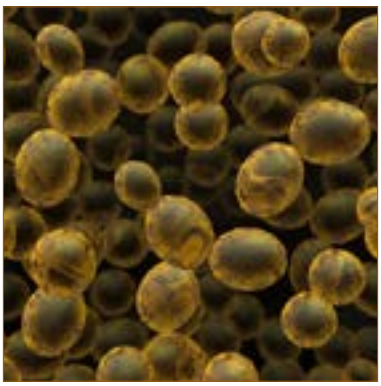
Scientific Tracks & Abstracts
May 14, 2018

Yeast Congress 2018



World Yeast Congress

May 14-15, 2018 | Montreal, Canada

Lack of G1/S control in *swi6Δ* mutants destabilizes the genome of *S. cerevisiae* via replication stress-induced DSBs and Rad51-mediated illegitimate recombination

Skoneczna Adrainna
IBB PAS, Poland

The protein *Swi6* in *Saccharomyces cerevisiae* is a cofactor in two complexes that regulate the transcription of the G1/S transition genes. It also ensures proper oxidative and cell wall stress responses. Our previous study identified *SWI6* among genes linked to oversensitivity to radiomimetic zeocin, i.e., genes important for surviving double-strand break (DSB) stress. The *swi6*Δ/*swi6*Δ strain belongs to a very limited group of knock-out strains with high sensitivity to DSBs induced both chemically and by the *in vivo* overexpression of homing endonucleases. This group also comprises strains lacking XRS2 or RAD52, whose products are crucial in DSB repair. Moreover, one of our previous whole-genome screens also identified the *swi6*Δ/*swi6*Δ strain as a spontaneous mutator, indicating an important role of *Swi6* in maintaining genome stability not only under genotoxic stress but also during unperturbed cell growth. Results we have got recently showed that *swi6*Δ mutants are genetically unstable and the source of this instability is the replication block that leads to double-strand break (DSB) formation. The cellular pathway that enables the repair of replication-born DSBs is the Rad51-dependent illegitimate recombination. Using this repair pathway increases the chance to survive DNA damage because it allows replication to resume. However, it also leads to genome rearrangements, which subsequently generate the division problems, which again leads to poor growth and increased mortality. We also noticed the differences between *swi6*Δ haploid and *swi6*Δ/swi6Δ diploid yeast cells in the molecular outcomes of replication block, which are not limited to different scenarios of replication block resolution but include different adverse effects of the absence of the *Swi6* protein in haploid vs. diploid cells on mutation frequency in the forward mutation assay. The overexpression of *SWI4* or PAB1 partially suppresses the *swi6*Δ cells oversensitivity to genotoxic agents. However, only overexpression of one of them can overcome another *swi6*Δ mutation phenotypic hallmark; the DNA content aberrations can be cured only by the overproduction of *SWI4* and not by PAB1.

Speaker Biography

Skoneczna A has completed her PhD from Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Poland. She is the Professor of Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland. She leads her group in the Laboratory of Mutagenesis and DNA Repair. She has over 30 publications that have been cited over 460 times, and her publication H-index is 12 and has been serving as a reviewer of reputed journals, as well as in National Science Centre and The National Centre for Research and Development.

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Heterologous expression of cyclic nucleotide-metabolizing enzymes for drug discovery using *Schizosaccharomyces pombe* and PKA-repressed reporters

Charles S Hoffman
Boston College, USA

The fission yeast *Schizosaccharomyces pombe* cAMP/PKA pathway is ideal for chemical genetics as it is not essential, thus allowing one to replace either the *S. pombe adenylyl* cyclase (AC) that produces cAMP or the phosphodiesterase (PDE) that hydrolyzes cAMP with genes encoding related proteins from other organisms. Our strain collection includes strains expressing 15 of the 21 mammalian PDE genes, all 10 of the mammalian AC genes, and both wild type and mutationally-activated forms of the human GNAS Gα that stimulates the activity of the mammalian transmembrane ACs. In addition, the *S. pombe fbp1* gene is transcriptionally-repressed by PKA such that an *fbp1-ura4* reporter can be used to detect PDE inhibitors by their ability to confer 5FOA-resistant growth, while *fbp1*-GFP and *fbp1*-luciferase reporters can be used to detect AC and/or GNAS inhibitors that confer increased reporter expression. One advantage of this screening platform is that compounds identified in these screens are cell permeable. In the case of the PDE inhibitors, hit compounds must be highly selective for binding as a promiscuously-binding compound would likely inhibit cell growth. Prior screens for PDE inhibitors have identified PDE4 and PDE7 inhibitors that display anti-inflammatory activity in mammalian cell culture, a PDE4/7 inhibitor that induces apoptosis in CLL cells, a PDE4/8 inhibitor that elevates testosterone production by Leydig cells, and a PDE11 inhibitor that elevates cortisol production by adrenocortical cells. Our most recent HTS has been for inhibitors of GNAS or AC9, as the mutationally-activated is found in McCune-Albright patients, as well as in many patients with pancreatic intraductal papillary mucinous neoplasms and associated adenocarcinomas. Current efforts are underway to profile the activity of these putative AC and GNAS inhibitors.

