**Can you begin with an overview of your research interests? What drew you to neurodegenerative disease?**

**MM:** My interests include how cells use specific proteins to receive, relay and incorporate extracellular signals, and engage the required energetics for the cell to respond accordingly. When cells no longer respond correctly to these cues, the risk of disease increases. Specifically, I am interested in understanding how the mutant huntingtin protein (HTT) controls the selective death of neurons to cause Huntington's disease (HD). I was drawn to this field by Dr James Gusella's discovery of the Huntington disease gene, by methods that opened the flood gates to find genes for many other inherited disorders. I have also witnessed the devastation that neurodegenerative diseases can have on family and friends.

**Which questions do you seek to answer?**

**MM:** The mutation in HTT is an expansion of a stretch of glutamines (polyQ) that alters the normal structure and function of HTT. We still don’t fully understand the normal function of HTT, or the events that initiate the disease. Nor do we know how the expanded polyQ alters the molecular structure of HTT, or why the longer Q-length inversely correlates with the age of symptomatic onset.

My initial question was whether I could use the haploid genetics and unique life cycle of *Dictyostelium discoideum* to learn about the function of genes that cause brain disease. Unfortunately, many genes that cause neurodegeneration are essential during embryonic development, so deleting the gene makes the study of its function very difficult. In *D. discoideum* we can circumvent this because development follows growth. Thus, if the gene isn’t essential for growth, but it is for development, we can often spot defects visually, allowing us to determine the protein’s function. The other question was whether we could get *D. discoideum* cells that lack their own HTT to make human HTT, and if the protein would be functional. To this end, I started using *D. discoideum* to study how polyQ expansion in human HTT alters structure/function.

**Has a multidisciplinary approach proven important to the success of your projects?**

**RW:** Yes, it is essential to engage scientists from both basic and clinical fields to ensure that fundamental work is undertaken with an eye to usefulness.

**AK:** Through collaboration, mutations in human leucine-rich-repeat kinase 2 gene (*LRRK2*) have been found to be the most frequent cause of late-onset Parkinson’s disease (PD). We have successfully used related proteins from *D. discoideum*, to understand the structure and regulatory mechanism of LRRK2. The structure of *D. discoideum* Roco4 kinase was obtained for wild-type and PD mutants, and explains PD-related increased LRRK2 kinase activity.

**What are the challenges associated with developing novel therapeutic approaches for HD?**

**MM:** Any HD research group will tell you that the normal function of the huntingtin protein plays a role in defining the specificity of neuronal loss. However, elucidating the cellular function that leads to the loss of stratial neurons (a part of the brain that helps coordinate movement) has been no easy task.

HTT is present in all cells, so why does the polyQ mutation seemingly target a specific neuronal subtype? How will therapeutics target only mutant HTT or neurons? Many...
questions have to be asked when trying to establish therapeutic approaches. My goal is to determine the earliest of disease-causing function(s) that are fully dependent upon the polyQ mutation in HTT.

Have you seen success in this area to date?

MM: We found that normal human HTT compensates for the \textit{D. discoideum} protein to restore cell directionality. However, polyQ-expanded HTT cannot, and confers poor directionality to cells, much like \textit{D. discoideum} \textit{hht} knockout cells. More research is needed to determine whether the polyQ mutation causes the protein to do more of something, less of something, or something altogether new.

Do you have plans for future projects or do you wish to further investigate areas of your current research?

RW: We are looking to set up an international consortium of scientists to take advantage of the experimental techniques that \textit{D. discoideum} offers, in order to address neurological disorders. This consortium could lead to breakthroughs in epilepsy, PD and many other important conditions.

AK: Multiple PD mutants result in an increase in LRRK2. The aim of our research is to elucidate the complex mechanism of LRRK2 activation, to better understand PD. Several kinase inhibitors have been identified that are selective for LRRK2 and are brain penetrant; however, long-term use leads to kidney abnormalities. To explore alternative approaches, it will be essential to understand molecular activation, identify regulators, and characterise the cellular function of LRRK2.

\textbf{NEURODEGENERATIVE DISEASE} is characterised by the progressive loss of function, and eventual death of neurons. Understanding the events which lead to this neuronal pathogenesis is a challenge being tackled by scientists worldwide, with the ultimate goal of developing long-term therapies.

Knowledge of two major neurodegenerative diseases, Alzheimer’s (AD) and Huntington’s (HD) has been advanced by Dr Michael Myre, Instructor of Neurology at the Center for Human Genetic Research (CHGR), Massachusetts General Hospital. These diseases, among numerous similar neurodegenerative conditions, have devastating impacts, with many feeling they have lost their loved ones even before death.

Myre’s current work focuses on HD, which is caused by the expansion of a polyglutamine (polyQ) domain in huntingtin – a large, ubiquitously expressed protein. The enigmatic nature of the protein presents major challenges in the field. Its normal function is yet to be fully clarified, largely owing to the fact it shares no significant similarity with any other known protein, and when deleted it causes early embryonic death in major animal models. Myre works to understand huntingtin function via its orthologues in lower organisms, and potential targets for the treatment of the disease.

Myre is laying the foundations for HD research and appreciates the continued support he receives: “The HD faculty and investigators at the CHGR have been extremely supportive of my innovative ‘outside the box’, but scientifically compelling, approach to understanding the function of huntingtin,” he enthuses.

