

Mycology and Fungal Infection

November 16-17, 2017 Atlanta, Georgia, USA

Scientific Tracks & Abstracts Day 1





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Genomics, transcriptomics, proteomics, and biochemistry of white-rot basidiomycete *Trametes hirsuta 072*

Andrey R Pavlov¹, Tatiana V Tyazhelova¹, Konstantin V Moiseenko¹, Daria V Vasina¹, Olga V Mosunova¹, Tatiana V Fedorova¹, Lilya G Maloshenok¹, Elena O Landesman¹, Sergei A Bruskin³, Nadezhda V Psurtseva⁴, Alexei I Slesarev², Sergei A Kozyavkin² and Olga V Koroleva¹

¹A N Bach Institute of Biochemistry Russian Academy of Sciences, Russia
 ²Fidelity Systems, Inc., USA
 ³N I Vavilov Institute of General Genetics Russian Academy of Sciences, Russia
 ⁴Komarov Botanical Institute, Russia

Wood-rotting fungi are organisms with the highest natural capacity to degrade lignocellulose substrates, which is enabled by complex systems of extracellular enzymes, whose expression and secretion depend on the nature of the environment. We sequenced and assembled the complete genome of the white rot saprotrophic fungus *Trametes hirsuta 072* (Basidiomycota, Polyporales) providing a framework for studies of gene transcription, translation, and secretion of essential fungal proteins. The genome sequence is assembled in 13 chromosomes and a circular mitochondrion, and it is partially annotated. Our transcriptomic and proteomic studies are focused primarily on ligninolytic oxidases and enzymes providing degradation of cellulose and hemicellulose. Our results identified seven laccase genes containing exons and introns, including the respective promoter regions. We found 18 ligninolytic peroxidase genes encoding nine putative lignin peroxidases, seven putative short manganese peroxidases, and two putative versatile peroxidases. The expression of the genes at various conditions of the fungal growth was studied on the transcriptomic and proteomic levels. Only a few genes encoding ligninolytic, cellulolytic, and hemicellulolitic enzymes were expressed in large quantities providing specific response of the fungus to the particular conditions of growth.

Biography

Andrey R Pavlov is currently working as a Research Scientist in Fidelity Systems, Inc., Gaithersburg, Maryland, USA.

ppav2002@yahoo.com



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High-throughput screening to identify regulators of meiosis-specific gene expression in *Saccharomyces cerevisiae*

Yona Kassir Technion-Israel Institute of Technology, Israel

Transcriptional regulation is a key mechanism that controls the fate and response of cells to diverse signals. Therefore, the identification of the signal transduction pathways as well as the DNA-binding proteins, which mediate these signals, is a crucial step in elucidating how cell fate is regulated, and how can we perturb it. In the talk, I will discuss bioinformatics and functional high-throughput genomic approaches. Our model system is the budding yeast *Saccharomyces cerevisiae*, and the *IME1* gene that encodes the master regulator of meiosis. High throughput technology, based on fluorescent reporters (R-SGA), allows the screening of an array of all viable yeast gene deletion mutants. This protocol promoted the identification of too many potential transcription factors, and signal transduction factors. The main problem we faced was to discriminate between false and true regulators. Bioinformatic analysis identified potential cis-regulatory sequences with perfect homology to known transcription factors (TF). However, these consensuses and their corresponding TFs were found to be nonfunctional in the R-SGA analysis. Moreover, many TFS were shown to bind to a non-perfect site. The most rewarding approach was to examine a TF only if its known upstream regulators were also found in the screen. Specific examples will be given. In conclusion, our results support the view that although bioinformatic analysis can provide a useful guide, functional assays are required for accurate identification of TF-binding site interactions in complex promoters.

