exoS-lacZ*/pBTK30 mutant #1 and 4: PAK*exoS-lacZ*/pBTK30 mutant #2. The bands specific to each mutant, marked with red dots were purified and sequenced.

Table II. Epistasis analysis in over-expression mutants

Mutant #	Phenotype in *exoS-lacZ**	Phenotype in Δ*gacA exoS-lacZ**	Phenotype in Δ*retS exoS-lacZ**	Net phenotypic change
129	B1		W1	5
58, 59	B1		W1	5
145	W1	B1		5
127	B2		W1	4
128	B2		W1	4
149	B2		W1	4
2	W1	B2		4
44	W1	B2		4
84	W1	B2		4
109	W1	B2		4
118	W1	B2		4
141	W1	B2		4
42,47	W1	B2		4
28	W2	B1		4
116	W2	B1		4
66	W1	B3		3
143,147	W1	B3		3
122	W2	B2		3
120,142	W1	W3		2
17	W2	B3		2
140	W2	B3		2
29	W2	W2		0

*Phenotypic scores are W, white, for down-regulating mutants and B, blue, for up-regulating mutants. Numbering is as follows: 1=very strong phenotype, 2=mediocre phenotype, 3=lesser, but still distinctly distinguishable from wild type.
**Net phenotypic change was calculated by counting number of possible phenotypic classification between the phenotype induced by overexpression in *exoS-lacZ* and the phenotype in the deletion mutant of opposing phenotype.

ing phenotypes are shown in Figure 8. To test if *exoS* inhibition was calcium-dependent in mutant #2, we grew our *exoS-lacZ* reporter strain and our transposon mutant #2 under varying calcium conditions and observed resulting *exoS-lacZ* expression. We found that transposon mutant two phenotype was highly modulated by calcium levels, but that in the absence of calcium, this ilvI disruption still caused slight decrease in *exoS-lacZ* expression. This suggests the regulation of *exoS* expression by *ilvI* is partially, but not entirely attributable to the alteration of calcium levels due to lack of chelation by acetolactate.

Discussion

Through two screening methods, overexpression library and transposon mutagenesis, using reporter strain PAK *exoS-lacZ*, a variety of genes were identified that alter *ExoS* expression, and therefore may regulate the GacS/GacA two-component signaling and biofilm formation. Through epistasis analysis and biofilm assays, a large number of gene products that either up or down regulate *exoS-lacZ* were pared down into a smaller subset which seem relevant to GacS/GacA regulation. These genes include *panC, rimI, spoT, algR, spuG, pvdJ, spoT,* and *ilvI.*

PanC, isolated in overexpression mutants #42 and #47, is involved in pantoate synthesis. Although it is unclear why overexpressing such a gene may result in decreased expression of *exoS,* we found a potential connection. Using neighbor group analysis on microbesonline.org, we identified *panC* as a gene co-localized in bacterial species closely related to *P. aeruginosa* with *retS*. *RetS* encodes a protein kinase known to form inactive heterodimers with GacS and ultimately prevent activation of the GacS/GacA two-component signaling pathway [1]. Colocalization of genes in bacteria correlates with related function of the genes' products. While the gene order may change, any related functions of the gene products likely will not. The link established through neighbor group analysis between *panC* and *retS* suggests that *panC* may be a regulatory element of the GacS/GacA signaling pathway. The strong GacA dependence score as well as the strong biofilm phenotype of over expression mutant #47 also support this hypothesis (only one duplicate segment was tested, so #42 was not). In future work, subcloning or creating a deletion mutant could discriminate between effects of overexpressing *panC* and overexpressing the other genes present on overexpression segment #42 and #47. Creating double deletion mutants could test for epistasis and quantifying *rsmY* and *rsmZ* levels could verify the regulatory role of *panC* in GacS/GacA signaling.

Another gene of interest, *rimI*, was isolated in two distinct overexpression plasmids #122 and 141 as COG-*rimI*, or the cluster of orthologous groups containing ribosomal-protein-alanine acetyltransferases. This implies that the identified genes are not the originally identified *rimI* gene of another bacterial species, but rather

Table III. Transposon-disrupted genomic segments conferring up or down regulation of *exoS*.

Mutant #	Phenotypic Classification*	ORF	gene name	gene function	Biofilm Phenotype
1	W3	PA14_70470	*spoT*	co-transfer metabolic process	+
2	W3	PA14_62160	*ilvI*	acetolactate synthase	
5	W1+**		*lacZ*	disruption of reporter construct	
6	W3	PA14_01150	COG5435	unknown	
9	W1		*intI 1****	class I integron	+
11	W2	PA14_64230	*retS*	inhibitor or GacS	+
13	W3	PA14_33630	*pvdJ*	nonribosomal peptide synthetase gene	(+)
15	W1+**		*lacZ*	disruption of reporter construct	
16	W1	PA14_46960	*ggt*	gamma-glutamyltranspeptidase precursor	+
18	W3	PA14_70470	*spoT*	co-transfer metabolic process	+
24	W1		*intI 1****	class I integron	+
25	W1+**			sequence undetermined	
31	B2	PA14_11600	*gldG*	possible ABC transporter component, motility	-
33	B2	PA14_03950	*spuG, spuH*	polyamine transfer potein	-
39	B1	PA14_12010	*proA*	probable gamma-glutamyl phosphate reductase	-

*Phenotypic categorization scores are W, white, for down-regulating mutants and B, blue, for up-regulating mutants. Numbering is as follows: 1=very strong phenotype, 2=mediocre phenotype, 3=lesser, but still distinctly distinguishable from wild type.
**W1+ indicates a mutant which displayed no *exoS-lacZ* expression at al
****intI1* is not in the PAK genome. It is an integrase that likely came in on our transposon.
"-" biofilm phenotype indicates a Δ*gacA*-like phenotype, while "+" denotes a Δ*retS*-like phenotype. "(+)" corresponds to a normal biofilm phenotype despite inoculation with less-dense culture. No mark indicates a wild-type biofilm phenotype

they have nearly identical sequence, structure, and likely function to the original gene. *RimI* has been identified by neighbor group analysis to be co-localized with *hfq*, a gene that encodes an RNA chaperone protein responsible for regulating mRNA stability and for small regulatory RNA stability. In application to the GacS/GacA pathway, one small RNA, *rsmY*, which is transcribed in response to phosphorylated response regulator GacA, requires *hfq* for its stability [4]. This potential connection between *rimI* and *hfq* is supported by epistasis results, which suggest that the downregulation of *ExoS* due to *rimI* ortholog overexpression is dependent on GacA, the transcriptional regulator of *rsmY*.

A final gene of interest identified as a complete open reading frame of over-expressed segment #28, is *algR*. This gene is a response regulator of the LytR/AlgR two component signaling pathway. This segment containing *algR*, when overexpressed, displayed a reduced *exoS-lacZ* expression phenotype, which was strongly dependent on the presence of GacA. The biofilm phenotype of this mutant was also particularly strong, further suggesting that activation of the GacS/GacA is occurring. This result also suggests that there may be cross-regulation between the LytR/AlgR two-component pathway and the GacS/GacA two-component pathway. This cross-regulation most likely would occur at the level of transcriptional regulation by *algR*, since the response regulator is the typical signaling branching point of two-component signaling pathways. *algR*, known to be involved in alginate biosynthesis and regulation of mucoidy, would provide an interesting connection between two-component pathways that signal for different cell processes. Alternatively, the connection between AlgR/LytR and GacS/GacA could occur through quorum sensing. AlgR directly represses the Rhl quorum-sensing circuit in a way, which enhances biofilm maturation, and therefore an AlgR overexpression mutant would be expected to have a strong biofilm phenotype [7]. While alginate synthesis and mucoidy have not yet been linked to GacS/GacA signaling, it seems plausible that repression of the Rhl quorum-sensing circuit enhances biofilm maturation in part through GacS/GacA signaling, explaining mutant #28's repression of negative reporter *exoS-lacZ*.

While some genes isolated from over expression screening seem to connect to GacS/GacA signaling, many more genes isolated as disrupted genes in transposon mutagenesis are explainable. The fact that we were able to verify our odd white mutants as disruptions of the *exoS-lacZ* reporter construct verifies our screening process and satisfactorily explains the complete loss of β-galactosidase expression. The disruption of *retS* in transposon mutant #11 also provided a nice verification of our screening process. The weaker phenotype (W2) displayed by this mutant as opposed to the clean deletion phenotype (W1) can likely be contributed to a partial loss of function, as the insertion was fairly late in the *retS* open reading frame.

One gene isolated via transposon disruption was *spuG*, which codes for a component of the spermidine ABC-transport system. Mutation of *spuDEFGH*, the operon which encodes this spermidine uptake system, is known to abolish expression of the *exsCEBA* operon. Disruption of *spuG* must have induced greater expression of the related spermidine gene, which increased T3S expression, thus increasing expression of *ExoS*. Our *spuG* transposon disruption mutant reconfirms this known connection between spermidine uptake and *ExoS* expression, but does not establish much in terms of the regulation of GacS/GacA signaling. The weak biofilm phenotype displayed by this mutant may indicate that entering an acute infection state, and increasing *ExoS* expressed, results in the downregulation of chronic infection genes, and therefore the downregulation of biofilm formation genes.

Another gene, *pvdJ*, involved in pyoverdine synthesis, was found to be disrupted in transposon mutant #13. *pvdJ* is part of the pyoverdine region and codes for a nonribosomal peptide synthetase gene. *pvdJ,* due to its role in pyoverdine synthesis, is likely involved in the sequestration of iron. The availability of iron has been shown to profoundly impact biofilm formation by *P. aeruginosa* [11]. Although little is known specifically about the role of *pvdJ* in biofilm formation, the gene is known to be down-regulated in the late stages of biofilm development. Perhaps its disruption somehow promotes GacS/GacA signaling and as a result, downregulates *ExoS* expression and promotes biofilm formation. For some reason, this transposon mutant did not grow as robustly in culture as the PAK*exoS-lacZ* strain or any of the other transposon mutants. Perhaps *pvdJ* disruption negatively impacts growth. Although its biofilm looked fairly similar to that of PAK*exoS-lacZ*, we believe the ring atttachement present corresponds to less bacteria in the well as the inoculation cultures and the cultures after incubation were visibly different densities. Either way, this *pvdJ* transposon disruption mutant suggests an interesting connection between the regulation of biofilms by pyoverdine through iron sequestration and by GacS/GacA signaling.

Interesting connections are also implicated in the gene *spoT,* twice disrupted in our screen for transposon mutants in mutant #1 and #18. *spoT* is involved in synthesizing ppGpp, which is synthesized in response to high cell-density or starvation in a given environment. Perhaps this mutant does not respond in the typical way to high cell density, and therefore is not as likely to be found in the planktonic state, where you would normally see *exoS* expression. Since *spoT* is known to be involved in cell density dependent cell signaling, it seems a likely candidate as a contributor to the regulation of GacS/GacA signaling and biofilm formation. It also seems logical that a transposon disrupted *spoT* mutant would have an increased biofilm phenotype, as its down regulating *ExoS* phenotype would suggest, because these mutants would not be as inhibited by cell density. As a sidenote, three genes located in overexpression mutant genomic segments, *lpdV, recO* (and others in its segment), and *panC* were all found to be co-localized with *spoT* using near neighbor analysis. This result suggests agreement between overexpression screening and transposon screening and adds support for the relevance of *spoT* in GacS/GacA regulation.

A final gene, *ilvI*, disrupted in transposon mutant #2, codes for the large subunit of acetolactate synthase. The effect on *exoS* expression resulting from disruption of acetolactate synthase function may be related to the reaction of acetolactate synthase and Ca^{2+}. *exoS* expression is calcium dependent, with highest *exoS* expression occurring in depleted calcium conditions. If no acetolactate is made, calcium levels will be higher and result in less *exoS* expression. Growing transposon mutant #2 on calcium supplemented plates showed that the *ExoS* phenotype was highly modu-

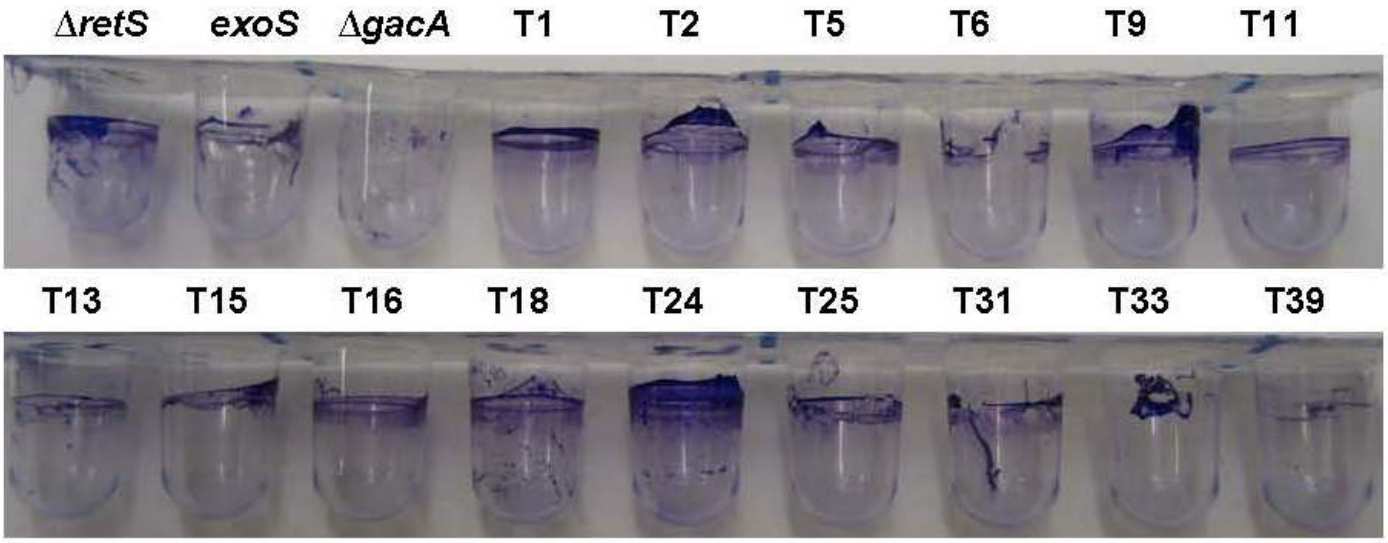

Figure 5. Biofilm phenotypes of transposon mutants. *exoS* represents a wild-type PAK biofilm phenotype, Δ*retS* represents a strong biofilm phenotype, while Δ*gacA* represents a weak biofilm phenotype. T1-T25 are mutants which down regulate *ExoS* expression and T31-39 are mutants which up regulate *ExoS* expression.

A.

Transposon Mutant #2: BTK30::*ilvI*

B.

	(-) Ca^{++}	0.025M Ca^{++}	0.125M Ca^{++}	0.25M Ca^{++}
ilvI (T2)	B3	W3	W1	W1
ExoS	B2	B2	B3	B3
RetS	W1	W1	W1	W1
GacA	B1	B1	B1	B2

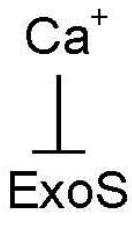

Figure 6. A. The resulting blue/white phenotypes of transposon mutant #2, on plates with varying levels of calcium. **B.** A summary of the blue/white phenotypes seen when control strain were grown on various levels of calcium. *ExoS=exoS-lacZ*; T2=disruption *ilvI; RetS*=deletion *retS; GacA*=deletion

lated by calcium concentration (changed from B3 with no calcium to W1 in 0.0125M Ca^{2+}.) However, its phenotype in the absence of calcium was not equivalent to our *exoS-lacZ* control. It was less blue (W3 instead of W2). Therefore we believe that disruption of *ilvI* may downregulate *exoS* via a net increase in intracellular calcium, however not all of its downregulating effects can be attributed to modulation of calcium levels. Using nearest neighbor analysis, we found that *ilvI* was also found to be co-localized in related bacteria with *hfq*, suggesting another possible connection to GacS/GacA signaling.

