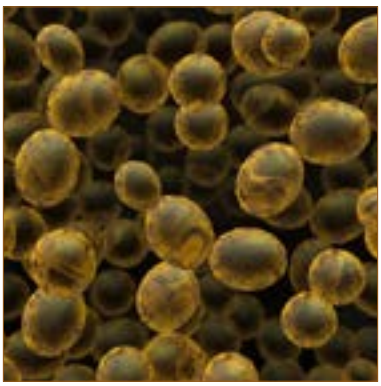
Keynote Forum
May 14, 2018

Yeast Congress 2018



World Yeast Congress

May 14-15, 2018 | Montreal, Canada

James R Broach

Penn State College of Medicine, USA

Coping with stress: Lessons from yeast

Yeast cells subjected to many different stresses elicit an acute transcriptional stress response mediated by the Msn2 transcription factor, which alters expression of both a stress specific cohort of genes as well as a common cohort of genes that changes expression in a stereotypic fashion upon exposure to any of a wide variety of stresses. We have shown by dynamic single cell analysis that stresses regulate Msn2 activity through cytoplasmic to nuclear relocalization but do so in an unusual way: stresses induce increased frequency of bursts of short-lived, recurrent periods of Msn2 nuclear localization with different stresses eliciting different patterns of bursts. Moreover, genetically identical cells subjected to an identical stress can behave quite differently. We have proposed that this idiosyncratic behavior allows populations of cells to "hedge their bet" as to what will be the optimal strategy for surviving ensuing stress. We have used computational modeling and single cell analysis to determine that bursting is a consequence of the noise in the stress signaling pathways, amplified by the small number of Msn2 molecules in the cell. Moreover, we have applied genome wide chromatin immunoprecipitation and nucleosome profiling to address how different stresses determine where Msn2 binds under a particularly stressful condition and thus what genes are regulated by that stress and how that binding affects and is affected, by nucleosome positioning and other transcription factor binding. These results provide an *in vivo* validation of indirect cooperativity of transcription factor binding, mediated by partial unwinding of nucleosomes by one transcription factor to allow access for a second transcription factor to a previously occluded binding site. Finally, we have addressed the "bet hedging" hypothesis by showing that persistence of the Msn2-mediated stress response yields cell growth arrest and have identified the targets responsible for that growth arrest. We have applied experimental evolution paradigms to address the relative fitness of cells exhibiting stochastic stress response versus those with a uniform response. In short, our results indicate that the stress response is complex and that complexity is critical for cell survival.

Speaker Biography

James R Broach is a distinguished Professor and Chair of the department of Biochemistry and Molecular Biology at Penn State College of Medicine, Director of the Institute for Personalized Medicine. He was Professor of molecular biology at Princeton University from 1984-2012, where he served as an Associate Director of the Lewis Sigler Institute for Integrative Genomics and Co-Director of the Center for Computational Biology. He has served as Chair of the Genomics, Computational Biology and Technology Study Section at NIH as well as Chair of Numerous Special Emphasis Genomics Panels. He was Co-Founder and Director of Research for Cadus Pharmaceuticals from 1992 to 2000. He is a Fellow of the American Academy of Microbiology and of the American Association for the Advancement of Science. He has published more than 175 articles in the area of Molecular Biology and Genomics and holds a number of patents in Drug Discovery Technologies.

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Makkuni Jayaram

University of Texas at Austin, USA

The yeast plasmid: A hitchhiker on chromosomes

The native 2-micron plasmid of yeast is remarkable for its nearly chromosome-like stability. This selfish DNA element is optimized for its maintenance at an average copy number of 40-60 molecules per cell nucleus. A plasmid coded partitioning system, comprised of two partitioning proteins and a cis-acting partitioning locus is responsible ensuring the equal or nearly equal segregation of replicated plasmid copies into mother and daughter nuclei. Cumulative results from a variety of genetic, cell biological and biochemical experiments suggest that the partitioning proteins promote the physical association of plasmid molecules to yeast chromosomes. This chromosome tethering is reminiscent of a similar strategy used by the episomes of mammalian gamma herpes and papilloma viruses for propagation in infected cells during long-term latency. Our analyses using fluorescence-tagged single-copy derivatives of the 2-micron plasmid suggest that plasmid sisters formed by replication tether to sister chromatids in a symmetric fashion, thus elevating the plasmid to nearly chromosome status in 1:1 segregation. We are currently mapping potential plasmid-localizing sites on chromosomes using genome-wide approaches

