USU Science Review

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Composite of fixed WT Fibroblasts, stained for filamentous actin

ABOUT THIS PUBLICATION

USU Science Review, a student-led publication, has two principal functions: to foster intellectual discourse in the Uniformed Services University community, and to provide students with opportunities to develop their writing and editing skills.

This inaugural Issue features faculty and student submissions from the Molecular and Cellular Biology PhD program. Future issues will highlight and recognize the broad scientific work of the university, ranging from molecular biology to neuroscience, infectious disease, and global health. *USU Science Review* will be published biannually, with fall and spring Issues.

USU Science Review was created by Rohini Manickam, a 4th year Molecular and Cell Biology PhD candidate, with close support from Laura Bauman, Faculty Advisor and Assistant Dean; and Claire Pak, School of Medicine Communications Specialist through the Henry Jackson Foundation.

We thank the authors for their time and support of this inaugural issue, as well as the many faculty members and students who offered helpful feedback and spirited conversations. Future issues will be managed and edited by an editorial team of graduate students who have advanced to candidacy - we invite interested students to reach out to ususciencereview-ggg@usuhs.edu. We additionally extend a warm welcome to faculty and student writers interested in submitting to this periodical.

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History of the Field

Thousands of scientific research papers are published every year, shedding light on the latest discoveries and novel modes of thought in our fields. With our hyperfocus on the latest products of science, we should not lose appreciation for the seminal discoveries and evolution of ideas that led us to our current scientific landscape. These stories from faculty experts share a historical perspective, including timelines of discoveries with technological, political and economic challenges, providing appreciable context and reminding us that our individual contributions continue to drive this collective undertaking of discovery.

DEATH, TAXES, AND ACTIN

Dr. Jeremy Rotty

Actin is one of the most abundant proteins in the eukaryotic lineage. It has been largely conserved from yeast to humans, and is so ubiquitous that we might as well amend that old saying about life's certainties to "death, taxes, and actin." Labs all over the world use actin as a standard, a loading control. We now take actin for granted. This makes it all the more remarkable that when my grandmother was born, nobody had any idea that it existed.

Albert Szent-Györgyi, Ilona Banga, and Brúnó Straub prepared biochemical fractions of rabbit skeletal muscle in Szeged, Hungary as World War II raged. Szent-Györgyi was already world-renowned. He had won the Nobel Prize in 1937 at the age of 44 for his work on vitamin C and the Krebs cycle. So of course, in the following year, he completely switched fields and began working on the biochemistry of muscle contraction. Myosin was already established as the contractile element of muscle. Through a combination of serendipity, hard work, and breathtaking interpretation, Szent-Györgyi's group discovered that a separate protein was required to 'activate' myosin, so they named this protein actin. This work would launch thousands of careers and co-headline (1) every lecture on the cytoskeleton in institutions the world over.

And for years, few people knew anything about the discovery. Frankly, there were more pressing matters to attend to than the scientific literature.

IT WAS ALREADY DIFFICULT TO GET RESULTS PUBLISHED IN WARTIME EUROPE, AND DOUBLY SO IN AXIS-ALIGNED COUNTRIES LIKE HUNGARY.

Exponentially more so for Albert Szent-Györgyi, who (according to his cousin Andrew) refused to publish in German, the lingua franca for European scientists. Szent-Györgyi was fiercely opposed to the Nazi regime, and he played a role in undermining Nazi influence in Hungary. For this he was confined to house arrest, which he escaped. Szent-Györgyi proceeded to stay one step ahead of the Gestapo for the remainder of the war. Remarkably, he found time to write up his work on 'acto-myosin,' which he published in 1945 in a Scandinavian journal. As Szent-Gyorgyi explained, "I expected to be killed so I wrote up my observations on muscle, which I did not want to be lost"(2). After the war, Szent-Györgyi was nominated for another Nobel Prize, and he won a Lasker Award for his contributions to muscle physiology.

Nearly twenty years after the discovery of actin in muscle, numerous labs converged on the same finding: Every eukaryotic cell expressed actin, from amoeba to humans. It is crucial for the cellular protrusions that allow cell migration to occur. Organelles are not organized properly without actin. Microtubules make up vesicular tracks, but actin provides the force for vesicular fission and fusion. Macrophages could not phagocytose pathogens without actin. Indeed, even the basic morphology of a cell is compromised if actin is disrupted. The discovery of small molecule inhibitors of actin helped spark many of these observations.

But how did actin monomers assemble into actin filaments?

In the 1970s and 80s a tremendous amount of insight into the biochemistry of actin began to accumulate. Among the research papers contributing to these new insights was a 1976 paper by Albrecht Wegner titled simply "Head to tail polymerization of actin," which stands out as a major influence for the model that would come to be known as 'treadmilling.' This model would be refined through the years with elegant and careful biochemistry, with rate and kinetic constants that beautifully described how ....

1. The structure and dynamic instability of microtubules being the other headliner.
2. Szent-Györgyi, Lost in the twentieth century. Annual Review of Biochemistry, 1963.

actin monomers could polymerize into polarized structures with a 'plus' and 'minus' end. The ideas of actin nucleation (the number of actin monomers required to drive polymerization) and the 'critical concentration' (the concentration of free actin monomer required for spontaneous nucleation) helped thoroughly round out the biological understanding of actin filament dynamics. These observations, which came rapidly and en masse, stood on the foundation provided by the first generation of actin biologists, many of whom were muscle physiologists.

While this era represents a sort of Golden Age of Actin, many fundamental questions remained. Why is the half-life of an actin filament so much shorter in cells than in a test tube? The discovery of cofilin and other actin-depolymerizing factors in the 1980s explains this. There is more than enough actin monomer in cells that it should all spontaneously polymerize – why doesn't it? Profilin and thymosin β4 bind monomeric actin and impair its addition to the 'plus end' of the actin filament. It rapidly became clear that actin and myosin weren't an isolated pair, but rather part of a larger ecosystem (which included actin-associated proteins) that underpins cellular dynamics.

The dawn of the 1990s saw exciting work using fluorescent actin and live cell microscopy that provided direct evidence of actin incorporation into cellular protrusions in migrating cells. In addition, Listeria monocytogenes revealed clues along the way, as the actin-rich 'comet tails' they assemble during their life inside mammalian cells provided a fertile ground for discovering fundamental principles about the actin cytoskeleton. The discovery of the first actin nucleating enzyme, the seven-subunit branched actin-polymerizing Arp2/3 complex, was an exciting development that rapidly led to a greater mechanistic understanding of numerous cellular behaviors. This discovery catalyzed the search for other actin nucleators into the 2000s. The formins, of which there are fifteen very different isoforms in mammals, can nucleate as well as elongate linear actin filaments. Some isoforms have additional functions, like filament bundling! Some formins can also influence microtubules! Meanwhile, structural studies were visualizing both the actin monomer and other actin-binding proteins (like Arp2/3 complex), yielding information that could be used in biochemical structure function studies, and to refine mechanistic models of complex cellular behaviors.

By the time I started graduate school in 2005, there was a robust, multi-disciplinary field ranging from structural biology to live cell microscopy, using every conceivable model system available to the biological community. The subsequent years have been punctuated by a steady drumbeat of findings that have expanded our understanding of how actin is assembled and disassembled, and which actin regulators are required and which are not for a given cellular process. Recent advances identified actin's role in gene transcription, DNA repair, and organelle structure. It seems that almost every cellular process is touched in some way by the actin cytoskeleton. These developments are also relevant to the bedside. Human patients are affected by polymorphisms in actin-associated proteins, which give rise to immunological disorders called 'actinopathies'.

It is tempting to think that this flow of events was inevitable, given how important actin is to so many processes. It is true that if Albert Szent-Györgyi and his colleagues hadn't discovered actin someone else would have. But it still had to be discovered. In an era in which we 'brute-force' our way to conclusions, it may be equally beneficial for us to take our lead from the early days of actin, to tread thoughtfully and trust serendipity. Maybe we should look more closely when something makes us say, "Huh. That's weird." Serendipity just may be the first step toward inevitability.

ABOUT DR. JEREMY ROTTY

SINCE 2017, DR. ROTTY HAS BEEN AN ASSISTANT PROFESSOR IN THE DEPARTMENT OF BIOCHEMISTRY AT USU WHERE HE STUDIES CELL MIGRATION, CYTOSKELETAL REGULATION AND EXTRACELLULAR MATRIX SENSING.

THE PREGNANCY PARADOX

Dr. Gabriela Dveklser

The ability to carry a pregnancy to term without complications, particularly in species in which the maternal immune system is in direct contact with fetal tissue throughout the pregnancy, continues to puzzle immunologists. How is the maternal immune system able to accommodate the fetus, which has half of its genes from the father, without rejecting it as if it were a transplant? While we still do not know the full answer to this question, much has been learned in recent years regarding the "immunology of pregnancy" by observing what goes wrong in pathological pregnancies and by studying reasons for pregnancy loss that cannot be explained by fetal genetic abnormalities, fetal or maternal infections, hormonal imbalances, maternal high blood pressure, or exposure to toxins.

The earliest hypotheses regarding the paradoxical nature of pregnancy were prompted by advances in the understanding of immunological tolerance, pioneered by Sir Peter Medawar in 1953. Dr. Medawar postulated several reasons why the maternal immune system did not reject the fetus: (1) complete anatomic separation of the mother and fetus; (2) lack of antigenic potential by the fetus; (3) inertness or unresponsiveness of the maternal immune system. Although later investigations from different laboratories disqualified the first two hypotheses; the third hypothesis, while also proven wrong, has guided the field of reproductive immunology. Since the idea of maternal immune system unresponsiveness was first proposed, investigators have determined that pregnancy represents a state of immunological tolerance during which the mother must tolerate the semi-allograft fetus. A growing body of evidence suggests that the fetus must also tolerate the mother to ensure a successful pregnancy outcome.

It is now well accepted that the maternal immune system is constantly exposed to and recognizes foreign paternal/fetal antigens, and that a series of local (uterine tissue-fetal interface) and systemic immunological adaptations,

known collectively as 'maternal-fetal tolerance,' are required for a successful pregnancy. Interestingly, fetal cells are not just found during pregnancy in the maternal circulation. Fetal stem-cell-like cells have been shown to migrate through the placenta into the maternal circulation and can take up residence in tissues of the mother's body, persisting for decades. The persistence of fetal cells in the mother long after pregnancy ends is known as "fetal microchimerism," and has been associated with increased susceptibility to certain autoimmune diseases like rheumatoid arthritis and systemic sclerosis.

Interestingly, fetal cells are not just found during pregnancy in the maternal circulation. Fetal stem-cell-like cells have been shown to migrate through the placenta into the maternal circulation and can take up residence in tissues of the mother's body, persisting for decades. The persistence of fetal cells in the mother long after pregnancy ends is known as "fetal microchimerism," and has been associated with increased susceptibility to certain autoimmune diseases like rheumatoid arthritis and systemic sclerosis.