In conclusion, several candidate genes identified though over expression screening and transposon mutagenesis may alter expression of our *exoS-lacZ* reporter strain through regulation of the GacS/GacA signaling pathway. In this way, such genes may also be involved in the regulation of biofilm formation, as a result of GacS/GacA signaling. Biofilm formation requires coordinated expression and repression of a multitude of genes, so studying the regulation of pathways like GacS/GacA give us insight into how large scale gene expression changes are coordinated in *Pseudomonas aeruginosa*. This preliminary screen for GacS/GacA regulators identified genes which could be important in understanding how GacS/GacA two-component signaling is regulated and its role in the transition between planktonic and biofilm states.

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A game of switches:

Epigenetic regulation of variegating cell wall genes in yeast

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One of the mechanisms that pathogenic microorganisms use to evade detection by the host is variegation, the process of reversibly turning particular genes on or off, which generates diverse protein expression at the cell surface. Many cell wall genes in Saccharomyces cerevisiae variegate. This cell-surface variation is regulated by binding of trans-acting factors, such as histone acetyltransferases (HATs) and histone deacetylases (HDACs), to histone proteins, which alter their interaction with the gene promoters. Each gene that variegates had been replaced at its normal chromosome site with either a GFP or a URA3 construct driven by its normal promoter to report visually the gene's activity. This study investigated the phenotypic consequences of knocking out each of eleven potential trans-acting factors (HATs and HDACs) on each of eight variegating yeast cell wall genes. The cell wall genes tested here are FIT2, FIT3, AGA1, SAG1, DSE1, DSE2, FIG1, and FIG2. The potential trans-acting factors examined are GCN5, SAS2, SPT10, HOS1, HOS3, HST3, HST4, SET3, SIR2, SIR3, and SIR4. We identified several trans-acting factors, the on and off switches, that directly or indirectly affected the switching of each of the eight variegating cell wall genes. Furthermore, our findings suggest that epigenetic regulation — the mechanism of histone modification — generates cell surface variations in yeast. The ability for cells to exhibit a diverse cell surface expression is a heritable characteristic, which may confer benefits to individual cells within a genetically homogenous population.

Introduction

Variegation, or the heterogeneous gene expression from a genetically identical population, enables organisms to adapt to a harsh, fluctuating environment and to withstand selective pressure. Such variability has been known to sustain fitness through generating diversity in the population [11]. Variegation is prevalent among pathogenic microorganisms, which are often able to survive because of it, in response to the fluctuating host environment. Variegation of cell-surface antigens, in particular, enables cells to evade detection by the host immune system.

A common motif in various microorganisms is often that one family member of the cell surface gene is expressed while the rest are silenced; this motif accounts for the cell-to-cell variations in the population. For example, *Plasmodium falciparum*, a well-known malaria parasite, has a var antigen-encoding gene family: while one var gene is expressed, other members within the same cell serve as a silent reservoir for diversity [18]. In another instance, *Trypanosome brucei,* the causative agent of African sleeping sickness, consecutively expresses different members of the variant surface glycoprotein (VSG) family in a single cell [14]. By reversibly turning particular genes on or off, pathogens generate heterogeneous protein expression at the cell surface, thereby reducing recognition by the host defense, as some individual cells in a population will fail to elicit an immune response. Because many of cell wall genes in *Saccharomyces cerevisiae* are highly conserved yet—interestingly—have been known to variegate, here we study these variegating cell wall genes in order to gain better insight on the cell surface variations in other microorganisms, which could include both pathogenic and non-pathogenic biota.

Both genetic and epigenetic regulations are mechanisms that account for variegating gene expression. Moreover, these regulations play deterministic roles in the activation and silencing of gene activity. Gene mutations and genome rearrangements modify the DNA sequence, generating cell-to-cell variations [2]. For example, a bacterial cell has a 1/1000 chance of carrying a duplicated portion of the genome [1]. Conversely, epigenetic regulation does not involve a sequence change: in this case, a gene variegates owing to interaction between its promoter and any upstream regulatory elements that interact with the promoter, such as the proteins that package the DNA [11]. Therefore, epigenetic regulation is similar to genetic regulation because both are heritable, whereas they differ because only epigenetic regulation is reversible. Epigenetic changes control how organisms generate different types of cells, under circumstances such as embryonic development, cell renewal, and cellular response to environmental stress [3]. In particular, epigenetic regulation—one of many underlying sources of cell heterogeneity—may be accomplished by histone modification, nucleosome remodeling, and DNA methylation [10]. Here, we study the role of histone modification and its impact on cell wall gene transcription.

Table 1. Variegating cell wall genes tested here and their functions.

GENE	FUNCTION*
FIT2	**Mannoprotein incorporated into the cell wall via a glycosylphosphatidylinositol (GPI) anchor, involved in retaining siderophore-iron in the cell wall**
FIT3	**Mannoprotein incorporated into the cell wall via a glycosylphosphatidylinositol (GPI) anchor, involved in retaining siderophore-iron in the cell wall**
AGA1	**Anchorage subunit of a-agglutinin of a-cells**
SAG1	**Alpha-agglutinin of alpha-cells, binds to Aga1p during agglutination**
DSE1	**Daughter cell-specific protein, may participate in pathways regulating cell wall metabolism**
DSE2	**Daughter cell-specific secreted protein similar to glucanases, degrades cell wall from the daughter side causing daughter to separate from mother**
FIG1	**Integral membrane protein required for efficient mating; may participate in or regulate the low affinity Ca2+ influx system, which affects intracellular signaling and cell-cell fusion during mating**
FIG2	**Cell wall adhesin, expressed specifically during mating; may be involved in maintaining cell wall integrity during mating**

***Information listed above was obtained from *Saccharomyces* Genome Database.**

Table 2. Potential *trans* acting factors tested here and their functions

Gene Name	Protein (*Saccharomyces* Genome Database)	Predicted Effect of Gene Deletion on Transcriptional Activity
GCN5	Histone acetyltransferase (HAT)	**Decrease**
SAS2	HAT	**Decrease**
SPT10	Putative HAT	**Decrease**
HOS1	Histone deacetylase (HDAC)	**Increase**
HOS3	HDAC	**Increase**
HST3	HDAC	**Increase**
HST4	HDAC	**Increase**
SET3	HDAC	**Increase**
SIR2	HDAC	**Increase**
SIR3	HDAC	**Increase**
SIR4	**HDAC**	**Increase**

Table 3. FACS data of top trans-acting factor null mutants

Strain Name	% Cells Fluorescent	Fluorescence Intensity	Coefficient of Variation	Observed	Expected	Obs=Exp?
BY4742	0	1.8	39.18	uninducible	uninducible	✓
eft2::GFP	99.02	77.09	33.52	constitutive	constitutive	✓
fit2::GFP	52.06	24.94	221.89	variegating	variegating	✓
fit2::GFP gcn5::KanMX4	99.73	100.11	80.81	++-, increase	decrease	
fit2::GFP hos3::KanMX4	91.8	41.17	167.53	++-, increase	increase	✓
fit2::GFP sir3::KanMX4	10.91	6.16	339.35	--+, decrease	increase	
fit2::GFP sir4::KanMX4	8.7	5.18	329.99	--+, decrease	increase	
fit3::GFP	32.23	11.88	245.64	variegating	variegating	✓
fit3::GFP gcn5::KanMX4	92.94	49.65	90.05	++-, increase	decrease	
fit3::GFP sas2::KanMX4	18.6	5.04	109.04	---, decrease	decrease	✓
fit3::GFP set3::KanMX4	84.01	22.4	96.54	++-, increase	increase	✓
fit3::GFP sir3::KanMX4	19.18	6.17	228.23	--0, decrease	increase	
aga1::GFP	57.67	10.41	79.28	variegating	variegating	✓
aga1::GFP gcn5::KanMX4	0.38	2	59.82	---, decrease	decrease	✓
aga1::GFP hst3::KanMX4	83.91	15.44	64.59	++0, increase	increase	✓
aga1::GFP sir2::KanMX4	0.01	1.89	38.12	---, decrease	increase	
aga1::GFP sir3::KanMX4	0.03	1.96	41.29	---, decrease	increase	
sag1::GFP	98.49	38.04	57.5	variegating	variegating	
sag1::GFP gcn5::KanMX4	0.62	2.06	68.29	--0, decrease	decrease	✓
sag1::GFP sir2::KanMX4	0.01	1.58	43.44	---, decrease	increase	
sag1::GFP sir3::KanMX4	0	1.65	38.22	---, decrease	increase	
sag1::GFP sir4::KanMX4	0	1.61	36.34	---, decrease	increase	
dse1::GFP	99.93	127.33	43.88	variegating	variegating	✓
dse1::GFP set3::KanMX4	99.71	101.58	46.26	0-0, decrease	increase	
dse1::GFP sir2::KanMX4	99.95	186.68	35.97	0+0, increase	increase	✓
dse1::GFP sir3::KanMX4	99.72	181.38	38.53	0+0, increase	increase	✓
dse2::GFP	99.69	69.08	35.84	variegating	variegating	✓
dse2::GFP set3::KanMX4	99.46	53.04	35.56	0-0, decrease	increase	
dse2::GFP sir2::KanMX4	99.91	102.36	43.61	0+0, increase	increase	✓
dse2::GFP sir3::KanMX4	99.78	97.52	37.27	0+0, increase	increase	✓
dse2::GFP sir4::KanMX4	98.76	91.91	38.4	0+0, increase	increase	✓

This table displays the FACS data for the top trans-acting factor null mutants of each variegating cell wall gene promoter shown in Figure 2(b). The negative control was BY4742, which did not exhibit fluorescence; the positive control was *eft2::GFP*, which exhibited constitutive *GFP* fluorescence. The "+," "-," and "0" indicate an increase, decrease, or no change of fluorescence from a 30% cutoff based on the three parameters (% cells fluorescent, fluorescence intensity, and coefficient of variation) shown for the variegating controls (*fit2::GFP*, *fit3::GFP*, *aga1::GFP*, *sag1::GFP*, *dse1::GFP*, and *dse2::GFP*).

Histone deacetylases participate in silencing of the members from the *FLO* gene family in yeast, raising the question of how many other cell wall genes are also controlled by the mechanism of histone modification [8]. The unpublished work by Dr. Sherwin Chan uncovered the number of other cell wall genes that also variegate and may be controlled by the mechanism of histone modification (personal communication).

This study examined two of the many types of histone modification, namely, the ones mediated by acetylation and deacetylation of the N-terminal tail. The trans-acting factors, histone acetyltransferase (HAT) and histone deacetylase (HDAC), post-translationally modify the histones by the addition or removal of acetyl groups (CH_3CO^-) from the N-terminal lysine residues [12, 16]. When a HAT adds a negatively charged acetyl group to the histones, histone-DNA affinity is reduced. The histones bind less tightly to the promoter, thereby activating gene expression. Conversely, when an HDAC removes an acetyl group that has been previously added by a HAT, gene expression is repressed [20].

Moreover, this study investigated the phenotypic consequences on eight different variegating yeast cell wall genes (Table 1), the target genes, by knocking out each of eleven potential trans-acting factors (HATs and HDACs) (Table 2). All variegating genes tested here are non-essential. Each gene had been replaced at its normal chromosome site with either a *GFP* or a *URA3* construct driven by its normal promoter to report visually the gene's activity. Using growth assays, flow cytometry, and fluorescence microscopy, we identified several trans-acting factors, the on and off switches, that affected the switching of each of eight variegating cell wall genes. Our finding suggests that epigenetic regulation, or more specifically, the mechanism of histone modification, generates cell surface variations in yeast. The ability for cells to exhibit a diverse cell surface expression by variegating non-essential genes is a heritable characteristic, which may confer benefits to individual cells within a genetically homogenous population.

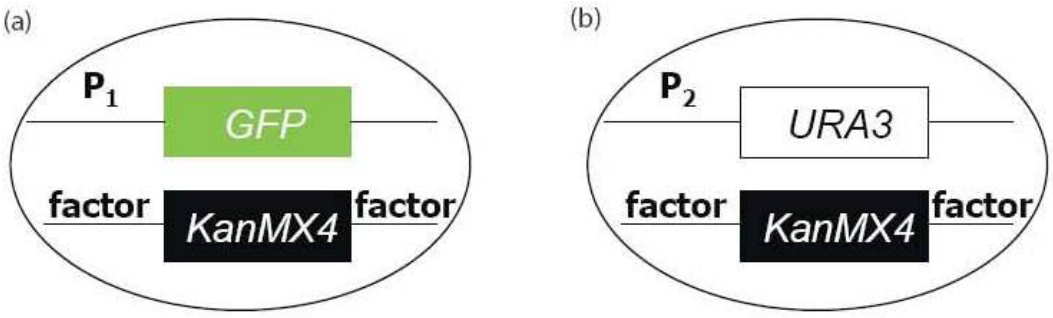

Figure 1. Expression Constructs Used in This Study.
This figure illustrates the expression constructs used to study epigenetic regulation at P1 (*FIT2, FIT3, AGA1, SAG1, DSE1, DSE2*) through *GFP* fluorescence microscopy and P2 (*FIG1, FIG2*) through URA/5-FOA analysis. In both (a) and (b) specific potential transacting factors had been knocked out by fully replacing their ORF, but not their promoters, with a *KanMX4* deletion cassette. Construct (a) was generated by placing *GFP* behind promotor P1; construct (b) was generated by placing *URA3* behind promoter P2.

Materials and Methods

General culture conditions

Saccharomyces cerevisiae strains (see below) were maintained on plates containing YPD [2% Bacto peptone (Oxoid, Cambridge, UK), 1% yeast extract (Oxoid), 2% D-glucose (Sigma-Aldrich, St. Louis, MO)] and 2% (wt/vol) agar (Sigma-Aldrich). When selection was needed, Geneticin [G418 (Sigma-Aldrich)] was added to the media to a final concentration of 300ug/ml; the media were solidified with 2% (wt/vol) agar. 5-FOA plates were poured using a 5-FOA (USBiological, Swampscott, MA) concentration of 1g/L and solidified with 2% (wt/vol) agar. Experimental yeast cultures for flow cytometry or fluorescence microscopy were inoculated from overnight starting cultures grown from single colonies, and cultured to mid-log phase (OD_{600}~2.0) in liquid YPD medium at 30°C, 120 r.p.m. Experimental yeast cultures for URA/5-FOA growth assay were inoculated from overnight starting cultures grown from single colonies, and cultured to mid-log phase (OD_{600}~2.0) in non-selective liquid SC medium [0.15% yeast nitrogen base (Becton Dickinson, Franklin Lakes, NJ), 0.5% ammonium sulfate (J. T. Baker, Deventer, Holland), 0.1% Inositol (Sigma-Aldrich), and 0.2% AA power mix (Sigma-Aldrich)] at 30°C, 120 r.p.m.

Yeast strains and constructions of trans-acting factor null mutants

Variegating genes: The target genes to be tested for variegation had their promoter fused to *GFP* or *URA3* at the normal ATG for each gene. These constructs were integrated into the chromosome at the site of the normal resident gene. The strains and evidence for their variegation were done in collaboration with Dr. Sherwin Chan.

