PROTEIN MISFOLDING MEETING:
crossroads between biology, cancer, and neurodegeneration

MAY 20-21,
2019
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DAY 1 | MAY 20TH

8h30 • 9h30	**Registration**
9h30	**Opening Ceremony**
	Session 1 – Cellular quality control mechanisms
10h	Charles Boone (University of Toronto, Canada) ***Talk Large-scale Mapping of Genetic Networks in Yeast and Human Cells***
10h30	Henning Ulrich (IQ, USP, Brazil) ***Talk Purinergic and calcium signalling in a Huntington's disease model***
11h	Daniel Jarosz (Stanford University, USA) ***Talk Quality control and aggregation in an aging proteome***
11h30	Ronald Kopito (Stanford University, USA) ***Talk Protein quality control in the endoplasmic reticulum***
12h	**Discussion**
12h30 • 14h30	**Lunch and poster presentation**
	Session 2 – Protein misfolding in biology and ageing
14h30	Daniel Kaganovich (The Alexander Silbermana Institute of Life Sciences, Jerusalem/ Georg-August-Universität Göttingen, Germany) ***Talk Stress Granules maintain metabolic balance during stress and aging***
15h	Harm Kampinga (University of Groningen, The Netherlands) ***Talk Heat Shock Proteins and Protein Aggregation Diseases***
15h30	Andrew Dillin (University of California, USA) ***Talk Central Nervous System Control of Peripheral Proteostasis and Metabolism***
16h	Thomas Nystrom (University of Gothenburg, Sweden) ***Talk Identifying new players involved in spatial sequestration of protein aggregates***
16h30	**Discussion**
17h	**Coffee break**
	Session 3 – Protein misfolding as a target for intervention
17h30	Christian Griesinger (Max Planck, Germany) ***Talk NMR spectroscopy to investigate liquid liquid phase separation in immunity***
18h	Sergio Ferreira (IbqM, UFRJ, Brazil) ***Talk An exercise-related miokine protects the brain in Alzheimer models***
18h30	**Lightning talks (3 minutes presentations of selected abstracts)**
19h	**Discussion**

DAY 2 | MAY 21ST

Session 4 – Protein misfolding in cancer

9h30 — Carolina Panis (UNIOESTE, Brazil)
Talk Unraveling the biology of breast cancer by proteomics

10h — Jerson Lima da Silva (IbqM, UFRJ, Brazil)
Talk Misfolding and Aggregation of Mutant p53: A Promising Target against Cancer

10h30 — Mauricio Menacho Marquez (CONICET, Rosario, Argentina)
Talk Characterization of the role of alpha-synuclein in melanoma: exploring the relationship between cancer and neurodegenerative diseases

11h — Anderson de Sá Pinheiro (IQ, UFRJ, Brazil)
Talk NSD3 or Pdp3 expression remodels yeast metabolism leading to aerobic glycolysis and glutaminolysis

11h30 — **Discussion**

12h • 14h — **Lunch and poster presentation**

Session 5 – Protein misfolding in neurodegeneration

14h — Debora Foguel (IbqM, UFRJ, Brazil)
Talk The role of inflammation in amyloid diseases: neutrophils and neutrophil extracellular traps (NET)

14h30 — Tiago Outeiro (Georg-August-Universität Göttingen, Germany)
Talk Alpha-synuclein acts in the nucleus and deregulates gene expression: insight into the molecular basis of Parkinson's disease

15h — Elis Eleutherio (IQ, UFRJ, Brazil)
Talk Functional links between Sod1 mutants, oxidative stress and Amyotrophic Lateral Sclerosis risk factors

15h30 — Yraima Cordeiro (FF, UFRJ, Brazil)
Talk Alpha-synuclein and prion protein: understanding the aggregation profile to search for therapeutic compounds

16h — Luciana Pizzatti (IQ, UFRJ, Brazil)
Talk In-Depth omics strategies applied for Parkinson disease biomarker discovery

16h30 — **Discussion**

17h — **Coffee break**

17h30 • 19h — **Final discussion – challenges and opportunities**

19h — **Closing ceremony**

PROTEIN MISFOLDING MEETING:
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ABSTRACTS | SPEAKERS

NSD3s or Pdp3 expression remodels yeast metabolism leading to aerobic glycolysis and glutaminolysis

Germana B. Rona[1], Natalia P. Almeida[1], Gilson C. Santos Jr[2], Tatiana K. S. Fidalgo[3], Fabio C. L. Almeida[4], Elis C. A. Eleutherio[1], Anderson S. Pinheiro[1]

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NSD3s, the PWWP domain-containing, short isoform of the human oncoprotein NSD3, displays high transforming properties. Overexpression of human NSD3s or the yeast protein Pdp3 in *Saccharomyces cerevisiae* induces similar metabolic changes, including increased growth rate and sensitivity to oxidative stress accompained by deacreased oxygen consumption, suggesting a shift from respiration to fermentation. Here, we set out to elucidate the biochemical pathways leading to the observed metabolic phenotype by analyzing the alterations in yeast metabolome in response to NSD3s or Pdp3 overexpression using ^{1}H NMR metabolomics. We observed an increase in aspartate and alanine, together with a decrease in arginine levels, upon overexpression of NSD3s or Pdp3, suggesting an increase in the rate of glutaminolysis. In addition, certain metabolites, such as glutamate, valine, and phosphocoline were either NSD3s- or Pdp3-specific, indicating that additional metabolic pathways are adapted in a protein-dependent manner. The observation that certain metabolic pathways are differentially regulated by NSD3s and Pdp3 suggests that, despite the structural similarity between their PWWP domains, the two proteins act by unique mechanisms and may recruit different downstream signaling complexes. This work establishes for the first time a functional link between the human oncoprotein NSD3s and cancer metabolic reprogramming.

Keywords: NSD3, Pdp3, PWWP, histone, NMR, metabolomics

Financial Support: CNPq, CAPES, FAPERJ, and Brown University.

Unraveling the biology of breast cancer by proteomics

Carolina Panis

Universidade Estadual do Oeste do Paraná UNIOESTE

Proteomic screening has been one of the most powerful tools employed in the last years that allowed major advances in our understanding about tumor biology. Advances have been reached regarding tumor biology and clinic pathological aspects of several tumors, including breast cancer. Here we discuss the relevance of proteomics to point out putative biomarkes in this field, and its importance concerning the discovery of proteins that are directly related with breast cancer clinico pathological aspects. The use of label-free proteomics as a tool for understanding disease spreading, molecular sub-types behavior, and treatment toxicity will be further addressed.

Large-scale Mapping of Genetic Networks in Yeast and Human Cells

Charlie Boone

University of Toronto, Canada

We've generated a comprehensive genetic network in yeast cells, testing all possible 18 million gene pairs for genetic interactions. Negative interactions connected functionally related genes, mapped core bioprocesses, and identified pleiotropic genes, whereas positive interactions often mapped general regulatory connections among gene pairs rather than shared functionality. The global network illustrates how coherent sets of genetic interactions connect protein complex and pathway modules to map a functional wiring diagram of the cell. We are now utilizing CRISPR-Cas9 technology to conduct genome-wide screens and map genetic and chemical-genetic interactions in human cells. In particular, we are exploring the NGLY1 pathway in order to gain insights into the degradation of misfolded glycoproteins.

