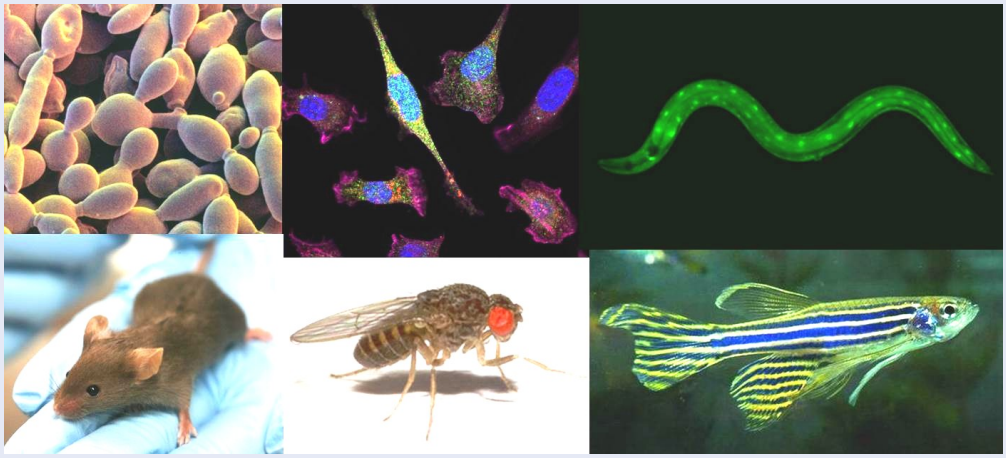
MND Australia Research Update

Isabella Lambert-Smith
Australian Rotary Health PhD Scholar, University of Wollongong

September 2014

Modelling MND: How do we explore the disease process in the lab



Neurodegenerative diseases are notoriously complex, which of course makes them an important focus in medical research, but also makes them tricky to study. Addressing this, many models of disease have been developed that have given us incredible insight into mechanisms of neurodegeneration.

One of the most simple models in wide use is the organism commonly known as brewer's or baker's yeast. Yeast share many similar cellular features with higher organisms, including humans, and are so well characterised and easy to use that they are well-suited for large-scale studies of basic biological processes. For more neurone-specific studies, cells that are derived from either human or other mammalian neurones are used in a method known as cell culture. The latest advances here have been in the development of induced pluripotent stem cells (IPSCs), which come from MND patients themselves and thus carry the full genetic make-up that led to disease in the first place.

For validation of findings from these sorts of studies, it is necessary to examine the mechanisms in a functional nervous system, which is where organisms such as the nematode worm, fruit fly, zebrafish, as well as mouse and rat models have become integral in MND research.

RNA vs proteins in *C9orf72*-linked MND

A genetic defect in the *C9orf72* gene is currently recognised to be the most common genetic cause of MND and also frontotemporal dementia (FTD). The abnormality in this gene is an expanded repeat of a sequence of six DNA nucleotides, the building blocks that make up DNA. The main question researchers have been asking concerning this *C9orf72* genetic defect is whether it causes disease through the repeat RNA that results from the repeat DNA, or by the abnormally short proteins (dipeptide repeat proteins, DPRs) that are produced from the RNA. Each of these scenarios involve quite different disease pathways, and many researchers have had trouble designing the right kinds of experiments to distinguish between them.

Recently, however, Sarah Mizielinska and collaborators at University College London and the Max Planck Institute have carried out a very well designed study to dissect out the main disease-causing mechanism in *C9orf72*-linked disease. They generated neurone cell culture and fruit fly models to test the toxicity of either the repeat RNA alone, or repeat RNA that could then synthesise the short DPRs. The results these researchers obtained strongly implicate the DPRs as the main toxic culprit, rather than the repeat RNA itself. However, the toxic contribution of the repeat RNA cannot be ruled out entirely, as other studies have produced evidence indicating its role in neurodegeneration. Given that the *C9orf72* genetic defect is causal in both MND and FTD, affecting different types of neurones in each case, it is possible that other factors come into play that are specific to subpopulations of neurones. Because repeat-containing RNA and proteins have also been implicated in other neurodegenerative diseases as well as several cancers, the insight provided by Mizielinski's study and others like it will help significantly in identifying the specific disease-mechanisms involved.

MND Research Shorts

- *A new mouse model of MND induced by the neurotoxin BMAA has been developed by researchers at the University of California, Irvine. This will enable studies into the pathological effects of BMAA in a fully functional nervous system.*
- *Researchers in Seoul, Republic of Korea, have discovered the neuroprotective effects of a novel compound called JGK-263. This compound inhibits the activity of GSK-3β, a protein that has been implicated in motor neurone degeneration through its action in promoting cell death.*
- *Computational modelling of motor neurone degeneration by the collaborative effort of researchers in Bordeaux, France and New York, USA, adds to existing evidence that dysfunctional mitochondria play a part in MND. These cellular structures are essential for energy production, which is particularly important for energy-hungry motor neurones.*
- *Maria Nikodemova and others in Wisconsin, USA, have identified key differences between glial cells in the worst affected regions of the spinal cord and those in unaffected regions in a rodent model of MND. This suggests that variability in the characteristics of glia in different central nervous system regions could be an important consideration in MND pharmacological intervention.*

UNC13A: A new genetic link between MND and FTD

The significant overlap between MND and FTD, including the *C9orf72* genetic mutation, led a large group of collaborating researchers from various institutes in the UK, Europe and the USA to search for further evidence of a common genetic basis for these diseases. Using published genetic data from MND and FTD patients, they identified common genetic variants in the *UNC13A* gene. This gene encodes a member of a family of proteins involved in neurotransmitter release and thus neurone-to-neurone communication. Changes in the function of this protein thus disrupt biochemical signalling between neurones, and have also been found to trigger structural changes in existing neuronal networks in the brain. This gene therefore may prove to be an effective therapeutic target, putting a focus on a fundamental aspect of neuronal function; relaying messages throughout the body via neurone-to-neurone communication.

