Signalling a change

DR MAREK MICHALAK

Dr Marek Michalak leads a young team of biochemists whose cutting edge research into protein folding in the endoplasmic reticulum has exciting implications for new treatments

To begin, can you give a synopsis of your primary research interests and goals?

My research focuses on the understanding of two fundamental biological processes that affect virtually every aspect of cellular physiology: calcium signalling and protein folding. Consequently, our research has implications for a large number of human diseases. The main objectives are to establish a role for chaperones – proteins that assist in folding of other proteins – in heart diseases and neuropathies. We investigate how to exploit these molecules to slow the progression of degenerative ailments, and our ultimate goal is to provide insights into mechanisms and interventional strategies for protein folding disorders.

What would you say are some of the most exciting findings to come out of your research group to date?

Over the past two decades, our team has made a number of significant observations. We are best known for our work that has brought understanding to the role of chaperones, protein folding and calcium homeostasis in the pathogenesis of human diseases. Our research has helped define the molecular mechanisms of protein mis-folding and their role in regulating calcium homeostasis, which has a significant and far-reaching impact. Major contributions of my laboratory are in the identification and characterisation of several membrane associated chaperones and discovery of their role in cardiac and neuronal pathology. These chaperones play a critical role in the control of protein folding and are therefore key molecules directly involved in control of protein folding diseases.

Why does a failure of the protein folding mechanism cause disease?

Defects in protein folding mechanisms may be due to non-functional chaperones, folding enzymes or folding sensors. These may also be secondary, where cellular homeostasis is in trouble. Most of the studies in this area focus on chaperone substrates and thus there is no obvious association between a human disease with a mutation and a particular chaperone. This is something we are exploring; we mostly see a relationship between modified chaperones and neuropathies.

Can you explain the benefits of the cutting edge technologies you employ in your research?

We use high throughput techniques and analyses of gene expression and protein profiling to help discover new molecules affecting normal and pathological states, working with computing engineers to model and design drugs and molecules that may help develop therapies. We also employ sophisticated equipment and imaging techniques that allow detection of sensitive and critical molecule-molecule interactions, and help visualise live cell protein interactions and movement of molecules in different cellular compartments.

Considering that dysfunctional proteins arise because of aggregation and deposition of mis-folded proteins in cells, what is the reason for the extraordinary diversity in the symptoms of the associated diseases?

One way to look at this is that protein aggregation may have dual effects: you may have a loss-of-function effect and a gain-of-function effect. For example, 1α antitrypsin disease results from a mis-folded 1α antitrypsin protein/enzyme, which then accumulates in the liver leading to liver failure and cirrhosis (gain-of-function). The enzyme is not released from the liver and, therefore, cannot perform its function on other organs ie. leading to hereditary lung emphysema (loss-of-function). We do not study this system but it is a good example of the diversity of symptoms.

What is the significance of your focus on the calcium binding chaperones over other chaperones that work independently of calcium signalling?

In general, proteins do not fold correctly in the absence of calcium. That said, some chaperones must bind calcium ions to perform their functions, others may not. The role of calcium binding to chaperones in their ability to support protein folding remains an open question.

Can you outline the next stages of your research?

Our main focus right now is on better understanding the role of molecular chaperones in neuropathies. We work very hard to link chaperones' role in regulation of the immune system and their impact on the nervous system. This is where the relationship between autoimmunity and neuropathies occurs. This also directly relates to complete heart block patients. Drugs known to affect calcium homeostasis are also being tested for impact on protein folding. Our work provides a unique blend of basic research with a high relevance to many clinical applications.

Reshaping the molecular world

Research into the endoplasmic reticulum has implications for the treatment of many human conditions. A project at the **University of Alberta** is using novel genetic and proteomic technologies to uncover the intricacies of protein folding

IT IS WIDELY accepted that congenital heart disease and neurodegenerative conditions such as Alzheimer's, as well as systemic diseases such as cancer, are currently more prevalent than at any other time in human history. Consequently, the need for a greater understanding of the cellular processes that govern the health of those afflicted and those at risk has never been greater. Fortunately, new research is indicating that biological processes involving protein molecules could hold the key to better diagnosis and treatment of some of the most devastating diseases of the 21st Century.

Working at the forefront of this research is Professor Marek Michalak of the University of Alberta in Canada. He has spent over twenty years building a passionate team committed to expanding knowledge of cellular mechanisms. In particular, his lab in the Biochemistry Department of the Faculty of Medicine & Dentistry has focused on the synthesis and denaturation of protein molecules and their highly specific, three-dimensional (3D) shapes, which allow proteins to carry out specialised roles in the body. Michalak explains that proteins are as vital as they are wide ranging: "They come in many thousands of shapes to support their functional role in the cell and organism, and they are involved in virtually every aspect of our lives including energy production, regulation of our behaviour and disease prevention".

Necessary chaperones

Given that proteins play such a crucial part in the functioning of healthy cells, it may not come as a surprise that evidence from previous genetic studies – identifying over 16,000 mutations in polypeptide chains – have suggested links between these mutations and human diseases. Moreover, the majority of these mutations affect protein folding, the process by which a protein structure assumes its functional shape or conformation. "Before proteins can carry out their function they must be folded correctly so they can recognise their partners, perform their functions and find the appropriate locations in the cell," elucidates Michalak. Thus, mutations that disrupt the protein's ability to fold correctly can render it dysfunctional. As a result, a quality control mechanism that can efficiently identify and eliminate mis-folded proteins is fundamental for normal cell function.

