10th Conference on Yeast Biology

Model Yeasts to Fungal Pathogens
Bringing Together Leaders: Mechanisms to Systems approach

February 8-11, 2018

Venue: Convention Centre JNU, New Delhi

SCIENTIFIC PROGRAMME

Organized by
School of Life Sciences, JNU and Amity University Gurgaon
General Information

REGISTRATION
Registration desk would be open on Feb 7th between 7pm and 8pm in the Cafeteria. Registration desk would be open on all conference days at the JNU Convention Centre. A conference kit would be provided during registration. Please display the name badges at all times during the conference including meals.

PROGRAMME AND ABSTRACT BOOK
A printed Programme book containing the Scientific Programme would be provided at the time of registration along with the conference kit. The complete Abstract Book would be provided in pdf form, and can be downloaded from the 10CYB conference website.

PAYMENT RECEIPTS AND CERTIFICATES
Payment receipts would be provided at the time of registration. Please remember to collect the receipts from Bhawna Gohar.
A certificate of attendance/presentation would be provided from Feb 10th at the Help Desk. Please remember to collect before your departure.

HELP DESK & TRAVEL INFORMATION
A help desk would be open on all conference days at the JNU Convention Centre. The departure details would be available in the help desk. Please check and ensure that the departure details are correct. You may contact Bhawna Gohar at the help desk for assistance during the conference.

SCIENTIFIC SESSIONS
The conference scientific sessions would be held from Feb 8th at 9am to Feb 11th at 1pm in the JNU Convention Centre, Auditorium II. However, all conference events on Feb 9th would be held in the auditorium of the Amity University, Gurgaon.

POSTER SESSIONS
Poster sessions would be held over two days in the Foyer of the JNU Convention Centre.
Poster Session A, Feb 8th: P1-P40
Poster Session B, Feb 10th: P41-P79
Please check your Programme book for your poster number.
All posters must be mounted on the board by 8:45am on the day of your presentation, and would remain all through the day. Posters must be removed after dinner on both days.

ACCOMMODATION
Accommodation for all invited speakers have been arranged in the guest houses in and around JNU. Accommodation for students/post-docs have been arranged in the Human Development Resources Centre hostel in JNU campus close to the JNU Convention Centre.
DEPARTURE AND CHECK-OUT
If you depart during the day, please remember to bring your bag/s to the conference site, and check-out before leaving the guest house in the morning.

TRANSPORTATION
Transportation from guest houses to the conference site has been arranged. Student volunteers would come to the guest house and the HRDC hostel to coordinate pick-ups. Please check your departure details at the help desk and ensure that a taxi has been organized. All efforts would be made to provide pooled transportation.
Organizing Committee

Prof. Rajendra Prasad, Amity University
Prof. K. Natarajan, JNU (Convenor)
Prof. Alok Mondal, JNU
Prof. Sneha Sudha Komath, JNU
Dr. Sneh Lata Panwar, JNU
Dr. Kaustuv Datta, UDSC
Dr. Naseem Gaur, ICGEB

Abstract Screening
Dr. K. Ganesan, IMTECH
Prof. Alok Mondal, JNU

Abstract Book
Poonam
Manjit
Shalini
Surbhi

Session Anchors
Atanu
Surbhi
Shabnam
Farah
Pranjali
Darakshan
Anshuman
Sudish
Edwina
Manjit

Housing and Transport
Atanu
Manjit
Sumit
Basharat
Anshuman
Subhash
Kiran
Anoop
Yash
Ritika
Ravishankar
Sudish

Registration
Deepshikha
Neha
Shailja
Surya
Subhash
Bhawna

Session Coordinators
(Organizing presentations)
Atanu
Basharat
Ravishankar

Auditorium Technical
(Mike, Projector, Pointer)
Poonam
Shalini
Jawinder
Sumit
Shivani
Prerna
Anshuman

Poster Sessions
Basharat
Manjit
Shivani
Prerna
Shabnam
Rafia
Subhash

Food and Catering
Poonam
Shalini
Shivani
Prerna
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<tr>
<th>Time</th>
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<tbody>
<tr>
<td>Feb 7, 2018</td>
<td>Arrivals, Registration and Dinner</td>
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<tr>
<td>Feb 8, 2018;</td>
<td>Day 1</td>
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<tr>
<td>Venue:</td>
<td>Convention Centre, Jawaharlal Nehru University</td>
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<tr>
<td>7:30am-8:30am</td>
<td>Registration and Breakfast</td>
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<tr>
<td>8:45am-10:00am</td>
<td><strong>Inaugural Session</strong></td>
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<tr>
<td>9:00am-9:25am</td>
<td><strong>Welcome:</strong> Prof. K. Natarajan</td>
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<td><strong>Opening remarks:</strong> Prof. Rajendra Prasad</td>
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<td>Prof. S.K. Goswami, Dean, SLS</td>
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<td>Prof. R.P. Singh, Rector III, JNU</td>
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<td><strong>Inaugural Address:</strong> Prof. M. Jagadesh Kumar, Vice Chancellor, JNU</td>
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<td><strong>Vote of thanks:</strong> Dr. Sneh Lata Panwar, SLS</td>
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<td>9:30-9:50</td>
<td><strong>Inaugural Talk</strong></td>
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<td>Prof. Asis Datta, NIPGR &amp; professor emeritus, JNU</td>
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<td></td>
<td><strong>Aminosugar metabolism: A universal virulence determinant in both plant and human pathogens</strong></td>
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<td>Chairperson: Prof. Alok Bhattacharya, SLS, JNU</td>
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<tr>
<td>Session I</td>
<td>10:00-11:35 <strong>Chromosome dynamics</strong></td>
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<td>Chairperson: Dr. Durgadas Kasbekar, CDFD</td>
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<td>10:00-10:20</td>
<td><strong>Making sense of G/C-rich sequences in meiotic chromosome synapsis and recombination in Saccharomyces cerevisiae</strong></td>
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<td>K. Muniyappa, IISc</td>
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<td>10:25-10:45</td>
<td><strong>Fungal centromeres - beyond the point</strong></td>
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<td>Kaustuv Sanyal, JNCASR</td>
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<td>10:50-11:10</td>
<td><strong>Hsp90: An emerging master regulator of homologous recombination</strong></td>
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<td>Sunanda Bhattacharya, University of Hyderabad</td>
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<tr>
<td>11:15-11:35</td>
<td><strong>Multiple roles of Candida albicans DNA polymerase eta (Poln/Rad30) in genome stability, morphogenesis and fungal drugs sensitivity</strong></td>
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<td></td>
<td>Narottam Acharya, ILS, Bhubaneswar</td>
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<tr>
<td>11:40-12:10</td>
<td><strong>Tea/Coffee</strong></td>
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| Session II | 12:15-1:30 | Gene Expression and Chromatin  
Chairperson: Dr. Jagmohan Singh, CSIR-IMTECH |
| --- | --- | --- |
| 12:15-12:35 | A single nucleosome dynamics regulates the highly expressed pol III-transcribed genes  
Purnima Bhargava, CSIR-CCMB |
| 12:40-1:00 | Pichia pastoris: opportunities and challenges  
P.N.Rangarajan, IISc |
| 1:05-1:25 | Gene-gene and gene-environment interactions elucidate the architecture of the genotype-phenotype map  
Himanshu Sinha, IITM |
| Lunch | 1:30-2:30 |
| Session III | 2:30-4:05 | Cell Signalling and Stress Responses  
Chairperson: Dr. Purnima Bhargava, CSIR-CCMB |
| 2:30-2:50 | Chaperone network based on cellular response reveals the non-optimal response to perturbed proteostasis  
Kausik Chakraborty, CSIR-IGIB |
| 2:55-3:15 | GPI anchor biosynthesis and Ras signaling in Candida albicans  
Sneha Sudha Komath, JNU |
| 3:20-3:40 | Mechanism underlying role of Hsp70s in protecting cells from protein inclusion toxicity  
Deepak Sharma, CSIR-IMTECH |
| 3:45-4:05 | Human peroxiredoxin I maintains peroxidase-active forms to combat redox stress  
Adesh Saini, Shoolini University |
| Poster Session 1 | 4:15-6:15 | Posters 1 to 40 – Tea/Coffee will be served |
| Session IV | 6:20-7:30 | Gene Expression and post-transcriptional regulation  
Chairperson: Prof. Kaustuv Sanyal, JNCASR |
| 6:20-6:40 | Functions for essential pre-mRNA splicing factors in constitutive and alternative splice-site choice  
Usha Vijayraghavan, IISc |
| 6:45-7:05 | Messenger RNA Surveillance in Eukaryotic Cells: safeguarding the cells and regulating the gene expression  
Biswa Dip Das, Jadavpur University |
<p>| 7:10-7:30 | Suppressor mutation in helix 32 of 18S rRNA alters the defective fidelity of translation start site selection associated with hyper GTPase Activating |</p>
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<th>Time</th>
<th>Session</th>
<th>Chairperson</th>
<th>Topic</th>
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| 7:30-7:50     | Student Talks (6 min each) | Prof. Kaustuv Sanyal, JNCASR | Protein (GAP) function of eIF5  
Pankaj Alone, NISER, Bhubaneswar |
|               |         |             | Suppressor genetics identifies role of the non-catalytic ATP-binding site of Cdr1p in inter-domain crosstalk  
Atanu Banerjee, JNU |
|               |         |             | DNA damage response in the pathogenic yeast *Candida glabrata*  
Kundan Kumar, CDFD |
| Dinner        | 8:00-10:00 | Cafeteria  |                                                                                         |

**Feb 9, 2018; Day 2**

**Venue: Amity University-Manesar**

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<th>Time</th>
<th>Session</th>
<th>Chairperson</th>
<th>Topic</th>
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<tr>
<td>7:30</td>
<td>Departure for Amity</td>
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<td>Buses Depart from Aravali Guest house and Academic staff college</td>
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<tr>
<td>Breakfast</td>
<td>9:00-9:45</td>
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| 10:00-11:35   | Session V | Dr. Nishant K.T., IISER-Tvm | Fungal Pathogenesis  
Chairperson: Dr. Nishant K.T., IISER-Tvm |
| 10:00-10:20   |         |             | The phosphatidylinositol 3-kinase is required for iron homeostasis in the pathogenic yeast *Candida glabrata*  
Rupinder Kaur, CDFD |
| 10:25-10:45   |         |             | The seven-transmembrane receptor protein Rta3 in *Candida albicans*: Potential for antifungal therapy  
Sneh Lata Panwar, JNU |
| 10:50-11:10   |         |             | Epidemiology of invasive candidiasis and challenges in its diagnosis  
Shivaparakash M Rudramurthy, PGIMER |
| 11:15-11:35   |         |             | Ceramide synthases: roles in fungal pathogenicity and implications in drug development  
Ashutosh Singh, Lucknow University |
| Tea/Coffee    | 11:35-12:05 |             |                                                                                         |
| 12:10-1:20    | Session VI | Prof. K. Muniyappa, IISc | Organelle Dynamics/Biogenesis  
Chairperson: Prof. K. Muniyappa, IISc |


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<tr>
<th>Time</th>
<th>Title</th>
<th>Presenter, Institution</th>
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| 12:10-12:30| **Ultrastructure regulation of early secretory pathway components**  
Dibyendu Bhattacharya, ACTREC         |                                        |
| 12:35-12:55| **Mge1, nucleotide exchange factor for Hsp70, consociate abiotic stresses response and regulation of mitochondria functions**  
Naresh Sepuri, University of Hyderabad |                                        |
| 1:00-1:20  | **Regulators of mitochondrial ribosome assembly/activity in response to cellular energy requirements**  
Kaustuv Datta, University of Delhi, South Campus |                                        |
| Lunch      | **Student Talks (6 min each)**  
Chairperson: Prof. Sunanda Bhattacharya, University of Hyderabad |                                        |
| 2:30-3:00  | **Disruption of catabolite repressor Mig1 increases growth, lateral hyphal branching and cellulase expression in P. funiculorum NCIM1228**  
Anmoldeep Randhawa, ICGEB |                                        |
|            | **LncRNA: Novel regulator of amino acid uptake in Saccharomyces cerevisiae**  
Ankita Awasthi, Gautam Budha University, G.Noida |                                        |
|            | **Systematic Mutational Analysis Revealed Critical Requirement of the Multifunctional SAGA Chromatin Modifying complex in Candida albicans Stress response**  
Poonam Poonia, JNU |                                        |
| Session VII| **Metabolic Regulation and Systems biology**  
Chairperson: Prof. P.N.Rangarajan, IISc |                                        |
| 3:00-4:35  | **Glutathione degradation and cellular signalling**  
Anand Bachhawat, IISER-M |                                        |
|            | **Gluconeogenesis enables the self-organization of metabolically specialized populations and division of labor within an isogenic microbial community**  
Sunil Laxman, InStem |                                        |
|            | **Variation in mitochondrial activity underlies phenotypic outcome and drug resistance in yeast**  
Riddhiman Dhar, IIT-Kgp |                                        |
|            | **Complexity of transcription factor binding sites: going beyond weight matrices in yeast**  
Rahul Siddharthan, IMSc |                                        |
<table>
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<tr>
<th>Time</th>
<th>Session IX</th>
<th>Topic</th>
<th>Chairperson</th>
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<tr>
<td>9:00-10:35</td>
<td></td>
<td>Anti-fungal agents and Drug Resistance Mechanisms</td>
<td>Dr. Mukund Deshpande, CSIR-NCL</td>
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<td>9:00-9:20</td>
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<td>ABC Transporter CDR6 governs azole resistance via TOR signaling in <em>Candida albicans</em></td>
<td>Rajendra Prasad, Amity University, Gurgaon</td>
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<td>9:25-9:45</td>
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<td>Molecular mechanisms of Amphotericin B resistance</td>
<td>K. Ganesan, CSIR-IMTECH</td>
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<td>9:50-10:10</td>
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<td>Hybrid histidine kinase 3 (HHK3): An interesting molecular target for developing antifungal agent</td>
<td>Alok Mondal, JNU</td>
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<tr>
<td>Time</td>
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<tr>
<td>10:15-10:35</td>
<td>Combating azole drug resistance in <em>Candida albicans</em>: targeting and molecular dissection studies of <em>TAC1</em>, a transcription activator of <em>CDR</em> genes</td>
<td>Dibyendu Banerjee, CSIR-CDRI</td>
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<tr>
<td>Tea/Coffee</td>
<td>10:40-11:10</td>
<td>Filamentous Fungi and Interaction with Host</td>
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<td>Chairperson: Dr. Rupinder Kaur, CDFD</td>
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<td>11:15-11:15</td>
<td>Neurospora heterokaryons with complementary <em>Dp</em> and <em>Df</em> in their constituent nuclei: Why we made them, and what we found</td>
<td>Durgadas Kasbekar, CDFD</td>
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<td>11:40-12:00</td>
<td>Unveiling the puzzle of higher virulence of <em>Metarhizium</em> isolates from custard apple field against insect pest</td>
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<td>Mukund Deshpande, CSIR-NCL</td>
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<td>12:05-12:25</td>
<td>Use of root endophyte fungus <em>Piriformospora indica</em> as a Plant Probiotic</td>
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<td>Atul Johri, JNU</td>
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<td>12:30-12:50</td>
<td>Host mimicry: a fungal oxylipin with a dual role in signaling and pathogenesis</td>
<td>Rajesh Patkar, MSU, Baroda</td>
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<td>12:55-1:15</td>
<td>Stress response in <em>Ustilago maydis</em>: Insights to in planta survival of the pathogen</td>
<td>Anupama Ghosh, Bose Institute</td>
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<td>Lunch</td>
<td>1:15-2:15</td>
<td>Cafeteria</td>
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<td>2:15-2:55</td>
<td>Student Talks (6 min each)</td>
<td>Chairperson: Dr. Rupinder Kaur, CDFD</td>
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<td>Defect in translation initiation fidelity alters differential protein expression pattern and adversely affect cellular physiology in <em>Saccharomyces cerevisiae</em></td>
<td>Anup Kumar Ram, NISER</td>
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<td><em>eIF4G</em> (<em>Tif4631p</em>): A major player in nucleating the DRN proteome</td>
<td>Upasana Saha, Jadavpur University</td>
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<td>Regulation of mitochondrial function by a yeast clade specific putative helicase <em>YDR332w</em> in <em>Saccharomyces cerevisiae</em></td>
<td>Jaswinder Kaur, University of Delhi, South Campus</td>
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<td>Mitochondria and iron homeostasis in <em>Candida albicans</em></td>
<td>Edwina Thomas, JNU</td>
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</table>
| Session XI | 3:00-4:10 | **Epigenetic regulation**  
Chairperson: Prof. Krishnaveni Mishra, UoH |
|------------|-----------|--------------------------------------------------|
|            | 3:00-3:20 | Cdc23/Mcm10 primase generates the lagging strand-specific ribonucleotide imprint in fission yeast  
Jagmohan Singh, CSIR-IMTECH |
|            | 3:25-3:45 | Yeast Sen1 is required for survival under stress conditions by regulation of cell wall flocculins  
Raghuvir Tomar, IISER-B |
|            | 3:50-4:10 | Understanding the mechanism of heterochromatin organization in *Schizosaccharomyces pombe*  
Altaf Bhat, University of Kashmir |
| Poster Session 2 | 4:15-6:15 | Posters 41 to 79—Tea/Coffee will be served |
|            | 6:15-7:00 | **Student Talks** (6 min each)  
Chairperson: Prof. Krishnaveni Mishra, UoH |
|            |           | A tRNA modification pathway senses the metabolic state of the cell and integrates it with cell cycle progression  
Ritu Gupta, InStem |
|            |           | Revealing unique meiotic role of microtubule plus end directed motors, Cin8 and Kip3 in maintaining genome integrity in *S. cerevisiae*  
Priyanka Mittal, IIT-B |
|            |           | Bioprospecting yeast for lignocellulosic ethanol production  
Ajay Kumar Pandey, ICGEB |
|            |           | The effect of various transmembrane deletion mutations in CaGPI2, an accessory sub-unit of GPI-GnT complex in *Candida albicans*  
Anshuman, JNU |
| Evening Lecture | 7:15-7:45 | **The Jewel in the Crown: Phanigiri**  
Prof. Naman Ahuja, School of Arts and Aesthetics, JNU  
Chairperson: Prof. Krishnamurthy Natarajan, JNU |
<p>| Dinner | 8:00-10:00 | Cafeteria |</p>
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<td>7:30 onwards</td>
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<td>Breakfast</td>
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<tr>
<td>9:00-10:00</td>
<td>Session XII</td>
<td>Yeast Biotechnology and Biofuel</td>
<td>Chairperson: Dr. K. Ganesan, CSIR-IMTECH</td>
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<td>Developing yeast strains for biofuel production</td>
<td>Naseem Gaur, ICGEB</td>
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<td>A simplified biorefining process for second generation ethanol</td>
<td>Ajay Sharma, IOCL</td>
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<td>9:50-10:00</td>
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<td>Student Talk (6 min) Non-conventional yeasts: a platform for production of sustainable food, fuel and valuable chemicals</td>
<td>Shaikh Khurshed Akhtar, IICT</td>
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<td>10:00-11:35</td>
<td>Session XIII</td>
<td>Regulation of Cell cycle</td>
<td>Chairperson: Dr. Dibyendu Bhattacharya, ACTREC</td>
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<td>Sculpting the ring to make a cut: contractile ring structure and mechanism of cell division</td>
<td>Mithilesh Mishra, TIFR</td>
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<td>Investigation of the role of Nse1, a subunit of the Smc5/6 complex, in maintenance of chromosome stability</td>
<td>Shikha Laloraya, IISc</td>
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<td>Understanding the role of Wat1, a WD repeat containing protein during the cell cycle checkpoint and TOR1 dependent stress response pathway in fission yeast <em>S. pombe</em></td>
<td>Shakil Ahmed, CSIR-CDRI</td>
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<td>Crossing the G2-M boundary: Novel perspectives on the role of MAPKs</td>
<td>Geetanjali Sundaram, University of Calcutta</td>
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<tr>
<td>11:40-12:00</td>
<td>Concluding Session</td>
<td>Closing remarks</td>
<td>Chairperson: Prof. Rajendra Prasad</td>
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<td>Lunch</td>
<td>12:30-1:30 Cafeteria</td>
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<td>Departure</td>
<td>2:00pm onwards</td>
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Poster Presentations
The complete Abstract Book is available online at https://jnu.ac.in/YeastBiology

P1 Nucleosome bridging by Sir4 is critical for SIR complex spreading and Gene Silencing
Ehsaan Abdullah, Romana Rashid, Shahid Banday, Zeenat Farooq and Mohammad Altaf
Chromatin and Epigenetics Lab, Department of Biotechnology, University of Kashmir, Srinagar, Jammu and Kashmir 190006, India

P2 Identification and characterization of a novel membrane protein Lem3 (Ligand effect modulator 3) in Candida albicans
Pranjal Agarwal and Sneh Lata Panwar
Yeast Molecular Genetics Laboratory, School of Life Sciences, Jawaharlal Nehru University, New Delhi-110067, India

P3 Relevance of seven transmembrane receptor proteins in endoplasmic reticulum stress response of Candida albicans
Darakshan Ali, Shabnam Siricaik, Edwina Thomas and Sneh Lata Panwar
Yeast Molecular Genetics Laboratory, School of Life Sciences, Jawaharlal Nehru University, New Delhi-110067, India

P4 Monoterpenoid perillyl alcohol compromise metabolic flexibility of Candida albicans by restraining glyoxylate cycle
Moiz A. Ansari1, Zeeshan Fatima1, Kamal Ahmad2 and Saif Hameed1
1Amity Institute of Biotechnology, Amity University Haryana, Gurgaon (Manesar)-122413, India. 2Center for Interdisciplinary Research in Basic Sciences, Jamia Millia Islamia, New Delhi-110025, India

P5 CgVps34, a class III phosphatidylinositol 3-kinase regulates ion homeostasis in Candida glabrata
Fizza Askari, Vandana Sharma, Rajaram Purushotham and Rupinder Kaur
Laboratory of Fungal Pathogenesis, Centre for DNA Fingerprinting and Diagnostics, Nampally Hyderabad-500001, India

P6 Regulation of Heterochromatin organization and silencing by inner nuclear membrane proteins in Saccharomyces pombe
Shahid Banday, Zeenat Farooq, Romana Rashid, Ehsaan Abdullah, and Mohammad Altaf
Chromatin Biology and Epigenetics lab, Department of Biotechnology, University of Kashmir, India

P7 Uptake and intracellular fate of nona-arginine cell penetrating peptide in yeast
Riddhi Banerjee1, Rachayaeta Deb1, Karabi Roy2, Sunanda Chatterjee2, Shirisha Nagotu1
1Organelle Biology and Cellular Ageing Lab, Department of Biosciences and Bioengineering, Indian Institute of Technology Guwahati, Guwahati-781039, Assam, India. 2The Peptide Laboratory, Department of Chemistry, Indian Institute of Technology Guwahati, Guwahati-781039, Assam, India

P8 Antagonistic influences of transcription factors Atf1 and Per1 on Schizosaccharomyces pombe cell cycle: A possible new “ON-OFF switch” for regulation of periodic transcription
Sohini Basu, Protiti Maiti Ghosh, Sushobhana Bandypadhyay and Geetanjali Sundaram
Department of Biochemistry, University of Calcutta, 35, Ballygunge Circular Road, Kolkata-700019, WB, India

P9 Cell surface-associated aspartyl proteases in Candida glabrata: the potent immune response modulators
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P10 Azole tolerance mechanism in the pathogenic yeast Candida glabrata
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P11 Co-relation between ERMES and ERES in budding yeast
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P12 Size and Shape Regulation of the Nucleolus
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P13 Exploring the Nuclear Machinery governing the Turnover of Ribosomal and Other Non-Coding RNAs in Saccharomyces cerevisiae
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P14 Cellular role of a zinc transporter in Neurospora crassa
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P15 Non-redundant roles for the evolutionarily conserved TAF6 Histone-fold and the HEAT domains for transcriptional activation
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P16 An export retarding element in SKS1 mRNA governs its nuclear retention, promotes its degradation by the nuclear EXOSOME/DRN and thereby tunes its physiological repertoire
Subhadeep Das and Biswadip Das
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P17 A novel NE-ER protein maintains the integrity of nuclear pore complexes in yeast
Imlitosh Jamir, Pallavi Deolal, Krishnaveni Mishra
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P18 Characterizing the dual targeting/function of the peroxisomal protein Pex30
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P19 Activity of cell surface-associated asparty proteases is pivotal to intracellular survival of Candida glabrata
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P20 Hsp90 modulates the nuclear translocation of Rad51 upon DNA damage
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P21 Structural and functional studies of Drp1, a Rint1 family protein in fission yeast S. pombe
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P22 A novel role for the MAPK Spc1 in sensing and combating aberrations in CDK activity in Schizosaccharomyces pombe
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P23 Genetic background affects MSUD efficiency in Neurospora
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P24 Magnesium deprivation affects cellular circuitry responsible for drug resistance and virulence in Candida albicans
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P25 Tunicamycin-induced endoplasmic reticulum stress and Hog1 MAPK pathway in Candida albicans
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P26 Gcn4p is stabilized by methionine, during growth in amino acid starved conditions
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P27 Oleaginous yeasts as suitable platform for production of value added biomolecules
Shruti D. Kothari, Annamma A. Odaneth and Arvind M. Lali
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P28 Impact of ABC transporter and sphingolipids in inhibitor tolerant yeast for biofuel production
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P29 Caffeine induces apoptosis in Saccharomyces cerevisiae LEA1 deficient cells
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P30 Functional role of fission yeast splicing factor Spprp18 in constitutive and stress regulated alternative splicing
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P31 Bioprospecting and bioengineering of yeast cell for biofuel production
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P32 Molecular phylogenetic analysis and expression profiling of ATP-binding cassette transporters gene family in human pathogen Candida glabrata in response to anti- mycotic drugs
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P33 Chemical genomics screening in Saccharomyces cerevisiae indicates fludioxonil mediates toxicity by targeting Ssd1
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P34 MTG3, a putative GTPase involved at a late step of mitochondrial ribosome biogenesis in Saccharomyces cerevisiae
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P35 DNA damage response in the pathogenic yeast Candida glabrata
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P36 3D models of Cdr1p and Mdr1p multidrug efflux proteins: a step forward into a new era of antifungal efflux mechanism understanding and inhibitor design
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P37 Arsenic tolerance in Papiliotrema laurenitii strain RY1 isolated from Kombucha tea
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P38 Induction of apoptosis-like cell death and clearance of stress-induced intracellular protein aggregates: dual roles for Ustilago maydis metacaspase Mca1
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P39 Genome-wide analysis reveals diverse transcriptional networks coordinated by HSF1 regulate various cellular machineries of Candida albicans
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P40 Transcriptional landscape of carbohydrate active enzymes regulation in Penicillium sp.
by genome and transcriptome analysis
Nandita Pasari, Mayank Gupta, Syed Shams Yazdani
International Centre for Genetic Engineering and Biotechnology, New Delhi

P41 Phospholipids are important in determining sensitivity in fungal cells
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P42 Is NADP-glutamate dehydrogenase important in yeast-hypha reversible transition of dimorphic zygomycete Benjaminiella poirasi?
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P43 Strategies for production of Microbial oil using oleaginous yeasts
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P44 Histone fold domain mediated heterodimerization specifies the selective association of TAF12 paralogs with TFIIID and SAGA complexes
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P45 Identification, isolation and functional characterisation of magnesium transporter of endophytic fungus Piriformospora indica
Durga Prasad and Atul Kumar Johri
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P46 Unravelling the sub-compartment specific mitochondrial protein misfolding induced stress response pathways
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P47 Role of the WW domain-containing protein Ifu5 in hypoxic response in Candida albicans
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P48 Molecular basis of substrate polyspecificity of the Candida albicans Mdr1p multidrug/H+ antiporter
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P49 Biochemical characterization of the caspase-like subunit, GPI8, of the GPI-transamidase complex in Candida albicans
Sudish Kumar Sah and Sneha Sudha Komath
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P50 Role for CgHog1 kinase in iron homeostasis in Candida glabrata
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P51 Ras1 activates the first step of GPI biosynthesis in Candida albicans
Subhash Chandra Sethi, Priyanka Jain, V A Pratyusha and Sneha Sudha Komath*
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P52 A comparative study of two integrative approaches, YLEX and CRISPR/Cas9, in Yarrowia lipolytica
Kurshed Aktar Shaikh, Vishwanath Khadye, Annamma Anil, and Arvind M Lali
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P53 The Fission Yeast Sirtuin Hst4 is downregulated by Dbf4 dependent kinase and SCF ubiquitin ligase to mediate replication stress response
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P54 Probing the functional role of S. pombe splicing factor SpPrp16 and understanding its role in splice-site recognition
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P55 Biofuel Production Through Combined Approach of Consolidated Bio-Processing and Enzyme Mining
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P56 Towards establishing NNS complex as the primary exosome-specificity-factor (ESF) in Saccharomyces cerevisiae
Pragyan Singh and Biswadip Das*
Department of Life Science and Biotechnology, Jadavpur University, Kolkata
P57 Elucidating the role of cytosolic Hsp70 machinery in folding and degradation of ERAD substrate
Priyanka Singh, Deepika Gaur and Deepak Sharma
CSIR-IMTECH, Sector-39A, Chandigarh-160036

