Malleable metabolism

Professor Vasu Appanna’s wide-ranging metabolic investigations reveal the mechanisms of abiotic toxins in disease and the importance of oxidative metabolism in stem cell differentiation

Could you provide an insight into your focus on metabolism?

I am a biochemist by training, and I utilise both microbial and mammalian models as my operational theatre. Communication amongst enzymes/proteins in metabolic networks or conjugating together to effect a specific task is an area of great interest to me. Metabolism gives a precise status of the being of an organism at a given time; how it is really doing – as opposed to its genetic makeup which relates only to unprocessed information (possibilities). It is more like a makeup which relates only to unprocessed output – what you see; hence, my fascination with this process.

Why is the tricarboxylic acid (TCA) cycle so important to human wellbeing?

The TCA cycle is the engine that drives energy production in all oxygen-dependent organisms. It provides the essential ingredients to fuel the synthesis of adenosine triphosphate, the universal energy currency. Its regulation and machinations with other networks are essential to our being, since energy dictates our development, ageing, diseases – basically, our existence.

What does your finding that dimethyl sulphoxide (DMSO)-treated cells have an increased level of TCA cycle metabolites reveal regarding stem cell differentiation?

The increased level of TCA cycle metabolites in response to DMSO – an agent that causes the differentiation of stem cells into endodermal and mesodermal tissues – is indicative of increased mitochondrial metabolism. Our functional studies have elucidated increased mitochondrial biogenesis and oxidative phosphorylation, and the key role they play in promoting differentiation. The discovery of natural metabolites involved in stem cell differentiation may play a vital role in bioengineering tissues and organs, in situ with minimal negative impact, to overcome a number of disorders.

Could you highlight your key findings regarding the hepatic response to aluminium (Al) toxicity?

The toxicity of Al stems from its similarity to other ions in the cell, such as iron (Fe) and magnesium. While in trace amounts such ions are critical to enzymatic function, Al has no known biological role. By substituting for Fe in a variety of TCA cycle enzymes and complexes of the electron transport chain, Al has the noxious ability to arrest central metabolism, leading to dysfunctional energy homeostasis.

Mysteries of the metabolic powerhouse

The complex interactions that produce energy for intracellular processes, how they change in response to stress and the role they play in steering stem cells towards their fates are under scrutiny at Laurentian University in Ontario, Canada

METABOLISM FUELS THE biological changes that govern the growth and wellbeing of all organisms, from microbes to the largest mammals. Central to healthy metabolism is the tricarboxylic acid (TCA) cycle which produces adenosine triphosphate (ATP), the molecular unit of currency for cellular energy.

Using proteomics and metabolomics techniques, Professor Vasu Appanna of the Faculty of Science and Engineering at Laurentian University in Ontario, Canada, is dedicated to unravelling the processes of the TCA cycle as key to understanding the bases of health and disease. Over the last few years, in response to the ever-increasing bioavailability of aluminium (Al) in soil and waterbodies from industrial expansion and acid rain, and concerns over its effects on environmental and human health, Appanna has explored how Al and resulting oxidative stress affect the proper functioning of the TCA cycle and energy metabolism.

METABOLIC RESPONSES TO A MULTIFACETED STRESSOR

Applying blue native polyacrylamide gel electrophoresis (BN-PAGE) allows Appanna to examine both the enzymatic activity of undoctored proteins in the conformations in which they occur naturally in living organisms and also see their convoluted interactions unfold, in situ and non-invasively. The technique crucially ensures that all biomolecules involved are included. “BN-PAGE permits the separation of protein mixtures, while allowing them to retain their native state, and has rendered it possible to observe the dysfunctional state of the TCA cycle under environmental challenge as it develops in vivo,” Appanna reveals.

Being able to see the effects of Al toxicity holistically, in the real setting of the network of metabolic pathways, has led Appanna to several key findings. One study of Al effects on liver cells found that Al disrupts the mitochondrial metabolism and leads to dysfunctional beta-oxidation, so promoting the accumulation of lipids and thus liver disease and obesity – turning the liver into a ‘fat machine,’ as Appanna describes it.

In another investigation examining the effects on astrocytes, Appanna found that Al exposure resulted in drastic changes to the TCA cycle, reducing aerobic energy production in the form of ATP and inducing dysfunctional mitochondrial metabolism. Effectively, this starves the brain of both oxygen and energy, encouraging the production of lipids and reducing oxidation of fatty acids – astrocytes exposed to Al were replete with fat deposits.