Biography

Yona Kassir is currently working as a Professor Emeritus at Technion-Israel Institute of Technology, Israel.

ykassir@tx.technion.ac.il



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Prion-like properties of a yeast g protein receptor involved in regulation of mating

Tatiana Chernova¹, Aysha Rashid¹, Sindhu Subramanian¹, Yury Chernoff² and Keith D Wilkinson¹ ¹Emory University School of Medicine, USA ²Georgia Institute of Technology, USA

-protein-coupled receptors (GPCRs) are integral membrane proteins that initiate responses to extracellular Jstimuli by mediating ligand-dependent activation of cognate heterotrimeric G proteins. Ste18 is a gammasubunit of a G-protein receptor that is conserved in evolution and plays a key role in a variety of cellular processes, including pheromone-signaling pathway that is crucial for the yeast mating. We demonstrate that Ste18 possess prion-like properties. Upon overproduction, Ste18 forms detergent-resistant amyloid-like aggregates and promotes formation of [PSI⁺], a prion isoform of Sup35/eRF3. Ste18 mutants, defective in anchoring to plasma membrane, are not able to form detergent-resistant aggregates or induce [PSI⁺] prion, while a mutant, deficient in signal transduction but not in membrane anchoring, is able to do so. These data show that prion-like properties of Ste18 depend on its association with a membrane and resemble our previous results for another protein, Lsb2 (see Chernova et al., 2017 Cell Reports 18: 751-761), whose prion properties depend on association with a peripheral actin cytoskeleton. Our findings emphasize the significance of a specific intracellular location for prion formation. Ste18 is short-lived, ubiquitinated, and degraded by a proteasome. Levels of Ste18 protein are increased when proteasome function is impaired, suggesting that Ste18 may form aggregates in response to proteotoxic stress when proteasome is malfunctioning Potential involvement of prion-like aggregation in regulation of G-protein dependent signaling and yeast mating will be discussed in the light of our data and recent developments, suggesting the role of protein aggregation in diseases and in regulation of some biological processes.

Biography

Tatiana Chernova received her PhD in Microbiology from Institute of Agricultural Microbiology, Academy of Agricultural Sciences, Pushkin, St. Petersburg, Russia in 1986, and performed postdoctoral studies at University of Illinois (Chicago, USA) and Winship Cancer Center, Emory University School of Medicine (Atlanta, USA). She is an Assistant Professor at Department of Biochemistry, Emory University School of Medicine (Atlanta, USA). She has published 27 peer-reviewed papers, that are cited 2050 times, with typically more 100 citations per year in the last 10 years. Her expertise is in protein postranslational modifications (including ubiquitination), misfolding and degradation, yeast prions and glycobiology.

tcherno@emory.edu



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Scientific Tracks & Abstracts Day 2





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A comparative study on the growth of *Aspergillus* species on formulated culture media from food crop wastes and sabouraud dextrose agar

Janet Uchechukwu Itelima University of Jos, Nigeria

In developing countries where conventional media used for the isolation and growth of microorganisms are very expensive and sometimes inaccessible to researchers, there is a growing interest regarding the utilization of agro-waste materials and other organic wastes for the formulation media used for cultivating microorganisms. A comparative study was carried out to test the suitability of formulated culture media from food crop waste materials (vam, sweet potato and potato peels) and that of a conventional medium for cultivating Aspergillus species isolated from different foodstuffs. Three formulated media which included Yam Glucose Agar (YPGA), Sweet Potato Peels Glucose Agar (SPPGA) and Potato Peels Glucose Agar (PPGA) were prepared and used in comparison with Sabouraud Dextrose Agar (SDA) which is a conventional culture medium. One gram of each of the foodstuffs was disinfected using 1% Sodium hypochlorite solution (1% NaOCl) for one minute, followed by three successive rinses in sterile distilled water after which they were coarsely crushed and plated directly unto sterilized Sabouraud Dextrose Agar (SDA). To inhibit bacteria growth, antibiotic drug (gentamicin) in solution, was added to the media. The Aspergillus species isolated from foodstuffs which included Aspergillus niger, Aspergillus flavus Aspergillus tamari and Aspergillus fumigatus were aseptically inoculated in duplicate into the three different formulated culture media including SDA which served as a control. The cultures were incubated at room temperature (25°C) for five days. The diameter of the fungal isolates on both the control medium and formulated media was measured in mutual perpendicular direction to ascertain the redial growth, starting from the second day to the fifth day of incubation. The four species of Aspergillus species isolated from different food stuffs grew profusely on the different formulated media with the exception of YPGA which yielded poor radial growth of the fugal isolates. Although the percentage radial growth of each of the organism on SPPGA and PPGA did not differ significantly (p>0.05) from each other, A. niger and A. fumigatus yielded maximum percentage radial growth of (100%) each on SPPGA and PPGA, while A. flavus and A. tamari yielded (100%) each only on SPPGA. Fugal growth on YPGA gave lowest percentage radial growth of 50.7, 50.2, 48.6 and 43.5% for A. niger, A. fumigatus, A. flavus and A. tamari respectively. All the species of Apergillus yielded 100% radial growth on the control (SDA). As the formulated media, especially (SPPGA and PPGA) compared favourably with the conventional medium (SDA) in the terms of the radial growth exhibited by the different species of Apergillus, it is therefore a clear indication that they could be good alternative culture media for the cultivation of these fungal isolates.