P58 Fungicidal geraniol modulates CDR1 efflux pump activity in Candida albicans and synergizes with fluconazole
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P59 Expression analysis of the thermomyces lanuginosus lipase gene
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P60 Chromatin and transcriptional control of iron homeostasis in Candida albicans
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P61 Piriformospora indica: A plant probiotic beyond host specificity
Nidhi Verma1, Abhimanyu Jogawat1, Om Prakash1, Durga Prasad1, Meenakshi Dua2 and Atul Kumar Johri1
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P62 MRX8: A potential switch that regulates mitochondrial function in response to cellular energy requirements
Pandey DK, Verma Y, Mehra U, Nair A and Datta K
Department of Genetics, University of Delhi South Campus, New Delhi, India

P63 Identification of motifs associated with NBD of ABC transporter family and subfamilies
Poonam Vishwakarma1,2, 3, Atanu Banerjee4, 5, Ritu Pasrija1, 3 Rajendra Prasad5* and Andrew M. Lynn1*
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P64 Metabolic engineering of natural isolates of Saccharomyces cerevisiae for second generation biofuel production
Farnaz Yusuf, Anup Kumar Singh and Naseem A. Gaur
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P65Suppressor genetics identifies role of the non-catalytic ATP-binding site of Cdr1p in inter-domain crosstalk
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P66 DNA damage response in the pathogenic yeast Candida glabrata
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P67 Disruption of catabolite repressor Mig1 increases growth, lateral hyphal branching and cellulase expression in P. funiculosa NCIM1228
Anmoldeep Randhawa1, Olusola A Ogunyewo1, Danish Eqbal1, Mayank Gupta1 and Syed Shams Yazdani1, 2, *
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P68 LncRNA: Novel regulator of amino acid uptake in Saccharomyces cerevisiae
Ankita Awasthi, Vikrant Nain, Rekha Puria
School of biotechnology, Gautam Buddha University, Greater Noida- 201308
P69 Defect in translation initiation fidelity alters differential protein expression pattern and adversely affect cellular physiology in Saccharomyces cerevisiae
Anup Kumar Ram², Amol R Suryawanshi³ and Pankaj V Alone¹
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P70 eIF4G (Tif4631p): A major player in nucleating the DRN proteome
Upasana Saha, Satarupa Das, Biswadip Das
Department of Life Science and Biotechnology, Jadavpur University, Kolkata – 700032

P71 Regulation of mitochondrial function by a yeast clade specific putative helicase YDR332w in Saccharomyces cerevisiae
Jaswinder Kaur, Ritika kapila and Kaustuv Datta
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P72 Mitochondria and iron homeostasis in Candida albicans
Edwina Thomas, Shivani Rod and Snehlata Panwar
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P73 A tRNA modification pathway senses the metabolic state of the cell and integrates it with cell cycle progression
Ritu Gupta and Sunil Laxman
Institute for Stem Cell Biology and Regenerative Medicine, NCBS-TIFR Campus, Bangalore

P74 Revealing unique meiotic role of microtubule plus end directed motors, Cin8 and Kip3 in maintaining genome integrity in S. cerevisiae
Priyanka Mittal, S.K. Ghosh
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P75 Bioprospecting yeast for lignocellulosic ethanol production
Ajay Kumar Pandey and Naseem A. Gaur
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P76 The effect of various transmembrane deletion mutations in CaGPI2, an accessory sub-unit of GPI-GnT complex in Candida albicans
Anshuman, Snehlata Singh and Sneha Sudha Komath
School of Life Sciences, Jawaharlal Nehru University, New Delhi, 110067

P77 Sphingolipids of Candida glabrata and its impact on drug susceptibility
Gaurima Shahi¹,², Mohit Kumar¹,², Nitesh Kumar Khandelwal¹, Naseem A. Gaur³, Rajendra Prasad¹
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P78 Functional insights into half size ABC transporter protein CaABCG2 of PDR subfamily of Candida albicans
Nitesh Kumar Khandelwal¹, Suman Sharma², Neeraj Chauhan³, Rajendra Prasad¹,²
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P79 Characterization and Antifungal susceptibility of clinical isolates from the different cohort of patients from Haryana hospitals
Ashok kumar¹, Ruchikav Bagga², M.R. Shivaprakash², arunolké cha alok Mondal, Rajendra Prasad¹
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Invited Speakers

and

Student’s Talk
Making sense of G/C-rich sequences in meiotic chromosome synapsis and recombination in *Saccharomyces cerevisiae*

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A growing body of work suggests that G/C-rich regions are enriched in mitotic and meiotic double-strand break sites (DSBs), and G-quadruplex structures are proposed to have a more general role in meiosis: for example, by promoting meiotic homologous recombination. This idea is consistent with genome-wide computational studies in *Saccharomyces cerevisiae* that demonstrate overlap between G4 motifs and preferred meiotic DSB sites. There is also good evidence that these motifs fold into highly stable G-quadruplex structures, both *in vitro* and *in vivo*, and modulate cellular processes such as DNA replication, gene regulation, recombination and epigenetics. Moreover, G-quadruplex DNA has been implicated in the alignment of four sister chromatids by forming parallel G-quadruplexes during meiosis; however, the underlying mechanism is the least understood aspect of meiosis. Several recent studies have demonstrated that *S. cerevisiae* Hop1 and Red1 proteins, the two-major meiosis-specific components of the synaptonemal complex axial element promote intra- and inter-molecular pairing of double-stranded DNA molecules through arrays of contiguous guanine residues. Additional studies have revealed novel insights into the molecular mechanisms that underlie meiotic chromosome pairing and non-homologous end-joining of broken DNA molecules. I will discuss the recent advances in our understanding of the mechanistic basis of meiotic chromosome synapsis and recombination, focusing primarily on developments in *S. cerevisiae*, where the regulation is beginning to be understood.
Fungal centromeres – beyond the point

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Since the discovery of the 125-bp long “point” centromeres in *Saccharomyces cerevisiae* in 1980, centromeres have been identified in many eukaryotic organisms. Apart from a few budding yeasts like *S. cerevisiae*, centromeres in most other fungal species span a longer region that ranges from 3 kb to more than 300 kb. Unlike point centromeres, these “regional” centromeres may or may not have conserved sequence elements and thus either genetically or epigenetically specified. We are interested in the evolution of centromere structure and function across the fungal kingdom. We cloned and characterized centromeres of closely related species of *Candida* and *Cryptococcus* that belong to the fungal phyla of Ascomycota and Basidiomycota respectively. We observed rapid divergence of the centromere DNA sequence and the organization of DNA sequence elements in *Candida* species although the length of centromeric chromatin remains around 3 kb across the species examined. We also demonstrated that centromeres in *Candida albicans* are epigenetically regulated. On the other hand, centromeres are long, retrotransposon-rich and repetitive in *Cryptococcus* species. Strikingly, we observed that RNAi plays a role in shaping the centromere structure in *Cryptococcus* species. Thus, in spite of the rapid progress in this field due to advent of improved sequencing technologies, the finding the common determinants of the centromere structure and function remains elusive.
**Invited Talk**

**Hsp90: An emerging master regulator of homologous recombination**

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Recent studies show that Hsp90 chaperone machinery is not only restricted to the cytoplasm, rather extends its service to the nucleus wherein it modulates important nuclear functions such as chromatin remodeling, DNA transcription and DNA repair. Our laboratory has demonstrated that Hsp90 regulates the homologous recombination (HR) pathway at multiple levels. Hsp90 has been shown to negatively regulate *RAD53* (DNA damage signaling kinase) transcription and thereby attenuates DNA damage response pathway. We find that inhibition of Hsp90 leads to significant decrease in the frequency of gene targeting efficiency. Further study revealed that Hsp90 maintains genome integrity by providing stability towards two important players of HR namely Rad51 and Chl1 (Chromosome Loss 1). We have demonstrated that Hsp90 inhibitor 17-AAG causes destabilization of Chl1 protein and enhances significant disruption of sister chromatid cohesion which is one of the major reasons for defect in HR mediated DSB repair. Besides, such condition completely abrogates Rad51 mediated gene conversion. We identified a separation of function mutant of *HSP90* which does not affect the stability of Rad51 protein, however, affects Rad51 nuclear translocation, i.e. DNA damage induced foci formation. Taking that study further, we have shown that dynamic interaction between Hsp90 and Rad51 regulates the nuclear import of Rad51.
**Invited Talk**

Multiple roles of *Candida albicans* DNA polymerase eta (Polη/Rad30) in genome stability, morphogenesis and fungal drugs sensitivity

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Polη is a unique DNA polymerase that promotes error-free replication bypass of UV induced cyclobutane pyrimidine dimers but possesses limited activity on undamaged DNA template. While Polη mediated TLS, required for genome stability has been explored extensively; the significance of functionality of Polη in synthesizing undamaged DNA has not been studied in any *in vivo* system yet. In this study we provide *in vivo* evidences for both TLS and non-TLS mediated roles of Polη in genome stability, morphogenesis and fungal drug resistance in *Candida albicans*. Unlike yeast, *C. albicans* is heterozygous in carrying Polη genes, and both the alleles equally suppressed UV sensitivity and UV induced mutagenesis as displayed by *S. cerevisiae rad30A* strain. Contrary to *S. cerevisiae*, homozygous deletion of *CaRAD30* alone resulted in increasing susceptibility to diverse array of DNA damaging agents, ascertaining that Polη plays a major role in stabilizing *C. albicans* genome. Surprisingly, the Carad30Δ or strain harboring catalytically inactive Polη did not exhibit any hyphal development in the presence of UV and cisplatin while the wild type strain profusely form DNA damage induced filamentation. Further, we confirmed that no common regulatory pathway of morphogenesis operates in *C. albicans* in response to various genotoxic agents. Additionally, a direct role of Polη in serum induced hyphal development has been established. Deletion of *RAD30* sensitized the strains to amphotericin B but its mere presence resulted in azole drug tolerance in DNA damaging conditions. As both the fungal drugs manipulate cell membrane permeability and do not act directly on genomic DNA; role of Polη in drug resistance is a new paradigm to explore it as a target for multimodal therapy.
Invited Talk

A single nucleosome dynamics regulates the highly expressed pol III-transcribed genes

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Despite differences in the constituents of transcription machinery as well as promoter architectures of the three eukaryotic RNA polymerases (pols), assembly of the transcription pre-initiation complex (PIC) for all takes place approximately 30 bases upstream of the transcription start site (TSS). The PIC formation is target of several classical as well as epigenetic mechanisms of regulation. Of the three, pol III transcription system is highly efficient as it transcribes the target genes at very high rate both in vitro and in vivo. Pol III in the budding yeast transcribes ~280 different genes. Short pol III-transcribed genes are constitutive and nucleosome-free and therefore considered refractory to regulation. Nevertheless, transcription by pol III is regulated according to the growth conditions and external stimuli like nutrient availability, although no classical activators and repressors are known for these genes in the budding yeast. Work from our lab has shown that chromatin structure and its remodeling regulate expression of pol III-transcribed genes in vivo.

The dynamics of a single nucleosome under different conditions regulates many of the pol III-transcribed genes from their downstream (DS) rather than the upstream (US) end where PIC assembles. Though the genes reside in the nucleosome-free regions, mobility of the DS nucleosome regulates the accessibility of the gene terminator and the rate of transcription. This DS nucleosome is under the influence of several chromatin remodelers, which show direct link to the transcription activity of the genes. The higher levels of histone variant H2A.Z in the DS than the US nucleosome makes it particularly amenable for remodeling. Therefore, a network of H2A.Z chaperones maintains the precise H2A.Z levels in the DS nucleosome, in a gene-specific manner. The results show the precision conferred by a single nucleosome in regulating these short genes, which are very important for maintaining the cellular translational activity.
**Pichia pastoris: opportunities and challenges**

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**Pichia pastoris: the global scenario**

*Pichia pastoris*, a methylotrophic yeast has emerged as an important host for the production of recombinant proteins and human therapeutics. *P. pastoris* gained global attention when Merck paid 400 million USD to acquire the New Hampshire-based GlycoFi to boost its move into biosimilar products in the year 2006. The GlycoFi technology was a *P. pastoris*-based platform for selective glycosylation of proteins. Tilmann Gerngross and Charles Hutchinson of Dartmouth’s Thayer School of Engineering, New Hampshire, USA, formed GlycoFi in the year 2000, based on their glycoengineering technology to genetically engineer *P. pastoris* cells to produce humanized proteins or proteins whose glycosylation was more similar to those expressed in humans than yeast. In addition to its exploitation as an expression platform for the production of recombinant proteins as well as metabolic engineering, *P. pastoris* is also being used as a model system to understand gene expression as well as peroxisome biogenesis.

**Pichia pastoris: the Indian scenario**

In India, *P. pastoris* shot into limelight, when it was used as an expression platform by biotech companies in India to produce Hepatitis B virus surface antigen to develop indigenous recombinant Hepatitis B vaccine. This effort led to dramatic reduction in the cost of the vaccine paving way for the Government of India to introduce it in the Universal Program of Immunization. Our laboratory used *P. pastoris* for the production of Hepatitis B surface antigen and the recombinant yeast strains were given to two Indian companies for the production of recombinant Hepatitis B vaccine. These strains are still being used by the two companies for the manufacture of both monovalent (BEVAC and ELOVAC-B) and pentavalent vaccine (ComBEFIVE). Till May 2016, these two companies have manufactured/sold ~400 million doses of these vaccines and have informed IISc that they are still using the yeast strain provided by IISc and will continue to use it in the years to come.

Following successful tech transfer, a basic research program was initiated to understand the mechanism of transcriptional regulation of Alcohol oxidase I (AOXI), whose promoter is widely used for methanol-inducible expression of recombinant proteins. Thus far, we have identified three transcription factors (*Mxr1p*, *Rop1p* and *Trm1p*) and several others are being investigated. We also identified a metabolic enzyme that has novel moonlighting functions in the nucleus. Our goal is to understand the metabolic pathways and regulatory circuits of respiratory yeasts such as *P. pastoris* which appear to be distinctly different from those of fermentative yeasts such as *Saccharomyces cerevisiae*. The basic and applied research work being carried out will be presented.
**Invited Talk**

**Gene-gene and gene-environment interactions elucidate the architecture of the genotype-phenotype map**

**Himanshu Sinha**

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One of the fundamental questions in biology is how the genotype regulates the phenotype. A large number of studies indicate that the effect of a genetic locus on the phenotype is context-dependent, i.e. it is influenced by the genetic background and the environment in which the phenotype is measured. Large-scale genome-wide yeast deletion collection studies have uncovered modules of genes that interact to regulate specific molecular processes, and as a result, have unveiled a highly interconnected architecture of genetic regulation. Similarly, screening efforts of these deletion collections have unveiled high environment dependence of the effects of different genes, indicating that gene-environment interactions are also highly abundant. However, most studies, in both model organisms and humans, that map the genetic regulation of phenotypic variation in complex traits primarily identify additive loci with independent effects. Does this reflect an absence of the contribution of genetic interactions to phenotypic variation in natural populations or is it a consequence of the technical limitations in mapping gene-gene and gene-environment interactions? In this talk, I will enumerate how studies from my laboratory have identified gene-gene and gene-environment interactions that regulate phenotypic variation in complex traits. These studies help explain missing heritability of these traits and enhance our understanding of the mechanisms underlying genetic robustness and adaptability that shape the architecture of the genotype-phenotype map.
Chaperone network based on cellular response reveals the non-optimal response to perturbed proteostasis

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Protein folding abnormalities are rampant in pathology of diseases. Proteotoxicity is surprising given the plethora of cellular machinery dedicated to aid protein folding. This is partially explained by the loss of cellular ability to re-program transcriptional outputs in accordance with proteostasis demands. We have used \textit{S. cerevisiae} to understand the response of cells when challenged with different proteostasis impairments, by removing one protein quality control (PQC) gene from the system. Using 14 PQC deletions, we investigated the transcriptional response and find the mutants were unable to upregulate pathways that could complement the function of the missing PQC gene. To our surprise, cells have inherently a limited scope of response that is not optimally tuned; with transcriptomic responses being decorrelated with respect to the sign of their epistasis. We conclude that this non-optimality in proteotoxic response may limit the cellular ability to reroute proteins through alternate and productive machineries resulting in pathological states. We posit that epistasis guided synthetic biology approaches may be helpful in realizing the true potential of the cellular chaperone machinery.
GPI anchor biosynthesis and Ras signaling in *Candida albicans*

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My lab has been working to understand the molecular details of glycosylphosphatidylinositol (GPI) anchor biosynthesis in the human pathogen, *C. albicans* pathway. Building on previous work from my lab this presentation will attempt to explain the unusual Ras dynamics that is observed in a hyperfilamentous GPI anchor mutant of *C. albicans* and its relationship with ergosterol.

Conventionally, ergosterol deficiency and higher membrane dynamics are associated with poor ability to form true hyphae that is essential for infection and virulence of pathogenic fungi. Yet ergosterol depletion via downregulation of lanosterol 14-α-demethylase (*ERG11*) in the *Cagpi19* mutant of *C. albicans* is accompanied by hyperfilamentation resulting from an upregulation of Ras signaling. Using a combination of steady state anisotropy, fluorescence lifetimes and fluorescence correlation spectroscopy (FCS), we show that membrane-specific probes experience faster dynamics in the membranes of *CaGPI19* mutants, and accumulation of sterol intermediates do not compensate for packing defects due to ergosterol depletion. Yet, dynamics of Ras1 is significantly reduced in the membranes of these cells as assessed using FCS. We also demonstrate that overexpression of Ras1 is dynamically a very different event from constitutive activation of Ras1 by a Gly13Val mutation. The latter can also be mimicked by depletion of Hsp90, which we refer to as “Ras1 hyperactivation”. Ras1 hyperactivation results in significantly slower Ras1 dynamics due to actin polymerization. In *Cagpi19* too Ras1 is hyperactivated and causes actin polymerization. Treatment with actin depolymerization agents substantially improve Ras1 dynamics in these and other strains that show Ras1 hyperactivation.

Based on these results, we propose a model for Ras1 hyperactivation in *C. albicans* and present its implications for hyphal morphogenesis in this pathogenic fungus.
Mechanism underlying role of Hsp70s in protecting cells from protein inclusion toxicity

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The mechanism underlying Hsp70s role in toxicity associated with intracellular accumulation of toxic protein inclusions is under intense investigation. The current study dissected the role of chaperoning and non-chaperoning action of yeast cytosolic Ssa Hsp70 isoforms on α-synuclein mediated cellular toxicity. We first explored the functional specificity of four highly homologous Ssa Hsp70s with regard to α-synuclein toxicity. Interestingly though highly homologous, stress inducible Hsp70s showed better protective response than their constitutive counterparts. We found that the protective action was mediated through enhanced autophagy and not due to chaperoning function of Hsp70s. The present study further explores the relative contribution of anti-oxidative response versus autophagy in protecting cells from α-synuclein toxicity.
Human peroxiredoxin I maintains peroxidase-active forms to combat redox stress

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Peroxiredoxins (Prxs), scavenge cellular peroxides, to form disulfides which can be recycled by cellular reductants. However, under oxidative stress, their active-site Cys can become hyperoxidised, resulting in loss of peroxidase activity. *Saccharomyces cerevisiae* deficient in human Prx (hPrx) orthologue *TSA1* can be complemented with *hPRXI* but not by *hPRXII*, suggesting their phylogenetic conservation. But it is not clear how the disulfide and hyperoxidation states of the hPrx vary under oxidative stress in the yeast model. Herein, we used *tsa1tsa2Δ* yeast strain, which is sensitive to redox stress, to express *hPRXI* or *hPRXII* and understand the relationship between the peroxidase active state of hPrx and its in vivo relevance. We found that hPrxI in yeast exists as a mixture of disulfide-linked dimer and reduced monomer but becomes hyperoxidised upon elevated oxidative stress. In contrast, hPrxII was predominantly dimeric in unstressed cells and was converted to a non-hyperoxidised, peroxidase-inactive monomer even with mild oxidative stress. Interestingly, we found that plant extracts containing antioxidants provided further protection against the growth defects of the *tsa1tsa2Δ* strain expressing hPrx and preserved the peroxidase-active forms of the Prxs. Antioxidants in extracts also helped to protect the hPrxs in the mammalian cells to consume more H$_2$O$_2$ and reduce hyperoxidation. Based on these findings we hypothesize that to manage oxidative stress inside the cells hPrxs needs to be maintained in a redox state that permits redox cycling and peroxidase activity.
Invited Talk

Functions for essential pre-mRNA splicing factors in constitutive and alternative splice-site choice

Usha Vijayraghavan
Messenger RNA Surveillance in Eukaryotic Cells: safeguarding the cells and regulating the gene expression

Subhadeep Das, Debasish Sarkar, Sunirmal Paira and **Biswa Dip Das**

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Messenger RNA surveillance safeguards the cells by preferentially degrading aberrant mRNAs. In addition, these degradation machineries also play a vital role in the regulation of eukaryotic gene expression at the post-transcriptional level, which dictates the “physiological repertoire” of the cellular transcriptome. In *Saccharomyces cerevisiae*, nuclear RNA exosome/DRN controls the cellular repertoire of almost two hundred non-aberrant mRNAs (referred to a special mRNAs). Our recent findings show that exosome/DRN regulates the unfolded protein response pathway and glucose adaptation response in baker’s yeast by preferentially degrading *HAC1* and *SKSI* mRNAs, two non-aberrant messages encoding two major components of these pathways respectively. In case of regulation of UPR response, the pre-*HAC1* mRNA undergoes a rapid and kinetic $3'\rightarrow 5'$ decay by exosome in absence of ER stress leading to a precursor pool, the majority of which lack an intracellular targeting element, BE. Stress causes a diminished decay thus leading to the production of an increased abundance of pre-*HAC1* mRNA population carrying an intact BE, which facilitates more efficient recruitment to Ire1p foci leading to the increased splicing and translation of the message, thus causing a massive production of Hac1p leading to an increased response. *SKSI* mRNA, which encodes a key component of the glucose adaptation response is exported very slowly. This slow export is critical for their rapid nuclear degradation. We identified a *cis*-acting RNA export retarding element in *SKSI*, which caused its slow export and rapid degradation in nucleus. Comparative stability analyses of full length and various deleted versions of *SKSI* in normal and DRN deficient yeast strains revealed that the element is located somewhere between 829 and 1034 nt *SKSI* mRNA sequence. Thus, a kinetic mRNA decay provides a novel paradigm for mRNA targeting, degradation and regulation of gene expression.
**Invited Talk**

Suppressor mutation in helix 32 of 18S rRNA alters the defective fidelity of translation start site selection associated with hyper GTPase Activating Protein (GAP) function of eIF5

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In eukaryotic translation, the recognition of AUG start codon and selection of Open Reading Frame (ORF) on the mRNA is the key fundamental step carried out by the 5’ to 3’ scanning 40S ribosome along with Met-tRNA_{Met} and several translation initiation factors to decode the genetic code. The factor eIF5 plays critical role in maintaining the fidelity of AUG start codon selection by providing GTPase activating protein (GAP) function through its N-terminal domain (NTD) to hydrolyse the GTP into GDP and P_i by the eIF2 ternary complex. However, in *Saccharomyces cerevisiae* the dominant negative translation initiation fidelity defective eIF5^{G31R} mutant causes preferential utilization of UUG as initiation codon due its hyper GTPase activity and termed as Suppressor of initiation codon (Su^{-}) phenotype. Consistently, the eIF5^{G31R} mutant utilizes upUUG initiation codons at the 5’ regulatory region present between uORF1 and main GCN4 ORF and repressed GCN4 expression (Gcn^{-} phenotype). To gain insights into the role of rRNA in selection of the start codon, a suppressor screen was performed to identify 18S rRNA mutations that suppress the preferential UUG start codon recognition associated with the eIF5^{G31R} mutant. A C1209U mutation in the helix 32 of 18S rRNA was observed to suppress Sui^{-} phenotype of both eIF5^{G31R} and a separate eIF2β^{S264Y} mutant. The C1209U mutation suppresses Gcn^{-} phenotype associated with eIF5^{G31R} mutant as well as Gcd^{-} phenotype (constitutive de-repression of GCN4 expression) associated with eIF2β^{S264Y} mutant. We propose that the C1209U mutation in helix 32 of 18S rRNA impairs transition of 40S ribosomal complex from scanning prompting ‘Open/P_{OUT}’ to scanning arrested ‘Closed/P_{IN}’ state that enhance stringency of start codon selection while bypassing selection of near cognate UUG start codon.
Suppressor genetics identifies role of the non-catalytic ATP-binding site of Cdr1p in inter-domain crosstalk

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Candida drug resistance 1 (Cdr1), a PDR subfamily ABC transporter mediates efflux of xenobiotics in Candida albicans and is a prime factor contributing to multidrug resistance in the fungal pathogen. One hallmark of this transporter is its asymmetric nature, characterized by peculiar alterations in its nucleotide binding domains. As a consequence, there exists only one canonical ATP-binding site; the other being atypical/non-catalytic. Despite extensive investigations, the function of the non-catalytic ATP-binding site remains poorly understood. In this study, we report result of suppressor screens for two drugs–susceptible transmembrane domain mutants, L529A and V532D of Cdr1p which identified suppressors in the atypical site. While the suppressor for L529A mapped to the highly conserved glutamine of the signature sequence (L529A-Q1005H), the suppressor for V532D mapped to a site very close to D-loop (V532D-W1038S). However, our experiments suggest that instead of ATPase activity, long range allosteric interactions play key role in the restoration mechanism. Of note, the regions identified in the present study have been highlighted in suppressor screens for intracellular loop mutants as well, further implying their relevance in inter-domain communication. Analysis of the ABCG5/ABCG8-based 3D-model of Cdr1p suggests that both W1038S and Q1005H mutations lead to loss of certain interactions at the level of non-catalytic ATP-binding site, which plausibly rewires the crosstalk with the TMDs. Together, we propose that the non-catalytic ATP-binding site of Cdr1p plays a pivotal role in the transport mechanism; through an impact on inter-domain crosstalk.
DNA damage response in the pathogenic yeast *Candida glabrata*

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*Candida glabrata* is an opportunistic human fungal pathogen which causes superficial and systemic infections in immunocompromized individuals. *C. glabrata* cells are able to survive and replicate in the macrophage hostile environment by inhibiting phagolysosomal acidification and altering cytokine production. Furthermore, *C. glabrata* is known to remodel its own chromatin and survive macrophage-induced reactive oxygen species (ROS) stress. We have previously reported that macrophage-internalized *C. glabrata* cells show differential levels of core histone proteins. A major goal of the current study is to elucidate the molecular basis underlying rendered histone H4 levels in the macrophage-internalized *C. glabrata* cells. Towards this, we have shown that *C. glabrata* respond to several stresses by reducing histone H4 levels and a mutant lacking two histone H4-encoding ORFs showed resistance to DNA damage caused by methyl methanesulfonate (MMS). As the DNA damage resistance phenotype was not found in the histone H3 single or double deletion strains, we concluded that the resistance was specific to histone H4. These findings along with other factors regulating the response of wild-type *C. glabrata* cells to DNA damage will be presented.
The phosphatidylinositol 3-kinase is required for iron homeostasis in the pathogenic yeast *Candida glabrata*

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*Candida glabrata* is a haploid budding yeast which accounts for up to 30% of total Candida bloodstream infections. It phylogenetically is closer to *Saccharomyces cerevisiae* than to other pathogenic Candida species, and lacks two key fungal virulence factors viz., hyphal formation and secreted proteolytic activity. A major goal of our research is to elucidate the strategies that *C. glabrata* employs to evade the host immune response. Using the human macrophage model system, we have shown that *C. glabrata* is able to prevent acidification of the phagolysosome and replicate in the nutrient-poor internal milieu of macrophages. We have also identified, using the signature-tagged mutagenesis approach, a set of 56 genes that are required for survival and/or proliferation of *C. glabrata* cells in human THP-1 macrophages. We have characterized, in detail, one of the identified genes, *CgVPS15*, which codes for a putative regulatory subunit of the phosphoinositide 3-kinase (PI3K). We have shown that CgVps34 constitutes the catalytic subunit of PI3K, and phosphorylates the third hydroxyl group of phosphatidylinositol to produce phosphatidylinositol-3-phosphate. These findings along with a role of PI3K in vesicular trafficking, iron homeostasis and virulence of *C. glabrata* will be presented.
The seven-transmembrane receptor protein Rta3 in *Candida albicans*: Potential for antifungal therapy

