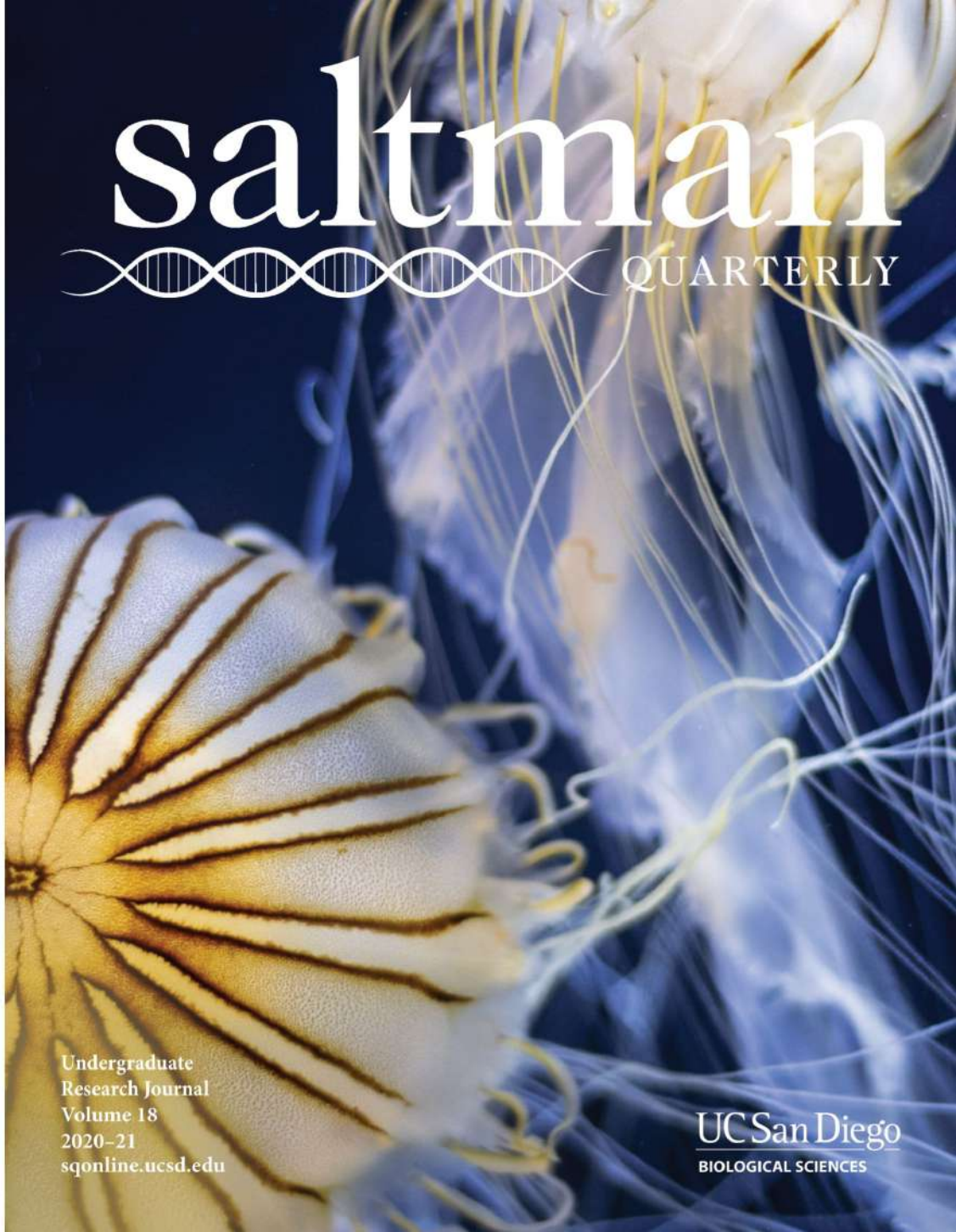
saltman QUARTERLY



Undergraduate
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UC San Diego
BIOLOGICAL SCIENCES

In an effort to engage the UC San Diego Community, *Saltman Quarterly* holds an annual photo contest. The winners of this contest have their images featured on the cover and interior pages of the journal.

FRONT COVER

Compass Jellyfish swimming at the Birch Aquarium in San Diego.
Photo by Sam Zilberman

INSIDE COVER

A Cethosia hypsea butterfly at the butterfly garden in Singapore's Changi Airport.
Photo by Zina Patel

BACK COVER

A puffer fish peeking from behind a rock at the Seattle Aquarium in Washington.
Photo by Zina Patel

GENEROUSLY UNDERWRITTEN BY

THE SALTMAN FAMILY

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UC San Diego
BIOLOGICAL SCIENCES

LETTER from the EDITORS

Dear Reader,

Science in the last few decades has been filled with unreserved hope and possibility. We have made advances in the last century that we could not have possibly even dreamt of before. Among many, a perception of security exists in the notion that science is infallible. Yet, despite all of the strides in scientific advancements we have had thus far, the pandemic has made it beyond apparent how truly vulnerable we can still be. The fact that a mere virus shattered our outlook on invincibility within science and exposed our fragility is absolutely jarring.

What many fail to realize though, is that one of the most fundamental drivers of science and research is in its opportunities for failure. As frustrating as failure may be, adaptability rises from it, and it is through adaptability that we come to a place with greater understanding and knowledge of all that is happening.

The pandemic has made changes in every aspect of our world, including the way we conduct research. Watching UCSD transform itself in order to cope with the public health challenges COVID-19 presented serves as a defining model on what adaptability should look like. Many of UCSD's research teams had to completely alter their workplaces and research focuses to accommodate what the community needed to face the global pandemic. This stands as an important reminder to the science community on how flexibility is crucial in generating progress.

Adaptability to a changing world is something *Saltman Quarterly* also experienced. Expanding our content creation to social media platforms and beyond what was once just our print publications is something we realized was crucial in an era of excess information. Whether it's in research or media, adjusting your sails as the winds shift is key.

Warmly,

Andra Thomas
Editor-in-Chief,
Saltman Quarterly 2020-21

It's been a year, hasn't it? It's more likely than any previous year that you're reading this on a screen. Maybe you can't feel the physical weight of a year's worth of hard work, but I hope that every digital page you flip imprints some impression of the thought and care that went into every line and every shade.

While campuses shut down, classes and socials gracelessly transitioned to Zoom, and microbiology labs were reduced to writing lab reports on research papers we read, the *Saltman Quarterly* staff kept going. And it was really, really hard. Between social and political upheaval climaxing to uproot our trust in what others call justice, against personal and familial crises, core staff meetings continued every Monday night. Articles and illustrations might have been uploaded late, but they were uploaded. Pitches were sculpted into drafts, and drafts were crafted into the final versions you will soon see before you.

Keeping in mind *Saltman Quarterly*'s decades-long commitment to delivering only the best content, valuing accessibility to readers and scientific integrity above all else, we kept working hard. Maybe we didn't get here the way we hoped, but we got here, and in the end we've gone above and beyond what we initially believed we were capable of.

This issue is something that is a precious achievement to over a hundred people. So to every writer, illustrator, photographer, outreach member, editor, advisor: thank you so much for staying with *Saltman Quarterly* throughout this challenging year. It was an honour to work with you all, and I'm grateful we were all here to support each other to produce this volume.

Happy Reading,

Salma Sheriff
Editor-in-Chief,
Saltman Quarterly 2020-21

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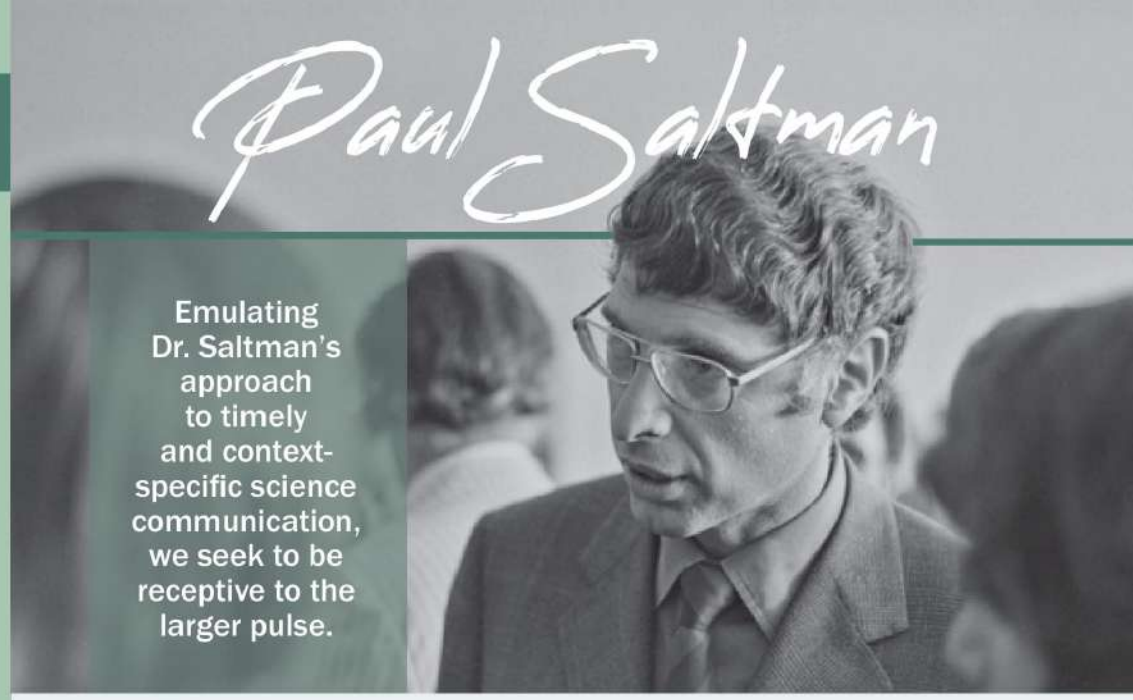
Paul Saltman



Emulating Dr. Saltman's approach to timely and context-specific science communication, we seek to be receptive to the larger pulse.

It seems like the light at the end of the tunnel is now visible; we are almost at the cusp of putting this pandemic behind us. As of April 18, 2021, almost 130 million American adults, or a little over half of the US adult population, have received their first dose of the COVID-19 vaccine. Rewinding to last April, we see a different world, with people struggling to adapt and systems scrambling to recalibrate. For the public, the mere thought of a vaccine—let alone a finished product doled out in the millions daily—was abstraction at best. Marked by self-isolation for many, the year in between has seen sweeping changes and unprecedented developments.

In January 2020, a month after the first ever COVID-19 case appeared in Wuhan, China, the SARS-CoV-2 virus was sequenced, sparking the race to develop an effective vaccine. Multinational pharmaceutical companies, governments, and other institutions worked around the clock and collaborated to expedite vaccine development. By the end of 2020, the first doses were already being administered in Europe and the US. The accelerated timeline, however, proved to be a source of concern for many. Some, understandably, had questions about the safety of the vaccine, given that it hadn't undergone long-term trials, while others were concerned about its effectiveness. The curiosity, concern, and interest of the general public regarding the COVID-19 vaccine reaffirmed the importance of and need for accessible science communication. Our work here at *Saltman Quarterly* took on greater meaning as we aspired to bridge the gap between scientists and the public. Dr. Paul Saltman's lifelong dedication to science education suddenly came into sharper focus.

> Dr. Saltman cherished his role as an educator, and was committed to the success of his students.

Dr. Paul Saltman, to whom we dedicate this undergraduate research journal we've worked so hard on, was a pioneering scientist who also strove to make science comprehensible for people of all backgrounds. Dr. Saltman started off his scientific career at CalTech, where he earned a BS in Chemistry in 1949 and a PhD in Biochemistry in 1953. He then joined the University of Southern California Keck School of Medicine as a Biochemistry instructor that same year, eventually becoming a professor in 1961. After spending 14 years at USC, in 1967, he joined UC San Diego as Provost of Revelle College. He would spend the next 32 years of his life serving the UC San Diego community in different capacities, including as Vice Chancellor of Academic Affairs and a research and teaching professor in the biology department, until he passed away in 1999.

Dr. Saltman cherished his role as an educator, and was committed to the success of his students. He believed that an effective teacher needed to have "knowledge, skills, the ability to comprehend the process of human understanding, and the ability to inspire students and excite them with the notion of learning." His empathetic nature and concern did not go unnoticed by his students. One student mentioned, "Paul

He believed that an effective teacher needed to have 'knowledge, skills, the ability to comprehend the process of human understanding and the ability to inspire students and excite them with the notion of learning.'

Saltman is the most charismatic, altruistic, caring and noble person I have ever known. I have never respected anyone more than I respect him." He was honored with multiple teaching awards, based on feedback from his students, such as Excellence in Teaching Awards from the University of Southern California and from Revelle, Muir, Warren and Thurgood Marshall Colleges at UCSD, as well as the first Career Teaching Award from the San Diego Division of the UC Academic Senate.

Just before his passing, in June 1999, the Paul D. Saltman Endowed Chair in Science Education at UC San Diego was established to recognize a senior faculty member for their commitment to biology education.

It was his love for and interest in science education that led Dr. Saltman to develop initiatives that would allow people without scientific backgrounds to understand scientific topics that at first glance, often seemed complex. He created a number of interdisciplinary courses for non-science majors, including a series called Frontiers of Science, as well as a "Food and Nutrition" course. In 1993, noticing the lack of easy-to-understand guides about nutrition and dieting in the market, he published the UC San Diego Nutrition Book, which was written to "demystify the science of nutrition" and allow the general public to understand "what's in food and how our body uses those nutrients."

Dr. Saltman regularly appeared on radio and television programs, breaking down and delivering scientific jargon to the masses in a clear, intelligible manner. He penned articles on nutrition, based on his own research in the field, for a variety of publications, from academic journals to popular magazines. He was also on the editorial boards for a number of scientific journals.

Dr. Saltman's legacy lives on with *Saltman Quarterly*. Started by students after Dr. Saltman's passing, this program is carrying forward his spirit and passion for communicating science to a broader audience. Our interdisciplinary team of writers, editors, designers, photographers, and artists come together to create different avenues for science communication. With our two print publications, *Saltman Quarterly* and *Under the Scope*, our interactive website, quarterly Insider, dynamic media team, and connected community outreach team, we are bringing stories of biology research, both here and elsewhere, out of esoteric circles and into the public eye. Emulating Dr. Saltman's approach to timely and context-specific science communication, we seek to be receptive to the larger pulse; for example, during the early part of the pandemic, our media and illustration teams collaborated to create Viral Virtual Updates, a series about the biology of coronaviruses, PPE, differences between airborne and droplet transmission, and more.

Dr. Saltman's guiding principles and abiding faith in science education will stay with us as we continue innovating new ways of science communication and storytelling.

WRITTEN BY

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Noorhan is a Human Biology student at John Muir College. She will be graduating in 2021.

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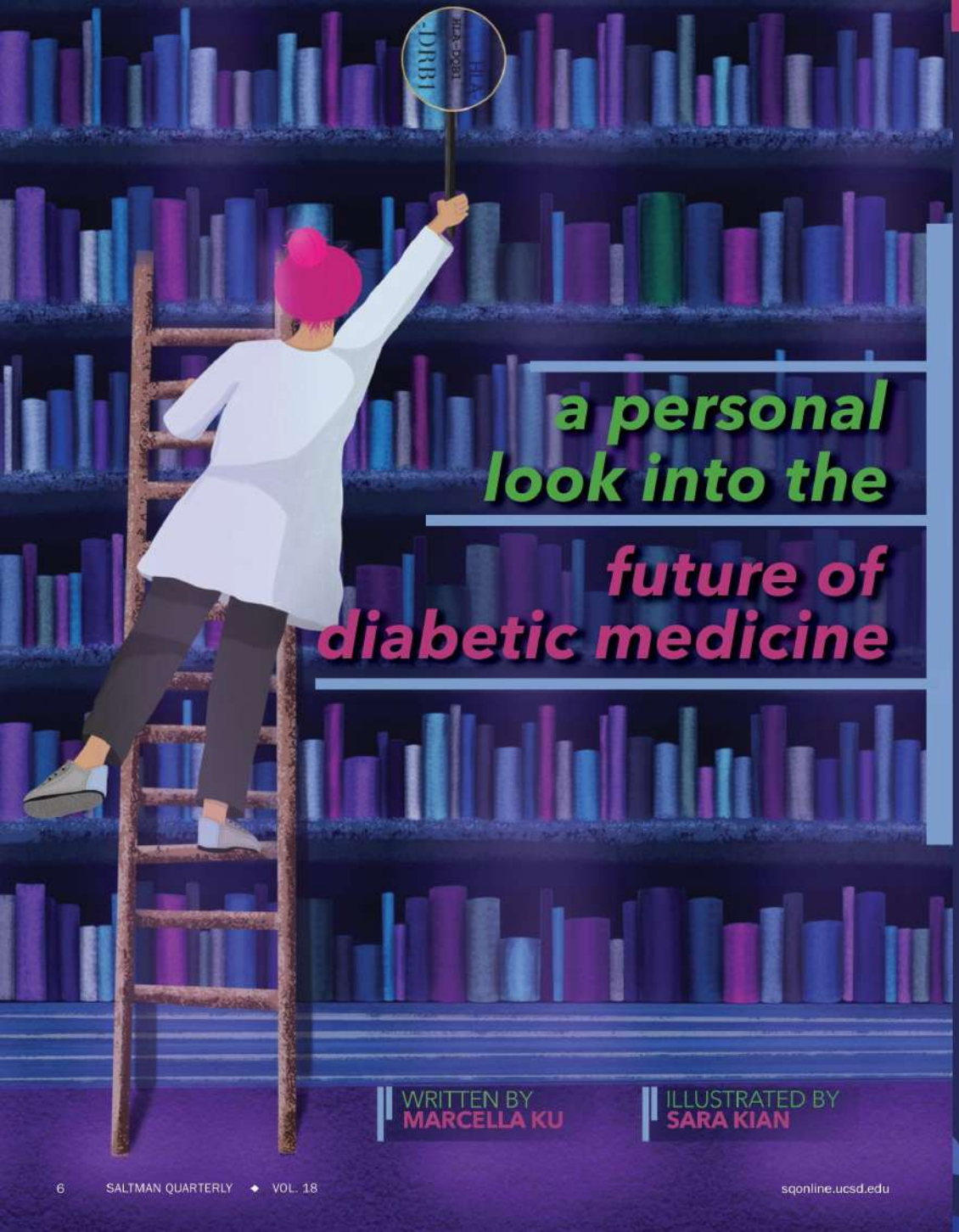
Features

UC San Diego is at the forefront of scientific discovery and exploration as a hub of biological research. The Features section highlights some of the groundbreaking work accomplished by researchers affiliated with the UC San Diego campus.

Male and female Nephila spiders (a.k.a. Banana spiders) hanging in a web in Panama City, Panama.

Photo by Bailey Munro



a personal look into the future of diabetic medicine

WRITTEN BY
MARCELLA KU

ILLUSTRATED BY
SARA KIAN

The fight against one of the nation's leading diseases has just gotten a lot more personal. Diabetes mellitus is a chronic disease that compromises the body's ability to regulate blood glucose, leading to a variety of complications that may worsen over time. Each person has a diverse health background, so every person's diabetic diagnosis falls within a broad range of conditions. Diabetes is not merely a single-faceted disease but rather is a broad spectrum of disease subtypes or less common versions of the disease.This spectrum of disease conditions in diabetes could contain unique diabetic cases specific to each person's genetic code and this poses a major problem for current treatment methods which provide more general therapies that may not be optimal for every individual. While still in infancy, personalized medicine is coming into the spotlight as a next generation cure alongside a shift in diabetic research towards the genomic level—the most individualized approach one can take—to deliver targeted therapies.

Dr. Amit Majithia, an Assistant Professor of Endocrinology at the Department of Medicine and Pediatrics at the UC San Diego School of Medicine, researches novel genetic methods of detection and treatment for insulin resistance.[1] Currently, he directs his efforts towards providing a more personalized therapeutic approach for type 2 diabetic patients. The Majithia lab uses a multi-disciplinary approach that combines computational bioinformatics and large-scale genomic techniques in order to further understand and classify genetic variants, or different versions of the same gene that vary in nucleotide sequence. The Majithia lab is specifically looking for genetic variants that are linked to an increase in insulin sensitivity by analyzing the functions of the genes and matching them to specific phenotypes through a parallel-line bioassay technique[1] which is an experimental technique that measures a target substance in various dilutions.[2] Through the functional characterizations of the missense genomic variants, a more efficient understanding of their impact on the function of genes related to diabetes can be made in order to provide deeper insights into diabetic treatment.

Currently, diabetic patients use traditional testing methods such as blood tests on a case-by-case basis when testing for the presence of potential, diabetically-linked variants. Rather than pursuing this old-fashioned route of testing, Dr. Majithia and his team have turned to the construction of a "lookup table" in which every possible mutation is synthesized and tested before it is discovered in a person. This is accomplished by using synthetic biological techniques and parallel cellular assays that are centered around identifying causes and susceptibilities of disease. The main genetic variants targeted are those that are hypothesized to correspond to an increase in insulin sensitivity through missense variations that produce proteins with altered functions.[1] Through this new, proposed research method, a patient who is diagnosed as either pre-diabetic or diabetic can have their DNA sample taken for whole genome sequencing. Unique genetic variants that are linked to increased insulin sensitivity[1] are identified from the patient's DNA sample, and functional characterization of these missense variants is done in order to determine the best course of treatment. As further mutations are discovered via patient samples, the "lookup table" enables researchers to cross-check previously unknown genetic variants in patients to synthetically tested variants, thereby quickening the timeline for clinical testing and treatment.

One of the genes the Majithia lab studied, peroxisome proliferator-activated receptor gamma (PPARG), contained certain mutations which were linked to an increased risk of type 2 diabetes and lipodystrophy, the abnormal loss and distribution of fat.[3] PPARG is one of many genes that were functionally classified in the Majithia lab in an effort to characterize and encode a table of all 9,595 single-amino acid substitutions found in peroxisome proliferator-activated receptor gamma (PPARγ), which is a nuclear receptor that is encoded by the PPARG gene.[3] Under Dr. Majithia's lab, fifty-seven other missense variants of PPARG were analyzed, out of which six variants were classified as pathogenic, or related to disease.[3] In other words, these six particular variants were correlated with a higher probability of susceptibility to lipodystrophy and type 2 diabetes. With the sequencing of more protein encoding sequences like PPARG, more missense variants are on track to be identified.The characterization of novel variants will ultimately provide researchers with a more comprehensive understanding of specific genes that are correlated with diabetes.

Researchers at Dr. Majithia's lab realized that current treatment and diagnostic techniques had the potential to provide a baseline for upgraded treatment and diagnostic technologies as they looked into the current mechanisms of continuous glucose monitoring (CGM) sensors. CGM readings are primarily used before and after meals in order to inform patients of their glucose levels.These readings allow patients to plan meals and insulin intake accordingly. Dr. Majithia noticed that current diabetic devices such as insulin pumps provide patients with constant information on blood glucose levels which could allow patients to increase their capacity to manage their diabetes. However, the standard CGM methods do not provide the most reliable and up-to-date results. Following this line of thought, the Majithia lab repurposed CGM sensors to provide further, reliable readings with consistent analysis of blood glucose levels that are now used in technologies for type 1 diabetic patients. This is done by improving the accuracy of CGM readings by showing a wider range of blood glucose readings that presents less restricted rate of change arrows.[4] Many current CGM devices' rate of change arrows are utilized

for convenience as they present rounded off number readings, and through repurposing these arrows, they can provide much more reliable information for patients to determine their insulin dosages.[4]

For type 2 diabetes, traditional testing currently involves a blood sampling technique that requires patients to prick their fingers throughout the day to obtain blood glucose readings. Similarly to the continuous monitoring technology in an EKG, a new method of diabetes monitoring for type 2 diabetic patients could potentially be developed that adopts the CGM method, in which patients monitor their glucose levels before and after meals. Instead of multiple finger pricks throughout the day, a single finger prick is sufficient for this newly improved blood test. Finger prick blood testing uses test strips that contain the enzyme glucose oxidase, which reacts with a patient's blood.[5] The reaction between blood and glucose oxidase generates an electric impulse that is measured by the blood glucose monitoring device; the more glucose there is in the blood, the greater the magnitude of the current (reflected as a numerical value in the device).[5] This blood glucose level number is taken together with measurements of blood pressure and cholesterol levels to yield a comprehensive reading that determines how much insulin a patient should inject daily. Thus, in utilizing an improved CGM method that is traditionally found in type 1 diabetic patients' technologies, type 2 diabetic patients may also be able to receive accurate, up-to-date readings in regards to their blood glucose levels and therefore be able to make better informed decisions in their everyday insulin treatment. It should be noted that treatment for type 2 diabetic patients does not focus solely on insulin administration but also can involve the use of CGMs to help patients make informed, lifestyle decisions.