Speaker Biography

Jayaram's research is focused on the *Saccharomyces cerevisiae* plasmid 2-micron circle—a small, high-copy extrachromosomal selfish DNA element with chromosome-like stability. Plasmid persistence is accomplished by a deceptively simple partitioning system consisting of two plasmid-coded proteins and a cis-acting partitioning locus. The partitioning system promotes the tethering of plasmid sisters formed by replication to sister chromatids, and 1:1 plasmid segregation by a hitchhiking mechanism. Copy number maintenance utilizes DNA amplification promoted by the plasmid-coded Flp site-specific recombinase. Amplification is initiated by a replication-coupled DNA inversion reaction. Plasmid gene expression circuitry is fine-tuned for prompt amplification response when needed, without the risk of runaway increase in copy number. Our research interests span mechanisms of (a)DNA rearrangements mediated by Flp and other site-specific recombinases, (b)chromosome-coupled plasmid segregation, and (c)*in vivo* regulation of Flp levels/activity to prevent inappropriate plasmid amplification. In summary, we wish to unveil the interplay of plasmid- and host-encoded mechanisms that promote their nearly conflict-free coexistence over evolutionary times.

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Kyoungtae Kim

Missouri State University, USA

Yeast dynamin plays a key role in the Endosome-to-Golgi traffic

Yeast dynamin (Vps1) has been implicated in recycling traffic from the endosome to the *trans-Golgi network* (TGN). We previously revealed a genetic interaction of Vps1 with Ypt6 and all components of the GARP tethering complex that anchors an incoming vesicle to TGN membrane. The present study identified a 33 amino acid segment of Vps51, a GARP subunit that interacts with Vps1. Based on sequence homology between Vps51 and its mammalian homolog Ang2; we identified two key residues of Vps51, E127 and Y129 that bind Vps1. The replacement of these residues led to severe defects in endosome-to-TGN transport of Snc1, providing evidence of the physiological relevance of the interaction of Vps51 with Vps1 for the traffic. Furthermore, our functional analysis revealed that Vps1 acts upstream of Vps51 and that the absence of Vps1 resulted in defects in targeting of Vps51 and its binding partner Tlg1 to the TGN. The present study also reveals that Vps1 physically interacts with *Ypt6*. Interestingly, severe defects in retrograde trafficking caused by loss of Ypt6 were rescued by overexpression of Vps1 and vice versa. Furthermore, overexpression of Vps1 GTPase mutants was not sufficient enough to rescue abnormal Snc1 recycling in ypt6Δ cells. These results suggest that the GTP binding and hydrolysis of Vps1 is essential for this trafficking pathway and that Vps1 and Ypt6 may function parallel. Finally, this study shows that Vps1 interacts with two SNARE proteins, Vti1 and Snc2, functioning for endosome-derived vesicle fusion at the TGN, pointing to a novel role of Vps1 in the late stage of the endosome-to-Golgi traffic. Therefore, we propose that Vps1 and Ypt6 converge on the GARP tethering machinery for efficient tethering/fusion at the TGN.

Speaker Biography

Kyoungtae Kim is a Professor at Missouri State University in Springfield, MO. He received his BA and MA in Biological Science at Kyungpook National University in Taegu, Korea. He went on to obtain his PhD in Biology at Florida State University in Tallahassee, Florida, and completed his Post-Doctoral at Washington University in St. Louis, Missouri, where he studied Cell Biology and Physiology. He is now located at Missouri State University where his research focuses on Diverse Cellular Processes including Endocytic Pathway, Intracellular Trafficking of Proteins and Membranes, Membrane Organization, Nanomaterial Traffic and Nanomaterial-Mediated Global Gene Expression Pattern Changes.