Speaker Biography

Charles S Hoffman received an SB in Life Sciences from MIT and completed his PhD in Molecular Biology and Microbiology from the Tufts University Sackler School of Graduate Biomedical Sciences. He has conducted his Post-doctoral studies at the Harvard Medical School, Department of Genetics, where he began his studies of glucose/cAMP signaling and transcriptional regulation of the *fbp1* gene in *Schizosaccharomyces pombe*. He has been a faculty member of the Boston College, Biology Department since 1990, and has published more than 60 papers and book chapters. He is an Associate Editor for Current Genetics and *G3 Genes, Genomes, Genetics*, and is a Member of the Luxuriant Flowing Hair Club for Scientists and the Scotch Malt Whisky Society of America.

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Macroporous materials for biodegradation of phenol derivatives

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Chlorophenols (CPs) and cresols are used as a precursor in the production of higher chlorophenols and dyestuffs, and as a preservative. The taste and odour thresholds levels of CPs in water are 0.1µg/L and 10µg/L, respectively. Contamination levels of CPs in ground water are located in the range 0.15-100mg/L. The EU has set maximum concentration limits for pesticides and their degradation products of 0.1µg/L and 0.5µg/L, respectively. People working in industries which produce textiles, leather products, domestic preservatives, and petrochemicals are most heavily exposed to CPs. The International Agency for Research on Cancers categorised CPs as potential human carcinogens. Also CPs are very hazardous to the environment and animals. Existing multistep water purification processes for CPs such as hydrogenation, ion exchange, liquid-liquid extraction, adsorption by activated carbon, forward and inverse osmosis, electrolysis, sonochemistry, UV irradiation, and chemical oxidation are not always cost effective and can cause the formation of more toxic or mutagenic derivatives. Bioremediation of CP derivatives applying microorganisms results in 60 to 100% decontamination efficiency and the process is more environmentally friendly compared to existing physicochemical methods. Microorganisms immobilised onto a substrate show many advantages over free bacteria systems, such as higher biomass density, higher metabolic activity, and resistance to toxic chemicals. They also enable continuous operation, avoiding the requirement for biomass-liquid separation. *Pseudomonas sp., Rhodococcus sp., Acinetobacter sp. and Bacillus sp. illustrated* a high capacity to degrade phenol derivatives and other nondegradable organic compounds. The work is devoted to the development of a bioremediation system for phenol derivatives based on macroporous materials, which can be used efficiently for wastewater treatment. Conditions for the preparation of the macroporous material from specific bacterial strains (*Pseudomonas mendocina* and *Rhodococcus koreensis,* Acinetobacter sp and Arthrobacter chlorophenolicus) were optimised. The concentration of bacterial cells was kept constant; the difference was only the type and concentration of crosslinking agents used e.g. glutaraldehyde, or novel polymers (0.5 to 1.7 wt/v %). SEM image analysis of the material indicated a monolithic macroporous structure; 4-chlorophenol (4CP), 2-chlorophenol (2CP), m-cresol and phenol were chosen as a model system to estimate the enzymatic activity of the cryogel. The viability of the cross-linked bacteria was checked using Live/Dead assay and Laser Scanning Confocal Microscopy and colorimetric assay MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), which revealed the presence of viable bacteria with the novel cross-linkers, whereas the control material cross-linked with glutaraldehyde, contained mostly dead cells. Degradation rates of the CPs, phenol and m-cresol are compared with free bacteria. Elasticity modulus of macroporous materials was evaluated using Texture analyser. Efficiency of free bacteria suspension and macroporous material based on bacteria was tested on degradation of phenol derivatives. Monitoring the turbidity of the solution at 600nm were concluded that most of free bacteria died within 6-12h due to high toxicity of 4CP and 2CP, however commercial strains were resistant to p- and m-cresols and were therefore exploited without additional adaptation. CP resistant strains were grown in minimal salt media (MSM) within two weeks. The 3-D-bioreactors based on these bacteria and free bacterial suspension (*Pseudomonas mendocina, Rhodococcus koreensi*, Acinetobacter sp. and Arthrobacter chlorophenolicus A6) were used for 4CP, 2CP and m-cresol degradation in batch mode at an initial concentration of 50 mg/L in MSM at 30oC. The concentrations of the phenol derivatives were estimated using spectrophotometric assay using aminoantipyrine and K3[Fe(CN)6] at alkaline

