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Plenary Speakers

Label-free proteomics a tool for understanding the role of post-translational protein modifications in cell physiology: Insights into the regulation of sperm function

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As a result of the 'omics revolution we are now gaining an understanding of the molecular composition of biological systems at a rate that would have been unbelievable, even a decade ago. There are many reasons for this sudden change, not least improvements in the sensitivity and accuracy of mass spectrometers, the new generation of which are not only more compact and sophisticated than ever before but also have much improved user interfaces that open up their application to a broader scientific church. Spermatozoa are not only important cells from both biological and clinical viewpoints but also they are prefect models for demonstrating the analytical power of such instruments. These cells can be obtained in large numbers in an absolutely pure state and possess a physiology that is entirely driven by post-translational modifications to their protein complement. One of the strategies we have employed to resolve the molecular mechanism controlling sperm function involves comparing the peptide profiles of spermatozoa in different physiological states using label-free methods. Typically tryptic peptides are enriched and then separated by reversed phase liquid chromatography. Eluted peptides are then viewed through either extracted ion-chromatograms or via the generation of *in silico* (survey) maps that plot the mass: charge ratio of each peptide against its elution profile to give an overall picture of the peptide composition of a given sample. Such survey scans can be accurately compared and peptides identified that change position (in terms of mass and charge) in response to a particular stimulus or pathology. Such methods have served to demonstrate the signal transduction pathways associated with epididymal maturation, motility activation capacitation, reactive oxygen species generation and sperm egg recognition. The analytical power of a new generation of mass spectrometers will revolutionize our understanding molecular mechanisms regulating cell function.

Key words: Mass spectrometers, molecular mechanisms, proteomics.

Melatonin: A pleiotropic hormone?

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Melatonin (N-acetyl-5-methoxytryptamine) is synthesized mainly by the pineal gland and regulates mammalian circadian rhythms. It is a highly conserved molecule found in organisms from unicells to vertebrates. Melatonin is a potent free radical scavenger and an anti-oxidant and highly effective against oxidative stress. Melatonin is involved in many physiological systems including the cardiovascular system. Melatonin effectively protects the heart against ischaemia-reperfusion damage, having both acute and long-term effects. Although these effects were initially attributed to its free radical scavenging abilities and anti-oxidant activity, recent evidence suggested a role for the melatonin receptor, since its beneficial effects are abolished by luzindole, a melatonin receptor blocker. In addition, melatonin has potent anti-adrenergic actions, involving nitric oxide, guanylyl cyclase and PKC, which have been shown to participate in melatonin-induced cardioprotection. Simultaneous administration of melatonin and inhibitors of nitric oxide synthase or guanylyl cyclase abolished cardioprotection. Recent studies showed that melatonin also protected mitochondrial integrity and functional capacity by virtue of its free radical scavenging actions and it was suggested that it may directly interact with the mitochondrial permeability transition pore, keeping it in a closed confirmation. There is continued interest in discovering new cardioprotective agents of high potency and low toxicity. Melatonin fulfills most of these criteria but all studies thus far have been done on animals and only one clinical trial is currently in progress where melatonin is used as an adjunct in patients with acute myocardial infarction undergoing angioplasty. Clearly the outcome of such a study may play a pivotal role in the decision to use melatonin in patients with ischaemic heart disease or to use the drug prophylactically.

Key words: Melatonin, pleiotropic, hormone, cellular functions.

Why medical physiologists need to know (some) comparative physiology

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Medical physiologists may be tempted to assume that what is normal physiology for humans is normal physiology for other mammals, and the converse. The limits of human function are not the limits of physiological capacity; knowledge of function in other animals reveals the potential scope of human function. Knowledge of function in other animals also allows identification of appropriate (and inappropriate) animal models for exploring human function in circumstances in which human experimentation would be unethical. Knowledge of the physiological differences between humans and other animals helps prevent the potentially-catastrophic extrapolation of observed responses in other animals to humans, and avoidance in future of events like the TGN1412 disaster at Northwick Park, UK.

Key words: Comparative physiology, animal models.