Trans-acting genes: *S. cerevisiae* strains BY4742 (S288C MATα leu2 his3 lys2 ura3) and derivative single deletion mutants were obtained from Euroscarf (Frankfurt, Germany). The deletion library of single deletion mutants purchased contained the *KanMX4* cassette in place of the ORF of each nonessential gene in the parent strain BY4742.

Genomic DNA was first isolated from the trans-acting factor deletion mutants as described in [9]. We used the members of the deletion library (Eurosarf) as the source to PCR the deletion cassettes in which each potential trans-acting factor was replaced by *KanMX4* (Sigma-Aldrich). The BY4742 strain with *GFP* inserted behind the individual variegating promoter (Table 1) was then transformed with each of the deletion cassettes (Table 2), according to the transformation procedures described in [7]. The constructs used in this study are shown in Figure 1. Three separate transformants from each transformation plate were streaked out onto fresh YPD+G418 plates and incubated at 30°C for two days for strain purification.

For selection of the cells in which the *KanMX4* cassette was integrated into the genome, transformants were plated onto YPD+G418 plates. To confirm that the integration occurred at the correct location in the genome, colony PCR was performed. Colonies taken from each transformant were lysed by heating up the samples in the microwave for 1min at full power and directly adding them to the PCR master mix (TaKaRa, Shiga, Japan) with the checking primers [forward primer that annealed to the potential trans-acting factor, reverse primer that annealed to the *KanMX4* cassette]. PCR products were then visualized by agarose gel electrophoresis with a 0.6% gel.

Flow cytometry

Expression data were obtained by using a FACSCalibur flow cytometer (Becton Dickinson) with a 488-nm argon excitation laser and a 515–545-nm emission filter (FL1). Samples of mid-log phase cells (2×10^7 cells) in YPD were washed and resuspended in double-distilled water (ddH_2O). Data were analyzed by using CellQuest Pro OS X software (Becton Dickinson). Cell samples were assayed at a low flow rate until 20,000 cells had been collected within a small forward scatter and side scatter gate to minimize fluorescence variation due to cell size. Correction for autofluorescence was done

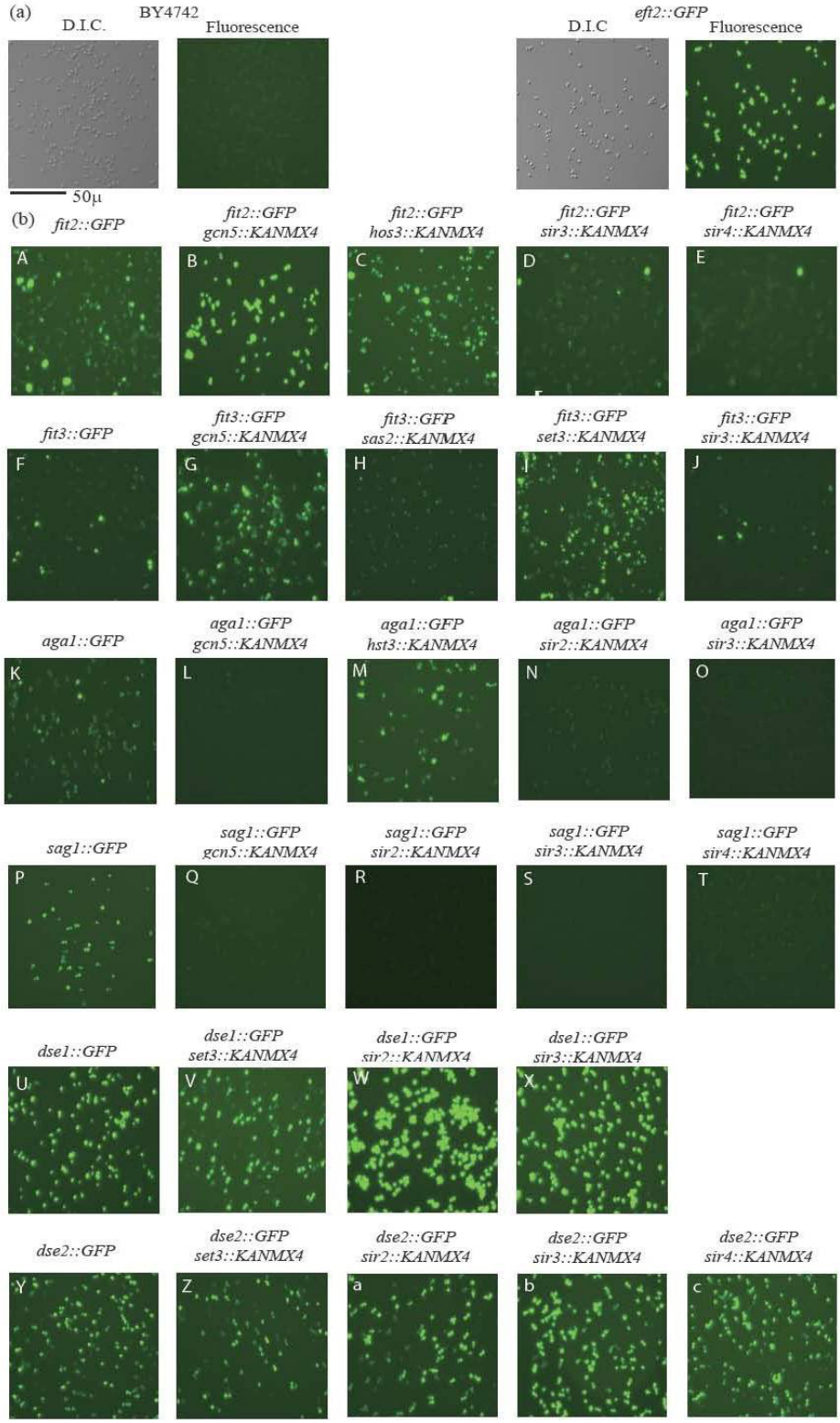

Figure 2. Epigenetic Regulation of *FIT2*, *FIT3*, *AGA1*, *SAG1*, *DSE1*, and *DSE2* by HATs and HDACs in YPD Liquid Medium

(a) Both D.I.C. and *GFP* fluorescent images are shown for the negative control (BY4742) and the positive control (*eft2::GFP*) in YPD liquid medium.
(b) *GFP* fluorescent images are shown for both variegating controls and trans-acting factor null mutants of each variegating gene (*FIT2*, *FIT3*, *AGA1*, *SAG1*, *DSE1*, and *DSE2*) in YPD liquid medium. All strains carried the *GFP*-tagged construct to visualize the cell-surface protein expression on a cell-by-cell basis. Only the images of null mutants with the most pronounced phenotypes for each variegating cell wall gene are shown. Each field has approximately the same number of cells; the number of green spots reflects the fraction of cells expressing *GFP*.

by subtracting the fluorescence value of the parental strain BY4742, which served as the non-GFP tagged negative control, from that of the experimental samples. Cell-to-cell heterogeneity was then determined by the coefficient of variation, CV [(standard deviation)/mean x 100%]. The gate for "on" cells was set based on the autofluorescence of the negative control, BY4742.

Fluorescence microscopy

Samples of mid-log phase cells (2 x 10^7 cells) in YPD were washed with ddH_2O and resuspended in a p-phenylene diamine solution [10mg p-phenylene diamine in 1.5ml ddH_2O and 1ml 10x PBS]. The samples were examined at a magnification of 40x with a Nikon Eclipse 800 fluorescence microscope (Nikon, Tokyo, Japan) fitted with an ORC lamp (Nikon). Images were captured with an Orca ER 12-bit camera (Hamamatsu, Tokyo, Japan). The filter used was a GFP filter cube (Nikon) with an excitation wavelength of 475nm and an emission wavelength of 510nm. The Differential Interference Contrast (D.I.C.) images were taken at an exposure time of 180 ms; the GFP fluorescent images were taken at 100 ms. Images were analyzed with the Openlab software (Improvision, Coventry, UK).

Growth assay in URA/5-FOA medium

Samples of mid-log phase cells (2 x 10^7 cells) in non-selective SC medium were washed and resuspended in ddH_2O, reaching a final OD_{600}~1.0. The suspension was serially diluted ten-fold for four successive times. The resulting series of dilutions ranged from OD~1 to OD~10^{-4}. 10ul of each diluted sample was then spotted onto SC-URA, SC, and SC+5-FOA media. The plates were incubated at 30°C for 3 days.

Results

Cell wall genes in yeast variegate

Many yeast cell wall genes have been found to variegate. In order to demonstrate that the cell wall genes in this study (Table 1), the target genes, variegate, *GFP* fluorescence microscopy was used to visualize the gene's activity in strains having a *GFP* inserted behind the promoter of each target gene (Figure 1, (a)). *GFP* expression was regulated by these target gene promoters. As a negative control, BY4742, the parent strain with no *GFP* gene inserted anywhere in its genome, was analyzed through imaging. No *GFP* fluorescence was detected (Figure 2, (a)). As a positive control, the fluorescence generated by *GFP* expression was analyzed when transcription of the *GFP* gene was controlled by the constitutively active promoter P_{EFT2}. *EFT2* encodes an elongation factor [15]. The expression of *GFP* was homogeneous under the regulation of the *EFT2* promoter (Figure 2, (a)). When strains with variegating gene promoters carrying any of these *GFP* reporters were grown in YPD liquid medium, the clonal population of cells arising from a single haploid cell was not homogeneous. Some cells expressed *GFP*, whereas other cells from the same culture expressed *GFP* at a lower magnitude or failed to express *GFP*, as seen in both flow cytometry (Table 3) and fluorescence imaging (Figure 2, (b), A, F, K, P, U, and Y). Genes *FIT2*,

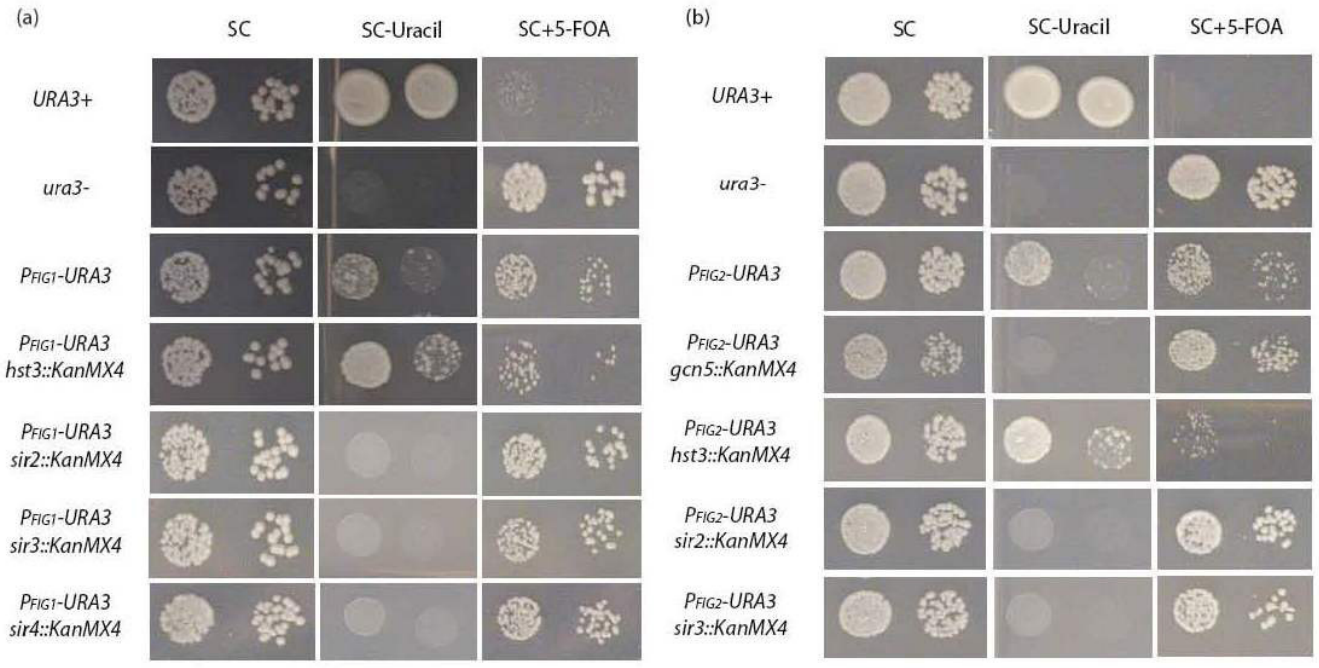

Figure 3. Effect of Trans-acting Factors on Expression of FIG1 and FIG2 promotors

All strains were grown in non-selective SC liquid medium and then plated onto SC, SC-Uracil, and SC+5-FOA solid medium in 1X and 0.1X dilutions. Strains carried *URA3* expressed from the *FIG1* promoter (a) and the *FIG2* promoter (b) in the presence of the *KanMX4* knockout of the trans-acting factor indicated to the left of each panel. The *URA3+* strain used as a positive control for *URA3* expression grew on medium lacking uracil and failed to grow on medium containing 5-FOA, which was toxic only when cells synthesized uracil. The *ura3*-strain used as a negative control grew only on medium containing 5-FOA. Phenotypes of null mutations in each of the eleven trans-acting factors were examined; only the knockouts that had the most pronounced phenotypes are shown.

FIT3, AGA1, SAG1, DSE1, and *DSE2,* but not *FIG1* or *FIG2*, variegated in YPD liquid medium.

To determine whether other cell wall genes, *FIG1* and *FIG2*, variegate in SC medium, the *URA3* cassette was used. Chromosomal *FIG1* and *FIG2* ORFs were replaced with the *URA3* ORF to generate P_{FIG1}-*URA3* and P_{FIG2}-*URA3* alleles (Figure 1, (b)). In cells that carried these constructs, active transcription from the *FIG1* or *FIG2* promoters resulted in the expression of the *URA3* gene and the cells were therefore prototrophic for uracil (Ura^+) and 5-FOA sensitive. 5-FOA was toxic only when cells synthesized uracil. On the other hand, cells in which the *FIG1* or *FIG2* promoters were silenced were auxotrophic for uracil (Ura^-) and 5-FOA^R (Figure 3, (a) and (b)). A clonal population of cells bearing the chromosomal P_{FIG1}-*URA3* or P_{FIG2}-*URA3* allele, when grown initially under non-selective conditions, contained some cells that were Ura^+, 5-FOA sensitive and others that were Ura^-, 5-FOA^R (Figure 3, (a) and (b)). This heterogeneous cell-surface protein expression, like the *GFP* fluorescence analysis of cell wall genes (*FIT2, FIT3, AGA1, SAG1, DSE1*, and *DSE2*), shows that a population derived from a single cell contained some cells that expressed the gene and some that did not.

Variegating cell wall genes are under epigenetic regulation

To identify specific trans-acting factors that affected the *GFP* expression of the target genes *FIT2, FIT3, AGA1, SAG1, DSE1*, and *DSE2* in YPD liquid medium, fluorescent images of the null mutants were examined. Because null mutations in many trans-acting factors led to a dramatic increase in *GFP* expression of the target genes, the wild-type form of these trans-acting factors behaved as strong repressors, or off switches (Figure 2, (b), B, C, G, I, M, W, X, a, b, and c). For instance, there was a significant rise in the *GFP* reporter activity of *fit3::GFP gcn5::KanMX4* (Figure 2, (b), G) compared to the *GFP* expression of the variegating control (Figure 2, (b), F). On the other hand, null mutations in other trans-acting factors led to a dramatic decrease in *GFP* expression of the target genes, so the wild-type form of these trans-acting factors behaved as strong activators, or on switches (Figure 2, (b), D, E, H, J, L, N, O, Q, R, S, T, V, and Z). For example, there was a signficant drop in the *GFP* reporter activity of *fit3::GFP sir3::KanMX4* (Figure 2, (b), J) in contrast to *GFP* expression of the variegating control (Figure 2, (b), F).

