

28th
SMYTE
September 23-27, 2010
Heritage Village, Manesar, Gurgaon



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Heritage Village, Manesar, Gurgaon

ABSTRACT BOOK

**28th Small Meeting on Yeast Transport and Energetics
(SMYTE)**

New Delhi, INDIA

September 23-27, 2010

**Sponsored by
School of Life Sciences
Special Centre for Molecular Medicine
Jawaharlal Nehru University**

The Organizing Committee

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Conference Secretariat

Professor Rajendra Prasad

Jawaharlal Nehru University

New Delhi-110067

Email:smyte28@mail.jnu.ac.in

Programme at a glance

23RD SEPT, 2010 (THURSDAY)	
12.30 – 2.00 PM	Lunch Venue: Heritage Village
4:00 – 5.30 PM	Registration with Tea/ Coffee
5.30 – 6:00 PM	Opening Remarks
6:30 – 7:00 PM	Nico van Uden Lecture <i>Chair: Rajendra Prasad</i>
7:00 – 7:30 PM	Euro-India Co-operation <i>Phillipe de Taxis du Poët</i>
7:30 PM	Cocktail / Dinner Venue: Heritage Village

24TH SEPT, 2010 (FRIDAY)	
9:00 – 10:30 AM	Ion Homeostasis in Yeast <i>Chairs: Hana Sychrova and Antonio Pena</i>
10:30 – 11:00 AM	Tea / Coffee Break
11:00 – 1:30 PM	Environmental Sensing, Metabolism and Signalling <i>Chairs: Johan M. Thevelein and Nilanjan Roy</i>
1:30 – 2:30 PM	Lunch Venue: Heritage Village
2:30 – 5:30 PM	Transporters of Yeast and Cancer Cells-I <i>Chairs: Andre Goffeau and Richard Cannon</i>
5:30 – 7:30 PM	Poster Session Tea / Coffee Break
7:30 PM	Cocktail / Dinner Venue: Heritage Village

25TH SEPT, 2010 (SATURDAY)	
8:00 AM	Group photo and day tour, Delhi Lunch in city
8:00 PM	Cocktail / Dinner Venue: Heritage Village

26TH SEPT, 2010 (SUNDAY)	
8:00 – 10:30 AM	Membrane Transporters and Regulation <i>Chairs: Joachim F. Ernst and Anand Bachhawat</i>
10:30 – 11:00 AM	Tea / Coffee Break
11:00 – 1:30 PM	Transport and Antifungal Drugs <i>Chairs: Joachim Morschhäuser and Mukund Deshpande</i>
1:30 – 2:30 PM	Lunch Venue: Heritage Village
2:30 – 5:00 PM	Transporters of Yeast and Cancer Cells-II <i>Chairs: Milan Hofer and Suresh Ambudkar</i>
5:00 – 5:30 PM	Indo-German Research Opportunities <i>Dr. Torsten Fischer</i>
5:30 – 7:00 PM	Poster Session Tea / Coffee Break
7:00 PM	SMYTE Dinner Venue: Heritage Village

27TH SEPT, 2010 (MONDAY)	
8:30 – 10:30 AM	Structure, Function and Gene Regulation <i>Chairs: Rajini Rao and Parag Sadhale</i>
10:30 – 11:00 AM	Tea / Coffee Break
11:00 – 1:30 PM	Transporters of Yeast and Cancer Cells-III <i>Chairs: Rama Shanker Verma and Patrick van Dijck</i>
1:30 – 1:45 PM	Conclusions Invitation to SMYTE 29, Mexico
1:45 – 2:30 PM	Lunch and Depart Venue: Heritage Village

Scientific Programme

23RD SEPT, 2010 (THURSDAY)		
12.30 – 2.00 PM		Lunch Venue: Heritage Village
4:00 – 5.30 PM		Registration
5.30 – 6:00 PM		Opening Remarks
6:30 – 7:00 PM	T1	Nico van Uden Lecture <i>Chair: Rajendra Prasad</i> New Developments in Multidrug Resistance in Cancer <u>Michael Gottesman</u>
7:00 – 7:30 PM		Euro-India Co-operation <u>Phillipe de Taxis du Poët</u> First Secretary, Head of Science and Technology, Delegation of the European Union to India
7:30 PM		Cocktail / Dinner Co-sponsored by the Delegation of the European Union to India Venue: Heritage Village

24TH SEPT, 2010 (FRIDAY)		
8:30 – 10:30 AM		Ion Homeostasis in Yeast <i>Chairs: Hana Sychrova and Antonio Pena</i>
	T2	Disruption of Ion Homeostasis by Antifungal Drugs <u>Rajini Rao</u>
	T3	Charge Balance matters: Lessons from modelling potassium homeostasis <u>Maik Kschischo</u>
	T4	Real Time measurements of ion fluxes across the plasma membrane of <i>S. cerevisiae</i> and of cytosolic pH: Comparison of Trk1p and Trk2p <u>Jost Ludwig</u>
	T5	Role of Na ⁺ /H ⁺ antiporter and Na ⁺ -ATPase in <i>Candida glabrata</i> physiology <u>Hana Sychrova</u>
10:30 – 11:00 AM		Tea / Coffee Break

<p>11:00 – 1:30 PM</p>		<p>Environmental Sensing, Metabolism and Signalling <i>Chairs: Johan M. Thevelein and Nilanjan Roy</i></p> <p>T6 The peculiar behavior of <i>S. cerevisiae</i> during the late stages of wine fermentation <u>Catarina Prista</u></p> <p>T7 <i>mrg19</i> increases chronological lifespan of <i>S. cerevisiae</i> by altering glutamate metabolism <u>Nilanjan Roy</u></p> <p>T8 On the road for the identification of the IP₃ receptor in <i>Saccharomyces cerevisiae</i> <u>Rogelio Lopes Brandão</u></p> <p>T9 Chronological life-span extension is blocked by ammonium in amino acid starved cells, associated to autophagy inhibition and to cell death induction <u>Maria João Sousa</u></p> <p>T10 Nutrient sensing mechanisms for rapid activation of the PKA pathway in yeast <u>Johan M. Thevelein</u></p>
<p>1:30 – 2:30 PM</p>		<p>Lunch Venue: Heritage Village</p>
<p>2:30 – 5:30 PM</p>		<p>Transporters of Yeast and Cancer Cells-I <i>Chairs: Andre Goffeau and Richard Cannon</i></p> <p>T11 Characterizing specificities of breast cancer resistance protein ABCG2 over P-glycoprotein ABCB1: selective inhibitors and additional ABC signature <u>A. Di Pietro</u></p> <p>T12 Development of natural product and small molecule modulators of multidrug resistance-linked ABC drug transporters <u>Suneet Shukla</u></p> <p>T13 Drug discovery using fungal ABC transporters expressed in <i>Saccharomyces cerevisiae</i> <u>Richard Cannon</u></p> <p>T14 Evolution and phylogenetic relationships between permeases transporting inorganic cations in Hemyascomycetes and Zygomycetes <u>Angel Dominguez</u></p>

	T15	Heterologous expression of ABC transporters in <i>Saccharomyces cerevisiae</i> <u>Andre Goffeau</u>
	T16	Transcriptional regulation of the multidrug efflux pump MDR1 in <i>Candida albicans</i> <u>Joachim Morschhäuser</u>
5:30 – 7:30 PM		Poster Session Tea / Coffee Break
7:30 PM		Cocktail / Dinner Venue: Heritage Village

25TH SEPT, 2010 (SATURDAY)

8:00 AM		Group photo and day tour, Delhi Lunch in city
8:00 PM		Cocktail / Dinner Venue: Heritage Village

26TH SEPT, 2010 (SUNDAY)

8:00 – 10:30 AM		Membrane Transporters and Regulation <i>Chairs: Joachim F. Ernst and Anand Bachhawat</i>
	T17	Multiple roles for the transcriptional regulators Rds2 and Ert1 in <i>Saccharomyces cerevisiae</i> <u>Bernard Turcotte</u>
	T18	Evolution and Function of Novel Transcriptional Regulators in Fungal Genomes <u>K. Natarajan</u>
	T19	Role and regulation of the ABC transporter Pdr18p in yeast adaptive response to auxin-like herbicides <u>Tânia R. Cabrito</u>
	T20	Glutathione transport and utilization in fungi <u>Anand Bachhawat</u>
	T21	Regulation of protein-O-mannosylation in <i>Candida albicans</i> <u>Joachim F. Ernst</u>
10:30 – 11:00 AM		Tea / Coffee Break

<p>11:00 – 1:30 PM</p>		<p>Transport and Antifungal Drugs <i>Chairs: Joachim Morschhäuser and Mukund Deshpande</i></p> <p>T22 Antifungals and chemosensitizers to combat multidrug resistance in <i>Candida albicans</i> <u>Slawomir Milewski</u></p> <p>T23 Real-time identification of basic events underlying the action of surface-active antifungals on the yeast <i>Saccharomyces cerevisiae</i> <u>Dana Gášková</u></p> <p>T24 Antifungal drug discovery: a sticky pursuit <u>Neeraj Chauhan</u></p> <p>T25 Signature motifs in the nucleotide-binding domains of ABC transporter CaCdr1p are functionally asymmetric <u>Antresh Kumar</u></p> <p>T26 Targeting chitin metabolism for antifungal agents development: Issues and challenges <u>Mukund Deshpande</u></p>
<p>1:30 – 2:30 PM</p>		<p>Lunch Venue: Heritage Village</p>
<p>2:30 – 5:00 PM</p>		<p>Transporters of Yeast and Cancer Cells-II <i>Chairs: Milan Hoefler and Suresh Ambudkar</i></p> <p>T27 The interplay between drug resistance and virulence in <i>Candida glabrata</i> involves the ABC transporter <i>CgCDRI</i> <u>Dominique Sanglard</u></p> <p>T28 Small molecular metal chelate can overcome multidrug resistance (MDR) in cancer through alteration of redox status <u>S. K. Choudhuri</u></p> <p>T29 Structural and functional characterization of secondary drug transporter Mdr1p of <i>Candida albicans</i>: A Rational Mutational analysis <u>Khyati Kapoor</u></p> <p>T30 Signaling Interface of the Yeast Multidrug Transporter Pdr5: Role of the Q-loop Residues <u>Neeti Ananthaswamy</u></p>

	T31	The mechanism of action of multidrug resistance-linked ABC drug transporters <u>Suresh Ambudkar</u>
5:00 – 5:30 PM		Indo-German Research Opportunities <u>Dr. Torsten Fischer</u> Head of the DFG Offices DFG Office India (Lead)
5:30 – 7:00 PM		Poster Session Tea / Coffee Break
7:00 PM		SMYTE Dinner Co-sponsored by DFG-Büro Indien Venue: Heritage Village

27TH SEPT, 2010 (MONDAY)		
8:30 – 10:30 AM		Structure, Function and Gene Regulation <i>Chairs: Rajini Rao and Parag Sadhale</i>
	T32	Insights into the glucose-mediated endocytosis of yeast Jen1 lactate transporter <u>Sandra Paiva</u>
	T33	The effect of ploidy on genome stability and virulence in <i>Candida albicans</i> and <i>Candida dubliniensis</i> <u>Kaustav Sanyal</u>
	T34	Study of a putative virulence factor of <i>Candida albicans</i> that does not affect morphogenesis in <i>Candida albicans</i> <u>Parag Sadhale</u>
	T35	Quantitative Assessment of Membrane Potential Changes by Fluorescent Probe in Yeast <u>Jaromír Plášek</u>
10:30 – 11:00 AM		Tea / Coffee Break

Programme

11:00 – 1:30 PM		<p>Transporters of Yeast and Cancer Cells-III <i>Chairs: Rama Shanker Verma and Patrick van Dijk</i></p> <p>T36 Vinca alkaloids induced multiple drug resistance reversing potential of black tea polyphenols in human cancer cells <u>Yogeshwer Shukla</u></p> <p>T37 Promiscutities and specificities of MDR transporters of pathogenic <i>Candida albicans</i> <u>Nidhi Puri</u></p> <p>T38 Heterologous expression of the <i>Candida albicans</i> general amino acid permeases in yeast shows that they function both as transporters and as trasceptors <u>Patrick van Dijk</u></p> <p>T39 Specific dipeptides induce persistent signaling and deficient vacuolar sorting of the yeast amino acid transeptor Gap1 <u>Griet Van Zeebroeck</u></p> <p>T40 Identification of new transport systems for folic acid and methotrexate in normal human T-lymphocytes and leukemic cells <u>Rama Shanker Verma</u></p>
1:30 – 1:45 PM		<p>Conclusions Invitation to SMYTE 29, Mexico</p>
1:45 – 2:30 PM		<p>Lunch and Depart Venue: Heritage Village</p>

Oral Presentations

T1**New Developments in Multidrug Resistance in Cancer**

Gottesman, Michael M.

Laboratory of Cell Biology, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, Maryland, USA 20892

Cancer which has spread from its tissue of origin and can no longer be treated by surgery or radiation remains the major cause of death from this disease. Some disseminated tumors are resistant to chemotherapy (intrinsic resistance), but in many cases chemotherapy is effective in reducing the tumor burden, but all too often the cancer relapses after becoming multidrug-resistant (MDR) (acquired resistance). We and others have studied both intrinsic and acquired resistance using established lines of cultured cancer cells, and approximately 380 genes that contribute to drug resistance have been identified using this approach.

Over expression of ATP-dependent efflux pumps that can pump out anti-cancer drugs, especially ABCB1 (P-glycoprotein), ABCG2 (BCRP), and ABCC1 (MRP) are commonly seen in MDR in cultured cells. Efforts to reverse ABCB1-mediated resistance have had minimal effect in clinical trials for several possible reasons, including difficulty in measuring functional forms of P-gp and the presence in human populations of at least one major haplotype with altered substrate and inhibitor specificity. It is also likely that other MDR genes contribute to clinical drug resistance.

To evaluate the clinical role of 380 genes whose expression can cause drug-resistance in cultured cancer cells, we have developed a Taqman Low Density Array chip which gives very sensitive, reproducible, and quantitative information about mRNA levels for these genes. Using this tool, we are examining expression in human cancers and correlating these with response to chemotherapy. Preliminary results suggest that for individual cancers, specific subsets of drug-resistance genes correlate with poor response to treatment (intrinsic resistance). Many of these genes are potential targets for intervention to improve therapy. In addition, patterns of expression of drug-resistance genes are quite different between cancers from patients and cultured cancer cells, underscoring the need for better in vitro models to study drug-resistance in cancer.

References:

1. Szakács, G. *et al.*, 2006 *Nat. Rev. Drug Discov.* 5, 219-234.
2. Kimchi-Sarfaty, C. *et al.*, 2007 *Science* 315, 525-528.
3. Orina, J. N. *et al.*, 2009 *Mol. Cancer Ther.* 8, 2057-2066.

T2

Disruption of Ion Homeostasis by Antifungal Drugs

Rao, Rajini

*The Johns Hopkins University School of Medicine, Department of Physiology
725 N. Wolfe Street, Baltimore MD 21205 USA*

The limited categories of antifungal agents and emergence of resistance to existing antimycotics have prompted a search for compounds with alternative modes of action. In addition, a better mechanistic understanding of the cellular events that mediate antifungal activity would improve their therapeutic potential by guiding combination therapy with other established drugs and lead to safer and more innovative treatments. Baker's yeast offers a sophisticated toolkit of experimental approaches unparalleled among other fungal organisms. We have developed *Saccharomyces cerevisiae* as a model organism for exploring the effect of a range of antimycotic agents, including azoles, amiodarone and essential oils at a cellular and molecular level. We used compartment-specific cellular probes to follow temporal changes in metabolic activity, membrane potential, Ca^{2+} and pH as a function of toxicity. We find that a number of membrane active compounds trigger a dysregulation of ion homeostasis leading to cell death. These ionic effects were independently confirmed by genome wide transcriptional profiling and drug hypersensitivity screens of the yeast haploid deletion library. Our studies reveal a novel functional link between ergosterol and V-ATPase activity that helps explain azole toxicity and synergistic interactions between fluconazole and amiodarone. We find a striking overlap in the transcriptional response between calcium stress, inhibition of the TOR pathway and membrane active drugs such as amiodarone (an anti-arrhythmic drug) and carvacrol (a component of oregano and other essential oils). Taken together, our studies reveal that ion homeostatic pathways are excellent targets for antifungal drug development.

T3

Charge Balance matters: Lessons from modelling potassium homeostasis

Kschischo, Maik¹; Kahm, Matthias¹; Clara, Navarrete² and Jose, Ramos²

¹*University of Applied Sciences Koblenz, RheinAhrCampus, Remagen, Germany, D-53424* and ²*University of Cordoba, Avenida Menendes Pidal s/n^o, 14071 Cordoba*

Potassium is the most abundant cation in living cells and is involved in a variety of essential cellular processes including translation, endocytosis and even cell cycle regulation. Changes of external and internal K^+ concentrations change the membrane potential required for the transport of molecules across the plasma membrane, affect the pH and osmolarity of the cytosol and induce changes of the cell volume. The complex interplay between these variables is still not well understood. Therefore, a mathematical model for potassium homeostasis in *Saccharomyces cerevisiae* has been developed that covers the thermodynamic constraints on the operation of the major potassium transport systems and the proton ATPase Pma1. The model predictions for starvation experiments were compared against experimental data: For cells grown in a medium with high K^+ and shifted to K^+ free medium, a decrease of the intracellular K^+ content and cell volume was measured. Model simulations indicated that the loss of potassium is always accompanied by the uptake or intracellular production of net positive charges. These net positive charges decelerate the loss of potassium under conditions of K^+ starvation. The most likely explanation of our experiments is the uptake of NH_4^+ ions in exchange for the loss of potassium. An extended model incorporating NH_4^+ ions can quantitatively explain the data. In addition, the model predicts time courses for the membrane voltage and for intracellular pH and allows to monitor the contribution of the individual transport systems to the overall K^+ and K^+ fluxes.

T4

Real Time measurements of ion fluxes across the plasma membrane of *S. cerevisiae* and of cytosolic pH: Comparison of Trk1p and Trk2p

Ludwig, Jost¹; Holweg, Saskia¹; Kahm, Matthias²; Kschicho, Maik² and Lichtenberg-Fraté, Hella¹

¹AG Molekulare Bioenergetik, IZMB, Rheinische Friedrich-Wilhelms Universität, Bonn, Germany and ²Fachhochschule Koblenz, RheinAhrCampus, Remagen Germany

Sacharomyces cerevisiae is able to maintain relatively stable intracellular $[K^+]_i$ over a wide range of extracellular $[K^+]_o$ from a few μM up to 1 M. Uptake of K^+ is mainly mediated by the uptake systems Trk1p and Trk2p. Deletion of TRK1 leads to loss of the ability of *S. cerevisiae* cells to grow on low K, whereas deletion of TRK2 exerts only minor effects on growth. From these results it has been concluded, that Trk1p is a high affinity K-uptake system, whereas Trk2p is a low or medium affinity K-transporter. However, it is not clear, whether the observed differences between the deletion strains reflect different activities of Trk1p and Trk2p or are due to different expression levels.