"Structure dynamics and kinetics of folding and recognition in proteins by NMR"

S. Prathihar[1], C. Smith[1,2], K. Chakrabarti[1], L. Wong[1], J. Kühn[3], S. Pirkuliyeva[3], D. Lee[1], T.M. Sabo[1], S. Ryzanov[1,4], L. Antonschmidt[1,4], A. Martinez Hernandez[5], H.Y. Agbemenyah[6], S. Shi[7], R. Dervisoglu[1], A. Fischer[6], G. Eichele[5], D. Lee[1], D. Becker[1], S. Becker[1], A. Leonov[1,4], R. Benz[8], L. Andreas[1], J. Wienands[3], A. Giese[7], and C. Griesinger[1,4]

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Kinetics of protein dynamics will be discussed on examples of folded on unfolded proteins (1). Protein recognition will be described with a new mathematical method to distinguish conformational selection and induced fit (2) which includes a concept for the measurement.
Further, the role of partially disordered proteins in droplet formation is investigated. The adaptor protein SLP65 which interacts with CIN85 (3). The two proteins bound to vesicles are essential for B cell activation. The tripartite phase separation can be reconstituted in vitro at physiological conditions.
We are additionally interested in a class of IDPs that are important in neuro- and cellular degeneration, which form oligomers and fibrils. Interference with these aggregates specifically on the oligomer level with DPP compounds proves to be a valid concept for treatment of devastating diseases such as Parkinson's, Alzheimer's, Creutzfeldt Jacob disease and Type II diabetes mellitus (4). Direct interaction between the preferred oligomer modulator anle138b and aSyn oligomers in membranes can be shown by DNP enhanced solid state NMR.

(1) C. Smith et al. *Proc. Natl. Acad. Sci. USA.* 113, 3296-74 (2016); C. Smith et al. *Angew. Chem. Int. Ed.* 54, 207-10 (2015)

(2) Paul, F. and T.R. Weikl, *Plos Computational Biology*, 2016. **12**(9).

(3) M. Engelke et al. Science signaling: 7 (339) ra79 (2014); J. Kühn et al. Science signaling: 9 (434) ra66 (2016)

(4) C.W. Bertoncini et al. PNAS 102, 1430-1435 (2005); P. Karpinar et al. EMBO J 28, 3256-3268 (2009); J. Wagner et al. Acta Neuropath. 125, 795-813 (2013); A.A. Deeg, Biochim. Biophys. Act. 1850 (9), 1884-1890 (2015); S. Shi, J. Neuropath. Exp. Neurol. 74(9) 924-933 (2015); J. Wagner et al. Act. Neuropath.130, 619-631 (2015); A. Hernandez et al. EMBO Mol. Med. 10, 32-47 (2018)

Quality control and aggregation in an aging vertebrate proteome

Yiwen Chen, Itamar Harel, Anne Brunet, and Daniel F. Jarosz

Age is the greatest risk factor for many neurodegenerative pathologies caused by protein aggregation. Yet because model vertebrates have long lifespans, examining the intersection of genetic risk factors with age has been challenging. To address this problem we took advantage of the African turquoise killifish *Nothobranchius furzeri*, the shortest-lived vertebrate that can be bred in captivity. Over a six-month lifespan this fish manifests age-dependent phenotypes and pathologies including neuronal death and cognitive decline. We established a robust protocol for isolating protein aggregates from fish tissues and analyzed the soluble and insoluble proteomes of young and old animals. These experiments revealed tissue-specific changes in proteostasis and aggregation during aging. These changes were accelerated in telomerase mutant animals. Many proteins that aggregated in an age-dependent manner harbor disordered, prion-like domains and are linked to age-related degenerative disease. Biochemical studies suggest that some of these proteins adopt an infectious prion-like conformation in old tissues (e.g. the brain), but not in matched tissues from young animals. Taking advantage of the genetic and biochemical tractability of this model organism, we are now examining whether age-related changes in proteostasis and aggregation act as a driving forces in aging.

Stress Granules maintain metabolic balance during stress and aging

Daniel Kaganovich

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Metabolic flexibility allows cells to survive starvation by reconfiguring energy producing routes in accordance with changing fuel availability. Starvation response spans several orders of operational complexity and temporal regulation. Short term starvation leads to PPAR-driven transcription activation, which in turn results in increase in fatty acid metabolism in mitochondria. During long term starvation, however, cells face the consequences of Fatty Acid Oxidation, such as oxidative damage, and must therefore reroute fatty acids towards Lipid Droplets. Stress Granules appear following long-term starvation. Their precise function in starvation, however, is not known. Our data provide evidence that Stress Granules both upregulate and downregulate Fatty Acid metabolism during stress. We show that Stress Granule formation is triggered by the PPAR response, which is in turn regulated by Stress Granules. Over longer periods of starvation, Stress Granules recruit a mitochondrial porin, thereby blocking Fatty Acid import into mitochondria and promoting Lipid Droplet biogenesis. The subsequent decrease in FAO during long-term starvation reduces oxidative damage and rations fatty acids for longer potential use.

The role of inflammation in amyloid diseases: neutrophils and neutrophil extracellular traps (NET)

Debora Foguel

IBQM, UFRJ Brazil

Neutrophil Extracellular Traps (NET) are chromatin derived structures decorated with neutrophil enzymes such elastase and myeloperoxidase. Our group has shown for the first time that amyloid fibrils (AF) composed of transthyretin (TTR) were able to induce NET in vitro. NET formation trigged by amyloid fibrils is dependent on the formation of reactive oxygen species (ROS) by NOX2, an isoform of NADPH oxidase present in phagocytes. The aim of the present work is to determine whether TTR-composed amyloid fibrils induce NET formation in vivo and to dissect the role of NOX2 in this process. Amyloid fibrils were prepared by incubating TTR-A25T, a highly amyloidogenic variant of TTR, in PBS for 15 days. Six-weeks-old C57BL/6 wildtype (WT) and knockout (KO) for NOX2 mice were used for *in vivo* experiments. 50 µg of amyloid fibrils were injected into peritoneal cavity. Four hours after injection, peritoneal cavity was washed with 3 mL of PBS-EDTA and cellular fraction were staining with Hematoxilin/Eosin for cell counting. Soluble fraction of peritoneal wash was used to measure NET formation. Our data show that both WT and NOX2 KO mice were able to recruit neutrophils to the same extent after fibril injection, but not lymphocyte or macrophage suggesting that NOX2 is not necessary for neutrophil recruitment as expected. Interestingly, extracellular DNA, a marker of NET formation, was only found in the peritoneal wash of WT mice injected with amyloid fibrils, suggesting that NOX2 is important to trigger NET formation in vivo as well. Our data show that amyloid fibrils are able to recruit neutrophil *in vivo* and triggers NET release. The connection of NET formation to the development of amyloid diseases are being evaluated.