While previously Al toxicity had been implicated in such conditions as Parkinson’s, Alzheimer’s and liver disease, Appanna’s studies were the first to find the precise mechanism of its action. He and his team are hopeful that this new proteomic-level understanding of metal toxicity will help in the design of effective chelators to suppress Al activity and in the development of treatments to rescue protein function and diminish oxidative stress.
Our research group has applied a functional proteomics approach to identify the biomolecular targets of Al toxicity. We’ve discovered that Al disrupts mitochondrial function, and thus energy production. Alpha-ketoglutarate (KG), an intermediate of the TCA cycle, is pooled to detoxify reactive oxygen species (ROS); it is also an antioxidant and has therapeutic potential. Succinate produced as consequence of this interaction can signal anaerobiosis. Channeling KG to combat ROS reduces its availability for carnitine biosynthesis, a vehicle that shuttles fatty acids for their breakdown in the mitochondria. Thus, Al toxicity and ROS convert the liver into a fat machine and decrease energy production. These findings have important implications for fatty liver disease and obesity.

**What have you found regarding the role of Al toxicity in neurological disease?**

While the target may change, the biochemical effects of Al remain the same. Dyslipidaemias, altered iron homeostasis and a dysfunctional energy metabolism characterise Al toxicity in astrocytes as well as hepatocytes. Owing to their long processes, astrocytes play a key role in the physical structuring of the brain. Al interferes with creatine kinase and profilin-2, key modulators of their star-like morphology – so astrocytes exposed to this metal adopt a globular shape. This impairs neuronal stability, which may provoke neurological disorders in addition to Al-induced lipogenesis and diminished energy production. The latter has a huge negative impact on brain function.

**What could we do to reduce Al toxicity/bioavailability?**

We have identified a microbe – *Pseudomonas fluorescens* – which can tolerate and precipitate high concentrations of Al out of solution. This form of bioremediation is cost-effective and environmentally friendly compared with currently available physicochemical processes.

**Your work on how environmental stress affects mammalian cells has identified key networks implicated in obesity and neurological disorders. What are your hopes for applying your research to the clinic?**

One of the key findings of our work is the role of keto acids, such as alpha-KG and pyruvate, in alleviating oxidative stress in biological systems. Supplementation with these compounds may alleviate the symptoms of such disorders, as has been shown with burn victims and patients with ocular disorders. Indeed, oxidative stress is a primary factor underlying the progress of numerous neurological diseases.

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**HOMOCYSTEINE DETECTION KIT**

The amino acid homocysteine is a blood plasma marker of risk for a variety of diseases, such as atherosclerosis and Alzheimer’s. Appanna has developed a kit which simplifies the way in which levels of homocysteine are detected, and is now being validated in partnership with the Advanced Medical Research Institute of Canada against patient plasma samples.

**BIOMINING**

Developed in collaboration with companies in India and the US, Appanna’s biomining products condition microbes to remove metals from industrial and domestic effluent. The products have achieved excellent results in recovering metals from gold tailings, Al and other metal wastes. Low cost and straightforward, they are now being further tailored to biomine metals from low-grade ores and to produce biofuel from landfill sites. Appanna is currently looking for further industrial opportunities to expand their applications.

**BIOTECHNOLOGY VENTURES**

In addition to cooperating with laboratories of various scientific disciplines in Chile, France, the US and India, Appanna is collaborating with industry in two development projects: one concerning a biomining process and the other a kit for detecting homocysteine.

More recently, Appanna has explored the intricacies of metabolic network function in mouse stem cell differentiation. He posits that mitochondrial energy metabolism is a pivotal factor in programming action and thus guiding stem cells into their different ultimate forms: “if one is to understand living systems, one has to decipher the intricate details of the metabolic network,” he asserts. “Its modulation and interaction with other cellular processes hold the key to unravelling our being.”

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**INTELLIGENCE**

**THE VASU APPANNA LABORATORY: METABOLISM IN CELLULAR SYSTEMS**

**OBJECTIVES**

To uncover how environmental stress affects microbial and mammalian cells. The former has led to discoveries on biomining and bioremediation while the latter has helped identify some biochemical networks leading to obesity and neurological disorders.

**KEY COLLABORATORS**

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**KEY PARTNERS**

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**PROFESSOR VASU APPANNA** obtained his BSc at the University of Bombay, India, his MSc from the University of Wales, UK, and his PhD from the University of Waterloo, Canada. He has served as Department Chair and Dean of the Faculty of Science and Engineering for numerous years. Appanna has published over 100 peer-reviewed articles and trained more than 125 highly qualified personnel. In addition, he is the editor of different journals and Academic Editor of *PLoS ONE*, and sits on various organisations and boards, including the Applied Medical Research of Canada.

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**Mitochondria**: the energy powerhouse.