There are also novel analytic methods being researched that focus on patterns hidden within blood glucose monitor data rather than on individual blood glucose values. Diagnostics and testing go hand in hand in the Majithia lab, where efforts are focused on developing more accurate and precise diagnostic tools that can identify more diabetic subtypes. Glucotypes are measurements of glucose variability patterns taken from comparisons of input from CGM systems and diabetic diagnostic measurements, such as daily insulin secretions and levels of insulin resistance.[6] These glucotypes are used to formulate meaningful diagnoses of specific type 2 diabetes subtypes and represent a form of technology that could provide each patient with a diagnosis of a specific diabetic subtype rather than a generic diagnosis of type 2. The Majithia lab analyzes genetically-linked and pharmacologically sourced blood glucose data from both non-diabetic and diabetic patients and uses algorithms to generate more personalized and accurate glucotypes for each individual. Subtyping also has the potential to streamline categorization of the complications that correlate with different diabetic subtypes by providing further specificity about the type of diabetes and its symptoms. Both of these developments will further clarify the understanding of diabetes and generate more personalized methods of treatment.

Genetic testing is another practice that can potentially provide personalized diagnoses of diabetes, thereby helping to illuminate once indistinguishable diabetic types for patients. Through previously mentioned methods, such as the Majithia lab's construction of a genomic 'lookup table', an individual may receive a more specific diagnosis of the subtype of diabetes they have. In specific diabetic cases that produce rare genetic diagnoses such as monogenic diabetes, or diabetes caused by a single point mutation, genetic testing can be used to categorize what subtype[7] of diabetes the individual has. In cases concerning newborn and young children, new genetic technologies are being employed to provide more accurate diabetic diagnoses such as the diabetic subtype maturity-onset diabetes of the young (MODY).[7] Oftentimes, patients with MODY are misdiagnosed with type 1 diabetes and placed on standard insulin treatments which are not effective courses of therapy for MODY. Genetic testing, however, is more likely to help elucidate a patient's specific condition, allowing patients to successfully switch from an insulin based treatment to a new class of drugs like sulfonylurea (SU), which are better suited to alleviate the complications of MODY. Personalized diagnosis through genetic testing is the first of many steps that will enable doctors to provide patients with more reliable descriptions of their specific forms of diabetes.

Personalized treatment for diabetes has already begun to take root in the pharmacogenomic sector. Pharmacogenomics is the study of how the genetic makeup of an individual reacts to certain drugs.[7] Every individual's genetic makeup is different, and thus each person will generate slightly different responses towards each drug. Pioneering work on more personalized pharmacogenomics has been aided by current research on genetic variants of diabetes, which take the form of genomic libraries. These massive tables of data, such as the PPARG table that Dr. Majithia and his colleagues created, provide researchers with comprehensive genotypic and phenotypic data that are useful for the detection of treatment response patterns and drug responsiveness in diverse groups of patients.[7] With more accurate genomic data that help tailor treatment for the individual, there could be decreased risks of adverse drug-related events, leading to more optimal treatment.

Personalized therapy is still in its early stages, and there is much more research and testing that needs to be done in order to implement it clinically. Despite the research that still needs to be conducted, the efforts of Dr. Majithia and his lab have advanced the progress towards technological and genomic advancement of personalized medicine for diabetes. With the proper biotechniques, diabetes research rooted in personalized medicine could establish a model for how to approach other diseases. Current treatments for diabetes are still far too generalized to provide optimized treatment for each individual. In taking a genetic, more personalized route, however, better treatment methods and more accurate diagnoses can be provided to patients. The future is personal and there is so much more to explore about the individuality of each body.

Figure 1: The Genetic Individuality of Diabetes
Diabetes is a unique disease that can be affected by a person's genetic makeup and influenced by their lifestyle and environmental factors. As such, an accurate diagnosis of diabetes goes beyond the general categorizations of Type 1 and Type 2.

Figure 2: Technological Diabetic Monitoring
Modern diabetic technologies use mobile apps that synchronize with continuous glucose monitoring patches to monitor blood glucose levels throughout the day.

References

1. Research | Majithia Lab | UC San Diego School of Medicine. medschoolucsdedu. [accessed 2021 Apr 21]. https://medschool.ucsd.edu/som/medicine/divisions/endocrinology/research/labs/majithia/research/Pages/default.aspx.
2. Stegmann DR. 2021 Feb 9. Parallel-line potency assays. PLA 30 – Software for Biostatistical Analysis. [accessed 2021 Apr 21]. https://www.bioassay.de/analytical-methods/experiments/parallel-line-potency-assays/.
3. Majithia AR, Tsuda B, Agostini M, Gnanapradeepan K, Rice R, Peloso G, Patel KA, Zhang X, Broekema MF, Patterson N, et al. 2016. Prospective functional classification of all possible missense variants in PPARG. *Nature Genetics*. 48(12):1570–1575. doi:10.1038/ng.3700.
4. Majithia AR, Wiltschko AB, Zheng H, Walford GA, Nathan DM. 2017. Rate of Change of Premeal Glucose Measured by Continuous Glucose Monitoring Predicts Postmeal Glycemic Excursions in Patients With Type 1 Diabetes: Implications for Therapy. *Journal of Diabetes Science and Technology*. 12(1):76–82. doi:10.1177/1932296817725756.
5. MIT School of Engineering |» How do glucometers work? 2011. *Mit Engineering*. https://engineering.mit.edu/engage/ask-an-engineer/how-do-glucometers-work/.
6. What Is Glucotyping? New Method of Detecting Glucose Dysregulation. 2018 Aug 18. *Diabetes In Control* A free weekly diabetes newsletter for Medical Professionals. [accessed 2021 Apr 21]. http://www.diabetesincontrol.com/what-is-glucotyping/
7. Xie F, Chan JC, Ma RC. 2018. Precision medicine in diabetes prevention, classification and management. *Journal of Diabetes Investigation*. 9(5):998–1015. doi:10.1111/jdi.12830.

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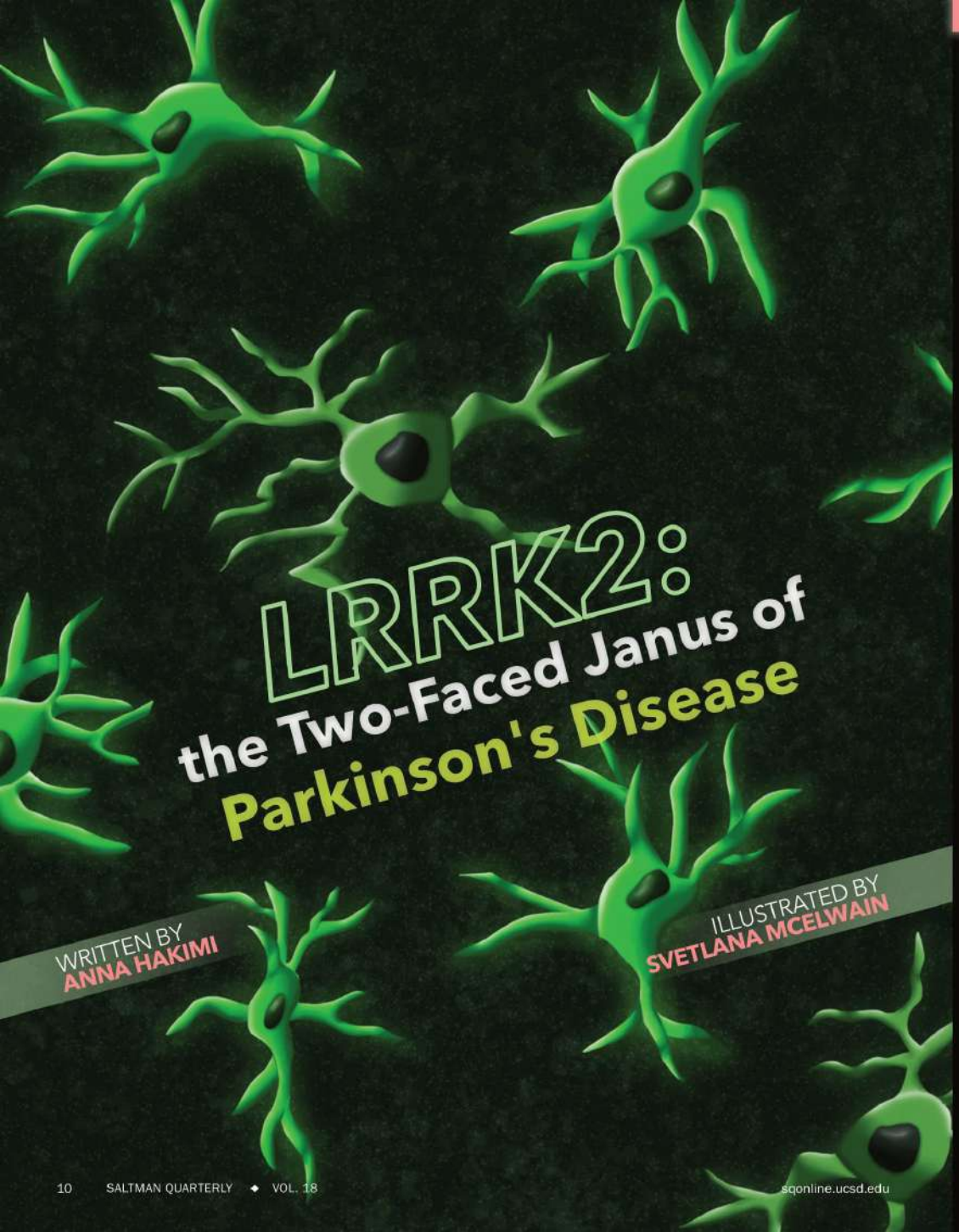


LRRK2: the Two-Faced Janus of Parkinson's Disease

WRITTEN BY
ANNA HAKIMI

ILLUSTRATED BY
SVETLANA MCELWAIN

The Reck-Peterson, Villa and Leschziner groups at UC San Diego discovered that the LRRK2 protein, a mutated subset in Parkinson's patients, has profound impacts on molecular transport in the cell and could influence the onset of Parkinson's disease by playing a role in substantia nigra neuronal death.

The gift of movement is granted to most of us at birth. However, this physical ability can be diminished by numerous pathologies, including the infamous Parkinson's disease. This particular motor disorder typically manifests during the later stages of one's life and consists primarily of an inability to elicit intentional movement. Normally, motor control is regulated by dopamine signaling between neurons in two regions of the brain: the basal ganglia and substantia nigra. However, when populations of substantia nigra neurons are destroyed, the basal ganglia neurons no longer receive these essential dopamine signals that permit movement initiation. As a result, patients with Parkinson's experience hypokinesia, or an inability to initiate and efficiently perform various motor tasks.

Current research on Parkinson's revolves around understanding the cause of substantia nigra neuron death. Researchers are currently examining the precise mechanics of neuronal death, as well as the role of supportive brain cells, known as glial cells, in maintaining the health of substantia nigra neurons. However, a novel and fascinating approach to elucidating Parkinson's explores the role of molecular transport in neuronal decay and may bring insight to future treatments for this devastating disease.

Molecular transport is vital for proper cellular function in all cell types. Materials must be delivered to certain locations in the cell in a timely manner since organelles and vesicles cannot passively move to the target cellular location. For this reason, vesicular or organellar cargoes are linked to molecular motors that physically travel along long filament polymers known as microtubules, which are commonly referred to as cellular highways. Molecular motors traffic their cargoes to target locations in the cell by traveling along these highways.

In neurons, numerous processes require molecular transport. For example, synaptic neurotransmitter release requires the trafficking of vesicles containing the neurotransmitter of interest to the axon terminal. Mitochondria are trafficked throughout neurons to ensure that the energy levels required to sustain neuronal function are met. Endoplasmic reticula act as crucial calcium stores in neurons and must be localized to areas that require calcium for signaling processes. Finally, molecular transport systems remove cellular toxins and damage in cells, so impeding molecular transport could prove lethal to neurons.

Molecular transport systems are especially important in neurons due to their elongated morphology. Materials are constantly shuttled between the neuronal cell body, or the soma, and axon terminals. The two types of molecular motors involved in the transport system are kinesin and dynein. Generally, kinesins move materials towards the negative end of the microtubule, from the soma to the axon terminals, while dyneins function in the reverse. Clearly, a lack of coordinated molecular transport impedes neuronal function and health.

A particular protein known as the Leucine Rich Repeat Kinase 2 (LRRK2) has been found to influence molecular transport and is mutated in a subset of patients with Parkinson's disease. The mysterious LRRK2 belongs to a group of enzymes known as kinases, which initiate cellular transduction signals by phosphorylating amino acids on other cellular proteins. Phosphorylation of proteins can cause a wide variety of effects, including changing protein-protein interactions. While LRRK2's normal function in a healthy cell is not yet known, the kinase is known to oligomerize, or form long protein chains by binding to other identical LRRK2 kinases. These LRRK2 oligomers can then wrap around microtubules in a helical manner.[1] Like the dual-faced Roman deity Janus, personified to preside over duality and transitions, LRRK2 function remains mysterious in both healthy and diseased cells.

Dr. Samara Reck-Peterson, Professor of Cellular and Molecular Biology and the Division of Biological Sciences at UC San Diego and a Howard Hughes Medical Institute Investigator, is currently exploring wildtype and mutant LRRK2's roles in cellular function and Parkinson's disease respectively. The investigation of LRRK2 has been an ongoing project between the Reck-Peterson group and several other UC San Diego research laboratories. LRRK2 oligomers were first observed by the laboratory of Dr. Mark Ellisman, and later, the laboratories of Dr. Andres Leschziner and Dr. Elizabeth Villa solved the three-dimensional structures of single LRRK2 subunits[1] and LRRK2 oligomers on microtubules respectively.[2] Using electron microscopy, microtubule-associated oligomers were resolved in the native cell environment and a high-resolution structure of LRRK2 in isolation was determined as well.

The LRRK2 kinase exists in two conformations: closed and open. In its closed conformation, the kinase is active and can phosphorylate its molecular targets. By comparing the structures of the microtubule-associated LRRK2 oligomers with LRRK2 in isolation, Drs. Leschziner and Villa's group discovered that the kinase domain was closed in the long linear oligomer chains that wrap around microtubules in a helical manner,[1,2] while Dr. Reck-Peterson's group discovered that these oligomeric chains form obstructions that

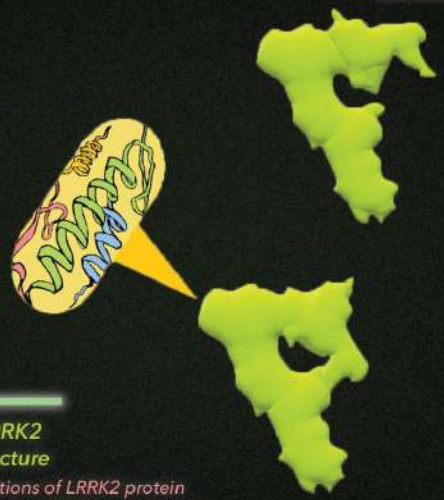
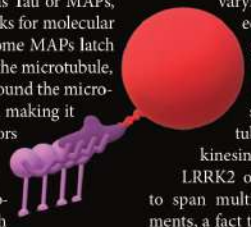


Figure 1. LRRK2 Protein Structure

Two conformations of LRRK2 protein monomer. Upper: LRRK2 in open and inactive conformation. Lower: LRRK2 in closed and active conformation.

can block molecular motor movement along a microtubule.[1] It is hypothesized that these oligomeric chains can perhaps obstruct molecular motors from delivering their cargoes to their target site in the cell, jeopardizing cellular health.

Other molecules linked to neurodegenerative diseases, such as Tau or MAPs, can also generate roadblocks for molecular motors. While Tau and some MAPs latch onto one small portion of the microtubule, LRRK2 oligomers wrap around the microtubule in a helical fashion, making it harder for molecular motors to step past them.[1]



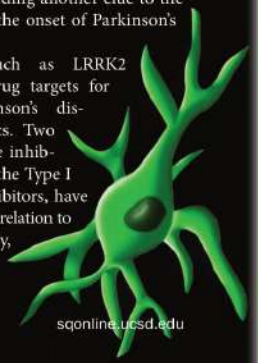
LRRK2 oligomers are also unique in that they obstruct the movement of both dynein and kinesin. While kinesin and dynein motors both transport cargoes along the same microtubular highways, their structural differences allow each motor to step along microtubules and navigate microtubular roadblocks in distinct ways. Microtubules themselves usually consist of thirteen protofilament chains interconnected together to form a hollow, tubular structure. Each protofilament is built from dimers of two subunits, alpha tubulin and beta tubulin.

Kinesin and dynein have different patterns of stepping along microtubules. Kinesin is composed of two identical motor heads connected by short linker chains to the central stalk of the motor complex. The kinesin motor heads take small alternating steps along a single microtubule protofilament, stepping from one tubulin dimer to the next. In contrast, the dynein motor is much larger, can take steps of varying sizes, and is not restricted to moving along a single protofilament. This greater flexibility in dynein's movement allows it to sidestep small and localized microtubular obstructions, which kinesin is unable to do.

LRRK2 oligomers are large enough to span multiple microtubule protofilaments, a fact that perhaps explains why it can so potently inhibit both dynein and kinesin. While normal LRRK2 can also form microtubular oligomers, four mutations in LRRK2 that are linked to Parkinson's disease correlate with the oligomer formation becoming more probable.[1] The exact mechanism of how these mutations impact LRRK2's ability to oligomerize is not yet known, though it is hypothesized that the mutated kinase may perhaps spend more time in its closed conformation. A growing theory on the cause of Parkinson's disease concerns microglia, or macrophage-like glial cells that work to remove and repair damaged parts of a neuron. These microglia cells release chemical signals into the extracellular fluid to accelerate neuronal repair mechanisms. However, in order for the signals to take effect, molecular motors are required to transport these signals within the microglia to the release terminals upon appropriate stimulation. If molecular transport is inhibited in the microglia that support adjacent substantia nigra neurons, damage could accumulate in these nigral cells and lead to their programmed death. Thus, some researchers apart from the Reck-Peterson group have begun to postulate that rather than a direct malfunction in the nigral neurons, microglial dysfunction may instead lead to the substantia nigra neuron death that is characteristic of Parkinson's disease.

LRRK2's role as a kinase is also being actively researched. Recently, Rab GTPases have been identified as LRRK2 phosphorylation targets.[1] There are about sixty types of Rab GTPases known in humans, and their unifying function is to signal for the fusion of lipid bilayer membranes. For example, Rab GTPases ensure the fusion of membranes during vesicular exocytosis, a process by which chemical messengers are released from a cell into the extracellular environment. Rab GTPases also mediate the linkage between a cargo-filled vesicle and molecular motor. In order to link a cargo to a motor, adaptor proteins are used to connect vesicle-linked Rabs to motors. One hypothesis currently being examined is whether the LRRK2 phosphorylation of Rabs alters the ability of the motors to link to their cargoes. An examination of this hypothesis will reveal whether LRRK2 impacts motor-directed cargo transport in ways other than the microtubular oligomer roadblocks in the cell, perhaps lending another clue to the kinase's role in the onset of Parkinson's disease.

Kinases such as LRRK2 are excellent drug targets for possible Parkinson's disease therapeutics. Two groups of kinase inhibitors, known as the Type I and Type II inhibitors, have been explored in relation to LRRK2. Generally, Type I inhibitors lock kinases in their closed, active conformation, while Type II inhibitors lock kinases in their open, inactive conformation. The inhibitors accomplish this by binding to the ATP-binding pocket at several conserved motifs either in the closed or open kinase, locking the kinase in the given conformation.[3]



Kinases are active in their closed conformation and inactive in their open conformation. Therefore, it was hypothesized that Type II inhibitors might rescue molecular motor movement on microtubules by keeping the kinase in its open and inactive conformation, inhibiting its ability to oligomerize. Dr. Reck-Peterson's lab directly tested this hypothesis by using pure proteins in vitro, and it was found that LRRK2 treated with Type II inhibitors no longer acted as a microtubular roadblock for kinesin and dynein motors. In contrast, Type I inhibitors caused the kinesin motor to be blocked at even higher levels by LRRK2 oligomers. The Reck-Peterson group also found that in eukaryotic cell culture, Type II inhibitors decrease LRRK2's ability to form oligomer filaments around microtubules.[1]

As the Reck-Peterson, Leschziner and Villa groups at UC San Diego look into the future of LRRK2 and Parkinson's research, many questions remain to be answered regarding the exact role of LRRK2 in Parkinson's development. The three labs, along with two collaborating labs in Germany, have recently received over seven million dollars in funding from Aligning Science Across Parkinson's Disease to explore this question. The groups plan to determine how both wild type and mutant LRRK2 impact intracellular trafficking, how the mutant may lead to Parkinson's disease, and how wild type LRRK2 functions in normal cells. It remains to be determined whether the mutated forms of LRRK2 disrupt the substantia nigra neurons by jeopardizing the health of the nigral neurons themselves, or the health of the supporting microglia. By understanding the role of the mysterious and two-faced LRRK2 in substantia nigra neuronal death, researchers will come yet another step closer to solving the puzzling mystery of Parkinson's disease and restoring patients' ability to efficiently move.

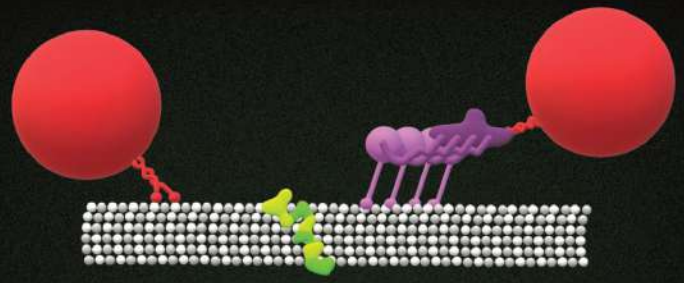


Figure 2. Microtubule: Highway of the Cell

Six microtubule protofilaments. LRRK2 oligomer (green) obstructs the movement of both dynein (purple) towards the positive end of the microtubule, and kinesin (red) towards the negative end. Kinesin motor heads are connected by short linkers to the central stalk of the motor complex, while the dynein motor domains exhibit larger movement flexibility.

References

1. Deniston CK, Salogiannis J, Mathea S, Snead DM, Lahiri I, Matyszewski M, Donosa O, Watanabe R, Böhning J, Shiau AK, Knapp S, Villa E, Reck-Peterson SL, Leschziner AE. 2020. Structure of LRRK2 in Parkinson's disease and model for microtubule interaction. *Nature*. 588(7837):344-349. doi: 10.1038/s41586-020-2673-2. Epub 2020 Aug 19. PMID: 32814344; PMCID: PMC7726071. https://pubmed.ncbi.nlm.nih.gov/32814344/
2. Watanabe R, Buschauer R, Böhning J, Audagnotto M, Lasker K, Lu TW, Boassa D, Taylor S, Villa E. 2020. The In Situ Structure of Parkinson's Disease-Linked LRRK2. *Cell*. 2020 Sep 17;182(6):1508-1518.e16. doi: 10.1016/j.cell.2020.08.004. Epub. PMID: 32783917; PMCID: PMC7869717. https://pubmed.ncbi.nlm.nih.gov/32783917/
3. Roskoski R. 2015. Classification of small molecule protein kinase inhibitors based upon the structures of their drug-enzyme complexes. *Elsevier*. http://www.brimr.org/Reprints/149.pdf

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botox: smoothening wrinkles soothing moods

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Common responses to stress include eating more ice cream, watching more TV shows, and the more physiologically relevant development of clenching every muscle in your body. Today, stress is seen as a well-established accompaniment to muscle tension.

The relationship between stress and muscle tension is multifaceted. While both are interlinked, a hypothesis called the facial feedback mechanism goes so far as to say that muscle tension can exacerbate feelings of stress. The Facial Feedback Hypothesis, first introduced by Charles Darwin in 1872, essentially proposed that facial expressions, which are manifestations of muscle contractions, have a direct effect on mood. Some people claim that if you smile enough, you will start to feel happy.[1] The facial feedback hypothesis supports this thought and also suggests the reverse: if you frown, tense up, or employ any other bodily expression associated with negative emotions, those negative emotions will likely be reflected in one's internal state or mood.[1] This suggests that the body has memories associated with certain muscle tensions. Because the body already associates stress-related muscle tension with negative affective states like stress and depression, continuing to tense our muscles even when we do not feel these negative emotions can send signals back to the brain, a development that can turn a positive mood negative.[2]

Researchers have opened new avenues to study the relationship between the facial feedback loop and depressive moods.[3] Promising research in this area has potentially found a way to alleviate depression through an unorthodox method of reducing muscle contractions—Botox. This novel treatment method involves the administration of Botox, which is a compound more widely known for its role in cosmetics rather than for its salutary applications.