Key words: Transthyretin, Neutrophil Extracelular Traps, NADPH oxidase
FAPERJ, CNPq and CAPES

Functional links between Sod1 mutants, oxidative stress and Amyotrophic Lateral Sclerosis risk factors

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Amyotrophic Lateral Sclerosis (ALS) is an age-associated disorder caused by the degeneration of motor neurons. More than 100 mutations in Cu/Zn superoxide dismutase (Sod1) have been reported in familial and sporadic ALS, with almost all inherited as a dominant trait. Although much has been known about ALS-Sod1 homodimers, the investigation of Sod1 heterodimers remains poorly unexplored in the literature. In this study, a non-invasive imaging approach named Bimolecular Fluorescence Complementation (BiFC) was used to analyze heterodimeric combination of WT and the mutant Sod1 proteins (A4V, L38V, G93A and G93C) as well as to investigate aggregation and dynamics properties in human neuroglioma cells (H4). According to our results Sod1 WT and mutant can dimerize and homodimers of Sod1 WT do not aggregate. We also verified that cells expressing ALS-Sod1 heterodimers showed a larger number of inclusions per cell than those expressing homodimers; the increase was more outstanding in cells expressing A4V Sod1. Moreover, the WT/A4V Sod1 aggregates showed to be the most stable. To eliminate the endogenous Sod1 influence and better evaluate the effect of ALS Sod1 expression, human Sod1 WT and mutants were expressed in *sod1Δ* yeast cells. Yeast and human Sod1 perform the same roles in both organisms, meaning that the expression of a human gene is able to replace for that of the yeast. In addition, yeast cells have long served as an advantageous model to study oxidative stress response. By using the yeast model submitted to chronological ageing, we concluded that only human heterodimers showed decreased antioxidant activity, increasing oxidative damage and impacting longevity. The yeast model showed clearly that an oxidative stress induces ALS Sod1 aggregation; cells expressing human Sod1 heterodimers showed more inclusions than when expressing homodimers. Recently it was reported a new role for Sod1: in response to an oxidative stress, Sod1 goes to the nucleus, which is important for maintaining genomic stability. According to our results, ALS mutations impair human Sod1 localization into the nucleus and antioxidant response, which might be involved with the disease mechanism of ALS.

Heat Shock Proteins and Protein Aggregation Diseases.

Harm H. Kampinga

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Protein aggregates hallmark nearly all age-related neurodegenerative diseases, including Alzheimer's disease (AD), Parkinson's disease (PD), Amyotrophic lateral sclerosis (ALS), and several polyglutamine (PolyQ) such as Huntington's disease (HD) and different forms of Spinocerebellar Ataxias (SCA1,2,3,6,7). The collapse of cellular protein homeostasis can be both cause and consequence of this protein aggregation. Boosting components of the cellular protein quality control system has been a widely investigated strategy to counteract protein aggregates or their toxic consequences. Heat shock proteins (HSP) play a central role in regulating protein quality control. Experimental evidence will be presented that suggests that in different individual diseases, the disease-associated proteins may be mediated by different pathways and that they require alternative elements of and handling by the HSP-chaperone network. Inversely, it will be shown how mutations in some HSPs can lead to protein-aggregation diseases in neuronal and (cardio)muscular tissues (so-called chaperonopathies) due to either direct loss-of-chaperone function of via dominant-negative effects on protein quality control networks.

Purinergic and Calcium Signalling in Huntington's Disease Models

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Huntington's disease (HD) is an autosomal dominant inherited disease caused by at least 35 repetitions of the N-terminal CAG trinucleotide (glutamine) in the Huntington's gene (Htt). We used as in vitro disease models induced pluripotent iPS cells obtained from HD patients and Htt-gene edited embryonic stem cells, which were induced to neuronal differentiation into GABAergic neurons. For this, huntingtin knockout (Htt-/-) cell lines were generated, which then were transfected with plasmids coding for the N-terminal of the Htt sequence with 23 (Q23) or 74 (Q74) repetitions of glutamine corresponding to wild type (WT) and mutated Htt, respectively. Spontaneous and receptor-induced intracellular calcium transient are known to determine neuronal cell fate. Our laboratory has characterized ionotropic (P2X) and metabotropic (P2Y) purinergic receptor-mediated intracellular calcium transients as triggers of neurogenesis and neuronal fate determination. Calcium oscillations were tracked by real-time fluorescence and luminescence microscopy to analyse the correlative relationship between calcium transient activity and rhythmic proneuronal transcription factor expression in embryonic stem cells after stable transfection with ASCL-1 or neurogenin-2 promoter-protein fused to the luciferase reporter gene. We show that pharmacological activity manipulation of L-type voltage-gated calcium channels and purinergic receptors induced a two-step process of neuronal differentiation. L-type channel-mediated augmentation of spike-like calcium oscillations first promoted stable expression of ASCL-1 in differentiating stem cells, which then further matured into GABAergic neurons after P2Y2 purinoceptor activation. In vitro

Misfolding and Aggregation of Mutant p53: A Promising Therapeutic Target against Cancer

Jerson L. Silva
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Protein misfolding results in grave degenerative diseases and cancer. Among the culprits involved in these illnesses are amyloids and prion-like proteins, which can propagate by converting normal proteins to the wrong conformation. p53 mutations are the most common genetic alterations found in cancers and are observed in more than 50% of all tumors. Mutant p53 not only undergoes misfolding but also aggregation, similar to that observed with amyloids, playing a crucial role in the development of cancer through loss of function, negative dominance and gain of function (1-3). Studies from our laboratory and others have demonstrated that the formation of prion-like aggregates of mutant p53 is associated with loss-of-function, dominant-negative and gain-of-function (GoF) effects (1,4). Compounds and peptides that have been described to inhibit mutant p53 aggregation also lead to a decline of tumor proliferation and migration (4-7). Thus, the misfolded and aggregated states of mutant p53 are formidable targets for the development of novel therapeutic strategies against cancer (6). (This work was supported by CNPq, FAPERJ, FINEP and CAPES)

References:

(1) Silva JL et al. *Trends Biochem. Sci.* **2014**, *39*, 260-267.
(2) Ishimaru D et al. *Biochemistry* **2003**, *42*, 9022-9027.
(3) Ano Bom AP et al. *J. Biol. Chem.* **2012**, *287*, 28152-28162.
(4) Silva JL et al. Acc Chem Res. 2018 51(1):181-190.
(5) Soragni A et al. *Cancer Cell* **2016**, *29*, 90-103.
(6) Ferraz da Costa DC et al. Oncotarget. 2018 Jun26;9(49):29112-29122
(7) Rangel LP et al. *J Biol Chem*. 2019 294(10):3670-3682.

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models of Huntington's disease (HD) showed increased basal intracellular calcium concentration together with augmented apoptosis rates and lacked spike-like calcium oscillations and P2Y2 receptor activity, being in agreement with deficiency of ASCL-1 expression activation and GABAergic differentiation. Our results suggest that HD may have developmental origins based on inefficient GABAergic differentiation, shedding new light on the mechanisms underlying neurogenesis of inhibitory neurons.

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In-Deph omics strategies applied for Parkinson's disease biomarker discovery

Profa Dra Luciana Pizzatti

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Instituto de Química (IQ)

Laboratório de Biologia Molecular e Proteômica do Sangue (LABMOPS- LADETEC)

Parkinson's disease (PD) is the most common debilitating neurodegenerative motor disorder, affecting millions of people worldwide. The diagnosis is not well established yet, and the knowledge of clinical symptoms and their variations is the most important requirement to define PD. Previous studies have suggested that dysbiosis of the intestinal microbiota can be related to several neurodegenerative diseases, including PD, however the exact mechanism of the gut and brain axis as well as alterations in the dormant microbiota present in blood of PD patients were not yet discovered. The PD diagnosis is based only on clinical symptoms such as bradikinesia, resting tremor or muscular rigidity and for this reason many patients are being misdiagnosed leading to inappropriate treatments. This proves the importance of identifying biomarkers for an accurate diagnose as well as for new treatment development. Regarding the biomarker's identification, omics strategies allows the identification of the expression profiles present in a given biological sample unequivocally, providing valuable information about signaling pathways presents in different groups of patients. Our group is interested in a research design that includes clinical features and high-resolution mass spectrometry strategies for metabolomics and proteomics as well as metagenomics approaches combined with big data mining to identify putative biomarkers of each clinical PD phenotype.

Characterization of the role of alpha-synuclein in melanoma: exploring the relationship between cancer and neurodegenerative diseases.