\textbf{ALZHEIMER’S DISEASE}

As the most common form of dementia, the estimated worldwide prevalence of AD is approaching 30 million people, and with figures expected to quadruple over the next 40 years, the need for novel therapeutics is intensifying. A terminal disease, the course of AD averages seven to 10 years, and it is characterised by amyloid plaques – accumulations of insoluble protein aggregates in the brain. The main component of these plaques is the amyloid-beta (A\textsubscript{\textgamma}) peptide, derived from the amyloid precursor protein (APP), via the sequential action of \textalpha- and \textgamma-secretase. APP was the first gene in which mutations were found to cause early-onset familial Alzheimer’s disease (FAD). Mutations in two other genes have also been found: \textit{PSEN1} and \textit{PSEN2}, which encode presenilin (PS), the catalytic part of the \textgamma-secretase complex.

In 2010, Myre revealed incredible insights into PS. He showed that \textit{D. discoideum} has highly diverged \textgamma-secretase subunits which can process human APP, proving that this key regulatory pathway is evolutionarily conserved over hundreds of millions of years. By creating single and double mutants for genes encoding the \textgamma-secretase components of \textit{D. discoideum}, working with Dr Alan Kimmel of the National Institutes of Health (NIH), Myre’s team showed that \textit{D. discoideum} has a highly diverged PS which regulates growth and cell fate specification. The researchers also demonstrated that regulation of phagocytosis requires an
INTELLIGENCE
THE UNDERLYING PATHOGENIC MECHANISMS THAT LEAD TO THE DEVELOPMENT OF HUNTINGTON’S DISEASE

OBJECTIVE
To develop robust biochemical and physiological in vivo assays by phenotypic mining in a huntingtin-deficient model organism, ultimately elucidating the normal function of huntingtin.

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MICHAEL A MYRE obtained his PhD from the University of Toronto in Dr Dan O’Day’s lab where he studied the role of calmodulin and calmodulin-binding proteins in the social amoeba Dictyostelium discoideum. In 2004, his interest in neurodegenerative disease led him to do a postdoctoral fellowship with Dr Wilma Wasco in the Mass General Institute for Neurodegenerative Disease (MIND) at Massachusetts General Hospital, working on Alzheimer’s disease drug discovery. In 2009, he joined Dr James Gusella at the Center for Human Genetic Research and developed Dictyostelium discoideum as a novel approach to understand Huntington’s disease. Myre holds the Harvard Medical School Faculty rank of Instructor.

active γ-secretase, a pathway suggested to occur in Drosophila and mammalian cells and crucial to innate immunity. The seminal findings also reinforce D. discoideum as a model for identifying novel PS signalling targets, providing an effective system for high-throughput screening aimed at uncovering novel therapeutics for AD.

By contributing to understanding of the function and regulation of PS-dependent γ-secretase cleavage, Myre’s work will facilitate new approaches for treating AD. It also provides a fascinating insight into the conserved nature of cell function, showing that PS has been conserved across millennia from D. discoideum to humans.

THE MYSTERY OF HD
Although HD has been extensively studied, the normal function of the underlying protein remains a mystery. A growing body of evidence suggests that genes which regulate aspects of the disease do exist, including the severity of symptoms and age of onset. Myre explains how he is using D. discoideum to identify these: “The Dictyostelium genome is haploid, meaning it is made up of single copies of each chromosome. We’re using this advantage to perform large-scale screens for genes that alter or shift polyQ-dependent functions back towards a healthy cell. If we can locate them, and they’re conserved among humans, they’ll be targets for therapeutics”.

Screening of the D. discoideum genome revealed a gene with sequence homology to the human huntingtin gene (HTT). Myre then generated a viable htt-null mutant and delineated a number of the resultant phenotypes. He describes the rationale behind this approach, and its benefits over other research: “Understanding huntingtin normal function will be significant towards developing an effective treatment. Many established models (eg. Drosophila) have used an amino-terminal fragment that results in perturbation of many cellular processes, but removes the polyQ tract from its normal context”.

The huntingtin-deficient cells showed many pleiotropic defects, ie. multiple, seemingly unrelated, phenotypic traits were affected. The cells did not exhibit typical polarised morphology and streamed poorly to form aggregates by accretion rather than chemotaxis. They also had a defect in the contractile vacuole (CV), making them hypersensitive to hypotonic conditions. This affected cyclic adenosine monophosphate (cAMP) signal transduction and, in turn, development, which was delayed and asynchronous and produced small bodies with defective spores. The finding that huntingtin is critical to the maintenance of osmolarity and ion balance is particularly significant, as ion homeostasis is crucial for proper neuronal function.

These results are consistent with studies in mammals which show that huntingtin is a multifunctional protein with roles in diverse biochemical processes. The next step is delineating which functions are conserved in humans and determining if they are modified by expansion of the polyQ tract. This will provide sorely needed insights into the mechanism by which mutant huntingtin causes HD.

HUMAN VALIDATION
Continuing to use D. discoideum as a model system, Myre expects to rapidly gain new insights into normal huntingtin function, which will ultimately facilitate specific validation studies in human systems.

Using innovative approaches in D. discoideum, Myre has made significant headway into improving understanding of neurodegenerative disease. The discovery that huntingtin-deficient D. discoideum cells show highly defective chemotactic behaviour, coupled to cytoskeletal and membrane deficits under low ionic conditions, suggests conserved mechanisms for the protein in axon guidance and neuritic extensions. By expanding on these findings, Myre hopes to transform the field and find new treatments for some of the most devastating known diseases.