conditions, and by HPLC analysis (C18 column, methanol/ aqueous acetic acid 1%, 75/25 at flow rate of 1 ml/min) HPLC data did not indicate the appearance of side products of degradation of phenol and its derivatives such as catechol and 3.4-dihydroxybenzoic acid. The immobilised bacteria can be reused several times, which opens the opportunity for developing cost-effective processes for wastewater treatment. *Pseudomonas mendocina, Rhodococcus koreensi* and Acinetobacter sp. we're not effective for 2CP and 4CP degradation (18-40% over 20-25 days), however it were efficient for complete decomposition of phenol and cresol.

Speaker Biography

Berillo Dmitriy is the Maria Curie Research Fellow at School Pharmacy & Biomolecular Sciences at University of Brighton (UK) (since September 2016). Dr Berillo was a visiting PhD student and then the postdoctoral researcher at Biomaterials and Biosensors group at Lund University (Sweden) in 2008-2009 and 2010-2014, respectively. Dr Berillo main research interest is the preparation of 3D-scaffolds for regenerative medicine. His work was related to cryogels preparation based on noncovalent interactions: polyelectrolyte complex formed between chitosan-gelatin; self-assembly of Fmoc-diphenylalanine into nanofibers under cryoconditions; scaffolds based on metal-polymer coordinated complexes; enzymatically cross-linked casein and gelatin under cryoconditions, stimuli-responsive cryogels, which have potential for biomedical application. Dr Berillo developed a synthetic nontoxic polymer for mild crosslinking of bacterial cells and a preparation of conditions for bacteria immobilization into cryogels. The project resulted in a method of 3D-bioreactor preparation, which can be used for several biotechnological processes. He is working under a water treatment from heavy metal ions using cryogels. Since January 2015 to September 2016, Dr Berillo hold a position of Senior researcher at Laboratory of Biosensors and Bioinstruments at Nazarbayev University (Astana, Kazakhstan), where he focused on electrodes modification with biorecognition elements (mAb MPT64, mAb CEA, mAb IL6, mAB ECPKA and aptamers to MPT64). The ultimate purpose of the project was the adaptation of various types of biosensors (SPR Biocore X100, SPR (SPIRIT), Impedance, Quartz crystal microbalance and Capacitive Biosensors) for early diagnosis of breast cancer and *M. tuberculesis*. He was awarded the Maria Curie Research Individual Fellowship in 2016. The Maria Curie project is related toenviromental microbiology for purification of water from stable organic toxic compounds (phenols, chlorophenols and cresols) using 3D-bioreactor composed of structured alive bacterial cells.

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The roles of checkpoint related protein phosphatases and regulators in regulating morphogenesis and virulence in *Candida albicans*

