PP3C-13
Misfolding of heterologously-produced eukaryotic translation initiation factor 5A

P. Gentz, G. Blatch and R. Dorrington
Rhodes University, Grahamstown, SOUTH AFRICA

Introduction: The function of eukaryotic translation initiation factor 5A (eIF5A) is dependent on hypusine, which is formed by the post-translational modification of a highly conserved lysine residue (Lys51 in yeast) involving deoxyhypusine synthase (DHS) and deoxyhypusine hydroxylase (DOHH). DHS, DOHH and hypusinated eIF5A have become targets for anti-cancer therapies, particularly as eIF5A is thought to play a role in the translation of specific mRNAs encoding proteins involved in the onset of the G1-S phase in the cell cycle. Structural data of eIF5A and archaeal homologues has been derived from crystals produced from heterologously-expressed unhypusinated eIF5A.
Methods: Affinity chromatography was used to purify heterologously-produced eIF5A (EceIF5A). The oligomeric state of EceIF5A was determined using gel filtration, native PAGE and cross-linking experiments. Site-directed mutagenesis of specific residues in EceIF5A was used to identify residues involved in maintaining the oligomeric state. The biological activity of these mutants was determined in a yeast complementation system.
Results: EceIF5A exists as a dimer in solution. The formation of this dimer is dependent on the highly conserved Cys39 residue, but not Lys51, which is required for dimerisation of the native protein in yeast. These and other results indicate that eIF-5A produced in *E. coli* is misfolded with important implications for the interpretation of current structural data.
Conclusions: Evidence of misfolding in heterologously produced eIF5A implies limited information from crystal structures derived from this protein. There is a need for structural data on hypusinated eIF5A to understand its function *in vivo*.

PP3C-14
Correlation between protein misfolding, immunity and neurodegeneration

M. Gruden[1], R. Sewell[2], V. Sherstnev[1] and L. Morozova-Roche[3]
[1]P. K. Anokhin Institute of Normal Physiology, RAMS, Moscow, RUSSIA, [2]Welsh School of Pharmacy, Cardiff University, Cardiff, UK, [3]Department of Medical Biochemistry and Biophysics, Umeå University, Umeå, SWEDEN

Introduction: The expression of misfolded proteins effects in development of aggregates which are hallmarks of various neurodegenerative diseases. Moreover, recently it has been demonstrated that intermediate species of aggregates as soluble oligomers of a number of amyloidogenic proteins may induce cell death (apoptosis). This fact rises the question concerning immunological protection of nervous cells against oligomeric toxicity.
Methods: AFM was used for characterization of amyloidogenic species, as also die mapping. Abs were determined using ELISA and Dot-blot analysis in patient sera with diagnose Alzheimer's disease and Parkinson's disease.
Results: Comparative investigations of sera antibodies (Abs) production to precisely characterized various species ranging from oligomers to protofilaments and fibrils of disease specific proteins ($A\beta_{(25-35)}$, α-synuclein) and abundant proteins (human lysozyme, insulin) in progressive Alzheimer's disease and Parkinson's disease were performed. We have revealed specific correlations between nature of misfolded protein species, Abs production to them and early outset of disease.
Conclusion: These studies provide new insights on the role of immune networks that protect neuronal cells from damage and apoptotic death during progressive neurodegeneration and usage of data to new therapeutic approaches targeted against misfolding diseases.

PP3C-15
DRPLA aggregates are highly dynamic

J. Hinz and Z. Ignatova
Department of Cellular Biochemistry, Max-Planck-Institute of Biochemistry, Martinsried, GERMANY

Introduction: The molecular basis of slowly progressing hereditary neurodegenerative disease dentatorubralpallidoluysian atrophy (DRPLA) is associated with a mutation that abnormally increases in-frame the number of glutamine residues in the atrophin-1 over a threshold value of 30–40 repeats.
Methods: Using fluorescence imaging of cells and FRAP live imaging we study the dynamic localization and mobility of the aggregates in HEK293 and differentiated and undifferentiated N2a cell line. The behaviour of the full length atrophin-1 with polyQ-stretch in the non-pathological (Q = 14) and pathological (Q = 71) length (FL14, FL71) and with truncated NLS sequence (NLS14, NLS71) fused downstream the GFP protein was examined. To quantitatively assess the partitioning of the aggregate species between the detergent-labile and detergent-resistant fraction we perform fractionation assays followed by an immunodetection.
Results and conclusions: $\triangle$NLS71 forms readily hyperfluorescent loci, even at the early time points of expression with a clearly defined SDS-resistance pointing out to an early appearance of the fibrillar phenotype. However, these early species are less densily packed with a higher degree of mobility within the aggregate loci. The FL71 expressing cells show a mixed population of cytoplasmic and nuclearly localized aggregates, whereas prolonged expression results in mainly cytoplasmic localization and fusion to one large aggregate. For the non-pathological variants $\triangle$NLS14 and FL14 we observed in addition to cells with homogenous GFP fluorescence in substantial fraction of cells (and intriguingly in all differentiated cells) hyperfluorescent foci. The latter showed a fast recovery of the fluorescence and lack of insoluble fraction after fractionation suggesting spatial localization within the cytoplasm.

PP3C-16
CA XII expression in HT-29 colon carcinoma cells treated with TNF-alpha and TGF-beta

R. Iliksi Sagkan[1], F. Kockar[2] and A. Sengul[1]
[1]Department of Immunology, GATA, Ankara, TURKEY, [2]Department of Molecular Biology, Balikesir University, Balikesir, TURKEY

Introduction: Carbonic anhydrases (CA, EC 4.2.1.1) are a family of metalloenzymes catalyzing the hydration of CO_2 to bicarbonate and a proton. α-Cas are involved in essential cellular functions such as pH regulation, respiration, gluconeogenesis, lipogenesis, ureagenesis, tumor progression. So far, 15 catalytically active carbonic anhydrase isozymes are described in mammals. hCA XII is one of the transmembrane human carbonic anhydrase isoforms. hCA XII is present in many normal tissues and overexpressed in some tumors.
Aim: The aim of this study was to investigate CA XII expression in HT 29 colon carcinoma cell line upon cytokine (TNF-α, TGF-β).
Methods: The expression of CA XII in colon cells was detected by real-time fluorescent quantitative PCR.
Results: There was no significant difference in the expression level of CA XII between cytokine treated and non-treated colon carcinoma cells. While TNF-α treatment increased proliferation TGF-β had a significant anti-proliferative effect in cells.