The mediators that foster and sustain maternal-fetal tolerance are many, and much remains to be learned about this complex phenomenon that is essential for life. Adaptive immune cells, including T-regulatory cells; innate immune cells, including macrophages and natural killer cells; and hormones secreted by the trophoblast cells of the placenta including progesterone, estrogen, and the pregnancy-specific glycoproteins (the focus of research in my laboratory) are required, and their functions and importance may differ depending on the trimester of pregnancy. Importantly, there must be a balance in the innate and adaptive immune responses during pregnancy, by which the mother's immune system remains vigilant against external threats such as viruses, bacteria, and parasites that otherwise could compromise the health of both the mother and the baby, while it tolerates the semi-allogeneic fetus. Disruptions or alterations in the activity of different immune cell subsets sometimes (but not always) triggered by infections, are often associated with adverse pregnancy outcomes.

Preeclampsia, a pregnancy-associated disorder that is unique to humans, is defined by the gestational onset of hypertension and proteinuria, and frequently results in the delivery of premature babies. Although the exact etiology of early-onset preeclampsia is not clear, one favored hypothesis is that through loss of maternal tolerance toward the fetus, the placentation process is impaired, resulting in failure of sufficient trophoblast invasion into the uterus and uterine spiral artery remodeling. At present, the only cure for preeclampsia is delivery of the baby, but this disease leaves sequelae for both the mother and the offspring. Mothers who suffer from preeclampsia have an increased risk of developing hypertension, ischemic heart disease, and stroke. While initially unnoticed; there is also an increased risk of cardiovascular disease, and higher rates of allergy, in the offspring of preeclamptic mothers, even if the delivery occurred at term.

Recently, studies of the effects of maternal immune responses on the offspring have been expanded. Using rodents and non-human primates, researchers showed that maternal immune activation accompanied by an increase in pro-inflammatory cytokines results in alterations in neurodevelopment, including marked reductions in frontal gray and white matter in the offspring. Therefore, a better understanding of the cause of failed maternal tolerance and of the immune responses mounted during pregnancy will require targeted therapeutic interventions to restore tolerance, with the aim of protecting the mother and the offspring without compromising the life of the mother, although

this may not always be possible. Another interesting notion recently explored in more detail and confirmed using animal models is that the human immune system is shaped in utero. Prenatal maternal stress in mice was shown to cause preterm birth and to affect neonatal immunity across generations. Furthermore, exposure to bacteria during pregnancy, even with a microorganism that does not infect the placenta, can have permanent and tissue-specific impacts on the offspring's immunity that could predispose the individual to immune-related diseases such as Crohn's.

Innovations including novel animal models of pregnancy-related diseases that better mimic human pathologies, the use of organoids to study the fetal-placenta interface, more sensitive imaging techniques, and the construction of "placentas on a chip" are enabling new discoveries that will help researchers to better understand the different players (cells, receptors, and soluble mediators) and the specific changes in the immune system required for pregnancy success. In addition, the observation that pregnant women suffering from certain autoimmune diseases, including multiple sclerosis, suffer less disease activity and fewer relapses has prompted the investigation of pregnancy-related factors of placental origin that can potentially be used as therapeutics.

About Dr. Gabriela Dveksler

Dr. Dveksler is a Professor in the department of Pathology. Her lab focuses on the elucidation of the biological roles and receptors of a family of placentally-secreted proteins known as "pregnancy-specific glycoproteins". They found that these proteins play a role in the induction of immune tolerance, placental angiogenesis and in trophoblast invasion of the uterus by interacting with TGF-beta, heparan sulfate proteoglycans and specific integrins, respectively.

THE INTEGRATED STRESS RESPONSE: FROM DISCOVERY TO PHARMACEUTICAL INTERVENTION

DR. SARA YOUNG-BAIRD

Maintenance of cellular health and function is an essential characteristic of life. For example, in response to external and internal stress, our cells rely upon the reprogramming of gene expression to restore core cellular processes back to a functional, stable state. Central to this process is the Integrated Stress Response (ISR), the mechanistic features of which were originally characterized by Alan Hinnebusch at the National Institutes of Health beginning in 1984 (1). This stress-activated, cell-adaptive pathway helps to maintain cellular health largely through the modulation of protein synthesis (i.e., translation of messenger RNA to protein). The overarching goals of my research program are to understand how 1) protein synthesis is regulated during the ISR and 2) dysregulation of protein synthesis contributes to disease. It's an exciting time to be in the ISR field – innovative advances in technology have allowed us to monitor translation with next generation sequencing ("RiboSeq" or "ribosome profiling") and therapeutics targeting the ISR are in human safety clinical trials. A brief description of this central cellular stress response pathway and therapeutic potential of targeting the ISR in disease is described below.

A key component of the ISR is the eukaryotic translation initiation factor 2 (eIF2). This essential protein complex is conserved in all three domains of life: archaea (aIF2), bacteria (IF2), and eukarya (eIF2; the focus of our research and this article). The main role of eIF2 is to help identify and select the translation initiation start site, meaning that eIF2 activity is required for all general protein synthesis. The eIF2 complex was characterized as a central hub

for protein synthesis regulation during the cellular stress response through the combined efforts of multiple groups in the 1970s and 80s (reviewed in 2). These seminal studies showed that phosphorylation of the α subunit of eIF2 results in a decrease in eIF2 activity and a downstream reduction in protein synthesis levels during ISR activation. Elegant yeast genetic studies by the Hinnebusch lab also identified mRNAs that are paradoxically translationally upregulated in response to nutrient deprivation as a part of the ISR. The best characterized of these mRNAs is GCN4 that encodes a transcriptional activator of amino acid biosynthesis, tRNA synthetases, and additional pathway-specific stress-responsive factors (reviewed in 3). Combined these observations have led to the longstanding idea that activation of the ISR allows the cell to conserve precious energy resources while adapting to a variable environment and maintaining cellular health.

This conserved regulatory scheme was expanded upon in mammalian cells where many additional players in the ISR pathway, including the mammalian ortholog of GCN4, known as ATF4, have been characterized (reviewed in 4). By this time, multiple mammalian eIF2 kinases, which are each activated in response to different cellular stresses, had also been identified. The integration of multiple stress signals on a specific molecular pathway, via eIF2α phosphorylation and its downstream translational effects, was collectively referred to as the Integrated Stress Response by David Ron in 2002 (5). We have also learned, perhaps unsurprising given its essential nature, that eIF2 activity and its impacts on protein synthesis play crucial roles in human development and physiology. For example, X-linked mutations in the γ subunit of eIF2 cause MEHMO syndrome, a severe developmental disorder that presents with Mental (intellectual) disability, Epilepsy, Hypogenitalism, Microcephaly, and Obesity and is characterized by inappropriate ISR activation, including chronically low levels of overall protein synthesis, even in the absence of a cellular stress (6). Modulation of eIF2 activity and dysregulation of the ISR are also implicated in multifactorial diseases, including neurodegenerative disorders, diabetes, and cancer (reviewed in 7). The profound impact of ISR signaling on human health and disease has spurred the development of several innovative drugs designed to modulate eIF2 activity. ISRIB, the most promising ISR-targeting therapeutic to date, was identified in 2013 in a cell-based screen for inhibitors of ATF4 translation and ISR activation (8), highlighting the importance of our understanding of global and gene-specific translation regulation mechanisms. In less than a decade, ISRIB has been shown by several independent studies to rescue the cellular phenotypes of ISR-related genetic diseases like MEHMO syndrome, and promising data from animal models indicate that it may be useful as a pharmaceutical treatment for traumatic brain injury and other stress-inducing human conditions (6 & 7). Patient recruitment has also recently begun for a Phase I clinical trial of ISRIB in the treatment Amyotrophic Lateral Sclerosis (ALS).

> *It is a testament to the powerful synergy of rigorous basic science research with downstream small molecule screening and characterization that the field has advanced from protein complex identification to disease treatment in less*

Furthermore, the history of the ISR field is an important reminder that basic science research is fundamental and necessary to lay the groundwork for improving human health and developing novel disease therapeutics.

About Dr. Sara Young-Baird

Dr. Young-Baird recently joined the USU Department of Biochemistry in January 2022. Her lab studies how our cells sense and respond to stress, with a particular focus on how disruption of these processes drive human disease.

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DEGRADATION DIGEST

Dr. Barrington Burnett

DISCOVERY OF THE CELLULAR DEGRADATION MACHINERY

Protein turnover is now universally recognized as essential to maintaining cellular homeostasis by regulating cell growth, development, and survival. Although this is accepted science today, until the 1940s, very little was known about protein synthesis and even less about protein breakdown. The discovery of the DNA double helical structure in the 1950s and the breaking of the genetic code also ushered in a new era for protein metabolism research, since the fate of newly synthesized proteins needed to be reconciled with proteins already present. In initial forays to understand the protein dynamics, Rudolf Schoenheimer treated adult rats with 15N-labeled L-leucine, and studied the distribution of isotope-labeled proteins throughout the body. His work showed that protein composition in tissues was dynamic, with newly synthesized proteins replacing older ones. The discovery of the lysosome in the 1950s uncovered how cytosolic proteins are degraded, and launched an exciting new field dedicated to revealing the regulatory mechanisms governing protein degradation.

SELECTIVE PROTEIN DEGRADATION

By the early 1980s, complementary breakthroughs by the laboratories of Avram Hershko and Aaron Ciechanover (Israel Institute of Technology, Haifa, Israel) and Irwin Rose (University of California, Irvine, USA) provided novel insight into the way proteins are selected for degradation by the proteasome. In a series of seminal studies, Hershko and his colleagues identified three types of enzymes (E1 ubiquitin-activating enzyme, E2 ubiquitin carrier enzyme, and E3 ubiquitin protein ligase enzymes) that facilitate protein ubiquitination, and showed in exquisite detail how ubiquitin conjugation facilitates recognition of proteins by the proteasome for subsequent degradation. For their pioneering work, Ciechanover, Hershko, and Rose received the 2004 Nobel Prize in Chemistry. Since then, a myriad of biological functions of the ubiquitin system have been decoded, including selective regulation of critical cellular proteins. A combination of genetic, biochemical, and cell biological studies with mammalian cells and the yeast Saccharomyces cerevisiae was central to these studies was pivotal in demonstrating how the proteome was so tightly regulated and why the regulation was required for proper functioning of the cell cycle, DNA repair, protein synthesis, transcription, and stress responses.

The ubiquitin proteasome system is now known to constitute nearly 4% of the entire human genome. The proteasome is responsible for catalyzing the rapid degradation of approximately 90% of cellular proteins, including many rate-limiting enzymes, transcription factors, and other regulatory proteins. Proteolysis via the UPS is a tightly regulated, rapid, and effective mechanism for degrading specific proteins; and in many cases, degradation occurs only in response to particular cellular stimuli. The single E1 enzyme transfers ubiquitin to the E2s, and a small repertoire of E2s directly transfers ubiquitin to either an E3 or to a lysine residue on the substrate. The E2s have only limited substrate specificity; therefore, E3s are primarily responsible for substrate recognition and are very attractive therapeutic targets.