To confirm the microscopy results described above, Fluorescent Activated Cell Sorting (FACS) analysis was done on the null mutants (Table 3). All three parameters (% cells fluorescent, fluorescence intensity, and coefficient of variation) obtained from FACS were used to examine the effect of the null mutations on *GFP* expression of the target genes. An arbitrary cutoff of 30% change from the variegating control was used: an increase of 30% or more from the variegating control indicated an increase in number, intensity, or both parameters, of *GFP*-expressing cells, whereas a decrease from the control indicated the opposite. Furthermore, the null mutant that generated such an increase had the wild-type function of a repressor. The null mutant that created such a decrease had the wild-type function of an activator. FACS data (Table 3) obtained were consistent with the *GFP* fluorescence microscopy images of their corresponding null mutants (Figure 2).

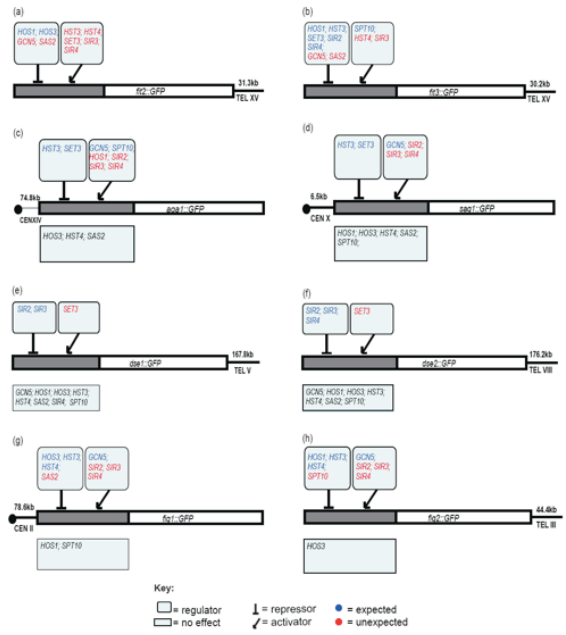

Figure 4. Summary of the Effect of Trans-acting Factors on Variegating Genes *FIT2, FIT3, AGA1, SAG1, DSE1, DSE2, FIG1*, **and** *FIG2*

Epigenetic regulation of variegating promotors by potential trans-acting factors was determined by the data from microscopy, FACS and URA3-FOA assays. These potential trans-acting factors are categorized into three groups: activators, repressors, and ones that had no effect on the promoter. The chromosomal location of each reporter construct with respect to the centromere or tellomere is shown.

As described earlier, *FIG1* and *FIG2* could not be tested for variegation in YPD using the *GFP* construct. Therefore, to identify specific trans-acting factors that affected the expression of the target genes *FIG1* and *FIG2*, constructs with *FIG1* and *FIG2* promoters fused to *URA3* were used (Figure 1, (b)). The *URA3+* strain, the positive control for the *URA3* expression, grew on medium lacking uracil and failed to grow on medium containing 5-FOA. The *ura3-* strain, the negative control for the *URA3* expression, grew only on medium containing 5-FOA. Both P_{FIG1}-*URA3* and P_{FIG2}-*URA3* expression constructs served as variegating controls, revealing both the repressed (Ura-, 5-FOA resistant) and the activated (Ura+, 5-FOA sensitive) states of the target promoters. For the *FIG1* and *FIG2* mutant strains, many trans-acting factors acted as repressors, the off switches, while others behaved as activators, the on switches (Figure 3).

The strongest example of a trans-acting factor mutant from the *FIG1* mutant strains is P_{FIG1}-*URA3 hst3::KanMX4* (Figure 3, (a)). Null mutation in trans-acting factor *HST3* led to an increase in *URA3* expression of the *FIG1* mutant, so the wild-type *HST3* behaved as a repressor, or off switch, for target gene *FIG1*. When compared to the *FIG1* variegating control, P_{FIG1}-*URA3 hst3::KanMX4* grew better on medium lacking uracil and grew less robustly on medium containing 5-FOA (Figure 3, (a)).

The strongest example of a trans-acting factor mutant from the *FIG2* mutant strains is P_{FIG2}-*URA3 gcn5::KanMX4* (Figure 3, (b)). Null mutation in trans-acting factor *GCN5* led to a decrease in *URA3* expression of the *FIG2* mutant, so the wild-type *GCN5* acted as an

activator, or on switch. When compared to the *FIG2* variegating control, P_{FIG2}-*URA3 gcn5::KanMX4* grew less robustly on medium lacking uracil and grew better on medium containing 5-FOA (Figure 3, (b)).

For the ease of visualization, Figure 4 summarizes the data for the total of eighty-eight mutants constructed. The arrows do not necessarily indicate a direct interaction between the trans-acting factors and the promoters, but rather, the knockout of that gene resulted in silencing of the target gene. Similarly, the bars do not suggest a direct silencing by the indicated trans-acting factors, instead, the knockout of that gene led to activation of the target gene (see discussion).

Discussion

This study has demonstrated that promoters of target genes *FIT2, FIT3, AGA1, SAG1, DSE1, DSE2, FIG1,* and *FIG2* are under epigenetic regulation by the trans-acting, chromatin-binding factors tested (summarized in Figure 4). Many of the target genes responding to these trans-acting factors, the on and off switches, behaved as reported in the literature, whereas others behaved differently.

The best example of the mutants that responded as expected to the null mutations in trans-acting factors was *dse1::GFP sir2::KanMX4* (Figure 2, (b), W). *SIR2*, an HDAC, has been demonstrated to act as a silencing factor [4, 19]. Therefore, a higher *GFP* expression than the variegating control was expected in *sir2* null mutants. The *dse1::GFP sir2::KanMX4* strain showed an increase in *GFP* expression; this result was consistent with the reported function of the Sir2 protein (Figure 2, (b), W and Figure 4, (e)).

The best example of the mutants that did not respond as expected to the null mutations in trans-acting factors was fit3::GFP *gcn5::KanMX4. GCN5*, a HAT, has been identified as a transcriptional activator [5, 13]. Therefore, a lower *GFP* expression than the variegating control was expected in *gcn5* null mutants. However, the *fit3::GFP gcn5::KanMX4* strain showed an increase in *GFP* expression; the result was inconsistent with the reported function of the Gcn5 protein (Figure 2, (b), G and Figure 4, (b)).

These inconsistencies were not uniform: a single promoter behaved as expected with some trans-acting factors but not others. For example, the promoter of target gene *FIT3* behaved as expected with *SET3* (Figure 2, (b), I and Figure 4, (b)), but not as expected with *GCN5, SAS2*, and *SIR3* (Figure 2, (b), G, H, J and Figure 4, (b)). In addition, the promoter of target gene *AGA1* behaved as expected with *GCN5* and *HST3* (Figure 2, (b), L, M and Figure 4, (c)), but not as expected with *SIR2* and *SIR3* (Figure 2, (b), N, O and Figure 4, (c)).

However, it is important to note that there were some parallel consistencies as well as parallel inconsistencies among different promoters in response to a particular set of trans-acting factors. For instance, *DSE1* and *DSE2* are homologous genes involved in cell separation during cell division [6]. These promoters shared a group of repressors (*SIR2* and *SIR3*) as well as an activator, *SET3* (Figure 4, (e) and (f)). Both promoters behaved as expected with *SIR2* and *SIR3*; in the meantime, they both did not behave as expected with *SET3*. For another example, *FIT2* and *FIT3* belong to the same family, which controls the retention of siderophore-iron in the cell wall [17]. These promoters shared a group of repressors (*GCN5, HOS1*, and *SAS2*) as well as a group of activators (*HST4* and *SIR3*) (Figure 4, (a) and (b)). Both promoters behaved as expected with *HOS1* while not as expected with *GCN5, SAS2, HST4*, and *SIR3*. Other homologous pairs in this study, *AGA1* and *SAG1* (Figure 4, (c) and (d)) and *FIG1* and *FIG2* (Figure 4, (g) and (h)) also exhibited the pattern of parallel consistencies and inconsistencies.

The inconsistency between these results and those previously reported in the literature could be explained if the trans-acting factors did not directly interact with the target gene being tested. The control of transcription could be mediated not only by factors that directly bind at upstream promoter elements but also by other factors that influence the binding of these factors to the promoter region [11]. For example, *GFP* reporter activity decreased in null mutant *fit3::GFP gcn5::KanMX4* (Figure 2, (b), G), which was not as expected. Because *GCN5* encodes a HAT, an activator, its null mutant was expected to have reduced *GFP* reporter activity when compared to the variegating control. However, the null mutation caused a dramatic increase instead. This inconsistency suggests that HATs such as *GCN5* regulated *FIT3* promoter activity indirectly, either through a single gene or a series of genes in a pathway, where they served as net repressors (Figure 5, (c)).

The same possibility exists for the HDACs: the trans-acting factors that did not behave as expected interacted with the target promoters indirectly, where the HDACs behaved as net activators (Figure 5, (f)). Other examples of inconsistencies are shown in Figure 2, (b), B, D, E, H, J, N, O, R, S, T, V and Z.

For the results that were consistent with those previously reported in the literature, there was still insufficient evidence to suggest a direct interaction between the trans-acting factors and the target promoters. For instance, comparing to the variegating control (Figure 2, (b), F), *GFP* reporter activity increased in null mutant *fit3::GFP set3::KanMX4* (Figure 2, (b), I) as expected. Because trans-acting factor *SET3* codes for an HDAC, which has the wild-type function of a repressor, *GFP* reporter activity in the *set3* null mutant was expected to increase. Therefore, the *GFP* fluorescence of the null mutant was consistent with what we expected.

Such consistency seems to imply a direct interaction between the trans-acting factors and the target promoters. However, the same null mutant phenotype could be explained by both models: one that suggests a direct interaction where the HDAC was a direct repressor (Figure 5, (d)), and the other that suggests an indirect interaction where the HDAC acted as a net repressor (Figure 5, (e)). The same possibility exists for HATs: the consistencies could be explained by pathways where HATs either acted as direct activators (Figure 5, (a)) or served as net activators (Figure 5, (b)). Many other examples are shown in Figure 2, (b), C, L, M, Q, W, X, a, b, and c.

Despite the consistencies and inconsistencies described above, the true interactions between the trans-acting factors and variegating promoters remain unclear. Future experiments such as Chromatin Immuno Precipitation (ChIP) would help determine the direct binding between the modified histone protein and the target promoter *in vivo*. The first candidates to be tested would be the null mutants that exhibited the expected effect on the target gene expression and thus are mostly likely to have direct protein-DNA

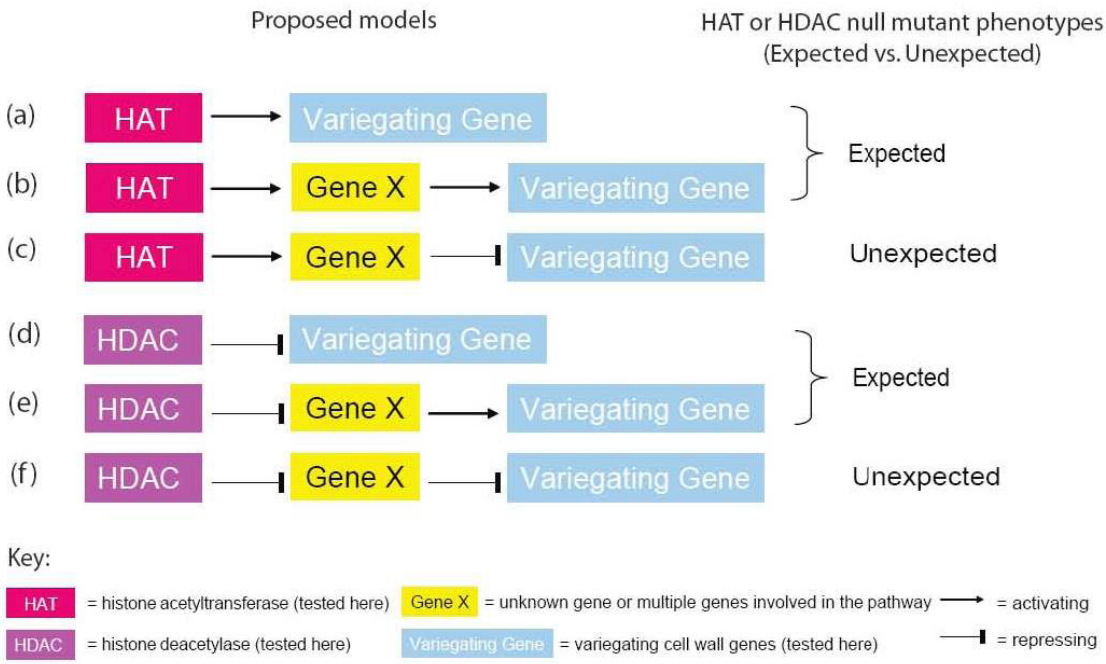

Figure 5. Models for Regulation of Variegating Gene Expression by Histone Modification.

(a) and (b) A HAT *directly or indirectly activates* the variegating gene expression, giving an *expected* null mutant phenotype when the HAT is knocked out.
(c) a HAT *indirectly represses* the variegating gene expression, giving an *unexpected* null mutant phenotype when the HAT is knocked out.
(d) and (e) An HDAC *directly or indirectly represses* the variegating gene expression, giving an *expected* null mutant phenotype when the HDAC is knocked out.
(f) An HDAC *indirectly activates* the variegating gene expression, giving an *unexpected* null mutant phenotype when the HDAC is knocked out.

binding. Building on the results obtained from this study, future efforts should be directed toward investigating pathways involving these trans-acting, chromatin-binding factors and the variegating genes which they regulate.

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Shoe-mounted PVDF piezolelectric transducer for energy harvesting[1]

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Small, portable power sources are needed as alternatives to batteries to supply MEMS, microelectronic devices, sensors, and other low power consumption electronics. Energy can be harvested parasitically from ambient motions such as human movements, and, in particular, footfalls. The compressive forces of a heel strike are harnessed in a shoe-mounted transducer to provide the tensile forces best suited to excite the piezoelectric polymer PVDF to generate a charge that is stored to do work. A total electromechanical efficiency of 1% and an average power of 0.06 mW was found.

Introduction

The increasing prevalence and portability of As compact, low power electronics become more prevalent in everyday use and as their increasing portability requires reliable power sources. Compared to batteries, , aambient energy harvesting devices show much potential as power sourcesover batteries. Indeed, by relying on energy scavenged from the environment, such electronics are no longer restricted by the periodic maintenance that batteries demand. In particular, energy harvested parasitically from human movements has garnered much discussion attention [1]. Perhaps the most energy- abundant and readily- utilized form of ambient human power is walking.

One of the more notable, existing methods of harnessing this energy from footfalls in previous work has been through the mechanical stress of piezoelectric materials incorporated into shoes. Specifically, transducers designed to harness the energy dissipated in the bending ball of the foot have been constructed as layered staves of piezoelectric polyvinylidene fluoride (PVDF) [4, 5, 6, 7]. As a polymer, PVDF has the advantages of beingis strong and flexible, but it has relatively low charge coefficients [2]. Thus, in order to be successful functional in an energy harvesting system, it must be paired with a structure that optimally converts thedirects the input mechanical energy to a useful direction and also uses every inch of the polymer to its fullest potential.