Before discussing specific research methods, it is important to understand the characteristics and mechanisms involved in muscle contraction. For us, voluntary muscle contractions seemingly occur without much thought; if you intend to move your arm, your arm moves. However, every conscious movement is actually the result of an intricate series of steps. More specifically, the interactions between neurons and muscle fibers actually help initiate muscle movement.

Neurons communicate with muscle fibers through chemicals called neurotransmitters. One such neurotransmitter is acetylcholine. Here, acetylcholine triggers a series of changes that result in an action potential, or the rapid change of charge, in a muscle fiber, and ultimately leads to muscle contraction.

Neuronal axon terminals contain vesicles that carry acetylcholine. Acetylcholine gets transported from the axon terminal to the presynaptic membrane, where it diffuses across the synaptic cleft and binds to receptors on the muscle fiber. Here, the presynaptic membrane refers to the portion of the axon terminal that faces the muscle fiber, and the synaptic cleft is the space between a neuron and a muscle fiber. To first bring the acetylcholine-containing membranes closer to the presynaptic membrane, synaptotagmin, a protein in the axon terminal, pinches the basement membrane of the terminal towards the oncoming vesicle. The basement membrane itself contains proteins called syntaxin and SNAP-25. On the vesicle membranes is a protein called synaptobrevin. The interaction between syntaxin, SNAP-25, and synaptobrevin creates a helical bundle, forming the SNARE complex. The SNARE complex brings the vesicles containing acetylcholine closer to the presynaptic membrane, allowing for fusion between vesicle and membrane and for the exocytosis of acetylcholine out of the synaptic terminal and towards the muscle fiber, where it can then bind to receptors and initiate a series of changes that trigger a muscle action potential and ultimately a muscle contraction.[4]

Although muscle contractions serve an important function in voluntary physical movement, certain beauty products have moved to capitalize on muscle paralysis. At first glance, we may think that all paralysis is medically undesirable; after all, it takes away a muscle's ability to contract, and a body's ability to move.

A certain form of flaccid paralysis, however, represents an essential form of treatment in the cosmetic industry, and even young people are keen to undergo such treatments as preventative measures. To achieve results, many are using the botulinum neurotoxin, commonly known as Botox. Botox is a neurotoxin that interferes with the formation of the SNARE complex, hinders acetylcholine release, and impairs muscle contraction. The result is a reduced appearance of wrinkles in the skin in the forehead, around the eyes, and above the upper lip. Stopping muscle contraction is even a useful tool outside of the beauty industry, as Botox can be used to treat muscle twitches, migraines, and hyperhidrosis.[5]

Botulinum toxin is a neurotoxin that prevents the formation of a functional SNARE complex. Different isotopes of this toxin target different SNARE proteins, but the end result is similar: inhibited ability to contract muscle fibers. Without a SNARE complex present, acetylcholine containing vesicles are prevented from fusing with the synaptic terminal membrane. Consequently, after Botox administration, there is no acetylcholine released from the neuron, and muscle contraction does not occur.

Botulinum toxin was first isolated from a bacterium called *Clostridium botulinum* found on incorrectly preserved food, and has been FDA approved as a medication since 1989. Though it has existed as a medical treatment for quite some

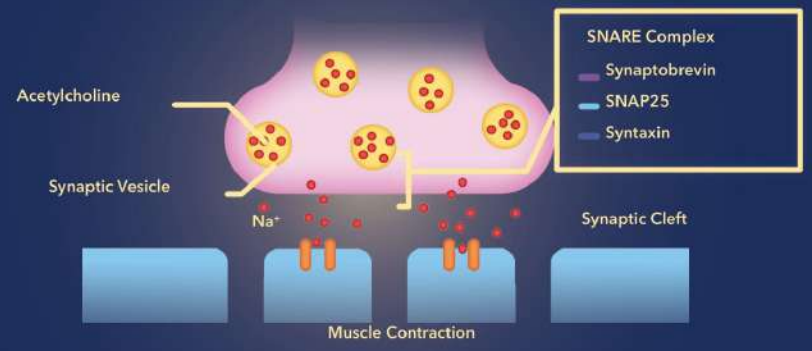

Figure 1. The Neuromuscular Junction
Acetylcholine from synaptic vesicles travels across the synaptic cleft, triggering a series of events that culminates in muscle contraction.

time, researchers at UC San Diego like Dr. Ruben Abagyan are still discovering novel ways to use botox as a therapeutic—namely, as a potential treatment for depression.[6]

Dr. Abagyan's research recalls the connection between muscle tension and mood that is bridged by the facial feedback hypothesis. This research builds on the notion that if muscles can no longer contract or remain tense as they do when the body is in depressive and stressful states, then perhaps the mind will also no longer receive signals that it should be depressed or stressed. Thus, Dr. Abagyan thought interrupting the facial feedback loop might help alleviate depression.

One way to stop such frown-associated facial contractions is through the use of Botox. Because Botox can inhibit muscle contraction, the injection of Botox into targeted facial locations can physically prevent individuals from frowning or wrinkling their forehead, both actions which manifest from tensed up muscles. This may work to reduce the negative effects of muscle tension, and may work to alleviate depressive emotions. On a more general level, Dr. Abagyan also thought that if facial muscles provide feedback that affects mood and emotions, it is possible that the contraction and tension of muscles throughout the body could also influence emotional states. Theoretically, a negative whole body state could contribute to a depression-enhancing feedback effect in the mind.

In his study, Dr. Abagyan injected Botox in patients who had been clinically diagnosed with depression. Across various patients, Botox was specifically administered into *corrugator* and *procerus muscles* (which produce the "angry wrinkle" on the forehead), upper and lower limbs, neck and eyelid muscles, or into the urinary bladder.

After six weeks, this placebo-controlled study showed that patients given a one-time Botox injection showed an improved score on the Hamilton Depression Rating Scale by an average of 47.1%, including patients who had taken prior depression medications with no satisfactory results. In contrast to these results, the placebo group showed only a 9.2% score improvement on average. Additionally, while current treatments for depression work differently for each patient and can take up to 4 to 6 weeks to become fully effective, reported antidepressant effects of Botox directly followed Botox administration. Results were self reported according to the Hamilton Depression Rating Scale, a 57-point scoring system that checks for symptoms of depression.[6]

The specific location of the Botox injection was found to be somewhat insignificant: patients reported similar anti-depressant effects whether they received the injection in the face, limbs, or other areas of the body. This suggests that though Dr. Abagyan's initial focus was to target facial muscles, preventing whole body muscle contractions produced similar antidepressant effects. Among the hypothesized explanations for these results is the facial feedback mechanism. It is possible that by reducing the amount of bodily tension from contracted muscles in the body and limbs, fewer signals that encourage negative thoughts are sent back to the brain. If this is indeed the mechanism by which depressive symptoms were alleviated, data may support Dr. Abagyan's belief that disrupting the Facial Feedback Loop stops the circularity of negative thoughts and tensed muscles.

One criticism of this treatment is that instead of treating depression via the facial feedback hypothesis and associated body muscle tension pathways, Botox mitigates the condition by removing either cosmetic sources of depression or alleviating symptoms caused by chronic pain. In other words, instead of treating depression by disrupting the facial feedback loop, Dr. Abagyan may have simply alleviated feelings of negative self-worth associated with common signs of aging or symptoms of chronic pain that decrease quality of life.

The nature of the experiments involving Botox poses unique challenges for data evaluation and collection. For instance, blind studies are impossible to carry out as the administration of Botox can change a patient's face drastically. So far, only firsthand accounts have been used to gauge efficacy. While this is not a foolproof method of determining effectiveness, accounts can be checked against statements given by other patients in control or placebo groups.

Depression is a major problem that afflicts many adults today. Because current treatments are often costly and require more than a month to reach full efficacy, it becomes increasingly important to research new avenues of antidepressant therapy. Current depression treatments have also been associated with a myriad of unwanted side effects, a development that can make the process of treating depression disheartening.[7] This preliminary study carried out by Dr. Abagyan shows the potential of Botox as a relief for depressive symptoms, but further research will need to be conducted in order to demonstrate conclusively whether or not Botox can enter the field as a viable treatment for depression. Factors such as cost, side effects, and patient reluctance to administer Botox also need to be considered if any serious advancements are to take place.

Due to the preliminary nature of Dr. Abagyan's studies and the prominent role patient self-reporting occupied in the experimental conclusions, the most we can say concerning Botox is that its administration is correlated with antidepressant effects. So far, however, Botox has shown a promising start as an unorthodox method of treatment as it seems to be at least somewhat helpful to some suffering with depression. Additional rigorous studies examining botox's capacity to mitigate depression could lay the groundwork for an unconventional line of treatment for depression. Given the mounting stresses of daily life, it is now more important than ever to find antidepressant treatments that actually work.

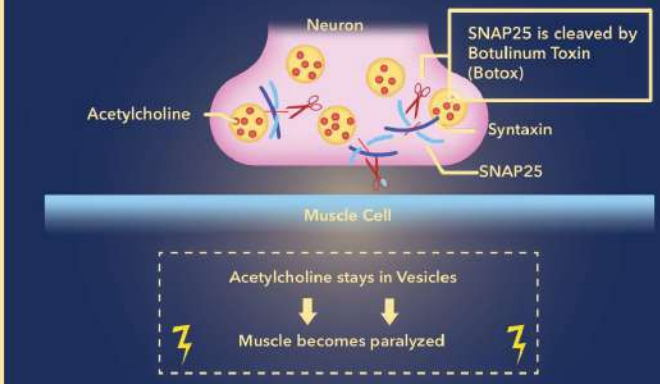

Figure 2. Botox in the Neuromuscular Junction
Botox inhibits the release of acetylcholine by disrupting the formation of the SNARE complex. This ultimately impairs muscle contraction.

Disclaimer: Dr. Ruben Abagyan is the co-founder of Molsoft, LLC, a drug discovery company, and owns equity.

References

1. Söderkvist, S., Ohlén, K., & Dimberg, U. How the Experience of Emotion is Modulated by Facial Feedback. *Journal of Nonverbal Behavior* 42, 129-151 (2018).
2. Jacobsen, L. N. et al. Bodily symptoms in moderate and severe depression. *Nord J Psychiatry* 60, 294-298 (2006).
3. Finzi, E., Rosenthal, N.E. Emotional proprioception: Treatment of depression with afferent facial feedback. *Journal of Psychiatric Research* (2016), doi: 10.1016/j.jpsychires.2016.06.009..
4. Washbourne, P. et al. Genetic ablation of the t-SNARE SNAP-25 distinguishes mechanisms of neuroexocytosis. *Nature Neuroscience* 5, 19-26 (2002).
5. Bhidayasiri, R., Truong, D. Expanding use of botulinum toxin. *Journal of the Neurological Sciences* 235, 1-9 (2005).
6. Makunts, T., Wollmer, M.A., & Abagyan, R. Postmarking safety surveillance data reveals antidepressant effects of botulinum toxin across various indications and injection sites. *Scientific Reports* 10, 12851 (2020).
7. Nalçakan, A.D. et al. Antidepressant awareness and stigmatizing attitudes toward depression and antidepressants, a comparison between first and sixth-year medical students. *International Journal of Social Psychiatry* (2021), doi: 10.1177/0020764020985545

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FISHING FOR GENOMES

targeting population genetics

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Successive generations of organisms may experience many changes in their physiology, behavior, and development. While most traits, such as beak shape or coat color, can be perceived by the human eye, the actual changes that produce variation in these traits occur at a molecular level.[1] These molecular differences can be used in genetic engineering to confer adaptive advantages for organism survival.[2] Researchers who study population genetics compare the genetics between organisms of interest to understand the molecular forces driving evolutionary change. These studies generally involve assessing the differences in DNA in a species over time and correlating these differences with variations in phenotype and variable survival rates.[3]

The Rennison Lab at the Ecology, Behavior and Evolution Section within the Division of Biological Sciences at UC San Diego aims to examine model systems that highlight the genetic differences in evolution. Dr. Andreas Haerer, a post-doctoral researcher in the Rennison Lab, focuses on studying alterations in the gut microbiome and how they affect ecology and evolution of the host. He has published a paper characterizing the relationship between gut microbiome changes and the parallel evolution of the hosts. Parallel evolution is the process by which similar traits arise independently of one another.

One of his studies examines the relationship between diets of different fish species and the differences in their gut microbiomes. In his controlled experiment, Dr. Haerer aimed to identify changes in diet and habitat that would result in changes of the gut microbiome of cichlid fish. Initially, the ancestors of the cichlid fish colonized the same lake. However, the fish were eventually separated into two different lake environments with variable conditions. Within these lakes, ecological divergence and speciation occurred as a result of adaptation to different niches. Over several generations, the shore water fish and the open water fish developed distinct gut microbiomes. The fish in these respective environments additionally have habitat-specific diets which are also associated with distinctive gut microbiomes. Diet-specific microbes have the potential to modulate digestion, intake of food, and health; as such, these respective microbes may confer selective advantages on these fish, such as more efficient processing of food and resistance to disease.[3] From these initial observations, Dr. Haerer hypothesized that parallel changes in the gut microbiomes of the fish across the lakes are associated with parallel changes in diet and habitat.

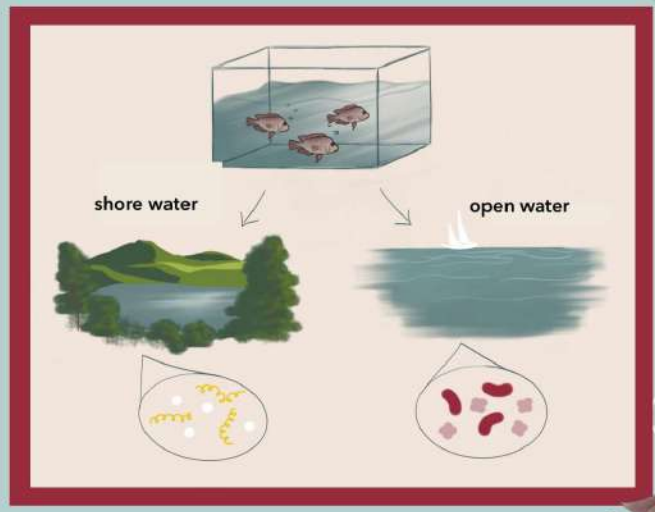
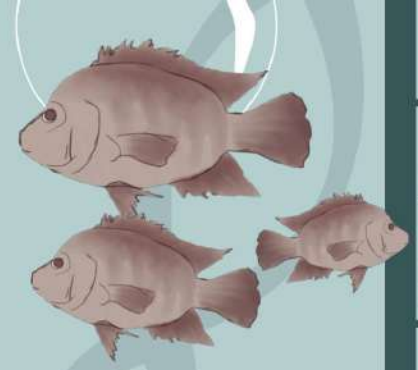


Figure 1. Correlations between microbial composition, diet, and habitat

Fish that initially originated from the same environment diverged into separate lake environments with distinct conditions. Dr. Haerer hypothesized that parallel changes in diet and habitat are associated with parallel changes in the gut microbiomes of the fish.



To test this hypothesis, Dr. Haerer and his team traveled to Nicaragua to collect fish from local lakes as samples for the study. Dr. Haerer and colleagues used a pair-wise study to analyze the fish in pairs: fish from each lake were compared to control fish in external analyses to assess whether the lake and diet resulted in a significant difference in microbial composition between the fish. The team used 16s rRNA sequencing to identify microbes based on the unique sequences of the 16s rRNA gene, which is highly conserved across microbial species. In addition to collecting fish samples from Nicaragua, Dr. Haerer obtained water samples to ensure the respective microbes in the surrounding environment of the fish could be determined. After sequencing the fish gut samples to characterize the microbial composition of the fish, Dr. Haerer analyzed the fish diets through stable isotope ratio analysis. This type of analysis identifies nitrogen and carbon isotope data, which can indicate where the fish feed in the lake in a three month period—by the shore versus in the open water.[4]

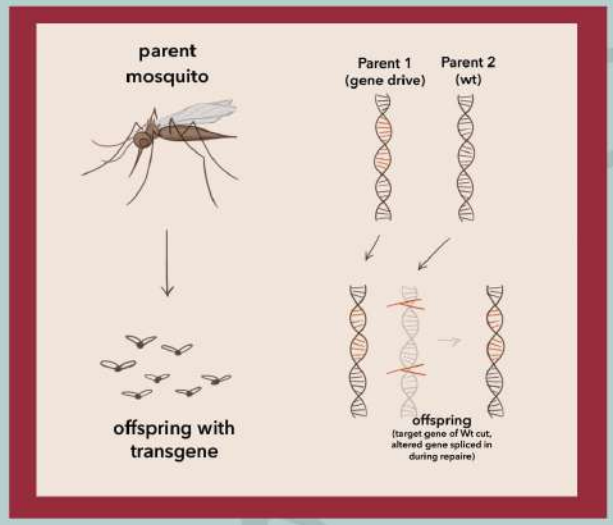
From the analyses, Dr. Haerer was able to identify a positive correlation between differences in diet and the variation in gut microbial composition. While Dr. Haerer found differences in the gut microbiota across species of fish and correlations between the gut microbiome and diet, his team did not find strong evidence for parallelism of gut microbiota changes. As such, he concluded that perhaps there was insufficient differentiation in diet to promote alterations in gut microbial composition.

The research conducted by the Rennison Lab contributes to the understanding of the evolution of organismal traits. In the future, the Rennison Lab hopes to continue pioneering research to explore the mechanisms by which microbes can provide evolutionary benefits to their hosts and how microbes subsequently impact their hosts' evolutionary trajectory.

Understanding the genetic changes responsible for phenotypic change can be important for preserving a species or aiding in preventing the spread of disease. The Akbari Lab at UC San Diego is particularly interested in mitigating the transmission of malaria, which is often spread by mosquitoes. Dr. Andie Smidler, a researcher in the Akbari Lab, is currently using population genetics to combat malaria by making mosquitoes incapable of transmitting the disease. The Akbari Lab uses the CRISPR/Cas9 system to study malaria. CRISPR/Cas9 is a form of genetic engineering technology that enables the genome to be edited or altered.

Figure 2. Gene drives propagate desired genetic changes to future generations

A gene drive serves to convert heterozygotes to homozygotes for the gene of choice such that the desired gene is always passed on to the offspring. The gene drive is introduced into selectively mated organisms. It contains guide RNA, which tags certain sections of DNA to be cut. The Cas9 protein subsequently cuts the double stranded DNA at a specific recognition sequence.



Although utilizing the novel CRISPR/Cas9 system opens a myriad of possibilities, there are many challenges to genetic engineering research, including ethical considerations, practical experimental issues, and environmental effects. The effects of releasing organisms genetically modified with CRISPR/Cas9 into the environment are relatively unstudied. As such, the researchers took ethical and practical considerations into account when designing their experiment. In their studies, researchers at the Akbari Lab found that there are many different ways to reduce the spread of malaria.

One of these methods involves introducing mosquitoes to a gene that has the ability to kill the malaria parasite; however, in this case, the researchers found that all mosquitoes must be introduced to this specific gene in order to eradicate malaria. In order to increase gene transmission, the researchers introduced a gene drive, a system which uses aspects of the CRISPR/Cas9 system on a larger scale, into the offspring of selectively mated mosquitos. The gene drive itself contains the Cas9 protein, which cuts the double stranded DNA at a specific recognition sequence. This system also contains the guide RNA, which tags certain sections of DNA to be cut, and homology arms, which facilitate chromosome recombination and interactions between the gene drive and organism DNA. A gene drive serves to convert heterozygotes to homozygotes for the gene of choice such that the desired gene is always passed on when present.

Another technique for eradicating malaria involves a sterilization technique to suppress mosquito populations. Under normal circumstances, female mosquitoes only mate once and are unable to reproduce after that initial mating.[5] By sterilizing the male mosquitoes through targeted radiation, thereby ensuring no offspring are produced, Dr. Smidler hypothesized that future generations of mosquitoes would be suppressed, which would reduce the spread of malaria. Dr. Smidler also found that the CRISPR/Cas9 genetics engineering system can be used to cut the gene associated with fertility in male mosquitoes, thus sterilizing them.

However, this system also brings up ethical concerns, as eliminating the progression of a species may disrupt natural selection and may have unintended consequences on the ecosystem. These consequences may include species extinction, the elimination of a source of food for other organisms, and the proliferation of pests that mosquitoes may consume. Moreover, there remain concerns regarding the safety and feasibility of genomically edited organisms. Organisms with altered genes may not be able to survive in their natural habitat and may cause harmful effects on the environment and other organisms.[6,7]

Additionally, simply sterilizing male mosquitoes may not address the issue of malaria completely, as female mosquitoes still retain the ability to bite others and spread malaria on their own. To combat this problem, the Akbari Lab plans to use a gene drive to render the gene associated with malaria nonfunctional in female mosquitoes, in order to reduce the spread of this deadly disease. By mitigating malaria with a multifaceted approach, the Akbari Lab hopes to eliminate malaria by reducing the genetic material that is passed onto offspring and preventing transmission of malaria from female mosquitoes.

Genetic variation can be used in a broad scope of applications, including eradication of progressive diseases. By combining studies of genetic, immunological, and microbial differences with methods that harness these findings on a larger scale, we can use population genetics to reduce both deadly diseases and their associated mortalities. We can also increase the survival rates of certain protected species by understanding their evolutionary trajectories. By examining the changes and hereditary nature of the microbiomes in fish species over time, Dr. Haerer and the Rennison Lab were able to identify the molecular mechanisms that affected ecosystem dynamics. Similarly, the Akbari Lab was able to harness molecular and population genetics mechanisms to investigate methods through which ecosystem dynamics can be altered.

References

1. Bonsall M, Charlesworth B. 2010. Genetics and the causes of evolution: 150 years of progress since Darwin. Philosophical transactions of the *Royal Society of London. Series B, Biological sciences*, 365(1552), 2427–2429.
2. Karalis DT, Karalis T, Karalis S, Kleisiari AS. 2020. Genetically Modified Products, Perspectives and Challenges. *Cureus*, 12(3), e7306.
3. Valdes AM, Walter J, Segal E, Spector TD. 2018. Role of the gut microbiota in nutrition and health. *BMJ*, 361:k2179.
4. Zimmo S, Blanco J, Nebel S. 2012. The Use of Stable Isotopes in the Study of Animal Migration. *Nature Education Knowledge* 3(12):3.
5. Degner EC, Harrington LC. 2016. Polyandry Depends on Postmating Time Interval in the Dengue Vector Aedes aegypti. *The American Journal of Tropical Medicine and Hygiene*, 94(4), 780–785.
6. Conrad DF, Hurles ME. 2007. The population genetics of structural variation. *Nature Genetics*, 39 (7 Suppl), S30–S36.
7. Caplan AL, Parent B, Shen M, Plunkett C. 2015. No time to waste—the ethical challenges created by CRISPR. *EMBO Rep*, 16:1421-1426.