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The ability of *C. albicans* to form complex microbial communities called biofilms on mucosal surfaces and indwelling medical devices is a source of persistent fungal infections. Biofilms protect *C. albicans* from antifungals and help in the evasion of host immune system, thereby making it a serious threat in clinical settings. *RTA3*, a plasma membrane localized seven transmembrane protein, is unique to the fungal kingdom and is regulated by Tac1, a well-known activator of drug efflux pumps. Herein, gene manipulations and global transcriptome analysis reveal a novel role for *RTA3* in promoting biofilm formation by regulating expression of Bcr1 and its target adhesin genes. We show that rta3Δ/Δ mutant is biofilm-defective in a rat venous catheter model of infection and that *BCRI* overexpression rescues this defect, indicating that Bcr1 functions downstream of Rta3 to mediate biofilm formation in *C. albicans*. Lipid asymmetry on the plasma membrane is critical for maintaining cell integrity and for regulating intracellular signaling events. Accordingly, we also uncovered a novel role for *RTA3* in influencing susceptibility to miltefosine, a broad-spectrum antifungal, and affecting plasma membrane phosphatidylcholine asymmetry. The identification of novel *RTA3*-dependent signaling and gene regulation network governing biofilm formation and plasma membrane asymmetry will provide important insights into *C. albicans* pathogenesis. Taken together, we propose that targeting *RTA3* may have therapeutic implications leading to the development of biofilm-specific antifungals.
**Epidemiology of invasive candidiasis and challenges in its diagnosis**

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Fungi has emerged as major cause of invasive infections since last two decades, especially due to a growing population of immunosuppressed patients in hospital settings. Majority of those infections are caused by the yeasts. *Candida spp.* is commonest yeast causing those infections. *Candida albicans* was once considered as the leading species of invasive candidiasis. In the recent years, the incidence of non-albicans species of *Candida* is increasing compared with that of *Candida albicans*, and several species, such as *Candida tropicalis, Candida glabrata, Candida parapsilosis*, and *Candida krusei*, are emerging as resistant species. The incidence of ICU acquired candidemia in Indian ICU’s is 6.5/1000 ICU admissions with 40% mortality. Recently, the novel *Candida* species, *Candida auris* has emerged as the multi-resistant species posing difficulty in detection and management of patients infected with this agent. Delayed diagnosis and delay in initiation of antifungal agents is associated with high mortality. Diagnosis of these infection in clinical setting is challenging and relies mainly on conventional culture techniques such as blood cultures, serological and histopathological examination which are usually time consuming. There is a need for the rapid and non-culture-based diagnostics. Though detection of serum biomarker, (1,3)-β-D-glucan helps in the diagnosis it cannot differentiate the type of yeast species involved in the disease. Other techniques that have been developed includes PCR based assays, T2 magnetic resonance, DNA microarrays and analysis of IL-17 as marker of invasive candidiasis. All these techniques have some advantages as well as limitations warranting further research in this area.
Ceramide synthases: roles in fungal pathogenicity and implications in drug development

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Secondary infections caused by human pathogenic fungi are a major cause of high morbidity and mortality in clinics. Limited antifungal remedies available till date are unable to counter this major clinical problem, resulting in over a million new cases in patients, especially with primary HIV infection. Recent studies have identified sphingolipids as a key regulator of pathogenesis in fungi. Earlier studies have mainly focused on complex sphingolipids like glucosylceramides and inositolphosphorylceramides, the biosynthetic pathways of both have shown promising results towards development of antifungal targets. In this presentation, I would like to discuss the novel roles of ceramide synthases, enzymes catalyzing early steps of sphingolipid biosynthesis, in pathogenesis and virulence of fungi. Recent results obtained by our lab and several others show that ceramide synthases, being at the epicenter of the sphingolipid biosynthesis, could very well serve as the much need targets to develop antifungals. Here I will discuss some key aspects of identification and characterization of ceramide synthases from 3 pathogenic fungi namely Cryptococcus neoformans, Candida albicans and Aspergillus fumigatus.
Invited Talk

Ultrastructure regulation of early secretory pathway components

Dibyendu Bhattacharyya

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Present discourse will focus on recent studies of our lab on ultrastructure regulation of two components of early secretory pathway, ER and Golgi. COPI vesicles mediate Golgi-to-ER recycling, but the COPI vesicle delivery sites at the ER are poorly defined. We explored this issue using the yeast Pichia pastoris, which has a small number of ER export sites (ERES) adjacent to Golgi stacks. Our results indicate that such ER arrival sites (ERAS) are closely associated with ERES. We infer that ERES and ERAS form bidirectional transport portals in the ER. Secondly, the mechanism that regulates cisternal stacking of Golgi apparatus is still not completely understood, primarily due to the lack of any cross species universal factors that may mediate such function. Although (GRASPs) has been implicated in cisternal stacking in metazoans, but the lack of functional GRASP homolog in budding yeast and plants raises the possibilities of existence of other potential universal factors with similar functions. Potential class of molecules that may mediate such adhesive role for cisternal stacking, possibly mediate dimerization with other similar molecules residing on different Golgi cisterna. Multiple such dimerization events collectively may bring two neighboring Golgi cisternae together to form a stack. In our present study, we tested this hypothesis in the budding yeast Pichia pastoris.
Invited Talk

Mge1, nucleotide exchange factor for Hsp70, consociate abiotic stresses response and regulation of mitochondria functions

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Recent findings that all signalling emanating from abiotic stresses converge on mitochondrial functions is an important addition to the repertoire of mitochondria’s activity as a hub for energy production, redox regulation and many metabolic pathways. A manifestation of the convergence of abiotic stresses on mitochondria is reflected by the unique capability of Mge1, a nucleotide exchange factor of Hsp70, to adapt to both oxidative and thermal stresses. We find that previously identified oxidative (Mge1 M155L) and thermal (Mge1 H167L) resistant mutants extend their resistance to other abiotic cellular insults like osmotic, salinity, thermal and oxidative. Intriguingly, differing from single mutants, Mge1 harboring both the above mutations (Mge1**) exhibit enhanced sensitivity to all abiotic stresses. RNAseq studies reveal that Mge1** cells are defective in oxidative phosphorylation and amino acid metabolism besides respiration. However, Mge1** induce several pathways like mitophagy, autophagy and unfolded response genes. Most importantly, we observe weaker interaction of Mge1** with complex IV of ETC that is crucial for mitochondrial signalling. Our high throughput studies on Mge1** suggest that Mge1 serves as a harbinger for abiotic stress and help modulate mitochondrial and cellular functions.
Regulators of mitochondrial ribosome assembly/activity in response to cellular energy requirements

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Mitochondrial genome encodes components of OXPHOS complex. Their translation and subsequent assembly are tightly coupled to cellular energy requirements. Biogenesis and activity of mitochondrial ribosome (mitoribosome) is critical as numerous human diseases are caused due to defects in mitochondrial translation. Mitoribosomes have a reduced rRNA size, compensated by an increase in the mitochondrial ribosomal protein numbers. Mitoribosomal proteins, assembly factors and regulators are either species specific or universally conserved. *MTG3* belongs to circularly permuted class of GTPase that is conserved from yeast to humans containing a central GTPases pocket flanked by N and C terminal domain and is essential for cellular respiration. We have shown that *MTG3* associates with both small and large subunit of mitochondrial ribosome and is involved at a late step in their biogenesis. Our studies also indicate that *MTG3* associates with the ribosome via the C-terminus independent of the bound nucleotide, although, *MTG3* requires guanine nucleotide binding as well as hydrolysis to carry out its *in vivo* function at the late step of mitoribosome biogenesis. *MRX8*, a YihA class of GTPase, predicted to function in translation, has orthologues in bacteria, yeast and vertebrates including humans but none in invertebrates. We have shown Δ*mrx8* cells have compromised cellular respiration. Consistent with a role in translation regulation, we have shown Mrx8p is localized to the mitochondrial matrix and associates with the 74S monosome. Mutations in *MRX8* that abolished nucleotide binding were not able to support cellular respiration whereas, contrary to expectation, mutants wherein the protein is predicted to be locked in a GDP-bound form weren’t compromised. Thus *in vivo* function of Mrx8p might involve communication of NTP/NDP cellular pools to mitochondrial ribosomes. Consistent with conservation in function, human orthologue of Mrx8p restored cellular respiration in Δ*mrx8* cells.
Disruption of catabolite repressor Mig1 increases growth, lateral hyphal branching and cellulase expression in *P. funiculosum* NCIM1228

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Bioprospecting for high-grade saccharifying secretomes identified a filamentous fungus *Penicillium funiculosum* NCIM1228 having repertoire of superior cellulases than presently available commercial formulations. A necessary genetic intervention needed to make it an industrial workhorse was the alleviation of carbon catabolite repression (CCR). Mig1/Cre, the chief mediator of CCR in fungi and a transcriptional regulator, maintains carbon homeostasis by negatively regulating genes involved in alternate carbon source utilization. Upon detailed genotypic and phenotypic analysis, we observed that NCIM1228 harbours a truncated yet functional allele of Mig1. We found Mig1 homolog of NCIM1228 has a non-sense mutation at 134th amino acid position, making a large C-terminal portion of Mig1 of total 415 amino acid size dispensable for carbon repression in NCIM1228. We further observed that basal level expression of two major classes of cellulases, namely, cellobiohydrolase and endoglucanase was regulated by Mig1¹³⁴ in NCIM1228, whereas other two major classes, namely, xylanases and β-glucosidase were marginally regulated. By homologous recombination, we replaced Mig1¹³⁴ functional allele with a null allele Mig1⁸⁸. *P. funiculosum* harbouring Mig1⁸⁸ showed derepressed expression of cellobiohydrolyses and endoglucanases class of proteins, however marginally affected expression of α-glucosidases and xylanases. Surprisingly, CCR disrupted *P. funiculosum* Mig1⁸⁸ showed better growth and glucose uptake than parent strain, contrary to other filamentous fungi reported. Using microscopic techniques, we unraveled that compact colonies of CCR disputed strain of *P. funiculosum* NCIM1228 were indeed result of compromised hyphal tip elongation and profuse lateral branching of mycelia.
LncRNA: Novel regulator of amino acid uptake in *Saccharomyces cerevisiae*

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Cellular amino acid uptake is critical for TOR Complex 1 (TORC1) activation and cell growth across eukaryotes. Uptake of branched-chain amino acids by *Saccharomyces cerevisiae* from media containing a preferred nitrogen source is mediated by the permeases such as BAP2, TAT1 etc. The transcriptional activity of permeases is a highly regulated partially understood mechanism. Intriguingly, the two key permeases BAP2 and TAT1 are present in close proximity in yeast genome. Transcriptional surveys, in organisms have revealed that genes encoded in close proximity tend to be expressed together through the activities of long non-coding RNAs (lncRNAs). lncRNA coordinate expression of pair of genes in tandem largely by acting as an antisense transcript overlapping the upstream gene, originated from the bidirectional promoter involved in regulation of expression of downstream gene. Our objective is to identify regulatory lncRNAs in TOR signaling modulating amino acid uptake. For the same, we combined transcriptomics approach with functional studies in *S. cerevisiae*. NGS based whole transcriptome sequencing of cells treated with TOR inhibitor, Torin2, were critically analyzed for identification of lncRNAs. The inhibition of TOR signaling induced the expression of lncRNA, transcribed from intergenic region of two amino acid transporter genes TAT1 and BAP2. This lncRNA was antisense to the upstream amino acid permease gene TAT1. The decreased expression of genes TAT1 and BAP2 in Torin2 indicates the role of noncoding RNA in regulating expression of these genes. Apparently, the Torin2 induced polarization of BAP2 promoter reduced expression of both BAP2 and TAT1, through induced expression of lncRNA antisense to TAT1. An improved growth of loss of function mutant of lncRNA compared to WT strain upon inhibition of TOR signalling was observed. The reduced survival of loss of function mutant of lncRNA in the presence of toxic amino acid analogues further corroborates on the regulatory role of lncRNA in uptake of amino acids by permeases. Further studies are going on to illustrate whether this lncRNA function as antisense RNA to ORF in vicinity and is transcribed by the biphasic promoters or it regulates the expression of ORF in vicinity by facilitating or inhibiting the recruitment of transcription factors required for expression of ORF in vicinity (epigenetic regulation). This is the first report showing TOR mediated regulation of amino acid transport by lncRNA.
Systematic mutational analysis revealed critical requirement for the multifunctional SAGA chromatin modifying complex in *Candida albicans* stress response

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Multisubunit coactivator complexes play critical roles in eukaryotic transcriptional regulation. SAGA is an evolutionarily conserved transcriptional regulatory complex. SAGA complex has a modular architecture bearing histone acetyl transferase (HAT), histone deubiquitinase (DUB), TBP delivery (SPT) and activator interaction modules. A central aspect is whether entire SAGA complex functions as a whole or if each module functions autonomously. Here, we have dissected the requirement of the SAGA complex for growth and stress response of the human fungal pathogen *Candida albicans*. We constructed null mutants for 10 non-essential subunits of the complex (*GCN5, ADA2, SPT7, SPT20, SPT8, SPT3, TAF12L, SGF73, UBP8, and SUS1*). These mutants were tested for growth under various stress conditions. Overall, our genetic screening data showed that all of the mutants, except the *ubp8Δ* mutant, had general growth defects in rich medium. Our data also showed that the HAT module mutants (*gcn5Δ* and *ada2Δ*) and the SPT module mutants (*spt7Δ, spt20Δ, spt3Δ* and *spt8Δ*) showed growth defects in media imposing iron limitation or oxidative stress. Although each subunit of the two modules is required, the severity of the phenotypes was different for each module. Interestingly, while *UBP8*, encoding the catalytic subunit of histone H2B deubiquitinase was dispensable for growth, deletion of the other subunits (*sus1Δ* and *sgf73Δ*) of the DUB module had differential effects on growth under stress conditions. Furthermore, the *gcn5Δ, ada2Δ, spt7Δ, spt20Δ* and *taf12lΔ* mutants exhibited a constitutive pseudohyphal morphology but could not be induced to produce true hyphae. In contrast, the *sgf73Δ* mutant produced constitutive, long pseudohyphae but could be induced to produce true hyphae. Thus, overall our study revealed that the SAGA complex functions in a module-specific manner for stress response and filamentation in *C. albicans*. 
Glutathione degradation and cellular signalling

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Glutathione, an unusual tripeptide, is the most abundant small molecular weight thiol compound of living cells. Discovered more than 125 years ago, it plays many vital roles that includes its function as the cells redox buffer. The degradation of glutathione was first described in 1952 with the discovery of the α-glutamyl transpeptidase enzyme. For more than 5 decades, this enzyme, which acts on non-cytosolic pools of glutathione was thought to be the only enzyme involved in glutathione degradation in living cells. In 2007, however, using a genetic strategy in yeasts, we first showed that organisms possess alternate enzymes of glutathione degradation. Since then, we have discovered two other new enzymes that can carry out the degradation of glutathione. These new enzymes, interestingly, were all cytosolic and have led to new understanding on the roles of glutathione degradation. One role that will be discussed in detail here is the role in calcium signalling. Yeasts have two channels that pump calcium into the cytoplasm, Cch1p and Yvc1p. Yvc1p is a yeast vacuolar TRP channel, while Cch1p is a plasma membrane L-type channel. Yvc1p and Cch1p were found to be activated by redox through glutathionylation. The uncovering of the mechanisms by which these signalling pathways are regulated by redox has led to new insights on the role of glutathione degradation in cellular signalling.
Gluconeogenesis enables the self-organization of metabolically specialized populations and division of labor within an isogenic microbial community.

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Under specific conditions, isogenic cell populations can transition into what appear to be specialized, spatially organized communities. However, the fundamental metabolic principles underlying the transition of unicellular organisms to a facultative multicellular state are poorly explored. We wanted to understand the development of these communities from a metabolism perspective, and explain the organizing principles of such communities. Using \textit{Saccharomyces cerevisiae} as our model system, we find that specific amino acids are critical for the development of spatially well-organized cellular communities, with the process itself being dependent upon gluconeogenesis. We show that starting from an overall gluconeogenic state, colonies develop and organize into populations containing distinct, diametrically opposite metabolic states that are spatially very well separated. This community organization depends upon the ability of a cell to carry out gluconeogenesis, and also upon the ability to switch to an alternate metabolic state. A simple physical model provides specific prerequisites that can lead to the spontaneous self-organization of such communities. This requires stochastic switching between metabolic states, as well as interactions between the cells of two distinct states. Finally, we identify a key metabolic resource to be a critical commodity that drives the development of these self-organized communities. This resource is produced by a subset of cells and shared with the others, to allow the distinct metabolic states to result in opposite outcomes. Our findings provide a general model of how isogenic cell populations can self-organize into specialized communities, driven by a key metabolic determinant.
Variation in mitochondrial activity underlies phenotypic outcome and drug resistance in yeast

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Non-genetic variations lead to phenotypic heterogeneity in isogenic populations. One such common phenotypic heterogeneity is variation in growth rate, which has important implications for stress tolerance and drug resistance in microbes and in cancer. To understand the causes of such heterogeneity, we set up a high throughput microscopy method for growth rate distribution measurement and screened ~1500 gene knockouts in yeast S. cerevisiae. We found that mutants associated with mitochondrial electron transport chain and ATP synthesis function tend to increase the slow growing subpopulation within an isogenic population. Mitochondrial membrane potential (MMP) varied among individuals in an isogenic population but not mitochondrial content. High MMP cells were enriched for slow growers and had significant reduction in mtDNA content. Finally, variation in mitochondrial activity was also associated with variability in stress response and drug tolerance. Taken together, this study identified variation in activity of an organelle as a major cause of phenotypic heterogeneity.
Invited Talk

Complexity of transcription factor binding sites: going beyond weight matrices in yeast

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Transcription factor binding sites are typically characterised by position weight matrices, which are independent multinomial distributions over nucleotides over the positions within the site, typically taken to be 8-20 base pairs long. In previous work we considered "dinucleotide matrices" as an alternative and showed that, in yeast for many transcription factors, predictive power is improved by using such matrices as well as by considering flanking sequence that shows little "signal" in the PWM sequence logo. Recently we have considered an alternative approach to analysing high-throughput ChIP-seq data by clustering peaks based on similarity, and showed that diverse variations on known TF motifs emerge from this process including significant "signal" in flanking regions within individual clusters. While this paper focused on human (ENCODE) data, in this talk we will discuss results on yeast.
Regulation of nuclear shape and size

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Nuclear shape and size is cell-type specific. Change in nuclear shape is seen during cell division, development and pathology. The nucleus is bound by a double-membrane nuclear envelope that is perforated with nuclear pore complexes, which facilitate the exchange of molecules between nucleus and cytoplasm. The outer and inner membrane contain several integral and peripherally associated proteins that participate in multiple functions of the cell including chromatin association, gene expression, spindle assembly, RNA metabolism. Many of these proteins are also involved in maintaining the shape and rigidity of the nucleus. Despite our growing knowledge of the importance of nuclear membrane proteins in nuclear transactions and cytoplasmic communication, a clear understanding of all the key components of the nuclear membrane and how they are organized in the nuclear membrane is lacking. We have initiated a genetic screen for nuclear shape/organization defects in *S. cerevisiae* and have identified novel proteins and pathways that are involved in the organization and maintenance of nuclear shape. In a parallel study we have taken a sequence comparison approach to identify the basic components of the nuclear membrane that are conserved across the eukaryotic kingdom in an attempt to define the basal requirements for a functional NE. Through this study, we have identified several proteins that are *S. cerevisiae* specific and others that are conserved across the fungal kingdom and several that are conserved in all eukaryotes giving us a picture of the evolving nuclear proteome. The talk will give an overview of our attempts to understand the structural basis of nuclear shape, size and NE organization.
The baker’s yeast Msh4-Msh5 complex binds to DSB hotspots at a distance from the chromosome axis to promote meiotic crossing over

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Meiotic crossovers facilitate homologous chromosome segregation at Meiosis I. In the baker’s yeast and mammals, the Msh4-Msh5 complex acts along with other repair factors including the Mlh1-Mlh3 endonuclease to generate the majority of meiotic crossovers initiated from programmed DNA double strand breaks (DSBs). To understand the mechanistic role of Msh4-Msh5 in meiotic crossing over, we performed genome wide ChIP-sequencing and cytological analysis of the Msh5 protein in cells synchronized for meiosis. We found that the initial recruitment of Msh4-Msh5 occurs following DSB resection. A two-step Msh5 binding pattern was observed: an early weak binding at DSB hotspots followed by enhanced late binding upon formation of double Holliday junction structures. The peak Msh5 binding showed a positive correlation with DSB hotspots and inverse correlation with the binding sites of the chromosome axis protein Red1. Not all DSB hotspots are enriched for Msh5 binding. Within the chromosome axis regions Msh5 preferentially binds strong DSB hotspots that are away from the axis. These data suggest that Msh5 enriched DSB hotspots located at a distance from the chromosome axis are preferentially repaired through Msh4-Msh5 dependent pathway, while Msh5 depleted DSB hotspots close to the axis are likely to be repaired through other pathways. These results provide insights into the assembly of the Msh4-Msh5 complex during meiosis and its role in crossing over.
Invited Talk

Replication stress response in fission yeast *S. pombe*: cross talk between checkpoint, chromatin regulators and the replisome

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DNA replication stress is a major source of genome instability as it causes mutations, chromosome rearrangements, and missegregation and compromise cell viability. Numerous studies have indicated that cell has evolved several mechanisms to detect, prevent and counter the deleterious effects of replication stress. It is known that DNA replication and DNA damage checkpoints are activated on replication stress to slow or stall DNA replication and promote DNA repair. Recent studies have indicated that chromatin regulators may play active part in replication stress response. In *Schizosaccharomyces pombe*, a sirtuins family histone deacetylase, Hst4, functions in the maintenance of genome stability by regulating histone H3 lysine56 acetylation and promoting cell survival during replicative stress. However, its molecular functions in DNA damage survival is unclear. Our data indicate that *hst4* deficiency in the fission yeast causes S phase delay and DNA synthesis defects. I will present data showing a novel functional link between hst4 and the replisome component mcl1. We have observed that on induction of replication stress (MMS treatment), when Hst4 is down regulated, Mcl1 levels also decrease. We have uncovered the novel Hst4-Mcl1 axis for regulation of DNA replication on replicative stress. Our results indicate that this pathway might be conserved in mammalian cells. I will discuss our results indicating crosstalk between chromatin regulator and the replication stress regulators. The knowledge of such regulatory mechanism involving sirtuins during replicative stress will be useful in designing therapeutics against diseases, such as cancer where sirtuins and human homolog of Mcl1, And-1 are deregulated.
Evolution of the centromere and its associated CENP-A protein in two asexual Candida species- Candida tropicalis and Candida parapsilosis

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The centromere is an essential chromosomal element, which recruits the kinetochore to manoeuvre the chromosomes to opposite poles during cell division. This process of chromosome segregation is highly conserved throughout eukaryotes and is impetus to maintain the genome integrity. However, centromeres are remarkably diverse in sequence and structure even in related species. In spite of rapid divergence of centromere sequence, functional centromeric chromatin constitutes CENP-A/Cse4 nucleosome, which is a centromeric histone H3 variant. Studies revealed that CENP-A lays the foundation of kinetochore assembly and CENP-A loading at the centromere after each round of replication is critical for centromere function. Surprisingly, CENP-A possesses a hyper-variable N-terminal tail, which is adaptively evolving even in related species. Recent studies posit that N-terminal tail of CENP-A is required for centromere seeding in human and fission yeast. However, the reason for accelerated evolution of the centromere sequence and its associated CENP-A protein rather than a conserved structure remains largely unknown.

Candida species are deadliest human pathogens and Candida albicans is the major one causing candidiasis. Recently, non-albicans Candida species particularly Candida tropicalis and Candida parapsilosis are emerging as a serious threat globally. Besides its pathogenicity, Candida species are exquisite model organisms to study the evolution of the centromere and its associated CENP-A protein. The centromeres of two related Candida species were previously reported to be a fast-evolving genomic locus in a chromosome. Moreover, the regulation of CENP-A loading at the centromere has been significantly rewired in related Candida species. In this presentation, I shall discuss the rapid evolution of centromeres and its role in CENP-A loading at the centromere in C. tropicalis. In addition, the divergence of CENP-A homolog both in sequence and length of related Candida species will also be discussed.
**Invited Talk**

**ABC transporter CDR6 governsazole resistance via TOR signaling in *Candida albicans***

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Candida genome harbors 26 putative ATP-binding cassette (ABC) proteins which according to the nomenclature adopted by the Human Genome Organization (HUGO), belongs to ABCB (MDR), ABCC (MRP), ABCD (ALDP), ABCF (YEF3), ABCE (RL1) and ABCG (PDR) subfamilies. Some of the members belonging to PDR, MDR and MRP families are multi drug transporters and play a major role in clinical drug resistance. Notably, majority of members of ABC superfamily are not drug transporters and are involved in performing diverse functions. We have been systematically dissecting role of each member of ABC superfamily of *C. albicans*. This presentation focuses on *CDR6*, a full ABC member protein, which could efflux xenobiotics such as plant alkaloid, berberine; fluorescent rhodamine 123, and a herbicide paraquat, however, it shows a surprising phenotype in which the loss of *CDR6* resulted in elective increase resistance against antifungal azoles. The FRAP data confirmed that *CDR6* null mutant exhibited increased plasma membrane rigidity, resulting in reducedazole accumulation. Further transcriptional profiling revealed that ribosome biogenesis genes were majorly up-regulated in the null mutant. We show that *CDR6* null mutant cells grown on rapamycin display hyperactivation of *TOR1*, which lead to Hsp90 dependent calcineurin stabilization and thereby increased azole resistance. Our study uncovers a novel mechanism of azole resistance in *C. albicans* *CDR6* null mutant, involving increased membrane rigidity and TOR signaling.
Molecular mechanisms of Amphotericin B resistance

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Amphotericin B (AmB) is a commonly used polyene drug for treating invasive fungal infections. It kills fungi by forming large, extramembranous fungicidal sterol sponge that depletes ergosterol from lipid bilayers. Leakage of intracellular ions due to pore formation is a secondary effect of AmB. Though AmB resistance is rare, it is seen in a significant percentage of pathogenic Candida species and filamentous fungi. The reported AmB resistance mechanisms mainly involve reduction in ergosterol content or alterations in cell wall. Since ergosterol interacts physically as well as functionally with sphingolipids, we speculated that sphingolipids, like ergosterol, might be involved in modulation of AmB resistance. Indeed, we have found that deletion of FEN1 and SUR4 genes, encoding fatty acid elongases of the sphingolipid biosynthetic pathway, rendered S. cerevisiae and C. albicans cells sensitive to AmB. These mutants are impaired in cell wall integrity, which possibly increased their AmB sensitivity. Inhibition of sphingolipid biosynthesis by myriocin sensitized wild-type cells to AmB, and addition of phytosphingosine, an intermediate in the sphingolipid pathway, reversed this sensitivity, further confirming the role of this pathway in modulating AmB susceptibility.