Jinrong Feng
Nantong University, China

Phosphorylation and dephosphorylation of the checkpoint kinase CaRad53 is crucial for fungal cells in response to genotoxic stresses. The catalytic subunit CaPph3 of protein phosphatase 4 (PP4) forms a complex with the regulatory subunit CaPsy2, which dephosphorylates activated CaRad53 during adaptation to and recovery from MMS-mediated DNA damage. We show here that the N-terminal Y33A mutation of CaPsy2 blocks the interaction between CaPph3 and CaRad53, the deactivation of CaRad53 and the morphologic switch in recovery from genotoxic stress. In *Saccharomyces cerevisiae*, the ScPph3-ScPsy2-ScPsy4 complex functions to dephosphorylate γH2A. Here, we also show that CaPsy4 is a functional homolog of ScPsy4, but not involved in the deactivation of CaRad53 or CaHta, the ortholog of H2A. However, deletion of CaPSY4 causes *C. albicans* cells a sensitivity to genotoxic reagents and a defect in DNA damage-induced filamentation. In S. cerevisiae, ScTip41 and ScTap42 are two regulators of CaPph3. In *C. albicans*, we show that deletion of CaTIP41 causes cells to be sensitive to DNA damaging agents, MMS and cisplatin. In addition, cells lacking CaTIP41 show a delay in the recovery from MMS-induced filamentation to yeast form, decreased total PP2A activity and a defect in deactivation of CaRad53 during recovery from DNA damage. We also show that CaTip41 interacts with CaPph3, CaPsy2 or CaTap42. And deletion of CaTIP41 promotes the interaction between CaTap42 and CaPph3. Finally, *C. albicans* cells lacking CaPPH3, CaPSY2, CaPSY4 or CaTIP41 and CaTAP42, and the cells carrying the Y33A mutation of CaPSY2, show increased virulence to mice. Therefore, CaPph3 and its regulators play negative roles in regulating the DNA damage-induced filamentation and the virulence in *C. albicans.*

Speaker Biography

Jinrong Feng has completed his PhD from Tianjin University, China. Currently, he is an Associate Professor at School of Medicine, Nantong University. He has mainly focused on checkpoint related protein phosphatases in *Candida albicans*. He has published over 10 papers in reputed journals.

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Antifungal photodynamic therapy: An overview

Wanessa de Cássia Martins Antunes de Melo
Faculty of Guanambi, Brazil

Fungi have become increasingly recognized as major pathogens in critically ill patients. The incidences of superficial and systemic fungal infections have increased markedly and caused a public health problem. Some factors may contribute to rise the occurrence of fungal infections, highlighting that antifungal treatment is limited to a very small number of drug substances; in many cases the treatment is ineffective, especially, due the arising of resistant fungal strains; very often the treatment is prolonged and serious side effects and drug–drug interactions are common. These problems have renewed the search for alternative treatment modalities, and antimicrobial photodynamic therapy (aPDT) seems to be a potential candidate. Several studies have been show that dermatophytes and yeasts can be effectively inhibited in vitro and *in vivo* by aPDT, causing a minimal damage to host cells. This therapy employs a photosensitizer (PS), visible light, and molecular oxygen to produce cytotoxic reactive oxygen species (ROS) that lead to apoptosis. So, my presentation aims to raise awareness of this area of research, which has the potential to make a significant impact in future treatment of fungal infections.

Speaker Biography

Wanessa Melo has completed her PhD at the age of 29 years in Science with especial focus in microbial infection at Universidade de São Paulo-Brazil. Part of her PhD was realized under Dr. Michael Hamblin supervision at Massachusetts General Hospital and Havard Medical School – Boston-MA. Her postdoctoral was performed at Universidade Estadual de São Paulo – Brazil, evaluating the activity of photodynamic therapy against fungal biofilms. Currently, she is professor-research at Faculdade de Guanambi- Brazil, where she develops several studies in photodynamic therapy area.

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Study on Yeast Lifespan for Aging- and Calorie Restriction- related Genes

Hyoung-Sam Heo[1] and **Hae Young Chung**[2]
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Aging is a physiological process caused by time-dependent, progressive changes in multiple biological systems, which induces the increased incidence of age-related diseases. Among anti-aging strategies, calorie restriction (CR) is a widely accepted anti-aging paradigm. Recently, high-throughput technologies are applied to find aging/CR-associated genes. Given that high-throughput methods generate huge amounts of data, it is necessary to study how these CR-related bio-molecules work, interact, and exert their influence in terms of systemic view. Here, we propose database of aging and calorie restriction (CR) related genes. We first screened mouse genes that are related to both aging and CR. We then investigated the orthologs of the common genes in yeast and used these results to confirm and measure functions and life-spans using yeast knockout strains. We suggested a systematic framework and database for further understanding of aging process.

Speaker Biography

Hae Young Chung has completed his PhD at the age of 30 years from Toyama University and postdoctoral study from University of Texas Health Science Center at San Antonio. He is the director of Molecular Inflammation Research Center for Aging Intervention. He has published more than 400 papers in reputed journals and has been serving as an editorial board member of AGE.

