

8th Meeting of the Århus Stress Group

Wednesday, september 10, Economy Lecture Halls, room 025, Building 1324

Program:

- 9:00 **Welcome**
- 9:15 **Dissecting the mechanisms of environmental stress adaptation: a systems biology approach**
Scott Hayward, School of Biosciences, Birmingham University, Birmingham
- 9:45 ***C. elegans* - Stress and ageing**
Anders Olsen, Institute of Molecular Biology, Aarhus University
- 10:15 Coffee break
- 10:45 **Heat shock protein translocation and expression response is attenuated in response to repeated eccentric exercise**
Kristian Vissing, Department of Sport Science, University of Aarhus
- 11:05 **Globin-mediated redox reactions and their protection against oxygen lack**
Angela Fago, Department of Biological Sciences, University of Aarhus
- 11:25 **Identification of cellular stress and metabolic imbalance in human skin fibroblasts using proteomics**
Johan Palmfeldt, Research Unit for Molecular medicine, Aarhus University Hospital
- 11:45 Lunch
- 13:00 **Electron Transfer Flavoprotein: Ubiquinone Oxidoreductase; a potential novel site for generation of reactive oxygen species in mitochondria**
Rikke Olsen, Research Unit for Molecular medicine, Aarhus University Hospital
- 13:20 **Effects of physicochemical stress on developmental competence and cryotolerance of porcine embryos**
Lin Lin, Faculty of Agricultural Sciences, Dept. Genetics and Biotechnology, University of Aarhus
- 13:40 **Thermal adaptation and environmental stress: from selection experiments to gene expression studies and field releases**
Volker Loeschcke, Department of Biological Sciences, University of Aarhus
- 14:00 Coffee break
- 14:30 **Can field populations of the enchytraeid, *Cognettia sphagnetorum*, adapt to increased drought stress?**
Kristine Maraldo, Department of Terrestrial Ecology, National Environmental Research Institute, University of Aarhus
- 14:50 **Psychosocial work stress, depression and common method bias**
Henrik Kolstad, Department of Occupational Medicine, Århus University Hospital
- 15:10 **Metabolomic signatures of inbreeding**
Kamilla Sofie Pedersen, Dept. of Biological Sciences and Faculty of Agricultural Sciences, Dept. Genetics and Biotechnology, University of Aarhus
- 15:30 Final discussion
- 16:00 End

Dissecting the mechanisms of environmental stress adaptation: a systems biology approach

Scott Hayward, School of Biosciences, Birmingham University, Birmingham

The protection of poikilotherms from cold is widely regarded as being causally linked to desaturase regulated changes in the unsaturation of membrane phospholipids. We previously provided the first formal test of this 'lipid hypothesis' in animals, using the model organism *C. elegans*. We demonstrated that whilst genetic disruption of desaturase activity, and thus lipid unsaturation, significantly reduced cold tolerance, animals retained a substantial component of their stress tolerant phenotype. To further dissect the cold stress response, and identify what other mechanisms contribute to acquired cold tolerance, we took full advantage of the molecular tractability of the *C. elegans* model system. Microarray experiments identified more than 200 genes whose transcript abundance directly followed the cold tolerance phenotype – i.e. cold upregulated and recovery downregulated. In addition, metabolomic analyses suggested that both glycerol and trehalose contribute to cold adaptation in this species. Finally, using the *C. elegans* gene interaction network package 'WormNet', we characterized cold stress response gene clusters, as well as identifying other potential candidate genes not picked up in the array experiment. From this systems biology approach a combined list of more than 300 genes was generated, and then targeted in a large-scale RNAi screen. This study has identified a number of key physiological mechanisms contributing to cold adaptation, yet the manipulation of single genes within these mechanisms has a very limited impact on cold tolerance. We have characterized compensatory gene interactions that can account for this, suggesting a high level of robustness in thermal stress responses.

***C. elegans* - Stress and ageing**

Anders Olsen, Institute of Molecular Biology, Aarhus University

The soil nematode *Caenorhabditis elegans* has become a prominent model organism for studying aging and many age-related diseases. We use *C. elegans* to study the relationship between cancer and aging. To prevent cancer, cells are equipped with surveillance systems that detect damage and stop cells from dividing. These surveillance systems are collectively called cellular checkpoints. We have shown that inactivation of certain checkpoint proteins can increase stress resistance and lifespan of the nematode. Furthermore, we have found that inactivation of certain checkpoint proteins, including p53, also causes resistance to the chemotherapeutic drug hydroxyurea (HU). HU stalls replication by depleting the dNTP pool. To further our understanding of how checkpoint proteins may determine lifespan we completed a *C. elegans* whole genome RNAi screen for genes that cause resistance to HU when inactivated. This screen isolated approximately 50 potential checkpoint genes that cause resistance to HU. Subsequent analysis has demonstrated that among these 50 genes several dramatically increase the lifespan of the nematode when inactivated. Many of the genes found in the nematode are also found in humans and checkpoint proteins are particularly well conserved.