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Ninety percent of cancers are caused by environmental factors of which most are chemicals

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Cancers are due to permanent or semi-permanent disruption of gene control. The repertoire of genes involved in cancer have been defined to a large extent and involve processes such as mitosis, apoptosis, angiogenesis, aerobic glycolysis, DNA-repair and metastasis. Permanent disruption is caused by covalent, nucleotide sequence changes while semi-permanent, epigenetic changes, do not involve sequence alteration in the DNA but rather methylation of DNA and structural changes in histones. These changes can be reversed but nevertheless play a carcinogenetic role. More epigenetic changes have been found in cancer cells than classical mutations suggesting that there could be far more carcinogens than suspected. In order to formulate preventive strategies, we ask what percentage of human cancers is caused by environmental chemicals. We propose as much as 60% which is higher than international consensus. There is general consensus that smoking causes 30% of cancers and that cigarette smoke contains at least 30 different carcinogens of which the worst is benz{a}pyrene. Ethanol has also been shown to cause 10% of cancers. We believe that the remaining 20% of the 60% is due to carcinogenic and epicarcinogenic effect of ensembles of man-made molecules, especially hormone disrupters like BPA (found in polycarbonate baby bottles). We also suspect that these molecules which are "in the face" of the public could also be involved in breast and prostate cancers, which make up 20% of all cancers world-wide. Results from the US show up to 200 man-made chemicals in the average American's blood. Nobody knows the harm these chemicals could be doing, especially during embryogenesis. We believe cancer prevention also involves science-based legislation for better control of cancer causing molecules in the immediate environment of millions of people. South Africa is taking a lead with legislation against trans fats and BPA in baby bottles. The role of CANSA in furthering research into environmental chemicals that may cause cancer, such as uranium in drinking water, acrylamide in crisps, plasticisers, trans fats and the ratio of omega-6/omega-3 fatty acids, will be discussed.

Key words: Cancer, environmental factors, CANSA.

The role of mitochondrial DNA in pluripotency and during differentiation

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During oogenesis and preimplantation development, mitochondrial DNA (mtDNA) replication is strictly regulated so that, by the blastocyst stage, the inner cell mass cells, which have the potential to give rise to all cells of the body, possess fewer copies of mtDNA than the trophectodermal cells. The inner cell mass cells also give rise to embryonic stem cells and, in their undifferentiated state, they possess significantly fewer copies and maintain immature mitochondria. This continual reduction results in the establishment of the ‘mtDNA-set point’ from which all pluripotent embryonic stem cells

can acquire the specific numbers of mtDNA to match the requirement for OXPHOS-derived ATP of specific cell types once they have fully differentiated. As embryonic stem cells differentiate, there are several distinct mtDNA replication events, which are driven by nuclear-encoded mtDNA-specific replication factors. However, one replication event appears to be critical to the continued survival of the cell whilst another is associated with the accumulation of mtDNA mass later during differentiation. Nevertheless, reprogrammed somatic cells generated through somatic cell nuclear transfer, cell fusion techniques and induced pluripotency do not appear to regulate mtDNA replication in a similar manner. They have significantly higher numbers of mtDNA copies in their pluripotent state or are primed to trigger premature mtDNA replication. During differentiation, they fail to achieve the two key mtDNA replication events observed in embryonic stem cells and have aberrant patterns of expression for the nuclear-encoded replication factors. Nevertheless, by culturing induced pluripotent stem cells with inhibitors of *de novo* DNA methylation, which act on the nuclear-encoded mtDNA replication factors, we have been able to mimic the mtDNA replication events of embryonic stem cells. These outcomes demonstrate that the regulation of mtDNA replication during differentiation and development is dependent on the epigenetic control of the nuclear-encoded mtDNA replication factors.

Key words: Mitochondrial DNA, pluripotency.

Endocrine disrupting chemicals: Are South Africans at risk?

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Pollutants in our water, food and air have a direct or indirect effect on consumers of the polluted resource. Most environmental pollutants are due to human activities such as residential waste, industrial waste, pharmaceuticals, mining effluents, agricultural effluents, and breakdown products of man made products such as plastics and paint. However, some environmental pollutants are due to products of natural origin such as the microbial toxins and phytotoxins. Over the past few decades it has become known that some of the pollutants in the environment act on animals by mimicking or inhibiting the action of normal hormones. These pollutants are called the endocrine disrupting chemicals (EDCs). EDCs can affect processes such as embryonic development, maturation, metabolism and fertility of a population and can result in the decline of population numbers. My study will focus on the occurrence and effects on EDCs in South African water bodies. For monitoring specific known EDCs, such as the steroids, ELISAs that can specifically measure the low levels of these steroidal compounds in water were developed. *In vitro* methods were also developed to monitor specific cellular processes and enzymatic pathways, the immune pathways, and female and male reproductive processes. Water collected from all over South Africa was assayed using these *in vitro* methods. Some South African water bodies contain high levels of EDCs. The EDCs levels found at some sites were high enough to induce male lower vertebrates to start producing female specific proteins. The EDC levels also have effects on the immune system and steroidogenesis *in vitro*. Drinking water treatment processes in the major city centers removed the EDCs. EDCs occur in South African water bodies and can impact consumers if treatment processes at drinking water plants are insufficient for their removal.

Key words: Endocrine disrupting chemicals, water safety, pollutants.