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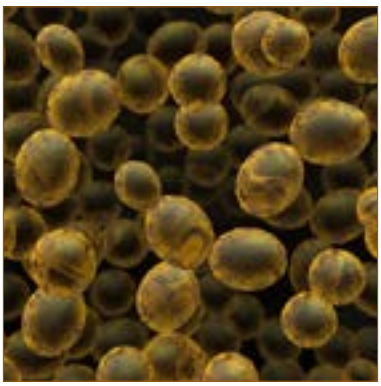

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Telomere position effect epigenetic conversions and paused replication forks

Krassimir Yankulov, Hollie Rowlands, Ririthiv Dhavarasa and **Ashley Chen**
University of Guelph, Canada

The transmission of epigenetic marks on histones and DNA is an integral part of eukaryotic DNA replication. This transmission culminates in the reconstitution of pre-existing chromatin structures or, alternatively, in an epigenetic conversion of the replicated locus. The subtelomeric genes of *S. cerevsiae* can be active or "silenced" and infrequently alternated between these two states. This phenomenon is referred to as Telomere Position Effect (TPE). The active or silenced states are determined by chromatin structures, which resemble euchromatin and heterochromatin in metazoans. We have a good understanding of the processes that establish and maintain these chromatin structures, but have very superficial understanding of the processes that lead to a conversion of the epigenetic state of these genes. Recently, we have developed an assay for the quantitative assessment of the frequency of epigenetic conversions at the telomeres of S. cerevisiae. We have documented that the destruction of Chromatin Assembly Factor-1 (CAF-1) or the helicase *RRM3* substantially reduce the frequency of conversions. CAF-I is a histone chaperone, which reassembles nucleosomes after the passage of the replication forks. RRM3 encodes a DNA helicase that helps the resumption of replication of paused replication forks. Of note, subtelomeric DNA contains multiple RRM3-dependent replication pausing sites. Current models suggest that both Rrm3p and CAF-1 are recruited to replication forks via an interaction with the Proliferating Cell Nuclear Antigen (PCNA, POL30) and that this interaction is regulated by the DBF4-Dependent Kinase, DDK. In this presentation, we propose to use TPE as model for replication-coupled epigenetic conversions. We will present our recent studies on the role of two kinases that phosphorylate (CDK and DDK) on the stability and activity of CAF-I.

Speaker Biography

Krassimir Yankulov has completed his PhD from the Imperial Cancer Research Fund, London, England in 1994 and also completed his Post-doc at the Amgen Institute, Toronto, Canada. Since 1998, he is a Professor at the Department of Molecular and Cellular Biology at the University of Guelph in Canada, Ontario. His main focus of research is on epigenetics in yeast. He has published over 40 publications that have been cited over 2000 times. He is serving as an Editorial Board Member of *Frontiers* in *Genetics* and of *PLoS One.*

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Specific synthesis of trehalose and polyols are protective factors against environmental stress in *Candida albicans*

J C Argüelles, R Sánchez-Fresneda, V Pujante, A Argüelles and **J P Guirao-Abad**
University of Murcia, Spain

Candida albicans still remains the most prevalent fungal pathogen in humans. The MAP-kinase HOG1 pathway plays an essential role in the pathobiology of this opportunistic yeast, including the colonization of the gastrointestinal tract in mouse or the defensive response against several environmental injuries. The latter, encompass mechanisms to face both oxidative and osmotic stress treatments. Here, we show that one of the main components of this defensive response consists of the intracellular protective accumulation of the non-reducing disaccharide trehalose and two polyols, glycerol and D-arabitol, an accumulation that occurs in a stress-specific dependent manner. Thus, oxidative exposures promoted a marked increase in both trehalose and D-arabitol in the wild type strain, RM-100 (and several standard genetic backgrounds), whereas the glycerol content remained virtually unaffected with respect to basal (untreated) levels. In contrast, osmotic challenges induced the significant storage of glycerol accompanied by minor changes, or even a slight drop, in the intracellular content of trehalose and D-arabitol. We examined the hypothetical role in this process of the MAP kinase Hog1, which regulates the protective responses in C. albicans against both oxidative and osmotic stress. Interestingly, unlike glycerol synthesis, the stress-induced trehalose accumulation was always Hog1-independent, whereas the ability to synthesize D-arabitol was only partially dependent on a functional Hog1 pathway, at least under our experimental conditions.