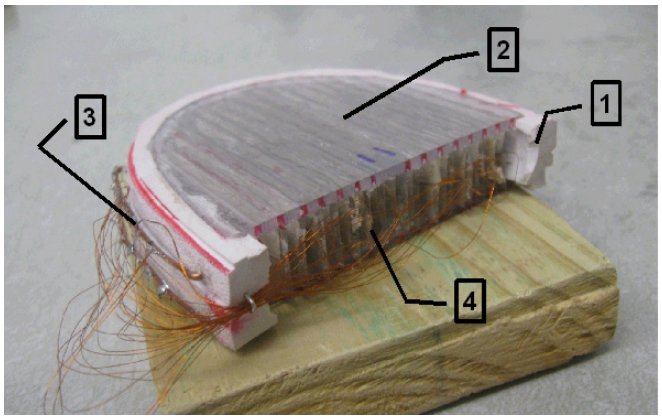

Figure 1. Shoe insert composed of (1) rubber cutout, (2) polycarbonate plates, (3) copper terminals, and (4) unimorph strips.

The aim of this paper is to describe a new, efficient design for a shoe-mounted PVDF piezoelectric transducer. To beginFirst, the design of this structure is described in detail. Experimental results demonstrating the power output and efficiency of the system are presented. A model to describe the peak performance of the transducer is then described and its expected values are compared with actual results. Finally, applications of the transducer as well as possible improvements are discussed. discussion on the improvement of the transducer and its applications is provided.

Material

Piezoelectric polyvinylidene fluoride is manufactured as thin films that which are stretched to align the polymer chains and electrically poled. These thin filmsy are then coated with silver electrodes on both surfaces of the filmsides to act as capacitor plates to accumulate the charge of the stressed PVDF. Under stress, the polar molecules align due to the semicrystalline nature of the PVDF in such a way as to generate an electric field and a potential across the surfaces of the film. Of the three possible modes available to excite the PVDF, mode 31 is focused on here because more electrical energy , for the same mechanical input, can be harnessed for the same mechanical input in this mode than in any of the other modes [3]. Mode 31 involves a stretching along the direction that the PVDF was mechanically stressed during fabrication (the stretch direction). Some of the applicable constants for PVDF are given in Table 1.

Overview of Design

The predominantly compressive forces of a heel strike have been harnessed using a heel insert to provide the tensile forces best suited to excite the PVDF. by means of a heel insert. The advantage

of situating the transducer beneath the heel of the foot instead of fuarther forward near the ball lies in the factis that there is more energy dissipated in this location. The wearer's body weight initially falls wholly on the heel and is only gradually transferred forward with the step. The heel insert, Figure 1, was constructed around a horseshoe-shaped piece of rubber material cut out from the heel of a sneaker. Two horizontal heel-shaped polycarbonate plates were glued at their curved edges to the top and bottom rubber edges of the shoe's heel cutout. Fifteen elongated, rectangular unimorph strips were in turn glued vertically between the two plates , along shallow front-to-back grooves cut in the polycarbonate. The rubber cutout serves both to protect the strips from excess compression – , i.e. to dissipate the forces that which the strips do not absorb, and to maintain the natural feel of the shoe. Indeed, Tthere is little to no difference in the sensation of walking with the transducer mounted in the sneaker.

The unimorph strips themselves were each constructed of one 0.5 inch tall, 52µm thick silver laminated PVDF film (Measurement Specialties) bonded with cyanoacrylate to the side of a slightly wider and longer 4 mil thick PET plastic film substrate (the strips vary in length from 1 to 2.25 inches with the changing space available in the cutout from 1 to 2.25 inches).). This particular substrate was chosen for its stiffness and spring-like qualities following after much experimentation with different materials. Other plastics failed to return to their original shape after deformation, began to craze, or were too thick to be bent under a reasonable force. During a heel strike, the polycarbonate plates are compressed together, in turn bending all of the PET strips aligned between them. The bending plastic strips induce a strain in the bonded PVDF film, which is offset from the neutral axis. Compared to bending solitary PVDF strips, this unimorph configuration substantially increases the electrical response of the PVDF.

Each piece of film was glued to the substrate in the same orientation, with the stretch direction aligned vertically and the positively chargedpoled side of the film facing away from the substrate. A narrow copper wire was bonded to the inner electrode of each laminate with conductive epoxy. Another wire was taped to the outer electrode. These leads from each strip were connected to two copper terminals on the outer edge of the shoe insert so that all of the charge generators would act in a parallel configuration to maximize the generated current [3].

The back ends of the unimorph strips were purposefully cut slightly too large to fit perfectly straight between the two polycarbonate plates, causing them to remain very slightly bent when the shoe insert is not in compression. This pre-bending ensures that each of the strips bends in the same direction under compression so that each piece of film undergoes a tensile strain and that the sign of the voltage produced from each strip is the same equivalent. This is important because a strip bending out of unison will cancel the voltage produced by another, decreasing the effectiveness of the transducer.

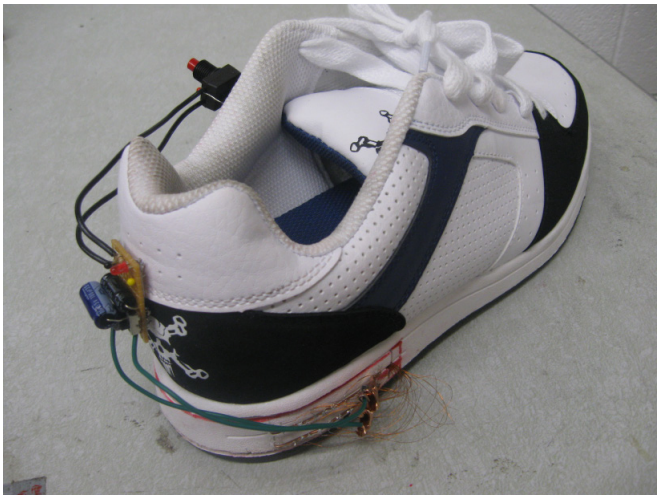

Figure 5. Complete shoe with transducer inserted in the heel and capacitor circuit attached.

Power and Efficiency

Two methods were usedutilized to determine the power output of the transducer system. In the first case, the potential drop across a known load resistor was measured. In order to determin identifye the resistor that best matched to the impedance of the transducer, the power output for various loads was measured while the transducer was compressed under a constant force. The results from this experiment, depicted in Figure 2, show that the power peaks at load of about 500 kΩ.

The fairly high resistance required to match the transducer's impedance can be explained by imagining an equivalent circuit composed of the piezoelectric charge generator, a capacitor for the system's internal capacitance, and a resistor to model the dielectric leakage across the PVDF. For low frequency applications, however, the internal film resistance is very high and can be ignored [8]. The structure's low net capacitance of 17 nF requires that the matching resistance be large.

The best load value determined, the voltage from the transducer across a 470 kΩ resistor at a 1 Hz frequency, shown in Figure 3, was recorded and the root mean square voltage calculated, giving an average power of 0.06 mW. The peak voltage was measured at 21 V, for a peak power of 0.94 mW. The sharp initial peaks are caused by the fairly coherent compression of the strips as the weight of the body falls on the heel. The subsequent rolling transfer of weight forward towards the ball of the foot allows the strips to straighten, generating the ensuing oppositely signed voltage. The negative spikes between steps can be attributed to individual strips returning to their fully extended positions and also to small tensile forces within the heel.

In the second method, the energy stored on a bucket capacitor was calculated. The capacitor was connected to the transducer circuit through a full-wave bridge rectifier. With a 1 µF capacitor (1 Hz excitation), the peak voltage reaches 9.6 V over a 1 second period, yielding an average power of 0.05 mW. This result is slightly lower than that found with the resistive load because the 1 µF capacitor

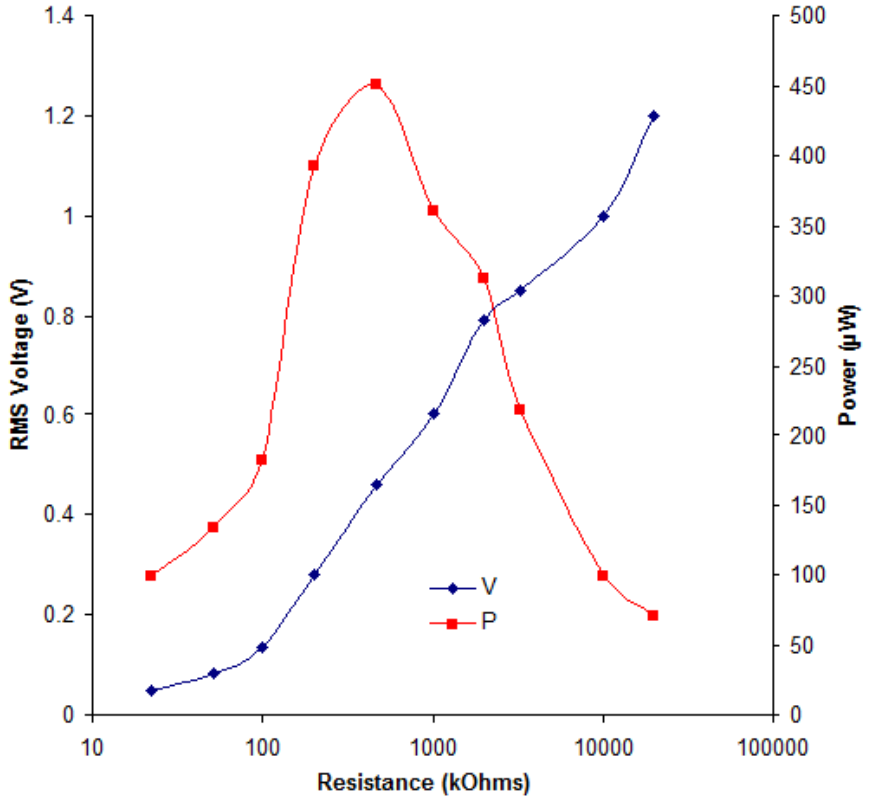

Figure 2. Dependence of power on load resistance, showing best resistance match.

does not match the impedance of the transducer as closely match the impedance of the transducer. Tests were also conducted with a larger 100 µF capacitor to demonstrate the effects of a larger storage medium. Predictably, the stored energy was about an order of magnitude lower. This highlights the need forecessity for efficient power conditioning when converting the output energy to a useful form.

In addition, the electromechanical efficiency of the entire shoe insert was calculated by comparing the energy required to compress the strips inside the shoe insert with the energy generated during a corresponding period. The net energy required to compress a single 1 inch long unimorph strip a distance of 1mm was 0.2 mJ. This value can be extrapolated to the case of the complete transducer , for a total mechanical input of 5.9 mJ. Using the average power generated by the transducer over a one second period, the efficiency of the transducer is found to be approximately 1%. This measurement takes into account losses caused by the imperfect compression of the multiple strips along with losses in the transfer of power from the PVDF to the load. For comparison, the PVDF stave developed by the MIT Media Lab achieved an electromechanical efficiency of 0.5% [4]. This calculation, however, used the open circuit voltage from the transducer to determine the raw electrical output. This approach proved to be extremely difficult in the case of the heel-mounted transducer because its much lower capacitance was not large enough to sustain a voltage long enough to be measured accurately. A dual unity gain buffer amplifier circuit was constructed out of two high power op-amps in an attempt to measure the potential, but the maximum supply voltage was not high enough to avoid capping. Very high power op-amps were not considered due to financial considerations. An accurate measurement of the open circuit voltage cwould possibly yield possibly give an approximate, raw electromechanical efficiency of 2%. This value is based on an expected peak open circuit voltage estimated from the slopes of truncated plots generated by a National Instruments data acquisition device.

System Model

We developed a theoretical It is advantageous to develop a model to describinge the strain along the length of the PVDF strips in order tothat we used for comparisone with experimental results. The strips aligned in the shoe insert can be approximated as bending beams. Thus, the strain in the PVDF can be calculated in terms of the height of the strip (H), the displacement in height (d), and the distance from the neutral axis to the center of the PVDF film (x),

$$S_1 = \frac{\Delta L}{L} = \frac{x}{\rho} = \frac{2xy}{y^2 + \left(\frac{H-d}{2}\right)^2} \approx \frac{4x\sqrt{2Hd - d^2}}{H^2}$$

where ρ is the radius of curvature of the bending strip. In expressing the deflection of the strip (y) in terms of the displacement, the

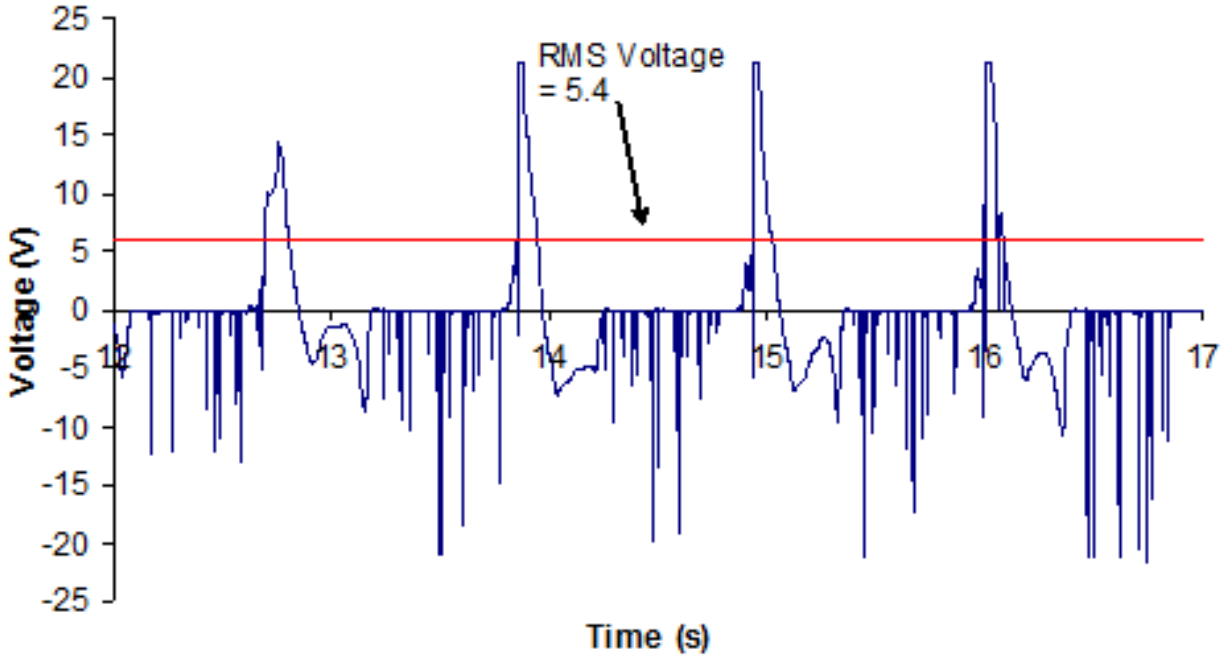

Figure 3. Voltage waveform from transducer with 470 kΩ load.