Biography

Janet Uchechukwu Itelima has her expertise in Applied Microbiology and passion in research related to Applied Microbiology, Biotechnology, and Plant Science, lecturing, and community services. She has obtained her PhD and she is currently an Associate Professor of Applied Microbiology. She is an Academic Staff of the Department of Plant Science and Technology, Faculty of Natural Sciences University of Jos, Nigeria. She has published 35 papers both nationally and internationally. She has also written two books. She is deeply involved in motivating students on how to obtain academic excellence. She has attended workshops and conferences both nationally and internationally, where she presented papers, chaired sessions and served in advisory committee.

janetitelima@yahoo.com





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Genome shuffling of mangrove endophytic *Aspergillus luchuensis* MERV10 for improving the cholesterol-lowering agent lovastatin under solid state fermentation

Hind A Al-Zahrani, Mervat Morsy Abbas Ahmed El-Gendy and Ahmed Mohamed Ahmed El-BondLly University of Jeddah, Saudi Arabia

In the screening of marine mangrove derived fungi for lovastatin productivity, endophvtic 4s/>ergi//us *luchuensis* MERV10 exhibited the highest lovastatin productivity (9.5 mg/Eds) in solid state fermentation (SSF) using rice bran. *Aspergillus luchuensis* MERV10 was used as the parental strain in which to induce genetic variability aker application of different mixtures as well as doses of mutagens followed by three successive rounds of genome shuffling. Four potent mutants, UN6, UN28, NEII, and NE23, with lovastatin productivity equal to 2.0-, 2.11-, 4 .95-, and 2.11-fold higher than the parental strain, respectively, were applied for three rounds of genome shuffling as the initial mutants. Four hereditarily stable recombinants (F3/3, F3/7, F3/9, and F3/13) were obtained with lovastatin productivity equal to TO.8, 5 7.0, 49.7, and 11.0 m@gds, respectively. Recombinant strain F3/7 yielded 57.0 mg/gels of lovastatin, which is 6-fold and 2.85-fr>ld higher, respectively, than the initial parental strain and the highest mutants UN28 and NE23. It was therefore selected for the optimization of lovastatin production through improvement of SSF parameters. Lovastatin procluctivity was increased! 32-fold through strain improvement method included mutations and three successive rounds of genome shuffling followed by optimizing SSF factors.