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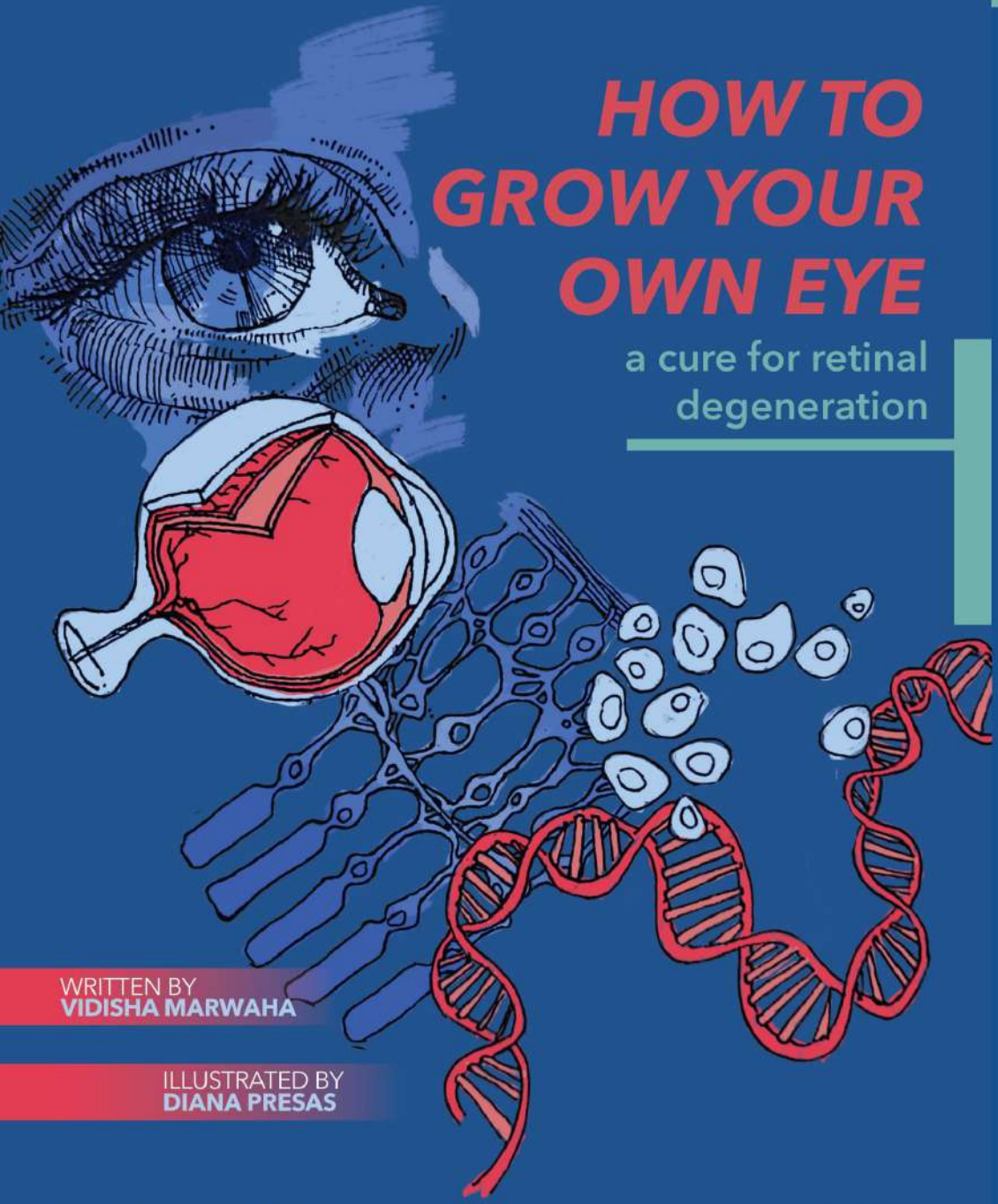

HOW TO GROW YOUR OWN EYE

a cure for retinal degeneration

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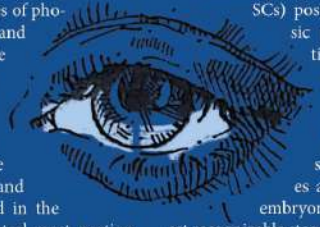
DIANA PRESAS

Over 1.3 billion people are afflicted with some form of vision impairment, which can mean these individuals require glasses or have a level of blindness. While the decline of vision with age is generally considered to be a naturally occurring and inevitable process, not all instances of vision impairment can be dismissed as products of aging. Instead, some afflictions result from various pathologies and conditions that develop in the eye. Retinal diseases are a class of ocular diseases that can damage the retina, the thin layer of tissue located at the back of the eyeball. Retinal diseases are relatively common and affect over 200,000 people in the United States alone. Retinal problems are very serious because if left untreated, they can lead to permanent blindness.[1]

Before expanding on retinal diseases, it is important to characterize the retina and understand how it functions. In the retina, light signals are transformed into electrical signals. Various interconnected cell types, such as photoreceptor cells, bipolar cells, horizontal cells, and retinal ganglion cells, work together to accomplish this key process. The retina sends the information collected by these cells through the optic nerve to the brain, a process that enables sight. In the human retina, two types of photoreceptor cells, rods and cones, are responsible for dim light vision and daylight vision (including color), respectively. Rods are located mainly in the peripheral retina, and cones are concentrated in the macula, the small, central-most portion of the retina that provides high-resolution vision. Photoreceptor cells transmit visual signals to bipolar cells which then pass these messages on to retinal ganglion cells. Ultimately, all the signals are gathered in the optic nerve and transmitted to the brain.2 Retinal degeneration is a type of progressive neurologic disorder that can be caused by genetic mutations, environmental or pathologic retinal damage, or a combination of both. This group of disorders is characterized by different types of retinal cell loss. Age-related macular degeneration and retinitis pigmentosa could involve a reduction in the retinal pigment epithelium or photoreceptor cells, while glaucoma involves retinal ganglion cell death.[3]

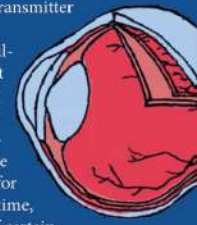


Currently, the process of retinal degeneration is both incurable and not completely understood. However, Karl Wahlin, Assistant Professor of Ophthalmology at UC San Diego, aims to study the root causes of retinal degenerative diseases by experimenting with stem cell-derived retina tissue. Dr. Wahlin's research explores the directed differentiation of pluripotent stem cells (PSCs), naïve cells that have the ability to mature into any one of numerous cell types, and their application in examining retinal development and disease. One subsidiary but vital aim of Dr. Wahlin and his lab is to produce fully functional retinal structures from PSCs.

Human pluripotent stem cells (hPSCs) possess two key intrinsic properties that distinguish them from all other cell types. First, they display the potential to differentiate into all somatic cell lineages and even developing embryonic tissue. In its earliest recognizable stage of growth, developing embryonic cells are capable of becoming tissue or even multi-layered organs. Secondly, hPSCs are able to maintain long telomeres, regions of nucleic acid that protectively tail DNA, allowing hPSCs to be replicatively immortal and making them a reliable, replenishable source of cells for differentiation and translational research.[4] Thus, PSCs, characterized by their unlimited proliferation capacity and ability to give rise to any cell type in the body, are a promising source for cell replacement therapy. Researchers at the Wahlin lab evaluated the directed differentiation of hPSC-derived retinal cells. Through their efforts, the researchers found that hPSCs can differentiate into retinal pigment epithelium, retinal neurons, and photoreceptor cells. In other words, which can organize into self-forming, multi-layered retinal tissues, essentially giving rise to their own 3D-mini-retinas.

During embryogenesis, the eye field first appears as an optic vesicle originating from the diencephalon. The distal tip then inverts itself to form a double-layered optic cup, with the outer layer forming the retinal pigment epithelium and the inner layer becoming the neural retina.[5] Rather than optic cups, the Wahlin lab uses stem cell-derived 3D-retinas that are single-layered sheets of the neuroepithelium, which are similar to optic vesicles. These lack both an optic stalk and an adjacent retinal pigment epithelium. As these vesicles mature, they are simply referred to as "retina cups" or "mini-retinas". These 3D-mini-retinas offer exciting opportunities to study the detailed mechanisms of retinal degeneration and provide new models for drug discovery and cell-based therapeutics.

The Wahlin lab makes use of pre-existing procedures to develop conditions that support the growth of these mini-retinas. However, they have modified what is known as the forced aggregate protocol, a method for cultivating stem cell tissue, for generating hPSC-derived 3D-retinas. At Wahlin lab, the protocol was modified for growth medium composition, O_2 concentration, and aggregate size. The method followed at the Wahlin lab lead to floating 3D-optic vesicle-like structures within twelve days, and in long-term cultures, leads to advanced photoreceptor development, including rod and cone outer segments and neurotransmitter expression.



Retinal cups are cultured in three different cell lines. The cell line is a general term that applies to a defined population of cells that can be maintained in culture for an extended period of time, retaining the stability of certain

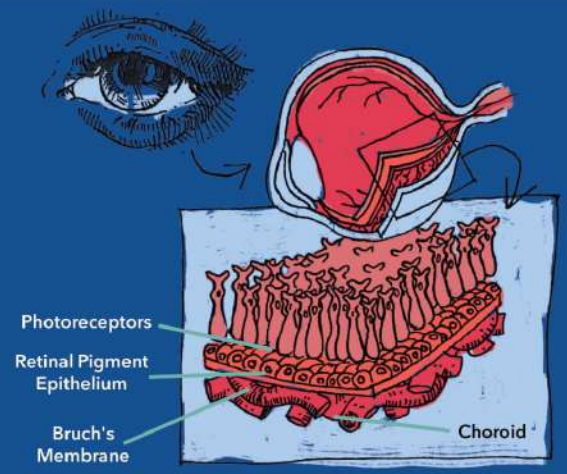


Figure 1. Layers of the Retina

Four layers of the retina are depicted here: photoreceptors, which include both rod cells and cone cells, the retinal pigment epithelium, Bruch's membrane, and the choroid.

phenotypes and functions. Cell lines are usually clonal, meaning that the entire population originates from a single common ancestor cell.[5] Because of the clonal nature of cell lines, researchers avoided cultivating cell-line-specific structures as such structures would create biases in the composition of the tissue. Thus, in order to ensure that the optic vesicle formation was not cell-line specific, researchers worked in parallel with three hPSC lines: the IMR90.4, EP1iPSC, and H7 ESC lines.

The generation of moderately sized optic vesicles takes seventeen days. The time taken to cultivate vesicles was the first step researchers worked to optimize. Based on the hypothesis that growth medium composition, O_2 concentration, and aggregate size of optic vesicle could each impact cell differentiation, the researchers at the Wahlin Lab systematically optimized each of those parameters.

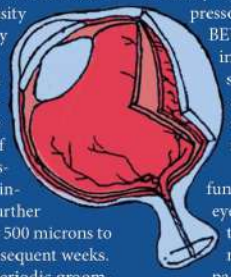
In regards to the expansion media, cells were cultured in a growth medium solution that was designed to support the expansion of a population of microorganisms or cells via proliferation. A Neural Induction Medium is a defined, serum-free medium for the neural induction of human embryonic stem cells and induced PSCs. This medium enables the highly efficient generation of neural progenitor cells, the precursor cells to retinal neurons. To optimize the medium composition, the lab found a serum-free formulation optimized to grow and maintain undifferentiated embryonic stem cells, which ended up being valuable in supporting vesicle formation.

Next, the researchers at Wahlin lab sought to optimize O_2 concentration. They found that hypoxia, or low levels of oxygen, can improve the survival, pluripotency, and proliferation of hPSCs. Relative to aggregates initiated in 20% O_2 (normal oxygen levels or normoxia), aggregates maintained in 5% O_2 (hypoxia) conditions for an additional day demonstrated increased viability. By day eight, hypoxic aggregates had more vesicles per aggregate and were larger in size than their normoxic counterparts.

Finally, the researchers examined the concentrations at which stem cells could be cultured. The vesicles were ultimately maintained at low density since at high density they often coalesced into caterpillar-like chains with necrotic cores. Poor quality vesicles with an opaque appearance or with signs of necrosis were regularly discarded. Vesicles that were insufficiently excised were further trimmed to approximately 500 microns to prevent overgrowth in subsequent weeks.

After one month of periodic grooming in the form of removal of non-retinal cup-like structures and trimming of overgrown vesicles, the 3D-translucent retinal cups appeared relatively homogeneous, with minor differences in shape and size.

The next step in the process of producing fully functional retinal structures from pluripotent stem cells was to check for necessary elements of a retina in the retinal cups: an important feature in the retina is the synaptic ribbons. Release of the neurotransmitter glutamate at the ribbon synapse facilitates information transfer from photoreceptor cells to bipolar cells. Conventional neurons encode information by changes in the rate of action potentials, but for complex senses like vision, this is not sufficient. Ribbon synapses enable neurons to transmit light signals over a dynamic range of several orders of magnitude in intensity. Synaptic ribbons are found in mature rods and cones and are essential for retinal function. In the outer plexiform layer of the retina, ribbons form a tripartite junction with bipolar and horizontal cell dendrites.[5] In order to ensure that the presence of these structures was preserved in the retinal cups, an immunohistochemistry (IHC) test was conducted for postsynaptic density-95 (PSD95) and C-terminal binding protein (CtBP2). IHC stainings use the principle of antibody-antigen binding specificity to detect target proteins in cells. The CtBP2 antibody recognizes a transcriptional repressor and a synaptic protein, RIBEYE. The presence of RIBEYE indicates the presence of ribbon synapses since RIBEYE interactions are required for the synaptic ribbon to bind to the retina.



Moreover, the synaptic function of photoreceptors in the eyecup was assessed using electrophysiological recordings to measure total membrane capacitance. Photoreceptors were clamped at -65 mV and then depolarized to -10 mV to trigger vesicle release. Stimulus-evoked spikes in capacitance unaccompanied by simultaneous changes in either the membrane or series resistances were taken as evidence of vesicle exocytosis.

The retinal cups were also tested for All-trans retinoic acid (ATRA). Although ATRA has well-documented boosts in retinal development, its prolonged presence can hamper maturation. Based on this, researchers treated retinal cups with 500 nM ATRA every day from 20 days after retina formation until 120 days before photoreceptor maturation. Shortly thereafter, small sprouts began to emerge from the retinal cup surface, and after 160 days, photoreceptor outer segment-like structures were present. Variability in the length and onset of cup formation was observed. The segment growth was self-limiting, reaching a terminal length of approximately 39 µm, a range similar to that reported in vivo. Unlike the cone-rich fovea in vivo, rods and cones in retinal cups were generally evenly dispersed across the mini-retinas. The retinal pigment epithelium also frequently grew on retinal cups opposite to the retina or as independent spheroids with honeycomb-shaped polygonal morphologies.[5]

hPSCs have become invaluable tools to investigate the different stages of retinal degeneration and to help tailor therapeutic strategies of the future. Although the Wahlin Lab's retinal cups are not prepared to serve as a cure for retinal degenerative diseases without thorough testing on animals and/or humans, the research conducted to develop the retinal cups is a significant stepping stone on our path towards a cure. While the retinal cups do not cater to all retinal degenerative diseases, they do provide a good model for researchers to study the causes of these diseases. In cases of advanced degeneration, hPSCs that have differentiated into retinal cells could be transplanted into the eye to replace lost cells or to support remaining photoreceptors through cytoplasmic exchanges. While significant roadblocks need to be addressed, the rapid development of more physiologically relevant cellular models that accurately capture the biological complexity of the retina in vitro, like the Wahlin Lab's stem cell-derived retina structures, bring these expectations closer to reality.

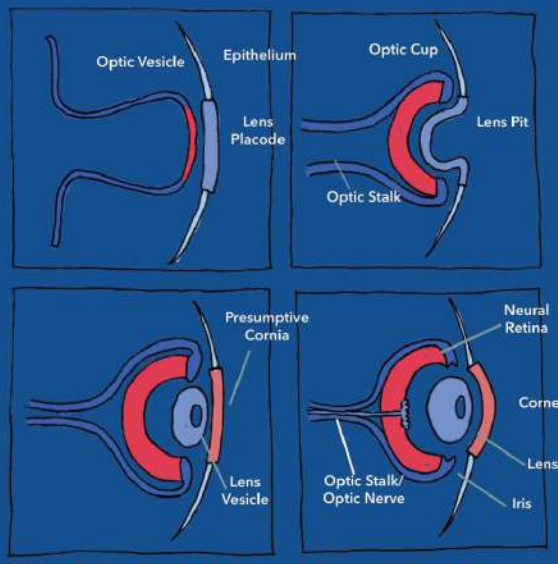


Figure 2. Formation of the 3D mini retina cups from the Optic Vesicle

The optic vesicle forms the foundation for what eventually becomes the optic cup, which includes the optic stalk, the optic nerve, the neural retina, and iris. Epithelial cells differentiate to form the cornea and lens.

References

1. Davidson, F., Steller, H. Blocking apoptosis prevents blindness in Drosophila retinal degeneration mutants. *Nature* 391, 587–591 (1998). https://doi.org/10.1038/35385

2. Hoon, Mrinalini, et al. "Functional Architecture of the Retina: Development and Disease." *Progress in Retinal and Eye Research*, Pergamon, 28 June 2014, www.sciencedirect.com/science/article/pii/S135094621400038X?casa_token=QQq1TD0bBocAAAAA%3AL-T9Op5yxB39MsIW9MErBLsJ0b-jsQ9xm59nOLwLB36lauakwB-pqKA8pz_lMWOjPW3QmxQjIKnzg.

3. Huang, Y., Enzmann, V. & Ildstad, S.T. Stem Cell-Based Therapeutic Applications in Retinal Degenerative Diseases. *Stem Cell Rev and Rep* 7, 434–445 (2011). https://doi.org/10.1007/s12015-010-9192-8

4. Singh, R., Cuzzani, O., Binette, F. et al. Pluripotent Stem Cells for Retinal Tissue Engineering: Current Status and Future Prospects. *Stem Cell Rev and Rep* 14, 463–483 (2018). https://doi.org/10.1007/s12015-018-9802-4

5. Wahlin, K.J., Maruotti, J.A., Sripathi, S.R. et al. Photoreceptor Outer Segment-like Structures in Long-Term 3D Retinas from Human Pluripotent Stem Cells. *Sci Rep* 7, 766 (2017). https://doi.org/10.1038/s41598-017-00774-9

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Research

Biology students at UC San Diego often choose to enrich their educational experience by joining labs and conducting their own research. This section showcases original research manuscripts and review papers produced and written entirely by undergraduate students.

Leafcutter bee on a blade of grass.

Photo by Bridget Spencer

A CORTEX-SPECIFIC PROMOTER FOR TARGETED TRANSDUCTION WITH ADENO-ASSOCIATED VIRAL VECTORS

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background

Gene therapy has grown in popularity as a potential treatment strategy for a variety of human diseases.[1] Adeno-associated virus (AAV) vectors have been shown to be effective gene delivery candidates based on their safety and ability to sustain expression of a transfected gene over extended time periods in the brain.[1] In particular, the AAV-PhP.eB viral capsid vector is known to be effective at transducing the central nervous system (CNS) in mice when delivered intravenously (IV).[2] However, more research is needed to identify cell-specific promoters that can target gene expression to specific brain regions. In this study, we investigated whether using a 2.2kb Ple394 promoter in the AAV-PhP.eB vector influences the specificity of expression of an eGFP transgene to a specific region of the brain. The Ple394 promoter derives its name from the Pleiades Promoter Project, which developed an array of promoters as tools to enhance the study of gene therapies and targeted gene expression.[3]

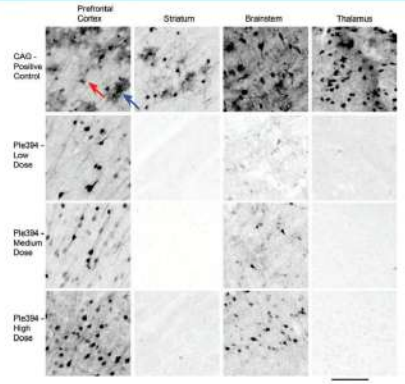
methods

One mouse received 150µL of phosphate-buffered saline (PBS) as a negative control. Four mice received 3x1010 vector genomes (vg) of PhP.eB-CAG-eGFP as positive control viral capsid. The CAG promoter is a pancellular chicken beta-actin promoter, which is known to be a ubiquitous promoter and is expressed in most mouse tissues. Eight additional mice received PhP.eB-Ple394-eGFP vectors as the treatment group. Of these eight, three received 6.25x1010 viral genomes (low dose), three received 1.25x1011 viral genomes (medium dose), and two received 2.5x1011 viral genomes (high dose). Vector was delivered in 150µL intravenous injections. A waiting period of four weeks followed. The animals were perfused with phosphate-buffered saline, euthanized, and dissected. The brains were fixed with paraformaldehyde and sagittally sectioned using a sliding microtome to give sections of 40 µm thickness. Immunohistochemistry was performed using DAB-labeling with rabbit anti-eGFP and Donkey anti-Rabbit antibodies, and the sections were viewed with an Axioscan microscope at 10x.

results

Immunostain results showed that the ubiquitously expressed PhP.eB-CAG-eGFP vector transduced both neurons and astroglia throughout the brain (Figure 1). Transduced neurons labeled by eGFP presented as small cell bodies with extended axonal and dendritic processes, while astroglia presented as roughly circular regions of diffuse processes. In contrast, transfection with the vector containing the Ple394 promoter showed almost no gene expression in astroglia and non-cortical brain regions such as striatum and thalamus. However, at all vector concentrations, the Ple394 promoter caused strong GFP expression in the cortical regions such as the prefrontal cortex. Only at the very highest concentration did this vector also show expression in the brainstem (Figure 1). Overall, the Ple394 images reveal strong transduction of cortical neurons and markedly reduced expression in non-cortical areas.

figure 1. ple394 results in cortex-specific gene expression after IV injection of AAV-PhP.eB. An immunostain against eGFP was done on 40µm thick sagittal brain sections from mice that received intravenous injections of either AAV-PhP.eB-CAG-eGFP (CAG-Positive Control) or AAV-PhP.eB-Ple394-eGFP at low (Ple394-Low Dose), medium (Ple394-Medium Dose), or high dosages (Ple394-High Dose). The brain sections are roughly 1.4 mm from midline. Prefrontal cortex is an on-target area. Striatum, brainstem, and thalamus are off-target areas. Red arrow indicates an eGFP stained neuron. Blue arrow indicates an eGFP stained astroglial cell. Scale bar: 100µm



discussion

Our experiment showed that the Ple394 promoter expressed strongly in cortical neurons and eliminated gene expression in regions such as the thalamus and striatum. However, some off-target expression remained in regions such as the brainstem. In the future, more research with larger samples will be needed to determine whether this dramatic effect is fully reproducible and whether the limited amount of off-target expression is biologically relevant or deleterious. Ultimately, we would wish to determine whether the Ple394 promoter can be used to treat defects in human cortical gene expression such as those found in specific human neurological diseases.

references

1. Dunbar, C. E. et al. "Gene therapy comes of age." *Science* 359, no. 6372 (2018).

2. Chan, K.Y. et al. "Engineered AAVs for efficient noninvasive gene delivery to the central and peripheral nervous systems." *Nature Neuroscience* 20, no. 8, 1172-1179 (2017).

3. Portales-Casamar, E. et al. "A regulatory toolbox of MiniPromoters to drive selective expression in the brain." *Proceedings of the National Academy of Sciences* 107, no. 38, 16589-16594 (2010)

FUNCTION OF SLEEP

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introduction

Do you remember your dream last night? Was it bizarre, weird, or random? Researchers have always been fascinated by the connection between sleep and dreaming. Sleep has been divided into two broad stages: rapid eye movement sleep (REM sleep) and non-rapid eye movement sleep (NREM sleep). REM sleep has always been associated with active dreaming. The history of this linkage dates back to 1958, when Dement and Kleitman first linked "eye-movement periods with minimal ocular activity" to passive dreams, while "frequent and large eye movements" were associated with active dreams.[1] Dement termed this stage of sleep "rapid eye movement sleep," later shortened to REM sleep.[2,3] In addition to REM sleep, there is NREM sleep, where sleepers' brain waves are usually slow and of high amplitude. Their breathing and heart rate are usually slow, with minimal body movements and less vivid dreams.[4]

Although scientists are aware of the different stages of sleep, they are still puzzled by the function of each one. Many theories have tried to explain the necessity of sleep. A few mainstream theories are the Synaptic Homeostasis Hypothesis by Tononi and Cirelli, Information Consolidation Theory by Born and Wilhelm, and Reverse Learning Theory by Crick and Mitchison.[5,6,7] Each of these theories offers insights from a different perspective.

synaptic homeostasis hypothesis

According to Tononi and Cirelli, learning during wakefulness is the process of changing the strength and number of synaptic connections for the long term.[5] Their synaptic homeostasis hypothesis states that synaptic strength increases while learning during daytime, and resets during sleep at night to prepare for the next day. This hypothesis is based on a common paradigm called long-term potentiation (LTP): learning signals like glutamate first activate AMPA receptors, which then subsequently allow calcium ions to enter nearby NMDA receptors. The additional insertion of AMPA receptors into the plasma membrane ultimately makes neurons more sensitive to future signaling.

