



Society for Experimental Biology Annual Main Meeting 28th June – 1st July 2009, Glasgow, UK

C1 – PROTEIN FOLDING AND GENERAL MOLECULAR CELL BIOLOGY

C1.1

09:00 Wednesday 1st July 2009

The routes so far trod and where we may go next to inhibit protein folding by the molecular chaperone heat shock protein 90

Marissa Powers (The Institute of Cancer Research)

HSP90 has a versatile role in maintaining cellular homeostasis, interacting with a diverse set of client proteins to ensure their conformational stability and activity or when necessary facilitating their degradation via the proteasome. HSP90 has emerged as an exciting target in cancer drug discovery because amongst its many clients are a number of oncogenic proteins involved in signal transduction and gene expression. Therefore, targeting HSP90 offers the possibility to simultaneously inhibit multiple cancer-driving pathways and antagonise all hallmarks of the cancer phenotype. In addition, cancer cells are stressed by a range of endogenous and exogenous factors, and are thus more dependent on HSP90 than are normal cells. The function of HSP90 is dependent on ATP-binding and hydrolysis by its intrinsic N-terminal ATPase domain. These processes require a chaperone cycle and are reliant on the orchestrated interaction of several co-chaperone proteins. Inhibition of HSP90 ATPase activity leads to client degradation, cell cycle arrest and apoptosis. The HSP90 inhibitor 17-AAG (tanespimycin) has shown promising clinical activity and provided proof-of-concept for targeting HSP90. Our laboratory has been involved for several years in work designed to understand the structure or function of HSP90 in cancer cells and in the discovery and development of HSP90 inhibitors for cancer treatment. Here we will give an overview of HSP90 and its accessory proteins, outline our perspective on the development of novel inhibitors of HSP90 ATPase activity and describe future approaches which can be taken to modulate the activity of this chaperone in novel ways for cancer therapy.

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C1.2

10:30 Wednesday 1st July 2009

Protein folding location determines binding of manganese versus copper

Steve Tottey (Newcastle University), Nigel Robinson (Newcastle University), Kevin Waldron (Newcastle University), Susan Firbank (Newcastle University), Conrad Bessant (Cranfield University)

Proteins can, of course, selectively bind different metals based upon the nature, number and geometric arrangement of the binding residues, the size and charge of the metal-binding pocket amongst other factors. However, because proteins are flexible this selection is imperfect and this is especially true of nascent unfolded proteins. Some metals form more stable protein complexes than others. As described in the Irving-Williams series, divalent copper and zinc typically form more stable complexes than manganese. Metallochaperones insert the correct metals into some proteins but metallochaperones have not been found for the vast majority. Thus, the cellular mechanisms which manage acquisition of the correct metal by the vast majority of nascent proteins remain ill-defined. Cyanobacteria have exceptional requirements for copper and manganese to support photosynthesis and we have explored the selective binding of these metals to two proteins. Here we uncover a cellular mechanism which overrides the inherent binding preference of a protein to control its metal content. The results explain why the cytosol must contain only tightly bound copper and zinc.

Waldron, K.J. & Robinson, N.J. How do bacterial cells ensure that metalloproteins get the correct metal? *Nature Reviews Microbiology*, 2009, 7, 25–35.

Tottey, S., Waldron, K.J., Firbank, S.J., Reale, B., Bessant, C., Sato, K., Cheek, T.R., Gray, J., Banfield, M.J., Dennison, C., & Robinson, N.J. Protein-folding location can regulate manganese-binding versus copper- or zinc-binding. *Nature* 2008, 455, 1138–1142.

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C1.3**11:00 Wednesday 1st July 2009****Communication mechanisms between nascent polypeptides and the ribosome**

Cheryl Woolhead (University of Glasgow)

The targeting of membrane proteins is a challenging cellular process in which highly hydrophobic transmembrane domains must remain in an insertion competent conformation while they are transferred from the ribosome to the lipid bilayer through the aqueous milieu of the cell. This process is universally controlled by the signal recognition particle (SRP), which docks at the ribosome exit tunnel sequestering signal sequences as they emerge and targeting them to the membrane insertion machinery. In mammalian cells this process is stringently controlled and both membrane and secretory proteins are targeted co-translationally by SRP to the Sec61 complex for insertion or translocation respectively. However in bacteria SRP recognizes only membrane proteins, some of which are thought to be targeted co-translationally and some post-translationally. In addition bacteria have developed numerous ways of inserting membrane proteins utilizing both the SecY and YidC proteins in the membrane. Therefore despite considerable conservation in the structures of SRP and the ribosome between eukaryotes and prokaryotes it appears that the early stages of targeting dictate quite different mechanisms for membrane protein integration. This presentation will discuss the communication mechanisms which operate between the ribosome and the nascent peptide, during early stages of translation, which affect the way in which proteins fold and are subsequently targeted.