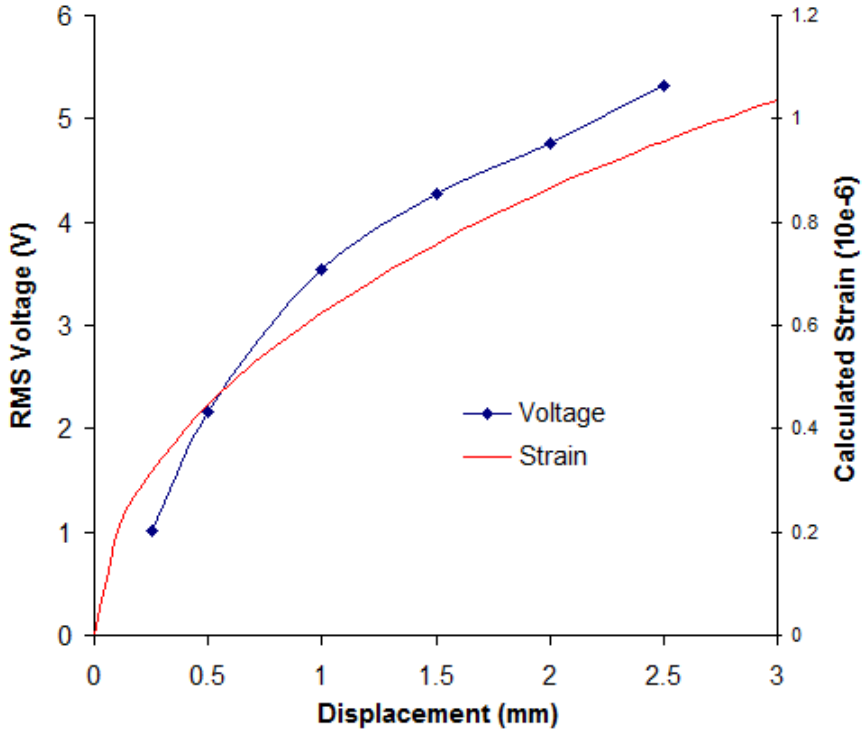

Figure 4. Experimental output voltage plotted with strain as a function of displacement to show correlation. The voltage is a linear function of strain.

bending strip was approximated as an isosceles triangle. In order to test and validate this approximation, a single strip, identical to the ones incorporated into the shoe insert, was compressed at several different displacements. The experimental data in Figure 4 is accompanied by a graph of the strain to show the correlation. The voltage increases steadily with displacement but begins to level off as the displacement nears the height of the strip. However, displacement of this magnitude (not shown on the graph) is impractical as it would result in destruction of the strips.

A dependent formula, developed by M. Toda [9] and derived from the piezoelectric fundamental equations (constitutive relations), gives the transducer's expected peak power based on the strain of the PVDF strip.

$$P_{peak} = \frac{|V^2|}{R} = \frac{(e_{31} A S_1 \omega)^2 R}{(1+\omega^2 C^2 R^2)}$$

where,

$$e_{31} = d_{31} Y$$

Using the constant values and physical characteristics given in Table 1, the expected peak power of the shoe transducer dissipated across a 470 kΩ load (1 Hz excitation, 3 mm displacement) is calculated to be 0.198 mW. This prediction is comparable with the measured peak power of 0.94 mW determined above, but it does, however, underestimates it. It should be noted that the formula predicts a higher peak power with a larger load. As the experimental results from above saw a decrease in power beyond 500 kΩ, this suggests that other factors , not captured by the modeling equation are at play, such as imperfect compression of the strips.

Discussion

An image of the shoe insert assembled in the heel of the sneaker is included shown in Figure 5. As the total power generated from this design is on the order of a few dozen microwatts, it is impractical for use as a power source for conventional personal electronics such as cell phones or audio players. However, it is sufficient for many MEMS, microelectronic devices, and other very low power applications.

The logical next step to increase the output of this kind of system is to add more PVDF material between the plates. An attempt was made to bond multiple laminates to a single substrate, but this approach suffered from slippage between the layers, (reducing the strain,) and from difficulties maintaining electrical insulation. Consequently, increasing the number of unimorph strips remains the best option. The width of the space inside the insert is 60 mm, and with a conservative total strip width of 0.5 mm, 120 individual strips could potentially be fit into the cutout. The eight fold increase in voltage that this could easily allow would provide about 4 mW of power. Moreover, this denser packing of the PVDF films would bring the efficiency up to 8% because the compressive load of the heel strike is underutilized. With fewer strips, much of the energy is still absorbed by the rubber heel. The additional strips would have little effect on the feel of the shoe as the force required to compress each is relatively small. An eEven more promising option is to situate, the vertical strips might be situated throughout the sole of the shoe, harnessing the full force of a footstep and perhaps replacing the function of the dissipative rubber sole. However, an increase in complexity of this type would require very accurate construction, not to mention assembly of large numbers of individual strips.

An increase in PVDF material such as that described would bring the total active area to only about 0.1 square meters. Indeed, the ratio of the square root of average generated power per unit area can be considered a metric for the comparison of piezoelectric PVDF energy harvesting systems. This ratio for the cutout transducer is more than double the value calculated for a PVDF stave, an increase that agrees with the other efficiency calculations above.

Besides adding to the concentration and mechanical efficiency of PVDF in piezoelectric transducers, efficient electrical energy conversion is vital to obtaining useful power from piezoelectric energy harvesting. Advances in more efficient power conditioning efficiency, and electrical power interfaces, better storage devices, and low power consumption electronics will undoubtedly lend more focus to this area.

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An alternative screening tool for prostate adenocarcinoma:

Biomarker discovery

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Prostate Cancer is the second major cause of cancer related death in the US. Prostate Specific Antigen (PSA) levels in the serum are used as the clinical standard for early detection of the disease. However, due to lack of specificity of PSA for prostate cancer, needle biopsy is invariably used to verify the presence of the tumor. This has resulted in a rise in the number of unnecessary biopsies performed, which, in addition to being invasive, are also associated with additional clinical complications. Thus, there is an urgent need to develop markers that can supplement PSA in early detection of prostate cancer. Looking at this from the patient's perspective, such a test needs to be non-invasive and adaptable in the clinical setting. We begin this biomarker search by interrogating the post-DRE (digital rectal exam) urine metabolome of men who presented elevated PSA levels warranting a prostate biopsy (median PSA: 6.5 ng/mL, range: [4.1, 31.9]). HPLC, ^{1}H-NMR and mass spectrometry (GC-MS) were used to quantify the metabolic profiles in the urine. Over 75 metabolites have been identified from the ^{1}H-NMR spectra. Jointly, the MS analyses discovered 557 biomolecules, of which 216 were identified. The three technologies provide coincident and complementary biomolecule measurements resulting in a thorough view of the urine metabolome. The samples were selected such that 50% received a positive biopsy result with biopsy Gleason grades ranging from 6 to 9 among the positive cases. We perform both supervised and un-supervised analysis to determine metabolite alterations that could be used to better predict biopsy outcome. Further we define candidate metabolites whose levels are altered in organ-confined disease and delineate pathways that are altered during prostate cancer development.

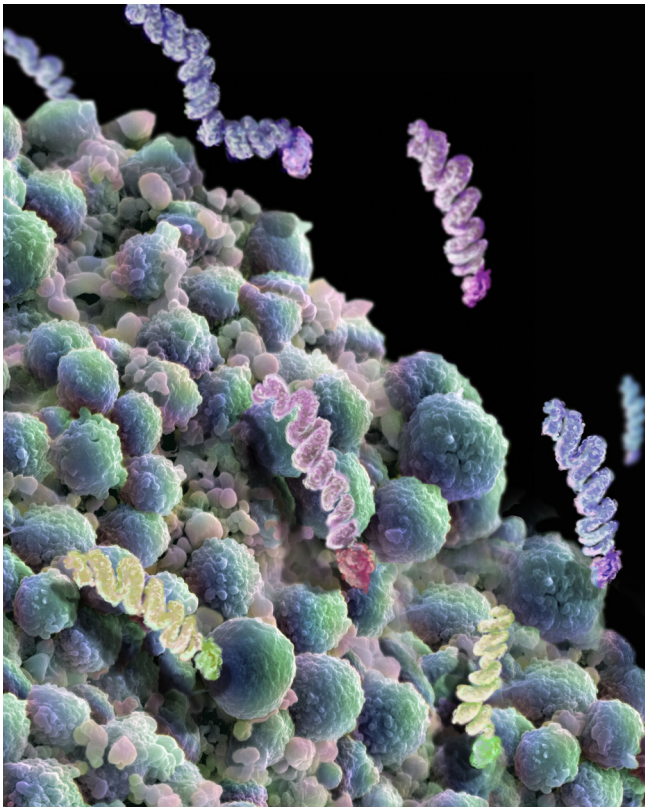
Introduction

Prostate cancer (prostate adenocarcinoma, PCa) is the most common non-skin cancer in American men, with about 230,110 new cases annually in recent years. PCa is the cause of about 40,000 deaths annually, only second to lung cancer in number of deaths in males.1 The survival of patients with prostate cancer (PCa) depends on early diagnosis and the ability to monitor the progression and regression of malignancy. Currently, the best method for the diagnosis of prostate cancer is the triad of digital rectal examination (DRE), transrectal ultrasound (TRUS) guided biopsy, and serum prostate-specific antigen (PSA) measurement. However, PSA measurements alone are unreliable and there are several limitations and disadvantages associated with these procedures. Thus, other biomarkers are needed to facilitate the early detection of prostate cancer.

The reasons for the appearance of PSA in the blood are poorly understood, because this is a pathological response rather than a normal physiological process. Ergo, elevated serum PSA levels can appear due to a number of situations unrelated to cancer, such as benign prostatic hyperplasia (BPH). Since BPH becomes more prevalent with advancing age, prostate cancer is often found against a BPH background. Data from prostate cancer prevention trials showed that prostate cancer was detected in about 25% of patients who had normal PSA levels and normal examinations, suggesting that increased PSA may be related more to BPH volume than to prostate cancer [2, 3]. Also, recent research shows that with time the tumor volume detected by PSA has decreased, so that PSA no longer correlates with cancer volume [4]. Digital rectal examination (DRE) only reveals tumors when their volume approaches 1 cc. The positive identification of prostate cancer is currently possible only with invasive biopsy. These considerations suggest that PSA measurements alone are untrustworthy and that there is a need for alternative biomarkers that can lead to minimally invasive, inexpensive, rapid, accurate and sensitive techniques for early diagnosis of prostate cancer.

Metabolomics (Metabolic profiling) is a relatively new field of research in which the total pool of metabolites in cells, body fluids (urine, plasma, serum, seminal fluid etc.,) or tissues from different patient groups is subjected to comparative analysis [5]. Biological samples usually range from highly invasive and specific (i.e., tissue biopsy), to more general biological samples that are easy to collect and are noninvasive for human patients (i.e. urine). Urine is often used as a biological fluid for investigation due to its ease of collection, repeatability, rich metabolite composition, and the often-higher metabolite concentrations relative to blood plasma or serum. The major technologies that have been used for this process include nuclear magnetic resonance (NMR) spectroscopy, high performance liquid chromatography (HPLC) and mass spectrometry (MS) [6,7]. These techniques are further enhanced by multiple methods of data analysis, ranging from metabolite quantification and pattern recognition studies to multivariate statistical analyses [8, 9]. The position of metabolomics is shown in respect to the other -omic approaches and the influence of the environment (Figure 1) [5]. In addition, a scheme is shown for producing pattern recognition models capable of deducing metabolic profiles. The initial phase usually involves the acquisition of a large dataset in terms of both the variables (metabolites) and observations (subjects), commonly using either ^{1}H NMR spectroscopy or mass spectrometry. However, the use of pattern recognition techniques is an integral part of the approach if the innate metabolic variation associated with different individuals is to be separated from that induced by the insult or physiological stimulus. Following the generation of a database from metabolic responses, these reactions can then be used to build a predictive pattern recognition model that is capable of predicting class membership (e.g. clustering according to the gene deleted).

NMR spectroscopy is especially suitable for metabolomics as it requires little to no sample preparation; moreover, it is rapid, nondestructive, noninvasive, and highly reproducible. Furthermore, its simple data set conversion provides a complete overview of a biological sample in one experiment. Although NMR has many advantages including relatively high-throughput sampling, and new developments to improve sensitivity, such as cryo-cooled probe head technology, the technique still has lower sensitivity compared to MS methodologies. In this study NMR was also combined with HPLC to better identify metabolites with little extrinsic contribution. For MS-based metabolomics, it is generally necessary to carry out a separation step, usually using liquid chromatography (LC) or chemical derivatization in conjunction with gas chromatography (GC) before the MS detection [10,11]. Moreover, MS is highly sensitive, with detection limits surpassing the femtomole range, allowing the detection of up to several thousand metabolites in biofluids. However, challenges exist in MS such as the non-uniform detection caused by variable ionization efficiency. Because of the very diverse range of metabolites found within the cell or biofluids in concurrence with the large dynamic range of metabolite concentrations, these technologies have to be used in combination with one another, to generate a near complete metabolomic description. In this study, we have explored the use of ^{1}H NMR spectroscopy and GC-coupled MS detection for the 'global' metabolite analysis of human urine samples from PCa biopsy positive and biopsy negative patients with the ultimate goal of developing a new screening tool for prostate cancer with urine and possibly the clinical treatment of prostate cancer with known biomarkers.



Representation of prostate cancer cells.

Experimental

Materials

Urine samples were collected from human male patients as part of the ongoing translational studies at University of Michigan Prostate SPORE under approved protocols and stored at -80^{O}C until needed for analysis. Fourteen samples total were used: (seven biopsy +, seven biopsy -) with equal amounts split for both parts of the experimentation (NMR+ GC-MS).

Deuterated water or Deuterium oxide (D2O), Disodium hydrogen phosphate (Na_2HPO_4), Sodium dihydrogen phosphate (NaH_2PO_4), 2, 2-Dimethyl-2-sila-pentane-5-sulfonate-d_6 sodium salt (DSS-d_6), Methanol(CH_3OH), N,N-Dimethyl-formamide (DMF), and N-tert-Butyldimethylsilyl-N-methyltrifluoroacet amide (MTBSTFA) were purchased from Sigma-Aldrich (St. Louis, MO, USA) and used as supplied.

Thin walled, 5mm OD, precision NMR tubes suitable for 750 MHz measurements were purchased from WILMAD-LABGLASS (Buena, NJ, USA).

Test mix

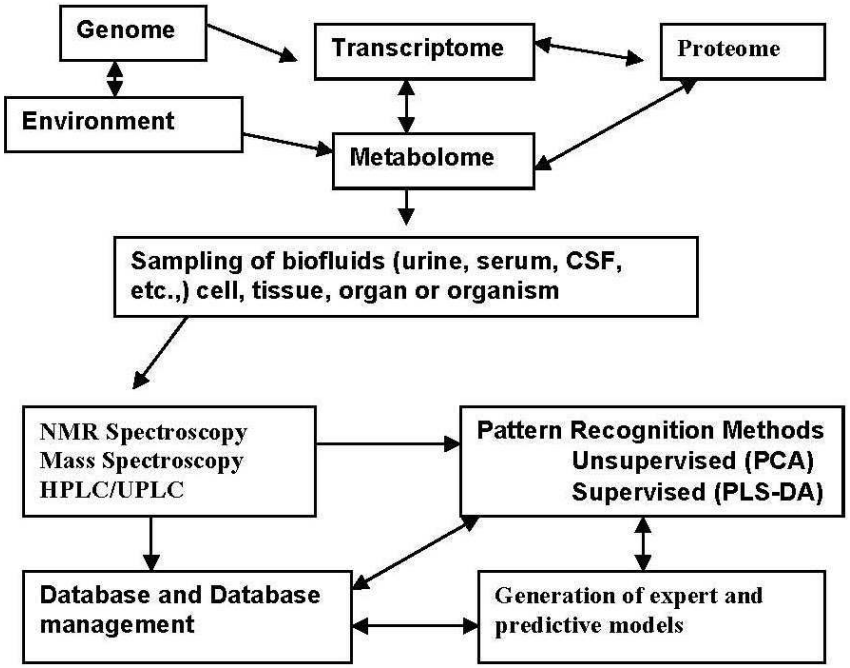


Figure 1. Schematic view of the NMR and MS- based metabolomics workflow.