Heat shock protein translocation and expression response is attenuated in response to repeated eccentric exercise

Kristian Vissing, Department of Sport Science, University of Aarhus

Muscle functional impairments, enzyme leakage and muscle soreness development produced after a first exposure eccentric exercise is attenuated when eccentric exercise is repeated – a phenomenon referred to as the repeated bout effect. In a recent study, we observed protein contents of HSP27 and $\alpha\beta$ -crystallin to increase in the cytoskeleton in response to single-bout eccentric exercise in humans. This indicates that a HSP translocation response is induced to protect sarcomeric proteins from denaturation upon muscle damaging exercise and suggests that the translocation response might be modulated when a muscle is re-exposed to eccentric exercise. The purpose of the present study was therefore (1), to compare the HSP protein contents in cytosolic and cytoskeletal muscle cell fractions and the HSP gene expression responses to concentric and eccentric exercise in human skeletal muscle, and (2), to investigate if such a HSP system response exhibited a repeated bout effect. A repeated bout effect from eccentric exercise was established from our protocol. HSP translocation and expression responded exclusively to eccentric exercise and the overall HSP responses were attenuated when eccentric exercise was repeated. Our data strongly support a role of HSP's system components – especially HSP27 to be involved in repeated bout adaptation.

Globin-mediated redox reactions and their protection against oxygen lack

Angela Fago, Department of Biological Sciences, University of Aarhus

The physiological role of globin proteins in vertebrates is not only limited to the well-known oxygen transport and storage functions of the hemoglobin in red blood cells and the myoglobin in heart and skeletal muscle, but also includes a wide variety of other functions. These include the preserving or scavenging of vasoactive nitric oxide (NO) generated within blood vessels, formation of S-nitrosothiols involved in cellular signalling, and several redox reactions favoured by hypoxic conditions, such as the reduction of endogenous nitrite into NO for the control of vasodilation and mitochondrial respiration, the scavenging of toxic peroxynitrite and the reduction of cytochrome c, possibly preventing cellular apoptosis. Although the biological significance of many of these reactions remains controversial, the four major vertebrate globins, hemoglobin, myoglobin, neuroglobin and cytoglobin, clearly show distinctive patterns of reactivity due to differences in heme-iron coordination and reducing potentials, thiol groups accessibility and presence of external and internal cavities for non-covalent binding of cofactors or ligand diffusion. Specifically, our data indicate that neuroglobin seems to be the most likely candidate among globins for the catalysis of most such redox-reactions, a feature that may relate to its most ancient phylogenetic origin.

Identification of cellular stress and metabolic imbalance in human skin fibroblasts using proteomics

Johan Palmfeldt, Research Unit for Molecular medicine, Aarhus University Hospital

Mitochondrial proteins are of central importance for many metabolic activities and are key regulators of apoptosis. The present study quantified mitochondrial proteins in cells from cultivated human skin fibroblasts, which is suitable for studies of internal and external cellular stress. The applied proteomics methodology, nanoLC-MS/MS analysis was sensitive enough to detect proteins from all the central pathways of the mitochondrial metabolism. When the fibroblast cultures were exposed to metabolic stress, by cultivation in galactose medium, the levels of NAD⁺-dependent isocitrate dehydrogenase of the citric acid cycle was increased together with several components of respiratory complex I, IV and V, confirming the applicability of galactose cultivation for detection of respiratory chain defects. Consistent data was also obtained for the following stress related pathways: protein quality control, antioxidant systems and apoptosis.

Effects of physicochemical stress on developmental competence and cryotolerance of porcine embryos

Lin Lin, Faculty of Agricultural Sciences, Dept. Genetics and Biotechnology, University of Aarhus

An innovative technique named high hydrostatic pressure (HHP) treatment has been recently reported to improve the cryosurvival of gametes or embryos in certain mammalian species, including mouse, porcine and bovine. The data indicate that sublethal shock of mammalian gametes and embryos may improve their resistance against damages caused by subsequent in vitro interventions. In various experimental systems spermatozoa, oocytes and preimplantation stage embryos were exposed initially for one or two hours to either high hydrostatic pressure (HHP). In my present study, HHP and osmotic stress induced by NaCl, sucrose, and trehalose were tested, optimized and compared. After stress treatment and a certain period of recovery time, the oocytes were used for somatic nuclear transfer or cyropreservation and parthenogenetic activation. Both HHP and osmotic stress could improve the developmental competence and cryotolerance of porcine embryos. After transferring the embryos derived from HHP treated oocytes into recipient sow, health piglets were born, indicating that full term development was allowed after the stress treatment. So far, the biological mechanisms are still unknown. The induction of heat shock proteins (HSPs) might be one of the possibilities. Further research is needed to find the optimal initial stress that may be different according to species and developmental stages. More molecular analysis is also needed to find out what happened in this process. The new principle, i.e. to improve the stress tolerance by a sublethal stress may outline a completely new strategy in mammalian embryology with remarkable theoretical and practical consequences.