PROTEIN DEGRADATION IN HUMAN HEALTH AND DISEASE

While advances in cancer cell biology have illuminated the detailed landscape of cellular proteolysis (how specific proteins are targeted for degradation in the central nervous system) the mechanism through which the degradation machinery adapts to neuronal dysfunction remains elusive. It is not surprising that complex mechanisms have evolved to control the proteasome assembly, composition, and activity in response to development and stress. Dysregulation of the ubiquitin proteasome system is associated with a number of neuropathies, including many neurodegenerative diseases, ischemic brain damage, and cognitive disorders. While it is clear that the degradation machinery is compromised following brain injuries, the molecular mechanism and pathological contribution of the UPS to the primary and/or secondary effects of brain injuries are poorly defined, and lack mechanistic insight.

While ubiquitin-mediated degradation is responsible for the clearance of the majority of intracellular proteins, the lysosome is responsible for recycling large organelles and destroying internalized viruses and bacteria. Lysosomal proteolysis is required to generate the antigens by degrading the invading pathogens, demonstrating the importance of this degradation machinery in adaptive immunity. In addition to clearing pathogens and providing molecular cues for the immunes system, lysosomes clean clear cells or accumulated trash. This process, referred to as autophagy (self-eating), was first described by Yohsinori Ohsumi in 1992 and has been strongly linked to aging and overall health. Recent evidence suggest that autophagy decreases with aging, resulting in the toxic accumulation of proteins and organelles. Attempts to slow the aging process by jump starting autophagy as gained tremendous interest over the past two decades, since it was shown that drinking red wine (active ingredient: resveratrol) induces autophagy and generally improves health and well-being. Despite the overwhelming enthusiasm, efficacy of resveratrol in a number of clinical trials remain mixed. Nevertheless, the consumption of red wine has not been severely impacted.

THERAPEUTIC OPPORTUNITIES TO TARGET PROTEIN DEGRADATION

The degradation system displays considerable plasticity that allows it to adapt rapidly and dynamically to multiple cellular challenges. Harnessing cellular proteolysis to selectively degrade toxic proteins or alternatively, hinder the turnover of deficient proteins, remains the long-term goal of researchers in the field. Proteasome inhibitors have been developed in the last few years, and pioneering work by Alfred L Goldberg led to the discovery of selective proteasome inhibitors. The original aim of the effort to develop proteasome inhibitors was to slow the progression of muscle wasting diseases. Dr. Goldberg showed that inhibiting the proteasome was an excellent way to study the function. His team identified MG132, which remains the most widely used proteasome inhibitor in basic research and bortezomib (Velcade®), which was fast-track FDA-approved for the treatment of multiple myeloma.

The success of bortezomib encouraged further biotech investment into tapping protein degradation to treat human diseases. Small-molecule drug discovery efforts are traditionally built around targeting the activity site of enzymes. However, it is estimated that up to 85% of intracellular proteins are "undruggable," with no known accessible binding sites for small molecules. Unfortunately, the enzymes involved in protein ubiquitination are either too critical to indiscriminately inhibit, or rely on protein-protein interactions, which stalls the advancement of small molecule screens to identify selective inhibitors of protein degradation. This challenge has prompted investigators to develop and deploy creative new technologies to screen for small-molecule drugs that overcome the limitation of current drug discovery strategies.

CURRENT OPPORTUNITIES TO ADVANCE OUR UNDERSTANDING OF PROTEIN DEGRADATION PATHWAYS

Our growing understanding of key features of selective cellular proteolysis offers an attractive therapeutic avenue for further exploration. Today we are much closer to being able to tag an undesirable protein for degradation, such as toxic protein aggregates associated with many neurodegenerative diseases. PROteolysis TArgeting Chimeras (PROTACs), which are heterobifunctional small molecules that redirect select proteins to the proteasome for degradation, are currently making their way through various stages of clinical development.

Unraveling the mechanistic pathways involved in targeted protein degradation has opened up other opportunities for drug discovery and development. These efforts require sensitive methods to detect and measure the degradation of endogenous proteins in response to the compounds. The emergence of new methods to monitor protein synthesis and turnover, such as Click-iT, Bioluminescence Resonance Energy Transfer (BRET), and AlphaLisa®, have provided new opportunities to refine how we investigate protein ubiquitination and proteasome degradation. Robust and scalable, these assays now allow access to the ubiquitin proteasome system for drug discovery and development. These methods are rapidly advancing our understanding of targeted protein degradation, and are effective tools to accelerate the pace of drug development within this rapidly growing field.

About Dr. Barrington Burnett

Dr. Burnett is an Associate Professor & Vice Chair of Research in the Department of Anatomy, Physiology and Genetics. Dr. Burnett's lab focuses on identifying neurodegenerative disease-causing genes and pathways. They use a combination of cell and molecular biology approaches to define disease-specific pathogenic mechanisms and advance preclinical assessment of novel treatment options in rodent models of disease.

Student Scientific Reviews

Literature reviews are crucial resources for scientists. This section provides an opportunity for USU students to provide critical analysis of published literature and present a current comprehensive summary of their field of interest.

The Link Between Autophagosomes and a TCR Dependent Cysotolic Complex

Imran Hussain

T lymphocytes are a critical component of the adaptive immune response. In addition to their crucial roles in anti-pathogen immunity, T cells are implicated in the pathogenesis of autoimmunity, cancer, and certain infectious diseases (e.g., leprosy). Therefore, understanding the signaling pathways that govern T cell activation is important for illuminating possible therapeutic targets. This essay will discuss fundamentals of the T cell receptor to NF-κB pathway; its connection to autophagosomes and selective autophagy adaptors; and the fate of the signaling adaptor BCL10 post-TCR activation.

The POLKADOTS signalosome: A signal transduction hub for NF-κB activation When an antigen is presented to a T cell, the T cell receptor (TCR) is activated, and the intracellular signals that follow induce transcription of NF-κB-regulated genes. NF-κB is a family of heterodimeric transcription factors that function downstream of many cellular receptors. Antigen receptor-dependent activation of NF-κB is important for T cell proliferation, survival, and differentiation. TCR-ligated effector T cells trigger the assembly of filament-like oligomers containing multiple coalesced BCL10 and MALT1 heterodimers. We call these assemblies Punctate and oligomeric killing or activating domains transducing signals, or POLKADOTS (1-2). POLKADOTS are highly dynamic cytosolic polymers that manage the signaling intermediates regulating NF-κB activity (8-10). Indeed, prior to the terminal steps of NF-κB activation, POLKADOTS recruit TRAF6 and the IKK complex, which are known proximal activators of NF-κB (3-4).

Macroautophagy and autophagy receptors such as p62 and NBR1 Autophagy is a lysosome-dependent macromolecular and organelle turnover pathway. Macroautophagy is one type of autophagy that employs an autophagosome, which is a double membrane vesicle that forms around a portion of the cytosol containing "cargo," degrading the macromolecules enclosed within its lumen. This process can be non-selective (e.g., it may occur under starvation conditions; or as a result of misfolded protein buildup, oxidative stress, infection, etc.) or selective (7). The latter mechanism usually requires both the ubiquitination of the substrate(s) and a selective autophagy adaptor. Selective autophagy adapters are proteins that serve as molecular bridges by simultaneously binding to: 1) polyubiquitin chains tagged to the substrate and 2) autophagosome receptors, such as LC3. Autophagy adapters are useful for the selective degradation of signaling intermediates that have a regulatory role over transcription factors and that ultimately control key gene expression programs.

Sequestosome-1/p62 Sequestosome-1 (also known as "p62") is one such autophagy receptor. It contains a PB1 domain at the N-terminus that enables oligomerization, an Ubiquitin-associated domain (UBA) at the C-terminus, and a LC3-interaction region (LIR). Beside its role in autophagy, p62 interacts with subunits of the proteasome (11).

NBR1. Neighbor of BRCA1 gene 1 (NBR1) is another autophagy adaptor that has an anatomy similar to that of p62. It contains a PB1 domain, a UBA domain, and a LIR motif. The PB1 domain affords NBR1 the ability to hetero-oligomerize with p62. It is likely that p62 and NBR1 have a set of non-overlapping substrates (12-13).

Connection of macroautophagy to TCR-activated effector T cells Based on observations gathered during imaging experiments, there seems to be a connection between regions of POLKADOTS and LC3+ autophagosomes. POLKADOTS-associated BCL10 colocalizes with vesicular markers of the autophagy-lysosome degradation processes (3 and 6). Moreover, previous data demonstrates that treatment with the lysosome inhibitor Bafilomycin A1 blocks BCL10 degradation, yet enhances NF-κB activation. The concept of TCR-induced autophagy (TCR-IA) naturally arises from these data.