The synaptic homeostasis hypothesis is backed by substantial experimental evidence that GluA1-containing AMPA receptors in the hippocampus have higher expression levels after wakening than after sleep. The axon-spine interface (ASI), the area between the presynaptic terminal and the postsynaptic terminal of neurons, decreases during sleep for mice, and the number of dendritic spines, the protrusions from dendrites that receive signals, decreases more rapidly during sleep than waking hours, with comparable formation rates between the two states. All of the above suggest that sleep is a process of reducing synaptic strength and resetting the brain.[8,9,10,11]

However, this theory has some pitfalls. There is no well-accepted metric for synaptic strength—spine volume, spine density, and ASI are all valid. Tononi and Cirelli did not specify why they chose ASI as the metric and why they cited ASI decrease as evidence for their theory. In addition, the theory mentions that not all synapses are affected: only smaller and weaker ones are down-regulated, preserving large ones that might store important memories. The theory also contradicts another study that shows this night-resetting effect only in large synapses.[5,12] Hence, researchers still need to address several uncertainties and gaps in the theory.

memory consolidation theory

Memory consolidation theory is based on the two-stage memory model that states that memories are first stored in the hippocampus as short-term ones. Then the memories are transferred to the neocortex, where they are permanently stored as long-term memories. Born and Wilhelm raised the theory that during slow-wave sleep (SWS), the deepest stage of NREM sleep, the brain reprocesses memories and transfers them to the neocortex.[6] Researchers have found strong evidence to support this theory. Studies show rats with spatiotemporal patterns of daytime neuronal firing reactivated during sleep, indicating that the rat's brain is indeed reprocessing the daytime memory. When human subjects were presented with odor they experienced at daytime learning during SWS, their memories were effectively enhanced. Also, human's odor-induced memory reactivation during SWS enhanced memories even without any REM sleep, meaning that SWS plays a direct role in memory consolidation.[13,14,15,16,17,18]

The findings are promising and exciting, but they are not conclusive. First, researchers are not certain if the odor-induced hippocampal activation during SWS detected by fMRI is due to the odor cue, some other neural conditions, or simply chance.[6] In addition, the human odor-induced memory reactivation experiment mentioned before woke subjects up immediately after the odor cue completed. Thus, there is the possibility that SWS was not complete, weakening the conclusion that SWS is a key part for memory consolidation. It is important to acknowledge these imperfections so future researchers can refine the theory.

reverse learning theory

Crick and Mitchison think the purpose of sleep, specifically REM sleep, is to actively detect and suppress certain memories to modulate storage space in the brain. This is based on the assumption that mammal brain cortices are like computer networks: like a computer, the brain likely generates "parasitic," or useless memories that would impede future storage—therefore, it needs to be cleaned periodically.[7] This cleaning process, termed "reverse learning," happens during sleep because the brain needs to temporarily stop receiving and sending signals. The mechanism proposed is that the brain needs to randomly activate memories to look for parasitic ones. For this purpose, random memory-related dreams are generated during REM sleep. The strongest evidence comes from John Hopfield and another group of researchers, who independently came up with the reverse learning model and showed it indeed improved neural networks.[19]

There are many implications and pitfalls to this theory. Some scientists propose that schizophrenia may be related to defects in reverse learning, since failed REM sleep can lead to hallucinations, delusions, and obsessions—all of which are symptoms of schizophrenia. This theory also has implications for A.I., since this learning-and-forgetting method can resolve memory overloading in neural networks. A major pitfall, however, is that this theory is extremely hard to test, and so far the only empirical evidence comes from computers, making scientists question whether or not the reverse learning theory fully explains the mechanism of human dreams.

conclusion

Although all the theories mentioned above have their drawbacks, they are bold steps on the road to understanding more brain functions. As research on sleep and dreaming continues to get more attention, we will have both the technology and the data to unravel the secrets of sleep in the near future, and potentially link sleep deficits to illnesses like depression, autism, and schizophrenia.

references

1. Dement W, Wolpert EA. The relation of eye movements, body motility, and external stimuli to dream content. *J Exp Psychol*. 55(6), 543-553 (1958).
2. Dement WC. Recent studies on the biological role of rapid eye movement sleep. *Am J Psychiatry*. 122, 404-408 (1965).
3. Cohen HB, Duncan RF, II, Dement WC. Sleep: the effect of electroconvulsive shocks in cats deprived of REM sleep. *Science*. 156, 1746-8 (1967).
4. Martin JM. et al. Structural differences between REM and non-REM dream reports assessed by graph analysis. *PLoS One*. (2020).
5. Cirelli C. Sleep, synaptic homeostasis and neuronal firing rates. *Curr Opin Neurobiol*. 44:72-79 (2017).
6. Born J, Wilhelm I. System consolidation of memory during sleep. *Psychol Res*. 76(2):192-203 (2012).
7. Crick F, Mitchison G. The function of dream sleep. *Nature*. 304(5922):111-114 (1983).
8. Vyazovskiy V, Cirelli C, Pfister-Genskow M, Faraguna U, Tononi G. Molecular and electrophysiological evidence for net synaptic potentiation in wake and depression in sleep. *Nat. Neurosci*. 11:200-208 (2008).
9. de Vivo L. et al. Ultrastructural evidence for synaptic scaling across the wake/sleep cycle. *Science*. 355:507-510 (2017).
10. Maret S, Faraguna U, Nelson AB, Cirelli C, Tononi G. Sleep and waking modulate spine turnover in the adolescent mouse cortex. *Nat. Neurosci*. 14:1418-1420 (2011).
11. Yang G, Gan WB. Sleep contributes to dendritic spine formation and elimination in the developing mouse somatosensory cortex. *Dev. Neurobiol*. 72:1391-1398 (2012).
12. Diering GH. et al. Homer1a drives homeostatic scaling-down of excitatory synapses during sleep. *Science* 355:511-515 (2017).
13. Pavlides, C., Winson, J. Influences of hippocampal place cell firing in the awake state on the activity of these cells during subsequent sleep episodes. *The Journal of Neuroscience*. 9, 2907-2918 (1989).
14. Wilson, M. A., McNaughton, B. L. Reactivation of hippocampal ensemble memories during sleep. *Science*, 265, 676–679 (1994).
15. Sutherland, G. R., McNaughton, B. Memory trace reactivation in hippocampal and neocortical neuronal ensembles. *Curr.Opin.Neurobiol.* 10, 180–186 (2000).
16. Ribeiro, S. et al. Long-lasting novelty-induced neuronal reverberation during slow-wave sleep in multiple forebrain areas. *PLoS Biology*, 2, E24. (2004).
17. O'Neill, J., Pleydell-Bouverie, B., Dupret, D., Csicsvari, J. Play it again: reactivation of waking experience and memory. *Trends in Neurosciences*, 33, 220-229 (2010).
18. Diekelmann, S., Bu¨chel, C., Born, J., Rasch, B. Labile or stable: opposing consequences for memory when reactivated during waking and sleep. *Nature Neuroscience*, 14, 381-386 (2011).
19. Hopfield, J.J., Feinstein, D.I., Palmer, R.G. 'Unlearning' has a stabilizing effect in collective memories. *Nature* 304,158-159(1983).

THE MUTUAL DEPENDENCE BETWEEN CIRCADIAN RHYTHMS AND GLUCOCORTICOIDS IN CORTICOSTEROID THERAPY

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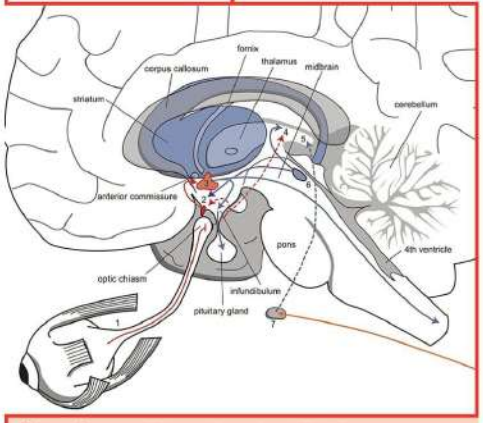

figure 1. suprachiasmatic nucleus in brain. In the figure above, the SCN lies in the shaded region above the optic chiasm, where the left and right optic nerves cross over.

introduction

Looking closely at natural phenomena, one realizes a common theme—rhythms. The Earth rotates on its axis, revolving around the sun, and the sun in turn rotates on its own axis and revolves around the milky way galaxy. Daily rhythms are vital to helping organisms prepare for the day as well as regulate important functions. These are known as Circadian Rhythms (*Latin: Circa-diem, about a day*). Given that bodily functions are governed by biochemical reactions, the circadian rhythm controls the timely release of a plethora of chemical compounds. This review seeks to establish the importance of timing in corticosteroid administration to treat autoimmune diseases, due to the mutual dependence between circadian rhythms and glucocorticoids.

circadian rhythm 101

The circadian clock is in the suprachiasmatic nucleus (SCN) of the brain.[1] This cluster of nuclei sits atop the optic chiasm, and it receives light input from the retina to time activities such as melatonin release from the pineal gland. Since a rise in melatonin causes sleepiness, the circadian regulation of melatonin regulates sleep. The clock can entrain—or adjust—to different time zones so it does not go out of sync.

Surprisingly, in addition to the SCN, there are circadian molecular clocks all over the body. But what exactly comprises the clock? In mammals, the clock is really a synchronized oscillation in levels of the molecules cryptochrome (CRY) and period (PER). In most mammalian cells, the CLOCK:BMAL1 heterodimer serves as a transcription factor, upregulating the transcription of PER and CRY. During the day, transcription of PER and CRY proceeds until dusk, when the protein products begin to suppress CLOCK:BMAL1 in a negative feedback loop. During the day, light degrades PER and CRY, allowing transcription to resume, thus establishing rhythmic transcription. In order to learn if body cells outside the SCN exhibit circadian clocks, Yoo et. al used a Luciferase bioluminescent reporter tied to the mPER2 gene promoter of the circadian rhythm; they found that mouse cells from the cornea, liver, lung, pituitary gland, retrochiasmatic area, and (dissected) tail continued to display bioluminescent light rhythms.

Scientists hypothesize that the SCN uses glucocorticoids as messengers to regulate subordinate clocks throughout the body. In a study dealing with the effects of glucocorticoids on lung tissue, researchers used the PER2:LUC reporter gene to find that Murine Clara cells exhibited sensitivity to glucocorticoids. Clara cells are tall, columnar, nonciliated cells that line respiratory bronchioles. They express glucocorticoid receptors as well as circadian rhythm genes.[3] Furthermore, removing these cells from the tissue sample caused other lung cells to lose rhythmicity.[3] In direct evidence supporting the hypothesis that glucocorticoids may influence circadian rhythms, rat fibroblasts have expressed clock genes in response to dexamethasone, a glucocorticoid. Liver, kidney, and heart cells all showed fluctuations in their rhythms in response to dexamethasone, while the SCN did not.[4] It would make no sense for the SCN to accidentally self-regulate while attempting to regulate peripheral clocks, and this is confirmed above, as glucocorticoids act only on peripheral clocks without affecting the SCN.

a link between the circadian rhythm and immunity

The immune system is another domain governed by the circadian rhythm. In a study investigating the effects of BCG vaccine administration at different times of day, researchers found that volunteers vaccinated in the morning displayed stronger trained and adaptive immunity. Those injected in the morning showed higher levels of cytokines—cell secretions that broadly regulate immunity—in response to an administration of *Staphylococcus aureus* and *Mycobacterium tuberculosis*.[5]

glucocorticoids in autoimmune disease treatment, and the circadian clock tie-in

Autoimmune diseases occur when the immune system attacks self-molecules following a breakdown of immunologic tolerance to autoreactive immune cells.[6] Although there is no cure, patients are frequently prescribed synthetic glucocorticoids such as prednisone to make up for a lack of endogenous cortisol. An example is Rheumatoid Arthritis (RA), which, simply put, is joint inflammation.

The Hypothalamus Pituitary Axis (HPA) is an endocrine linkage that, among many functions, detects and combats inflammation. The HPA axis is governed by the SCN as well as positive and negative feedback loops. Levels of interleukin-6 (IL-6), a proinflammatory cytokine, rise just before dawn and fall as the day progresses. Cortisol is the body's natural anti-inflammatory agent, and it is downregulated when IL-6 is on the rise, allowing for inflammation to take place. The HPA axis reacts to the increased inflammation by increasing cortisol levels, which alleviates inflammation.

In order to study the action of the HPA axis, researchers simulated inflammation by injecting IL-6 into eighteen healthy males. As expected, the HPA axis released cortisol to combat the inflammation.

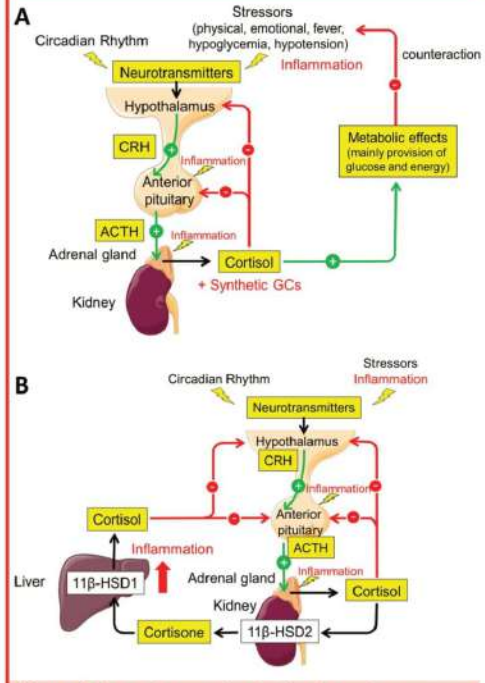

figure 2. hpa axis negative feedback regulator. Note that cortisol is a negative regulator of the feedback loop, thereby shutting off its own production.

But this response began to diminish with repeated injections of IL-6. This finding is consistent with the fact that RA patients suffer from chronically dampened levels of cortisol release by the HPA axis. The severe, prolonged inflammation caused by the disease dampens glucocorticoid release by the HPA axis. In fact, administering a glucocorticoid like prednisone with the goal of alleviating pain may backfire. Prednisone administered at 7AM was less effective, and we may speculate that it actually fed the negative feedback loop, prematurely terminating cortisol release (Figure 2).[10] This results in increased inflammation.

To combat this, research has shown that a single morning dose of GC does not interfere with the natural rise and fall of cortisol.[9] In practice, slow-release prednisone at 2AM was able to effectively suppress IL-6 release, while also leaving cortisol levels unaffected.[10] Thus, timing prednisone administration is critical so as not to interfere with the body's natural cortisol rhythm, which is, in part, circadian-controlled. Thus, the circadian timing of prednisone administration cannot be overlooked.

future directions

It would be wise for physicians to take into account the timing of glucocorticoid release before they administer prednisone. Slow release prednisone pills like Rayos are helpful in this regard. For example, taking Rayos at 10 pm results in a 2 am release. This ensures that the natural circadian cortisol rhythm is unperturbed. It is also vital for future studies to further elucidate the time-dependent dosage of glucocorticoids as these drugs cause harmful side effects down the line for many patients due to the instabilities they create.

references

1. Vitaterna, M. H., Takahashi, J. S., & Turek, F. W. (2001). Overview of circadian rhythms. *Alcohol research & health : the journal of the National Institute on Alcohol Abuse and Alcoholism*, 25(2), 85–93.

2. Yoo, S. H., Yamazaki, S., Lowrey, P. L., Shimomura, K., Ko et.Al, (2004). PERIOD2::LUCIFERASE real-time reporting of circadian dynamics reveals persistent circadian oscillations in mouse peripheral tissues. *Proceedings of the National Academy of Sciences of the United States of America*, 101(15), 5339–5346.

3. Gibbs, J. E., Beesley, S., Plumb, J., Singh, D., Farrow, S., Ray, D. W., & Loudon, A. S. (2009). Circadian timing in the lung; a specific role for bronchiolar epithelial cells. *Endocrinology*, 150(1), 268–276.

4. Balsalobre A, Brown SA, Marcacci L, Tronche F, Kellendonk C et.Al. Resetting of circadian time in peripheral tissues by glucocorticoid signaling. *Science*. 2000 Sep 29;289(5488):2344-7.

5. de Bree LCJ, Mourits VP, Koeken VA, Moorlag SJ, Janssen R et. Al. Circadian rhythm influences induction of trained immunity by BCG vaccination. *J Clin Invest*. 2020 Oct 1;130(10):5603-5617.

6. Smith, D.A. Germolec, D.R. Introduction to immunology and autoimmunity. Environ. *Health Perspex*. 107(Suppl 5). 1999.

7. Son GH, Chung S, Kim K. The adrenal peripheral clock: glucocorticoid and the circadian timing system. *Front Neuroendocrinol*. 2011 Oct;32(4):451-65.

8. Spies, C. M., Straub, R. H., Cutolo, M., & Buttgereit, F. (2014). Circadian rhythms in rheumatology--a glucocorticoid perspective. *Arthritis research & therapy*, 16 Suppl 2(Suppl 2), S3.

9. Crofford LJ, Kalogeras KT, Mastorakos G, Magiakou MA, Wells J et.Al. Circadian relationships between interleukin (IL)-6 and hypothalamic-pituitary-adrenal axis hormones: failure of IL-6 to cause sustained hypercortisolism in patients with early untreated rheumatoid arthritis. *J Clin Endocrinol Metab*. 1997;

10. Clarke L, Jessop DS, Hunt LP, Straub RH, Perry MG, Kirwan J. Alleviation of morning joint stiffness by low-dose prednisone in rheumatoid arthritis is associated with circadian changes in IL-6 and cortisol. *Int J Clin Rheumatol*. 2011

NOVEL NON-SSRI TREATMENTS FOR OBSESSIVE COMPULSIVE DISORDER: PHARMACOTHERAPIES TARGETING CSTC SIGNALING

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introduction

Obsessive-Compulsive Disorder (OCD) is a psychiatric illness characterized by intrusive thoughts, designated as obsessions, and repetitive compulsions, such as motor-related fixations.[1] Symptoms of OCD include checking behavior—compulsive interactions with switches or locks—and fixation on symmetry, hygiene, or routine tasks. Standard clinical approaches to OCD are cognitive behavioral therapy (CBT) and treatment with selective serotonin-reuptake inhibitors (SSRIs).[1] CBT aims to reduce anxiety by retraining responses to unwanted obsessional thoughts, replacing checking behavior with safer interactions, for example; this psychiatric approach is routinely supplemented with SSRIs, which increase extracellular concentrations of serotonin via inhibition of the serotonin (5-HT) transporter. However, roughly forty to sixty percent of patients diagnosed with OCD are inadequately treated with this clinical approach.[1] OCD patients also experience a high rate of comorbidity with bipolar disorder (BP - OCD), among other conditions, which further complicates treatment with the standard CBT-SSRI approach.

Dysfunction of the cortico-striato-thalamo-cortical (CSTC) pathway loops is implicated in the emergence of OCD symptoms.[2] This pathway is primarily responsible for regulation of reward processing, executive motor function, and habit formation; it is also targeted by SSRIs and other alternative treatments.

Treatment-resistant patients typically repeat SSRI trials with increased potency or dosage. Continued treatment failure will lead clinicians to prescribe alternative pharmacotherapies; the mechanisms of action of these treatments range widely, from dopamine agonism to serotonin agonism to glutamate antagonism, visualized in Figure 1. Each of these pharmacotherapies act selectively on their target receptor, yet each produces symptom relief. By studying changes in neurotransmitter signaling in patients and the response to each of these treatments, this paper will review alternative therapies and what their mechanisms of action suggest about the physiology of CSTC pathway loops.

dopamine signaling implicated in OCD model reward processing and compulsions

Treatment with the dopamine agonist aripiprazole in addition to SSRI/CBT therapy produces relief of OCD symptoms in a general OCD patient group; aripiprazole also displays high potency for striatal D_2Rs.[3,4] In patients experiencing comorbidity with bipolar disorder, co-administration of D_2R agonists and SSRIs was especially effective in alleviating high-risk symptoms, primarily suicidal ideations.[5]

Murray and colleagues investigated anterior cingulate cortex (ACC) and nucleus accumbens (NAc) dopamine signaling in OCD patients and a healthy volunteer group treated with the dopamine antagonist amisulpride and agonist pramipexole, which selectively bind D_2 and D_3 dopamine receptors ($D_{2/3}$R), in order to study impaired dopamine signaling in patients.[6] They found that both $D_{2/3}$R agonism and $D_{2/3}$R antagonism partially reduced error during prediction of reward, which is typically exacerbated in OCD patients.[6] This paper suggests that this "bidirectional" effect may be due to other neurotransmitter circuits being implicated as a result of impaired dopamine signaling.

In mice, administration of a $D_{2/3}$R agonist increased compulsive checking behavior, while $D_{2/3}$R antagonism reduced checking.[7] Compulsive behavior under extended $D_{2/3}$R agonism was also associated with reduced D2R expression in the dorsal striatum. Perani et al. further demonstrates, with PET imaging, a reduction in D_2 receptor availability in the ventral striatum of OCD patients; impaired D_2R activity throughout the basal ganglia is also observed to occur alongside decreased 5-HT_2R expression throughout the frontal cortex, cingulate gyrus, and parietal cortex, suggesting strong cross-regulation of CSTC dopamine and serotonin circuitry between these regions.[8]

selective serotonin signaling elicits obsessive-compulsive (OC) symptoms

Serotonin-2 (5-HT_2), 5-HT_{1B}, and 5-HT_{1D} receptors have all been implicated in OCD. Clomipramine and fluoxetine, a tricyclic

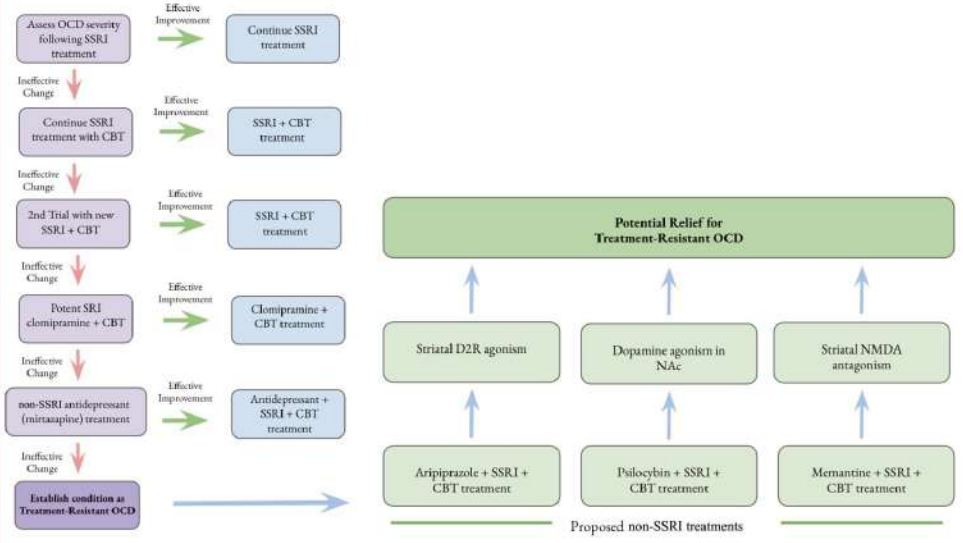

figure 1. Sequential exploration of therapeutic options for treatment-resilient OCD.

antidepressant and SSRI, respectively, are typically used for treatment, and in mice these drugs downregulate 5-HT_{1B}R signaling in the orbitofrontal cortex (OFC), relieving OCD symptoms.[9] OFC 5-HT_{1B}R activation is shown to be both sufficient and necessary for the emergence of symptoms.[9] Researchers also observed that an agonist that selectively binds 5-HT_{1D} receptors, sumatriptan, heightens symptoms, while 5-HT_{1A}R agonism failed to produce the same effect.[10] This implicates 5-HT_{1D}R hyperactivity as an additional pathology for OCD; high 5-HT_{1D}R populations in the substantia nigra may play a role in dysregulation of CSTC circuitry.

Reduced 5-HT_2R binding in the frontal and cingulate cortex of OCD patients, as demonstrated by Perani et al, supports the hypothesis that SSRI treatment is mediated by attenuating hypoactivity of cortical 5-HT_2R expression.[8] The mechanism of action for SSRI treatment is not fully understood, but it may partially function by restoring 5-HT_{2C} signaling; in animal studies, a selective 5-HT_{2C} antagonist potently reverses the treatment effects of the SSRI fluoxetine.[11] Reduced 5-HT_2R expression also occurs alongside increased 5-HT_1R expression in OCD patients, further speaking to the complicated physiology of CSTC circuits.

The indolamine psilocybin is a low-selectivity serotonin agonist that increases prefrontal cortex (PFC) serotonin signaling and CSTC 5-HT_2R expression, while decreasing PFC dopamine signaling.[12] Administration of low doses of psilocybin in a small sample study reduced severity of OCD symptoms in patients.[13] SSRI drugs typically take a few weeks to produce relief, but psilocybin produces faster increases in 5HT_{2A}R occupancy with high-potency binding; the strength of this serotonergic agonist may produce relief in patients insufficiently treated with SSRIs.[14]

glutamate dysregulation affects corticostriatal signaling

Expression of a genetic variant of glutamatergic gene SCL1A1 is hExpression of a genetic variant of glutamatergic gene SCL1A1 is heightened in the population of patients with treatment-resistant OCD, pushing scientists to investigate the implications of impaired glutamate signaling. SCL1A1 encodes the postsynaptic glutamate transporter EAAT3; in the brain, EAAT3 inhibits NMDA receptor (NMDAR) response and disruption of EAAT3 produces stronger NMDAR activation.[15] In mice with OCD-like behaviors, corticostriatal synapses showed increased NMDAR activity, as well as increased $mGluR_5$ activity.[16]

Administration of NMDAR antagonists, like memantine, also helps alleviate treatment-resistant conditions. Memantine was successful in double-blind treatment-resistant OCD trials, showing much greater efficacy for the treatment-resistant group versus the treatment-compliant group.[17] Memantine co-treatment with SSRIs is a strong strategy for treating patients expressing variants of glutamatergic genes, particularly SCLA1, since SSRIs alone fail to mediate glutamate hyperactivity in CSTC circuits.

conclusion

Each potential therapy demonstrates aspects of improvement over SSRI-CBT treatment alone. Aripiprazole treatment shows great potential for treating high-risk OCD symptoms and comorbidity. Administration of psilocybin can serve as a fast-acting alternative to SSRI treatment with increased potency. Memantine shows the most promise in treatment-resistant conditions, by correcting additional pathologies and attenuating SCL1A1-associated hyperglutamatergic activity. Further study into CSTC signaling changes in OCD patients after drug treatment are warranted to better understand the role of these novel pharmacotherapies; as a number of these studies demonstrate, serotonin, dopamine, and glutamate circuits are closely interconnected in the CSTC pathway, and altering expression of any of these neurotransmitters can cause OCD symptoms. Corresponding research may also elucidate which factors are associated with resistance to treatment in OCD patients, potentially leading to a restructuring of the current clinical approach.

references

1. Wilhelm, S., Tolin, D., and Steketee, G. "Challenges in Treating Obsessive-Compulsive Disorder: Introduction." *Journal of Clinical Psychology* 60, 1127-1132 (2004). https://pubmed.ncbi.nlm.nih.gov/15389623/

2. Calzà, J. et al. "Altered Cortico-Striatal Functional Connectivity During Resting State in Obsessive-Compulsive Disorder." *Frontiers in Psychiatry* vol. 10 (2019). https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6524661/

3. Muscatello, M et al. "Effect of Aripiprazole Augmentation of Serotonin Reuptake Inhibitors or Clomipramine in Treatment-Resistant Obsessive-Compulsive Disorder: a Double-Blind, Placebo-Controlled Study." *Journal of Clinical Psychopharmacology* 134, 174-179 (2014). https://doi.org/10.3410/f.718508460.793497753.