To analyse the activities of Trk1p and Trk2p, we expressed TRK1 and 2 in the TOK1 locus of a *trk1,2* strain under the control of either a strong (*Candida albicans* P_{CDR1}) or a weak promoter (*C.a.* P_{CDR2}) and examined net K^+ - and H^+ -fluxes by time resolved flux measurements with ion selective electrodes. By expressing ratiometric pHluorin in these strains, we complementary monitored intracellular pH changes.

Transient K^+ -uptake accompanied by H^+ -extrusion and decrease in $[H^+]_i$ were observed with all strains ($\Delta trk1,2 tok1::P_{CDR1/2}$ -TRK1/2) after adding KCl to starved cells and energizing the cells with glucose. Both, K-uptake and H-extrusion were dependent on $[K^+]_o$, indicating that H^+ -efflux compensates K^+ uptake (and vice versa) to maintain charge balance. Surprisingly, only small differences in K^+ -net fluxes and net uptake were observed, when TRK1 and TRK2 were expressed under the control of either the strong or the weak promoter. Only at $[K]_o \geq \sim 0.5$ mM a slightly higher net K^+ -uptake was observed with Trk1p.

The results indicate that both, Trk1p and Trk2p, represent K^+ -uptake systems with similar transport properties. Thus, the observed major role of Trk1p in high affinity K^+ -uptake is obviously caused by the higher expression level of TRK1 (as compared to TRK2) in *S. cerevisiae*, and not by different properties of the two K^+ -uptake systems.

T5

Role of Na^+/H^+ antiporter and Na^+ -ATPase in *Candida glabrata* physiology

Krauke, Yannick and Sychrova, Hana

Dept. Membrane Transport, Institute of Physiology AS CR, v.v.i.Videnska 1083, 142 20 Prague 4, Czech Republic

To maintain high intracellular concentration of K^+ and low concentration of toxic Na^+ and Li^+ cations yeast species possess two types of systems to export surplus alkali metal cations, Na^+ -ATPases and Na^+/H^+ antiporters. Our *in silico* search revealed the existence of corresponding genes in all *Candida* species. *C. glabrata* is more tolerant to alkali metal cations than *S. cerevisiae*, but its genome contains only one copy of genes encoding the putative ATPase (*CgENAI*) and antiporter (*CgCNHI*).

To assess the role of these two putative transporters in *C. glabrata* physiology, 1) both genes were expressed in *S. cerevisiae* and transport properties of *CgEna1* and *CgCnh1* transporters were characterized in detail; 2) *C. glabrata* ATCC2001 knock-out mutant strains were constructed and their phenotypes tested; and 3) expression of both genes upon various conditions was estimated. In *S. cerevisiae*, both transporters had a broad substrate specificity, however, the *Cnh1* antiporter was not an efficient exporter of Li^+ what is in contrast to most other yeast Na^+/H^+ antiporters. *C. glabrata* mutants were viable, the *cnh1Δ* mutant showed a reduced tolerance to high external concentration of K^+ but not of Na^+ . On the contrary, the *ena1Δ* strain was sensitive to Na^+ but not to surplus K^+ . Cation efflux measurements confirmed the diminished ability of *cnh1Δ* to export potassium, and the efflux of Na^+ was reduced in *ena1Δ* cells. Observed mutant phenotypes were confirmed by reintegration of corresponding genes to the *C. glabrata* genome. RT-PCR results showed that the expression of *CgENAI* is highly regulated whereas *CgCNHI* is expressed at low and constitutive level. Obtained results suggest that the role of *Nha1/Cnh1* antiporters and *Ena* ATPases is different in *S. cerevisiae* and *C. glabrata*. In *S. cerevisiae*, both transporters cooperate and are involved in efflux of Na^+ and K^+ , whereas in *C. glabrata* they fulfil more specialized roles, *CgCnh1p* being important for potassium homeostasis and *CgEna1p* for sodium and lithium detoxification.

This work was supported by MRTN-CT-2004-512481 and MSMT LC531.

T6

The peculiar behavior of *S. cerevisiae* during the late stages of wine fermentation

Viana, Tiago Loureiro-Dias; Maria C. and Prista, Catarina
CBAA, ISA-UTL, 1349-017 Lisboa, Portugal

During wine fermentation, yeasts are submitted to a wide gradient of stress situations. After a short stage of active duplication, yeasts reach soon the stationary phase and most of the fermentation is carried out by resting cells. As fermentation proceeds, sugar concentration decreases and ethanol concentration increases, pH drops and temperature rises enhancing the deleterious effect of the other forms of stress. While transforming must into wine, the physiological state of the yeast suffers important changes.

A *S. cerevisiae* strain selected from a commercial starter was inoculated in white grape must. The fermentation proceeded at 15°C, 30°C and 25°C (commonly used for white wine and red wine production and lab standard studies, respectively) up to the point when no sugar was detected by standard methods. Five situations throughout fermentation were selected to collect cells.

The efficient performance of the cells was accessed by measuring cell viability and metabolic activity, pH_{in} and H^+ extrusion and H^+ influx through the plasma membrane, as well as the effect of ethanol on the last two parameters.

At the lower fermentation temperature, cells exhibited the lowest pH_{in} ($\text{pH}_{\text{in}} \approx 5$), inability to extrude H^+ and very low H^+ permeability. At higher fermentation temperatures, this effect was less evident. Nevertheless, for the three temperatures used, the effect of ethanol on H^+ permeability was always less evident in stationary phase cells.

Our results reinforce the idea that at late stages of fermentation, cells behave amazingly as compared with the well-recognized behavior of *S. cerevisiae*. “Older” cells are able to cope with extreme stress conditions deleterious to “younger” cells without significant loss of viability and keeping their capacity to ferment grape must.

This work was partially financed by Project POCTI/AGR/47891/2002, T.Viana is PhD fellow (SFRH/BD/65236/2009) FCT Portugal.

T7

***mrg19* increases chronological lifespan of *S. cerevisiae* by altering Glutamate metabolism**

Mittal, Nitish^{1, 2}; Wuster, Arthur²; Janga, Sarath Chandra²; Meena, Chuttan Lal³; Jain, Rahul³; Babu, Madan M.² and Roy, Nilanjan¹

¹Department of Biotechnology, ³Department of Medicinal Chemistry, National Institute of Pharmaceutical Education and Research Sector 67, S. A. S. Nagar, Punjab 160062, India and ²MRC-Laboratory of Molecular Biology, Hills Road, Cambridge CB2 0QH, UK

Integrity of mitochondrial functionality is a key determinant of longevity in several organisms. In particular, reduced mitochondrial ROS (mtROS) production leading to decreased mtDNA damage is believed to be a crucial aspect of longevity. The generation of low mtROS was thought to be due to low mitochondrial oxygen consumption. However, recent studies have shown that higher mitochondrial oxygen consumption could still result in low mtROS and contribute to longevity (1). This increased mitochondrial efficiency (i.e., low mtROS generated despite high oxygen consumption) was explained due to mitochondrial biogenesis, which provides more entry points for the electrons to the electron transport chain (ETC), thereby resulting in low mtROS production. In our previous study, we provide evidence for the existence of an alternative pathway to explain the observed higher mitochondrial efficiency in the long-lived *mrg19* mutant of *S. cerevisiae*. Though we observe similar amounts of mitochondria in *mrg19* and wild type (wt) yeast, we find that *mrg19* mitochondria have higher expression of ETC components per mitochondria in comparison to the wt. These findings demonstrate that more efficient mitochondria due to increased ETC per mitochondria can also produce less mtROS (2).

Genome expression profile analysis provides evidence of higher glucose up-take in *mrg19* cells and the excess glucose is utilized for the glutamate biosynthesis through anaplerotic reactions. Further, our study also demonstrates that glutamate transporter activity is down regulated in *mrg19* cells. These results suggest that *mrg19* cells mimics nitrogen depletion condition (low glutamate) and to maintain the homeostasis, cells utilizes glucose for *de-novo* glutamate biosynthesis that results in higher expression of TCA cycle and leads to longevity. Present study demonstrates mechanistic insight for longevity in *mrg19* cells as well as the relationship of nitrogen and carbon metabolism. We anticipate that similar mechanisms might also be responsible for the lifespan extension on amino acid restricted condition in higher organisms.

References:

1. Kharade, SV *et al.*, (2005) FEBS Lett., 579, 6809-6813.
2. Mittal, N *et al.*, (2009) Aging Cell, 8, 643-653.

T8

On the road for the identification of the IP₃ receptor in *Saccharomyces cerevisiae*

Brandão, Rogelio Lopes; Perovano, Eduardo; Bouillet, Leoneide and Castro, Ieso de Miranda

Laboratório de Biologia Celular e Molecular – Núcleo de Pesquisas em Ciências Biológicas – Universidade Federal de Ouro Preto 35.400-000 – Ouro Preto, MG – Brazil

In the last few years, we have found evidences for a clear connection between glucose-induced calcium signaling and plasma membrane ATPase activation in *Saccharomyces cerevisiae* cells (Trópia *et al. Bioch. Biophys. Res. Comm.* 343, 1234-1243, 2006; Pereira *et al FEMS Yeast Res.*, 8, 622-630, 2008). In spite of the fact that a gene with homology to IP₃ receptor has not yet been demonstrated in yeast, it seems to us that there must exist other proteins that by interacting with IP₃, regulate the calcium availability in the cytosol. In this work, we show different strategies that we are using to try to identify the protein that binds IP₃ and, most probably, acting on the calcium vacuolar channel Yvc1 controls the intracellular level of calcium.

Supported by CNPq, FAPEMIG and UFOP.

T9

Chronological life-span extension is blocked by ammonium in amino acid starved cells, associated to autophagy inhibition and to cell death inductionSantos, Júlia¹; Sousa, Maria João² and Leão, Cecília¹¹*Life and Health Sciences Research Institute, University of Minho, Braga, Portugal* and ²*Molecular and Environmental Research Centre (CBMA)/ Department of Biology, University of Minho, Braga, Portugal*

In extreme starvation conditions, as in water suspensions, cells can attain a quiescent state being able to survive for a long time, a phenotype known as life-span extension. Addition of glucose to cell suspension in water in the absence of other nutrients can induce cells to exit this quiescent state and commit to an apoptotic cell death program (1). In this work we show that ammonium stimulates cell death in amino acid-deprived auxotrophic cells of *Saccharomyces cerevisiae* transferred to water, whereas this was not observed when the cells were completely starved for nitrogen. Ammonium effect appears not to dependent of on its metabolization as its analogue methylamine produced an identical outcome and the effect did not depend on glutamine synthase activity. Ammonium-induced cell death was accompanied by an increase in ROS production, chromatin condensation, exposure of phosphatidylserine on the outer surface of the plasma membrane, DNA strand breaks and PI+ staining. However, cell death was not decreased in the presence of Z-vad-FMK caspase inhibitor, and no caspase activation could be detected using double staining with Zvad-FMK-FITC/PI. The induction of autophagy observed in cells transferred to water is inhibited by the presence of ammonium, correlating with the faster loss of cell viability observed under the latter conditions. Ammonium-induced cell death is involved in different human disorders that are accompanied by hyperammonemia. However, the precise molecular mechanisms triggering ammonium-induced cell death remain poorly understood. In addition, deprivation of essential amino acids has been employed as a strategy in cancer therapy but resistance is often found. Our results showing that ammonium can stimulate cell death in amino acid-deprived cells, open the possibility to use *Saccharomyces cerevisiae* as an useful model for the identification of signalling pathways and of new therapeutic targets for these diseases.

This work was developed in the scope of the projects PTDC_AGR-ALI-71460/FCT and PTDC/AGR-ALI/102608/2008/FCT. JS holds a PhD fellowship (SFRH / BD / 33314 / 2008) from Fundação para a Ciência e a Tecnologia.

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T10

Nutrient sensing mechanisms for rapid activation of the PKA pathway in yeast

Thevelein, Johan¹; Bonini, Beatriz; Castermans, Dries; Kriel, Johan; Nag, Harish; Peeters, Ken; Popova, Yulia; Rubio-Teixeira, Marta; Schepers, Wim and Zeebroeck, Van Griet

Laboratory of Molecular Cell Biology, Institute of Botany and Microbiology, Katholieke Universiteit Leuven, Department of Molecular Microbiology, VIB, Kasteelpark Arenberg 31, B-3001 Leuven-Heverlee, Flanders, Belgium

In yeast the Protein Kinase A pathway can be rapidly activated by a variety of nutrients under appropriate conditions. Glucose triggers a spike in the cAMP level, followed by activation of PKA and phosphorylation of many target proteins causing a.o. mobilization of reserve carbohydrates, repression of stress-related genes and induction of growth-related genes (1). Glucose is sensed by Gpr1, a GPCR that activates adenylate cyclase through Gpa2. This activation depends on phosphorylation of glucose in a way that is not well understood but probably acts through the Ras proteins. We have now obtained strong evidence that several intermediates of glycolysis can trigger activation of the Ras proteins.

Amino acids added to nitrogen-starved yeast cells, trigger rapid activation of the PKA pathway without increase of cAMP. The amino acids are sensed by the Gap1 transceptor (transporter/receptor), which is subsequently inactivated by ubiquitination, endocytic internalization and hydrolysis in the vacuole (2). We have discovered that specific gamma-glutamyl dipeptides cause persistent activation of the PKA target trehalase because they cause internalized Gap1 to accumulate in endosome like vesicles, indicating that internalized Gap1 can still signal to the PKA pathway. This has now been confirmed further by the finding that regular amino acids can also cause persistent trehalase activation in specific mutants of the endocytic machinery. The Pho84 transceptor mediates phosphate induced activation of the PKA pathway (3). We have now obtained a mutant allele in a putative phosphorylation site of Pho84 that triggers constitutive activation of the PKA pathway and remarkably, also of the classical PHO repression pathway of secreted phosphatase.

Recent work has also provided evidence that a transceptor system is involved in sulfate activation of the PKA pathway in sulfate-starved cells. Several sulfate analogues have been obtained that are able to activate the PKA target trehalase and lose their activating effect in a strain lacking the putative transceptor system.

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T11

Characterizing specificities of breast cancer resistance protein ABCG2 over P-glycoprotein ABCB1: selective inhibitors and additional ABC signature

Macalou, S.¹; Robey, R.W.²; Nicolle, E.³; Boumendjel, A.³; Carrupt, P.A.⁴; Bates, Susan² and Pietro, Di Attilio¹

¹*Institute of Protein Biology and Chemistry, UMR5086 CNRS-University of Lyon, France;* ²*Medical Oncology Branch, Center for Cancer Research, NIH, Bethesda, MD, USA;* ³*Molecular Pharmacochimistry Dpt, UMR5063 CNRS-University of Grenoble, France and* ⁴*School of Pharmaceutical Sciences, Universities of Geneva and Lausanne, Switzerland*

Cancer cell resistance to chemotherapeutics is often related to overexpression of the multidrug efflux pumps P-glycoprotein/ABCB1 and breast cancer resistance protein/ABCG2. The two transporters display highly-overlapping substrate spectra whereas the inhibitor ones appear more restricted. From its early discovery, ABCB1 has been extensively studied and up to third-generation inhibitors have been reported, of which very few such as LY335979/ Zosuquidar appear to be selective, while GF120918/Elacridar and others are not.

The aim was to investigate the specificities of more-recently discovered ABCG2 by optimizing selective inhibitors, especially among flavonoids, and characterizing their interaction mechanism. Hydrophobic derivatives of flavones (1), rotenoids (2) and acridones (3) appeared specific and allowed us to build up a molecular model from 3D-QSAR analyses (4), (5). This specific inhibitory site appeared adjacent to the catalytic transport site, where GF120918 was able to bind and modify ATPase activity.

Another approach was to characterize the role of a second, additional, ABC signature/C motif only found in ABCG2 within the linker region between the cytosolic nucleotide-binding domain and the transmembrane domain. Alanine-scanning mutagenesis of the five constituting residues indicated that this additional signature/motif behaved similarly to the canonical one found in all ABC transporters: some mutations fully abolished the stimulation of ATPase activity coupled to substrate binding to the catalytic transport site and efflux, as well as the related drug resistance of cell growth. This demonstrates an original coupling mechanism between ATP hydrolysis and drug efflux, which might constitute a new ABCG2-specific target for therapeutic strategy.

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Development of natural product and small molecule modulators of multidrug resistance-linked ABC drug transporters

Shukla, Suneet¹; Silverton, Latoya¹; Zaher, Hani²; Ware, A. Joseph² and Ambudkar, Suresh V.¹

¹Laboratory of Cell Biology, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, Maryland and ²Department of Pharmacokinetics, Dynamics, and Metabolism, Pfizer Global Research and Development, Ann Arbor, Michigan

The human ATP-binding cassette (ABC) transporters, ABCB1 (P-glycoprotein, Pgp), ABCG2 and ABCC1 (MRP1) are the drug efflux pumps that transport cytotoxic anticancer agents thereby conferring resistance to multiple chemotherapeutic agents in most cancers. Although many innovative therapies have been developed to combat cancer, multidrug resistance (MDR) continues to be the major obstacle to successful chemotherapeutic treatment. For several years, it appeared that direct inhibition of ABC transporters would be the cheapest and most efficient way to combat this problem. Unfortunately, progress in finding a potent, selective inhibitor to modulate ABC transporters and restore drug sensitivity in multidrug-resistant cancer cells has been slow and challenging. Candidate drugs should ideally be selective, potent and relatively non-toxic. In an effort to find effective non-toxic agents that modulate the function of ABCB1, ABCG2 and ABCC1, we have screened a number of natural product compounds and found that curcumin, a nutraceutical spice component, inhibits the function of these three transporters in cultured cells as well as in an *ex vivo* rat brain capillary model of the blood-brain barrier. Consistent with these results, oral administration of curcumin (200 mg/Kg) with doxorubicin (7.5 mg/Kg) inhibited the growth of Pgp-expressing tumors in a xenograft mouse model. Additional studies in wild type and *Abcg2*^{-/-} mice showed that oral curcumin also increased the apparent plasma half life and C_{max} of unchanged plasma sulfasalazine, an ABCG2-specific substrate, by inhibiting ABCG2 function. Thus, this study provides the first *in vivo* evidence demonstrating the ability of curcumin to inhibit both Pgp and ABCG2-mediated function. Recently, we observed that the curcumin effect can be potentiated 3 to 4-fold by treatment with 10 μM piperine, which is an active ingredient of black pepper. Piperine appears to increase the bioavailability of curcumin by blocking its metabolism. Based on these studies, we propose that a combination of curcumin and piperine may be used as an adjuvant to improve the efficiency of chemotherapy in the clinic and may also be used to enhance drug exposure when the rate-limiting step of drug absorption is impacted via active efflux by ABC drug transporters.