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Monique Lacroix

INRS-Institute Armand-Frappier, Canada

Yeast membrane constituents and their potential beneficial effect against Colorectal cancer

Colorectal cancer (CRC) is one of the third most commonly diagnosed cancers in westerns countries. Diet and life style have important rules as preventive methods and still seem to be the most efficient approach. Consumption of diet containing agents with CRC preventive properties could reduce the risks of CRC incidence. Cancer prevention properties could be obtained via cancer cells cytotoxicity, apoptosis and antioxidant and via enzymatic modulation. QR is a phase II detoxification enzyme recognized to protect against toxic metabolites involved in the first stage of carcinogenesis process and can decrease chemical carcinogenicity compounds by transforming them to compounds with less toxicity. β-glucuronidase enzyme can release carcinogenic compounds in the colon. A reduction in its activity can lead to a reduced exposure to carcinogenic substance. *Saccharomyces boulardii and Kluyveromyces marxianus* are well-known yeasts in food industry. Their membranes are composed of insoluble (47%) and soluble glucan (36%) and mannoprotein (0.45%). Our studies showed that mannoprotein of *K. marxianus* exhibit the most relevant antioxidant activity probably due to the presence of aromatic amino acids and thiol groups but only insoluble glucan from both yeast species can induce Quinone Reductase (QR) enzyme activity. Cell wall extracts of both yeast cells, are able to inhibit the growth of HT 29 cells and colon cancer cells by more than 50% and extracts of S. boulardii show the lowest IC50 values. *In vivo* studies with rats demonstrated that ingestion of crude insoluble glucan (0·5 mg kg-1 day-1), obtained from S. boulardii cell wall exhibited colon cancer prevention properties and enzymatic modulation is one of the mechanism observed. An induction of more than 68% of the QR specific activity and a reduction of more than 50% of the β-glucuronidase activity was found. Also, a reduction of more than 45% in the total count of aberrant crypt (AC), 50% of aberrant crypt foci (ACF) and a 73% reduction of the total number of ACF containing 4-5 AC per focus in the animal colon was observed. Extraction of *S. boulardii* and *K. marxianus* yeast cell wall via simple and fast extraction can be proposed for the development of a new nutraceuticals product against colon cancer.

Speaker Biography

Monique Lacroix has completed her BSc and MSc in Food Sciences Technology in 1980 and 1982 respectively and PhD in Nutrition in 1986. She is a Professor at INRS-Institute Armand-Frappier, Canada and Director of the Research Laboratories in Sciences Applied to Food and of the Canadian Irradiation Centre. She is a Fellow of the International Academy of Food Science and Technology (IAFoST) for her outstanding representatives of international food science and technology. She received 4 awards for her most cited publications in Food Sciences and for the best 10 research Partnership with Industries for two partnerships. She has served as an expert member of several United Nations Research Networks on Food Safety and on Nano Biopolymer using Gamma Irradiation. She is also member of three Canadian networks: Canadian Food Processing Networks, Research Network on Dairy Products in Québec and of the Institute of Nutraceuticals and Functional Foods. She is author of more than 264 publications, 10 patents and 21 book chapters. Until today, she has supervised 14 Post-doc, 98 graduated students of 2nd and 3rd cycles and more than 315 trainees that come from all over the world. She has been invited to present more than 188 conferences in major congresses, including United Nations Conferences as a Member of Expert Committees.