4. Mamo, M.D. et al. "Differential Effects of Aripiprazole on D 2 , 5-HT 2 , and 5-HT 1A Receptor Occupancy in Patients With Schizophrenia: A Triple Tracer PET Study." *American Journal of Psychiatry* 164, 1411-1417 (2007). https://ajp.psychiatryonline.org/doi/full/10.1176/appi.ajp.2007.06091479.

5. Lai, J. et al. "Aripiprazole Augmentation in Managing Comorbid Obsessive-Compulsive Disorder and Bipolar Disorder: a Case with Suicidal Attempts." *Neuropsychiatric Disease and Treatment* 13, 87-90 (2017). https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5207469/.

6. Murray, G. et al. "Dopaminergic drug treatment remediates exaggerated cingulate prediction error responses in obsessive-compulsive disorder." *Psychopharmacology* 236, 2325-2336 (2019). https://pubmed.ncbi.nlm.nih.gov/31201476/

7. Eagle, D. et al. "The Dopamine D2/D3 Receptor Agonist Quinpirole Increases Checking-like Behaviour in an Operant Observing Response Task with Uncertain Reinforcement: A Novel Possible Model of OCD." *Behavioural Brain Research* 264, 207-229 (2014). https://www.sciencedirect.com/science/article/pii/S0166432813007894.

8. Perani, D. et al. "In Vivo PET Study of 5HT2A Serotonin and D2 Dopamine Dysfunction in Drug-Naive Obsessive-Compulsive Disorder." *NeuroImage* 42, 306-314 (2008). https://www.sciencedirect.com/science/article/abs/pii/S1053811908005041?via%3Dihub.

9. Shanahan, N. A., Velez, L. P., Masten, V. L., and Dulawa, S. C. "Essential Role for Orbitofrontal Serotonin 1B Receptors in Obsessive-Compulsive Disorder-like Behavior and Serotonin Reuptake Inhibitor Response in Mice." *Biological Psychiatry* 70, 1039-1048 (2011). https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3310222/.

10. Aouizerate, B. et al. "Updated overview of the putative role of the serotoninergic system in obsessive-compulsive disorder." *Neuropsychiatric Disease and Treatment* vol. 1, 231-243 (2005). https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2416754/.

11. Rodriguez, M. et al. "Behavioral Effects of a Novel Benzofuranyl-Piperazine Serotonin-2C Receptor Agonist Suggest a Potential Therapeutic Application in the Treatment of Obsessive–Compulsive Disorder." *Frontiers* 22 (2017). https://www.frontiersin.org/articles/10.3389/fpsyt.2017.00089/full.

12. Sakashita, Y. et al. "Effect of Psilocin on Extracellular Dopamine and Serotonin Levels in the Mesoaccumbens and Mesocortical Pathway in Awake Rats." *Biological and Pharmaceutical Bulletin* 38, 134-138 (2015). https://pubmed.ncbi.nlm.nih.gov/25342005/

13. Moreno, F. A., Wiegand, C.B., Taitano, E. K., and Delgado, P. L. "Safety, Tolerability, and Efficacy of Psilocybin in 9 Patients With Obsessive-Compulsive Disorder." *The Journal of Clinical Psychiatry* 67, 1735-1740 (2006). https://doi.org/10.4088/jcp.v67n1110

14. Madsen, M.K. et al. "Psychedelic Effects of Psilocybin Correlate with Serotonin 2A Receptor Occupancy and Plasma Psilocin Levels." *Neuropsychopharmacology* 48, 1328-1334 (2019). https://www.nature.com/articles/s41386-019-0324-9.

15. Li, M.H. et al. "Amphetamine and Methamphetamine Increase NMDAR-GluN2B Synaptic Currents in Midbrain Dopamine Neurons." *Neuropsychopharmacology* 42, 1539-1547 (2017). https://www.nature.com/articles/npp2016278

16. Escobar, A. P., Wendland, J. R., Chávez, A. E., and Moya, P. R. "The Neuronal Glutamate Transporter EAAT3 in Obsessive-Compulsive Disorder." *Frontiers* 10 (2019). https://www.frontiersin.org/articles/10.3389/fphar.2019.01362/full

17. Ghaleiha, A., et al. "Memantine Add-on in Moderate to Severe Obsessive-Compulsive Disorder: Randomized Double-Blind Placebo-Controlled Study." *Journal of Psychiatric Research* 47, 175-180 (2012). https://www.sciencedirect.com/science/article/abs/pii/S0022395612002956?via%3Dihub.

SMALL MOLECULE EXTRACELLULAR ELECTRON SHUTTLES: MECHANISM OF ELECTRON TRANSFER AND BIOTECHNOLOGICAL APPLICATIONS

FISHER PRICE

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extracellular electron transfer and microbial natural products

Oxygen is an essential part of the electron transport chain, functioning as the terminal electron acceptor in aerobic respiration.1 Without oxygen, an aerobic cell is unable to complete the final step of electron transfer, causing the transfer process to halt. When this occurs, the intermembrane proton gradient is not maintained, and ATP synthase is unable to generate ATP for the cell.[1] The resulting lack of oxygen inhibits necessary cellular pathways and the cell eventually dies due to asphyxiation. However, some bacteria have developed ingenious ways of circumventing this issue. Apart from more traditional forms of low-oxygen respiration, such as the use of high-affinity terminal oxidases by microaerophilic bacteria, several groups of bacteria have evolved unique means of respiration that utilize oxygen indirectly.[2] This indirect process, known as extracellular electron transfer, can occur through a variety of different methods. Some methods include external longitudinal electron transfer by cable bacteria (over centimeter scale distances), conductive nanowires by dissimilatory metal reducing bacteria, and the use of exogenous humic substances by various soil and marine sediment bacteria.[3,4,5] However, one recently discovered type of extracellular electron transfer is performed by endogenously-produced redox-active natural products.

Microbial natural products (also known as secondary metabolites) are organic molecules produced outside of a microorganism's primary metabolism.[6] Natural products have various roles, acting as anything from signaling molecules to antibiotics.[6] They have also been a fruitful source for modern drug discovery.[7] However, one group of natural products, small-molecule extracellular electron shuttles (SMEES), blurs the line between primary and secondary metabolites. While most natural products do not contribute directly to growth or metabolism, SMEES have been shown to directly participate in cellular respiration and survival in oxygen limited environments.[8] While several classes of SMEES have been identified, phenazine secondary metabolites are the best characterized endogenously produced extracellular electron shuttles (Figure 1).[8,9] Phenazine metabolites, especially those produced by the pathogenic bacteria *Pseudomonas aeruginosa*, have been shown to transfer electrons from oxygen deficient areas (such as in the center of bacterial biofilms), to areas with high oxygen (such as the periphery of biofilms) in order to perpetuate the electron transport chain when oxygen is limiting.[9]

mechanism of phenazine mediated extracellular electron transfer

Phenazine natural products are an anomaly in the world of microbial chemical ecology. While most characterized bacterial natural products have yet to be linked to their ecological roles, phenazines have been tied to several.[10] Phenazines are known to function as virulence factors, participate in biofilm formation, late-stage quorum sensing, extracellular metal reduction, and antibacterial activity.[10] However, their ability to shuttle electrons out of the cell under oxygen limiting conditions while stabilizing the intracellular redox state is the most unique aspect of these molecules.[11] While scientists first reported in 1931 that *Pseudomonas aeruginosa* produced an unidentified diffusible, redox active substance in low-oxygen conditions, a mechanism for phenazine-mediated extracellular electron transfer (PMEET) was not proposed until late 2020.[9,12]

PMEET has been best studied in *Pseudomonas aeruginosa* biofilms, where phenazines stimulate bacterial swarming motility and contribute directly to colony morphology.[13] Once a biofilm has been formed, cells deep within the biofilm are quickly deprived of oxygen due to normal metabolic activity. In an article by Saunders, *et al.* two multi-step mechanisms for PMEET have been proposed (Figure 2).[9] Analogous to the intracellular electron transport chain, the steps involve transferring electrons from molecules with a low redox potential to those with a higher redox potential. Several phenazine metabolites—phenazine-1 carboxamide (PCN), phenazine-1 carboxylic acid (PCA), and pyocyanin (PYO), listed in order of increasing redox potential—act in concert to transfer electrons from the interior of the biofilm to the

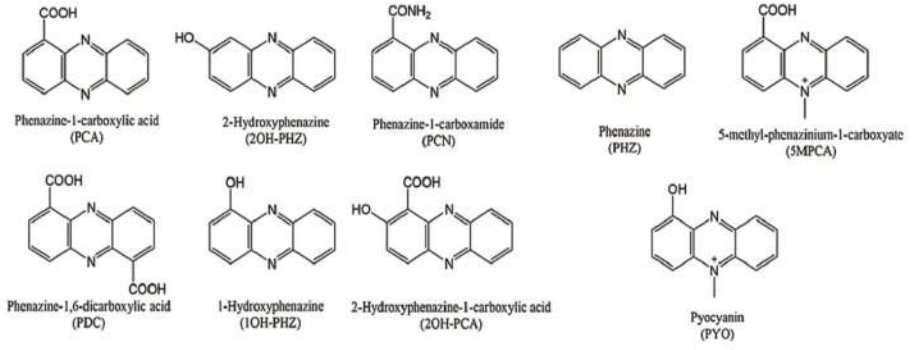


figure 1. representative structures of phenazine natural products produced by pseudomonads. The core phenazine moiety, as shown in the bottom left by PHZ, is the core of all phenazine metabolites. The various functional groups observed in each molecule directly affect the characteristics of the metabolite, such as hydrophobicity and redox potential. *Bilal (2017) Microbial Biotechnology.*[8]

periphery of the biofilm where oxygen is available to be the final acceptor of electrons.[9]

The two proposed mechanisms both begin with a low-potential phenazine (either PCN or PCA) removing electrons from an oxygen-deprived cell by a currently unknown mechanism.[9] The next step involves reduced PCN or PCA molecules freely diffusing throughout the biofilm until they reach the periphery.[9] Then, reduced low-potential phenazines transfer electrons onto PYO, a higher potential phenazine metabolite.[9] Pyocyanin is preferentially retained at the periphery of the biofilm due to intercalation with extracellular DNA (eDNA), a major component of biofilms.[14] From there, PYO transfers electrons to molecular oxygen, forming water.[9] Pyocyanin then binds with eDNA and the cycle starts again. The second proposed method follows a similar pattern as the first, with just one different step. Rather than freely diffusing throughout the cell, reduced PCN or PCA intercalate with eDNA and transfer electrons onto the eDNA complex.[9] Electrons are transferred through stacked pi-orbitals of DNA until they reach intercalated PYO, which then acquires electrons and uncouples from the eDNA double helix.[9] Both methods of PMEET have been shown to be energetically favorable, fast and efficient.[9] Individual experiments suggest that both methods likely occur endogenously and simultaneously.[9] Additionally, this study was the first time eDNA has been identified as not just a major structural component of biofilms, but also as an important component of biofilm metabolism.[9]

biotechnological and biopharmaceutical applications of SMEES

Small molecule extracellular electron shuttles are used in several biotechnological and biopharmaceutical applications. One of the most important applications of SMEES is their use in microbial fuel cells. Microbial fuel cells (MFCs) are essentially batteries made from bacterial cultures in which the electrochemical energy produced by bacterial metabolism is harnessed to power electrical devices.[15] MFC current generation has been shown to benefit from both the endogenous production and exogenous addition of SMEES.[15] MFCs represent an exciting form of sustainable energy as energy production does not rely on external stimuli. However, they are currently hampered by high electrical resistances which constrain electrical outputs.

Apart from microbial fuel cells, SMEES have found use in the bioremediation of toxic heavy metals and crude oil.[16] They have also been used as agricultural probiotics, stimulating growth of beneficial agricultural microbial symbionts and suppressing harmful ones.[17] Furthermore, some pharmaceuticals contain chemical moieties analogous to those of known SMEES.[18]

Redox-active natural products are a diverse group of organic molecules produced by a wide range of bacteria. Many redox active natural products may function as electron shuttles. Future research, namely environmental metabolomics and metagenomics, could potentially reveal that small molecule extracellular electron transfer is a widespread strategy for survival by indirect aerobic respiration.

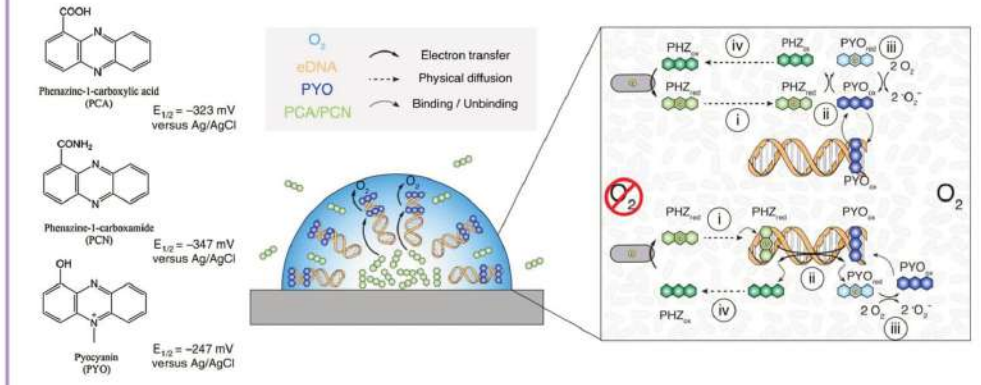


figure 2. proposed mechanism of phenazine mediated extracellular electron transfer. (A) Structures and redox potentials of the electron shuttles PCA, PCN, and PYO. (B) Organization of several phenazines, eDNA, and oxygen concentration in a Pseudomonas aeruginosa biofilm. (C) Two proposed mechanisms for phenazine mediated extracellular electron transfer. The top without DNA charge transfer and the bottom with. Steps are explained in text. PHZ indicates a lower redox phenazine, either PCA or PCN. Saunders (2020) Cell[9]

references

1. Anraku, Y. Bacterial electron transport chains. *Annual review of biochemistry*, 57(1), 101-132. (1988).

2. Morris, R. L., & Schmidt, T. M. Shallow breathing: bacterial life at low O 2. *Nature Reviews Microbiology*, 11(3), 205-212. (2013).

3. Schauer, R., et. al. Succession of cable bacteria and electric currents in marine sediment. *The ISME journal*, 8(6), 1314-1322. (2014).

4. Steidl, R. J., Lampa-Pastirk, S., & Reguera, G. Mechanistic stratification in electroactive biofilms of Geobacter sulfurreducens mediated by pilus nanowires. *Nature Communications*, 7, 12217. (2016).

5. Shi, L., et. al. Extracellular electron transfer mechanisms between microorganisms and minerals. *Nature Reviews Microbiology*, 14(10), 651-662. (2016).

6. Schmidt, R., et. al. Microbedriven chemical ecology: past, present and future. *The ISME journal*, 13(11), 2656-2663. (2019).

7. Pelaez, F. The historical delivery of antibiotics from microbial natural products— can history repeat?. *Biochemical pharmacology*, 71(7), 981-990. (2006).

8. Bilal, M., et. al. Engineering Pseudomonas for phenazine biosynthesis, regulation, and biotechnological applications: a review. *World Journal of Microbiology and Biotechnology*, 33(10), 191. (2017).

9. Saunders, S. H., et. al. Extracellular DNA promotes efficient extracellular electron transfer by pyocyanin in Pseudomonas aeruginosa biofilms. *Cell*, 182(4), 919-932. (2020).

10. Price-Whelan, A., Dietrich, L. E., & Newman, D. K. Rethinking Secondary Metabolism: physiological roles for phenazine antibiotics. *Nature chemical biology*, 2(2), 71-78. (2006).

11. Friedheim, E. A. Pyocyanine, an accessory respiratory enzyme. *The Journal of experimental medicine*, 54(2), 207. (1931).

12. Ramos, I., Dietrich, L. E., Price-Whelan, A., & Newman, D. K. Phenazines affect biofilm formation by Pseudomonas aeruginosa in similar ways at various scales. *Research in microbiology*, 161(3), 187-191. (2010).

13. Bellin, D. L., et. al. Electrochemical camera chip for simultaneous imaging of multiple metabolites in biofilms. *Nature communications*, 7(1), 1-10. (2016).

14. Rabaey, K., Boon, N., Höfte, M., & Verstraete, W. Microbial phenazine production enhances electron transfer in biofuel cells. *Environmental science & technology*, 39(9), 3401-3408. (2005).

15. Dasgupta, D., Kumar, A., Mukhopadhyay, B., & Sengupta, T. K. Isolation of phenazine 1, 6-di-carboxylic acid from Pseudomonas aeruginosa strain HRW. 1-S3 and its role in biofilm-mediated crude oil degradation and cytotoxicity against bacterial and cancer cells. *Applied microbiology and biotechnology*, 99(20), 8653-8665. (2015).

16. Dahlstrom, K. M., McRose, D. L., & Newman, D. K. Keystone metabolites of crop rhizosphere microbiomes. *Current Biology*, 30(19), R1131-R1137. (2020).

17. Borrero, N. V., et. al. Phenazine antibiotic inspired discovery of potent bromophenazine antibacterial agents against Staphylococcus aureus and Staphylococcus epidermidis. *Organic & Biomolecular Chemistry*, 12(6), 881-886. (2014).

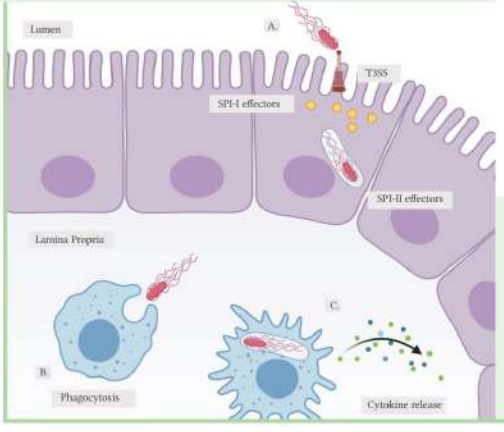
18. Singh, S., et al. "In vitro evaluation of a new drug combination against clinical isolates belonging to the Mycobacterium abscessus complex." *Clinical Microbiology and Infection* 20.12 (2014): O1124-O1127.

WXXXE SIGNATURE MOTIF CONTAINING BACTERIAL EFFECTORS INTERACT WITH THE HOST ENGULFMENT PATHWAY TO CONTROL PATHOGENESIS

SAREH KARIMILANGI
M.S. Biology, UC San Diego 2021, Das Lab, George Palade Laboratories for Cellular and Molecular Medicine

introduction

Despite many prevention efforts, foodborne illnesses remain a serious global health threat.[1] The Centers for Disease Control and Prevention (CDC) recently reported that approximately 48 million people in the United States become ill with foodborne infections annually; this number includes both hospitalizations and deaths.[2] While there are numerous pathogens known to cause foodborne illnesses, *Salmonella* continues to be one of the leading causes, infecting a wide range of hosts through contaminated food and water. Healthy individuals recover within a few days, but those with weak or compromised immune systems—children, the elderly, and individuals with chronic disease—experience severe infection, or salmonellosis.[2] In order to improve treatment and prevention of salmonellosis, it is crucial to examine the host immune response to *Salmonella* infection.



salmonella secretes bacterial effector proteins for entry and survival in host cell

During the infection process (Figure 1), *Salmonella* will depend on its Type III secretion system (T3SS) to produce bacterial effector proteins.[3] These proteins are encoded by genes that fall within large gene cassettes known as *Salmonella* pathogenicity islands (SPIs).[3] The effector proteins allow the bacteria to permeate the host tissue, subdue the host immune system, and to survive and replicate inside the host cell.[3,4] *Salmonella* first invades epithelial cells by expressing effector protein genes such as SopB, SopE, and SopE2 within the *Salmonella* pathogenicity island 1 (SPI-1).[5] Once *Salmonella* has crossed the epithelial lining, the pathogen is engulfed by phagocytes such as macrophages in the lamina propria, which are thin layers of connective tissue beneath the epithelium that form part of the mucous membrane.[4] As it enters the mucous membrane, *Salmonella* encodes for another set of effector proteins in the *Salmonella* pathogenicity island 2 (SPI-2).[4]

recognition of bacterial pathogens and activation of host immune response

When a macrophage encounters a pathogen such as *Salmonella*, the macrophage detects the pathogen through specialized cell surface receptors known as pattern recognition receptors (PRRs).[4] These receptors sense foreign ligands such as pathogen-associated molecular patterns (PAMPs), which contain molecular motifs conserved within a group of microbes.[4] Upon pathogen recognition, PRRs induce the activation of downstream signaling cascades in the macrophage, which then mounts an appropriate immune response.[3] In a recent study, Das et al. identified Brain Angiogenesis Receptor 1 (BAI1) as a PRR that binds to lipopolysaccharides (LPS) of gram-negative bacteria.[3] Upon binding, BAI1 recruits engulfment and cell motility protein 1 (ELMO1), a cytosolic microbial sensor protein that ultimately leads to the reorganization of the actin cytoskeleton to allow for the engulfment of certain pathogens.[3]

figure 1. *salmonella* entry into the host cell.

(A) *Salmonella* employs T3SS to inject SPI-I effector proteins, promoting invasion into the host epithelial cell. Upon entry, *Salmonella* will express SPI-II effector proteins to increase bacterial survival by forming the SCV. (B) In the lamina propria, *Salmonella* will encounter macrophages where they will be engulfed via phagocytosis. (C) Once engulfed, the macrophage will secrete cytokines to activate the host inflammatory response. Internalized *Salmonella* will again release SPI-II effectors to evade immune response. (created with biorender.com)

some pathogenic bacteria evade immune response through hijacking host cell machinery

Once the bacteria are internalized by a macrophage, the fate of the bacterium depends on its virulence factor or its ability to infect the host.[4] Non-pathogenic bacteria are internalized into a compartment known as the early phagosome, which is formed through the invagination of the plasma membrane.[5] Soon this compartment undergoes swift maturation into a late phagosome through a succession of membrane trafficking events.[6] The phagosome then fuses with the lysosome that contain different hydrolytic enzymes to form the phagolysosome, where the internalized bacteria are eventually degraded.[6] However, pathogenic bacteria such as *Salmonella* can avoid this fate by disrupting the trafficking mechanisms within the macrophage in order to survive and replicate.[6] To protect itself, the internalized pathogen redirects the maturation of the phagosome to create a modified phagosome known as the *Salmonella* containing vacuole (SCV).[5,6] The *Salmonella* bacteria replicate inside the SCV, which has many features of the late endosome, including maintaining an acidic pH essential for the induction of SPI-2 T3SS bacterial effector proteins.[6] In addition to this, *Salmonella* will block the fusion of the lysosome with the SCV and interactions with the endocytic pathway to prevent degradation of the bacterium.[5]

bacterial effector proteins interact with cytosolic microbial sensor elmo1 through a conserved WXXXE motif to disrupt the host inflammatory response

Our recent findings indicate that ELMO1 assists macrophages in sensing pathogens and commensals to predict pathogenic infection through differential regulation of the host immune response. Considering that *Salmonella* bacterial effector SifA is important in maintaining SCV integrity for bacterial survival through disruption of the host immune response, our lab wanted to assess whether SifA interacts with ELMO1 to control pathogenesis.

Previous studies have found that ELMO1 interacts with the *Shigella* effector protein IpgB1, which is involved in entry into host cells through membrane ruffling.[3] Since then, several known T3SS effectors including *Salmonella* SifA and SifB have been categorized into a single family of effector proteins that all contain a

figure 2. multiple sequence alignment of bacterial effector protein family with a conserved wxxxe motif.