Biography

Hind AA Al-Zahrani is currently working as a Research Scientist at University of Jeddah, Saudi Arabia.

hend.alzahrani.1@gmail.com



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Young Researchers Forum Day 2





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Heme promotes transcriptional and demethylase activities of Gis1, a member of the histonendemethylase JMJD2/KDM4 family

Tianyuan Wang The University of Texas, USA

The yeast Gis1 protein is a transcriptional regulator belonging to the JMJD2/KDM4 subfamily of demethylases that contain a JmjC domain, which are highly conserved from yeast to humans. They have important functions in histone methylation, cellular signaling, and tumorigenesis. Besides serving as a cofactor in many proteins, heme is known to directly regulate the activities of many proteins ranging from transcriptional regulators to potassium channels. This study reports a novel mechanism of heme regulation of Gis1 transcriptional and histone demethylase activities. We found that two Gis1 modules, the JmjN+JmjC domain and ZnF, can bind to heme specifically *in vitro*. *In vivo* functional analysis showed that the ZnF, not the JmjN+JmjC domain, promotes heme activation of transcriptional activity. Likewise, measurements of the demethylase activity of purified Gis1 proteins showed that full-length Gis1 and the JmjN+JmjC domain both possess demethylase activity. However, heme potentiates the demethylase activity of full-length Gis1, but not that of the JmjN+JmjC domain, which can confer heme activation of transcriptional activity in an unrelated protein. These results demonstrate that Gis1 represents a novel class of multi-functional heme sensing and signaling proteins, and that heme binding to ZnF stimulates Gis1 demethylase and transcriptional activities.

Biography

Tianyuan Wang is a PhD student in Department of Biological Sciences at the University of Texas at Dallas, under the guidance of Dr. Li Zhang. The Zhang lab is interested in investigating the molecular mechanisms underlying heme signaling in eukaryotic cells. She is currently focusing on the study of heme regulation on yeast transcriptional factor Gis1, which is highly homologous to the mammalian JmjC domain-containing KDM4B protein. Her research interests also include heme regulation of KDM4 subfamily demethylase activity and heme availability in lung cancer initiation and tumorigenicity. Prior to joining graduate school at the University of Texas at Dallas, she has obtained her Bachelor's degree in Biological Sciences at China Agricultural University, where she worked as a Research Assistant and was invoved in the project proteomic analysis of Arabidopsis response to environmental stress.

Tianyuan.Wang@utdallas.edu





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The hnRNP-like yeast termination factor Nab3 can employ heterologous low complexity domains in place of its essential low complexity domain

Travis Loya Emory University, USA

Many RNA-binding proteins possess domains with biased amino acid content. A common property of these low complexity domains (LCDs) is that they assemble into an ordered amyloid form, juxtaposing RNA recognition motifs in a subcellular compartment in which RNA metabolism is focused. Yeast Nab3 is one such protein that contains RNA-binding domains and a low complexity, glutamine/proline-rich, prion-like domain that can self-assemble. Nab3 also contains a region of structural homology to human hnRNP-C that resembles a leucine zipper which can oligomerize. We determined that the LCD and the human hnRNP-C homology domain of Nab3 are experimentally separable, as cells are viable with either segment, but not when both are missing. In exploiting the lethality of deleting these regions of Nab3, we tested if heterologous prion-like domains known to assemble into amyloid can substitute for the native sequence. These results suggest there are different cross-functional classes of amyloid-forming LCDs and that appending merely any assembly-competent LCD to Nab3 does not restore function or rescue viability. As LCD's are known to be mediators of RNA granule formation *in vivo*, we are also exploring the subcellular localization of wild-type and mutant Nab3's in response to sugar deprivation. Wild-type Nab3 localizes to granules during sugar deprivation, while LCD mutants show a loss of localization, showing this to be an LCD-mediated process. Analysis of Nab3 has provided insights into the diversity of LCD mediated interactions as well as a means of dissecting their function in the cell.