This work was supported by the Intramural Research Program of the NIH, National Cancer Institute, Center for Cancer Research.

T13

Drug discovery using fungal ABC transporters expressed in *Saccharomyces cerevisiae*

Cannon, Richard¹; Hayama, Kazumi²; Lamping, Erwin¹; Niimi, Kyoko¹; Keniya, Mikhail¹; Niimi, Masakazu¹; Holmes, Ann¹; Abe, Shigeru² and Monk, Brian¹

¹*Sir John Walsh Research Institute, University of Otago, Dunedin, New Zealand* and ²*Teikyo University Institute of Medical Mycology, Tokyo, Japan*

Saccharomyces cerevisiae presents several advantages for the screening of compound libraries to identify inhibitors of fungal ABC transporters. Drug efflux pumps from pathogenic fungi are expressed in *S. cerevisiae* at high levels and correctly trafficked to the plasma membrane, giving a strong drug resistance phenotype. Cells expressing fungal ABC proteins are easily cultured and conveniently used in medium- or high-throughput drug resistance- or fluorescence-based screening assays.

Candida albicans is a major opportunistic fungal pathogen of the immunocompromised. Treatment of candidiasis patients with azole antifungals such as fluconazole (FLC) can, however, lead to drug resistance. Clinically significant, high-level azole resistance in *C. albicans* is most often due to overexpression of ABC protein Cdr1p. We have used heterologous expression of *C. albicans* Cdr1p in *S. cerevisiae* to identify a potent Cdr1p inhibitor.

AD/CDR1, formed by cloning *C. albicans* CDR1 in *S. cerevisiae* AD, was 600-fold more FLC resistant (MIC = 300 µg/ml) than AD. An in-house 1.8 million member combinatorial D-octapeptide library was screened for peptides that chemosensitized AD/CDR1 to FLC (40 µg/ml). A peptide derivative, RC21, was identified that inhibited efflux of the Cdr1p substrate rhodamine 6G from AD/CDR1 cells and inhibited the Cdr1p ATPase activity of AD/CDR1 plasma membrane fractions (IC₅₀ = 1.25 µM). We determined that RC21 was a specific Cdr1p inhibitor; it did not chemosensitize *S. cerevisiae* cells expressing other fungal ABC transporters including Cdr2p, or the major facilitator superfamily transporter Mdr1p, to FLC. The analysis of *S. cerevisiae* suppressor mutants revealed domains of Cdr1p likely to interact with RC21. Importantly, RC21 chemosensitized azole-resistant *C. albicans* clinical isolates to FLC and itraconazole, both *in vitro* and in an *in vivo* oral candidiasis infection model. *S. cerevisiae* thus provides a valuable screening platform for the identification of fungal transporter protein inhibitors.

This work was supported by the JHSF and NIH grant DE016885.

T14

Evolution and phylogenetic relationships between permeases transporting inorganic cations in Hemyascomycetes and Zygomycetes

Muñoz, Elisa¹ and Domínguez, Ángel²

¹*Department of Cellular Biology and Pathology and* ²*Department of Microbiology and Genetics, IMB, Cietus, University of Salamanca, Salamanca, Spain*

We have carried out a comparison between permeases transporting inorganic cations in the model Hemyascomycetes and the three recent sequenced model Zygomycetes *Phycomyces blakesleeanus*, *Mucor circinelloides* and *Rhizopus oryzae*. In most of the cases we have detected expanded families of transport proteins. However, although it has been described an ancient whole-genome duplication (WGD) for several gene families in *Rhizopus*, our results suggest that this is not the case for cation transporters.

T15**Heterologous expression of ABC transporters in *Saccharomyces cerevisiae***

Goffeau, Andre

ISV/UCL, Croix du Sud, 5/15, 1349, Louvain la Neuve, Belgium

Overexpression in *Saccharomyces cerevisiae* of polytopic plasma membrane P-type ATPases genes such as *PMA* from yeast or plants leads to traffic jam and proliferation of endoplasmic structures which impairs growth.

We will describe two conditions which allows overexpression of polytopic ABC transporters from yeast or murine plasma membrane which are fully functional

The constitutive promoter of *PDR5* activated by the *pdr1-3* mutation in the transcription factor gene *PDR1* allows heterologous expression of the *CDR1* gene from the pathogen *Candida albicans*. The cloned *CDR1* gene confers antifungal resistance to the super-sensitive host *Saccharomyces cerevisiae* deleted in several endogenous PDR genes (collaboration with Anabelle Decottignies, UCL and Brian Monk, Otago).

The inducible *Met25* promoter allows transient expression of the murine Abca1 gene in *Saccharomyces cerevisiae*. The cloned ABCA1 protein is fully functional as shown by modified sensitivity of yeast growth to lipid-dependent antifungals such as amphotericin A, filippin and papuamycin. The cloned murine ABCA1 gene which has no ortholog in yeast thus confers unique properties to the outer leaflet of the yeast plasma membrane (collaboration with Tom Bocer, Stan Ulaszewski, Wroclaw and Giovanna Chimini, Marseille).

T16

Transcriptional regulation of the multidrug efflux pump *MDR1* in *Candida albicans*

Morschhäuser, Joachim

T17

Multiple roles for the transcriptional regulators Rds2 and Ert1 in *Saccharomyces cerevisiae*

Turcotte, Bernard¹; Liang, Maggie¹; Forest, Audrey²; Jacques, Pierre-Etienne²; Mitra, Shuvadeep¹; Soontorngun, Nitnipa³ and Robert, François²

¹*Department of Medicine, McGill University, Montréal, Québec, Canada, H3A 1A1*; ²*Institut de recherches cliniques de Montréal, Montréal, Québec, Canada, H2W 1R7* and ³*King Mongkut's University of Technology Thonburi, School of Bioresources and Technology, Bangkok, Thailand 10150*

In *Saccharomyces cerevisiae*, a shift from glucose to a nonfermentable carbon source, such as ethanol, results in massive reprogramming of gene expression. A master regulator of this adaptation to an alternate carbon source is the kinase Snf1. It is activated under low glucose conditions resulting in phosphorylation of various substrates including the zinc cluster proteins Cat8 and Sip4. These proteins are transcriptional regulators that play a major role in gluconeogenesis. We have previously shown that in the presence of ethanol, Rds2, another zinc cluster protein, binds to and regulates expression of several genes of the gluconeogenic and glyoxylate pathways including *PCK1* encoding PEP carboxykinase, a key enzyme for gluconeogenesis. Rds2 also controls expression of regulatory genes such as *SIP4*. Moreover, our results show that the zinc cluster protein Ert1 binds to and regulates expression of some genes (e.g. *PCK1*) implicated in gluconeogenesis. In addition to ethanol, both *RDS2* and *ERT1* play a role in the presence of glucose. Unlike *CAT8* and *SIP4*, *RDS2* and *ERT1* are expressed in the presence of glucose. Gluconeogenic genes are normally expressed at extremely low levels in the presence of glucose. However, our results show that these genes do play a role in conferring resistance to the antifungal drug ketoconazole and that this resistance is mediated by Rds2. Using genome-wide location analysis with high resolution microarrays, we observe binding of both Rds2 and Ert1 at centromeres in the presence of glucose but not ethanol. This observation suggests that Rds2 and Ert1 have additional functions under glucose conditions. A model will be presented to account for these observations.

Evolution and Function of Novel Transcriptional Regulators in Fungal Genomes

Natarajan, Krishnamurthy; Singh, RanaPratap; Prasad, Himanshu; Sinha, Ishani and Agarwal, Neha

School of Life Sciences, Jawaharlal Nehru University, New Delhi 110067, India

Transcriptional control is a critical step in gene regulation. Bulk of the transcriptional regulators function by DNA-binding and consequent activation and/or repression of transcription. With the availability of numerous fungal genome sequences, it has become possible to effectively combine large throughput computational screen followed by genetic and genomic approaches to examine transcriptional regulator evolution and function. We carried out systematic identification of putative transcriptional regulators using sensitive computational approaches such as HMMER, tBLASTN and BLASTP, which yielded over a thousand transcriptional regulator sequences from the 62 fungal genomes encompassing ascomycetes, basidiomycetes, chytrid, microsporidia and zygomycetes. In this talk I will present the transcriptional regulator sequence evolution in the various genomes and discuss experimental results that led to the conclusion of the role of CAP2, a bZIP/CBF domain protein, as a master regulator of iron homeostasis.

T19

Role and regulation of the ABC transporter Pdr18p in yeast adaptive response to auxin-like herbicides

Cabrito, Tânia R.¹; Teixeira, Miguel C.¹ and Sá-Correia, Isabel¹

¹IBB – Institute for Biotechnology and Bioengineering, Centre for Biological and Chemical Engineering, Instituto Superior Técnico, 1049-001 Lisboa, Portugal

The widespread use of herbicides has led to the emergence of resistant weeds. The understanding of the molecular mechanisms underlying herbicide resistance is crucial to deal with this problem. *Saccharomyces cerevisiae* has been used as a model system to gain insights into the mechanisms underlying resistance to the widely used auxin-like herbicide 2, 4-D (1). Among other mechanisms, the yeast drug: H⁺ antiporter Tpo1p and its homolog in *Arabidopsis thaliana*, ORF At5g13750, were shown to confer 2,4-D resistance in *Saccharomyces cerevisiae* and to decrease the intracellular accumulation of the herbicide (2).

In this work, *PDR18* gene (ORF *YNR070w*), encoding a new pleiotropic drug resistance transporter of the ATP-binding cassette (ABC) superfamily was found to confer yeast resistance to the auxin-like herbicides 2,4-D and MCPA, among other xenobiotic compounds. Yeast exposure to 2,4-D leads to a 6-fold transcriptional activation of *PDR18*. This up-regulation in cells challenged with 2, 4-D was found to be fully dependent on Nrg1, a transcription factor involved in the response to carbon source availability and stress, and, less significantly, on Yap1 and Pdr3, two of the main regulators of the Pleiotropic Drug Resistance (PDR) network in yeast. A tentative network structure underlying this complex transcriptional control is proposed. The role of Pdr18p in yeast tolerance to 2,4-D correlates with the observation that *PDR18* expression is required to reduce the intracellular concentration of ¹⁴C-2,4-D. Clues on the physiological role of Pdr18p are being obtained by comparison of the metabolomes of the wild type and Δ *pdr18* strains.

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Glutathione transport and utilization in fungi

Bachhawat, Anand, K¹; Thakur, Anil¹; Kaur, Jaspreet¹; Kaur, Hardeep¹; Ganguli, Dwaipayan¹ and Kumar, Chitranshu¹

¹*Institute of Microbial Technology, Sector 39-A, Chandigarh- 160036*

Glutathione, γ -glutamyl-cysteinyl-glycine, a tripeptide with an unusual γ -glutamyl linkage, is the principle redox buffer of almost all eukaryotic cells, and is present at high concentrations in living cells. Although glutathione degradation has been earlier thought to be always initiated in all living cells by the enzyme γ -glutamyl transpeptidase, we have recently demonstrated that an alternative pathway exists in *S. cerevisiae* that involves 3 previously uncharacterized proteins that we have named as Dug1p, Dug2p and Dug3p, and which appear to form a complex for glutathione degradation. In addition to the Dug complex is a high affinity glutathione transporter, Hgt1p that is also required for the utilization of exogenous glutathione. Interestingly, homologues of the Dug complex appear to be present in all fungi, barring the fission yeast, *S. pombe*, while homologues of the glutathione transporter, Hgt1p appear to be present in all fungi except *C. glabrata*. Investigations into the glutathione metabolism pathways in these and other yeasts, as well as more detailed investigations into the Dug complex and into the transporter protein, Hgt1p, has led to some interesting new insights of glutathione utilization in fungi.

T21

Regulation of protein-*O*-mannosylation in *Candida albicans*

Cantero, P. D.; Szafranski, E. and Ernst, F. Joachim

*Institut f. Mikrobiologie, Molekulare Mykologie, Heinrich-Heine-Universitaet
Duesseldorf, Germany*

Most secretory proteins in *C. albicans* are *O*-mannosylated at serine or threonine residues by protein-*O*-mannosyltransferases (Pmt proteins). Isoforms 1, 2, 4, 5 and 6 of Pmt proteins *O*-mannosylate largely different target proteins and these modifications are required for virulence and antifungal sensitivity of *C. albicans*. We have begun to explore differential regulation of *PMT* genes encoding the 5 Pmt isoforms under various growth conditions and in different mutant backgrounds. The transcript level of *PMT1* but not the other *PMT* genes was strongly increased in cell wall mutants and in the presence of inhibitors of the cell wall and *N*-glycosylation. This regulatory pattern was reproduced in *PMT1* promoter fusions to the *RLUC* reporter suggesting that *PMT1* is de-repressed on the transcriptional level if the cell wall structure is impaired. By deletion analysis we identified sequences within the *PMT1* promoter that are necessary for tunicamycin-mediated transcriptional upregulation. In contrast, in the presence of a rhodanine-type Pmt1 inhibitor the *PMT2* and *PMT4* transcript levels were strongly upregulated. This upregulation was not reflected by a *PMT2* promoter fusion to the *RLUC* reporter suggesting that the expression of *PMT2* and *PMT4* is upregulated on a post-transcriptional level, which is consistent with short 5'-intergenic regions of both *PMT* genes. Results will be presented linking expression of *PMT* genes to upstream signalling pathways including the Cek1 kinase and the upstream Msb2 membrane sensor.

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Antifungals and chemosensitizers to combat multidrug resistance in *Candida albicans*

Milewski, Slawomir¹; Lacka, Izabela¹; Wakiec, Roland¹ and Prasad, Rajendra²

¹Department of Pharmaceutical Technology and Biochemistry, Gdansk University of Technology, Gdansk, Poland and ²School of Life Sciences, Jawaharlal Nehru University, New Delhi, India

The relatively narrow repertoire of effective antifungal chemotherapeutic agents is further limited, due to the consequences of multidrug resistance (MDR) of human pathogenic fungi. Several strategies have been developed to deal with the problem, including: (a) use of antifungals not extruded by fungal drug transporters or acting extracellularly, for example 5-fluorocytosine, BAY 10-8888, cell penetrating peptides (CPPs), polyene macrolides or benanomycin; (b) antifungals of enhanced activity against MDR fungi: nikkomycin and FMDP-peptides; (c) combined action of antifungals + chemosensitizers restoring the wild-type sensitivity of MDR cells to established drugs.

In our studies we have focused on approaches (b) and (c). Taking the former, we discovered that *C. albicans* strains overexpressing Cdr1p/Cdr2p demonstrated enhanced susceptibilities to antifungal small peptides transported by oligopeptide permeases. Mechanism of this phenomenon was found to be a consequence of the increased plasma membrane electrochemical gradient stimulating oligopeptide uptake by permeases acting as ligand/H⁺ symporters. Oligopeptide antifungals are thus especially promising agents to treat infections caused by MDR human pathogenic yeasts.

In the framework of another project we conducted screening for compounds able to restore the original sensitivity of MDR *C. albicans* to fluconazole. The screening procedure resulted in identification of heterocyclic dyes acting at low concentrations (less than 2 µg ml⁻¹) as chemosensitizers of fungal MDR caused by overexpression of ABC- and/or MFS-type drug transporters. Nile Blue and Methyl Red were found to be the most effective in the case of Cdr1p/Cdr2p cells, while for the Mdr1p cells, Nile Blue, Sudan III, Methylene Blue and Pyragoll Red. Consequently, Nile Blue was selected as an universal chemosensitizer. None of the tested compounds inhibited the ATPase activity of Cdr1p/Cdr2p efflux pumps, but most of them appeared good substrates of one or both types of candidal drug transporters.

T23

Real-time identification of basic events underlying the action of surface-active antifungals on the yeast *Saccharomyces cerevisiae*Gášková, Dana¹; Kodedová, Marie¹ and Sigler, Karel²¹ Charles University, Faculty of Mathematics and Physics, Institute of Physics, Ke Karlovu 5, 121 16 Prague 2, Czech Republic and ² Institute of Microbiology, Acad. Sci. Czech Republic, Vídeňská 1083, 142 20 Prague 4

Ideally, antifungals should interact with specific targets present in the fungal cells and absent in higher eukaryotes. This is often not true and management of fungal infections is thus markedly limited by undesirable side effects of the antifungals and also by the rapid development of drug resistance against most of the currently used antifungal drugs, such as fluconazole etc. The discovery of new antifungal agents with different mechanisms of action overcoming the problem of resistance to current anti-infective drug therapies and with acceptable toxicity is thus urgently needed. One of the ways of circumventing intracellular resistance mechanisms of the yeast is to use drugs that target directly the cell surface structures, i.e. are surface-active. Both cell wall and membrane can be attacked, resulting ultimately in cell permeabilization and death. This mode of action can reduce opportunities for adverse effects, or the emergence of drug resistance caused by intracellular drug and target modification and by the induction of drug efflux pumps.

We developed a technique for detecting with high sensitivity and in real time the range, sequence and persistence of individual events in yeast damage by surface-active drugs. We monitor the evolution of diS-C₃(3) staining of cells after their variously long exposure to a surface-active antifungal compound and its removal by multiple washing. For distinguishing different kinds of damage to the cells we use the differences between the diS-C₃(3) staining of **intact** (though hyperpolarized and/or depolarized) and **permeabilized** cells. An indispensable diagnostic tool of our assay indicating cell integrity is the use of protonophore CCCP in combination with H⁺-ATPase blocker DM-11.

By using known agents with different mechanisms of cell permeabilization, we present here this efficient strategy for identifying the mechanism of damage to the cell surface structures.

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Antifungal drug discovery: a sticky pursuit

Desai, Chirayu; Mavrianos, John and Chauhan, Neeraj

Public Health Research Institute, New Jersey Medical School, University of Medicine and Dentistry of New Jersey (UMDNJ), 225 Warren Street Newark NJ 07103 USA

Candida glabrata, a commensal of humans, is the second most common cause of fungal infections in humans after *Candida albicans* and is responsible for about 20 - 24 % of all *Candida* blood stream infections (BSI) in the United States. Despite being classified in the same genus, *C. glabrata* and *C. albicans* are quite distinct and the former is much more closely related to *Saccharomyces cerevisiae*, suggesting that the interactions of these two *Candida* species with their mammalian host may have evolved independently. *C. glabrata* is also a serious clinical problem because it is innately less susceptible to the azole class of antifungal drug used for treating patients.