For this reason, much of the Michalak team's efforts have centred on the endoplasmic reticulum (ER), an intracellular membrane system on the surface of which, proteins are synthesised and accurately folded under the control of chaperones. Further to their role in protein folding, chaperones are also involved in intracellular signalling and communication, all important functions for helping to maintain homeostasis within the cell and preventing the development of disease.

Signals to the heart

For several years, the group has been working towards understanding two chaperone proteins present in the ER: calreticulin and calnexin, both of which are calcium binding proteins that are widely researched by biochemists and cell biologists across the world because of their fundamental importance for maintaining normal cell function. They have been thoroughly explored and characterised at the University of Alberta. By investigating the structure and function of these proteins using cutting-edge genomic and proteomic technologies, the researchers have made great strides in developing an understanding of how problems with the protein folding mechanism can have serious consequences for the development of diseases such as congenital heart defects and arrhythmias commonly found in children.

Of particular interest to the team is a disorder known as heart block, where electrical signals

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between the upper and lower chambers of the heart are disrupted, causing a restriction of blood flow to vital organs. By investigating how chaperone function is disrupted in this condition, Michalak's lab have uncovered an important connection between the calcium regulated chaperones and heart function: "We discovered that modulation of calcium signalling in the heart and likely, although not confirmed, protein folding in the immune system, contributes significantly to the pathology," Michalak reflects. In establishing a link between chaperone proteins in the ER of immune cells and cardiac pathology the team has been able to not only demonstrate the seriousness of mis-folded protein structures for congenital disease, but also its potential relevance in a wide range of other disorders. Having a deeper understanding of these associations, opens up the possibility of radical new treatment modalities for heart block.

The right and wrong response

Michalak's group has begun to explore the relationship between chaperons and the inflammatory disease multiple sclerosis (MS). MS affects the brain and spinal cord, such that sufferers experience crippling muscle weakness and spasms alongside bouts of severe fatigue and bladder problems. These symptoms are caused by generalised disruption to the nervous system, resulting in inflammation of tissues and subsequent removal of myelin protein from the outside of nerve cells. Myelin is a protein that surrounds the axon of nerve cells as a sheath and promotes efficient transmission of nerve impulses along the axon. In cases of MS, the immune response is inappropriately activated, causing host white blood cells – such as T-lymphocytes – to attack the myelin protein in the sheaths and disrupting nerve impulse transmission.

Using animal models, the researchers made the important discovery that cellular calnexin deficiency can lead to dysfunction of the peripheral nerves due to degradation of myelin on the neuron sheaths. Since calnexin is an ER-associated molecular chaperone, these results have led Michalak's team to conclude that changes in ER homeostasis have a direct impact on the ability of the nerve cells to transmit signals, which then leads to the debilitating symptoms of several neuropathies.

No 'magical hands'

While the team has been able to identify the significance of calnexin in the production of many of the proteins that are released from, or retained on, the surface of the cell, the effect of these proteins can be detrimental as well as beneficial. In the healthy state, the investigators found that the correctly synthesised and folded proteins assist with myelin production, ensuring that a protective sheath is maintained on peripheral neurons. However, in a pathological state, an immune response can stimulate the production of unwanted pro-inflammatory molecules. This underlines the delicate balance between the molecular chaperones and the immune response. "There are no 'magical hands' holding and folding polypeptides," notes Michalak. "The process is highly regulated, involving modification of the proteins, interaction with functional and structural partners and engagement of specific chaperones."

New therapies

These insights into the misfolding of proteins, their regulatory chaperones and the contribution of calcium signalling, have furthered understanding of fundamental cellular processes affecting many aspects of human health. These foundations are paving the way for the development of therapeutic and management strategies for many conditions for which there are currently few, if any, successful treatments. Looking to the future, there is potential scope to apply knowledge about these links between protein folding and efficient neuron function, in the development of treatments for Alzheimer's or Huntingdon's disease, for example, two other neurodegenerative conditions that could have causes related to chaperone dysfunction. As Michalak suggests, the next stage will be to utilise this knowledge to create new targeted therapies to compensate for chaperone dysfunction: "The key is to develop molecular chaperones that can assist in protein folding and prevent protein aggregation. We must find ways of controlling chaperones and their substrates with small molecules, nucleic acid tools, carbohydrate and metabolic manipulations," he asserts.

Thanks to this pioneering research, the Biochemistry Department at the University of Alberta is a global leader in this field. Moreover, Michalak's recent University Cup award, the highest academic honour bestowed at the university, is testament to his remarkable success at studying cells at the molecular level, as well as attracting funding and students from all over the world.

INTELLIGENCE

MICHALAK LAB

OBJECTIVES

To understand two fundamental biological processes that affect virtually every aspect of cellular physiology: calcium signalling and protein folding. The research has implications for a large number of diseases including vascular disease such as heart attack, neurodegenerative diseases such as Alzheimer's and systemic diseases such as cancer. The objectives are to establish a role for membranes associated molecular chaperones in congenital heart diseases and specific neuropathies, providing insights into mechanisms and interventional strategies for these and other protein folding disorders.

KEY COLLABORATORS

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DR MAREK MICHALAK is a Distinguished University Professor at the Faculty of Medicine & Dentistry, University of Alberta, a Fellow of the Royal Society of Canada and 2012 University Cup winner. He completed his MSc degree in Cell Biology at the University of Warsaw, Poland, and a PhD degree in Biochemistry at the Nencki Institute for Experimental Biology, also in Warsaw, Poland. He received his postdoctoral training at the University of Toronto, Canada, and at the Swiss Federal Institute of Technology Zurich. Michalak joined the Faculty of Medicine & Dentistry in 1987. He served as Chair of the Department of Biochemistry (2005-2009) and most recently as a Vice-Dean (Research), Faculty of Medicine & Dentistry (2009-2013).

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