Guanabenz: leading the way to tackle neuronal stress

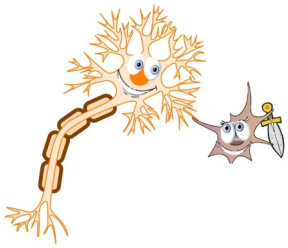
Recent studies in MND patients and animal models indicate an important role for neuronal stress caused by abnormal protein accumulation in a cellular compartment known as the endoplasmic reticulum (ER). This ER stress triggers a response in the cell that involves activation of the key protein eIF2α, which then represses protein production to enable stress recovery. While evidence from a previous study by Nancy Bonini and colleagues using a TDP-43 MND fly model suggests that prolonged activity of eIF2α is harmful for neurones, a recent study by Hong-Qi Jiang and his group from Harbin and Weifang in China indicates that keeping eIF2α activated may be protective in MND. A compound that promotes active eIF2α, Guanabenz, has previously been tested for its effectiveness in nematode worm and zebrafish models of MND. Jiang and his collaborators tested Guanabenz in a SOD1 MND mouse model, and found that it extended lifespan, delayed symptom onset and attenuated motor neurone loss. The fact that different studies have generated such contradictory results may be due to genetic differences between the model systems used. What is clear is that the ER stress pathway may be a promising therapeutic target to tackle MND.

Molecular police and protein quality control

Amongst individuals with MND there is considerable variability in the severity of disease, even between those carrying the same disease-causing genetic mutation. Marianna Marino and her colleagues in Milan, Italy and Sheffield in the UK have found similar variability between mouse models of MND carrying the same *SOD1* genetic mutation. These mouse models differ in their genetic background, differences that are likely to influence the disease mechanisms triggered by mutant *SOD1*. Wanting to investigate this, Marino's group compared the molecular profile between two of these mouse strains. They discovered differences in disease progression that correlated with the abundance of abnormal aggregated proteins in neurones, as well as with the levels of a family of "molecular police" that maintain quality control, preventing protein accumulation and disposing of abnormal proteins. Slower disease progression was observed in the mouse strain exhibiting proper protein quality control, highlighting the role of defective protein maintenance in motor neurone degeneration.

Neuronal support cells: not so supportive in MND

There have been some studies in the past reporting on an MND mechanism in which neuronal loss is triggered by signalling molecules and toxic factors released from glia, the cells that normally protect and support neurones. Hans-Georg König and his team in Dublin, Ireland, investigated the role of a pro-death signalling molecule called Bid in motor neurone degeneration. Bid is important in normal tissue and organ maintenance as a way to eradicate diseased cells while not affecting healthy cells. They found that levels of Bid in glia in the spinal cord increased significantly during disease progression in SOD1 mice. Moreover, glia rich in Bid had an abnormally toxic effect on motor neurones, demonstrating a new role for Bid and its family of related proteins in glia-mediated MND.



A loop-hole in *FUS*-linked MND

A newly discovered type of genetic mutation in the *FUS* gene, other genetic mutations in which account for ~4-5% of inherited MND, has shed intriguing light on a particularly aggressive form of *FUS*-linked MND. Stefano Dini Modigliani and colleagues in Rome, Italy, found that this genetic mutation leads to increased expression of *FUS* and a more severe disease course. They demonstrated that this occurs because the identified *FUS* mutation affects its interaction with certain types of microRNA, small molecules of RNA that "silence" genes to control gene expression. *FUS* and the microRNA form a regulatory loop in which cellular levels of the FUS protein are kept in check. The detrimental result of this genetic mutation in patients highlights the importance of well-regulated *FUS* expression in normal neuronal function. This group's discovery opens up the possibility of targeting or using modified microRNA in the treatment of *FUS*-linked MND.

Stress granules are a continuing theme in MND

Accumulating evidence implicates an important role for stress granules (SGs) in MND. These short-lived protein- and RNA-containing structures are important for cell recovery from insults and resulting stress states, allowing non-essential cellular activities to slow down or stop while prioritising recovery. Work carried out by Leeanne McGurk and others in the Nancy Bonini lab, University of Pennsylvania, provides further support for SG involvement. This group has previously reported that the toxicity caused by the MND-associated protein TDP-43 can be alleviated by modification of SG-associated pathways in fly, yeast and mammalian models. In particular, the protein PABP-1, which is a central component of SGs, was found to interact with TDP-43 and to be an important modifier of toxicity. What this group has now discovered is a link between PABP-1, TDP-43 and the *C9orf72* genetic mutation, which is estimated to account for ~40% of inherited MND cases. They found a significantly higher incidence of PABP-1 accumulation in TDP-43-containing protein aggregates in the motor neurones of people carrying the *C9orf72* mutation than in individuals with sporadic MND. Importantly, this finding zones in on PABP-1 as a new research focus that may prove to be a key player in many forms of MND.