C. glabrata owes its success as a pathogen, in part, to a large repertoire of adhesins present on the cell surface. Our current knowledge of *C. glabrata* adhesins and their role in the interaction between host and pathogen is limited to work with only a single family of epithelial adhesins (Epa proteins). Here we report identification of a family of glycosyl phosphatidylinositol (GPI) anchored cell wall proteins in *C. glabrata* by in silico analysis. These proteins are absent in both *S. cerevisiae* and *C. albicans* suggesting that *C. glabrata* has evolved different mechanism(s) for interaction with host cells. In the current study we present data on the characterization of Pwp7p (PA14 domain containing Wall Protein) and Aed1p (Adherence to Endothelial cells) of this family in the interaction of *C. glabrata* with Human Umbilical Vein Endothelial Cells (HUVECs). Deletion of *C. glabrata* genes *PWP7* and *AEDI* results in significant reduction in adherence to endothelial cells compared to the wild type parent. These data indicate that *C. glabrata* utilizes these proteins for adherence to endothelial cells in vitro. Based on these data, we hypothesize that these proteins are a promising therapeutic target and may be exploited in the development or identification of new antifungal drugs.

T25

Signature motifs in the nucleotide-binding domains of ABC transporter CaCdr1p are functionally asymmetricKumar, Antresh¹ and Prasad, Rajendra¹¹*School of Life Sciences, Jawaharlal Nehru University, New Delhi, India*

Nucleotide Binding Domains (NBDs) of multidrug transporter of *Candida albicans*, Cdr1p possess unique divergent amino acids in their conserved motifs. For example, NBD1 possesses conserved signature motif (VSGGE) while the same motif is divergent (LNVEQ) in NBD2.

We report here the contribution of these conserved and divergent signature motifs of Cdr1p in ATP catalysis and drug transport. For this, we mutagenized signature motif residues by either replacing them with alanines or replacing residues with equiposition residues of another signature motif. We also swapped an entire motif with either a conserved or degenerated signature motif. We observed that conserved and equipositional residues of NBD1 and NBD2, and swapped signature motif mutants showed high susceptibility to all the tested drugs with simultaneous abrogation in ATPase and R6G efflux activities. However, some of the mutants displayed selective increase in susceptibility to the drugs. Notably, none of the mutant variants and Cdr1p showed any difference in drug and nucleotide binding. Our mutational analyses show that not only certain conserved residues of NBD1 Signature sequence (S304, G306 and E307) are important in ATP hydrolysis and R6G efflux but a few divergent residues (N1002 and E1004) of NBD2 signature motif have also evolved to be functionally relevant and are not interchangeable. Taken together, our finding show that signature motifs in the Cdr1p-NBDs are non-interchangeable and further suggest that both the signature motifs of Cdr1p are non-exchangeable.

Targeting chitin metabolism for antifungal agents development: Issues and challenges

Chaudhary, Preeti¹; Tupe, Santosh¹ and Deshpande, Mukund¹

¹*Biochemical Sciences Division, National Chemical Laboratory, Pune-411008*

Chitin, an integral component of fungal cell wall is absent in higher plants and vertebrates. Hence, chitin synthesis appears to be the most promising target pathway for development of novel antifungal agents. However, chitin synthase inhibitors like nikkomycin and polyoxin have problems of selectivity, cell permeability, different susceptibility of fungal species, stability in the cell and variable response in animal models. The low cell permeability and intracellular instability of these inhibitors is attributed to the presence of dipeptide in their scaffold. Furthermore, many fungi contain upto 10 chitin synthases. This multiplicity makes drug designing more difficult against them. The limited knowledge about structure-function relationship of chitin synthases is also a major concern in developing target specific drugs.

The 14 compounds were synthesized by replacing peptide of nikkomycin scaffold with 1,2,3-triazole. The 1,2,3-triazole unit may be considered as surrogate for peptide bond as these triazoles have atom placement and electronic properties similar to the peptide bond. HPLC analysis indicated that the modified compounds were more hydrophobic with increased cellular uptake and enhanced stability. All synthesized compounds inhibited growth of *Candida albicans*, *Cryptococcus neoformans* and *Fusarium oxysporum* with MIC values in the range of 0.003–0.305 μmol , with 70-95% *in vitro* chitin synthase inhibition in *Benjaminiella poitrasii*, a dimorphic zygomycetous fungus.

The affinity of chitin synthase towards its substrate can be increased through multivalent interactions between multiple UDP-binding motifs. Hence appropriate dimeric/multimeric inhibitor should be more potent than corresponding monomer. Therefore, 7 dimeric inhibitors, which had two uridine molecules connected with 1, 2, 3-triazole linker with varied aliphatic spacer in-between were synthesized. It was seen that chitin synthase inhibition increased with increase in the length of spacer.

Similarly, hybrid molecules with dual inhibitory action against chitin synthase and glucosamine-6-phosphate synthase, an enzyme which brings both C and N pathways together in chitin synthesis, can be an effective strategy to tackle the problem of resistance against one mechanism. The strategic details will be discussed during presentation.

T27

The interplay between drug resistance and virulence in *Candida glabrata* involves the ABC transporter *CgCDR1*Sanglard, Dominique*Institute of Microbiology, University of Lausanne and University Hospital Center, Lausanne, Switzerland*

Candida glabrata develops rapidly resistance to azole antifungals. We showed that the transcription factor CgPdr1p is involved in azole resistance via upregulation of ABC-transporter genes (among which *CgCDR1*, *CgCDR2* and *CgSNQ2*). Using a collection of resistant clinical isolates (77), we recently reported the existence of a high diversity of GOF (gain-of-function) mutations (57) in *CgPDR1* conferring high expression of ABC transporter genes in azole-resistant isolates. Genome-wide changes in gene expression driven by seven individual *CgPDR1* alleles containing different GOF mutations (hyperactive alleles) were compared to wild-type alleles. Our microarray experiments revealed a high number of genes (ranging from 80 to 400 genes) differentially regulated by individual *CgPDR1* hyperactive alleles. Enrichment of specific biological processes (response to stimuli, amino acid metabolism and cell wall biogenesis) was observed upon expression of specific *CgPDR1* alleles. Intriguingly, we observed a poor overlap in the number of coordinately expressed genes from all hyperactive alleles. Only two genes, *CgCDR1* and *PUP1* (for *PDR1* Upregulated, encoding a mitochondrial protein), were commonly upregulated by all tested hyperactive alleles.

Because azole-resistant isolates originated from patient samples, we probed whether azole resistance would a negative impact on virulence. We found however the opposite in an intravenous mice model: azole-resistant isolates were more virulent than parent azole-susceptible isolates. This feature was shown to be dependent on GOF mutations in *CgPDR1*. Because *CgCDR1* and *PUP1* were the two genes commonly upregulated by all GOF mutations in *CgPDR1*, mutants of both genes were tested for virulence. We found that deletion of these genes in an azole-resistant strain led to a drastic reduction of virulence, thus demonstrating the relevance of these *CgPDR1*-dependent genes in virulence.

Collectively, our data demonstrate that distinct *CgPDR1* GOF mutations have differentiated effects on target genes. This work allowed the identification two *C. glabrata* virulence factors, *CgCDR1* and *PUP1*. Importantly, this study shows that the overexpression of *CgCDR1* and *PUP1* does not only mediate azole resistance but also virulence of *C. glabrata*. Therefore, by developing resistance to azoles, *C. glabrata* also acquires enhanced capacities in its interaction with the host. Such a negative combination for the host highlights that *C. glabrata* infections and resistance development should be carefully monitored.

Small molecular metal chelate can overcome multidrug resistance (MDR) in cancer through alteration of redox status

Choudhuri, S. K.; Chatterjee S.; Majumder S. and Mookerjee A

Department of Invitro carcinogenesis and cellular chemotherapy, Chittaranjan National Cancer Institute, Calcutta, India

Multidrug resistance (MDR) is a major obstacle to successful application to chemotherapy and to overcome MDR in cancer innumerable resistance modifying agents (RMA) have so far been developed. Most of the RMAs have been proved either to be toxic or ineffective. MDR in cancer follows a number of pathways and in a number of drug resistant cases, γ -Glutamyl cysteine glycine (GSH) is over-expressed to counteract the elevated level of reactive oxygen species (ROS). We hypothesized that ROS generating non toxic and small molecule capable of depleting cellular GSH may overcome MDR in cancer. In quest of a small and non toxic molecule capable of altering the redox level by depleting GSH, we synthesized a novel copper complex viz., copper *N*-(2-hydroxyacetophenone) glycinate (CuNG) which was initially noted to be a potent RMA and later found to be an immunomodulator in mice model. The CuNG at non toxic doses increased the life span of drug resistant Ehrlich ascites carcinoma bearing mice tremendously (T/C% = 453) by alteration the level of ROS, depleting GSH and deactivating multidrug resistant protein, like MRP and P-gp. CuNG stimulated ROS generation and reduced MRP1 expression in EAC/Dox cells while only temporarily depleted glutathione (GSH) within 2 h in heart, kidney, liver and lung of EAC/Dox bearing mice, which were restored within 24 h. The level of liver Cu was observed to be inversely proportional to the level of GSH.

In the present investigation, we also found that the level of ROS generation is lowered in EAC/Dox cells, which contain high levels of intracellular GSH compared to their drug sensitive counterparts. Moreover, low levels of ROS favor the expression of ABC transporters like P-gp on cancer cell surface. We also found that an oxidative environment favors reduced MRP1 expression on EAC/Dox cells. Interestingly, CuNG treatment resulted in elevated ROS generation by EAC/Dox cells and reduced surface MRP1 expression. We observe that by suppressing surface MRP1 expression, CuNG converts EAC/Dox cells susceptible to doxorubicin (dox) induced killing. The resistant cells accumulate dox and undergo apoptosis. Based on our studies; we conclude that CuNG may be a promising candidate to sensitize drug resistant cancers in the clinic.

Structural and functional characterization of secondary drug transporter Mdr1p of *Candida albicans*: A Rational Mutational analysis

Kapoor, Khyati¹; Rehan, M.²; Lynn, Andrew M.² and Prasad, Rajendra¹

¹*School of Life Sciences, Jawaharlal Nehru University, New Delhi, India and*

²*School of Information Technology, Jawaharlal Nehru University, New Delhi, India*

Over-expression of secondary multidrug transporter Mdr1p in *Candida albicans* is linked to frequently encountered azole resistance in hospital isolates. The traditional measure of conservation is not a useful parameter in mapping functionally important residues in the case membrane proteins. For example, hydrophobically conserved stretches usually form the helical transmembrane (TM) regions of the protein and are responsible for membrane localization, which individually have limited effect on binding and transport. To circumvent this problem, in this study, we have employed a membrane environment based computational approach to predict structurally and functionally critical residues of CaMdr1p. Information theoretic scores which are variants of Relative Entropy (Modified Relative Entropy RE_M) were calculated from Multiple Sequence Alignment (MSA) by considering the distinct physico-chemical properties of TM and inter-TM regions separately. We have compared traditional conservation and standard RE with our improved method and shown that RE_M is a better index for predicting the importance of a residue. Also, by employing a distance plot from a 3D deduced model of CaMdr1p, we could predict the role of these functionally critical residues in maintaining apparent inter-helical interactions to provide the desired fold for the proper functioning of CaMdr1p. Additionally, to identify the residues critical for specific function, we further exploited information theoretic measure (Cumulative RE). CaMdr1p is a drug-proton antiporter and thus belongs to DHA1 family, we contrasted DHA1 (antiporters) with Sugar Porter family (SP) (symporters) to identify residues selectively conserved in DHA1 vis-à-vis SP family. Thus an extrapolation of our method could predict the residues important for antiport mechanism and for substrate specificity for the entire DHA1 family.

In both cases the residues with high scores were replaced with alanine or leucine by site-directed mutagenesis. The mutant variants exhibited hypersensitivity towards drugs which was also supported by abrogated substrate efflux and was not attributed to their poor expression or surface localization. Thus, by information theoretic measures, we have been able to pinpoint critical residues important to maintain the structure for all MFS transporters in general and CaMdr1p in particular. Taken together, we could separately identify the MFS wide function-specific residues for CaMdr1p and also identify the function-specific residues by comparing the two major subfamilies of MFS transporters.

Signaling Interface of the Yeast Multidrug Transporter Pdr5: Role of the Q-loop Residues

Ananthaswamy, Neeti¹ and Golin, John¹

¹ *Department of Biology, The Catholic University of America, Washington D.C, USA*

Pdr5 (Pleotropic drug resistance-5) is a 160 kDa, multidrug, ABC transporter in *Saccharomyces cerevisiae* that facilitates the efflux of drugs and xenobiotics. Like all ABC transporters Pdr5 is composed of two domains; a pair of transmembrane domain (TMDs) that contain substrate binding pockets and a pair of nucleotide binding domains (NBDs) that power substrate transport. In most ABC transporters the NBD contains several conserved motifs including Walker A, Walker B, Q-loop, A-loop, H-loop and signature that facilitate positioning of ATP at the ATP binding site and its hydrolysis. Crystal structures of the NBDs of several bacterial ABC transporters such as Sav1866 demonstrate that the two ATP binding sites are formed by the hybrid of both the NBDs. At each ATP binding site, an ATP molecule is sandwiched between Walker A, Walker B, Q-loop and A-loop of one NBD and signature and D-loop of the other. In fungal transporters like Pdr5, one of the ATP binding sites is made up of residues that are conserved among the ABC superfamily and this canonical site is known to bind and hydrolyze ATP. The other ATP binding site, however, is made up deviant residues including the Q loop that are conserved only in the Pdr5 family of fungal ABC transporters.

The drug efflux cycle involves the binding of the drug to the TMDs and ATP to the NBDs. Hydrolysis of ATP triggers a conformational change at the TMDs that result in drug efflux. Biochemical and genetic data along with X-ray structure of Sav1866 suggest a transition interface between the TMDs & NBDs in which the Q loop of one NBD makes contact with TMDs via the intercellular loops. Previously, the Q loop was implicated in communication between the TMDs and the NBDs in several ABC transporters. In order to determine its role in Pdr5, we evaluated the role of the conserved Q loop residues, E244G in the deviant NBD1 and the corresponding canonical residue mutation Q951G present in NBD2. Surprisingly, mutation of the conserved residues exhibited only mild drug hyper sensitivity and retained significant ATPase activity. We also investigated the phenotype of the double mutant E244G/Q951G. THE drug phenotype double mutant showed a greater than additive sensitivity, indicating a functional overlap between the two Q loops¹. Interestingly, the reduced ATPase activity of the double mutant was equal to the single mutants. It is likely, therefore that the deviant and canonical Q-loop residues are involved in interdomain communication in an overlapping fashion (1).

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T31

The mechanism of action of multidrug resistance-linked ABC drug transporters

Ambudkar, Suresh; Ohnuma, Shinobu; Chufan, Eduardo and Sauna, Zuben
Laboratory of Cell Biology, Center for Cancer Research, National Cancer Institute, NIH, Bethesda, MD 20892, USA

The human ATP-binding cassette (ABC) transporters, ABCB1 (P-glycoprotein, Pgp), ABCG2 and ABCC1 (MRP1) are the drug efflux pumps that transport cytotoxic anticancer agents thereby conferring resistance to multiple chemotherapeutic agents in most cancers. Recent studies with Pgp are focused on elucidation of the catalytic cycle of ATP hydrolysis by studying reaction intermediates of the ATPase reaction and the coupling between ATP hydrolysis and drug transport. Site-directed mutagenesis guided by data mining of non-redundant ABC domains resulted in identification of amino acid residues critical for ATP binding and/or hydrolysis. We have demonstrated that although two ATP molecules may initially bind to the two NBDs of Pgp, only one is driven to a tightly bound reaction intermediate state which constitutes the E•S state of the ATPase reaction and occlusion of nucleotide at this step provides the “power-stroke” for the movement of drug-substrate from a high-affinity to a low-affinity site in the transmembrane domains. Furthermore, the non-hydrolysable ATP analogs can be exploited to elucidate the catalytic cycle of ATP hydrolysis. 5' -Fluorosulfonyl-benzoyl 5' -adenosine (FSBA) is a reactive ATP analog that covalently modifies several residues in the nucleotide-binding sites of kinases and ATPases. Incubation of Pgp with FSBA inhibited ATP hydrolysis ($IC_{50} = 0.21$ mM) as well as the binding of 8-azido-[α - ^{32}P]-ATP ($IC_{50} = 0.68$ mM). FSBA-mediated inhibition of ATP hydrolysis was irreversible, suggesting covalent modification of Pgp. However, protection of the ATP sites with excess ATP prior to treatment with FSBA resulted in stimulation of ATP hydrolysis. Furthermore, FSBA inhibits the photo cross-linking of Pgp with [^{125}I]-iodoarylazidoprazosin ($IC_{50} = 0.17$ mM). Finally, mass spectrometric analysis showed that FSBA cross-links to sites in the NBDs but not in the transmembrane domains. Thus, FSBA is a novel reagent that interacts with both the transport-substrate and ATP-binding sites of Pgp, but fluorosulfonyl-mediated cross-linking occurs only at the NBDs.

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Insights into the glucose-mediated endocytosis of yeast Jen1 lactate transporter

Vieira, Neide^{1*}; Léon, Sébastien^{2*}; Gomes, Jéssica¹; Cunha, Carina¹; Haguenaer-Tsapis Rosine²; Casal Margarida¹ and Paiva, Sandra²

*both authors contributed equally for this work

¹*Department of Biology, Molecular and Environmental Biology Centre, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal and* ²*Institut Jacques Monod, CNRS-Université Paris Diderot-Paris 7, 75013 Paris, France*

The intracellular trafficking of plasma membrane proteins, such as receptors and transporters, in eukaryotic cells, is a highly regulated process. Ubiquitylation of cell surface transporters acts as a signal triggering their internalization, and subsequent degradation in the lysosome/vacuole, and, in yeast, this modification is mediated by the HECT ubiquitin ligase (E3) Rsp5p, usually in lysine residues of the target protein. Despite the prevailing role of Rsp5 in ubiquitylation of yeast plasma membrane proteins, there is no evidence for a direct interaction between the ligase and these cargoes. The majority of Rsp5 substrates do not carry proline rich sequences (PY elements) that are recognized directly by the tryptophan rich domains (WW) of Rsp5 and may depend on PY containing adaptor proteins for their ubiquitylation by Rsp5. Several yeast transporters have been shown to rely on arrestin-like adaptor proteins to undergo ubiquitylation. These PY motif-containing arrestin-like proteins are found in many species and were suggested to regulate protein trafficking. Jen1 monocarboxylate permease of *Saccharomyces cerevisiae* was shown to display glucose-regulated endocytosis, dependent on both phosphorylation and ubiquitylation (1), (2). In this work, we show that an arrestin like protein is involved in glucose induced downregulation of Jen1. Moreover we have demonstrated that phosphorylation of Jen1-Ser606, is also required for Jen1 endocytosis, upon glucose treatment.