Speaker Biography

J C Argüelles has completed his PhD in Biology (1987) at the University of Murcia and Post-doctoral studies from Institute of Biomedicine (CSIC, Madrid, Spain) and from the Laboratory of Molecular Cell Biology at the Catholic University of Leuven (Belgium). He is currently working as Professor of Microbiology and has published more than 50 papers in reputed journals and has been serving as an Editorial Board Member. Furthermore, he is also engaged in the Social and Humanistic Features of Science, has published two books on Scientific Historiography; participated on Forums on the Dissemination of Science and is a writer of popular science articles in some leading newspapers.

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Regulation of the mitochondrial functions by phosphorylation in the yeast *Saccharomyces* cerevisiae

Lemaire Claire
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The mitochondrion is an organelle of which the most important function is to provide energy to the cell generated by oxidative phosphorylation catalyzed by the respiratory enzymes. In humans, deregulation of mitochondrial functions, particularly with regard to the respiratory chain, is associated with several pathologies. The activity of the respiratory enzymes may be modulated in response to metabolic demand and various types of stress. Several levels of regulation may be conceived, including post-translational modifications such as phosphorylation. The steadily increasing number of identified mitochondrial phosphoproteins suggests that reversible protein phosphorylation could be an important level of regulation in mitochondria. However, this hypothesis cannot be tested without quantitative data on variations in the abundance of mitochondrial proteins and their level of phosphorylation under different growth conditions. The yeast *Saccharomyces cerevisiae* is a powerful tool for studying various energetic and physiological states. We realized for the first time a quantitative study of both protein abundance and phosphorylation levels in yeast mitochondria under respiratory (lactate) and fermentative (glucose or galactose) conditions. Protein abundances were quantified using a label-free method. The phosphoproteome was analyzed quantitatively using the multiplex stable isotope dimethyl labeling procedure. Label free quantitative analysis of protein accumulation revealed significant variation of 176 mitochondrial proteins. We highlighted significant differences of the proteome between the two fermentative substrates.This study enlarges significantly the map of yeast mitochondrial phosphosites as 670 phosphorylation sites were identified, of which 214 were new and quantified. Above all, we showed that 90 phosphosites displayed a significant variation according to the medium. This proteomic and phosphoproteomic study is the first extensive study providing confident quantitative data on mitochondrial phosphosites responses to different carbon substrates in the yeast *S. cerevisiae* mitochondria. The significant changes observed in the level of phosphorylation according to the carbon substrate open the way to the study of the regulation of mitochondrial proteins by phosphorylation in fermentative and respiratory media. In addition, the identification of a large number of new phosphorylation sites show that the characterization of the yeast mitochondrial phosphoproteome is not yet completed.

Speaker Biography

Lemaire Claire is expert in the biochemistry of membrane proteins. Her scientific interests have always been focused on energy-transducing systems and in particular those evolved in organelles. She began her career in the photosynthesis field on the assembly and regulation of photosynthetic complexes (Institute of Physico-Chemical Biology, Paris). She then joined the C.N.R.S. (French National Center for Scientific Research) where she has acquired an excellent appreciation of the mitochondrial system through the study of the biogenesis of respiratory complexes in yeast and human using various biochemical and genetic approaches. These last years, she has developed a new research project with her group focusing on the regulation of the mitochondrial functions by post-translational modifications.

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Cannabinoids biosynthesis using recombinant cannabinoid synthase enzymes expressed from industrial *yeast Pichia pastoris*

Mingyang Sun, Oleg Tyurin, Jasleen Bains, Neil Von Wittgenstein, Richard Peet and **Malcolm Kavarana**
Teewinot Life Sciences Corporation, USA