A BLAST search of amino acid sequences in enteric pathogenic bacteria exhibit homologues that share a common amino acid sequence Trp-xxx-Glu or WxxxE motif (image courtesy of Das Lab).

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SifA     STM,SE              TELRKGHLDGWKAQEKATYLAAKIQ
         SEST                SEWRKGNLDEWKTQEKATYLAAKIQ
         YF                  NINQGDKFDMWKKEERTTYLSAVIN
SifB     STM,SE,SEST,ST,SN   AMAEKGNLCDWKEQERKAAISSRIN
IpgB1    SF,EC               DSNSGNQLFCWMSQERTSYVSSMIN
IpgB2    SF,EC               EQI-GENITDWKNDEKKVYVSRVVN
Map      EP,EC               KQTGSSDTQQWFKQEQITFLSRAVN
         CR                  KQTGNGDTQQWFRQEQITFISKTVN
         EHEC                KQTGSSDTQQWFKQEQITFLSRTVN
EspT     EC                  KQTRSGDTQQWFQQEQTTYISRTVN
         CR                  LKN-EGKMNEWMREECICFVSRDVN
EspM     EC,CR               RQS-TKDIDEWIKDERIVYPVRVIN
TrcA     EPEC                RQN-TKDINGWIKDERIVYPSRVIN
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common WxxxE motif.[6] Following the original classification, our lab has used BLAST to identify more enteric bacterial proteins that share this common motif; we found that these effector proteins are present in pathogenic bacteria but not in commensals (Figure 2). Moreover, preliminary studies have found that mutations in the WxxxE motif in *Salmonella* SifA affect its interaction with ELMO1.[3,7]

The interaction between ELMO1 and other WxxxE effector proteins from *Shigella* and *E.coli* suggests that ELMO1 plays an important role in pathogenesis. Further research is needed to evaluate the interaction of ELMO1 with bacterial effectors secreted by pathogenic bacteria and to elucidate how these pathogens can hijack the host signaling machinery and impact the host inflammatory response.

references

1. Nyachuba D. G. (2010). Foodborne illness: is it on the rise?. *Nutrition reviews*, 68(5), 257–269. https://doi.org/10.1111/j.1753-4887.2010.00286.

2. Centers for Disease Control and Prevention. (2020). Foodborne Germs and Illnesses. Retrieved from https://www.cdc.gov/foodsafety/foodborne-germs.html

3. Das, S., Sarkar, A., Choudhury, S. S., Owen, K. A., Castillo, V., Fox, S., Eckmann, L., Elliott, M. R., Casanova, J. E., & Ernst, P. B. (2015). ELMO1 has an essential role in the internalization of Salmonella Typhimurium into enteric macrophages that impacts disease outcome. *Cellular and molecular gastroenterology and hepatology*, 1(3), 311–324. https://doi.org/10.1016/j.jcmgh.2015.02.003

4. Ehrbar, K., Friebel, A., Miller, S. I., & Hardt, W. D. (2003). Role of the Salmonella pathogenicity island 1 (SPI-1) protein InvB in type III secretion of SopE and SopE2, two Salmonella effector proteins encoded outside of SPI-1. *Journal of bacteriology*, 185(23), 6950–6967. https://doi.org/10.1128/jb.185.23.6950-6967.2003

5. Alto, N. M., Shao, F., Lazar, C. S., Brost, R. L., Chua, G., Mattoo, S., McMahon, S. A., Ghosh, P., Hughes, T. R., Boone, C., & Dixon, J. E. (2006). Identification of a bacterial type III effector family with G protein mimicry functions. *Cell*, 124(1), 133–145. https://doi.org/10.1016/j.cell.2005.10.031

6. Bulgin, R., Raymond, B., Garnett, J. A., Frankel, G., Crepin, V. F., Berger, C. N., & Arbeloa, A. (2010). Bacterial guanine nucleotide exchange factors SopE-like and WxxxE effectors. *Infection and immunity*, 78(4), 1417–1425. https://doi.org/10.1128/IAI.01250-09

7. Sayed, I. M., Suarez, K., Lim, E., Singh, S., Pereira, M., Ibeawuchi, S. R., Katkar, G., Dunkel, Y., Mittal, Y., Chattopadhyay, R., Guma, M., Boland, B. S., Dulai, P. S., Sandborn, W. J., Ghosh, P., & Das, S. (2020). Host engulfment pathway controls inflammation in inflammatory bowel disease. *The FEBS journal*, 287(18), 3967–3988. https://doi.org/10.1111/febs.15236

8. Handa, Y., Suzuki, M., Ohya, K., Iwai, H., Ishijima, N., Koleske, A. J., Fukui, Y., & Sasakawa, C. (2007). Shigella IpgB1 promotes bacterial entry through the ELMO-Dock180 machinery. *Nature cell biology*, 9(1), 121–128. https://doi.org/10.1038/ncb1526

9. Mellouk, N., & Enninga, J. (2016). Cytosolic Access of Intracellular Bacterial Pathogens: The Shigella Paradigm. *Frontiers in cellular and infection microbiology*, 6, 35. https://doi.org/10.3389/fcimb.2016.00035

THE IMPORTANCE OF PROTEIN-PROTEIN INTERACTIONS IN DRUG DISCOVERY AND INHIBITION OF MYCOLIC ACID PRODUCTION IN MYCOBACTERIUM TUBERCULOSIS

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introduction

Tuberculosis (TB), a disease which primarily targets the lungs, is caused by the human pathogen *Mycobacterium tuberculosis* (*Mtb*). TB spreads when an infected person coughs, sneezes, or speaks, passing the bacteria onto someone else. *Mtb* is deposited into the lungs of the newly infected individual (resulting in pulmonary TB) and begins to proliferate, with a possibility of moving to other parts of the body such as the spine, brain, or kidneys.[1] Pulmonary TB symptoms include a bad cough, chest pain, and coughing up blood or phlegm, while symptoms of TB infection in other parts of the body vary depending on the organ in question.[2]

About 10 million individuals per year are still infected with TB, resulting in 1.5 million deaths worldwide.

drug resistance

As drug resistance becomes increasingly prevalent, researchers have the responsibility of developing novel drugs to fight multidrug resistant (MDR) and extensively drug resistant (XDR) TB. With the emergence of mycobacterial resistance, more than 500,000 individuals throughout the world become infected with resistant TB every year. These types of infections are very complicated and expensive to treat, especially in developing countries and underserved areas. Therefore, there is a high demand for novel and affordable antimycobacterial drugs. In order to develop new anti-tubercular drugs, it is essential to recognize the important role mycolic acids play in the virulence of *Mtb*. Mycolic acids, integral structural components of the *Mtb* cell wall, are long-chain fatty acids that are produced by fatty acid biosynthesis (FAB). *Mtb* employs both Type I and II fatty acid synthase pathways (FAS I and FAS II), producing various mycolic acids for a rigid and waxy cell wall.

FAS I and FAS II mycolic acid production

FAS I is one giant multifunctional system of various protein domains, also referred to as a mega-enzyme, whereas FAS II is a system made up of multiple separate protein domains. *Mtb* employs the FAS pathways to form mycolic acids by moving an acyl substrate, which is attached to the acyl carrier protein (acpM), from one domain to the next. Each enzyme, also known as a partner protein, forms catalytically relevant interactions with the acpM and catalyzes its respective reaction. There are four iterative reactions involved in the FAS pathways: a condensation step, a reduction of a beta ketone, a dehydration step, and a final reduction of the enoyl intermediate into the final product.

protein-protein interactions

According to a study by Veyron-Churlet, et al., the protein-protein interactions in the FAS II system of *Mtb* are extremely important for future drug development. Since these interactions are of great importance for the biosynthesis of the mycolate layer of the mycobacterium, they are also essential for the survival of the mycobacterium. These findings point to potential sites of inhibition in the FAS II pathway, which can possibly reduce the growth of *Mtb*.[3]

However, due to the very transient nature of the interactions between the acpM and its partner proteins, studying these interactions has not yet been successful.

In order to deal with the transient nature of some protein-protein interactions, the Burkart lab at University of California, San Diego has developed various biochemical tools that can be used to physically link two proteins together while they are interacting with each other. This covalently attaches the proteins to one another, completely eliminating the difficulty of evaluating transient interactions. These tools had previously been used for studying the *E.coli* system. Now, the Burkart lab strives to implement these

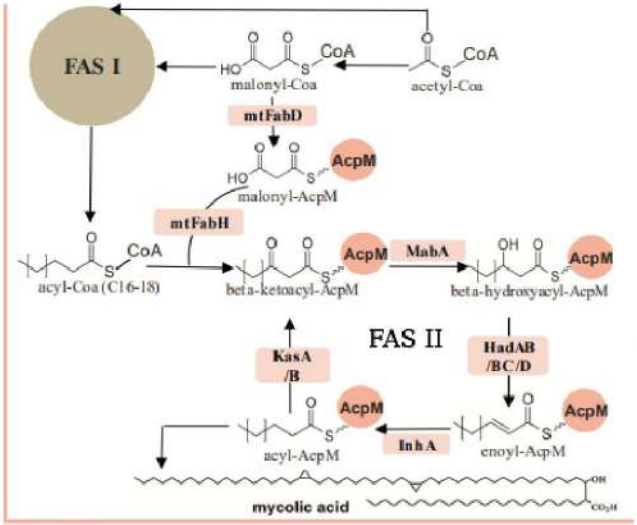

figure 1. FAS I and FAS II in mycobacterium tuberculosis. The FAS I and FAS II metabolic pathways of Mycobacterium tuberculosis show the formation of crucial structural components, mycolic acids.

techniques into the *Mtb* FAS II system, and study the protein-protein interactions of acpM and some of its partner proteins.[4]

The ketosynthase enzymes of *Mtb* FAS II, KasA and KasB, are two of the targets in this ongoing project. These enzymes perform the condensation step of the pathway to form a new carbon-carbon bond. Although both KasA and KasB catalyze the same reaction, there is a slight difference between the two enzymes. KasA, the essential elongation enzyme, catalyzes the initial elongations of the acyl molecule, and KasB, the nonessential elongation enzyme, is involved in the full elongation of the molecule (two carbons at a time until completion).

Other targets of interest of this current project are the recently identified dehydratase enzymes of *Mtb* FAS II, HadAB and HadBC, which perform the dehydration reaction to produce the trans-enoyl chain in the pathway.[5]

As a tightly bound heterodimer, HadAB consists of two monomers, HadA and HadB, each with its specific function; HadB contains a critical catalytic dyad consisting of Histidine and Aspartate, whereas HadA has the ability to bind the fatty acyl substrate in its channel for catalysis to take place. HadAB is involved in the initial elongation of short mycolic acids, and HadBC, the other dehydratase of *Mtb*, catalyzes the elongation of mycolic acids to completion.[4] The dehydration step is also a very important part of mycolic acid production, and therefore, an integral part of *Mtb* viability and survival. According to Belardinelli and Morbidoni, drugs currently used in the clinical treatment of TB, isoxyl and thiacetazone, affect the dehydration step of FAS II, catalyzed by HadAB and HadBC. However, the Had heterodimers are still a target for novel anti-tubercular drug development due to the rising drug resistance in *Mtb*.[6,7]

conclusion

Mtb continues to be a worldwide health crisis. In particular, many developing countries are still facing the harshest effects of *Mtb* resistance against the current treatments. To fight this crisis, ongoing research strives to elucidate the protein-protein interactions involved in the fatty acid biosynthesis in *Mtb*. The potential findings of this research will aid in developing novel treatments and drug discovery.

references

1. How TB Spreads. (2016, March 11). Retrieved August 02, 2020, from https://www.cdc.gov/tb/topic/basics/howtbspreads.htm

2. Signs & Symptoms. (2016, March 17). Retrieved August 02, 2020, from https://www.cdc.gov/tb/topic/basics/signsandsymptoms.htm

3. Veyron-Churlet, R., Guerrini, O., Mourey, L., Daffé, M., & Zerbib, D. (2004). Protein-protein interactions within the Fatty Acid Synthase-II system of Mycobacterium tuberculosis are essential for mycobacterial viability. *Molecular Microbiology*, 54(5), 1161-1172. doi:10.1111/j.1365-2958.2004.04334.x

4. Konno, S., La Clair, J., & Burkart, M. (2018, November 27). Trapping the complex molecular machinery OF Polyketide and fatty acid Synthases with Tunable Silylcyanohydrin crosslinkers. Retrieved April 22, 2021, from https://onlinelibrary.wiley.com/doi/full/10.1002/anie.201806865

5. Cantaloube, S., Veyron-Churlet, R., Haddache, N., Daffé, M., & Zerbib, D. (2011). The Mycobacterium Tuberculosis FAS-II Dehydratases and Methyltransferases Define the Specificity of the Mycolic Acid Elongation Complexes. *PLoS ONE*, 6(12). doi:10.1371/journal.pone.0029564

6. Belardinelli, J. M., & Morbidoni, H. R. (2012). Mutations in the essential FAS II β-hydroxyacyl ACP dehydratase complex confer resistance to thiacetazone inMycobacterium tuberculosisandMycobacterium kansasii. *Molecular Microbiology*, 86(3), 568-579. doi:10.1111/mmi.12005

7. Dong, Y., Qiu, X., Shaw, N., Xu, Y., Sun, Y., Li, X., . . . Rao, Z. (2015). Molecular basis for the inhibition of β-hydroxyacyl-ACP dehydratase HadAB complex from Mycobacterium tuberculosis by flavonoid inhibitors. *Protein & Cell*, 6(7), 504-517. doi:10.1007/s13238-015-0181-1

Senior Honors Theses

UC San Diego's Senior Honors Thesis Program allows undergraduate biology majors to work one-on-one with faculty mentors to pursue independent lab research. These are the abstracts of all the exceptional research projects conducted by honors students this past year.

Spotted lagoon jellyfish at the Seattle Aquarium in Washington.

Photo by Zina Patel

Earl Warren College
Human Biology Major

AYSHA ALANI

PI: Eric Schmelz, Ph.D., UCSD, Division of Biological Sciences

Potential signals and pathways fueling maize antibiotic biosynthesis

To ensure food security, maize (Zea mays) is a model crop investigated for genetically and biochemically based stress resistance mechanisms. Specialized metabolic pathways typically function as defenses and can critically limit the spread of fungal diseases; however, a majority of these antibiotics remain poorly understood. I use pangenome diversity, analytical chemistry and genetic mapping to understand defenses derived from the sesquiterpene volatile ß-selinene. Metabolite-based co-expression analyses in association panels and metabolite-led mapping efforts reveal that beyond ß-costic acid, diverse arrays of selinene derivatives exist with multiple germplasm dependent metabolite family sizes. A selinene diol, termed Kudtdiol, was discovered in roots along with a dozen related unknown analytes. Genetic mapping efforts revealed a cytochrome P450 (CYP) on chromosome 1 is nearly certain to partially drive selinene-based antibiotic biosynthesis. Bioassays with maize terpene synthase 21 mutants (tps21) lacking selinene derivatives now confirm a critical protective role of the pathway.

Sixth College
Human Biology Major

LIKITHA ARADHYULA

PI: Trey Ideker, Ph.D., UCSD School of Medicine, Department of Medicine

Mechanisms of drug-autophagy interactions in cancer

Autophagy is a cellular process that allows for proper degradation of cellular components that can then be reused by the cell. Its role in cancer is complex and context-dependent; it can suppress tumorigenesis in healthy cells or, alternately, promote survival in established tumors. Thus, the potential of autophagy modulation as a cancer therapeutic approach is limited due to a need for a better understanding of these complexities. We use DrugCell, a deep learning interpretable model of cancer cell biology mechanisms, to identify drug-autophagy interactions across multiple cancer cell lines. Furthermore, we test several predicted drug-autophagy pairs and show the effects on autophagic flux and resulting survival rate.

John Muir College
Ecology, Behavior, and
Evolution Major

ABEGAIL RIO BIGASIN

PI: Deirdre Lyons, Ph.D., Scripps Institution of Oceanography

Exploring nematocyst sequestration and regeneration in cerata of *Berghia stephanieae*

Regeneration is widely distributed across many animal species, but retention of functional capabilities after the regeneration of a lost body part is variable. There are four different phyla that are able to regenerate and are also able to sequester nematocysts, including the Mollusca. One nudibranch species of the phylum mollusca, Berghia stephanieae, is able to regenerate almost all parts of its body, including the tissue that sequesters nematocysts. This structure is the ceras, a structure emerging from the animal's main body that engages in the sequestration of its prey's nematocysts (unique organelles that are created only in species of the phylum Cnidaria) into its cnidosacs at the tips of the cerata. Here, we demonstrate the effects of starvation on (a) growth rate and (b) functionality in regenerating cerata in the nudibranch Berghia stephanieae. After removing cerata, starved animals regenerate all cerata at a slower rate than animals that are fed.

Revelle College
Biochemistry & Cell
Biology Major
Humanities Minor

JAIDEEP CHAKLADAR

PI: Weg Ongkeko, M.D., Ph.D., UCSD School of Medicine, Department of Surgery

Pan-cancer analysis of immune-associated mechanisms induced by tobacco smoke

The effect of tobacco smoke on immune-associated (IA) carcinogenesis is not well defined on a pan-cancer scale. This project aims to characterize features of the immune landscape that are common and unique to a panel of five smoking-induced cancers. Patient sequencing data from tumor and adjacent normal samples from The Cancer Genome Atlas were analyzed to determine IA genes that are differentially expressed in cancer patients who are also smokers. These genes were further correlated to patient prognosis, immune and cancer-associated pathway expression, and genomic alterations. We found that prognostically-relevant smoking-mediated IA gene dysregulation was most common in head and neck cancer and lung adenocarcinoma patients. Dysregulated genes and direction of gene dysregulation were not consistent between cancers. However, the functional consequences and genomic alterations leading to IA gene dysregulation were common in some cases, suggesting differing effects of smoking carcinogens on the genome that lead to similar downstream consequences.

John Muir College
Physiology &
Neuroscience Major

ACHOL CHOWDHURY

PI: Matthew Shtrahman, M.D., Ph.D., UCSD School of Medicine, Department of Neurosciences

Studying temporal pattern separation in mice through a novel behavioral paradigm

The hippocampus is a critical brain region involved in memory formation. In particular, the hippocampal dentate gyrus is thought to be important for distinguishing similar, but distinct, experiences in order to form new memories in a process called pattern separation. Memories are constructed from sensory information describing our environment and experiences. These sensory inputs are rarely static, but rather are encountered as a stream of continuously changing information. How the hippocampus processes temporally dynamic information to form memories is not understood. We aim to investigate the role of the hippocampal dentate gyrus in performing pattern separation of temporally varying inputs. This study attempts to visualize this process of temporal pattern separation using two photon calcium imaging in mice that are head fixed on a treadmill as the mice attempt to differentiate auditory patterns or songs in a time dependent manner.

Roger Revelle College
Human Biology Major

KYPROS DERESCHUK

PI: Weg M. Ongkeko, M.D., Ph.D., UCSD School of Medicine, Department of Surgery

The oral fungal microbiome is implicated in etiology-specific head and neck squamous cell carcinoma prognosis

Previous studies have identified the importance of smoking and human papillomavirus (HPV) on head and neck squamous cell carcinoma (HNSCC) carcinogenesis. As the microbiome has been emerging as a potentially important modulator of carcinogenesis, this study aims to characterize the effects smoking and HPV have on the fungal oral microbiome and investigate how these etiologies may alter HNSCC development. RNA-seq data from cancer and adjacent normal samples was obtained from The Cancer Genome Atlas (TCGA) and aligned to a library of fungal reads to determine microbial abundance. Fungal microbial abundance was correlated to patient prognosis, cancer and immune-associated gene and pathway expression, and genomic and transcriptomic alterations. We identified microbial signatures correlated to upregulation of oncogenic pathways, worse prognosis, and downregulation of tumor-suppressive pathways. Abundance of these pro-cancer microbes were augmented in HPV and smoking cohorts. Anti-cancer microbial signatures were discovered, having lower abundance in non-HPV and non-smoking cohorts.

John Muir College
Human Biology Major
Psychology Minor

NGUYEN DO

PI: Louise C. Laurent M.D.,Ph.D., UCSD School of Medicine, Department of Obstetrics, Gynecology, & Reproductive Sciences

Discovery and verification of extracellular miRNA biomarkers for non-invasive prediction of Pre-eclampsia in Asymptomatic Women

Preeclampsia is a common cause of preterm birth and maternal mortality. It is a pregnancy complication characterized by new-onset high blood pressure and protein in urine. Many patients that develop preeclampsia do not have known risk factors. Thus, effective and non-invasive screening for high-risk pregnancies may be clinically useful. For our project, we study the utility of extracellular miRNAs (exRNAs) biomarkers in blood and other biofluids to identify asymptomatic patients at high risk for preeclampsia. We identified candidate miRNA biomarkers for prediction of preeclampsia in maternal serum. One of the Late window bivariate biomarkers was of particular interest (hsa-miR-516b-5p/hsa-miR-155-5p). Our aim is to validate these biomarkers of interest in an independent set of patients. We have successfully extracted the exRNAs from 886 serum samples of this new cohort and are halfway in the progress of completing RNA libraries. The results can help us confirm the presence of certain biomarkers.

Roger Revelle College
Physiology &
Neuroscience Major

RYAN GHASSEMI

PI: Shannon Lauberth, Ph.D., UCSD, Division of Biological Sciences, Section of Molecular Biology

Investigating the role of non-coding RNAs in non-canonical RNA-binding proteins

Gene transcription is the evolutionarily conserved process by which DNA is used as a template to produce RNA. Depending on where transcription occurs in the genome, the RNA transcripts produced may carry the coding instructions for proteins or fall into the category of "non-coding RNA" (ncRNA), whose functions have been increasingly characterized in recent years. When transcriptional levels deviate from the normal, cellular homeostasis can be shifted, resulting in abnormal protein expression and potentially disease. Prior research has shown that a class of ncRNA produced from enhancer regions can bind to and influence the function of proteins that help activate transcription. The goal of my project is to assess the role of ncRNA in modulating transcription and cellular homeostasis through binding to proteins that regulate these activities. In studying the mechanisms that alter transcriptional activity, we aim to identify ways in which a molecular transition to disease can be prevented.

Earl Warren College
Human Biology Major
Business Minor

PEYTON GRAVES

PI: Amir Zarrinpar, M.D., Ph.D., UCSD, Department of Medicine

Role of bacterial bile acid deconjugation on host exercise performance

Exercise is an environmental factor that drives gut microbiome composition and function, but it is unclear how the microbiome influences host exercise performance. Bacterially modified bile acids (BAs) are signaling molecules that mediate host-microbiome interactions and affect host energy homeostasis. Using a novel mechanism to functionally manipulate the microbiome, we investigate how bacterial BA biotransformations affect host exercise performance. The Zarrinpar Lab has engineered a native E. coli to express bile salt hydrolase (BSH) (EcAZBSH+), which increases BA deconjugation and alters host fecal and serum BA pool composition. Metabolic phenotyping reveals that mice colonized with EcAZBSH+ engage in significantly more voluntary wheel running compared to controls despite no change to overall cage activity. These mice also demonstrate increased grip endurance in the inverted grid test. To elucidate the mechanism underlying these changes, further efforts will investigate whether these differences are driven by altered reward-motivated behavior, skeletal muscle physiology, or both.

Roger Revelle College
Molecular & Cell
Biology Major

LINDSEY GRIFFIN

PI: Eyal Raz, M.D., UCSD School of Health Sciences, Department of Medicine

The role of chronic lung inflammation in lung carcinogenesis

Aeroallergens are extremely prevalent indoors and outdoors around the world, which drive the immunological cascades resulting in allergic reactions. As commonly known, people who experience allergies affecting the lungs may develop asthma. However, it is unclear whether the chronic inflammatory response observed in asthma may increase the risk of developing lung cancer. Here we are proposing experiments to test this association in different mouse models of lung cancer. We are evaluating the effect of aeroallergens and the resulting chronic lung inflammation on lung cancer incidence in specific cohorts of mice. We expect to find that aeroallergen-induced chronic lung inflammation promotes lung carcinogenesis.

Earl Warren College
Human Biology Major

CALVIN HARRIS

PI: Eric Alexander Schmelz, Ph.D., UCSD, Division of Biological Sciences

Maize dolabralexins: activity and biosynthesis of newly discovered root antibiotics

Global agriculture places a heavy reliance on maize (Zea mays) production; however, pest and pathogen attack remain problematic resulting in annual yield losses approaching 20%. As a layer of innate immunity, crops such as rice, oats and maize deploy diverse root terpenoid antibiotics functioning as potent antifungal agents. To better understand maize antifungal metabolites and biosynthetic pathways, I worked to isolate and identify dominant root metabolites and candidate end products in a newly discovered diterpenoid pathway, termed dolabralexins. High-performance liquid chromatography enabled purification and NMR structural elucidation of 16-nordolabranolic acid (NDA), a novel maize metabolite. Antimicrobial assays using two Fusarium species demonstrated that NDA is an important and relevant antifungal defense at physiologically relevant concentrations common to maize roots. Analyses of mutant plants blocked in dolabralexin production confirmed a key role in disease resistance, highlighting that many critical defense mechanisms remain to be discovered to potentially deploy in related crops.