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The effect of ploidy on genome stability and virulence in *Candida albicans* and *Candida dubliniensis*

Chakraborty, Uttara¹; Kakade, Pallavi²; Aiyaz, Mohamed³; Mugasimangalam, Raja³; Sadhale, Parag² and Sanyal, Kaustuv¹

¹Molecular Mycology Laboratory, Molecular Biology & Genetics Unit, Jawaharlal Nehru Centre for Advanced Scientific Research, Jakkur, Bangalore 560064, India and ²Department of Microbiology & Cell Biology, Indian Institute of Science, Bangalore 560012, India and ³Genotypic Technology, 4th Cross, RMV 2nd Stage, Bangalore 560094, India

Candida albicans and *Candida dubliniensis* are strictly diploid asexual pathogenic yeasts which do not exhibit meiosis and therefore do not exist in the haploid state. As a result, the significance of their preferred diploid state in genome stability and virulence is poorly understood. Generation of a pseudodiploid hybrid between the two species can be a useful tool to study the effect of reduced ploidy on the biology of these pathogens. We constructed a stable tetraploid somatic hybrid of *C. albicans* and *C. dubliniensis*, by PEG mediated spheroplast fusion, and induced chromosome loss to obtain pseudodiploid progenies with ploidy levels lower than those of the parents. The unique centromere sequences of the different chromosomes of each species (1), (2) were used as markers to identify the presence of specific chromosomes in the hybrid genome. The effect of an altered ploidy on virulence was tested in a murine model of systemic infection. Interestingly, our results indicate that the centromere identity of chromosomes of each species is maintained in the tetraploid and pseudodiploids, suggesting that the hybrid genomes can propagate stably. In addition, the virulence of the progeny hybrids is significantly diminished in the mouse challenge experiments although, that of the tetraploid hybrid (with the diploid genome content of each species) did not show any significant difference as compared to the diploid *Candida* species. We thereby propose that diploidy is important for virulence in both *C. albicans* and *C. dubliniensis*.

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Study of a putative virulence factor of *Candida albicans* that does not affect morphogenesis in *Candida albicans*

Sen, Manimala¹; Shah, Bhavin¹; Rakshit, Srabanti²; Singh, Vijender¹, Padmanabhan, Bhavna¹, Ponnusamy, Manikandan²; Pari, Koteppa³; Vishwakarma, Ram³; Nandi, Dipankar² and Sadhale, Parag P.¹

Department of Microbiology and Cell biology; ²*Department of Biochemistry, Indian Institute of Science, Bangalore, 560012, India and* ³*Piramal Life sciences India Ltd, Mumbai-400063, India*

Candida albicans, a human fungal pathogen, undergoes morphogenetic changes that are associated with virulence. We have previously shown that UDP galactose epimerase does not affect virulence. We report here characterization of a paralog of UDP-galactose-4-epimerase in *C. albicans*. We show that although annotated as a paralog of UDP-galactose-4-epimerase it does not encode a functional galactose epimerase but its deletion shows constitutive pseudohyphal morphology, altered gene expression, and increased sensitivity to antifungal drugs. The mutant also forms biofilms consisting mainly of hyphal cells that show less turgor. We present the detailed study with this mutant strain indicating that the gene encoding this galactose epimerase paralog contributes to virulence by affecting interactions with the host immune system.

Quantitative Assessment of Membrane Potential Changes by Fluorescent Probe in Yeast

Plášek, Jaromír¹; Gášková, Dana¹ and Höfer, Milan²

¹Faculty of Mathematics and Physics, Charles University in Prague, Prague, Czech Republic and ²Institute of Cellular and Molecular Botany, University of Bonn, 53115 Bonn, Germany

The use of lipophilic cationic fluorochromes for the examination of membrane potential is a technique suitable for cells which are too small to allow for direct membrane potential measurements with microelectrodes, and/or for monitoring mean properties of high numbers of cells. Unfortunately, this indirect technique of membrane potential monitoring is notorious for a very high rate of artefacts, as well as for a low chance to convert measured fluorescence intensities to a true membrane potential scale. We have published earlier a couple of papers on a fluorescent probe 3,3'-dipropylthiadicarbocyanine, diS-C₃(3), which is the member of a slow-dye family. In current presentation, we will evaluate key factors that may influence the membrane-potential-dependent performance of this dye. Then a theory will be introduced that makes it possible to convert changes of equilibrium diS-C₃(3) fluorescence intensities measured under certain well-defined circumstances into underlying membrane potential differences, scaled in the units of millivolts. An experimental protocol enabling such quantification employs a fact that both the excitation and emission spectrum of diS-C₃(3) fluorescence shifts towards longer wavelengths upon the binding of this fluorochrome to proteins and other cytosolic components. Consequently, the spectral unmixing of synchronously-scanned diS-C₃(3) fluorescence spectra could be used as a tool for the assessment of the ratio of intra- to extracellular dye concentrations, which ratio is found in Nernst equation describing the equilibrium dye redistribution between the cells and the medium. A practical application of this approach will be demonstrated with the assay on the pH-induced depolarization of *Rhodotorula glutinis* yeast cells (*Rhodospodium toruloides*, ATCC 26194).

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Vinca alkaloids induced multiple drug resistance reversing potential of black tea polyphenols in human cancer cells

Shukla, Yogeshwer¹ and Singh, Madhulika¹

¹*Proteomics Lab Indian Institute of Toxicology Research (CSIR), MG Marg, PO Box 80, Lucknow-226001*

Multi-factorial drug resistance is the main stay of chemotherapy failure in various cancer treatments. Over-expression of *MDR1* gene-encoded permeability glycoprotein (P-gp) is known to play a pivotal role in the development of this resistant phenotype. This phenomenon attributed to the P-gp capability to extrude various drugs of different structures and mechanisms out of the cytoplasm. Modulator agents of different generations (e.g. verapamil, cyclosporin) are capable of inhibiting P-gp. In solid tumors, clinical trials using these drugs are not so convincing because of toxicity and poor specificity. The role of P-gp is proposed as an important goal in the design of chemotherapy strategies. We explored the potential of tea polyphenols which would be helpful in finding novel MDR reversal agents and consequently, chemosensitizers.

Black tea polyphenols (BTP), are promising chemopreventive agent, has been evaluated for their potential to modulate the vinca alkaloids (vinblastine and vincristine; known inducers of *MDR1* gene) induced over-expression of P-gp in human cancer cells viz. K562, PC-3, DU-145, HepG2, and MCF-7. BTP significantly reversed the over-expression of P-gp protein induced by increasing doses of vinca-alkaloids, in time and dose-dependent manner. The ability of BTP to sensitize selected resistant cells to the cytotoxicity of vinblastine (VBL) was also evaluated. The results showed that 50% sensitization of VBL resistant K562, PC-3, MCF-7, DU-7 and HepG2 cells (after 72 h) by BTP concentrations 0.5%, 1.5%, 1.5%, 1.0% 0.5% and 1.0%, respectively. Along with these, BTP restored the doxorubicine accumulation in resistant cells to a level comparable to sensitive cells, in dose-dependent manner. The present investigation suggests that BTP can significantly inhibit drug resistance and may have utility as a dietary adjuvant in the treatment of cancer via reversal of multiple drug resistance.

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T37

Promiscuities and specificities of MDR transporters of pathogenic *Candida albicans*Puri, Nidhi¹ and Prasad, Rajendra¹¹*Membrane Biology Laboratory, School of Life Sciences, Jawaharlal Nehru University, New Delhi-110067, India*

The promiscuity of multidrug transporters, CaCdr1p belonging to ATP-binding cassette protein (ABC) or CaMdr1p of Major Facilitator Superfamily (MFS) of *Candida albicans* is well known. Interestingly, both CaCdr1p and CaMdr1p are functionally similar but differ mechanistically in terms of their mode of energy coupling to drug transport (1) and also in their lipid specificities (2). Notwithstanding the differences in energy coupling and lipid preferences elicited by CaCdr1p and CaMdr1p, both transporters exercise promiscuity towards substrates they export. Therefore, considerable attention is being devoted towards assessment of the structural and functional features in these proteins which could explain their diverse substrate specificity spectrum. Understanding SAR between these proteins and host of xenobiotics they export is expected to help in designing improved strategies to develop modulators/inhibitors of these clinically relevant pump proteins.

In this study, we have explored the structure activity relationships of substrates of two major, promiscuous, multidrug transporters of an opportunistic human pathogen *Candida albicans* namely, CaCdr1p and CaMdr1p. To differentiate between substrates and non-substrates, the susceptibilities of the *Saccharomyces cerevisiae* strains over expressing CaCdr1p or CaMdr1p were determined for 67 structurally diverse xenobiotics. A comparison of physico-chemical indices of these tested compounds enabled identification of molecular descriptors such as, degree of hydrophobicity (MLogP), geometrical descriptor (DISPv), molecular edge descriptor (MDEC.12 and MDEC.13) and 3D Morse descriptors, that allowed their segregation into substrates and non-substrates for both the transporter proteins (3). Taken together, present study provides first evidence of chemical basis of substrate specificities of two clinically relevant multidrug transporters of an opportunistic human pathogen *C. albicans*.

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Heterologous expression of the *Candida albicans* general amino acid permeases in yeast shows that they function both as transporters and as traseptors

Van Dijck, Patrick^{1,2}; Kraidlova, Lucie^{1,2,3} and Sychrova, Hana³

¹VIB department of Molecular Microbiology, K.U. Leuven, ²Laboratory of Molecular Cell biology, Kasteelpark Arenberg 31, B-3001 Leuven, Belgium and ³Institute of Physiology AS CR, v.v.i., Prague, Czech Republic

Candida albicans is a human fungal pathogen that can be found in virtually any host niche. In order to be able to sense its environment very well, it expresses a large number of nutrient transporters and sensors. There is evidence that amino-acid sensing and uptake is very important for *C. albicans* growth and virulence. This is clear from the fact that the *Candida* genome expresses six genes with high homology to the *S. cerevisiae* general amino acid permease Gap1. Gap1 is not only required for amino-acid transport but also for sensing the presence of external amino acids and thereby activating signal transduction pathways that induce many intracellular changes. Our aim is to elucidate the role of each individual CaGap permease in amino-acid uptake and sensing, in cell morphology, virulence and pathogenicity. Heterologous expression of each of these CaGAP genes in specific yeast transporter mutants shows that they have different specificities. Only one of them, *CaGAP2*, encodes an amino-acid permease with very broad substrate specificity, i. e. similar to *ScGap1p*. *CaGap1* and *CaGap6* permeases have rather broad substrate specificity whereas the others transport a limited number of amino acids. *CaGap2*, *CaGap1* and *CaGap6* can also activate the PKA signaling pathway upon amino-acid addition to nitrogen-starved *S. cerevisiae* cells. This indicates that also in *C. albicans*, these transporters may function as nutrient sensors.

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T39

Specific dipeptides induce persistent signaling and deficient vacuolar sorting of the yeast amino acid transceptor Gap1Van Zeebroeck, Griet^{1,2}; Rubio-Teixeira, Marta^{1,2} and Thevelein, Johan M.^{1,2}¹Laboratory for Molecular Cell Biology, Institute of Botany and Microbiology-KULeuven and ²Department of Molecular Microbiology, Flemisch Interuniversity Institute for Biotechnology –VIB10 Kasteelpark Arenberg 31, B-3001 Leuven, Belgium

Addition of a nitrogen source to *Saccharomyces cerevisiae* cells starved for nitrogen on a glucose containing medium triggers activation of protein kinase A (PKA) targets through a pathway which requires for sustained activation both a fermentable carbon source and a complete growth medium (Fermentable Growth Medium induced (FGM) pathway). Activation of the PKA targets by amino acids requires the general amino acid permease, Gap1. Gap1 transceptor combines amino acid transport and receptor functions.

In a large-scale screen of amino acid analogs, we identified three specific γ -glutamyl-bound dipeptides, L-Glu- γ -(γ -Abu), L-Glu- γ -L-Ala and L-Glu- γ -Gly, that, in contrast to the classical agonists, cause persistent activation of the PKA pathway and prevent delivery of Gap1 to the vacuole. They are the first examples of dipeptides transported by Gap1. The three dipeptides slow down internalization and cause Gap1 to accumulate in endosome-like structures, which fail to fuse with the vacuole. The three dipeptides can be used by cells as the only nitrogen source in a Gap1-dependent manner. A specific mutation in the amino acid binding domain of Gap1 overrides the sorting defect, persistent signaling and transport of these dipeptides, suggesting that these phenotypes result from continued interaction between Gap1 and the dipeptides after internalization of the transceptor. Together, our results point to the induction of a particular signaling conformational state of Gap1 in the presence of the dipeptides that also makes it incompetent for recognition as vacuolar cargo after internalization.

T40

Identification of new transport systems for folic acid and methotrexate in normal human T-lymphocytes and leukemic cells

Verma, Rama Shanker

Stem Cell and Molecular Biology Laboratory, Department of Biotechnology, Indian Institute of Technology Madras, Chennai-600036, India

Methotrexate (MTX) alone and combination with other drugs have been used in the treatment of cancer and in Rheumatoid arthritis's for a long time. Conceptually, it is accepted that MTX and folic acid are transported by folate receptors (FRs) in cancerous cells, but the exact mechanism of MTX uptake in human leukemia is unknown. In our laboratory, different transport systems for FA and MTX has been identified, in MOLT4, K562, Hut78 leukemia cells and normal human T cells. In MOLT4, uptake of MTX was higher than FA, similar to that of K562, Hut78 and normal T cells. Uptake of FA and MTX was significantly inhibited by anions, suggesting anion-dependent transport system. FA uptake was found to be energy dependent whereas MTX uptake was energy independent. RT-PCR and immunofluorescence results demonstrated the presence of reduced folate carrier as well as proton coupled folate transporter and absence of FR in MOLT4 and normal T cells. Existence of two separate and independent carrier-mediated transport systems for the uptake of FA and MTX in normal and leukemic human T cells will be discussed.

Poster Presentations

P1**Utilization of cystine in *C. glabrata* and identification of *CTNI* as a novel cystine transporter**

Yadav, Amit¹ and Bachhawat, Anand¹

¹*Institute of Microbial Technology, Sector- 39A, Chandigarh, India*

Candida glabrata is an opportunistic yeast pathogen, which has recently emerged as a second most common cause of mucosal and systemic fungal infections. To gain insights in to the sulphur assimilatory pathways of *C. glabrata* its *MET15* gene was disrupted, followed by growing the *met15Δ* strain on different sulphur sources. It was found that *C. glabrata met15Δ* strain was a strict organic sulphur auxotroph like *S. cerevisiae* and could grow on both cysteine and methionine (forward and reverse transsulphuration pathways present) but unlike *S. cerevisiae*, *C. glabrata* failed to utilize glutathione as a sulphur source. Inability to utilize glutathione was due to the absence of a glutathione transporter (Hgt1p) in *C. glabrata* which when introduced in *C. glabrata* restored its growth defect.

C. glabrata was also found to utilize cystine, while *S. cerevisiae* lacked this ability. To investigate the utilization of cystine in *C. glabrata* genomic library of *C. glabrata* made in *S. cerevisiae* specific vector was transformed in *S. cerevisiae met15Δ* strain followed by selecting transformants on cystine. Through this genomic library approach we fished out *CTNI* gene as being responsible for the growth on cystine. Ctn1p is a 12 transmembrane domain protein which when disrupted in *C. glabrata* abrogated its growth on cystine, which was restored by reintroducing *CTNI* gene.

Nitrogen limitation triggers ROS production and cell cycle arrest during alcoholic fermentation in *Saccharomyces cerevisiae*

Ferreira, Ana Mendes¹; Sampaio-Marques, Belém²; Barbosa, Catarina¹; Costa, Vítor³; Faia, Arlete Mendes¹; Ludovico, Paula² and Cecília Leão^{2*}

¹*Institute for Biotechnology and Bioengineering, Centre of Genomics and Biotechnology, (IBB/CGB-UTAD), Universidade de Trás-os-Montes e Alto Douro, 5001-801 Vila Real, Portugal.* ²*Life and Health Sciences Research Institute (ICVS), School of Health Sciences, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal* and ³*IBMC, Instituto de Biologia Molecular e Celular, Universidade do Porto, 4150-180 Porto, Portugal*

The nitrogen limitation has strong effects on yeast physiology and metabolism, being of utmost importance to elucidate the boundary levels of nitrogen in fermentation media that have minimum effect on yeast alcoholic fermentation. On the other hand, uncovering the underlying effects nitrogen limitation can be translated into the prediction of fermentation problems during alcoholic beverage production, particularly wine making.

In the present work, reactive oxygen species (ROS), plasma membrane integrity and cell cycle were evaluated as stress biomarkers in cells of *Saccharomyces cerevisiae*, during alcoholic fermentation in nitrogen-limiting media. The results indicated that nitrogen-limitation leads to an increase of ROS production, where superoxide anion seems not to play a relevant contribution. Together with these effects an increase of loss of plasma membrane integrity and a persistent arrest of cells in G0/G1 cell cycle phases were observed. Moreover, under these conditions it appears that autophagy, evaluated by *ATG8* expression, is being induced, suggesting that it might be essential to allow cell-survival. Conversely, nitrogen feed permitted cells to re-enter cell cycle by abolishing oxidative stress and decreased autophagy. Altogether the results provide new insights on the understanding of wine fermentations under nitrogen-limiting conditions.

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P3

The role of transmembrane helix 9 in substrate recognition by the fungal high affinity glutathione transporters

Thakur, Anil¹ and Bachhawat, Anand K¹

¹*Institute of Microbial Technology (CSIR), Sector 39 A, Chandigarh, India*

Hgt1p is a high affinity glutathione transporter from *Saccharomyces cerevisiae*. To obtain insights into substrate recognition and translocation, we have subjected all 21 residues of transmembrane helix 9 (TMD9) to alanine scanning mutagenesis. F523 was found to be critical for glutathione recognition, since F523A mutants showed a 4-fold increase in K_m without affecting expression or localization. F523 and the previously identified polar residue Q526 were on the same face of the helix suggesting a joint participation in glutathione recognition, while two other polar residues, S519 and N522 of TMD9, although also oriented in the same face, did not appear to be involved. The size and hydrophobicity of F523 were both key features of its functionality as seen from mutational analysis. These findings indicate a key role for TMD9 for substrate recognition in Hgt1p.