We also used an overexpression screen to identify genes affecting AmB resistance. PMP3 gene, encoding plasma membrane proteolipid 3 protein, upon overexpression increased, and upon deletion decreased AmB resistance. It does not affect AmB resistance by modulating ergosterol content or cell wall integrity. However, PMP3 overexpression mediated increase in AmB resistance requires a functional sphingolipid pathway. Moreover, AmB sensitivity of strains deleted in PMP3 can be suppressed by the addition of phytosphingosine, confirming the importance of this pathway in modulation of AmB resistance. Potent inhibitors of sphingolipid pathway that are not toxic to humans, if developed, can be used to sensitize pathogenic fungi to AmB, thereby enhancing its therapeutic efficacy.
Hybrid histidine kinase 3 (HHK3): An interesting molecular target for developing antifungal agent

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The hybrid histidine kinases 3 (HHK3) is a ubiquitous sensor kinase in fungi that regulates the downstream HOG/p38 MAPK. In addition to their role in osmoadaptation, HHK3 are also involved in hyphal morphogenesis, conidiation, virulence and cellular adaptation to oxidative stress. However, the molecular mechanism regarding their role in these processes remained obscure. Although they function as osmosensor, they lack membrane anchoring domain and mostly localize in the cytoplasm. Structurally, HHK3 orthologs are quite distinct from other HHK as they contain HAMP domain repeats (poly-HAMP) in the N-terminal region of the molecule. Recent studies from our laboratory indicate that the histidine kinase activity of HHk3 is regulated by the upstream poly-HAMP module through an in-line mechanism and the protein undergo shape changes mainly perpendicular to the long axis of the protein which is achieved through the concerted motion of the HAMP domains. It appears that the poly-HAMP in HHK3 functions as a dynamic switch. Thus, the poly-HAMP module appears to function both as sensor and signal conversion module in HHK3. Absence of HHK3 in human genome makes it an ideal target for developing antifungal agent. The antifungal agent e.g. fludioxonil which is extensively used in agricultural practices, targets HHK3. To understand the fungicidal action of fludioxonil, we employed the chemical genomics approach which revealed a role of vesicle trafficking in this process. Inhibition of HHK3 activity by fludioxonil severely affects hyphal growth by impeding vesicle trafficking. We propose that HHK3 regulates hyphal growth, virulence, conidiation by modulating vesicle trafficking.
Combating azole drug resistance in *Candida albicans*: targeting and molecular dissection studies of *TAC1*, a transcription activator of CDR genes

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The up-regulation of ABC transporters Cdr1p and Cdr2p that efflux antifungal azole drugs from inside the cells is a leading cause of Multi Drug Resistance (MDR) in *Candida albicans*. Previously, *TAC1* was identified as the transcriptional activator of *CDR1* and *CDR2* genes having no known human homologs. We carried out the molecular dissection study of *TAC1* in order to understand the functional regulation of ABC transporter genes *CDR1/2*. The N-terminal DNA Binding Domain (DBD) of Tac1p interacts with the Drug Responsive Element (DRE) present in the upstream promoter region of *CDR1/2*. The interaction between DBD and DRE recruits Tac1p to the promoter of *CDR* genes. The C-terminal Acidic Activation Domain (AAD) of Tac1p interacts with TATA box Binding Protein (TBP) and thus recruits TBP to the TATA box of *CDR1/2*. Taking cue from a previous study involving a *TAC1* deletion strain that suggested that Tac1p acts as a xenobiotic receptor; in this study, we identified the Middle Homology Region (MHR) of Tac1p as a xenobiotic binding domain (XBD). Further, by conducting an *in silico* screening of ZINC compound database, we identified two compounds namely Z1 and Z3 that were able to disrupt the Tac1p-DRE interaction, resulting in the sensitization of fluconazole resistant clinical isolates of *C. albicans*, *in-vitro*. We also verified that Z1 and Z3 could cause down-regulation of ABC transporter genes *CDR1* and *CDR2* that explains the drug sensitivity that was observed.
Neurospora heterokaryons with complementary $Dp$ and $Df$ in their constituent nuclei: Why we made them, and what we found

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Heterokaryons are multinucleate mycelia (fungal cells) in which not all nuclei have the same genotype. They are easy to make in *Neurospora crassa*. For example, inoculating together two auxotrophic strains bearing mutations in different genes (say, aux-1 and aux-2) on minimal medium allows only the complementing [aux-1 + aux-2] heterokaryon formed by anastomosis to grow. However, a Df strain is ordinarily inviable therefore this approach cannot be used to construct [Dp + Df] heterokaryons where Dp and Df are, respectively, nuclei bearing a duplication of a large chromosomal segment and its complementary deficiency. Making [Dp + Df] heterokaryons would enable us to screen the deleted genome segment for “nucleus-limited genes”. That is, genes for which nuclei bearing a null allele ($\Delta$) are not complemented by the wild-type nuclei (+) in a [(+) + ($\Delta$)] heterokaryon.

Using an alternative approach, that involved introgressing *N. crassa* translocations into the pseudohomothallic species *N. tetrasperma*, we constructed the first [Dp + Df] heterokaryons in any system. They can now be compared via RNA-seq with sibling [T + N] heterokaryons, where T is the translocation and N the normal sequence genotype. The T and N genotypes do not have any deficiency. These studies led us to make the discovery that during ascus development the partitioning of nuclei into ascospores can occasionally be dissociated from the post-meiotic mitosis. We also uncovered a novel type of transmission ratio distortion.
Unveiling the puzzle of higher virulence of *Metarhizium* isolates from custard apple field against insect pest

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An important mechanism for insect pest control should be the use of fungal entomopathogens. In our search for indigenous virulent strains of entomopathogenic *Metarhizium anisopliae*, we observed that isolates from soils associated with custard apple (*Annona squamosa*) had higher virulence (>90% mortality of *Helicoverpa armigera* larvae at 1/10th the concentration) than strains isolated from fields with tomato plants. The possible mechanism of this improved virulence was investigated. Proteomics analysis revealed the presence of two insecticidal cyclopeptides (E and F), of custard apple origin, in *M. anisopliae* strains. Further, transcriptomic, and genomic evidence showed that *M. anisopliae* strains acquired five genes from the custard apple. These genes, including those coding for cyclic hexapeptide synthase, non-ribosomal peptide synthetase and plant cyclotide, were absent in the strains isolated from tomato fields. The data suggests that the higher virulence exhibited by *M. anisopliae* strains can be attributed to the genes acquired from custard apple during their endophytic existence. These virulent endophytes may have been released into the rhizosphere through plant debris. Further, the *Metarhizium* strains associated with chillies (*Capsicum annuum*), papaya (*Carica papaya*) and neem (*Azadirachta indica*), plants that have insecticidal properties, also showed higher virulence, similar to those associated with custard apple. These findings give a direction in the search for indigenous strains with higher virulence to save crops from insect pests. This could prove an alternative to genetically modifying the organisms in the lab to increase their potency. Given the social and cultural resistance against the acceptance of transgenic crops, as well as of genetically modified insect pathogens, the use of indigenous entomopathogens naturally transformed into more virulent strains is a viable and cost-effective strategy in agricultural biotechnology to save crops from insect pests.
Use of root endophyte fungus *Piriformospora indica* as a plant probiotic

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The growth and development of plants are highly dependent on nutrient availability in rhizosphere soil. However, nutrient availability is often a limiting factor. Plants have developed various strategies like plant- fungal interaction for the acquisition of important nutrients from soil. Membrane transporters of fungal partner are required for this transfer nutrients from the soil to the plant. Sulfur and phosphate are two important nutrients required for the growth of plants and are key components of many metabolic pathways. The deficit of these two nutrients in agricultural soil is a major challenge. In this study, we identified and functionally characterized two genes *PiSulT* encoding sulfate transporter and *PiPT* encoding high affinity phosphate transporter from an root endophytic fungus *Piriformospora indica*. We also determined the role of these transporters in the transfer of nutrients from fungus to the host plant and its impact on plant health. The major challenge with mycorrhizal fungi is that they cannot be cultivated axenically, and due to the unavailability of a stable transformation system, the transporters of AMF could not be genetically manipulated therefore their use in the agriculture field to improve the crop yield was hampered. However, *P. indica* can be grown axenically and a stable transformation system is now available to study the gene function. Therefore, this fungus can be genetically manipulated to be used in the agriculture field. In the present study, our work highlights the importance of *P. indica* membrane transporters in the improvement of sulfur and phosphate nutrition to host plant particularly under nutrient deprived conditions. Our study will open new vistas in order to apply *P. indica* as biofertilizer in the sulphate and phosphate deficit field to improve crop production.
Host mimicry: a fungal oxylipin with a dual role in signaling and pathogenesis

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Oxylipins, the oxygenated lipids derived from polyunsaturated fatty acids, play a crucial role at the plant-fungus interface. While plant oxylipins, such as jasmonic acid (JA), are involved in development and disease resistance, those of fungal origin are known to play roles in pathogenesis and/or synthesis of toxins. We recently showed that the rice-blast fungus Magnaporthe oryzae produces its own JA, and converts it into 12-hydroxyjasmonic acid (12OH-JA) using the Abm monoxygenase, to suppress the plant immunity at the time of host invasion¹. However, other than a critical role in evading host immunity, it was hitherto unclear why M. oryzae produces a so-called plant hormone. We found that M. oryzae failed to form a normal appressorium (infection structure) with an appropriate length of germ tube when endogenous JA biosynthesis was blocked. Importantly, exogenously added JA not only rescued the JA-deficient mutant phenotype but also induced appressorial development on a non-inductive surface. This suggested that fungal JA was likely involved in appressorial development in M. oryzae. Indeed, our further studies (unpublished) show that JA likely plays a crucial role, in concert with cAMP, in the signaling pathway required for appressorium development. We propose that Magnaporthe oxylipin JA has a dual function specifically during fungal pathogenic development and in suppression of host immunity.
Stress response in *Ustilago maydis*: Insights to in-planta survival of the pathogen

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*Ustilago maydis* is a biotrophic plant pathogen that infects host plant *Zea mays*. The pathogen is the cause of smut disease in maize. Like every other pathogen, the fungus experiences a number of adverse stress conditions while attempting to invade and subsequently colonizing its host. However, the fungus adopts quite interesting and unique strategies to circumvent the host inhibitory environment in order to make it more habitable. It is believed that a major part of these strategies involve secretory proteins that are the primary contact points with the host extracellular environment. Besides, intracellular defense signaling events helping the fungus to survive host response to pathogen invasion are nevertheless worth mentioning. The present talk will highlight some of these in-host survival and disease establishment strategies in *U. maydis*. A discussion would also be made on the possible functional redundancy in a group of secreted proteins that pose a severe hurdle in deciphering their individual role in the pathogenicity of the fungus. In addition, a focus will also be made on the recently identified unconventional function of a metacaspase (Mca1) homologue of the fungus in maintaining intracellular protein homeostasis under conditions of stress. The protein could be identified with a unique mechanism to use its N-terminal low complexity region to locate the stress-induced intracellular protein aggregates and clear that through its proteolytic activity. Not to mention, the protein could also be found to be partially involved in the in-planta pathogenic development of the fungus.
Defect in translation initiation fidelity alters differential protein expression pattern and adversely affect cellular physiology in *Saccharomyces cerevisiae*

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In eukaryotic translation, the decoding of genetic code into a linear sequence of polypeptide chain begins with the selection of the Open Reading Frame (ORF) on mRNA, accomplished by assembly of translation initiation factors, tRNA$_i$ on 40S ribosome and scanning the mRNA for AUG start codon from 5’ to 3’ direction. The initiation factor eIF5 plays an important role in translation start site selection by providing the GAP (GTPase activating protein) function. However, in yeast the dominant negative translation initiation fidelity defective eIF5$_{G31R}$ mutant causes preferential utilization of UUG as initiation codon and termed as Suppressor of initiation codon (Sui¯) phenotype and also shows severe slow growth phenotype. The strong Sui¯ phenotype of eIF5$_{G31R}$ mutant causes repression of GCN4 expression (Gcn¯ phenotype) by utilizing upUUG codon from the 5’ upstream regulatory region of GCN4 transcript. Bioinformatics analysis of yeast genome suggests more than ~10% of the mRNA has either in-frame or out-of-frame UUG codon at the 5’ UTR region. We are hypothesizing that the dominant negative eIF5$_{G31R}$ mutant alters the translation of these mRNAs and severely affects the cellular physiology. To test this, a differential protein expression pattern of the eIF5$_{G31R}$ mutant was compared with its isogenic wild type strain using 2-Dimentional gel electrophoresis and iTRAQ technique followed by mass-spectrometric analysis. Out of approximately 1300 proteins identified to show differential protein expression pattern, 26 proteins showed significant up-regulation and some were shown to associate with DNA replication stress, micro-autophagy and protein degradation pathway. Whereas, 7 proteins showed significant down-regulation and one of them associated with pyrimidine nucleotide biosynthesis. The eIF5$_{G31R}$ mutant showed high ROS activity and sensitivity to DNA damaging agents. This altered protein expression pattern shown by eIF5$_{G31R}$ mutant might be a reason for cellular stress and slow growth phenotype.
**eIF4G (Tif4631p): A major player in nucleating the DRN proteome**

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The error-prone nuclear mRNA biogenesis leads to the generation of a spectrum of aberrant messages. To limit their abundance, mRNA surveillance mechanisms efficiently degrade those faulty messages and thereby safeguarding the cells from their detrimental effects. In the nucleus of *S. cerevisiae*, nuclear exosome, TRAMP and DRN (Decay of mRNA in Nucleus) selective degrades diverse classes of faulty messages. Recent studies established that in addition to TRAMP, DRN also acts as an alternative exosome cofactor, which assists nuclear exosome to target and degrade specific classes of aberrant messages. Importantly, DRN requires nuclear cap-binding complex Cbc1p/2p, and two shuttling proteins Tif4631p and Upf3p. However, whether these proteins exists in the form of multi-protein cofactor complex (like TRAMP) is currently not known. In this work we made a holistic approach using a combination of biochemical and genetic approach to (i) define this protein-complex and (ii) to investigate the role of Tif4631p/eIF4G and its various active functional domains (namely RRM1, RRM2, RRM3, CBC, MIF4G & PAB) in the DRN function as well as in the complex formation. Our findings indicate that the RRM1 (RNA Recognition Motif 1) & PAB (Poly-A Binding) domains (both positioned towards the N-termini of Tif4631p sequence) display crucial roles in the DRN function to assist exosome to recruit specific target mRNAs and its subsequent degradation. In addition, employing a c-terminally TAP-tagged Tif4631p protein and its selective deletion constructs we showed that a multiprotein–complex consisting of seventeen known and unkown proteins consistently co-purifiy along with Tif4631p-TAP. Interestingly, Co-imunoprecipitation studies indicated that Cbc1/2p, Rrp6p and Nrd1p forms three major proteins, those are always associated with Tif4631p. Our results thus indicate that perhaps DRN exists in a novel multiprotein complex, which is nucleated by Tif4631p. Currently the identity of the other interacting candidates are in progress.
Regulation of mitochondrial function by a yeast clade specific putative helicase YDR332w in Saccharomyces cerevisiae

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Mitochondrial gene expression requires dedicated translation machinery distinct from its cytosolic counterpart as well as its bacterial ancestor. Mitochondrial has their own ribosome, synthesize seven of proteins involved mostly in oxidative phosphorylation. In comparison to bacterial ribosomes, mitochondrial ribosomes contain a smaller rRNA species compensated by a larger set of ribosomal proteins. Ribosomal proteins as well as their regulators are either universally conserved in eukaryotes or those that are species specific. Species specific proteins diversity in the mitochondrial ribosome/regulators is thought to are predicted to increase the ability of the mitochondrial ribosome to appropriately respond to niche specific carbon and environmental cues, thus aiding the organism to establish and maintain its unique environmental niche. DEAD/H box helicases are universal regulator of RNA metabolism including splicing, translation and ribosome biogenesis. YDR332w (IRC3) is essential DEAD/H box proteins. We have shown that upon deletion of YDR332w (IRC3) cells rapidly lose the ability to utilize glycerol as the sole carbon source followed by loss in intact mtDNA. We have also shown that Ydr332wp (Irc3p) co-fractionates partially with both the small and large subunit of the mitochondrial ribosome on a sucrose gradient. Consistent with a role in mitochondrial translation, cells harboring temperature sensitive alleles of YDR332w (IRC3) have reduced levels of both small and large mitochondrial ribosomal subunit. We are currently exploring the molecular and genetic partners that aid Ydr332p (Irc3p) in regulating mitochondrial function, specifically its role in mitochondrial translation.
Mitochondria and iron homeostasis in *Candida albicans*

**Edwina Thomas**, Shivani Ror and Sneh Lata Panwar

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*C. albicans* sustains a complex regulatory circuit to adapt to the diverse biological niches offered by the host in the bloodstream and gastrointestinal tract. In the bloodstream, iron is actively sequestered by host transferrin leading to an iron depleted habitat, whereas low host absorption leads to an excess of bioavailable iron in the GI tissues. Notably, loss of mtDNA in the model yeast *S. cerevisiae* leads to a signature iron starvation transcriptional response and decreased function of cellular iron-sulfur cluster containing proteins. In a previous study from our laboratory, we characterized *FZO1*, a key component of the mitochondrial biogenesis apparatus, in order to analyze the pleiotropic effects of dysfunctional mitochondria in *C. albicans* (Thomas E et al., 2013). Several iron regulon genes (involved in iron assimilation/transport) upregulated in *fzo1Δ/Δ* such as *SIT1*, *RBT5*, *CFL2* and *CFL5* are shown to be direct gene regulatory targets of the transcription factors, Sef1 and Sfu1 in *C. albicans*. Therefore, the objective of the current study is to delineate the regulatory mechanism of perturbed iron homeostasis in *fzo1Δ/Δ* mitochondrial mutant and explore its implications on associated cellular functions. Our results provide the basis of a model wherein, defective iron-sulfur cluster biogenesis in cells with dysfunctional mitochondria serves as a signal for transcription factor, Sef1, enabling it to localize in the nucleus thereby causing constitutive expression of the iron regulon in the *fzo1Δ/Δ* cells. We also examine pathogenicity factors in this mutant such as cell wall characteristics, hyphal morphogenesis and *in vivo* virulence in animal model as Fzo1 is a fungal specific protein and a target candidate for antifungal drug development.
**Invited Talk**

**Cdc23/Mcm10 primase generates the lagging strand-specific ribonucleotide imprint in fission yeast**

This work is dedicated to the memory of Amar Klar

Balveer Singh, Kamlesh K. Bisht, Jagpreet Singh Nanda, Avinash Chandra Kushwaha, Udita Upadhyay, Amar J.S. Klar and Jagmohan Singh

The developmental asymmetry of fission yeast daughter cells strictly derives from inheriting “older Watson” versus “older Crick” DNA strand from the parental cell, strands that are complementary but not identical with each other. A novel DNA strand-specific “imprint”, installed during DNA replication at the mating-type locus (*mat1*), imparts competence for cell type inter-conversion to one of the two chromosome replicas. Defining the biochemical nature of the imprint and the mechanism of its installation has been under investigation for decades to decipher this unusual, chromosomal mechanism of asymmetric cell division. The catalytic subunit of DNA Polymerase α (Pola) was the first factor genetically implicated in the imprinting process. Based on its well-known biochemical function, Pola might install the *mat1* imprint during lagging strand synthesis. Surprisingly, Pola site-directed mutations defective in domains of putative homing, restriction endonuclease or even in Okazaki fragment synthesis did not compromise its imprinting function. Rather, our detailed genetic and molecular analyses of earlier steps of DNA replication reveal a novel and more direct role played by the Cdc23/SpMcm10 primase activity in installing the imprint in cooperation with Pola.
Yeast Sen1 is required for survival under stress conditions by regulation of cell wall flocculins

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Yeast cells can grow on many surfaces including medical devices, cells and tissues. Pathogenic yeasts adhere in the form of flocks to form drug-resistant biofilms. The flocculation property of yeast is also useful in biotechnological applications. Adhesion/flocculation of yeast occurs due to expression of a special class of cell wall proteins, called adhesins/flocculins which allow them to survive in stressful environmental conditions.

The signalling mechanism that regulates the expression of flocculins is not very well understood. Normally FLO genes that encode flocculins are repressed by the binding of global repressor complex, Cyc8-Tup1 which in association with histone deacetylases, maintains positioning of de-acetylated nucleosomes. Under stress conditions, Swi2/Snf2 and histone acetylase complexes occupy the promoters to activate the expression of FLO genes. We have identified the role of Yeast Sen1, a RNA/DNA helicase in regulation of FLO genes and redox homeostasis. We propose that cooperation among Sen1, Tup1, histone modifications and Swi2 is essential for the expression of FLO genes.
Understanding the mechanism of heterochromatin organization in *Schizosaccharomyces pombe*

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Heterochromatin in the fission yeast *Schizosaccharomyces pombe* is clustered at the nuclear periphery and interacts with a number of nuclear membrane proteins. However, the significance, and the factors that sequester heterochromatin at the nuclear periphery are not fully known. We have recently identified an inner nuclear membrane protein complex Lem2-Nur1, essential for heterochromatin-mediated gene silencing. We will discuss the mechanistic details of how Lem2-Nur1 complex and other factors that associate with Lem2-Nur1 in regulating heterochromatin mediated gene silencing.
A tRNA modification pathway senses the metabolic state of the cell and integrates it with cell cycle progression

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The decision of a cell to enter the cell cycle can be viewed primarily as a metabolic problem. Cells must appropriately sense available nutrients, and regulate their metabolic outputs, to enter and eventually complete the cell division process. However, specific mechanisms that couple metabolism with cell cycle progression are poorly understood. Our earlier work had suggested that a specific tRNA modification, which incorporates a thiol group on uridines present at the wobble-codon recognizing position (s2-U), acts to sense amino acid availability (Laxman et al Cell 2013). This tRNA modification appears to integrate amino acid homeostasis with cellular translational capacity. In this study, we observe that in the absence of these s2-U tRNA modifications, cells switch their metabolism to an apparent “starvation state”. By combining translational reporter assay, along with targeted, quantitative metabolite analysis and flux experiments, we find that this metabolic reprogramming results in an altered accumulation and utilization of amino acids and nucleotides. Furthermore, this tRNA modification dependent metabolic switch altered the cell cycle progression, and also results in hypersensitivity to deoxyribonucleotide depleting agent, hydroxyurea. Collectively, our results suggest that this s2-U tRNA modification plays a critical role in amino acid sensing, and coupling amino acid and nucleotide homeostasis with entry into the cell cycle.
Revealing unique meiotic role of microtubule plus end directed motors, Cin8 and Kip3 in maintaining genome integrity in *S. cerevisiae*

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Faithful chromosome segregation relies mainly on the formation of a bipolar spindle, assembly of a kinetochore complex that attaches the chromosomes to this spindle and the movement of chromosomes towards the spindle poles. Kinesin motors provide the molecular forces that are required for several of these events and thereby control the process of chromosome segregation. Current literature suggests that the microtubule plus end directed motors such as Cin8 and Kip1 of kinesin-5 family or Kip3 of kinesin-8 family have roles at different intensities such as microtubule sliding and plus end clustering when they act on anti-parallel or parallel microtubule, respectively which helps in maintaining the spindle length and a clustered configuration of all the kinetochores during chromosome segregation. Kip3 also has a role in the kinetochore microtubule plus-end depolymerization which helps in synchronous movement of the sister chromatids towards the poles. With respect to the differences in the organization of the kinetochore ensemble, orientation and movement of the chromosomes along the spindle and cycles of spindle assembly and disassembly between mitosis and meiosis, we studied the role of these motors in meiosis. Unlike mitosis, we found that the motor function is crucial for assembly of a mature kinetochore in meiosis. Owing to the functional redundancy among the motors, we also studied the double along with the single mutants. Surprisingly, we found that in *cin8Δ kip3Δ* double mutant the mitotic growth is not perturbed whereas the viability of the spores (byproducts of cell undergoing meiosis) drops drastically suggesting their unique role in meiosis. Further investigation revealed that the double mutant arrests transiently at anaphase I and as the cells enter into meiosis II, chromosome breakage near the centromeric region was observed. Albeit the mechanism behind the loss of chromosome integrity is still under investigation, overall our study indicates that the kinesin motors may influence the kinetochore-microtubule interface and thus the gross chromosome segregation uniquely in meiosis more than they do so in mitosis.
Bioprospecting yeast for lignocellulosic ethanol production

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Lignocellulosic hydrolysate is a mixer of C6/C5 sugars and inhibitors (Furans, weak acids and phenolics) generated during the pretreatment. Therefore, robust yeast isolates with characteristics of C6/C5 fermentation and pretreatment inhibitor tolerance are pre-requisite for lignocellulosic ethanol production. Moreover, use of thermotolerant yeast isolates will reduce cooling cost, contaminations during fermentation, and also required for developing SSF and SSCF processes. Therefore, we evaluated the growth and fermentation performances of yeast isolates isolated from diverge natural habitats. The growth and fermentation performances were evaluated at 30°C and 40°C along with tolerance towards pretreatment inhibitors (Furfural, HMF, Acetic acid and Ethanol). K. marxianus NGY8 and O. thermophila NGY11 were able to grow on wide range of C6/C5 sugars including arabinose and cellobiose. S. cerevisiae NGY10 isolate, isolated from sugarcane bagasse distillery waste, produced maximum ethanol yield of 47.59 ± 2.34 g/l and 46.8 ± 3.11 g/l with the efficiency of 94.11% and 93.73% at 30°C and 40°C respectively, in 24 hours with glucose as a carbon source. This isolate produced ethanol yield of 8.17 ± 0.14 g/l with fermentation efficiency of 93.23% at 40°C, when rice straw enzymatic hydrolyzate was used as carbon source and displayed furfural (1.5 g/l), HMF (3.0 g/l), acetic acid (0.2% v/v) and ethanol (10.0% v/v) tolerant phenotypes. Together, NGY10 isolate could be potential yeast isolate for lignocellulosic ethanol production, C5 metabolic engineering and developing strategies for SSF and SSCF processes.
The effect of various transmembrane deletion mutations in CaGPI2, an accessory sub-unit of GPI-GnT complex in Candida albicans

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Candida albicans is an opportunistic fungal pathogen which causes candidiasis in immunocompromised patients. The biosynthesis of GPI-anchor, which is a glycolipid post translational modification, occurs in the endoplasmic reticulum in which the first step involves the transfer of GlcNAc (N-acetylglucosamine) from UDP-GlcNAc to phosphatidylinositol. This step is catalysed by a multi subunit complex i.e. a GPI-GnT complex (Glycosylphosphatidylinositol-N-acetyglycosaminyltransferase) which has one catalytic subunit (Gpi3) and five accessory subunits (Gpi1, Gpi2, Gpi15, Gpi19 and Eri1). Previous studies from our lab on C. albicans suggest that the GPI-GnT complex cross talks with both ergosterol biosynthesis and Ras signalling pathways (Yadav et al., 2014). The downregulation of Gpi19 subunit in C. albicans results in hyperfilamentation while that of Cagpi2 results in just the opposite phenotype. Detailed analyses of these mutants had shown that CaGPI2 and CaGPI19 negatively regulate each another. In addition to this, CaGPI2 positively regulates Ras signalling while CaGPI19 is mutually co regulated with ERG11, involved in the ergosterol biosynthesis pathway (Yadav et al., 2014). My work specifically examines how different truncation mutations of GPI-GnT subunits of complex affect GPI-GnT activity as well as cross talk with ergosterol biosynthetic pathway and Ras signaling. Using TMHMM (transmembrane prediction by Hidden Markov Model) analysis CaGpi2 was predicted to have six transmembrane (TM) domains. A number of truncation constructs lacking one or more putative TM domains were made and analyzed. One construct lacking three TM domains from the N-terminus was azole sensitive while those lacking one or two N-terminal domains were not. Similarly, constructs lacking one, two or three domains from the C-terminus showed no azole sensitivity.
Evening Lecture

The Jewel in the Crown: Phanigiri

Prof. Naman Ahuja

School of Arts and Aesthetics, Jawaharlal Nehru University, New Delhi

An astonishingly beautiful sculpture of prince Siddharth discarding his turban in a symbolic gesture of giving up his inheritance was recovered a few years ago from Phanigiri, an ignored ancient Buddhist stupa and monastic site in Telangana. The sculpture rivals the finest known sculptures from Amaravati which are kept at the British Museum in a highly protected environment. The Indian sculpture is widely regarded as the show-stopper of the exhibition India and the World which is currently on at the CSMVS in Mumbai. Its beauty and spiritual message apart, Prof. Ahuja will tell us about the subtly important social and political message of this sculpture.
Developing yeast strains for biofuel production

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Microbial cell factories are excellent alternative for manufacturing bio-based chemicals including biofuel molecules. The advantage of microbial cell factories is that they can use low value materials (agricultural waste) as carbon source for growth and synthesis of desired products. Thus, microbial cell factories have the potential to make major contributions to economy by extracting value from waste materials. To this end, we exploited synthetic biology and metabolic engineering approaches to develop genetically modified C5/C6 co-utilizing and FAFE producing strains. *S. cerevisiae* strains secreting cellulases were generated and evaluated in SSF processes. Novel biocatalyst for biofuels were identified by metagenomics analysis of the gut microbiomes of herbivorous animals. To understand the role of membrane lipids and transporters in microbial cell factories development, we generated and analysed library of ABC transporters and sphingolipid pathway mutants.
A simplified biorefining process for second generation ethanol- Simultaneous saccharification and co-fermentation of acid pre-treated rice straw using co-culture of wild type and genetically engineered *Saccharomyces cerevisiae*