Mauricio Ariel Menacho Marquez
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Both, cancer and Parkinson disease (PD), are a consequence of the interaction between genes and environmental factors. The key difference is that the biological processes leading to these pathologies occur in different cellular environments, leading to cell division or death. Recent studies suggested that alpha-synuclein (AS), one of the key regulators in PD, although toxic to dopaminergic neurons, is protective for advanced melanoma cells.In our work we began to explore the biological role of AS in tumoral cells derived from melanoma. First, by bioinformatic approaches we confirmed an increased expression of AS in melanoma samples. Indeed, we found that AS expression could have prognostic value for tumor patient outcome.In our melanoma models we identified that AS was expressed at considerably high levels and that part of AS is bound to membrane. By using shRNA technologies, we were able to modulate the levels of AS in mouse and human melanoma cells. Growth studies done with these cells indicated that reduced expression of AS leads to proliferative defects. Not only melanoma cell growth was affected by a decrease in AS levels; our preliminary data also indicate that reduced expression of AS leads to changes in actin cytoskeleton architecture and tubulin fibers organization. Interestingly, cells with reduced level of AS showed increased mitochondrial activity/number as observed by cytoplasmic formazan deposits.Altogether, our data indicate a putative role for AS in processes associated to melanoma growth and development.

Protein Quality Control In The Endoplasmic Reticulum

Ron R. Kopito

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The ubiquitin proteasome system (UPS) maintains the integrity of the proteome by selectively degrading misfolded or mis-assembled proteins, but the rules that govern how conformationally defective proteins in the secretory pathway are selected from the structurally and topologically diverse constellation of correctly folded membrane and secretory proteins for efficient degradation by cytosolic proteasomes is not well understood. I will present the development of a parallel pooled genome-wide CRISPR-Cas9 forward genetic analysis using a highly quantitative and sensitive protein turnover assay to generate a fine-grained genomic map of protein quality control machinery in the secretory pathway. This analysis revealed a previously unrecognized collaboration between membrane-associated and cytosolic ubiquitin conjugation machinery. Mining this resource revealed an unexpected connection between ER protein quality control and ubiquitin fold modifier 1 (UFM1), a small, metazoan-specific, ubiquitin-like modifier involved in the biosynthetic translocation of proteins into the ER.

An exercise-related miokine protects the brain in Alzheimer models

Sergio T. Ferreira
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Defective brain hormonal signaling has been associated with Alzheimer's disease (AD), a disorder characterized by synapse and memory failure. Irisin is an exercise-induced myokine released on cleavage of the membrane-bound precursor protein fibronectin type III domain-containing protein 5 (FNDC5), also expressed in the hippocampus. We found that FNDC5/irisin levels are reduced in AD hippocampi and cerebrospinal fluid, and in experimental AD models. Knockdown of brain FNDC5/irisin impairs long-term potentiation and novel object recognition memory in mice. Conversely, boosting brain levels of FNDC5/irisin rescues synaptic plasticity and memory in AD mouse models. Peripheral overexpression of FNDC5/irisin rescues memory impairment, whereas blockade of either peripheral or brain FNDC5/irisin attenuates the neuroprotective actions of physical exercise on synaptic plasticity and memory in AD mice. By showing that FNDC5/irisin is an important mediator of the beneficial effects of exercise in AD models, our findings place FNDC5/irisin as a novel agent capable of opposing synapse failure and memory impairment in AD.

"Identifying new players involved in spatial sequestration of protein aggregates"

Thomas Nyström
Department of Microbiology and Immunology, Institute of Biomedicine, The Sahlgrenska Academy at the University of Gothenburg, Sweden

Spatial sorting to discrete quality control sites in the cell is a process harnessing the toxicity of aberrant proteins. Using a genome-wide screen, we found that the yeast t-snare phosphoprotein, syntaxin5 (Sed5), acts as a key factor in mitigating proteotoxicity and the spatial deposition and clearance protein inclusions associated with the disaggregase Hsp104. Hsp104-associated aggregates co-localize with Sed5-associated vesicles as well as components of the ER, trans-Golgi network, and endocytic vesicles, transiently during proteostatic stress explaining mechanistically how misfolded/aggregated proteins formed at the vicinity of the ER can hitchhike towards vacuolar IPOD sites. Many inclusions became associated with mitochondria and such association appears required for efficient clearance of aggregated proteins.

Alpha-synuclein acts in the nucleus and deregulates gene expression: insight into the molecular basis of Parkinson's disease

Tiago Fleming Outeiro
University Medical Center Goettingen, Goettingen, Germany

Alpha-synuclein (aSyn) is a central player in Parkinson's disease (PD) but the precise molecular mechanisms underlying its pathogenicity remain unclear. It has recently been suggested that nuclear aSyn may modulate gene expression, possibly via interactions with DNA. However, the biological behavior of aSyn in the nucleus and the factors affecting its transcriptional role are not known. Here, we investigated the mechanisms underlying aSyn-mediated transcription deregulation by assessing its effects in the nucleus and the impact of phosphorylation in these dynamics. We found that aSyn induced severe transcriptional deregulation, including the downregulation of important cell cycle-related genes. Importantly, transcriptional deregulation was concomitant with reduced binding of aSyn to DNA. By forcing the nuclear presence of aSyn in the nucleus (aSyn-NLS), we found the accumulation of high molecular weight aSyn species altered gene expression and reduced toxicity when compared to the wild-type or exclusively cytosolic protein. Interestingly, nuclear localization of aSyn, and the effect on gene expression and cytotoxicity, was also modulated by phosphorylation on serine 129. Thus, we hypothesize that the role of aSyn on gene expression and, ultimately, toxicity, may be modulated by the phosphorylation status and nuclear presence of different aSyn species. Our findings shed new light onto the subcellular dynamics of aSyn and unveil an intricate interplay between subcellular location, phosphorylation, and toxicity, opening novel avenues for the design of future strategies for therapeutic intervention in PD and other synucleinopathies.

Alpha-synuclein and prion protein: understanding the aggregation profile to search for therapeutic compounds

Yraima Cordeiro

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Transmissible spongiform encephalopathies (TSEs) and Parkinson´s Disease (PD) are neurodegenerative disorders that arise because of protein misfolding and aggregation, being the prion protein (PrP) the culprit of TSEs and alpha-synuclein (a-syn) related to PD pathogenesis. It has been described that PD shares a similar pathogenic mechanism with TSEs, i.e. abnormally folded a-syn can be transmitted from cell-to-cell and induce misfolding of native a-syn. Thus, as PD can be a prion-like disease and since there is no available treatment for TSEs and PD, we focus on the selection and characterization of compounds that modulate protein misfolding and aggregation. We stablished a cell-free protocol for amplification of recombinant PrP misfolding and conversion into scrapie-like forms and adapted the protocol for a-syn aggregation. Identification of bioactive compounds able to inhibit this conversion is a promising strategy towards therapy; besides, characterization of the final species in the presence of ligands aids characterizing the molecular mechanism of inhibition. We verified that our most promising anti-prion compound, a trimethoxy-chalcone, which efficacy was previously verified in scrapie-infected cell lines, significantly decreased rPrP conversion in this new method and was also effective against a-syn aggregation. Additionally, this method allowed us to obtain homogeneous rPrP fibrils for three-dimensional structure characterization (solid state NMR). Thus, this method provides a simplified, safe, and economical approach to screen and assess the mechanism of action of anti-prion and anti-PD compounds, as well as it can generate high-quality fibrillar species for further structural biology analysis.