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Characterization of a mutant with improved glycerol growth

Souffriau, Ben^{1,2} and Thevelein, Johan M.^{1,2}

¹Laboratory of Molecular Cell Biology, Institute of Botany and Microbiology, Katholieke Universiteit Leuven, Belgium and ²Department of Molecular Microbiology, VIB, Belgium

The biochemical role of glycerol in fungi has been extensively studied. Glycerol is important for lipid synthesis, in osmoregulation, oxidative and heat stress protection and redox balance maintenance. Furthermore it has also industrial importance in wine taste and bioethanol applications. As a waste product of biodiesel its industrial applications are under exploration. Uptake of glycerol occurs by Fps1-mediated diffusion under conditions of low osmolarity, but has also an active component attributed to *STL1* (1).

In this study a *Saccharomyces cerevisiae* (CEN.PK) mutant was obtained after evolutionary engineering in medium with 10 mM glycerol. The mutant displays an improved growth on low concentrations (10 mM) of glycerol, but not on high concentrations (200 mM). Microarray analysis revealed a 25-fold increase in expression of the glycerol/H⁺ symporter *STL1*. Tetrad analysis showed a 2:2 segregation of the glycerol growth phenotype. qPCR analysis in the spores suggests linkage between the increase in *STL1* expression and the improved growth on low glycerol concentrations. These results are in accordance with the findings of Ferreira *et al.* (2005) where the role of *STL1* in active glycerol uptake is established. It is known that glycerol permeation is also mediated by Fps1 by a first-order kinetics mechanism. For this reason it is likely that glycerol uptake is not limiting growth at higher concentrations. This could explain why the phenotype of improved glycerol growth is only visible at low glycerol concentrations.

Another peculiar finding is that glycerol growth can be drastically improved by addition of organic acids as glucuronate, galacturonate, malate and lactate as well in the mutant strain as in the wild-type. Sequence data did not show any mutation in the *STL1* ORF, nor in the promoter or terminator (750bp) regions. However, the microarray data revealed that the 6 most up-regulated genes are situated in an 18 kb region surrounding *STL1*, suggesting a DNA multiplication event in the mutant.

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P5

Application of membrane proteins: The phosphate:H⁺-symporter plasma-membrane protein, Pho84, in a biosensor system.

Basheer, S.¹; Samyn, D.²; Hedström, M.³; Thakur, M.S.¹; Persson, B.L.^{2, 4} and Mattiasson, B.³

¹Department of Fermentation, Technology and Bioengineering, Central Food Technology Research Institute, Mysore 570 013, India; ²School of Natural Sciences, Linnaeus University, 391 82 Kalmar, Sweden; ³Department of Biotechnology, Center for Chemistry and Chemical Engineering, Faculty of Engineering, Lund University, Sweden and ⁴Laboratory of Molecular Cell Biology, Institute of Botany and Microbiology, Katholieke Universiteit Leuven, and Department of Molecular Microbiology, Flanders Institute of Biotechnology, Kasteelpark Arenberg 31, 3001 Leuven-Heverlee, Flanders, Belgium

Affinity biosensors based on protein-substrate recognition have almost exclusively been constructed by using soluble proteins that have been immobilized on the transducer surface of the sensor. In contrast, the cell membrane harbors a protein diversity of staggering proportions (approximately 30% of all proteins), but has so far not been applied extensively in biosensors. If the intention is to exploit the structural properties and unique dynamics of membrane proteins in constructing biosensors, then intact membrane proteins must be used. The high affinity phosphate permease Pho84 of *Saccharomyces cerevisiae* (1), is an integral plasma-membrane transport protein belonging to the family of phosphate:H⁺ symporters (PHS) (2) of the Major Facilitator Superfamily. Based on our experience in heterologous expression and purification of the Pho84 protein, we have constructed a affinity capacitance biosensor with the Pho84 protein as the sensory unit. The capacitance measurements were performed by using the Dropsense® electrode as the working electrode in the electrochemical flow cell. Measurements were made using KH₂PO₄ as the analyte. The total capacitance was measured at the working electrode/analyte interface and was continuously monitored. The behavior of the capacitive measurements depends upon the interaction between phosphate ions and the immobilized Pho84 membrane protein. Capacitance signals were expected as a result of the conformational changes in the protein due to the binding of phosphate ions. Upon injecting KH₂PO₄, there is a clear concentration dependent difference between the control electrodes (without the protein) as compared to the response registered when Pho84 is immobilized on the electrode surface. In our study we show for the first time that it is possible to construct a biosensor based upon the Pho84 inorganic phosphate transporter, as such that both the phosphate sensing and the substrate selectivity have been retained, even though the protein is not in its natural lipid environment. This development may give us the tools to study mutations *in vivo* and establish a phosphate analogue database.

Detection of protein-protein interactions in the plasma membrane of yeast *Saccharomyces cerevisiae*

Strachotová, Dita^{1, 2}; Holoubek, Aleš²; Benda, Aleš³; Váchová, Libuše¹ and Palková, Zdena²

¹*Institute of Microbiology, v.v.i., Academy of Sciences of the Czech Republic, Prague, Czech Republic;* ²*Department of Genetics and Microbiology, Charles University, Prague, Czech Republic and* ³*J. Heyrovský Institute of Physical Chemistry, Academy of Sciences of the Czech Republic, Prague, Czech Republic*

Yeasts are non-motile unicellular organisms. Nevertheless, they can create organized structures, colonies, in which cells communicate and cooperate. Our previous studies on yeast colony development revealed that colonies periodically change pH of their surroundings. Alkalization of an agar medium is accompanied by production of volatile ammonia that acts as the long-range signal (1). Membrane proteins Ato1-3p play an important role in the development of yeast colonies, which is indicated by the steep increase in their expression during the acid-to-alkali transition. Deletion of any ATO gene affects alkalization and leads to decrease in ammonia production. Although the exact function of these proteins has not yet been postulated we propose that they may function as ammonium/H⁺ antiporters (2).

Here, we present a novel approach for FRET-based detection of protein-protein interactions in the plasma membrane of yeast *Saccharomyces cerevisiae* using FLIM. FRET (Fluorescence Resonance Energy Transfer) is one of major techniques used to confirm protein-protein interactions within cells. When FRET between fluorescence donor and acceptor occurs, donor fluorescence lifetime shortens. Monitoring the lifetime shortening of donor fluorescence due to FRET using FLIM (Fluorescence Lifetime IMaging) technique brings a possibility to localize and visualize the protein-protein interaction directly in living cells. Using these approaches, we show that particular Ato proteins interact with each other in the plasma membrane of cells from colonies occurring in the alkali developmental phase.

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P7

Novel synthetic antimicrobial peptides display fungicidal effect against various fungi and in *Candida albicans* the effect is via disruption of cell wall and accumulation of reactive oxygen species

Maurya, Indresh Kumar¹; Sanwal, Hina¹; Sharma, Monika¹; Pathak, Sarika²; Chaudhary, Preeti³; Tupe, Santosh³; Deshpande, Mukund³; Chauhan, Virander Singh² and Prasad, Rajendra¹

¹Membrane Biology Laboratory, School of Life Sciences, Jawaharlal Nehru University, New Delhi-110067, India and ²International Centre for Genetic Engineering and Biotechnology, New Delhi-110067, India and ³Biochemical Sciences Division, National Chemical Laboratory, Pune -.411008, India

In the present study, we for the first time, have investigated the antifungal activity of two novel *de novo* designed and synthesized cationic, amphipathic antimicrobial peptides containing a non protein amino acid α , β -didehydrophenylalanine. We observed that the low haemolytic synthetic antimicrobial peptides VS2 and VS3 could irreversibly inhibit the growth of various *Candida* species and multidrug resistance strains overexpressing drug efflux pumps belonging to either ATP binding Cassette (ABC) or Major Facilitator superfamily (MFS) of proteins. The VS peptides also display fungicidal activity against both filamentous and non-filamentous fungi (Human pathogen, Plant pathogen and Saprophyte). The kinetics of *C. albicans* cells killing showed that it was a rapid and time dependent process. The confocal fluorescent microscopy of FITC tagged-VS peptides confirmed their energy independent, rapid entry into the cytoplasm which corroborated with the killing time kinetics. The entry of these peptides coincided with the damage of cell wall structure as was revealed by scanning and transmission electron microscopy. VS peptides prevented hyphae development of *Candida* cells in both liquid and solid hyphae inducing media. The entry of peptides into *Candida* cells induced the accumulation of Reactive Oxygen Species (ROS) and necrosis. Notably, combination of antifungal drugs (fluconazole (FLC), Cycloheximide) with these peptides resulted in synergistic killing of *Candida* cells *in vitro*. The fraction inhibitory concentration index (FICI) of less than 0.5 confirmed the synergistic interactions between the peptides and antifungal drugs. Taken together, these synthetic antimicrobial peptides represent a good template for further improved design and development as antifungal agents.

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Bragg sensors and its potential applications in yeast studies

Alberto, Nélia¹; Abe, Ilda¹; Tang, Chunhjiu¹ and Pinto, Joao¹

¹*I3N, Physics Department, Aveiro University, Aveiro, Portugal*

In recent years, optical fiber Bragg grating technology has been increasingly applied in many different scientific and engineering applications. They exhibit great advantages when compared with conventional devices in terms of immunity to electromagnetic radiation, sensitivity, geometric versatility, dimensions and system integration. Although conventional measuring techniques are adequate and reliable, they give bulk rather than localized results. In addition, they are often not adequate for real-time and in-situ measurements.

Optical fiber long period gratings, fiber Bragg gratings and tilted fiber Bragg gratings have been exploited in many chemical and biochemical applications. Multi-parameter optical sensors based on tilted fiber Bragg gratings have been proposed for monitoring refractive index, strain and temperature. Typically, resolutions up to 2×10^{-5} , $4 \mu\text{e}$ and 0.1 C have been achieved. The response of optical fiber long period gratings have also been reported to measure changes of the refractive index of external media relatively to variations of wavelength and in transmission. Higher sensitivities have been achieved with layers of a few tens of nanometers deposited onto the fiber. In the present communication, potential applications of fiber Bragg gratings in yeast studies will be analyzed and discussed. In particular, its feasibility for metabolic and energy regulation and stress responses and transport studies will be considered in terms of refractive index, temperature and microstrain variations.

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P9

Mode of action of chalcone derivatives on the yeast *Saccharomyces cerevisiae*Łacka, Izabela¹; Kodedová, Marie²; Gášková, Dana² and Milewski, Sławomir¹¹*Department of Pharmaceutical Technology and Biochemistry, Gdansk University of Technology, Gdansk, Poland and* ²*Charles University, Faculty of Mathematics and Physics, Institute of Physics, Ke Karlovu 5, 121 16 Prague 2, Czech Republic*

Chalcones (1,3-diaryl-2-propen-1-ones) of natural or synthetic origin, like other flavonoids, exhibit a number of different biological effects, including antimicrobial and anticancer activity. It has been shown that some of the chalcone derivatives inhibit the drug extrusion activity of the yeast drug transporter Pdr5p and it is also known that the mode of antifungal action of some chalcones is related to their effect on the fungal cell wall (1), (2).

Using a set of five isogenic mutant strains, we tested the effects of six oxathiolone fused chalcone derivatives, on membrane potential and the activity of Pdr5p, Snq2p and Yor1p MDR pumps in *Saccharomyces cerevisiae* by the fluorescence diS-C₃(3) method and disc diffusion test. Depending on their concentration and small changes in chemical structure, all chalcone derivatives caused hyperpolarization of the membrane, the biggest effect was shown in cells treated with AMG-148 and AMG-154. The most effective compound, AMG-148, perturbed the membrane in the concentration-dependent manner, starting from the very low level (0.1 μM) by forming very narrow leaks which allowed outflow of cations (predominantly K⁺) from the cytosol. Moreover, AMG-148 caused damage of the cell wall. This effect was probably caused by inhibition of membrane enzymes – chitin and β(1→3)glucan synthase. Most of the tested compounds also inhibited transport of the fluorescence probe by Pdr5p and Snq2p pumps.

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P10

Residues contributing to substrate selectivity in the Pho84 phosphate:H⁺ symporter of *Saccharomyces cerevisiae*.

Samyn, D.¹; Ruiz-Pavon, L.¹ and Persson, L. B.¹

¹ *School of Natural Sciences, Linnaeus University, 391 82 Kalmar, Sweden*

In *Saccharomyces cerevisiae*, under phosphate-limited growth conditions, phosphate uptake is mainly dependent upon the Pho84 phosphate:H⁺ symporter. The high-affinity phosphate permease Pho84 of *Saccharomyces cerevisiae* (1), is an integral plasma-membrane transport protein belonging to the family of phosphate:H⁺ symporters (PHS) (2) of the Major Facilitator Superfamily. With the 3D crystal structure of the *E. coli* glycerol-3-phosphate/phosphate (GlpT) antiporter resolved, our lab has used this transporter as a template to model the Pho84 transporter (3). This model, together with a multiple-sequence-alignment (MSA) of members belonging to different species, has been the basis for selecting several amino acid residues which could be important in the selectivity and transport of inorganic phosphate (Pi) across the membrane. The selection of residues for site-directed mutagenesis was based on several criteria involving side-chain properties, location in our theoretical model (i.e. facing towards in the “binding pocket”). All residues selected are highly conserved in the MSA, and some can be allocated to a known signature-sequence motif **TLCFFR168 FWLGF~~G~~I~~G~~GD178** YPLSATIMSE, shared by proton-coupled phosphate transporters from plants, fungi, bacteria, and mammals. This motif contains the strongly conserved R168 and D178 residues. All mutated genes were cloned in the pU6H2Myc vector, which served as the template for the homologous recombination cassette. External phosphate determinations and growth spot-tests in either high (10mM) or low (200μM) K₂HPO₄ conditions were performed as an initial screening method. Expression levels of the mutant proteins were checked with immunoblotting and immunologically detected with either cMyc or Pho84 c-terminal antibodies. In order to determine the real contribution of the selected residues, we performed ³²P-uptake studies. Our results indicate that some of the selected residues seem to contribute to phosphate uptake. Whether this contribution is an immediate consequence of one or more residues is under investigation.

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P11

Alanine scanning of transmembrane domains of ABC transporter CaCdr1p, of *Candida albicans*; their role in drug recognition and transport

Rawal, Manpreet Kaur¹; Puri, Nidhi¹ and Prasad, Rajendra¹

¹*School of Life Sciences, Jawaharlal Nehru University, New Delhi, India*

Candida albicans is a dimorphic, opportunistic pathogenic fungus, most frequently associated with fungal infections in humans. To combat increasing incidences of multidrug resistance (MDR) in *Candida albicans*, in particular, azole resistance, proper understanding of the molecular mechanisms of drug resistance is needed. One of the major mechanism documented for the occurrence of MDR is over-expression of *CaCDRI* encoding a major multidrug efflux membrane protein CaCdr1p belonging to the ABC (ATP Binding Cassette) super-family of proteins. CaCdr1p comprises of two cytoplasmic nucleotide binding domains (NBDs) and two transmembrane domains (TMDs). Each TMD comprises of six transmembrane segments (TMS). TMDs exhibit relatively high sequence variability than NBDs and probably determine the broad substrate specificity of CaCdr1p and ensure substrate translocation across the membrane. To formulate strategies to rationally design modulators/inhibitors of CaCdr1p, which could block the pump activity, an understanding of the protein structure and function is essential.

In vitro site directed mutagenesis in combination with functional assays of the mutants is expected to provide information about the structural basis of transporter functions and identification of the putative substrate/drug binding sites. Earlier by employing site directed mutagenesis, it was revealed that TMS5 and TMS11 of CaCdr1p play an important role in drug recognition and transport (1, 2). To map the entire drug binding pocket and to investigate the role of all the TMS in CaCdr1p function in general and substrate specificity, in particular, we are subjecting all the 12 TMSs to alanine scanning. Thus more than 100 mutant variants of different TMS have been constructed and characterized. Although the phenotypes of individual mutant variant were varied, some generalizations can be made. For example, replacement at specific positions seemed to affect the drug resistance profile or transport activity or both. This presentation will discuss some of the possible role of these TMS residues of CaCdr1p in substrate specificity and drug efflux.

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Role of the Fps1 aquaglyceroporin in ethanol sensitivity of *Saccharomyces cerevisiae* and protection effect of acetic acid

Trindade, Dário¹; Afonso, Andreia¹; Piper, Peter W.²; Côrte-Real, Manuela¹ and Sousa, Maria João¹

¹CBMA/Departamento de Biologia, Universidade do Minho, Braga and

²Department of Molecular Biology and Biotechnology, University of Sheffield, Sheffield, UK

The Fps1 aquaglyceroporin is a yeast member of the MIP family of channel proteins and well described as a glycerol facilitator (1) being also able to mediate flux of other small molecules. Mollapour and Piper identified Fps1p as an important element in acetic acid resistance of yeasts; a process that involves Fps1p removal from the plasma membrane after phosphorylation by the Hog1p (2). Our previous results demonstrated that *S. cerevisiae* cells presented increase resistance to high ethanol concentrations when simultaneously treated with low concentrations of acetic acid (Veira et al., unpublished results). Herein we evaluated the possible involvement of Fps1 in this protection. Tolerance of *S. cerevisiae* BY4741 and *fps1Δ* to 14% (v/v) ethanol in the absence or presence of 16 mM acetic acid, was assessed both by CFU counting and PI staining. Also, we directly assessed Fps1 response to the presence of ethanol by using a C-terminally GFP tagged Fps1p. Both strains displayed no significant difference when treated only with ethanol, and though acetic acid increased ethanol tolerance of both strains, this phenotype was even more evident for *fps1Δ*. While ethanol stress induced an Fps1p rearrangement in the membrane, changing from an even to a spot distribution, combined treatment with acetic acid led not only to a similar Fps1p membrane rearrangement but also to its removal from the plasma membrane. The results suggested that although ethanol, either alone or in combination with acetic acid, can trigger an Fps1p response, this seems not relevant for the higher tolerance to ethanol or for the protection afforded by acetic acid. Next, the possible contribution of an acetic acid-activated Hog1 mechanism to the acetic acid protection phenotype was evaluated. The *hog1Δ* strain in spite of being more sensitive to ethanol alone, exhibited an increased tolerance to the combined treatment with acetic acid, as observed for *fps1Δ*. Therefore, the mechanism responsible for acetic acid-induced protection of ethanol toxicity is also not dependent on the direct action of Hog1.

This work was developed in the scope of the project PTDC/AGR-ALI/102608/2008/FCT.