**Invited Talk**

**Ajay Kumar Sharma**, Manas Ranjan Swain, Ajit Singh, A S Mathur, R P Gupta, S K Puri

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Present work focus on development of economic viable biorefinery process for ethanol production from surplus agriculture residue of rice straw. To obtain high ethanol titer, sulphuric acid pretreated rice straw was simultaneous saccharified and fermented using co-culture of wild type and genetically engineered *Saccharomyces cerevisiae* at 25% solid loading using 15 FPU/gm solid biomass of Cellic Ctec3 (Novazyme) enzyme and ethanol titer 57 g/l obtained. To increase ethanol titer and reduce enzyme loading, fed batch simultaneous saccharification and fermentation was performed at 29% solid loading of whole slurry pretreated rice straw with enzyme loading at 12.38 FPU/gm solid biomass. This resulted into ethanol titer of 68 g/l in 72 hr with 80% efficiency. Different nutrients and their impacts on bio-ethanol production were also investigated to reduce final delivery cost of ethanol. The result shows that only MgSO4 at 2 g/l is sufficient to achieve ethanol titer of 68 g/l. The wild type inhibitor and thermo tolerant robust *Saccharomyces cerevisiae* DBTIOC-S24 strain able to convert xylose into value added product xylitol. Which further make process economic viable due to its higher cost. The developed process of ethanol production is easy to upscale at the industrial scale with a lower process time and cost.
Non-conventional yeasts: a platform for production of sustainable food, fuel and valuable chemicals

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Yeast, being a unicellular organism, combines the ease of genetic manipulation and growth capacity of prokaryotic cells with eukaryotic system’s subcellular organization, latter enabling post translational modification and secretion. Conventional yeasts mainly *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* have been extensively used as microbial cell factories over the years. However, non conventional yeasts mainly but not limited to *Pichia pastoris*, *Yarrowia lipolytica*, *Kluveromyces lactis*, etc. are gaining importance because of their considerable properties like, ability to utilize cheaper and wider range of substrates, high production of organic and fatty acids, high lipid accumulation, high protein secretion and lastly development of newer and efficient genetic manipulation tools making them genetically tractable. These extraordinary properties of non conventional yeasts coupled with emerging genetic manipulation tools have driven our interest to utilize them as a microbial platform for production of industrially relevant biocommodities. DBT-ICT CEB is involved extensively in the development of sustainable and green technologies for production of food, feed, fuel and high value chemicals. Working towards this aim, we are currently developing oleaginous yeasts for production of proteins, aroma chemicals, high-value lipids from various low-cost substrates. Efforts are underway to develop molecular biology, fermentation and downstream capabilities to be able to use Yarrowia lipolytica as a unique platform for all applications.
Sculpting the ring to make a cut: contractile ring structure and mechanism of cell division

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Cytokinesis in many eukaryotes involves the contraction of a tension-producing actomyosin-based contractile ring that constricts the membrane to divide the cell. However, the detailed mechanism of contractile ring organisation and contraction is not fully understood. A sliding filament mechanism where myosin pulls actin filaments like those in the sarcomere has been proposed decades ago, however, contractile rings are far more disordered and the components of the ring are highly dynamic. We have recently established, for the first time, an experimental system to study contraction of the ring to completion in vitro. We show that contractile rings undergo rapid contraction in an adenosine triphosphate (ATP) and myosin-II dependent manner in the absence of other cytoplasmic constituents. Surprisingly, neither actin polymerisation nor its disassembly is required for contraction of the ring although addition of exogenous actin cross-linking proteins blocks ring contraction. Furthermore, we use cryosectioning and cryo-focused ion beam milling to gain access to natively-preserved actomyosin rings for direct three-dimensional imaging by electron cryotomography. Our results show that the ring is composed of straight, overlapping actin filaments that “saddle” the septal membrane, but they do not make contact with the membrane or gather at nodes. Correlative cryo-fluorescence light microscopy and electron cryotomography on vitreous cryosections further reveal that myosin does not form thick oligomeric filaments in the ring as it does in vitro. Finally, by tuning parameters and properties of the ring’s components to match data from electron cryotomography, we show that coarse-grained simulations could help reveal the mechanism of ring constriction.
Investigation of the role of Nse1, a subunit of the Smc5/6 complex, in maintenance of chromosome stability

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Smc (Structural maintenance of chromosomes) proteins are key architects of the genome, conserved from bacteria to humans. Eukaryotic Smc proteins form a heterodimer and further associate with non-Smc subunits to form Smc complexes e.g. Cohesin, Condensin or the Smc5/6 complex. Smc-protein dependent chromosome organization also affects many chromosomal processes such as cohesion, condensation, segregation, DNA double strand break repair, and gene expression. The Smc5/6 complex is an as yet unnamed but conserved Smc complex that consists of Smc5, Smc6 and six Nse (Non-smc element) subunits critical for DNA repair. I will discuss our recent work on the Nse subunits of the budding yeast Smc5/6 complex, particularly ScNse1, a RING-domain containing putative ubiquitin E3 ligase. We investigated the contribution of the budding yeast Nse1 RING-domain by isolating a mutant nse1-103 bearing substitutions in conserved Zinc-coordinating residues of the RING-domain that is hypersensitive to genotoxic stress and temperature. The nse1-103 mutant protein was defective in interaction with Nse3 and other Smc5/6 complex subunits, Nse4 and Smc5. Chromosome loss was enhanced, accompanied by a delay in the completion of replication and a modest defect in sister chromatid cohesion, in nse1-103. The nse1-103 mutant was synthetic sick with rrm3Δ (defective in fork passage through pause sites), this defect was partially rescued by inactivation of Tof1, a subunit of the fork protection complex that enforces pausing. The temperature sensitivity of nse1-103 was partially suppressed by deletion of MPH1, encoding a DNA-helicase. Our findings demonstrate that the budding yeast Nse1 RING-domain organization is important for interaction with Nse3, which is crucial for completion of chromosomal replication, cohesion, and maintenance of chromosome stability.
Invited Talk

Understanding the role of Wat1, a WD repeat containing protein during the cell cycle checkpoint and TOR1 dependent stress response pathway in fission yeast S. pombe

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DNA double strand breaks (DSBs) are critical lesions that can lead to chromosomal aberrations and genomic instability. In response to DNA damage, Chk1, a serine/threonine kinase is responsible for cell cycle arrest to prevent damaged cells from progressing through the cell cycle. The disruption of wat1, a WD repeat containing protein leads to the phosphorylation of Chk1. The double deletion of chk1 and wat1 pose a grave effect on the survival of fission yeast cells and exhibit high level of nuclear fragmentation that results in the accumulation of Rad22 YFP foci. Furthermore, we observed elevated level of ROS generation in wat1 null mutant suggesting its role in the stress response pathway. We have shown that Wat1 protein is phosphorylated at Serine 116 residue which under goes hyper-phosphorylation in response to osmotic stress. Co-immunoprecipitation studies suggest that Wat1 interacts with the C-terminal region of Tor1 protein that includes the Kinase domain of Tor1. More importantly, residue E2322 of FATC domain of Tor1 protein was observed to interact via hydrogen bond formation with Arg216 and Tyr217 of Wat1 protein and the deletion of FATC domain of Tor1 affects its interaction with Wat1 protein suggesting that this domain plays a role in stabilizing the Tor1 interaction with Wat1. The tor1 and wat1 mutants that fail to interact with each other also fail to phosphorylate the Gad8 protein suggesting that the interaction of Wat1 with Tor1 in heterotrimeric complex facilitate proper binding of Gad8 and hence promote its phosphorylation. We have also shown that Wat1 phosphorylation is essential for proper Gad8 function.
Crossing the G2-M boundary: Novel perspectives on the role of MAPKs

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Mitogen Activated Protein Kinases (MAPKs) play vital roles in multiple cellular processes and represent prominently pursued targets for development of therapeutic regimes. It has been known for nearly two decades that the p38MAPK homolog Spc1 (S. cerevisiae Hog1 ortholog) plays a major role in controlling the G2/M transition in the fission yeast Schizosachcharomyces pombe. The intriguing pattern that has emerged from the various reports about its influence on mitosis is that Spc1 can have contrasting effects on mitotic entry decisions. It has in fact been demonstrated to contribute to both mitotic promotion and delay. A moderate increase in Spc1 activity has been shown to promote mitotic entry (in response to nutrient stress) while a higher increase leads to activation of the G2/M checkpoint (in response to oxidative, genotoxic or heat stress) in S. pombe. In the first case, its function resembles the role of mammalian ERK, while in the next, the functional similarity is with the mammalian p38 MAPK. A detailed mechanistic explanation for the observed dose dependent conflicting outcome of the regulation of mitotic entry by Spc1 was not available though. Here we present results that shed some light on the mechanism of switching of Spc1 from a mitotic promoter to inhibitor in S. pombe cells. We also show that the Spc1 dependent pathway for delaying mitosis represents an important backup mechanism for controlling mitotic entry in S. pombe cells.
Posters Abstract
(P1-P76)
Nucleosome bridging by Sir4 is critical for SIR complex spreading and gene silencing

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Assembly of silent chromatin in the budding yeast Saccharomyces cerevisiae requires the SIR complex, composed of the Sir2, Sir3, and Sir4 proteins. The SIR complex spreads along the chromatin fiber from defined nucleation sites through interactions with nucleosomes that contain deacetylated H4K16 and unmethylated H3K79. Here we examine the relative contribution of the Sir3 and Sir4 proteins to SIR complex spreading and silencing. We show that in vitro Sir3 has little or no nucleosome bridging activity while Sir4 can efficiently bridge Sir3-bound mono-nucleosomes. Consistent with these results, the spreading of Sir3 to extended subtelomeric heterochromatin in vivo, which occurs when Sir3 is over-expressed, requires Sir3-Sir4 interactions at nearly all loci, except at two regions between the silent mating type cassettes and telomeres. This Sir4-independent spreading of Sir3 occurs over nucleosomes that contain acetylated H4K16 and methylated H3K79 and does not silence the underlying mating type cassette genes. However, silencing can be restored upon the substitution of H4K16 with arginine, which mimics deacetylated lysine, thus indicating that a primary function of Sir3-Sir4 interaction involves the spreading of Sir2-dependent H4K16 deacetylation. Our results reveal distinct modes of Sir3-nucleosome binding and suggest that only a particular mode of binding results in silencing.
Identification and characterization of a novel membrane protein Lem3 (Ligand effect modulator 3) in *Candida albicans*

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Transport of phospholipids across the yeast plasma membrane for maintaining membrane asymmetry has been amply addressed in *S. cerevisiae*. Parallel studies in *C. albicans* in this context have been limited. Membrane asymmetry is the balanced action of flippases and floppases, proteins that direct the inward and outward transbilayer movement of phospholipids, respectively. As a consequence, phospholipids are differentially distributed across a membrane. Phosphatidylcholine (PC) is predominantly distributed in the outer leaflet, whereas phosphatidylethanolamine (PE) and phosphatidylserine (PS) are distributed in the inner leaflet. In *S. cerevisiae* LEM3 (ligand effect modulator 3) plays a significant role exclusively in the transbilayer translocation of PC as well as the alkylphosphocholine drugs such as miltefosine and edelfosine across the plasma membrane. It is an essential contributor to the plasma membrane flippase activity, and is associated with catalytic subunits encoded by *DNF1* and *DNF2*. As the molecular components involved in maintaining membrane asymmetry remain uncharacterized in *C. albicans*, we sought to elucidate the role of *CaLEM3* in this fungus. Herein, we show that *lem3Δ/Δ* cells displayed an increased resistance to alkylphosphocholine drug miltefosine. Concurrent with the increased resistance to miltefosine, *lem3Δ/Δ* cells also displayed decreased internalization of NBD-labelled phosphatidylcholine, compared to the wild type, suggesting a role for *LEM3* in miltefosine transport. Interestingly, *lem3Δ/Δ* cells also displayed increased susceptibility to azole antifungals and cell wall damaging agents, indicating its importance in maintaining proper membrane environment. Additionally, the mutant also displayed a defect in the bud- to- hyphae transition. Our data so far demonstrates a requirement for *LEM3* expression for normal transport of NBD-PC across the plasma membrane, pointing to a role of this gene in maintaining the asymmetric distribution of PC across the plasma membrane in *C. albicans*.
Relevance of seven transmembrane receptor proteins in endoplasmic reticulum stress response of *Candida albicans*

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*C. albicans* genome shows the presence of three Rta-like genes, named *RTA2*, *RTA3* and *RTA4*. These *RTA* genes are shown to bear homology to proteins of the Rta1-like family of *S. cerevisiae*. All these *RTA* genes have a 7 TMD topology, similar to GPCRs. Interestingly, these proteins are unique to the fungal kingdom and do not have plant or human orthologs, hence can be used as a drug target for developing new antifungals. In this study, we are particularly interested in assigning functions to *RTA2* and *RTA4*, two downstream effector molecules of the calcineurin pathway. Deletion of *RTA2* renders cell susceptible to tunicamycin (TM), while deletion of *RTA4* does not affect susceptibility to ER stressors. Interestingly, *rta2Δ/Δrta4Δ/Δ* cells show increased susceptibility to tunicamycin, compared to their parent strains. Furthermore, to explore a link between the *RTA* genes and the ER stress induced unfolded protein response pathway (UPR), we monitored the expression of a subset of *HAC1*-dependent UPR target genes that allows the cell to tolerate ER stress caused by protein misfolding. The deletion mutants displayed impaired induction of UPR target genes in a mechanism largely independent of *HAC1* splicing and Hac1 protein levels. Our data indicate that the activity of *RTA2* and *RTA4* is requisite for activating *HAC1* dependent UPR in response to ER stress. This, study assigns an additional role to *RTA2* and a novel role to *RTA4* in *C. albicans*, two proteins that may potentially represent new targets for antifungal therapy.
Monoterpenoid perillyl alcohol compromise metabolic flexibility of *Candida albicans* by restraining glyoxylate cycle

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The rise of the immunocompromised conditions with concomitant resistance in *Candida albicans* against current therapeutic drugs is compelling us to sort out new drugs with novel targets. The metabolic pathways such as glyoxylate cycle (GC) enable *C. albicans*, to endure under glucose scarce conditions prevalent in the hostile niche. Thus, its key enzymes (Isocitrate lyase; ICL1 and malate synthase; MLS1) are the attractive targets against *C. albicans* as they are absent in humans. Previously, we have reported the antifungal efficacy of a natural monoterpenoid, perillyl alcohol (PA), present in the lemon grass and peppermint, which inhibits calcineurin signaling and potential virulence traits. The present study led to the identification of a potent GC inhibitor in PA. We explored that PA phenocopied ICL1 deletion mutants and were hypersensitive under low carbon utilizing conditions. The effect of PA on GC was further elaborated by docking analyses, which reveals the *in-silico* binding affinity of PA with ICL1 and MLS1 when compared to their known inhibitors, 3-nitropropionate and bromopyruvate respectively. We further explored that PA binds to the active sites of both proteins with higher negative binding energy in comparison to their known inhibitors. Enzyme kinetics by Lineweaver-Burk plots has revealed that PA inhibits ICL1 and MLS1 enzymes in competitive and non-competitive manner respectively. Moreover, a semi-quantitative RT-PCR analysis shows that PA not only inhibits the ICL1 but MLS1 mRNA expressions as well. Furthermore, lower hemolytic activity (10.6%) depicts its non-lethal behavior on human blood cells. Lastly, we have established the antifungal potential for PA against *C. albicans* infections by enhanced survival of *Caenorhabditis elegans* model. Further studies are warranted for PA to be considered as practicable drug candidate.
CgVps34, a class III phosphatidylinositol 3-kinase regulates ion homeostasis in Candida glabrata

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Candida glabrata is an opportunistic human fungal pathogen. It causes life threatening diseases in immunocompromised patients and is associated with a high mortality rate of about 50%. One of the important virulence factor of C. glabrata is the class III phosphatidylinositol 3-kinase encoded by CgVPS34 gene. We have previously shown that CgVps34 phosphorylates phosphatidylinositol (PI) to phosphatidylinositol-3-phosphate (PI3P) and is required for vesicular trafficking, autophagy, intracellular survival and virulence of C. glabrata. In addition, CgVps34 has recently been implicated in maintenance of iron homeostasis by regulating the retrograde trafficking of the high affinity iron permease CgFtr1 from the plasma membrane to the vacuole. The current study is aimed at delineation of the role of CgVps34 in trafficking of other ion transporter and identification of protein that interact with the CgVps34 lipid kinase. We will present these findings along with the role of CgVps34 in copper and zinc homeostasis.
Regulation of heterochromatin organization and silencing by inner nuclear membrane proteins in *Saccharomyces pombe*

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The long DNA molecule within Eukaryotic cell is condensed with the help of histone proteins that form the nucleosome. The chromatin is not a static molecule it needs to regulate the different topological states and execute different functions. The chromatins within the cell exist as euchromatin and heterochromatin. Heterochromatin in the fission yeast *Schizosaccharomyces pombe* is clustered at the nuclear periphery and interacts with a number of inner nuclear membrane proteins. However, the significance and the factors that sequester heterochromatin at the nuclear periphery are not fully known. Here, we report that an inner nuclear membrane protein complex Lem2-Nur1 is essential for heterochromatin-mediated gene silencing. We found that Lem2 is physically associated with another inner nuclear membrane protein, Nur1, and deletion of either *lem2* or *nur1* causes silencing defect at centromeres, telomeres, and rDNA loci. We analyzed the genome-wide association of Lem2 using ChIP sequencing and we found that it binds to the central core region of centromeres, in striking contrast to Chp1, a component of pericentromeric heterochromatin, which binds H3K9me-rich chromatin in neighboring sequences. The recruitment of Lem2 and Nur1 to silent regions of the genome is dependent on H3K9 methyltransferase, Clr4. Finally, we show that the Lem2-Nur1 complex regulates the local balance between the underlying Snf2/HDAC-containing repressor complex (SHREC) histone deacetylase complex and the anti-silencing protein Epe1. These findings uncover a novel role for Lem2- Nur1 as a key functional link between localization at the nuclear periphery and heterochromatin-mediated gene silencing.
Cell-penetrating peptides (CPPs) comprise a group of peptides of about 8 to 30 amino acids in length that can be efficiently taken up by cells and mediate the uptake of the conjugated cargo (Milletti, 2012). Different mechanisms of cellular uptake of CPPs depending on their nature, concentration and experimental conditions have been proposed in literature (Madani et al., 2011). However, the two most common uptake mechanisms are direct entry/energy-independent pathway and various endocytosis mechanisms (Madani et al., 2011).

In this study, we analyzed the uptake of nona-arginine CPP in *Saccharomyces cerevisiae* cells using two methods: fluorescence microscopy and flow cytometry. The effect of different conditions like pH, temperature, and peptide concentration was studied. In addition to this, we also report the subcellular localization of the peptide after internalization. Furthermore, we investigated the influence of endoosmolytic agents and endocytic inhibitors on the entry of the peptide into the cell. Our results suggest that the efficiency of internalization of the peptide in yeast depends on conditions like pH, temperature and media compositions. However, no significant alteration in fluorescence intensity after a particular time point also suggests a probable saturation point for the entry of the peptide. These findings will enable us to understand in detail the mechanism of entry of arginine CPP in yeast cells which can be used as a promising tool for several cell biology related studies.
Antagonistic influences of transcription factors Atf1 and Pcr1 on Schizosaccharomyces pombe cell cycle: A possible new “ON-OFF switch” for regulation of periodic transcription

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Control of cell cycle phase transition involves periodic transcription of genes encoding important regulators of cell cycle, growth, proliferation, and apoptosis. The transcription of these genes is dependent on several transcription factors, which activate phase specific transcription of genes. bZIP domain containing transcription factors are found in all organisms and are associated with many cellular processes. Aberrations in their function have been shown to lead to cancer and various other diseases. These transcription factors are also known to regulate expression of many cell cycle related genes. Recent reports from our group have shown that the bZIP transcription factor Atf1, in Schizosaccharomyces pombe has a significant control over cell cycle related transcription and its overexpression results in override of both the G2-M and G1-S checkpoints. An increase in activity of this same transcription factor is indispensable for cellular survival during stress conditions. These observations seem paradoxical because, an Atf1-dependent increase in the expression of cell division promoting genes will oppose activation of checkpoints, necessary to ensure repair and cell survival during stress. Hence, cells must possess a mechanism to selectively inhibit the cell cycle accelerating functions of Atf1 during stress. Even in unperturbed cells, temporal restriction of the cell cycle related functions of Atf1 would be necessary to ensure proper progression from one phase to another. The present work highlights the role that another bZIP transcription factor Pcr1 can play here. We show that Pcr1 can specifically antagonize the cell cycle related functions of Atf1. Our results point towards the existence of a novel switch (dependent on a balance of Atf1 and Pcr1 activities) for regulation of transcription program of the cell cycle.
Cell surface-associated aspartyl proteases in *Candida glabrata*: the potent immune response modulators

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*Candida glabrata*, haploid budding yeast is an opportunistic fungal pathogen of humans. *C. glabrata* accounts for up to 30% of total bloodstream infections caused by Candida *spp.* worldwide and is associated with a mortality rate up to 50%. One of the major virulence factors in *C. glabrata* is a family of eleven glycosylphosphatidylinositol (GPI)-linked aspartyl proteases, also designated as ‘Yapsins’ which is required for vacuolar homeostasis and survival in low pH environment, survival in macrophages and in the murine model of systemic candidiasis. Here, we present RNA-Seq findings showing cell wall related genes to be differentially regulated in the mutant lacking all eleven yapsins, *Cgyps1-11Δ*. Consistent with RNA-Seq results, we show that cell wall of the *Cgyps1-11Δ* mutant contains diminished β-glucan and mannann, and increased chitin content. Cell wall components of fungal pathogens *i.e.* β-glucan, mannann and chitin, are known to modulate immune responses in the host upon infection. Therefore, we next determined the transcriptional response of human THP-1 macrophages to infection with *C. glabrata* wild-type and the mutant *Cgyps1-11Δ*. Macrophages infected with the *Cgyps1-11Δ* mutant showed a subdued and differential response compared to macrophages infected with wild-type *C. glabrata* cells. These findings along with the role for CgYapsins in suppression of the innate immune response in THP-1 macrophages and murine model of systemic candidiasis will be presented.
Azole tolerance mechanism in the pathogenic yeast *Candida glabrata*

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Pathogenic fungi have become a serious human health problem world-wide, and invasive candidiasis, in particular, is associated with a high mortality rate. Treatment of infections caused by *Candida glabrata*, an opportunistic human fungal pathogen, is limited by its inherent low susceptibility towards the most commonly used azole antifungals. Fluconazole, a triazole, inhibits ergosterol biosynthesis in yeast cells by targeting the cytochrome P450-dependent C14 α-lanosterol demethylase enzyme, encoded by the *ERG11* gene. To better understand the molecular basis underlying innate and acquired azole resistance in *C. glabrata*, a Tn7 insertion mutant screen for altered growth profiles in the presence of fluconazole was carried out. This screen identified a set of 27 novel genes that are required for tolerance to, fluconazole in *C. glabrata*. Of 27 identified genes, disruption of a subset of genes also led to increased susceptibility to the echinocandin antifungal, caspofungin, which inhibits the β-glucan synthase enzyme, with β-glucan being a major fungal cell wall component. Our study has shown a vacuolar membrane-resident phosphatidylinositol 3-phosphate 5-kinase (CgFab1) to be a novel determinant of azole tolerance in *C. glabrata*. Further, we have demonstrated the CgFab1 kinase to be pivotal to vacuole homeostasis, biofilm formation and virulence.
Co-relation between ERMES and ERES in budding yeast

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In Eukaryotic Cell, inter-organelle communication occurs by two major pathways – secretory via vesicular trafficking (ER-Golgi) and non-secretory via Direct membrane contact sites formed between adjacent organelles. Endoplasmic reticulum-Mitochondria Encounter Structures (ERMES) are membrane contact junction formed between ER and mitochondria plays a major role in lipid transport and calcium exchange and homeostasis. The main objective of this project is to check for interplay between vesicular and non-vesicular pathways of inter-organelle communication. Endoplasmic reticulum exits sites (ERES) are sites at which secretory pathway originates, while Endoplasmic Reticulum-Mitochondria Encounter Structures (ERMES) are the direct contact junction where non-vesicular mode of exchange occurs between ER and Mitochondria. As ERES are distinct and well-studied structures in *P. pastoris*, however not much is known about the existence of ERMES and their role in this organism. Thus, the relation between ERMES and ERES in *Pichia pastoris* is investigated in this work. When the ERMES resident proteins are fluorescently labelled and co-expressed along with candidate ERES markers, a juxtaposition localization or overlap between the two sites are observed. These observations have put forward spatial proximity between ERMES and ERES. In addition to this, positional overlap between two pathways can also be attributed to functional interplay between them, which needs to be studied in details.
Size and shape regulation of the nucleolus

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Size and shape regulation of non membranous organelles is a poorly understood field. Nucleolus, a non membranous organelle undergoes significant morphological alteration in malignancy. Nucleolar hypertrophy is considered to be a hallmark feature of cancer cells. Despite the biological importance of the nucleolus, the understanding of its assembly and organization is lacking. Though various reports have come up with respect to size control mechanism of the nucleolus, a lot still remains unexplored. A high throughput study carried out in Saccharomyces cerevisiae and Drosophila melanogaster identified molecular modules involved in size regulation of the nucleolus. The cross species analysis classified the factors responsible for size control of the nucleolus into various classes. In budding yeast the nucleus and nucleolus undergoes size and shape alteration upon certain cues. Different set of genes like the cell cycle related genes have an effect on the size and shape of the nucleolus. Kinesins, which are microtubule motors, play role during mitosis. They facilitate spindle elongation, spindle assembly, microtubule polymerization and organization. Kar3 and Cin8 are minus and plus end motors respectively in the budding yeast Saccharomyces cerevisiae. These genes show significant alteration in size and shape of the nucleolus upon deletion. Essential genes like Prp45, involved in pre mRNA splicing also have an effect on the nucleolar size and shape. We intend to study the role of these genes in nucleolar size regulation and also study their effect through the various stages of cell cycle.
Exploring the nuclear machinery governing the turnover of ribosomal and other non-coding RNAs in *Saccharomyces cerevisiae*

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In *Saccharomyces cerevisiae*, the aberrant mRNPs arising due to error-prone nuclear biogenesis events are rapidly eliminated by the Eukaryotic nuclear RNA exosome, and its two cofactors, TRAMP and DRN. In this study, we address the functional relationship within these nuclear decay components in the turnover of non-coding RNAs. The general turnover of ribosomal RNAs was previously shown to be exclusively carried out by the exosome component Rrp6p independent of the core Exosome, which raises an intriguing question concerning the involvement of core exosome subunits in the process. Hence, a functional contribution of the core exosome in the general turnover of rRNAs still remains an enigma.