PROTEIN MISFOLDING MEETING:
crossroads between biology, cancer, and neurodegeneration

ABSTRACTS | PARTICIPANTS

Heterodimer formation of WT and ALS-associated mutant Sod1: implications for aggregation, activity and ROS mediated toxicity

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ABSTRACT

Amyotrophic Lateral Sclerosis (ALS) is an age-associated disorder caused by the degeneration of motor neurons. Approximately 20 % of familial ALS (fALS) is caused by mutations in Cu/Zn superoxide dismutase (Sod1), which induce structural destabilization and/or oxidative damage leading to the misfolding and aggregation of Sod1 into a neurotoxic species. In this study, we aimed to analyze the effects of heterodimeric combinations of WT and mutant Sod1 proteins (A4V, L38V, G93A and G93C) in human cells, by using a live-cell imaging approach. Interestingly, we found that although both WT and mutant Sod1 can dimerize, the WT protein does not aggregate. In contrast, co-expression of WT and the ALS-associated Sod1 mutants resulted in the formation of a larger number of inclusions per cell than observed in cells co-expressing either WT or mutant Sod1 homodimers. The number was greater in cells expressing A4V Sod1. Strikingly, the inclusions formed between WT and A4V Sod1 were the most stable. To eliminate the contribution of endogenous Sod1, and better evaluate the effect of ALS-associated mutant Sod1 expression, we expressed human Sod1 WT and mutants in *sod1Δ* yeast cells. By using the yeast model, submitted to chronological aging, we concluded that only human heterodimers showed decreased antioxidant activity, increased oxidative damage and impacted longevity. We observed that oxidative stress induced ALS-associated mutant Sod1 aggregation, and that Sod1 heterodimers formed more inclusions than homodimers. In addition, we also found that ALS-associated mutant Sod1 reduced nuclear localization and, consequently, impaired the antioxidant response, suggesting this change in localization may contribute to disease in certain forms of familial ALS. Overall, our study provides novel insight into the molecular underpinnings of ALS and may open novel avenues for the design of future therapeutic strategies.

KEYWORDS: Sod1, Amyotrophic Lateral Sclerosis, Aging, Heterodimers

Effects of 6-hidroxidopamine on neural cells in the crab *Ucides cordatus*

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Introduction: Invertebrates have been used to study neurodegeneration due to easy manipulation and maintenance, low cost, short cell life cycle and well described central nervous systems (CNS). Specifically, crustaceans have low rate mutations, and because crabs dwell in mangroves with high concentration of metals, neuronal degeneration deserves study in this animal model. Objective: To evaluate the effects of the neurotoxin 6-hydroxydopamine (6-OHDA) on cell cultures of neurons and glial cells from cerebral ganglia in the crab *Ucides cordatus*. Material and Methods: We conducted imunohistochemistry and Western blotting to verify the content and localization of tyrosine hydroxylase (TH) and alpha-synuclein in the CNS. Next, we analyzed in cell cultures neuron (NeuN) and glial (GFAP, CNPase and IB4) markers, besides TH and alpha-synuclein in control and experimental groups (1, 3 or 5 days of exposure to 6-OHDA). Discuss and Results: *Ex vivo* results showed TH- and alpha-synuclein-positive cells in CNS regions associated with sensorial processing, motor control, memory and neurogenesis, suggesting that these molecules can modulate these functions in crustaceans. The *in vitro* study showed, in all experimental conditions, that there were less TH-positive, oligodendrocytes-like cells, and mature neurons than the time-matched control groups. We also observed more astrocyte-like, microglial-like and alpha-synuclein-positive cells than the time-matched control groups. The *in vitro* results showed a neurodegeneration standard similar to vertebrates. Conclusions: These results showed TH and alpha-synuclein in the crustacean CNS, and that 6-OHDA affects neural cells similarly to vertebrates. Therefore, *Ucides cordatus* is appropriate to study neurodegeneration, focusing on cellular physiology and evolution.
Keywords: neurodegeneration, cell culture, crustacean, 6-hidroxydopamine.

Multiple Time Scale Dynamics Depiction of Cellular Retinoic Acid-Binding Protein 2 (CRABP2) and its complex with Retinoic Acid

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Retinoic acid (RA) is the major vitamin A metabolite that regulates gene transcription through the activation of several nuclear receptors. CRABP2 enables AR to perform its biological functions by delivering it to Retinoic Acid Receptors (RARs). The goal of this work was to characterize the role of CRABP2 dynamics in the CRABP2:RA interaction using Nuclear Magnetic Resonance (NMR) as the main tool. NMR spectra were collected on ^{15}N-labeled-CRABP2$_{1-137}$ samples at 298K on Bruker 600MHz/800MHz/900MHz spectrometers. Fast and slow backbone dynamics of free and RA-bound CRABP2 were derived from ^{15}N longitudinal (R_1), transverse(R_2) relaxation rates and ^{15}N–{^{1}H} NOE, and ^{15}N Carr-Purcell-Meiboom-Gill relaxation dispersion (CPMG)-based experiments, respectively. The interaction between CRABP2 and RA was characterized by a combination of NMR titration experiments and Surface Plasmon Resonance Spectroscopy (SPR). Even though R_1, R_2 and ^{15}N–{^{1}H} NOE values were uniform throughout both apo and holo-CRABP2, CRABP2 self-association triggered by RA was an outcome of relaxation data. Holo-CRABP2 self-association interface was mapped by NMR titration experiments and residues clustered on the portal region of iLBPs (α1-loop-α2/βC-βD/βE-βF) showed chemical shift perturbations (CSPs) as a function of holo-CRABP2 concentration increase. Numerous residues of apo-CRABP2 displayed conformational exchange dynamics in the µs-ms time scale. Sixteen residues constituted the core group that showed the most similar fluctuation parameters (k_{ex} = 320 ± 40s^{-1}/p_B = 0.2 ± 0.03). A smaller subset of residues showing fast exchange fluctuation was identified in holo-CRABP2 (k_{ex} >2000s^{-1}/p_B ~ 0.05). Even though RA suppressed conformational exchange on protein segments associated to its entrance/release to the binding cavity (portal and gap regions), indicating a correlation between CRABP2 µs-ms dynamics and RA binding, the comparison of NMR titration, SPR and CPMG results suggested that CRABP2 slow backbone dynamics (µs-ms) appear to be neither related to RA binding nor to RA release from the binding cavity.

Keywords: Retinoic acid, Cellular Retinoic Acid-Binding Protein 2, dynamics, NMR.