The cannabinoid synthase enzymes Tetrahydrocannabinolic acid (THCA) synthase and Cannabidiolic acid (CBDA) synthase were recombinantly produced in yeast *Pichia pastoris* Mut+ strain. The coding regions of THCA synthase and CBDA synthase genes were codon optimized for *Pichia* expression. Both synthase genes were operably linked to the methanol inducible AOX1 promoter, an N-terminal alpha mating factor secretion signal, and a C-terminal 6x His-tag. These elements provide for inducible expression of the genes and simple processing/purification of the encoded enzymes. Each synthase construct was cloned into Invitrogen's pPIC3.5K plasmid. The recombinant plasmids were transformed into *Pichia* strain GS-115. *Pichia* clones transformed with multiple copies of each construct were selected based on their resistance to varying amount of geneticin concentrations. Gene copy numbers were further verified with RT-PCR. Fermentation conditions were optimized by investigating the impact of pH, temperature, methanol feed, and fermentation medium composition on cell growth and enzyme yield. The fermentation conditions were further optimized in a pilot scale, 14-liter fermenter. Based on these results, production was successfully scaled up to 500-liter fermenters. The Teewinot enzyme production system produced active THCA synthase and CBDA synthase enzymes. The THCA synthase converts chemically synthesized CBGA into ∆9-THCA and CBCA or chemically synthesized Cannabigerovarinic acid (CBGVA) into Tetrahydrocannabivarin (THCVA) and CBCVA in a bioreactor. The ratio of ∆9-THCA to CBCA and THCVA to CBCVA is dependent on reaction conditions including pH. The CBDA synthase enzyme converted chemically synthesized CBGA into CBDA, CBCA, and THCA or chemically synthesized CBGVA into CBDVA, CBCVA, and THCVA. Once again, the molar ratios of CBDA, CBCA, and THCA or the molar ratios of CBDVA, CBCVA, and THCVA produced in the bioreactor were dependent on reaction conditions such as pH. Each of the biocatalytically-produced cannabinoids was purified to greater than 99.5% purity. The identity and structure of each biocatalytically-produced cannabinoid was confirmed by HPLC, mass spectral, and NMR analysis.

Speaker Biography

Mingyang Sun has completed his Master's Degree in Synthetic Biology from Concordia University, Montreal. He is a Co-inventor of several US patents on cannabinoid biosynthesis and the Vice President of Teewinot Laboratories Inc, subsidiary of Teewinot Life Sciences Corporation.

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Using natural yeast isolates to understand the function of an orphan metabolite

Nicole Paczia
University of Luxembourg, Luxembourg

2-Hydroxyglutarate (2HG) is an atypical metabolite that accumulates in neurometabolic diseases as well as in certain types of cancer. The mechanisms through which 2HG leads to cell transformation or neurodegeneration remain, however, poorly understood. Compared to the research on 2HG in mammalian systems, and despite certain advantages of yeast as a model organism for biomedical research, only a very limited number of studies reported on the occurrence and metabolism of 2HG in yeast. An extensive study performed over the last three years in our lab, revealed a panoply of new findings on 2HG metabolism of Saccharomyces cerevisiae. Among those the fact that the yeast phosphoglycerate dehydrogenases Ser3 and Ser33 convert α-ketoglutarate to D-2HG in addition to their primary metabolic role, which consists in catalysing the first step of the serine synthesis way converting 3-phosphoglycerate to 3-phosphohydroxypyruvate. Our results also show, however, that the two identified D-2HG producing enzymes do not represent the only sources of this metabolite in yeast Within our study, the two dehydrogenases Dld2 and Dld3 were both shown to convert D-2HG to α-ketoglutarate in vitro. Targeted metabolome analyses and biochemical characterisation led additionally to the original finding that DLD3 is actually an FAD-dependent trans-hydogenase that converts D-2HG to α-ketoglutarate, using pyruvate as a hydrogen acceptor. Based on our findings, we were for the first time able to propose a central carbon network of Saccharomyces cerevisiae integrating the metabolite D-2HG and connecting its metabolism to the mitochondrial respiratory chain. In the present research project we aimed to further elucidate the metabolic network involved in 2HG formation and degradation in yeast. Using targeted metabolome analysis and high-throughput growth phenotyping, we analysed the accumulation of D-2HG in genotyped natural yeast isolates. The analysis of strains carrying copy number variations of the gene DLD3 confirmed that it is the main regulator of 2HG, but also showed evidence for the presence of additional regulators.

Speaker Biography

Nicole Paczia obtained her doctorate from the University of Bielefeld (Germany) in 2012, and worked as a postdoctoral researcher at the Institute for Bio- and Geosciences 1 (Research center Jülich), before starting as a research associate at the Luxembourg centre for systems biomedicine (LCSB). In 2016, Dr. Paczia was awarded a CORE Junior Fellowship by the Luxembourg National Reseach Fund (FNR), which allowed her to establish a Junior Research group within the group for Enzymology and Metabolism, headed by Dr. Carole Linster at the LCSB. She has published more than 10 papers in reputed journals, and holds two patents.