Thermal adaptation and environmental stress: from selection experiments to gene expression studies and field releases

Volker Loeschke, Department of Biological Sciences, University of Aarhus

We are studying adaptation to genetic and environmental stresses and their evolutionary implications with particular emphasis on thermal adaptation and the heat shock response using *Drosophila* as a model organism. To achieve our research goals, we study correlated responses in lines selected for resistance to various environmental stresses as heat, cold, starvation and desiccation as well as lines selected for increased lifespan. Results on the phenotypic level are related to results on the DNA level, studying gene regulation in the same stress selected lines using Affymetrix gene chips at different time points after being exposed to a heat or cold hardening treatment. To complement these expression studies, we study the relation of DNA sequence variation in candidate genes to variation in resistance traits in natural populations and selection lines. Finally, we use selection and hardened/acclimated lines to study fitness in the wild using release-recapture experiments to bridge the gap between laboratory experiments and studies of thermal adaptation in the wild.

Can field populations of the enchytraeid, *Cognettia sphagnetorum*, adapt to increased drought stress?

Kristine Maraldo, Department of Terrestrial Ecology, National Environmental Research Institute, University of Aarhus

The ability to evolve increased drought tolerance in response to climate change was investigated in the enchytraeid, *Cognettia sphagnetorum*. Populations exposed to reduced precipitation or increased night time temperature for more than six years were collected in mixed Calluna/grass heathland at the Mols Laboratory, Denmark. The level of prolonged drought and increased temperature corresponded to a predicted climate change scenario and has been applied since 1999. In autumn 2005, enchytraeids were sampled in 3 cm intervals down to 9 cm depth and total number, biomass, diversity and soil organic matter were determined. The drought treatment resulted in a significant reduction of the density and biomass of enchytraeids, as well as changes in the species composition. In total, five different genera were found at the site in all three treatments (control, temperature and drought). *C. sphagnetorum* was the dominant species, especially in the upper 0-3 cm, and was clearly affected by the drought treatment. *C. sphagnetorum* from all plots were cultured in the laboratory to rear second or third generation adults. Results showed that populations of drought treated plots had not developed an increased drought resistance compared to populations of control or warming plots even after several years of a putative severe selection. Lack of adaptive potential in *C. sphagnetorum* suggests that more frequent periods with drought in the future will have a very strong negative influence on enchytraeid density, biomass and diversity.

Psychosocial work stress, depression and common method bias

Henrik Kolstad, Department of Occupational Medicine, Århus University Hospital

Stressful work has been identified as an important risk factor for distress and depression in epidemiological studies. However, most studies have relied on individuals' perceptions of exposure and outcome and the results are thus expected to be inflated towards erroneously high values by common method bias. We examine this bias by comparing self-reports with an objective measure of stressful work. The distressed workers reported levels of job demands, decision authority and skill discretion that systematically deviated towards the worse by 10% from the work-unit averages (p values <0.01). These estimates were not weakened by restricting analyses to work units with low exposure variability. Self-reported stressful work was associated with distress (overall odds ratio 2.0, 95% CI 1.5-2.6). No associations were seen between job demands, decision authority, or skill discretion and distress if workers were classified by their work-unit average. The between work unit exposure variance components varied between 10% and 20% of the total variance. The strong association observed between self-reported stressful work and distress may reflect causality or common method bias. The fact that the distressed workers consistently reported more adverse working conditions than their non-distressed colleagues with high agreement in their reporting, speaks in favour of the latter interpretation. This emphasises the need for independent and objective exposure estimates and group based analyses are a straightforward solution. The work-units of this study captured a significant part of the total exposure variability and identification of groups with homogenous work conditions will be a major challenge and should be defined by any work information robust to response style or psychological reactivity.

Metabolomic signatures of inbreeding

Kamilla Sofie Pedersen, Dept. of Biological Sciences and Faculty of Agricultural Sciences, Dept. Genetics and Biotechnology, University of Aarhus

We have investigated the effects of inbreeding on the *Drosophila melanogaster* metabolome. Metabolite fingerprints in outbred and inbred lines were studied by NMR spectroscopy after exposure to benign temperature, heat- or cold-stress. Both in the absence and presence of temperature stress, metabolite levels were significantly different among inbred and outbred lines. Apart from affecting mean metabolite levels, inbreeding led to an increased between-line variation in metabolite profiles compared to outbred lines. In contrast to previous observations revealing interactions between inbreeding and environmental stress on gene expression patterns and life history traits, the effect of inbreeding on the metabolite profile was similar across the different temperature treatments.