A high-affinity DNA hairpin binds to the prion protein globular domain leading to partial unfolding and liquid phase separation

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Prion diseases are neurodegenerative disorders characterized by the accumulation of PrP^{Sc} (scrapie) in the CNS. Previously identified molecules that inhibit the accumulation of PrP^{Sc} in prion-infected cells range from small organic compounds to biological macromolecules. Any ligand that binds with high affinity and stabilizes PrP^{C} (cellular) is expected to block the cellular to scrapie conversion and prevent prion diseases. Using the SELEX technique, we selected two 25-mer single-stranded DNA aptamers (A1 and A2) that binds PrP^{C} tightly. The aim of this work was to study the structure of A1 and A2 and their interaction with PrP^{C}. A1 and A2 structure and their interaction with recombinant murine PrP^{C} C-terminal domain (PrP^{C}_{90-231}) were investigated by isothermal titration calorimetry (ITC), circular dichroism (CD), nuclear magnetic resonance (NMR), small-angle X-ray scattering (SAXS), and differential interference contrast (DIC) microscopy. By ITC, a nanomolar and a micromolar affinity were estimated for PrP^{c}_{90-231}:A1 and PrP^{c}_{90-231}:A2, respectively. For A1, this interaction is maintained even in the presence of 500 mM NaCl, highlighting its high specificity. CD and ^{1}H NMR showed evidence for Watson-Crick GC pairing in A1, which is consistent with the formation of a DNA hairpin structure. SAXS data showed that A1 adopts a globular structure with a Rg of 1.77 nm, while A2 is mostly extended. CD experiments were performed to investigate the effect of aptamer binding on PrP^{c}_{90-231} structure. Surprisingly, aptamer interaction triggered PrP^{C}_{90-231} partial unfolding, however with different degrees. DIC showed that PrP^{C}_{90-231} undergoes liquid-liquid phase separation and that A1 and A2 binding leads to a dose-dependent increase in number and reduction in size of liquid droplets (11.26 – 3.94 μm). Altogether, our results suggest that A1 adopts a compact hairpin conformation. Interaction of aptamers with PrP^{c}_{90-231} leads to partial unfolding depending on protein:DNA stoichiometry and modulates liquid-liquid phase transition.

Keywords: prion protein, DNA, aptamer, phase-separation.

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Unraveling the binding specificity of the PWWP domains of NSD3s and Pdp3 to histone tails containing different methylation marks

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NSD3 (*Nuclear Receptor Binding SET-domain containing protein 3*) is a human histone-methyl transferase frequently amplified in 15% of breast cancer cases. NSD3 is composed of a catalytic SET domain, five PHD finger domains, and two PWWP domains, responsible for NSD3 interaction with methylated chromatin. NSD3s, the short splicing isoform of NSD3, contains a single N-terminal PWWP domain and is sufficient for oncogenic transformation. Pdp3 (*PWWP domain-containing protein 3*) is a member of the NuA3b acetyl-transferase complex in *Sacharomyces cerevisiae*. Pdp3 is formed by a PWWP domain only, which bears sequence similarity to that of NSD3s. The goal of our work was to investigate the structure and binding selectivity of NSD3s-PWWP and Pdp3-PWWP to histone-derived, methyl-lysine-containing peptides. The binding affinity of recombinant, purified NSD3s-PWWP and Pdp3-PWWP to nine different 15-mer histone peptides containing different methylation marks was determined by fluorescence spectroscopy. The intrinsic fluorescence spectra of NSD3s-PWWP and Pdp3-PWWP showed maxima at ~340 nm, indicating that the tryptophan residues are relatively solvent exposed. Increasing concentrations of histone peptides led to a suppression of fluorescence spectra until saturation, suggesting that the PWWP sequence motif is part of the binding site. From binding isotherms, we estimated affinities (K_d) for each histone peptide. NSD3s-PWWP and Pdp3-PWWP displayed the highest affinity to H3K36me3-containing peptide, highlighting the role of PWWP domains as specific methylation mark readers. 2D [^{1}H, ^{15}N] HSQC spectra of NSD3s-PWWP and Pdp3-PWWP revealed features of folded proteins, including a large chemical shift dispersion of amide resonances. Nevertheless, both proteins showed low solubility at conditions ideal for NMR measurements. Thus, we are currently optimizing buffer conditions for NMR resonance assignment and structure determination. Finally, peptide binding increased the thermodynamics stability of both NSD3s-PWWP and Pdp3-PWWP.

Keywords: NSD3, Pdp3, PWWP, histone, structure, NMR.

Financial Support: CNPq, CAPES, FAPERJ, and Brown University.

Evaluation of the antioxidant activity of Mn^{2+} coordination compounds and their potential therapeutic use against alpha-synuclein aggregation

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INTRODUCTION: Parkinson's disease (PD) is a neurodegenerative disease and has been related to α-synuclein (αSyn) aggregation and oxidative stress. Although the molecular events of aSyn aggregation are still unclear, it is well-known the association of oxidative stress with PD. Aiming to reduce the damages and to increase cell tolerance against oxidative stress and its consequences, many studies have been focused on discoveries of new antioxidant compounds. Therefore, compounds that either prevent oxidative stress or inhibit αSyn toxicity are expected to constitute a potential drug against PD. **GOAL:** Evaluate the ability of four Mn^{2+} coordination compounds in protecting cells against oxidative stress and toxicity caused by αSyn aggregation. **MATERIALS AND METHODS:** Yeast cells were subjected to compounds treatments to assess cytotoxicity. Cells were previously submitted to compounds treatment before being exposed to 2.0 mM H_2O_2/1h and then plated to evaluate cells protection. To assess the cytotoxicity of compounds in human (H4) cells, the MTT assay was performed and the IC_{50} calculated. Using ThT assay, the compounds were tested for their ability to reduce αSyn aggregation *in vitro.* The capacity of compounds to reduce αSyn oligomerization and aggregation in H4 cells was measured using flow citometry and fluorescence microscopy respectively. **RESULTS:** Our results indicate that all compounds did not inhibit yeast growth after 1h of treatment. However, after 24h of treatment compounds start to inhibit yeast growth at 25 µM. MD1 and derivatives presented a strong capacity to protect the wild type cells against H_2O_2 stress. MD1 also rescues the ability to growth of *S. cerevisiae* cells expressing αSyn. In the presence of both compounds ThT fluorescence was reduced. At last but not least, MD1 reduced both alpha-synuclein oligomerization and aggregation in human cell cultures. **CONCLUSIONS:** Our results suggest that MD1 and derivatives might be a potential alternative to prevent αSyn damages.

Financial Support: CAPES, FAPERJ and CNPq
Keywords: Mn^{2+} coordination compounds, Oxidative Stress, Parkinson Disease.

Title: Microglia-Neuron Interaction in Parkinson's Disease: The Role of Cytokine CCL21

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Summary: Chemokines are cytokines with function of promoting chemotaxis of leukocytes into the blood and lymph nodes. Among them, CCL21 was described as overexpressed in the context of malignant neoplasia such as glioblastoma and neurodegenerative diseases like Alzheimer's and Multiple Sclerosis, promoting the activation of microglia at the lesion site. We intent to identify in this project the role of the CCL21 and its receptor CCR7 in the recruitment and activation of microglia during neurodegeneration in an *in vitro* Parkinson Disease (PD) model. As model we used 3,4-dihydroxyphenylacetaldehyde (DOPAL) which is a metabolite of dopamine that interacts with lysine amino acids of α-synuclein (αS) and induces its aggregation into neurotoxic oligomers. We used primary cultures of dopaminergic neurons and microglia from the mesencephalon and cortex of Swiss mice respectively and treated these microglia with conditioned medium (CM) of neurons treated previously with DOPAL. As results, we observed by MTT assay that 50μM of DOPAL could reduce 50% of neurons viability with no effect on microglial viability and confirmed by TUNEL assay that these neurons were being taken to an apoptosis pathway. When treated with DOPAL, microglia had a 30% increase in phosphorylated αS expression and neurons had a significant increase in CCL21 expression, both seen by immunocytochemistry. We observed by BrdU incorporation and transwell assays that CM of neurons treated previously with DOPAL increases microglial proliferation on 20%, migration on 300%, and induces microglial activation to a proinflammatory phenotype (M1). Those effects are lost when the CM is incubated with both neutralizing Anti-CCL21 or Anti-CCR7 antibodies. We observed by immunocytochemistry that primary mice and human microglia from brain tissue of neurosurgery patients from University Hospital Clementino Fraga Filho, express the receptor CCR7. Although the results are still preliminary, they suggest that CCL21/CCR7 pathway should be involved in neuron-microglia interaction in PD.