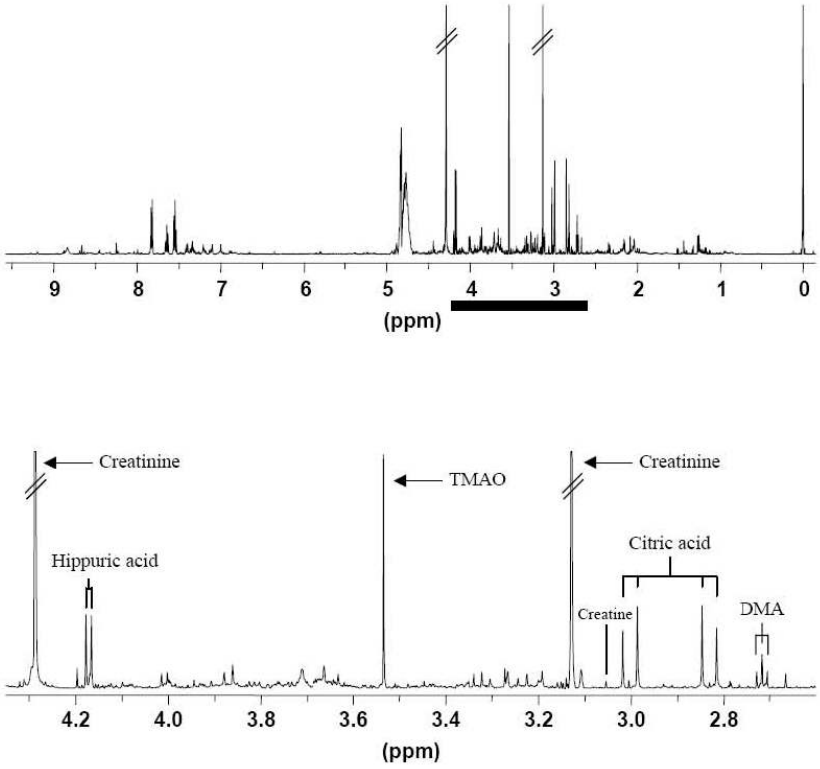


Figure 2. ^{1}H-NMR spectrum of urine from a healthy volunteer (upper panel) and an expansion of a part of this spectrum (lower panel) recorded at 750 MHz. DMA = dimethylamine; TMAO = trimethylamine N-oxide.

GC test standards (Sarcosine, Alanine, Homocysteine, Methionine, and Glycine) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

GC-MS Sample preparation and derivatization method

A GeneVac EZ2- evaporator drying unit was used to dry the samples down. The pressure was initially maintained at 400 mbar for 10 minutes then reduced to 100mbar for 20 minutes before finally being reduced to 7mbar for the remaining 45 minutes. The temperature was set at a constant maximum of 65^{O}C throughout and revolution of the unit was set to 900rpm. Samples were dried twice with additions of 100 µL of DMF (residual water remover) each time. Following the second drying period, DMF (100µL) and the derivatizing agent, MTBSTFA, (100µL) were added to the sample. The sample was vortexed vigorously using a Vortexmeter (Fisher brand) for 30 seconds and placed in Isotemp 125D Fisher Scientific sandbath for 60 minutes at 60^{O}C.

Control samples were prepared following the same guidelines as the urine sample preparation, but with the substitution of water for the urine.

Each of the 14 samples was analyzed three times to observe inter-injection variability. To reduce errors from trends, the order of the analysis was randomized and 10-minute blanks of 2µL ethyl acetate (EA) were injected between each sample to prevent carryover. A GC standard test mix and control samples were also run at regular points throughout the study.

Chromatography and Mass Spectrometry

The samples were analyzed on an Agilent 6890N gas chromatograph equipped with a DB-5MS (15m x 0.2mm x 0.33µm) polysiloxane column, 7683B Injector, electron ionization detector and a 5975 Inert XL MSD mass spectrometer (Agilent Ltd., USA). A volume of 1µL of the derivatized sample was injected into the system.

Helium gas at a constant pressure of 25psi was used as the carrier gas. The injector temperature was set at 250^{O}C. The GC oven temperature was initially maintained at 40^{O}C for 3 minutes, rose to 70^{O}C at 60^{O}C/min. (held for 2 minutes), and then programmed to 285^{O}C at a rate of 20^{O}C/min., ending at a temperature of 300^{O}C , which was maintained for 15 minutes. The electron ionization detector was operated at 300^{O}C throughout the analysis. The mass spectrometer transfer line was set at 325^{O}C. The mass spectrometer was operated in electron impact (EI) ionization mode over a mass range of m/z 50–650, with a multiplier voltage of 2106V and a data collection rate of 1.47 scans per second. The EI source was operated at 70eV and a temperature of 230^{O}C with the quadrupole region at 150^{O}C.

NMR sample preparation

The urine samples were prepared by reconstituting the lyophilized urine pellets in 650µl of phosphate buffer of pH 7.0 (0.1M Na_2HPO_4/NaH_2PO_4 solution in D2O, containing 0.005% 2-Dimethyl-2-sila-pentane-5-sulfonate-d6 sodium salt (DSS-d_6) as a reference standard) in Eppendorf micro centrifuge tubes. After the solution had been spun for 10 minutes at 15000rpm (25ºC), 600µl of the supernatant was transferred into 5mm NMR tubes for subsequent analysis. A selected group of biopsy-positive (4) and biopsy-negative (4) samples were also subjected to HPLC fractionation in order to reduce complexities of the urine spectra. Reverse phase HPLC was used with a Thermo-Finnigan system with a Surveyor pump, C-18 analytical column, Water in 0.1% Formic Acid + Methanol in 0.1% Formic Acid as the mobile phases, with the gradient going from 95/5 to 5/95.

NMR acquisition

The buffered urine samples were analyzed on a Bruker DMX - 750 MHz spectrometer equipped with a 5mm TXI, cryoprobe head operating at 298 K. ^{1}H-NMR spectra were acquired using a standard one-dimensional Bruker NOESY pre-saturation pulse program (relaxation delay— 90^{o}— τ_1 — 90^{o}— τ_m — 90 — acquire-FID), where τ_m = 100ms. The residual water resonances were suppressed by pre-saturation during the relaxation delay of 2s and mixing time (τ_m). Sixty-four transients were collected into 32K complex data points using a spectral width of 9000Hz. The acquisition time was 4.5 minutes and, including a preacquisition delay of 3 minutes and automatic shimming, the total run time was 12 minutes per sample. The data were Fourier transformed after multiplying by an exponential window function with a line broadening of 0.5Hz, and the spectra were manually corrected for phase and baseline using Bruker XwinNmr Software (version 3.5).

Data Reduction and Analysis

Each NMR spectrum (32K data points) was binned to 4K data points from 0 to 10ppm. The region of water signal (4.6 – 6.00ppm) was excluded because the intense water signal, which is affected by water pre-saturation, does not carry additional information and skews the analysis. Similarly, the region of chemical shift reference, DSS (-0.5 to 0.5ppm) was excluded. This data 'binning' simplifies statistical analysis and reduces the effects of small, pH-related variations in chemical shifts. All NMR spectra were normalized to a constant integrated intensity of 100 units to compensate for large variations in urine concentration.

The NMR data were mean-centered and principal component analysis (PCA) [12] was carried out to identify similarities and differences between the two sets of urine samples. Data formatting and data pre-processing for the NMR data was carried out using in-house software written in EXCEL and MATLAB. Statistical analysis was performed using the in-house software written in MATLAB (version 7, The MathWorks, Natick, MA, USA).

Results and Discussion

A ^{1}H-NMR spectrum provides a characteristic 'fingerprint' of almost all proton-containing metabolites. In these spectra, the spectral parameters chemical shift (δ, ppm), spin-spin coupling (J, Hz), and signal intensity are important for biofluid analysis. Using these spectral parameters, body fluid ^{1}H-NMR spectra can be used for identification and quantification of proton-containing metabolites allowing for the diagnosis of diseases.^{1}H-NMR spectra of urine from patients with biopsy positive (disease) and biopsy negative (control) were recorded. An example of a urine spectrum from a healthy

volunteer and an expansion of a part of this spectrum are shown in Figure 2. Although many resonances in ^{1}H-NMR spectrum of urine can be assigned directly based on their chemical shifts, signal multiplicities and by adding authentic material, the complexity of urine NMR spectra is such that, on horizontal and vertical expansion, several thousand peaks are observed from hundreds of endogenous metabolites representing many biochemical pathways.

Conventional measurement of the major NMR signals can be used to detect biochemical changes, but the complexity of the spectra and the presence of natural biological variation across a set of samples often make it difficult to detect meaningful patterns of change by eye. Generally, it is necessary to use a combination of data reduction techniques and complex multivariate statistical analysis methods in order to access the latent biochemical information present in the spectra. Chemometric techniques such as principal component analysis (PCA) [12] and partial least squares discriminant analysis (PLS-DA) [13] are some of the more well-established techniques that have been used in ^{1}H-NMR metabolomics analysis of complex biological NMR spectra with success. [14,15].

PCA of ^{1}H-NMR spectra of urines from patients with biopsy positive (disease) and biopsy negative (controls)

PCA was used for the statistical analysis of the data. It was applied to the binned data (9.7 – 6.05 ppm, i.e. data sets of 1264 for NMR variables) and each of these data sets was reduced to 2 or 3 linear combinations of buckets. Each linear combination of buckets can be thought of as a direction in 1250-dimensional space for NMR. The first PCA component corresponds to the direction of maximum variance of the data. The second component corresponds to the direction of maximum variance subject to the constraint that it is orthogonal to the first, and so on. The coefficients that define how the buckets are combined to create the linear combinations are called 'loadings'. The values for the linear combination of each sample are called 'scores'. The scores plot of PC1, often called first component, against PC2 (second component) is a simple 2D visualization of the main variation in the data and can be used to see which samples are similar and which are dissimilar. The corresponding loadings plots for each principle component indicate which parts of the spectra are responsible for the observed patterns in the scores plots. A 2D PCA scores plot PC1 vs. PC2 for the NMR data (9.7 – 6.05 ppm) is shown in Fig. 3. PCA of ^{1}H-NMR spectra of urines from patients with biopsy positive (disease) and biopsy negative (controls).

PCA analysis of the reduced spectra indicated good separation when comparing urine from biopsy positive to biopsy negative patients. We correctly separated 7 biopsy positive urine (disease) samples (blue diamonds in the figure 3) from 7 biopsy negative (control) urine samples (pink squares) as shown in figure 3. The

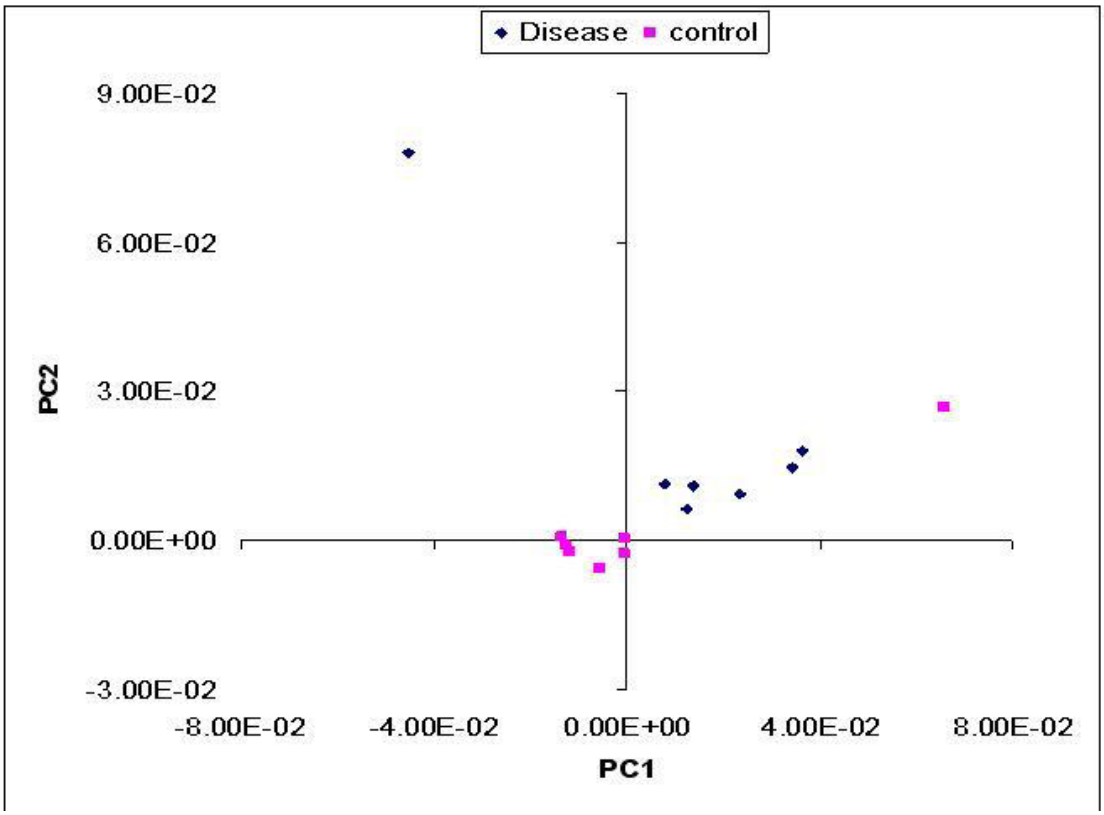


Figure 3. PCA score plot (PC1 vs PC2) from the NMR data (9.7 – 6.05 ppm)
The pink square plots represent the biopsy negative control samples. The blue diamond plots represent the biopsy positive diseased samples. We see a typical orthogonal split with certain unexpected outliers.

biopsy negative urine data set displayed variables that were more tightly grouped, indicating less variability from one sample to the next. The dataset for biopsy positive samples had more variability, possibly due to additional systemic alterations in patients bearing more advanced PCA. One data point lay outside of the disease cluster and this value is to be considered an outlier. The control dataset displayed tight clustering for 86% of the data, with one sample lying outside of the data cluster and this sample appeared in the region defined as being not far from biopsy positive (reason unknown). Overall, PCA could successfully distinguish between a biopsy positive and biopsy negative patient line and a normal one when using the first and second principal components (PC1 and PC2).

Loadings responsible for spectral differences between urine samples

In light of the fact that separation between urine from biopsy positive and biopsy negative (controls) occurred when using unsupervised PCA, we attempted to identify which molecules were responsible for the differences in the observed spectral patterns. The loadings plot displays NMR chemical shifts of molecules that are responsible for the separation of data sets along the two principal component factors described in figure 4. The loadings plot for PC1 is shown in Fig. 4 (NMR data 9.7 - 6.05 ppm). There are at least 17 different molecules that are responsible for the separation. Once specific regions of highly differential molecules are determined and tabulated, the next step is to learn more about the metabolite and its biochemical pathways, in relationship to prostate cancer progression. The loading plot shown in figure 4 shows that 2 sets of peaks drive the separation: set I consists of the molecules hippurate, 1-methyl nicotinate, adenosine diphosphate, adenine and set II consists of tyrosine, histidine, serotonine, 3-methyl histidine, inosine formate, 1-methyl histidine, 1-methyl nicotinamide, adenosine triphosphate and adenosyl methionine, all identified by HMDB (human metabolome database from Calgary, Canada) and University of Wisconsin at Madison, USA database searches.