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C1.4**11:30 Wednesday 1st July 2009****Development of a tractable model for membrane protein folding**

David Brockwell (University of Leeds)

By contrast with the wealth of knowledge obtained about the folding of water soluble proteins, little is known about the molecular mechanism by which membrane proteins fold. This chasm in knowledge has arisen from (i) the difficulty in expressing membrane proteins in sufficient quantity to allow systematic biophysical analysis and (ii) the rarity of model proteins that undergo reversible refolding/unfolding in vitro so that the methods developed to study soluble proteins can be applied to their membrane-bound counterparts. To address this, we have exploited the experimental tractability of the 161 residue outer membrane protein PagP from *E. coli* to perform a detailed biophysical analysis of the folding mechanism of a β -barrel membrane protein. Experiments that measure the stability, activity and folding kinetics of PagP in the presence of unilamellar vesicles will be described and the effect of mutations on these processes will be discussed.

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C1.5**12:00 Wednesday 1st July 2009****The phytohormone salicylic acid influences autolysis in ruminant-ingested plant cells**

Alison H. Kingston-Smith (IBERS Aberystwyth University), Teri E. Davies (IBERS Aberystwyth University), Edward Kyme (IBERS

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Fresh forage ingested by grazing cattle is subject to a combination of stresses including heat, anoxia and attack by micro-organisms culminating in autolysis: self-mediated breakdown of protein and DNA. Understanding the stress responses of ingested forage will allow us to breed improved forages which will release less pollutant nitrogen when used as cattle feed. The plant signalling compound salicylic acid (SA) is implicated in regulating responses to biotic and abiotic stresses such as those experienced by plant cells post-ingestion. To test the hypothesis that SA is involved in control of autolysis, *Arabidopsis* wild type (Col-0) has been compared with *sid2* which contains a mutation in the SA biosynthetic gene isochorsimate synthase (*ICS1*). Incubation in an aqueous environment at 39 °C in the absence of oxygen for 6 h resulted in a decrease in leaf protein of approximately 50% in Col-0 but not in *sid2*. This difference was not associated with differential activities of the most abundant serine or cysteine proteases. It is possible that changes in lower abundance proteases were undetected. The SA signal transduction pathway includes induction of the oxidative burst, programmed cell death (PCD), and expression of defence-related proteins. DNA-based characterisation techniques are being used to reveal if decreased proteolysis in *sid2* mutants is due to incomplete execution of an SA mediated PCD pathway when plant cells are exposed to ingestive stress.

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C1.6**13:30 Wednesday 1st July 2009****Substrate specificity of the PDI family of ER oxidoreductases**

Neil Bulleid (University of Manchester)

The lumen of the endoplasmic reticulum is the site for folding and assembly of proteins entering the secretory pathway. Most proteins destined for secretion contain one or more disulphide bonds. The formation of these disulphides is catalysed by members of the protein disulphide isomerase family of which there are at least 17 members. What role individual family members play in this important post-translational modification is unclear. We have been trying to determine whether there is a distinct substrate specificity for each of these enzymes. Our most recent results indicate that there are some proteins which require a specific PDI family member. In addition, substrates that associate with the ER localised chaperone BiP also show selectivity towards PDI family members. These results indicate that the multitude of PDI-like proteins has evolved to assist the folding of distinct sets of proteins entering the secretory pathway.

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C1.7**14:30 Wednesday 1st July 2009****Characterisation of novel platelet surface thiol isomerase enzymes**

L. Holbrook (Institute of Cardiovascular and Metabolic Research, University of Reading), K. Jones (Institute of Cardiovascular and Metabolic Research, University of Reading), N.A. Watkins (Department of Haematology, University of Cambridge), W.H. Ouwehand (Department of

Haematology, University of Cambridge), Jon M. Gibbins (Institute of Cardiovascular and Metabolic Research, University of Reading)

The thiol isomerase family of proteins, which are important components of the mammalian protein secretory pathway, consists of 17 structurally different members. Increasing evidence indicates key regulatory roles for these proteins on the surface of several cell types. Within platelets, two cell-surface proteins have been characterised: protein disulphide isomerase (PDI) and endoplasmic reticulum protein 5 (ERp5). PDI and ERp5 are present on the surface of resting platelets and their levels increase following platelet activation. In addition, specific inhibition of these enzymes diminishes platelet aggregation, secretion, adhesion and integrin activation in response to physiological agonists.