The aim of the present study is to investigate whether Rrp6p and the core exosome subunits collaborate in the process of general turnover of non-coding RNAs by comparing the steady state levels of various non-coding RNAs in normal strains and strains deficient in components of either TRAMP, DRN or exosome subunits. Our data shows that the steady state levels of small non-coding RNAs such as 5S, 5.8S rRNAs, snRNAs and snoRNAs were enhanced when *RRP6* was knocked out, whereas their level remained unaltered when the core exosome components, *RRP4* and *RRP41*, DRN component *CBC1* and TRAMP component *TRF4* were inactivated. This finding suggests that Rrp6p independent of nuclear exosome, TRAMP or DRN plays a crucial role in the general turnover of these non-coding RNAs. The information derived from this study would augment our present conception of the degradation process and general turnover of non-coding RNAs in eukaryotes, which may have an impact on the RNA machinery governing the human genetic diseases associated with abnormal ribosome biogenesis.
Cellular role of a zinc transporter in *Neurospora crassa*

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In the filamentous fungus *Neurospora crassa*, four cation diffusion facilitator (CDF) family members trm-25 trm-32, trm-56 and trm-29 have been identified. We studied cell functions of the trm-56, also called as NcZrg-17, in *N. crassa*. The deletion mutant of the NcZrg-17 gene showed several phenotypes such as reduced growth, stunted aerial hyphae, and dense hyphal branching in zinc deficient media, indicating that it plays multiple roles during the vegetative growth in *N. crassa*. Moreover, the ΔNcZrg-17 mutant showed early conidiation, inappropriate conidiation in submerged culture and delayed conidial germination indicating that NcZrg-17 gene plays a pivotal role in vegetative development. We are also investigating the role of NcZrg-17 in circadian rhythm and its possible interaction with the calcium signaling pathway.
Non-redundant roles for the evolutionarily conserved TAF6 histone-fold and the HEAT domains for transcriptional activation

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RNA PolII transcription is regulated by a multitude of factors, including large, multisubunit complexes. TFIID and SAGA are transcriptional regulatory complexes, consisting of five shared TAFs. TAF6 has an evolutionarily conserved histone H4-like histone fold domain (HFD) at its N-terminal region, and a conserved HEAT repeat domain in the C-terminal region. How these conserved TAF6 domains contribute to transcriptional activation has not been understood. Using an unbiased genetic screen in *Saccharomyces cerevisiae*, we isolated and characterized several TAF6 mutants bearing amino acid substitutions in the HFD, middle region or the HEAT domain. Transcriptional activation by the Gcn4 and Gal4 activators was substantially impaired in the *taf6* mutants. Chromatin immunoprecipitation assays showed that the *taf6-HFD* and the *taf6-HEAT* domain mutations independently led to abrogation of TFIID and SAGA complex promoter occupancy *in vivo*. Using high copy suppression analysis, we uncovered non-overlapping genetic interactions between the HFD and HEAT domain. Coimmunoprecipitation assays showed that the association of the core TAF subunits TAF9, TAF5, TAF12 and the mutant TAF6 to TFIID was not impaired in the HFD and the HEAT mutants. Interestingly, the association of Spt7 and the core TAFs with the SAGA complex was impaired in the HEAT domain mutant, but not in the histone fold mutant. Molecular modeling and in-vitro GST pull down assays indicated that the mutant amino acid residues were involved in intra- and inter-molecular interactions, and the mutations impaired TAF6-TAF9 HFD dimerization in vitro providing a structural basis for the TAF6 HFD function. Together, our results suggested that the HF domain and the HEAT domain are independently required for TAF6 function. Because TAF6 mutations in the HF domain have been implicated in neurogenerative disorders, structure-function analyses in yeast would uncover a molecular mechanism of TAF6 pathogenesis in human disease.
An export retarding element in *SKS1* mRNA governs its nuclear retention, promotes its degradation by the nuclear exosome/DRN and thereby tunes its physiological repertoire

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The nuclear exosome/DRN degrades a wide spectrum of non-aberrant messages in addition to a diverse aberrant mRNAs in *Saccharomyces cerevisiae*. The rapid degradation of these otherwise normal messages was primarily caused due to their natural propensity of slow export and consequent nuclear retention. The property of slow export was attributed to a hypothetical cis-acting export retarding element(s) present in their transcript body. In this study, we identified an export retarding cis-element in a model non-aberrant mRNA, *SKS1*. *SKS1* is highly susceptible to the exosome/DRN action owing to its inefficient export and subsequent retention in the nucleus. An extensive deletion analysis by eliminating various segments of this transcript revealed a 194 nt long RNA segment encompassing the 826 to 1020 nt region of *SKS1* ORF, which appears to harbor the cis-element responsible for its slow export and nuclear retention. Deleting this segment led to the loss of the susceptibility to the nuclear exosome/DRN and the slow export of resulting transcript as revealed from the in situ localization of the deleted version of the message by confocal microscopic analysis. In order to further validate our hypothesis, we will fuse this element to the 3'-end of a *CYC1* message (otherwise exported efficiently and is insensitive to nuclear decay) to see whether this fusion converts the chimeric mRNA into export inefficient and susceptible to nuclear exosome and DRN. Thus, a kinetic and differential export leads to the fine modulation of the subsequent decay and stability of this transcript, which provides a novel paradigm for regulation of eukaryotic gene expression. This unique regulatory mechanism may throw new light on the mechanism of pseudohyphal growth, which underlies pathogenesis of various pathogenic fungal species.
A novel NE-ER protein maintains the integrity of nuclear pore complexes in yeast

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Eukaryotic cells contain multiple membrane-enclosed organelles. This compartmentalization ensures that the cellular processes are spatially separated. The nucleus houses the chromatin, bordered by a double membrane bilayer. The outer nuclear membrane is continuous with the endoplasmic reticulum (ER), called the perinuclear ER. Nuclear pore complexes (NPC) perforate the nuclear membrane and allow for regulated nucleocytoplasmic transport. The NPCs assemble at the sites where pores are created as a result of fusion of inner and outer nuclear membrane. The exact mechanisms governing site selection and insertion are not clearly understood. Deletion of Uip4p, an uncharacterized protein identified as an interactor of Ulp1p, was found to alter the shape of nuclear membrane and cause clustering of nuclear pores at the nuclear envelope. We found that nucleoporins belonging to several sub-complexes of the NPC were mislocalized upon altering levels of Uip4p. Our studies show that Uip4 localizes to the ONM and ER membrane. Although Uip4 is excluded from the pores, several nucleus-related functions are affected when Uip4p levels are perturbed. Our findings related to the effect of altered levels of Uip4p on the nuclear structure and function will be presented. This work has uncovered a novel role for a previously uncharacterized protein in Saccharomyces cerevisiae.
Characterizing the dual targeting/function of the peroxisomal protein Pex30

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Peroxisomes are dynamic cellular organelles found in most eukaryotic cells whose number and function can vary according to the requirements of the cell. They are involved in β-oxidation of fatty acids and neutralizing harmful reactive oxygen species (ROS) produced by the cell (Deb and Nagotu, 2017). The conundrum associated with peroxisome biogenesis is dependent on a group of peroxisomal proteins known as peroxins (Pex). Yeast mutants lacking peroxisomes regenerate peroxisomes after complementation with the wild-type version of the mutated gene (Kragt et al., 2005). Studies now show that several peroxisomal membrane proteins (PMPs) can be targeted to the ER and subsequently lead to the formation of pre-peroxisomes which mature to fully formed peroxisomes (Hettema et al., 2014). Although it is apparent that the ER plays a primary role in trafficking key peroxins essential for de novo peroxisome biogenesis, the function of ER-resident proteins and the ER structure itself in peroxin trafficking and the formation of peroxisomes has just begun to be understood (Kim and Hettema, 2015). Previous studies have reported that Pex30 not only associates with peroxisomes but also localizes to the ER and regulates de novo biogenesis of peroxisome from the ER. Pex30 interacts with reticulon proteins that are essential to maintain tubular structure of ER (Mast et al., 2016). Our study aims to understand the importance of this dual localization of Pex30 in both peroxisome inducing and non-inducing growth. For this we have constructed Saccharomyces cerevisiae strains expressing Pex30-GFP and GFP-Pex30. Only a fraction of the Pex30 was observed to be co-localized with peroxisomes labelled with DsRed-SKL. Our in silico analysis also revealed several post-translational modification sites in Pex30. We aim at characterizing mutants affected in these modifications and understand the role of these modifications in the function and localization of the protein.
Activity of cell surface-associated aspartyl proteases is pivotal to intracellular survival of *Candida glabrata*

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*Candida glabrata* is a unicellular, non-dimorphic, opportunistic pathogen which is ranked as the second most common cause of invasive candidiasis across the world after *C. albicans*. The major virulence factors associated with *C. glabrata* include biofilm formation, colony switching, and expression of cell wall adhesins (EPA proteins) and GPI (Glycosylphosphatidylinositol)-linked aspartyl proteases. *C. glabrata* contains a total of eleven GPI-linked cell surface associated aspartyl proteases also known as yapsins which are encoded by CgYps 1-11 genes. A typical yapsin is comprised of one α and β subunit, linked to each other by a disulfide bond which is flanked by a loop region of different length in each yapsin. Each subunit contains a conserved catalytic motif containing aspartic acid residue essential for the proteolytic activity. The deletion of eleven yapsins in *C. glabrata* is known to lead to highly diminished survival in human and mice macrophages. To examine if the catalytic activity of CgYps is required for intracellular survival and virulence of *C. glabrata*, we have predicted the catalytically active aspartate residue through in silico analysis and mutated them to alanine. We have examined the ability of catalytically dead *C. glabrata* to perform the known functions of yapsins. The role of catalytic sites in CgYps proteins in intracellular survival *in vitro* and pathogenicity of *C. glabrata in vivo* will be presented.
Hsp90 modulates the nuclear translocation of Rad51 upon DNA damage

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The subcellular redistribution of Rad51 in response to DNA damage is one of the regulatory events in HR. However, the present knowledge towards its nuclear import is contradictory and poorly understood. Here we have demonstrated that the dynamic interaction between Hsp90 and Rad51 is crucial for DNA damage induced nuclear import of Rad51. Using in-silico studies involving Hsp90 and Rad51 protein docking we have identified two strong interacting points, namely; R670Hsp90-E108Rad51 (salt-bridge) and K637Hsp90-M142Rad51 (H-bond). We have generated two mutant strains: rad51E108L or rad51M142L. Our study shows that the mutant Rad51¹E108L protein fails to translocate to the nucleus upon MMS treatment, resulting in hyper sensitivity to MMS. Additionally, mutation at the corresponding R670th residue of yHsp90 with isoleucine also abrogates Rad51 translocation to the nucleus upon MMS treatment. Studies involving the repair of HO endonuclease mediated DNA double strand break revealed significant loss in the recruitment of Rad51¹E108L to the broken junction, resulting in complete loss of gene conversion efficiency. We quantitatively measured the association between Rad51¹E108L and Hsp90 using purified proteins. We find that Rad51¹E108L binds very tightly to Hsp90 compared to the wild type Rad51. We reason that due to stronger association between Rad51¹E108L and Hsp90 the former protein remains locked in the cytoplasm along with Hsp90 and as a result it shows Δrad51 phenotype. These results highlight that the reversible interaction between Hsp90-Rad51 is one of the pre-requisites for the faithful repair by homologous recombination.
Structural and functional studies of Drp1, a Rint1 family protein in fission yeast S. pombe

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Cell division is an indispensible mechanism, in which the cell doubles its genomic content. Several factors interfere with the normal process of cell cycle leading to DNA damage. However, it is very difficult to retain the genomic integrity of the cell or the organisms. To maintain the normal cellular process and genomic stability, cells have control mechanism that ensures the fidelity of cell division in cells known as checkpoints. Although extensive studies have been shown that fission yeast Drp1 (damage responsive protein1), a RINT1/TIP1 family protein participates in G2/M checkpoint control, their role in DNA repair still remains unknown. During this study, we have deciphered the minimum essential region of Drp1 for interaction with Rad50 through immunoprecipitation. We observed that the N-terminal region of Drp1 is an essential for the cells survival. We have also isolated a temperature sensitive mutant allele of drp1 gene (drp1-654) which shows sensitivity to DNA damaging agents such as MMS (methyl methane sulfonate), UV and sensitive to double strand break inducing agent bleomycin. Moreover, several interacting partners of Drp1 gene has been isolated using yeast two hybrid approach. Further characterization of these genes will reveal the mechanism of Drp1 in maintaining the genomic integrity.
A novel role for the MAPK Spc1 in sensing and combating aberrations in CDK activity in *Schizosaccharomyces pombe*

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The mitogen activated protein kinase (MAPK) Spc1 (human p38 homolog) has multi facet role in regulating cell division in *S. pombe* as well as in controlling the core environmental stress response (CESR). Since p38 is a well-established target for cancer therapy, understanding its role in regulation of the cell cycle is very important. Recent reports from the lab have shown that Spc1 can detect changes in the optimal activity of key members of the core cell cycle regulatory machinery in *S. pombe*. Cdc2 (Cyclin dependent kinase) hyperactivation resulting from such perturbations can trigger a Spc1 dependent alternative backup mechanism for delaying mitosis. This pathway is activated by perturbations in the balance of Cdc25 (Cdc2 activator) and Wee1 (Cdc2 inhibitor) activities in *S. pombe* and activated Spc1 then targets the 14-3-3 protein, Rad24 (Cdc25 inhibitor) leading to inactivation of Cdc2 and mitotic inhibition. Detailed investigation of this new mechanism is important to understand how Spc1 co-ordinates the response to environmental changes with cell cycle progression in *S. pombe*. Here we present results that help to understand the intricacies of this novel mechanism.
Genetic background affects MSUD efficiency in *Neurospora*

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Meiotic silencing by unpaired DNA (MSUD) silences any gene that remains unpaired during meiosis and was discovered in the OR genetic background of *Neurospora crassa*. MSUD was weaker when crosses were made between OR-derived testers and wild-isolated strains (e.g., the novel B/S background derived from the *N. crassa* wild-isolated strains Bichpuri-1a and Spurger 3A). One hypothesis to explain this is that sequence heterozygosity between OR and B/S strains can result in self-silencing of some MSUD genes. Alternatively, the OR background might be atypically conducive for MSUD. To address these possibilities, we created novel testers in the B/S background and found that the silencing of unpaired gene was less efficient in crosses made in this background. Crosses heterozygous for an ::réc transgene produced relatively few round ascospores in the B/S genetic background and ::réc-homozygous crosses showed inappropriate silencing of a paired gene. Our results suggest that the difference in silencing efficiency between OR and B/S is not due to sequence heterozygosity. Similar results were found in the crosses made in the *N. tetrasperma* 85 genetic background. Results of Novak and Srb (Can. J. Genet. Cytol. 15: 685-693, 1973) suggested that the efficiency of MSUD in the T-220 background of *N. tetrasperma* might be similar to that of in *N. crassa* OR. If such is the case, then the difference in MSUD efficiency between OR and B/S1 in *N. crassa* will be paralleled by that between T-220 and 85 in *N. tetrasperma*, and suggest a trans-species polymorphism in MSUD efficiency. We are creating tester strains to test the efficiency of MSUD in the T220 background.
Magnesium deprivation affects cellular circuitry responsible for drug resistance and virulence in *Candida albicans*

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The management of invasive fungal infection diagnosis and treatment has become more challenging with the evolution of multidrug resistance (MDR). The failure of current antifungal drug regime compels to develop novel strategies which could be more effective against most prevalent human fungal pathogen, *Candida albicans*. The ability of *C. albicans*, to sense and adapt to changes in the host environment is essential for their survival and confers the basis of their success as dreadful pathogen. One such significant environmental factor that *C. albicans* must overcome is magnesium (Mg) limitation. Withholding the nutrient supply to the invading fungi could be a potential strategy as *C. albicans* has to struggle for the limited micronutrients present in the hostile niche. In this study we deciphered the effect of Mg deprivation on the drug resistance and virulence of *C. albicans*. We found that Mg chelation leads to enhanced drug susceptibility of common antifungals. To gain insights into the possible mechanisms involved, we explored the role of Mg on membrane homeostasis. We found remarkable differences in ergosterol levels and fluorescence microscopy images of propidium iodide intake confirm membrane perturbation. We also observed enhanced cellular leakage and altered Na⁺/K⁺ transport. Moreover, Mg deprivation leads to disrupted pH homeostasis and showed enhanced cell sedimentation rate. We further tested the genotoxicity under Mg deprivation and observed enhanced DNA damage as revealed by DAPI staining confirming indispensability of Mg to sustain genotoxic stress. Furthermore, Mg deprivation inhibited potential virulence traits including morphological transition, biofilm formation and displayed diminished capacity of *C. albicans* to adhere both to the polystyrene surface and buccal epithelial cells. Considering the restricted growth of *C. albicans* in Mg deficient environment, metal chelation strategies could be adopted to boost the effectiveness of existing antifungals.
Tunicamycin-induced endoplasmic reticulum stress and Hog1 MAPK pathway in Candida albicans

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Endoplasmic Reticulum (ER), which is an important cellular organelle where most proteins fold and mature, maintains an optimized environment filled with chaperones, glycosylation enzymes and oxidoreductases. The load of misfolded protein increases inside the ER lumen when the cells are challenged with conditions such as hypoxia, nutrient deprivation or calcium starvation. As a consequence, an adaptive response collectively termed as the unfolded protein response (UPR) is triggered by sensors residing on the ER membrane. The UPR pathway has been shown to play a crucial role in antifungal resistance and virulence; therefore, the pathway may serve as a target for developing new antifungals. Candida albicans, a human pathogenic fungus, faces ER stress when it invades human tissues, which increases demand on the secretory pathway in these fungal pathogens. In S. cerevisiae, the high osmolarity glycerol (HOG) pathway plays a role in ER stress resistance, while the role of this pathway in ER stress remains uncharacterized in C. albicans. We are particularly interested in exploring the role of the HOG MAPK pathway during ER stress in C. albicans. We show that strains lacking the MAPK Hog1p and other components of the HOG pathway displayed sensitivity to the ER stressors; tunicamycin and DTT. This effect seems to be mediated by the basal activity of the Hog1p MAPK as tunicamycin exposure does not trigger the phosphorylation of Hog1p and its nuclear import. Besides, all these effects seem to be independent of the classical Hac1p-mediated UPR pathway. Moreover, tunicamycin exposure leads to overproduction of glycerol, the osmoprotectant, and that this is dependent on a functional HOG pathway.
**Gcn4p is stabilized by methionine, during growth in amino acid starved conditions**

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Amino acid starvation leads to translational repression via induction of stress factors. We recently showed that complementation of the starvation medium with a single amino acid, methionine, significantly relieves this repression. Despite overall starvation, methionine addition stimulates an overall anabolic transformation, which requires the activity of Gcn4p, a transcription factor that is the primary regulator of amino acid biosynthesis under starvation. Gcn4p accumulates when methionine is uniquely supplemented, but not with other non-sulphur amino acids. This accumulation was transcription independent, suggesting post-transcriptional regulation of Gcn4p amounts. We dissect this phenomenon, as well as the role of the well-characterized Gcn2p-eIF2 axis in Gcn4 translation, to understand how Gcn4p is regulated by methionine. We find that methionine dependent Gcn4p accumulation continued even in the absence of Gcn2p, and reduced eIF2 phosphorylation. We show that Gcn4p undergoes high turnover, and methionine specifically inhibited the degradation component of Gcn4p, leading to stabilization of protein levels. Through a combination of direct assays for Gcn4p ubiquitinylation and site directed mutagenesis of Gcn4p residues implicated in proteasomal degradation, we demonstrate that methionine uniquely and specifically modulates the cellular protein degradation response. This thereby modulates the Gcn4p mediated transcriptional and metabolic programs differently during starvation, dependent upon methionine availability. This regulation of Gcn4p stability and turnover suggests a broader regulation of regulated protein turnover in a methionine dependent manner, leading to altered growth responses.
Oleaginous yeasts as suitable platform for production of value added biomolecules

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Microbial oils from oleaginous yeasts attract increasing interest as they offer a renewable and cheap feedstock for the production of biodiesel, food ingredients and other products. These yeasts use fermentable sugars to produce 20-70% of their biomass as TAGs that have composition very similar/superior to vegetable oil. *Lipomyces lipofer* is considered an important candidate in production of TAGs as it can accumulate large amounts of oil and has a broad adaptability to various carbon sources that are cheap and vastly available. These include waste oil, acid oil, crude glycerol, etc. Growth in media with excess carbon source and deficiency of nitrogen leads to significant lipid enrichment in the cells. The present study describes targeted efforts in utilizing *L.lipofer* for accumulation and enrichment of intracellular lipid from a variety of carbon sources including glucose, glycerol, acid oil, etc. The average production of oil is 9.4g/L for different substrates. Bioconversion of acid oil to TAGs using *L.lipofer* was confirmed on the basis of equivalent chain number (ECN) of the molecule using HPLC-ELSD. Further characterization with LCMS validated the results.
Impact of ABC transporter and sphingolipids in inhibitor tolerant yeast for biofuel production

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Microbial cell factories have the potential to make major contributions to the circular economy by extracting value from current waste streams. To this end microbial cell factories are becoming increasingly flexible, efficient production platforms for various chemicals, materials, and fuels using a variety of renewable feedstocks. For any microbial cell, factory substrates have to be taken up to build the factory (cell biomass), fuel the bioprocess and export the product (efflux system). Translocation of molecules across membranes requires the action of proteins known as transporters. Membrane transporters are the gate keepers of the cell controlling what is allowed in and out. A greater understanding of these proteins will allow us to manipulate their properties, ultimately leading to increased productivity of cell factories.

In this study, we identified and deleted 23 putative ABC transporters and sphingolipid pathways genes of Candida glabrata, and evaluated for fermentation inhibitor tolerance. Deletion of putative transporter genes CAGL0J04862g(cgSNQ2), ΔCAGL0C03289g(cgYBT1), CAGL0E03982g(cgVMR1) and CAGL0K00363(cgSTE6), which displayed a varying degree of tolerance to fermentation inhibitors like ethanol, furfural, and acetic acid. Interestingly, deletion of ΔCAGL0G00242g (cgYOR1), ΔCAGL0C03289g (cgYBT1) and ΔCAGL0M02387g (cgPXA1) produced higher ethanol yield at 30°C and 40°C as compared to the wild type strains. Notably, sphingolipid K/O CAGL0K02805g (cgAUR1) of C. glabrata also displayed lower yield of ethanol, which reveals the impact of sphingolipid in bioethanol production. These data point out to the unusual roles of ABC transporters and sphingolipids of yeast in bioethanol production.
Caffeine induces apoptosis in *Saccharomyces cerevisiae* LEA1 deficient cells

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LEA1 (looks exceptionally like U2A∗) specific component of U2snRNP, plays crucial role in first step of splicing *in vivo* by participating in spliceosome assembly with U2 snRNA base pairing with both the pre mRNA branch point sequence and U6 snRNA. It is highly conserved across metazoans. Cells lacking LEA1 (*lea1*) harbour low levels of various mRNA and accumulate pre mRNA to different levels. In order to gain insights into role of accumulated RNA in cell growth, we examined the sensitivity of *lea1*Δ cells under different environmental conditions including caffeine. Interestingly, *lea1*Δ cells showed hypersensitivity to caffeine along with increased accumulation of pre-transcripts of certain RNAs. Further studies revealed hypersensitivity of *lea1*Δ cells to oxidative stress induced by H2O2. The treatment of *lea1*Δ cells with antioxidants restored their growth. An exposure to caffeine resulted in highly fragmented nuclei and an increased expression of Caspase gene MCA1 in *lea1*Δ cells. This implies that sensitivity of *lea1*Δ cells to caffeine results from induced apoptosis. Caffeine is an established inhibitor of TOR signalling pathway and largely affects functions mediated by TORC1. Thus, to illuminate on role of TOR signalling, we examined expression profile of TORC1 and TORC2 marker genes in *lea1*Δ cells. A highly perturbed expression of marker genes indicated the possible role of TOR signalling in regulation of cell growth under intracellular stress conditions induced by accumulated unprocessed RNA. This is the first study correlating TOR signalling with splicing defect.
Functional role of fission yeast splicing factor SpPrp18 in constitutive and stress regulated alternative splicing

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Fission yeast (Schizosaccharomyces pombe) genome is rich in short introns and have abundant multi-intronic transcripts, degenerate splice signals and SR proteins, these intronic features together makes it a suitable model organism for study roles for core associated spliceosomal factors in alternative splice-site choice and intron retention. We have analyzed functionally important domains of Schizosaccharomyces pombe SpPrp18 and found five-alpha helix bundle structure for its C terminal domain. Mutational analyses showed vital functions for residues in the conserved region and in helix five. To examine in vivo functions the slow growing missense mutant prp18-5 was generated. This mutant protein was unstable above 40ºC and had compromised secondary structure. Using genome wise splicing data from wild type and mutant (prp18-5) cells we inferred a stringent reproducible subset of S. pombe alternative events. This prompted us to study the functional role of core spliceosome factor SpPrp18, for these alternate splice choice events and allowed us to investigate the relationship to growth phase and stress. We found that wild-type cells at log and stationary phase of growth showed ats1+ exon 3 skipped and intron 3 retained transcript isoforms. We showed that the non-consensus 5'ss in ats1+ intron 3 caused SpPrp18 dependent intron retention. We also validated the use of an alternative 5'ss in dtd1+ intron 1 and of an upstream alternative 3'ss in DUF3074 intron 1. Our investigation showed the dtd1+ intron 1 non-canonical 5'ss yielded an alternative mRNA whose levels increased during stationary phase. Further the analysis of the relative abundance of these splice isoforms during mild thermal, oxidative and heavy metal stress was done. We show stress-specific splice patterns for ats1+ and DUF3074 intron 1 some of which were SpPrp18 dependent. This study thus provides leads to study environmental signal dependent effects in bringing about rapid changes in cellular transcript isoforms.
Bioprospecting and bioengineering of yeast cell for biofuel production

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Due to increased oil demand, depleting fossil fuels and greenhouse gas emissions, biofuels production are getting much attention. The fatty acid based biofuels (fatty acids ethyl ester/biodiesel, fatty acids methyl ester, fatty alcohol, etc.) produced from microbial cells have emerged as ideal alternatives to fossil oils, with substantial advantages over plant, animal and algae oils. *Saccharomyces cerevisiae* is a most studied industrial model microorganism and also its fatty acid production capacity has been increased by sequential metabolic engineering approach. But still the cost of the process limits its industrial production therefore, more research is required to increase its production. So, here we are addressing this issue by two independent approaches; bioprospecting of yeasts for high lipid production and improving biodiesel production by bioengineering of yeast strain.