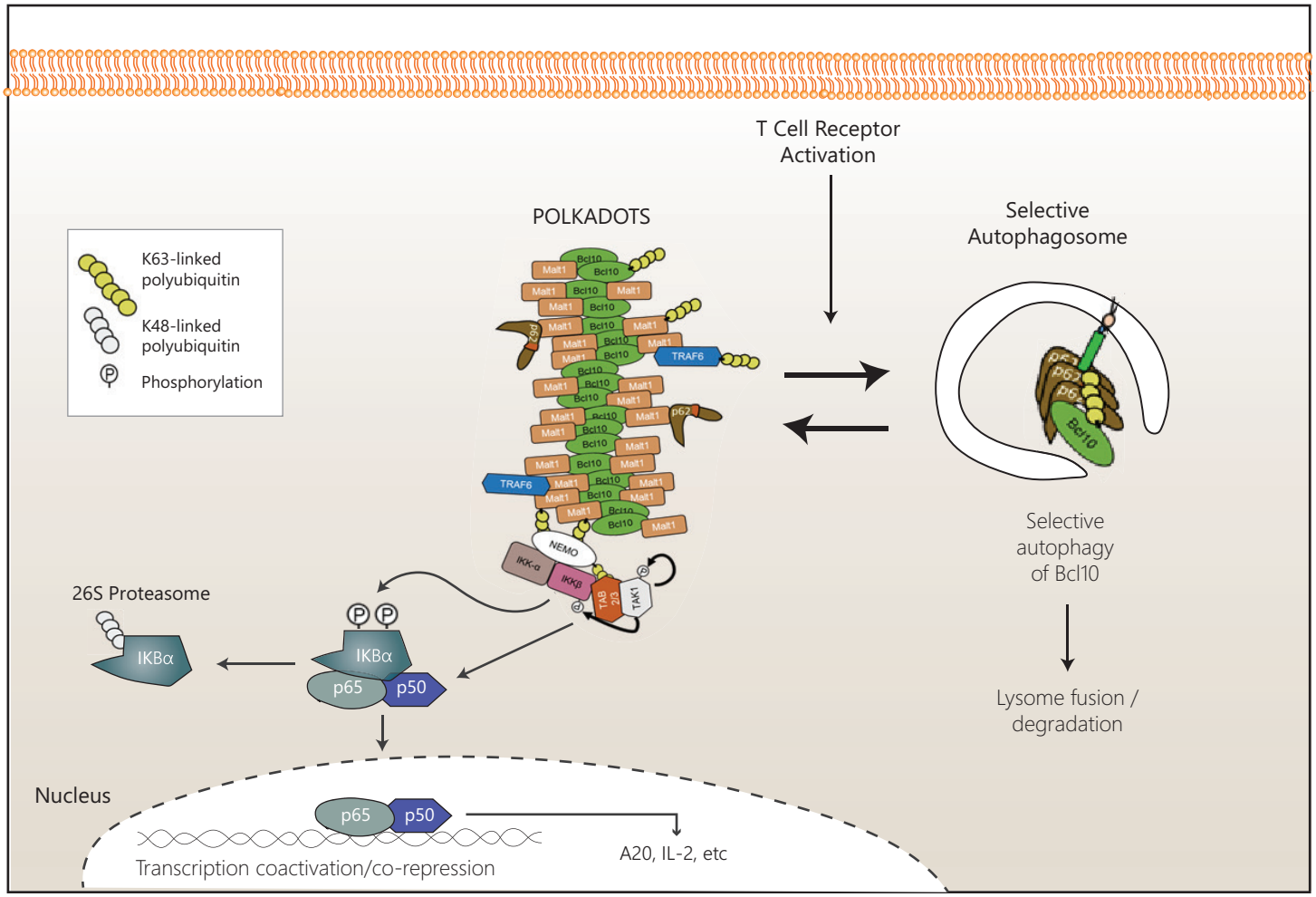

Figure 1: Schematic of working model of the POLKADOTS singalosome and selective autophagosome downstream of T cell receptor activation

Curiously, autophagosome formation is impaired in the double genetic null (p62-/- & NBR1-/-) T cells. This suggests that both p62 and NBR1 may potentially act as adaptors that anchor POLKADOTS to autophagosomes, but that neither of these adaptors facilitate the degradation of BCL10 or are critical for NF-κB activation. As for the significance of POLKADOTS-localized autophagosomes, they likely serve to sequester POLKADOTS-signaling intermediates apart from BCL10, many of which are ubiquitinated (14-16). However, the fate of these other signaling effectors remains an open question.

Is TCR-dependent BCL10 degradation dictated by autophagy adapters? The proper timing and magnitude of BCL10 degradation at the POLKADOTS is important for fine-tuning NF-κB activation. Interestingly, BCL10 has been implicated as a potential selective autophagy substrate in TCR-activated effector T cells. Beginning at about 20 minutes post TCR activation, BCL10 is degraded by selective macroautophagy, whereas MALT1 levels remain stable. A shRNA knockdown of p62 in a murine T cell line results in impaired TCR-dependent degradation of BCL10, suggesting that p62 is required for the selective autophagic turnover of BCL10 (3). In contrast, more recent experiments performed with CRISPR-generated p62 genetic null cell lines suggest that p62, as well as NBR1, is dispensable for total cellular BCL10 clustering, BCL10 degradation, and NF-κB activation. One possible reasons for this discrepancy could be the induction of genetic compensatory mechanisms when using CRISPR-generated genetic null lines—the CRISPR cell lines potentially fail to recapitulate the phenotype due to the involvement of nonsense-mediated decay rather than the RNAi pathway (5). Regardless, whether or not p62 and NBR1 are critical for the degradation of a specific pool of BCL10—that is, the POLKADOTS-associated BCL10—remains uncertain. To answer this question, one must overcome the technical challenges involved in isolating intact POLKADOTS filaments in activated T cells. One way to approach this question is to separate activated T cell lysates under non-denaturing conditions with a sucrose-gradient fractionation or HPLC.

POLKADOTS are an important cytosolic complex in activated effector T cells, and have a regulatory relationship to autophagosomes that depends on autophagy adaptors, p62, and NBR1. The autophagic degradation of the BCL10 core at the POLKADOTS is one way this relationship is manifested, but autophagy acting on other NF-κB regulators cannot be excluded. Understanding the molecular details of TCR-IA will elucidate the mechanisms underpinning TCR-activation to NF-κB in effector T cells.

Imran Hussain is a 6th year MCB PhD Candidate under the mentorship of Dr. Brian Schaefer in the Department of Microbiology and Immunology. He is studying how autophagy adapters and related machinery dictate the TCR-receptor-to-NF-kappaB pathway.

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Integrins: Finding a Strong Binding Partner

Matthew Stinson

Integrins are ubiquitously expressed, heterodimeric transmembrane receptors for a variety of extracellular matrix components. Non-covalent interactions between α- (18 types) and β- (8 types) subunits form a total of 24 cell- and tissue-type dependent integrins, which are synthesized and heterodimerize in the endoplasmic reticulum and Golgi before they are transported to the cell surface [1-2]. In order to interact with the microenvironment, integrins must be in their active, unfolded conformation. Generally, active integrins must be stimulated either by intracellular signals, such as talin and kindlin binding to the cytoplasmic tail, or by mechanical forces induced by the ECM itself (i.e., strong adhesion pulling open partially active integrins). Thus, integrins are capable of "inside-out" and "outside-in" signaling. These signals can compete for access to adaptor and regulatory proteins, making it challenging to map out exact pathways. Since cells exist in complex, mutable 3-D environments, establishing basic understandings of ECM-integrin-function pipelines is important.

Integrins are well-characterized as linkers between the extracellular environment and the cytoskeleton. Actin-binding proteins like vinculin and tensin can be recruited after talin binds to the integrin tail, allowing for the development of weak nascent adhesions to fully formed, mature focal adhesions that require diverse adapter and regulatory complexes. Depending on the complexes recruited, the cytoskeleton can form a variety of context-based morphologies. A classic example is the recruitment of paxillin by kindlin, which in turn activates a master regulator of membrane protrusion, Rac1. Rac1 is a RHO GTPase, along with Rho and Cdc42, which are all responsible for various forms of cell migration. Rac1 activates the Arp2/3 complex, which nucleates F-actin and induces spreading on an ECM substrate [3]. ROCK and Myosin II are phosphorylated, strengthening adhesion and pulling against the surface to create tension. This is just one example of an adhesion complex. Integrin binding operates on a spectrum: weak adherence prevents cells from generating traction and can even cause them to float off, whereas an abundance of integrin clusters in mature focal adhesions can form stress fibers, slowing cell migration almost to a halt.

Leukocytes contradict many of the central dogmas of cell migration. For example, they do not form stress fibers. This phenomenon is still not understood[4]. Furthermore, on average, immune cells are significantly smaller and migrate faster than other cell types[5]. Given their role in host defense and inflammatory regulation, this is intuitive. Leukocytes also inherently expand the traditional roles of integrins. Beyond forming adhesions, leukocyte-specific integrins such as αMβ2 can bind iC3b to initiate a complement cascade and ICAM-1 to initiate extravasation[6-7]. Leukocytes also tend to be more rounded and rely on higher rear ROCK-myosin II contractility, associated with ameboid migration, compared to the mesenchymal migration of fibroblasts and epithelial cells[8]. Bone marrow-derived macrophages are a fantastic modeling system for studying connections between ECM-integrin-immune functions because they are functionally diverse and are known to migrate in mesenchymal and amoeboid fashion, depending on context. They are also relatively understudied. For example, a still-cited 1988 study of murine macrophages that express α6β1 integrin (one of the integrins required for binding the basement membrane ECM component laminin), concluded that they do not adhere to laminin unless stimulated with PMA[9]. However, we have found that not only do macrophages adhere to and interact with laminin 111, but it activates a complex signaling pathway that raises many questions about the established understanding of integrin, ROCK, and Myosin II signaling and how they affect migration velocity, persistence, spreading, and adhesion.

RNA sequencing data indicate that our murine BMBMs only express a single integrin for binding laminin, α6β1, which could suggest low adhesion potential. In comparison, macrophages express many integrins that bind the RGD domain in fibronectin. Fibronectin is used in many macrophage studies, as it induces strong spreading, adhesion, polarity, and persistent migration. In mesenchymal cells, it is also generally understood that more persistent, polarized cells are faster[10]. In contrast, macrophages plated on laminin 111 were faster, less spread, less polar, and less persistent than macrophages on fibronectin. Adhesion and migration both require myosin II activation to anchor cells to substrates and contract at the rear of the cell, but at high levels, it can cause cells to ball-up like the laminin cells. Through a series of inhibitor experiments for Myosin II (Blebbistatin) and ROCK (Y-27632), which is upstream of Myosin II, we found that Myosin II and ROCK appear to have distinct functions in the context of macrophage morphology and migration on laminin. Blebbistatin treatment generally causes cells to spread out, but in this context, it appeared only to decrease the velocity of our macrophages on laminin. Y-27632 treatment, on the other hand, induced spreading, polarity, and persistence on laminin, but macrophages still migrated faster than on fibronectin and had low adhesion. This was surprising, since ROCK is upstream of Myosin Light Chain Kinase (MLCK), and Blebbistatin inhibits Myosin II-Actin binding. Also, blocking 2 RGD binding integrins, αvβ3 and αvβ5, with Cilengitide created further confounding results when macrophages on fibronectin became faster and less persistent (more laminin-like), but not less adhesive. Blocking α6 integrin completely inhibited adhesion to laminin 111, suggesting that macrophages do adhere to laminin primarily through this integrin.

These data suggest that the integrins expressed on the surface of a macrophage recruit specific regulatory and cytoskeletal elements that impact their migration and morphology. Rather than following traditional understandings of adhesion, spreading, velocity, and persistence, mBMDMs appear to preferentially respond to laminin 111, despite having fewer integrins. Additionally, inhibiting αvβ3 and αvβ5 appears to increase Myosin II and ROCK activity to an extent reminiscent of laminin-plated cells, but it does not affect their adhesion. Therefore, we hypothesize that αvβ3 and αvβ5 may play a role in attenuating ROCK and Myosin II activity. However, it remains to be seen what scaffolding and regulatory proteins are specifically recruited and activated in response to laminin and fibronectin binding. We have resolved many of the downstream actomyosin signaling processes and corresponding morphology, but the effect on immune responses is also unknown. Fibronectin is considered an anti-inflammatory ECM component, but now that macrophages have been shown to adhere to laminin, additional studies should be conducted to evaluate the impact of laminin on immune function and signaling.

Polarity: intrinsic asymmetry observed in cells, either in their shape, structure, or organization of cellular components. Most epithelial cells, migrating cells and developing cells require some form of cell polarity for their function.