Biography

Travis Loya is a third year graduate student in the Biochemistry, Cell, and Developmental Biology program in the Laney graduate school at Emory University. He has participated in multiple short reviews for F1000 as well as published five manuscripts and one review article during his time in the lab of Dr. Danny Reines. He plans to graduate in 2018 and move on to an academic post-doctoral position continuing to explore the emerging field of low complexity domain containing proteins and their roles in biology.

tloya2@emory.edu



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Characterizing protein interactions promoting heme regulation of the JMJC domaincontaining protein Gis1 in yeast

Purna Chaitanya Konduri University of Texas, USA

Heme plays versatile and fascinating regulatory roles for fundamental biological processes. Heme serves as a signaling molecule for oxygen levels in yeast as heme function is entwined with molecular oxygen levels. Heme and oxygen regulate the expression of many genes in eukaryotes by modulating activity of regulatory proteins. In yeast, Gis1 is a DNA-binding transcriptional regulator belonging to the JMJD2/KDM4 subfamily of demethylases. It is highly homologous to the mammalian JmjC domain-containing protein JMJD2B, which plays an important role in histone demethylation, oxygen regulation, and hormonal signaling. Notably, recent experiments in our lab showed that heme regulates Gis1 transcriptional and demethylase activities. Biochemical studies indicate that heme binds directly to Gis1 (JmjN+JmjC domain, ZnF) and JMJD2B proteins. This study aims to dissect the molecular interactions promoting heme regulation of Gis1 activity by characterizing Gis1-interacting proteins. Our Affinity Purification Mass Spectrometry (AP-MS) studies indicate that Gis1 interacts with different sets of proteins under conditions of hypoxia, low heme, and high heme. Together, our results show that Gis1 represents a novel class of transcriptional regulators, with multiple interacting partners playing a role in mediating heme signaling.

Biography

Purna Chaitanya Konduri has completed her BE in Biotechnology from PES Institute of Technology, Bangalore, India in 2008 and MS in Molecular and Cell Biology from UT Dallas in 2014. She is currently pursuing her PhD in Molecular and Cell Biology at UT Dallas in Dr. Li Zhang's Lab. She is working on understanding the mechanism underlying heme regulation of transcription factors in yeast. Specifically, she works on characterizing protein interactions involved in heme regulation of JMJC-domain containing transcription factor Gis1. She has co-authored in a journal article accepted in Nucleic Acids Research and also in a book chaper.

pxk121430@utdallas.edu



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E-babe- bioremediation potential of fungi isolated from uranium mine in Brazil

Ednei Coelho University of São Paulo, Brazil

The Osamu Utsumi was one of the most important uranium (U) mine in Brazil and since its activities had ceased in 1995 the decommissioning process has been considered a important environmental challenge. The aim of this study was to identify and evaluate the bioremediation potential of fungal species recovered from soil, water and sediment samples collected from uranium mine. A total of 65 fungi were isolated and molecularly identified using ITS region (rDNA). *Penicillium* was the most prevalent genus isolated (53%). The characterization of the samples showed that the U concentration was high in all substrates (soil: 58 to 268 mg/kg; water: 4.46 to 1.05 mg/L; sediment: 283 to 488 mg/kg). The pH of the water samples was 3.2 and the water activity (Aa) of the soil samples was 0.98. Fourteen fungal isolates showed the U minimum inhibitory concentration (MIC) of 2000 mg/L, while 51 isolates were able to grow up to the maximum concentration tested (2000 mg/L). The uranium tolerance index showed that *Talaromyces amestolkiae* was the most tolerant species. However, *Trichoderma koningiopsis* demostrated the best U biosorption capacity, removing 5.8 mg of uranium per gram of live biomass. Our finds indicate that fungi isolated from U-contaminated sites presents great metal tolerance and high bioaccumulation capacity, which makes them potential candidates for bioremediation.

Biography

Ednei Coelho has completed his Master's degree in Microbiology at the University of São Paulo (USP), has experience in Microbiology, with emphasis on Mycology, mycotoxins, gamma radiation and electron beam, physical-chemical food analysis, HPL/CLAE analysis. Currently holds a PhD in Microbiology at the Institute of Biomedical Sciences of the University of São Paulo (ICB-USP), where he works with isolation and identification of fungi in a contaminated uranium mine, which will later be used in bioremediation.

ednei@usp.br