Here, we systematically engineered the yeast for high-level biodiesel production by rewiring the lipid metabolism by increasing the precursor supply, increasing the cofactor supply, and disrupting the nonessential pathway. Additionally we identified few yeast isolates which produce very high lipid naturally.
Molecular phylogenetic analysis and expression profiling of ATP-binding cassette transporters gene family in human pathogen Candida glabrata in response to antifungal drugs

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Different classes of antifungal drugs are available to treat candidiasis however occurrence of multidrug resistance (MDR), a phenomena where a cell develop resistance towards various structurally and functionally unrelated drugs becomes a serious threat to the treatment. Among several mechanisms which contribute to MDR in \textit{Candida} species, the overexpression of drug-efflux pumps belonging to the ABC (ATP-binding cassette) and Major facilitator superfamily (MFS) are the most frequent cause of resistance in clinical isolates. Recent reports suggested that ABC transporters not only involved in transport function but also affects several cellular function and virulence capacity in \textit{Candida} species. These finding suggested an urge to study ABC transporter proteins in depth to understand their physiological relevance not only in MDR but also in other vital cellular functions. Our bioinformatics analysis reveals that \textit{C. glabrata} genome contains 25 ABC protein coding genes. Further by phylogenetic and topological analysis, we categorized these proteins into seven subfamilies including PDR, MDR, MRP, ALDp, YEF3 and RLI. Out of these 25, only 18 proteins consist of transmembrane domains (TMDs) and considered as transport proteins and belong to PDR, MDR, MRP, and ALDp. In these transporter families, PDR is the largest family with 7 members. Here, we attempted to identify and categorize ABC proteins and their expression profile by transient exposure of anti-fungal drugs in \textit{C. glabrata}. This study would provide an important platform for further investigation and functional characterization of ABC proteins in \textit{C. glabrata}.
Chemical genomics screening in *Saccharomyces cerevisiae* indicates fludioxonil mediates toxicity by targeting Ssd1

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Hybrid histidine kinase 3 (HHK3), a ubiquitous sensor kinase in fungi, constitutes an important molecular target for developing antifungal agents. Fungicidal agent e.g. fludioxonil which is extensively used in agricultural practices, is known to target hybrid histidine kinase 3 and imitates osmostress conditions. However, the elaborate mode of action of fludioxonil remains obscure. To better understand this, we had earlier created a *Saccharomyces cerevisiae* model by heterologous expression of ClNIK1 and used the genome wide deletion library to identify genes that buffer against fludioxonil toxicity. In this study, we used the same chemical genomic platform to identify the genes whose deletion confers resistance to fludioxonil. One of the identified genes is SSD1 which encodes a RNA binding protein. Ssd1 is phosphorylated by Cbk1 which helps in the expression of downstream effector molecules like UTH1, SIM1, SRL1 at the budding sites. Fludioxonil did not cause constitutive activation of HOG pathway in ssd1Δ strain. Similarly, the fragmentation of vacuole was not observed in ssd1Δ upon fludioxonil treatment. Upon upregulation of regulatory genes like Cbk1 and Sit4 in wildtype it was observed that Cbk1 overexpression helped the cells overcome toxicity of fludioxonil. Among the downstream molecules regulated by Ssd1 UTH1 overexpression showed heightened resistance to fludioxonil. Therefore, overexpression of CBK1 or UTH1 or phosphomimic SSD1 helps override the toxicity of fludioxonil. This work reports key molecules involved in the mode of action of fludioxonil.
MTG3, a putative GTPase involved at a late step of mitochondrial ribosome biogenesis in Saccharomyces cerevisiae

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Mitochondria utilize dedicated ribosome molecules that are distinct from either its cytosolic counterpart or ancestral bacterial ribosomes. During the course of evolution, size of rRNA has reduced in the mitochondrial ribosome in comparison to its bacterial ancestor. Reduction in rRNA domain is likely been compensated by an increase in the mitochondrial ribosomal protein numbers in comparison to its bacterial ancestor as well as addition of domains to the ancestral bacterial ribosomal proteins. Ribosome biogenesis is a multistep process micro-managed by assembly factors including GTPase that utilize energy released upon nucleotide hydrolysis to promote its biogenesis. MTG3 belongs to circularly permuted class of GTPase that is conserved from yeast to humans containing a central GTPases pocket flanked by N and C terminal domain. Deletion of MTG3 leads to defects in utilization of glycerol as sole carbon source and accumulation of 15S rRNA precursor. We have shown that MTG3 associates with both small and large subunit of mitochondrial ribosome and is involved at a late step in their biogenesis. Our studies also indicate that MTG3 associates with the ribosome via the C-terminus independent of the bound nucleotide. However, MTG3 requires guanine nucleotide binding as well as hydrolysis to carry out its in vivo function at a late step during mitochondrial ribosome biogenesis. We have also shown Mtg3p in a molecular complex with Mtg2p, a large mitoribosomal subunit assembly factor indicating its role in coordination of both small and large subunit biogenesis. We are currently trying to determine the nearest neighbor that Mtg3p is associated with how they regulate a late step in mitochondrial ribosome biogenesis.
DNA damage response in the pathogenic yeast *Candida glabrata*

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*Candida glabrata* is an opportunistic human fungal pathogen which causes superficial and systemic infections in immunocompromized individuals. *C. glabrata* cells are able to survive and replicate in the macrophage hostile environment by inhibiting phagolysosomal acidification and altering cytokine production. Furthermore, *C. glabrata* is known to remodel its own chromatin and survive macrophage-induced reactive oxygen species (ROS) stress. We have previously reported that macrophage-internalized *C. glabrata* cells show differential levels of core histone proteins. A major goal of the current study is to elucidate the molecular basis underlying rendered histone H4 levels in the macrophage-internalized *C. glabrata* cells. Towards this, we have shown that *C. glabrata* respond to several stresses by reducing histone H4 levels and a mutant lacking two histone H4-encoding ORFs showed resistance to DNA damage caused by methyl methanesulfonate (MMS). As the DNA damage resistance phenotype was not found in the histone H3 single or double deletion strains, we concluded that the resistance was specific to histone H4. These findings along with other factors regulating the response of wild-type *C. glabrata* cells to DNA damage will be presented.
3D models of Cdr1p and Mdr1p multidrug efflux proteins: a step forward into a new era of antifungal efflux mechanism understanding and inhibitor design

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Candida species are commensal yeasts which can turn into a filamentous pathogen form under favorable conditions with a death rate over 50% in case of systemic candidiasis leading to more than 50,000 deaths per year worldwide. The infection rate has grown over the years with the use of antifungals which led to the development of multidrug resistant (MDR) strains by induction of the overexpression of the two multidrug efflux pumps Cdr1p and Mdr1p. Cdr1p is a member of the ABC (ATP-Binding Cassette) exporters while Mdr1p is part of the MFS (Major Facilitator Superfamily) antiporters. Both families are composed of membrane proteins among which some are involved in the efflux of structurally-unrelated cytotoxic compounds. ABC transporters display the same global topology, made of 2 halves either identical or different, and linked together in a single polypeptide chain or 2 distinct. Each moiety contributes to drug binding and export through a transmembrane domain (TMD), coupled to 2 nucleotidobinding domains (NBD), which, by hydrolyzing ATP, allow the protein to reset to its initial conformation after drug efflux. Cdr1p, with its reverse organization where the NBD precedes the TMD contrary to most of the other ABC transporters, is topologically close to the human ABCG subfamily among which the homodimer G2 is involved in anticancer-drug resistance and the heterodimer G5/G8 is involved in cholesterol transport. MFS antiporters are composed of two halves made of 6 or 7 transmembrane helices each for the DHA1 (Drug:H+ antiporter family 1) or DHA2 respectively. Mdr1p belongs to the DHA1 family and is structurally related to the MDR conferring MFS Glycerol-3-phosphate transporter (GlpT) from E. coli. As ABCG5/G8 with Cdr1p and GlpT with Mdr1p respectively share the same topology and have enough homology, we built 3D models that we validated back and refined by looking at the location of 252 alanine mutants generated in the TMD for both transporters and characterized for their implication in several substrates transport. For Cdr1p these results highlight the key role of TM1, 2, 8 and 11, which are forming the core of the main drug binding pocket. For Mdr1p a similar approach led us to identify the implication of TM7, 10 and 11 in the drug binding pocket. As in Cdr1p, it also highlights the implication of peripherical residues in the transport of substrates of different nature. The comparison of these results with crystallographic structures of substrate-specific MFS transporters not involved in MDR phenotype shows that the involvement of such peripherical residues is limited to the multidrug efflux pumps only and may be the key of their polyspecificity. Altogether these findings open an exciting era in the understanding of the molecular mechanism by which such proteins pump drugs out of the cells and to design potent inhibitors by a structure-based approach.
Arsenic tolerance in *Papiliotrema laurentii* strain RY1 isolated from Kombucha tea

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Arsenic is a metalloid which exists in inorganic compounds mainly in two forms arsenate (valency +V) and arsenite (valency +III) which are bio hazards and major pollutants of groundwater in India. Arsenite is more toxic than arsenate. The yeast *Papiliotrema laurentii* strain RY1 (Old name: *Cryptococcus laurentii* strain RY1) isolated from Kombucha tea was found to be arsenic tolerant. The MIC and MBC values of arsenite was found to be 10mM and 100mM for the yeast. The growth pattern of the yeast was studied in presence of arsenite. The morphology of yeast in presence of arsenite was investigated by Scanning Electron Microscopy. The arsenic binding property of the yeast was confirmed by X-Ray Diffraction and Fourier-Transform Infrared spectroscopy. The arsenic accumulation in the yeast was confirmed by Atomic Absorption Spectroscopy. The over expression of aquaporin and metallothionein at the RNA level suggested a probable mechanism for the uptake and detoxification of arsenite. To the best of our knowledge, this is the first report of the arsenic tolerance of *Papiliotrema laurentii* strain RY1 which has the potential to be used as a biosorbent and / or bioaccumulator against the toxic ion.
Induction of apoptosis-like cell death and clearance of stress-induced intracellular protein aggregates: dual roles for *Ustilago maydis* metacaspase Mca1

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Caspases are cysteine dependent aspartate proteases which depend on the catalytic activity of two conserved Histidine and Cysteine residues that together form the catalytic dyad. In multicellular eukaryotes caspases play major role in the regulation and execution of the programmed cell death machinery. Metacaspases are structural homologs of the caspases and in single cell eukaryotes regulate apoptosis. Here we show *U. maydis* metacaspase in addition to playing a conventional role in the induction of cell death also takes part in the dissipation of insoluble protein aggregates and thus helps maintain protein quality control during stress induced conditions that the fungus faces while invading its host. The study also reveals a role of *U. maydis* metacaspase in the virulence of the fungus through host infection assays with the *mca1* deletion strain.
Genome-wide analysis reveals diverse transcriptional networks coordinated by HSF1 regulate various cellular machineries of Candida albicans

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The master regulator of thermal stress response, Hsf1, is also an essential determinant for viability and virulence in the human pathogenic yeast, C. albicans. Our recent studies highlighted that apart from ubiquitous roles of Hsf1, it has myriad non-heat shock responsive roles essential under iron deprivation and drug defense. In this study, we further explored the normal cellular functioning of Hsf1 by profiling its genome-wide occupancy using chromatin immune-precipitation coupled to high-density tiling arrays under normal and iron deprived conditions. A comparison of Hsf1 recruitment under these two conditions revealed that Hsf1 occupies 661 gene promoters of varied functions under both the conditions, however, elicited variability in the intensity of binding. Additionally, in 67 gene promoters Hsf1 showed exclusive occupancy under normal conditions while in 30 genes Hsf1 showed exclusive binding only upon iron deprivation. Interestingly, in this study motif analysis also divulged a novel motif comprising -nGTGnGTGnGTGn- where HSF1 showed strong occupancy at a significant number of sites on several promoters at basal temperatures apart from the previously known consensus of three-inverted nGAAn repeats. Expectedly, promoters of the Heat Shock Proteins (HSPs) such as HSP104, HSP90, HSP78, HSP70 and HSP60 showed constitutive binding but promoter occupancy was also perceived on several other genes related to the core stress responses pathways under basal conditions. For instance, Hsf1 binding was observed on several genes of oxidative and osmotic stress response, cell wall integrity, iron homeostasis and mitochondrial genes, hyphae development and multidrug transporters. It may hence, be perceived that by binding to and regulating the major chaperones, stress responsive genes and also certain regulators of drug resistance constitutively, Hsf1 is imperative regulative various cellular machineries of the cell. Thus, the current study provides a framework for understanding the novel aspects of how Hsf1 coordinates diverse cellular functions.
Transcriptional landscape of carbohydrate active enzymes regulation in *Penicillium sp.* by genome and transcriptome analysis

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Plant lignocellulosic material is a storehouse of solar and chemical energy in the form of β 1→4 linked glucose moieties. Certain bacteria and fungi thrive on it by producing cellulolytic enzymes specific to the feed. These microbes have been used in industry for cellulolytic enzyme production which is used for production of fermentable sugars from plant biomass. Over the years these strains have been improved by random mutagenesis and selection of the hypercellulolytic strains. Rational engineering for improvement of its enzyme production requires deeper understanding of the pathway involved in induction of these enzymes. Information about the signalling molecules and key transcription factors involved in its regulation is still lacking. Cellulolytic organism of *Penicillium sp.* was screened in our lab for its outstanding hydrolytic capabilities. It outperformed other cellulolytic strains and industrial enzyme cocktails in terms of lignocellulose hydrolysis. Also the enzyme cocktail secreted by this organism was found to specific to the carbon substrate used as feed. Further improvement of this strain requires deeper understanding of the induction system. Its genome and transcriptome were sequenced and analyzed for the kind of genes it encodes for. The expression pattern of carbohydrate active enzymes (CAZymes) and transcription factors (TFs) was followed in presence of different carbon substrates which were either rich in cellulose (avice) or hemicelluloses (wheat bran). Also we used wheat straw (abundant in India) to get the exact repertoire of TFs involved in its regulation. Correlation matrix of the TFs and CAZymes helped us in identifying a subset which was involved in regulation. Further validation was done using qPCR, which reduced the numbers. Improvement was done using proteome sequencing of the nuclear extract obtained at different time points and carbon substrates. Promoter region of one of the major CAZymes secreted by this organism cellobiohydrolase (CBHI) was analyzed. EMSA studies helped in identifying the stretch responsible for TF binding. Further analysis of the transcriptional rewiring occurring in presence of different substrates will help us in improvement of the system.
Phospholipids are important in determining sensitivity in fungal cells

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Lipid rafts (microdomains) are highly enriched in sphingolipids and ergosterol/cholesterol and are characterized by their insolubility in non-ionic detergents at low temperatures. Recently, presence of the microdomains, was verified in various organisms including plants, humans, bacteria and yeast too. These are discreet sites in plasma membrane for performing specialized functions like entry of pathogen, cell signalling, protein sorting, virulence and drug resistance. In yeasts, different membrane proteins of varied functions (including drug exporters) are shown to be influenced by imbalances in composition of lipid raft components, thus affecting their activity and localization of raft markers like Pma1p. But, we have extended this knowledge by also including the importance of phospholipids in membrane. We checked the activity and translocation of plasma membrane transporters in altered phospholipids background by making the genetic mutants in S. cerevisiae background. The phospholipids including: PE, PC, PI and PS (Phosphatidylserine) present in the plasma membranes were modulated by mutating their genes PE requires PSD1 (YNL169C) and PSD2 (YGR170W); whereas PC needs OPI3 (YJR073C) and CHO2 (YGR157W). To our surprise, even phospholipids affected the activity of these transporters. It would be interesting to explore the role of phospholipids on targeting of these proteins too.
Is NADP-glutamate dehydrogenase important in yeast-hypha reversible transition of dimorphic zygomycete *Benjamiella poitrasii*

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*Benjamiella poitrasii*, a dimorphic non-pathogenic zygomycete shows glucose, pH and temperature dependent yeast (Y) - hypha (H) morphological transition. The biochemical correlation of relative proportion of NAD- and NADP- dependent glutamate dehydrogenases (GDHs measured as NADP-/NAD-GDH ratio) with morphology was reported first time in *B. poitrasii*. Furthermore, one NAD-GDH and two form specific NADP-GDH isoenzymes were also reported in *B. poitrasii*. At the molecular level, one NAD- (*BpNADGDH*) and two separate genes coding for NADP-GDH isoenzymes in *B. poitrasii* (*BpNADPGDH I and II*) have been identified. Full length genes, *BpNADGDH* (2.643 Kbp), *BpNADPGDH I* (Yeast form specific, 1.365 Kbp) and *BpNADPGDH II* (H form specific, 1.368 Kbp) were isolated and characterized. Under normal dimorphism triggering conditions (glucose and temperature), hyphal form specific *BpNADPGDH II* was not expressed in Y5 (yeast form monomorphic mutant). However, it was induced in the presence of ethanol, leading to Y-H transition in mutant Y5. The effect of ethanol was reverted by myo-inositol with subsequent repression of *BpNADPGDH II* gene. On the hand, the transformation with *BpNADPGDH II* gene also induced germ tube formation in mutant Y5.

Further, purified NADPGDH isoenzymes were evaluated for their potential as an antifungal drug targets. The structural analogous of 2-ketoglutarate (NADP-GDH substrate), dimethyl esters of isophthalic acid (DMIP), were found to inhibit BpNADPGDH isoenzymes. The inhibition kinetics suggested that DMIP compounds were competitive inhibitors of NADP-GDH. The DMIP compounds (DMIP 1, 7 and 8) inhibiting hyphal from specific NADPGDH (*BpNADPGDH II*), also inhibited Y-H morphological transition in *B. poitrasii*. Furthermore, the effect of DMIP compounds on GDH activity and Y-H transition in human pathogenic *Candida albicans* strains are in progress.
Strategies for production of microbial oil using oleaginous yeasts

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The ability of non-conventional oleaginous yeasts to accumulate lipids by assimilation of diverse carbon substrates has projected them as prospective cell factories for Microbial oil production. The process of oil accumulation in oleaginous yeast is a response shown by the organism to the stress conditions induced by depletion of a nutrient source. *Yarrowia lipolytica,* a model oleaginous yeast accumulates 37.5% of oil with respect to its dry cell biomass in glucose deprived of proteinaceous ancillaries, contrasting to a meagre 11.2% in its growth medium. When growth media was replaced by glucose at high C: N ratios in a second stage, the oil yield increased from 0.06 to 0.15g/g of glucose. This increase in the oil yield is ~50% of the theoretical maximum values, prompting the need for more inclusive strategies for yield improvement. *In situ* recovery of oil using solvents has been previously reported for algal systems. This however has limitations on account of solvent toxicity and mass transfer limitations across the two phases. Continuous online capture of extracellular oil from the culture media and probably the cell surface was carried out by contacting the broth to an adsorbent bed with a capacity of 100 mg of oil per ml of adsorbent. This approach was observed to aid glucose consumption and have an overall yield of 0.33 g of oil per g of glucose after 72 hrs of fermentation. The oil yields achieved are by far the best reported for microbial oil production and present a viable approach that could make this technology commercially possible.
Histone fold domain mediated heterodimerization specifies the selective association of \textit{TAF12} paralogs with TFIIID and SAGA complexes

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TBP-associated factors (TAFs) are evolutionary conserved proteins that play a major role in eukaryotic transcription. A subset of TAFs (TAF5, TAF6, TAF9, TAF10 and TAF12) is shared between TFIIID and SAGA complexes. In addition to the 13 canonical TAFs in each eukaryote that are conserved in evolution, higher eukaryotes also contain a number of alternative TAFs that function in a cell-type and tissue-specific manner. However, it is not understood how the alternative TAFs are selectively associated with TFIIID or the SAGA complex. Previous studies from our lab have shown that the TAF12 variants TAF12 and TAF12L selectively associate with TFIIID and SAGA complex in \textit{C. albicans}. In this study, we have dissected the molecular requirements for the interaction of the two TAF12 variants. To determine the selectivity of their interaction, we depleted TAF12 and TAF12L one at a time and carried out coimmunoprecipitation assays, and found that neither of the variants could substitute for the other in the TFIIID and SAGA complex. Furthermore, we found that the expression of the Histone fold domains alone is sufficient for the function and incorporation of the two TAF12 variants into their respective complexes. \textit{In vitro} pull down assays with recombinant proteins showed that histone fold domain was sufficient for TAF12L and TAF12 heterodimerization with the histone fold domains of SAGA-specific Ada1 and TFIIID-specific Taf4 respectively. Surprisingly, while the TAF12L HFD specifically interacted with HFD of Ada1 only, the TAF12 HFD interacted with the HFD of both Ada1 and Taf4. We next tested interactions of full length proteins and found that unlike the HFDs, the full length TAF12L and TAF12 specifically interacted with ADA1 and TAF4 respectively. To determine the \textit{in vivo} requirement for the interaction of the TAF12 variants, we cloned and expressed the two genes in \textit{C. albicans}. The genetic complementation assays showed that the \textit{CaTAF12L} construct with the \textit{CaTAF12} HFD was able to fully rescue the growth defect of TAF12 depletion, but the \textit{CaTAF12} construct with the \textit{CaTAF12L} HFD was unable to do so indicating tight specificity. Furthermore, the chimeric \textit{CaTAF12} constructs bearing \textit{CaTAF12L} \(\alpha\)-L1, \(\alpha\)-L2, \(\alpha\)-L3 or \(\alpha\)C helices were also able to fully rescue the growth defect indicating that the different segments of the HFD have a cumulative function in determining the specificity of the interaction of the TAF12 with the TFIIID complex. On the other hand the \textit{CaTAF12L} constructs with \textit{CaTAF12} \(\alpha\)-L1, \(\alpha\)-L3 or \(\alpha\)C were able to only partially rescue TAF12 depletion. Interestingly, the \textit{CaTAF12L} construct bearing \textit{CaTAF12} \(\alpha\)-L2 region was able to fully rescue the growth defect of \textit{CaTAF12} depletion indicating that at least one of the major determinant of the specificity of the TAF12 variants lies in the \(\alpha\)-L2 region of the HFD. Thus overall our integrated genetic, biochemical and mutational study established that the \textit{CaTAF12} variants have an inherent specificity for heterodimerization and the regions that define this specificity are primarily located in the conserved HFD region of two proteins.
Identification, isolation and functional characterisation of magnesium transporter of endophytic fungus *Piriformospora indica*

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Magnesium is the most abundant divalent cation in living cells yet, by comparison to most other macro and micronutrients, but little is known about its role in biology. In bacteria, Mg$^{2+}$ uptake primarily occurs via the constitutively expressed *CorA* transport system. *Saccharomyces cerevisiae* has two genes, designated *ALR1* and *ALR2* that encode proteins with a low degree of similarity to *CorA* responsible for magnesium transportation in it. The *Piriformospora indica* fungus promotes nutrient uptake, allows plants to survive under water, temperature and salt stresses, and confers systemic resistance to toxins, heavy metal ions, insects and pathogenic organisms. Because of these virtues *P. indica* has been proven as plant biofertilizer, probiotic and biohardening tool. Our present study showed that the *P. indica* helps the plant to uptake the magnesium under deprived condition whenever, it is under colonized state with Plant roots. Plants colonised with the *P. indica* were healthy, taller and there leaf contained more chlorophyll in comparison to non-colonised *P. indica* plants. Blast analysis showed us that *P. indica* has two magnesium transporter (*Pimgt1* and *Pimgt2*) which are homologue of *ALR1* and *ALR2*. Both the genes were found of approximately 1.9 kb in size and contains the signature tag GMN related to magnesium transportation in *Saccharomyces cerevisiae*. Semi quantitative PCR study revealed that *Pimgt1* is high affinity transporter. The complemented magnesium mutant strain of *Saccharomyces cerevisiae* regained its growth when grown on MN media containing under 4 mM magnesium. Our study on this magnesium transporter will help in better understanding of this agro-friendly fungus.
Unravelling the sub-compartment specific mitochondrial protein misfolding induced stress response pathways

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Mitochondria, is one of the complex organelle present in all the eukaryotic cells. It has its own genome which encodes 1% of total mitochondrial proteins and the remaining 99% of proteins are encoded by the nucleus, translated in the cytoplasm and are imported to mitochondria as predominantly unfolded precursor proteins, for mitochondrial biogenesis (Walter Neupert and Johannes M. Herrmann, 2007. Annu. Rev. Biochem. 76:723–4). The co-ordination between the mitochondrial genome-encoded proteins (8 proteins in yeast, few of the different components of ETC sub-complexes) and nuclear DNA-encoded proteins is crucial for the correct assembly of the electron transport chain. Furthermore, due to sub-compartmentalization of mitochondria into two compartments, inter-membrane space and matrix, with different oxido-reductive environment, the proper intra-organelle sorting and folding of proteins are also complicated and requires continuous quality control.

To decipher the details of mitochondrial sub-compartment specific unfolded protein response pathways, we targeted different misfolded protein with specific misfolding propensities and checked the response upon targeting to different sub-compartment of mitochondria, in yeast S. cerevisiae. We have observed sub-compartment specific growth phenotype, alteration in mitochondrial morphology and gene expression upon misfolding stress. It clearly shows that mitochondrial sub-compartments have different mechanisms of protein quality control to handle misfolding induced stress. Further study need to be done to elucidate the molecular mechanism of sub-mitochondrial unfolded protein response in yeast, which would pave the way to understand the molecular mechanisms which might exist in the higher eukaryotes like mammalian system to handle misfolding induces stress and pathological conditions.
Role of the WW domain-containing protein Ifu5 in hypoxic adaptation in Candida albicans

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Human gut and other body organs are home to prokaryotic and eukaryotic commensal microbes. Candida albicans is such a fungal commensal residing mostly in gut of healthy individuals but immune suppression turns them pathogenic and allows propagation into blood stream. Environmental niches of various body organs present challenges to inhabiting microbes like, variable oxygen tension, nutrient limitation, extreme pH etc. Efg1 is a master regulator having role in adaptation to hypoxia, regulation of cell wall integrity, filamentation and biofilm. This study demonstrates a role for the Tac1-regulated WW domain-containing protein Ifu5 in hypoxic adaptation in Candida albicans. Localization study shows that Ifu5 is ubiquitously distributed between the nucleus and cytoplasm. Transcriptional profiling of the ifu5Δ/Δ cells showed significant up regulation of EFG1 and EFG1 dependent genes. Concurrent with this, ifu5Δ/Δ cells displayed abrogated filamentation and defects in biofilm formation in normoxia and hypoxia. Additionally, we also analyzed the role of the WW domain in the aforementioned phenotypes by mutating both the conserved tryptophan residues. We show that WW domain is important for biofilm formation under hypoxia. Taken together, our study assigns a role to Ifu5 in hypoxic adaptation by C. albicans.
Molecular basis of substrate polyspecificity of the *Candida albicans* Mdr1p multidrug/H⁺ antiporter

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The molecular basis of polyspecificity of Mdr1p, a major drug/H⁺ antiporter of *Candida albicans*, is not elucidated. We have probed the nature of the drug-binding pocket by performing systematic mutagenesis of the 12 transmembrane segments. Replacement of the 252 amino acid residues with alanine or glycine yielded 2/3 neutral mutations while 1/3 led to the complete or selective loss of resistance to drugs or substrates transported by the pump. Using the GlpT-based 3D-model of Mdr1p, we categorized these critical residues depending on 1°/ their structural impact (“S” group), 2°/ exposure to the lipid interface (“L” group), 3°/ buried but not facing the main central pocket, inferred as critical for the overall H⁺/drug antiport mechanism (“M” group) and finally 4°/ buried and facing the main central pocket (“B” group). Among “B” category, 13 residues were essential for the binding of a large majority of drugs/substrates, while 5 residues displayed specificity towards substrates, implying their role in governing polyspecificity (P group). 3D superposition of the substrate-specific MFS Glut1 and XylE with the MDR substrate-polyspecific MdfA and Mdr1p revealed that the B group forms a common substrate-binding core while the P group residues are only found in the 2 MDR MFS, distributed into 3 areas around the B core. This specific pattern has let us to conclude that the structural basis for polyspecificity of MDR MFS is the extended capacity brought by residues located at the periphery of a binding core to accommodate compounds differing in size and type.
Biochemical characterization of the caspase-like subunit, GPI8, of the GPI-transamidase complex in Candida albicans

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Glycosylphosphatidylinositol (GPI) anchor is a glycolipid anchor found in all eukaryotes. It is attached as a post translational modification in the lumen of the endoplasmic reticulum (ER) to a protein having a C-terminal GPI attachment signal sequence (SS). GPI anchored proteins serve a variety of functions in the plasma membrane and cell wall for example, as enzymes (e.g. alkaline phosphatase, acetylcholinesterase), receptors (e.g. folate receptor, CD14), cell adhesion molecules (e.g. NCAM), coat proteins or prion proteins. The signal sequence of the proteins destined to be GPI anchored must be recognized and cleaved by the transamidase complex to generate the mature GPI anchored protein. The GPI transamidase complex in yeast and human consists of five subunits, Gpi8 (PIG K in human), Gaa1 (GPA1), Gpi16 (Pig T), Gab1 (PIG U) and Gpi17 (PIG S), all of which are localized in the ER membrane. Mechanism of transamidation, contribution of each subunit for completion of transamidation and also the stoichiometry of each subunit in an active transamidase complex are poorly understood. Here, we have generated CaGPI8/gpi8 strain of Candida albicans, a pathogenic fungus. In metabolic labeling experiments, CaGPI8/gpi8 cells showed accumulation of complete precursor lipid (CP2) along with reduced level of GPI anchored proteins than wild type cells. In addition, rough ER fraction of CaGPI8/gpi8 cells was unable to cleave the peptide substrate (a mimic of GPI anchor signal peptide) while rough ER fraction from the wild type could, which led us to conclude that GPI8 is required for the transamidation reaction specifically for recognition and cleavage of the GPI anchor signal peptide. Moreover, CaGPI8/gpi8 cells were resistant to cell wall perturbing agents like SDS and CFW, suggesting that cell wall integrity pathways have been up regulated in the mutant to counteract the deficiency of GPI anchored proteins.
Role for CgHog1 kinase in iron homeostasis in *Candida glabrata*

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*Candida glabrata* is the second most prevalent *Candida* species in Candida blood stream infections. Being phylogenetically more close to the non-pathogenic yeast *Saccharomyces cerevisiae* compared to other Candida spp, *C. glabrata* shares key components of the iron acquisition and homeostatic machinery with *S. cerevisiae*. Iron is an essential element for growth under both in vitro and in vivo conditions. In our laboratory, we are interested in investigating the molecular mechanisms underlying the regulation of iron homeostasis under iron-deplete and iron-replete conditions in *C. glabrata*. Towards this, we have demonstrated the CgHog1 MAPK (mitogen-activated protein kinase) to be a key determinant of iron homeostasis and virulence in *C. glabrata*. We have shown that *C. glabrata* cells respond to high external iron levels through phosphorylation of the CgHog1 kinase at threonine and tyrosine residues at 174\textsuperscript{th} and 176\textsuperscript{th} positions. To identify substrates of the CgHog1 kinase, we have performed the CgHog1 protein interactome analysis in cells grown under iron-limiting and iron-surplus conditions. These findings along with a role of CgHog1 in the transcriptional response of *C. glabrata* to changes in the environmental iron content will be presented.
Ras1 activates the first step of GPI biosynthesis in *Candida albicans*

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Glycosylphosphatidylinositol (GPI) anchor biosynthesis is a multi-step pathway found in all eukaryotes. GPI anchored proteins are involved in a variety of functions like adhesion, host cell attachment, invasion, biofilm formation etc. in *Candida albicans*. The first and committed step of the pathway i.e., formation of GlcNAc-PI involves an enzyme complex (GPI-GnT) of six subunits viz. CaGpi1, CaGpi2, CaGpi3, CaGpi15, CaGpi19 and CaEri1. We previously reported that there is cross-talk between GPI anchor biosynthesis, Ras signaling and ergosterol biosynthesis in which CaGPI2 controls hyphal morphogenesis through Ras1 and CaGPI19 seems to regulate azole drug response via CaERG11. Interestingly, both CaGPI2 and CaGPI19 negatively co-regulate each other. Further investigations on how Ras signaling cross-talks with GPI biosynthesis in *C. albicans* suggest that Ras activates GPI-GnT activity which is exactly opposite to what is seen in *Saccharomyces cerevisiae*. Out of the two Ras proteins, Ras1 alone can activate GPI-GnT activity while Ras2 cannot. Different forms of Ras1 activates GPI-GnT activity too *in vivo*. This inference is further strengthened by the fact that Ras1 physically interacts with CaGpi2 which is confirmed by FRET. CaGpi2 influences Ras signaling pathway and hyphal morphogenesis through its effect on Hsp90. In other words, Ras1 is activated upon overexpression of CaGpi2 due to a downregulation of Hsp90 *in vivo*. Understanding the mechanism of interactions between Ras signaling and GPI biosynthesis pathway will enable us in targeting any of these two to combat the virulence of this human pathogenic fungus.
A comparative study of two integrative approaches, YLEX and CRISPR/Cas9, in *Yarrowia lipolytica*