Persistence: when migrating randomly with no directional cue over longer time intervals, the cells execute random changes in direction. A less persistent cell changes direction from a straight path more often than a persistent cell.

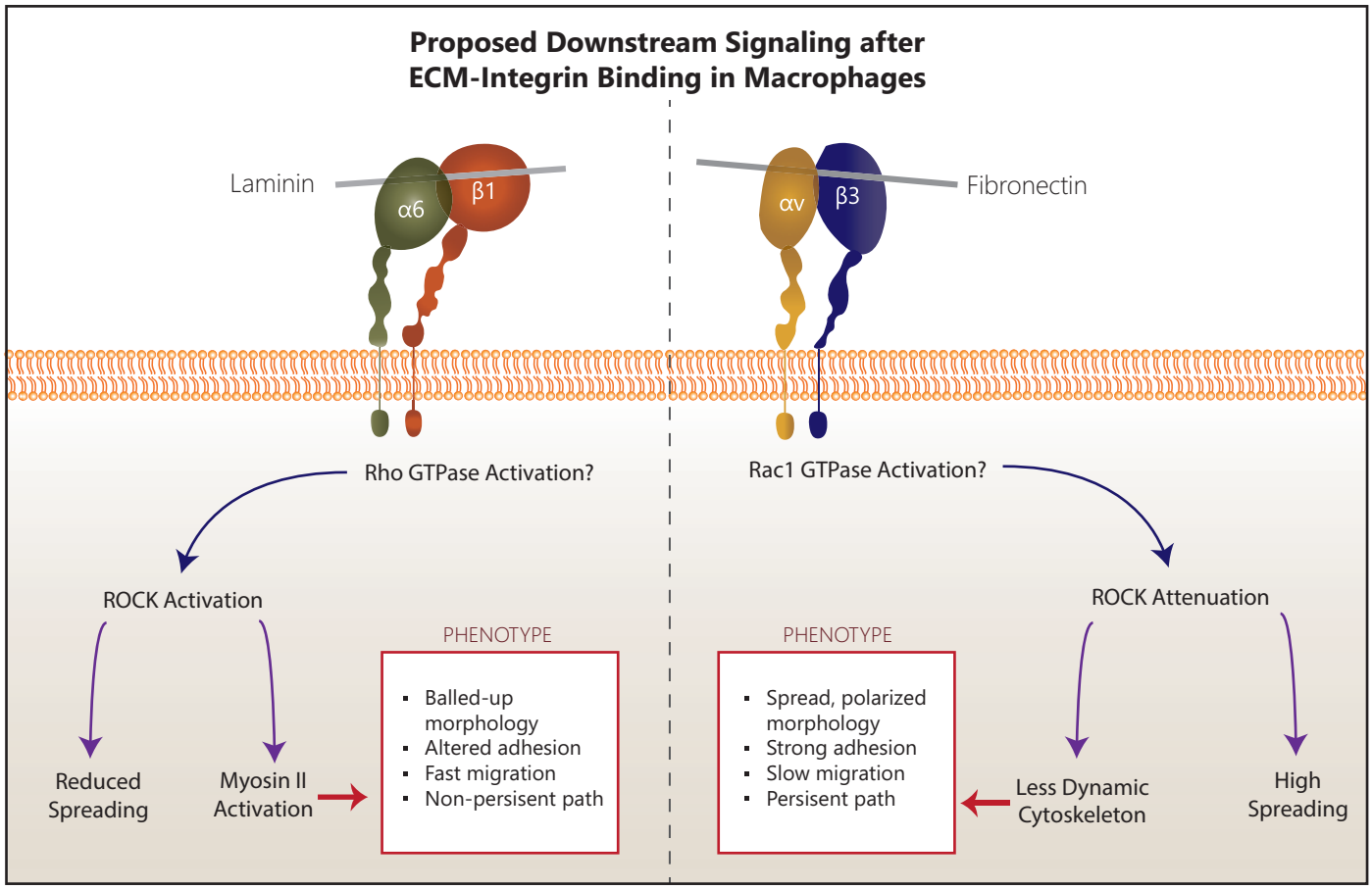

Figure 1: Overview of phenotypic differences between macrophages plated on laminin versus fibronectin extracellular matrix, with the proposed signaling downstream of the corresponding integrin receptor -ECM engagement

Matthew Stinson is a 5th year PhD candidate in the USU Biochemistry Department in Dr. Jeremy Rotty's lab. He is investigating the molecular mechanisms behind ECM components differentially influencing integrin binding and downstream signaling as an avenue to tune macrophage behavior and inflammatory regulation.

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Putting Ras Mutations Under the Microscope

Nancy Sealover

RAS proteins are small GTPases that are active when bound to GTP and inactive when bound to GDP. The genes KRAS, NRAS, and HRAS encode four highly homologous protein isoforms: (KRAS4B, KRAS4A, NRAS, and HRAS). RAS genes are mutated in approximately 25% of all cancers and respond poorly to standard chemotherapy. Additionally, RAS proteins are notoriously difficult to directly target pharmacologically because they lack candidate binding pockets for inhibitor compounds. For over 40 years, scientists have searched for adequate approaches to treat RAS-mutated cancers. The greatest challenge is that although RAS appears to be a single therapeutic target, multiple levels of complexity occur within the diversity of RAS-mutations that require careful characterization and consideration.

The first indication that RAS genes are not all biologically equivalent was discovered during development and testing of farnesyl transferase inhibitors (FTIs) as anti-RAS therapeutics. RAS proteins are lipid-modified by farnesylation in their C-terminal CAAX motif prior to membrane localization. Scientists hypothesized that by targeting the process of RAS farnesylation, FTIs would accomplish inhibition of all RAS isoforms. FTIs showed preclinical promise in HRAS-mutated cancers but, disappointingly, had no benefit in clinical trials for patients with KRAS-mutated pancreatic cancer. Subsequent analysis revealed that FTIs uniquely inhibit HRAS, while KRAS and NRAS can be alternatively geranylgeranylated, and thus are resistant to FTIs. The lesson that RAS proteins should not be treated equally when developing and testing therapeutics has only increased in significance over time as scientists discovered that each RAS protein has unique biological activity toward its primary effector kinases, phosphoinositide 3-kinase (PI3K) and RAF. HRAS only strongly interacts with one RAF isoform (CRAF), while mutant KRAS strongly activates all RAF proteins (ARAF, BRAF, and CRAF) through high affinity interactions (1). However, mutant KRAS was specifically identified as a weak activator of PI3K signaling, and requires SOS2-mediated activation of HRAS and NRAS wild-type proteins to fully activate PI3K in KRAS-mutated cancers (2). Therefore, identifying biochemical and signaling vulnerabilities is a significant component in understanding therapeutic windows at the level of RAS isoform complexity.

Within each RAS gene, mutations primarily occur at one of three mutational hotspots—Gly12, Gly13, or Gln61 (commonly referred to as G12, G13, or Q61)—and manifest a second level of RAS protein complexity: codon-specificity. Mutations at specific codons with RAS genes dysregulate RASGTP/RASGDP cycling to promote RASGTP loading and activity. The mechanism leading to increased RASGTP is unique to each hotspot and is a product of differences in nucleotide exchange (GDP for GTP) and GTP hydrolysis (cleavage of GTP gamma phosphate) which can be intrinsic or GAP-mediated (3). While mutations at all three hotspots decrease GAP-mediated GTP hydrolysis,

Geranylgeranylation and farnesylation are two different types of prenylation, which is a type of posttranslational modification of proteins by lipids.

Q61 mutants show markedly reduced intrinsic GTP hydrolysis, decreasing their sensitivity to inhibition of upstream proximal receptor tyrosine kinase signaling using SOS1 or SHP2 inhibitors (4). Thus, the codon hotspot specificity of RAS-mutations has therapeutic implications for targeting RAS-mutated cancers.

A growing body of research indicates that a third level of specificity exists: allele- and tissue-specificity. Within each codon hotspot, multiple specific allele mutants can occur; for example, a mutation at G12 can result in a G12C, G12D, G12V, or G12R mutant, etc. Each allele-specific mutant has begun to be characterized, revealing therapeutic vulnerabilities. Recently, it was shown that in KRAS-mutated cancers, KRASG12R-mutated cells showed defective PI3K signaling and were therefore unable to induce KRAS-dependent micropinocytosis or suppression of autophagy compared to G12D and G12V mutants, revealing a sensitivity of KRASG12R mutants to inhibitors of autophagy (5). In addition, it was through structural characterization of allele-specific mutants that the first inhibitor to bind directly to mutant RAS was developed. In 2013, the Shokat lab discovered that in the inactive GDP-bound state, the mutated cysteine of KRASG12C was exposed in a previously undiscovered pocket in the mutant KRASG12C protein structure, which could be exploited with an inhibitor to lock KRASG12C proteins in the inactive state (6). Finally, in a surprising result, studying allele-specific RAS-mutations has begun to answer the question of why specific RAS-mutations occur more or less frequently than others in different tissue types. A unique example of this was recently published, indicating that in KRASQ61-mutated cancers, KRASQ61E allele-mutants occur less frequently than would be expected by predicted rates of random mutagenesis due to impaired metabolic function and the production of actin stress fibers (7). These observations indicate that while specific RAS-mutations are more common in tumors from specific tissues, the development of these tumors is a more dynamic process of tumor initiation than the simple occurrence of any RAS mutation. Investigation of the phenotypic, biochemical, and biological strengths and vulnerabilities of RAS allele-specific mutants will contribute to the understanding of RAS-mutation frequency and how to target the RAS-mutated cells that become tumors in specific tissues.