In this study, we used whole-genome microarray based expression profiling of megakaryocyte mRNA to identify additional thiol isomerases that are expressed in platelets. Specific antibodies were then used to confirm the presence of selected thiol isomerases.

We report the identification of 5 previously uncharacterised platelet thiol isomerases: ERp72, ERp57, ERp44, ERp29 and TMX3. Of these 5, ERp72, ERp57, ERp44 and ERp29 are secreted by human platelets and re-associate with the cell surface suggesting that they may play a role in the modulation of activation associated cell-surface changes. The transmembrane enzyme, TMX3 is recruited to the cell surface but is not secreted. Inhibition of these cell surface enzymes not only reduced platelet thrombus formation in a dose dependent manner under physiological flow conditions but also substantially reduced clot formation and retraction in human plasma. These data implicate novel platelet thiol isomerases in the regulation of normal platelet function.

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C1.8

15:30 Wednesday 1st July 2009

Inflammatory arthritis and HLA-B27: Is there a role for protein misfolding?

Simon Powis (University of St Andrews)

HLA-B27 is a major histocompatibility complex (MHC) class I molecule, which functions by presenting short antigenic peptides to T cells of the immune system, allowing the detection and elimination of virally infected cells. Uniquely, HLA-B27 is also strongly associated with a group of inflammatory arthritic conditions, known as spondyloarthropathies, the most well studied of which is ankylosing spondylitis (AS). In AS, around 90% of affected individuals express the HLA-B27 molecule. However, the precise role that HLA-B27 plays in AS remains unknown.

Recently, data has been obtained by various groups indicating that protein misfolding may play a significant role in the pathogenesis of AS. Thus HLA-B27 misfolding in the endoplasmic reticulum may contribute to the formation of heavy chain dimers, which may trigger an ER stress response, resulting in the release of proinflammatory cytokines. A potentially distinct population of heavy chain dimers, which forms at the cell surface, may also be recognized aberrantly by immune receptors. An understanding of the folding pathway of MHC class I molecules and the various chaperones and accessory molecules that co-operate to load antigenic peptides is therefore of key importance in understanding the biology of HLA-B27.

In this presentation the data supporting HLA-B27 misfolding will be reviewed, alongside recent advances in how MHC class I molecules fold. Our own studies of HLA-B27 dimer formation, performed in

collaboration with Dr. Antony Antoniou (UCL, London) will also be presented. Finally, new data on the presence of novel HLA-B27 dimers on the small secretory vesicles known as exosomes will be shown.

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C1.9

16:00 Wednesday 1st July 2009

HLA-B27 misfolding and dimerisation

Helen Fussell (National Health Service Blood and Transplant, Colindale Blood Centre, London), Izabela Lenart (University College London), Darren Nesbeth (University College London), David Guiliano (University College London), Keith Gould (University College London), Simon Powis (University of St. Andrews), Antony N. Antoniou (University College London)

HLA-B27 exhibits a strong association with a group of inflammatory arthritic diseases known as the Spondyloarthropathies (SpAs). Despite the association being known for over 30 years, the role of HLA-B27 in disease pathogenesis remains undetermined. HLA-B27 is an MHC class I molecule which assembles within the endoplasmic reticulum (ER) via transient interactions with ER resident chaperones and functions to present peptides derived from endogenous antigens to cytotoxic T cells. Recently, the ability of HLA-B27 to misfold has been hypothesised to participate in the disease process, especially the ability to form heavy chain (HC) dimers. The aims of this study were to determine; (1) why HLA-B27 exhibits this enhanced tendency to misfold and (2) the dimerisation process.