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May 15, 2018

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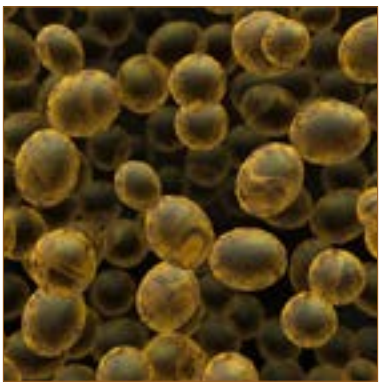

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Terrance G Cooper

University of Tennessee Health Science Center, USA

The ins and outs of nitrogen-responsive gene regulation in *Saccharomyces cerevisiae*

Yeast cells have evolved to maintain steady internal nitrogen homeostasis in the face of continuous and drastic transitions in its environmental nitrogen supply. Cells are able to take full advantage of luxurious nitrogenous environments, while retaining the ability to successfully cope with those that are more austere. This Nitrogen Catabolite Repression (NCR) sensitive control is achieved through the regulation of the GATA-binding transcription activators, Gln3 and Gat1. In nitrogen replete conditions Gln3 and Gat1 are efficiently sequestered in the cytoplasm and as a result, NCR-sensitive transcription is minimal. As nutritional conditions deteriorate, Gln3 and Gat1 relocate to the nucleus and dramatically increase GATA factor-mediated transcription of the genes required to import and catabolize poor nitrogen sources scavenged from the environment. TorC1 kinase complex was originally thought to be the principle contributor to NCR-sensitive Gln3 regulation. However, Gln3 responds to 5 distinct physiological conditions each exhibiting a unique set of regulatory requirements. This argued that NCR-sensitive control was more complex than appreciated. Using amino acid substitutions throughout the disordered Gln3 protein, we show that nitrogen-responsive TorC1 control only partially accounts for NCR-sensitive regulation. Uncharged tRNA-activated, Gcn2 kinase-mediated General Amino Acid Control (GAAC) is equally critical with the Gcn2 and TorC1 kinases functioning independently and in opposition to one another. Epistasis experiments indicate Gcn2 likely functions upstream of Ure2, whereas the 14-3-3 proteins Bmh1/2, also required for nuclear Gln3 localization, likely function downstream. Nuclear Gln3 import is also more complex than previously appreciated requiring two additional Nuclear Localization Sequences (NLS) in addition to the previously reported NLS1 as well as a newly identified Ure2 Relief Sequence. A third level of Gln3 regulation is imposed within the nucleus. In high glutamine, Gln3 exits from the nucleus in the absence of binding to its GATA targets within NCR-sensitive promoters. In contrast, as glutamine levels decrease, GATA binding becomes requisite for Gln3 to exit from the nucleus. It is only through the concerted actions of this full array of regulatory components that NCR can effectively manage intra-cellular homeostasis in the face of unreliable environments.

Speaker Biography

Terrance G Cooper investigated Avian Oil Droplets as an undergraduate and obtained his MS in Chemistry studying carboxylase enzyme mechanisms at Wayne State and his PhD at Purdue University. He first discovered that Π-oxidation occurs in peroxisomes rather than mitochondria with Magasanik at MIT. He investigated the mechanism of carbon catabolite repression in E. coli. While there he and Patricia Whitney discovered yeast urea amidolyase to be a multifunctional protein consisting of urea carboxylase and allophanate hydrolase. Moving to the University of Pittsburgh, he and his students elucidated the reactions of the allantoin degradative pathway, proposed nitrogen catabolite repression (NCR) as controlling nitrogen-responsive gene expression and he authored "*The Tools of Biochemistry*". He learned the intricacies of yeast genetics from Sye Fogel and cloning from John Carbon. His group identified, mapped and cloned and sequenced the allantoin pathway structural and four GATA-transcriptional regulatory genes. As Harriet S Van Vleet Professor at the University of Tennessee, he founded and directed the Molecular Resource Center and was Chair of Microbiology and Immunology for 15 years. His students identified the promoter structures of the NCR-sensitive genes, binding sites for their four regulatory transcription factors and now the regulatory pathways controlling Gln3 localization and intra-nuclear regulation. He served 17 years on and chaired NIH and ACS study sections, chaired the AAMC Council of Academic Societies, served on the AAMC Executive Committee, Multiple Editorial Boards and as Treasure and American Representative to the International Conference of Yeast Genetics and Molecular Biology. He is currently a member of the UT Board of Trustees.