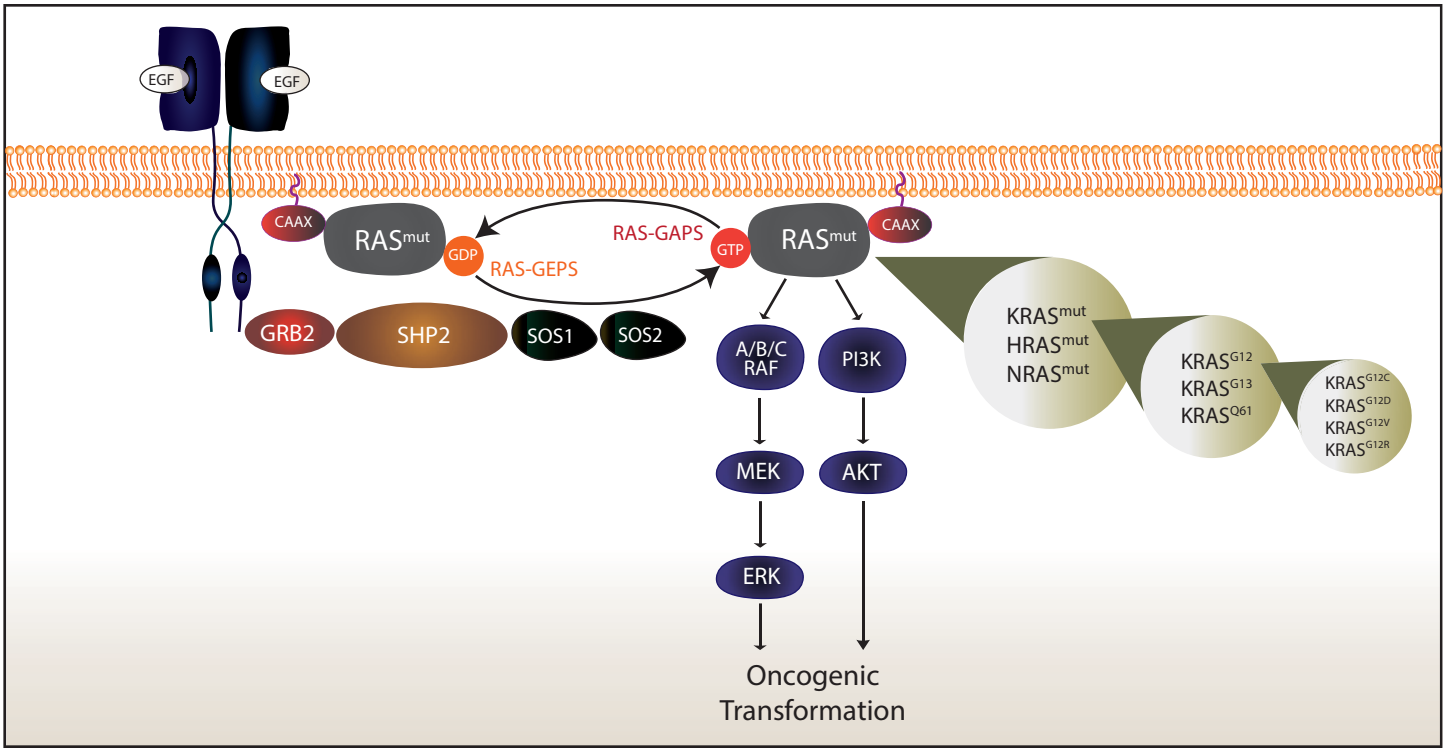

Figure 1: When a ligand binds to a receptor tyrosine kinase and initiates receptor activity, receptors recruit adaptor proteins such as GRB2, which in turn recruit proximal receptor proteins such as SHP2 and SOS1/2. SOS1 and SOS2 are RAS guanine nucleotide exchange factors (GEFS) and facilitate nucleotide exchange of GDP for GTP on RAS, activating RAS proteins. RAS further activates the RAF-MEK-ERK and PI3K-AKT pathways, supporting oncogenesis of tumor cells when RAS is mutated. RAS-GTPase activating proteins (GAPs) revert RAS proteins back to the GDP bound state by cleaving the gamma phosphate of GTP in a process called GTP hydrolysis.

Studying the unique biological effects of RAS mutations between RAS family members, RAS hotspot mutations, and individual mutant RAS alleles is essential to understanding therapeutic vulnerabilities in RAS-mutated cancers. RAS mutations alone or combined with secondary changes (5, 8) create a heterogeneous signaling environment that may alter therapeutic efficacy. Careful characterization of RAS mutants is imperative to the effort to target RAS in cancer and to treating patients with RAS mutations from the perspective of personalized medicine.

This article was adapted from a FOCUS publication in Science Signaling written by Nancy Sealover and Dr. Robert Kortum (9).

Nancy Sealover is a 4th year PhD candidate who works in the USU Pharmacology Department in Dr. Robert Kortum's lab. Ms. Sealover studies the use of Tipifarnib and KRASG12C inhibitors alone and in combination therapies to treat pediatric and adult RAS-mutated cancers.

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Conferences

In the past six months, USU students and faculty attended scientific meetings on a variety of topics in locations all over the world. Here, they recount their personal experiences and newfound knowledge, and share reviews of the conferences they attended.

45TH ANNUAL CONFERENCE ON SHOCK

1

Major Simon Tallowin MRCS Msc RAMC
3rd year MCB PhD Candidate
Toronto, Canada June 4-7

In June, I was fortunate to attend the 45th Annual Conference on SHOCK in Toronto. The meeting is the calendar highlight of the SHOCK Society, a multi-disciplinary group founded in Chicago in 1977 with the mission of integrating basic science and clinical disciplines in trauma, sepsis and shock research. The Society are responsible for publishing a prominent journal in the field (Shock: Injury, Inflammation, and Sepsis; Impact Factor: 3.553) and so I was anticipating high quality presentations and was not disappointed.

Highlights included an update on multi-omic studies in polytrauma, a panel session on microvascular response to injury, and the visually stunning keynote speech from Professor Paul Kubes on imaging of a 3D-modeling of vascular resuscitation techniques and the use of digital twins in predictive models. One surprising but valuable takeaway was the renewed focus on the fundamentals of preclinical research. For example, there was significant debate around the translational relevance of specific animal models of injury and sepsis, with strong evidence-based recommendations generated of relevance to my own research.

As a conference itself what struck me was the welcoming and collegial atmosphere. Networking opportunities included an evening social and a morning fun run along the banks of Lake Ontario. The balance of scientific and clinical delegates no doubt fostered an environment of collaboration, aiding innovation and acting as a catalyst for the translation of preclinical insights to the bedside. There were also ample opportunities for young investigators to present with notable genuine encouragement from the senior Society leadership.

As a surgeon and scientist-in-training I have at times felt like I have a foot in two very distinct camps, each with their own priorities and cultures, but never being wholly rooted in either. At the SHOCK Society however, it felt different. The research was simultaneously fundamental and scientifically robust yet remained very clinically-relevant. Therefore, for anyone interested in the biology of critical illness I can strongly recommend you attending. The next meeting is in Portland, Oregon in June 2023 - I hope to see you there!

2 3RD INTERNATIONAL SYMPOSIUM ON INFECTIOUS DISEASES OF BATS

Dr. Brian Schaefer, Deptartment of Microbiology
Colorado State University, July 24-27

The conference was organized by Dr. Tony Schountz, Professor of Microbiology, Immunology, and Pathology at Colorado State. Over 200 attendees from around the world traveled to Colorado for this in-person conference (which also included a few virtual presentations from speakers who could not attend). There were nine oral presentation sessions and one poster session that covered a variety of topics including bat immunology and bat ecology; and sessions focused on specific disease-causing pathogens carried by bats, including coronaviruses, filoviruses, paramyxoviruses, and lyssaviruses. The sessions on paramyxoviruses and lyssaviruses were of primary interest to me, since my lab uses mouse models to study immune responses to and develop novel therapeutics for these viral pathogens.

USU representation at this meeting was very strong. Celeste Huaman, a 4th-year EID student in my lab, and Dr. Moushimi Amaya, a Scientist in Dr. Broder's group, delivered excellent oral presentations in the lyssaviruses and paramyxoviruses sessions, respectively. Additionally, EID student Marana Tso (Broder lab) delivered a poster presentation that attracted considerable interest.

Because this meeting covered material on topics outside of my primary focus, I learned a great deal. For example, Peng Zhou from the Wuhan Institute of Virology, China, gave a virtual presentation titled On the evolution of bat type I interferons, which provided considerable insight into why bats may be able to harbor many viruses that cause severe disease in other mammals, without experiencing disease signs themselves. He presented data from his group showing that bats express interferon genes that are quite divergent from those expressed in many other mammals, including humans. In particular, their interferon-triggered innate immune responses to viral infection tend to be less inflammatory, and thus less pathological, as compared to immune responses in humans.

The conference also included a live session of the This Week in Virology (TWiV) podcast with Vincent Racaniello and Brianne Barker. I enjoyed meeting Dr. Racaniello and discussing his (very successful) efforts to bring recent developments in virology to a very broad audience. Having often been frustrated by the media's inability to convey current scientific advances with anything more than an often-inaccurate sound bite, I found it very encouraging to see scientists such as Dr. Racaniello setting up their own media enterprises to convey new developments in science in a way that is accurate, interesting, and accessible by audiences with little to no science background.

Overall, this conference provided a very interesting mix of bat biology and virology, with many excellent presentations. The conference center at Colorado State was well-suited for an event of this size, and there were many opportunities to meet/have discussions with colleagues. Fort Collins, CO, was a very good location for this meeting, offering many outdoor activities and a variety of dining options close to the conference center.

3 GORDON RESEARCH CONFERENCE ON CALCIUM SIGNALING

Benjamin Tripoli, 4th year MCB PhD Candidate
Ventura, CA June 19-24

I have attended a few scientific conferences, but almost all of them have been virtual due to the Covid-19 pandemic. Having now attended a scientific conference in person, I can say that a conference is a great place to get valuable feedback on your project, learn about new techniques and scientific findings, and meet colleagues from within your area of research. This past June, I attended the Gordon Research Conference on Calcium Signaling in Ventura, CA, along with Dr. Jeremy Smyth and NES PhD candidates Eugene Berezovski and Mariya Prokhorenko. The conference was highly specific to calcium signaling research, and thus attracted a small audience of approximately 100 attendees.

My experience was that the small size made for better discussions, and it was easier to talk with the presenters after the sessions ended. Much of the research in the field addressed resolving structures of calcium channels and understanding the interplay between the mitochondria as a calcium store and the endoplasmic reticulum (ER), as well as how these calcium signaling processes are mechanistically regulated. There is much to understand about the interplay between calcium movement in the cell, especially between the ER and the mitochondria. One question that I wanted to see addressed at this conference was how STIM and ORAI, the two proteins involved in the Store Operated Calcium Entry (SOCE) pathway, are regulated. Specifically, what is the thought regarding how these two proteins are spaced correctly to physically interact with each other so that the pathway can proceed. Sadly, this was not discussed in great length at this conference, but as a lab, we discussed some theories involving phosphatidylinositol 4,5-bisphosphate (PiP2).

I had the opportunity to present my research findings to all in attendance via a ten-minute oral presentation, with some time for questions. This was important to me, because I needed some practice explaining my work and presenting my findings, so the opportunity to present to this audience was invaluable. I also presented a poster at a separate conference session, and this was a good experience as well, as presenting a static poster is much different than presenting a PowerPoint. Many of the questions that followed my presentation were specific to our model organism and how to best interpret the results we presented. I probably need to do a better job providing background to introduce the model organism and explain our analysis methods. I learned that people genuinely enjoy the videos our lab makes to help show our data, so I will always keep a video in my presentations moving forward.

After attending the conference, I believe that my path forward is to complete an assay that allows me to observe calcium flux through the ORAI channels in cardiomyocytes, as this assay is a key piece of my dissertation work. Attending this conference was a beneficial experience for me as a graduate student because it provided an opportunity to present my findings and gain access to the most up-to-date work in my field.