Proteomics reveals protein patterns related to the resistance of gastric cancer to perillyl alcohol

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Introduction: Gastric cancer (GC) is the fifth most common malignant neoplasia and the third leading cause of cancer death worldwide. One of the major challenges for GC treatment is in dealing with the development of drug resistance, urging the need for novel chemotherapeutic agents. Perillyl alcohol (POH), a naturally occurring monoterpene, has shown promising antitumor response in different types of cancer, such as breast, pancreas, and lung. Even though a noticeable improvement in health has been reported by several clinical trials in different types of cancers, eventually tumors obtain resistance to POH and ultimately leading to death. Here we investigate alterations that make gastric cancer cells resistant to POH.

Material and Methods: To achieve this, we generated a resistant gastric cancer cell culture by gradually increasing the concentration of POH in the cells medium. We then used a Q-Exactive mass spectrometer to compare the proteomic profiles of the resistant culture obtained from the medium with POH, a resistant culture cultivated in a medium without POH, and non-resistant gastric cancer cells.

Discussion and results: Our results shortlisted several proteins that could be further linked to resistance mechanisms. By referring to other works, we noticed that these same proteins were found in higher abundancy in the more aggressive gastric cancer type and in a POH-resistant glioblastoma. Some examples of proteins related to the resistance are: carbonic anhydrase 2, 14-3-3 proteins, and S100 proteins. Moreover, the resistant cell lines share a proteomic profile that is more compatible to aggressive types of cancer and to carcinogenesis processes when compared to the non-resistant cell line.

Keywords: gastric cancer, perillyl alcohol, chemo-resistance, cancer proteomics.

N-Terminal acetylation of α-synuclein affects the thermodynamic stability of fibrils and favors phospholipid membrane-induced fibrillation

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Alpha-synuclein (aSyn) is a 14 kDa natively unfolded protein abundantly expressed in neurons that is thought to play a central role in a group of neurodegenerative disorders collectively known as synucleinopathies, including Parkinson´s disease (PD). N-terminal acetylation is the predominant post-translational modification (PTM) found in aSyn in both normal and PD individuals. Nevertheless, virtually most of the studies on aSyn properties have been conducted using non-acetylated bacterially expressed protein. Herein we utilized biochemical and computational techniques to understand the impact of N-terminal acetylation on the fibrillation of aSyn stimulated or not by the presence of negatively charged small unilamellar phospholipid vesicles (SUV). Our data indicate that N-terminal acetylation significantly increased the rate of fibrillation of aSyn as well as promoted a decrease in the thermodynamic stability of the mature fibril. In the presence of SUV, N-terminally acetylated aSyn exhibited an elevated rate of fibrillation at lower lipid/protein ratio relative to the non-acetylated protein. These results were discussed in light of both the membrane binding affinity probed by circular dichroism and conformation of the protein at lipid membrane obtained by using molecular dynamic simulation. Taken together, our results show the relevance of this PTM in aggregation and amilodoigenic properties of aSyn.

Keywords: Alpha-synuclein; N-terminal acetylation; phospholipid vesicles.

The cold shock domain of the glycine-rich protein AtGRP2 shows sequence selectivity and folds upon binding its cognate DNA

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AtGRP2 (*Arabidopsis thaliana glycine rich protein 2*) is a glycine-rich, RNA-binding protein that plays a key role in flowering time regulation in *Arabidopsis thaliana*. AtGRP2 consists of an N-terminal cold shock domain (CSD) and two C-terminal retroviral CCHC-type zinc knuckles interspersed with glycine-rich regions. Despite the wealth of information on AtGRP2 function, the molecular mechanisms underlying its biological role is largely unknown. Here, we used a combination of biophysical technics to investigate the structure and systematically evaluate the binding selectivity of AtGRP2-CSD. Circular dichroism (CD) spectra of recombinant, purified AtGRP2-CSD showed a minimum at 200 nm, indicating the prevalence of a random coil structure. In contrast, 1D ^{1}H NMR spectra exhibited features of a folded protein, including a large amide chemical shift dispersion and methyl resonances below 0 ppm. 2D [^{1}H, ^{15}N] HSQC spectra revealed the presence of an unfolded state in equilibrium with the native, folded protein with exchange kinetics happening in the slow time regime. AtGRP2-CSD conformational equilibrium is shifted toward the folded state by temperature decrease and by oligonucleotide binding. Using multidimensional, triple resonance NMR, we unambiguously assigned 100% of the folded state and 48% of the unfolded state resonances. Three-dimensional structure determination of AtGRP2-CSD is currently being performed. We investigated the binding specificity of AtGRP2-CSD by fluorescence spectroscopy using a set of 25 different 7-mer DNA oligonucleotide sequences. AtGRP2-CSD maximum fluorescence emission occurred at 349 nm, indicating that the single tryptophan residue is solvent exposed. Increasing concentrations of oligonucleotides led to suppression of fluorescence spectra, suggesting that Trp37 is part of the binding site. DNA binding occurred with affinities ranging from low nM - μM, suggesting that AtGRP2 selectively interacts with certain DNA sequences. Remarkably, AtGRP2-CSD bound to cognate DNA with a Hill "*n*" coefficient of 0.4, suggesting negative cooperativity arising from folding upon binding events.

Keywords: AtGRP2, glycine-rich, RNA-binding, NMR, fluorescence
Financial Support: CNPq, CAPES, FAPERJ, and Brown University

New method for prion protein aggregation *in vitro* as a useful tool to select and study therapeutic compounds against prion diseases

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The conversion of the cellular prion protein (PrP^{C}) into the aggregated scrapie isoform (PrP^{Sc}) is the molecular basis for the transmissible spongiform encephalopathies (TSEs), incurable neurodegenerative diseases. In these disorders, PrP^{Sc} seeds its formation from PrP^{C}. While PrP^{C} is soluble and protease-sensitive, PrP^{Sc} is insoluble and partially protease-resistant. Molecules able to inhibit this conversion would be promising towards therapy. The aim of this work is the establishment of a fast and economical cell-free protocol for PrP aggregation *in vitro* which could be useful to screen and select drug candidates and to investigate their mechanism of action. We induced the *de novo* aggregation of soluble, recombinant full-length PrP ($rPrP^{23–231}$) upon shaking in the presence of guanidine and urea. We then used the aggregates formed to seed soluble $rPrP^{23–231}$ conversion under non-denaturing conditions. All the conversion processes were probed by thioflavin T (ThT) fluorescence. We analyzed the morphology of the aggregates by transmission electron microscopy (TEM) and tested their proteolytic resistance by treatment with proteinase K (PK). The seeded conversion under non-denaturing condition was reproducibly achieved with soluble $rPrP^{23–231}$ concentrations as low as 2 μM and reached plateau within 8 to 12 hours at the seed concentration of 0.02 μM. We also observed a reduction of the time to half-maximum conversion ($T_{1/2}$) as seed:rPrP ratio increased. Regarding the TEM analyses, rPrP aggregates produced in non-denaturing condition exhibited a branched, irregular shape, according to literature data. The PK digestion test indicated that rPrP aggregates produced *in vitro* are partially protease-resistant. As published by our group (Ferreira, Ascari *et al*, 2018), the anti-prion chalcone J8 significantly decreased rPrP conversion in this method. This method thus provides a useful approach to search for anti-prion compounds and assess their underlying mechanism of action without the need to work with PrP infectious form.

Keywords: prion, protein, aggregation, conversion, therapy.