John Muir College
Biochemistry &
Cell Biology Major

JENNIFER HARRISON

PI: Enfu Hui, Ph.D., UCSD, Section of Cell and Developmental Biology

Dual-ITIM and dual-ITSM variants and their effect on SHP-2 recruitment and PD-1 signaling

The immune checkpoint programmed-cell-death-1 (PD-1) has been successfully targeted for cancer immunotherapy, but its mechanism remains incompletely understood. PD-1 inhibits T cells by recruiting the tyrosine-protein-phosphatase SHP-2 via two motifs: immunoreceptor tyrosine-based inhibitory motif (ITIM) and immunoreceptor tyrosine-based switch motif (ITSM). However, it is not clear how ITIM and ITSM collaborate to recruit SHP-2 and how the motif diversity contributes to PD-1 function. This project seeks to measure SHP2 recruitment and inhibitory functions of engineered PD-1 with degenerated motifs: dual-ITIM and dual-ITSM, using both cell-free membrane reconstitution and cell culture assays. My preliminary data showed that dual-ITSM PD-1 variant is comparable to wild-type PD-1 in both SHP2 recruitment and T cell inhibition, whereas dual-ITIM PD-1 variant is inferior in both aspects. My data supports the notion that ITSM is the major PD-1 signaling motif and raises new questions about the role of ITIM.

Thurgood Marshall
College
Molecular & Cell
Biology Major

MAX HERRERIAS

PI: Karl Willert, Ph.D., UCSD School of Medicine, Department of Cellular & Molecular Medicine

A characterization and isolation of the recombinant human WNT9A protein

The Wnt signaling pathway is critical for development of all metazoans. Dysregulation of this pathway is associated with many pathological conditions, such as diabetes, cancer, neurodegeneration, and birth defects, to name a few, highlighting the need to better understand the fundamental processes that regulate Wnt signaling. In the Wnt signaling pathway, members of the WNT family of secreted growth factors interact with their cognate receptors, called Frizzleds (FZDs), to control axis-formation, cell proliferation, and cell fate specification. Previously, the Willert laboratory identified Wnt9a and its receptor Fzd9 as critical for hematopoietic stem cell (HSC) development in both zebrafish and humans. My goal is to isolate human WNT9A so that its specific effects on HSC development can be further understood. Here, I show the steps I have taken to fractionate and partially purify recombinant WNT9A and detect its activity in cell-based signaling studies.

Roger Revelle College
Biochemistry &
Cell Biology &
Humanities Majors

INGRID HEUMANN

PI: David Cheresh, Ph.D., UCSD, Sanford Consortium for Regenerative Medicine

Cellular stress promotes cancer stem-like cell phenotypes via β3 induction

Cancer stem-like cells (CSCs) are a subpopulation of undifferentiated cells that contribute to tumor progression and drug resistance. Notably, CSCs express αvβ3, a heterodimeric surface-signaling molecule. Previous studies have demonstrated αvβ3 expression is both necessary and sufficient to drive CSC phenotypes. Furthermore, acquired resistance to chemotherapy in cancer cells is preceded by an induction of αvβ3 expression; however, the molecular underpinnings leading to αvβ3 expression remain undefined. Here we investigate cellular stress as a regulator of β3 expression, αvβ3 surface-presentation, and β3-dependent stem-like phenotypes. We report both hypoxia and oxidative stress induce β3 expression as determined by western blot and qPCR analysis. Additionally, we generated human pancreatic cancer models to evaluate β3-mediated effects on tumor progression and drug resistance. Cell lines were engineered to express ectopic β3 or a doxycycline-inducible shRNA silencing β3 expression. These models represent biological tools that will help elucidate the relationship between αvβ3 and CSCs.

Roger Revelle College
Biochemistry & Cell
Biology Major
Chemistry Minor

YU-TING HUANG

PI: Gerald Joyce, M.D., Ph.D., Salk Institute for Biological Studies

Co-variation mutagenesis reveals structural evolution in an RNA polymerase ribozyme

The class 1 RNA polymerase ribozyme was evolved in the laboratory to copy other RNA molecules as a model for the origin and early evolution of life before the invention of protein enzymes. Analysis of polymerase ribozyme sequences suggests that the ribozyme underwent significant structural evolution as the polymerase activity improved. Here we performed targeted co-variation mutagenesis on the ribozyme to characterize these structural changes and demonstrate their importance to the improved polymerase activity over the course of directed evolution. Combined with biophysics and bioinformatics experiments from a collaborating laboratory at Yale University, our results show how evolution remodeled the core of the class I polymerase to provide an RNA enzyme that can copy RNA. These results also suggest how to design new ribozymes and new directed evolution experiments that would enable the class I polymerase to copy itself and ultimately replicate autonomously.

Roger Revelle College
Biochemistry & Cell
Biology Major

DARREN LAM

PI: Randolph Y. Hampton, Ph.D., UCSD, Division of Biological Sciences

Uncovering the novel UPS pathway required for Lys1-V26D degradation

The ubiquitin-proteasome system (UPS) is a major component of protein quality control (PQC) that degrades misfolded proteins and maintains proteostasis throughout the cell. E3 ubiquitin ligases determine the substrate specificity of each UPS pathway. Accordingly, the PQC field has dedicated significant effort to identifying novel E3s. Our lab previously developed a screen to generate new UPS substrates. Remarkably, one of these substrates, Lys1-V26D, was not degraded by any of the classical UPS pathways. This result suggests that Lys1-V26D is instead degraded by a new, unidentified UPS pathway. Here, we screen a 94-strain null collection to uncover the UPS pathway required for Lys1-V26D degradation. The UPS provides the cell with an extensive proteostasis network; failure to degrade misfolded proteins produces incredible toxicity, observed in neurodegenerative diseases such as Alzheimer's and Parkinson's disease. Thus, a novel UPS pathway will allow us to further understand protein degradation's essential role in maintaining cellular health.

Roger Revelle College
Physiology &
Neuroscience Major

JAISEN LIM

PI: Don W. Cleveland, Ph.D., UCSD School of Medicine, Department of Cellular and Molecular Medicine

Mechanisms underlying synergy between gain of toxicity and loss of function in C9orf72 ALS

Amyotrophic Lateral Sclerosis is a progressive and debilitating disease characterized by degeneration of both upper and lower motor neurons. A hexanucleotide GGGGCC repeat expansion in a non-coding region of the C9orf72 gene is the most common cause of familial ALS. The repeat expansion causes ALS through a combination of gain of toxicity from and intranuclear RNA foci and/or poly-dipeptide repeat proteins (DPRs) encoded by repeat-containing RNAs and loss of C9ORF72 protein function from the affected allele. The project undertaken here focused on investigation of potential synergy between gain of toxicity and C9ORF72 loss of function, and on elucidation of their mechanisms of interaction, using ALS patient iPSC-derived motor neurons and a novel C9orf72 ALS mouse model that was generated (and which recapitulates motor neuron disease phenotypes, thereby providing an in vivo approach to testing these questions).

Earl Warren College
Human Biology &
Global Health Majors

MICHELLE LIN

PI: Mark Lawson, Ph.D., UCSD School of Medicine, Department of OB/GYN and Reproductive Sciences

Regulation of glucose transporters and other genes in kisspeptin cell lines

Reproduction is controlled by three major cell types in the hypothalamus and pituitary: kisspeptin neurons regulate GnRH neurons, which in turn regulate the gonadotropes in the anterior pituitary. Previous research has shown that the regulation of reproduction is influenced by nutrient availability, so examining glucose and its transporters may help better define the regulation of kisspeptin and GnRH, and thus hormone synthesis and secretion in gonadotropes. This research aims to determine the effect of a glucose dose response on glucose transporters and other related genes of the signal transduction pathway within two different kisspeptin cell lines. While there doesn't appear to be significant changes in the glucose dose response of GLUT1 or GLUT4, results suggest that there may be a dose-dependent upregulation of target gene TXNIP, as well as downregulation in the dose response of target genes c-Fos and Egr1.

Roger Revelle College
Human Biology Major

STEPHANIE LUEDTKE

PI: Anthony J. O'Donoghue, Ph.D., Skaggs School of Pharmacy and Pharmaceutical Sciences

Quantification of proteolytic activity in human breast milk after pasteurization

Human breast milk is seen as the gold standard of infant nutrition and contains specific bioactive peptides that cater to the nutritional and immunological needs of the infant. These peptides are generated from the enzymatic degradation of proteins by proteases that are present in the milk. In the US, milk banks must pasteurize donated milk before distributing to needful mothers. The effect of heat pasteurization (62.5C for 30min) on enzymatic activity of proteases has not been studied. Pasteurization was predicted to decrease enzyme activity of proteases in donor breast milk. We determined that several proteases are completely inactivated by pasteurization while others show either no change or partial reduction. The results indicate that there are significant differences in enzyme activity between pasteurized donor milk and fresh, non-treated milk. Therefore, infants who primarily feed on donor milk will lack key peptides that may be important for nutrition and immune defense.

Roger Revelle College
Cell Biology &
Biochemistry Major
Cognitive Science Minor

YASHASWAT SINGH MALHOTRA

PI: Pradipta Ghosh, M.D., UCSD SOM, Departments of Medicine and Cellular and Molecular Medicine

Reprogramming macrophages to resist atherosclerosis

Macrophages, a.k.a "big eaters" are key cells in our immune system. In hyperlipidemic conditions, they scavenge harmful oxidized lipids (oxLDLs) that accumulate in the aortic wall and transform into foam cells within atherosclerotic plaques. We have stumbled upon a novel atherogenic signaling pathway in macrophages which presents a hitherto unforeseen avenue to manipulate atherosclerosis. The pathway is initiated by the multi-modular G protein activator and a potent inhibitor of cAMP, GIV (a.k.a Girdin). Because cAMP is a versatile anti-atherogenic second messenger, we hypothesized that GIV plays a proatherogenic role and its inhibition will be therapeutic. Preliminary studies showed exactly that—while control mice developed plaques on a western diet, mice without GIV in their macrophage were protected. In vitro studies revealed that oxLDL-challenged macrophages rapidly increased cellular cAMP and cleared up the lipid in the absence of GIV but transformed into lipid-laden foam cells in its presence. Going forward, we seek to dissect the molecular mechanisms underlying the pro-atherogenic role of GIV and how might we manipulate this new pathway pharmacologically to reduce atherosclerosis.

Thurgood Marshall
College
Neurobiology Major

KEVIN W. MAZO

PI: Karl J. Wahlin, Ph.D., UCSD Health, Department of Ophthalmology

MicroRNA expression profiles in early-stage human retinal organoids

During differentiation, genes are coordinately expressed to drive cells towards a fate. MicroRNAs (miRNAs), a class of small non-coding RNA, serves as 'fine tuners' of differentiation by inhibiting translation or degrading messenger RNA. During development of human retinal organoids (RO), miRNAs changes are evident during the transition of a stem cell line from pluripotency into neural progenitor cells, from neural progenitors to retinal progenitor cells (RPC) and finally into retinal ganglion cells (RGCs). To identify significantly differentially expressed miRNAs, we harvested differentiated ROs and isolated RNAs. RNAseq was performed to determine the mRNA and miRNA profiles. Bioinformatic analyses from samples collected between days 0 (D0) and 45 (D45) revealed dynamic miRNA expression patterns, including that of the Let-7 miRNA family. Many microRNAs are predicted to target mRNAs involved in Axon Guidance, Neurotrophin Signaling, and Regulation of Actin Pathways. Together, these are likely to participate in determining retinal cell fate.

Sixth College
Molecular Biology Major
Psychology Minor

SWAPNIL MITTAL

PI: Joseph G. Gleeson, M.D., UCSD School of Medicine, Department of Neurosciences

Dissecting Zika virus: role of NS2B-NS3 protease in congenital Zika Syndrome

Zika Virus (ZIKV) epidemic in the Americas caused a dramatic rise in congenital microcephaly during its 2016 outbreak. In this paper, we study the effects of ZIKV protease NS2B-NS3 which has been previously shown to cause cell proliferation defects and apoptosis in human neural progenitor cell culture. The protease is implicated in cleavage of cytoskeletal factor septin-2 (PMID 30713029) which causes dysregulation of cell division leading to neuronal cell death which may be sufficient to cause microcephaly. To model the prenatal effects of the protease, we generated transgenic mice carrying the stop-floxed NS2B-NS3 protease which conditionally begins expression during neuronal development when activated by Nestin-Cre. Understanding these molecular mechanisms of ZIKV syndrome can uncover new drug targets and possible therapeutics.

Eleanor Roosevelt
College
Biochemistry & Cell
Biology Major

JAMES PAI

PI: Omar S. Akbari, Ph.D., UCSD, Division of Biological Science, Department of Cell & Developmental Biology

Developing and optimizing pgSIT in the malaria mosquito, *Anopheles gambiae*

Precision-guided sterile insect technique (pgSIT) is a novel CRISPR-based genetic sterilization technique to sterilize males and remove females for mass SIT vector control campaigns. Our lab has successfully demonstrated pgSIT in Aeges aegypti, and the goal of this work is to adapt pgSIT to the primary human African malaria vector Anopheles gambiae. pgSIT relies on mass release of sterile males, which when mated to wild-type females, causes females to be infertile. This makes complete male sterilization and female killing/incapacitation the cornerstones of this technology. During the course of my work, I have assisted in successfully adapting pgSIT to Anopheles gambiae, and have made great progress towards identifying and characterizing a novel candidate female-specific flight gene target to improve pgSIT female-killing characteristics. In all, our work has demonstrated that the adaptation of pgSIT to this critical malaria vector will likely be achievable to help control malaria transmission.

Eleanor Roosevelt
College
Molecular Biology Major

MARINA RAMSEY

PI: Chengbiao Wu, Ph.D., UCSD School of Medicine, Department of Neurosciences

A novel mutant nerve growth factor (NGF R100W) as a treatment for peripheral sensory neuropathy

Nerve growth factor (NGF) has both trophic and nociceptive functions; thus, its ability to reverse neurodegeneration is limited by the side-effect of pain. A single residue (R100W) switch in NGF was identified from individuals with Hereditary Sensory and Autonomic Neuropathy V (HSAN V), a disease involving loss of pain sensation but normal cognitive function. Our recent study has revealed that local injection of NGF R100W effectively restored pain sensation in a mouse model of Charcot Marie Tooth 2B peripheral sensory neuropathy (CMT2B). To further define the trophic effect of NGF R100W, we hypothesized that NGF R100W injection promoted the re-growth of sensory nerve fibers in CMT2B mice. Computation of nerve density in hindpaw epithelial tissue showed that, similar to wtNGF, local injection of NGF R100W injection was able to restore nerve growth ipsilaterally, but not contralaterally. These data suggest that NGF R100W retained its trophic function with a localized effect.

NEIL SHENDE

Earl Warren College
Biochemistry & Cell Biology Major
Mathematics Minor

PI: Weg Ongkeko, M.D., Ph.D., UCSD School of Medicine, Department of Surgery

Characterization of archaeal species in head and neck cancer

Archaea are single-celled prokaryotic organisms which are abundantly found on Earth as well as in humans, but while we know much about bacterial and eukaryotic organisms and their role in human diseases, we know very little about archaea. In recent years methanogenic archaea have been discovered in oral, colon, and vaginal regions of the human body, but the extent of their influence on disease is not yet clear. Given the presence of archaea in the oral cavity, we sought to characterize their presence in head and neck cancer. Using RNA-seq data from TCGA, we identified differentially abundant microbes in cancer and adjacent normal tissue samples. Further, we were able to correlate archaeal species with survival in head and neck cancer, and associate it with HPV status in patients. Further studies with tissue cultures and cell lines could help clarify the effects of these microbes, and could also help us understand their role in the human body.

NEVILLE TARAPOREVALA

Thurgood Marshall College
Ecology, Behavior & Evolution Major
Cognitive Science Minor

PI: Deirdre Lyons, Ph.D., Scripps Institute of Oceanography

Using laboratory culture of the nudibranch Berghia stephanieae to study reproductive development and feeding behavior

Nudibranch molluscs are established neurobiological models, but most species cannot be cultured in the lab. The aeolid Berghia stephanieae can be cultured through multiple generations, making transgenesis feasible. To enable this, we are studying their reproductive development and feeding behavior. Isolating age- and size-matched individuals at sequential timepoints allows us to determine the precise timing of reproduction. Berghia are translucent, and gonads become visible externally a few weeks before adults begin laying egg masses. Berghia isolated one month before appearing gravid can produce fertilized eggs, indicating earlier sperm exchange. Berghia, known for solely eating Exaiptasia, can consume the model cnidarian Nematostella vectensis. Berghia live at standard salinities (1.024 sg), but die at the preferred salinity of Nematostella (1.010 sg). Both species tolerate 1.020 sg, allowing for long-term co-culture. This precise knowledge of reproductive timing and expanded feeding open new research avenues for Berghia as a model system.

NATHANIEL TSAI

Roger Revelle College
Molecular Biology Major
Film Studies Minor

PI: Eric Bennett, Ph.D., UCSD, Division of Biological Sciences

Orphan protein degradation of MSH6

To maintain a healthy and functional state, eukaryotic cells sustain a high level of proteome organization through the segregation of protein subunits into sub-cellular components and the proper assembly of larger multi-protein complexes. Protein subunits that fail to correctly assemble in their cognate complexes are referred to as orphan proteins and must be recognized and degraded to maintain a stable proteome and avoid potentially cytotoxic effects. MutSα is a DNA mismatch repair complex heterodimer composed of subunits MSH2 and MSH6, that recognizes errors in DNA replication and aids in maintenance of genomic integrity. Here I report that the MutSα subunit MSH6 is highly unstable when expressed out of complex and is targeted for degradation by the proteome through ubiquitylation. Thus, this work establishes the MutSα complex as a model for orphan protein degradation and will facilitate future work aimed at the identification of novel components of orphan protein degradation pathways.

LILIT VARDANYAN

Thurgood Marshall College
Human Biology Major
Chemistry Minor

PI: Michael D. Burkart, Ph.D., UCSD, Department of Chemistry & Biochemistry

Protein-Protein interactions of the FAS II system in *Mycobacterium tuberculosis*

Mycobacterium tuberculosis (Mtb), the causative agent of tuberculosis (TB), employs the type II fatty acid synthase (FAS-II) metabolic pathway to produce long-chain fatty acids, precursors for mycolic acids and crucial structural components of the mycobacterial cell wall. Drug resistant TB infections continue driving the need for new antibiotics that target essential pathways. Our lab has used mechanistic crosslinking of the transient acyl carrier protein (ACP) with FAS-II enzymes in E. coli; here we aim to extend these studies to the Mtb FAS-II system. We investigate the protein-protein interactions between Mtb ACP (AcpM) and its dehydratase, HadBC. Results demonstrate that two fluorescently labeled probes previously used to crosslink E. coli FAS-II proteins do not react with HadBC, confirming the need to develop probes specific for Mtb FAS-II. Future work will investigate crosslinking the Mtb ketosynthase protein with other probes evaluated in the E. coli system and new probe development for HadBC.

LINDSAY WONG

Roger Revelle College
Molecular Biology Major

PI: Weg Ongkeko, M.D., Ph.D., UCSD School of Medicine, Department of Surgery

Analysis of the immune landscape in virus-induced cancers using a novel integrative mechanism discovery approach

The mechanisms of viral-induced carcinogenesis are extraordinarily complex and could potentially be immune-associated (IA). Using RNA and DNA-sequencing data from The Cancer Genome Atlas, we aim to computationally explore whether different virus-induced tumors exhibit similar IA dysregulations. Using a variety of computational analyses, we identified the most clinically relevant IA genes dysregulated in five virus-induced cancers (HPV-induced head and neck cancer, HPV-induced cervical cancer, EBV-induced stomach cancer, HBV-induced liver cancer, HCV-induced liver cancer) and developed an algorithm to identify pathways implicated in IA gene dysregulation. Our results revealed that IA dysregulations vary with cancer and virus type, but all five virus-induced cancers shared dysregulated oncogenic signatures and IA pathways. Furthermore, using our algorithm, we discovered potential mechanisms for genomic alterations to induce IA gene dysregulations. Our study offers a new approach to identifying mechanisms of cancer pathogenesis, which may be invaluable to discovering new immunotherapy targets for virus-induced cancers.

SEKO LI

Earl Warren College
Human Biology Major
Business Minor

PI: Derek Welsbie, M.D., Ph.D., Shiley Eye Institute, Department of Ophthalmology

Activation of Tau signaling in retinal ganglion cells after optic nerve injury

Glaucoma is a neurodegenerative disease involving the death of neurons called retinal ganglion cells (RGCs) and remains one of the leading causes of irreversible blindness. Other neurodegenerative diseases, such as Alzheimer disease, are characterized by the pathology and hyperphosphorylation of tau, a microtubule-associated protein. We investigated the hypothesis that tau also plays an important role in RGC death. We used histology and immunofluorescent staining to determine whether tau is phosphorylated in mice retina after optic nerve injury. Based on immunostaining of several different phospho-tau antibodies, we identified three potential phosphorylation sites that are activated in RGCs after axon injury. Further studies will be conducted to determine the change in tau signaling in cells with CRISPR-Cas9 knockouts of various kinases, such as dual leucine zipper kinase (DLK), that are proven to be involved in RGC death. Investigation of these interactions may provide a better understanding of the mechanisms of RGC death.

TAT HEI TSIN (DEXTER)

Earl Warren College
Biology: Bioinformatics Major
Computer Science Minor

PI: Kay Tye, Ph.D., Salk institute for Biological Studies

Liquid delivery system for mice behavioral training

In the field of systems neuroscience, to record neural activity of mice under certain stereotyped behavior consistently, scientists need to train experimental subjects extensively for a specific task. With rewards like sucrose and repetitions over days, animals can perform tasks accurately under an experimental setup. Therefore, training needs to be done efficiently and consistently so unwanted external variables are not introduced into the environment and affect a training animal's judgment. My senior honor thesis project includes a flexible liquid delivery system that could accommodate multiple training sessions simultaneously. I designed and implemented fully customized hardware and software components, integrating Arduino and Matlab code. In this pipelined process, users would only need to input their desired parameters to obtain accurate training data summary after training sessions.

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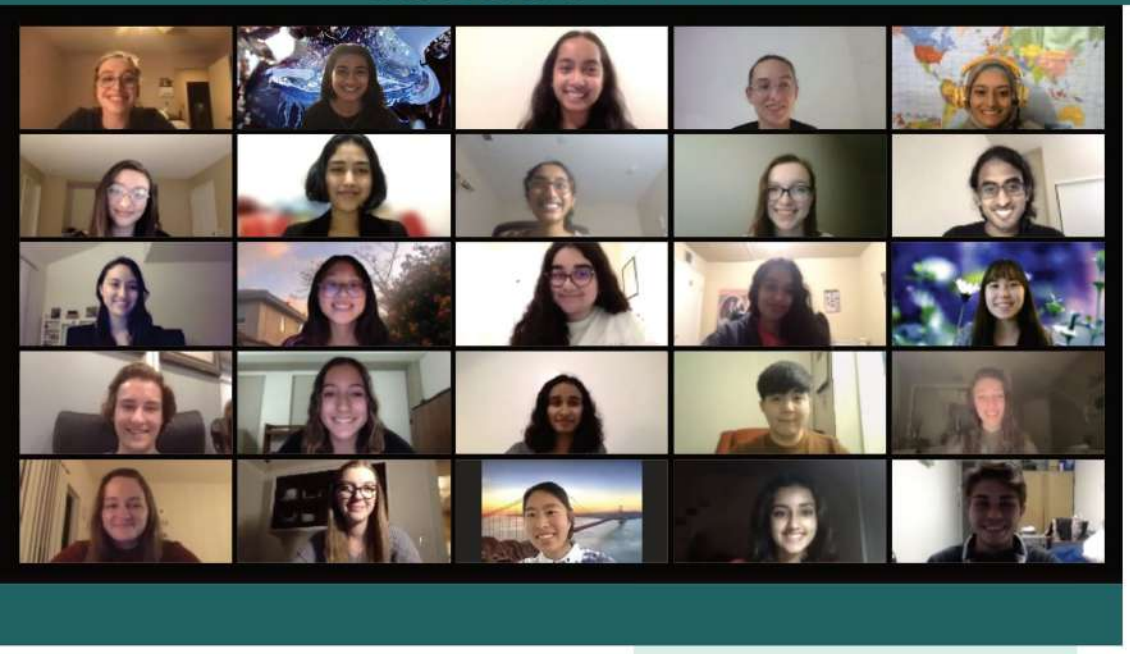

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