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P13

***N*-substituted derivatives of nystatin of improved selective toxicity are active against multidrug-resistant yeast**

Milewska, Maria J.¹; Salewska, Natalia¹; Boros, Joanna²; Borowski, Edward² and Milewski, Sławomir²

¹*Department of Organic Chemistry and* ²*Department of Pharmaceutical Technology & Biochemistry, Gdansk University of Technology, 11/12 Narutowicza Street, 80-233 Gdansk, Poland*

One of the topics of our studies is an improvement of the chemotherapeutic index of polyene macrolide antibiotics by their rational chemical modification. We have previously shown that mammalian toxicity of Amphotericin B can be strongly reduced due to the chemical modification of the amino group of mycosamine residue present in most polyene macrolides. Now we have taken advantage of the same rationale for the construction of derivatives of nystatin A₁. Several novel compounds were synthesized by Michael-type addition of *N*-substituted maleimides, reductive alkylation with aromatic and alkyl aldehydes, Amadori rearrangement of sugar derivatives or *N*-acylation. The synthesized compounds were tested for antifungal *in vitro* activity, hemolytic activity and induction of potassium leakage from fungal and mammalian cells. *N*-substituted derivatives of nystatin demonstrated 4 – 8 times higher MICs than the mother antibiotic, however some of them showed substantial reduction of lytic activity against human erythrocytes and in consequence, an improved chemotherapeutic index. Potassium efflux from fungal cells was induced at much lower concentrations of nystatin derivatives than from erythrocytes. Multidrug-resistant clinical *Candida albicans* strains demonstrated similar susceptibility to novel derivatives of nystatin as their drug-sensitive counterparts.

P14

Protective effect of antioxidants on amyloid induced toxicity in *Saccharomyces cerevisiae*

Verma, Meenakshi¹; Sharma, Abhishek¹; Bhadra, Ankan¹ and Taneja, Vibha¹
¹*Functional Genomics Unit, Institute of Genomics and Integrative Biology, Delhi, India*

Amyloidosis is a group of protein misfolding disorders, characterized by abnormal accumulation of insoluble fibrous protein aggregates in various organs. Broadly these proteins can be characterized as glutamine/asparagine-rich (Q/N) such as huntingtin (Huntington disease) and alpha-synuclein (Parkinsons disease) and non-glutamine/asparagines-rich (non-Q/N) such as Prion protein (Prion disease), Amyloid beta protein (Alzheimer's disease). Interestingly, yeast and other fungi have endogeneous prions which have been exploited to understand the prion- amyloid phenomena (1). Yeast has already been established as a model for Huntington's, Alzheimer's and Parkinson's diseases (2).

The etiology of pathogenesis involved with these diseases is still not very clear, but some probable mechanisms have been hypothesized. Oxidative stress has been shown to be implicated with various neurodegenerative processes including amyloidosis (3). Antioxidants prevent oxidative stress in neurons and have been shown to play a role in amyloid toxicity. We have studied the effect of various antioxidants for their potency to ameliorate this stress in Huntington and Prion diseases in yeast model. Three of these antioxidants clearly cause ~2-fold decrease in aggregation of both Q/N and non Q/N-rich amyloid proteins. **One of these molecules appears to act through Endosomal Sorting Complex Required for Transport (ESCRT)**. The findings and the importance of ESCRT complex in amyloid formation will be presented in the meeting.

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P15

Impact of conserved glutamic acid residues on phosphate transport of *Saccharomyces cerevisiae* Pho89 inorganic phosphate transporterAndersson, Michael and Persson, Bengt L.*School of Natural Sciences, Linnaeus University, Sweden*

In order to adapt to a fluctuating nutrient environment, unicellular organisms need to have specialized nutrient sensing and uptake mechanisms. The acquisition of inorganic phosphate in *Saccharomyces cerevisiae* is mediated via a H⁺-coupled low-affinity system composed of the phosphate transporters Pho87, Pho90, and Pho91, and a high-affinity system composed of the H⁺-coupled Pho84 and the Na⁺-coupled Pho89 permeases.

Under phosphate-limited growth condition the Pho84 transporter will be mainly responsible for the phosphate uptake, with the Pho89 contribution being of a minor role (1). The low affinity system- transporters and the Pho84 are members of the major facilitator superfamily (MFS) having a pH optimum for Pi uptake of 4, 5 and transports Pi in a proton dependent manner. Pho89 is distinct from the other transporters for that it has a pH optimum of 9.5, which is far from the natural habitat of *Saccharomyces cerevisiae*, and is a member of the NaPiIII (PiT) family where several human transporters as PiT2 also are members.

Until now, the Pi uptake of Pho89 has only been characterized on a physiological level. Here we report the preliminary findings of our investigation, using radiolabeled phosphate uptake studies, if the same amino acid residues shown to be of importance for Pi uptake in hPiT2 also have a conserved function for Pi uptake in Pho89.

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A transcriptome and lipidome analyses of *Candida albicans* establishes ergosterol biosynthetic pathway as a major potential antifungal target of curcumin

Sharma, Monika¹; Dhamgaye, Sanjiveeni¹; Singh, Ashutosh¹; Manoharlal, Raman¹; Roth, Mary²; Welti, Ruth² and Prasad, Rajendra¹

¹Membrane Biology Laboratory, School of Life Sciences, Jawaharlal Nehru University, New Delhi-110067, India and ²Kansas Lipidomics Research Center, Division of Biology, 510 Ackert Hall, Kansas State University, Manhattan, KS 66506-4901, USA

We previously demonstrated that purified polyphenol curcumin (CUR) inhibits the growth of various *Candida* species and multidrug resistant strains. Here, to further investigate the mechanism underlying CUR antifungal activity, we performed genome-wide transcriptome and lipidome analyses of *Candida albicans* following CUR treatment. Our microarray data revealed that almost 25% of total open reading frames responded to CUR treatment by up- or down-regulation. Among the responsive classes of genes, the lipid metabolic pathways, namely the genes involved in ergosterol (*ERG*), sphingolipids, and fatty acid metabolism, were most noteworthy. Several *ERG* genes, including its transcription factor *UPC2*, were down-regulated. *HMG1*, which encodes the regulatory enzyme HMG-CoA reductase, was also down-regulated. In addition, the transcripts of many mitochondrial and oxidative stress-related genes were affected; corroborating our earlier results that CUR treatment leads to the accumulation of reactive oxygen species. To validate the transcriptome data, lipidome analysis of CUR-treated *Candida* cells was conducted through high throughput mass spectroscopy-based lipid profiling. The lipidome data largely matched the transcriptome observations and revealed that the ergosterol depletion was accompanied by a parallel accumulation of its precursors such as squalene, lanosterol, and farnesol. Taken together, these findings indicate that CUR targets several steps of the ergosterol biosynthetic pathway, leading to the accumulation of ergosterol intermediates and resulting in ergosterol depletion. The resulting perturbations in the plasma membrane affect mitochondrial function, leading to oxidative stress and cell death. Thus, our data provide the first novel mechanistic insight into the mode of action of CUR.

P17

Towards expression and functional characterization of recombinant phosphate transporter Pho89 protein in *Pichia pastoris*Sengottayan, Palanivelu and Persson, Bengt L.*School of Natural Sciences, Linnaeus University, Kalmar, Sweden*

In *Saccharomyces cerevisiae*, the high-affinity Pho89 plasma membrane phosphate transporter belongs to the sodium-phosphate III family of proteins. It is a Na⁺/Pi co-transporter and catalyzes a sodium-dependent phosphate uptake at alkaline pH. In response to phosphate limiting conditions, the phosphate responsive signaling pathway (*PHO*) induces the transcription of genes involved in acquisition, uptake and storage of phosphate.

The primary amino acid sequence (574 amino acid residues) of Pho89 protein predicts a membrane topology involving 12 transmembrane-spanning domains (TMDs) structure with proposed periplasmic N - and C - termini and has a large internal hydrophilic loop connecting TMDs 7 and 8. The Pho89 protein exhibits significant amino acid sequence homology with the mammalian type III Na⁺/-Pi transporters (PiT-1 and PiT-2) and the Pho4 phosphate transporter of *Neurospora crassa*. To gain insights into the structural and functional characterization of the Pho89 protein, we aim for expression of the Pho89 protein in a heterologous expression system using the methylotrophic yeast *Pichia pastoris*.

In this study, the *PHO89* gene of *S. cerevisiae* was cloned into the yeast expression vector pPICZB, resulting in the fusion of a His₆ tag sequence at the C-terminus of the protein. The pPICZB+*PHO89*-His₆ construct were linearized with *SacI* to target integration at the *AOX1* locus in the *Pichia* genome. After transformation into yeast and screening by immunoblotting analysis, we have obtained yeast clones for the Pho89 protein expression. Further expression and purification studies of the Pho89 protein are currently in progress.

Transport and metabolism of glutathione in *Candida albicans*

Desai, Prashant¹; Thakur, Anil¹; Ganguli, Dwaipayan¹; Morschhäuser, Joachim² and Bachhawat, Anand¹

¹ *Institute of Microbial Technology (CSIR), Sector 39A, Chandigarh, India and*
² *Institut für Molekulare Infektionsbiologie, Universität Würzburg, Josef-Schneider-Str. 2, D- 97080 Würzburg, Germany*

Glutathione, a tripeptide thiol molecule, is an essential metabolite in yeasts. In addition to its role as a redox buffer and in dealing with different stress responses, it can also be used as a sulphur source. We have constructed a *met15Δ/met15Δ* strain that shows partial organic sulphur auxotrophy to evaluate the ability of *Candida albicans* to utilize exogenous glutathione. We observed that *C. albicans* can utilize glutathione from the extracellular environment. In *S. cerevisiae*, the Dug proteins (Dug1p, Dug2p and Dug3p) are required for degradation of glutathione. *C. albicans* has homologues to these proteins. By disrupting one of the components of the complex, we observed the requirement of the DUG complex in *C. albicans* for degradation of exogenous glutathione. *C. albicans* has eight members of the oligopeptide transporter (*OPT*) family, Ca*OPT1*-Ca*OPT8*. In *S. cerevisiae* and *S. pombe*, members of the *OPT* family have been shown to function in glutathione transport. *MET15* gene deletions were created in a *C. albicans* mutant lacking *OPT1-OPT5*, but Opt1p-Opt5p did not seem to be involved in the transport of glutathione to any significant extent. We are now attempting to identify the potential glutathione transporters by complementation of a *C. albicans* genomic library in a *S. cerevisiae hgt1Δ met15Δ* strain that lacks the ability to grow on glutathione as a sulphur source owing to the absence of the glutathione transporter, Hgt1p. Using this strategy we have identified possible candidate proteins that are being investigated.

P19

Regulatory Logic of Iron Homeostasis in Response to Iron Deprivation is Mediated by a Bipartite CBF-bZIP Domain Transcriptional Regulator in *Candida albicans*

Singh, RanaPratap; Prasad, Himanshu; Sinha, Ishani; Agarwal, Neha and Krishnamurthy, Natarajan

School of Life Sciences, Jawaharlal Nehru University, New Delhi 110067, India

Transcriptional regulators respond to various external cues by activating or repressing transcription of the target genes. We identified and analyzed the full repertoire of bZIP family of transcriptional regulators in the *Candida* clade genomes. Comparative analyses showed that while the sequence orthologs for each of the known *S.cerevisiae* bZIP sub-family members were present in the *Candida* clade genomes, the repertoire and orthology of YAP sub-family were strikingly different. The *S.cerevisiae* genome encoded eight YAP proteins compared to only four YAP proteins, named CAP1-CAP4, in *Candida* clade genomes.

Here, we present the functional dissection of *CAP2* that has an evolutionarily conserved 17-amino acid HAP4L/CBF domain in addition to bZIP domain, hence named *HAP43* previously. We found that the *cap2Δ/Δ* mutant was severely impaired for growth under iron deprivation conditions imposed by the iron chelator, BPS. The *cap2Δ/Δ* mutant was also attenuated for virulence with a high statistical significance in a mouse model of candidiasis. Thus *CAP2* is likely important in iron homeostasis during infection. Consistent with its requirement in response to iron-deprivation, the *CAP2* level is tightly regulated by iron availability; it is induced steadily upon BPS treatment while repressed upon addition of FeCl₃. Microarray analyses showed that *CAP2* is a master regulator of the genes involved in iron homeostasis during iron-deprivation. Our mutational data showed that the bZIP as well as HAP4L domains in *CAP2* contribute independently to its function during iron-stress. Real-time qRT-PCR analyses showed that the expression of two other subunits of the CBF complex in *C. albicans*, called *HAP2* and *HAP32*, are induced in a *CAP2*-dependent manner in iron-deprivation and *hap32Δ/Δ* mutant conferred BPS-sensitive phenotype. Our analyses also showed that the *CAP2* is epistatic to *SFUI*, previously shown to be a repressor of iron uptake in *C. albicans*. Taken together, these results support the primacy of the *CAP2* regulatory function in iron-deprivation.

Role of proline residues of the TM domains in substrate translocation by Hgt1p, a high affinity glutathione transporter of *Saccharomyces cerevisiae*

Gupta, Shiffalli¹ and Bachhawat, Anand¹

¹*Institute of Microbial Technology, Chandigarh, India*

Hgt1p, a 799 amino acid membrane protein is a high affinity glutathione transporter from *Saccharomyces cerevisiae*. Hgt1p belongs to a relatively novel and poorly characterized family of oligopeptide transporters (OPT), majority of whose members have still unknown substrate specificity. In this part of work efforts have been made to investigate the possible role of proline residues in substrate binding and transport by Hgt1p. We selected a total of 12 proline residues (proline residues present in the TMDs, controversial loops and the conserved, non TMD prolines) and subjected them to alanine mutagenesis. Functional characterization was carried out by the dual complementation cum toxicity assay. P292A, P555A, P704A and P705A were found to be severely affected in function. Residues P147A, P525A, P672A and P708A showed a moderate effect, while P351A, P371A, P552A and P589A showed only a mild effect. As observed, defect in functionality could be due to a defect in protein expression level or an inability in trafficking to the plasma membrane or a defect in the ability of cell to transport glutathione. The mutants were examined for both protein expression and trafficking defect. Mutants P525A and P589A showed a significant decrease in protein expression level, indicating a defect in protein expression. All the severely affected mutants were localized on the plasma membrane similar to wild type except for P292A, which showed a predominant intracellular signal, with a small signal at the plasma membrane indicating a severe defect in localization of the protein to the plasma membrane.

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P21

Zinc and copper plasma membrane transporters an “in silico” analysis

Muñoz Elisa

Poster Session

P22

Cation transporters affected by defects in glycosylation in *Candida albicans*

Perez M. Angeles

P23

The expression of *Arabidopsis thaliana* gene encoding for glutathione transport in yeastAngra, Deepti and Murray, James A. H.*Cardiff School of Biosciences, Life Sciences Building, Museum Avenue, Cardiff CF10 3AX, United Kingdom*

Plants like other eukaryotes have the ability to transfer peptides across membranes, and have been shown to have a number of peptide transporters. The sulphur-containing tripeptide glutathione is a key antioxidant and stress signaling molecule important for growth, development and in several plant defence against abiotic and biotic stress. Glutathione is synthesized in two steps, the first generating γ -glutamyl cysteine (γ -GS) occurring in the membrane-bound plastid and the second in the plastid and cytosol generating glutathione. Cytosolic glutathione therefore originates in the plastid, exported either as glutathione or as the γ -GS intermediate. We recently identified and cloned a novel family of three related glutathione transporters from *Arabidopsis* At5g19380, At4g24460 and At5g12170. This family of transporters is plant-specific, and the only known related protein is the PfCRT (*Plasmodium falciparum* CQ like transporter) of malaria, which is a putative drug/metabolite transporter and confers resistance to the anti-malarial compound chloroquine. *Saccharomyces cerevisiae* is known to grow using, methionine, cysteine or glutathione as sole source of sulphur. To investigate further the function of this novel family in glutathione transport, we are expressing them in the yeast *Saccharomyces cerevisiae*. We are using complementation of the yeast mutant *hgt 1*, deficient in its own glutathione transporter, to characterize the putative *Arabidopsis* glutathione transporters and comparing these to malaria PfCRT expressed in yeast.

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List of participants

Ambudkar Suresh			T12	T31	
Ananthaswamy Neeti				T30	
Andersson Michael					P15
Angra Deepti					P23
Bachhawat Anand	T20	P1	P3	P18	P20
Barbosa Catarina					P2
Bhadoriya Sneh Lata					
Bhadra Bhaskar					
Boer Victor					
Brandão Rogelio Lopes			T8		
Cabrito Tânia R				T19	
Cannon Richard				T13	
Chauhan Neeraj				T24	
Choudhuri S.K				T28	
Desai Prashant R					P18
Deshpande Mukund				T26	P7
Dev Rahul					
Dhamgaye Sanjiveeni					P16
Dominguez Angel				T14	
Ernst Joachim				T21	
Ganesan K.					
Garcia Eloy					
Garcia Sonsoles					
Gášková Dana			T23	T35	P9
Gerber Susanne					
Goffeau Andre				T15	
Gottesman Michael				T1	
Gottesman Susan					
Gupta Shiffalli					P20
Hameed Saif					
Hoefler Milan				T35	
Hoefler Radka					
Izabela Lacka				T22	P9
Kapoor Khyati				T29	
Kschischo Maik				T3	T4
Kumar Anil					
Kumar Antresh				T25	
Kumar Indresh					P7
Leaeo Jorge					
Leaeo Maria					
Leão Cecília				T9	P2
Lichtenberg-Fraté Hella				T4	
Ludovico Paula					P2
Ludwig Jost				T4	
Mahanty Sarmishtha					
Mendes-Ferreira Ana					P2

Author Index

Milewska Maria J.								P13
Milewski Slawomir					T22		P9	P13
Misra Prashant K.								
Morschhäuser Joachim							T16	P18
Mukhopadhyay Gauranga								
Muñoz Elisa							T14	P21
Naik Surabhi								
Natarajan Krishnamurthy							T18	P19
Paiva Sandra							T32	
Pathan Ejaj								
Pavon Lorena Ruiz								P10
Pena Antonio								
Perez M. Angeles								P22
Pietro Di Attilio							T11	
Pinto João Lemos								P8
Plasek Jaromir							T35	
Prasad Rajendra	T22	T25	T29	T37	P7		P11	P16
Prasad Tulika								
Prista Catarina							T6	
Puri Nidhi							T37	P11
Rao Rajini							T2	
Rawal Manpreet Kaur								P11
Roy Nilanjan							T7	
Sachdeva Pulkit								
Sadhale Parag							T33	T34
Samyn Dieter							P5	P10
Sanglard Dominique							T27	
Santo Regiane Espãrito								
Sanyal Kaustav							T33	
Seigler Karel								
Sengottaiyen Palanivelu								P17
Shah Peer Abdul Haseeb								
Sharma Monika							P7	P16
Shukla Shipra								
Shukla Suneet							T12	
Shukla Yogeshwer							T36	
Singh Ashutosh								P16
Singh RanaPratap							T18	P19
Souffriau Ben								P4
Sousa Maria João							T9	P12
Srinivasan M.								
Strachotova Dita								P6
Sychrova Hana					T5		T38	
Taneja Vibha								P14

Author Index

Thakur Anil	T20	P3	P18
Thevelein Johan		T10	P4
Thomas Edwina			
Tupe Santosh Genba		T26	P7
Turaga VN Ramachander			
Turcotte Bernard		T17	
Van Dijck Patrick		T38	
Van Zeebroeck Griet		T10	T39
Verma Meenakshi			P14
Verma Rama Shanker		T40	
Yadav Amit Kumar			P1

List of Participants

Ambudkar Suresh V.