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Cost effective production of cellulases is a pre-requisite for economic viability of bio-ethanol industry as cellulases accounts for more than 20% of total bio-ethanol production cost. Fungal expression systems dominate the cellulase market with *Trichoderma reesei* being gold standard for cellulase production. However to increase the biomass hydrolytic potential, fungal secretomes are supplemented with additional enzymes like β-glucosidase and ancillary proteins. Also fungal systems require special cultivation and induction conditions thus hampering the production cost efficiency. Hence, to address this issue the work aims at developing yeast based expression platform as they are capable of synthesizing and secreting protein at higher concentrations in comparison to bacteria and their fermentations are comparatively simpler when compared to fungi. *Yarrowia lipolytica*, oleaginous yeast, because of its high secretion capabilities (upto 1 g/l) has the potential to become the industrial workhorse for enzyme production. To tailor biomass specific secretome of *Yarrowia lipolytica* a fast, simple and efficient genome integration tool is desired. Therefore two different integrative approaches based on YLEX kit and CRISPR/Cas9 tool were used and compared. Firstly, Endo5A gene was integrated and expressed from its chromosome using pYLSC1 integrative vector provided with YLEX kit. To demonstrate CRISPR/Cas9 functioning in *Yarrowia lipolytica* green fluorescent protein (GFP) was integrated at XPR2 loci within its genome. In order to compare both the techniques a thermotolerant β-glucosidase was successfully integrated and expressed in *Yarrowia lipolytica*. The total enzyme activity of the supernatant was determined and compared. Though efficient, CRISPR/Cas9 tool very easily out compete YLEX kit when it comes to simple and multiple genome modifications.
**The Fission yeast sirtuin Hst4 is downregulated by Dbf4 dependent kinase and SCF ubiquitin ligase to mediate replication stress response**

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Sirtuins are NAD+ dependent class III histone deacetylases which functions in cellular processes such as cell survival, apoptosis, gene transcription, DNA damage and repair. *S. pombe* Hst4 is a deacetylase that deacetylates H3K56 residue and plays a vital role in the DNA damage response. Our previous reports show that Hst4p is downregulated during DNA damage stress. However, the mechanism of this regulation is not known. The DNA damage response pathway consists of checkpoint sensors and effectors which senses the damage and signals for DNA repair and thus contributes to genomic integrity. Here, we show that Hst4 is targeted for degradation by ATR/Rad3 checkpoint sensor kinase, however the degradation is independent of effector replication checkpoint kinase Cds1. Further, we found Hst4 is targeted by ubiquitin dependent proteolysis by a major E3 ligase SCF in response to MMS treatment. DDK kinase Hsk1/Cdc7 is the major regulator of DNA replication and is conserved from yeast to human. We found the levels of Hst4 being stabilized in the *hsk1-89* mutant cells on MMS treatment. Domain mapping analysis has revealed the role of C-terminal residues in the degradation of Hst4 which harbours the Hsk1 kinase phosphorylation sites. We also show by sensitivity assays that dynamic regulation of Hst4 is important to mediate replication stress response in *S. pombe*. Further work is undergoing to understand the effect of Hst4 regulation on replication fork stability. This study helps in understanding the dynamic regulation of chromatin factors during vital processes such as DNA replication, DNA damage response and repair, thus, promoting genome stability.
Probing the functional role of *S. pombe* splicing factor SpPrp16 and understanding its role in splice-site recognition

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The two transesterification reactions of nuclear pre-mRNA splicing occur precisely at the conserved phosphodiester bond at the exon-intron junction. These reactions result in ligated exons and excised lariat. Study of fission yeast splicing enables us to understand the co-evolution of spliceosome to recognize splice sites that are more degenerate and present in a short-intron rich genome similar to other fungal genomes. Hence, studies on pre-mRNA splicing in fission yeast allows us to examine diversity in the dynamics of this gene regulatory process. Prp16 is a DExD box RNA helicase whose budding yeast homolog promotes spliceosomal rearrangements that allows second step catalysis. Here, using in depth bio-informatic analysis from transcriptome deep sequencing data, the global splicing profile in *spprp16* and *spprp16F528S* strains was assessed. This confirmed a critical and nearly global role for SpPrp16 in splicing of fission yeast pre-mRNA. Comparative analysis of SpPrp16 dependent vs independent splicing events showed the intronic 5'SS consensus, particularly minor variations in the frequency of specific nucleotide could discriminate between these substrates. Since in budding yeast splice sites, U6 snRNA-5’SS and U2 snRNA-BS base pairing interactions play a critical role in active site interaction and transition from first to second step of splicing, these interactions were assessed using some selected SpPrp16 dependent and independent introns, expressed from plasmid mini gene transcripts. These data suggest global dependence on Prp16 for splicing is dictated by the strength of splice site-snRNA interactions in the genome. The *in vitro* RNA unwinding activity of wild type SpPrp16 and two different mutant proteins were also investigated as was their *in vitro* ATP hydrolyzing ability and RNA binding capabilities. *In vitro* studies together with the *in vivo* splicing studies observed for transcripts hints the involvement of some SpPrp16 residues in indispensable spliceosomal interactions and others in its intrinsic enzymatic activity.
Biofuel production through combined approach of consolidated bio-processing and enzyme mining

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Over reliance on natural resources for transportation purposes is leading to rapid depletion of available fossil fuels and its current impact on the universal scenario is accelerating researches on alternative strategies for fuel supply such as, bioethanol. On a global scale, 1.3x10^10 Mt (dry weight) of terrestrial plants are produced annually (Demian et al, 2005). The hydrolysis and conversion of these raw materials into commodity products such as ethanol has great economic potential. Consolidated bioprocessing (CBP), which combines enzyme production, saccharification and fermentation in a single step, has gained increased recognition as a potential bioethanol production system. Microorganisms with the ability of CBP are not present naturally. It requires a highly engineered biocatalyst developed for several process-specific characteristics.

We have identified strong cellulolytic enzymes from fungal and bacterial sources, to express in yeast using synthetic biology approaches. Degradation of cellulosic substrates by the engineered yeast strains expressing the recombinant cellulases have been achieved in terms of their specific activities and their fermentation abilities are being explored. Parallelly to introduce alternatives of industrially used expensive cellulases, the search for efficient yet cheaper enzymes is required. Herbivorous animals consume lignocellulosic biomass in bulk which is converted to free sugars by the gut microbiome. It is expected that these microbiomes are rich source of C5/C6 assimilations. Three fungal strains were isolated and identified to be Aspergillus flavus, Aspergillus niger (elephant excreta) and Aspergillus fumigatus (rhinoceros excreta). These isolates had optimum growth rate at 30 and 37°C and secreted diversified enzymes on lignocellulosic biomass. The highest cellulase activity of 1.09, 0.90, 0.68, and 1.19 FPU/mg of was shown by A. niger with wheat straw, wheat bran, rice straw, and rice bran, respectively. While, A. flavus showed optimal activity of 0.8 FPU/mg with wheat straw, and A. fumigatus showed 1.05 and 1.25 FPU/mg of with similar carbon sources.
Towards establishing NNS complex as the primary exosome-specificity-factor (ESF) in *Saccharomyces cerevisiae*

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The yeast Nrd1-Nab3-Sen1 (NNS) complex, interacts with the nuclear exosome and C-terminal domain (CTD) of RNA polymerase II (RNAPII) via its CTD-interacting domain (CID) and is postulated to participate in the nuclear mRNA surveillance by acting as the primary Exosome-specificity-factor (ESF). We investigate the relationship between NNS complex with the nuclear exosome and its cofactors, TRAMP and DRN by examining its relative contributions in the degradation of diverse classes of aberrant nuclear mRNAs generated at various phases of mRNP biogenesis. Our results revealed that NNS actively participates in degradation of all kinds of aberrant messages including mis-packaged, splicing-defective, 3’-extended transcripts and export-defective aberrant and non-aberrant messages. Remarkably, a specific deletion in the RNAPII interacting domain of *NRD1* (*nrd1ΔCID*), which fail to interact with RNAPII cannot support the rapid decay of mis-packaged mRNAs, splicing-defective messages and abnormally long 3’-extended transcripts but did support the decay of the export defective transcripts. On the other hand, a specific mutant in *PCF11*, another CID containing gene cannot support the recruitment of Nrd1p on to the export defective messages. Collective results suggest that NNS complex is involved in the degradation of various nuclear aberrant messages by acting as the primary ESF. This complex, when recruited via RNAPII during the early half of mRNP-biogenesis, helps TRAMP to assist nuclear exosome to selectively recruit the mis-packaged, splice-defective and 3-extended aberrant messages. During the late phase of mRNA biogenesis, the NNS complex is still required to assist DRN complex to selectively recruit the export-defective messages to nuclear exosome for subsequent decay, but the co-transcriptional recruitment of NNS to export defective messages is dependent on Pcf11p, instead of RNAPII. We believe that this differential recruitment of the NNS complex onto diverse aberrant messages dictates the choice of either TRAMP or DRN as the cofactor for the nuclear exosome.
Elucidating the role of cytosolic Hsp70 machinery in folding and degradation of ERAD substrate

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Cells have evolved complex interconnected network called protein quality control (PQC) system against misfolded proteins. Chaperones have their essential but distinct involvement in this quality control system for protein folding and degradation of various toxic protein aggregates. One of such pathway is Endoplasmic Reticulum Associated Degradation (ERAD) where endoplasmic reticulum quality control checks nearly all secreted proteins, and proteins that fail to achieve its native state are sorted out for degradation. Pma1*(D378T) is among widely studied ERAD substrate in Saccharomyces cerevisiae. Pma1 is a 100 kDa polytopic plasma membrane protein essential for maintaining electrochemical proton gradient. Substitution of the D378T residue of Pma1 leads to dominant lethal effect as the wild type Pma1 oligomerizes with the misfolded form and targeted for degradation causing depletion on plasma membrane. In this study using S.cerevisiae as a model organism the primary aim is to provide insight into molecular regulatory mechanism underlying roles of cytosolic Hsp70 in ERAD. As the Hsp70 activity and specificity is regulated by its different co-factors, we have investigated the role of various co-chaperones in Pma1* mediated growth defect. For this we made knockout of these co-chaperones and found that Sse1 (nucleotide exchange factor) knockout strain rescues Pma1* mediated growth defect. We have also checked the degradation rate for Pma1* in sse1Δ strain and found to be increased marginally. Our future endeavor would be to understand the role of Sse1 in Pma1* folding or degradation.
**Fungicidal geraniol modulates CDR1 efflux pump activity in *Candida albicans* and synergizes with fluconazole**

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Among the several mechanisms of multidrug resistance (MDR), overexpression of efflux pumps including CDR1 and MDR1, are the predominant mechanisms contributing to candidal infections. Therefore inhibiting or modulating the function of drug efflux transporters continues to draw attention as effective strategy to combat MDR. We have previously reported the antifungal potential of Geraniol (Ger), a naturally derived monoterpenoid from Palmarosa oil, against *C. albicans*. In the present study, we have investigated its effect on efflux pump (CDR1 and MDR1) activity. The effect of Ger was investigated by examining Rhodamine 6G dye (R6G) efflux in the overexpressing *Candida* cells for CaCdr1p and CaMdr1p. The accumulation assay by fluorescent dyes Nile red and R6G confirms the specific effect on CaCdr1p transporter. The RT-PCR results have although shown no change in expression of *CDR1*, however, confocal microscopy images depict CaCdr1p mislocalization in presence of Ger. Moreover, modulatory role of Ger by kinetic studies have revealed the competitive inhibition for rhodamine 6G efflux in CaCdr1p overexpressing cells as evident from increase in apparent Km without affecting the V_max value. The labeling with Rhodamine B demonstrates altered mitochondrial potential in Ger treated cells which was commensurate with our previous study showing dysfunctional mitochondria. Furthermore the effect of Ger on efflux pump activity, ergosterol depletion on fluconazole sensitive and resistant clinical matched pair of isolates Gu4/Gu5 correlated well with our findings. We also estimated phenotypic virulence marker such as extracellular phospholipase activity which was considerably diminished in presence of Ger. Additionally, we observed that Ger was not only fungicidal in nature but synergistic (FICI<0.5) with fluconazole. Furthermore, hemolytic activity (20%) depicts its non lethal behavior on human blood cells. Together, the modulation of efflux pump by Ger represents a promising approach for combinatorial treatment for candidiasis.
Expression analysis of the *Thermomyces lanuginosus* lipase gene

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Lipases represent the most widely used class of enzymes in biotechnological applications and organic chemistry as they bring about a wide range of bioconversion reactions. The increasing demand for thermostable lipases with high activity and stability, have paved the way for extensive research in this direction. Choosing the best expression system requires evaluating many options from yield to glycosylation, proper folding, to economics of scale up and this selection is function of the target protein to be expressed. With the aim of looking for a host with high-volume/lowcost application in industry, a synthetic lipase gene (TL-LIP from *Thermomyces lanuginosus*) was expressed in different hosts of *E. coli*, *Bacillus* and *Yarrowia lipolytica*. Cloning and purification in all the expression system was optimized. Expression was found to be highest in the order of *Yarrowia lipolytica* (secretory vector pYLSC)> *E. coli* (BL21 Shuffle) > *Yarrowia lipolytica* vector pYLEX > *E. coli* (XL1 Blue) > *Bacillus subtilis* secretory vector. The hp4d promoter in *Yarrowia* system is a strong promoter with benefit of high secretion capacity and high transformation efficiency. Also, it does not require an *E. coli* transformation step like other mentioned expression systems. With growing scientific interest in *Y. lipolytica* and numerous industrial applications, it has a huge potential for enzyme production in the market. Currently, we are working on lowering the costs of production for meeting the cost requirements of commercial enzymes.
Chromatin and transcriptional control of iron homeostasis in *Candida albicans*

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Intracellular iron homeostasis needs to be exquisitely controlled in living systems. The human fungal pathogen *Candida albicans* has evolved multiple pathways to mobilize iron from intracellular and extracellular sources in iron poor environments. Under iron replete environments, however, the expression of iron uptake system genes are turned off, and paradoxically, the genes encoding iron requiring proteins are induced. Key transcription factors HAP complex, Cap2 and Sef1 are essential for regulation of iron homeostasis under iron poor conditions and robust virulence in *C. albicans*. In this study, we identified SAGA histone modifying complex to be critical for iron homeostasis gene regulation. Our genetic data showed that SAGA is required for robust growth under iron limiting condition, for control of iron homeostasis gene expression, and for histone H3-Lys9 acetylation of promoters. Using ChIP assay, we found that each of the Core HAP complex subunits Hap2-Hap3-Hap5 and Sef1 are recruited to both low-iron induced (*FRP1*) and repressed (*ACO1*) gene promoters. However, Cap2 is recruited to the low-iron repressed (*ACO1*) gene promoter. Moreover, Sef1 promotes Hap5 recruitment at both low-iron induced (*FRP1*) and repressed (*ACO1*) gene promoters suggesting that Sef1 is epistatic to HAP complex. Hap5, Sef1 and Cap2 are required for hyperacetylation of histone H3-Lys9 at *FRP1* promoter and to maintain the hypoacetylated state of the *ACO1* promoter under low iron conditions. Consistent with the transcriptional states, RNAP II occupancy was enhanced by Hap5 and Sef1 at *FRP1* and *ACO1* promoters under low-iron and high-iron conditions respectively. Together, this study has uncovered an elaborate mechanism for adaptation to iron deprivation conditions that is controlled by the SAGA complex and the transcription factors Sef1, trimeric HAP complex and Cap2 to maintain iron homeostasis in *Candida albicans*. 
Piriformospora indica: A plant probiotic beyond host specificity

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P. indica was isolated from roots of the orchid plants found in the Thar Desert, Rajasthan, India. P. indica has a wide range of host plants from bryophytes to angiosperms and monocots to dicots. Unlike AMF, P. indica can be cultivated axenically. P. indica helps the host plants to cope up with biotic and abiotic stresses. Because of these qualities, P. indica has been termed as plant probiotic. The biochemical, molecular and physiological changes occur during colonization leads to enhance defence mechanism of host plant. The growth promotion activity of P. indica has been also found to be associated with plant survival under various stress conditions. For satisfying the demand of exponentially growing population there is need to improve crops for sustainable food production without compromising the environmental factors. Involvement of P. indica in high salt tolerance, disease resistance, proline content, chlorophyll content, phosphate uptake and growth-promoting activities leading to enhancement of host plant yield make it a potential tool for nutrient fortification of cereal crops in an ecologically beneficial way, while minimizing risk of environmental damage. Thus, using P. indica as a plant probiotic may be a new hope for improving crop yield.
Identification of motifs associated with NBD of ABC transporter family and subfamilies

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ABC transporter proteins are involved in active transport, both in prokaryotes and eukaryotes. These proteins structurally consist of four domains: two nucleotide binding domains (NBD) and two transmembrane domains (TMD). NBD is conserved during the evolution, and consist of five motifs: Walker A, Walker B, switch region which contains a histidine loop, the signature motif and the Q-motif. Various studies suggested the role ABC transporters in various inherited human diseases, tumor resistance, cystic fibrosis and in clinical drug resistance. Taking into account recent structural data which highlighted the presence of additional motifs in NBD which interact with coupling helices of TMD and role of N and C terminal motifs within NBDs. We carried out an extensive computational analysis to unravel important conserved regions which plausibly play role in the interaction of NBD with TMD. Results of sequence analysis of ABC proteins from all taxa revealed a well conserved new motif having signature PxxTVxENxxF located in each NBDs between Q-loop and ABC signature sequence. 3D-structure analysis of ABC transporters highlighted its interfacial position with the intercellular loop 1 (ICL1). Considering its wide occurrence, we examined the importance of this motif in one representative multidrug ABC transporter of \textit{Candida albicans}, Cdr1p. The motif residues were subjected to site-directed mutagenesis and replaced by alanines both individually as well as in combinations. The GFP tagged version of mutant variant proteins were overexpressed in \textit{S. cerevisiae}. While some of the single alanine mutant showed differential susceptibility towards certain drug substrates of Cdr1p, the motif of NBD2 when replaced with alanine resulted in a misfolded variant incapable of proper localization to the plasma membrane pointing to an important role of this motif in inter-locking the NBD and TMD together in a proper manner. We, thus, unveil an essential structural motif in ABC superfamily transporters.
MRX8: A potential switch that regulates mitochondrial function in response to cellular energy requirements

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Saccharomyces cerevisiae generates ATP either through fermentation or respiration depending upon availability of carbon sources. During fermentation, activity geared towards ATP production occurs in the cytosol and mitochondrial activity is maintained at a minimal level whereas during respiration ATP generation takes place in mitochondria. This “shift” requires extensive metabolic reprogramming of the cell. The metabolic state of yeast during respiration closely resembles differentiated mammalian cells; while proliferative cells are akin to yeast undergoing fermentation. Dysregulation of these metabolic states in differentiated mammalian cells has been linked to disease states including cancer. Altered gene expression of nuclearly encoded mitochondrial proteins in response to glucose is well established. However, mitochondrial genome expression and its regulatory factors remain unexplored. Mitochondrial ribosomal proteins, assembly factors and regulators are either species specific or universally conserved. MRX8, a YihA class of GTPase, predicted to function in translation, has orthologues in bacteria, yeast and vertebrates including humans but none in invertebrates. We have shown Δamrx8 cells have compromised cellular respiration. Consistent with a predicted role in translation regulation, we have shown Mrx8p is localized to the mitochondrial matrix and associates with the 74S monosome. Mutations in MRX8 that abolished nucleotide binding were not able to support cellular respiration whereas, contrary to expectation, mutants wherein the protein is predicted to be locked in a GDP-bound form weren’t compromised. Thus in vivo function of Mrx8p might involve communication of NTP/NDP cellular pools to mitochondrial ribosomes. Consistent with conservation in function, human orthologue of Mrx8p restored cellular respiration in Δamrx8 cells.
Metabolic engineering of natural isolates of *Saccharomyces cerevisiae* for second generation biofuel production

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Growing concern regarding the depletion of fossil fuels, rising energy demands and the global climate change due to emission of greenhouse gases has intensified the interest in second generation biofuel production. Biofuels offer a promising cleanest renewable alternative source of energy to meet the energy consumption across the globe. Xylose is the main pentose and second most abundant sugar after glucose in lignocellulosic materials. The rapid co-fermentation of both glucose and xylose is important for the efficient conversion of lignocellulosic biomass into fuels. *Saccharomyces cerevisiae* lacks xylose metabolizing pathway therefore, efforts are being made to engineer *S. cerevisiae* strains for efficient xylose fermentation to enable the lignocellulosic bioethanol production process economically feasible. The present study aims to construct a xylose fermenting yeast strains with engineered oxido-reductative/xylose isomerase pathway for xylose metabolism. This study also demonstrated the effect of inducible and constitutive promoters on efficiency of xylose assimilations and fermentation. The engineered strain was able to grow on xylose as sole carbon source with the maximum ethanol yield of 0.26g/g xylose and productivity of 0.07g/l/h at 96 hours. The cofactor specificity of xylose reductase (XR) and xylitol dehydrogenase (XDH) has been changed by site directed mutagenesis to overcome redox imbalance and reduce xylitol production.
Sphingolipids of *Candida glabrata* and its impact on drug susceptibility

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Although lipid metabolic pathways are fairly well established in yeast, our knowledge of lipid compositional profile, particularly in pathogenic species, is rather limited. Fungal lipids are important on two accounts; firstly, they possess lipids, particularly sphingolipids, which are unique to *Candida species* and are absent in mammalian host hence are novel drug targets. Secondly, the functionality of MDR export proteins is dependent upon optimal lipid environment implying their role in clinical drug resistance. The comprehensive high-throughput Lipidomics combined with genetic approaches is being applied to human pathogenic haploid Candida glabrata to assess strategies aimed at disrupting *Candida* lipids and particularly functional interactions between lipids, virulence, and MDR determinants. Complex sphingolipids are unique to fungi and hence provides a good target for antifungal drugs. *C. glabrata* being the second most pathogenic human fungal after albicans drawing a considerable amount of attention of researchers. Phylogenetically, *C. glabrata* is closer to *S. cerevisiae*. Despite it, some of the genes of sphingolipid pathway are essential in case of *S. cerevisiae* but are non-essential in *C. glabrata*. In our study, we have made knockout mutants of some sphingolipid pathway genes cgIPC1 (catalyzes the transfer of Phosphoinositol from phosphatidylinositol to ceramide to form IPC), cgDPL1 (Dihydrosphingosine phosphate Lyase, degrades phosphorylated long chain bases), cgISC1 (Inositol phosphosphingolipid phospholipase C, catalyze the degradation of complex phospho-sphingolipids) and cgIPT1 (Inositol phosphoryl transferase, catalyzes synthesis of most abundant sphingolipid, mannosyl diinositol diphosphoceramide [M(IP)2C] from mannosyl inositol phosphoceramide [MIPC] by using fusion PCR based method. Gene deletion cassette was constructed by fusing dominant marker NAT1 with the flanking UTR regions of the gene. Cassettes are then transformed and allowed for homologous recombination, which replaces the gene with NAT1. The knockout was selected on a nourseothricin drug plate and confirmed by gene specific PCR. The preliminary analysis of some of the sphingolipid mutants revealed interesting phenotypes. For instance, loss of [M(IP)2C] in the ipt1 disruptant resulted in altered susceptibility towards azoles and cell wall perturbing agents. The other mutants such as cgIPC1 and cgISC1 became highly susceptibility to H$_2$O$_2$, acetic acid, caffeine, and SDS. The analysis of some of these mutants will be presented.
Functional insights into half size ABC transporter protein CaABCG2 of PDR subfamily of Candida albicans

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Candida albicans genome comprises extremely diverse class of 26 putative ABC proteins coding genes, which are belongs 7 subfamilies including PDR, MDR, MRP, ALDP, YEF3 and RLI. PDR subfamily is largest ABC subfamily, having 9 members, contains full (two TMD and two NBD) and half size (one TMD and one NBD) transporters. The overexpression of rapid drug extrusion pumps encoding genes of PDR subfamily such as CDR1 and CDR2 is most predominant mechanism by which the human pathogen Candida albicans acquires MDR. Other members of PDR subfamily such as CDR3 and CDR4 do not have much demonstrable role in drug susceptibility but do participate as phospholipid translocators and maintain membrane lipid asymmetry. While some of the transporters role in the development of drug tolerance is well established but the existence of battery of transporters in Candida genome points to their relevance in its cellular physiology which remains to be uncovered. This study focuses on an uncharacterized PDR subfamily transporter (orf19.3120/CaABCG2) that is a homologue of human ABCG2 transporter protein, which is very relevant in breast cancer. We show that similar to CDR1 and CDR2 proteins, CaABCG2 is localized in plasma membrane and its deletion does not affect cell surface morphology and hyphal formation. CaABCG2 overexpression or deletion did not impact on drug susceptibilities in C. albicans. Transcriptional profiling of the CaABCG2Δ/Δ mutant revealed significant (≥2-fold) down-regulation and up-regulation of 191 and 941 genes, respectively as compared to WT. These genes are involved in different functions such as biofilm formation, lipid metabolism, metabolic pathways, oxido-reductase activity, virulence and hyphal development etc. High throughput MS-based lipidome analysis revealed that the CaABCG2p levels affect lipid homeostasis and deletion of it resulting in significantly decrease in LPC (lysophosphatidylcholine) and LPE (lysophosphatidylethanolamine) content as compared to WT. Interestingly, we observed that the survival of CaABCG2Δ/Δ strain in presence of macrophage is significantly reduced as compared to WT. Together, our study proposes novel roles of the CaABCG2 in lipid homeostasis and host pathogen interaction. Further investigation of gene categories which are differentially regulated in CaABCG2Δ/Δ may provide novel insights into indirect role of this ABC transporter.
Characterization and Antifungal susceptibility of clinical isolates from the different cohort of patients from Haryana hospitals

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Among the human pathogens, fungi are referred to as “hidden killers”, as more people die from the invasive fungal diseases than from tuberculosis or malaria. Candidemia is considered as the major cause of invasive fungal infections worldwide with incidence ranging from 2-14 cases per 100000 persons and 6.87 cases per 1000 ICU patients. The developing countries currently struggle which have 4-15 times higher rate of candidemia. Over the past two decades many non- *Candida albicans*, *Candida* (NCAC) species have emerged as significant pathogens of clinical importance. The NCAC species are a very heterogeneous group of organisms that are fundamentally different from each other and from *C. albicans* at the biological level. This diversity in the range of *Candida* species now associated with human infection has provided new challenges in the diagnosis and treatment of Candidiasis and in the study of their virulence and biology. In the early 20th century, *C. albicans* was considered as the only *Candida* species of medical importance, while *C. parapsilosis*, *C. tropicalis* and *C. auris* were considered as occasional pathogens. The prevalence of fungal infections and their resistome in fungal isolates from hospitals particularly from Haryana region remain poorly monitored. The lack of data on resistome and fungi biome has serious implications for the management of mycoses. For this study, we collected samples directly from the repository of the hospitals, we only collected *Candida* positive samples for our analysis. This was done in collaboration with the participating hospitals in Haryana e.g. Fortis Health Care, Gurgaon, Medanta -the Medicity, Gurgaon, Micro Pathology Services P.Ltd,Gurgaon and Kalawati Hospital, Rewari during the period from December - 2015 to March- 2017. A total of 200 samples were collected from the repository of the different hospitals which were earlier recovered from blood, urine, CSF, sputum, high-vaginal swabs, BAL etc. The order of prevalence was; *C. albicans* (68 %), *C. tropicalis* (16 %), *C. glabrata* (4 %), *auris* (8%) and rest were *C. parapsilosis*, *C. dublinicencias*, *C. kefyr*. Out of 200 samples 27 % samples were found to be drug resistant, 9 % displayed intermediate or Drug Dose Dependent resistance. Over all sensitivity profile of *Candida* species revealed that 23 % were resistance to fluconazole and variconazole. Among the five anti-fungal tested, caspofungin and miconazole showed higher susceptibility against all *Candida* species. The lack of data on resistome has serious implications for the management of mycoses. This study is an attempt to fill the existing gap between awareness of fungal infections in Haryana region.