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Projections for the production of enzymes of industrial interest by *Humphreya coffeata*

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Mushrooms have been widely studied for their production of extracellular enzymes with lignin-degrading ability. Enzymes are biological catalysts of great importance at cellular level, but also, they are of great interest at industrial level since they are key for certain reactions to occur, as they increase the rate of reactions without changing the equilibrium. Typically, enzymes are produced during fermentation processes of microorganism. However, low efficiencies and high costs are usually associated with their production. Therefore, bioprospecting for new microorganism for the production of enzymes is an important topic of research. In particular, the basidiomycete *Humphreya coffeata*, a native white-rot fungus found in Colombia, has not been largely explored for its ability to produce biologically active metabolites. Given the natural growth conditions of this fungus, it is likely that lignin-degradative enzymes are produced, such as: pectinases, amylases, laccases and cellulases. With this project, we aim at exploring the ability of *H. coffeata* to produce these four lignin-degradative enzymes under submerged fermentation conditions. First, the effect on fungal biomass and enzymatic production were assessed using two different flasks geometries and four different growth media, according to literature reports. Fungal biomass production was measured by dry weight, while enzymatic activity was determined using specific protocols depending on the kind of enzyme that wanted to be evaluated. In general, the enzymatic extract of each medium was added to a substrate solution, depending on the evaluated enzyme and either change in viscosity or absorbance values were recorded. The results of these evaluations showed that the geometry of the flask did not affect enzymatic production. On the other hand, greater enzymatic activities were found for pectinases and cellulases than for amylases and laccases. In fact, for the latter enzyme, we have not been able to determine the enzymatic activity under submerged fermentation; even after evaluating the addition of different waste/by-products of food industry to the culture media. However, we found that when adding ABTS-like inductor under solid fermentation conditions, some degradation occurred, suggesting that laccases were produced.

Speaker Biography

L Carmona Saldarriaga has completed her under graduation in Process Engineering from Universidad EAFIT, Medellin, Colombia. Currently, she is pursuing her Master's Degree in Engineering and Bioprocesses at the same institution. She enjoys doing research and has worked on several projects at the University, such as: establishing the working conditions for the production of a biopolymer from *Auerobasidium pullulans.* She received an award at COLAEIQ Conference in 2016. Other projects she has worked on are mainly related to other areas such as: biotechnology, materials and chemical processes. Currently, she is an Assistant Researcher for the company Cementos Argos S.A.S, at the Alternative Materials Department.

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Notes:

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World Yeast Congress

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Humanization of yeast genes with multiple orthologous human genes reveal principles of functional divergence in paralogs

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The most deeply evolutionarily conserved human genes encode essential cellular machinery whose failures are linked to diverse diseases, from cancer to cardiovascular disease. Recent systematic studies have discovered extensive genetic polymorphism in these genes yet studying how these variations contribute to cellular function and overall human health remains a challenge. The remarkable extent to which protein-coding genes are still functionally equivalent between humans and yeast emphasizes the power even of distant organism for studying human gene function. We recently created hundreds of humanized yeast strains (>200) such that human genes can complement a lethal growth defect conferred by loss of the corresponding yeast gene with little or no effect on growth. Humanizability is not well-explained by sequence similarity between the human and yeast genes but is instead a property of specific protein complexes and pathways. We have further extended this work replacing the entire set of shared essential genes (>500 human genes) in yeast that have several co-orthologs in humans assaying for functional complementation. We find that duplicated human genes tend to differentially replace their yeast ortholog, rarely observing broad ability to replace within gene families. These results suggest that within-species paralogs do indeed diverge in function at a higher rate than between species orthologs. Thus, by extending the scope of humanization assays to include those yeast genes that have more than one human ortholog, we have successfully added 90 new human genes to our tested set (Total 310 - a 73% increase).

Speaker Biography

Aashiq H Kachroo did his PhD at the Indian Institute of Science, Bangalore, INDIA on the molecular evolution of new functions in bacteria. He did his first postdoctoral training at the University of Texas at Austin, USA with Dr. Makkuni Jayaram, studying the mechanisms of site-specific DNA recombination. In his second postdoctoral research at the University of Texas at Austin, USA with Dr. Edward Marcotte, he focused on understanding deep homologies in essential genes across vast evolutionary distances (yeast and humans) towards the development of humanized yeast. He is currently an Assistant professor at Concordia University, Montreal, Canada. His research interests span mechanisms of evolution of novel gene functions in bacteria, site-specific DNA recombination, and 'humanization' of critical pathways in yeast.

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