4 NATIONAL NEUROTRAUMA SOCIETY 2022 SYMPOSIUM

Nicholas Breehl, 4th year MCB PhD Candidate
Atlanta, GA June 26-29

This was my second experience at a scientific conference, but my first as a graduate student. As a MCB student who joined a laboratory with a primary focus on the brain, I found the learning curve to be steep. This conference offered an opportunity to continue learning about the many facets of neurotrauma research. I was able to attend sessions on topics that varied considerably, such as neutrophil response, biomarkers, and neurodegeneration, where I could listen to leaders in the field discuss their unique approaches to specific topics that I had only read about.

I work in Dr. Sharon Juliano's laboratory, where we study multimodal TBI (blast and Closed-Head Impact Model of Engineered Rotational Acceleration) in a gyrencephalic brain. We have become interested in the role of astrocytes with the glymphatic system – a unique pathway that aids in the clearance of interstitial toxins. Astrocytes express aquaporin-4 (AQP4), a water channel protein integral to glymphatic waste removal.

I participated in a poster session where I shared that our data revealed an increase in GFAP, a marker for reactive astrocytes, and AQP4 immunofluorescence after injury in several regions of the brain, which lasts for up to six months. To better understand this pathology, I would need to characterize this phenotype in more detail. Serendipitously, a gentleman introduced himself with his business card and I recognized his name, Dr. Jeffrey Iliff, as the author of many of the publications I had read to learn about the principles of the glymphatic system. His lab recently developed a technique for the analysis of perivascular spaces represented by AQP4, and he invited me to initiate a collaboration. With this new protocol, I will adapt code to semi-automate quantification of the ratio change in polarization of AQP4 in injured vs control brains. Polarization, in this context, is something that many may not consider, but it is a topic that was reiterated during a seminar at the conference. Essentially, astrocytes are known to direct their AQP4 to the end feet of their arborations to surround vessels. It may be that our multimodal injury conditions are jeopardizing the organization of this protein leading to a depolarized state and thereby impacting the health of the vessel microenvironment. If I hadn't attended the conference, I would not have had the opportunity to make this connection and expand my understanding of AQP4.

5 MEOISIS GORDON CONFERENCE

Marnie Skinner, 5th year MCB PhD Candidate. Transfer from JHU
Colby Sawyer College, New Hampshire June 5-10

This past June, I attended the biennial Meiosis Gordon Research Symposium (GRS) and the Gordon Research Conference (GRC) on Diverse and Conserved Molecular Mechanisms Preventing Aneuploidy During Gamete Production. This was my first time attending this conference and it provided me with a great opportunity to get to meet individuals within the meiosis community. The small size of the conference, approximately 150 attendees, and the way it was scheduled provided me a chance to talk to many of the principal investigators (PI's) present and get to learn more about the work that they do beyond what they covered during their presentations. My primary research focus is on centriole and centrosome biogenesis during mammalian spermatogenesis and I chose to attend this conference to hear more about meiotic research and broaden my knowledge about the field outside of what I primarily focus on. I did have an opportunity to talk directly with some PI's about their work concerning spindle pole assembly in other organisms and how that may relate to my thesis project. The conversations were very insightful and provided me with some additional information to consider while moving forward with my research. However, my biggest takeaway from the conference was not one talk or interaction in particular, but rather the opportunity to get to know my peers in the meiosis community. Many of the PI's have been attending this conference since they too were in graduate school and many met each other as a result of this conference and the community it helps provide. I hope to remain involved in the meiosis community as my career progresses and I am very glad to have begun building these relationships with fellow graduate students already. Many of us would not have met so soon, if at all, without this conference setting bringing us together. The Gordon research symposiums and conferences cover a variety of topics in addition to meiosis, and I would highly recommend these events to anyone looking for an opportunity to interact with a more specific research community.

6 CSHL TRANSLATIONAL CONTROL

Anthony Erb, 2nd year MCB Student
Cold Spring Harbor, NY September 6-10

The 2022 Cold Spring Harbor Laboratory (CSHL) Translational Control conference helped me to form a personal perspective on the direction of this important field of scientific research. With approximately 454 participants and presentations of mostly unpublished research on diverse topics ranging from the mechanism and regulation of translation, to strong associations between translational disruptions in human diseases, I feel excited and hopeful as a young graduate student to have meaningful scientific investigations of my own in this field.

If I were to highlight just one of the many interesting and insightful directions that the field of translation is heading it would be elucidating the various regulatory mechanisms of upstream open reading frames (uORFs) on fine-tuning protein synthesis. Located in the 5'-untranslated region of about 50% of all mRNAs, these regulatory sequences contain cognate start codons upstream of the main open reading frame that encodes the functional protein. Excellent examples, in which the mechanism of uORF regulation has been described, include activating transcription factor 4 (ATF4) and C/EBP-homologous protein (CHOP). Ultimately, one of the most inspiring presentations was given by Dr. Judith Frydman who highlighted the role of a functional uORF in regulating translation of the HTT mRNA in Huntington's disease. Important to note is that the regulatory mechanism of uORFs for certain transcripts differ, supporting the notion that we as a scientific community could potentially help patients with a variety of diseases by better understanding this method of maintaining cellular proteostasis.

This was the first academic conference that I have ever attended (albeit virtually) but I could feel a sense of dedication when those attending in-person had a coffee break at 9 PM on the first day. All I could do was smile at the thought of caffeine that late. However, that same dedication shined through on many of the presentations and posters developed for this conference. This model of hard work is commendable and something I hope to emulate during my time at USUHS.

18TH EUROPEAN MEETING ON COMPLEMENT IN HUMAN DISEASE

7

Rohini Manickam, 4th year MCB Candidate
Bern, Switzerland August 26-29

The annual European Meeting for Complement in Human Disease (EMCHD) highlights the latest contributions in the field of complement system research. Historically, the focus was on developing complement therapies for extremely rare immunodeficiencies driven by genetic aberrations in complement system proteins. Only one such therapeutic, which was approved in 2007, is currently available. In recent years, the field has expanded to focus on understanding the complement system's role in driving pathogenesis in broader inflammatory contexts such as autoimmune diseases, cancers, Alzheimer's Disease, and acute infections like Covid-19. I study the function of cytoskeletal proteins in macrophages in the lab of Dr. Jeremy Rotty. Using some recent data that suggests that an actin-binding protein Phactr4 may be involved in complement-mediated phagocytosis (a critical functional endpoint of the complement immune response), I presented a poster at EMCHD and sought feedback from the field.

The conference started with a teaching day for junior scientists and graduate students with specialized workshops tailored to their interests. One of my more translationally focused workshops included a conversation with Dr. Martin Trendelenburg, an internal medicine physician in the Basel University Hospital who also runs a complement system research group. Although there is evidence that dampening the hyperactivation of complement can be beneficial to controlling extended inflammatory states in patients, and hospitals have complement activation testing panels available, Dr. Trendelenburg cannot convince his colleagues to run these tests when he believes they could be helpful. He believes that physicians are reluctant to treat complement activation related symptoms, highlighting the need for additional approved therapeutics and more clinical data.

This conversation certainly set the stage for the following days of the conference, with research talks and poster sessions that included translational and animal model research exploring the rationale for inhibiting specific complement system pathways in certain pathological contexts. One particular standout session was given by Dr. Younglun Lou of Aarhus University, who shared his lab's work in spatially resolved transcriptome analysis and their international collaborative effort behind Human Cell Atlas and Human Protein Atlas. These will be public resources for a complete body-wide mapping of RNA and protein profiles across diverse single-cells and tissues, and will be a powerful tool beyond the complement field.

This small conference of approximately 500 attendees was extremely welcoming toward new researchers in this growing field. I appreciated the single program of presentations, the new connections I made, and the spirited conversations at my poster presentation. I was especially excited to re-connect with a Bethesda based friendly face, Dr. Claudia Kemper. Dr. Kemper, a complementologist at the NIH, is a recognized leader in the field who also teaches the USU Introductory Immunology lecture on the complement system.

The conference's social activities took advantage of beautiful Switzerland, with a welcome dinner at an historic Bern restaurant and a trip to Lucerne for a dinner cruise. My first conference experience was generously funded by the GEO/VPR Travel Award, which I highly recommend to fellow students. Next year's meeting, the 29th International Complement Workshop, will take place in Newcastle, UK, and I hope to attend if my project takes me there!

8 17TH TRANSGENIC TECHNOLOGY MEETING

Dr. Philip Jordan, Department of Biochemistry and Molecular Biology (BIO)
Helsinki, Finland September 17-20

The 17th Transgenic Technology Meeting (TT2022), overseen by the International Society for Transgenic Technologies, was organized by Dr. Satu Kuure (University of Helksinki), Dr. Reetta Hinttala (University of Oulu), and Dr. Søren Warming (Genentech). Drs. Kuure, Hinttala, and Warming specialize in development and optimization of transgenic technologies.

TT2022 was originally scheduled for April of 2022, but rescheduled to September due to issues related to COVID-19. The conference was then moved from Levi, Finland, to the more accessible Finnish capital, Helsinki. The silver lining of the pandemic is that the scientific community has become very good at holding virtual and hybrid conferences, workshops, symposia, and interest groups. Additionally, the virtual option expands accessibility and affordability so that more people can participate. Because of my recent lab move to USU, I was unable to attend in person, but TT2022's virtual component was so well-managed that I almost felt like I was there in person. I was invited to present at the conference, and I was able to deliver the presentation virtually (at 3 AM EST on Sunday!) and answer questions from the live audience in Helsinki and from virtual attendees from around the globe. We take virtual meeting technology for granted, but I still think it's cool that a presenter in Maryland, USA can engage live with scientific peers in Finland and around the world without leaving home. It's a very small world.

TT2022 offered so many interesting presentations that I do not want to highlight one specific area. Instead, I will summarize the topics/themes that the presenters covered. The first sessions revolved around creating animal models that mimicked patient-specific mutations, with the aim of characterizing and developing personalized treatments. There were sessions that explained exciting in vivo genome editing advances, all of which revolve around using powerful technologies such as CRISPR-Cas9 gene targeting to create conditional alleles, knock-in alleles, and transgenes. There was also discussion on how to effectively create your own transgenic animals with optimized strategies and new technologies. It was fantastic to learn about these topics, and my lab is adopting several new approaches to create transgenic animals in-house. So, watch this space!

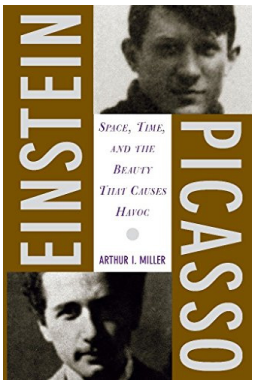
For my NES research colleagues at USU, there was an entire section dedicated to neuroscience, covering animal models and treatments of schizophrenia, ischemic stroke, and ALS. There were presentations discussing the creation of new animal models and approaches, such as a method for rapid genetic manipulation of gene expression in embryonic nervous systems to reduce the numbers of mice used in research. There were also presentations covering large animal transgenesis topics, including some that focused on the generation of transgenic marmosets as non-human primate models for brain disorders and neuroscience research.