To address the misfolding ability of HLA-B27, we employed rapid acidification/alkylation to determine cysteine residue accessibility. Our analysis revealed that HLA-B27 HC exists as distinct populations, possessing accessible cysteine residues. The accessibility of the cysteine residues can be influenced by the structurally important residues at position(p) 114–116. Removal of the non-structural cysteine residues at p67, 308 and 325 revealed that the HLA-B27 HC was still prone to misfolding. To determine the mechanism of the dimerisation process we sought to determine the role of ER resident chaperones. Our analysis revealed that calnexin has an important modulating role in dimerisation.

We propose that HLA-B27 misfolding is a result of enhanced exposure and accessibility of cysteine residues to the oxidising environment of the ER and that HC-dimers can be modulated by ER resident chaperones.

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C1.10

16:15 Wednesday 1st July 2009

Folding and assembly of MHC class II molecules

Marcel Van Lath (Durham University), Adam M. Benham (Durham University)

The Major Histocompatibility Complex (MHC) is central to the adaptive immune response and is associated with a number of autoimmune diseases such as rheumatoid arthritis, multiple sclerosis, SLE, myasthenia gravis and type-1 diabetes. Human MHC class II molecules (HLA-DP, HLA-DQ and HLA-DR) are expressed on professional antigen presenting cells (APCs), where they mainly present

extracellular peptides to CD4+ T cells. Although the HLA-DR requirement for the accessory molecules invariant chain (Ii) and HLA-DM is well established, less is known about how allelic variability affects HLA-DR assembly in the endoplasmic reticulum (ER), or whether HLA-DP and HLA-DQ assemble in the same way as HLA-DR. Here, we ask if different DRB chains influence the stability of the DRab complex against the same intracellular background. We also compare the assembly of HLA-DR with HLA-DP and HLA-DQ. Our findings raise the possibility that, under certain circumstances, HLA-DP may present non-classical peptides to the immune system.

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C1.11

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DNA content, immunophenotype, proliferation, plasticity and clonogenic potential of human adipose-derived stem cells after short-, medium- or long-term culture

Nance B. Nardi (Universidade Luterana do Brasil), Roberto C. Chem (Santa Casa de Porto Alegre), Luis A. Costa (Santa Casa de Porto Alegre), Carolina Franke (Santa Casa de Porto Alegre), Melissa Camassola (Universidade Luterana do Brasil)

The adipose tissue contains a stromal population composed of microvascular endothelial cells, smooth muscle cells and stem cells, which can be enzymatically isolated. The adherent, mesenchymal-like cells that establish under appropriate culture conditions, termed adipose tissue-derived stem cells (ADSCs), have extensive proliferative potential and may undergo multilineage differentiation. They are currently considered as one of the most promising type of adult stem cell for therapeutic applications. In this study, we characterized human ADSCs submitted to different periods of culture: short-term (1–9 passages), medium-term (10–19 passages) and long-term (>20 passages) ($n=5$ cultures each). Doubling population indices were significantly higher for long-term and particularly for medium-term cultures when compared to short-term cultures. The clonogenic potential of the cultures, analyzed by limiting dilution, was lower in older cultures. Cell plasticity was analyzed by inducing adipogenic, chondrogenic and osteogenic differentiation of cultures. The differentiation potential was similar in short- and medium-term cultures, but showed a decrease in cultures older than 22 passages. The immunophenotype, typical of ADSCs, was the same in the three types of culture. The DNA content was analyzed by flow cytometry, and was also unaltered ($2n$) in all cultures. These results show that human ADSCs can be maintained in culture for prolonged periods of time, but that long-term cultures lose some of the potential for therapeutic applications, as shown by decreased plasticity and clonogenic potential.

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C1.12

Poster Session – Tuesday 30th June 2009

UV-absorbing compounds extracted from the Persian sturgeon caviar and *Artemia urmiana* cysts and their UV protective effects on human skin fibroblasts

Saber Khodabandeh (Tarbiat Modares University Marine Biology Group), Mehrdad Noruzinia (Tarbiat Modares University Hematology Group)