The metabolites concentrations from set II appear to be negatively correlated with the metabolites concentrations from set I concentration: patients with high PC2 scores have relatively high concentrations of set I metabolites and lower concentrations of set II metabolites. Conversely, volunteers with negative PC2 scores have relatively low concentrations in set I and higher concentrations in set II metabolites. Using the valuable information from the loading plot, we can now gather a general scientific understanding as to which molecules are fluctuated in cancer patients with respect to the normal ones. Additionally, since multiple runs with checkpoints were initiated, we can assume sufficient accuracy. Finally, the loading plot is based on an accurate orthogonal variability as expressed in the score plot, which makes this data more reliable.

2D NMR Methods

It is well-known that 2D NMR experiments improve the ability to interpret spectra because of the higher resolution provided by dispersing the spectral peaks. However, 2D NMR methods have

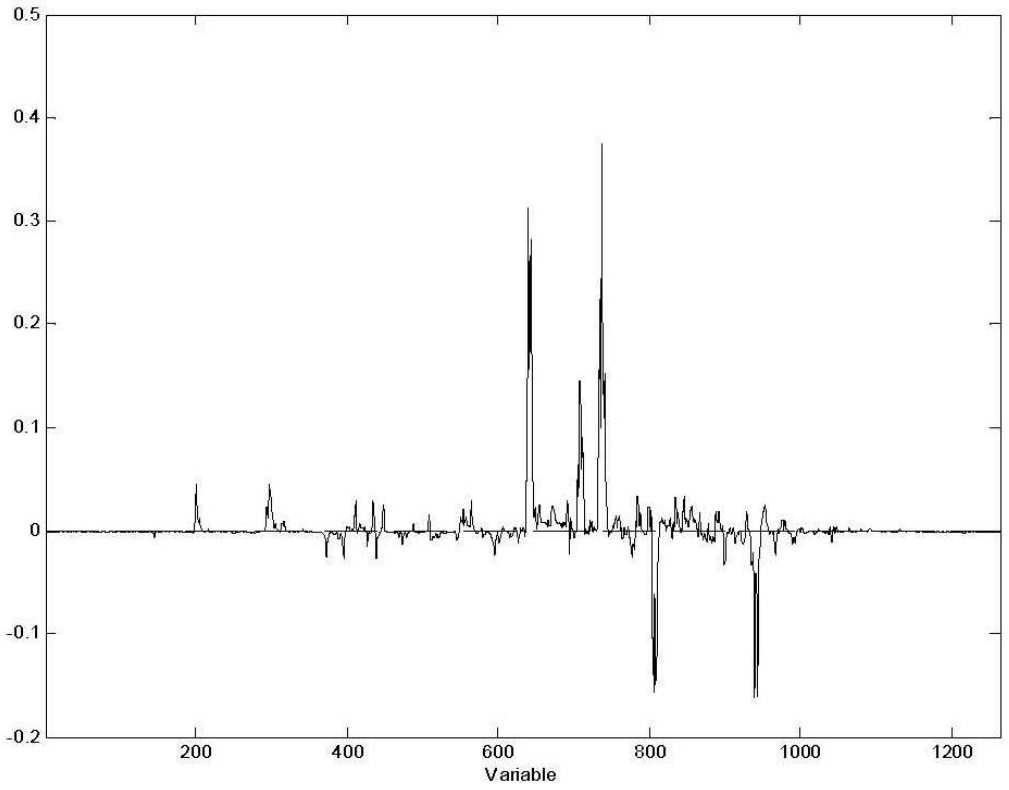


Figure 4. PCA loading plot for the NMR data (9.7 – 6.05 ppm). Set II metabolites are negatively correlated and can be seen in regions such as the one from 800 to 1000. Set I metabolites are positively correlated and can been seen in regions such as the one from 600 to 800.

not been widely used in metabolomics to date because of their increased acquisition time, data size, and complexity in data analysis. Nevertheless, a small but growing number of papers report using 2D approaches in metabolomics studies. [16,17]. The most commonly used experiments include correlation spectroscopy (COSY), total correlation spectroscopy (TOCSY), 2D-J spectroscopy, and heteronuclear single quantum coherence (HSQC) spectroscopy. With modern NMR spectrometers and modified pulse programs, these experiments are readily accessible.

2D J-Resolved Spectroscopy

J-resolved (JRES) spectroscopy is a simple homonuclear 2D experiment in which J-coupling information is removed from the first spectral dimension and separated into a second dimension. The 1D projection of the 2D spectrum retains only chemical shift information, leading to a substantial simplification of the spectra. This technique has been successfully applied to many body fluids including cerebrospinal fluid (CSF), seminal body fluid, blood plasma, and urine. [18]. We have used this technique (figure 5) to reduce the complexity of ^{1}H-NMR spectra of urine and enhance the identification of metabolites in our present study.

Gas Chromatography-Mass Spectroscopy

We have investigated in this study whether gas chromatography (GC) separation in combination with Electron Impact (EI) ionization mass spectrometry (MS) detection can be combined for enhanced metabolite profiling from urine. We show that although GC sample preparation is much more involved than for NMR, hundreds of metabolites can reproducibly be detected and analyzed by GC-MS. A sample set of urines (both biopsy positive and negative) from fourteen human volunteers studied by ^{1}H-NMR was also analyzed independently by GC-MS, and subsequent chemometric analysis of the two datasets showed some interesting features. Since in contrast to NMR every peak in GC corresponds to a single metabolite, the electron ionization spectra can be used to quickly identify metabolites of interest if their reference spectra are present in a searchable database.

The Wilcoxon test is a nonparametric method used to differentiate between two related groups. It mathematically computes the difference between a set of samples, and examines the variations. Table 1 depicts the list of highly differential metabolites detected by GC-MS. A comparison between NMR data and MS data indicates an overlap as shown in Figure 6. Using both technologies, two specific metabolites were detected as highly elevated in prostate cancer patients when compared to controls.

Concluding remarks

In a relatively small pilot study with ^{1}H-NMR and GC-MS based analysis of urine obtained from patients, strong capabilities exit of distinguishing between biopsy (+) and biopsy(-) using novel methods. Our findings further strengthen the hypothesis that a

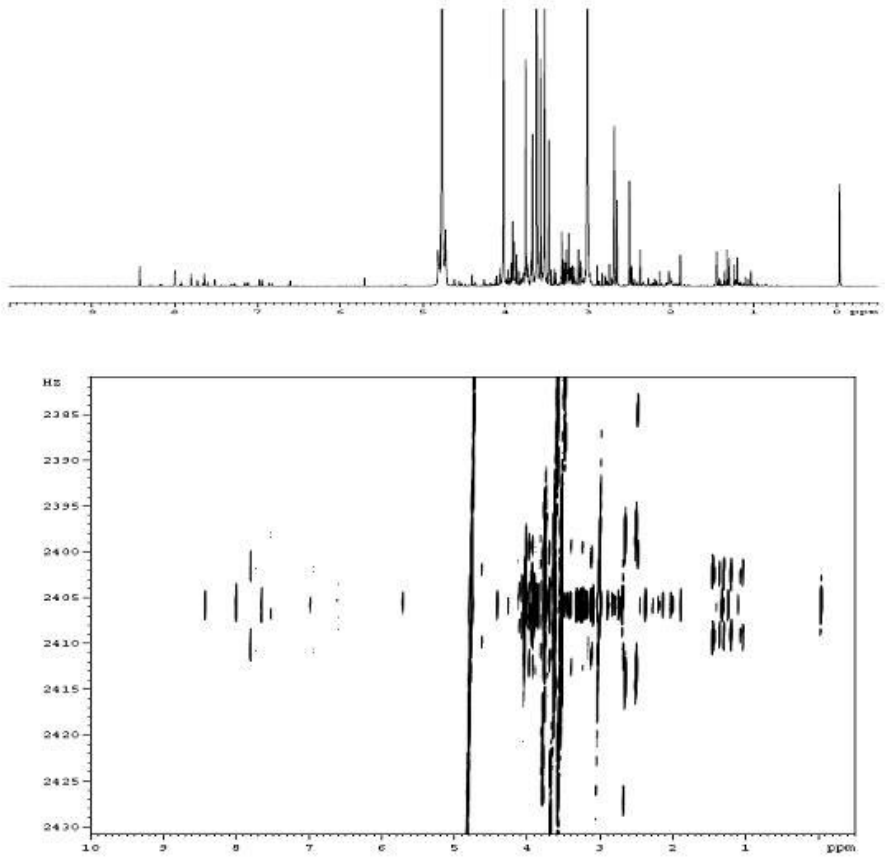


Figure 5. The 2D J-resolved spectrum of biopsy negative urine sample (bottom panel) and its 1D projection with multiplicity removed resulting in substantially simplified spectra (upper panel).

GC-MS data analysis:

Metabolites	Pvalue	Z-value	Change
1-methylimidazoleacetate.32350.200	0.0538	1.9286	Dn_BX+
glycylproline.22171.200	0.0383	2.0714	Dn_BX+
hypoxanthine.3127.200	0.1004	1.6429	Dn_BX+
xanthosine.15136.200	0.0538	1.9286	Dn_BX+
3-hydroxyphenylacetate.1413.50	0.0538	-1.9286	Up_BX+
3-hydroxypyridine.21169.50	0.1004	-1.6429	Up_BX+
allantoin.22808.50	0.0741	-1.7857	Up_BX+
fumarate.1643.50	0.0538	-1.9286	Up_BX+
glycerophosphorylcholine..GPC..15990.200	0.0538	-1.9286	Up_BX+
guanidine.22287.50	0.0184	-2.3571	Up_BX+
hippurate.15753.200	0.1004	-1.6429	Up_BX+
mandelate.22160.50	0.0538	-1.9286	Up_BX+
N-acetylleucine..32995.200	0.0383	-2.0714	Up_BX+
N-acetyltyrosine.32390.200	0.1004	-1.6429	Up_BX+
salicyluric.glucuronide..33384.201	0.0741	-1.7857	Up_BX+
sorbose.563.50	0.0383	-2.0714	Up_BX+
threote.27738.50	0.1004	-1.6429	Up_BX+

Table 1. Wilcoxon Matched Pair Test (Differential analysis)

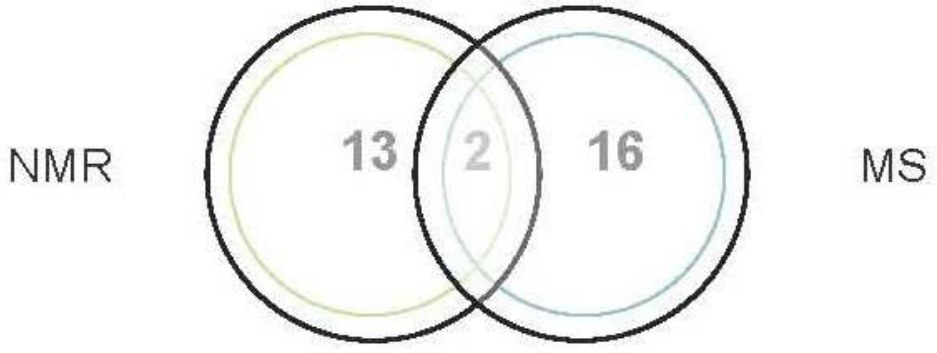


Figure 6. Venn Diagram of Consistent Metabolites.

global analysis of urine using multiple technologies may be the right step towards developing an effective urine screening technique for early cancer detection, possible warranted further studies for clinical technique. As for the two molecules that were found to be highly differential in both technologies, in depth biological invasion assays are in progress to determine whether these molecules influence toxicity. Further experiments (unpublished data) have confirmed the cancer-causing potential of one of these two molecules. Introduction of the said metabolite into benign tissue induced invasion and cancer progression.

Currently, NMR studies with a higher frequency magnet (900 Mhz) and more comprehensive MS studies have been completed. A sample size of 100 urine specimens was used for the extension. A simple summer study with fourteen men yielded astounding results, indicating that men with prostate cancer have specific molecular differences in their overall metabolic activities. These differences can be isolated to label prostate cancer with certain markers that can serve as predictors and preventers of the disease in the long run. Because of the appealing results in the basic study done during the summer, a more extensive survey of the population is being concluded including a larger sample population (100+), which ultimately lead to more reliable results and repeatability.

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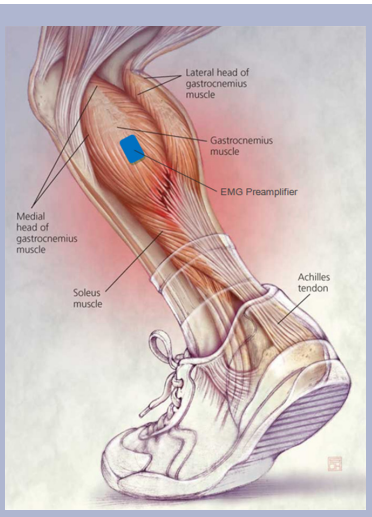
EMG of the Human Calf Muscle in Bipedal Forward Motion

By Jesse Thornburg, MIT Class of 2011, Mechanical Engineering



Numerous leg motions, specifically those which involve elevating the heel, require neurological stimulation of the calf muscles. Athletes seek to exercise these muscles by standing exercises, walking, jumping, and running. This study used an electromyographic (EMG) preamplifier to record voltage signals in the calf, specifically looking at the way these signals vary for different states of forward motion. Standing, walking, and running were compared, with the latter two activities being further observed at different inclines. In total, EMG in the calf was recorded over seven states of bipedal activity. The mean voltage signal was observed to increase with both speed and incline. Increases in speed also caused the signals' oscillations to become less uniform and their sinusoidal behavior to become less distinct. This shift to more sporadic signals is a phenomenon which merits future study.

Improving MIT IS&T's Call Resolution Time

Paul Kominers, MIT Class of 2012, Economics

MIT's Information Services and Technology Call Center provides robust software and hardware not only for members of the immediate MIT Community but also for familes thereof. It handles a significant daily call volume and is constantly looking for ways to meet and improve on its efficiency goals. Using simple multiple-server queueing models, we found that a reorganized schedule would allow the Call Center to maintain its current staffing levels but bring wait times under their goal of 30 seconds average per customer.

Testing the Feasibility and Socioeconomic Impacts of the "Mediterranean Solar Plan" in the Middle East / North Africa Region

Erik Fogg, November 2008, Political Science

This paper argues that the Mediterranean Solar Plan is likely to emulate the Club of Rome-sponsored DESERTEC plan, and also that all willing MENA countries would be able to accommodate the solar project, and successfully construct, maintain, and protect solar collection resources. These countries will benefit immensely from the plan, which includes economic deals beyond simple export of electricity. Trade agreements with the EU and incentives to liberalize markets, along with a recent trend towards liberalization, welfare, and free trade, will prevent solar power from becoming a "new oil;" electricity export revenues, sustainable sources of energy, and desalinization benefits from solar thermal power generation will all lead to increases in GDP and standard of living, increases in government provisions and drinkable water, a long-term alternative (or replacement) to oil, and decreases in unemployment. The paper will conclude with policy recommendations for the Secretariat of the Union for the Mediterranean that can be implemented to maximize the benefits felt by both the European Union and MENA region countries.

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