Although I could not attend in person, I thought that TT2022 was a fantastic conference. Even as a virtual participant, I made many new contacts and established some collaborations as well. One reason why TT2022 was such a successful virtual event was effective platforms and tools, including a conference phone app that allowed attendees to reach out to other attendees and start conversations. I took full advantage of this tool, and it almost made up for missing out on the collegial conversations that one enjoys when sharing a beer with fellow attendees at an in-person event….. TT2023 will be held in Houston, TX. I am determined to get there next November, in person.

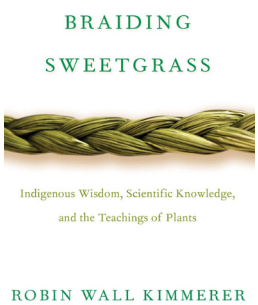
Book Club

Content for scientists, curated by scientists.
The USU community anonymously shares reviews of books and podcasts they enjoyed for the next like-minded individual on the hunt for a good read/listen.

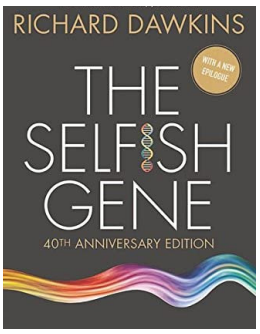
FOR THE READERS



The title immediately suggests a lionization of Einstein and Picasso, who in many or most circles are considered the standout scientific and artistic figures of the 20th century. But this is less an individual tribute than an examination of how these two firmly viewed their disciplines as pursuits in practical problem solving during times that demanded it. And therein lay their genius: transforming the non-obvious into the obvious through relativism and realism. It's an altogether incisive - and fun, easy - read for the scientist and artist alike.

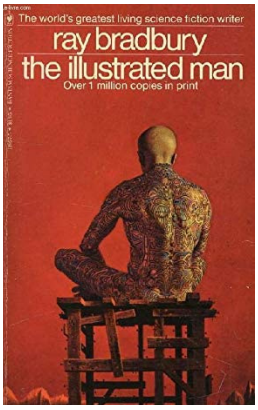


In *Braiding Sweetgrass,* Kimmerer brings together her multi-faceted lenses of knowledge as a trained botanist and a member of the Citizen Potawatomi Nation. We can take away much from indigenous wisdom as it connects to western science, gratitude, and how humans relate to the land. Great bedtime read.

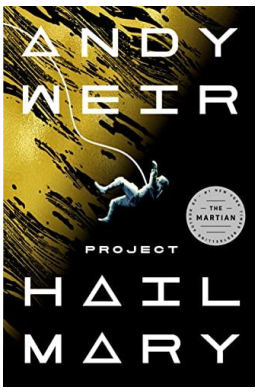


Though published in the 70s, this new-to-me classic focuses on British evolutionary biologist, Richard Dawkin, and his re-imaging of Darwin's theory of natural selection with a genetic scope. I found this read to be refreshing, grounding and occasionally bewildering.

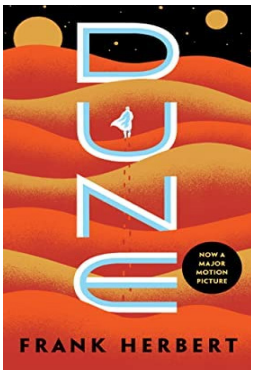
This collection of compelling short stories shares raw and honest portrayals of pregnancy and motherhood and lack thereof. Some anecdotes navigate especially painful terrain, which highlights our society's obsession with women's most personal decisions. Warning: may incite melancholy, but may also make you feel seen.



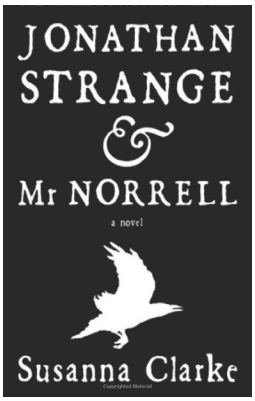
The Illustrated Man is a series of related short stories that were compiled into a cohesive novel. This makes it a super easy read! There is a good mix of uplifting and thought-provoking stories in it for those who enjoy futurism and sci-fi.



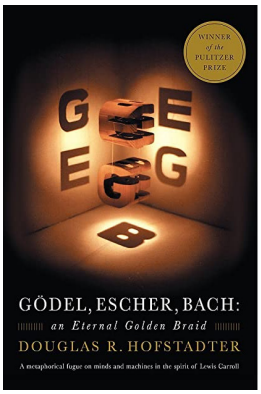
The less you know going in, the better. A man wakes up, memoryless, in a white room with only two desiccated corpses, a robot voice and surgeon arms to keep him company. The author is clearly a huge physics and biology nerd, so he knows his stuff. You'll constantly try to figure out what's going on alongside him, as well as going "I know what that is!" when he does science.



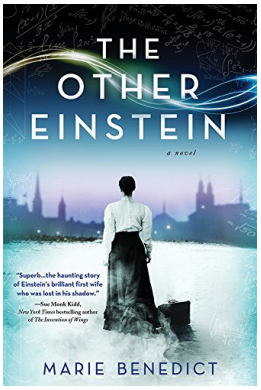
The world building and writing by Herbert made this one of the most influential sci-fi novels of all time. It still reads as strange and fascinating now as it did in the sixties and the sequel, *Dune Messiah,* comes just as highly recommended.



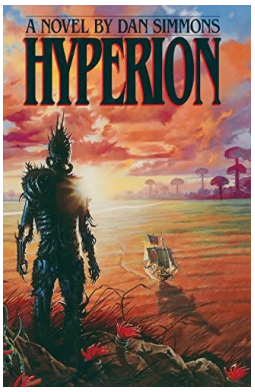
This fiction offers an alternative history in which magic is real and treated as a science, with a compelling historical lore. It's like if Harry Potter was a Georgian Era magician who helped protect Europe from Napoleon.



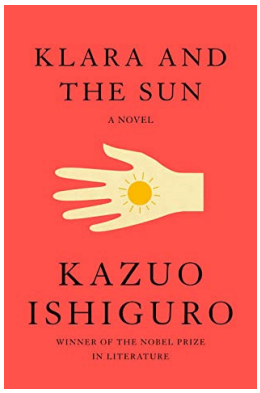
GEB (as it's known by its cult following) is one of the strangest books I've ever read. One page has 50 lines of computer code; 100 pages later, the author offers hand drawn images of protein transcription and translation. Through pattern matching, this book challenges you to explore how different aspects of reality such as math, art, and music, are connected. You may be persuaded to find surprising similarities between a musical canon and logical recursion. Hofstadter will certainly make you think.



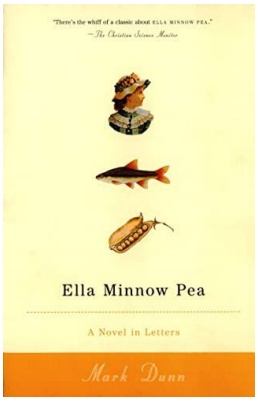
This book is a historical fiction created from the letters between Albert Einstein and his first wife, a fellow physicist named Mileva Maric. It is well-written and engaging while maintaining its impact as a reminder that we often learn revisionist history.



The Hyperion Series is epic sprawling sci-fi/fantasy at it's best. The first book follows seven people on a pilgrimage to the world of Hyperion, home to a metal spiked being with the ability to grant one wish. There is action, political intrigue, and borderline horror elements. After ending on a cliffhanger, the second book has a satisfying conclusion that feels like it has a six-hour straight climax of wrapping up every plot thread. The third and fourth books have a time skip, and follow mostly new characters, but it retains much of the great adventure and clever plotting of the first two.

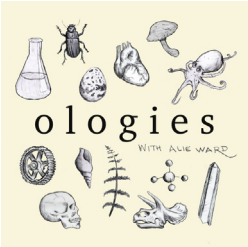


Klara and the Sun has an interesting narrator and hero: an artificially intelligent robot who is a companion to a human child. Klara's voice has the perfect mix of naivety and intelligence in her journey from the AI store to life with her human. Her detached tone makes you feel the absolute opposite as the plot spins out of control and you inevitably want to bundle up Klara and bring her home. This delicate story makes you feel sorrow, hope, and brings up important questions about humanity.



Ella Minnow Pea takes place on an island that is the birthplace of the man who came up with the phrase "The quick brown fox jumps over the lazy dog", which has all the letters of the alphabet. When a storm starts plucking letters from the sign, the island council decides to start banning use of the lost letters, saying it is a sign from the phrase creator. The awe of getting through entire pages before realizing you never saw certain letters is incredible and the author does a great job in handling the slow loss of letters!

FOR THE LISTENERS



Ologies with Alie Ward. Each episode Alie brings on a different professional in the field of the ology that episode is themed after. Episodes are are well done and fun, but can tend to be long since the theme switches each one.

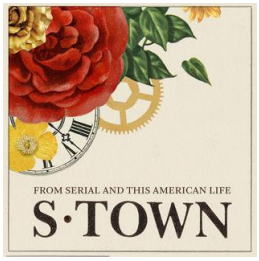
Financial Feminist. Toni Dunlap is intelligent and well-spoken, and she brings on wonderful guests that speak about different areas such as MLMs, racial injustice and investing.

The New Yorker: Fiction brings on well-known authors to read and discuss their favorite pieces of fiction. Because each episode varies in length, this is a great way to pick the perfect podcast for your long morning commute or short afternoon run. The analyses are high-quality but a far cry from being stuffy academic debates. I find these episodes to be a great way to just let your mind wander.

Conan O' Brien Needs a Friend is a quality comforting comedy podcast that's the perfect balance of funny and serious talk at a great pace. (Noteworthy triplicate submission!)

Scoundrel: History's Forgotten Villains. Engaging historical accounts of people who altered history by just being the worst. Favorite episode so far highlights John Taylor, the man who almost single-handedly ended the Baroque Period by being a heinously unscrupulous ophthalmologist.

The Magnus Archives is a "weekly horror fiction anthology podcast examining what lurks in the archives of the Magnus Institute, an organisation dedicated to researching the esoteric and the weird."



S-Town was started after an Alabama resident contacted radio journalist Brian Reed to investigate an alleged murder. The series covers many difficult and controversial topics.

The Adan Buxton Podcast. In this highly rated production, British Comedian Buxton interviews friends and celebrities.

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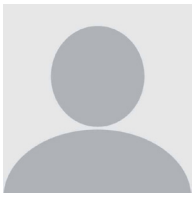

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We welcome any inquiries or feedback
to the *USU Science Review* team at
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to producing the second Issue in Spring of 2023.

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