Brain expressed x-linked (BEX3) and its transitions from intrinsic disorder to the formation of ordered 3D fold and liquid-liquid phase separation

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Keywords: BEX3, intrinsically disordered protein, disorder-to-order transition, liquid-liquid phase separation, RNA-binding protein.

ABSTRACT

Brain Expressed X-linked 3 (BEX3) protein belongs to a family composed of five members in humans and is highly expressed during neuronal development. In addition to be expressed only in placentae mammals, BEX3 plays a role in neuronal death and tumour development. Unlike mouse BEX3, the human homolog lacks a low complexity region close to the N-terminus spanned by 13 interleaved residues of asparagine and histidine. Biophysical techniques showed that mouse BEX3 auto-associates in an intrinsically disordered oligomer *in vitro*. Rat BEX3 shuttles to the nucleus and directly enhances *trkA* transcription promoting NGF-mediated neuronal survival and differentiation. However, there are no structural and functional studies of the human homolog. Here we show that human BEX3 can form liquid-like droplets *in vitro* while retaining well-folded regions in the presence of bacterial RNA. Performing a combination of biochemical and biophysical assays, including nuclear magnetic resonance and circular dichroism spectroscopies, SEC-HPLC, microscopy, small-angle X-ray scattering and intrinsic fluorescence of tryptophan, we found that human BEX3 undergoes disorder-to-order transition upon binding to a base-paired RNA.

Label-free Proteomic Analysis in Bone Marrow Mesenchymal Stromal Cells from patients with Myelodysplastic Syndrome revealed New Insights into the Leukemic Evolution

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Introduction: Myelodysplastic syndrome (MDS) comprises a heterogeneous group of clonal bone marrow disorders with increased risk of evolution to acute myeloid leukemia (AML). The key component of the microenvironment is bone marrow mesenchymal stromal cells (BM-MSCs). The bone marrow microenvironment has been indicated as an important contributor to MDS pathogenesis. However, the molecular and cytogenetic alterations in BM-MSCs involved in MDS pathogenesis remain unclear.

Objectives: Investigate proteins, processes and signaling pathways in BM-MSCs using high-resolution label-free proteomic approach and analyze cytogenetically BM-MSCs from MDS patients to characterize alterations involved in the evolution from MDS to AML.

Material and Methods: Bone marrow samples were obtained from 25 MDS patients and 20 healthy donors (HD). Mononuclear cells were cultured in DMEN-low glicose and FBS. The MSCs were collected after 3 passage for protein extraction and cytogenetic analyses. The proteomic analysis was performed using the label-free strategy. The peptides generated were identified by qualitative and quantitative bidimensional chromatography nanoUPLC and nanoESI-MSE experiments performed in the Synapt HDMS mass spectrometry. *In silico* analysis was conducted in MetaCore data base.

Discussion and Results: BM-MSCs from MDS patients had no clonal chromosomal abnormalities. Venn diagram analyses showed 46 proteins in BM-MSCs-MDS subtypes, suggesting a common molecular signature for BM-MSCs-MDS compared with BM-MSCs-HD. The primary biological processes altered were cytoskeletal modeling, cell cycle and immune response. c-MYC is a possible key

protein, acting as an up-regulator associated with common and exclusive proteins at initial and advanced MDS stages.

Conclusions: We conducted the first study using label-free proteomic analysis to characterize alterations in BM-MSCs in MDS pathogenesis. The primary biological processes altered in BM-MSCs were cytoskeletal modeling, cell cycle, immune response. The number of proteins that may be regulated by c-MYC increased as disease progressed. Our results suggest that BM-MSCs play an important role in MDS pathogenesis.

Keywords: Myelodysplastic syndrome, label-free proteomics, bone marrow mesenchymal stromal cells.

Neutrophil Extracellular Traps induction by Amyloid Fibrils depends on NADPH oxidase

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Neutrophil Extracellular Traps (NET) are chromatin derived structures decorated with neutrophil enzymes such elastase and myeloperoxidase. Our group has shown that amyloid fibrils (AF) are able to induce NET *in vitro*. NET formation trigged by amyloid fibrils in human isolated neutrophils is dependent on the formation of reactive oxygen species (ROS) by NOX2, an isoform of NADPH oxidase present in phagocytes. The aim of the present work is to determine whether TTR or α-sinuclein -composed amyloid fibrils induce NET formation *in vitro* and *in vivo* in mice lacking NOX2 and to dissect the role of NOX2 in this process. Amyloid fibrils were prepared by incubating TTR-A25T or α-sinuclein in PBS for 15 days. Six-weeks-old C57BL/6 wildtype (WT) and knockout (KO) for NOX2 mice were used for *in vitro* and *in vivo* experiments. For in vitro experiments neutrophils were purified from mouse bone marrow in Percoll gradient. For *in vivo* experiments 50 µg of amyloid fibrils were injected into peritoneal cavity. Four hours after injection, peritoneal cavity was washed with 3 mL of PBS-EDTA and cellular fraction were staining with Hematoxilin/Eosin for cell counting. Soluble fraction of peritoneal wash was used to measure NET formation. Our data show that amyloid fibrils were able to induce NET in murine neutrophils only in WT animal. Both WT and KO mice were able to recruit neutrophils to the same extent after fibril injection suggesting that NOX2 is not necessary for neutrophil recruitment. Interestingly, extracellular DNA, a marker of NET formation, was only found in the peritoneal wash of WT mice injected with AF, suggesting that NOX2 is important to trigger NET formation *in vivo* as well. Our data show that amyloid fibrils are able to induce NET in a NADPH oxidase dependent manner and to recruit neutrophil *in vivo*.

Palavra chave: Amyloid Fibrils, Neutrophil Extracelular Traps, NADPH oxidase
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Evaluation of recombinant prion protein interaction with Cu(II) and nucleic acids: insights into the pathophysiology of prion disorders

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Transmissible spongiform encephalopathies (TSEs), also known as prion diseases, form a group of devastating neurodegenerative disorders. Their pathology is associated with conformational changes in cellular prion protein (PrP^{C}), converting to the infectious form PrP scrapie (PrP^{Sc}). Although the PrP^{C} to PrP^{Sc} conversion mechanism is not completely understood, it is proposed that a catalyst is required to modulate the process. Nucleic acids and copper [Cu (II)] ions have been reported to affect PrP aggregation when tested separately. As interaction with the isolated ligands has been deeply investigated, we aimed to evaluate whether the combination of both ligands, DNA and Cu (II), could affect PrP conversion to scrapie-like forms and try to understand the conversion mechanisms. We used murine recombinant PrP (rPrP) to evaluate the effect of a double-stranded DNA aptamer (21-mer) with high affinity for rPrP binding and Cu(II). The influence of both ligands in the protein secondary structure was analyzed by Fourier-transform infrared spectroscopy (FTIR) and circular dichroism (CD). Complex size distribution was measured by Nanoparticle Tracking Analysis (NTA) and Dynamic light scattering (DLS). Complexes cytotoxicity were evaluated in cell culture assays. rPrP aggregate morphology was determined by transmission electron microscopy (TEM) and differential interference contrast (DIC) microscopy. Our results indicate that rPrP aggregation is induced by DNA and Cu(II) in combination. Heterogeneous aggregates are formed as a result of individual and combined ligands interaction. Complex size differences and changes in the secondary structure content of rPrP were observed after the addition of both molecules, and these alterations depend on the order of ligand addition. Cytotoxicity are still under evaluation. Although we can provide preliminary evidence that DNA and Cu(II) interact at the same time with rPrP, we still need to prove the formation of PrP:Cu(II):DNA ternary complexes.