Laboratory of Cell Biology
NCI, NIH, Building 37, Room 2E18
37, Convent DR MSC 4255
Bethesda MD 20892, USA
ambudkar@mail.nih.gov

Ananthaswamy Neeti

Catholic University of America
Biology, 620 Michigan Ave N.E.
Washington DC 20064, USA
a.neeti@gmail.com

Andersson Michael

Linneaus University
School of Natural Science
Kalmar, Sweden
michael.andersson@hik.se

Angra Deepti

Cardiff School of Biosciences
Life Sciences Building
Museum Avenue
Cardiff CF10 3AX
United Kingdom
angrad@cardiff.ac.uk

Bachaawat Anand

Institute of Microbial Technology
Yeast Molecular Biology Division
Sector - 39A, Chandigarh, 160036
India
anand@imtech.res.in

Barbosa Catarina

Universidade de Trás-os-Montes e
Alto Douro
Institute for Biotechnology and
Bioengineering,
Centre of Genomics and
Biotechnology, (IBB/CGB-UTAD)
Quinta de Prados, P.O. BOX 1013
Vila Real, 5001-801, Portugal
crbarbosa@utad.pt

Bhadoriya Sneha Lata

Jawaharlal Nehru University
School of Life Sciences
New Mehrauli Road
New Delhi, 110067
India
sneh@mail.jnu.ac.in

Bhadra Bhaskar

DuPont Knowledge Centre, BCS&E
DS9, ICICI Knowledge Park
Hyderabad 500078
India
Bhaskar.Bhadra@ind.dupont.com

Boer Victor

Viktor.Boer@dsm.com

Brandao Rogelio

Universidade Federal de Ouro Preto
Núcleo de Pesquisas em Ciências
Biológicas
Campus do Morro do Cruzeiro
Ouro Preto 35.400-000
Brazil
rlbrand@nupeb.ufop.br

Cabrito Tania

Institute for Biotechnology and
Bioengineering
Center for Biological and Chemical
Engineering,
Biological Science Research Group,
Instituto Superior Técnico
Av. Rovisco Pais, Lisboa 1049-001
Portugal
tania.cabrito@ist.utl.pt

Cannon Richard

University of Otago
Oral Sciences, PO Box 647
Dunedin, 9054
New Zealand
richard.cannon@otago.ac.nz

Chauhan Neeraj

Public Health Research Institute
New Jersey Medical School
University of Medicine and
Dentistry of New Jersey (UMDNJ)
225 Warren Street
Newark NJ 07103
USA
chauhan1@umdnj.edu

Choudhuri S.K.

Department of In vitro
carcinogenesis and cellular
chemotherapy
Chittaranjan National Cancer
Institute
37 S P Mukherjee Road
Calcutta-700 026
India
soumitra01@yahoo.com

Desai Prashant R

Institute of Microbial Technology
Yeast Molecular Biology Division
Sector - 39A
Chandigarh 160036
India
prashant@imtech.res.in

Deshpande Mukund

National Chemical Laboratory
Biochemical Sciences Division
Dr. Homi Bhabha Road
Pune 411008
India
mv.deshpande@ncl.res.in

Dev Rahul

School of Life Sciences
New Mehrauli Road
New Delhi 110067
India
rahuldev14@gmail.com

Dhamgaye Sanjiveeni

Membrane Biology Laboratory
School of Life Sciences
Jawaharlal Nehru University
New Delhi 10067
India
sanjiveeni02@gmail.com

Di Pietro Attilio

CNRS and University of Lyon
Institute of Protein Biology and
Chemistry
Passage du Vercors, 7 Lyon, 6
France
a.dipietro@ibcp.fr

Dominguez Angel

University of Salamanca
Alfonso X el Sabio
Salamanca 37007, Spain
ado@usal.es

Ernst Joachim

Heinrich-Heine-Universitaet
Inst. f. Mikrobiologie/ Molekulare
Mykologie
Univ.-str. 1/26.12.01
Duesseldorf 40225, Germany
joachim.ernst@uni-duesseldorf.de

Ganesan K.

Institute of Microbial Technology
Yeast Molecular Biology Division
Sector - 39A, Chandigarh, 160036
India
ganesan@imtech.res.in

Garcia Eloy

University of Salamanca
Cellular Biology and Pathology
Alfonso X el Sabio
Salamanca 37007, Spain
emuto@usal.es

List of Participants

Garcia Sonsoles

University of Salamanca
Cellular Biology and Pathology
Alfonso X el Sabio
Salamanca 37007, Spain

Gaskova Dana

Inst. Phys., Charles Univ
Dept. Biophysics
Ke Karlovu 5
Prague 121 16
Czech Republic
gaskova@karlov.mff.cuni.cz

Gerber Susanne

Theoretical Biophysics
Humboldt University of Berlin
Berlin 10115, Germany
gerber.sj@gmail.com

Goffeau Andre

Université catholique de Louvain
Institut des Sciences de la Vie
Croix du Sud 4/15
Louvain-la-Neuve 1348
Belgium
agoffeau@hotmail.com

Gottesman Michael

National Institutes of Health
Laboratory of Cell Biology, Cancer
Research Center, NCI
37 Convent Drive, Bldg. 37, Ste
2108A
Bethesda 20892-4258, USA
gottesmanm@mail.nih.gov

Gottesman Susan

Laboratory of Cell Biology
NCI, NIH, Building 37, Room 2E18
37, Convent DR MSC 4255
Bethesda MD 20892, USA
susang@helix.nih.gov

Gupta Shiffali

Institute of Microbial Technology
Yeast Molecular Biology Division
Sector - 39A Chandigarh 160036
India
shiffalli@imtech.res.in

Hameed Saif

Membrane Biology Laboratory
School of Life Sciences
Jawaharlal Nehru University
New Delhi 10067, India
saifhameed@yahoo.co.in

Hofer Milan

IZMB
Kirschallee 1
53115 Bonn
Germany
unb121@uni-bonn.de

Hofer Radka

Izabela Lacka

Gdansk University of Technology
Department of Pharmaceutical
Technology and Biochemistry
Narutowicza 11/12
Gdansk 80 233, Poland
lackaiza@wp.pl

Kapoor Khyati

Membrane Biology Laboratory
School of Life Sciences
Jawaharlal Nehru University
New Delhi 10067, India
kapoor.khyati@gmail.com

Kumar Anil

Yeast Genetics Laboratory
School of Life Sciences
Jawaharlal Nehru University
New Delhi 10067, India
anilnehra@gmail.com

Kumar Antresh

Membrane Biology Laboratory
School of Life Sciences
Jawaharlal Nehru University
New Delhi 10067
India
omraj2003@gmail.com

Kumar Indresh

Membrane Biology Laboratory
School of Life Sciences
Jawaharlal Nehru University
New Delhi 10067
India
inderwinner@gmail.com

Leão Cecilia

University of Minho
Life and Health Sciences Research
Institute (ICVS)
School of Health Sciences Campus
de Gualtar
Braga 4710-057
Portugal
cleao@ecsaude.uminho.pt

Lichtenberg-Fraté Hella

University of Bonn
IZMB Kirschallee 1
Bonn 53115
Germany
h.lichtenberg@uni-bonn.de

Ludovico Paulo

University of Minho
Life and Health Sciences Research
Institute (ICVS)
School of Health Sciences Campus
de Gualtar
Braga 4710-057
Portugal
pludovico@ecsaude.uminho.pt

Ludwig Jost

Universität Bonn
IZMB - Mol. Bioenerg. Kirschallee 1
Bonn 53115
Germany
jost.ludwig@uni-bonn.de

Mahanty Sarmishtha

Yeast Genetics Laboratory
School of Life Sciences
Jawaharlal Nehru University
New Delhi 10067
India
dulu_mahanty@yahoo.co.in

Maik Kschischo

University of Applied Science
Koblenz
Mathematics and Technology
Suedallee
Remagen 53424, Germany
kschischo@rheinahrampus.de

Mendes-Ferreira Ana

Universidade de Trás-os-Montes e
Alto Douro
Institute for Biotechnology and
Bioengineering,
Centre of Genomics and
Biotechnology, (IBB/CGB-UTAD)
Quinta de Prados, P.O. BOX 1013
Vila Real, 5001-801, Portugal
anamf@utad.pt

Milewska Maria J.

Gdansk University of Technology
Department of Pharmaceutical
Technology and Biochemistry
Narutowicza 11/12
Gdansk 80 233
Poland
mjm@chem.pg.gda.pl

List of Participants

Milewski Slawomir

Department of Pharmaceutical
Technology and Biochemistry
Organic Chemistry
Narutowicza 11/12
Gdansk 80 233, Poland
slamilew@pg.gda.pl

Misra Prashant K

Institute of Microbial Technology
Yeast Molecular Biology Division
Sector - 39A Chandigarh 160036
India
prashant@imtech.res.in

Morschhäuser Joachim

Universität Würzburg
Institut für Molekulare
Infektionsbiologie
Josef-Schneider-Str. 2
Würzburg 97080
Germany
joachim.morschhaeuser@mail.uni-
wuerzburg.de

Mukhopadhyay Gauranga

Special Centre for Molecular
Medicine
Jawaharlal Nehru University
New Delhi 10067
India
garunga@hotmail.com

Muñoz Elisa

University of Salamanca
Cellular Biology and Pathology
Alfonso X el Sabio
Salamanca 37007
Spain
emuto@usal.es

Naik Surabhi

Yeast Genetics Laboratory
School of Life Sciences
Jawaharlal Nehru University
New Delhi 10067, India
surabhin_15@yahoo.com

Natarajan Krishnamurthy

School of Life Sciences
Jawaharlal Nehru University
New Delhi 10067
India
nat0200@mail.jnu.ac.in

Paiva Sandra

University of Minho
Biology Campus de Gualtar
Portugal 4710-051
spaiva@bio.uminho.pt

Pathan Ejaj

National Chemical Laboratory
Biochemical Sciences Division
Pashan Pune 411008, India
ejaj.pathan@yahoo.co.in

Pavon Lorena Ruiz

Linnaeus University
School of Natural Sciences
Norravägen 49
Kalmar 39 182, Sweden
lorena.ruiz@lnu.se

Pena Antonio

Universidad Nacional de México
Instituto de Fisiología Celular
Molecular Genetics, Ciudad
Universitaria
Mexico City 4510, Mexico
apd@ifc.unam.mx

Perez M. Angeles

University of Salamanca
Department of Human Anatomy and
Histology
Alfonso X el Sabio
Salamanca 37007
Spain
mapec@usal.es

Pinto João Lemos

Professor, Physics Department
University of Aveiro, Aveiro
Portugal
jlp@fis.ua.pt

Plasek Jaromir

Charles University
Faculty of Mathematics and Physics
Ke Karlovu 3
Prague 12116
Czech Republic
plasek@karlov.mff.cuni.cz

Prasad Rajendra

Membrane Biology Laboratory
School of Life Sciences
Jawaharlal Nehru University
New Delhi 10067
India
rp47jnu@gmail.com

Prasad Tulika

Jawaharlal Nehru University
Advanced Instrumentation Research
Facility
Near JNU Post Office
New Delhi 110067, India
prasadtulika@hotmail.com

Prista Catarina

Instituto Superior de Agronomia
CBAA
Tapada da Ajuda
Lisbon 1349-017, Portugal
cprista@isa.utl.pt

Puri Nidhi

Membrane Biology Laboratory
School of Life Sciences
Jawaharlal Nehru University
New Delhi 10067
India
npuri79@gmail.com

Rao Rajini

Johns Hopkins University School of
Medicine
Physiology 725 N. Wolfe St
Baltimore 21205
USA
rrao@jhmi.edu

Manpreet Kaur

Membrane Biology Laboratory
School of Life Sciences
Jawaharlal Nehru University
New Delhi 10067
India
manpreet.rawal@gmail.com

Roy Nilanjan

Department of Biotechnology
National Institute of Pharmaceutical
Education and Research
Sector 67 S. A. S. Nagar
Punjab 160062, India
Nilanjanroy@niper.ac.in

Sachdeva Pulkit

Panjab University
Biotechnology, UIET
Chandigarh 160014
India
mailpulkitsachdeva@gmail.com

Sadhale Parag

Indian Institute of Science
Microbiology and Cell Biology
CV Raman Road
Bangalore 560012, India
parag.sadhale@gmail.com

List of Participants

Samyn Dieter

Linneaus University
School of Natural Science
Kalmar, Sweden
dieter.samyn@lnu.se

Sanglard Dominique

University of Lausanne and
University Hospital Center
Institute of Microbiology
Rue Bugnon 48
Lausanne 1011
Switzerland
Dominique.Sanglard@chuv.ch

Santo Regiane Esp rito

Federal University of Ouro Preto
NUPEB
Campus do Morro do Cruzeiro
Ouro Preto 35400-000, Brazil
rlbrand@nupeb.ufop.br

Sanyal Kaustav

JNCASR
Molecular Biology & Genetics Unit
Jakkur
Bangalore 560064, India
sanyal@jncasr.ac.in

Seigler Karel

Inst. Microbiol., CR Academy of
Sciences
Lab. Cell Biol. Videnska 1083
Prague 14220
Czech Republic
sigler@biomed.cas.cy

Sengottaiyen Palanivelu

Linnaeus University
Biochemistry Norrg rd
Kalmar SE-39182, Sweden
Palanivelu.Sengottaiyan@lnu.se

Shah Peer Abdul Haseeb

Membrane Biology Laboratory
School of Life Sciences
Jawaharlal Nehru University
New Delhi 10067, India
hasb789biotech@gmail.com

Sharma Monika

Membrane Biology Laboratory
School of Life Sciences
Jawaharlal Nehru University
New Delhi 10067, India
monikabiotech@gmail.com

Shukla Shipra

Special Centre for Molecular
Medicine
Jawaharlal Nehru University
New Delhi 10067, India
shipsmail@gmail.com

Shukla Suneet

Laboratory of Cell Biology
NCI, NIH, Building 37, Room 2E18
37, Convent DR MSC 4255
Bethesda MD 20892, USA
shuklas@mail.nih.gov

Shukla Yogeshwar

Proteomics Lab
Indian Institute of Toxicology
Research (CSIR)
MG Marg, PO Box 80
Lucknow-226001, India
yogeshwer_shukla@hotmail.com

Singh Ashutosh

Membrane Biology Laboratory
School of Life Sciences
Jawaharlal Nehru University
New Delhi 10067, India
ashutosh.singh29@gmail.com

Singh RanaPratap

Jawaharlal Nehru University
School of Life Sciences
New Mehrauli Road
New Delhi 110067, India
rana.pratap@gmail.com

Souffriau Ben

KULeuven
Laboratory of Molecular Cell
Biology
Kasteelpark Arenberg 31 bus 2438
Heverlee 3001, Belgium
ben.souffriau@mmbio.vib-
kuleuven.be

Sousa Maria JoÃ£o

University of Minho
Biology
Campus de Gualtar
Braga 4710-057, Portugal
mjsousa@bio.uminho.pt

Srinivasan Malathi

Central Institute of Medicinal and
Aromatic Plants
Research Center
Allalassandra, GKVK Post
Bangalore 560065, India
m.srinivasan@cimap.res.in

Sychrova Hana

Inst. Physiology AS CR
Dept. Membrane Transport
Videnska 1083 Prague 4, 14220
Czech Rep.
sychrova@biomed.cas.cz

Taneja Vibha

Institute of Genomics and
Integrative Biology
Functional Genomics
Mall Road, Delhi 110008, India
vibha.taneja@igib.in

Thakur Anil

Institute of Microbial Technology
Yeast Molecular Biology Division
Sector - 39A Chandigarh 160036
India
athakur@imtech.res.in

Thevelein Johan

VIB and KULeuven
Molecular Microbiology and
Biology
Kasteelpark Arenberg 31
Leuven-Heverlee 3001, Belgium
johan.thevelein@mmbio.vib-
kuleuven.be

Thomas Edwina

Yeast Genetics Laboratory
School of Life Sciences
Jawaharlal Nehru University
New Delhi 10067
India
edwinathomas@gmail.com

Tupe Santosh Genba

National Chemical Laboratory
Biochemical Sciences Division
Homi Bhabha Road, Pashan
Pune 411009, India
sgtupe@yahoo.co.in

Turaga VN Ramachander

DuPont Knowledge Centre, BCS&E
DS9, ICICI Knowledge Park
Hyderabad 500078, India
ramachander.turaga@ind.dupont.com

Turcotte Bernard

Room H5.74 Royal Victoria
Hospital McGill University
Medicine 687 Pine Ave
West Montreal H3A 1A1
Canada
bernard.turcotte@mcgill.ca

List of Participants

Van Djick Patrick

VIB, K.U. Leuven
VIB department of Molecular
Microbiology
Laboratory of Molecular Cell
Biology
Kasteelpark Arenberg 26
Leuven B-2996
Belgium
patrick.vandijck@mmbio.vib-
kuleuven.be

Van Zeebroeck Griet

KULeuven
Biologie
Kasteelpark Arenberg
Heverlee 3001
Belgium
griet.vanzeebroeck@mmbio.vib-
kuleuven.be

Verma Meenakshi

Institute of Genomics and
Integrative Biology Functional
Genomics
Mall Road, Delhi 110008, India
minsvrm06@gmail.com

Verma Rama Shanker

Stem Cell and Molecular Biology
Laboratory
Department of Biotechnology
Indian Institute of Technology
Madras
Chennai-600036, India
verma_r98@yahoo.com

Yadav Amit Kumar

Institute of Microbial Technology
Yeast Molecular Biology Division
Sector - 39A Chandigarh 160036
India
amity@imtech.res.in



**School of Life Sciences &
Special Centre for Molecular Medicine
Jawaharlal Nehru University**