Ultraviolet radiation (UVA=320–400 nm, UVB=280–320 nm and UVC=200–280 nm) has significant effects on aquatic animal eggs and larval survival, DNA damage, and oxidative stress level. UV radiation can also accelerate human skin aging and increase the incidence of skin cancer. Mycosporine-like amino acids (MAAs) are ultraviolet-absorbing ($\lambda_{max}=309–360$ nm) molecules that are synthesized by cyanobacteria, algae, fungi and bacteria. These compounds also have been extracted from several marine plants and animals. In this study, the UV-absorbing compounds extraction from Persian sturgeon caviar and *Artemia urmiana* cysts and their protective effects on human skin fibroblast against UV light were investigated. We identified three UV-absorbing compounds (mycosporine-glycine, shinorine and palythine) as MAAs in the cysts and caviar. Mycosporine-glycine and palythine were the most common MAAs among in *Artemia* cysts and caviar, respectively. We observed the protective effects of these compounds on human fibroblast from UV-induced death. The mycosporine-glycine was more effective than shinorine and palythine. Our results suggest that accumulation of MAAs in *Artemia* cysts can protect them during long dormancy period and can also protect the sturgeon embryos from UV radiation during developments. We also observed that these MAAs can possess the UV protective effects on human fibroblast in laboratory condition and they can use in cosmetics products as sunscreen.

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C1.13

Poster Session – Tuesday 30th June 2009

Interplays of metalloproteinase and tissue inhibitors of metalloproteinase in the rapid atrial pacing atria

Chih-Sheng Lin (Department of Biological Science and Technology, National Chiao Tung University, Taiwan)

Extracellular matrix remodeling in cardiac atrium has been characterized in atrial fibrillation (AF) and considered to be a major contributor to AF persistence. In the AF disease, our aim is to test whether altered expression of extracellular matrix metabolized enzymes or their interplays is associated with the disease. In the porcine atria with AF induced by rapid atrial pacing for 3–4 weeks, a significantly greater percentage of ECM, increased matrix proteins (including collagen, fibronectin-1 and fibrillin-1), and the changes of the activity of matrix metalloproteinase (MMPs) and tissue inhibitors of MMPs (TIMPs) were identified. The significant increase of MMP-9, but not MMP-2, in its latent form and mRNA level was demonstrated to be responsible for the significantly increased gelatinase activity in the atria with AF. Furthermore, the inhibitory activity of glycosylated TIMP-1 and -3, but not TIMP-2, in the AF tissues was markedly elevated. Of remarkable interest, TIMP-1 was found to be mostly colocalized with gelatinase activity over the AF tissues, implying the coexistence of gelatinase activity and TIMP-1; however, TIMP-3 appeared only partial colocalization, revealing that TIMP-1 and TIMP-3 may play a differential role in inhibiting the gelatinase *in vivo*. Together with the results found in the fibrillating atria, we concluded that the MMPs/TIMPs interplay may contribute to the atrial ECM remodeling of AF. Upon further study, we have demonstrated that MMPs/TIMPs balancing regulation in heart is highly related to angiotensin converting enzyme (ACE)-angiotensin II axis and angiotensin converting enzyme 2 (ACE2)-angiotensin 1–7 axis pathways.

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C1.14**Poster Session – Tuesday 30th June 2009****Cloning, expression and purification of plant ornithine delta-aminotransferase**

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Ornithine δ -aminotransferase (OAT, EC 2.6.1.13) is a pyridoxal phosphate-dependent enzyme catalyzing the transamination of L-ornithine with 2-oxoglutarate. In plants, OAT has been found for example in pea and wheat. The physiological role of OAT is related to proline and arginine biosynthesis. Proline is known to have an important role in the adaptation of plant cells to drought and salinity stress.

In this work, a cDNA coding for pea OAT was obtained by reverse transcription of pea seedling mRNA followed by PCR amplification. A PCR product of about 1500 bp, whose identity was verified by DNA sequencing (GenBank accession number EU414030), was cloned and expressed in *E. coli* to obtain C-terminally 6xHis-tagged recombinant protein. The cDNA was digested by *NcoI* and *XhoI*, ligated into the pET28b + plasmid vector and then used for transformation of T7 Express Competent *E. coli* cells. Different cultivation and extraction conditions were evaluated. Recombinant PsOAT was partially purified under native conditions by chromatography on IDA Sepharose charged with Ni²⁺ ions followed by ion exchange chromatography on Resource Q.

Basic molecular and kinetic properties of PsOAT were characterized. SDS-PAGE demonstrated a molecular mass value of 50 kDa. The identity of PsOAT was confirmed by in-gel digestion followed by MALDI-TOF MS or LC-MS/MS. Activity assay was performed using a spectrophotometric method based on the formation of a colored compound by the reaction of pyrroline-5-carboxylate with ninhydrin. A specific activity value of 0.3 nkat mg⁻¹ was measured in the lysate, which increased to 27.4 nkat mg⁻¹ after the affinity purification.