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Kyoungtae Kim

Missouri State University, USA

Functional up regulation of ribosome biogenesis in yeast treated with silver or CdSe/ZnS nanoparticles

Nanoparticles are commercially used in everyday products including zinc sunscreen and water resistant fabrics and surfaces, but in the future they may be used in the targeted treatment of cancer, printable monitoring systems and affordable phones. Understanding the effects of nanoparticles on biological organisms is crucial for the responsible use of these technologies. We investigated the effects of silver (Ag) and cadmium (CdSe/ZnS) nanoparticles on the budding *yeast Saccharomyces cerevisiae* using growth assays, FUN-1 staining for metabolic activity, RNAseq and RTPCR. Our growth assay showed that Ag has an inhibitory effect with its concentrations above 5µg/ml, whereas SdSe/ZnS had no effect on cell growth. Interestingly, cells treated with 5µg/ml Ag showed no metabolic defects. Hundreds of the same genes in both Ag and CdSe/ZnS treated cells were differentially expressed according to our transcriptome investigation, the majority of which are responsible for ribosomal biogenesis and nucleotide binding. Furthermore, we validated the RNAseq results using an RTPCR assay. The resulting expression profile leads us to suspect that Ag and CdSe/ZnS nanoparticle exposure creates a stress environment in the cell.

Speaker Biography

Kyoungtae Kim is a Professor at Missouri State University in Springfield, MO. He received his BA and MA in Biological Science at Kyungpook National University in Taegu, Korea. He went on to obtain his PhD in Biology at Florida State University in Tallahassee, Florida, and completed his Post-Doctoral at Washington University in St. Louis, Missouri, where he studied Cell Biology and Physiology. He is now located at Missouri State University where his research focuses on Diverse Cellular Processes including Endocytic Pathway, Intracellular Trafficking of Proteins and Membranes, Membrane Organization, Nanomaterial Traffic and Nanomaterial-Mediated Global Gene Expression Pattern Changes.

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Liza A Pon

Columbia University, USA

Role for lipid droplet biogenesis and microlipophagy in adaptation to lipid imbalance in yeast and in a mouse model for human disease

The immediate responses to inhibition of phosphatidylcholine (PC) biosynthesis in yeast are altered phospholipid levels, slow growth, and defects in the morphology and localization of ER and mitochondria. With chronic lipid imbalance, yeast adapt. We find that lipid droplet (LD) biogenesis is up-regulated in yeast undergoing lipid imbalance and is required for adaptation to lipid imbalance. We confirmed that the Unfolded Protein Response, a stress response pathway that is activated by accumulation of unfolded ER proteins, is activated by this lipid stress. We also find that LDs form at ER aggregates, contain polyubiquitinated proteins and an ER chaperone, and are degraded in the vacuole by a process resembling microautophagy. This process, microlipophagy, is required for restoration of organelle morphology and cell growth during adaptation to lipid stress. Microlipophagy does not require a core macroautophagy gene, ATG7, but does requires ESCRT components. It also requires a newly identified class E VPS protein that localizes to ER and is up-regulated by lipid imbalance. In complementary studies, we detect elevated lipid droplet biogenesis, ER stress, and defects in ER proteins that are essential for excitation contraction coupling in a mouse model for a congenital muscular dystrophy produced by defects in PC biosynthesis. Using super-resolution microscopy, we find that unfolded ER proteins are associated with lipid droplets. Thus, the ER proteostasis pathway that we identified in yeast occurs in mammalian cells and may contribute to protein quality control in human disease

Speaker Biography

Liza Pon studied mitochondrial function in steroid hormone biosynthesis as a pre-doctoral student in the laboratory of N.R. Orme-Johnson at Tufts University (1982-1987). As an NRSA Postdoctoral Fellow with Gottfried Schatz at the University of Basel, she studied protein import into mitochondria (1987 -1990). Dr. Pon established her own laboratory in 1990 at Columbia University, where she is currently Professor of Pathology and Cell Biology and the Institute of Human Nutrition, and Director of the Confocal and Specialized Microscopy Shared Resource. The focal point of her research is organelle quality control, interaction of mitochondria with the cytoskeleton and other organelles, and how these processes affect cellular fitness and lifespan.

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