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C1.15**Poster Session – Tuesday 30th June 2009****Cloning PKS and NRPS genes from the fungus *Colletotrichum falcatum***

Asifa Munawar (School of Biological Sciences, University of Bristol)

Polyketides (PKs) and nonribosomal peptides (NRPs) are two classes of natural product with tremendous medicinal value. PKs and NRPs are synthesized by multidomain enzymes called PK synthases (PKSs) and NRP synthetases (NRPSs).

We are exploring PK and NRP synthesis in the sugarcane pathogen *Colletotrichum falcatum*. Degenerate primers based on specific PKS enzymatic domains have been employed to amplify sequences from the *C. falcatum* genome, and the cloned PCR products have been used to probe a *C. falcatum* genomic library. A number of lambda clones containing three different genes has been isolated. One gene encodes a PKS-NRPS that aligns most closely with the equisetin synthetase of *Fusarium heterosporus*. The second gene aligns closely with a highly reducing PKS gene from *Cochliobolus heterostrophus*. The third gene

encodes a protein that aligns most closely with a NRP siderophore synthetase gene from *Aspergillus fumigatus*. The full-length genes are being constructed by homologous recombination in yeast and functionally characterised by chemical analysis of novel metabolites produced by their heterologous expression in *A. oryzae*.

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C1.16**Poster Session – Tuesday 30th June 2009****Investigating the expression and function of chaperones and oxidoreductases in the GI tract**

Adam M. Benham (Durham University); Graeme R. Watson (Durham University); Sanjika Dias-Gunasekara (Durham University); Anjan Dhar (County Durham Darlington NHS Foundation Trust, Bishop Auckland Hospital); Yks Viswanath (James Cook University Hospital, Middlesbrough)

A host of chaperones and “foldases” are required for protein folding in the endoplasmic reticulum (ER), including ER oxidoreductases (Eros) and protein disulfide isomerases (PDIs). Ero and PDI are essential for the formation of disulfide bonds in proteins (Benham et al., 2000), and therefore help control the output of secreted proteins in specialised cell types. Humans express over 19 PDI proteins and two related Ero proteins, Ero1a and Ero1b (Eros). Ero1a is induced by hypoxia whereas Ero1b is upregulated by the unfolded protein response. Although the general properties of PDI and Eros are being well investigated, less is known about how these enzymes function in different tissues in vivo. Here, we address this question in the gastrointestinal (GI) tract.

Our data indicate that Ero1a is expressed in the epithelial cells of the oesophagus, whereas Ero1b is strongly expressed in the stomach and in the pancreas. In the stomach, high expression of Ero1b occurs in the enzyme producing chief cells (Dias-Gunasekara et al., 2005). The implications of the expression of these proteins in cancer of the GI tract will be discussed.

Benham, A.M., Cabibbo, A., Fassio, A., Bulleid, N., Sitia, R. & Braakman, I. 2000. EMBO J. 19: 4493–4502.

Dias-Gunasekara et al. 2005. J. Biol. Chem. 280: 33066–33075.

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C1.17**Poster Session – Tuesday 30th June 2009****Dissecting the activation of the unfolded protein response by misoxidised MHC class I molecules**

Adam M. Benham (Durham University), Andrew J. Lemin (Durham University)

Major Histocompatibility Complex (MHC) class I molecules are of central importance to the adaptive immune system. They usually present short peptides, derived from intracellular pathogens, to cytotoxic T cells, enabling the destruction of infected cells. However, the MHC class I molecule HLA-B27 is also strongly linked to the rheumatic condition ankylosing spondylitis. HLA-B27 has the unusual tendency to form disulfide linked heavy chain dimers, instead of the usual heavy chain-beta2 microglobulin complex. Misfolded HLA-B27

heavy chain dimers have been proposed to induce an unfolded protein response (UPR) in the endoplasmic reticulum, which may exacerbate the inflammatory response in this arthritic disease.

Here, we investigate the ability of misoxidised MHC class I heavy chains to induce the UPR in different cell lines, compared with chemical UPR instigators, including DTT and tunicamycin. By monitoring the activation of the transcription factor XBP1, we find that there are variations in the ability of different cell lines to induce,

and resolve, the UPR. Our work suggests that it may be possible to target specific misfolded molecules without compromising ER protein folding in general in healthy cells and tissues.

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